BioMimetic PHARMACEUTICALS

GEM 21S™

A GROWTH-FACTOR ENHANCED MATRIX
(BONE VOID FILLER WITH WOUND HEALING ADJUNCT)

ORIGINAL PRE-MARKET APPROVAL (PMA) APPLICATION

PMA APPLICATION NO. P040013
(IDE NO. G010340)

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VOL. I

BioMimetic
PHARMACEUTICALS

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1.0 INTRODUCTION

The GEM 21S™ device is provided to the user in a kit form that is composed of the following three individually marketed components, two of which are purchased as bulk product that are processed to BioMimetic specifications:

1) Vitoss™ synthetic beta tri-calcium phosphate ("β-TCP") granulate particles, that is a sterile, porous bone void filler for the repair of bony defects supplied by Orthovita, Inc.;

2) Becaplermin, a sterile solution of highly purified recombinant human platelet-derived growth factor ("rhPDGF-BB"), supplied by Chiron Corporation; and

3) A blunt-end cannula that is provided in the kit as an accessory to aid in the delivery of the rhPDGF-BB solution and is purchased as a finished component.

These GEM 21S™ components are marketed pursuant to a cleared premarket notification ("510(k) notice"), an approved biologics license application ("BLA"), and a 510(k) exemption, respectively. Information regarding the approval/clearance of becaplermin and Vitoss is provided in the following table:

Approval/ Clearance Number	Product Name	Decision Date
BLA Ref No. 96-1422/CBER STN 103692	Becaplermin	December 16, 1997
K994337	Vitoss Scaffold Synthetic Cancellous Bone Void Filler	December 14, 2000

Letters of authorization from Orthovita and Chiron permitting the Agency access to these FDA files in support of this PMA have been included in the PMA. The blunt end needle included in the kit is supplied to BioMimetic by Kendall, a Division of Tyco Healthcare Group Growth Enhanced Matrix ("GEM 21S™").

1.1 Intended use/Indication for Use

GEM 21S™ is intended for use as a bone replacement and regeneration system. This device is indicated to treat osseous defects resulting from:

- periodontal disease;
- cystectomy;
- apicoectomy;
- deficient alveolar ridges; and
- tooth extraction.

1.2 Technological Characteristics

GEM 21S™ is a synthetic bone matrix that incorporates an adjunctive wound-healing agent. The device consists of two FDA cleared or approved products: (1) Beta-Tricalcium Phosphate ("ß-TCP"), which Orthovita, Inc. ("Orthovita") markets as Vitoss™ Scaffold Synthetic Cancellous Bone Void Filler ("Vitoss") (K994337); and (2) a highly purified

recombinant human platelet-derived growth factor ("rhPDGF-BB") called Becaplermin for which Chiron Corporation ("Chiron") obtained approval under BLA#96-1422 and which Chiron supplies to Johnson & Johnson to market in a gel form under the trade name Regranex® pursuant to BLA#96-1408.

1.3 Principles of Operation

The sterile ß-TCP component of the device physically fills the bone defects and provides a biocompatible, osteoconductive, and three-dimensional scaffold to facilitate new bone formation. The rhPDGF-BB component (0.3 mg of Becaplermin per milliliter in a sodium acetate buffer) enhances the physical and mechanical actions of the ß-TCP component by promoting bone and periodontal ligament cell proliferation (mitogenesis), cell migration (chemotaxis) into the wound and matrix, and promoting revascularization (angiogenesis) of the surgical site. GEM 21S™ is resorbed and replaced by autologous bone during the healing process.

1.3.1 Mode of Use

In order to implant the GEM 21S™ device, the clinician exposes the bony defect with a periosteal flap and removes the granulation tissue. Next, the clinician conditions the tooth root surfaces using a tetracycline paste or another root conditioning agent, e.g., citric acid, if the patient is allergic to tetracycline. The clinician fully saturates the β-TCP particles with the rhPDGF solution to rehydrate it. The clinician estimates how much product is needed depending upon the size of the void to be filled. The clinician then carefully packs the GEM 21S™ product into the bone void so that it is in direct contact with well-vascularized bone without overfilling the defect. Finally, the clinician sutures the periosteal flaps to achieve primary closure. The

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GEM 21S™ is a single use product so any remaining β-TCP or rhPDGF that is not used to treat an individual patient is to be discarded after the procedure.

1.4 Functional Characteristics

GEM 21S™ physically fills the bone defects in order to provide a biocompatible, osteoconductive, and three-dimensional scaffold to facilitate new bone formation. It also stabilizes the blood clot and prevents the collapse of scar tissue into the bone void, thus leading to bone formation and regeneration. The rhPDGF-BB component enhances the physical and mechanical actions of the ß-TCP component by promoting angiogenesis, cellular in-growth into the periodontal osseous defect and scaffold, and revascularization of the wound site. GEM 21S™ is resorbed and replaced by autologous bone during the healing process.

1.5 Description of Device Functional Components

BioMimetic Pharmaceuticals, Inc. obtains β-TCP and rhPDGF, which are the functional components of GEM 21S™, in bulk from Orthovita and Chiron, respectively. BioMimetic is incorporating the descriptions of β-TCP and rhPDGF from their premarket submissions as authorized by their manufacturers.

1.5.1 ß-TCP

The ß-TCP component of GEM 21S™ is the same material as Orthovita's 510(k) cleared Vitoss (K994337), which is indicated for filling "bony voids or gaps of the skeletal system (i.e., the extremities, spine, and pelvis)". Vitoss's labeling is included as reference. Vitoss is a synthetic and resorbable porous calcium phosphate bone void filler for the repair of bony defects. It has a similar chemistry to human cancellous bone. It also has a molecular structure that resembles the multidirectional interconnected porosity of human

cancellous bone. Vitoss conforms to ASTM Standard F 1088-87 (R1992) "Standard Specification for Beta-Tricalcium Phosphate for Surgical Implantation". The β-TCP component of GEM 21S™ is chemically and physically identical to Vitoss, which is also sterilized by gamma radiation, except that it consists of only the smaller size Vitoss particle.

Moreover, the β-TCP component of GEM 21S™ has the same intended use as the cleared Vitoss: physically filling bone defects that are from traumatic injury to the bone or are surgically created. Although GEM 21S™'s proposed indication is for periodontal use while Vitoss is cleared for orthopedic use, the same β-TCP product serves the same function of providing a scaffold for bone generation in both GEM 21S™ and Vitoss. Thus, the effectiveness of GEM 21S™ as a bone void filler has already been established.

The safety of β-TCP in periodontal applications is well established. The FDA's Dental Products Advisory Panel voted unanimously to reclassify periodontal devices composed of TCP as Class II on May 22, 2003. As FDA pointed out at that Advisory Panel meeting, there have been no known published reports of adverse responses to TCP products in humans during more than 25 years of clinical use, including more than 20 years of use as a periodontal bone defect filler. A review of FDA's Manufacturer and User Facility Device Experience Database (MAUDE), conducted on March 8, 2004, revealed that there have been no reported adverse events regarding Vitoss, which shows the safety of the β-TCP component of GEM 21S™.

1.5.2 rhPDGF-BB

The rhPDGF component of GEM 21S™ is Becaplermin, for which Chiron obtained approval (BLA #96-1422) (hereinafter the terms "rhPDGF-BB" and "Becaplermin", which is its chemical name, will be used interchangeably). Johnson & Johnson markets Chiron's

Becaplermin in gel form (0.01%) under the trade name Regranex for the treatment of slowly healing diabetic, neuropathic cutaneous wounds in the lower extremities, which are refractory to standard medical treatment. Labeling for Regranex is included as reference.

A number of different isoforms of PDGF occur naturally in the body, including PDGF-BB, PDGF-AB, PDGF-AA, PDGF-C and PDGF-D. PDGF, which is an endogenous peptide, is produced in low or undetectable levels in normal cells, but its production can be increased by factors released at the site of injury (e.g. TGF-ß, TNF-a, thrombin). Chiron produces Becaplermin by recombinant DNA technology by inserting the gene for the B chain of platelet-derived growth factor (PDGF) into the yeast, *Saccharomyces cerevisiae*. Becaplermin has a molecular weight of approximately 25 kilodaltons ("kDa") and is a homodimer composed of two identical polypeptide chains, each compound of 109 amino acids, that are bound together by disulfide bonds.

BioMimetic will obtain the Becaplermin concentrate in bulk directly from Chiron and will dilute the Becaplermin in 20mM of sodium acetate buffer (pH of 6.0), which is the same buffer in which Chiron supplies the Becaplermin, under aseptic conditions so that there are 0.3 milligrams of Becaplermin per milliliter of sodium acetate buffer (hereinafter "0.3 mg/ml"). The becaplermin is aseptically filled using standard Becton Dickinson Hypak Syringes with 0.5 ml of the rhPDGF-bb.

Becaplermin (rhPDGF-BB) has the same intended use in GEM 21S™ and Regranex, *i.e.,* stimulating healing by regenerating tissue. Although GEM 21S™ is indicated as a system for regeneration of periodontal defects while Regranex is approved for the healing/regeneration of diabetic foot ulcers, the Becaplermin in both products promotes cellular

in-growth. FDA's approval of Regranex demonstrates Becaplermin's safety and effectiveness as a wound healing agent.

In addition, the amount of Becaplermin in GEM 21S™ is significantly less than the total amount of Becaplermin that a patient would receive during Regranex treatment. Each gram of Regranex Gel contains 100 μg of Becaplermin. The amount of Becaplermin in the 0.5 ml rhPDGF solution in the GEM 21S™ kit is 0.15 mg but the amount of the combined β-TCP and rhPDGF product used depends on the size of the individual patient's periodontal defect and thus, not all of the product will be used in each patient. Moreover, Regranex is approved for chronic use involving multiple applications, *i.e.,* at least 140 daily applications during a 20 week period, whereas the GEM 21S™ is intended for a single application. Thus, the concentration of Becaplermin in GEM 21S™ is only a small fraction of the total amount of Becaplermin delivered during Regranex treatment.

Furthermore, more than 100 published studies during the last 15 years have demonstrated the safety and effectiveness of rhPDGF in promoting tissue regeneration. As also explained in detail in the clinical study report, BioMimetic conducted a clinical trial of GEM 21S™ that not only confirmed the safety and effectiveness of Becaplermin as a wound healing agent when used adjunctively to β-TCP in periodontal bone defects, but also showed that β-TCP plus rhPDGF-BB is more effective and at least as safe as β-TCP alone in regenerating periodontal bone.

A Summary of the Safety and Effectiveness Data

2. General Information

2.1 Device Generic and Trade Name

A Growth-factor Enhanced Matrix
(Bone void filler with wound healing adjunct)

GEM 21S™

2.2 Applicant's Name and Address

BioMimetic Pharmaceuticals, Inc.
330 Mallory Station No. A1
Franklin, TN 37067
Telephone: (615) 844-1280
Fax: (615) 844-128

2.3 Indications for Use

GEM 21S™ is intended for use as a bone replacement and regeneration system. This device is indicated to treat osseous defects resulting from:

- periodontal disease;
- cystectomy;
- apicoectomy;
- deficient alveolar ridges; and
- tooth extraction.

2.4 Contraindications

Standard bone graft contraindications apply. GEM 21S™ is not intended to provide structural support during the healing process and is contraindicated in cases where structural support of the defect is not possible.

GEM 21S™ is contraindicated in the presence of one or more of the following clinical situations:

(1) untreated acute infections at the surgical site;

(2) untreated malignant neoplasm(s) at the surgical site;

(3) patients with know hypersensitivity to either ß-TCP or rhPDGF-BB;

(4) metabolic or systemic bone disorders that affect bone or wound healing;

(5) intraoperative soft tissue coverage is not planned or possible; and

(6) conditions in which general bone grafting is not possible.

2.5 Warnings and Precautions

The labeling for GEM 21S™ contains warnings and precautions.

2.5.1 Warnings

- The following adverse events may occur due to a bone surgical procedure and/or the administration of local anesthesia: swelling, pain, bleeding, hematoma, dizziness, fainting, difficulty breathing, eating, or speaking, sinusitis, headaches, increased tooth mobility, superficial or deep wound infection; cellulites, wound dehiscence, neuralgia and loss of sensation locally and peripherally and anaphylaxis. Patients being treated with GEM 21S™ may experience some of these adverse events. Occurrence of one or more of these conditions may require an additional surgical procedure and may also require removal of the bone void filler.

- Complete postoperative wound closure is essential. GEM 21S™ must not be used to repair bone defects where soft tissue coverage cannot be achieved.

2.5.2 Precautions

- GEM 21S™ is intended for use by clinicians familiar with surgical grafting techniques. GEM 21S™'s radiopacity is comparable to that of bone and diminishes as it is resorbed. This moderate radiopacity may mask underlying pathological conditions and must be considered when evaluating X-rays.

- It is not known if GEM 21S™ interacts with other medications.

- The use of GEM 21S™ with other drugs has not been studied.

- Carcinogenesis and reproductive toxicity studies have not been conducted.

- The use of GEM 21S™ in pregnant women has not been studied; it is not known whether rhPDGF-BB is excreted in nursing women.

- Safety and effectiveness of GEM 21S™ in pediatric patients below the age of 16 years have not been established.

- GEM 21S™ is a prescription device. No specialized training is required .specific to the use of the GEM 21S™ device beyond that required to perform bone grafting procedures in general.

- Careful consideration should be given to alternative therapies prior to performing bone grafting in patients: (1) Who have severe endocrine-induced bone diseases (e.g. hyperparathyroidism); or (2) Who are receiving immunosuppressive therapy.

- **The GEM 21S™ bone grafting material is intended to be packed into periodontal osseous defects. It must not be injected systemically.** GEM21S™'s radiopacity is comparable to that of bone and diminishes as it is resorbed. This moderate radiopacity may mask underlying pathological conditions and must be considered when evaluating x-rays.

2.6 Device Description

GEM 21S™ is described briefly in this Section.

2.6.1 Device Components

GEM 21S™ is a combination of the following two individually marketed components:

(1) VitOss, an osteoconductive, biocompatible and resorbable synthetic beta tri-calcium phosphate (β-TCP), that is a sterile, porous bone void filler for the repair of bony defects. The β-TCP particle size of the 510(k)-cleared VitOss device ranges from 250 to 2000 μm. The β-TCP used in the GEM 21S™ has been sieved to a particle size range of 250 to 1000 μm to accommodate smaller bone voids such as those encountered in periodontal surgeries; and

(2) Becaplermin (rhPDGF-BB), a highly purified recombinant human platelet-derived growth factor.

GEM 21S™ is supplied in "kit" form with individual sterile components for single use and contains:

(1) a perio-cup of 0.5 cc of sterile β-TCP;

(2) a Hypak syringe containing a 0.5 ml of dilute (0.3 mg/ml) rhPDGF-BB sterile solution (Becaplermin) (in 20 mM NaAc Buffer) in identical volumetric proportion with β-TCP; and

(3) an accessory blunt needle (or blunt end cannula).

The kit is currently available in a single volume and a single concentration.

2.6.2 Device Operation

Following debridement and root planning, the clinician fully saturates the β-TCP (VitOss) particles with the Becaplermin solution. The root surface is conditioned with tetracycline, the hydrated graft is packed into the osseous defect, and standard surgical techniques are used to complete the procedure. Surrounding tissue flaps are then secured with interdental sutures to achieve complete coverage of the surgical site. Standard medical practices are followed for postoperative patient management.

2.6.3 Principles of Operation

GEM 21S™ is a periodontal implant used to physically fill intra-osseous bone defects and acts as a bone regeneration system to facilitate new bone formation into the space occupied by the resorbing implant. The bioresorbable β-TCP component of GEM 21S™ serves as a scaffold to guide the three-dimensional regeneration of bone in a clinical defect site into which it is implanted. The rhPDGF-BB (Becaplermin) component of the GEM 21S™, which is added to the β-TCP scaffold, is a wound healing agent that promotes cellular in-growth into the intra-osseous defect and scaffold and revascularization of the wound site.

2.7 Marketing History

GEM 21S™ has not yet been marketed. However, a prospective, randomized, blinded clinical investigation ("RCT") performed in the US on the GEM 21S™ resulted in a no serious and/or unanticipated adverse events attributable to GEM 21S™. Furthermore, the two components comprising GEM 21S™, VitOss (β-TCP), and Regranex ("Becaplermin"), are each separately marketed. Consequently, the product marketing history presented below concerns these two individual components of GEM 21S™.

VitOss, for use as a bone void filler in orthopedic and periodontal applications, received marketing approvals in Australia (March 2001) and the European Union (October 2000). VitOss received marketing approval in United States (December 2000 and August 2003) for orthopedic applications, only. Regranex gel (Becaplermin), for use in the treatment of diabetic ulcers, received marketing approvals in the European Union (March 1999), Australia (August 1999), Canada (December 1998), and the United States (December 1997). VitOss and Regranex have not been withdrawn from these markets for any reasons. Furthermore, a review of FDA's Manufacturer and User Facility Device Experience Database (MAUDE), conducted on February 5, 2004, showed no records of adverse device experience with VitOss, confirming the continued safe use of this bone void filler.

2.8 Potential Adverse Effects of Device on Health

The potential adverse events arising from the use of GEM 21S™ for periodontal applications, as well as each of the device's components, are discussed below.

<u>GEM 21S™ β-TCP Component</u>

β-TCP has been successfully used in orthopedic applications without adverse effects. For orthopedic applications, the following complications have been reported to result from bone grafting surgical procedures where anesthesia was used and are considered to be unlikely complications for β-TCP when used in periodontal applications but that can not be ruled out: superficial and deep wound infection with osteomyelitis, loss of reduction, cyst recurrence, hematoma, and cellulitis. During the May 22, 2003, FDA Dental Advisory Panel, FDA identified several potential risks associated with the use of β-TCP in dental procedures, dental surgical and treatment procedures including: infection of soft tissue and/or bone, adverse tissue reaction, incomplete or lack of bone formation, failure to osseointegrate, device migration or extrusion and weakness of newly formed bone. At that same panel meeting, FDA identified several special controls that lessen these risks including clear labeling indications and instructions for use, compliance with recognized standards to ensure sterility and biocompatibility of these devices, and animal or human testing to assess performance characteristics. The Dental Advisory Panel voted unanimously to reclassify β-TCP as Class II based upon the identified device risks that could be mitigated through special controls recommended by FDA.

GEM 21S™ Becaplermin Component

A review of the clinical safety of Becaplermin gel, which is prescribed at a higher dosage than that contained in GEM 21S™, has been published. (1). The report was based on six well-controlled human clinical studies that included patients with lower extremity diabetic neuropathic ulcers. Patients receiving Becaplermin gel (n=538), placebo (n=278) or good ulcer care alone (n=190) had a similar incidence of ulcer-related adverse events such as infection, cellulitis, or osteomyelitis. The most common adverse event reported in these dermatological trials was erythematous rashes, which occurred in 2% of patients treated with Becaplermin gel and placebo, and none in patients receiving good ulcer care alone. No such reaction was reported in the GEM 21S™ clinical trial

The incidence of cardiovascular, respiratory and musculoskeletal and central and peripheral nervous system disorders were similar across all treatment groups. Mortality rates were also similar across all treatment groups. Patients with Becaplermin gel did not develop neutralizing antibodies against Becaplermin.

GEM 21S™ Periodontal Use

The primary risks associated with the use of GEM 21S™ in periodontal bone regeneration are associated with periodontal surgical procedures itself and include infection, swelling, pain, bleeding, and increased tooth mobility.

2.9 Summary of Nonclinical Studies

The nonclinical studies are summarized below:

2.9.1 Safety/Biocompatibility of GEM 21S™

Published data regarding the safety of GEM 21S™'s components and biocompatibility testing of the device are summarized below.

2.9.1.1 Published Studies of the Safety of the GEM 21S™ Components (β-TCP and PDGF)

Published studies regarding the safety of ß-TCP and PDGF are summarized in this Section.

β-TCP

β-TCP is a purified, multicrystalline, porous form of calcium phosphate. The $Ca:PO_4$ ratio of β-TCP is 1.5 and is similar to that found in bone mineral (1-7). A number of animal and human studies have shown that β-TCP is compatible with host tissue and elicits no adverse reactions (2-16). Ingrowth of bone into the β-TCP bone substitute has been observed in numerous animal models for the treatment of various types of defects in numerous locations of the skeleton, as well as for human periodontal defects (17-26). In over 25 years of use as a bone void filler, there are no known reports in the literature of unfavorable responses to β-TCP. Additionally, there are no known interactions with drugs. The β-TCP used in GEM 21S has undergone biocompatibility testing in conformance with the ISO 10993 standard and has been determined to be biocompatible.

PDGF

Several published in vitro and in vivo studies containing data demonstrating that PDGF, when applied at the low, safe levels provided for in the GEM 21S™ device, has a beneficial effect when used in a single application. Specifically, these published studies demonstrate that:

(1) PDGF is naturally present in the bone matrix and is produced locally at fracture sites (27-30) and is a necessary component in the promotion of normal fracture repair (31);

(2) PDGF provides for enhanced repair of craniofacial bone defects using only 2-3 ng of PDGF per day in chitosan-TCP sponges, supported improved bone regeneration (32);

(3) Platelet Rich Plasma, which contains small amounts of PDGF, when combined with allograft, increases the rate of bone formation in clinical use (33);

(4) Clinical use of low amounts of PDGF (50μg/ml) was found to regenerate bone and to be safe when applied in a single application to periodontal bone defects. The study used a combination of growth factors to include IGF (34);

(5) PDGF enhanced bone regeneration even when used in conjunction with the current therapeutic 'Gold Standard' of Demineralized Bone Matrix (DBM) (35);

(6) The pleotrophic effects of PDGF on both the bone and periodontal ligament (PDL) make it well suited for enhancing regeneration of all the components of the periodontium *i.e.,* the supporting tissues around teeth. Several investigators have demonstrated the potent effects of PDGF on the proliferative activity of periodontal ligament fibroblasts *in vitro* (36-39);

(7) PDGF stimulates gingival fibroblast hyaluronate synthesis, a prerequisite for the formation of large aggregates of proteoglycans that provide the lattice for the extracellular matrix (40). The growth factor has also been shown to promote periodontal ligament fibroblast proliferation beneath expanded polytetrafluorethylene (ePTFE) in protected fenestration defects in dogs (41-43);

(8) Clinical studies to assess the clinical significance of PRP, in reconstruction of large mandibular discontinuity defects in patients treated with autograft plus PRP gel demonstrated increases in both the rate of bone formation and bone density as evaluated clinically, radiographically and histologically compared to patients who received the autograft without PRP (44). These investigators confirmed increased platelet counts in PRP, increased PDGF and TGF-β concentrations in the PRP gel, and the presence of PDGF and TGF-β receptors on the surface of cells within iliac crest autografts; and

(9) A clinical study of the safety and preliminary effectiveness of rhPDGF-BB (1.0 mg/ml) in bone allograft in treating patients possessing intra-osseous and/or furcation periodontal lesions demonstrated a favorable outcome clinically, radiographically, and histologically following application of the rhPDGF-BB product. The PDGF/allograft treatment was found to result in periodontal regeneration, including restoration of bone, cementum, and periodontal ligament. No adverse effects of the treatment were reported (45).

In summary, there is an extensive body of scientific publications that support: (1) the role and effectiveness of PDGF in stimulating bone cell proliferation and recruitment (chemotaxis) to include bone and regeneration; and (2) the acceptable safety profile of the single application PDGF contained in the GEM 21S™ as demonstrated by published clinical data on the safe use of PDGF at levels far exceeding the low levels of PDGF contained in GEM 21S™.

2.9.2 Biocompatibility Testing of GEM 21S™

Tripartite biocompatibility testing in accordance with the standard EN ISO 10993-1:1997 "Biological evaluation of medical devices - Part 1: evaluation and testing" was conducted on the GEM 21S™. All studies were performed in accordance with Good Laboratory Standards (GLPs). The following tests were performed.

- *In vitro* Genotoxicity: Bacterial Reverse Mutation (Ames) test with Salmonella and E. coli strains using saline and DMSO extracts. No evidence of mutagenicity to Salmonella and E. coli strains were observed.
- Sensitization in 15 Guinea Pigs using saline and cottonseed oil (CSO) extracts. Saline and cottonseed oil extracts from the device were individually injected intradermally into guinea pigs and occlusively patched. Following a recovery period, a challenge patch was placed. The sites were evaluated at 24 to 96 hours. No evidence of delayed dermal contact sensitization was observed for either extract.
- *In vitro* Cytotoxicity, MEM elution. An extract of β-TCP and Becaplermin (PDGF) was made using minimal essential medium, and flowed over a confluent monolayer of mouse fibroblasts. The confluency of the monolayer, percent lysis, and cellular characteristics were analyzed to determine potential cytotoxicity. The 1X MEM test extract showed mild or no evidence of causing mild cell lysis or toxicity. All samples exposed to the 1X MEM test extracts had a biological response of less than or equal to grade 2 (mild reactivity).
- Intracutaneous reactivity in rabbits that were intracutaneously injected with saline and cottonseed oil extracts from the device. As compared to blank vehicles as the control, there was no evidence of significant irritation or toxicity from the extracts. There was evidence of slight irritation from the 0.9% sodium chloride USP solution injected intracutaneously into rabbits. The Primary Irritation Index Characterization for the cottonseed extract was negligible and slight for saline.
- Acute systemic toxicity in mice that were injected intravenously or intraperitoneally with saline and cottonseed oil extracts from the device. Under the conditions of this study, there was no mortality or evidence of systemic toxicity from the extracts.
- Muscle Implantation Study in the Rabbit. The test article was prepared and then was surgically implanted in muscle tissue of the rabbit. The muscle tissue was evaluated for evidence of irritation or toxicity. At 4 weeks, the

macroscopic reaction was not significant as compared to the comparative control material and not significant as compared to the USP negative control implant material. Microscopically, the test article was classified as a non-irritant as compared to the comparative control material and a slight irritant as compared to the USP negative control article. Additionally, the comparative control was considered a slight irritant as compared to the USP negative control article.

The biocompatibility studies described above demonstrate the biocompatibility of GEM 21S™. There was no evidence of mutagenicity, delayed dermal contact sensitization, systemic toxicity and only slight to mild reactivity and irritation. An independent, board-certified toxicologist assessed the results of the GEM 21S™ tests and concluded that the device is safe for its intended use.

Furthermore, β-TCP has been used clinically for over 25 years with no published unfavorable adverse responses. 1/ Becaplermin gel (PDGF) (Regranex®, has been FDA approved for nearly four years for at least 140 daily applications (20 weeks) to surgically excised wounds that extend into the subcutaneous tissue or beyond in the lower extremities of diabetics. Both components have a history of safe clinical use.

As noted previously, PDGF is a natural endogenous protein that lacks genotoxic potential. Additionally, it has a very low degree of absorption and a short half-life in plasma (46). When administered topically onto surgically excised wounds or subgingivally in periodontal defects, Becaplermin (PDGF) is quickly cleared [half-life of about four (4) hours] and has an insignificant effect on endogenous plasma PDGF concentrations (46, 47). For these reasons, Becaplermin is not considered to be a potential reproductive toxin or a systemic carcinogen. In the January 31, 2002 IDE supplement, the Company explained why carcinogenicity testing is not required for the GEM 21S™. On April 24, 2002, FDA approved the IDE without requiring carcinogenicity testing.

In summary, the extensive published data on the safety of VitOss and Becaplermin, FDA's clearance or approval of each of the individual components of GEM 21S™ (VitOss and Becaplermin) based, in part on the biocompatibility of these products, and the ISO 10993 testing conducted by BioMimetic on the combination of these components demonstrate that GEM 21S™ is toxicologically safe and biocompatible.

2.9.3 Laboratory Studies

BioMimetic has conducted Binding and Release Kinetics testing of PDGF and PDGF residence time in rats.

1/ FDA approved a PMA for Miter, Inc.'s Peri-Oss, which is a TCP product, on March 24, 1981 (P800035). The PMA remains active as evidenced by FDA's approval of a PMA supplement for this device on December 8, 2003.

2.9.3.1 Binding and Release Kinetics of PDGF

To determine the binding and release kinetics of rhPDGF-BB (Becaplermin, Chiron Corp.) to various ceramic-based osteoconductive bone substitutes and the effectiveness of released rhPDGF-BB upon osteoblastic cell proliferation (bioavailability) BioMimetic conducted laboratory studies using various ceramic bone substitutes with primary emphasis on the beta tricalcium phosphate ("β-TCP") based materials, VitOss™ and ChronOS™. Binding studies were conducted under conditions intended to mimic the clinical situation where the surgeon saturates the ceramic bone substitute with a predetermined volume of rhPDGF-BB solution prior to placement in the defect during surgery. For all binding experiments, a working volume of 75 uL of rhPDGF-BB (at either 0.2 or 0.5 mg/ml) and 100 uL volumes of ceramic bone substitute. Experiments to determine the rhPDGF-BB release profile were conducted by incubating the rhPDGF-BB bound to ceramic bone substitutes in vials containing either phosphate buffered saline (PBS) or MEM:Ham's F12 media containing 10% fetal bovine serum and sodium azide. The biological activity of the released rhPDGF-BB was determined by application of the releasate onto cultured human MG63 osteoblast cells and examination of the uptake of ^{3}H-thymidine into newly synthesized DNA.

For all materials, 0.075 ml of rhPDGF-BB solution completely saturated 0.1 cc of bone substitute. RhPDGF-BB bound to all materials examined with maximal binding observed within 10 minutes. Between 25-50% of rhPDGF-BB that was originally applied in solution remained bound to all materials after washing with sodium acetate buffer. VitOss™ and ChronOS™ displayed similar binding characteristics. Similar binding characteristics were observed when binding was performed in vials or in open dishes. Bound rhPDGF-BB was released from all materials. VitOss™ and ChronOS™ displayed similar release profiles. For VitOss™ in serum containing media, 50% of the rhPDGF-BB was released within 30 minutes, over 90% was released by 24 hours and all detectable protein was released in 48 hours. Releasates from either PBS or serum conditions stimulated cell proliferation in a similar pattern as mass of rhPDGF-BB released from these materials over time. Proliferation of osteoblast-like cells increased by 5- to 18-fold when exposed to the rhPDGF-BB releasate from VitOss™.

Theoretical projection of these *in vitro* data to *in vivo* models would indicate a bolus release of rhPDGF-BB of about 70% within minutes to hours after implantation of the ceramic bone substitute material saturated with a sodium acetate buffer containing rhPDGF-BB. This bolus release is followed by release of the remaining 30% over a 2 to 3 day period. The rhPDGF-BB released initially as a bolus as well as the rhPDGF-BB released over the following few days would be expected to be biologically active based on these studies.

2.9.3.2 Study of PDGF Residence Time in Rats

A residence time study in rats was conducted using rhPDGF-BB (Becaplermin, Chiron Corp.) and demineralized, freeze-dried human allograft bone matrix (DFDBA) and VitOss™ (β-TCP). The purposes of this study were: (1) to compare the binding of rhPDGF-BB to DFDBA and β-TCP; and (2) to determine the residence time, or release profile, for rhPDGF-BB absorbed to either β-TCP or allograft in an in vivo craniofacial bone defect model (skeletally mature rats).

VitOss™ β-TCP and DFDBA were saturated with iodinated rhPDGF-BB, the radioactivity of the combination materials was characterized and implanted in calvarial defects of rats. Radioactivity at the defect sites was measured at different time-point intervals during one week.

To determine in vivo residence time, a mixture of (^{125}I)-rhPDGF-BB and unlabeled rhPDGF-BB and β-TCP or DFDBA was administered to rats at a concentration of 50,000 cpm/animal. This mixture was composed of 0.05 cc of β-TCP or DFDBA added to 375. uL of a 0.25 mg/mL solution of radiolabeled and unlabeled rhPDGF-BB. Cranial defects were surgically created in anesthetized rats, the rhPDGF-BB, β-TCP or DFDBA grafts were implanted, and the wounds were closed. Radioactivity at the implant sites was measured at different time-point intervals over one week.

Data from these laboratory studies demonstrates that on average 45% and 40% of the rhPDGF-BB applied in a solution bound to β-TCP and allograft, respectively. When applied to calvarial defects in rats, approximately 95% of the rhPDGF-BB was released from both β-TCP and allograft implant sites within 48 hours. Approximately 2-5% of the rhPDGF-BB remained in both groups at one week. There were no significant differences between either VitOss™ or allograft groups. This study demonstrated that the binding and release (residence time) of rhPDGF-BB to β-TCP and DFDBA is comparable.

2.9.4 Canine Study

Between September 2001 and March 2003, BioMimetic sponsored a randomized, control study in canines at the State University of New York, Buffalo, New York. The purpose of this study was to assess the periodontal regenerative properties of rhPDGF-BB ("PDGF") in combination with β-TCP, rhPDGF-BB in combination DFDBA (allograft), and DFDBA (allograft) alone. Two (2) separate doses of PDGF, one at 0.3 mg/mL and one at 1.0 mg/mL, were evaluated in the study.

Using a beagle dog model of horizontal Class III furcation defects, a model that provides for 'critical size' defects that would not otherwise heal on their own, fifteen (15) adult animals contributed 60 treated defects for the study. Defect sites received one of the following treatments: (1) β-TCP alone; (2) β-TCP plus 0.3 mg/mL PDGF; (3) β-TCP plus 1.0 mg/mL PDGF; (4) DFDBA alone; (5) DFDBA plus 0.3 mg/ml PDGF; (6) DFDBA plus 1.0 mg/ml PDGF; and (7) surgery only, no graft. Treatments were histomorphometrically assessed at two and four months for: (1) length of complete new attachment apparatus (CNAA); (2) new bone fill (NB); (3) connective tissue fill (CT); and (4) void (VO). Specifically, six (6) defects per treatment group were biopsied at two months for a total of 42 sites and six defects in treatment groups 1, 2, and 3 were biopsied at four months for a total of 18 sites. All histomorphic assessments were performed blinded to the treatment.

One-way analysis of variance was used to test for overall difference among treatment groups, and pair wise comparisons were made using the Student's t-test. Significant differences between groups were found at the two-month assessment point. β-TCP, when combined with rhPDGF-BB for the treatment of periodontal bone loss, resulted in greater periodontal regeneration than current products or procedures, β-TCP alone, bone allograft or

periodontal surgery without grafts. Of note is that β-TCP with 0.3 mg/ml PDGF was significantly better than the current gold standard of DFDBA. The 0.3 mg/ml concentration of rhPDGF was at least equally as effective as the 1.0 mg/ml concentration when mixed with β-TCP. Furthermore, β-TCP was observed to be more effective than allograft when mixed with rhPDGF-BB. There was no increase in ankylosis or root resorption in the rhPDGF groups. Sites receiving rhPDGF-BB exhibited improved healing as shown by a decrease in ankylosis vs. control sites. This finding may result from the fact that rhPDGF-BB is mitogenic and chemotactic for periodontal ligament cells.

At the four-month assessment point, which only included treatment groups receiving β-TCP alone, β-TCP plus 0.3 mg/mL PDGF, and β-TCP plus 1.0 mg/mL PDGF, there remained significant differences in periodontal regeneration. β-TCP alone, as a result of extensive ankylosis, resulted in 36% regeneration, while the sites treated with β-TCP containing rhPDGF-BB had a mean regeneration of 58% and 49% in the 0.3 and 1.0 mg/ml rhPDGF-BB concentrations. β-TCP alone; (2) β-TCP plus 0.3 mg/mL PDGF; (3) β-TCP plus 1.0 mg/mL PDGF Substantial bone fill was present in all three treatment groups. β-TCP alone resulted in 70% bone fill, β-TCP plus 0.3 mg/ml rhPDGF yielded 100% fill while the 1.0 mg/ml rhPDGF group had 75% fill.

2.9.5 Immunological Testing

The biocompatibility studies described above demonstrate the biocompatibility of GEM 21S™. There was no evidence of mutagenicity, delayed dermal contact sensitization, systemic toxicity or mortality, and only sight to mild reactivity and irritation in these studies.

Furthermore, β-TCP has been used clinically for more than years with no published unfavorable adverse responses. Becaplermin gel (Regranex®, has been FDA approved for nearly four years for at least 140 daily applications (20 weeks) to surgically excised wounds that extend into the subcutaneous tissue or beyond in the lower extremities of diabetics.

As noted previously, PDGF is a natural endogenous protein that lacks genotoxic potential. Additionally, it has a very low degree of absorption and a short half-life in plasma. [42]. When administered topically onto surgically excised wounds or subgingivally in periodontal defects, Becaplermin (PDGF) is quickly cleared (half-life of about four hours) and has an insignificant effect on endogenous plasma PDGF concentrations. [42, 51] For these reasons, Becaplermin is not considered to be a potential reproductive toxin or a systemic carcinogen. Thus, carcinogenicity testing was not conducted on GEM 21S™.

In summary, the extensive published data on the safety of VitOss and Becaplermin coupled with the ISO 10993 testing conducted by BioMimetic on the combination of these components demonstrate that GEM 21S™ is toxicologically safe and biocompatible.

2.9.6 Stability Studies

BioMimetic's conducted shelf life studies of GEM 21S and found that the device maintains its integrity for two (2) years under its conditions of storage.

2.10 Sterilization

The individual components of the GEM 21S™ kit are provided sterile while the finished kit is not terminally sterilized after final assembly and packaging of the following three components, which are separately sterilized before kitting.

2.10.1 Filled β-TCP Perio-Cups

Filled β-TCP containers (perio-cups) are terminally sterilized by irradiation at Penn's contract sterilizer, Isotron plc, a FDA-registered contract sterilizer. The sterilization cycle for the filled β-TCP perio-cups is being validated in accordance with the requirements outlined in Method 1 of ISO Standard ISO 11137 for substantiation of a 25 kGy sterilization dose. The sterilization method produces a 10^{-6} or higher Sterility Assurance Level ("SAL").

2.10.2 Sterile rhPDGF-BB

rhPDGF-BB is aseptically processed when manufactured. No terminal sterilization process is applied. The aseptic filling of rhPDGF-BB into Hypak syringes is supported by the following validation packages:

- Sterile filtration validation; and,
- Media fill validation of aseptic filling process.

The aseptic fill validation studies will follow standard Installation Qualification ("IQ"). Operational Qualification ("OQ"), and Process Qualification ("PQ") documentation.

2.10.3 Sterile 23 Gauge Blunt Needle

The 23 gauge blunt needle is an "off-the-shelve" product that is supplied sterile by Kendall and is not further processed by BioMimetic or any of its contract vendors. Kendall terminally sterilizes this accessory by irradiation (gamma) before receipt.

2.11 Microbiological Testing

Release testing of the GEM 21S™ incorporates microbiological testing, including bioburden and sterility testing.

2.12 Immunological Testing

The biocompatibility studies described above demonstrate the biocompatibility of GEM 21S™. There was no evidence of mutagenicity, delayed dermal contact sensitization, systemic toxicity or mortality, and only sight to mild reactivity and irritation in these studies.

Furthermore, β-TCP has been used clinically for more than years with no published unfavorable adverse responses. Becaplermin gel (Regranex®, has been FDA approved for nearly four years for at least 140 daily applications (20 weeks) to surgically excised wounds that extend into the subcutaneous tissue or beyond in the lower extremities of diabetics.

As noted previously, PDGF is a natural endogenous protein that lacks genotoxic potential. Additionally, it has a very low degree of absorption and a short half-life in plasma. [42]. When administered topically onto surgically excised wounds or subgingivally in periodontal defects, Becaplermin (PDGF) is quickly cleared (half-life of about four hours) and has an insignificant effect on endogenous plasma PDGF concentrations. [42, 51] For these reasons, Becaplermin is not considered to be a potential reproductive toxin or a systemic carcinogen. Thus, carcinogenicity testing was not conducted on GEM 21S™.

In summary, the extensive published data on the safety of VitOss and Becaplermin coupled with the ISO 10993 testing conducted by BioMimetic on the combination of these components demonstrate that GEM 21S™ is toxicologically safe and biocompatible.

3.1 Summary Of Clinical Studies

The pivotal clinical study of GEM 21S™ is described in this section.

3.1 Introduction

A single multi-center controlled clinical trial was performed in the United States to demonstrate the safety and effectiveness of GEM 21S™ in the management of periodontal defects and to assess the regenerative capability of GEM 21S™ on bone and soft tissue. As described in more detail below, this study compared results of treatment in three groups: low concentration, high concentration, and control. The effect of treatment on bone and soft tissue regeneration was assessed using Clinical Attachment Level ("CAL") and radiographic bone measurements. The duration of the study for each patient was almost six months (24 weeks).

The clinical study described in this section was performed pursuant to an approved Investigational Device Exemption ("IDE") Application, (G010340), which was conditionally approved on February 28, 2002 and approved without conditions on April 22, 2002. The IDE investigation was approved for 180 patients at 12 sites. The first patient was enrolled in the study on May 10, 2002 and the last patient visit was conducted on May 7, 2003.

3.2 Protocol Summary

The clinical study was carried out at 11 investigational sites in the United States. A total of 180 subjects were enrolled in the clinical trial, evenly divided between the three treatment groups. The three treatment groups were defined as follows:

Group I: GEM 21S™ with sodium acetate buffer containing 0.3 mg/mL rhPDGF-BB ("low concentration")

Group II: GEM 21S™ with sodium acetate buffer containing 1.0 mg/mL rhPDGF-BB ("high concentration")

Group III: β-TCP with sodium acetate buffer alone ("control")

<u>Eligibility Criteria</u>

Subjects for this study were recruited from existing subject databases at each investigational site, referrals, and screening of volunteers responding to advertisements.

To be <u>included</u> in this study, subjects must have met all of the following criteria:

a. Aged 25-75,
b. No evidence of Localized Aggressive Periodontitis,
c. Treatment site with the following characteristics:
   - Probing pocket depth ≥7 mm at baseline,
   - After surgical debridement, ≥4 mm vertical bone defect with at least 1 bony wall,

- Sufficient keratinized tissue to allow complete tissue coverage of defect, and
- Radiographic base of defect ≥3 mm coronal to the apex of the tooth.

d. Give signed informed consent and be willing to comply with the follow-up visit schedule.

Subjects were excluded from the study if any of the following were true:

- Failure to maintain adequate oral hygiene during the lead-in phase,
- Woman pregnant or planning to become pregnant,
- History of oral cancer or HIV in the last 6 months,
- History of previous periodontal surgery on the study tooth within the last year,
- Study tooth exhibiting mobility of greater than Grade II,
- Study tooth exhibiting a Class III furcation defect,
- Clinical or radiographic signs of untreated acute infection at the surgical site, apical pathology, root fracture, severe root irregularities, cemental pearls, cemento-enamel projections not easily removed by odontoplasty, untreated carious lesions at the cemento-enamel junction (CEJ) or on the root surface, subgingival restorations and/or restorations with open margins at or below the CEJ,
- Weekly or more frequent use of smokeless chewing tobacco, pipe or cigar smoking, or smoking more than 20 cigarettes/day in the last 6 months,
- Allergy to yeast-derived products, or
- Using an investigational therapy with the past 30 days.

Study Visits

Patients in the study attended the following visits:

- Visit 1 (up to 6 months pre-surgery): Eligibility screening and informed consent
- Visit 2 (3 months pre-surgery): Scaling and root planning if necessary
- Visit 3 (2 months pre-surgery): Scaling and root planning if necessary
- Visit 4 (14 days pre-surgery): Baseline evaluation
- Visit 5: Periodontal surgery and device placement
- Visits 6-9 (post-surgical days 3-5, 6-9, 12-15, and 19-24): Wound healing assessment, pain assessment
- Visits 10-13 (post-surgical weeks 6, 12, 18 and 24): Clinical measurements and radiographs

Adverse events were ascertained at each post-operative visit and concomitant medications were recorded.

Primary Effectiveness Endpoint

The primary effectiveness endpoint for the study was a change in clinical attachment level (CAL) *between* baseline and 6 months. There were two hypotheses for this endpoint, to be evaluated sequentially:

1. Clinically meaningful efficacy: The mean change in CAL between baseline and 6 months was compared to a historically established level of clinical efficacy (1.5 mm) using a one-sample t-test.

2. Comparative efficacy: The mean change in CAL between baseline and 6 months was compared between Group I (low concentration) and Group III (control) using a two-sample t-test and a one-sided p-value of 0.05.

Secondary Effectiveness

The secondary effectiveness endpoints for the study were as follows:

1. Comparison of linear bone growth (LBG) and percent of original bone defect filled with new bone (%BF) based on radiographic measurements (Groups I and II versus Group III).
2. Area under the curve (AUC) for change in CAL, incorporating baseline, 3 month and 6 month data (Groups I and II versus Group III).
3. Change in CAL between baseline and 6 months (Group II versus Group III).
4. Pocket depth reduction (PDR) change between baseline and 6 months (all groups).
5. Gingival recession (GR) change between baseline and 6 months (all groups).
6. Wound healing (WH) during the first three weeks post-operatively (all groups).

The following secondary effectiveness endpoints were added post-hoc in order to compare GEM 21S™ outcomes with those of previously approved products:

1. 95% lower confidence bound for change in CAL (both treatment groups at 3 and 6 months): a 95% lower bound in excess of 2.7 mm is considered to substantiate effectiveness (based on Emdogain PMA)
2. 95% lower confidence bound for change in CAL (both treatment groups at 3 and 6 months): a 95% lower bound in excess of 1.1 mm is considered to substantiate effectiveness (based on PepGen P-15 PMA)
3. 95% lower confidence bound for %BF in Group I at 6 months: a 95% lower bound in excess of 15% will be considered to substantiate effectiveness (based on Parashis, *et al.*)
4. 95% lower confidence bound for LBG in Group I at 6 months: a 95% lower bound in excess of 0.5 mm will be considered to substantiate effectiveness (based on Persson, *et al.*)

Two composite outcomes were also defined based on results from previous studies, with success criteria as follows:

1. CAL gain ≥2.7 mm and LBG ≥1.1 mm at 6 months.
2. CAL gain ≥2.7 mm and %BF ≥14.1% at 6 months.

Safety

Safety was monitored throughout the clinical trial by recording information on all adverse events. Adverse events were classified by the investigator according to severity (mild, moderate, severe) and relation to device (not related, unlikely to be related, likely related, definitely related). The investigator also recorded information on the action taken as a result of the adverse event. To assist in adverse event identification, investigators reviewed radiographs for evidence of ankylosis, root resorption, or other abnormal changes to the bony architecture.

3.3 Population Characteristics

Subjects were enrolled at 11 investigational sites in the United States. One hundred ninety five (195) subjects were enrolled into the study, of which 180 were randomized. Of the 15 subjects that were not randomized, 4 were excluded during screening and 11 were excluded during surgery. The 180 eligible patients were randomized into 3 groups, as defined above; 60 into Group I, 61 into Group II, and 59 into Group III.

Of the 180 randomized subjects, 178 completed the study. One subject from Group II (02-03) was lost to follow-up after Visit 10 (Week 6). One subject from Group III (04-12) withdrew from the study after Visit 6 (Day 3-5), but agreed to return for the 6 month visit clinical examination. Thus, 6 month outcomes are available for 179 subjects. No subjects were withdrawn from the study for non-compliance and no subjects withdrew from the study due to adverse events.

Baseline characteristics by treatment group can be found in **Table 3-1.** As shown in this table, there are no statistically significant differences between the treatment groups with respect to baseline characteristics.

Table 3-1: Baseline Characteristics by Treatment Group

	Group I (N=60) N (%)	Group II (N=61) N (%)	Group III (N=59) n (%)	p-value
Gender=Male	29 (48)	41 (67)	38 (64)	0.07
Race=Caucasian	33 (55)	37 (61)	37 (63)	0.39
Age (years) Mean±SD	49.4±10.2	50.4±13.0	52.8±9.5	0.22
Current Smoker	12 (20)	19 (31)	12 (20)	0.26
General Medical Abnormality	39 (65)	42 (69)	49 (83)	0.07
Dental Abnormality	16 (27)	11 (18)	15 (25)	0.48
CAL (mm) Mean±SD	9.1±1.8	8.8±1.6	8.8±1.5	0.50
PD (mm) Mean±SD	8.6±1.3	8.2±1.3	8.3±1.2	0.17
1-2 Wall Defect	46 (76)	49 (80)	45 (76)	0.30

	Group I (N=60) N (%)	Group II (N=61) N (%)	Group III (N=59) n (%)	p-value
Multi-rooted Defect	35 (58)	33 (54)	30 (51)	0.71
Vertical Bone Defect Depth (mm) Mean±SD	6.0±1.6	5.7±1.4	5.7±1.4	0.36
Width of Osseous Defect (mm) Mean±SD	3.7±1.3	3.5±1.1	3.7±0.9	0.61
Base of Defect to Root Apex (mm) Mean±SD	6.5±2.5	7.0±2.7	7.7±2.8	0.04

3.4 Results & Analysis

The test results are summarized below. An analysis of those results is also provided below.

3.4.1 Procedural Outcome

Primary flap closure was achieved in 100% of Group I subjects, and 98% of Group II and III subjects.

Good or excellent containment of study medication in the lesion was achieved in 92% of Group I subjects, 98% of Group II subjects, and 95% of Group III subjects. Good or excellent soft tissue closure was achieved in 100% of Group I and III subjects and 98% of Group II subjects.

3.4.2 Post-Procedure Outcomes

No days of work were missed due to the surgical procedure in 90% of Group I subjects, 92% of Group II subjects, and 98% of Group III subjects. Sutures were removed in less than 10 days in 20% of Group I subjects, 17% of Group II subjects, and 16% of Group III subjects. Good oral hygiene was maintained at all visits by 75% of subjects in each group.

3.4.3 Effectiveness

Table 3-2 summarizes the clinical outcome measurements (mean±SD CAL, PD, and GR) by treatment group and visit. These measurements are used to compute improvements from baseline, as presented in subsequent tables.

Table 3-2: Clinical Outcomes by Treatment Group and Visit

Outcome	Group I (N=60)	Group II (N=61)	Group III (N=59)
Clinical Attachment Level (CAL)			
Baseline	9.1±1.8	8.8±1.6	8.8±1.5
12 Weeks	5.4±1.6	5.5±1.5	5.5±1.7
24 Weeks	5.4±1.7	5.2±1.6	5.3±1.6
Pocket Depth (PD)			
Baseline	8.6±1.3	8.2±1.3	8.3±1.2
12 Weeks	4.4±1.3	4.1±1.1	4.1±1.1
24 Weeks	4.3±1.3	4.0±1.1	4.1±1.1
Gingival Recession (GR)			
Baseline	0.5±1.2	0.6±1.4	0.5±1.1
12 Weeks	1.0±1.4	1.4±1.4	1.4±1.2
24 Weeks	1.2±1.4	1.3±1.5	1.2±1.3

Primary Endpoint - CAL Gain

Clinically Meaningful Efficacy

Table 3-3 shows the mean CAL gain between baseline and 24 weeks for each treatment group. In addition, this table shows the 95% lower confidence bound for the mean and the one-sample t-test p-value comparing the mean with 1.5 mm, the historically established level of clinical effectiveness specified in the protocol.

Table 3-3: CAL Gain between Baseline and 24 Weeks by Treatment Group

CAL Gain	Group I (N=60)	Group II (N=60)	Group III (N=59)
Mean±SD (mm)	3.7±1.7	3.7±1.6	3.5±1.4
Median	4.0	3.5	3.0
Range (mm)	-2.0 to 7.0	-1.0 to 9.0	0.0 to 7.0
95% LCB	3.3	3.2	3.1
p-value	<0.001	<0.001	<0.001

As shown in **Table 3-3**, all three treatment groups had mean CAL gain well in excess of the established 1.5 mm level. This, the results for all treatment groups were considered to be clinically meaningful. These values also exceed the result reported in the Emdogain PMA (2.7 mm) and the PepGen P15 PMA (1.1 mm).

Comparative Efficacy

Table 3-4 shows the mean CAL gain between baseline and 12 weeks and between baseline and 24 weeks for Groups I and III. In addition this table shows the two-sample t-test p-value (one-sided) comparing Groups I and III at each time point.

Table 3-4: CAL Gain at Weeks 12 and 24 for Groups I and III

CAL Gain	Group I (N=60)	Group III (N=59)	p-value
Week 12			
Mean±SD (mm)	3.8±1.4	3.3±1.5	0.04
Median	3.0	3.0	-----
Range (mm)	1.0 to 8.0	-1.0 to 6.0	-----
Week 24			
Mean±SE (mm)	3.7±1.7	3.5±1.4	0.20
Median	4.0	3.0	-----
Range (mm)	-2.0 to 7.0	0.0 to 7.0	-----

As shown in **Table 3-4**, the difference in CAL gain at 12 weeks between Groups I and III (3.8 mm versus 3.3 mm) was statistically significant (p=0.04). Between Week 12 and Week 24 the CAL gain in Group I remained stable, while the CAL gain in Group III improved by 0.2 mm. Accordingly, the difference in CAL gain at Week 24 between Group I and Group III (3.7 mm versus 3.5 mm), while still numerically superior, is no longer statistically significant (p=0.20).

Secondary Endpoints – LBG and %BF

Of the 180 randomized subjects, 174 had data available for the radiographic analysis (60 in Group I, 58 in Group II, and 56 in Group III). **Table 3-5** shows the mean LBG and %BF between baseline and 24 weeks for each treatment group. In addition, this table shows the two-sample t-test p-value (one-sided) comparing Groups I and II with Group III, and the 95% lower confidence bound for the mean.

Table 3-5: LBG and %BF at 24 Weeks by Treatment Group

	Group I (N=60)	Group II (N=58)	Group III (N=56)
Linear Bone Growth (LBG)			
Mean±SD (mm)	2.52±1.96	1.53±1.61	0.89±1.71
Median (mm)	2.17	1.15	0.70
Range (mm)	-0.22 to 9.36	-1.80 to 6.97	-6.66 to 5.06
p-value	<0.001	0.02	-----
95% LCB	2.02	1.10	0.43
Percent Bone Fill (%BF)			
Mean±SE (%)	56.0±46.4	33.9±32.2	17.9±48.2

	Group I (N=60)	Group II (N=58)	Group III (N=56)
Median (mm)	49.5	33.0	19.7
Range (%)	-3.9 to 254.9	-22.8 to 108.1	-235.3 to 86.4
p-value	<0.001	0.02	-----
95% LCB	44.0	25.4	5.0

As shown in **Table 3-5**, for both LBG and %BF, Group I was superior to Group II and both were superior to Group III (Group I versus Group III: p<0.001; Group II versus Group III: p=0.02). In fact, for both LBG and %BF the mean for Group I was approximately three times the mean for Group III.

The literature-based thresholds for effectiveness were determined to be 0.5 mm for LBG and 15% for %BF. As shown in **Table 3-5**, the 95% lower confidence bounds substantially exceeded these thresholds for both Groups I and II. In contrast, the 95% lower confidence bounds for Group III (0.43 for LBG and 5% for %BF) do not exceed these thresholds.

Secondary Endpoint – Clinical and Radiographic Composite

To assess the cumulative beneficial effect for clinical and radiographic outcomes, two composite endpoints were defined with success criteria as follows:

1. CAL gain ≥ 2.7 mm and LBG ≥ 1.1 mm at 6 months
2. CAL gain ≥ 2.7 mm and %BF ≥ 14.1% at 6 months

These composite endpoints are presented in **Table 3-6**. In addition, this table shows the chi-square test p-value (one-sided) for the comparisons between Groups I and II and Group III.

Table 3-6: Composite Clinical and Radiographic Endpoints

	Group I N (%)	Group II N (%)	Group III N (%)
CAL+LBG	N=60	N=58	N=56
Composite Success	37 (62)	22 (38)	17 (30)
p-value	<0.0001	0.20	-----
CAL+%BF	N=60	N=60	N=59
Composite Success	(70)	(55)	(45)
p-value	0.003	0.13	-----

As shown in **Table 3-6**, 62% of subjects in Group I experienced success with respect to CAL gain and LBG, as compared to 38% for Group II and 30% for Group III. The difference between Group I and Group III was highly significant (p<0.001). Likewise 70% of subjects in Group I experienced success with respect to CAL gain and %BF, as compared to 55% for Group II and 45% for Group III (p=0.003).

Secondary Endpoint – AUC for CAL Gain

In order to measure the cumulative results from the two time points (12 and 24 weeks), an Area Under the Curve (AUC) analysis was performed for CAL gain. This measurement is in units of "mm-weeks" (mm of CAL gain multiplied by number of weeks of follow-up). **Table 3-7** shows the CAL Gain AUC for all three treatment groups, as well as the two-sample t-test p-value (one-sided) for comparison of Groups I and II with Group III.

Table 3-7: CAL Gain AUC by Treatment Group

CAL Gain AUC	Group I (N=60)	Group II (N=60)	Group III (N=59)
Mean±SD (mm-weeks)	67.5±25.1	61.8±22.4	60.1±24.2
Median (mm-weeks)	63.0	59.6	58.2
Range (mm-weeks)	11.5 to 140	0.5 to 117	0.1 to 112
p-value	0.05	0.35	-----

As shown in **Table 3-7**, the CAL Gain AUC for Group I (67.5 mm-weeks) was significantly better than that for Group III (60.1 mm-weeks), with a p=0.05. This demonstrates that subjects in Group I maintained an overall level of improvement that was superior to that experienced by subjects in Group III. Referring back to the CAL gain values in **Table 3-3**, it is clear that, while both treatment groups ultimately achieved a similar degree of improvement, subjects in Group I improved more quickly than subjects in Group III.

Secondary Endpoint – CAL Gain Group II versus Group III

The primary effectiveness endpoint is a comparison of CAL gain at 24 months between Groups I and III. The comparison of CAL gain at 24 months between Groups II and III was made a secondary endpoint since it is only of interest if the low concentration product is effective and if there is a significant advantage to using the high concentration product instead of the low concentration. **Table 3-8** shows mean CAL gain at 24 weeks for Groups II and III, along with the two-sample t-test p-value (one-sided) for this comparison.

Table 3-8: CAL Gain between Baseline and 24 Weeks for Group II and Group III

CAL Gain	Group II (N=60)	Group III (N=59)
Mean±SE (mm)	3.7±0.2	3.5±0.2
Median (mm)	3.5	3.0
Range (mm)	-1.0 to 9.0	0.0 to 7.0
p-value	0.29	-----

As shown in **Table 3-8**, there is no significant difference in CAL gain at 24 weeks between Groups II and III (p=0.29).

Secondary Endpoints – GR and PDR

Tables 10-9A and 10-9B summarize the results for gingival recession (GR) and pocket depth reduction (PDR) at 12 and 24 weeks, respectively, for all three treatment groups. These tables also show the two-sample t-test p-values (two-sided) for the comparisons between Groups I and II and Group III.

Table 3-9A: GR and PDR at 12 Weeks by Treatment Group

	Group I (N=60)	**Group II (N=60)**	**Group III (N=59)**
Gingival Recession (GR)			
Mean±SD (mm)	0.5±1.0	0.7±1.0	0.9±1.2
Median (mm)	0.0	1.0	1.0
Range (mm)	-3.0 to 4.0	-1.0 to 3.0	-2.0 to 5.0
p-value	0.04	0.46	-----

	Group I (N=60)	**Group II (N=60)**	**Group III (N=59)**
Pocket Depth Reduction (PDR)			
Mean±SD (mm)	4.2±1.4	4.1±1.0	4.2±1.2
Median (mm)	4.0	4.0	4.0
Range (mm)	1.0 to 8.0	1.0 to 6.0	2.0 to 7.0
p-value	0.80	0.67	-----

Table 3-9B: GR and PDR at 24 Weeks by Treatment Group

	Group I (N=60)	**Group II (N=60)**	**Group III (N=59)**
Gingival Recession (GR)			
Mean±SD (mm)	0.7±0.8	0.6±1.0	0.7±1.0
Median (mm)	0.5	0.0	0.0
Range (mm)	-1.0 to 3.0	-1.0 to 3.0	-2.0 to 3.0
p-value	0.95	0.81	-----
Pocket Depth Reduction (PDR)			
Mean±SD (mm)	4.4±0.2	4.3±0.2	4.2±0.2
Median (mm)	4.0	4.0	4.0
Range (mm)	1.0 to 7.0	2.0 to 9.0	1.0 to 9.0
p-value	0.38	0.66	-----

As shown in **Table 3-9B**, there were no significant differences between treatment groups with respect to GR and PDR at 24 weeks. As shown in **Table 3-9A**, consistent with the

results for CAL gain, however, there was a statistically significant difference for GR at 12 weeks between Group I and Group III (0.5 mm versus 0.9 mm; p=0.04).

Secondary Endpoint – Wound Healing

A wound-healing scale modified from the index described by Lobene *et al.* (1986) was used to assess wound healing during the first three weeks post-surgery. This scale ranges from 0 (absence of inflammation) to 5 (severe inflammation).

Table 3-10 shows the wound healing scores for each of the 4 early post-surgical visits, for each treatment group. This table also shows the Cochran-Mantel-Haenszel p-value for comparison of Groups I and II with Group III.

Table 3-10: Wound Healing by Treatment Group

Wound Healing Score	Group I N (%)	Group II N (%)	Group III N (%)
Visit 6 (3-5 days)	**N = 60**	**N = 60**	**N = 56**
0	8 (13)	10 (17)	8 (14)
1	36 (60)	27 (45)	32 (57)
2	10 (17)	18 (30)	11 (20)
3	5 (8)	5 (8)	5 (9)
4	1 (2)	0 (0)	0 (0)
p-value	0.91	0.66	-----
Visit 7 (6-9 days)	**N = 59**	**N = 59**	**N = 58**
0	18 (30)	18 (30)	25 (43)
1	26 (44)	26 (44)	27 (47)
2	11 (19)	10 (17)	2 (3)
3	3 (5)	5 (8)	3 (5)
4	1 (2)	0 (0)	1 (2)
p-value	0.10	0.10	-----

Wound Healing Score	Group I N (%)	Group II N (%)	Group III N (%)
Visit 8 (12-15 days)	**N = 60**	**N = 59**	**N = 58**
0	34 (57)	28 (48)	27 (47)
1	18(30)	19 (32)	22 (38)
2	2 (3)	11 (19)	7 (12)
3	5 (8)	1 (2)	2 (3)
4	1 (2)	0 (0)	0 (0)
p-value	0.81	0.89	-----
Visit 9 (19-24 days)	**N = 60**	**N = 60**	**N = 58**
0	43 (72)	36 (60)	32 (55)
1	12 (20)	19 (32)	19 (33)
2	3 (5)	2 (3)	3 (5)
3	1 (2)	3 (5)	3 (5)
4	1 (2)	0 (0)	1 (2)
p-value	0.14	0.44	-----

As shown in **Table 3-10**, by Visit 9 (approximately 3 weeks post-surgery), completing healing was experienced by 72% of subjects in Group I, 60% in Group II, and 55% in Group III. Although the Cochran-Mantel-Haenzsel test (which takes into account the ordinal nature of the wound healing score) p-value did not achieve statistical significance (p=0.14), a chi-square test contrasting complete healing (Grade 0) with incomplete healing (Grades 1-4), showed that the difference between Groups I and III was, indeed, statistically significant (p=0.03).

Comparison with Historical Efficacy

Table 3-11 summarizes the results for the outcome measures for which historical efficacy thresholds were established. For CAL Gain, LBG and %BF, the mean and 95% lower confidence bound are presented, along with the historically established threshold.

Table 3-11: Outcome Measures Compared with Historical Efficacy

	Mean (95% Lower Confidence Bound)		
	Group I	Group II	Threshold
CAL Gain at 3 months	3.8 (3.4)	3.4 (3.1)	1.1 or 2.7
CAL Gain at 6 months	3.7 (3.3)	3.7 (3.2)	1.1 or 2.7
LBG at 6 months	2.52 (2.02)	1.55 (1.10)	0.5
%BF at 6 months	56.0 (44.0)	33.9 (25.4)	15

As shown in **Table 3-11**, in all cases the outcome measures observed in this study far exceed the historical efficacy threshold, indicating that GEM 21S™ is superior to products previously approved for the same indication as well as to standard clinical practice for this patient group.

3.4.4 Safety

Adverse events (AEs) were collected throughout the study by patient inquiries during scheduled patient visits. **Table 3-12** summarizes the overall number and incidence of adverse events, as well as the number and incidence of severe AEs, serious AEs, and treatment related AEs.

Table 3-12: Summary of Adverse Events

Number of Events	Group I N=60	Group II N=61	Group III N=61	p-value
# AEs	88	93	89	-----
# Severe AEs	0	2	2	-----
# Serious AEs	1	1	2	-----
# Related AEs	7	6	5	-----
Number of Subjects	n (%)	n (%)	n (%)	-----
# Subjects with AEs	44 (73)	42 (69)	39 (66)	0.69
# Subjects with Severe AEs	0 (0)	2 (3)	2 (3)	0.47
# Subjects with SAEs	1 (2)	1 (2)	2 (3)	0.70
# Subjects with Related AEs	7 (12)	6 (10)	5 (8)	0.84

As shown in **Table 3-12**, 44 subjects (73%) in Group I experienced 88 adverse events (AEs), 42 subjects (69%) in Group II experienced 93 AEs, and 39 subjects (66%) in Group III experienced 89 AEs. There were no significant differences in the incidence of adverse

events across the three treatment groups. There were no treatment-related serious adverse events and no subjects discontinued study participation due to adverse events.

Table 3-13 lists all AEs with incidence ≥2%, by treatment group and body system.

Table 3-13. Adverse Events by Body System and Treatment Group

Body System	Preferred Term	Group I N=60	Group II N=61	Group III N=59
Body as a whole				
	Accidental injury	2 (3.3%)	2 (3.3%)	1 (1.7%)
	Allergic reaction	0 (0.0%)	1 (1.6%)	3 (5.1%)
	Back pain	5 (8.3%)	2 (3.3%)	1 (1.7%)
	Cyst	0 (0.0%)	2 (3.3%)	0 (0.0%)
	Flu syndrome	2 (3.3%)	3 (4.9%)	3 (5.1%)
	Headache	5 (8.3%)	3 (4.9%)	7 (11.9%)
	Malaise	0 (0.0%)	0 (0.0%)	2 (3.4%)
Digestive				
	Periodontal abscess	1 (1.7%)	1 (1.6%)	2 (3.4%)
	Stomach ulcer	0 (0.0%)	2 (3.3%)	0 (0.0%)
	Surgical site reaction	35 (58.3%)	35 (57.4%)	32 (54.2%)
	Tooth disorder	4 (6.7%)	7 (11.5%)	1 (1.7%)
	Tooth pain	3 (5.0%)	4 (6.6%)	4 (6.8%)
Musculoskeletal				
	Muscle pain	3 (5.0%)	1 (1.6%)	0 (0.0%)
Respiratory				
	Respiratory disorder	2 (3.3%)	1 (1.6%)	3 (5.1%)
	Sinusitis	3 (5.0%)	0 (0.0%)	0 (0.0%)
Skin/Appendages				
	Herpes simplex	2 (3.3%)	0 (0.0%)	1 (1.7%)

As shown in **Table 3-13**, the most frequently experienced adverse event for all treatment groups was pain at the surgical site, which was an expected sequelae following the periodontal procedure employed for this trial. There were no differences in incidence of pain across the three treatment groups.

3.4.5 Multivariate Analysis

Subgroup analyses showed that increased LBG, improvement in %BF and higher CAL gains were observed in non-smokers compared to smokers, and subjects with three or circumferential apical bone walls compared with subjects with one or two apical bone walls. Improved effectiveness outcomes were seen in subjects with baseline areas of defect >21 mm^2, those who were ≤50 years of age, and non-Caucasians.

Additional analyses were performed to examine differences in CAL outcomes adjusting for demographic characteristics. In these analyses the following results were noted:

- No statistically significant differences were observed between treatment Groups I and III when comparisons were adjusted for age, gender, race and current smoking status ($p = 0.42$, ANCOVA model two-sided test).
- The study center by treatment interaction was found not to be statistically significant ($p = 0.12$, ANCOVA model two-sided test).
- No statistically significant differences were observed in the among-group comparison ($p = 0.70$, one-way ANOVA model).
- There was no observed linear concentration trend ($p = 0.41$, linear contrast ANOVA model).

3.5 Summary

Effectiveness

As shown in **Table 3-14**, below, Group I (β-TCP plus 0.3 mg/ml PDGF) achieved statistically beneficial results for gain in clinical attachment levels and less gingival recession at three (3) months as well as gain in linear bone growth and increased bone fill at six (6) months, compared to the active control Group III which received β-TCP alone. The clinical significance of these results is confirmed by comparison to historical benchmarks of effectiveness for other approved treatments. Furthermore, the beneficial effects of GEM 21S™ were observed in all types of defects, including one to three wall and circumferential defects. These results address an unmet clinical need in that GEM 21S™ provided a clear benefit even in the most severe cases where β-TCP alone was ineffective. Thus, GEM 21S™ provided a more predictable treatment option for all types of defects than the active β-TCP control.

Table 3-14. Summary of Clinical and Radiographic Effectiveness of GEM 21S™

Summary of GEM 21S™ Effectiveness				
Endpoint		Group I	Group II	Group III
CAL Gain (mm): 3 months		3.8 (p=0.04)	3.4 (p=0.40)	3.3
CAL: AUC Analysis (mm x wk)		67.5 (p=0.05)	61.8 (p=0.35)	60.1
CAL (mm): 95% LCB at 6 months (vs 2.7 mm for Emdogain & 1.1 mm for PepGen P-15)		3.3	3.2	3.1
GR (mm): 3 months		0.5 (p=0.04)	0.7 (p=0.46)	0.9
LBG (mm): 6 months		2.5 (p<0.001)	1.5 (p=0.02)	0.9
%BF: 6 months		56.0 (p<0.001)	33.9 (p=0.02)	17.9
Composite Analyses (% Success)	CAL-LBG	61.7% (p<0.001)	37.9% (p=0.20)	30.4%
	CAL-%BF	70.0% (p=0.003)	55.2% (p=0.13)	44.6%

Safety

There were no statistically significant differences observed in the incidence of AEs (all causes) among the three treatment groups. The most frequently experienced AE for all treatment groups was pain at the surgical site, which was an expected sequelae following routine periodontal surgeries.

Seven (7) subjects in Group I, six (6) subjects in Group II and five (5) subjects in Group III experienced AEs that were assessed by the investigator as likely or definitely related to the investigational product, none of which were considered serious (see below).

Four (4) subjects experienced four (4) adverse events classified as "serious" (SAEs), none of which were directly attributable to the test product(s).

No subject discontinued participation in the study due to an adverse event.

In conclusion, the safety analysis did not identify any increased risk for the test products.

3.11 Additional Information Requested by FDA

This section includes the following additional information that FDA requested at the February 3, 2004 pre-PMA meeting: (1) a Kappa analysis regarding intra-examiner reproducibility and inter-examiner consistency for the pivotal study; (2) a meta-analysis comparing GEM 21S™ to current treatments periodontal bone defects; and (3) a reassessment of CAL gain due to the outlier patient.

3.11.1 Kappa-Analysis

The PMA also summarizes the results of the Kappa analysis regarding intra-examiner reproducibility and inter-examiner constancy of probing measurements, which FDA requested. The Kappa score was 0.9357 ($p<0.0001$) for intra-examiner reproducibility. The Kappa score was 0.8901 ($p<0.0001$) for inter-examiner consistency. Thus, the probing measurements were both reproducible within each investigator and consistent across investigators.

3.11.2 Meta-Analysis

Comparisons of the GEM 21S™ clinical results were made to the clinical outcome findings ("meta-analysis") conducted by the American Academy of Periodontology and reported by Reynolds et al. (48) and Giannobile et al. (49) in the December 2003 issue of the *Annals of Periodontology*. The lead authors of each of these articles compared GEM 21S™ (β-TCP+0.3mg/ml PDGF) to the results reported in their respective published articles.

In the first analysis, Dr. Mark Reynolds, the lead author of the article on bone substitutes, concluded that the GEM 21S™ clinical study patient population was comparable to the patient population described in the published meta-analysis, based on their baseline characteristics. The author compared GEM 21S™'s and current periodontal bone grafting materials' clinical attachment levels, probing depths, and linear bone fill. The results are summarized in the following table.

Treatment Modality	CAL Gain (MM)	PD Reduction (MM)	Tissue Fill (MM)
OFD	1.43	2.34	0.83
ALL	1.62	2.90	2.18
AUT	2.56	1.40	2.19
CER	2.76	3.17	2.11
COR	2.33	3.18	2.83
GLA	2.31	3.37	2.56
Emdogain	2.67	3.68	1.10
PEP GEN	1.10	3.2	-
GEM 21S™	**3.7**	**4.4**	**2.52**
B-TCP Alone	3.5	4.2	0.89

Abbreviations:
OFD=open flap debridement; ALL=allograft; AUT=autograft; CER=ceramic; COR=coralline; GLA=Bioactive glass

The author concluded that the GEM 21S™ study results demonstrated its clinical effectiveness in the treatment of periodontal bone defects and provided favorable outcomes compared to these historical clinical measures.

Drs. William Giannobile and Martha Somerman, the authors of the article on enamel matrix derivatives and growth factors, compared the clinical and radiological effects of GEM 21S™ to those reported in their article for Emdogain and open flap debridement, both of which are current standards of care. The results are summarized in the following table.

Treatment Modality	Linear Bone Gain (MM)	Bone Fill (%)	CAL Gain (MM)
OFD	0	0	2.1
Emdogain	1.1	14.5	2.9
Allograft	0.5	15.3	0.5
GEM 21S™	**2.5**	**56**	**3.7**
B-TCP Alone	0.9	17.9	3.5

The authors concluded that GEM 21S™ is safe and effective and was likely to provide superior results in clinical applications where bone augmentation is warranted.

Both meta-analyses showed that GEM 21S™ compares favorably to the current treatment options for periodontal bone defects.

3.11.3 CAL Recalculation Due to Patient Outlier

An assessment of patient enrollment indicated that a single patient at site number 9 (patient 09-21) was an outlier in the database where the absolute value of CAL gain declined from 3 to 6 months. This patient was enrolled in Group I (0.3 mg/ml). An assessment of this patient's radiographs was performed. It was noted that the radiograph for this patient at the time of surgery indicated an untreated acute apical infection at the surgical site. The use of GEM 21S in an untreated acute infection at the surgical site is a contraindication for the device. Thus, the rationale for this outlier analysis is justified on the basis of the radiographic evidence.

The revised CAL gain for Group I between surgery and 12 weeks is shown in the table below. The results have been computed to two decimal points to better illustrate the effects of the revision.

Table 34 clearly shows that the exclusion of the single outlier patient improves the superiority assessment. The CAL gain has improved from 3.78 mm to 3.81. Importantly, the p value of Group I vs. Group III has been improved from 0.041 to 0.032, both of which are statistically significant.

Table 1. Adjusted CAL Gain for Group I Between Surgery and 12 Weeks

Patient Effect: Change in CAL Gain at 12 Weeks Group I			
12 Weeks		**12 Weeks Revised (Patient 09-21 Excluded)**	
CAL Gain	**p-Value+**	**CAL Gain**	**p-Value+**
3.78 (SD=1.44)	**0.041***	3.81 (SD=1.43)	**0.032***
Ref.: Statistical Tables 11b2 and 11b2a			

+ vs. Group III (Not covariate-adjusted)
* Statistically significant

A similar improvement can be seen in the 24 week results, below. In the following table, the original CAL gain of 3.73 mm has been revised upward to 3.83 mm, indicating that the CAL gain has, in fact, improved from the 12 week time point.

Adjusted CAL Gain for Group I Between Surgery and 24 Weeks (Same as Table 9)

Patient Effect: Change in CAL Gain at 24 Weeks Group I			
24 Weeks		**24 Weeks Revised (Patient 09-21 Excluded)**	
CAL Gain	**p-Value+**	**CAL Gain**	**p-Value+**
3.73 (SD=1.69)	0.419	3.83 (SD=1.52)	0.282
Ref.: Statistical Tables 11b1 and 11b1a			

+ vs. Group III

At 24 weeks, the original assessment appeared to indicate that the change in CAL for Group between 12 and 24 weeks had declined from 3.8 mm to 3.7 mm. In contrast, the reanalysis of the data with the exclusion of a single patient with an acute infection indicates a slight improvement in CAL between 12 and 24 weeks. In all cases, Group I continued to be improved compared to Group III.

A similar effect was seen by removing the entire site from the analysis as shown in the table below. By excluding Site 09, the CAL gain at 24 weeks has been revised from 3.73 mm (original analysis) to 3.85 mm. Further, this revision shows an improvement in CAL gain from 12 weeks (3.78 mm) to 24 weeks (3.85 mm).

Table 2. Adjusted CAL Gain for Group I Between Surgery and 24 Weeks

Center Effect: Change in CAL Gain at 24 Weeks Group I			
24 Weeks		**24 Weeks Revised (Site 09 Excluded)**	
CAL Gain	**p-Value+**	**CAL Gain**	**p-Value+**
3.73 (SD=1.69)	0.419	3.85 (SD=1.51)	0.341
Ref.: Statistical Tables 11b1 and 11b1b			

+ vs. Group III

The recalculation improved the significance of CAL gain at 12 weeks from a p value of 0.041 to 0.021 (both statistically significant); improved the 24 week CAL Gain from 3.72 mm to 3.83 mm; and revised the statistical significance for the Area Under the Curve ("AUC") analysis from p=0.054 to p=0.033, a statistically significant finding.

3.12 Conclusions Drawn From the Studies

This section contains a summary of the safety and effectiveness data regarding the GEM 21S™ and a risk benefit analysis of this device.

3.12.1 Safety

This study revealed no evidence of either local or systemic adverse effects. This is consistent with the GEM 21S™ biocompatibility data and the historical safe use of each individual component. There were no adverse outcomes attributable to the study device and the device. Thus, was found to be safe.

3.12.2 Effectiveness

GEM 21S™ was found to be an effective treatment for the restoration of soft tissue attachment level and bone as shown by:

- Significantly improved clinical attachment levels at three months compared to the active control, a finding that was consistent with the area under the curve analysis that showed an improvement in clinical attachment level gain between baseline and six months;

- Significantly improved linear bone growth and % bone fill compared to the active control;

- Significantly improved clinical outcomes as shown by the composite analysis of both soft and hard tissue measurements compared to the β-TCP alone active control;

- Exceeding established benchmarks of effectiveness both clinically and radiographically.

The effectiveness outcome measures, as shown in the clinical and statistical results above, together with extensive and confirmatory data from *in vitro*, animal and other human studies, provide a persuasive and powerful record on the benefits of GEM 21S™ in regeneration of both soft and hard tissue in periodontal defects. Thus, it is concluded that GEM 21S™ was shown to achieve clinical and radiographic effectiveness by six months for the treatment of moderate to severe intraosseous periodontal defects.

3.12.3 Summary

GEM 21S™ was shown to be safe and effective in the restoration of alveolar bone and clinical attachment around teeth with moderate to advanced periodontitis in a large, randomized clinical trial involving 180 subjects studied for up to 6 months.

As a fully synthetic product, the risk-to-benefit assessment establishes the GEM 21S™ device to have a sound and compelling clinically beneficial effect as measured by standard and accepted outcomes with minimal or no risks.

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Section 4: Principles of Operation of the Device

GEM 21S™ Summary Principles of Operation

4.1 Principles of Operation/Properties of the Device Relevant to its Indications for Use

GEM 21S™ is a bone void filler that consists of equal parts β-TCP and Becaplermin. Although the β-TCP and Becaplermin are combined before being implanted in the bone defect, each component performs different functions.

4.1.1 β-TCP

Orthovita's Vitoss, which is GEM 21S™'s β-TCP component, is the primary component of the device. β-TCP is a synthetic bone void filler that has a similar chemistry and molecular structure to human cancellous bone. This component provides a biocompatible, osteoconductive, and three dimensional scaffold to facilitate new bone formation. It also stabilizes the blood clot and prevents the collapse of scar tissue into the bone void.

More specifically, when the GEM 21S™ product is placed in direct contact with viable host bone, new bone grows in opposition to the β-TCP's calcium phosphate surfaces. As GEM 21S™'s β-TCP resorbs, bone and other connective tissues grow into the space previously occupied by the scaffold.

4.1.2 rhPDGF-BB

GEM 21S™ contains a diluted solution (0.3 mg/ml) of rhPDGF-BB from Chiron which enhances the physical and mechanical action of the β-TCP by promoting cellular in-growth into the periodontal bone defect and the β-TCP scaffold, thus providing an optimal bone healing environment.

The mechanisms of action of PDGF are well understood. Endogenous PDGF is one of five major growth factors that appear to initiate the cascade of events that lead to healing. [4,6] Naturally occurring PDGF is contained within the alpha granules of blood platelets where it is delivered to injured areas in the body. Once released from the platelets (or the recombinant protein is applied) it has been shown to establish the normal wound healing cascade, to include proliferation (mitogenesis) of osteoblasts and periodontal ligament fibroblasts as well as their directed migration (chemotaxis) and protein synthesis by binding to well characterized cell surface receptors. PDGF also initiates angiogenesis leading to capillary budding into the graft. In addition, PDGF is present in bone matrix and is produced locally at fracture sites. [60, 44, 43, 1,8] Cell surface receptors for PDGF are up regulated during fracture healing, which provides additional evidence that these proteins contribute to normal fracture healing.[1, 30] Thus, PDGF encourages the repair process by inducing cell chemotaxis, activation of inflammatory cells and increasing extracellular matrix deposition, cell mitogenesis, and protein synthesis.

There are a number of reports of *in vitro* studies demonstrating the potent effects of PDGF on the proliferative activity of periodontal ligament fibroblasts. [20, 57, 67, 73] PDGF stimulates gingival fibroblast hyaluronate synthesis, a prerequisite for the formation of large aggregates of proteoglycans that provide the lattice for the extracellular matrix. [3] PDGF has also been shown to promote periodontal ligament fibroblast proliferation beneath expanded polytetrafluorethylene (ePTFE) fenestration defects in dogs. [97]

The extensive pre-clinical literature, including animal studies in rats, dogs, miniature pigs and non-human primates, and clinical studies regarding rhPDGF-BB demonstrates that its application to general and periodontal bone defects, promotes periodontal regeneration (including new bone, periodontal ligament and cementum formation).

4.1.2 GEM 21S™

The mixing of β-TCP and rhPDGF-BB does not alter the properties of either component. Thus, GEM 21S™ provides the clinical benefits of both of its components.

Section 5: Sterilization of the Device

5.0 Information on Sterilization of the Device; Manufacturing

The components of the GEM 21S™ kit are provided to the user as sterile, while the tray used in the packaging and shipping of the kit are non-sterile.

5.1 Introduction and Overview

BioMimetic has a well-established Quality System that describes, implements, and monitors the good manufacturing practices the company follows in the manufacture of the GEM 21S™. The elements of this system are described in the sections below. The format and content of the information contained in this section follows the Food and Drug Administration's ("FDA" or the "agency") *Quality System Information for Certain Premarket Application Reviews; Guidance for Industry and FDA Staff (February 3, 2003)* 2/ and includes information regarding the company's compliance with major provisions of FDA's Quality System Regulation ("QSR") (21 C.F.R. Part 820). Representative procedures and forms that are in effect at the time of this submission are included within the Appendices to this section.

Individual manufacturing, packaging, sterilization, testing, and distribution processes for GEM 21S™ (and GEM 21S™ components) are performed under the direction of BioMimetic at contract vendors, including: (1) [redacted] the contract vendor who is responsible for the filling, packaging, and testing of the Beta Tricalcium

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2/ The February 2003 Guidance supersedes the draft *Guidance on Quality System Regulation Information for Various Premarket Submissions* released for comment on August 3, 1999.

Phosphate ("β-TCP") component of GEM 21S™ into perio-cups and the packaging, labeling, and release of the final GEM 21S™ Kit; [redacted] contact sterilization vendor who is responsible for sterilizing the filled β-TCP perio-cups; [redacted] the contract vendor who is responsible for the dilution of bulk rhPDGF-BB, filling of the diluted rhPDGF-BB into sterile Hypak syringes, and performing in-process analytical testing; an[redacted] the contract vendor who is responsible for conduct analytical and stability testing on the rhPDGF-BB finished product. Each of these processes, and BioMimetic's practices to ensure QSR compliance in the manufacture, packaging, sterilization, testing, and distribution of GEM 21S™, are described below.

Finally, GEM 21S™ is a kit which contains component parts, *i.e.*, rhPDGF-BB, β-TCP, and a blunt-end cannula, which BioMimetic sources only from FDA-registered/licensed suppliers.

5.2 Manufacturing Processes

BioMimetic is the designer and specification developer for the GEM 21S™ Kit. BioMimetic subcontracts the manufacture, packaging, sterilization, testing, and distribution of the GEM 21S™ Kit to different contract vendors ensuring: (1) filling, packaging, and testing of β-TCP component of GEM 21S™ into perio-cups; (2) sterilizing the filled β-TCP perio-cups; (3) dilution of bulk rhPDGF-BB, filling of the diluted rhPDGF into sterile Hypak syringes, and performing in-process analytical testing; (4) packaging, labeling and release of the final GEM 21S™ Kit; (5) analytical and stability testing on the finished product; and (6) distributing GEM 21S™ into the market. Each kit contains a filed β-TCP perio-cup; a syringe containing

diluted rhPDGF-BB, and a 23 gauge blunt needle (or blunt end cannula). As discussed below in Section 6.16, the kit itself in not terminally sterilized.

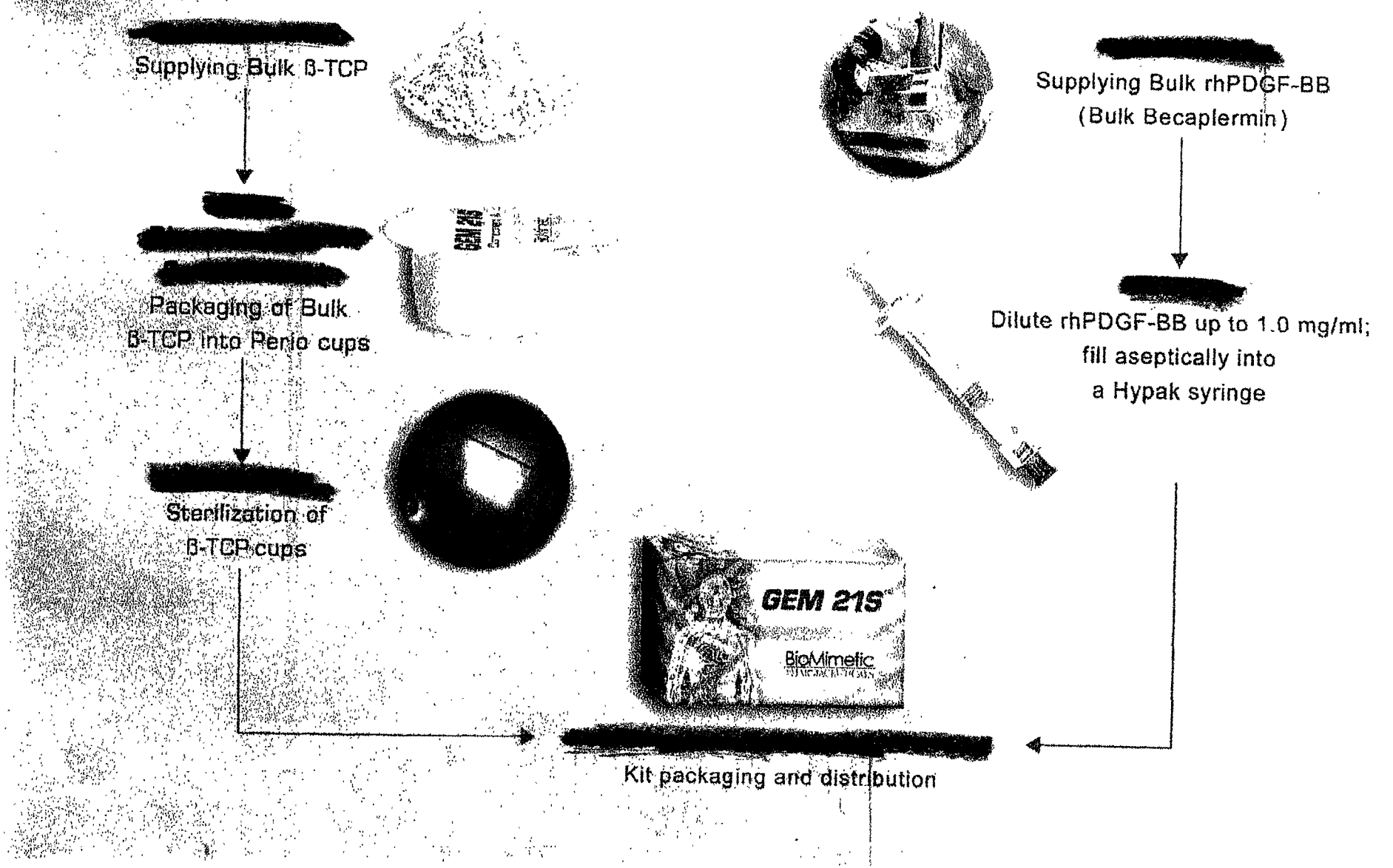
The manufacture and assembly of the GEM 21S™ kit is unique in that its production and components consists entirely of FDA licensed or marketed materials. It uses standard processes to produce the device and established testing procedures to evaluate its quality. BioMimetic does not directly manufacture or perform analytical testing of GEM 21S™. All the production and testing operations are performed by vendors specifically contracted by BioMimetic on the basis of their technical expertise, quality standards, and regulatory fitness in that all vendors:

- Have specific technical experience in the operations that they have been contracted to perform and have been assessed for their suitability to perform these functions;
- Have undergone an assessment on their quality systems; and,
- Have in place current and appropriate regulatory registrations, permits and certificates that allow them to perform their function in conformance with applicable laws and regulations.

The sterility for each of the components are assured through the overall manufacturing process, the quality systems applied and the adherence to recognized standards to include USP sterility testing for the aseptically filled rhPDGF-BB and sterilization validation of the β-TCP component in conformance with ISO 11307.

BioMimetic
PHARMACEUTICALS

GEM 21S™ MANUFACTURING FLOWCHART



Section 6: Nonclinical Laboratory Studies

Nonclinical Laboratory Studies

6.0 NONCLINICAL LABORATORY STUDIES

6.1 Introduction

As previously stated, the beta tricalcium phosphate ("ß-TCP") component of the GEM 21S™ device, *i.e.,* VitOss™, was 510(k)-cleared by the FDA as a osteoconductive synthetic bone void filler for the treatment of nonstability osseous defects resulting from surgery or traumatic injury to the bone. BioMimetic incorporates by reference all data and information in Orthovita, Inc.'s 510(k) notice for VitOss as authorized by Orthovita's letter of cross-reference.

The GEM 21S™ kit also contains a dilute solution of Becaplermin (.05 ml of 0.3 mg/ml) that enhances the recruitment of bone cells into the bone void filler facilitating bone regeneration. Becaplermin (also referred to as rhPDGF-BB or PDGF) is a synthetically produced and purified recombinant human platelet-derived growth factor that is a normal component in wound healing. A number of different isoforms of platelet-derived growth factor (PDGF) occur naturally in the body, such as PDGF-BB, PDGF-AB, PDGF-AA, PDGF-C and PDGF-D. The Becaplermin used in GEM 21 S™ is the same as the rh-PDGF-BB active ingredient used in the FDA, BLA-approved Regranex® product (FDA License No.1106), and has been subject to numerous *in vitro,* animal and human trials to demonstrate its safety and effectiveness as a wound healing agent. Additional information on the characteristics and manufacturing of Becaplermin are incorporated by reference.

More than 60 scientific articles have been published describing the contribution of each part of the GEM 21S™ device. Importantly, this substantive body of scientific evidence shows that each component of GEM 21S™ is safe and effective. The principal mode of action

of β-TCP is as an osteoconductive bone void filler. The device physically fills bone defects providing a matrix or scaffolding for bone formation. By filling the defect it also prevents collapse of the soft tissues into the bone defect and, due to its porous nature, facilitates stabilization of the blood clot. The product has a macroscopic structure and chemical and physical properties similar to human bone thus favoring bone ingrowth. This process does not require chemical action within or on the body nor does the synthetic bone substitute have to be metabolized to achieve bone regeneration. The rhPDGF-BB enhances the β-TCP's physical and mechanical actions by promoting cellular ingrowth into the osseous defect and bone matrix. Thus, rhPDGF-BB serves as an adjunct to the ß-TCP.

6.2 Published Studies on β-TCP

β-TCP is a purified, multicrystalline, porous form of calcium phosphate. The $Ca:PO_4$ ratio of β-TCP is 1.5 and is similar to that found in bone mineral (1.7). A number of animal studies have shown that β-TCP is compatible with host tissue and elicits no adverse reactions. [6, 47, 48, 64, 16, 58] Ingrowth of bone into the β-TCP bone substitute has been observed in numerous animal models for the treatment of various types of defects located throughout the skeleton.

In a study comparing non-resorbable hydryoxyapatite ("HA") to resorbable β-TCP, for use in surgically created defects in the mandible of sheep, the authors report that defects treated with β-TCP healed completely after four months while defects treated with HA did not heal completely, even after 12 months. [31] Additionally, the histologic results demonstrated that the β-TCP granules underwent remodeling and were replaced by host bone while the non-resorbable HA often exhibited fibrous encapsulation. At 12 months, the authors state that it was

not possible to distinguish the borders of the original defect treated with β-TCP, providing further evidence of excellent incorporation and remodeling of the β-TCP within the host bone.

In an animal study (caprine model) evaluating the use of β-TCP for treatment of tibial plateau fractures, the authors report that β-TCP performed as well as the gold standard (autologous bone) when evaluating stiffness of the regenerated bone. [98] Additionally, tibiae treated with autologous bone alone developed degenerative joint disease or plateau collapse, while those treated with β-TCP did not.

A study has been conducted comparing β-TCP, collagen sponge, demineralized freeze-dried bone allograft ("DFDBA") or hydroxyapatite (Interpore 200) in surgically created defects of miniature pigs. [12] The authors report that β-TCP demonstrated extensive substitution (replacement with host bone) during the healing period. Parallel with the decrease of β-TCP volume within the defect, the mean volume of bone matrix increased consistently, "reaching what was clearly the best level of all test fill materials (69%) after 24 weeks of healing, and in fact exceeding levels obtained with autograft alone (53%)". This finding indicates the usefulness of this material as a bone substitute due to its ability to facilitate host bone growth throughout the fill material by providing a substrate for matrix deposition, subsequently undergoing remodeling and finally being replaced by host bone.

β-TCP has been used in the treatment of periodontal defects in dogs and humans. [64, 16] β-TCP undergoes progressive degradation and replacement by calcified bone. [6, 16, 27, 62, 31, 12]

In a comparative study of proximal tibial marrow defects in nine adult Goettinger mini-pigs, The degradation kinetics of α- and β-TCP were evaluated. [56] Surgically created defects were prepared in the right and left tibiae, and defects on one side received β-TCP while the contralateral side was implanted with α-TCP. Both types of TCP were found to be osteoconductive, however, the β-TCP demonstrated faster degradation with better micro-osseous conduction and an enhanced tissue response compared to α-TCP.

A number of human clinical investigations have been conducted using β-TCP products. In a report of a prospective clinical study to compare combination grafts of β-TCP and autologous bone (iliac crest) to allograft and autologous bone (iliac crest), the authors report that both combination grafts were successful in achieving dorsal spondylodesis in adolescent idiopathic scoliosis (AIS). Bone mineral density was greater in the β-TCP group, with complete resorption of β-TCP after 8 ± 2 months. [62]

Numerous additional clinical studies provide further evidence of β-TCP's biocompatibility and usefulness as an osteoconductive scaffold for the treatment of intrabony defects resulting from periodontal disease. [65, 92, 36, 41, 84, 2, 86, 29, 78, 86] At six months post-surgery, for intrabony periodontal defects treated with β-TCP, the material has been shown to be well retained, provide flap support, reduce tooth mobility, and preserve function of the dentition. [92]

In a pilot study to assess the ability of TCP to maintain the alveolar ridge following tooth extraction, three patients with 10 fresh extraction sockets were treated with five TCP grafts, on one side, or with nothing (blood clot alone), on the contralateral side. [55]

Extraction sockets filled with TCP resulted in significantly better maintenance of alveolar ridge width and height compared to the control (contralateral) sites. The investigators concluded that the TCP resulted in more effective preservation of alveolar bone.

In over 25 years of use as a bone void filler, there are no known reports in the literature of either unfavorable host responses to β-TCP or interactions between β-TCP and any currently approved drugs.

β-TCP (VitOss™) Scaffold

The following section provides a brief description of the completed studies conducted on the VitOss™ component of GEM 21S™.

VitOss™ synthetic cancellous bone void filler is characterized by nano-sized particles and an extensive pore size distribution. VitOss™ is a porous, low-density construct prepared by fusing particles of β-TCP that average 1-2 μm in diameter. The use of nano-sized β-TCP primary particles increases the microporosity of the structure, resulting in a scaffold of approximately 90% interconnected void space, with pores ranging in size from 1 μm to 1000 μm. [21] This large increase in interconnected porosity, relative to other β-TCP constructs, potentially permits a more continuous supply of nutrients and extensive cellular and tissue ingrowth into the scaffold, with enhanced revascularization. These characteristic properties may facilitate a more rapid and complete bone repair. *In vivo* studies have shown that progressively smaller particles improve osteoconductive performance and by exposing more of its surface area to the biologic milieu, the material may be resorbed at a faster rate than more dense forms of β-TCP. [43]

A canine study was undertaken to assess the efficacy of VitOss™ synthetic cancellous bone void filler. [52] Blocks of VitOss™ were used in the repair of cylindrical metaphyseal defects measuring 10 mm by 25 mm in canine humeri. Bone ingrowth and scaffold resorption were quantified using standard histomorphometric techniques for specimens examined up to 1 year post-implantation. As early as three weeks, VitOss™ showed resorption throughout the defect site and some immature bone formation evenly throughout the site as well. Remodeling was essentially complete in six to 12 weeks, with bone density in the normal range and the scaffold almost completely resorbed by the end of that period. By 24 weeks, the implant's trabecular orientation approximated the normal stress patterns of adjacent bone. Histologically and radiographically, the bone within the defects were virtually indistinguishable from the surrounding bone at the 12-, 24-, and 52-week post-implantation time points. The stress-strain curve of VitOss™ was found to be nearly identical to the host bone at 24 and 52 weeks. X-ray diffraction (XRD) data confirms a 95% match to normal bone at 1 year.

6.3 Published Studies on PDGF

Set forth below is a summary review of several published *in vitro* and *in vivo* studies containing data demonstrating that PDGF, when applied at the low, safe levels provided for in the GEM 21 S™ device, has a beneficial effect when used in a single application. Specifically, these published studies demonstrate that:

(1) PDGF is naturally present in the bone matrix and is produced locally at fracture sites [44, 1, 8];

(2) PDGF provides for enhanced repair of craniofacial bone defects using only 2-3 ng of PDGF per day in chitosan-TCP sponges [46];

(3) PDGF is a chemotactic factor for bone cells and is naturally produced during early phases of fracture repair [44];

(4) PDGF is a necessary component in the promotion of normal fracture repair [30];

(5) Platelet Rich Plasma, which contains small amounts of PDGF, provides benefits to allograft in the rate of bone formation observed clinically [54];

(6) Clinical use of low amounts of PDGF (50μg/ml), in a single application to periodontal defects, was found to be safe and effective in the regeneration of bone, when used in combination with another growth factor, IGF [50]; and

(7) PDGF enhanced bone regeneration even when used in conjunction with the current therapeutic 'Gold Standard' of Demineralized Bone Matrix (DBM). [38]

The *in vitro* and *in vivo* literature has shown that the PDGF component in GEM 21S™ is sufficient to provide a beneficial affect that is equal to or better than that seen in marketed materials such as Autologous Growth Factors (AGF), without posing any of the risks associated with AGF. Furthermore, an acceptable safety profile of the single application PDGF

contained in the GEM 21S™ is supported by substantial published scientific evidence on the safe use of PDGF at levels far exceeding the low levels of PDGF contained in GEM 21S™. [83]

6.3.1 PDGF Mode of Action

Nearly 100 studies over 15 years have been published in the literature, demonstrating the regenerative effects of PDGF on bone and other components of the periodontium, in animals and humans. These studies have shown that PDGF can promote bone and periodontal regeneration *i.e.*, regeneration of bone, cementum and periodontal ligament (PDL).

Naturally occurring PDGF is contained within the alpha granules of blood platelets where it is delivered to sites of injury in the body. Once released from the platelets (or when the recombinant protein is applied) it has been shown to establish the normal wound healing cascade, to include the directed migration (chemotaxis) and proliferation (mitogenesis) of osteoblasts and periodontal ligament fibroblasts with subsequent protein synthesis, or matrix production . PDGF also initiates angiogenesis leading to capillary budding into the graft. PDGF is also present in bone matrix and is produced locally at fracture sites. [44, 1, 8] Further suggesting the role of these proteins in normal fracture healing, is the observation that cell surface receptors for PDGF are upregulated during fracture healing. [1, 30]

Furthermore, in direct comparative *in vitro* studies, it was found that PDGF had improved effects over other growth modulators, including bone morphogenic proteins (BMPs), on cell proliferation, migration and protein synthesis. [77]

6.3.2 Published PDGF Animal Studies

Numerous animal studies demonstrate the important physiologic role PDGF plays in bone regeneration and the effectiveness of rhPDGF-BB in stimulating bone cell recruitment (chemotaxis) and proliferation to assist in the regenerative process. The following summary points from these published studies provide evidence of PDGF's role in bone repair:

(1) Studies with transgenic mice have underscored the physiological role of PDGF as essential for bone development and regeneration. [1, 85] Spontaneous deletion of the alpha receptors for PDGF results in abnormal development of craniofacial bones and vertebrae, demonstrating the importance of PDGF in skeletal development; [79]

(2) PDGF has been shown to have significant effects on bone repair in tibial osteotomies in rabbits and to increase bone density in osteoporotic rodent skeletons. [63, 59] Systemic administration of rhPDGF-BB in ovarectomized rats has been shown to promote significant increases in osteoblast cell number, trebecular bone volume in both long bones and vertebrae, whole skeletal bone mineral density as judged by bone density scan, and strength of long bones and vertebrae as evaluated by 3-point bending and compression, respectively [59];

(3) The pleotrophic effects of PDGF on both the bone and periodontal ligament (PDL) make it well suited for enhancing regeneration of all the components of the periodontium *i.e.*, the supporting tissues around teeth. Several investigators have demonstrated the potent effects of PDGF on the proliferative activity of periodontal ligament fibroblasts *in vitro*. PDGF stimulates gingival fibroblast hyaluronate synthesis, a prerequisite for the formation of large aggregates of proteoglycans that provide the lattice for the extracellular matrix. [3] The growth factor has also been shown to promote periodontal ligament fibroblast proliferation beneath expanded polytetrafluorethylene (ePTFE) in protected fenestration defects in dogs; [97]

(4) Pre-clinical animal studies by several research groups have shown that the implantation of rhPDGF-BB alone or a combination of rhPDGF-BB and another growth factor, insulin-like growth factor I (IGF-I), has the capacity to stimulate new bone, as well as, periodontal ligament and cementum formation in periodontal lesions in dogs and non-human primates; [50, 51, 32, 77, 69]

(5) Cho and coworkers have shown that the addition of rhPDGF-BB to periodontal defects in dogs results in substantially greater regeneration than the use of guided tissue regeneration alone using ePTFE (Goretex) membranes. [17, 69] No adverse effects were observed;

(6) In other studies in the craniomaxillofacial field of study, Lee and coworkers have demonstrated that rhPDGF-BB added to a TCP/chitosan sponge stimulates bone fill in 8mm bone defects in the calvariae of adult rats. No adverse effects of the rhPDGF-BB/TCP/chitosan treatment were reported; [46]

(7) Nash and coworkers evaluated the effects of rhPDGF-BB on bone healing in tibial osteotomies in rabbits. The study found that rhPDGF-BB significantly enhanced normal bone wound healing in the bony environment. No adverse effects were observed; [63] and

(8) In an example of rhPDGF-BB's effect on bone formation in other skeletal sites, intravenous administration of rhPDGF-BB in ovarectomized, estrogen-deficient osteoporotic rats has been demonstrated to result in increased bone formation in

both the long bones and vertebrae. [89] The effect of rhPDGF-BB was generally equal to or greater than that achieved with Fosomax, Merck's bisphosphonate product.

In conclusion, the extensive pre-clinical literature, including animal studies in rats, dogs, miniature pigs, and non-human primates with rhPDGF-BB, demonstrates that application of PDGF to general bone defects, periodontal defects, or osteoporotic animals results in the filling of bone defects, periodontal regeneration (including new bone, periodontal ligament and cementum formation), and improved bone quality.

6.3.3 Published PDGF Clinical Studies

Naturally concentrated platelet-derived growth factors have been used clinically for the last five years employing commercially available systems to promote bone restoration for the treatment of bone voids and further support the role of PDGF in bone regeneration. This procedure involves the production of platelet-rich plasma [PRP, also referred to as autologous platelet gel (APG) after addition of a clotting agent] at the time of the surgical procedure.

Marx and co-workers recently performed a comprehensive clinical and analytical study assessing the mechanism of action and clinical significance of PRP in reconstruction of large mandibular discontinuity defects in humans. [53] The investigators confirmed increased platelet counts in PRP, increased PDGF and TGF-β concentrations in the PRP gel, and the presence of PDGF and TGF-β receptors on the surface of cells within iliac crest autografts. Furthermore, in an 88 patient human clinical trial they compared the clinical effectiveness of particulate iliac crest autograft alone to autograft plus PRP gel. All 88 patients received major reconstructive surgery resulting in 5 cm or greater discontinuity defects. The 44 defects treated

with the PRP/autograft demonstrated increases in both the rate of bone formation and bone density as evaluated clinically, radiographically and histologically, compared to the 44 patients who received autograft without PRP.

In a clinical trial evaluating a combination of rhPDGF-BB and rhIGF-I (insulin-like growth factor) in 38 human subjects possessing bilateral osseous periodontal lesions, PDGF/IGF-I treatment (0.15 mg/ml of each growth factor), was found to significantly increase alveolar bone formation compared to control subjects. No local or systemic safety issues were found as result of growth factor administration and no patients developed antibodies to the rhPDGF-BB protein. [37]

In a clinical trial, which was conducted in Brazil in nine human subjects possessing intra-osseous and/or furcation periodontal lesions (a total of 11 defect sites), the safety and preliminary effectiveness of rhPDGF-BB (1.0 mg/ml) in bone allograft was evaluated. Efficacy data demonstrated a favorable outcome clinically, radiographically and histologically following application of the rhPDGF-BB product. The PDGF/allograft treatment was found to result in periodontal regeneration, including restoration of bone, cementum, and periodontal ligament. No adverse effects of the treatment were reported. [13]

In summary, there is an extensive body of scientific publications that support: (1) the role and effectiveness of PDGF in stimulating bone cell recruitment (chemotaxis) and proliferation to result in bone and periodontal regeneration; and (2) the acceptable safety profile of a single application PDGF contained in the GEM 21S™ as demonstrated by published clinical data on the safe use of PDGF at levels far exceeding the low levels of PDGF contained in GEM 21S™.

6.4 Prior Laboratory Studies

In addition to the published studies supporting the characteristics of PDGF, BioMimetic Pharmaceuticals Inc. independently conducted *in vitro* and animal studies to further demonstrate relevant properties of GEM 21S™. Summaries of these studies are included in the following sections.

6.4.1 Binding and Release

The purpose of this *in vitro* study, which was conducted by BioMimetic at the Bone Tissue Engineering Center, Institute for Complex Engineered Systems, Carnegie Mellon University, Pittsburgh, PA, was to determine the binding and release kinetics of rhPDGF-BB (Becaplermin, Chiron Corp.) to various ceramic-based osteoconductive bone substitutes and the effectiveness of released rhPDGF-BB upon osteoblastic cell proliferation (bioavailability). Surface binding to ceramic materials can be expected to occur in two forms, non-specific ligand binding reversible by simple dilution and specific binding of ligands to a surface and interacting with competing ligands. Entrapment of molecules in the ceramic substrate will also play a role. Both binding and entrapment will determine the rate and magnitude of release of molecules from a carrier material.

The rhPDGF-BB binding and release studies included VitOss™ (OrthoVita, Inc.) of the specific size used in GEM 21S™ and the following ceramic bone substitutes for comparative purposes: (1) Pro Osteon® 200R (Interpore Cross International); (2) ChronOS™ (Mathys Medical); and (3) Calceon 6™ (Synthes). However, the studies primarily focused on the beta tricalcium phosphate ("β-TCP") based materials, VitOss™ and ChronOS™. Scanning electron microscopy ("SEM") was conducted on supplied ceramic samples to determine any micro- and nano particle differences between the TCP-based materials.

Binding Study Methods

Binding studies were conducted under conditions intended to mimic the clinical situation where the surgeon saturates the ceramic bone substitute with a predetermined volume of rhPDGF-BB solution prior to placement in the defect during surgery. Binding conditions were targeted to minimize manipulation, specialized equipment, time and labor in preparation for clinical usage of the device. For all binding experiments, a working volume of 75 uL of rhPDGF-BB (at either 0.2 or 0.5 mg/ml) and 100 uL volumes of ceramic bone substitute were used. The rhPDGF-BB was first iodinated (^{125}I) using a standard chloramines-T/sodium metabisulfate oxidation/reduction reaction and then the rhPDGF-BB and the ceramic bone substitutes were added to microcentrifuge tubes and incubated at 23°C (room temperature). Reactions were then terminated over intervals between 1 and 30 minutes by adding 1 mL of sodium acetate buffer. After centrifuging and discarding the supernatant, bound rhPDGF-BB in the material remaining in the microcentrifuge tubes was determined by measuring the radioactivity. Additionally, rhPDGF-BB added to microcentrifuge tubes that did not contain ceramic bone substitute was processed through the same procedures to assess any binding to the microcentrifuge tubes.

Release Study Methods

Experiments to determine the rhPDGF-BB release profile were conducted by incubating the rhPDGF-BB bound to ceramic bone substitutes in vials containing either phosphate buffered saline (PBS) or MEM:Ham's F12 media containing 10% fetal bovine serum and sodium azide. The vials of rhPDGF-BB bound ceramic bone substitutes were subsequently incubated at 37°C under a continuous rocking motion. At intervals between 0 and approximately

50 hours post-incubation, 50 uL aliquots of material were removed from the vials and an equivalent volume of PBS or serum media was added as replacement. The aliquots of removed material were measured for radioactivity.

Biological Activity Assessment Methods

The biological activity of the released rhPDGF-BB was determined by application of the releasate onto cultured human MG63 osteoblast cells and examination of the uptake of ^{3}H-thymidine into newly synthesized DNA. Sterilized samples were incubated with sterile preparations of unlabeled rhPDGF-BB. Under aseptic conditions, released rhPDGF-BB from bound materials was collected at 30 minutes, 4 hours, and 24 hours. PBS or serum media unexposed to rhPDGF-BB was used as baseline controls. The ^{3}H-thymidine incorporation assay involved seeding of approximately 2 x 10^{4} cells into microwells containing 0.5 mL 10% serum MEM:Ham's F12 media to which antimicrobials were added. Following 48 hours of culture, 0.5 uCi of ^{3}H-thymidine was added to the microwells and the cultures were incubated for an additional 24 hours. After rinsing, fixation and solubilization of cells, samples were counted for radioactivity.

Summary of Results

For all materials, 0.075 ml of rhPDGF-BB solution completely saturated 0.1 cc of bone substitute. RhPDGF-BB bound to all materials examined with maximal binding observed within 10 minutes. Between 25-50% of rhPDGF-BB that was originally applied in solution remained bound to all materials after washing with sodium acetate buffer. VitOss™ and ChronOS™ displayed similar binding characteristics. Similar binding characteristics were observed when binding was performed in vials or in open dishes. Bound rhPDGF-BB was released from all materials. VitOss™ and ChronOS™ displayed similar release profiles. For

VitOss™ in serum containing media, 50% of the rhPDGF-BB was released within 30 minutes, over 90% was released by 24 hours and all detectable protein was released in 48 hours. Releasates from either PBS or serum conditions stimulated cell proliferation in a similar pattern as mass of rhPDGF-BB released from these materials over time. Proliferation of osteoblast-like cells increased by 5- to 18-fold when exposed to the rhPDGF-BB releasate from VitOss™.

Theoretical projection of these *in vitro* data to *in vivo* models would indicate a bolus release of rhPDGF-BB of about 70% within minutes to hours after implantation of the ceramic bone substitute material saturated with a sodium acetate buffer containing rhPDGF-BB. This bolus release is followed by release of the remaining 30% over a 2 to 3 day period. The rhPDGF-BB released initially as a bolus as well as the rhPDGF-BB released over the following few days would be expected to be biologically active based on these studies.

A more detailed test report of this study is included.

6.4.2 Residence Time Study

A residence time study in rats was conducted using rhPDGF-BB (Becaplermin, Chiron Corp.) and demineralized, freeze-dried human allograft bone matrix (DFDBA) and VitOss™ (β-TCP). This study was conducted by BioMimetic at the Bone Tissue Engineering Center, Institute for Complex Engineered Systems, Carnegie Mellon University, Pittsburgh, PA. The purpose of this study was: (1) to compare the binding of rhPDGF-BB to DFDBA and β-TCP; and (2) to determine the residence time, or release profile, for rhPDGF-BB absorbed to either β-TCP or allograft in an in vivo craniofacial bone defect model (skeletally mature rats).

<u>Binding Study Methods</u>

For the binding studies rhPDGF-BB was iodinated (^{125}I) with using a standard Chloramine – T/sodium metabisulfate oxidation/reduction reaction. VitOss™ β-TCP and DFDBA were then saturated with of iodinated rhPDGF-BB in sodium acetate buffer and the volumes required for saturation were recorded. The combination materials' radioactivity was characterized and implanted in calvarial defects in anesthetized rats. A total of six rats were implanted with each test material. Radioactivity at the defect sites was measured at different time-point intervals during one week.

In Vivo Residence Time Study Methods

A mixture of (^{125}I)-rhPDGF-BB and unlabeled rhPDGF-BB and β-TCP or DFDBA was administered to rats at a concentration of 50,000 cpm/animal. This mixture was composed of 0.05 cc of β-TCP or DFDBA added to 375. uL of a 0.25 mg/mL solution of radiolabeled and unlabeled rhPDGF-BB. Cranial defects were surgically created in anesthetized rats, the rhPDGF-BB, β-TCP or DFDBA grafts were implanted, and the wounds were closed. Radioactivity at the implant sites was measured at different time-point intervals over one week. A total of six rats were implanted with each test material.

Results

The data demonstrate that on average 45% and 40% of the rhPDGF-BB applied in a solution, bound to β-TCP and allograft, respectively. When applied to calvarial defects in rats, approximately 95% of the rhPDGF-BB was released from both β-TCP and allograft implant sites within 48 hours. Approximately 2-5% of the rhPDGF-BB remained in both groups at one week. There were no significant differences between either VitOss™ or allograft groups. This study demonstrated that the binding and release (residence time) of rhPDGF-BB to β-TCP and DFDBA is comparable.

6.5 Canine Study

Between September 2001 and March 2003, BioMimetic Pharmaceuticals, Inc. sponsored a randomized, control study in canines at the State University of New York, Buffalo, New York. The purpose of this study was to assess the periodontal regenerative properties of 1) rhPDGF-BB ("PDGF") in combination with β-TCP; 2) rhPDGF-BB in combination DFDBA (allograft), or 3) DFDBA (allograft) alone. Two (2) separate doses of PDGF, one at 0.3 mg/mL and one at 1.0 mg/mL, were evaluated in the study.

Using a beagle dog model with horizontal Class III furcation defects, a model that provides for 'critical size' defects that would not otherwise heal on their own, fifteen (15) adult animals contributed 60 treated defects for the study. Seven treatment groups received treatment according to the experimental design described in the table, below. Treatments were histomorphometrically assessed at two and four months for: (1) length of complete new attachment apparatus (CNAA); (2) new bone fill (NB); (3) connective tissue fill (CT); and (4) void (VO). Specifically, six (6) defects per treatment group were biopsied at two months for a

total of 42 sites and six defects in treatment groups 1, 2, and 3 were biopsied at four months for a total of 18 sites. All histomorphic assessments were performed blinded to the treatment.

Table PDGF Canine Treatment Study Experimental Design

Group No.	No. of Sites	Treatment	Sites/Assessment Points
1	12	β-TCP alone	6 sites at week 8; 6 sites at week 16
2	12	β-TCP + 0.3 mg/mL PDGF	6 sites at week 8; 6 sites at week 16
3	12	β-TCP + 1.0 mg/mL PDGF	6 sites at week 8; 6 sites at week 16
4	6	DFDBA alone	6 sites at week 8
5	6	DFDBA + 0.3 mg/mL PDGF	6 sites at week 8
6	6	DFDBA + 1.0 mg/mL PDGF	6 sites at week 8
7	6	Surgery, no graft	6 sites at week 8

One-way analysis of variance was used to test for overall difference among treatment groups, and pairwise comparisons were made using the Student's t-test. The following significant differences between groups were found at the two-month assessment point:

- β-TCP, when combined with rhPDGF-BB for the treatment of periodontal bone loss, resulted in greater periodontal regeneration than current products or procedures, β-TCP alone, bone allograft or periodontal surgery without grafts. Of note is that β-TCP with 0.3 mg/ml PDGF was significantly better than the current gold standard of DFDBA.

- The 0.3 mg/ml concentration of rhPDGF was at least equally as effective as the 1.0 mg/ml concentration when mixed with β-TCP.
- β-TCP was more effective than allograft when mixed with rhPDGF-BB.
- The new bone matured (remodeled) normally over time (0-8-16 weeks) in all groups tested at these time points. There was no increase in ankylosis or root resorption in the rhPDGF groups. Sites receiving rhPDGF-BB exhibited improved healing as shown by a decrease in ankylosis vs. control sites. This finding may result from the fact that rhPDGF-BB is mitogenic and chemotactic for periodontal ligament cells.

At the four-month assessment point, which only included treatment groups 1, 2 and 3, there remained significant differences in periodontal regeneration. The following observations were made:

- β-TCP alone, as a result of extensive ankylosis, resulted in 36% regeneration, while the sites treated with β-TCP containing rhPDGF-BB had a mean regeneration of 58% and 49% in the 0.3 and 1.0 mg/ml rhPDGF-BB concentrations; and

- Substantial bone fill was present in all three treatment groups. β-TCP alone resulted in 70% bone fill, β-TCP plus 0.3 mg/ml rhPDGF yielded 100% fill while the 1.0 mg/ml rhPDGF group had 75% fill.

A detailed study report is attached. This study also was submitted to FDA on May 5, 2003 as a supplement to IDE G010340 in support of a protocol change to use the 0.3 mg/ml concentration as the primary endpoint. The supplement was approved on May 19, 2003.

6.6 Biocompatibility Studies

FDA's clearance or approval of each of the individual components of GEM 21S™ (VitOss and Becaplermin) demonstrates that these products are biocompatible. They also have a history of safe clinical use. An overview of the established safety and biocompatibility of VitOss and Becaplermin was provided in an Expert Report submitted in support of the GEM 21S™ European Technical Dossier for CE Marking. A copy of that Expert Report is included. To confirm that the combination of these components is biocompatible, BioMimetic conducted biocompatibility testing of GEM 21S™ consistent with the standard ISO 10993-1:1997 "Biological evaluation of medical devices - Part 1: Evaluation and testing Evaluation and testing". All studies were conducted in accordance with Good Laboratory Practices (GLP) and included the specific tests listed in the table, below. The biocompatibility studies were conducted with a combination of Becaplermin (1 mg/mL 20 mM NaAc Buffer) and VitOss Scaffold (ratio 1mL:1g) and have performed acceptably. Complete test reports are attached.

Table. Biocompatibility tests with GEM 21S™ (VitOss and Becaplermin)

ISO Reference	Title	Test Lab[1]	Report No.	Date	PMA Appendix
ISO 10993-3:1992	Genotoxicity: Bacterial reverse mutation study (Saline Extract)	NAMSA	V0023-211	1/14/02	7.7.5
ISO 10993-3:1992	Genotoxicity: Bacterial reverse mutation study (DMSO Extract)	NAMSA	V0023-212	1/14/02	7.7.6
ISO 10993-5:1999	Cytotoxicity study using the ISO elution method (1 X MEM Extract)	NAMSA	V0014-130	1/3/02	7.7.7
ISO 10993-6:1994	ISO muscle implantation study in the rabbit with histopathology (Surgical method, 4 weeks)	NAMSA	TI247-804, TH035-800, TI247-001	2/1/02	7.7.8
ISO 10993-10:1995	ISO sensitization study in the guinea pig (Maximization method)	NAMSA	TI261-300, TI261-301	1/25/02	7.7.9
ISO 10993-10:1995	ISO acute intracutaneous reactivity study in the rabbit (extracts)	NAMSA	TI251-800	1/8/02	7.7.10
ISO 10993-11:1995	ISO acute systemic toxicity study in the mouse (Extracts)	NAMSA	TI264-500	1/8/02	7.7.11

1. NAMSA Lab No: 01T 15267 00, Northwood, Ohio.

The results of individual studies for GEM 21S™ containing Becaplermin (PDGF) and the VitOss β-TCP are summarized below:

(1) *In vitro* Genotoxicity: Bacterial Reverse Mutation (Ames) test, Salmonella and E. coli strains, saline and DMSO extracts. Both extracts were considered to be nonmutagenic to Salmonella and E. coli strains.

(2) Sensitization in 15 Guinea Pigs; saline and cottonseed oil (CSO) extracts – Saline and cottonseed oil extracts from the device were individually

injected intradermally into guinea pigs and occlusively patched. Following a recovery period, a challenge patch was placed. The sites were evaluated at 24 to 96 hours. No evidence of delayed dermal contact sensitization was observed for either extract;

(3) *In vitro* Cytotoxicity, MEM elution -An extract of β-TCP and Becaplermin (PDGF) was made using minimal essential medium, and flowed over a confluent monolayer of mouse fibroblasts. The confluency of the monolayer, percent lysis, and cellular characteristics were analyzed to determine potential cytotoxicity. The 1X MEM test extract showed mild or no evidence of causing mild cell lysis or toxicity. The 1X MEM test extracts met the requirements of the test since all samples had a biological response of less than or equal to grade 2 (mild reactivity). The pH of the test medium was similar to the negative control medium at 48 hours;

(4) Intracutaneous reactivity in three rabbits, per extract, saline and cottonseed oil extracts– Saline and cottonseed oil extracts from the device were compared to blank vehicles, for erythema and edema in the rabbit. There was no evidence of significant irritation or toxicity from the extracts injected intracutaneously into the rabbits. There was evidence of slight irritation from the 0.9% sodium chloride USP solution injected intracutaneously into rabbits. The Primary Irritation Index Characterization for the cottonseed oil was negligible and slight for saline. The test animals injected with CSO appeared ungroomed 4 and 24 hours

after dosing, while the control animals injected with CSO appeared ungroomed 24 hours after dosing. This was considered an expected effect due to the unctuous nature of the extract. All other animals appeared clinically normal throughout the study;

(5) Acute systemic toxicity in five mice, per extract, saline and cottonseed oil extracts – Saline and cottonseed oil extracts from the device were injected intravenously or intraperitoneally in mice. Under the conditions of this study, there was no mortality or evidence of systemic toxicity from the extracts. Each test article met the test requirements; and

(6) Muscle Implantation Study in the Rabbit – The test article was prepared and then was surgically implanted in muscle tissue of the rabbit. The muscle tissue was evaluated for evidence of irritation or toxicity. Under the conditions of this study, the macroscopic reaction was not significant as compared to the comparative control material and not significant as compared to the USP negative control implant material. Microscopically, the test article was classified as a non-irritant as compared to the comparative control material and a slight irritant as compared to the USP negative control article. Additionally, the comparative control was considered a slight irritant as compared to the USP negative control article.

The biocompatibility studies described above demonstrate the biocompatibility of GEM 21S™. There was no evidence of mutagenicity, delayed dermal contact sensitization, systemic toxicity or mortality, and only sight to mild reactivity and irritation in these studies.

BioMimetic also demonstrated the biocompatibility of β-TCP in combination with PDGF using another commercially available β-TCP source, ChronOS™ (Mathys Medical). Test reports demonstrating the biocompatibility of the combination ChronOS β-TCP material, which were included in the Company's IDE (G010340), are incorporated by reference in this PMA application. The biocompatibility of the β-TCP/Becaplermin combination provides further evidence of the biocompatibilty of GEM 21S™'s β-TCP/Becaplermin combination.

Furthermore, β-TCP has been used clinically for more than years with no published unfavorable adverse responses. Becaplermin gel (Regranex®, has been FDA approved for nearly four years for at least 140 daily applications (20 weeks) to surgically excised wounds that extend into the subcutaneous tissue or beyond in the lower extremities of diabetics.

PDGF is a natural endogenous protein that lacks genotoxic potential. Additionally, it has a very low degree of absorption and a short half-life in plasma. [42]. When administered topically onto surgically excised wounds or subgingivally in periodontal defects, Becaplermin (PDGF) is quickly cleared (half-life of about four hours) and has an insignificant effect on endogenous plasma PDGF concentrations. [42, 51] For these reasons, Becaplermin is not considered to be a potential reproductive toxin or a systemic carcinogen. Thus, carcinogenicity testing was not conducted on GEM 21S™.

In summary, the extensive published data on the safety of VitOss and Becaplermin coupled with the ISO 10993 testing conducted by BioMimetic on the combination of these components demonstrate that GEM 21S™ is toxicologically safe and biocompatible.

GLP Statement

All biocompatibility testing described in this submission was conducted in full compliance with FDA's Good Laboratory Practice regulations, 21 C.F.R. Part 58. Certificates of quality assurance inspections conducted by the testing laboratory were included with each test report issued to BioMimetic Pharmaceuticals, Inc.

PERIODONTAL REGENERATION WITH PDGF TREATED OSTEOCONDUCTIVE SCAFFOLDS

EXECUTIVE SUMMARY

A randomized, control study in canines where the assessors were blinded was performed to determine the safety and effectiveness of two (2) separate doses of PDGF when used in conjunction with either β-TCP or allograft ("gold standard"). Fifteen adult beagle dogs contributed 60 treated defects using a recognized and standard model in seven (7) treatment groups. Treatment was assessed at 8 and 16 weeks.

This model provides for 'critical size' defects that would not otherwise heal on their own.

The results showed that β-TCP, when combined with PDGF, provided significantly better bone regeneration than allograft alone, the current gold standard. Also of significance was that β-TCP was more effective than allograft when combined with PDGF. It was found that the concentration of PDGF (0.3mg/ml and 1.0mg/ml) was equally effective when mixed with β-TCP. Finally, healing in the β-TCP + PDGF group was significantly improved in that the per cent connective tissue formed in these groups (scar) was eliminated ('0').

I. Study Dates

The study was conducted between September 2001 and March 2003.

II. Key Personnel/ Dates of Service/ Hours on Project

The Principal Investigator was: **Samuel E. Lynch, D.M.D., D.M.Sc.**
President, BioMimetic Pharmaceuticals, Inc.

Histologic Expert and Consultant: **Professor Robert Schenk**
University of Berne
Berne, Switzerland

Co-Investigators in the study were the following:

Dr. Rosemary Dziak
State University of NY at Buffalo, Buffalo, NY

Dr. Robert Genco
State University of NY at Buffalo, Buffalo, NY

Dr. Moon Il Cho
State University of NY at Buffalo, Buffalo, NY

Dr. Sebestiano Adreanna
State University of NY at Buffalo, Buffalo, NY

III. Specific Aims

The hypothesis of the present study was that rhPDGF-BB treatment of osteoconductive matrices, like β-TCP or DFDBA, will enhance the periodontal regenerative properties of these osteoconductive materials. The specific aims of the present study were to determine:

1. if β-TCP or DFDBA to which rhPDGF-BB has been adsorbed, promotes greater periodontal regeneration in the beagle dog animal model than unmodified β-TCP or DFDBA.

2. the concentration of rhPDGF-BB that, when added to β-TCP or DFDBA, stimulates the greatest amount of periodontal regeneration in the beagle dog animal model.

3. if any abnormal responses occur in periodontal defects particularly as it relates to healing as a result of the rhPDGF-BB treated β-TCP or DFDBA.

All three specific aims of this project were accomplished simultaneously using the beagle dog model with horizontal Class III furcation defects as previously conducted and described by Cho and Wikesjo in their studies on the effect of rhPDGF or BMP respectively (Park *et al*, 1995; Wikesjo *et al*, 1994). Fifteen adult beagle dogs contributed 60 treated defects.

IV. Treatment and Control Groups

Defects received either:

1. β-TCP (VitOss®)
2. β-TCP (VitOss®) plus rhPDGF-BB (0.3 mg/ml rhPDGF-BB)
3. β-TCP (VitOss®) plus rhPDGF-BB (1.0 mg/ml rhPDGF-BB)
4. Dog DFDBA
5. Dog DFDBA plus rhPDGF-BB (0.3 mg/ml rhPDGF-BB)
6. Dog DFDBA plus rhPDGF-BB (1.0 mg/ml rhPDGF-BB)
7. Sham surgery (treated by open flap debridement only, no graft)

Six (6) defects per treatment group were biopsied at two months (42 total sites). In addition, six defects in treatment groups 1, 2 and 3 were biopsied at four months (18 total sites).

TABLE 1. EXPERIMENTAL DESIGN

GROUP NO.	NO. OF TEST SITES	TREATMENT	TIME POINTS
1	12	β-TCP alone	8 & 16 weeks n=6 for 8 wk n=6 for 16wk
2	12	β-TCP + 0.3 mg/ml rhPDGF-BB	8 & 16 weeks n=6 for 8 wk n=6 for 16 wk
3	12	β-TCP + 1.0 mg/ml rhPDGF-BB	8 & 16 weeks n=6 for 8 wk n=6 for 16 wk
4	6	DFDBA alone	8 weeks
5	6	DFDBA + 0.3 mg/ml rhPDGF-BB	8 weeks

GROUP NO.	NO. OF TEST SITES	TREATMENT	TIME POINTS
6	6	DFDBA + 1.0 mg/ml rhPDGF-BB	8 weeks
7	6	Surgery, no graft	8 weeks

Accordingly, at 8 weeks there are 7 groups divided among 42 sites in 11 dogs. At 16 weeks, there are 3 groups divided among 18 sites in 4 dogs.

All forty-two (42) biopsy specimens obtained two months following treatment were evaluable while 15 of 18 biopsy specimens obtained four months post-surgical were evaluable. One specimen was lost due to difficulties encountered during processing and two were not evaluable due to infection that was present.

V. Analyses

Histomorphometric analyses were performed on the blinded slides by Professor Schenk and Dr. Lynch. The following parameters were assessed:

a) **Length of Complete New Attachment Apparatus (CNAA):**
Periodontal regeneration measured as the distance between the coronal level of the old bone and the coronal level of the new bone, including only that new bone adjacent to new cementum with functionally oriented periodontal ligament between the new bone and new cementum.

b) **New Bone Fill (NB):**
Measured as the cross-sectional area of new bone formed within the furcation.

c) **Connective Tissue Fill (CT):**
Measured as the area within the furcation occupied by fibrous connective tissue.

d) **Void (VO):**
The area of recession where there is an absence of tissue.

VI. Results

A. Clinical Observations

Clinically, all sites healed well. There was qualitative evidence that the sites treated with rhPDGF-BB healed more quickly, as indicated by the presence of firm, pink gingivae within one week post-operatively. However, no quantitative measurements of healing were obtained. There were no adverse events experienced in any treatment group as assessed by visual inspection of the treated sites. Upon clinical observation, there appeared to be increased gingival recession in groups that received β-TCP or DFDBA alone.

B. Histomorphometric Observations:

Histomorphometric assessment of the length of new cementum, new bone and new periodontal ligament (CNAA) as well as new bone fill, connective tissue fill (scar formation) and void space were evaluated and are expressed as percentages. In the case of CNAA, values for each test group represent the CNAA measurements (length in mm)/ total available CNAA length (in mm) x 100%. Bone fill, connective tissue fill and void space were evaluated and are expressed as percentages of the total furcation defect area.

One-way analysis of variance (ANOVA) was used to test for overall differences among treatment groups, and pair wise comparisons were made using the Student's t-test. Significant differences between groups were found upon analyses of the coded slides. Table 2 shows the results at two months.

TABLE 2. TWO MONTH HISTOMETRIC ANALYSES

GROUP NO.	TREATMENT	% BONE FILL	% CONNECTIVE TISSUE (SCAR)	% VOID	% CNAA
1	β-TCP alone	28.0 ± 29.5	36.0 ± 40.99	12.0 ± 17.9	37.0 ± 22.8 **
2	β-TCP + 0.3 mg/ml rhPDGF	84.0 ± 35.8 ‡, †	0.0 ± 0.0 +	8.0 ± 17.9	59.0 ± 19.1 *, †
3	β-TCP + 1.0 mg/ml rhPDGF	74.2 ± 31.7 ‡	0.0 ± 0.0 +	0.0 ± 0.0	46.0 ± 12.3 *
4	DFDBA alone	6.0 ± 8.9	26.0 ± 19.5	30.0 ± 27.4	13.4 ± 12.0
5	DFDBA + 0.3 mg/ml rhPDGF	20.0 ± 18.7	36.0 ± 13.4	18.0 ± 21.7	21.5 ± 13.3
6	DFDBA + 1.0 mg/ml rhPDGF	46.0 ± 23.0 ≠	26.0 ± 5.48	8.0 ± 13.04	29.9 ± 12.4
7	Sham Surgery, no graft	34.0 ± 27.0	48.0 ±35.64	10.0 ± 22.4	27.4 ± 15.0

‡ GROUPS 2 AND 3 SIGNIFICANTLY GREATER THAN GROUPS 1, 4 AND 7.
* GROUPS 2 AND 3 SIGNIFICANTLY GREATER (P<0.05) THAN GROUPS 4 AND 7.
\+ GROUPS 2 AND 3 SIGNIFICANTLY LESS (P<0.05) THAN GROUPS 4, 5, 6 AND 7
** GROUP 1 SIGNIFICANTLY GREATER (P<0.05) THAN GROUP 4.
† GROUP 2 SIGNIFICANTLY GREATER (P<0.05) THAN GROUP 5.
≠ GROUP 6 SIGNIFICANTLY GREATER THAN GROUP 4.

The mean percent periodontal regeneration (CNAA) in the surgery without grafts and surgery plus β-TCP alone groups were 27.4% and 37%, respectively. In contrast, β-TCP groups containing rhPDGF-BB exhibited significantly greater periodontal regeneration ($p<0.05$) than surgery without grafts or DFDBA alone, groups (59% and 46% respectively for the 0.3 and 1.0 mg/ml concentrations versus 27% for surgery alone and 13% for DFDBA alone). The groups containing 0.3 mg/ml rhPDGF-BB + β-TCP demonstrated significantly greater periodontal regeneration ($p<0.05$) than the same concentration of rhPDGF-BB groups combined with allograft (59% versus 21.5%).

Bone fill was significantly greater ($p<0.05$) in the β-TCP + 0.3 mg/ml rhPDGF-BB (84.0%) and the β-TCP + 1.0 mg/ml rhPDGF-BB (74.2%) groups than in the β-TCP alone (28.0%), surgery alone (34%) or DFDBA alone (6%) treatment groups. There was also significantly greater bone fill ($p<0.05$) for the β-TCP + 0.3 mg/ml rhPDGF-BB group compared to the DFDBA + 0.3 mg/ml rhPDGF-BB group (84% and 20% respectively).

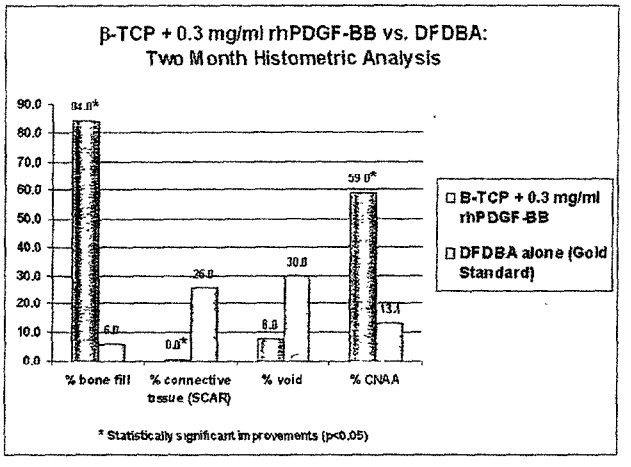


Fig 2

The group of analyses examining the 8-week data from the DFDBA groups (Groups 4, 5 and 6) and the surgery alone group (Group 7) demonstrated no statistically significant differences between the DFDBA groups and surgery alone for periodontal regeneration (CNAA). There was a trend toward greater regeneration for those sites treated with the 1.0mg/ml rhPDGF-BB enhanced DFDBA versus DFDBA alone. There was significantly greater bone fill ($p<0.05$) for sites treated with DFDBA + 1.0 mg/ml rhPDGF-BB than DFDBA alone (46 and 6% respectively). There was a trend toward greater bone fill for sites treated with DFDBA containing 0.3 mg/ml rhPDGF-BB compared to DFDBA alone or surgery alone. However, sites treated with DFDBA alone demonstrated less bone fill into the defect than surgery alone (6 and 34% respectively), with most of the defect being devoid of any fill or fill consisting of gingival (soft) connective tissue.

A comparison between results obtained 8 weeks post-surgical for the low dose rhPDGF-BB enhanced β-TCP and DFDBA alone are shown in Figure 2. These results show that rhPDGF-BB enhanced β-TCP yielded significantly greater percent bone fill as well as periodontal regeneration than DFDBA alone, currently the most widely utilized material in clinical practice.

At four months following treatment, there remained significant differences in periodontal regeneration. β-TCP alone, as a result of extensive ankylosis, resulted in 36% regeneration, while the sites treated with β-TCP containing rhPDGF-BB had a mean regeneration of 58% and 49% in the 0.3 and 1.0 mg/ml rhPDGF-BB concentrations. Substantial bone fill was present in all three treatment groups. β-TCP alone resulted in 70% bone fill, β-TCP plus 0.3 mg/ml rhPDGF yielded 100% fill while the 1.0 mg/ml rhPDGF group had 75% fill.

C. Histopathology Observations:

8-weeks post-surgical:

Fig 3A

Representative photomicrographs are shown in Figures 3, 4, and 5. Figure 3A shows results from a site treated with surgery alone (no grafts). This specimen demonstrates limited periodontal regeneration [new bone (NB), new cementum (NC) and periodontal ligament (PDL)] as evidenced in the area of the notches and extending a short distance coronally (arrows). Where regeneration is observed, the PDL is primarily of the type shown in Figure 3B, consisting of a fairly regular width of approximately 100 μm with good vascularity but loosely arranged somewhat irregularly oriented collagen fibers. The area of the furcation is occupied primarily by fibrous connective tissue (CT) with minimal new bone formation. Where new bone formation has occurred it is often in direct contact with the root surface (ankylosis).

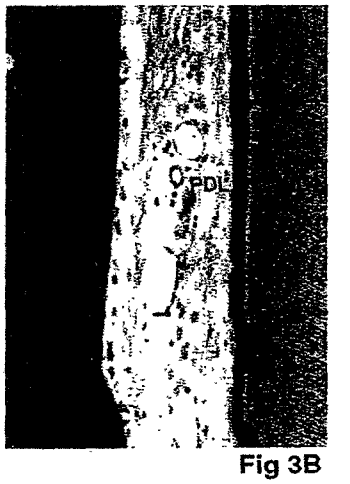


Fig 3B

For sites treated with β-TCP alone (Figure 4A) there is periodontal regeneration extending from the base of the notches for a short distance coronally (arrows). As was seen in the surgery alone specimens, minimal new bone formation was observed with the greatest area of the furcation being occupied by fibrous connective tissue. Periodontal regeneration that occurred resembled that obtained in the surgery alone specimen with little new cementum and a loosely organized PDL adjacent to new bone.

Fig 4B

In contrast, Figure 4B illustrates results obtained for sites treated with β-TCP + 0.3 mg/ml rhPDGF-BB. Significant periodontal regeneration is shown with new bone, new cementum, and periodontal ligament extending along the entire surface of the furcation (arrow at the base of one notch to the arrow at the base of the notch in the second root). Additionally, the area of the furcation is filled with new bone that extends the entire distance to the fornix of the furcation. The PDL space exhibits remarkably consistent width and vascularity although the collagen fibers within the coronal portion of the PDL space appear more loosely organized and more irregularly oriented than those at the apical portion of the PDL space. This finding may be the result of cells within the coronal portion of the PDL being less well differentiated than those at the apical portion of the PDL at this relatively early time point.

Fig 4C

Representative results for sites treated with β-TCP + 1.0 mg/ml rhPDGF-BB are shown in Figure 4C. While there is significant periodontal regeneration (arrows), it does not extend along the entire surface of the furcation. There is significant new bone formation within the furcation with fibrous connective tissue at the coronal portion of the defect. A small space which is void of any tissue (VO) at the fornix of the furcation is also observed. A higher magnification of the outlined area demonstrating periodontal regeneration is shown in Figure 4D. This area clearly demonstrates new bone and new cementum with perpendicularly oriented inserting collagen fibers.

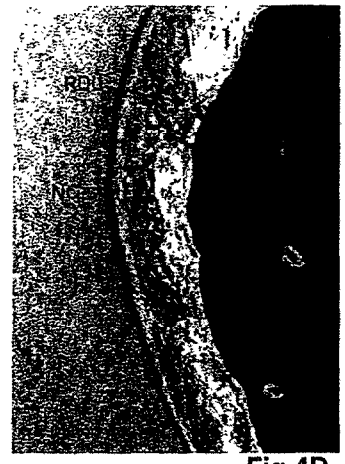


Fig 4D

Degradation of β-TCP occurs through processes of both dissolution and cell mediated degradation. High power magnifications of the cell mediated process may be seen in Figures 4E and F, representing specimens obtained from sites treated with rhPDGF-BB enhanced β-TCP. In Figure 4E the rather dense β-TCP particles (gray/blue in color) are partially dissolved and penetrated by capillaries (cap), fibroblasts (F) and macrophages (MØ). A higher power of the area outlined in this figure is shown in Figure 4F. The capillaries, fibroblasts, macrophages and a single giant cell are easily observed. The giant cell (GC) (to the left of the center) is loaded with β-TCP granules and there are heavily loaded macrophages to the right.

Fig 4E

Fig 4F

Finally, there were also β-TCP granules observed within newly formed bone matrix, in the rhPDGF-BB enhanced β-TCP treatment groups. A representative photomicrograph demonstrating this finding is shown in Figure 4G. The β-TCP granules are easily observed throughout the calcified matrix adjacent to fully enclosed osteocytes (oc). These findings clearly illustrate the biocompatibility of the growth factor enhanced β-TCP.

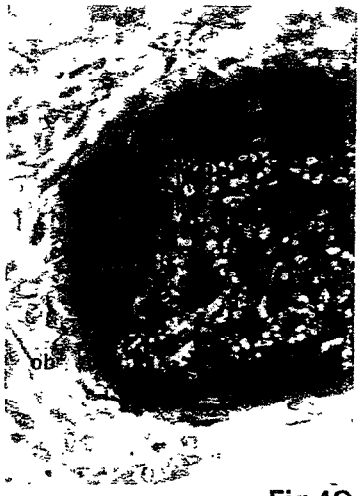


Fig 4G

Fig 5A

Fig 5B

Figures 5A, B and C illustrate results obtained for the allograft (DFDBA) treatment groups. Representative results for the DFDBA alone group (Figure 5A) shows poor periodontal regeneration that is limited to the area of the notches (arrows) extending only slightly in a coronal direction. New bone formation is limited and is surrounded by extensive fibrous connective tissue that extends coronally to fill a significant area within the furcation. Finally, a large void space extends from the coronal surface of the fibrous connective tissue to the fornix of the furcation.

Histologic results for the DFDBA + 0.3 and 1.0 mg/ml rhPDGF-BB are shown in Figures 5B and C, respectively. Both groups demonstrate greater periodontal regeneration, compared to DFDBA alone, that extends from the base of the notches in the roots for a short distance coronally (arrows). They also had greater bone fill within the area of the furcation, although there was significant fill of the furcation with fibrous connective tissue.

Figure 5D is a higher power representative photomicrograph for the DFDBA alone treatment group. The figure shows DFDBA lined by osteoblasts (ob) with new bone (NB) deposited along the surface of the particle. Note the presence of fibrous connective tissue (CT) surrounding the particles. This particular specimen also demonstrates a finding reported previously (Buser *et al.,* 1998) showing recalcification of an allograft particle. This is seen in the "stippled" area of the matrix that is a result of discrete spherical apatite-depositions around former canaliculi.

Fig 5C

Fig 5D

<u>16-weeks post-surgical:</u>
Evaluation of specimens at sixteen weeks was performed, primarily, in order to assess the long-term

effects of growth factor treatment. Since previous studies (Park *et al*, 1995) have shown that there may be substantial spontaneous healing of osseous defects in the dog model at this time point, treatment groups evaluated in this study included β-TCP alone or in combination with 0.3 and 1.0 mg/ml rhPDGF-BB. In all treatment groups, the bone underwent normal remodeling events progressing from the woven type bone observed in the 8-week specimens to bone that is more lamellar in appearance in the 16-week specimens.

A representative photomicrograph for the β-TCP alone treatment group is shown in Figure 6A. As was seen in the 8-week specimens for this treatment group, periodontal regeneration is limited primarily to the area of the root notches (arrows). There is increased bone formation in the area of the furcation, compared to the 8-week time point, but there continues to be a large area within the furcation that is occupied by fibrous connective tissue. Additionally, there is significant ankylosis present along much of the surface of the furcation, with direct contact between new bone and the root surface (lines). An area of root resorption may be observed at the area of the fornix of the furcation.

Fig 6A

Fig 6B

A representative photomicrograph for the β-TCP + 0.3 mg/ml rhPDGF-BB is shown in Figure 6B. There is periodontal regeneration present that extends from the base of the root notches along most of the surface of the furcation (arrows). There is a small area of ankylosis present in the area of the fornix of the furcation (lines). An area of the periodontal ligament space that is wider in appearance (upper box) than adjacent periodontal ligament space is observed along the superior extent of the furcation. The area appears to have undergone some remodeling, leading to a widened space. This appears to be a function of the dog model as there is an area (lower box) apical to the level of the original defect that also indicates evidence of spontaneous root resorption. Additionally, evidence of isolated root resorption was observed in the photomicrograph for the sham surgery and β-TCP alone treatment groups at 8 and 16 weeks (β-TCP alone). Therefore, in the absence of additional areas of evidence of root resorption, a single area within the site of treatment may be considered normal in this animal model.

Finally, Figure 6C shows a representative photomicrograph for the β-TCP + 1.0 mg/ml rh PDGF-BB treatment group. Again, there is evidence of periodontal regeneration extending from the notches in a coronal direction (arrows). There is less bone fill into the area of the furcation than was observed for the lower dose (0.3 mg/ml rhPDGF-BB) treatment group, with more of the area being occupied by fibrous connective tissue. There is ankylosis present at the fornix of the furcation extending apically for a short distance (lines). Within this region there are two small areas (boxes) indicative of root resorption. As was true in the 0.3 mg/ml rhPDGF-BB treatment group, there was no increase in root resorption in this treatment group over that observed for the β-TCP alone treatment group.

Fig 6C

VII. Conclusions

It can be concluded from this study that:

1. β-TCP, when combined with rhPDGF-BB for the treatment of periodontal bone loss, resulted in greater periodontal regeneration than current products or procedures, β-TCP alone, bone allograft or periodontal surgery without grafts. Of note is that β-TCP with 0.3 mg/ml PDGF was significantly better than the current gold standard of DFDBA.

2. The 0.3 mg/ml concentration of rhPDGF was at least equally as effective as the 1.0 mg/ml concentration when mixed with β-TCP.

3. β-TCP was more effective than allograft when mixed with rhPDGF-BB.

4. The new bone matured (remodeled) normally over time (0-8-16 weeks) in all groups. There was no increase in ankylosis or root resorption in the rhPDGF groups. Sites receiving rhPDGF-BB exhibited improved healing as shown by a decrease in ankylosis vs. control sites. This finding may result from the fact that rhPDGF-BB is mitogenic and chemotactic for periodontal ligament cells.

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DATE COMPLETED: **APRIL 28, 2003**

STUDY DIRECTOR: **DR. S. LYNCH**
BIOMIMETIC PHARMACEUTICALS, INC.
330 MALLORY STATION, NO. A1
FRANKLIN, TN 37067

STUDY SITES:
1. **STATE UNIVERSITY OF NY AT BUFFALO, BUFFALO, NY**
2. **UNIVERSITY OF BERN, BERN, SWITZERLAND**
3. **BIOMIMETIC PHARMACEUTICALS, INC., FRANKLIN, TN**

SIGNATURES

APPROVED BY:

DR. ROSEMARY DZIAK

APPROVED BY:

DR. ROBERT SCHENK

APPROVED BY:

DR. SAMUEL LYNCH

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SIGNATURES

APPROVED BY:

DR. ROSEMARY DZIAK

APPROVED BY: Robert Schenk

DR. ROBERT SCHENK

APPROVED BY: Samuel Lynch

DR. SAMUEL LYNCH

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I. INTRODUCTION

Recombinant human platelet-derived growth factor-BB, rhPDGF-BB or PDGF, has been shown to facilitate wound healing and bone regeneration in both animals and humans. It is approved in both the US and EU for chronic, repeated human use in topical applications to heal recalcitrant diabetic foot sores. PDGF has also been shown to be effective either singly or in combination with other growth factors for improving periodontal regeneration, i.e., regrowth of bone, cementum and ligament around teeth.

The synthetic bone matrix beta tricalcium phosphate (β-TCP or VitOss®) and demineralized freeze dried bone allograft (DFDBA) have also been shown to enhance bone regeneration in both animal models and in humans. β-TCP and DFDBA are considered osteoconductive materials in that they facilitate bone regeneration principally by providing a physical scaffold that facilitates clot stabilization and ingrowth of bone forming cells. Both materials are marketed in the US and EU for use in humans to treat bone defects in non-weight bearing applications. DFDBA in particular is currently considered a "gold standard" by clinicians for the treatment of bone and periodontal defects.

Recent studies on the utility of a PDGF+β-TCP system have demonstrated that PDGF can be adsorbed to β-TCP and DFDBA and that the growth factor is released *in vitro* and *in vivo*. Addition of PDGF to β-TCP or DFDBA has been shown to enhance *in vitro* osteoblast cell attachment and proliferation compared to untreated β-TCP or DFDBA.

As a result, a randomized, controlled study in canines where the assessors were blinded was performed to determine the safety and effectiveness of two separate doses of PDGF when used in conjunction with β-TCP.

II. PURPOSE

The hypothesis of the present study was that PDGF treatment used in conjunction with β-TCP (modern, synthetic bone void filler) or DFDBA (allograft "gold standard") will enhance the periodontal regenerative properties of these osteoconductive materials. The specific aims of the present study were to determine:

1. if β-TCP or DFDBA to which PDGF has been adsorbed, promotes greater periodontal regeneration in the beagle dog animal model than unmodified β-TCP or DFDBA.

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2. the concentration of PDGF that, when added to β-TCP or DFDBA, provides the greatest amount of periodontal regeneration in the beagle dog animal model.

3. if any abnormal responses; i.e., safety concerns, occur in periodontal defects particularly as it relates to healing as a result of the PDGF impregnated into β-TCP or DFDBA.

III. STUDY SITE

The study was conducted at the following locations:

1. BioMimetic Pharmaceuticals, Inc., Franklin, TN
2. State University of NY at Buffalo, Buffalo, NY
3. University of Bern, Bern, Switzerland

The study was IACUC approved. The animal welfare assurance number was A3354-01.

IV. STUDY DIRECTOR

The Study Director was: Samuel E. Lynch, D.M.D., D.M.Sc.
BioMimetic Pharmaceuticals, Inc.

Investigators in the study were the following:

Primary Investigator, State U of NY, Buffalo, NY
Dr. Rosemary Dziak
Co-investigators, State U of NY, Buffalo, NY
Dr. Robert Genco
Dr. Moon Il Cho
Dr. Sebestiano Adreanna
Primary Investigator (Histopathology), University of Bern, Bern, Switzerland
Dr. Robert Schenk

V. STUDY DATES

The "in life" portion of the study was conducted between January 2002 and January 2003. Histopathological analyses were completed in March 2003.

VI. MATERIALS AND METHODS

A. Materials Utilized: Test and Control Articles

The β-TCP (VitOss®) was supplied by OrthoVita, Inc., Malvern, PA and utilized a

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unique particle-size (0.25 mm – 1.0 mm) optimized for periodontal use. VitOss® is OrthoVita tradename for its synthetic, porous, osteoconductive, biocompatible, resorbable bone replacement device that conforms to ASTM Standard F 1088-87/92. VitOss® has been reported to be 80% resorbed within three months in the dog and replaced by autologous bone during the healing process. VitOss® has been cleared for use in humans by the FDA (K994337) as a bone void filler for use in skeletal defects. For the purpose of this study, material otherwise identical to commercial materials that met all applicable FDA standards were employed.

The DFDBA was supplied by Musculoskeletal Transplant Foundation (MTF). The material was dog allograft in order to minimize immunological response. It was made from the bones of a single dog that was killed following completion of another study that tested a surgical procedure that was deemed to have no effect on skeletal tissues and was processed in the same manner as human DFDBA in order to be representative of human material.

PDGF was supplied by BioMimetic Pharmaceuticals and was manufactured by Chiron, Inc, the only supplier of FDA-approved PDGF for human use. This PDGF was approved by the FDA as a wound healing product under the trade name of Regranex® and the materials employed in this study were from the same lots as those used in humans.

An agreement is in place between BioMimetics and Chiron Corporation for the supply of only clinical grade PDGF. This agreement between BioMimetics and Chiron assures that the PDGF will be of a quality that meets FDA standards and will be available for the proposed studies as well as future studies.

The test materials were supplied by the sponsor in a kit containing:

1. PDGF
   Syringes containing 0.5 ml of sterile PDGF at two separate concentrations prepared in conformance with FDA standards for human materials and according to current applicable Good Manufacturing Processes (cGMP). Concentrations tested included 0.3 mg/ml and 1.0 mg/ml.

2. β-TCP (VitOss®)
   Vials containing 0.5 cc of sterile particles, or

3. DFDBA
   2.0 ml syringes containing 1.0 cc of sterile, demineralized freeze-dried dog bone allograft

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Table 3. Two month histometric analyses

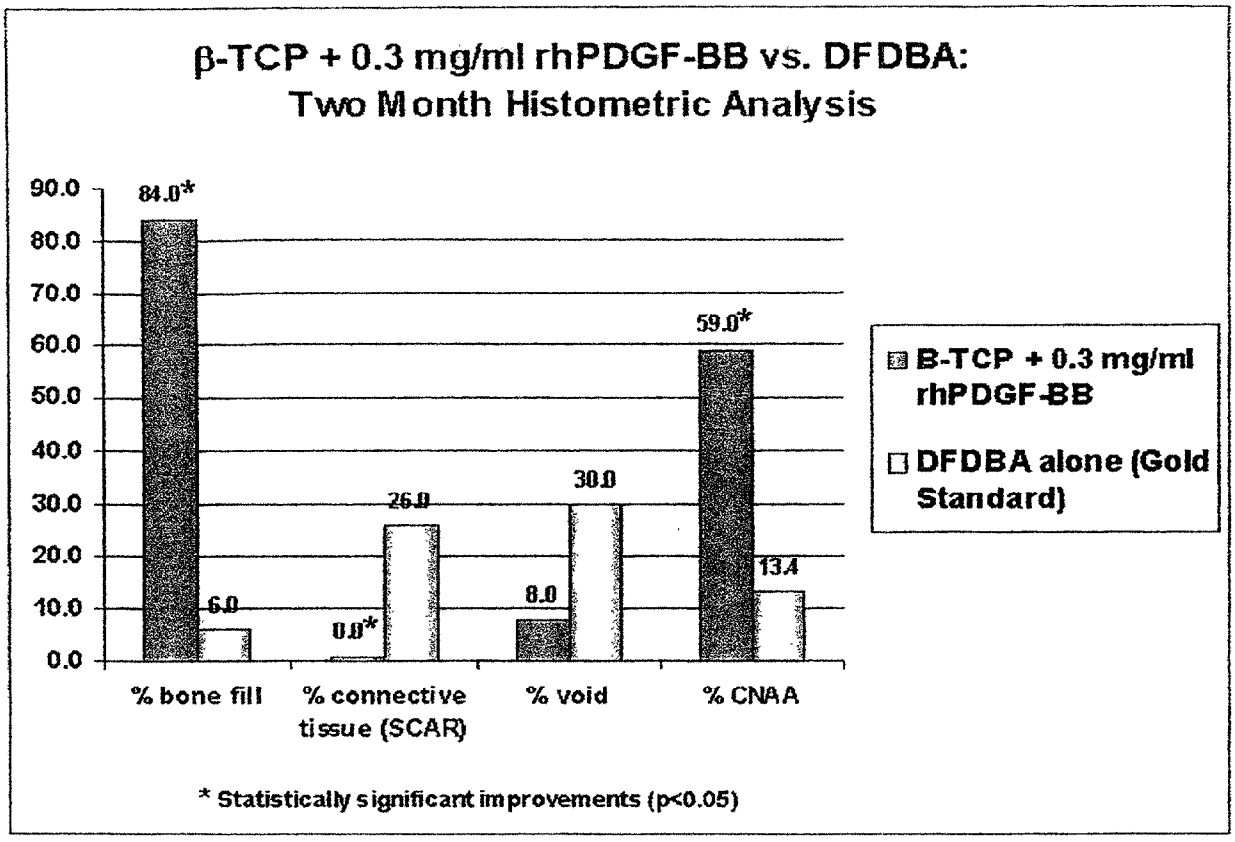
GROUP NO.	TREATMENT	PER CENT BONE FILL	PER CENT CONNECTIVE TISSUE (SCAR)	PER CENT VOID	PER CENT CNAA	COMMENTS
1	β-TCP alone	28.0 ± 29.5	36.0 ± 40.99	12.0 ± 17.9	37.0 ± 22.8**	
2	β-TCP + 0.3 mg/ml PDGF	84.0 ± 35.8‡, †	0.0 ± 0.0+	8.0 ± 17.9	59.0 ± 19.1*,†	Group No. 2 highest rank; statistically better than gold standard (allograft, Group 4)
3	β-TCP + 1.0 mg/ml PDGF	74.2 ± 31.7‡	0.0 ± 0.0+	0.0 ± 0.0	46.0 ± 12.3*	
4	DFDBA alone	6.0 ± 8.9	26.0 ± 19.5	30.0 ± 27.4	13.4 ± 12.0	'Gold Standard' of care. Did not perform as well as any β-TCP+PDGF groups ($p<0.05$)
5	DFDBA + 0.3 mg/ml PDGF	20.0 ± 18.7	36.0 ± 13.4	18.0 ± 21.7	21.5 ± 13.3	
6	DFDBA + 1.0 mg/ml PDGF	46.0 ± 23.0≠	26.0 ± 5.48	8.0 ± 13.04	29.9 ± 12.4	
7	Sham Surgery, no graft	34.0 ± 27.0	48.0 ±35.64	10.0 ± 22.4	27.4 ± 15.0	

SYMBOLS
‡ Groups 2 and 3 significantly greater than Groups 1, 4 and 7.
* Groups 2 and 3 significantly greater ($p<0.05$) than Groups 4 and 7.
\+ Groups 2 and 3 significantly less ($p<0.05$) than Groups 4, 5, 6 and 7
** Group 1 significantly greater ($p<0.05$) than Group 4.
† Group 2 significantly greater ($p<0.05$) than Group 5.
≠ Group 6 significantly greater than Group 4.

The mean percent periodontal regeneration (CNAA) in the surgery without grafts and surgery plus β-TCP alone groups were 27.4% and 37%, respectively. In contrast, β-TCP groups containing PDGF exhibited significantly greater periodontal regeneration ($p<0.05$) than surgery without grafts or DFDBA alone, groups (59% and 46% respectively for the 0.3 and 1.0 mg/ml concentrations versus 27% for surgery alone and 13% for DFDBA alone). The groups containing 0.3 mg/ml PDGF + β-TCP demonstrated significantly greater periodontal regeneration ($p<0.05$) than the same concentration of PDGF groups combined with allograft (59% versus 21.5%).

Bone fill was significantly greater ($p<0.05$) in the β-TCP + 0.3 mg/ml PDGF (84.0%) and the β-TCP + 1.0 mg/ml PDGF (74.2%) groups than in the β-TCP alone (28.0%), surgery alone (34%) or DFDBA alone (6%) treatment groups. There was also significantly greater bone fill ($p<0.05$) for the β-TCP + 0.3 mg/ml PDGF group compared to the DFDBA + 0.3 mg/ml PDGF group (84% and 20% respectively).

The group of analyses examining the 8-week data from the DFDBA groups and the surgery alone group (Groups 4, 5, 6, and 7) demonstrated no statistically significant differences between the DFDBA groups and surgery alone for periodontal regeneration (CNAA). There was a trend toward greater regeneration for those sites treated with the 1.0mg/ml PDGF enhanced DFDBA versus DFDBA alone. There was significantly greater bone fill ($p<0.05$) for sites treated with DFDBA + 1.0 mg/ml PDGF than DFDBA alone (46 and 6% respectively). There was a trend toward greater bone fill for sites treated with DFDBA containing 0.3 mg/ml PDGF compared to DFDBA alone or surgery alone. However, sites treated with DFDBA alone demonstrated less bone fill into the defect than surgery alone (6 and 34% respectively), with most of the defect being devoid of any fill or fill consisting of gingival (soft) connective tissue.



At four (4) months following treatment, there remained significant differences in periodontal regeneration. β-TCP alone, as a result of extensive ankylosis, resulted in 36% regeneration, while the sites treated with β-TCP containing PDGF had a mean regeneration of 58% and 49% in the 0.3 and 1.0 mg/ml PDGF concentrations. Substantial bone fill was present in all three treatment groups. β-TCP alone resulted in 70% bone fill, β-TCP plus 0.3 mg/ml PDGF yielded 100% fill while the 1.0 mg/ml PDGF group had 75% fill.

D. Histopathology Observations:

Representative photomicrographs are shown in Figures 2, 3, and 4. Figure 2A shows results from a site treated with surgery alone (no grafts). This specimen demonstrates limited periodontal regeneration [new bone (NB), new cementum (NC) and periodontal ligament (PDL)] as evidenced in the area of the notches and extending a short distance coronally (arrows). Where regeneration is observed, the PDL is primarily of the type shown in Figure 2B, consisting of a fairly regular

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width of approximately 100 μm with good vascularity but loosely arranged somewhat irregularly oriented collagen fibers. The area of the furcation is occupied primarily by fibrous connective tissue (CT) with minimal new bone formation. Where new bone formation has occurred it is often in direct contact with the root surface (ankylosis).

For sites treated with β-TCP alone (Figure 3A) there is periodontal regeneration extending from the base of the notches for a short distance coronally (arrows). As was seen in the surgery alone specimens, minimal new bone formation was observed with the greatest area of the furcation being occupied by fibrous connective tissue. Periodontal regeneration that occurred resembled that obtained in the surgery alone specimen with little new cementum and a loosely organized PDL adjacent to new bone.

In contrast, Figure 3B illustrates results obtained for sites treated with β-TCP + 0.3 mg/ml PDGF. Significant periodontal regeneration is shown with new bone, new cementum, and periodontal ligament extending along the entire surface of the furcation (arrow at the base of one notch to the arrow at the base of the notch in the second root). Additionally, the area of the furcation is filled with new bone that extends the entire distance to the fornix of the furcation. The PDL space exhibits remarkably consistent width and vascularity although the collagen fibers within the coronal portion of the PDL space appear more loosely organized and more irregularly oriented than those at the apical portion of the PDL space. This finding may be the result of cells within the coronal portion of the PDL being less well differentiated than those at the apical portion of the PDL at this relatively early timepoint.

Representative results for sites treated with β-TCP + 1.0 mg/ml PDGF are shown in Figure 3C. While there is significant periodontal regeneration (arrows), it does not extend along the entire surface of the furcation. There is significant new bone formation within the furcation with fibrous connective tissue at the coronal portion of the defect. A small space which is void of any tissue (VO) at the fornix of the furcation is also observed. A higher magnification of the outlined area demonstrating periodontal regeneration is shown in Figure 3D. This area clearly demonstrates new bone and new cementum with perpendicularly oriented inserting collagen fibers.

Degradation of β-TCP occurs through processes of both dissolution and cell mediated degradation. High power magnifications of the cell mediated process may be seen in Figures 3 E and F, representing specimens obtained from sites treated with PDGF enhanced β-TCP . In Figure 3E the rather dense β-TCP particles (gray/blue in color) are partially dissolved and penetrated by capillaries, fibroblasts and macrophages. A higher power of the area outlined in this figure is

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shown in Figure 3F. The capillaries, fibroblasts, macrophages and a single giant cell are easily observed. The giant cell (to the left of the center) is loaded with β-TCP granules and there are heavily loaded macrophages to the right.

Finally, there were also β-TCP granules observed within newly formed bone matrix, in the PDGF enhanced β-TCP treatment groups. A representative photomicrograph demonstrating this finding is shown in Figure 3G. The β-TCP granules are easily observed throughout the calcified matrix adjacent to fully enclosed osteocytes (oc). These findings clearly illustrate the biocompatibility of the growth factor enhanced β-TCP.

Figures 4 A, B and C illustrate results obtained for the allograft (DFDBA) treatment groups. Representative results for the DFDBA alone group (Figure 4A) shows poor periodontal regeneration that is limited to the area of the notches (arrows) extending only slightly in a coronal direction. New bone formation is limited and is surrounded by extensive fibrous connective tissue that extends coronally to fill a significant area within the furcation. Finally, a large void space extends from the coronal surface of the fibrous connective tissue to the fornix of the furcation.

Histologic results for the DFDBA + 0.3 and 1.0 mg/ml PDGF are shown in Figures 4 B and C, respectively. Both groups demonstrate greater periodontal regeneration, compared to DFDBA alone, that extends from the base of the notches in the roots for a short distance coronally (arrows). They also had greater bone fill within the area of the furcation, although there was significant fill of the furcation with fibrous connective tissue.

Figure 4 D is a higher power representative photomicrograph for the DFDBA alone treatment group. The figure shows DFDBA lined by osteoblasts (ob) with new bone (NB) deposited along the surface of the particle. Note the presence of fibrous connective tissue (CT) surrounding the particles. This particular specimen also demonstrates a finding reported previously (Buser *et al.*, 1998) showing recalcification of an allograft particle. This is seen in the "stippled" area of the matrix that is a result of discrete spherical apatite-depositions around former canaliculi.

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IX. CONCLUSIONS

It can be concluded from this study that:

1. β-TCP, when combined with PDGF for the treatment of periodontal bone loss resulted in greater periodontal regeneration than current products or procedures, β-TCP alone, bone allograft or periodontal surgery without grafts. Of note is that β-TCP with 0.3 mg/ml PDGF was significantly better than the current clinical gold standard, DFDBA.

2. The 0.3 mg/ml concentration of PDGF was at least equally as effective as the 1.0 mg/ml concentration when mixed with β-TCP.

3. β-TCP was more effective than allograft when mixed with PDGF.

4. The new bone matured (remodeled) normally over time (0-8-16 weeks) in all groups. There was no increase in ankylosis or root resorption in the PDGF groups. Sites receiving PDGF exhibited improved healing as shown by a decrease in ankylosis vs. control sites. This finding is consistent with reports that PDGF is mitogenic and chemotactic for periodontal ligament cells.

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Exhibit A

REFERENCES

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Other surgical supplies provided at the investigational site included:

- Sutures
- Antibiotics (Penicillin and Oxytetracycline)
- Chlorhexidine gluconate solution
- Surgical anesthetics, e.g. pentobarbital, acepromazine
- Surgical instrumentation and disposables

B. Material Preparation

At the time of the surgical procedure, the final implanted grafts were prepared by mixing the PDGF solution with the matrix materials as previously described (Jiang *et al*, 1999; Stephan *et al*, 2000; Camelo *et al in press*). Briefly, an amount of β-TCP or DFDBA to completely fill the osseous defect was aseptically placed into a sterile container. The PDGF solution sufficient to completely saturate the matrix was then added; the materials were mixed and allowed to sit on the surgical tray for approximately 10 minutes at room temperature prior to being placed in the osseous defect.

Based on our earlier studies, a 10 minute incubation time with the β-TCP material is sufficient to obtain maximum adsorption of the growth factor. This kit concept has been developed following discussions with the FDA, who have indicated that this approach would greatly simplify product shelf life/stability considerations.

The two doses tested used in this *in vivo* assessment are based on several considerations:

1) previous human experience using PDGF mixed with DFDBA for the treatment of both interproximal intrabony and Class II furcation periodontal defects (Nevins *et al in press* and Camelo *et al in press*);
2) previous experience using PDGF in the dog model used for the present studies;
3) successful completion of the ISO 10993 series of safety and biocompatibility tests on these concentrations showing complete safety as well as an expert toxicologist on the safety of the PDGF; and,
4) *in vitro* pharmacokinetic and bioactivity studies with osteoblastic cells which demonstrated significant effects on osteoblastic cell proliferation.

These are also the same doses that the sponsor is using in an ongoing randomized controlled multicenter human clinical trial.

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C. *In Vivo* dog studies

1. Introduction
All three specific aims of this project were accomplished simultaneously using the beagle dog model with horizontal Class III furcation defects as previously conducted and described by Cho and Wikesjo in their studies on the effect of PDGF or BMP, respectively (Park *et al*, 1995; Wikesjo *et al*, 1989). Fifteen adult beagle dogs contributed 60 treated defects

2. Rationale for Animal Model
The beagle dog is the most widely used animal model for testing putative periodontal regeneration materials and procedures (Wikesjo *et al* 1988, 1994, 1999; Cho *et al* 1995; Giannobile *et al* 1998; Clergeau, 1996). Plaque and calculus accumulation can induce gingival inflammation that may lead to marginal bone loss and the etiology of periodontitis in dogs and humans can be compared. In naturally occurring disease, however, there is a lack of uniformity between defects. Additionally, as more attention has been given to oral health in canine breeder colonies, it has become impractical to obtain animals with natural periodontal disease. Therefore, the surgically-induced horizontal Class III furcation model has become one of the most commonly used models to investigate periodontal healing and regeneration.

3. Creation of Defects, Application of Implant Materials & Treatment Groups

a. Defect Preparation
The "critical-size" periodontal defect model as described by numerous investigators (Wikesjo, 1988, 1994, and 1999; Giannobile, 1998, Cho, 1995 and Park, 1995) was utilized. Both mandibular quadrants in 15 male beagle dogs (2-3 years old) without general and oral health problems were used. One month prior to dosing, the animals were sedated with a subcutaneous injection of atropine (0.02mg/kg) and acepromazine (0.2mg/kg) approximately 30 minutes prior to being anesthetized with an IV injection of pentobarbital sodium (25mg/kg). Following local infiltration of the surgical area with Lidocaine HCl plus epinephrine 1:100,000, full thickness mucoperiosteal flaps were reflected and the first and third premolars (P1 and P3) were extracted. Additionally, the mesial portion of the crown of the 1st molar was resected.

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Alveolar bone was then removed around the entire circumference of P2 and P4, including the furcation areas using chisels and water-cooled carbide and diamond burs. Horizontal bone defects were created such that there was a distance of 5 mm from the fornix of the furcation to the crest of the bone. The defects were approximately 1 cm wide, depending on the width of the tooth. The roots of all experimental teeth were planed with curettes and ultrasonic instruments and instrumented with a tapered diamond bur to remove cementum. After the standardized bone defects were created the gingival flaps were sutured to achieve primary closure. The animals were fed a soft diet and received daily chlorhexidine rinses for the duration of the study.

b. Application of Graft Material

The periodontal defects of P2 and P4 in each mandibular quadrant of the 15 animals were randomized prior to treatment using sealed envelopes. About four weeks after defect preparation, animals were re-anesthetized as described above and full thickness flaps were reflected in both mandibular quadrants. A notch was placed in the tooth root surfaces at the residual osseous crest using a 1/2 round bur to serve as a future histologic reference point. The sites were irrigated with sterile saline and the roots were treated with citric acid as described previously (Cho, 1995; Park, 1995) for the purpose of decontamination and removal of the smear layer. During this period an amount of β-TCP or DFDBA sufficient to fill the periodontal defect was saturated with a solution of PDGF solution (0.3 or 1.0 mg/ml) and the PDGF/graft mixture was allowed to sit on the sterile surgical stand for about ten minutes. The PDGF saturated graft was then packed into the defect with gentle pressure to the ideal level of osseous regeneration.

After implantation of the graft material, the mucoperiosteal flaps were sutured approximately level to the CEJ using interproximal, interrupted 4.0 ePTFE sutures. Following suturing of the flaps chlorhexidine gluconate gel was gently placed around the teeth and gingivae.

c. Treatment and Control Groups

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Defects received either:

1. β-TCP (VitOss®)
2. β-TCP (VitOss®) plus PDGF (0.3 mg/ml PDGF)
3. β-TCP (VitOss®) plus PDGF (1.0 mg/ml PDGF)
4. Dog DFDBA
5. Dog DFDBA plus PDGF (0.3 mg/ml PDGF)
6. Dog DFDBA plus PDGF (1.0 mg/ml PDGF)
7. Sham surgery (treated by open flap debridement only, no graft)

Six (6) defects per treatment group were biopsied at two months (42 total sites). In addition, six defects in treatment groups 1, 2 and 3 were biopsied at four months (18 total sites).

Table 1. Experimental design

GROUP NO.	NO. OF TEST SITES	TREATMENT	TIME POINTS
1	12	β-TCP alone	8 & 16 weeks - n=6 for 8 wk - n=6 for 16wk
2	12	β-TCP + 0.3 mg/ml PDGF	8 & 16 weeks - n=6 for 8 wk - n=6 for 16 wk
3	12	β-TCP + 1.0 mg/ml PDGF	8 & 16 weeks - n=6 for 8 wk - n=6 for 16 wk
4	6	DFDBA alone	8 weeks
5	6	DFDBA + 0.3 mg/ml PDGF	8 weeks
6	6	DFDBA + 1.0 mg/ml PDGF	8 weeks
7	6	Surgery, no graft	8 weeks

Accordingly, at 8 weeks there are 7 groups divided among 42 sites in 11 dogs. At 16 weeks, there are 3 groups divided among 18 sites in 4 dogs (one dog received two treatment surgeries staggered eight weeks apart and thus contributed two sites to each the 8 and 16 week time points).

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d. Post-surgical Treatment
The surgical sites were protected by feeding the dogs a soft diet during the first four (4) weeks post-operative. To insure optimal healing, systemic antibiotic treatment with penicillin G benzathine was provided for the first two weeks and plaque control was maintained by daily local irrigation of the defect sites with 2% chlorhexidine gluconate throughout the experiment. Sutures were removed after three (3) weeks.

D. Data Collection

1. Rationale for Data Collection Points
The eight (8) week time point was chosen because this is the most common time point reported for this model in the literature and therefore there are substantial historical data. For example, Wikesjo *et al* and Giannobile *et al* (1998) also chose 8 weeks to assess the regenerative effects of BMP-2 and OP-1, respectively, in the same model. Additionally, Park *et al* (1995) evaluated the effect or PDGF applied directly to the conditioned root surface with and without GTR membranes in the beagle dog model at 8 weeks. These studies, indicate that the 8 week period optimal for illustrating potential significant healing effects among the various treatment modalities.

The sixteen (16) week time point was chosen to assess long-term effects of growth factor treatment. Previous studies (Park *et al*, 1995) suggest that by this time there is substantial spontaneous healing of the osseous defects. Nevertheless, it is possible to assess whether PDGF treatment leads to any unusual or abnormal tissue response, such as altered bone remodeling, tumorgenesis or root resorption. Thus the 16 week time point is used to determine safety.

2. Biopsies and Treatment Assessments
At the time of biopsy, the animals were perfused with 4% paraformaldehyde and sacrificed. The mandibles were then removed and placed in fixative. Periapical radiographs were taken and the treated sites were cut into individual blocks using a diamond saw. The coded (blinded) blocks were wrapped in gauze, immersed in a solution of 4% formaldehyde and shipped to Professor Robert Schenk for processing and analysis.

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During processing the biopsies were dehydrated in 100% ethanol and infiltrated and embedded in methylmethacrylate. Undecalcified sections of approximately 300 μm in thickness were obtained using a low speed diamond saw with coolant. The sections were glued onto opalescent acrylic glass, ground to a final thickness of approximately 80 μm, and stained with toludine blue and basic fuchsin. Step serial sections were obtained in a mesiodistal plane.

All forty-two (42) biopsy specimens obtained two months following treatment were evaluable while 15 of 18 biopsy specimens obtained four months post-surgical were evaluable. One specimen was lost due to processing and two were not evaluable due to infection that was present.

Histomorphometric analyses were performed on the blinded slides by Professor Schenk and Dr. Lynch. The following parameters were assessed:

a) *Length of Complete New Attachment Apparatus (CNAA)*
Periodontal regeneration measured as the distance between the coronal level of the old bone and the coronal level of the new bone, including only that new bone adjacent to new cementum with functionally oriented periodontal ligament between the new bone and new cementum.

b) *New Bone Fill (NB)*
Measured as the cross-sectional area of new bone formed within the furcation.

c) *Connective Tissue Fill (CT)*
Measured as the area within the furcation occupied by fibrous connective tissue.

d) *Void (VO)*
The area of recession where there is an absence of tissue.

3. Data Control
All data was entered into laboratory notebooks at the individual study sites.

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VIII. RESULTS

A. Clinical Observations

Clinically, all sites healed well. There was qualitative evidence that the sites treated with PDGF healed more quickly, as indicated by the presence of firm, pink gingivae within one (1) week post-operatively. However, no quantitative measurements of healing were obtained. There were no adverse events experienced in any treatment group as assessed by visual inspection of the treated sites. Upon clinical observation, there appeared to be increased gingival recession in groups that received β-TCP or DFDBA alone.

B. Radiographic Observations

Radiographically, there was evidence of increased bone formation at two months as judged by increased radiopacity in Groups 2, 3 (β-TCP + PDGF 0.3 and 1.0 mg/ml, respectively) and 6 (DFDBA + 1.0 mg/ml) compared to the other groups (Figures 1A-D). At four (4) months, there was evidence of increased bone formation in all groups compared to the two month time point. There was no radiographic evidence of any abnormal bone remodeling, root resorption or ankylosis in any group.

Table 2. Radiographic results. Rank order.

QUALITATIVE ASSESSMENT OF BONE FILL AT 8 WKS*	TREATMENT
1	β-TCP + 0.3 mg/ml PDGF
2	β-TCP + 1.0 mg/ml PDGF
3	DFDBA + 1.0 mg/ml PDGF
4	Surgery, no graft
5	DFDBA + 0.3 mg/ml PDGF
6	β-TCP alone
7	DFDBA alone

* 1= most fill; 7= least fill

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C. Histomorphometric Observations:

Histomorphometric assessment of the length of new cementum, new bone and new periodontal ligament (CNAA) as well as new bone fill, connective tissue fill (scar formation) and void space were evaluated and are expressed as percentages. In the case of CNAA, values for each test group represent the CNAA measurements (length in mm)/ total available CNAA length (in mm) x 100%. Bone fill, connective tissue fill and void space were evaluated and are expressed as percentages of the total furcation defect area.

One-way analysis of variance (ANOVA) was used to test for overall differences among treatment groups, and pairwise comparisons were made using the Student's t-test. Significant differences between groups were found upon analyses of the coded slides. Table 3 shows the results at two (2) months.

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Exhibit B

FIGURE LEGENDS

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FIGURE LEGENDS

Figure 1A: Radiographic appearance 8 weeks post-surgical for site treated with surgery alone. Note the radiolucency in the area of the furcation (*) indicating the lack of osseous fill into this site. A slight radiopacity is evident at the apical portion of the furcation (arrows) that may be indicative of some bone regeneration.

Figure 1B: Radiographic appearance 8 weeks post-surgical for site treated with β-TCP alone. The radiolucent area (*) indicates the absence of bone fill with some radiopacity observed at the base of the furcation (arrows) that may indicate limited new bone fill into the furcation.

Figure 1C: Radiographic appearance 8 weeks post-surgical for site treated with β-TCP + 0.3 mg/ml PDGF. Uniform radiopaque appearance is present throughout the furcation. There is no clear radiolucent area within the furcation as observed in Figures 1 A and B. Arrows indicate base of original defect.

Figure 1D: Radiographic appearance 8 weeks post-surgical for site treated with β-TCP + 1.0 mg/ml PDGF.

Figure 2A: Representative photomicrograph from the surgery alone treatment group at 8 weeks. Limited complete new attachment apparatus [new bone (NB), new cementum (NC) and periodontal ligament (PDL)] is observed extending from the base of the notches in a coronal direction (arrows). The area of the furcation is occupied primarily by soft connective tissue (CT) with limited amounts of new bone. OB: old bone.

Figure 2B: Representative photomicrograph of regenerated periodontal ligament (PDL) at 8 weeks in apical portion of surgery alone treatment group. Dentin (far right) and new cementum (NC), loose connective tissue, osteoblasts (ob) and new bone (NB) are present. Within the PDL there is vascularity and loosely arranged, mostly unorganized, collagen fibers.

Figure 3A: Representative photomicrograph from the β-TCP alone treatment group at 8 weeks post-surgical. Limited periodontal regeneration exists in the area of the root notches and extending coronally for a short distance (arrows). There is new bone (NB) present within the original defect, but most of the area is filled with soft connective tissue (CT). The old bone (OB) at the base of the original defect is observed apical to the root notches.

Figure 3B: Representative photomicrograph from the β-TCP + 0.3 mg/ml PDGF treatment group at 8 weeks post-surgical. Extensive periodontal regeneration is observed that extends from the base of the notch in one root to the base of the notch in the second root (arrows). There is also extensive new bone (NB) formation in the area of the original defect that extends coronally to the fornix of the

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furcation. A small amount of soft connective tissue (CT) is observed at the inferior central portion of the original defect. NC: new cementum; PDL: periodontal ligament; OB: old bone.

Figure 3C: Representative photomicrograph from the β-TCP + 1.0 mg/ml PDGF treatment group at 8 weeks post-surgical. Significant periodontal regeneration is present (arrows) but does not extend along the entire surface of the furcation. There is also significant new bone (NB) formation within the furcation that extends coronally more than half the distance of the original defect. Soft connective tissue (CT) may be seen coronal to the new bone with a small void (VO) area noted at the fornix of the furcation indicating some recession. NC: new cementum; PDL: periodontal ligament; OB: old bone.

Figure 3D: A higher power magnification of the area outlined in Figure 3C. New cementum (NC), periodontal ligament (PDL), and new bone (NB) are observed. The PDL is more mature and well organized. There is less vascularity than that observed in Figure 2B. The collagen fibers are well organized perpendicularly oriented inserting collagen fibers.

Figure 3E: Representative photomicrograph of specimen obtained from PDGF enhanced β-TCP treatment group. Dense β-TCP particles (gray/blue in color) are partially dissolved and penetrated by capillaries (cap), fibroblasts (F) and macrophages (M∅).

Figure 3F: High power magnification of area outlined in Figure 3E. The invading elements are clearly delineated. Note the giant cell (GC) loaded with β-TCP granules to the left of the center, and the heavily loaded macrophages (M∅) to the right.

Figure 3G: Representative photomicrograph for specimen obtained from PDGF enhanced β-TCP treatment group. β-TCP granules are seen throughout the calcified matrix (NB) adjacent to fully enclosed osteocytes (oc), with osteoblasts (ob) observed along the surface of the bone matrix. This illustrates biocompatibility of the growth factor enhanced matrix.

Figure 4A: Representative photomicrograph from the DFDBA alone treatment group at 8 weeks post-surgical. Limited periodontal regeneration is observed within the area of one root notch, and extending coronally for a short distance at the second root notch (arrows). Small amounts of new bone (NB) may be observed along the surface of residual DFDBA particles (dark red staining along lighter pink stained islands). The area of new bone formation is limited to the inferior aspect of the original defect, extending only slightly coronal to the area of one root notch. The remaining area within the furcation is occupied by dense soft connective tissue (CT) and void space (VO). OB: old bone.

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Figure 4B: Representative photomicrograph from DFDBA + 0.3 mg/ml PDGF treatment group 8 weeks post-surgical. There is greater periodontal regeneration observed (arrows) than for the previous group (DFDBA alone), however it is still quite limited. There is new bone (NB) formation present along DFDBA particles within the apical 1/3 of the furcation with the remainder of the furcation occupied by soft connective tissue (CT) and a small void space (VO) at the fornix of the furcation. OB: old bone.

Figure 4C: Representative photomicrograph from DFDBA + 1.0 mg/ml PDGF treatment group 8 weeks post-surgical. Moderate periodontal regeneration is observed (arrows) along with new bone (NB) formation on DFDBA particles extending coronally along the surface of both roots. The remainder of the furcation is occupied by soft connective tissue (CT). OB: old bone.

Figure 4D: Representative photomicrograph (high power) of DFDBA specimen. The DFDBA particle is lined by osteoblasts (ob) with new (NB) along the surface of the particle. Note that fibrous connective tissue (CT) surrounds the particles. Recalcification of the allograft particle is seen in the "stippled" area of the matrix where there are discreet spherical apatite-depositions around former canaliculi.

Figure 5A: Representative photomicrograph for the β-TCP alone treatment group at 16 weeks. Periodontal regeneration is limited to the root notches (arrows). There is an increase in the amount of bone regeneration within the area of the furcation compared to the same treatment group at 8 weeks, with normal remodeling from woven to lamellar bone. Significant ankylosis is observed along the surface of the furcation (lines). However, there is still a large area within the furcation that is occupied by fibrous connective tissue. An area of root resorption may be observed in the box at the fornix of the furcation. NB: new bone; CT: fibrous connective tissue.

Figure 5B: Representative photomicrograph for the β-TCP + 0.3 mg/ml rhPDGF-BB treatment group at 16 weeks. Periodontal regeneration extends from base of root notches along most of the surface of the furcation (arrows). Limited ankylosis is observed (line) in the area of the fornix of the furcation. A widened space (upper box) indicative of remodeling has lead to a widened periodontal ligament space. This finding may be a function of the model as there is an area (lower box) apical to the level of the original defect, indicating similar remodeling. NB: new bone; CT: fibrous connective tissue; OB: old bone.

Figure 5C: Representative photomicrograph for the β-TCP + 1.0 mg/ml rhPDGF-BB treatment group at 16 weeks. Periodontal regeneration is observed extending from the area of the root notches in a coronal direction (arrows). There is less bone fill into the area of the furcation compared to that observed for the 0.3

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mg/ml rhPDGF-BB treatment group, with a greater area being occupied by fibrous connective tissue. Ankylosis (lines) is also observed as well as discrete areas of root resorption (boxes). As was the case for the 0.3 mg/ml rhPDGF-BB, there was no increase in root resorption over that observed for the β-TCP alone treatment group. NB: new bone; CT: fibrous connective tissue.

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Exhibit C

PHOTO MICROGRAPHS

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Fig 1A

Fig 1B

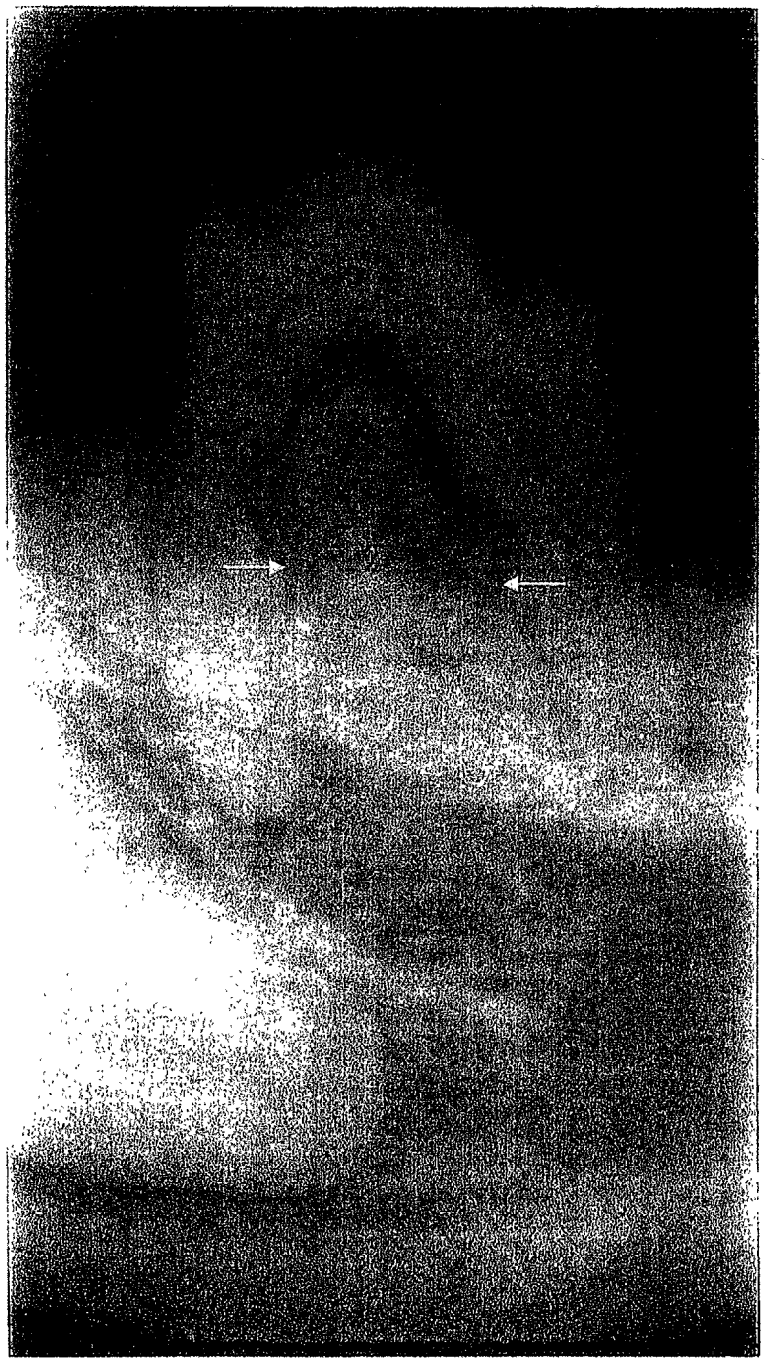

Fig 1C

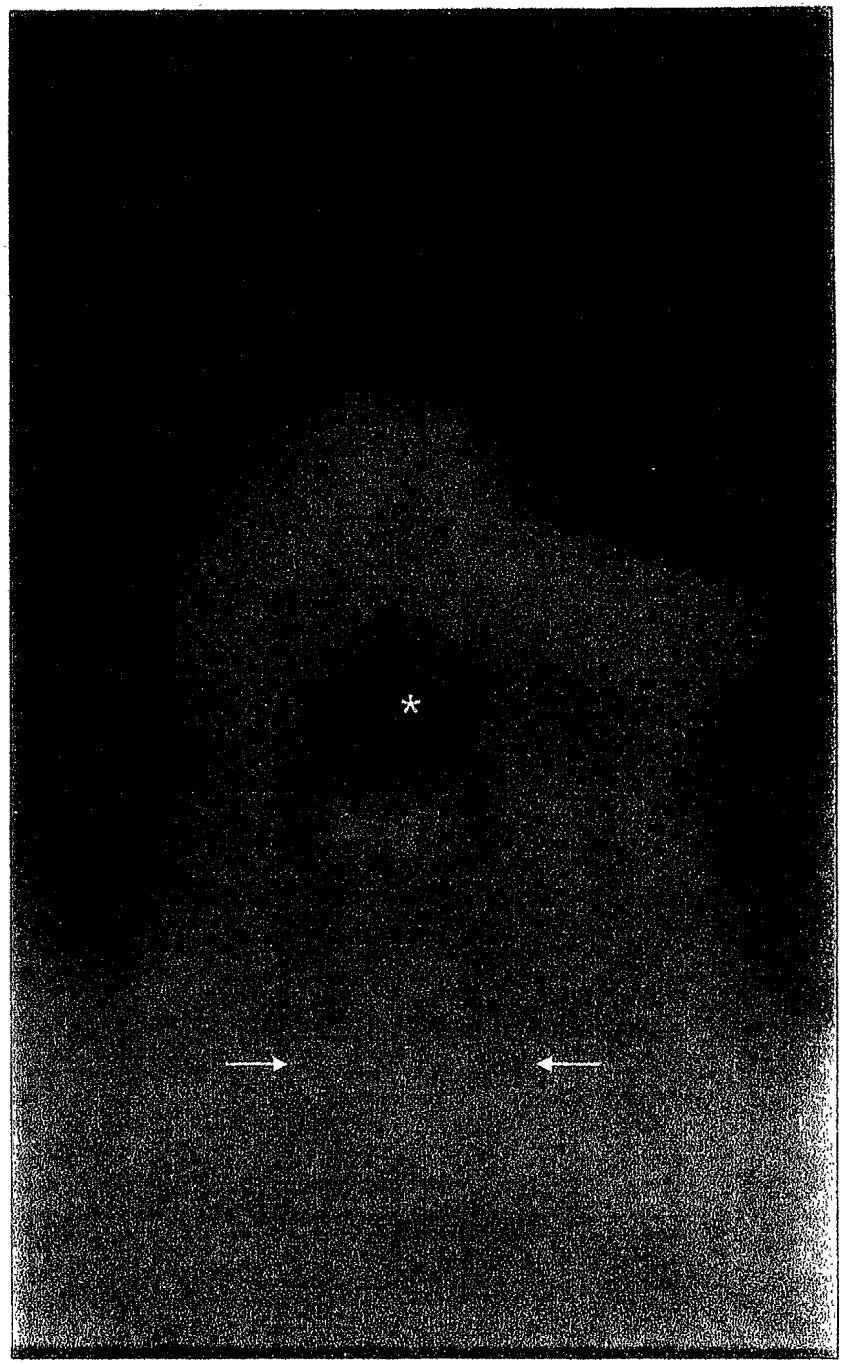

Fig 1D

Fig 2A

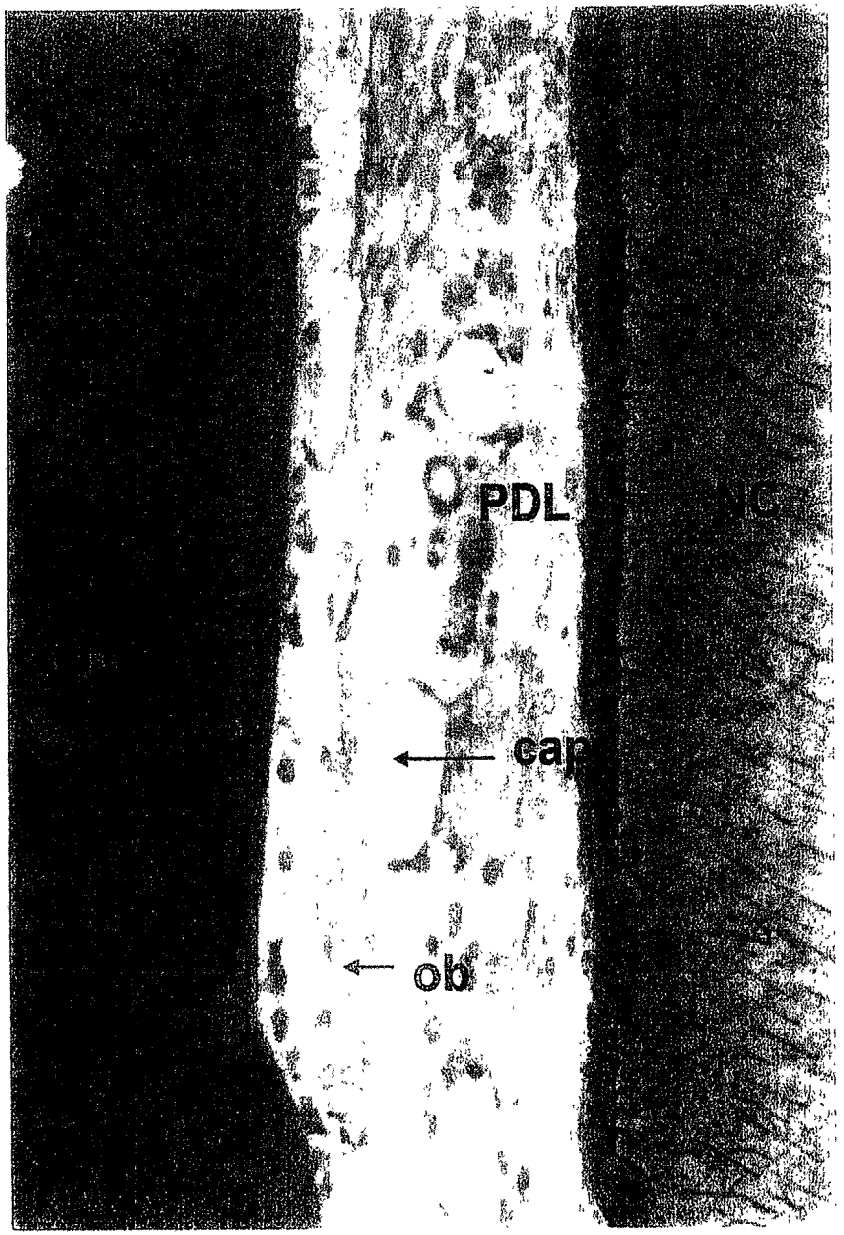

Fig 2B

Fig 3A

Fig 3B

Fig 3C

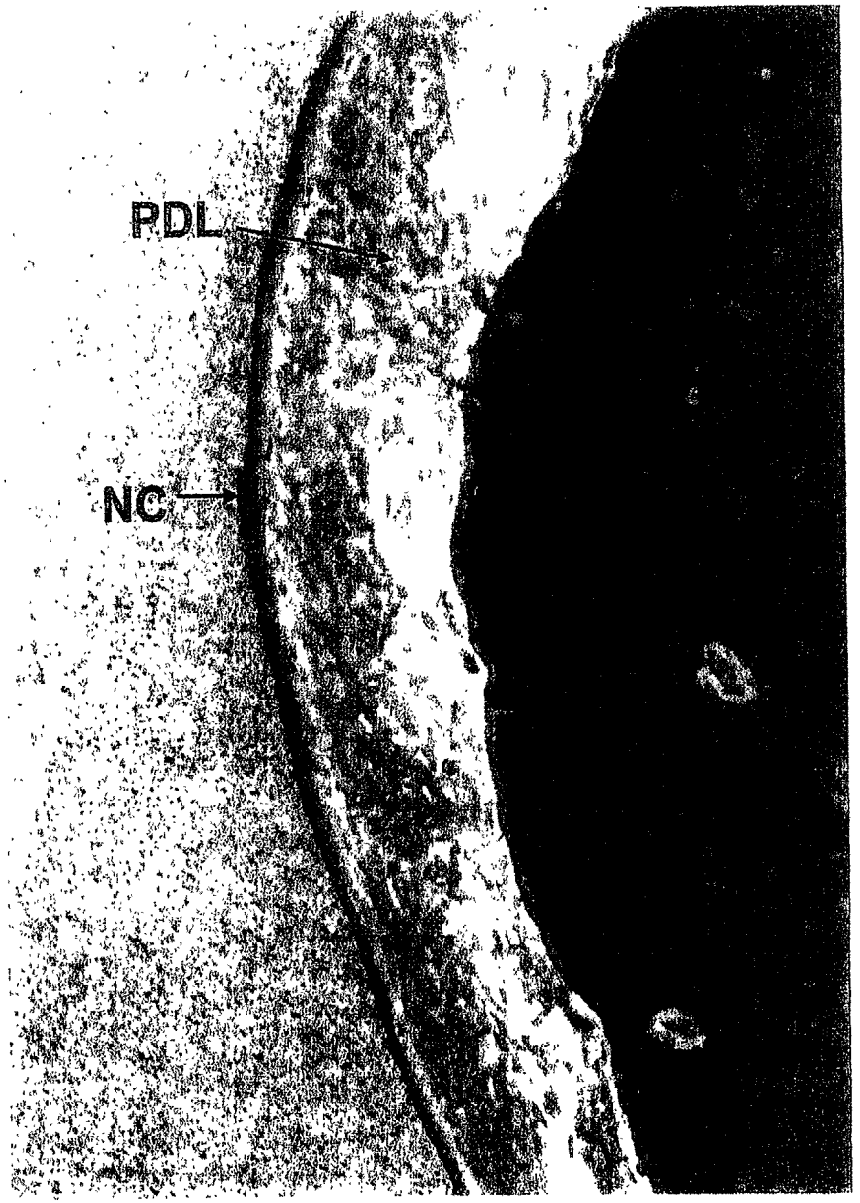

Fig 3D

Fig 3E

Fig 3F

Fig 3G

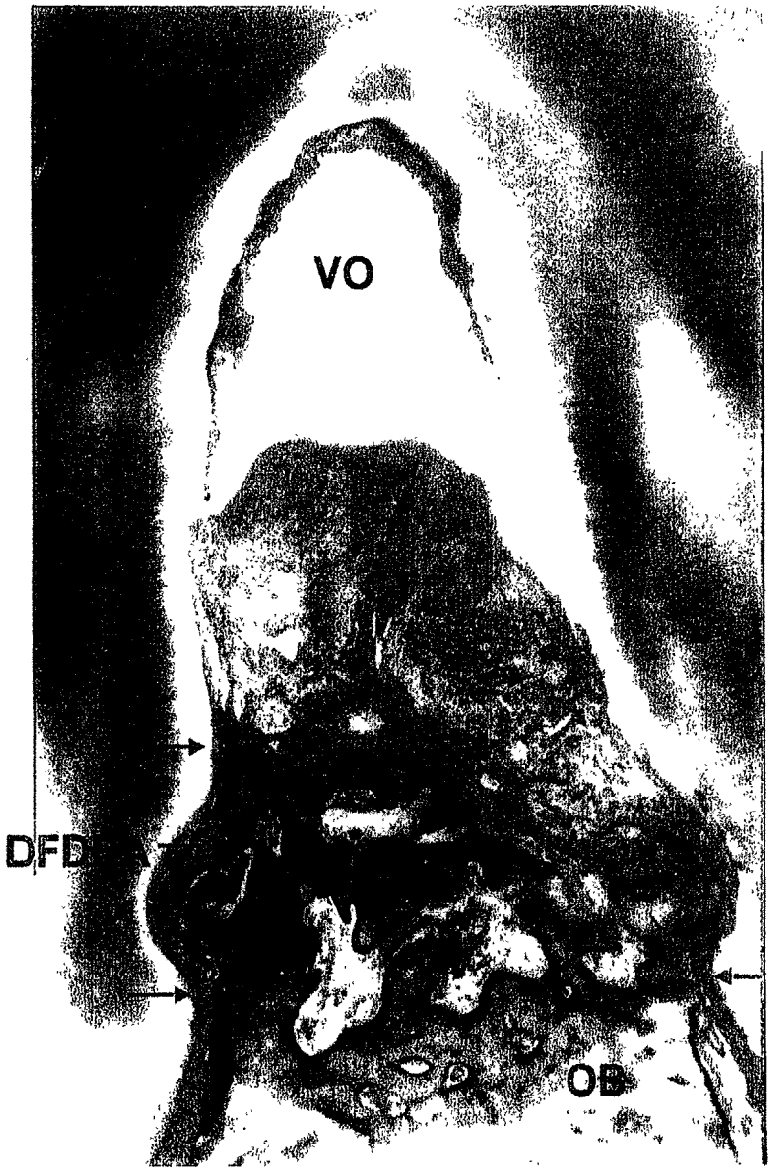

Fig 4A

Fig 4B

Fig 4C

Fig 4D

Fig 5A

Fig 5B

Fig 5C

Appendix 7.7.5: **Test Report V0023-211. Genotoxicity: Bacterial reverse mutation study (Saline Extract)**

STUDY TITLE:

GENOTOXICITY: BACTERIAL REVERSE MUTATION STUDY

(Saline Extract)

TEST ARTICLE:

rhPDGF-BB (Becaplermin -1 mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold

IDENTIFICATION NO.:

Lot #QA2217 and Lot 081098-146

TEST FACILITY:

NAMSA
2261 Tracy Road
Northwood, OH 43619-1397

SPONSOR:

BILL BEASLEY
BIOMIMETIC PHARMACEUTICALS
1616 WESTGATE CIRCLE
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BM001668

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SUMMARY

A *Salmonella typhimurium* and *Escherichia coli* reverse mutation standard plate incorporation study was conducted to evaluate whether a saline extract of rhPDGF-BB (Becaplermin -1 mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold, Lot #QA2217 and Lot 081098-146, would cause mutagenic changes in the average number or revertants for histidine-dependent *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, and in tryptophan-dependent *Escherichia coli* strain WP2*uvrA* in the presence and absence of S9 metabolic activation. This study was conducted to satisfy, in part, the genotoxicity requirement of the International Organization for Standardization: Biological Evaluation of Medical Devices, Part 3: Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity.

The saline test article extract was found to be noninhibitory to growth of tester strains TA98, TA100, TA1535, TA1537, and WP2*uvrA*. Separate tubes containing 2 ml of molten top agar supplemented with histidine-biotin solution for the *S. typhimurium* strains and with tryptophan for the *E. coli* strain were inoculated with 0.1 ml of culture for each of five tester strains, and 0.1 ml of the saline extract. A 0.5 ml aliquot of sterile Water for Injection (SWI) or S9 homogenate, simulating metabolic activation, was added when necessary. The mixture was poured across triplicate Minimal E plates. Parallel testing was also conducted with a negative control and five positive controls. The mean number of revertants of the triplicate test plates was compared to the mean number of revertants of the triplicate negative control plates for each of the five tester strains employed. The means obtained for the positive controls were used as points of reference.

Under the conditions of this assay, the saline test article extract was considered to be nonmutagenic to *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537, and to *Escherichia coli* strain WP2*uvrA*. The negative and positive controls performed as anticipated. The results of this study should be evaluated in conjunction with other required tests as listed in ISO 10993, Part 3: Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity.

Study and Supervisory Personnel:

Sally C. Ahmed, BS
Suzanne E. Brimacombe
Lionel D. Brock, BS
Cherise M. McCoy, BS

Study Director: Laura L. Tasse — Jan. 14, 2002

Laura L. Tasse, BS, ALAT
Study Director

Date Completed

/lag

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INTRODUCTION

A *Salmonella typhimurium* and *Escherichia coli* reverse mutation standard plate incorporation study was conducted to evaluate whether a saline test article extract would cause mutagenic changes in the average number of revertants for *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537, and *Escherichia coli* tester strain WP2*uvrA* in the presence and absence of S9 metabolic activation. This test was conducted to satisfy, in part, the requirements of the International Organization for Standardization (ISO) 10993, Part 3: Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity. Bacterial reverse mutation tests have been widely used as rapid screening procedures for the determination of mutagenic and potential carcinogenic hazards. The test article was received on November 20, 2001 and December 10, 2001. The preliminary toxicity screen began on December 18, 2001, and the testing ended on December 20, 2001.

The study, initiated by protocol signature on December 10, 2001, was conducted in accordance with the provisions of the FDA Good Laboratory Practice (GLP) Regulations, 21 CFR 58. A Certificate of Quality Assurance Inspections was issued with this report.

MATERIALS

The sample provided by the sponsor was identified and handled as follows:

Test Article:	rhPDGF-BB (Becaplermin - 1 mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold
Identification No.:	Lot #QA2217 and Lot 081098-146
Stability Testing:	In Progress (per sponsor)
Expiration Date:	Stable for duration of intended testing (per sponsor)
Storage Conditions:	rhPDGF-BB – refrigerated; Vitoss Scaffold - room temperature
Vehicle:	Saline (0.9% Sodium Chloride USP)
Preparation:	A 1.0 g portion of Vitoss Scaffold was saturated with a 1.0 ml portion of rhPDGF immediately prior to adding the vehicle. Based on the USP ratio of 4 g:20 ml, a 1.9 g portion of the prepared test article was covered with 9.5 ml of saline. A single preparation was extracted at 37°C for 24 hours. Vehicle without test material was similarly subjected to the extraction conditions for use as a negative control. The test extract and negative control were decanted.

	Test	Control
Condition of Extracts:	cloudy	clear

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METHODS

Test System:

Each *Salmonella typhimurium* tester strain contains a specific mutation in the histidine operon and other mutations that increase their ability to detect mutagens. In addition, the *Escherichia coli* contains a specific mutation in the tryptophan operon, and a deletion in the *uvrA* gene. These genetically altered *S. typhimurium* strains (TA98, TA100, TA1535, and TA1537) and *E. coli* strain (WP2*uvrA*) cannot grow in the absence of histidine or tryptophan, respectively. When placed in a histidine-free (for *S. typhimurium*) or tryptophan-free (for *E. coli*) medium, only those cells which mutate spontaneously back to their wild type state (histidine independent by manufacturing their own histidine, or tryptophan independent by manufacturing their own tryptophan) are able to form colonies. The spontaneous mutation rate (or reversion rate) for any one strain is relatively constant, but if a mutagen is added to the test system, the mutation rate is significantly increased.

Tester Strain	Mutations/Genotypic Relevance
S. typhimurium TA98	hisD3052, *rfa, uvrB,* frameshift, pKM101
S. typhimurium TA100	hisG46, *rfa, uvrB,* missense, pKM101
S. typhimurium TA1535	hisG46, *rfa, uvrB,* missense
S. typhimurium TA1537	hisC3076, *rfa, uvrB,* frameshift
E. coli WP2*uvrA*	trpE65, *uvrA*, missense

rfa	=	causes partial loss of the lipopolysaccharide wall which increases permeability of the cell to large molecules (i.e., crystal violet inhibition)
uvrB or *uvrA*	=	deficient DNA excision - repair system (i.e., ultraviolet sensitivity)
frameshift	=	base-pair addition/deletion
missense	=	base-pair substitution
pKM101	=	plasmid confers ampicillin resistance (R-factor) and enhances sensitivity to mutagens

Metabolic Activation:

Aroclor 1254 - induced rat liver (S9 homogenate) was used as metabolic activation. The S9 homogenate is prepared from male, Sprague Dawley rats. The rats are induced with one intraperitoneal injection of Aroclor 1254 (500 mg/ml) 5 days prior to sacrifice. Just prior to use, the S9 homogenate was mixed with a buffer containing 0.4 M $MgCl_2$/1.65 M KCl, 1.0 M Glucose-6-phosphate, 0.1 M NADP, 0.2 M sodium phosphate buffer, and sterile Water for Injection (SWI).

Preparation of Tester Strains:

Cultures of *Salmonella typhimurium*, TA98, TA100, TA1535 and TA1537, and *Escherichia coli*, WP2*uvrA*, were inoculated to individual Erlenmeyer flasks containing oxoid broth. The inoculated broth cultures were incubated overnight at 37 ± 2°C in an incubator shaker operating at 115-125 rpm for 10-12 hours.

Negative Control:

Saline (vehicle without test material) was tested with each tester strain to determine the spontaneous reversion rate. Each strain was tested with and without S9 activation. These data represented a base rate to which the number of revertant colonies that developed in each test plate were compared to determine whether the test article had significant mutagenic properties.

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Positive Control:

A known mutagen, Dexon (paradimethylaminobenzene diazosulfonic acid sodium salt), was used as a positive control to demonstrate that tester strains TA98, TA100, and TA1537 were sensitive to mutation to the wild type state. For tester strain TA1535, sodium azide was used as a positive control. For tester strain TA100, 2-aminofluorene was also used as a positive control. For tester strain WP2*uvrA*, 2-aminoanthracene and methylmethane-sulfonate were used as positive controls. Although metabolic activation was only required with 2-aminofluorene and 2-aminoanthracene to induce mutagenic results, all positive controls were tested with and without S9 homogenate.

Strain Characteristics and Strain Standard Plate Counts:

Strain characteristics were verified and viable counts were determined.

Spot Plate Inhibition Screen:

The saline test article extract was evaluated by a spot plate technique, modeled after the antimicrobial zone of inhibition test. This screen was used to evaluate the toxicity of the extract to determine whether dilution of the extract was required to provide an extract noninhibitory to the *Salmonella typhimurium* or to the *Escherichia coli*.

Separate tubes containing 2 ml of molten top agar supplemented with histidine-biotin solution for the *Salmonella typhimurium* and tryptophan for the *Escherichia coli* were inoculated with 0.1 ml of culture for each of the five tester strains. After mixing, the agar was poured across the surface of separate Minimal E plates labeled with lab number and appropriate tester strain. Once the agar solidified, sterile filter discs were placed in the center of the plates. A 0.1 ml aliquot of the saline test article extract was added to the filter discs on each of the labeled plates. Parallel testing was conducted with a negative control, and to demonstrate a positive zone of inhibition, 10X Dexon was utilized.

The plates were incubated at 37°C for 2 days. Following the incubation period, the zone of growth inhibition was observed and recorded. Only extracts that were noninhibitory to the tester strains were tested by the standard plate incorporation method.

Standard Plate Incorporation Assay:

Separate tubes containing 2 ml of molten top agar supplemented with histidine-biotin solution for the *Salmonella typhimurium* or with tryptophan for the *Escherichia coli* were inoculated with 0.1 ml of culture for each of the five tester strains and 0.1 ml of the saline test article extract. A 0.5 ml aliquot of SWI or S9 homogenate, simulating metabolic activation, was added when necessary. The mixture was poured across triplicate Minimal E plates labeled with lab number, appropriate tester strain, and S9 metabolic activation (when applicable). Parallel testing was also conducted with a negative control and five positive controls.

Histidine-free media plates (for *S. typhimurium*) and tryptophan-free media plates (for *E. coli*) were prepared in triplicate as follows:

1. Saline test article extract with and without S9 activation
2. Negative control with and without S9 activation
3. 1X Dexon (known mutagen) with and without S9 activation with strains TA98, TA100, and TA1537
4. 1X 2-Aminofluorene (known mutagen) with and without S9 activation with strain TA100
5. 1X Sodium azide (known mutagen) with and without S9 activation with strain TA1535
6. 1X 2-Aminoanthracene (known mutagen) with and without S9 activation with strain *WP2uvrA*
7. 1X Methylmethane-sulfonate (known mutagen) with and without S9 activation with strain WP2*uvrA*

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The plates were incubated at 37°C for 2 days. Following the incubation period, the revertant colonies on each plate were recorded. The mean number of revertants was determined. The mean number of revertants of the test plates were compared to the mean number of revertants of the negative control for each of the five tester strains employed.

For the saline extract to be evaluated as a test failure or "potential mutagen" there must have been a 2-fold or greater increase in the number of mean revertants over the means obtained from the negative control for any or all five tester strains. Each positive control mean must have exhibited at least a 3-fold increase over the respective negative control mean of the *Salmonella* tester strain employed, and at least a 2-fold increase over the respective negative control mean of the *E. coli* tester strain. Exceptions included conditions not intended to provoke a mutagenic response (e.g. 2-amino-anthracine and 2-aminofluorine without metabolic activation). The negative control results of each tester strain exhibited a characteristic number of spontaneous revertants based on historical data collected at NAMSA.

RESULTS

Strain Characteristics and Strain Standard Plate Count:

Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2*uvrA* exhibited appropriate genetic characteristics pertaining to this assay (see Table I).

Spot Plate Inhibition Screen:

No significant inhibition was observed (see Table II).

Standard Plate Incorporation Assay:

The results are summarized in Table III. In no case was there a 2-fold or greater increase in the mean number of revertants of tester strains TA98, TA100, TA1535, TA1537, and WP2*uvrA* in the presence of a saline test article extract. Each positive control mean exhibited at least a 3-fold increase over the respective mean of the *S. typhimurium* tester strain employed and at least a 2-fold increase over the respective mean of the *E. coli* tester strain.

Test Validity:

The data obtained from this study met NAMSA criteria for a valid assay.

Results and conclusions apply only to the test article tested. No further evaluation of these results is made by NAMSA. Any extrapolation of these data to other samples is the responsibility of the sponsor. All procedures were conducted in conformance with good laboratory practice and ISO 17025.

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CONCLUSION

Under the conditions of this assay, the saline test article extract was considered to be nonmutagenic to *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537, and to *Escherichia coli* strain WP2*uvrA*. The negative and positive controls performed as anticipated. This test was conducted to satisfy, in part, the requirements of the International Organization for Standardization (ISO) 10993, Part 3: Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity.

RECORD STORAGE

All raw data pertaining to this study and a copy of the final report are to be retained in designated NAMSA archive files.

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TABLE I

STRAIN CHARACTERISTICS AND STRAIN STANDARD PLATE COUNTS

Characteristics (expected)	Tester Strains				
	TA98	TA100	TA1535	TA1537	WP2*uvrA*
Ampicillin-TA98 & TA100 = (Resistant) TA1535, TA1537 & WP2*uvrA* = (Sensitive)	R	R	S	S	S
rfa Mutation; UV (Sensitive)	S	S	S	S	NA
uvrB/uvrA (No Growth)	NG	NG	NG	NG	NG
Histidine Requirement; (Growth)	G	G	G	G	NA
Tryptophan Requirement; (Growth)	NA	NA	NA	NA	G
Biotin (No Growth)	NG	NG	NG	NG	NA
L-tryptophan (No Growth)	NA	NA	NA	NA	NG
Purity (Pure)	PURE	PURE	PURE	PURE	PURE
Total Plate Count CFU's (10^{-7})	12	72	159	67	280
	14	89	162	85	290
Mean	13	81	161	76	285
Titer (Organisms/ml)	1.3×10^8	8.1×10^8	1.6×10^9	7.6×10^8	2.9×10^9

R = Resistant S = Sensitive NG = No Growth G = Growth NA = Not Applicable

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TABLE II

SPOT PLATE INHIBITION SCREEN RESULTS

	Zone of Inhibition (mm)				
	TA98	TA100	TA1535	TA1537	WP2*uvrA*
Saline negative control	0	0	0	0	0
Saline test article extract	0	0	0	0	0
Dexon positive control	45	39	48	46	26

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TABLE III

STANDARD PLATE INCORPORATION ASSAY - REVERSION RATES FOR TESTER STRAINS

	Salmonella typhimurium								*Escherichia coli*	
	TA98		TA100		TA1535		TA1537		WP2*uvrA*	
	CFTP	Mean	CFTP	Mean	CFTP	Mean	CFTP	Mean	CFTP	Mean
Saline w/o S9 negative control	46 46 42	45	153 172 170	165	8 15 11	11	7 12 7	9	32 23 20	25
Saline w S9 negative control	55 52 42	50	177 185 179	180	8 14 9	10	5 9 6	7	31 25 44	33
Saline test article extract w/o S9	46 41 41	43	166 141 163	157	9 6 20	12	11 9 12	11	25 23 20	23
Saline test article extract w S9	50 55 43	49	197 191 180	189	9 14 12	12	6 7 12	8	22 21 26	23
Dexon w/o S9 positive control	2000 2192 2048	2080	1296 1536 1408	1413			424 440 468	444		
Dexon w S9 positive control	1392 1664 1712	1589	1008 896 800	901			252 416 452	373		
2-aminofluorene w/o S9 positive control*			200 184 167	184						
2-aminofluorene w S9 positive control†			1888 1920 1888	1899						
Sodium azide w/o S9 positive control					3728 3712 5584	4341				
Sodium azide w S9 positive control					3776 4880 4032	4229				
2-aminoanthracene w/o S9 positive control*									35 31 33	33
2-aminoanthracene w S9 positive control†									87 93 118	99
Methylmethane-Sulfonate w/o S9 positive control									364 320 404	363
Methylmethane-Sulfonate w S9 positive control									400 312 324	345

CFTP = Counts from triplicate plates Mean = Mean of triplicate plates = Not Applicable
*Negative control for S9 †Positive control for S9

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CERTIFICATE OF QUALITY ASSURANCE INSPECTIONS

Phase Inspected	Date	Auditor	Reports to Management and Study Director(s)	Date
Strain Characteristics	Dec. 18, 2001	L. M. Byrd	Periodic Status Report	Jan. 7, 2002
Spot Plate Inhibition	Dec. 18, 2001	L. M. Byrd		
Final Report Review	Jan. 10, 2002	L. M. Byrd		

This study will be included in the next periodic status report as completed.

This study has been conducted in accordance with the provisions of the FDA Good Laboratory Practice Regulations (21 CFR, Part 58).

QA Representative: Lisa M Byrd — Date: Jan. 14, 2002

Lisa M. Byrd, CQA, ALAT
Auditor, Quality Assurance

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GLP COMPLIANCE NOTIFICATION

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Report To: BioMimetic Pharmaceuticals
1626 Westgate Circle
Brentwood, TN 37027
Attn: Samuel Lynch, DMD, MSc

Authorized Asst.: (if different from left)
Attn:

A. Test Article Identification: Vitoss Scaffold

1. Batch/Code/Lot (circle one) Identification: 081098-146

2. Storage Conditions: Room Temp. ✓; Refrig. ___; Freezer ___; Other: ___

3. Handling and Safety Precautions: None

4. Stability: The sponsor assures that the above material has been characterized for identity, strength, purity and composition as required by subpart F section 58.105 (see back). Stability testing is the responsibility of the sponsor and is subject to FDA audit; please indicate one of the following:

✓ Stability testing is in progress; article is stable for duration of intended testing.
___ Stability testing is complete and on file with sponsor. Expiration Date: ___

5. Disposition: Discard/Destroy ___; Return to Sponsor ✓; Retain* ___ *required for studies longer than 28 days

B. Control Article Submitted By Sponsor: No ✓; Yes ___; Identification: ___

1. Batch/Code/Lot (circle one) Identification: ___

2. Storage Conditions: Room Temp. ___; Refrig. ___; Freezer ___; Other: ___

3. Handling and Safety Precautions: ___

4. Stability: Characterization and stability information as above are also required for control articles.

___ Stability testing is in progress; article is stable for duration of intended testing.
___ Stability testing is complete and on file with sponsor. Expiration Date: ___
___ Marketed product stability characterized by its labeling.

5. Disposition: Discard/Destroy ___; Return to Sponsor ___; Retain* ___ *required for studies longer than 28 days

C. Mixtures of test or control articles with carriers require analysis to demonstrate proper concentration, homogeneity and stability. Sponsor will provide analytical methods ___; or will perform analysis on representative aliquots provided by NAMSA ✓. NA LLT 12-10-01

D. NAMSA Cost Estimate No. 1649-LLT (or attach list of tests) Sponsor's PO No. 01-1005

Name of Principal Investigator (Sponsor): Samuel Lynch Title: President

Signature: See additional paperwork for signature LLT 12-10-01 Date: ___ Phone: 615-844-1280

Sample received 12-10-01 LLT 12-10-01

To be completed at NAMSA Test Facility:

Lab No. OIT 15267 00 Study Director: Laura J. Dasse 12-10-01

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GLP COMPLIANCE NOTIFICATION

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Report To: BioMimetic Pharmaceuticals
1616 Westgate Circle
Brentwood, TN 37027

Attn: Bill Beasley

Authorized Asst.: _____
(if different from left) _____

Attn: _____

A. **Test Article Identification:** rh PDGF-BB (Becaplermin - 1mg/ml 20mM NaAc buffer)

1. Batch/Code/Lot (circle one) Identification: QA 2217
2. Storage Conditions: Room Temp.___; Refrig.___; Freezer___; Other: ~~-70°C to 95°C~~ 2°C to 8°C per sponsor LLT 11-21-01
3. Handling and Safety Precautions: See MSDS
4. Stability: The sponsor assures that the above material has been characterized for identity, strength, purity and composition as required by subpart F section 58.105 (see back). Stability testing is the responsibility of the sponsor and is subject to FDA audit; please indicate one of the following:

   X Stability testing is in progress; article is stable for duration of intended testing.
   ___Stability testing is complete and on file with sponsor. Expiration Date: _____

5. Disposition: Discard/Destroy___; Return to Sponsor X; Retain*___ *required for studies longer than 28 days

B. **Control Article Submitted By Sponsor:** No X; Yes___; Identification: _____

1. Batch/Code/Lot (circle one) Identification: _____
2. Storage Conditions: Room Temp.___; Refrig.___; Freezer___; Other: _____
3. Handling and Safety Precautions: _____
4. Stability: Characterization and stability information as above are also required for control articles.

   ___Stability testing is in progress; article is stable for duration of intended testing.
   ___Stability testing is complete and on file with sponsor. Expiration Date: _____
   ___Marketed product stability characterized by its labeling.

5. Disposition: Discard/Destroy___; Return to Sponsor___; Retain*___ *required for studies longer than 28 days

C. Mixtures of test or control articles with carriers require analysis to demonstrate proper concentration, homogeneity and stability. Sponsor will provide analytical methods___; or will perform analysis on representative aliquots provided by NAMSA___. N/A

D. NAMSA Cost Estimate No. 1649-LLT (or attach list of tests) Sponsor's PO No. 01-1005

Name of Principal Investigator (Sponsor): Samuel Lynch Title: President

Signature: Samuel Lynch Date: 11/13/01 Phone: 615-844-1280

Sample received 11-20-01 LLT 12-10-01

To be completed at NAMSA Test Facility:

Lab No. 01T-15267-00 Study Director Laura J Casse 12-10-01

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NAMSA™ GLP PROTOCOL V23

GENOTOXICITY: BACTERIAL REVERSE MUTATION STUDY

Sponsor:

Bill Beasley
BioMimetic Pharmaceutical Inc.
1616 Westgate Circle
Brentwood, TN 37027

Test Facility:

North American Science Associates, Inc. (NAMSA)
2261 Tracy Road
Northwood, OH 43619-1397

NAMSA Use Only

Lab No. 01T-15267-00
CEP No. 1649-LLT (Revised)

Approvals:

Protocol Submitted By (NAMSA): Laura L. Tasse
Laura L. Tasse, BS, ALAT
Study Director

Date Issued: October 29, 2001

Principal Investigator: (Sponsor)

Date Approved: 11/6/01

Study Director (NAMSA): Laura L. Tasse

Date Initiated: Dec. 10, 2001

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NAMSA™ GLP PROTOCOL V23

GENOTOXICITY: BACTERIAL REVERSE MUTATION STUDY

Purpose of the Study:

The purpose of the study is to evaluate whether an extract of the test material or a solubilized material will cause mutagenic changes in a tryptophan-dependent strain of *Escherichia coli* or in one or more strains of histidine-dependent *Salmonella typhimurium* in the presence or absence of S9 metabolic activation. The Bacterial Reverse Mutation Study will be used as a rapid screening procedure for the determination of mutagenic and potential carcinogenic hazards and should be used in conjunction with other tests that characterize potential genotoxicity properties. This study will be based on OECD guidelines and the requirements of the International Organization for Standardization: Biological Evaluation of Medical Devices - Part 3: Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity.

This study will be conducted in accordance with the requirements of the FDA Good Laboratory Practice (GLP) Regulations, 21 CFR 58.

Test Article:

The sponsor will submit the material to be tested. Detailed information about the test sample will be provided by the sponsor on the GLP Compliance Notification form furnished by NAMSA or on a similar attachment to the protocol.

The following is to be completed (with initials and dates in the margin) by the sponsor or study director. Further instructions may be attached to the protocol. The sample will be prepared as follows:

Test article form:

___ Soluble material (solid or liquid) - complete "Preparation of Soluble Material"
✓ Insoluble material - complete "Preparation of Extract"
___ Other (specify)_____

Preparation of Extract (for insoluble materials):

1. Ratio of test material to vehicle:

___ Material thickness less than 0.5 mm, use ratio of 120 cm^2:20 ml
___ Material thickness greater than or equal to 0.5 mm, use ratio of 60 cm^2:20 ml
✓ Irregularly shaped objects and/or sponsor option, use ratio of 4 g:20 ml
___ Other (specify)_____

2. Vehicle:

✓ 0.9% Sodium Chloride for Injection , USP
✓ Dimethyl sulfoxide (DMSO)*
___ 95% ethanol (EtOH)**
___ Other (specify)_____

-LT
12-10-01

*Dimethyl sulfoxide can be extracted at 37°C ± 2°C for 24 hours, but room temperature for 72 hours is recommended.
**95% ethanol can only be extracted at room temperature (various times can be used).

BM001683

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3. Conditions (use highest temperature that will not degrade material):

___ 121°C, 1 hour
___ 70°C, 24 hours
___ 50°C, 72 hours
✓ 37°C, 24 hours – saline only
✓ room temperature, 72 hours – DMSO only
___ Other (specify)_____

LLT 11-21-01

Preparation of Soluble Material:

1. ___ Solid:

One gram of the sample will be transferred to a 10 ml volumetric flask. Various sized flasks may be used to accommodate nature of test material utilizing 100 mg/ml or 10% w/v. Appropriate vehicle (specified below) will be added (q.s.) to the 10 ml (or appropriate) demarcation to achieve 100 mg/ml or a 10% (w/v) solution of the material.

2. ___ Liquid:

One milliliter of the sample will be transferred to a 10 ml volumetric flask. Various sized flasks may be used to accommodate nature of test material utilizing 100 mg/ml or 10% v/v. Appropriate vehicle (specified below) will be added (q.s.) to the 10 ml (or appropriate) demarcation to achieve 100 mg/ml or a 10% (v/v) solution of the material.

NOTE: GLP regulations 21 CFR 58.113 requires concentration analysis and stability determination for mixtures with carriers.

Vehicles:

___ 0.9% Sodium Chloride for Injection, USP
___ Dimethyl sulfoxide (DMSO)
___ 95% ethanol (EtOH)
___ Other (specify) _____

All preparations of soluble materials will be performed the day of test. In the event the material does not completely dissolve at these concentrations, serial dilutions will be prepared. The highest possible concentration that achieves complete dissolution of the material will be used for testing purposes.

Test System:

Each *S. typhimurium* tester strain contains a specific mutation in the histidine operon and other mutations that increase their ability to detect mutagens. The *E. coli* strain contains a mutation in the tryptophan operon and a deletion in the *uvrA* gene. These genetically altered *S. typhimurium* strains (TA98, TA100, TA1535, and TA1537) and *E. coli* strain (WP2*uvrA*) cannot grow in the absence of histidine or tryptophan, respectively. When placed in a histidine-free (for *S. typhimurium*) or tryptophan-free (for *E. coli*) medium, only those cells which mutate spontaneously back to their wild type state (histidine independent by manufacturing their own histidine, or tryptophan independent by manufacturing their own tryptophan) are able to form colonies. The spontaneous mutation rate (or reversion rate) for any one strain is relatively constant, but if a mutagen is added to the test system, the mutation rate is significantly increased.

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Tester Strain	Mutations/Genotypic Relevance
S. typhimurium TA98	hisD3052, *rfa*, *uvrB*, frameshift, pKM101
S. typhimurium TA100	hisG46, *rfa*, *uvrB*, missense, pKM101
S. typhimurium TA1535	hisG46, *rfa*, *uvrB*, missense
S. typhimurium TA1537	hisC3076, *rfa*, *uvrB*, frameshift
E. coli WP2*uvrA*	trpE65, *uvrA*, missense

rfa	=	causes partial loss of the lipopolysaccharide wall which increases permeability of the cell to large molecules (i.e., crystal violet inhibition)
uvrB or *uvrA*	=	deficient DNA excision - repair system (i.e., ultraviolet sensitivity)
frameshift	=	base-pair addition/deletion
missense	=	base-pair substitution
pKM101	=	plasmid confers ampicillin resistance (R-factor) and enhances sensitivity to mutagens

Metabolic Activation:

Aroclor 1254 - induced rat liver (S9 homogenate) will be used as metabolic activation. The material is prepared from male, Sprague Dawley rats. The rats are induced with one intraperitoneal injection of Aroclor 1254 (500 mg/ml) 5 days prior to sacrifice. The S9 homogenate is purchased from Organon Teknika Corporation, Box 15969, Durham, NC 27704-0969. Just prior to use, the S9 homogenate will be mixed with a buffer containing 0.4 M $MgCl_2$/1.65 M KCl, 1.0 M Glucose-6-phosphate, 0.1 M NADP, 0.2 M sodium phosphate buffer and sterile water.

Preparation of Tester Strains:

Cultures of *Salmonella typhimurium*, TA98, TA100, TA1535 and TA1537, and *Escherichia coli*, WP2*uvrA*, will be inoculated to individual Erlenmeyer flasks containing oxoid broth. The inoculated broth cultures will be incubated overnight at 37 ± 2°C in an incubator shaker operating at 115-125 rpm for 10-12 hours.

Preparation of Negative Control:

Negative control (vehicle without test material) will be utilized for each tester strain with and without S9 activation.

Preparation of Positive Controls:

A known mutagen, Dexon (paradimethylaminobenzene diazosulfonic acid sodium salt), will be used as a positive control to demonstrate that tester strains TA98, TA100, and TA1537 are sensitive to mutation to the wild type state. For tester strain TA1535, sodium azide will be used as a positive control. For tester strain TA100, 2-aminofluorene will be used as a positive control. For tester strain WP2*uvrA*, 2-aminoanthracene and methylmethane-sulfonate will be used as positive controls. Although metabolic activation is only required with 2-aminofluorene and 2 aminoanthracene to induce mutagenic results, all positive controls will be tested with and without S9 homogenate.

Strain Characteristics and Strain Standard Plate Counts:

Strain characteristics will be verified and viable counts will be determined.

Spot Plate Inhibition Screen:

The extract(s) or solubilized material(s) and negative control(s) will be evaluated by a spot plate technique modeled after the antimicrobial zone of inhibition test. This screen is used to evaluate extract or solution concentrations for toxicity which are noninhibitory to the *Salmonella* strains and the *E. coli* strain.

Separate tubes containing 2 ml of molten top agar supplemented with histidine-biotin (for *S. typhimurium*) or with tryptophan (for *E. coli*) will be inoculated with 0.1 ml of culture for each of the five tester strains. After mixing, the

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agar will be poured across the surface of separate Minimal E plates labeled with lab number, appropriate tester strain, and dose level (when necessary). Once the agar solidifies, sterile filter discs will be placed in the center of the plates. A 0.1 ml aliquot of the extract or solubilized material will be added to the filter discs on each of the labeled plates. Parallel testing will be conducted with a negative control. To demonstrate a positive zone of inhibition, 10X stock Dexon will be used.

The plates will be incubated at 37 ± 2°C for 2-3 days. Following the incubation period, the zone of growth inhibition will be recorded. If significant inhibition of the background lawn occurs, the extract or solubilized material concentration will be adjusted by preparing one or more dilutions and repeating the inhibition screen to find a nontoxic level.

Standard Plate Incorporation Assay:

Separate tubes containing 2 ml of molten top agar supplemented with histidine-biotin solution (for *S. typhimurium*) or tryptophan (for *E. coli*) will be inoculated with 0.1 ml of culture for each of the five tester strains, and 0.1 ml of the test material. A 0.5 ml aliquot of SWI or S9 homogenate, simulating metabolic activation, will be added when necessary. The mixture will be poured across triplicate Minimal E plates labeled with lab number, appropriate tester strain, and S9 metabolic activation (when applicable). Parallel testing will be conducted on a negative control and five positive controls.

Histidine-free media plates (for *S. typhimurium*) and tryptophan-free media plates (for *E. coli*) will be prepared in triplicate as follows:

1. Extract or solubilized material with and without S9 activation
2. Negative control with and without S9 activation
3. 1X Dexon (known mutagen) with and without S9 activation with strains TA98, TA100, and TA1537
4. 1X 2-aminofluorene (known mutagen) with and without S9 activation with strain TA100
5. 1X Sodium azide (known mutagen) with and without S9 activation with strain TA1535
6. 1X 2-aminoanthracene (known mutagen) with and without S9 activation with strain WP2*uvrA*
7. 1X Methylmethane-sulfonate (known mutagen) with and without S9 activation with strain WP2*uvrA*

The plates will be incubated at 37 ± 2°C for 2-3 days. After the incubation period, the revertant colonies on each plate (test, negative and positive) will be counted and recorded. The mean number of revertants will be calculated. All test methods are described in approved NAMSA Standard Operating Procedures.

Evaluation of Test Results:

The mean number of revertants of the triplicate test plates will be compared to the mean number of revertants of the triplicate negative control plates for each of the five tester strains employed. The means obtained for the positive controls are used as points of reference.

For a test material to be identified as a test failure or "potential mutagen" there must be a 2-fold or greater increase in the number of mean revertants over the means obtained from the negative control, for any/all five tester strains. If no 2-fold increase is present, the test material is considered nonmutagenic.

Any apparent "positive response" will be confirmed by demonstrating a dose-response relationship using three nontoxic dose levels of the test material. There should be a range of concentrations that produce a linear dose-response. In the event linearity cannot be established, the assay will be repeated with an appropriate change in dose levels. A test material will be judged mutagenic if it causes a dose-related increase in the number of revertants over a minimum of two increasing dose concentrations.

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Test Validity:

For any assay to be considered valid, it must meet the following criteria:

1. Strain characteristics: All *S typhimurium* tester strains (TA98, TA100, TA1535, and TA1537) must exhibit sensitivity to crystal violet (*rfa* mutation), and ultraviolet light (*uvrB*), and must exhibit no growth on biotin plates, and growth on histidine-biotin plates. Tester strains TA98 and TA100 must exhibit resistance to ampicillin (R-factor); tester strains TA1535 and TA1537 must exhibit sensitivity to ampicillin. Tester strain WP2*uvrA* must exhibit sensitivity to ultraviolet light, no growth on tryptophan deficient plates, growth on tryptophan supplemented media and sensitivity to ampicillin.

2. Strain Standard Plate Counts: A viable count on the working culture suspensions for each tester strain (TA98, TA100, TA1535, TA1537 and WP2*uvrA*) should not be less than 1×10^8 CFU/ml.

3. Spot Plate Inhibition Screen: Each prepared extract or solubilized material will be evaluated for inhibition or toxicity to the cells. A test sample that is noninhibitory to moderately noninhibitory to the tester strains will be tested by the standard plate incorporation method. In the event a test material is inhibitory, dilutions will be required to find a nontoxic level.

4. Standard Plate Incorporation Assay: Each positive control mean must exhibit at least a 3-fold increase over the respective negative control mean of the *Salmonella* tester strain employed, and at least a 2-fold increase over the respective negative control mean of the *E. coli* tester strain. Exceptions include conditions not intended to provoke a mutagenic response (e.g. 2-amino-anthracine and 2-aminofluorine without metabolic activation). The negative control results of each tester strain will exhibit a characteristic number of spontaneous revertants. Spontaneous reversion rates may vary, but should be consistent with the ranges specified (see Table II). Table II is meant as a guideline only. Negative control results for tester strains may fall outside of the range listed. In such an instance, the results should be evaluated with caution.

Species	Tester Strains	Number of Spontaneous Revertants
S. typhimurium	TA98	15-50
	TA100	120-240
	TA1537	3-28
	TA1535	10-35
E. coli	WP2*uvrA*	20-125

Report:

A report of the results of the assay will be prepared by NAMSA and will describe all methods used to generate and analyze data. The report will contain the bacterial tester strains employed and characterization including strain standard plate count, spot plate inhibition screen data, test conditions, individual and mean reversion rates (in tabular form), evaluation of results and conclusions.

Quality Assurance:

Inspections will be conducted at intervals adequate to assure the integrity of the study in conformance with 21 CFR 58.35(b)(3). The final report will also be reviewed for conformance to Section 58.185, Subpart J, of the GLP Regulations. A Certificate of Quality Assurance Inspections will be provided with the final report.

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Records:

Test article preparation, strain characteristics, standard strain plate counts, spot plate inhibition screen data, standard plate incorporation assay data, and dates of relevant activities will be recorded.

All raw data pertaining to this study and a copy of the final report will be retained in designated NAMSA archive files.

Proposed Dates:

The study dates will be scheduled by the study director following receipt of the sponsor-approved protocol and appropriate material for the study. Initiation of the study will be the date on which the study director signs the GLP protocol. Projected dates for starting the study (first treatment) and for the completion of the study (final report release) will be provided to the sponsor (or representative of the sponsor) and added to the protocol.

References:

Ames, B.N., McCann, J., and Yamasaki, E., "Methods for Detecting Carcinogens and Mutagens with the Salmonella/Mammalian-Microsome Mutagenicity Test," Mutation Research 31, (1975): 347-364.

Brusick, D.J., V.F. Simmon, H.S. Rosenkranz, V.A. Ray, and R.S. Stafford, "An Evaluation of the *Escherichia coli* WP2 and WP2*uvrA* Reverse Mutation Assay," Mutation Research 76, (1980): 169-190.

Maron, Dorothy M., Ames, Bruce N., "Revised Methods for the Salmonella Mutagenicity Test," Mutation Research, 113 (1983): 175-215.

ISO 10993-3. Biological Evaluation of Medical Devices, Part 3: Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity.

OECD Guideline for the Testing of Chemicals, Proposal for Replacement of Guidelines 471 Bacterial Reverse Mutation Test, Document Number 471.

Ortiz, A.I., M.T. Pollastrini, M. Barea, and D. Ordóñez, "Bacterial Mutagenic Evaluation of Luxabendazole, a New Broad Spectrum Antihelminic, with the *Salmonella typhimurium* Histidine and the *Escherichia coli* Tryptophan Reversions Tests," *Mutagenesis* 11 (1996): 27-31.

Test validation, Bacterial Mutagenicity Test: NAMSA lab number 98T-00785-00.

Protocol Changes:

Any changes necessary to the protocol after the sponsor approval or study initiation will be documented and approved by the study director as protocol amendments. Copies will be distributed to the sponsor, the raw data file, and the NAMSA Quality Assurance department.

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December 10, 2001

BILL BEASLEY
BIOMIMETIC PHARMACEUTICALS
1616 WESTGATE CIRCLE
BRENTWOOD, TN 37027

PROTOCOL AMENDMENT I

Test Article: rhPDGF-BB (Becaplermin - 1 mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold

Identification: Lot #QA2217 and Lot 081098-146

NAMSA Lab No.: 01T 15267 00

We have received appropriate test articles and approved protocols for the program to be conducted in accordance with the Good Laboratory Practice (GLP) Regulations on the material described above. Below is a projected schedule for the work to be performed.

NAMSA Code	Study	Estimated Start Date:	Estimated Report Release Date:
V0014-130	Cytotoxicity Study using the ISO Elution Method (Extract)	Dec. 13, 2001	Jan. 8, 2002
TI261-300	ISO Sensitization Test in Fifteen Guinea Pigs, Saline Extract		
TI261-301	ISO Sensitization Test in Fifteen Guinea Pigs, Cottonseed Oil Extract	Dec. 14, 2001	Jan. 25, 2002
TI251-800	ISO Acute Intracutaneous Reactivity Study in Three Rabbits Saline Extract, Cottonseed Oil Extract	Dec. 17, 2001	Jan. 8, 2002
TI264-500	ISO Acute Systemic Toxicity Study in Five Mice, Saline Extract, Cottonseed Oil Extract	Dec. 17, 2001	Jan. 8, 2002
V0023-211	Genotoxicity: Bacterial Reverse Mutation (Ames) Test, Salmonella and *E. coli* strains, Saline Extract		
V0023-212	Genotoxicity: Bacterial Reverse Mutation (Ames) Test, Salmonella and *E. coli* strains, DMSO Extract	Dec. 18, 2001	Jan. 15, 2002
TI247-804 TH035-800	ISO Surgical Muscle Implantation, in Three Rabbits (4 Weeks) With Histopathology	Dec. 19, 2001	Jan. 25, 2002

Laura L. Tasse

Laura L. Tasse, BS, ALAT
Study Director

Dec. 10, 2001
Date

LLT/dap

cc: QA (NAMSA)
GLP study file

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December 10, 2001

SAMUEL LYNCH, M.D.
BIOMIMETIC PHARMACEUTICALS
1616 WESTGATE CIRCLE
BRENTWOOK, TN 37027

PROTOCOL AMENDMENT II

Test Article:	rhPDGF-BB (Becaplermin – 1 mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold
Identification No.:	Lot #QA2217 and Lot 081098-146
Protocol:	V0014-130 Cytotoxicity Study using the ISO Elution Method (Extract) TI261-300 ISO Sensitization Test in Fifteen Guinea pigs (Saline extract) TI261-301 ISO Sensitization Test in Fifteen Guinea pigs (Cottonseed oil extract) TI251-800 ISO Acute Intracutaneous Reactivity Study in Three Rabbits TI264-500 ISO Acute Systemic Toxicity Study in Five Mice V0023-211 Genotoxicity: Bacterial Reverse Mutation (Ames) Test (Saline extract) V0023-212 Genotoxicity: Bacterial Reverse Mutation (Ames) Test (DMSO extract)
NAMSA Lab No.:	01T 15267 00

This amendment was written to clarify the preparation of the test article.

The two components of the test article must be combined just prior to extraction preparation. To do this, a 1:1 ratio of Vitoss (in grams) to rhPDGF-BB (in ml's) will be prepared. For example, a 2 g portion of the ChronOS will be saturated with 2 ml of the rhPDGF. If the frozen rhPDGF-BB is used, it must first be thawed prior to the preparation.

The resulting mixture will then be used for the extraction preparation (i.e. weight of the prepared test article to volume of extraction vehicle).

Laura L. Tasse

Laura L. Tasse, BS, ALAT
Study Director

Dec. 10, 2001
Date

cc: NAMSA QAU
GLP study file

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January 8, 2002

BILL BEASLEY
BIOMIMETIC PHARMACEUTICALS
1616 WESTGATE CIRCLE
BRENTWOOD, TN 37027

REVISED*

PROTOCOL AMENDMENT II

Test Article:	rhPDGF-BB (Becaplermin – 1 mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold
Identification No.:	Lot #QA2217 and Lot 081098-146
Protocol:	V0014-130 Cytotoxicity Study using the ISO Elution Method (Extract) TI261-300 ISO Sensitization Test in Fifteen Guinea pigs (Saline extract) TI261-301 ISO Sensitization Test in Fifteen Guinea pigs (Cottonseed oil extract) TI251-800 ISO Acute Intracutaneous Reactivity Study in Three Rabbits TI264-500 ISO Acute Systemic Toxicity Study in Five Mice V0023-211 Genotoxicity: Bacterial Reverse Mutation (Ames) Test (Saline extract) V0023-212 Genotoxicity: Bacterial Reverse Mutation (Ames) Test (DMSO extract)
NAMSA Lab No.:	01T 15267 00

This amendment was written to clarify the preparation of the test article.

The two components of the test article must be combined just prior to extraction preparation. To do this, a 1:1 ratio of Vitoss (in grams) to rhPDGF-BB (in ml's) will be prepared. For example, a 2 g portion of the Vitoss* will be saturated with 2 ml of the rhPDGF. If the frozen rhPDGF-BB is used, it must first be thawed prior to the preparation.

The resulting mixture will then be used for the extraction preparation (i.e. weight of the prepared test article to volume of extraction vehicle).

* This amendment was revised to correct the sponsor name and address, and to correct the name of the Vitoss in the second paragraph.

Laura L. Tasse

Laura L. Tasse, BS, ALAT
Study Director

Jan. 8, 2002
Date

cc: NAMSA QAU
GLP study file

Appendix 7.7.6: **Test Report V0023-212. Genotoxicity: Bacterial reverse mutation study (DMSO Extract)**

STUDY TITLE:

GENOTOXICITY: BACTERIAL REVERSE MUTATION STUDY

(DMSO Extract)

TEST ARTICLE:

rhPDGF-BB (Becaplermin-1 mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold

IDENTIFICATION NO.:

Lot #QA2217 and Lot 081098-146

TEST FACILITY:

NAMSA
2261 Tracy Road
Northwood, OH 43619-1397

SPONSOR:

BILL BEASLEY
BIOMIMETIC PHARMACEUTICALS
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SUMMARY

A *Salmonella typhimurium* and *Escherichia coli* reverse mutation standard plate incorporation study was conducted to evaluate whether a dimethyl sulfoxide extract of rhPDGF-BB (Becaplermin - 1 mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold, Lot #QA2217 and Lot 081098-146, would cause mutagenic changes in the average number of revertants for histidine-dependent *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, and in tryptophan-dependent *Escherichia coli* strain WP2*uvrA* in the presence and absence of S9 metabolic activation. This study was conducted to satisfy, in part, the genotoxicity requirement of the International Organization for Standardization: Biological Evaluation of Medical Devices, Part 3: Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity.

The dimethyl sulfoxide test article extract was found to be noninhibitory to growth of tester strains TA98, TA100, TA1535, TA1537, and WP2*uvrA*. Separate tubes containing 2 ml of molten top agar supplemented with histidine-biotin solution for the *S. typhimurium* strains and with tryptophan for the *E. coli* strain were inoculated with 0.1 ml of culture for each of five tester strains, and 0.1 ml of the dimethyl sulfoxide extract. A 0.5 ml aliquot of sterile Water for Injection or S9 homogenate, simulating metabolic activation, was added when necessary. The mixture was poured across triplicate Minimal E plates. Parallel testing was also conducted with a negative control and five positive controls. The mean number of revertants of the triplicate test plates was compared to the mean number of revertants of the triplicate negative control plates for each of the five tester strains employed. The means obtained for the positive controls were used as points of reference.

Under the conditions of this assay, the dimethyl sulfoxide test article extract was considered to be nonmutagenic to *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537, and to *Escherichia coli* strain WP2*uvrA*. The negative and positive controls performed as anticipated. The results of this study should be evaluated in conjunction with other required tests as listed in ISO 10993, Part 3: Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity.

Study and Supervisory Personnel:

Sally C. Ahmed, BS
Deedee M. Davis, BA
Suzanne E. Brimacombe
Lionel D. Brock, BS
Laura L. Tasse, BS, ALAT
Cherise M. McCoy, BS

Study Director: Laura L. Tasse — Jan. 14, 2002

Laura L. Tasse, BS, ALAT
Study Director

Date Completed

/rl

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INTRODUCTION

A *Salmonella typhimurium* and *Escherichia coli* reverse mutation standard plate incorporation study was conducted to evaluate whether a dimethyl sulfoxide test article extract would cause mutagenic changes in the average number of revertants for *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537, and *Escherichia coli* tester strain WP2*uvrA* in the presence and absence of S9 metabolic activation. This test was conducted to satisfy, in part, the requirements of the International Organization for Standardization (ISO) 10993, Part 3: Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity. Bacterial reverse mutation tests have been widely used as rapid screening procedures for the determination of mutagenic and potential carcinogenic hazards. The test article was received on November 20, 2001 and December 10, 2001. The preliminary toxicity screen began on December 18, 2001, and the testing ended on December 20, 2001.

The study, initiated by protocol signature on December 10, 2001, was conducted in accordance with the provisions of the FDA Good Laboratory Practice (GLP) Regulations, 21 CFR 58. A Certificate of Quality Assurance Inspections was issued with this report.

MATERIALS

The sample provided by the sponsor was identified and handled as follows:

Test Article:	rhPDGF-BB (Becaplermin-1 mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold
Identification No.:	Lot #QA2217 and Lot 081098-146
Stability Testing:	In Progress (per sponsor)
Expiration Date:	Stable for duration of intended testing (per sponsor)
Storage Conditions:	rhPDGF-BB – Refrigerated Vitoss Scaffold – Room temperature
Vehicle:	Dimethyl sulfoxide (DMSO)
Preparation:	A 1.0 g portion of Vitoss Scaffold was saturated with a 1.0 ml portion of rhPDGF immediately prior to adding the vehicle. Based on the USP ratio of 4 g:20 ml, a 1.9 g portion of the prepared test article was covered with 9.5 ml of DMSO. A single preparation was extracted at room temperature for 72 hours. Vehicle without test material was similarly subjected to the extraction conditions for use as a negative control. The test extract and negative control were decanted.

	Test	Control
Condition of Extracts:	cloudy	clear

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METHODS

Test System:

Each *Salmonella typhimurium* tester strain contains a specific mutation in the histidine operon and other mutations that increase their ability to detect mutagens. In addition, the *Escherichia coli* contains a specific mutation in the tryptophan operon, and a deletion in the *uvrA* gene. These genetically altered *S. typhimurium* strains (TA98, TA100, TA1535, and TA1537) and *E. coli* strain (WP2*uvrA*) cannot grow in the absence of histidine or tryptophan, respectively. When placed in a histidine-free (for *S. typhimurium*) or tryptophan-free (for *E. coli*) medium, only those cells which mutate spontaneously back to their wild type state (histidine independent by manufacturing their own histidine, or tryptophan independent by manufacturing their own tryptophan) are able to form colonies. The spontaneous mutation rate (or reversion rate) for any one strain is relatively constant, but if a mutagen is added to the test system, the mutation rate is significantly increased.

Tester Strain	Mutations/Genotypic Relevance
S. typhimurium TA98	hisD3052, *rfa, uvrB*, frameshift, pKM101
S. typhimurium TA100	hisG46, *rfa, uvrB*, missense, pKM101
S. typhimurium TA1535	hisG46, *rfa, uvrB*, missense
S. typhimurium TA1537	hisC3076, *rfa, uvrB*, frameshift
E. coli WP2*uvrA*	trpE65, *uvrA*, missense

rfa	=	causes partial loss of the lipopolysaccharide wall which increases permeability of the cell to large molecules (i.e., crystal violet inhibition)
uvrB or *uvrA*	=	deficient DNA excision - repair system (i.e., ultraviolet sensitivity)
frameshift	=	base-pair addition/deletion
missense	=	base-pair substitution
pKM101	=	plasmid confers ampicillin resistance (R-factor) and enhances sensitivity to mutagens

Metabolic Activation:

Aroclor 1254 - induced rat liver (S9 homogenate) was used as metabolic activation. The S9 homogenate is prepared from male, Sprague Dawley rats. The rats are induced with one intraperitoneal injection of Aroclor 1254 (500 mg/ml) 5 days prior to sacrifice. Just prior to use, the S9 homogenate was mixed with a buffer containing 0.4 M $MgCl_2$/1.65 M KCl, 1.0 M Glucose-6-phosphate, 0.1 M NADP, 0.2 M sodium phosphate buffer, and sterile Water for Injection.

Preparation of Tester Strains:

Cultures of *Salmonella typhimurium*, TA98, TA100, TA1535 and TA1537, and *Escherichia coli*, WP2*uvrA*, were inoculated to individual Erlenmeyer flasks containing oxoid broth. The inoculated broth cultures were incubated overnight at 37 ± 2°C in an incubator shaker operating at 115-125 rpm for 10-12 hours.

Negative Control:

DMSO (vehicle without test material) was tested with each tester strain to determine the spontaneous reversion rate. Each strain was tested with and without S9 activation. These data represented a base rate to which the number of revertant colonies that developed in each test plate were compared to determine whether the test article had significant mutagenic properties.

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Positive Control:

A known mutagen, Dexon (paradimethylaminobenzene diazosulfonic acid sodium salt), was used as a positive control to demonstrate that tester strains TA98, TA100, and TA1537 were sensitive to mutation to the wild type state. For tester strain TA1535, sodium azide was used as a positive control. For tester strain TA100, 2-aminofluorene was also used as a positive control. For tester strain WP2*uvrA*, 2-aminoanthracene and methylmethane-sulfonate were used as positive controls. Although metabolic activation was only required with 2-aminofluorene and 2-aminoanthracene to induce mutagenic results, all positive controls were tested with and without S9 homogenate.

Strain Characteristics and Strain Standard Plate Counts:

Strain characteristics were verified and viable counts were determined.

Spot Plate Inhibition Screen:

The DMSO test article extract was evaluated by a spot plate technique, modeled after the antimicrobial zone of inhibition test. This screen was used to evaluate the toxicity of the extract to determine whether dilution of the extract was required to provide an extract noninhibitory to the *Salmonella typhimurium* or to the *Escherichia coli*.

Separate tubes containing 2 ml of molten top agar supplemented with histidine-biotin solution for the *Salmonella typhimurium* and tryptophan for the *Escherichia coli* were inoculated with 0.1 ml of culture for each of the five tester strains. After mixing, the agar was poured across the surface of separate Minimal E plates labeled with lab number and appropriate tester strain. Once the agar solidified, sterile filter discs were placed in the center of the plates. A 0.1 ml aliquot of the DMSO test article extract was added to the filter discs on each of the labeled plates. Parallel testing was conducted with a negative control, and to demonstrate a positive zone of inhibition, 10X Dexon was utilized.

The plates were incubated at 37°C for 2 days. Following the incubation period, the zone of growth inhibition was observed and recorded. Only extracts that were noninhibitory to the tester strains were tested by the standard plate incorporation method.

Standard Plate Incorporation Assay:

Separate tubes containing 2 ml of molten top agar supplemented with histidine-biotin solution for the *Salmonella typhimurium* or with tryptophan for the *Escherichia coli* were inoculated with 0.1 ml of culture for each of the five tester strains and 0.1 ml of the DMSO test article extract. A 0.5 ml aliquot of SWI or S9 homogenate, simulating metabolic activation, was added when necessary. The mixture was poured across triplicate Minimal E plates labeled with lab number, appropriate tester strain, and S9 metabolic activation (when applicable). Parallel testing was also conducted with a negative control and five positive controls.

Histidine-free media plates (for *S. typhimurium*) and tryptophan-free media plates (for *E. coli*) were prepared in triplicate as follows:

1. DMSO test article extract with and without S9 activation
2. Negative control with and without S9 activation
3. 1X Dexon (known mutagen) with and without S9 activation with strains TA98, TA100, and TA1537
4. 1X 2-Aminofluorene (known mutagen) with and without S9 activation with strain TA100
5. 1X Sodium azide (known mutagen) with and without S9 activation with strain TA1535
6. 1X 2-Aminoanthracene (known mutagen) with and without S9 activation with strain *WP2uvrA*
7. 1X Methylmethane-sulfonate (known mutagen) with and without S9 activation with strain WP2*uvrA*

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TABLE I

STRAIN CHARACTERISTICS AND STRAIN STANDARD PLATE COUNTS

Characteristics (expected)	Tester Strains				
	TA98	TA100	TA1535	TA1537	WP2*uvrA*
Ampicillin-TA98 & TA100 = (Resistant) TA1535, TA1537 & WP2*uvrA* = (Sensitive)	R	R	S	S	S
rfa Mutation; UV (Sensitive)	S	S	S	S	NA
uvrB/*uvrA* (No Growth)	NG	NG	NG	NG	NG
Histidine Requirement; (Growth)	G	G	G	G	NA
Tryptophan Requirement; (Growth)	NA	NA	NA	NA	G
Biotin (No Growth)	NG	NG	NG	NG	NA
L-tryptophan (No Growth)	NA	NA	NA	NA	NG
Purity (Pure)	PURE	PURE	PURE	PURE	PURE
Total Plate Count CFU's (10^{-7})	12 14	72 89	159 162	67 85	280 290
Mean	13	81	161	76	285
Titer (Organisms/ml)	1.3×10^8	8.1×10^8	1.6×10^9	7.6×10^8	2.9×10^9

R = Resistant S = Sensitive NG = No Growth G = Growth NA = Not Applicable

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TABLE II

SPOT PLATE INHIBITION SCREEN RESULTS

	Zone of Inhibition (mm)				
	TA98	TA100	TA1535	TA1537	WP2*uvrA*
DMSO negative control	0	0	0	0	0
DMSO test article extract	0	0	0	0	0
Dexon positive control	45	39	48	46	26

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TABLE III

STANDARD PLATE INCORPORATION ASSAY - REVERSION RATES FOR TESTER STRAINS

	Salmonella typhimurium								*Escherichia coli*	
	TA98		TA100		TA1535		TA1537		WP2*uvrA*	
	CFTP	Mean	CFTP	Mean	CFTP	Mean	CFTP	Mean	CFTP	Mean
DMSO	39		168		11		9		21	
w/o S9	31	36	155	167	10	10	10	9	32	26
negative control	38		178		10		8		24	
DMSO	39		187		10		6		28	
w S9	53	44	186	193	11	11	6	6	27	29
negative control	39		205		11		5		32	
DMSO	40		132		10		8		38	
test article	41	41	163	153	8	11	12	9	26	30
extract w/o S9	42		165		14		8		26	
DMSO	43		143		8		11		41	
test article	42	45	153	168	10	10	10	10	39	38
extract w S9	50		209		13		10		34	
Dexon	2000		1296				424			
w/o S9	2192	2080	1536	1413			440	444		
positive control	2048		1408				468			
Dexon	1392		1008				252			
w S9	1664	1589	896	901			416	373		
positive control	1712		800				452			
2-aminofluorene			200							
w/o S9			184	184						
positive control*			167							
2-aminofluorene			1888							
w S9			1920	1899						
positive control†			1888							
Sodium azide					3728					
w/o S9					3712	4341				
positive control					5584					
Sodium azide					3776					
w S9					4880	4229				
positive control					4032					
2-aminoanthracene									35	
w/o S9									31	33
positive control*									33	
2-aminoanthracene									87	
w S9									93	99
positive control†									118	
Methylmethane-									364	
Sulfonate w/o S9									320	363
positive control									404	
Methylmethane-									400	
Sulfonate w S9									312	345
positive control									324	

CFTP = Counts from triplicate plates Mean = Mean of triplicate plates = Not Applicable

*Negative control for S9 †Positive control for S9

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GLP COMPLIANCE NOTIFICATION

This form is used for non-clinical studies to be conducted according to FDA Good Laboratory Practice Regulations of 21 CFR Part 58. Completion of this form identifies material that will be listed on a NAMSA Master Schedule Sheet available for FDA inspection. All information requested must be submitted to NAMSA before testing may begin.

Report To: BioMimetic Pharmaceuticals
1616 Westgate Circle
Brentwood, TN 37027

Authorized Asst.: (if different from left)

Attn: Samuel Lynch, MD, DMSc

Attn:

A. Test Article Identification: Vitoss Scaffold

1. Batch/Code/Lot (circle one) Identification: 081098-146

2. Storage Conditions: Room Temp. ✓; Refrig. ___; Freezer ___; Other: ___

3. Handling and Safety Precautions: None

4. Stability: The sponsor assures that the above material has been characterized for identity, strength, purity and composition as required by subpart F section 58.105 (see back). Stability testing is the responsibility of the sponsor and is subject to FDA audit; please indicate one of the following:

✓ Stability testing is in progress; article is stable for duration of intended testing.
___ Stability testing is complete and on file with sponsor. Expiration Date: ___

5. Disposition: Discard/Destroy ___; Return to Sponsor ✓; Retain* ___ *required for studies longer than 28 days

B. Control Article Submitted By Sponsor: No ✓; Yes ___; Identification: ___

1. Batch/Code/Lot (circle one) Identification: ___

2. Storage Conditions: Room Temp. ___; Refrig. ___; Freezer ___; Other: ___

3. Handling and Safety Precautions: ___

4. Stability: Characterization and stability information as above are also required for control articles.

___ Stability testing is in progress; article is stable for duration of intended testing.
___ Stability testing is complete and on file with sponsor. Expiration Date: ___
___ Marketed product stability characterized by its labeling.

5. Disposition: Discard/Destroy ___; Return to Sponsor ___; Retain* ___ *required for studies longer than 28 days

C. Mixtures of test or control articles with carriers require analysis to demonstrate proper concentration, homogeneity and stability. Sponsor will provide analytical methods ___; or will perform analysis on representative aliquots provided by NAMSA ✓. NA LLT 12-10-01

D. NAMSA Cost Estimate No. 1649-LLT (or attach list of tests) Sponsor's PO No. 01-1005

Name of Principal Investigator (Sponsor): Samuel Lynch Title: President

Signature: See additional paperwork for signature LLT 12-10-01 Date: ___ Phone: 615-844-1280

Sample received 12-10-01 LLT 12-10-01

To be completed at NAMSA Test Facility:

Lab No. 01T15267 00 Study Director Laura J. Dasse 12-10-01

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1001U

GLP COMPLIANCE NOTIFICATION

This form is used for non-clinical studies to be conducted according to FDA Good Laboratory Practice Regulations of 21 CFR Part 58. Completion of this form identifies material that will be listed on a NAMSA Master Schedule Sheet available for FDA inspection. All information requested must be submitted to NAMSA before testing may begin.

Report To: BioMimetic Pharmaceuticals
1616 Westgate Circle
Brentwood, TN 37027

Attn: Bill Beasley

Authorized Asst.: _____
(if different from left) _____

Attn: _____

A. Test Article Identification: rh PDGF-BB (Becaplermin - 1mg/ml 20mM NaAc buffer)

1. Batch/Code/Lot (circle one) Identification: QA2217

2. Storage Conditions: Room Temp.___; Refrig.___; Freezer___; Other: ~~-70°C to 75°C~~ 2°C to 8°C per sponsor LLT 11-21-01

3. Handling and Safety Precautions: See MSDS

4. Stability: The sponsor assures that the above material has been characterized for identity, strength, purity and composition as required by subpart F section 58.105 (see back). Stability testing is the responsibility of the sponsor and is subject to FDA audit; please indicate one of the following:

X Stability testing is in progress; article is stable for duration of intended testing.
___Stability testing is complete and on file with sponsor. Expiration Date: _____

5. Disposition: Discard/Destroy___; Return to Sponsor X; Retain*___ *required for studies longer than 28 days

B. Control Article Submitted By Sponsor: No X; Yes___; Identification: _____

1. Batch/Code/Lot (circle one) Identification: _____

2. Storage Conditions: Room Temp.___; Refrig.___; Freezer___; Other: _____

3. Handling and Safety Precautions: _____

4. Stability: Characterization and stability information as above are also required for control articles.

___Stability testing is in progress; article is stable for duration of intended testing.
___Stability testing is complete and on file with sponsor. Expiration Date: _____
___Marketed product stability characterized by its labeling.

5. Disposition: Discard/Destroy___; Return to Sponsor___; Retain*___ *required for studies longer than 28 days

C. Mixtures of test or control articles with carriers require analysis to demonstrate proper concentration, homogeneity and stability. Sponsor will provide analytical methods___; or will perform analysis on representative aliquots provided by NAMSA___. N/A

D. NAMSA Cost Estimate No. 1649-LLT (or attach list of tests) Sponsor's PO No. 01-1005

Name of Principal Investigator (Sponsor): Samuel Lynch Title: President

Signature: Samuel Lynch Date: 11/13/01 Phone: 615-844-1280

Sample received 11-20-01 LLT 12-10-01

To be completed at NAMSA Test Facility:

Lab No. 01T-15267-00 Study Director Laura J. Casse 12-10-01

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NAMSA™ GLP PROTOCOL V23

GENOTOXICITY: BACTERIAL REVERSE MUTATION STUDY

Sponsor:

Bill Beasley
BioMimetic Pharmaceutical Inc.
1616 Westgate Circle
Brentwood, TN 37027

Test Facility:

North American Science Associates, Inc. (NAMSA)
2261 Tracy Road
Northwood, OH 43619-1397

NAMSA Use Only

Lab No. 01T-15267-00
CEP No. 1649-LLT (Revised)

Approvals:

Protocol Submitted By (NAMSA): Laura L. Tasse
Laura L. Tasse, BS, ALAT
Study Director

Date Issued: October 29, 2001

Principal Investigator: (Sponsor)

Date Approved: 11/6/01

Study Director (NAMSA): Laura L. Tasse

Date Initiated: Dec. 10, 2001

/pak

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NAMSA™ GLP PROTOCOL V23

GENOTOXICITY: BACTERIAL REVERSE MUTATION STUDY

Purpose of the Study:

The purpose of the study is to evaluate whether an extract of the test material or a solubilized material will cause mutagenic changes in a tryptophan-dependent strain of *Escherichia coli* or in one or more strains of histidine-dependent *Salmonella typhimurium* in the presence or absence of S9 metabolic activation. The Bacterial Reverse Mutation Study will be used as a rapid screening procedure for the determination of mutagenic and potential carcinogenic hazards and should be used in conjunction with other tests that characterize potential genotoxicity properties. This study will be based on OECD guidelines and the requirements of the International Organization for Standardization: Biological Evaluation of Medical Devices - Part 3: Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity.

This study will be conducted in accordance with the requirements of the FDA Good Laboratory Practice (GLP) Regulations, 21 CFR 58.

Test Article:

The sponsor will submit the material to be tested. Detailed information about the test sample will be provided by the sponsor on the GLP Compliance Notification form furnished by NAMSA or on a similar attachment to the protocol.

The following is to be completed (with initials and dates in the margin) by the sponsor or study director. Further instructions may be attached to the protocol. The sample will be prepared as follows:

Test article form:

___ Soluble material (solid or liquid) - complete "Preparation of Soluble Material"
✓ Insoluble material - complete "Preparation of Extract"
___ Other (specify)_____

Preparation of Extract (for insoluble materials):

1. Ratio of test material to vehicle:

___ Material thickness less than 0.5 mm, use ratio of 120 cm^2:20 ml
___ Material thickness greater than or equal to 0.5 mm, use ratio of 60 cm^2:20 ml
✓ Irregularly shaped objects and/or sponsor option, use ratio of 4 g:20 ml
___ Other (specify)_____

2. Vehicle:

LLT 12-10-01

✓ 0.9% Sodium Chloride for Injection , USP
✓ Dimethyl sulfoxide (DMSO)*
___ 95% ethanol (EtOH)**
___ Other (specify)_____

*Dimethyl sulfoxide can be extracted at 37°C ± 2°C for 24 hours, but room temperature for 72 hours is recommended.
**95% ethanol can only be extracted at room temperature (various times can be used).

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3. Conditions (use highest temperature that will not degrade material):

___ 121°C, 1 hour
___ 70°C, 24 hours
___ 50°C, 72 hours
✓ 37°C, 24 hours – saline only
✓ room temperature, 72 hours – DMSO only
___ Other (specify)_____

LLT 11-21-01

Preparation of Soluble Material:

1. ___ Solid:

One gram of the sample will be transferred to a 10 ml volumetric flask. Various sized flasks may be used to accommodate nature of test material utilizing 100 mg/ml or 10% w/v. Appropriate vehicle (specified below) will be added (q.s.) to the 10 ml (or appropriate) demarcation to achieve 100 mg/ml or a 10% (w/v) solution of the material.

2. ___ Liquid:

One milliliter of the sample will be transferred to a 10 ml volumetric flask. Various sized flasks may be used to accommodate nature of test material utilizing 100 mg/ml or 10% v/v. Appropriate vehicle (specified below) will be added (q.s.) to the 10 ml (or appropriate) demarcation to achieve 100 mg/ml or a 10% (v/v) solution of the material.

NOTE: GLP regulations 21 CFR 58.113 requires concentration analysis and stability determination for mixtures with carriers.

Vehicles:

___ 0.9% Sodium Chloride for Injection, USP
___ Dimethyl sulfoxide (DMSO)
___ 95% ethanol (EtOH)
___ Other (specify) _____

All preparations of soluble materials will be performed the day of test. In the event the material does not completely dissolve at these concentrations, serial dilutions will be prepared. The highest possible concentration that achieves complete dissolution of the material will be used for testing purposes.

Test System:

Each *S. typhimurium* tester strain contains a specific mutation in the histidine operon and other mutations that increase their ability to detect mutagens. The *E. coli* strain contains a mutation in the tryptophan operon and a deletion in the *uvrA* gene. These genetically altered *S. typhimurium* strains (TA98, TA100, TA1535, and TA1537) and *E. coli* strain (WP2*uvrA*) cannot grow in the absence of histidine or tryptophan, respectively. When placed in a histidine-free (for *S. typhimurium*) or tryptophan-free (for *E. coli*) medium, only those cells which mutate spontaneously back to their wild type state (histidine independent by manufacturing their own histidine, or tryptophan independent by manufacturing their own tryptophan) are able to form colonies. The spontaneous mutation rate (or reversion rate) for any one strain is relatively constant, but if a mutagen is added to the test system, the mutation rate is significantly increased.

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Tester Strain	Mutations/Genotypic Relevance
S. typhimurium TA98	hisD3052, *rfa*, *uvrB*, frameshift, pKM101
S. typhimurium TA100	hisG46, *rfa*, *uvrB*, missense, pKM101
S. typhimurium TA1535	hisG46, *rfa*, *uvrB*, missense
S. typhimurium TA1537	hisC3076, *rfa*, *uvrB*, frameshift
E. coli WP2*uvrA*	trpE65, *uvrA*, missense

rfa	=	causes partial loss of the lipopolysaccharide wall which increases permeability of the cell to large molecules (i.e., crystal violet inhibition)
uvrB or *uvrA*	=	deficient DNA excision - repair system (i.e., ultraviolet sensitivity)
frameshift	=	base-pair addition/deletion
missense	=	base-pair substitution
pKM101	=	plasmid confers ampicillin resistance (R-factor) and enhances sensitivity to mutagens

Metabolic Activation:

Aroclor 1254 - induced rat liver (S9 homogenate) will be used as metabolic activation. The material is prepared from male, Sprague Dawley rats. The rats are induced with one intraperitoneal injection of Aroclor 1254 (500 mg/ml) 5 days prior to sacrifice. The S9 homogenate is purchased from Organon Teknika Corporation, Box 15969, Durham, NC 27704-0969. Just prior to use, the S9 homogenate will be mixed with a buffer containing 0.4 M $MgCl_2$/1.65 M KCl, 1.0 M Glucose-6-phosphate, 0.1 M NADP, 0.2 M sodium phosphate buffer and sterile water.

Preparation of Tester Strains:

Cultures of *Salmonella typhimurium*, TA98, TA100, TA1535 and TA1537, and *Escherichia coli*, WP2*uvrA*, will be inoculated to individual Erlenmeyer flasks containing oxoid broth. The inoculated broth cultures will be incubated overnight at 37 ± 2°C in an incubator shaker operating at 115-125 rpm for 10-12 hours.

Preparation of Negative Control:

Negative control (vehicle without test material) will be utilized for each tester strain with and without S9 activation.

Preparation of Positive Controls:

A known mutagen, Dexon (paradimethylaminobenzene diazosulfonic acid sodium salt), will be used as a positive control to demonstrate that tester strains TA98, TA100, and TA1537 are sensitive to mutation to the wild type state. For tester strain TA1535, sodium azide will be used as a positive control. For tester strain TA100, 2-aminofluorene will be used as a positive control. For tester strain WP2*uvrA*, 2-aminoanthracene and methylmethane-sulfonate will be used as positive controls. Although metabolic activation is only required with 2-aminofluorene and 2 aminoanthracene to induce mutagenic results, all positive controls will be tested with and without S9 homogenate.

Strain Characteristics and Strain Standard Plate Counts:

Strain characteristics will be verified and viable counts will be determined.

Spot Plate Inhibition Screen:

The extract(s) or solubilized material(s) and negative control(s) will be evaluated by a spot plate technique modeled after the antimicrobial zone of inhibition test. This screen is used to evaluate extract or solution concentrations for toxicity which are noninhibitory to the *Salmonella* strains and the *E. coli* strain.

Separate tubes containing 2 ml of molten top agar supplemented with histidine-biotin (for *S. typhimurium*) or with tryptophan (for *E. coli*) will be inoculated with 0.1 ml of culture for each of the five tester strains. After mixing, the

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agar will be poured across the surface of separate Minimal E plates labeled with lab number, appropriate tester strain, and dose level (when necessary). Once the agar solidifies, sterile filter discs will be placed in the center of the plates. A 0.1 ml aliquot of the extract or solubilized material will be added to the filter discs on each of the labeled plates. Parallel testing will be conducted with a negative control. To demonstrate a positive zone of inhibition, 10X stock Dexon will be used.

The plates will be incubated at 37 ± 2°C for 2-3 days. Following the incubation period, the zone of growth inhibition will be recorded. If significant inhibition of the background lawn occurs, the extract or solubilized material concentration will be adjusted by preparing one or more dilutions and repeating the inhibition screen to find a nontoxic level.

Standard Plate Incorporation Assay:

Separate tubes containing 2 ml of molten top agar supplemented with histidine-biotin solution (for *S. typhimurium*) or tryptophan (for *E. coli*) will be inoculated with 0.1 ml of culture for each of the five tester strains, and 0.1 ml of the test material. A 0.5 ml aliquot of SWI or S9 homogenate, simulating metabolic activation, will be added when necessary. The mixture will be poured across triplicate Minimal E plates labeled with lab number, appropriate tester strain, and S9 metabolic activation (when applicable). Parallel testing will be conducted on a negative control and five positive controls.

Histidine-free media plates (for *S. typhimurium*) and tryptophan-free media plates (for *E. coli*) will be prepared in triplicate as follows:

1. Extract or solubilized material with and without S9 activation
2. Negative control with and without S9 activation
3. 1X Dexon (known mutagen) with and without S9 activation with strains TA98, TA100, and TA1537
4. 1X 2-aminofluorene (known mutagen) with and without S9 activation with strain TA100
5. 1X Sodium azide (known mutagen) with and without S9 activation with strain TA1535
6. 1X 2-aminoanthracene (known mutagen) with and without S9 activation with strain WP2*uvrA*
7. 1X Methylmethane-sulfonate (known mutagen) with and without S9 activation with strain WP2*uvrA*

The plates will be incubated at 37 ± 2°C for 2-3 days. After the incubation period, the revertant colonies on each plate (test, negative and positive) will be counted and recorded. The mean number of revertants will be calculated. All test methods are described in approved NAMSA Standard Operating Procedures.

Evaluation of Test Results:

The mean number of revertants of the triplicate test plates will be compared to the mean number of revertants of the triplicate negative control plates for each of the five tester strains employed. The means obtained for the positive controls are used as points of reference.

For a test material to be identified as a test failure or "potential mutagen" there must be a 2-fold or greater increase in the number of mean revertants over the means obtained from the negative control, for any/all five tester strains. If no 2-fold increase is present, the test material is considered nonmutagenic.

Any apparent "positive response" will be confirmed by demonstrating a dose-response relationship using three nontoxic dose levels of the test material. There should be a range of concentrations that produce a linear dose-response. In the event linearity cannot be established, the assay will be repeated with an appropriate change in dose levels. A test material will be judged mutagenic if it causes a dose-related increase in the number of revertants over a minimum of two increasing dose concentrations.

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Test Validity:

For any assay to be considered valid, it must meet the following criteria:

1. Strain characteristics: All *S typhimurium* tester strains (TA98, TA100, TA1535, and TA1537) must exhibit sensitivity to crystal violet (*rfa* mutation), and ultraviolet light (*uvrB*), and must exhibit no growth on biotin plates, and growth on histidine-biotin plates. Tester strains TA98 and TA100 must exhibit resistance to ampicillin (R-factor); tester strains TA1535 and TA1537 must exhibit sensitivity to ampicillin. Tester strain WP2*uvrA* must exhibit sensitivity to ultraviolet light, no growth on tryptophan deficient plates, growth on tryptophan supplemented media and sensitivity to ampicillin.

2. Strain Standard Plate Counts: A viable count on the working culture suspensions for each tester strain (TA98, TA100, TA1535, TA1537 and WP2*uvrA*) should not be less than 1 x 10^8 CFU/ml.

3. Spot Plate Inhibition Screen: Each prepared extract or solubilized material will be evaluated for inhibition or toxicity to the cells. A test sample that is noninhibitory to moderately noninhibitory to the tester strains will be tested by the standard plate incorporation method. In the event a test material is inhibitory, dilutions will be required to find a nontoxic level.

4. Standard Plate Incorporation Assay: Each positive control mean must exhibit at least a 3-fold increase over the respective negative control mean of the *Salmonella* tester strain employed, and at least a 2-fold increase over the respective negative control mean of the *E. coli* tester strain. Exceptions include conditions not intended to provoke a mutagenic response (e.g. 2-amino-anthracine and 2-aminofluorine without metabolic activation). The negative control results of each tester strain will exhibit a characteristic number of spontaneous revertants. Spontaneous reversion rates may vary, but should be consistent with the ranges specified (see Table II). Table II is meant as a guideline only. Negative control results for tester strains may fall outside of the range listed. In such an instance, the results should be evaluated with caution.

Species	Tester Strain	Number of Spontaneous Revertants
S. typhimurium	TA98	15-50
	TA100	120-240
	TA1537	3-28
	TA1535	10-35
E. coli	WP2*uvrA*	20-125

Report:

A report of the results of the assay will be prepared by NAMSA and will describe all methods used to generate and analyze data. The report will contain the bacterial tester strains employed and characterization including strain standard plate count, spot plate inhibition screen data, test conditions, individual and mean reversion rates (in tabular form), evaluation of results and conclusions.

Quality Assurance:

Inspections will be conducted at intervals adequate to assure the integrity of the study in conformance with 21 CFR 58.35(b)(3). The final report will also be reviewed for conformance to Section 58.185, Subpart J, of the GLP Regulations. A Certificate of Quality Assurance Inspections will be provided with the final report.

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Records:

Test article preparation, strain characteristics, standard strain plate counts, spot plate inhibition screen data, standard plate incorporation assay data, and dates of relevant activities will be recorded.

All raw data pertaining to this study and a copy of the final report will be retained in designated NAMSA archive files.

Proposed Dates:

The study dates will be scheduled by the study director following receipt of the sponsor-approved protocol and appropriate material for the study. Initiation of the study will be the date on which the study director signs the GLP protocol. Projected dates for starting the study (first treatment) and for the completion of the study (final report release) will be provided to the sponsor (or representative of the sponsor) and added to the protocol.

References:

Ames, B.N., McCann, J., and Yamasaki, E., "Methods for Detecting Carcinogens and Mutagens with the Salmonella/Mammalian-Microsome Mutagenicity Test," Mutation Research 31, (1975): 347-364.

Brusick, D.J., V.F. Simmon, H.S. Rosenkranz, V.A. Ray, and R.S. Stafford, "An Evaluation of the *Escherichia coli* WP2 and WP2*uvrA* Reverse Mutation Assay," Mutation Research 76, (1980): 169-190.

Maron, Dorothy M., Ames, Bruce N., "Revised Methods for the Salmonella Mutagenicity Test," Mutation Research, 113 (1983): 175-215.

ISO 10993-3. Biological Evaluation of Medical Devices, Part 3: Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity.

OECD Guideline for the Testing of Chemicals, Proposal for Replacement of Guidelines 471 Bacterial Reverse Mutation Test, Document Number 471.

Ortiz, A.I., M.T. Pollastrini, M. Barea, and D. Ordóñez, "Bacterial Mutagenic Evaluation of Luxabendazole, a New Broad Spectrum Antihelminic, with the *Salmonella typhimurium* Histidine and the *Escherichia coli* Tryptophan Reversions Tests," *Mutagenesis* 11 (1996): 27-31.

Test validation, Bacterial Mutagenicity Test: NAMSA lab number 98T-00785-00.

Protocol Changes:

Any changes necessary to the protocol after the sponsor approval or study initiation will be documented and approved by the study director as protocol amendments. Copies will be distributed to the sponsor, the raw data file, and the NAMSA Quality Assurance department.

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December 10, 2001

BILL BEASLEY
BIOMIMETIC PHARMACEUTICALS
1616 WESTGATE CIRCLE
BRENTWOOD, TN 37027

PROTOCOL AMENDMENT I

Test Article: rhPDGF-BB (Becaplermin - 1 mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold

Identification: Lot #QA2217 and Lot 081098-146

NAMSA Lab No.: 01T 15267 00

We have received appropriate test articles and approved protocols for the program to be conducted in accordance with the Good Laboratory Practice (GLP) Regulations on the material described above. Below is a projected schedule for the work to be performed.

NAMSA Code	Study	Estimated Start Date:	Estimated Report Release Date:
V0014-130	Cytotoxicity Study using the ISO Elution Method (Extract)	Dec. 13, 2001	Jan. 8, 2002
TI261-300	ISO Sensitization Test in Fifteen Guinea Pigs, Saline Extract		
TI261-301	ISO Sensitization Test in Fifteen Guinea Pigs, Cottonseed Oil Extract	Dec. 14, 2001	Jan. 25, 2002
TI251-800	ISO Acute Intracutaneous Reactivity Study in Three Rabbits Saline Extract, Cottonseed Oil Extract	Dec. 17, 2001	Jan. 8, 2002
TI264-500	ISO Acute Systemic Toxicity Study in Five Mice, Saline Extract, Cottonseed Oil Extract	Dec. 17, 2001	Jan. 8, 2002
V0023-211	Genotoxicity: Bacterial Reverse Mutation (Ames) Test, Salmonella and *E. coli* strains, Saline Extract		
V0023-212	Genotoxicity: Bacterial Reverse Mutation (Ames) Test, Salmonella and *E. coli* strains, DMSO Extract	Dec. 18, 2001	Jan. 15, 2002
TI247-804 TH035-800	ISO Surgical Muscle Implantation, in Three Rabbits (4 Weeks) With Histopathology	Dec. 19, 2001	Jan. 25, 2002

Laura L. Tasse

Laura L. Tasse, BS, ALAT
Study Director

Dec. 10, 2001
Date

LLT/dap

cc: QA (NAMSA)
GLP study file

1000U

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December 10, 2001

SAMUEL LYNCH, M.D.
BIOMIMETIC PHARMACEUTICALS
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PROTOCOL AMENDMENT II

Test Article:	rhPDGF-BB (Becaplermin – 1 mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold
Identification No.:	Lot #QA2217 and Lot 081098-146
Protocol:	V0014-130 Cytotoxicity Study using the ISO Elution Method (Extract) TI261-300 ISO Sensitization Test in Fifteen Guinea pigs (Saline extract) TI261-301 ISO Sensitization Test in Fifteen Guinea pigs (Cottonseed oil extract) TI251-800 ISO Acute Intracutaneous Reactivity Study in Three Rabbits TI264-500 ISO Acute Systemic Toxicity Study in Five Mice V0023-211 Genotoxicity: Bacterial Reverse Mutation (Ames) Test (Saline extract) V0023-212 Genotoxicity: Bacterial Reverse Mutation (Ames) Test (DMSO extract)
NAMSA Lab No.:	01T 15267 00

This amendment was written to clarify the preparation of the test article.

The two components of the test article must be combined just prior to extraction preparation. To do this, a 1:1 ratio of Vitoss (in grams) to rhPDGF-BB (in ml's) will be prepared. For example, a 2 g portion of the ChronOS will be saturated with 2 ml of the rhPDGF. If the frozen rhPDGF-BB is used, it must first be thawed prior to the preparation.

The resulting mixture will then be used for the extraction preparation (i.e. weight of the prepared test article to volume of extraction vehicle).

Laura L. Tasse

Laura L. Tasse, BS, ALAT
Study Director

Dec. 10, 2001
Date

cc: NAMSA QAU
GLP study file

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January 8, 2002

BILL BEASLEY
BIOMIMETIC PHARMACEUTICALS
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BRENTWOOD, TN 37027

REVISED*

PROTOCOL AMENDMENT II

Test Article:	rhPDGF-BB (Becaplermin – 1 mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold
Identification No.:	Lot #QA2217 and Lot 081098-146
Protocol:	V0014-130 Cytotoxicity Study using the ISO Elution Method (Extract) TI261-300 ISO Sensitization Test in Fifteen Guinea pigs (Saline extract) TI261-301 ISO Sensitization Test in Fifteen Guinea pigs (Cottonseed oil extract) TI251-800 ISO Acute Intracutaneous Reactivity Study in Three Rabbits TI264-500 ISO Acute Systemic Toxicity Study in Five Mice V0023-211 Genotoxicity: Bacterial Reverse Mutation (Ames) Test (Saline extract) V0023-212 Genotoxicity: Bacterial Reverse Mutation (Ames) Test (DMSO extract)
NAMSA Lab No.:	01T 15267 00

This amendment was written to clarify the preparation of the test article.

The two components of the test article must be combined just prior to extraction preparation. To do this, a 1:1 ratio of Vitoss (in grams) to rhPDGF-BB (in ml's) will be prepared. For example, a 2 g portion of the Vitoss* will be saturated with 2 ml of the rhPDGF. If the frozen rhPDGF-BB is used, it must first be thawed prior to the preparation.

The resulting mixture will then be used for the extraction preparation (i.e. weight of the prepared test article to volume of extraction vehicle).

* This amendment was revised to correct the sponsor name and address, and to correct the name of the Vitoss in the second paragraph.

Laura L. Tasse
Laura L. Tasse, BS, ALAT
Study Director

Jan. 8, 2002
Date

cc: NAMSA QAU
GLP study file

Appendix 7.7.7: **Test Report V0014-130. Cytotoxicity study using the ISO elution method (1 X MEM Extract)**

STUDY TITLE:

CYTOTOXICITY STUDY USING THE ISO ELUTION METHOD

(1X MEM Extract)

TEST ARTICLE:

rhPDGF-BB (Becaplermin - 1 mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold

IDENTIFICATION NO.:

Lot #QA2217 and Lot 081098-146

TEST FACILITY:

NAMSA
2261 Tracy Road
Northwood, OH 43619-1397

SPONSOR:

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BM001715

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SUMMARY

An *in vitro* biocompatibility study, based on the International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 5: Tests for Cytotoxicity: *in vitro* Methods guidelines, was conducted on the test article, rhPDGF-BB (Becaplermin - 1 mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold, Lot #QA2217 and Lot 081098-146, to determine the potential for cytotoxicity. A single extract of the test article was prepared using single strength Minimum Essential Medium supplemented with 5% serum and 2% antibiotics (1X MEM). This test extract was placed onto three separate confluent monolayers of L-929 mouse fibroblast cells propagated in 5% CO_2. Three separate monolayers were prepared for the reagent control, negative control and for the positive control. All monolayers were incubated at 37°C in the presence of 5% CO_2 for 48 hours. The monolayer in the test, reagent control, negative control and positive control wells was examined microscopically at 48 hours to determine any change in cell morphology.

Under the conditions of this study, the 1X MEM test extract showed evidence of causing mild cell lysis or toxicity. The 1X MEM test extract met the requirements of the test since the grade was a grade 2 (mild reactivity). The reagent control, negative control and the positive control performed as anticipated.

Study and Supervisory Personnel:

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Michelle Weingate-Furiate, BA
Suzanne E. Brimacombe
Anne M. Schuler, BE
Melissa A. Cadaret, MS
Cherise M. McCoy, BS

Study Director: Laura L. Tasse — Jan. 3, 2002

Laura L. Tasse, BS, ALAT
Study Director

Date Completed

/ms

BM001717

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INTRODUCTION

The test article identified below was extracted, and the extract was subjected to an *in vitro* cytotoxicity study for biocompatibility based on the requirements of the International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 5: Tests for Cytotoxicity: *in vitro* Methods. The test was performed to determine whether leachables extracted from the material would cause cytotoxicity. The test article was received on November 20, 2001 and December 10, 2001. The cells were first exposed to the extract on December 14, 2001, and the observations were concluded on December 16, 2001.

The study, initiated by protocol signature on December 10, 2001, was conducted in accordance with the provisions of the FDA Good Laboratory Practice (GLP) Regulations, 21 CFR 58. A Certificate of Quality Assurance Inspections was issued with this report.

MATERIALS

The sample provided by the sponsor was identified and handled as follows:

Test Article:	rhPDGF-BB (Becaplermin - 1 mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold
Identification No.:	Lot #QA2217 and Lot 081098-146
Stability Testing:	In Progress (per sponsor)
Expiration Date:	Stable for duration of intended testing (per sponsor)
Storage Conditions:	rhPDGF-BB - Refrigerated Vitoss Scaffold - Room temperature
Extraction Vehicle:	Single strength Minimum Essential Medium supplemented with 5% serum and 2% antibiotics (1X MEM)
Test Article Preparation:	A 1.0 g portion of Vitoss Scaffold was saturated with 1.0 ml of rhPDGF immediately prior to pouring the vehicle used for extraction. Based on the USP ratio of 4 g:20 ml, a 2 g portion of the prepared test article was covered with 10 ml of 1X MEM. A single preparation was extracted at 37°C for 24 hours.
Negative Control Preparation:	High density polyethylene was used as the negative control. Based on the USP ratio of 60 cm^2:20 ml, a single 30.8 cm^2 portion of the control material was covered with 10 ml of 1X MEM. The preparation was subjected to the extraction conditions previously described for the test article.
Reagent Control Preparation:	A single aliquot of 1X MEM without test material was subjected to the same extraction conditions as described for the test article.
Positive Control Preparation:	The current NAMSA positive control, tin stabilized polyvinylchloride, was used to determine a cytotoxic end-point. Based on the USP ratio of 60 cm^2:20 ml, a single 60.8 cm^2 portion of the control material was covered with 20 ml of 1X MEM and extracted at 37°C for 24 hours. Serial dilutions were prepared (1:2, 1:4, 1:8, 1:16, 1:32) for an end-point titration.

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Condition of Extracts:

Test: cloudy pink
Reagent Control: clear
Negative Control: clear
Positive Control (undiluted): clear

METHODS

Test System Management:

L-929, mouse fibroblast cells, (ATCC CCL 1, NCTC Clone 929, of strain L, or equivalent source) were propagated and maintained in open wells containing single strength Minimum Essential Medium supplemented with 5% serum and 2% antibiotics (1X MEM) in a gaseous environment of 5% carbon dioxide (CO_2). For this study, 10 cm^2 wells were seeded, labeled with passage number and date, and incubated at 37°C in 5% CO_2 to obtain confluent monolayers of cells prior to use. Aseptic procedures were used in the handling of the cell cultures following approved NAMSA Standard Operating Procedures.

Experimental Procedure:

Triplicate culture wells were selected which contained a confluent cell monolayer. The growth medium contained in triplicate cultures was replaced with 2 ml of the test extract. Similarly, triplicate cultures were replaced with 2 ml of the reagent control, negative control and the undiluted and each titer of the positive control. Each well was labeled with the corresponding lab number, replicate number, dilution (as applicable) and the dosing date. The wells were incubated at 37°C in 5% CO_2 for 48 hours.

Following incubation, the cultures were examined microscopically (100X) to evaluate cellular characteristics and percent lysis.

Evaluation Criteria:

The confluency of the monolayer was recorded as (+) if present and (-) if absent. In addition, the color of the test medium was observed and compared to the negative control medium. A color shift toward yellow was associated with an acidic pH range and a color shift toward magenta to purple was associated with an alkaline pH range. Each culture well was evaluated for percent lysis and cellular characteristics using the following criteria:

Grade	Reactivity	Observations	
0	None	Discrete intracytoplasmic granules	No lysis
1	Slight	Not more than 20% of the cells are round, loosely attached, and without intracytoplasmic granules	Not more than 20% lysis
2	Mild	Not more than 50% of the cells are round and devoid of intracytoplasmic granules	Not more than 50% lysis
3	Moderate	Not more than 70% of the cell monolayer contains rounded cells	Not more than 70% lysis
4	Severe	Nearly complete destruction of the cell monolayer	Greater than 70% lysis

For the test to be valid, the reagent control and the negative control must have had a reactivity of none (grade 0) and the positive control must have been a grade 3 or 4. The test sample met the requirements of the test if the biological response was less than or equal to grade 2 (mild). The test would have been repeated if the controls did not perform as anticipated and/or if all three test wells did not yield the same conclusion.

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RESULTS

See Table I for results.

pH Observation: The test medium was similar to the negative control medium at 48 hours.

Results and conclusions apply only to the test article tested. No further evaluation of these results is made by NAMSA. Any extrapolation of these data to other samples is the responsibility of the sponsor. All procedures were conducted in conformance with good laboratory practice and ISO 17025.

CONCLUSION

Under the conditions of this study, the 1X MEM test extract showed evidence of causing mild cell lysis or toxicity. The 1X MEM test extract met the requirements of the test since the grade was a grade 2 (mild reactivity). The reagent control, negative control and the positive control performed as anticipated.

RECORD STORAGE

All raw data pertaining to this study and a copy of the final report are to be retained in designated NAMSA archive files.

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TABLE I

REACTIVITY GRADES FOR ELUTION TESTING

Well	Confluent Monolayer	Percent Rounding	Percent Cells Without Intracytoplasmic Granules	Percent Lysis	Grade	Reactivity
Test (1A)	(-)	30	30	30	2	Mild
Test (1B)	(-)	30	30	30	2	Mild
Test (1C)	(-)	30	30	30	2	Mild
Negative Control (1A)	(+)	0	0	0	0	None
Negative Control (1B)	(+)	0	0	0	0	None
Negative Control (1C)	(+)	0	0	0	0	None
Reagent Control (1A)	(+)	0	0	0	0	None
Reagent Control (1B)	(+)	0	0	0	0	None
Reagent Control (1C)	(+)	0	0	0	0	None
Positive Control (1A) 1:2 Dilution	(-)	60	60	60	3	Moderate
Positive Control (1B) 1:2 Dilution	(-)	60	60	60	3	Moderate
Positive Control (1C) 1:2 Dilution	(-)	60	60	60	3	Moderate

(+) = Present (-) = Absent

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CERTIFICATE OF QUALITY ASSURANCE INSPECTIONS

Phase Inspected	Date	Auditor	Reports to Management and Study Director(s)	Date
Dosing	Dec. 14, 2001	N. L. Alt		
Final Report Review	Jan. 2, 2002	N. L. Alt		

This study will be included in the next periodic status report as completed.

This study has been conducted in accordance with the provisions of the FDA Good Laboratory Practice Regulations (21 CFR, Part 58).

QA Representative: Nathan L. Alt, BS
Auditor, Quality Assurance

Jan. 3, 2002
Date

/ms

BM001722

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GLP COMPLIANCE NOTIFICATION

This form is used for non-clinical studies to be conducted according to FDA Good Laboratory Practice Regulations of 21 CFR Part 58. Completion of this form identifies material that will be listed on a NAMSA Master Schedule Sheet available for FDA inspection. All information requested must be submitted to NAMSA before testing may begin.

Report To: BioMimetic Pharmaceuticals
1616 Westgate Circle
Brentwood, TN 37027

Attn: Bill Beasley

Authorized Asst.: _____
(if different from left) _____

Attn: _____

A. Test Article Identification: rh PDGF-BB (Becaplermin - 1mg/ml 20mM NaAc buffer)

1. Batch/Code/Lot (circle one) Identification: QA2217

2. Storage Conditions: Room Temp.___; Refrig.___; Freezer___; Other: ~~-70°C to 75°C~~ 2°C to 8°C per sponsor LLT 11-21-01

3. Handling and Safety Precautions: See MSDS

4. Stability: The sponsor assures that the above material has been characterized for identity, strength, purity and composition as required by subpart F section 58.105 (see back). Stability testing is the responsibility of the sponsor and is subject to FDA audit; please indicate one of the following:

X Stability testing is in progress; article is stable for duration of intended testing.
___Stability testing is complete and on file with sponsor. Expiration Date:_____

5. Disposition: Discard/Destroy___; Return to Sponsor X; Retain*___ *required for studies longer than 28 days

B. Control Article Submitted By Sponsor: No X; Yes___; Identification:_____

1. Batch/Code/Lot (circle one) Identification:_____

2. Storage Conditions: Room Temp.___; Refrig.___; Freezer___; Other:_____

3. Handling and Safety Precautions:_____

4. Stability: Characterization and stability information as above are also required for control articles.

___Stability testing is in progress; article is stable for duration of intended testing.
___Stability testing is complete and on file with sponsor. Expiration Date:_____
___Marketed product stability characterized by its labeling.

5. Disposition: Discard/Destroy___; Return to Sponsor___; Retain*___ *required for studies longer than 28 days

C. Mixtures of test or control articles with carriers require analysis to demonstrate proper concentration, homogeneity and stability. Sponsor will provide analytical methods___; or will perform analysis on representative aliquots provided by NAMSA___. N/A

D. NAMSA Cost Estimate No. 1649-LLT (or attach list of tests) Sponsor's PO No. 01-1005

Name of Principal Investigator (Sponsor): Samuel Lynch Title: President

Signature: Samuel Lynch Date: 11/13/01 Phone: 615-844-1280

Sample received 11-20-01 LLT 12-10-01

To be completed at NAMSA Test Facility:

Lab No: 01T-15267-00 Study Director Laura J. Casse 12-10-01

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BM001723

V0014-130
R-02

NAMSA™ GLP PROTOCOL V14

CYTOTOXICITY STUDY USING THE ISO ELUTION METHOD

Sponsor:

Bill Beasley
BioMimetic Pharmaceutical Inc.
1616 Westgate Circle
Brentwood, TN 37027

Test Facility:

North American Science Associates, Inc. (NAMSA)
2261 Tracy Road
Northwood, OH 43619-1397

NAMSA Use Only

Lab No. 01T-15267-00
CEP No. 1649-LLT (Revised)

Approvals:

Protocol Submitted By (NAMSA): Laura L. Tassel
Laura L. Tassel, BS, ALAT
Study Director

Date Issued: October 29, 2001

Principal Investigator: (Sponsor)

Date Approved: 11/6/01

Study Director (NAMSA): Laura L. Tassel

Date Initiated: Dec. 10, 2001

/pak

BM001724

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NAMSA™ GLP PROTOCOL V14

CYTOTOXICITY STUDY USING THE ISO ELUTION METHOD

Purpose of the Study:

The objective of this study is to evaluate the biocompatibility of a test article extract using an *in vitro* mammalian cell culture test. This study is based on the requirements of the International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 5: Tests for Cytotoxicity: *in vitro* Methods.

This study will be conducted in accordance with the requirements of the FDA Good Laboratory Practice (GLP) Regulations, 21 CFR 58.

Test Article:

The sponsor will submit the material to be tested. The test material shall be representative of either the final product or a component of the final product that is to be tested. Detailed information about the test sample will be provided by the sponsor on the GLP Compliance Notification form furnished by NAMSA or on a similar attachment to the protocol.

The following is to be completed (with initials and dates in the margin) by the sponsor or study director. Further instructions may be attached to the protocol.

Ratio of Test Material to Extraction Vehicle:

___ Material thickness less than 0.5 mm - ratio of 60 cm^2:10 ml (based on the USP ratio 120 cm^2:20 ml)
___ Material thickness greater than or equal to 0.5 mm - ratio of 30 cm^2:10 ml (based on the USP ratio 60 cm^2:20 ml)
✓ Irregularly shaped objects and/or sponsor option - ratio of 2 g:10 ml (based on the USP ratio 4 g:20 ml)
___ Other (explain) _____

NOTE: Only a single test article will be prepared.

LLT 12-10-01

Extraction Vehicles:

✓ Single strength Minimum Essential Medium supplemented with 5% serum and 2% antibiotics (1X MEM)
___ Single strength Minimum Essential Medium, serum free, but supplemented with 2% antibiotics (MEM-SF)
___ 0.9% Sodium Chloride Irrigation, USP (SC)
___ Purified Water (PW)

If an SC or PW extract is prepared, a 50% concentration [1 part test extract to 1 part double strength Minimum Essential Medium supplemented with 10% serum and 4% antibiotics (2X MEM)] of the test extract and 2X MEM will be made prior to dosing the cell monolayers. The negative control and reagent control will be handled in the same manner.

BM001725

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Extraction Conditions:

The extraction conditions shall attempt to exaggerate the clinical use conditions so as to define the potential toxicological hazard; however, they should not in any instance cause physical changes such as fusion or melting, which results in a decrease in the available surface area. A slight adherence of the pieces can be tolerated.

LLT 12-10-01

✓ 37°C for 24 ± 2 hours
___ 50°C for 72 ± 2 hours
___ 70°C for 24 ± 2 hours
___ 121°C for 1 hour
___ Other (specify) _____

NOTE: The preferable extraction condition is 37°C for 24 hours with MEM to simulate physiological conditions. At temperatures greater than 37°C, MEM cannot be used. At temperatures greater than 50°C, MEM-SF cannot be used.

Control Articles:

Negative Control: high density polyethylene, will be prepared based on a ratio of 60 cm^2:20 ml extraction vehicle. A single preparation of the material will be made, and will be extracted using the same conditions as described for the test article.

Reagent Control: A single aliquot of the extraction vehicle without test material will be prepared using the same conditions as described for the test article.

Positive Control: Current NAMSA positive control material*, tin stabilized polyvinylchloride, will be prepared based on a ratio of 60 cm^2:20 ml extraction vehicle. A single preparation of the material will be made and extracted at 37°C for 24 hours. Serial dilutions will be prepared for an end-point titration procedure.

*NOTE: The current NAMSA positive control material has been qualified as an acceptable replacement for the USP recommended control material.

Test System and Justification:

Mammalian cell culture monolayer, L-929, mouse fibroblast cells, (ATCC CCL 1, NCTC Clone 929, of strain L, or equivalent source), will be used. *In vitro* mammalian cell culture studies have been used historically to evaluate cytotoxicity of biomaterials and medical devices (Wilsnack, *et al.*, 1973).

Test System Management:

L-929, mouse fibroblast cells, (ATCC CCL 1, NCTC Clone 929, of strain L, or equivalent source) will be propagated and maintained in open wells containing single strength Minimum Essential Medium supplemented with 5% serum and 2% antibiotics (1X MEM) in a gaseous environment of 5% carbon dioxide (CO_2). For this study, 10 cm^2 wells will be seeded, labeled with passage number and date, and incubated at 37°C in 5% CO_2 to obtain confluent monolayers of cells prior to use. Aseptic procedures will be used in the handling of the cell cultures following approved NAMSA Standard Operating Procedures.

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Methods and Route of Administration:

Each culture well will be selected which contains a confluent cell monolayer. The growth medium in triplicate cultures will be replaced with 2 ml of the test extract. Similarly, triplicate cultures will be replaced with 2 ml of the reagent control, negative control extract and the undiluted and each titer of the positive control. Each well will be labeled with the corresponding lab number, replicate number, dilution (as applicable) and the dosing date and incubated at 37°C in 5% CO_2 for 48 hours.

Following incubation, the cultures will be examined microscopically (100X) to evaluate cellular characteristics and percent lysis.

Evaluation Criteria and Statistics:

The confluency of the monolayer will be recorded as (+) if present and (-) if absent. In addition, the color of the test medium will be observed and compared to the negative control medium. Each culture well will be evaluated for percent lysis and cellular characteristics using the following criteria:

Grade	Reactivity	Observations	
0	None	Discrete intracytoplasmic granules	No lysis
1	Slight	Not more than 20% of the cells are round, loosely attached, and without intracytoplasmic granules	Not more than 20% lysis
2	Mild	Not more than 50% of the cells are round and devoid of intracytoplasmic granules	Not more than 50% lysis
3	Moderate	Not more than 70% of the cell monolayer contains rounded cells	Not more than 70% lysis
4	Severe	Nearly complete destruction of the cell monolayer	Greater than 70% lysis

For the test to be valid, the reagent control and the negative control must have a reactivity of none (grade 0) and the positive control must be a grade 3 or 4. The test sample meets the requirements of the test if the biological response is less than or equal to grade 2 (mild). The test will be repeated if the controls do not perform as anticipated and/or if all three test wells do not yield the same conclusion.

Report:

The final report will contain information on the cell line, culture medium methods, test and control results, and any additional pertinent observations.

Quality Assurance:

Inspections will be conducted at intervals adequate to assure the integrity of the study in conformance with 21 CFR 58.35(b)(3). The final report will also be reviewed for conformance to Section 58.185, Subpart J, of the GLP Regulations. A Certificate of Quality Assurance Inspections will be provided with the final report.

Records:

Material preparation, cell line and passage number, control and test well observations, and dates of relevant activities (such as the study initiation and termination) will be recorded.

All raw data pertaining to this study and a copy of the final report will be retained in designated NAMSA archive files.

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Proposed Dates:

The study dates will be finalized by the study director following receipt of the sponsor-approved protocol and appropriate material for the study. Initiation of the study will be the date on which the study director signs the GLP protocol. Projected dates for starting the study (first treatment) and for the completion of the study (final report release) will be provided to the sponsor (or representative of the sponsor) and added to the protocol.

References:

21 CFR 58 (GLP Regulations).

International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 5: Tests for Cytotoxicity: *in vitro* Methods.

United States Pharmacopeia (USP), current edition.

Wilsnack, R. E., "Quantitative Cell Culture Biocompatibility Testing of Medical Devices and Correlation to Animal Tests," *Biomaterials, Medical Devices and Artificial Organs* 4 (1976): 235-261.

Wilsnack, R. E., F. J. Meyer and J. G. Smith, "Human Cell Culture Toxicity Testing of Medical Devices and Correlation to Animal Tests," *Biomaterials, Medical Devices and Artificial Organs* 1 (1973): 543-562.

Protocol Changes:

Any necessary changes to the protocol after sponsor approval or study initiation will be documented and approved by the study director as protocol amendments. Copies will be distributed to the sponsor, the raw data file, and the NAMSA Quality Assurance department.

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December 10, 2001

BILL BEASLEY
BIOMIMETIC PHARMACEUTICALS
1616 WESTGATE CIRCLE
BRENTWOOD, TN 37027

PROTOCOL AMENDMENT I

Test Article: rhPDGF-BB (Becaplermin - 1 mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold

Identification: Lot #QA2217 and Lot 081098-146

NAMSA Lab No.: 01T 15267 00

We have received appropriate test articles and approved protocols for the program to be conducted in accordance with the Good Laboratory Practice (GLP) Regulations on the material described above. Below is a projected schedule for the work to be performed.

NAMSA Code	Study	Estimated Start Date:	Estimated Report Release Date:
V0014-130	Cytotoxicity Study using the ISO Elution Method (Extract)	Dec. 13, 2001	Jan. 8, 2002
TI261-300	ISO Sensitization Test in Fifteen Guinea Pigs, Saline Extract		
TI261-301	ISO Sensitization Test in Fifteen Guinea Pigs, Cottonseed Oil Extract	Dec. 14, 2001	Jan. 25, 2002
TI251-800	ISO Acute Intracutaneous Reactivity Study in Three Rabbits Saline Extract, Cottonseed Oil Extract	Dec. 17, 2001	Jan. 8, 2002
TI264-500	ISO Acute Systemic Toxicity Study in Five Mice, Saline Extract, Cottonseed Oil Extract	Dec. 17, 2001	Jan. 8, 2002
V0023-211	Genotoxicity: Bacterial Reverse Mutation (Ames) Test, Salmonella and *E. coli* strains, Saline Extract		
V0023-212	Genotoxicity: Bacterial Reverse Mutation (Ames) Test, Salmonella and *E. coli* strains, DMSO Extract	Dec. 18, 2001	Jan. 15, 2002
TI247-804 TH035-800	ISO Surgical Muscle Implantation, in Three Rabbits (4 Weeks) With Histopathology	Dec. 19, 2001	Jan. 25, 2002

Laura L. Tasse

Laura L. Tasse, BS, ALAT
Study Director

Dec. 10, 2001
Date

LLT/dap

cc: QA (NAMSA)
GLP study file

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December 10, 2001

SAMUEL LYNCH, M.D.
BIOMIMETIC PHARMACEUTICALS
1616 WESTGATE CIRCLE
BRENTWOOK, TN 37027

PROTOCOL AMENDMENT II

Test Article:	rhPDGF-BB (Becaplermin – 1 mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold
Identification No.:	Lot #QA2217 and Lot 081098-146
Protocol:	V0014-130 Cytotoxicity Study using the ISO Elution Method (Extract) TI261-300 ISO Sensitization Test in Fifteen Guinea pigs (Saline extract) TI261-301 ISO Sensitization Test in Fifteen Guinea pigs (Cottonseed oil extract) TI251-800 ISO Acute Intracutaneous Reactivity Study in Three Rabbits TI264-500 ISO Acute Systemic Toxicity Study in Five Mice V0023-211 Genotoxicity: Bacterial Reverse Mutation (Ames V0023-212 Genotoxicity: Bacterial Reverse Mutation (Ames
NAMSA Lab No.:	01T 15267 00

ChronOS vs. Vitoss

This amendment was written to clarify the preparation of the test article.

The two components of the test article must be combined just prior to extraction prep... of Vitoss (in grams) to rhPDGF-BB (in ml's) will be prepared. For example, a 2 g portion of the ChronOS will be saturated with 2 ml of the rhPDGF. If the frozen rhPDGF-BB is used, it must first be thawed prior to the preparation.

The resulting mixture will then be used for the extraction preparation (i.e. weight of the prepared test article to volume of extraction vehicle).

Laura L. Tasse

Laura L. Tasse, BS, ALAT
Study Director

Dec. 10, 2001
Date

cc: NAMSA QAU
GLP study file

1000U

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December 11, 2001

BILL BEASLEY
BIOMIMETIC PHARMACEUTICALS
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PROTOCOL AMENDMENT II

Test Article:	rhPDGF-BB (Becaplermin – 1 mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold
Identification No.:	Lot #QA2217 and Lot 081098-146
Protocol:	TI247-804, TH035-800 ISO Surgical Muscle Implantation in Three Rabbits (4 Weeks) with Histopathology
NAMSA Lab No.:	01T 15267 00

This amendment was written to clarify the following:

The two components of the test article must be aseptically combined just prior to implant. To do this, a 1:1 ratio of Vitoss (in grams) to rhPDGF-BB (in ml's) will be mixed. The frozen rhPDGF-BB sample will be used and must first be thawed prior to the preparation. The resulting mixture will then be used as the test sample. For implanting, the amount of the prepared sample that covers the end of a metal spatula will be placed in each test sample site.

As a comparative control condition, the Vitoss sample will be hydrated with 0.9% sodium chloride solution in a 1:1 ratio and will then be implanted. The preparation will be conducted aseptically. For implanting, the amount of the prepared sample that covers the end of a metal spatula will be placed in each comparative control site.

Additionally, USP polyethylene strips cut into 1 mm x 10 mm sections will also be implanted as a control condition. The USP strips will be steam sterilized prior to implant.

The following implant scheme will be followed:

Cranial

Left	Right
Comparative control	Test sample
Comparative control	Test sample
Comparative control	Test sample
Comparative control	Test sample
USP	USP
USP	USP

Caudal

Laura L. Tasse
Laura L. Tasse, BS, ALAT
Study Director

Dec. 11, 2001
Date

cc: NAMSA QAU
GLP study file

BM001731

1000U

Appendix 7.7.8: **Test Report TI247-804, TH035-800, TI247-001. ISO muscle implantation study in the rabbit with histopathology (Surgical method, 4 weeks)**

STUDY TITLE:

ISO MUSCLE IMPLANTATION STUDY IN THE RABBIT

WITH HISTOPATHOLOGY

(Surgical Method, 4 Weeks)

TEST ARTICLE:

rhPDGF-BB (Becaplermin - 1 mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold

IDENTIFICATION NO.:

Lot #QA2217 and Lot 081098-146

TEST FACILITY:

NAMSA
2261 Tracy Road
Northwood, OH 43619-1397

SPONSOR:

BILL BEASLEY
BIOMIMETIC PHARMACEUTICALS
1616 WESTGATE CIRCLE
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SUMMARY

The test article, rhPDGF-BB (Becaplermin - 1 mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold, Lot #QA2217 and Lot 081098-146, was prepared and then was surgically implanted in muscle tissue of the rabbit. The muscle tissue was evaluated for evidence of irritation or toxicity based on the requirements of the International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 6: Tests for Local Effects after Implantation.

Sterile implant samples and comparative controls were prepared aseptically. USP negative controls were sterilized by steam. Rabbits were implanted and were then euthanatized 4 weeks later. Muscle tissues were excised and the implant sites were examined macroscopically. A microscopic evaluation of representative tissue sites from each rabbit was conducted to further define any tissue response.

Under the conditions of this study, the macroscopic reaction was not significant as compared to the comparative control material and not significant as compared to the USP negative control implant material. Microscopically, the test article was classified as a nonirritant as compared to the comparative control material and a slight irritant as compared to the USP negative control article. Additionally, the comparative control was considered a slight irritant as compared to the USP negative control article.

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Oct 1, 2002
Date Completed

/lag

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INTRODUCTION

The test article identified below was evaluated for biocompatibility based on the requirements of the International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 6: Tests for Local Effects after Implantation. The purpose of the study was to evaluate the potential for a local irritant or toxic response to material implanted in direct contact with muscle tissue. The test article was received on October 3, 2001 and December 10, 2001. The animals were implanted on December 12, 2001, and tissues were explanted on January 9, 2002.

The study, initiated by protocol signature on December 10, 2001, was conducted in accordance with the provisions of the FDA Good Laboratory Practice (GLP) Regulations, 21 CFR 58. A Certificate of Quality Assurance Inspections was issued with this report.

MATERIALS

The sample provided by the sponsor was identified and handled as follows:

Test Article:	rhPDGF-BB (Becaplermin - 1 mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold
Identification No:	Lot #QA2217 and Lot 081098-146
Stability Testing:	In Progress (per sponsor)
Expiration Date:	Stable for duration of intended testing (per sponsor)
Storage Conditions:	Vitoss Scaffold - Room temperature rhPDGF-BB - Frozen
Negative Control Article:	USP polyethylene negative control reference strips were purchased by NAMSA from the offices of the US Pharmacopeial Convention and were cut into approximate 1 mm x 10 mm sections.
Preparation:	The test article was aseptically prepared by adding a 2 gram portion of Vitoss Scaffold to a 2 ml portion of thawed rhPDGF-BB. The comparative control article was aseptically prepared by adding a 2 gram portion of Vitoss Scaffold to a 2 ml portion of 0.9% sodium chloride USP solution. USP negative control sections were sterilized by steam. For the test article and comparative control article, an approximate 0.1 ml portion (amount that covered the end of a spatula) was placed in each appropriate site.
Sample Disposition:	Any remaining sample was returned to the sponsor.

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METHODS

Test System:

Species:	Rabbit (*Oryctolagus cuniculus*)
Breed:	New Zealand White
Source:	Myrtle's Rabbitry, Inc.
Sex:	Male
Body Weight Range:	3.5 kg to 4.3 kg at implantation
Age:	Young adult
Acclimation Period:	Minimum 5 days
Number of Animals:	Three
Identification Method:	Ear tag

Justification of Test System:

The rabbit is suggested as an appropriate animal model for evaluating polymer materials by the current ANSI/AAMI/ISO testing standards. The muscle tissue has been used historically because the response to implanted material is easily graded and compared to a known negative control material.

Duplication of Experimental Work:

By signature on the protocol, the sponsor confirmed that the conduct of this study did not unnecessarily duplicate previous experiments.

Animal Management:

Husbandry:	Conditions conformed to Standard Operating Procedures which are based on the "Guide for the Care and Use of Laboratory Animals."
Food:	PROLAB® High Fiber Rabbit Diet was provided daily.
Water:	Freely available, municipal (Toledo, OH) water was delivered through an automatic watering system.
Contaminants:	Reasonably expected contaminants in feed or water supplies did not have the potential to influence the outcome of this test.
Housing:	Animals were individually housed in stainless steel suspended cages identified by a card indicating the lab number, animal number, test code, sex, and date implanted.
Environmental:	The room temperature was monitored daily. The temperature range for the room was within a range of 61-72°F. The room humidity was monitored daily. The humidity range for the room was 30-70%. The light cycle was controlled using an automatic timer (12 hours light, 12 hours dark).
Facility:	NAMSA is an AAALAC International accredited facility and is registered with the United States Department of Agriculture. Additionally, NAMSA maintains an approved Animal Welfare Assurance on file with the National Institutes of Health, Office for Laboratory Animal Welfare.

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Personnel: Associates involved were appropriately qualified and trained.

Selection: Healthy animals were selected. To reduce the number of animals used for testing, and to comply with the directives of the NAMSA IACUC, rabbits on this study may have been used previously in an unrelated test model. Any previously evaluated test or control articles did not cause a response in the animals. Complete history of animal usage is traceable in laboratory records. Animals used for previous evaluations are identified in the report.

Experimental Procedure:

Rabbits were clipped free of fur over the paravertebral muscles. An intramuscular injection of a combination ketamine hydrochloride and xylazine (34 mg/kg + 5 mg/kg) general anesthetic was administered to each animal at a dose of 0.6 ml/kg body weight. Each rabbit was then injected subcutaneously with 0.02 mg/kg buprenorphine. After the general anesthetic had taken effect, the surgical site was scrubbed with a germicidal soap, wiped with 70% isopropyl alcohol, painted with povidone iodine and draped. The animals were placed on inhalant anesthetic for continued general anesthesia during the procedure.

An incision large enough to accommodate the samples was made through the skin over the dorsolumbar region of the vertebral column. The fascia was cut to expose the paravertebral muscle. A hemostat was gently inserted between the muscle fibers and opened to form a pocket into which the implant material was introduced. Four portions (approximately 0.1 ml per site) of the prepared test article and two USP negative control sections were implanted in the right paravertebral muscle of each rabbit. The sections were placed parallel to the spinal column and approximately 2.5 cm apart. In the opposite muscle, four portions (approximately 0.1 ml per site) of the prepared comparative control and two USP negative control sections were similarly implanted. The fascial incision was closed with 4-0 gut suture and the skin with stainless steel wound clips. A pressure bandage was applied.

The rabbits were returned to their respective cages and monitored for recovery from the anesthetic. Rabbits were observed daily for general health. Body weights were recorded prior to implantation and at termination.

On the day following surgery, another buprenorphine injection was administered subcutaneously at a dose of 0.02 mg/kg. Animals remained bandaged for 14 days; bandages were replaced as needed during this time. Wound clips were removed at 14 days after surgery.

At 4 weeks, the rabbits were euthanatized by an intravenous injection of a sodium pentobarbital based drug. The paravertebral muscles were dissected free and fixed in 10% neutral buffered formalin (NBF) to facilitate cutting. After fixation, the muscles were methodically cut cranial to caudal to locate the test, sponsor provided and negative control sites in each rabbit. All test, comparative control and negative control sites were accounted for. Capsule formation or other signs of irritation were scored using low magnification and the scores were recorded as follows:

0 No capsule, no adverse reaction (other than minimal hemorrhage)
1 Up to 0.5 mm capsule or reaction area
2 0.6 to 1.0 mm capsule or reaction area
3 1.1 to 2.0 mm capsule or reaction area
4 >2.0 mm capsule or reaction area

Evaluations and Statistics:

The tissue implant sites (test, comparative control and negative control) from each rabbit were excised, allowing a sufficient area around the site for proper histological preparation. Test, comparative control and negative control articles were removed from the sites. These sections were histologically processed (embedded, sectioned and stained in hematoxylin and eosin) for microscopic evaluation.

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Average macroscopic scores for test implants were compared to average scores of the control sites. Calculations were rounded off to the nearest 0.1. A Reaction Index difference of 0.0 to 0.5 in scores (test minus control) was regarded as "not significant," 0.6 to 1.0 as a "trace," 1.1 to 2.0 as "slight," 2.1 to 3.0 as "moderate" and ≥3.1 as "marked."

A microscopic evaluation of tissue sites from each rabbit was conducted to further define any tissue response. The histopathology evaluation was conducted by a qualified pathologist. The microscopic irritant response was graded as nonirritant, slight, moderate, or severe.

RESULTS

Clinical Observations: All animals appeared clinically normal throughout the duration of the study. Body weight data for individual rabbits were considered acceptable.

Macroscopic Observations: There was no visible reaction at any test or control site. This resulted in a macroscopic reaction of "not significant" tissue contact irritation.

The findings for the macroscopic evaluation are shown in Table I.

Microscopic Observations: The test article was a nonirritant as microscopically compared to the comparative control, and a slight irritant as compared to the USP negative control implant material. Additionally, the comparative control was a slight irritant as compared to the negative control. Individual results of the pathology findings appear in the microscopic evaluation report.

Results and conclusions apply only to the test article tested. No further evaluation of these results is made by NAMSA. Any extrapolation of these data to other samples is the responsibility of the sponsor. All procedures were conducted in conformance with good laboratory practice and ISO 17025.

CONCLUSION

Under the conditions of this study, the macroscopic reaction was not significant as compared to the comparative control material and not significant as compared to the USP negative control implant material. Microscopically, the test article was classified as a nonirritant as compared to the comparative control material and a slight irritant as compared to the USP negative control article. Additionally, the comparative control was considered a slight irritant as compared to the USP negative control article.

RECORD STORAGE

All raw data, paraffin blocks and tissue slides pertaining to this study and a copy of the final report are to be retained in designated NAMSA archive files.

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TABLE I

BODY WEIGHTS AND MACROSCOPIC OBSERVATIONS

Rabbit Number/ Gender	Weight (kg) Day 0	Weight (kg) Day 28	Test	USP Negative Control	Comparative Control
27041* Male	4.3	4.5	0 0 0 0	0 0 0 0	0 0 0 0
27148 Male	3.6	3.7	0 0 0 †	0 0 0 0	0 0 0 0
27035* Male	3.5	3.6	0 0 0 0	0 0 0 †	0 0 0 0
		Average:	0.0	0.0	0.0

*Previous use history traceable in laboratory records.
†Site not located.

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APPENDIX 1

ISO MUSCLE IMPLANTATION STUDY IN THE RABBIT (SURGICAL METHOD)
MICROSCOPIC EVALUATION OF IMPLANT SITES

Test Article: rhPDGF-BB (Becaplermin – 1mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold

Comparative Control: 0.9% sodium chloride solution and Vitoss Scaffold

Interval Implanted: Four (4) Weeks

	TEST			COMPARATIVE CONTROL		
Rabbit Number:	27041	27148	27035	27041	27148	27035
Inflammation						
Polymorphonuclear	0	0	0	0	1	0
Lymphocytes	2	1	1	2	2	2
Plasma Cells	1	1	1	1	1	1
Macrophages	1	1	1	1	1	1
Giant Cells	2	1	1	2	2	2
Necrosis	0	0	0	0	0	0
SUB TOTAL (X2)	12	8	8	12	14	12
Fibroplasia	2	2	2	2	2	2
Fibrosis	1	1	1	1	1	1
Fatty Infiltrate	0	0	0	0	0	0
SUB TOTAL	3	3	3	3	3	3
TOTAL	15	11	11	15	17	15
GROUP TOTAL	37			47		
AVERAGE*	TEST 12.3 (-) CONTROL 15.7 = 0.0					
*Used to determine Irritant Ranking Score shown below as the Conclusion. A negative difference was recorded as zero.						
Traumatic Necrosis	0	0	0	0	0	0
Foreign Debris	4	3	4	4	4	4
No. Sites Examined	4	3	4	4	4	4

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APPENDIX 1 (continued)

ISO MUSCLE IMPLANTATION STUDY IN THE RABBIT (SURGICAL METHOD)
MICROSCOPIC EVALUATION OF IMPLANT SITES

Test Article: rhPDGF-BB (Becaplermin – 1mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold

Interval Implanted: Four (4) Weeks

	TEST			USP NEGATIVE CONTROL PLASTIC		
Rabbit Number:	27041	27148	27035	27041	27148	27035
Inflammation						
Polymorphonuclear	0	0	0	1	1	1
Lymphocytes	2	1	1	1	1	1
Plasma Cells	1	1	1	0	0	0
Macrophages	1	1	1	1	1	1
Giant Cells	2	1	1	0	0	0
Necrosis	0	0	0	0	0	0
SUB TOTAL (X2)	12	8	8	6	6	6
Fibroplasia	2	2	2	0	0	0
Fibrosis	1	1	1	1	1	1
Fatty Infiltrate	0	0	0	0	0	0
SUB TOTAL	3	3	3	1	1	1
TOTAL	15	11	11	7	7	7
GROUP TOTAL	37			21		
AVERAGE* TEST 12.3 (-) CONTROL 7.0 = 5.3						
*Used to determine Irritant Ranking Score shown below as the Conclusion. A negative difference was recorded as zero.						
Traumatic Necrosis	0	0	0	0	0	0
Foreign Debris	4	3	4	0	0	0
No. Sites Examined	4	3	4	4	4	3

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APPENDIX 1 (continued)

ISO MUSCLE IMPLANTATION STUDY IN THE RABBIT (SURGICAL METHOD)
MICROSCOPIC EVALUATION OF IMPLANT SITES

Comparative Control: 0.9% sodium chloride solution and Vitoss Scaffold

Interval Implanted: Four (4) Weeks

	COMPARATIVE CONTROL			USP NEGATIVE CONTROL PLASTIC		
Rabbit Number:	27041	27148	27035	27041	27148	27035
Inflammation						
Polymorphonuclear	0	1	0	1	1	1
Lymphocytes	2	2	2	1	1	1
Plasma Cells	1	1	1	0	0	0
Macrophages	1	1	1	1	1	1
Giant Cells	2	2	2	0	0	0
Necrosis	0	0	0	0	0	0
SUB TOTAL (X2)	12	14	12	6	6	6
Fibroplasia	2	2	2	0	0	0
Fibrosis	1	1	1	1	1	1
Fatty Infiltrate	0	0	0	0	0	0
SUB TOTAL	3	3	3	1	1	1
TOTAL	15	17	15	7	7	7
GROUP TOTAL	47			21		
AVERAGE*	TEST 15.7 (-) CONTROL 7.0 = 8.7					
*Used to determine Irritant Ranking Score shown below as the Conclusion. A negative difference was recorded as zero.						
Traumatic Necrosis	0	0	0	0	0	0
Foreign Debris	4	4	4	0	0	0
No. Sites Examined	4	4	4	4	4	3

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APPENDIX 1 (continued)

Comments:

The test article consisted of distinct, slightly irregular basophilic granular material. A percentage of the material was translucent. Most of the material was extracellular with some remaining within the cytoplasm of macrophages and giant cells (Figure 1). The comparative control consisted of similar material (Figure 2). The comparative control was associated with a slight increase in the number of inflammatory cells as compared to the test article. The tissue response to the test and comparative control was within the expected range for their physical characteristics (granular). There was no evidence of a toxic leachable component for either the test article or comparative control.

Conclusion:

Under the conditions of this study, the test article was considered a
_X_ Nonirritant (0.0-2.9), __ Slight Irritant (3.0-8.9), __ Moderate Irritant (9.0-15.0), __ Severe Irritant (≥15.1)
to the tissue as compared to the comparative control.

Under the conditions of this study, the test article was considered a
__ Nonirritant (0.0-2.9), _X_ Slight Irritant (3.0-8.9), __ Moderate Irritant (9.0-15.0), __ Severe Irritant (≥15.1)
to the tissue as compared to the USP Negative Control Plastic.

Under the conditions of this study, the comparative control was considered a
__ Nonirritant (0.0-2.9), _X_ Slight Irritant (3.0-8.9), __ Moderate Irritant (9.0-15.0), __ Severe Irritant (≥15.1)
to the tissue as compared to the USP Negative Control Plastic.

Pathologist Tim Muench, DVM, MS, PhD Date 2-1-02
Tim Muench, DVM, MS, PhD

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APPENDIX 1 (continued)

Digital Images

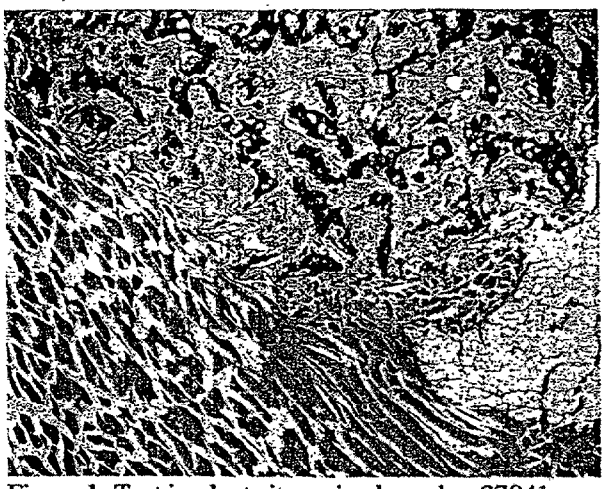

Figure 1. Test implant site, animal number 27041, 40X, HE. The test article induced a mild fibroplastic and fibrotic response. A low number of inflammatory cells and immature blood vessels penetrated into the test article.

Figure 3. Comparative implant site, animal number 27035, 40X, HE. The comparative control article induced a similar tissue response.

Figure 2. Test implant site, animal number 27041, 200X, HE. This image is of a higher magnification of the test implant site in Figure 1.

Figure 4. Comparative implant site, animal number 27035, 200X, HE. This image is of a higher magnification of the comparative control implant site in Figure 3. There were more inflammatory cells present in the comparative control implant sites as compared to the test implant sites.

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APPENDIX 1 (continued)

Digital Images (continued)

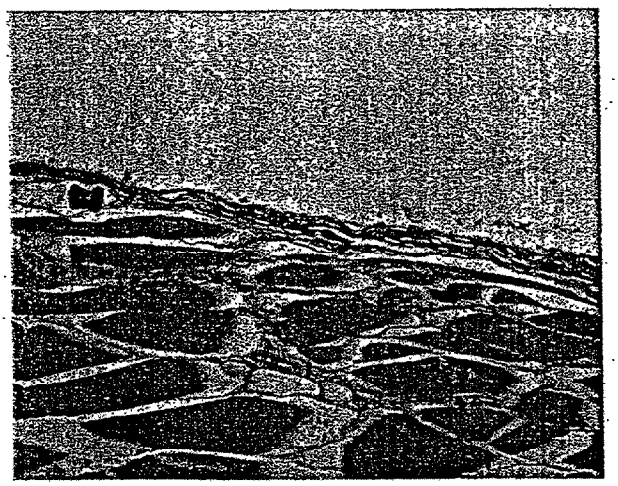

Figure 5. USP plastic implant site, animal number 27048, 200X, HE. The control implant-tissue interface consisted of a thin rim of fibrous tissue infiltrated by variable numbers of inflammatory cells.

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CERTIFICATE OF QUALITY ASSURANCE INSPECTIONS

Phase Inspected	Date	Auditor	Reports to Management and Study Director(s)	Date
Explant	Jan. 9, 2002	L. M. Byrd	Periodic Status Report	Jan. 7, 2002
Final Report Review	Feb. 1, 2002	L. M. Byrd		

This study will be included in the next periodic status report as completed.

This study has been conducted in accordance with the provisions of the FDA Good Laboratory Practice Regulations (21 CFR, Part 58).

QA Representative: Lisa M. Byrd — Feb. 1, 2002

Lisa M. Byrd, CQA, ALAT
Auditor, Quality Assurance

Date

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GLP COMPLIANCE NOTIFICATION

This form is used for non-clinical studies to be conducted according to FDA Good Laboratory Practice Regulations of 21 CFR Part 58. Completion of this form identifies material that will be listed on a NAMSA Master Schedule Sheet available for FDA inspection. All information requested must be submitted to NAMSA before testing may begin.

Report To: BioMimetic Pharmaceuticals
1626 Westgate Circle
Brentwood, TN 37027

Attn: Samuel Lynch, DMD, MSC

Authorized Asst.: (if different from left)

Attn:

A. Test Article Identification: Vitoss Scaffold

1. Batch/Code/Lot (circle one) Identification: 081098-146
2. Storage Conditions: Room Temp. ✓; Refrig. ___; Freezer ___; Other: ___
3. Handling and Safety Precautions: None
4. Stability: The sponsor assures that the above material has been characterized for identity, strength, purity and composition as required by subpart F section 58.105 (see back). Stability testing is the responsibility of the sponsor and is subject to FDA audit; please indicate one of the following:

   ✓ Stability testing is in progress; article is stable for duration of intended testing.
   ___ Stability testing is complete and on file with sponsor. Expiration Date: ___

5. Disposition: Discard/Destroy ___; Return to Sponsor ✓; Retain* ___ *required for studies longer than 28 days

B. Control Article Submitted By Sponsor: No ✓; Yes ___; Identification: ___

1. Batch/Code/Lot (circle one) Identification: ___
2. Storage Conditions: Room Temp. ___; Refrig. ___; Freezer ___; Other: ___
3. Handling and Safety Precautions: ___
4. Stability: Characterization and stability information as above are also required for control articles.

   ___ Stability testing is in progress; article is stable for duration of intended testing.
   ___ Stability testing is complete and on file with sponsor. Expiration Date: ___
   ___ Marketed product stability characterized by its labeling.

5. Disposition: Discard/Destroy ___; Return to Sponsor ___; Retain* ___ *required for studies longer than 28 days

C. Mixtures of test or control articles with carriers require analysis to demonstrate proper concentration, homogeneity and stability. Sponsor will provide analytical methods ___; or will perform analysis on representative aliquots provided by NAMSA ✓. NA LLT 12-10-01

D. NAMSA Cost Estimate No. 1649-LLT (or attach list of tests) Sponsor's PO No. 01-1005

Name of Principal Investigator (Sponsor): Samuel Lynch Title: President

Signature: See additional paperwork for signature LLT 12-10-01 Date: ___ Phone: 615-844-1280

Sample received 12-10-01 LLT 12-10-01

To be completed at NAMSA Test Facility:

Lab No. OIT 15267 00 Study Director Laura J. Tasse 12-10-01

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1001U

GLP COMPLIANCE NOTIFICATION

This form is used for non-clinical studies to be conducted according to FDA Good Laboratory Practice Regulations of 21 CFR Part 58. Completion of this form identifies material that will be listed on a NAMSA Master Schedule Sheet available for FDA inspection. All information requested must be submitted to NAMSA before testing may begin.

Report To: BioMimetic Pharmaceuticals
1616 Westgate Circle
Brentwood, TN 37027

Attn: Bill Beasley

Authorized Asst.: _____
(if different from left) _____

Attn: _____

A. Test Article Identification: rh PDGF-BB (Becaplermin - 1mg/ml 20mM NaAc buffer)

1. Batch/Code/Lot (circle one) Identification: QA2217

2. Storage Conditions: Room Temp.___; Refrig.___; Freezer___; Other: ~~-70°C to -75°C~~ 2°C to 8°C or per sponsor LLT 11-21-01 -75°C (*)

3. Handling and Safety Precautions: See MSDS

4. Stability: The sponsor assures that the above material has been characterized for identity, strength, purity and composition as required by subpart F section 58.105 (see back). Stability testing is the responsibility of the sponsor and is subject to FDA audit; please indicate one of the following:

X Stability testing is in progress; article is stable for duration of intended testing.
___Stability testing is complete and on file with sponsor. Expiration Date: _____

5. Disposition: Discard/Destroy___; Return to Sponsor X; Retain*___ *required for studies longer than 28 days

B. Control Article Submitted By Sponsor: No X; Yes___; Identification: _____

1. Batch/Code/Lot (circle one) Identification: _____

2. Storage Conditions: Room Temp.___; Refrig.___; Freezer___; Other: _____

3. Handling and Safety Precautions: _____

4. Stability: Characterization and stability information as above are also required for control articles.

___Stability testing is in progress; article is stable for duration of intended testing.
___Stability testing is complete and on file with sponsor. Expiration Date: _____
___Marketed product stability characterized by its labeling.

5. Disposition: Discard/Destroy___; Return to Sponsor___; Retain*___ *required for studies longer than 28 days

C. Mixtures of test or control articles with carriers require analysis to demonstrate proper concentration, homogeneity and stability. Sponsor will provide analytical methods___; or will perform analysis on representative aliquots provided by NAMSA___. N/A

D. NAMSA Cost Estimate No. 1649-LLT (or attach list of tests): Sponsor's PO No. 01-1005

Name of Principal Investigator (Sponsor): Samuel Lynch Title: President

Signature: Samuel Lynch Date: 11/13/01 Phone: 615-844-1280

Sample received 11-20-01 LLT 12-10-01

To be completed at NAMSA Test Facility:

Lab No. 01T-15267-00 Study Director Laura J. Cosse 12-10-01

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BM001749

NAMSA™ GLP PROTOCOL T247

ISO MUSCLE IMPLANTATION STUDY IN THE RABBIT

WITH HISTOPATHOLOGY

(Surgical Method, 4 Weeks)

Sponsor:

Bill Beasley
BioMimetic Pharmaceutical Inc.
1616 Westgate Circle
Brentwood, TN 37027

Test Facility:

North American Science Associates, Inc. (NAMSA)
2261 Tracy Road
Northwood, OH 43619-1397

NAMSA Use Only

Lab No. 01T-15267-00
CEP No. 1649-LLT (Revised)

Approvals:

Protocol Submitted By (NAMSA): Laura J. Tasse
Laura L. Tasse, BS, ALAT
Study Director

Date Issued: October 29, 2001

Principal Investigator: (Sponsor)

Date Approved: 11/6/01

Study Director (NAMSA): Laura J. Tasse

Date Initiated: Dec. 10, 2001

/pak

BM001750

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NAMSA™ GLP PROTOCOL TI247

ISO MUSCLE IMPLANTATION STUDY IN THE RABBIT

WITH HISTOPATHOLOGY

(Surgical Method, 4 Weeks)

Purpose of the Study:

The objective of the study is to evaluate the test article in direct contact with muscle tissue following surgical implantation. This study will be based on the requirements of the International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 6: Tests for Local Effects after Implantation.

This study will be conducted in accordance with the requirements of the FDA Good Laboratory Practice (GLP) Regulations, 21 CFR 58.

Test Article:

The sponsor will submit the test article to be evaluated. Detailed information about the test article will be provided by the sponsor on the GLP Compliance Notification form furnished by NAMSA or on a similar attachment to the protocol. The implant samples will be no larger than can be accommodated by the tissue (e.g., less than or equal to 15 mm x 10 mm x 2 mm in area). The test article should be manufactured, processed, cleaned and sterilized by the method intended for the final product. When provided sterile, the test article will be handled aseptically. In the event that the test article is not supplied sterile, the following is to be completed (with initials and dates in the margin) by the sponsor or study director. Further instructions may be attached to the protocol. The sample will be sterilized as follows:

___ The test facility will sterilize the test article by ethylene oxide (EO). Implant samples sterilized by EO will be degassed at least 4 days prior to implantation.
___ The test facility will sterilize the test article by steam (121°C).

The test facility will cut the samples to the appropriate size (if applicable). For each rabbit, a minimum of four sections of the test article will be prepared.

Control Article:

It is recommended that a comparative material be used as a control. The sponsor will submit a material comparable in size, shape, surface condition, manufacturing, processing and sterilization. Detailed information about the control article will be provided by the sponsor on the GLP Compliance Notification form furnished by NAMSA or on a similar attachment to the protocol.

___ sponsor provided control submitted
  ___ The test facility will sterilize the sponsor provided control article by ethylene oxide (EO). Implant samples sterilized by EO will be degassed at least 4 days prior to implantation.
  ___ The test facility will sterilize the control article by steam (121°C)

In the event that such a material is not supplied by the sponsor, the test facility will supply USP polyethylene strips purchased from the offices of the US Pharmacopeial Convention as the negative control. The strips will be cut into 1 mm x 10 mm sections (four per rabbit). Only if appropriate, the USP plastic strips will be flattened and cut to a similar shape as the test implant. The control articles will be sterilized in the same manner as the test article. If the test article is supplied sterile, the USP strips will be sterilized by steam.

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Test System:

Species:	Rabbit (*Oryctolagus cuniculus*)
Breed:	New Zealand White
Source:	Single USDA licensed supplier
Sex:	No particular gender is prescribed for this test, however, all rabbits will be of the same sex
Body Weight Range:	Not less then 2.5 kg at implantation
Age:	Young adults
Acclimation Period:	Minimum 5 days
Number of Animals:	Three
Identification Method:	Ear tag

Justification of Test System:

The rabbit is suggested as an appropriate animal model for evaluating polymer materials by the current ANSI/AAMI/ISO testing standards. The muscle tissue has been used historically because the response to implanted material is easily graded and compared to a known negative control material.

Duplication of Experimental Work:

By signature on this protocol, the sponsor confirms that the conduct of this study does not unnecessarily duplicate previous experiments.

Animal Management:

Husbandry:	Conditions will conform to Standard Operating Procedures which are based on the "Guide for the Care and Use of Laboratory Animals."
Food:	A commercially available, pelleted, rabbit feed will be provided daily.
Water:	Freely available, municipal water will be delivered through an automatic watering system.
Contaminants:	Reasonably expected contaminants in feed or water supplies should not have the potential to influence the outcome of this test.
Housing:	Animals will be individually housed in stainless steel suspended cages identified by a card indicating the lab number, animal number, test code, sex, and date implanted.
Environmental:	The room temperature will be monitored daily. The recommended temperature range for the room is 61-72°F. The room humidity will be monitored daily. The humidity range for the room is 30-70%. The light cycle will be controlled using an automatic timer (12 hours light, 12 hours dark).
Facility:	NAMSA is an AAALAC International accredited facility and is registered with the United States Department of Agriculture. Additionally, NAMSA maintains an approved Animal Welfare Assurance on file with the National Institutes of Health, Office for Laboratory Animal Welfare.
Personnel:	Associates involved will be appropriately qualified and trained.

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Selection: Healthy animals will be selected. To reduce the number of animals used for testing, and to comply with the directives of the NAMSA IACUC, rabbits on this study may have been used previously in an unrelated test model. Any previously evaluated test or control articles did not cause a response in the animals. Complete history of animal usage is traceable in laboratory records. Animals used for previous evaluations will be identified in the report.

Sedation, Analgesia or Anesthesia: It has been determined that the use of these agents will be necessary during the routine course of this procedure.

Veterinary Care: In the unlikely event that an animal should become injured, ill, or moribund, care will be conducted in accordance with current veterinary medical practice. If warranted for humane reasons, euthanasia will be conducted in accordance with the current report of the American Veterinary Medical Association's Panel on Euthanasia. The objective of the study will be given due consideration in any decision and the study sponsor will be advised.

IACUC: This protocol has been approved by NAMSA Institutional Animal Care and Use Committees (IACUC), and is reviewed at least annually by the same committees. Any significant changes to this protocol must be approved by the IACUC prior to conduct.

Methods and Route of Administration:

Rabbits will be clipped free of fur over the paravertebral muscles. An intramuscular injection of a combination ketamine hydrochloride and xylazine (34 mg/kg + 5 mg/kg) general anesthetic will be administered to each animal at a dose of 0.6 ml/kg body weight. Each rabbit will be injected subcutaneously with 0.02 mg/kg buprenorphine. After the general anesthetic has taken effect, the back will be scrubbed with a germicidal soap and wiped with 70% isopropyl alcohol. The surgical site will be painted with an antiseptic such as povidone iodine and draped. The animals will be placed on inhalant anesthetic for continued general anesthesia.

An incision large enough to accommodate the samples will be made through the skin over the dorsolumbar region of the vertebral column. The fascia will be cut to expose the paravertebral muscle. A hemostat will be gently inserted between the muscle fibers and opened to form a pocket in which the implant material will be introduced. Three test article and two USP negative control sections will be implanted in the right paravertebral muscle of each rabbit. The sections will be placed parallel to the spinal column and approximately 2.5 cm apart. In the opposite muscle, three sponsor provided control and two USP negative control sections will be similarly implanted. If a sponsor control is not submitted, the number of test and USP negative control sections implanted will be increased to four each. The fascial incision will be closed with 3-0 or 4-0 gut suture. The skin will be closed with 4-0 nonabsorbable suture or stainless steel wound clips. A pressure bandage will be applied. The rabbits will be returned to their respective cages and monitored for recovery from the anesthetic.

On the day following surgery, another buprenorphine injection will be administered subcutaneously at a dose of 0.02 mg/kg. Animals will remain bandaged for 10-14 days; bandages will be replaced as needed during this time. The skin sutures or wound clips will be removed 10-14 days after surgery. Body weights will be recorded prior to implant and at termination.

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Terminal Studies:

After 4 weeks, the rabbits will be weighed and euthanatized by an intravenous injection of a sodium pentobarbital based drug. The paravertebral muscles will be dissected free and fixed in 10% neutral buffered formalin (NBF) to facilitate cutting. After fixation, the muscles will be methodically cut cranial to caudal to locate the test and control sites. All test and control sites will be accounted for. Capsule formation or other evidence of irritation will be scored using low magnification and recorded as follows:

0 No capsule, no adverse reaction (other than minimal hemorrhage)
1 Up to 0.5 mm capsule or reaction area
2 0.6 to 1.0 mm capsule or reaction area
3 1.1 to 2.0 mm capsule or reaction area
4 >2.0 mm capsule or reaction area

Test and control articles will be removed from the sites. The tissue implant sites for each test and control from each rabbit will be histologically processed (embedded, sectioned and stained in hematoxylin and eosin) for evaluation by a qualified pathologist.

Evaluations and Statistics:

The recovery of a minimum of eight representative test sites and a minimum of eight control sites will be necessary for a valid test. The average macroscopic scores for test implants will be compared to average scores of the control sites. Calculations will be rounded off to the nearest 0.1. A difference of 0.0 to 0.5 in scores (test minus control) is regarded as "not significant," 0.6 to 1.0 as "trace," 1.1 to 2.0 as "slight," 2.1 to 3.0 as "moderate" and ≥3.1 as "marked."

The microscopic evaluation will be conducted by a qualified pathologist. Cellular changes will be graded according to severity (0-4). Any resulting irritant response indicated by a difference between test and control values will be graded as nonirritant, slight, moderate, or severe (see attached).

Report:

The final report will include a description of the methods employed, individual body weights, macroscopic scores, results, abnormal observations if noted, and the microscopic evaluation in the histopathology report.

Quality Assurance:

Inspections will be conducted at intervals adequate to assure the integrity of the study in conformance with 21 CFR 58.35(b)(3). The final report will also be reviewed for conformance to Section 58.185, Subpart J, of the GLP Regulations. A Certificate of Quality Assurance Inspections will be provided with the final report.

Records:

Method of sterilization, individual body weights, dates of relevant activities (such as the study initiation and termination), macroscopic and microscopic observations and results will be recorded.

All raw data, paraffin blocks and tissue slides pertaining to this study and a copy of the final report will be retained in designated NAMSA archive files.

The full address of any consultant retained by NAMSA will not be included in the report, but will be on file at NAMSA. NAMSA conducts routine audits to ensure that consultants are in compliance with applicable regulations.

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Proposed Dates:

The study dates will be finalized by the study director following receipt of the sponsor-approved protocol and appropriate material for the study. Initiation of the study will be the date on which the study director signs the GLP protocol. Projected dates for starting the study (first treatment) and for the completion of the study (final report release) will be provided to the sponsor (or representative of the sponsor) and added to the protocol.

References:

21 CFR 58 (GLP Regulations).

Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research, National Academy of Sciences (Washington: National Academy Press, 1996).

International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 6: Tests for Local Effects after Implantation

OLAW, Public Health Service Policy on Humane Care and Use of Laboratory Animals (NIH Publication).

United States Code of Federal Regulation (CFR) 9: The Animal Welfare Act.

United States Pharmacopeia (USP), current edition

Protocol Changes:

Any necessary changes to the protocol after sponsor approval or study initiation will be documented and approved by the study director as protocol amendments. Copies will be distributed to the sponsor, the raw data file, and the NAMSA Quality Assurance department.

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APPENDIX 1

ISO MUSCLE IMPLANTATION STUDY IN THE RABBIT (SURGICAL METHOD)
MICROSCOPIC EVALUATION OF IMPLANT SITES

Test Article: « 13»

Interval Implanted:

	TEST			USP NEGATIVE CONTROL PLASTIC		
Rabbit Number:						
Inflammation Polymorphonuclear						
Lymphocytes						
Plasma Cells						
Macrophages						
Giant Cells						
Necrosis						
SUB TOTAL (X2)						
Fibroplasia						
Fibrosis						
Fatty Infiltrate						
SUB TOTAL						
TOTAL						
GROUP TOTAL						
AVERAGE* TEST (-) CONTROL =						
*Used to determine Irritant Ranking Score shown below as the Conclusion. A negative difference was recorded as zero.						
Traumatic Necrosis						
Foreign Debris						
No. Sites Examined						

Conclusion:

Under the conditions of this study, the test article was considered a
___ Nonirritant (0.0-2.9), ___ Slight Irritant (3.0-8.9), ___ Moderate Irritant (9.0-15.0), ___ Severe Irritant (≥15.1)
to the tissue as compared to the USP Negative Control Plastic.

Comments:

Pathologist _____ Date _____

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December 10, 2001

BILL BEASLEY
BIOMIMETIC PHARMACEUTICALS
1616 WESTGATE CIRCLE
BRENTWOOD, TN 37027

PROTOCOL AMENDMENT I

Test Article: rhPDGF-BB (Becaplermin - 1 mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold

Identification: Lot #QA2217 and Lot 081098-146

NAMSA Lab No.: 01T 15267 00

We have received appropriate test articles and approved protocols for the program to be conducted in accordance with the Good Laboratory Practice (GLP) Regulations on the material described above. Below is a projected schedule for the work to be performed.

NAMSA Code	Study	Estimated Start Date:	Estimated Report Release Date:
V0014-130	Cytotoxicity Study using the ISO Elution Method (Extract)	Dec. 13, 2001	Jan. 8, 2002
TI261-300	ISO Sensitization Test in Fifteen Guinea Pigs, Saline Extract		
TI261-301	ISO Sensitization Test in Fifteen Guinea Pigs, Cottonseed Oil Extract	Dec. 14, 2001	Jan. 25, 2002
TI251-800	ISO Acute Intracutaneous Reactivity Study in Three Rabbits Saline Extract, Cottonseed Oil Extract	Dec. 17, 2001	Jan. 8, 2002
TI264-500	ISO Acute Systemic Toxicity Study in Five Mice, Saline Extract, Cottonseed Oil Extract	Dec. 17, 2001	Jan. 8, 2002
V0023-211	Genotoxicity: Bacterial Reverse Mutation (Ames) Test, Salmonella and *E. coli* strains, Saline Extract		
V0023-212	Genotoxicity: Bacterial Reverse Mutation (Ames) Test, Salmonella and *E. coli* strains, DMSO Extract	Dec. 18, 2001	Jan. 15, 2002
TI247-804 TH035-800	ISO Surgical Muscle Implantation, in Three Rabbits (4 Weeks) With Histopathology	Dec. 19, 2001	Jan. 25, 2002

Laura L. Tasse

Laura L. Tasse, BS, ALAT
Study Director

Dec. 10, 2001
Date

LLT/dap

cc: QA (NAMSA)
GLP study file

Appendix 7.7.9: **Test Report TI261-300, TI261-301. ISO sensitization study in the guinea pig (Maximization method)**

STUDY TITLE:

ISO SENSITIZATION STUDY IN THE GUINEA PIG

(MAXIMIZATION METHOD)

TEST ARTICLE:

rhPDGF-BB (Becaplermin - 1 mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold

IDENTIFICATION NO.:

Lot #QA2217 and Lot 081098-146

TEST FACILITY:

NAMSA
2261 Tracy Road
Northwood, OH 43619-1397

SPONSOR:

BILL BEASLEY
BIOMIMETIC PHARMACEUTICALS
1616 WESTGATE CIRCLE
BRENTWOOD, TN 37027

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SUMMARY

A guinea pig maximization test of rhPDGF-BB (Becaplermin -1 mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold, Lot #QA2217 and Lot 081098-146, was conducted to evaluate the potential for delayed dermal contact sensitization. This study was conducted based on the requirements of the International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 10: Tests for Irritation and Sensitization.

The test article was extracted in 0.9% sodium chloride USP (SC) and cottonseed oil, NF (CSO). Each extract was intradermally injected and occlusively patched to ten test guinea pigs (per extract) in an attempt to induce sensitization. The vehicle was similarly injected and occlusively patched to five control guinea pigs (per vehicle). Following a recovery period, the test and control animals received a challenge patch of the appropriate test article extract and the reagent control. All sites were scored at approximately 24, 48 and 72 hours after patch removal.

Under the conditions of this study, the SC and CSO test article extracts showed no significant evidence of causing delayed dermal contact sensitization in the guinea pig.

Study and Supervisory Personnel:

Mark J. Menzel
Brian Brooks
Diane L. Miller
Shawn L. St. Clair, BS
Kathrina C. Ratliff
Deedee M. Davis, BA
Sara S. Hartman, ALAT
Cathy L. Walters
Debra S. Dunn
Brian P. Stultz
Tammy L. Lindsey
Suzanne E. Brimacombe
Martha Oswanski, BS, LAT

Study Director: Laura L. Tasse, BS, ALAT
Study Director

Jan. 25, 2002
Date Completed

/lag

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INTRODUCTION

A guinea pig maximization test of the material identified below was conducted to evaluate the potential to cause delayed dermal contact sensitization. This study was conducted based on the requirements of the International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 10: Tests for Irritation and Sensitization. The test article was received on November 20, 2001 and December 10, 2001. Treatment began on December 14, 2001, and the observations were concluded January 10, 2002. The susceptibility of the Hartley guinea pig strain to a known sensitizing agent, 1-chloro-2,4-dinitrobenzene (DNCB), has been substantiated at NAMSA with this method under lab number 01T-06495-00 completed on October 11, 2001.

The study, initiated by protocol signature on December 10, 2001, was conducted in accordance with the provisions of the FDA Good Laboratory Practice (GLP) Regulations, 21 CFR 58. A Certificate of Quality Assurance Inspections was issued with this report.

MATERIALS

The sample provided by the sponsor was identified and handled as follows:

Test Article:	rhPDGF-BB (Becaplermin - 1 mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold
Identification No.:	Lot #QA2217 and Lot 081098-146
Stability Testing:	In Progress (per sponsor)
Expiration Date:	Stable for duration of intended testing (per sponsor)
Storage Conditions:	Vitoss Scaffold-Room temperature rhPDGF-BB - Refrigerated
Vehicles:	0.9% sodium chloride USP solution (SC) Cottonseed oil, NF (CSO)
Preparation:	A 1:1 ratio of Vitoss Scaffold (g) to rhPDGF (ml) was prepared for each extraction. A ratio of 4 g:20 ml, weight of prepared test article (1:1 preparation) to volume of vehicle was prepared for each extraction interval. The test article was extracted in CSO at 37°C for 72 hours. The vehicle (without test article) was similarly prepared to serve as the reagent control.

Condition of Extracts:

	SC Test	SC Control
Induction I:	clear with particulates	clear
Induction II:	clear with particulates	clear
Challenge:	clear with particulates	clear

	CSO Test	CSO Control
Induction I:	cloudy with particulates	clear
Induction II:	clear with particulates	clear
Challenge:	cloudy with particulates	clear

Additional Materials: Freund's Complete Adjuvant (FCA) was mixed 50:50 (v/v) with the chosen vehicle and used at induction I. A 10% (w/w) sodium lauryl sulfate (SLS)

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suspension in petrolatum was used for induction II. These materials were provided by the test facility.

METHODS

Test System:

Species:	Guinea pig (*Cavia porcellus*)
Strain:	Crl:(HA) BR
Source:	Charles River Laboratories
Sex:	Female (nulliparous)
Body Weight Range:	366 grams to 448 grams at identification
Age:	Young adult
Acclimation Period:	Minimum 5 days
Number of Animals:	Thirty
Identification Method:	Ear punch

Justification of Test System:

The Hartley albino guinea pig has been used historically for sensitization studies (Magnusson and Kligman, 1970). The guinea pig is believed to be the most sensitive animal model for this type of study. The susceptibility of the Hartley strain to a known sensitizing agent, 1-chloro-2,4-dinitrobenzene (DNCB) has been substantiated at NAMSA with this method.

Duplication of Experimental Work:

By signature on the protocol, the sponsor confirmed that the conduct of this study did not unnecessarily duplicate previous experiments.

Animal Management:

Husbandry:	Conditions conformed to Standard Operating Procedures which are based on the "Guide for the Care and Use of Laboratory Animals."
Food:	PROLAB® Guinea Pig Diet was provided daily.
Water:	Freely available, municipal (Toledo, OH) water was delivered through an automatic watering system.
Contaminants:	Reasonably expected contaminants in feed or water supplies did not have the potential to influence the outcome of this test.
Housing:	Animals were housed in groups in stainless steel suspended cages identified by a card indicating the lab number, animal numbers, test code, sex, animal code and first treatment date.
Environmental:	The room temperature was monitored daily. The temperature range for the room was within a range of 64-79°F.

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The room humidity was monitored daily. The humidity range for the room was 30-70%.

The light cycle was controlled using an automatic timer (12 hours light, 12 hours dark).

Facility: NAMSA is an AAALAC International accredited facility and is registered with the United States Department of Agriculture. Additionally, NAMSA maintains an approved Animal Welfare Assurance on file with the National Institutes of Health, Office for Laboratory Animal Welfare.

Personnel: Associates involved were appropriately qualified and trained.

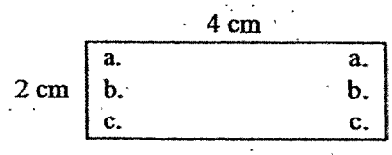
Selection: Only healthy, previously unused animals were selected.

Experimental Procedure:

The day prior to treatment, each animal was weighed, identified and the fur over the dorsoscapular region was removed with an electric clipper. Each animal was observed daily for general health.

Induction I:

The following day, the test animals were injected with the test article extract and the control animals were injected with the reagent control. Three rows of intradermal injections (two per row) were given to each animal within an approximate 2 cm x 4 cm boundary of the fur clipped area as illustrated below:



Control Animals:

a. 0.1 ml of 50:50 (v/v) mixture of FCA and the chosen vehicle
b. 0.1 ml of vehicle
c. 0.1 ml of a 1:1 mixture of the 50:50 (v/v) vehicle/FCA mixture and the vehicle

Test Animals:

a. 0.1 ml of 50:50 (v/v) mixture of FCA and the chosen vehicle
b. 0.1 ml of test extract
c. 0.1 ml of a 1:1 mixture of the 50:50 (v/v) vehicle/FCA mixture and the test extract

To minimize tissue sloughing the "a" and "c" injections were slightly deeper than "b". Site "c" was injected slightly more caudal than site "b".

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Induction II:

Six days after the injections, the same area used during induction I was clipped free of fur and treated with 0.5 to 1 gram of a 10% sodium lauryl sulfate (SLS) suspension in petrolatum. The suspension was massaged into the skin over the injection site to provoke a mild acute inflammation. The area was left uncovered. The day following SLS treatment, any remaining SLS residue was gently removed with a gauze pad. A 2 cm x 4 cm section of Whatman No. 3MM filter paper, saturated with 0.3 ml of freshly prepared test article extract (SC or CSO), was then topically applied to the previously injected sites of the test animals. The control animals were similarly patched with the appropriate reagent control. Each patch was secured with a nonreactive tape and the trunk of each animal was wrapped with an elastic bandage. Animal #5 (SC test) was found unwrapped approximately 24 hours after patch application. The animal was repatched using held-over extract. At 48 hours, the binders and patches were removed.

Challenge:

On the day prior to the challenge patch, the fur was removed from the sides and flank areas. At 14 days after the removal of the induction patch, the nonwoven cotton disk contained in a Hill Top Chamber® was saturated with 0.3 ml of the test article extract (SC or CSO) or reagent control. All patches were topically applied as indicated below:

	CHALLENGE SITE	
TREATMENT GROUP (n)	LEFT FLANK	RIGHT FLANK
Test (10 SC, 10 CSO)	Reagent Control	Test Extract
Control (5 SC, 5 CSO)	Reagent Control	Test Extract

Each patch was secured to the skin with semiocclusive hypoallergenic adhesive tape. The trunk of each animal was wrapped with an elastic bandage to maintain well-occluded sites for the 24 hour exposure. The sites were wiped gently with gauze after patch removal. At 24 hours after patch removal, the challenged sites and surrounding area were shaved.

Observations for dermal reactions were conducted at 2-4 hours following the shave and at 48 and 72 hours after challenge patch removal. Prior to scoring at each interval, sites were wiped with 35% isopropyl alcohol. Scores were recorded in accordance with the criteria shown below:

ERYTHEMA (ER)		EDEMA (ED)	
Reaction	Numerical Grading	Reaction	Numerical Grading
No erythema	0	No edema	0
Slight erythema	1	Slight edema	1
Well-defined erythema	2	Well-defined edema	2
Moderate erythema	3	Moderate edema	3
Severe erythema to slight eschar formation	4	Severe edema	4

The response, pattern, character, and duration of any test animal reactions were compared to any reactions in the control conditions. Any dermal inflammatory response at the test sites greater than that seen in any control condition was considered evidence of a potential allergic response.

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DEVIATION

Daily health observations were in advertently not recorded by the technologist on one day during the study. Records maintained by the Animal Resources staff indicated that all animals appeared normal on this day. This deviation was not believed to have adversely affected the outcome of the study.

RESULTS

Clinical Observations: Individual body weights are presented in Table I. All animals were observed with the expected dermal reactions associated with intradermal injections of FCA. Otherwise, all animals appeared clinically normal throughout the study.

Dermal Observations: Individual results of dermal scoring for the challenge phase appear in Table II. Only insignificant background reactions were noted. No evidence of sensitization was observed.

Results and conclusions apply only to the test article tested. No further evaluation of these results is made by NAMSA. Any extrapolation of these data to other samples is the responsibility of the sponsor. All procedures were conducted in conformance with good laboratory practice and ISO 17025.

CONCLUSION

Under the conditions of this study, the SC and CSO test article extracts showed no significant evidence of causing delayed dermal contact sensitization in the guinea pig.

RECORD STORAGE

All raw data pertaining to this study and a copy of the final report are to be retained in designated NAMSA archive files.

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TABLE I

INDIVIDUAL BODY WEIGHTS AND CLINICAL OBSERVATIONS

SC GROUP

Animal Number/ Group	Individual Observation	
	Pretreatment Body Weight (g)	Clinical Observations
1 Test	407	Animal appeared clinically normal throughout the study.
2 Test	388	Animal appeared clinically normal throughout the study.
3 Test	437	Animal appeared clinically normal throughout the study.
4 Test	448	Animal appeared clinically normal throughout the study.
5 Test	423	Animal appeared clinically normal throughout the study.
6 Test	394	Animal appeared clinically normal throughout the study.
7 Test	405	Animal appeared clinically normal throughout the study.
8 Test	388	Animal appeared clinically normal throughout the study.
9 Test	388	Animal appeared clinically normal throughout the study.
10 Test	402	Animal appeared clinically normal throughout the study.
11 Control	409	Animal appeared clinically normal throughout the study.
12 Control	390	Animal appeared clinically normal throughout the study.
13 Control	407	Animal appeared clinically normal throughout the study.
14 Control	417	Animal appeared clinically normal throughout the study.
15 Control	391	Animal appeared clinically normal throughout the study.

Note: As expected, dermal reactions were observed over the FCA injection sites. This was considered to be a typical finding for this study.

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TABLE I (continued)

INDIVIDUAL BODY WEIGHTS AND CLINICAL OBSERVATIONS

CSO GROUP

Animal Number/ Group	Individual Observation	
	Pretreatment Body Weight (g)	Clinical Observations
16 Test	419	Animal appeared clinically normal throughout the study.
17 Test	390	Animal appeared clinically normal throughout the study.
18 Test	401	Animal appeared clinically normal throughout the study.
19 Test	390	Animal appeared clinically normal throughout the study.
20 Test	409	Animal appeared clinically normal throughout the study.
21 Test	401	Animal appeared clinically normal throughout the study.
22 Test	403	Animal appeared clinically normal throughout the study.
23 Test	433	Animal appeared clinically normal throughout the study.
24 Test	416	Animal appeared clinically normal throughout the study.
25 Test	366	Animal appeared clinically normal throughout the study.
26 Control	383	Animal appeared clinically normal throughout the study.
27 Control	383	Animal appeared clinically normal throughout the study.
28 Control	405	Animal appeared clinically normal throughout the study.
29 Control	404	Animal appeared clinically normal throughout the study.
30 Control	406	Animal appeared clinically normal throughout the study.

Note: As expected, dermal reactions were observed over the FCA injection sites. This was considered to be a typical finding for this study.

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TABLE II

GUINEA PIG SENSITIZATION

DERMAL REACTIONS - CHALLENGE

SC GROUP

Animal Number/ Group	HOURS FOLLOWING PATCH REMOVAL											
	24				48				72			
	Site A		Site B		Site A		Site B		Site A		Site B	
	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED
1 Test	0*	0	0*	0	0	0	0	0	0	0	0	0
2 Test	0	0	0*	0	0	0	0	0	0	0	0*	0
3 Test	0*	0	0*	0	0*	0	0	0	0	0	0	0
4 Test	0	0	0*	0	0	0	0*	0	0	0	0	0
5 Test	0*	0	0*	0	0	0	0*	0	0	0	0	0
6 Test	0	0	0*	0	0	0	0*	0	0	0	0	0
7 Test	0*	0	0*	0	0	0	0	0	0	0	0	0
8 Test	0*	0	0*	0	0	0	0	0	0	0	0	0
9 Test	0*	0	0*	0	0	0	0	0	0	0	0	0
10 Test	0	0	0*	0	0	0	0	0	0	0	0	0
11 Control	0*	0	0*	0	0	0	0	0	0	0	0	0
12 Control	0	0	0*	0	0	0	0*	0	0	0	0	0
13 Control	0*	0	0*	0	0	0	0	0	0	0	0	0
14 Control	0	0	0	0	0	0	0	0	0	0	0	0
15 Control	0	0	0*	0	0	0	0*	0	0	0	0	0

ER = Erythema
ED = Edema

Site A = Left Flank = SC reagent control
Site B = Right Flank = SC test extract

*Nonconfluent erythema not considered to be related to sensitization.

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TABLE II (continued)

GUINEA PIG SENSITIZATION

DERMAL REACTIONS - CHALLENGE

CSO GROUP

Animal Number/ Group	HOURS FOLLOWING PATCH REMOVAL											
	24				48				72			
	Site A		Site B		Site A		Site B		Site A		Site B	
	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED
16 Test	0*	0	0*	0	0	0	0*	0	0	0	0	0
17 Test	0*	0	0	0	0	0	0	0	0	0	0	0
18 Test	0	0	0*	0	0	0	0	0	0	0	0	0
19 Test	0*	0	0*	0	0	0	0	0	0	0	0	0
20 Test	0*	0	0	0	0*	0	0	0	0	0	0	0
21 Test	0*	0	0*	0	0	0	0*	0	0	0	0	0
22 Test	0*	0	0*	0	0*	0	0*	0	0*	0	0	0
23 Test	0*	0	0*	0	0*	0	0	0	0	0	0	0
24 Test	0	0	0	0	0	0	0	0	0	0	0	0
25 Test	0*	0	0*	0	0	0	0	0	0	0	0	0
26 Control	0*	0	0*	0	0	0	0*	0	0	0	0	0
27 Control	0*	0	0*	0	0*	0	0	0	0	0	0	0
28 Control	0*	0	0*	0	0*	0	0*	0	0	0	0	0
29 Control	0*	0	0*	0	0*	0	0	0	0	0	0	0
30 Control	0*	0	0*	0	0	0	0	0	0	0	0	0

ER = Erythema
ED = Edema

Site A = Left Flank = CSO reagent control
Site B = Right Flank = CSO test extract

*Nonconfluent erythema not considered to be related to sensitization.

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CERTIFICATE OF QUALITY ASSURANCE INSPECTIONS

Phase Inspected	Date	Auditor	Reports to Management and Study Director(s)	Date
Test Article Preparation	Dec. 18, 2001	N. L. Alt	Periodic Status Report	Jan. 7, 2002
Induction II	Dec. 21, 2001	N. L. Alt		
Final Report Review	Jan. 25, 2002	L. M. Byrd		

This study will be included in the next periodic status report as completed.

This study has been conducted in accordance with the provisions of the FDA Good Laboratory Practice Regulations (21 CFR, Part 58).

QA Representative: Lisa M. Byrd
Lisa M. Byrd, CQA, ALAT
Auditor, Quality Assurance

Jan. 25, 2002
Date

/lag

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GLP COMPLIANCE NOTIFICATION

This form is used for non-clinical studies to be conducted according to FDA Good Laboratory Practice Regulations of 21 CFR Part 58. Completion of this form identifies material that will be listed on a NAMSA Master Schedule Sheet available for FDA inspection. All information requested must be submitted to NAMSA before testing may begin.

Report To: BioMimetic Pharmaceuticals
1616 Westgate Circle
Brentwood, TN 37027

Authorized Asst.: (if different from left)

Attn: Samuel Lynch, DMD, MSc

Attn:

A. Test Article Identification: Vitoss Scaffold

1. Batch/Code/Lot (circle one) Identification: 081098-146

2. Storage Conditions: Room Temp. ✓; Refrig.___; Freezer___; Other:

3. Handling and Safety Precautions: None

4. Stability: The sponsor assures that the above material has been characterized for identity, strength, purity and composition as required by subpart F section 58.105 (see back). Stability testing is the responsibility of the sponsor and is subject to FDA audit; please indicate one of the following:

✓ Stability testing is in progress; article is stable for duration of intended testing.
___ Stability testing is complete and on file with sponsor. Expiration Date:

5. Disposition: Discard/Destroy___; Return to Sponsor ✓; Retain*___ *required for studies longer than 28 days

B. Control Article Submitted By Sponsor: No ✓; Yes___; Identification:

1. Batch/Code/Lot (circle one) Identification:

2. Storage Conditions: Room Temp.___; Refrig.___; Freezer___; Other:

3. Handling and Safety Precautions:

4. Stability: Characterization and stability information as above are also required for control articles.

___ Stability testing is in progress; article is stable for duration of intended testing.
___ Stability testing is complete and on file with sponsor. Expiration Date:
___ Marketed product stability characterized by its labeling.

5. Disposition: Discard/Destroy___; Return to Sponsor___; Retain*___ *required for studies longer than 28 days

C. Mixtures of test or control articles with carriers require analysis to demonstrate proper concentration, homogeneity and stability. Sponsor will provide analytical methods___; or will perform analysis on representative aliquots provided by NAMSA ✓. NA LLT 12-10-01

D. NAMSA Cost Estimate No. 1649-LLT (or attach list of tests) Sponsor's PO No. 01-1005

Name of Principal Investigator (Sponsor): Samuel Lynch Title: President

Signature: See additional paperwork for signature LLT 12-10-01 Date: Phone: 615-844-1280

Sample received 12-10-01 LLT 12-10-01

To be completed at NAMSA Test Facility:

Lab No. OTT 15267 00 Study Director: Laura J. Dasse 12-10-01

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GLP COMPLIANCE NOTIFICATION

This form is used for non-clinical studies to be conducted according to FDA Good Laboratory Practice Regulations of 21 CFR Part 58. Completion of this form identifies material that will be listed on a NAMSA Master Schedule Sheet available for FDA inspection. All information requested must be submitted to NAMSA before testing may begin.

Report To: BioMimetic Pharmaceuticals
1616 Westgate Circle
Brentwood, TN 37027

Attn: Bill Beasley

Authorized Asst.: _____
(if different from left) _____

Attn: _____

A. Test Article Identification: rh PDGF-BB (Becaplermin - 1mg/ml 20mM NaAc buffer)

1. Batch/Code/Lot (circle one) Identification: QA2217

2. Storage Conditions: Room Temp.___; Refrig.___; Freezer___; Other: ~~-70°C to 95°C~~ 2°C to 8°C per sponsor LLT 11-21-01

3. Handling and Safety Precautions: See MSDS

4. Stability: The sponsor assures that the above material has been characterized for identity, strength, purity and composition as required by subpart F section 58.105 (see back). Stability testing is the responsibility of the sponsor and is subject to FDA audit; please indicate one of the following:

X Stability testing is in progress; article is stable for duration of intended testing.
___Stability testing is complete and on file with sponsor. Expiration Date: _____

5. Disposition: Discard/Destroy___; Return to Sponsor X; Retain*___ *required for studies longer than 28 days

B. Control Article Submitted By Sponsor: No X; Yes___; Identification: _____

1. Batch/Code/Lot (circle one) Identification: _____

2. Storage Conditions: Room Temp.___; Refrig.___; Freezer___; Other: _____

3. Handling and Safety Precautions: _____

4. Stability: Characterization and stability information as above are also required for control articles.

___Stability testing is in progress; article is stable for duration of intended testing.
___Stability testing is complete and on file with sponsor. Expiration Date: _____
___Marketed product stability characterized by its labeling.

5. Disposition: Discard/Destroy___; Return to Sponsor___; Retain*___ *required for studies longer than 28 days

C. Mixtures of test or control articles with carriers require analysis to demonstrate proper concentration, homogeneity and stability. Sponsor will provide analytical methods___; or will perform analysis on representative aliquots provided by NAMSA___. N/A

D. NAMSA Cost Estimate No. 1649-LLT (or attach list of tests) Sponsor's PO No. 01-1005

Name of Principal Investigator (Sponsor): Samuel Lynch Title: President

Signature: Samuel Lynch Date: 11/13/01 Phone: 615-844-1280

Sample received 11-20-01 LLT 12-10-01

To be completed at NAMSA Test Facility:

Lab No. 01T-15267-00 Study Director Laura J. Cosse 12-10-01

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BM001773

TI261-300
TI261-301
R-06

NAMSA™ GLP PROTOCOL T261

ISO SENSITIZATION STUDY IN THE GUINEA PIG

(Maximization Method)

Sponsor:

Bill Beasley
BioMimetic Pharmaceutical Inc.
1616 Westgate Circle
Brentwood, TN 37027

Test Facility:

North American Science Associates, Inc. (NAMSA)
2261 Tracy Road
Northwood, OH 43619-1397

NAMSA Use Only

Lab No. 01T-15267-00
CEP No. 1649-LLT (Revised)

Approvals:

Protocol Submitted By (NAMSA): Laura L. Tasse
Laura L. Tasse, BS, ALAT
Study Director

Date Issued: October 29, 2001

Principal Investigator: (Sponsor)

Date Approved: 11/6/01

Study Director (NAMSA): Laura L. Tasse

Date Initiated: Dec. 10, 2001

/pak

BM001774

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Lab No. 01T-15267-00

NAMSA™ GLP PROTOCOL T261

ISO SENSITIZATION STUDY IN THE GUINEA PIG

(Maximization Method)

Purpose of the Study:

The objective of the maximization test in the guinea pig is to identify the potential for dermal sensitization. The Magnusson and Kligman method has been effective in identifying a variety of allergens. This study will be based on the requirements of the International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 10: Tests for Irritation and Sensitization.

This study will be conducted in accordance with the requirements of the FDA Good Laboratory Practice (GLP) Regulations, 21 CFR 58.

Test Article:

The sponsor will submit the material to be evaluated. Detailed information about the test article will be provided by the sponsor on the attached GLP Compliance Notification form furnished by NAMSA or on a similar attachment to the protocol.

The following is to be completed (with initials and dates in the margin) by the sponsor or study director. Further instructions may be attached to the protocol. The sample will be prepared as follows:

1. Ratio of test article to extraction vehicle:
   - ___ Material thickness less than 0.5 mm - ratio of 120 cm^2: 20 ml
   - ___ Material thickness greater than or equal to 0.5 mm - ratio of 60 cm^2:20 ml
   - ✓ Irregularly shaped objects and/or sponsor option - ratio of 4 g:20 ml
   - ___ Other (explain)___

2. Extraction vehicle:
   - ✓ 0.9% sodium chloride USP solution (SC)
   - ✓ cottonseed oil, NF (CSO)
   - ___ Other (specify)___

LT
1-21-01

3. Extraction conditions:
   - ✓ 37°C, 72 hours (± 2 hours)
   - ___ 121°C, 1 hour (± 0.2 hours)
   - ___ 70°C, 24 hours (± 2 hours)
   - ___ 50°C, 72 hours (± 2 hours)
   - ___ Other (specify) ___

4. The test article itself is suitable for topical application at the challenge phase.
   - ___ Yes
   - ✓ No

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Control Article:

The vehicle used to prepare the extract will be prepared in the same manner as the extract (but without test article) to serve as the control measure. Untreated skin will serve as an additional control reference for scoring dermal reactions during the challenge phase.

Test System:

Species:	Guinea pig (*Cavia porcellus*)
Strain:	Crl:(HA) BR
Source:	Charles River Laboratories
Sex:	No particular gender is prescribed for this test. If females are used, they will be nulliparous and not pregnant
Body Weight Range:	300-500 grams at identification
Age:	Young adults
Acclimation Period:	Minimum 5 days
Number of Animals:	15 (per extract)
Identification Method:	Ear punch

Justification of Test System:

The Hartley albino guinea pig has been used historically for sensitization studies (Magnusson and Kligman, 1970). The guinea pig is believed to be the most sensitive animal model for this type of study. The susceptibility of the Hartley strain to a known sensitizing agent, 1-chloro-2,4-dinitrobenzene (DNCB) has been substantiated at NAMSA with this method.

Duplication of Experimental Work:

By signature on this protocol, the sponsor confirms that the conduct of this study does not unnecessarily duplicate previous experiments.

Animal Management:

Husbandry:	Conditions will conform to Standard Operating Procedures which are based on the "Guide for the Care and Use of Laboratory Animals."
Food:	A commercially available, pelleted, guinea pig feed will be provided daily.
Water:	Freely available, municipal water will be delivered through an automatic watering system.
Contaminants:	Reasonably expected contaminants in feed or water supplies should not have the potential to influence the outcome of this test.
Housing:	Animals will be housed in groups in stainless steel suspended cages identified by a card indicating the lab number, animal numbers, test code, sex, animal code and first treatment date.

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Environmental:	The room temperature will be monitored daily. The recommended temperature range for the room is 64-79°F. The room humidity will be monitored daily. The humidity range for the room is 30-70%. The light cycle will be controlled using an automatic timer (12 hours light, 12 hours dark).
Facility:	NAMSA is an AAALAC International accredited facility and is registered with the United States Department of Agriculture. Additionally, NAMSA maintains an approved Animal Welfare Assurance on file with the National Institutes of Health, Office for Laboratory Animal Welfare.
Personnel:	Associates involved will be appropriately qualified and trained.
Selection:	Only healthy animals will be selected.
Sedation, Analgesia or Anesthesia:	It has been determined that the use of these agents will not be necessary during the routine course of this procedure.
Veterinary Care:	In the unlikely event that an animal should become injured, ill, or moribund, care will be conducted in accordance with current veterinary medical practice. If warranted for humane reasons, euthanasia will be conducted in accordance with the current report of the American Veterinary Medical Association's Panel on Euthanasia. The objective of the study will be given due consideration in any decision and the study sponsor will be advised.
IACUC:	This protocol has been approved by NAMSA Institutional Animal Care and Use Committees (IACUC), and is reviewed at least annually by the same committees. Any significant changes to this protocol must be approved by the IACUC prior to conduct.

Test and Control Article Preparation:

Fresh extracts will be prepared at each phase of the study as previously indicated (see Test Article). If the test material is suitable for patching, a topical application of the test sample (2 cm x 2 cm patch) will be used at the challenge. The vehicle used to prepare the extract will be prepared in the same manner as the extract (but without test article) to serve as the control measure.

Methods and Route of Administration:

The day prior to treatment, 15 guinea pigs per extract (10 test, 5 control) will be weighed and identified. The fur from the dorsoscapular area of the animals will be removed with an electric clipper.

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Induction I:

Three pair of intradermal injections will be administered to the animals within an approximate 2 cm x 4 cm area over the dorsoscapular region as follows:

Control Animals:
a. 0.1 ml of 50:50 (v/v) mixture of Freund's Complete Adjuvant (FCA) and the chosen vehicle
b. 0.1 ml of vehicle
c. 0.1 ml of a 1:1 mixture of the 50:50 (v/v) FCA and the vehicle

Test Animals:
a. 0.1 ml of 50:50 (v/v) mixture of FCA and the chosen vehicle
b. 0.1 ml of test extract
c. 0.1 ml of a 1:1 mixture of the 50:50 (v/v) FCA and the test extract

To minimize tissue sloughing the "a" and "c" injections will be slightly deeper than "b". Site "c" will be injected slightly more caudal than site "b".

Induction II:

Six days later, the injection sites will be clipped free of fur again and treated with 0.5 to 1 g of a 10% (w/w) sodium lauryl sulfate (SLS) suspension prepared by mixing the powdered SLS with petrolatum. The day following the SLS treatment, any remaining SLS residue will be gently wiped from the area with gauze.

A 2 cm x 4 cm filter paper patch (3MM, Whatman), saturated with 0.3 ml of the extract preparation or vehicle, will be applied over the same injection area and secured with a nonreactive tape. The trunk of each animal will then be wrapped snugly with an elastic band for 48 hours (±2 hours).

Challenge:

At 13 days after unwrapping induction II wraps, the fur will be clipped from the sides and flanks of all guinea pigs. On the following day, a nonwoven cotton disk backed by a flexible chamber (e.g. Hill Top Chamber®) and semiocclusive hypoallergenic tape, will be saturated with 0.3 ml of freshly prepared test material extract and applied to the right flank or dorsum of each animal. In addition, the vehicle control will be patched to the left flank or dorsum of each animal. An approximate 2 cm x 2 cm section of test material itself (if appropriate) will be applied to the right flank.

The trunk of each animal will be wrapped for 24 hours (±2 hours). At patch removal the sites will be wiped with gauze. At 24 hours (± 2 hours) after patch removal, the challenged sites and surrounding area will be shaved. The sites will be examined for signs of any irritation or sensitization reaction, as indicated by erythema and edema at a minimum of 2 hours and a maximum of 4 hours following the shave and at 48 (±2 hours) and 72 (±2 hours) hours after removal of the dressings. Prior to scoring, each site will be wiped gently with a 35% isopropyl alcohol gauze sponge.

Should the original challenge results prove to be equivocal, the animals may be rechallenged with a fresh test extract and vehicle control approximately 7 days after the first challenge patch application. The rechallenge will be conducted in the same manner as the challenge but at virgin sites on the opposite flank. After the test is completed, all animals will be handled in accordance with IACUC approved NAMSA procedures.

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Evaluations and Statistics:

Daily challenge scores for reactions will be recorded at 24, 48 and 72 hours after patch removal in accordance with the following scheme:

ERYTHEMA (ER)		EDEMA (ED)	
Reaction	Numerical Grading	Reaction	Numerical Grading
No erythema	0	No edema	0
Slight erythema	1	Slight edema	1
Well-defined erythema	2	Well-defined edema	2
Moderate erythema	3	Moderate edema	3
Severe erythema to slight eschar formation	4	Severe edema	4

Any other observation relating to the site will be footnoted.

The responses will be compared within the test animal group and between test and control conditions. Control conditions are (1) the vehicle control solution on the test animals and (2) the test extract, control solution and biomaterial (if applied) on the control animals.

In the final analysis of data, consideration will be given to the overall pattern, intensity, duration, and character of reactions of the test as compared to the control conditions. Statistical manipulation of data is not applicable to this study. An effect interpreted as "irritation" is generally observed at 24 hours, but diminishes thereafter, and is also concurrently present as a transient response in the control animals. Closed patches typically show maximal sensitization readings 48 to 72 hours after patch removal in the test condition but not in the control condition. Grades of 1 or greater in the test group generally indicate sensitization, provided that grades of less than 1 are observed on the control animals. If grades of 1 or greater are noted on control animals, then the reactions of test animals which exceed the most severe control reaction are considered to be due to sensitization.

Background or artifactual reactions (e.g., from fur clipping, patch chamber edge, nonspecific FCA effects) will not be considered as evidence of a sensitization response. The treatment with FCA and occlusive dressings may lower the threshold level for skin irritation.

If the test group has a greater number of animals showing responses that are not greater than the control animals, a rechallenge may be conducted. The rechallenge will be conducted approximately 7 days after the first challenge at virgin sites on the opposite flank of the animals. Absence of dermal response at rechallenge may nullify earlier findings. Recurring observations in at least one of the same animals verify earlier findings.

Report:

A final report will be issued to include a description of the methods, the resulting data in tabular format and conclusions.

Quality Assurance:

Inspections will be conducted at intervals adequate to assure the integrity of the study in conformance with 21 CFR 58.35(b)(3). The final report will also be reviewed for conformance to Section 58.185, Subpart J, of the GLP Regulations. A Certificate of Quality Assurance Inspections will be provided with the final report.

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Records:

Test article preparation, animal weights, treatment procedures, dermal reaction scores, and dates of relevant test activities from study initiation to completion will be recorded.

All raw data pertaining to this study and a copy of the final report will be retained in designated NAMSA archive files.

Proposed Dates:

The study dates will be finalized by the study director following receipt of the sponsor-approved protocol and appropriate material for the study. Initiation of the study will be the date on which the study director signs the GLP protocol. Projected dates for starting the study (first treatment) and for the completion of the study (final report release) will be provided to the sponsor (or representative of the sponsor) and added to the protocol.

References:

21 CFR 58 (GLP Regulations).

Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research, National Academy of Sciences (Washington: National Academy Press, 1996).

International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 10: Tests for Irritation and Sensitization.

Magnusson, B. and A. Kligman, *Allergic Contact Dermatitis in the Guinea Pig* (Springfield: C.H. Thomas, 1970).

OLAW, Public Health Service Policy on Humane Care and Use of Laboratory Animals (NIH Publication)

United States Code of Federal Regulation (CFR) 9: The Animal Welfare Act.

Protocol Changes:

Any necessary changes to the protocol after sponsor approval or study initiation will be documented and approved by the study director as protocol amendments. Copies will be distributed to the sponsor, the raw data file, and the NAMSA Quality Assurance department.

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December 10, 2001

BILL BEASLEY
BIOMIMETIC PHARMACEUTICALS
1616 WESTGATE CIRCLE
BRENTWOOD, TN 37027

PROTOCOL AMENDMENT I

Test Article: rhPDGF-BB (Becaplermin - 1 mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold

Identification: Lot #QA2217 and Lot 081098-146

NAMSA Lab No.: 01T 15267 00

We have received appropriate test articles and approved protocols for the program to be conducted in accordance with the Good Laboratory Practice (GLP) Regulations on the material described above. Below is a projected schedule for the work to be performed.

NAMSA Code	Study	Estimated Start Date:	Estimated Report Release Date:
V0014-130	Cytotoxicity Study using the ISO Elution Method (Extract)	Dec. 13, 2001	Jan. 8, 2002
TI261-300	ISO Sensitization Test in Fifteen Guinea Pigs, Saline Extract		
TI261-301	ISO Sensitization Test in Fifteen Guinea Pigs, Cottonseed Oil Extract	Dec. 14, 2001	Jan. 25, 2002
TI251-800	ISO Acute Intracutaneous Reactivity Study in Three Rabbits Saline Extract, Cottonseed Oil Extract	Dec. 17, 2001	Jan. 8, 2002
TI264-500	ISO Acute Systemic Toxicity Study in Five Mice, Saline Extract, Cottonseed Oil Extract	Dec. 17, 2001	Jan. 8, 2002
V0023-211	Genotoxicity: Bacterial Reverse Mutation (Ames) Test, Salmonella and *E. coli* strains, Saline Extract		
V0023-212	Genotoxicity: Bacterial Reverse Mutation (Ames) Test, Salmonella and *E. coli* strains, DMSO Extract	Dec. 18, 2001	Jan. 15, 2002
TI247-804 TH035-800	ISO Surgical Muscle Implantation, in Three Rabbits (4 Weeks) With Histopathology	Dec. 19, 2001	Jan. 25, 2002

Laura L. Tasse

Laura L. Tasse, BS, ALAT
Study Director

Dec. 10, 2001
Date

LLT/dap

cc: QA (NAMSA)
GLP study file

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December 10, 2001

SAMUEL LYNCH, M.D.
BIOMIMETIC PHARMACEUTICALS
1616 WESTGATE CIRCLE
BRENTWOOK, TN 37027

PROTOCOL AMENDMENT II

Test Article:	rhPDGF-BB (Becaplermin – 1 mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold
Identification No.:	Lot #QA2217 and Lot 081098-146
Protocol:	V0014-130 Cytotoxicity Study using the ISO Elution Method (Extract) TI261-300 ISO Sensitization Test in Fifteen Guinea pigs (Saline extract) TI261-301 ISO Sensitization Test in Fifteen Guinea pigs (Cottonseed oil extract) TI251-800 ISO Acute Intracutaneous Reactivity Study in Three Rabbits TI264-500 ISO Acute Systemic Toxicity Study in Five Mice V0023-211 Genotoxicity: Bacterial Reverse Mutation (Ames) Test (Saline extract) V0023-212 Genotoxicity: Bacterial Reverse Mutation (Ames) Test (DMSO extract)
NAMSA Lab No.:	01T 15267 00

This amendment was written to clarify the preparation of the test article.

The two components of the test article must be combined just prior to extraction preparation. To do this, a 1:1 ratio of Vitoss (in grams) to rhPDGF-BB (in ml's) will be prepared. For example, a 2 g portion of the ChronOS will be saturated with 2 ml of the rhPDGF. If the frozen rhPDGF-BB is used, it must first be thawed prior to the preparation.

The resulting mixture will then be used for the extraction preparation (i.e. weight of the prepared test article to volume of extraction vehicle).

Laura L. Tasse

Laura L. Tasse, BS, ALAT
Study Director

Dec. 10, 2001
Date

cc: NAMSA QAU
GLP study file

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January 8, 2002

BILL BEASLEY
BIOMIMETIC PHARMACEUTICALS
1616 WESTGATE CIRCLE
BRENTWOOD, TN 37027

REVISED*

PROTOCOL AMENDMENT II

Test Article:	rhPDGF-BB (Becaplermin – 1 mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold
Identification No.:	Lot #QA2217 and Lot 081098-146
Protocol:	V0014-130 Cytotoxicity Study using the ISO Elution Method (Extract) TI261-300 ISO Sensitization Test in Fifteen Guinea pigs (Saline extract) TI261-301 ISO Sensitization Test in Fifteen Guinea pigs (Cottonseed oil extract) TI251-800 ISO Acute Intracutaneous Reactivity Study in Three Rabbits TI264-500 ISO Acute Systemic Toxicity Study in Five Mice V0023-211 Genotoxicity: Bacterial Reverse Mutation (Ames) Test (Saline extract) V0023-212 Genotoxicity: Bacterial Reverse Mutation (Ames) Test (DMSO extract)
NAMSA Lab No.:	01T 15267 00

This amendment was written to clarify the preparation of the test article.

The two components of the test article must be combined just prior to extraction preparation. To do this, a 1:1 ratio of Vitoss (in grams) to rhPDGF-BB (in ml's) will be prepared. For example, a 2 g portion of the Vitoss* will be saturated with 2 ml of the rhPDGF. If the frozen rhPDGF-BB is used, it must first be thawed prior to the preparation.

The resulting mixture will then be used for the extraction preparation (i.e. weight of the prepared test article to volume of extraction vehicle).

* This amendment was revised to correct the sponsor name and address, and to correct the name of the Vitoss in the second paragraph.

Laura L. Tasse

Laura L. Tasse, BS, ALAT
Study Director

Jan. 8, 2002
Date

cc: NAMSA QAU
GLP study file

Appendix 7.7.10: Test Report TI251-800. ISO acute intracutaneous reactivity study in the rabbit (extracts)

STUDY TITLE:

ISO ACUTE INTRACUTANEOUS REACTIVITY STUDY IN THE RABBIT

(Extracts)

TEST ARTICLE:

rhPDGF-BB (Becaplermin - 1 mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold

IDENTIFICATION NO.:

Lot #QA2217 and Lot 081098-146

TEST FACILITY:

NAMSA
2261 Tracy Road
Northwood, OH 43619-1397

SPONSOR:

BILL BEASLEY
BIOMIMETIC PHARMACEUTICALS
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SUMMARY

The test article, rhPDGF-BB (Becaplermin - 1 mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold, Lot #QA2217 and Lot 081098-146, was extracted in 0.9% sodium chloride USP solution and cottonseed oil, NF. These extracts were evaluated for intracutaneous reactivity based on the requirements of the International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 10: Tests for Irritation and Sensitization.

A 0.2 ml dose of the appropriate test article extract was injected by the intracutaneous route into five separate sites on the right side of the back of each rabbit. Similarly, the corresponding reagent control was injected on the left side of the back of each rabbit. The injection sites were observed immediately after injection. Observations for erythema and edema were conducted at 24, 48, and 72 hours after injection.

Under the conditions of this study, there was no evidence of significant irritation from the cottonseed oil, NF extract injected intracutaneously into rabbits. There was evidence of slight irritation from the 0.9% sodium chloride USP solution injected intracutaneously into rabbits. The Primary Irritation Index Characterization for the cottonseed oil extract was negligible while the saline extract was slight.

Study and Supervisory Personnel:

Deedee M. Davis, BA
Sara S. Hartman, ALAT
Brian Brooks
Suzanne E. Brimacombe
Martha Oswanski, BS, LAT

Study Director: Laura L. Tasse, BS, ALAT
Study Director

Jan. 8, 2002
Date Completed

/cal

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INTRODUCTION

The test article identified below was extracted and the extracts were evaluated for biocompatibility based on the requirements of the International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 10: Tests for Irritation and Sensitization. The purpose of the study was to determine whether leachables extracted from the material would cause local dermal irritant effects following injection into rabbit skin. The test article was received on November 20, 2001 and December 10, 2001. The animals were injected on December 14, 2001, and the observations were concluded on December 17, 2001.

The study, initiated by protocol signature on December 10, 2001, was conducted in accordance with the provisions of the FDA Good Laboratory Practice (GLP) Regulations, 21 CFR 58. A Certificate of Quality Assurance Inspections was issued with this report.

MATERIALS

The sample provided by the sponsor was identified and handled as follows:

Test Article:	rhPDGF-BB (Becaplermin - 1 mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold
Identification No:	Lot #QA2217 and Lot 081098-146
Stability Testing:	In Progress (per sponsor)
Expiration Date:	Stable for duration of intended testing (per sponsor)
Storage Conditions:	rhPDGF-BB – refrigerated Vitoss Scaffold – room temperature
Vehicles:	0.9% sodium chloride USP solution (SC) cottonseed oil, NF (CSO)
Preparation:	A 2 g portion of Vitoss Scaffold was saturated with a 2 ml portion of rhPDGF-BB for each extraction vehicle immediately prior to pouring. Based on a ratio of 4 g:20 ml, a 3.5 g portion of the prepared test article was covered with 18 ml of the vehicle. The prepared test article was extracted in SC and CSO at 37°C for 72 hours. The extraction vehicles without test article were similarly prepared to serve as reagent controls.

Condition of Extracts:

	Test	Control
SC:	clear with particulates*	clear
CSO:	cloudy with particulates	clear

*The test extract was filtered with a 0.8 µm filter disc to yield a clear, particulate free extract.

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METHODS

Test System:

Species:	Rabbit (*Oryctolagus cuniculus*)
Breed:	New Zealand White
Source:	Myrtle's Rabbitry, Inc.
Sex:	Female
Body Weight Range:	2.0 kg to 2.3 kg at selection
Age:	Young adult
Acclimation Period:	Minimum 5 days
Number of Animals:	Three
Identification Method:	Ear tag

Justification of Test System:

The intracutaneous injection test in rabbits is specified in the current ISO testing standards and has been used historically to evaluate biomaterial extracts.

Duplication of Experimental Work:

By signature on the protocol, the sponsor confirmed that the conduct of this study did not unnecessarily duplicate previous experiments.

Animal Management:

Husbandry:	Conditions conformed to Standard Operating Procedures which are based on the "Guide for the Care and Use of Laboratory Animals."
Food:	PROLAB® High Fiber Rabbit Diet was provided daily.
Water:	Freely available, municipal (Toledo, OH) water was delivered through an automatic watering system.
Contaminants:	Reasonably expected contaminants in feed or water supplies did not have the potential to influence the outcome of this test.
Housing:	Animals were individually housed in stainless steel suspended cages identified by a card indicating the lab number, animal number, test code, sex, and date dosed.
Environmental:	The room temperature was monitored daily. The temperature range for the room was within a range of 61-72°F. The room humidity was monitored daily. The humidity range for the room was 30-70%. The light cycle was controlled using an automatic timer (12 hours light, 12 hours dark).
Facility:	NAMSA is an AAALAC International accredited facility and is registered with the United States Department of Agriculture. Additionally, NAMSA maintains an approved Animal Welfare Assurance on file with the National Institutes of Health, Office for Laboratory Animal Welfare.
Personnel:	Associates involved were appropriately qualified and trained.

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Selection: Only healthy, previously unused, thin-skinned animals free of mechanical irritation or trauma that could interfere with the test were selected.

Experimental Procedure:

The day prior to treatment, each rabbit was weighed and clipped free of fur from the back and both sides of the spinal column to yield a sufficient injection area. The clipped area of the back was wiped with a 70% alcohol soaked gauze pad just before injection and allowed to dry. Three rabbits were prepared per pair of extracts. A 0.2 ml dose of test article extract was injected intracutaneously into five separate sites on the right side of the back of each rabbit; 0.2 ml of the reagent control was injected into five separate sites on the corresponding left side of the back. Injections were spaced approximately 2 cm apart. The appearance of each injection site was noted immediately after injection. The animals were returned to their respective cages following the procedure.

Observations for erythema and edema were conducted at 24, 48, and 72 hours after injection. Reactions were scored on a 0 to 4 basis. Any reaction at the injection sites was also noted. The reactions were evaluated according to the following subjective rating scale:

ERYTHEMA (ER)		EDEMA (ED)	
0	No erythema	0	No edema
1	Very slight erythema (barely perceptible)	1	Very slight edema (barely perceptible)
2	Well-defined erythema	2	Well-defined edema (edges of area well-defined by definite raising)
3	Moderate erythema	3	Moderate edema (raised approximately 1 mm)
4	Severe erythema (beet redness) to eschar formation preventing grading of erythema	4	Severe edema (raised more than 1 mm, and extending beyond exposure area)

For each animal, the erythema and edema scores obtained at each time interval were added together and divided by the total number of observations. This calculation was conducted separately for each test extract and reagent control. The score for the reagent control was subtracted from the score for the test extract to obtain the Primary Irritation Score. The Primary Irritation Score of each animal was added together and divided by the total number of animals to obtain the Primary Irritation Index (PII). The Primary Irritation Index was characterized by number and description as follows: 0-0.4 (negligible), 0.5-1.9 (slight), 2.0-4.9 (moderate), 5.0-8.0 (severe). Any adverse reaction noted in the test extract was compared to the corresponding reagent control.

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RESULTS

Results of scores for individual rabbits appear in Table I. All injection sites appeared normal immediately following injection. The Primary Irritation Index (PII) and Characterization for each extract are summarized below:

Extract	Animal Number	Test Score Average	Control Score Average	Primary Irritation Score	Primary Irritation Score Total	Primary Irritation Index Characterization
SC	27973	0.7	0.0	0.7	0.8	Slight
	27974	0.7	0.0	0.7		
	27971	0.9	0.0	0.9		
CSO	27973	0.5	0.1	0.4	0.2	Negligible
	27974	0.7	1.0	0.0		
	27971	0.9	0.7	0.2		

Results and conclusions apply only to the test article tested. No further evaluation of these results is made by NAMSA. Any extrapolation of these data to other samples is the responsibility of the sponsor. All procedures were conducted in conformance with good laboratory practice and ISO 17025.

CONCLUSION

Under the conditions of this study, there was no evidence of significant irritation from the cottonseed oil, NF extract injected intracutaneously into rabbits. There was evidence of slight irritation from the 0.9% sodium chloride USP solution injected intracutaneously into rabbits. The Primary Irritation Index Characterization for the cottonseed oil extract was negligible while the saline extract was slight.

RECORD STORAGE

All raw data pertaining to this study and a copy of the final report are to be retained in designated NAMSA archive files.

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TABLE I

ISO ACUTE INTRACUTANEOUS REACTIVITY OBSERVATIONS

Rabbit Number/ Gender	Body Weight (kg)	Extract	Scoring Interval											
			24 Hours				48 Hours				72 Hours			
			Test		Control		Test		Control		Test		Control	
			ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED
27973 Female	2.0	SC	0	0	0	0	1	1	0	0	0	0	0	0
			0	0	0	0	1	1	0	0	0	0	0	0
			0	0	0	0	1	1	0	0	0	0	0	0
			0	0	0	0	1	1	0	0	0	0	0	0
			0	0	0	0	1	1	0	0	0	0	0	0
27974 Female	2.0	SC	0	0	0	0	1	1	0	0	0	0	0	0
			0	0	0	0	1	1	0	0	0	0	0	0
			0	0	0	0	1	1	0	0	0	0	0	0
			0	0	0	0	1	1	0	0	0	0	0	0
			0	0	0	0	1	1	0	0	0	0	0	0
27971 Female	2.3	SC	1	0	0	0	1	1	0	0	0	0	0	0
			1	0	0	0	1	1	0	0	0	0	0	0
			1	0	0	0	1	1	0	0	0	0	0	0
			1	0	0	0	1	1	0	0	0	0	0	0
			0	0	0	0	1	1	0	0	0	0	0	0
27973 Female	2.0	CSO	0	0	0	0	1	1	1	0	0	0	0	0
			0	0	0	0	1	1	1	0	1	0	0	0
			0	0	0	0	1	0	0	0	1	0	0	0
			0	0	0	0	0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0	0	0	0	0
27974 Female	2.0	CSO	1	0	1	0	1	0	1	0	1	0	1	0
			1	0	1	0	1	0	1	0	1	0	1	0
			1	0	1	0	1	0	1	0	0	0	1	0
			0	0	1	0	1	0	1	0	1	0	1	0
			1	0	1	0	0	0	1	0	0	0	1	0
27971 Female	2.3	CSO	1	0	1	0	1	1	1	0	1	0	0	0
			1	0	1	0	1	1	1	0	1	0	0	0
			1	0	1	0	1	0	1	0	0	0	0	0
			1	0	1	0	1	0	1	0	0	0	0	0
			1	0	1	0	0	0	1	0	0	0	1	0

ER = Erythema
ED = Edema

SC = 0.9% sodium chloride USP solution
CSO = cottonseed oil, NF

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CERTIFICATE OF QUALITY ASSURANCE INSPECTIONS

Phase Inspected	Date	Auditor	Reports to Management and Study Director(s)	Date
Dosing	Dec. 14, 2001	N. L. Alt	Periodic Status Report	Jan. 7, 2002
Final Report Review	Jan. 7, 2002	L. M. Byrd		

This study will be included in the next periodic status report as completed.

This study has been conducted in accordance with the provisions of the FDA Good Laboratory Practice Regulations (21 CFR, Part 58).

QA Representative: Lisa M. Byrd, CQA, ALAT
Auditor, Quality Assurance

Jan. 8, 2002
Date

/cal

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GLP COMPLIANCE NOTIFICATION

This form is used for non-clinical studies to be conducted according to FDA Good Laboratory Practice Regulations of 21 CFR Part 58. Completion of this form identifies material that will be listed on a NAMSA Master Schedule Sheet available for FDA inspection. All information requested must be submitted to NAMSA before testing may begin.

Report To: BioMimetic Pharmaceuticals
1616 Westgate Circle
Brentwood, TN 37027

Attn: Samuel Lynch, OMD, MSC

Authorized Asst.: (if different from left)

Attn:

A. Test Article Identification: Vitoss Scaffold

1. Batch/Code/Lot (circle one) Identification: 081098-146
2. Storage Conditions: Room Temp. ✓; Refrig. ___; Freezer ___; Other: ___
3. Handling and Safety Precautions: None
4. Stability: The sponsor assures that the above material has been characterized for identity, strength, purity and composition as required by subpart F section 58.105 (see back). Stability testing is the responsibility of the sponsor and is subject to FDA audit; please indicate one of the following:

   ✓ Stability testing is in progress; article is stable for duration of intended testing.
   ___ Stability testing is complete and on file with sponsor. Expiration Date: ___

5. Disposition: Discard/Destroy ___; Return to Sponsor ✓; Retain* ___ *required for studies longer than 28 days

B. Control Article Submitted By Sponsor: No ✓; Yes ___; Identification: ___

1. Batch/Code/Lot (circle one) Identification: ___
2. Storage Conditions: Room Temp. ___; Refrig. ___; Freezer ___; Other: ___
3. Handling and Safety Precautions: ___
4. Stability: Characterization and stability information as above are also required for control articles.

   ___ Stability testing is in progress; article is stable for duration of intended testing.
   ___ Stability testing is complete and on file with sponsor. Expiration Date: ___
   ___ Marketed product stability characterized by its labeling.

5. Disposition: Discard/Destroy ___; Return to Sponsor ___; Retain* ___ *required for studies longer than 28 days

C. Mixtures of test or control articles with carriers require analysis to demonstrate proper concentration, homogeneity and stability. Sponsor will provide analytical methods ___; or will perform analysis on representative aliquots provided by NAMSA ✓. NA LLT 12-10-01

D. NAMSA Cost Estimate No. 1649-LLT (or attach list of tests) Sponsor's PO No. 01-1005

Name of Principal Investigator (Sponsor): Samuel Lynch Title: President

Signature: See additional paperwork for signature LLT 12-10-01 Date: ___ Phone: 615-844-1280

Sample received 12-10-01 LLT 12-10-01

To be completed at NAMSA Test Facility:

Lab No. OTT 15267 00 Study Director: Laura J. Tasse 12-10-01

NAMSA
Ensuring Medical Device Safety and Compliance

Corp. Hdqtrs: 2281 Tracy Road, Northwood, OH 43619-1397 / 419.866.9855 / Fax 419.698.2954
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1001U

GLP COMPLIANCE NOTIFICATION

This form is used for non-clinical studies to be conducted according to FDA Good Laboratory Practice Regulations of 21 CFR Part 58. Completion of this form identifies material that will be listed on a NAMSA Master Schedule Sheet available for FDA inspection. All information requested must be submitted to NAMSA before testing may begin.

Report To: BioMimetic Pharmaceuticals
1616 Hedgate Circle
Brentwood, TN 37027

Attn: Bill Beasley

Authorized Asst.: _____
(if different from left) _____

Attn: _____

A. Test Article Identification: rh PDGF-BB (beaplermin - 1mg/ml 20mM NaAc buffer)

1. Batch/Code/Lot (circle one) Identification: QA2217

2. Storage Conditions: Room Temp.___; Refrig.___; Freezer___; Other: ~~-70°C to 95°C~~ 2°C to 8°C per sponsor LLT 11-21-01

3. Handling and Safety Precautions: See MSDS

4. Stability: The sponsor assures that the above material has been characterized for identity, strength, purity and composition as required by subpart F section 58.105 (see back). Stability testing is the responsibility of the sponsor and is subject to FDA audit; please indicate one of the following:

X Stability testing is in progress; article is stable for duration of intended testing.
___Stability testing is complete and on file with sponsor. Expiration Date:_____

5. Disposition: Discard/Destroy___; Return to Sponsor X; Retain*___ *required for studies longer than 28 days

B. Control Article Submitted By Sponsor: No X; Yes___; Identification:_____

1. Batch/Code/Lot (circle one) Identification:_____

2. Storage Conditions: Room Temp.___; Refrig.___; Freezer___; Other:_____

3. Handling and Safety Precautions:_____

4. Stability: Characterization and stability information as above are also required for control articles.

___Stability testing is in progress; article is stable for duration of intended testing.
___Stability testing is complete and on file with sponsor. Expiration Date:_____
___Marketed product stability characterized by its labeling.

5. Disposition: Discard/Destroy___; Return to Sponsor___; Retain*___ *required for studies longer than 28 days

C. Mixtures of test or control articles with carriers require analysis to demonstrate proper concentration, homogeneity and stability. Sponsor will provide analytical methods___; or will perform analysis on representative aliquots provided by NAMSA___. N/A

D. NAMSA Cost Estimate No. 1649-LLT (or attach list of tests) Sponsor's PO No. 01-1005

Name of Principal Investigator (Sponsor): Samuel Lynch Title: President

Signature: Samuel Lynch Date: 11/13/01 Phone: 615-844-1280

Sample received 11-20-01 LLT 12-10-01

To be completed at NAMSA Test Facility:

Lab No. 01T-15267-00 Study Director Laura J. Asse 12-10-01

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10011J

NAMSA™ GLP PROTOCOL T251

ISO ACUTE INTRACUTANEOUS REACTIVITY STUDY IN THE RABBIT

Sponsor:

Bill Beasley
BioMimetic Pharmaceutical Inc.
1616 Westgate Circle
Brentwood, TN 37027

Test Facility:

North American Science Associates, Inc. (NAMSA)
2261 Tracy Road
Northwood, OH 43619-1397

NAMSA Use Only

Lab No. 01T-15267-00
CEP No. 1649-LLT (Revised)

Approvals:

Protocol Submitted By (NAMSA): Laura L. Tasse
Laura L. Tasse BS, ALAT
Study Director

Date Issued: October 29, 2001

Principal Investigator: (Sponsor)

Date Approved: 11/6/01

Study Director (NAMSA): Laura L. Tasse

Date Initiated: Dec. 10, 2001

/pak

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NAMSA™ GLP PROTOCOL T251

ISO ACUTE INTRACUTANEOUS REACTIVITY STUDY IN THE RABBIT

Purpose of the Study:

The objective of this study is to evaluate the local dermal irritant effects of leachables extracted from the test article following intracutaneous injection in rabbits. This study will be based on the requirements of the International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 10: Tests for Irritation and Sensitization.

This study will be conducted in accordance with the requirements of the FDA Good Laboratory Practice (GLP) Regulations, 21 CFR 58.

Test Article:

The sponsor will submit the material to be tested. Detailed information about the test article will be provided by the sponsor on the GLP Compliance Notification form furnished by NAMSA or on a similar attachment to the protocol.

The following is to be completed (with initials and dates in the margin) by the sponsor or study director. Further instructions may be attached to the protocol. The sample will be prepared as follows:

1. Ratio of test article to extraction vehicle:
   - ___ Material thickness less than 0.5 mm - ratio of 120 cm^2:20 ml
   - ___ Material thickness greater than or equal to 0.5 mm - ratio of 60 cm^2:20 ml
   - ✓ Irregularly shaped objects and/or sponsor option - ratio of 4 g:20 ml
   - ___ Other (explain)_____

LLT 11-21-01

2. Extraction vehicle:
   - ✓ 0.9% sodium chloride USP solution (SC)
   - ___ alcohol in saline 1:20 solution (AS)
   - ___ polyethylene glycol 400 (PEG)*
   - ✓ cottonseed oil, NF (CSO)
   - ___ Other (specify)_____

   NOTE: Due to the known pH of these vehicles, the pH of the test article extracts will not be determined.

   *If PEG is used, the PEG test extract and reagent control will be diluted with saline to provide 120 mg of PEG/ml.

3. Extraction conditions:
   - ✓ 37°C, 72 hours (± 2 hours)
   - ___ 121°C, 1 hour (± 0.2 hours)
   - ___ 70°C, 24 hours (± 2 hours)
   - ___ 50°C, 72 hours (± 2 hours)
   - ___ Other (specify) _____

Control Article:

Reagent controls (extraction vehicle without test material) will be prepared in the same way and at the same time as the test extracts.

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Test System:

Species:	Rabbit (*Oryctolagus cuniculus*)
Strain:	New Zealand White
Source:	Single USDA licensed supplier
Sex:	No particular gender is prescribed in this test
Body Weight Range:	2.0 kg or greater at selection
Age:	Young adults
Acclimation Period:	Minimum 5 days
Number of Animals:	Three per pair of extracts
Identification Method:	Ear tag

Justification of Test System:

The intracutaneous injection test in rabbits is specified in the current ISO testing standards and has been used historically to evaluate biomaterial extracts.

Duplication of Experimental Work:

By signature on this protocol, the sponsor confirms that the conduct of this study does not unnecessarily duplicate previous experiments.

Animal Management:

Husbandry:	Conditions will conform to Standard Operating Procedures which are based on the "Guide for the Care and Use of Laboratory Animals."
Food:	A commercially available, pelleted, rabbit feed will be provided daily.
Water:	Freely available, municipal water will be delivered through an automatic watering system.
Contaminants:	Reasonably expected contaminants in feed or water supplies should not have the potential to influence the outcome of this test.
Housing:	Animals will be individually housed in stainless steel suspended cages identified by a card indicating the lab number, animal number, test code, sex, and date dosed.
Environmental:	The room temperature will be monitored daily. The recommended temperature range for the room is 61-72°F. The room humidity will be monitored daily. The humidity range for the room is 30-70%. The light cycle will be controlled using an automatic timer (12 hours light, 12 hours dark).
Facility:	NAMSA is an AAALAC International accredited facility and is registered with the United States Department of Agriculture. Additionally, NAMSA maintains an approved Animal Welfare Assurance on file with the National Institutes of Health, Office for Laboratory Animal Welfare.
Personnel:	Associates involved will be appropriately qualified and trained.

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Selection: Only healthy, thin-skinned animals free of mechanical irritation or trauma that could interfere with the test will be selected. To reduce the number of animals used for testing, and to comply with the directives of the NAMSA Institutional Animal Care and Use Committee (IACUC), rabbits on this study may have been used previously in an unrelated test model. Any previously evaluated test or control articles did not cause a response in the animals. Complete history of animal usage is traceable in laboratory records. Animals used for previous evaluations will be identified in the report.

Sedation, Analgesia or Anesthesia: It has been determined that the use of these agents will not be necessary during the routine course of this procedure.

Veterinary Care: In the unlikely event that an animal should become injured, ill, or moribund, care will be conducted in accordance with current veterinary medical practice. If warranted for humane reasons, euthanasia will be conducted in accordance with the current report of the American Veterinary Medical Association's Panel on Euthanasia. The objective of the study will be given due consideration in any decision and the study sponsor will be advised.

IACUC: This protocol has been approved by NAMSA Institutional Animal Care and Use Committees (IACUC), and is reviewed at least annually by the same committees. Any significant changes to this protocol must be approved by the IACUC prior to conduct.

Methods and Route of Administration:

The day prior to treatment, each rabbit will be weighed and clipped free of fur from the back and both sides of the spinal column to yield a sufficient injection area. The clipped area of the back will be wiped with a 70% alcohol soaked gauze pad just before injection and allowed to dry. Due to concern with the crowding and subsequent obscuring of injection sites, the test and control sites will not be cranial and caudal on the same side of the back as defined in the ISO standards. Each test extract will be administered in five intracutaneous injections of 0.2 ml each on the right side of each rabbit's back. Five reagent control injections will be injected similarly on the left side of the back. No more than two test extracts and the corresponding reagent controls will be injected into each animal. Injections will be about 2 cm apart. The appearance of the injection sites will be noted immediately after injection.

Observations for erythema and edema will be noted for each injection site at 24 (± 2 hours), 48 (± 2 hours) and 72 (± 2 hours) hours after injection. Reactions will be scored on a 0 to 4 basis. Other adverse changes at the injection sites will also be noted. After the test is completed, all animals will be handled in accordance with IACUC approved NAMSA procedures. The reactions will be evaluated according to the subjective rating scale as shown below:

ERYTHEMA (ER)		EDEMA (ED)	
0	No erythema	0	No edema
1	Very slight erythema (barely perceptible)	1	Very slight edema (barely perceptible)
2	Well-defined erythema	2	Well-defined edema (edges of area well-defined by definite raising)
3	Moderate erythema	3	Moderate edema (raised approximately 1 mm)
4	Severe erythema (beet redness) to eschar formation preventing grading of erythema	4	Severe edema (raised more than 1 mm, and extending beyond exposure area)

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Evaluations and Statistics:

No statistical analysis of the data will be performed. For each animal, the erythema and edema scores obtained at each time interval will be added together and divided by the total number of observations. This calculation will be conducted separately for each test extract and reagent control. The score for the reagent control will be subtracted from the score for the test extract to obtain the Primary Irritation Score. The Primary Irritation Score of each animal will be added together and divided by the total number of animals. The value obtained is the Primary Irritation Index (PII). The Primary Irritation Index is characterized by number and description as follows: 0-0.4 (negligible), 0.5-1.9 (slight), 2.0-4.9 (moderate), 5.0-8.0 (severe). If the response in the initial test is equivocal, additional testing may be necessary. Any adverse reaction noted in the test extract will be compared to the corresponding reagent control.

Report:

The final report will include a description of the methods employed, individual dermal scores for each test and control injection site, and the assessment of the results (Primary Irritation Scores and the Primary Irritation Index).

Quality Assurance:

Inspections will be conducted at intervals adequate to assure the integrity of the study in conformance with 21 CFR 58.35(b)(3). The final report will also be reviewed for conformance to Section 58.185, Subpart J, of the GLP Regulations. A Certificate of Quality Assurance Inspections will be provided with the final report.

Records:

Test article and reagent control preparation data, dates of relevant activities (such as the study initiation and completion), the appearance of each injection site immediately after injection, individual dermal scores at 24, 48, and 72 hours, the Primary Irritation Score, and the Primary Irritation Index will be recorded.

All raw data pertaining to this study and a copy of the final report will be retained in designated NAMSA archive files.

Proposed Dates:

The study dates will be finalized by the study director following receipt of the sponsor-approved protocol and appropriate material for the study. Initiation of the study will be the date on which the study director signs the GLP protocol. Projected dates for starting the study (first treatment) and for the completion of the study (final report release) will be provided to the sponsor (or representative of the sponsor) and added to the protocol.

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References:

21 CFR 58 (GLP Regulations).

Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research, National Academy of Sciences (Washington: National Academy Press, 1996).

International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 10: Tests for Irritation and Sensitization.

OLAW, Public Health Service Policy on Humane Care and Use of Laboratory Animals.

United States Code of Federal Regulation (CFR) 9: The Animal Welfare Act.

United States Pharmacopeia (USP), current edition.

Protocol Changes:

Any necessary changes to the protocol after sponsor approval or study initiation will be documented and approved by the study director as protocol amendments. Copies will be distributed to the sponsor, the raw data file, and the NAMSA Quality Assurance department.

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December 10, 2001

BILL BEASLEY
BIOMIMETIC PHARMACEUTICALS
1616 WESTGATE CIRCLE
BRENTWOOD, TN 37027

PROTOCOL AMENDMENT I

Test Article: rhPDGF-BB (Becaplermin - 1 mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold

Identification: Lot #QA2217 and Lot 081098-146

NAMSA Lab No.: 01T 15267 00

We have received appropriate test articles and approved protocols for the program to be conducted in accordance with the Good Laboratory Practice (GLP) Regulations on the material described above. Below is a projected schedule for the work to be performed.

NAMSA Code	Study	Estimated Start Date:	Estimated Report Release Date:
V0014-130	Cytotoxicity Study using the ISO Elution Method (Extract)	Dec. 13, 2001	Jan. 8, 2002
TI261-300	ISO Sensitization Test in Fifteen Guinea Pigs, Saline Extract		
TI261-301	ISO Sensitization Test in Fifteen Guinea Pigs, Cottonseed Oil Extract	Dec. 14, 2001	Jan. 25, 2002
TI251-800	ISO Acute Intracutaneous Reactivity Study in Three Rabbits Saline Extract, Cottonseed Oil Extract	Dec. 17, 2001	Jan. 8, 2002
TI264-500	ISO Acute Systemic Toxicity Study in Five Mice, Saline Extract, Cottonseed Oil Extract	Dec. 17, 2001	Jan. 8, 2002
V0023-211	Genotoxicity: Bacterial Reverse Mutation (Ames) Test, Salmonella and *E. coli* strains, Saline Extract		
V0023-212	Genotoxicity: Bacterial Reverse Mutation (Ames) Test, Salmonella and *E. coli* strains, DMSO Extract	Dec. 18, 2001	Jan. 15, 2002
TI247-804 TH035-800	ISO Surgical Muscle Implantation, in Three Rabbits (4 Weeks) With Histopathology	Dec. 19, 2001	Jan. 25, 2002

Laura L. Tasse

Laura L. Tasse, BS, ALAT
Study Director

Dec. 10, 2001
Date

LLT/dap

cc: QA (NAMSA)
GLP study file

10600

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December 10, 2001

SAMUEL LYNCH, M.D.
BIOMIMETIC PHARMACEUTICALS
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PROTOCOL AMENDMENT II

Test Article:	rhPDGF-BB (Becaplermin – 1 mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold
Identification No.:	Lot #QA2217 and Lot 081098-146
Protocol:	V0014-130 Cytotoxicity Study using the ISO Elution Method (Extract) TI261-300 ISO Sensitization Test in Fifteen Guinea pigs (Saline extract) TI261-301 ISO Sensitization Test in Fifteen Guinea pigs (Cottonseed oil extract) TI251-800 ISO Acute Intracutaneous Reactivity Study in Three Rabbits TI264-500 ISO Acute Systemic Toxicity Study in Five Mice V0023-211 Genotoxicity: Bacterial Reverse Mutation (Ames) Test (Saline extract) V0023-212 Genotoxicity: Bacterial Reverse Mutation (Ames) Test (DMSO extract)
NAMSA Lab No.:	01T 15267 00

This amendment was written to clarify the preparation of the test article.

The two components of the test article must be combined just prior to extraction preparation. To do this, a 1:1 ratio of Vitoss (in grams) to rhPDGF-BB (in ml's) will be prepared. For example, a 2 g portion of the ChronOS will be saturated with 2 ml of the rhPDGF. If the frozen rhPDGF-BB is used, it must first be thawed prior to the preparation.

The resulting mixture will then be used for the extraction preparation (i.e. weight of the prepared test article to volume of extraction vehicle).

Laura L. Tasse

Laura L. Tasse, BS, ALAT
Study Director

Dec. 10, 2001
Date

cc: NAMSA QAU
GLP study file

NAMSA
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January 8, 2002

BILL BEASLEY
BIOMIMETIC PHARMACEUTICALS
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REVISED*

PROTOCOL AMENDMENT II

Test Article:	rhPDGF-BB (Becaplermin – 1 mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold
Identification No.:	Lot #QA2217 and Lot 081098-146
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This amendment was written to clarify the preparation of the test article.

The two components of the test article must be combined just prior to extraction preparation. To do this, a 1:1 ratio of Vitoss (in grams) to rhPDGF-BB (in ml's) will be prepared. For example, a 2 g portion of the Vitoss* will be saturated with 2 ml of the rhPDGF. If the frozen rhPDGF-BB is used, it must first be thawed prior to the preparation.

The resulting mixture will then be used for the extraction preparation (i.e. weight of the prepared test article to volume of extraction vehicle).

* This amendment was revised to correct the sponsor name and address, and to correct the name of the Vitoss in the second paragraph.

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Jan. 8, 2002
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BM001804

Appendix 7.7.11: Test Report TI264-500. ISO acute systemic toxicity study in the mouse (Extracts)

Confidential

TI264-500

Lab No. 01T 15267 00

P.O. No. 01-1005
GLP

STUDY TITLE:

ISO ACUTE SYSTEMIC TOXICITY STUDY IN THE MOUSE

(Extracts)

TEST ARTICLE:

rhPDGF-BB (Becaplermin - 1 mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold

IDENTIFICATION NO.:

Lot #QA2217 and Lot 081098-146

TEST FACILITY:

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BM001806

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SUMMARY

The test article, rhPDGF-BB (Becaplermin - 1 mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold, Lot #QA2217 and Lot 081098-146, was extracted in 0.9% sodium chloride USP solution and cottonseed oil, NF. These extracts were evaluated for systemic toxicity in accordance with the requirements of the International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 11: Tests for Systemic Toxicity.

A single dose of the appropriate test article extract was injected into each of five mice per extract by either the intravenous or intraperitoneal route. Similarly, five mice were dosed with each corresponding reagent control. The animals were observed immediately and at 4, 24, 48, and 72 hours after systemic injection.

Under the conditions of this study, there was no mortality or evidence of systemic toxicity from the extracts. Each test article extract met the test requirements.

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INTRODUCTION

The test article identified below was extracted and the extracts were evaluated for biocompatibility in accordance with the requirements of the International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 11: Tests for Systemic Toxicity. The purpose of the study was to determine whether leachables extracted from the material would cause acute systemic toxicity following injection into mice. The test article was received on November 20, 2001 and December 10, 2001. The animals were dosed on December 14, 2001, and the observations were concluded on December 17, 2001.

The study, initiated by protocol signature on December 10, 2001, was conducted in accordance with the provisions of the FDA Good Laboratory Practice (GLP) Regulations, 21 CFR 58. A Certificate of Quality Assurance Inspections was issued with this report.

MATERIALS

The sample provided by the sponsor was identified and handled as follows:

Test Article:	rhPDGF-BB (Becaplermin - 1 mg/ml 20 mM NaAc Buffer) and Vitoss Scaffold
Identification No:	Lot #QA2217 and Lot 081098-146
Stability Testing:	In Progress (per sponsor)
Expiration Date:	Stable for duration of intended testing (per sponsor)
Storage Conditions:	rhPDGF-BB – refrigerated Vitoss Scaffold – room temperature
Vehicles:	0.9% sodium chloride USP solution (SC) cottonseed oil, NF (CSO)
Preparation:	A 2 g portion of Vitoss Scaffold was saturated with a 2 ml portion of rhPDGF-BB for each extraction vehicle immediately prior to pouring. Based on a ratio of 4 g:20 ml, a 3.5 g portion of the prepared test article was covered with 18 ml of the vehicle. The prepared test article was extracted in SC and CSO at 37°C for 72 hours. The extraction vehicles without test article were similarly prepared to serve as reagent controls.

Condition of Extracts:

	Test	Control
SC:	clear with particulates*	clear
CSO:	cloudy with particulates	clear

*The test extract was filtered with a 0.8 µm filter disc to yield a clear, particulate free extract.

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Section 7: Bibliography

7.0 BIBLIOGRAPHY/REFERENCES/OTHER INFORMATION

A list of references is provided below. An annotated bibliography that includes all the references listed below is included as well as copies of selected references.

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Annotated Bibliography

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results	Conclusions
1	"Platelet-Derived Growth Factor Expression in Normally Healing Human Fractures" By: J.G. Andrew, J.A. Hoyland, A.J. Freemont, D.R. Marsh Synopsis: Biopsy material from 16 healing human fractures between 1 and 6 weeks after surgery for malunion were studied to better understand the role of Platelet Derived Growth Factor (PDGF), in vivo, in bone repair. Immunohistocehmistry (for PDGF) and in situ hybridization (for the PDGF A and B chains) were used to evaluate the possible role of PDGF during fracture healing.	16	N/A	-Grade 1 (fracture hematoma and granulation tissue): PDGF protein was detected in the early stages of fracture healing, but gene expression was not seen for PDGF A or B chains. PDGF A gene expression as well as PDGF protein was noted in endothelial cell cells as well as in "mesenchymal cells" . PDGF B gene expression was less. -Grade 2: (definitive matrix formation): PDGF A mRNA was seen in hypertrophic chondrocytes and osteoblasts while low levels of PDGF B mRNA were seen only in cuboidal osteoblasts. PDGF A gene expression was positive in the endothelial and perivascular cells of vessels, but PDGF B mRNA was absent. PDGF staining was present on all osteoblasts, although the staining of flat surface osteoblasts was variable. The staining was also most pronounced in osteoid immediately adjacent to osteoblasts and woven bone. -Grade 3: PDGF A gene was expressed in the chondrocytes and osteoblasts, but few expressed PDGF B mRNA. Osteoclasts were nearly all positive for PDGF A, but only a few were positive for PDGF B. The amount of PDGF in bone matrix was less, but PDGF A and B chains were positive in the round mononuclear cells of the trabeculae. Endothelium was positive for PDGF protein and gene (A and B) expression.	PDGF is expressed during normal human fracture repair. This study confirmed prior in vitro data that PDGF is an important natural modulator of bone wound repair and new blood vessel formation..
2	"An evaluation of Tricalcium Phosphate Implants in Human Periodontal Osseous Defects of Two Patients" By: W.T. Baldock, L.H.	2	N/A	-Clinical: Post-surgical healing was normal and without complications; primary interproximal wound closure in 10 of 13 implant sites was obtained; after 1 week, 3 sites lost interdental papillary soft tissue covering and TCP was visible until second week. By week 3 the	TCP alone is a clinically well tolerated material but was not shown to result in new bone formation in this study evaluating 2 patients. This finding suggests that TCP requires supplementation in order to optimize

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	Hutchens Jr., W.T. McFall, Jr., D.M. Simpson Synopsis: Thirteen defects in two patients were treated with mucoperiosteal flaps and implantation of TCP. The defects were then evaluated clinically and radiographically utilizing standardized probe placement and radiographic technique.			interdental gingival covering was evident. Probing pocket reduction of 4.5mm seen after 3-9 months. -Radiographic: A 1.8mm increase (fill) in the coronal position of the preoperative Hirschfield point in the defects seen. The implant material was radiographically visible during all observation periods. Demarcation between TCP implant material and bone and proximal tooth was evident. No radiographic evidence of root resorption or ankylosis seen. -Histologic Findings: TCP present in all specimens as vacuoles due to decalcification of implant material. No evidence of ongoing sequestration of TCP. TCP vacuoles separated by fibrous connective tissue from the osseous defect. There was limited evidence of bone replacement of the TCP vacuoles, but some new bone formation was seen on the margins of the adjacent host bone. Connective tissue attachment to the new and old cementum was seen and new cementum formed coronal to the apical reference notch in 3 specimens.	its role in bone regeneration.
3	"Platelet-derived Growth Factor Stimulates Hyaluronate but not Proteoglycan synthesis by human gingival fibroblasts in vitro" By: P. M. Bartold Synopsis: To determine the effects of Platelet Derived Growth Factor-BB on human gingival fibroblasts. Human gingival fibroblast	Not specified; listed as healthy donors	Present, dose response studies were carried out on triplicate cultures	-Mitogenicity of PDGF: Control cultures exposed only to serum showed a dose dependent response to FCS (0% FCS-little proliferation, 10% FCS-5 fold increase). The cells responded to PDGF with a marked increase in proliferation over the controls, the highest being a 10 fold increase over the control conditions of 0.2%FCS. -Effect of PDGF on proteoglycan synthesis: Increasing amounts of FCS stimulated the amounts of proteoglycans. The presence of PDGF in 0.2% serum had no stimulatory effect on the amount of proteoglycans synthesized by human gingival fibroblasts. -Effect of PDGF on glycosaminoglycan species:	PDGF was found to stimulate gingival fibroblast proliferation and other factors associated with early wound healing and repair, which may be significant in the process of repair and regeneration of periodontal tissue.

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	cultures were evaluated for their response to PDGF-BB, specifically as it related to the synthesis of important extracellular matrix components, proteoglycan and hyaluronate.			In all culture conditions, no significant differences were noted in the distribution of this proteoglycan component. -Effect of proteoglycan hydrodynamic size: Chromatography was used to determine the effects of PDGF on the size of proteoglycans synthesized. Two peaks were noted for the material secreted into the medium for cells cultured in FCS and PDGF, but only one peak for cells cultured in FCS. There were no differences in elution profiles for the cell layer associated material either. -Effect of PDGF on hyaluronate synthesis: The response to PDGF was dose-dependent and showed a significant increase over the control cultures exposed to 0.2% FCS alone. The stimulatory effect was evident within 24 hours of exposure to PDGF. -Specific Activity Determinations: The specific activity of glycosaminoglyans was assessed by dual labeling and analysis of marker ratios in digested disaccharides. The marker (S/H) ratio increased in proportion to the levels of FCS and PDGF present in the culture medium. The ratio for chondroitinase ABC disaccharides from cultures exposed to PDGF in 0.2% and 10% FCS was 1.4 times greater than that for control cultures in 0.2% FCS alone.	
4	"Osteoinductivity of Subcutaneous Ultraporous Beta-TCP with Isologous Bone Marrow in Rats" By: L. Bellencampi, T. Clineff, and E. Erbe Synopsis: In this study, β-TCP used alone or in combination with bone	Vitoss β-TCP; 48 female athymic nude rats	N=8 for each type of implant	Bone formation was evaluated at 14, 28, and 56 days after implantation. β-TCP + BMA samples and animals implanted with human DBM had significantly more alkaline phosphatase activity than β -TCP alone or UHMWPE at all time periods. β -TCP + BMA induced significantly more bone formation than other types of implants at all but one time point (day 56 v. DBM). Increased bone formation with time was observed with the β -TCP + BMA implant. Significantly	This study suggests that β -TCP used in combination with bone marrow aspirate is more effective at inducing bone formation than the porous ceramic alone, demineralized bone matrix or a protein extract obtained from demineralized bone.

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	marrow aspirate was compared to DBM and UHMWPE in a subcutaneous implant rat model for its ability to induce bone formation .			more new bone formation was found at 56 days compared to 14 and 28 days.	
5	"PLF and Iliac Crest Repair with a Synthetic Cancellous Bone in the Baboon" By: L. Bellincampi, E, Ledet, B. Sachs, T. Clineff, and E. Erbe Synopsis: β-TCP + bone marrow aspirate was compared to autologous bone used in posteolateral spinal fusion procedures performed in baboons. CT scan evaluations were performed over a 6 month period of time. Additionally, β-TCP was evaluated for its ability to facilitate healing of an iliac crest donor site.	Vitoss β-TCP; 3 skeletally mature baboons	N/A	CT scans revealed indications of early immature bone formation posteriorly with no detectable differences between autologous bone and β-TCP/BMA treatments. At 6 months, the formation of a fusion mass in all animals between the vertebral bodies indicated efficacy of the synthetic bone.	The results of this study show that the β -TCP scaffold was completely effective in promoting iliac crest repair and was equally effective as autologous bone grafts when used in combination with bone marrow aspirate for posterolateral fusion of vertebral bodies.
6	"Tissue reaction to intrabony ceramic implants" By: S.N. Bhaskar, D.E. Cutright, M.J. Knapp, J.D. Beasley, B. Perez, M. Driskell Synopsis: This studied investigated the histologic tissue reaction to ceramic	60 albino rats, 4 miniature pigs, 2 monkeys	N/A	-Rats: Implants placed into surgically created defects in the femur. One day after implantation, the area of the ceramic was surrounded by edema, neutrophils, and red blood cells. After 4 days, the ceramic pores had plasma cells and lymphocytes and fibroblastic proliferation. After 14 days, the periphery of the implant and alveoli showed fibroblastic proliferation and formation of bone (mediated by osteoblasts) and osteoid. From 28-56 days bone formation in the implant continue and bone marrow elements differentiated.	This study confirms that the phosphate-bonded ceramic is well tolerated by tissues, does not elicit a foreign-body reaction, is quickly infiltrated by connective tissue and bone marrow, and forms a latticework upon which osteoid is deposited.

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				disadvantage of CPC is its lack of macropores. Thus, biodegradation occurs layer by layer from outside to inside. Solutions to this problem include the addition of soluble particles into the cement.	
8	"IMMUNOLOCALIZATION AND EXPRESSION OF BONE MORPHOGENETIC PROTEINS 2 AND 4 FRACTURE HEALING" By: M.P. Bostrom, J.M. Lane, W.S. Berberian et.al. J. Orthop Res 1995	Study of the definition and characterization of bone morphogenetic proteins in an established rat fracture healing model. In each of 42 rats, femoral diphyseal fractures were created. Rats were divided into 7 groups of six animals. One group each was killed postfracture on days 2,4,6,8, 10,14, and 21. At sacrifice radiographs were taken to evaluate bone formation. The femora was removed and immunohistochemical analysis using monoclonal antibodies	N/A	Based on radiographic studies, calcification of the calluses were evident at day 10 postfracture. Immunohistochemical results analyzed both in terms of cellular and temporal expression of BMP-2 and BMP-4 and relative to intramembranous bone formation and endochondral ossification that proceeded from the initial fracture hematoma through chondrogenesis and subsequent formation of new bone. Staining was intracellular with the exception of extracellular matrix staining in the periosteum and the periosteoblastic areas of primitive woven bone. No other tissues contained stain. Intramembranous bone formation (*i.e.*, bone formation with cartilaginous intermediates): Positive staining was present immediately after fracture in the cambium cell layer of the persiosteum proximal and distal to the fracture site. These periosteal cells increased as fracture healing progressed and there was a minimal increase in the percentage of cells staining positively. By postfracture day 6, abundant staining was evident in osteoblasts lining primitive woven bone. Woven bone remodeled into lamellar bone showed a decrease in intensity of osteoblasts, however, the percentage staining positive remained constant. As the process of lamellar bone formation continued, the number of periosteal cells and percentage staining positive decreased. Endochondal Ossification: Within several days after the fracture, periosteal cells and osteoblasts	It appeared that the act of fracturing a bone not only stimulated the periosteal reaction seen histologically but also induced the synthesis of BMP 2 and BMP-4 by the same cells. As bone formation progressed, the number of more mature cell types increased and BMP-2 and BMP-4 decreased. It appeared that as undifferentiated primitive cells proliferated in the fracture hematoma, the synthesis of BMP-2 and BMP-4 was induced. Formation of osteoblastic lamellar bone, especially during remodeling, was not associated with a dominant BMP process. Data from the study, as well as the know osteoinductive capabilities of BMPs, suggest that BMP-2 and BMP-4 are important regulators of cell differentiation during fracture repair.

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		specific for BMP-2 and BMP-4		demonstrated intense staining for BMP-2 and BMP-4. As woven bone was replace with lamellar bone, staining decreased.	
9	"Factors in the fracture microenvironment induce primary osteoblast angiogenic cytokine production" By: P.J. Bouletreau, S.M. Warren, J.A. Spector, D.S. Steinbrech, B.J. Mehrara, and M.T. Longaker July 2002 *Angio Cytokine Production* Synopses: The purpose of this *in vitro* study was to investigate the effect of recombinant human platelet derived growth factor BB isoform (rhPDGF-BB) on the synthesis of vascular endothelial growth factor (VEGF) by neonatal rat osteoblasts, and determine whether PDGF works in concert with hypoxia to additively or synergistically induce VEGF production.	Neonatal rat osteoblast cultures incubated with various doses of rhPDGF-BB (1 to 100 ng/mL) under normoxic and hypoxic (<1% O_2 tension) conditions.	Neonatal rat osteoblast cultures incubated without rhPDGF-BB stimulation under normoxic and hypoxic.	rhPDGF-BB induced osteoblast VEGF mRNA expression in a dose-dependent manner, with 50 ng/ml producing a maximal 2.4-fold increase in VEGF mRNA expression compared with control. Peak VEGF mRNA expression occurred at 3 hours and gradually declined to 1.5-fold at 24 hours. rhPDGF-BB and hypoxia seemed to have an additive effect, resulting in a 3.7-fold increase in VEGF mRNA expression after 6 hours compared with control. Using inhibitors of RNA transcription (actinomycin D) and protein translation (cycloheximide) demonstrated that rhPDGF-BB-induced VEGF mRNA expression was transcriptionally mediated and not dependent on de novo protein synthesis. VEGF mRNA stability was not affected by rhPDGF-BB.	This *in vitro* study demonstrated that rhPDGF-BB transcriptionally increases osteoblasts VEGF mRNA expression *in vitro* in a time- and dose-dependent fashion. Furthermore, rhPDGF-BB and hypoxia act additively through different pathways to increase VEGF mRNA expression. Similar mechanisms may occur *in vivo*, at a site of skeletal injury, to induce neoangiogenesis and promote fracture repair.
10	"Is PDGF really important? Testing the	Patch Mutant Mouse	N/A	The PDGF receptor (PDGFR) alpha gene is deleted in the Patch Mouse, which was studied.	This article outlined the various roles PDGF plays in wound healing as well

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	hypotheses" By: Daniel F. Bowen-pope, Antje van Koppen, Gina Schatteman Synopsis: After discussing the various approaches that have already been utilized to study the function of PDGF, the consequences of the deletion of a PDGF receptor gene in the Patch mutant mouse was discussed. The effects of this deletion on the embryo were assessed in order to elucidate the function of the PDGF α receptor during development.			Ph/Ph embryos do not survive to birth, perhaps because of this. PDGFR-alpha is expressed by extraembyonic endoderm and primitive mesoderm. Lack of expression in Ph/Ph mutants doesn't prevent germ layer formation. As development proceeds, lack of PDGFR-alpha expression retards the proliferation of mesenchymal cells throughout the embryo. Many mesodermal derivatives that express PDGF-alpha in normal embryos are poorly developed in Ph/Ph embryos. This accounts for the kinking of the neural tube at this stage. About two-thirds of the embryos die at the end of this period. Embryos that survive show abnormalities in mesenchymal structures, including significant skeletal abnormalities. Other organ defects are seen in those organs that depend on a non-neurogenic subset of neural crest cells. High PDGFR-alpha expression is detected in the lens of the eye and lens development is abnormal in Ph/Ph embryos. Ph/Ph embryos also have a very poorly developed choroid plexus, and thus may not be able to produce enough cerebrospinal fluid to maintain adequate intraventricular pressure.	as other physiological .processes. In an attempt to better understand specific roles of the PDGF -α receptor during development, the authors evaluated Patch mouse embryos which lack this receptor. The results indicate that this PDGF receptor, and therefore presumable PDGF, is critical in the development of many mesordermal and some ectodermal derivatives, including the skeleton.
11	"Evaluation of Filling Materials in Membrane Protected Bone Defects: A Comparative Histomorphometric Study in the Mandible of Miniature Pigs" By: D. Buser, B. Hoffmann, J.P. Bernard, A. Lussi, D. Mettler, and R.K. Schenk Synopsis: This study	β-TCP, collagen sponge, HA and allograft bone; 12 adult miniature pigs	One filler was a blood clot (-) control and another was an autograft (+) control	Clinical and Radiographic: During healing, no signs of postoperative complications were noted. Following sacrifice, 1 animal in the 24 week group showed displacement of all miniscrews and signs of chronic infection in the bone (animal withdrawn from analysis) Qualitative Histologic Observations: -Blood Clot (negative control sites): Initially, bone formation in membrane-covered defects was seen. Mineralization followed and after 12 weeks, all the defects were almost completely filled with new bone. The original scaffold of woven bone was recognizable but was reinforced	The results of this study show that while sites filled with autograft demonstrated the best osteoconductive results in the early phase of healing, β-TCP showed a significantly higher percentage of bone fill at 24 weeks post-surgical, exceeding results obtained with autograft or any other tested bone substitute material. Thus, among the tested bone substitutes, β-TCP had the best osteoconductive properties.

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results	Conclusions
	evaluated 4 different bone-filling materials and their potential in membrane-protected defects with regard to biocompatibility, osteoconductivity, degradability, and substitution.			by layers of lamellar bone. The 3-fold increase in width of the trabeculae occurred at the expense of the marrow. After 24 weeks, remodeling became the dominant process and corticalization was achieved by continuous bone deposition that narrowed the vascular spaces. Most of the trabecular surface was subjected to either osteoclastic resorption or lamellar bone formation. -Autografts (positive control sites): Harvesting autografts resulted in cortical bone chips of various shapes/sizes. At 4 weeks, chips were evenly distributed within the defect area. At 12 weeks, it was noted that lamellar bone deposition had continued through the 2nd and 3rd month. At 24 weeks, ongoing remodeling activity resulted in a relative increase of the lamellar bone compartment at the expense of woven bone, but the grafted, non-vital remnants persisted. -β-TCP: At 4 weeks, bone formation exhibited an inconsistent pattern where bony ingrowth alternated with ceramic particles embedded in fibrous tissue only. At 12 weeks, bone had filled in all of the defects resulting in homogenous osseous structure. At 24 weeks, extensive bone remodeling became obvious. Cement lines, secondary osteons, and lamellar packets were frequently found within and on the surface of the trabeculae of cancellous bone. Some of the particle surface underwent resorption by osteoclast like giant cells. -Collagen Sponge: Had same healing pattern as negative control sites. After 4 weeks, no remnants of the collagen sponge could be detected and the central area showed a large portion of granulation tissue invaded by bone trabeculae, which consisted partially of osteoid. -Allografts: At 4 weeks, bone formation started and the central area beneath the membrane had	

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				only DFDBA particles embedded in granulation tissue. At 12 weeks, bone formation spread over the whole defect area and achieved an overall density similar to that of the controls. This bone included a considerable amount of grafted material and represents a mixture of recalcified DFDBA, woven bone, and lamellar bone deposits. At 24 weeks density and overall structure of the bony regenerate in this group closely resembled specimens examined at 12 weeks. -Coral-derived HA: At 4 weeks, coral-derived HA granules were evenly dispersed in the defect sites and were invaded by dense fibrous tissue and bony trabeculae. At 12 weeks, the coralline substrate appeared unchanged, but bony filling of its pores had become much denser. The overall remodeling activity was low. At 24 weeks, the composite of coralline filler and bony regenerate was virtually the same, but bone structure had matured.	
12	"Evaluation of Filling Materials in Membrane Protected Bone Defects: A Comparative Histomorphometric Study in the Mandible of Miniature Pigs" By: D. Buser, B. Hoffmann, J.P. Bernard, A. Lussi, D. Mettler, and R.K. Schenk Synopsis: This study evaluated 4 different bone-filling materials and their potential in membrane-protected	β-TCP, collagen sponge, HA and allograft bone; 12 adult miniature pigs	One filler was a blood clot (-) control and another was an autograft (+) control	Clinical and Radiographic: During healing, no signs of postoperative complications were noted. Following sacrifice, 1 animal in the 24 week group showed displacement of all miniscrews and signs of chronic infection in the bone (animal withdrawn from analysis) Qualitative Histologic Observations: -Blood Clot (negative control sites): Initially, bone formation in membrane-covered defects was seen. Mineralization followed and after 12 weeks, all the defects were almost completely filled with new bone. The original scaffold of woven bone was recognizable but was reinforced by layers of lamellar bone. The 3-fold increase in width of the trabeculae occurred at the expense of the marrow. After 24 weeks, remodeling became the dominant process and corticalization was	A series of currently used bone substitutes were compared histologically in a standard animal model. Among the tested bone substitutes, TCP had the best osteoconductive properties. The results of this study show that while sites filled with autograft demonstrated the best osteoconductive results in the early phase of healing, β-TCP showed a significantly higher percentage of bone fill at 24 weeks post-surgical, exceeding results obtained with autograft or any other tested bone substitute material.

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results	Conclusions
	defects with regard to biocompatibility, osteoconductivity, degradability, and substitution.			achieved by continuous bone deposition that narrowed the vascular spaces. Most of the trabecular surface was subjected to either osteoclastic resorption or lamellar bone formation. -Autografts (positive control sites): Harvesting autografts resulted in cortical bone chips of various shapes/sizes. At 4 weeks, chips were evenly distributed within the defect area. At 12 weeks, it was noted that lamellar bone deposition had continued through the 2nd and 3rd month. At 24 weeks, ongoing remodeling activity resulted in a relative increase of the lamellar bone compartment at the expense of woven bone, but the grafted, non-vital remnants persisted. -β-TCP: At 4 weeks, bone formation exhibited an inconsistent pattern where bony ingrowth alternated with ceramic particles embedded in fibrous tissue only. At 12 weeks, bone had filled in all of the defects resulting in homogenous osseous structure. At 24 weeks, extensive bone remodeling became obvious. Cement lines, secondary osteons, and lamellar packets were frequently found within and on the surface of the trabeculae of cancellous bone. Some of the particle surface underwent resorption by osteoclast like giant cells. -Collagen Sponge: Had same healing pattern as negative control sites. After 4 weeks, no remnants of the collagen sponge could be detected and the central area showed a large portion of granulation tissue invaded by bone trabeculae, which consisted partially of osteoid. -Allografts: At 4 weeks, bone formation started and the central area beneath the membrane had only DFDBA particles embedded in granulation tissue. At 12 weeks, bone formation spread over the whole defect area and achieved an overall density similar to that of the controls. This bone	

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				included a considerable amount of grafted material and represents a mixture of recalcified DFDBA, woven bone, and lamellar bone deposits. At 24 weeks density and overall structure of the bony regenerate in this group closely resembled specimens examined at 12 weeks. -Coral-derived HA: At 4 weeks, coral-derived HA granules were evenly dispersed in the defect sites and were invaded by dense fibrous tissue and bony trabeculae. At 12 weeks, the coralline substrate appeared unchanged, but bony filling of its pores had become much denser. The overall remodeling activity was low. At 24 weeks, the composite of coralline filler and bony regenerate was virtually the same, but bone structure had matured.	
13	Camelo M. Advances in periodontal regeneration procedures. *American Academy of Periodontology annual meeting*, September 2001.				
14	"Clinical, radiolographic and histologic evaluation of rhPDGF-BB in a osteoconductive bone matrix for the treatment of advanced osseous periodontal defects in humans" By: M. Camelo Synopsis: The objective of this human trial was to evaluate the safety and effectiveness of rhPDGF-BB in an osteoconductive	rhPDGF-BB in three amounts 0.5 mg/ml, 1.0 mg/ml and 5.0 mg/ml; 9 patients- 11 osseous defects.	Historical controls	This study was designed to evaluate the safety and preliminary effectiveness of rhPDGF-BB administered in a bone matrix to periodontal osseous defects, including both mandibular molar Class II furcation defects and interproximal intrabony defects. There were no safety issues associated with use of rhPDGF-BB administered in a bone matrix. Efficacy data demonstrated a favorable outcome clinically, radiographically and histologically following application of the rhPDGF-BB product. Clinical measurements of pocket depth (PD) and clinical attachment level (CAL) were significantly improved. Comparison to a meta analysis of sixteen previous bone grafting clinical trials, suggested	This human clinical trial indicates that rhPDGF-BB in a osteoconductive bone matrix for the filling of bone voids around teeth is safe. Further, the results suggest that better clinical, radiographic and histologic results, i.e. bone and periodontal regeneration, can be achieved with this device than has heretofore been possible.

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	matrix for treatment of osseous periodontal defects.			that rhPDGF-BB may lead to improved clinical outcomes compared to existing therapies. Radiographic analysis suggested bone fill in the defects and no abnormal changes in bone architecture or root configuration. Histologic analysis showed remarkable regeneration of bone, periodontal ligament and cementum in both furcation defects and interproximal angular defects, especially in the 1.0 mg/mL group.	
15	"Periodontal Regeneration can be Achieved in Human Class II Furcations using Purified Recombinant Human Platelet-Derived Growth Factor BB (rhPDGF-BB) with Bone Allograph" By: M. Camelo, Marc Nevins, R. Schenk and S. Lynch, M. Nevins 2003 *J Periodontol* Synopsis: The objective of this human trial was to evaluate the safety and effectiveness of rhPDGF-BB in an osteoconductive matrix for treatment of osseous periodontal defects.			This study was designed to evaluate the safety and preliminary effectiveness of rhPDGF-BB administered in a bone matrix to periodontal osseous defects, including both mandibular molar Class II furcation defects and interproximal intrabony defects. There were no safety issues associated with use of rhPDGF-BB administered in a bone matrix. Efficacy data demonstrated a favorable outcome clinically, radiographically and histologically following application of the rhPDGF-BB product. Clinical measurements of pocket depth (PD) and clinical attachment level (CAL) were significantly improved. Comparison to a meta analysis of sixteen previous bone grafting clinical trials, suggested that rhPDGF-BB may lead to improved clinical outcomes compared to existing therapies. Radiographic analysis suggested bone fill in the defects and no abnormal changes in bone architecture or root configuration. Histologic analysis showed remarkable regeneration of bone, periodontal ligament and cementum in both furcation defects and interproximal angular defects, especially in the 1.0 mg/mL group.	
16	"Evaluation of a Biodegradable Ceramic" By: H.U. Cameron, I MacNab, R.M. Pillar,	12 beagle dogs; for toxicity testing blocks of the material were	N/A	-Toxicity Testing: The local tissue reaction was benign and the implant was surrounded by and infiltrated with fibrous tissue. Rarely were foreign-body giant cells present, however no	Tricalcium phosphate does not produce any untoward tissue or systemic reaction and is thus safe for clinical use. The material also has

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results	Conclusions
	Synopsis: This study evaluates the porous biodegradable ceramic tricalcium phosphate (TCP) and reports on its effects regarding bone ingrowth. The material was implanted in beagle dogs and examined after sacrifice using radiography.	inserted subcutaneously and intramuscularly in each dog. Serum calcium and phosphate levels were monitored; for bone ingrowth studies, TCP cores were implanted into surgically created defects in the metaphyses of the femur in 12 dogs.		inflammatory cells were noted. -Bone Ingrowth: The reaction to the ceramic was benign. Where absorption of the ceramic was taking place, a layer of osteoblasts surrounded the bony trabeculae, while fibroblasts and giant cells were found in the ceramic trabeculae. After 4 months, much resorption had occurred, but at the same time considerable amounts of ceramic were still present. If the implant was placed outside the periosteum or placed subperiosteally and loosely held in place, no bone ingrowth occurred. If smooth cortical bone was roughened, the tendency for ingrowth was increased. When the ceramic was firmly fixed to smooth cortical bone, ingrowth was not marked, but when firmly fixed to raw bleeding bone, ingrowth occurred in each case.	some useful structural properties, but appears of greatest value as a bone graft supplement or substitute.
17	"Platelet-Derived Growth Factor-Modulated Guided Tissue Regenerative Therapy" By: Moon-II Cho, Wen-Lang Lin, Robert J. Genco Synopsis: The goal of the study was to develop an effective regenerative therapy to achieve periodontal regeneration of class III furcation defects. Lesions were protected by a barrier membrane to prevent cell	Beagle Dogs	N/A	-Early Stages of Periodontal Repair: Furcation defect areas were primary filled with granulation tissue comprised of numerous infiltrated inflammatory cells and blood vessels. Thin fibrous connective tissue formed on the root surface and bone. In the periodontal ligament (PDL) and root surface, labeled fibroblasts were in the coronal position. With time, the fibroblasts continued to form connective tissue on the root surface and bone (which replaced inflamed tissue). -Effects of Growth Factors on PDL Fibroblastic Cells: PDGF had strong chemotaxis and proliferation effects and moderate collagen synthesis effects. EGF had slight chemotaxis and proliferation effects and inhibitory collagen synthesis and differentiation effects. TGF-B had	PDGF-Modulated GTR therapy successfully and reproducibly promoted full periodontal regeneration more rapidly and effectively than GTR therapy, and without ankylosis. In short, PDGF-BB modulated GTR therapy may be capable of promoting periodontal regeneration of Class III furcation defects.

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	migration, PDGF-BB was used to promote migration of fibroblasts and their proliferation on root surfaces, and demineralized root surface was chosen as the area for PDGF-BB application.			no chemotaxis or differentiation effects, inhibitory proliferation effects, and moderate collagen synthesis effects. IGF-I had strong chemotaxis, proliferation and differentiation effects but no collagen synthesis effects. PDGF + IGF-I had the strongest chemotaxis and proliferation effects, but undetermined collagen synthesis and differentiation effects. -Use of demineralized root surface as the primary site for PDGF-BB Application: The number of silver grains representing I-EGF declined over time, but a considerable amount were still present at 8 days when compared to the first day of application. -PDGF-BB Modulated Regenerative Therapy: After 5 weeks of therapy, lesions were free of inflamed tissue/epithelium and completely filled with new connective tissue/bone. PDGF-BB modulated therapy did reveal ankylosis in all samples. After 8 weeks, lesions were full with varying amounts of bone, but ankylosis was noted. -PDGF-BB Modulated Guided Tissue Regenerative Therapy: Five weeks after reconstructive surgery, no significant difference in furcation defect repair was noted. As healing progressed, alveolar bone, fibrous connective tissue, and PDL replaced the areas. At 8 weeks, the lesions showed nearly complete regeneration with new bone and PDL formation. No significant root resorption or ankylosis was observed	
18	"Analytical Technique for Quantifying the Resorption of Calcium Phosphate Bone Void Fillers using Polarized-	Vitoss β-TCP; Large mongrel dogs	N/A	The β-TCP implant was visualized as pale pink on bright field microscopy. The H&E slides viewed with polarized light easily differentiated the implant from the bone. Three operators analyzed two separate images of bone and Vitoss	This study suggests that the resorption characteristics of a β-TCP bone void filler can be more accurately measured with this validated technique as opposed to staining alone. This

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results	Conclusions
	light Microscopy" By: T.D. Clineff, G.M. Gualtieri, J.G. Marx, and E.M. Erbe Synopsis: In this study an analytical technique was developed to quantify the resorption of a β-TCP bone void filler. The technique used a light microscope with a polarized filter, a digital camera, and image analysis software.			with 8 repetitions. The results of the study indicated that there were no significant differences between operators and that the quantification methods used were valid. An operator, blinded to explant time, quantified the amount of Vitoss at several explant times. This showed clear discrimination between time periods, further validating the technique.	study confirmed that Vitoss is well tolerated and an effective scaffold for osseous tissue repair.
19	"Surface Contour Analysis of Tibial Plateaus with Subchondral Bone Defects Supplemented with Autograft or Tricalcium Phosphate in a Caprine Model" By: A.R. Cross, E.J. Eschbach, D.D. Lewis, and D.L. Wheeler Synopsis: This study evaluated the ability of β-TCP or autogenous graft materials to maintain joint surface morphology when used to supplement a massive subchondral bone defect in a caprine model.	β-TCP; 16 adult goats	Autograft	One goat in the autogenous bone graft group died prematurely due to a pulmonary ailment, unrelated to surgery. Gross examination of the articular surface of the harvested experimental tibiae showed that 6 out of 7 in the autogenous graft group and 0 out of 8 in the TCP group had degenerative changes. Two of 7 goats in the autogenous graft group had large-scale collapse of the caudal tibial plateau. Mean and maximum surface deviations were much greater for the autogenous group than the TCP group.	β-TCP maintained normal articular surface morphology better than autogenous cancellous bone graft. Joint surface incongruity resulting from the greater surface deviation in the autogenous bone graft group may explain the higher incidence of degenerative change seen in this group. These results indicate that β-TCP may be a useful alternative grafting material in the repair of tibial plateau fractures.
20	"Differential Effect of	Cells from	Present, but	-Effect of Growth Factors on PDL cells: No	TGF-B1, although producing a mild

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results	Conclusions
	TGF-B1 and PDGF on Proliferation of Periodontal Ligament Cells and Gingival Fibroblasts" By: David K. Dennison, Dominic R. Vallone, Gerald J. Pinero, Barry Rittman, and Raul G. Caffesse Synopsis: Gingival fibroblastic cells and cells from the periodontal ligament (PDL) were studied to compare the effects of supplemental addition of TGF-B1 and PDGF on proliferation. Thymidine incorporation in cultures was measured after 24, 48 and 72 hours to determine relationships.	healthy donors (human gingival fibroblasts from biopsy sites, periodontal ligament fibroblasts from tissue cultured from middle 1/3 of the root of premolars extracted for orthodontic reasons or from impacted third molars).	unspecified number	significant effects after 24 hours of culture. After 48 hours, all factors were found to be significantly different from control by Scheffe's comparison. Addition of TGF-B1/PDGF elicited the greatest response (318% of the control) while TGF-B1 resulted in 260% and PDGF in 215% of the control response. After 72 ours, TGF-B1/PDG showed the greatest response, 571% of the control. TGF-B1 alone produced a response 388% of the control. The PDGF response was 144% of the control. -Effect of Growth Factors on Gingival Fibroblasts: After 24 hours, PDGF showed the greatest response at 190% of the control values. Cultures with TGF-B1/PDGF showed 175% response of the control and TGF-B1 showed 133% response of the control value, but insignificant. After 48 hours, PDGF showed a 171% response when compared to the control and TGF-B1/PDGF resulted in 164% response of the control. TGF-B1 resulted in 113% response of the control. After 72 hours, PDGF resulted in a response that was 162% of the control, TGF-B1/PDGf showed a response that was 150% of the control, and TGF-B1 showed a response 126% of the control. -Comparison of Gingival Fibroblasts to Periodontal Ligament Cells: TGF-B1 increased marker incorporation more in PDL cells than in gingival fibroblasts. The difference wasn't significant at 24 hours, but at 48 hours PDL cells incorporated 57% more marker and at 72 hours PDL cells incorporated 162% more marker. PDGF had a minimal effect on both cells, except at 72 hours when marker incorporation was greater for gingival fibroblasts than PDL cells. TGF-B1/PDGF increased PDL cell marker incorporation more than it did in gingival fibroblasts. At 24 hours there was no significant	increase in proliferation, is a poor stimulator of gingival fibroblasts. TGF-B1stimulates proliferation of PDL cells, and the proliferative response of PDL cells to PDGF is enhanced by TGF-B1. In short, there is a positive preferential mitogenic effect of TGF-B1 on PDL cells versus gingival fibroblasts.

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results	Conclusions
				difference between the cells, at 48 hours PDL cell incorporation of thymidine marker was 35 % greater than gingival fibroblasts, and at 72 hours, PDL cell incorporation of thymidine was 227% greater than gingival fibroblasts.	
21	"A New Resorbable Bone Void Filler – Vitoss™ Scaffold" By: David H. Dychala, Jeffrey G. Marx, Erik M. Erbe Synopsis: In an attempt to address some of the shortcomings of currently available synthetic bone substitutes, a novel resorbable bone void filler was developed.	Calcium phosphate materials prepared by a novel process	N/A	The materials studied were calcium phosphates prepared from aqueous solutions of salts and inorganic acids that are processed thermally (sintered) in the range of 800 ° C to 1250 ° C. The materials were characterized using X-ray diffraction (XRD), surface area (BET), scanning electron microscopy (SEM), and mercury porosimetry techniques. -Scanning Electron Micrograph analysis indicated the presence of multi-modal pore size distribution in the thermally treated calcium phosphate scaffolds. The material's macroscopic porosity is up to 1000μm. This is a biologically relevant size for bone ingrowth. The material also exhibits interconnected meso- and micro-porosity that will allow for fluid communication. -X-Ray Diffraction pattern analyses of the thermally treated calcium phosphate materials reveal that the primary crystal phase is β-tri Calcium Phosphate (TCP). The particle size was determined to be 74 nm and 102 nm for 800°C and 1100°C sintered materials. -BET analyses of the fired materials indicates specific surface areas 3.23-.41m^2/g for sintering temperatures 800°C-1250°C. -Mercury porosimetry indicates the smallest pore diameters were .03 and 1 μm in 800°C and 1100°C fired materials.	The β-TCP materials investigated display favorable physiochemical characteristics for bone void filling and osteoconduction. The synthesis method gives enough room for control of properties since factors like sintering temperature are variables available for tailoring the physical properties of the scaffold material.
22	"Porous Hydroxyapatite and Tricalcium Phosphate cylinders with Two Different Pore Size	β-TCP large pores/ β-TCP; small pores; 15 New Zealand	HA large pore/HA small pore	-Tricalcium Phosphate: After 2 weeks, the volume density of bone in the total implant area was 18.1% in the TCPS (TCP small pores) compared to 7.1% in the TCPL (larger pores)	The results of this study confirm that β-TCP is biocompatible and highly osteoconductive, performing far superior to HA. Additionally there is

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results	Conclusions
	Ranges Implanted in the Cancellous Bone of Rabbits" By: P.S. Eggli, W. Muller, and R.K. Schenk Synopsis: The purpose of this study was to evaluate the histophysiology of implant degradation by implanting TCP and HA cylinders in the cancellous bone of the distal femur and proximal tibia of rabbits.	White Rabbits		ceramic. The microscopically determined volume density of the proper implant material was 49.7% for TCPS and 49.3% for TCPL. After 2 weeks this reduced to 38% for TCPS but only 46.5% for TCPL. As seen by microradiographs, the outermost pores of the TCPL were partially filled with bone while bone formation within the TCPS cylinders spread through a greater depth. After 4 weeks, the bone volume densities reached 16.7% in TCPL and 40.9% in TCPS. There were still some open pores in the TCPL cylinder, but in the TCPS areas no empty pores were left. Resorption of TCPL was restricted to the peripheral zone while degradation of TCPS proceeded homogenously through the entire implant. In addition to osteoclast like cells lining the walls of the pores, macrophages filled with granular material were found within the pores. They only gave a weak reaction to acid phosphatase. Substitution of the implant by bone was still occurring at 4 months. The ceramic volume density was now 21% in TCPL and 14.5% in TCPS. Bone volume was increasing in TCPL but was less than it had been at 4 weeks for TCPS. The number of osteoclast like cells declined, but the clusters of cells with granular material were about 2 mm thick. In the last stage of the study, the implants were not completely substituted. At 6 months, less than 1/6 of the originally implanted TCPS persisted while 1/3 of the TCPL was present. -Hydroxyapatite: At 6 months the HAPL was observed when implant resorption was only 5.4%. Tissue had penetrated the whole depth of the cylinders but some pores still remained unopened. The total amount of bone formed was the smallest found in all test implants and declined considerably from the surface toward the center. The HAPS was studied after 4 months	evidence for osteoclast-like cell resorption of the β-TCP. Finally, smaller pore size of the β-TCP seems to result in more rapid bone ingrowth compared to the larger pore size.

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				and total amount of bone was similar to TCPL and TCPS while the density was much higher than HAPL. No resorption was seen. -Structure of Implant Material: TCPL, TCPS, and HAPS all showed a regular pore distribution with a homogenous diameter. All specimens showed surface irregularities (microporosity) because of the arrangement of calcium phosphate in a small diameter.	
23	"Comparison of Vitoss™ and Pro Osteon 500R in a Canine Model at 1 Year" By: E. Erbe, T. Clineff, M. Lavagino, L. Dejardin, and S. Arnoczky Synopsis: Blocks of Vitoss (β-TCP) and morsels of Pro Osteon 500R (HA coated calcium carbonate) were evaluated for a year in a canine model. The purpose of the study was to determine whether one material would facilitate faster ingrowth of new bone and resorb faster than the other material.	Vitoss β-TCP; large mongrel dogs	Pro Osteon hydroxyapatite	There was faster bone formation in the center of the Vitoss implants compared to the control (ProOsteon hydroxyapatite). Also, the resorption of Vitoss was significantly faster at all time periods compared to the control. The percent of bone in the center of the defect was similar to adjacent bone by 12 weeks in the Vitoss treated specimens. The Vitoss had resorbed 82% by 12 weeks and 90% by 1 year. Indications of cell-mediated phagocytosis of Vitoss were present as early as 3 weeks. At 1 year, Vitoss had 85% more bone in the defect than Pro Osteon 500 R.	The results of this study indicate that while both Vitoss and Pro Osteon 500R are capable of supporting bone growth into a defect in canine metaphyseal bone, Vitoss demonstrated accelerated ingrowth of new bone and implant resorption compared to Pro Osteon 500R.
24	"In vivo Evaluation of a Novel Calcium Phosphate Bone Substitute (Vitoss™ Scaffold) By: E.M. Erbe, L.M. Dejardin, S.P. Arnoczky, D.H. Dychala, T.D. Clineff, and G.M.	Vitoss β-TCP; 12 adult mongrel dogs	12 (adjacent native cancellous bone used for comparison)	All of the implants were well tolerated with no gross or microscopic evidence of inflammation. Radiographs demonstrated a progressive ingrowth and remodeling of the new bone over a 24-week period. Histological and BSE images showed excellent infiltration of new bone in the scaffold with mature lamellar bone being evident at 6 weeks. Resorption of the scaffold was	The results of this study indicate that the Vitoss calcium phosphate bone void filler supports bone ingrowth. The resorption of the scaffold appears to permit normal cancellous remodeling by 12 weeks.

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	Gualtieri Synopsis: The efficacy of Vitoss Scaffold as a bone void filler is evaluated in this in vivo study that assesses bone ingrowth and scaffold resorption in a canine cancellous bone model.			notable by 6 weeks while histomorphometric measurements showed a significant remodeling of the new cancellous bone by 12 weeks. Trabecular density was similar to the cancellous bone density at 12 weeks.	
25	"Potential of an Ultraporous Beta-Tricalcium Phosphate Synthetic Cancellous Bone Void Filler and Bone Marrow Aspirate Composite Graft" By: E.M. Erbe, J.G. Marx, T.D. Clineff, and L.D. Bellincampi Synopsis: This study assesses the efficacy of Vitoss as bone void filler in canines by quantifying bone ingrowth and scaffold resorption. It then assesses the ability of the Vitoss/BMA composite to act as a bone graft substitute.	Vitoss β-TCP; canines	N/A	In a study to assess the efficacy of Vitoss, at 3 weeks Vitoss showed resorption throughout the defect site and immature bone formation as well. Remodeling was complete within 6 to 12 weeks, with bone density in the normal range and the scaffold nearly all resorbed. By 24 weeks, the implant trabecular orientation approximated the normal stress patterns of adjacent bone. At 12, 24, and 52 week follow ups the bone within the defects was indistinguishable from the surrounding bone. Other studies on canines showed that femoral defects with implanted calcium phosphate cylinders with cultured autologous mesenchymal stem cells increased the development of new periosteal bone around the implants. This study and previous studies indicate that BMA cells absorbed into an appropriate scaffold, (like the new ultraporous β-TCP construct) may remain viable and make such a composite a true synthetic replacement for autogenous cancellous bone.	The results of this study suggest that Vitoss is a logical delivery vehicle for BMA-derived osteoinductive growth factors and osteogenic precursor cells.
26	"Use of a Synthetic Cancellous Bone Void Filler in Bony Defects" By: E.M. Erbe, J.G. Marx, and T.D. Clineff	Vitoss β-TCP; Mongrel dogs and rats	N/A	All the implants were well tolerated with no inflammation. The high resolution radiographs saw progressive ingrowth and remodeling of the new bone over the 52 week period. Resorption of the scaffold was seen by 6 weeks, and at 1 year less than 2% of the defect site was filled with	The results of this study indicate that Vitoss (β-TCP) supports osteoconductive bone ingrowth. The scaffold resorbs with time and permits normal cancellous bone remodeling by 12 weeks. Also, a Vitoss with bone

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results	Conclusions
	Synopsis: In vivo studies were performed in order to evaluate bone ingrowth and Vitoss resorption both in a canine metaphyseal surgical defect and an athymic rat model.			Vitoss. Histological images revealed excellent infiltration of new bone into the scaffold with mature lamellar bone evident at 6 weeks. The area of the bone in the middle of the implant was not much different from the native adjacent cancellous bone at 12 weeks. At all intervals (12, 24, and 52 weeks), the bone volume at the center was measured to be in the normal cancellous bone range. In the rat model, Vitoss with bone marrow aspirate (BMA) showed bone formation through a primary path, without any cartilage step.	marrow aspirate graft provides an osteoinductive and osteogenic response in the Urist rat model, over and above the Vitoss alone..
27	"Experimental Evaluation of Ceramic Calcium Phosphate as a Substitute for Bone Grafts" By: James W. Ferraro, M.D. Synopsis: The paper reports on preliminary studies using Synthos, a resorbable bioceramic material that is a single phase β-tricalcium phosphate.	β-TCP; 12 dogs	Used in study to confirm that the dogs could not repair their own defects, but number unspecified	After the insertion of the ceramic, the insterstices rapidly filled with clotted blood. After 3 months (postoperative) peripheral trabecular bone had replaced the TCP prosthesis in a concentric manner. In the gross autopsy, a progressive indistinctness at the bone prosthesis interface and tight adherence of the prosthesis to the adjacent bony structure was seen. Histological appearance indicates that ingestion by foreign body giant cells played a role, though passive dissolution of the material can occur. Bone spicules were seen first near the bone-prosthesis interface, possibly from rapid calcification of dense connective tissue with varied amounts of osteoid formation. The lacunae of the spicules showed TCP particles. Examination of the dogs one year later showed a total growth of bone across the prosthesis. Some TCP was found in these specimens as islands with areas of loose, cellular, dense connective tissue suggesting incomplete replacement by bone. Two animals had several prostheses placed in subcutaneous pockets adjacent to the bone, either as extraperiosteal or subperiosteal implants. Bone ingrowth occurred in these implants as well.	The β-TCP resorbable material can be placed into a defect to act as a bridge across a bony gap. As the adjacent tissue proliferates and grows within the interstices of the tricalcium phosphate block, the synthetic matrix is replaced by the animal's regenerating bone.

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				Tight fixation of the prosthesis to the adjacent bone is essential as it is with any bone graft. If movement occurs between a prosthesis and bone, the ingrowth is disturbed and a much slower replacement occurs or no ingrowth occurs at all.	
28	"BMP-2, BMP-4, and PDGF-BB stimulate chemotactic migration of primary human mesenchymal progenitor cells" By: J. Fiedler, G. Roderer, K.P. Gunther, and R.E. Brenner 2002 *J Cellular Biochemistry* Synopses: This *in vitro* study examines the chemotactic effects of recombinant human platelet derived growth factor BB isoform (rhPDGF-BB) and other growth factors on primary human mesenchymal progenitor cells (MPC) compared with *in vitro* differentiated MPCs, and mature osteoblasts.	Cultures of MPCs harvested from human bone marrow; MPCs differentiated *in vitro*; and osteoblasts harvested from cancellous bone fragments from hip/knee replacements (8 subjects) Chemotaxis filter separated upper wells containing cells from lower wells with growth factor dilutions (0.001 to 1.0 ng/ml each) of DMEM medium.	Chemotaxis filter separated upper wells containing cells from lower wells with DMEM medium only (control). Conditioned medium of human fetal osteoblasts (positive control)	rhPDGF-BB, recombinant human bone morphogenetic protein-2 (rhBMP-2), and recombinant bone morphogenetic protein-4 of *Xenopus laevis* (rxBMP-4) induced migration of primary human MPCs, but to a different degree and with different dose-response curve For primary MPCs, the increase in chemotactic index (CI) (ratio of average number of migrated cells in stimulated versus control wells) was up to 3.5-fold for rhBMP-2, 3.6-fold for rxBMP-4, and up to 22-fold for rhPDGF-BB compared to control, whereas recombinant human transforming growth factor beta 1 (rhTGF-β1) and basic fibroblast growth factor (rhbFGF) did not stimulate MPC migration in the concentration range tested. rhPDGF-BB stimulated migration in a dose-dependent manner with a maximal CI (22.4 ±4) at 1 ng/mL, and ~5 at 0.001 and 0.01 ng/mL. A potent migratory effect was seen with the conditioned medium that served as positive control. In contrast to primary MPCs, differentiated MPCs behaved similar to mature human osteoblasts in chemotactic response. rhPDGF-BB, rhBMP-2, and rhTGF-β1 stimulated migration from 2.2 to 2.4-fold each, while rxBMP-4 and rhbFGF reached only a CI of 1.7-1.6. Thus, *in vitro* differentiation of MPCs changed the responsiveness to growth factors towards that of primary osteoblasts.	This *in vitro* study showed that the chemotactic response depends on the growth factor and differentiation status of the cells. There is a positive chemotactic response of MPCs to rhBMP-2, rxBMP-4, and rhPDGF-BB, but a lack of directed migratory effect of rhTGF-β1and rhbFGF. *In vitro* differentiation of MPCs to osteoblast-like cells leads to loss of response to rxBMP-4 and rhPDGF-BB. The effect of rhBMP-2, rxBMP-4, and rhPDGF-BB as chemoattractive growth factors for primary human MPCs suggests a functional role for recruitment of MPCs during bone development and remodeling, as well as fracture healing. MPCs are targets for rhPDGF-BB and some other chemoattractive growth factors.

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				Checkerboard analysis indicated that cell migration seen in response to growth factors was due to true chemotaxis, not chemokinesis.	
29	"Human Intraosseous Healing Responses to the Placement of Tricalcium Phosphate Ceramic Implants" By: S. Froum and S.S. Stahl Synopsis: To study human intraosseous healing responses o tricalcium phosphate ceramic implants over the extended period of 18 months after graft placement using clinical and histologic parameters for evaluation.	5 periodontal lesions in 1 female patient	N/A	-Clinical Observation: Pocket depth at the 5 sites changed from 8.5-13.2 mm preoperatively to 4.3-7.2 mm postoperatively. Gingival recession was present at all sites that ranged from 1.9-3.0 mm. Clinical gain in closure ranged from 1.7-3.0 mm. -Histologic Observations: In the five sites studied, the gingival margin had receded apical to the marginal notch. Closure was by adhesion of the junctional epithelium to the level of the most coronal fiber/cementum insertion sites. Inflammation at the pocket sites varied from minimal to severe, but did not appear to be related to the presence or absence of graft particles. The specimens showed no evidence of osteogenesis at particle seams and the alveolar crest was remodeling. Cemento-dentoclastic activity was seen at the root surface, the resorbing site showed odontoclasts, and the connective tissue at this site was highly cellular. Fibrils appeared to project from the dentin border. Areas of surface root resorption were seen at the level of the junctional epithelium. Apical to this adhesion, cementogenesis occurred at the resorption site.	Histologic evaluation of the TCP implants showed limited presence of graft particles. It did not induce inflammation, nor did it appear to enhance osteogenesis or cementogenesis. Closure of lesions was done by junctional epithelium adhesions and some new connective tissue formation (root attachment). [These findings suggest that adjunction of TCP may be necessary to achieve the optimum environment for bone regeneration.]
30	"Expression of platelet-derived growth factor proteins and their receptor alpha and beta mRNAs during fracture healing in the normal mouse" By: H. Fujii, R. Kitazawa, S. Maeda, K. Mizuno, S.	12 week old mice	N/A	Closed fractures were made in the proximal tibiae of adult mice. On days 2,4,7,10,14,21 and 28 after fracture the bones were excised and examined using immunohistochemistry and in situ hybridization. -Days 2-4: Mesenchymal cells gathering at the fracture site expressed PDGF-BB and β receptor mRNA.	These data suggest that PDGF contributes to the promotion of the chondrogenine and osteogenic phases from early through late stages of fracture healing.

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	Kitazawa Synopsis:PDGF is abundant in bone tissue. This study was conducted to elucidate the role of PDGF- A and –B chains and PDGF receptors alpha and beta in fracture repair.			-Day 7: PDGF A and B chains and alpha and β receptors were observed on proliferating chondrocytes. -Days 14-21: During this period of bone formation, the predominant expression of the PDGF-BB and β receptor was observed on both osteoblasts and osteoclasts. -Day 28: There was diminished expression of PDGF A and B chains and PDGF receptors. The coordinate localization of both the PDGF (ligands) and receptors implies a paracrine and/or autocrine mechanism whereby PDGF participates in fracture repair.	
31	"Behavior of tricalcium phosphate and hydroxyapatite granules in sheep bone defects" By: A.M. Gatti, D. Zaffe, G.P. Poli Synopsis: Granules of hydroxyapatite and tricalcium phosphate were implanted in surgically created defects in the mandibles of sheep to evaluate in vivo behavior of the materials particularly as it relates to bone growth in and around the implanted materials. Portions of the jaw bone containing the original implanted sites were processed and evaluated under optical and scanning electron microscopes.	B-TCP; 3 sheep	HA (hydroxyl-apatite)	-A microradiographic study carried out in mandibular sections of the jaw 4 months after implantation show new bone deposition in the defects filled with the TCP granules. The newly grown bone closes the surgically created defect completely. The density of the new bone is lower than that of the old compact bone. In the defects filled with HA granules, little bone deposition was observed and instead there is still HA present within the defect that is surrounded by dense connective tissue. Where there is new bone present it is not closely associated with the HA granules. -Histologic observation shows the presence of haversian systems in the bone surrounding the TCP granules and woven bone. Big vascular cavities are seen near the granules and inside the new bone. -SEM analysis shows the TCP granules in close contact with the bone and shows that elongated osteocyte lacunae are present in the proximity of the bone. The SEM study of HA granules demonstrates few granules in contact with new bone and many surrounded by dense connective tissue. Many HA granules appear whole while some appear fragmented. There is little newly	The results show that these types of HA and TCP granules are biocompatible. However, β-TCP implantation into surgically created defects showed complete healing of the defects with no discernable margin to the original defects, while the HA implanted sites fail to heal completely resulting in a residual defect similar to the control, non-grafted site. Therefore, the TCP is superior in overall performance demonstrating biocompatibility as well as osteoconductivity.

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results	Conclusions
				formed bone. -A histomorphological study carried out shows TCP particles, at 4 months, surrounded by new bone, with some particles demonstrating bone deposition within the particle. Additionally, there is histologic evidence of bone remodeling and resorption processes present on TCP particles but not HA particles. -Analyses of the jaw 8 and 12 months after implantation show almost total disappearance of TCP and HA granules. The holes filled with HA granules are not closed, but the defects filled with the TCP granules are completely closed by new bone 8 and 12 months later. At 12 months post-surgical it is no longer possible to discern were TCP granules were implanted.	
32	"Comparative effects of platelet-derived growth factor-BB and insulin-like growth factor-I, individually and in combination, on periodontal regeneration in Macaca fascicularis" By: W.V. Giannobile, R.A. Hernandez, R.D. Finkelman, S. Ryan, C.P. Kiritsy, M. D'Andrea, S.E. Lynch Synopsis: This study characterizes the biological effects of PDGF and/or IGF-1 when used individually or in combination in non-human primate periodontal defects. The parameters measured	rhPDGF-BB and rhIGF-I; 10 Macaca Fascicularis primates with ligature induced periodontitis	10 (Control and treatment sites were within each animal) Treatment groups: methylcell-ulose gel alone (control), or methyl-cellulose gel containing 10 ug each PDGF-BB, IGF-I or both	-Histomorphometric analyses: rhPDGF-BB: 1 and 3 months following rhPDGF-BB application, increases were seen in all the parameters, although only % new attachment at week 12 was statistically significant. At week 4, % osseous defect fill was increased in treated sites 54% over controls. CNAA and % new attachment were increased in treated sites compared to controls by 68% and 46%. At 12 weeks, all measured parameters of periodontal regeneration were greater than controls following rhPDGF-BB application. Percent new attachment and % osseous fill increased at levels 162% and 111% of the controls. CNAA and new bone height increases were not significant. IGF-1: Only modest changes in all parameters were seen following application. At 4 weeks, bone height, CNAA, % new attachment, and % defect fill were changed at levels 58%, 40%, -1%, and 54% from vehicle values. rhPDGF-BB/IGF-1: At 4 weeks, bone height, CNAA, % new attachment, and % defect fill	The results from this study indicate that IGF-1 treatment did not significantly alter healing compared to surgery plus vehicle control sites in any parameter of periodontal regeneration. rhPDGF-BB treated sites exhibited significant regeneration of new attachment and demonstrated trends for treatment effect in other parameters measured. The combination of both growth factors resulted in significant regeneration of periodontal structures above vehicle at both 1 and 3 months.

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	include length of compete new attachment apparatus (CNAA), percent new attachment, new bone height, and percent osseous defect fill.			showed 179%, 196%, 87%, and 196% increases above vehicle. These increases showed greater effects than the additive responses of PDGF-BB and IGF-1 individually. At 12 weeks, 180%, 193%, 135%, 156%, were seen for % new attachment, % defect fill, CNAA and new bone height, all of which were not statistically significant. The increases for new bone height, CNAA and % defect fill were greater than the additive effects of PDGF-BB/IGF-1each. -Histology: At 4 weeks, vehicle and IGF-1 treated sites healed similarly with little evidence of regeneration and were filled with dense connective tissue. New bone was seen coronal to the original defect in rhPDGF sites. The combination of PDGF-BB/IGF-1 increased new cementum, new bone coronal to the original osseous defects, and an intermediary organized PDL with collagenizing fibers. At 12 weeks, vehicle treated sites showed minimal evidence for periodontal regeneration, IGF-1 sites showed dense connective tissue, and rhPDGF-BB sites showed mature new bone. rhPDGF/IGF-1 sites showed greatest evidence for periodontal regeneration with abundant new mature bone evident.	
33	Giannobile WV, Somerman MJ. Growth and Amelogenin-Like Factors in Periodontal Wound Healing. A Systematic Review. *Periodontal.*			The article is provided in Appendix 10.2.2.	
34	"Anti-apoptotic effects of IGF-1 and PDGF on human intervertebral disc cells in vitro"	Human intervertebral disc cells (25 subjects) cultured with	Human intervertebral disc cells cultured without growth	1% FBS (positive control) was associated with significantly greater percentage of apoptosis than 20% FBS (negative control) ($P<0.0001$). PDGF 100 ng/mL significantly reduced the	Data from this *in vitro* study demonstrated a significant reduction in the percentage of apoptotic human intervertebral disc cells after exposure to some doses of PDGF or IGF-I.

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results	Conclusions
	By: H.E. Gruber, H.J. Norton, and E.N. Hanley Jr. 2000 *Spine* Synopses: This *in vitro* study investigates whether platelet-derived growth factor (PDGF) and insulin-like growth factor-I (IGF-I) are effective in decreasing apoptosis in cultured human intervertebral disc cells.	various doses of PDGF (0.05 to 500 ng/mL), IGF-I (1 to 100 ng/mL), and insulin (100 ng/mL) plus 1% fetal bovine serum (FBS).	factors or insulin, but supplemented with 1% FBS (positive control) or 20% FBS (negative control).	percentage of apoptotic cells (0.18% ±0.11; P=0.0001 versus positive control). Exposure to IGF-I 50 ng/mL also significantly reduced the percentage of apoptosis to 0.49% ±0.26 (P=0.005); IGF-I 500 ng/mL was also significantly effective (0.09% ±0.04; P=0.0001). There was no difference in percentage of apoptotic cells to PDGF 100 ng/mL versus IGF-I 500 ng/mL. Insulin 100 ng/mL and lower levels of PDGF (1 or 10 ng/mL) and IGF-I (0.05 to 5 ng/mL) were ineffective in reducing the percentage of apoptotic cells. Only age showed a significant positive correlation with percentage apoptosis in the presence of PDGF 100 ng/mL (Spearman correlation coefficient = 0.427; P=0.02).	Thus, these growth factors can retard or prevent programmed cell death *in-vitro*, and may have future therapeutic potential in the treatment of disc degeneration.
35	"A Highly Porous Beta-Tricalcium Phosphate/Bone Marrow Graft Promotes Osteoinductivity in a Rat Subcutaneous Model" By: R. Gunzberg, and M. Szpalski Synopsis: This study evaluates the osteoinductive potential of a β-TCP bone marrow aspirate/(BMA) graft compared with a known osteoinductive substrate, human demineralized bone, using a well-	Vitoss B-TCP; Athymic nude rats	Polyethylene was used as a negative control	All implant materials were well tolerated and no unscheduled deaths occurred in the study. Vitoss with BMA elicited rapid bone formation without a cartilage intermediate. DBM was slow to form bone, with a cartilage intermediate. Vitoss neat showed its purely osteoconductive nature (zero bone formation) in a non-osseous site. Polyethylene had zero bone formation. At 56 days, new bone formation between the Vitoss/BMA and DBM samples was not significantly different. The presence of an enzyme marker for osteoblasts (alkaline phosphatase) was increased for Vitoss/BMA compared to DBM at all times, and was statistically significantly different at 28 days. The resorption of the β-TCP was statistically significant with each time period. Primary bone formation occurred in the Vitoss/BMA group	This study indicates that new generation ultraporous β-TCP's are good carriers for bioactive agents like bone marrow. The results using the Urist pouch model in rats illustrate that the composite graft, combining an osteoconductive synthetic matrix with bone marrow aspirate, may provide the advantages of autograft while avoiding its drawbacks.

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	established rat model.			while slower endochondral bone formation occurred in the DBM group.	
36	"THE USE OF TRICALCIUM (SYNTHOGRAFT). PART I: ITS USE IN EXTENSIVE PERIODONTAL DEFECTS" By: D.L. Hoexter J Oral Implatol 1983	β-TCP; Three (3) patients with extensive periodontal pathology (deep pockets and/or deterioration of osseous support). Defects exposed, packed with slurry of TCP, flap repositioned, sutured, and covered with dressing. Patients treated with broad spectrum antibiotic for 7 days post implantation.	Patients were their own control.	At 9 months post implantation, no repocketing occurred; teeth were stabilized and function maintained.	TCP observed to be biocompatible; provided good support and eliminated mobility with function maintained for at least 6 months. Preliminary prognosis hopeful in light of failure of graft materials within first few months. Histological and resorption profiles should be collected in the future.
37	"A Phase I/II Clinical Trial to Evaluate a Combination of Recombinant Human Platelet-Derived Growth Factor-BB and Recombinant Human Insulin-Like Growth Factor-I in Patients with Periodontal Disease" By: T.H. Howell, J.P. Fiorellini, D.W. Paquette, S. Offenbacher, W.V.	38 patients with advanced osseous periodontal defects	38 (Each patient's mouth had a control quadrant); contra-lateral quadrant received either LD_PDGF-BB/IGF-I [50μg/ml each of rhPDGF-BB and rhIGF-I in gel vehicle] or	-Safety: The LD-PDGF/IGF-1 and HD-PDGF/IGF-1 drug formulations were well tolerated. No serious adverse events, discontinuations, signs of uncontrollable tissue formation, or deaths were attributed to the drug or placebo. The most serious adverse effects noted were pain at the operative site, sensitivity, and edema; these were not unexpected post-surgical events and no measurable differences were found between treatment and control quadrants. There were 15 reports of postoperative pain, 2 reports of soft tissue dehiscence, and 1 report of tooth abscess, all of mild severity. There were 2	The results from the Phase I/II human clinical trial show that the rhPDGF-BB and rhIGF-I are safe for use when applied locally to bone defects during periodontal surgery. A single application of the HD-PDGF/IGF-1 is effective, resulting in a statistically significant improvement in bone growth and fill of periodontal defects compared to the current standard practice of full thickness flap reflection and debridement.

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	Giannobile, S.E. Lynch Synopsis: This human study assesses the safety of rhPDGF-BB and rhIGF-1 when applied locally to bone defects during periodontal surgery and accrues data to determine the dose of growth factor needed to stimulate periodontal regeneration.		HD PDGF-BB/IGF-I [150µg/ml each of rhPDGF-BB and rhIGF-I in gel vehicle]	reports of adverse events with moderate severity. Treatment emergent abnormal laboratory events were reported for 5 patients and included liver enzymes, lymphocytosis, and hematuria, all considered mild and were found at both baseline and 28-day timepoints (the patients entered into the study with abnormal levels prior to therapy and continued to display altered levels at one month. No patients developed antibodies to rhPDGF-BB or rhIGF-I after exposure to the drug. -Efficacy: Patient Assessment: For LD-PDGF/IGF-1, there were a total of 42 sites evaluated from 16 patients; the control therapies were evenly distributed-8 surgery alone, 8 surgery + placebo. For the HD-PDGf/IGF-1 subjects, 14 were evaluable (28 sites). Hard Tissue Endpoints: No significant differences were noted between LD-PDGF/IGF-1 subjects and the standard care or standard care plus vehicle subjects. In LD-PDGF/IGF-1 subjects, re-entry of the surgical site at 6 months proved to be to early and may have disturbed newly regenerated tissue. Thus, the HD-PDGF/IGF-1 patients were only re-entered at 9 months. This re-entry showed a 0.75 mm new mean bone height in the control group and 2.08 mm in the treated group. The % of osseous defect fill was calculated and there was no significant difference in LD-PDGF/IGF-1 subjects. In HD-PDGF/IGF-1 subjects, control defects resulted in an 18.5+/-7% bone fill, while the defects treated with the HD PDGF-BB/IGF-I resulted in 42.3+/-9% bone fill. Soft Tissue Assessments: Gains in attachment level were found for both test and control groups following periodontal surgery. For both LD-PDGF/IGF-1 and HD-PDGF/IGF-1 treated	

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				patients, no significant differences were found between the control and treatment groups. Trends of effect were found for both horizontal furcation probing depth and relative attachment level in HD-PDGF/IGF-1 treated patients.	
38	"PLATELET-DERIVED GROWTH FACTOR ENHANCES DEMINERALIZED BONE MATRIX-INDUCED CARTILAGE AND BONE FORMATION" By: R. Howes, J.M. Bowness, G.R. Grotendorst, G.R. Martin, H. Reddi Calficied Tissue International 1988	Demineralized bone matrix powder mixed with collagen. Bone matrix/collagen was implanted on one side of the panniculus carnosis of the thorax of rats. Bone matrix/collagen with PDGF or other growth factors was implanted on the other side of the thorax. Animals observed at 7, 10 or 14 days. Tissues analyzed for increase in production of mRNA, for collagen II, alkaline phosphatase activity and calcium content.	Thorax side without PDGF.	Levels of mRNA for collagen II increased by 2-fold in the implantation sites supplemented with PDGF at 7 days post implantation. This was, however, not observed at days 10 and 14 post implantation. Alkaline phosphatase (AP) activity and calcium content in implants in young rats we two times that of older rats. Exogenous PDGF did not increase the rate of bone formation in the young rats. In older rats, the PDGF increased AP and calcium content of the plaque.	PDGF had little or no effect on formation of cartilage and bone when bone matrix was implanted into young rats. However, PDGF increased cartilage and bone formation in the implants placed in mature rats. Other growth factors had no effect. PDGF may be more active due to its chemotactic and mitogenic characteristics and may act to recruit cells and stimulate bone formation.
39	"Pulse application of platelet-derived growth factor enhances formation	Fetal rat osteoblastic cells cultured in	Fetal rat osteoblastic cells cultured in	Compared with untreated control, continuous PDGF-BB 20 ng/mL produced a significant two-	This *in vitro* study demonstrated that multiple brief exposures to PDGF-BB enhance formation of a mineralized

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	of a mineralizing matrix while continuous application is inhibitory" By: S.C. Hsieh and D.T. Graves 1998 *J Cellular Biochemistry* Synopses: This *in vitro* study evaluates the effect of continuous and pulse treatments with platelet-derived growth factor BB isoform (PDGF-BB) on osteoblast proliferation and nodule formation in mineralizing cultures of fetal rat osteoblastic cells.	mineralizing media with various concentrations of pulsed and continuous PDGF-BB (0 to 50 ng/mL) over a range of time periods	mineralizing media without PDGF-BB	fold increase ($P \leq 0.01$) in osteoblastic cell proliferation on Days 3 and 7, but no significant difference on Day 28 and thereafter. The PDGF-BB-induced burst in osteoblastic cell number reached a plateau after 14 days. The effect of PDGF-BB on mineralized nodule formation depended on the timing and duration of exposure. Although PDGF-BB increased osteoblastic cell proliferation, continuous treatment with PDGF-BB caused a significant ($P \leq 0.01$) dose-dependent decrease in mineralized nodule formation. When osteoblastic cells were treated with multiple (Days 7 and 14), short-term (1 day) pulse exposures to PDGF-BB at the osteoblast differentiation stage, there was a significant 50% increase ($P \leq 0.01$) in mineralized nodule area. Longer-term (7 day) pulse exposures to PDGF-BB inhibited alkaline phosphatase activity if applied at early stages of osteoblastic cell culture (Days 0 to 7 and 7 to 14); little effect was observed if PDGF-BB was applied to cultures after Day 14. Based on modulation of alkaline phosphatase activity, longer-term exposure to PDGF-BB reduces mineralized nodule formation largely by inhibiting differentiated osteoblast function, while short-term exposure enhances proliferation without inhibiting the differentiated phenotype. Thus, the ultimate affect of PDGF-BB on bone formation is likely to reflect two processes: a positive effect through enhancing cell number or a negative effect by inhibiting differentiated function.	matrix while longer-term exposure is inhibitory. Thus, PDGF-BB may have anabolic as well as inhibitory effects on bone formation depending upon the length of exposure. These findings suggest that multiple, brief exposures to PDGF-BB would enhance bone formation *in vivo*, while prolonged exposure to PDGF-BB, which is likely to occur in chronic inflammation, would inhibit differentiated osteoblast function and limit bone regeneration.
40	"Modification of an osteoconductive anorganic bovine bone	Anorganic bovine bone matrix incubated	Anorganic bovine bone matrix without	PDGF-BB-treated matrix material (at all treatment concentrations) produced significantly greater ($P<0.05$) proliferation of cultured	The results of this *in vitro* study indicated that PDGF-BB can be adsorbed to anorganic bovine bone

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	mineral matrix with growth factors" By: D. Jiang, R. Dziak, S.E. Lynch, and E.B. Stephan August 1999 *J Periodontol* Synopses: This *in vitro* study investigates the interaction of osteoconductive anorganic bovine bone mineral matrix ("matrix") with platelet-derived growth factor BB isoform (PDGF-BB) and insulin-like growth factor-I (IGF-I), and determines if the combination of growth factors with the matrix can stimulate proliferation of neonatal rat (Sprague-Dawley) osteoblastic cells.	with various concentrations of radiolabeled PDGF-BB or IGF-I To measure proliferation, neonatal rat osteoblastic cells were cultured with matrix incubated with PDGF-BB (0.4 to 5.5 x 10^6 M) or IGF-I (0.9 to 14.2 x 10^6 M). To measure interaction dynamics, radiolabeled PDGF-BB (0.4 to 5.5 x 10^6 M) or IGF-I (0.9 to 16.06 x 10^6 M) were incubated with matrix over a range of time periods (1 min to 10 days).	PDGF-BB or IGF-I	osteoblastic cells compared to the matrix alone. In contrast, IGF-I adsorbed to the matrix did not significantly enhance cell proliferation. Both PDGF-BB and IGF-I adsorbed to the matrix in a concentration-dependent manner. There was a significant difference ($P<0.05$) between absorption of PDGF-BB at 0.4 x 10^6 M and all higher concentrations, but not between 2.8 and 5.5 x 10^6 M, suggesting that absorption of PDGF-BB was approaching saturation at these higher doses. PDGF-BB 2.5 x 10^6 M reached maximum absorption to the matrix by 15 minutes. The absorption curve of IGF-1 was linear at all concentrations without evidence of saturation. The affinity of IGF-I for the matrix was 10-fold greater than PDGF-BB. Approximately 50% of PDGF-BB and 10% of the IGF-I were released after 10 days. The percentage of PDGF-BB released from the matrix showed a time-dependent increase that was significant ($P<0.05$) between 1-hour incubation and 48-hour and 10-day incubations. The percentage of IGF-I released from the matrix did not change over time.	mineral matrix and enhances the osteogenic properties of the matrix. IGF-I was also adsorbed to the matrix, but was not readily released and did not produce significant effects in osteoblastic cell proliferation. However, the slow release of IGF-I may be clinically beneficial since IGF-I is important in long-term bone remolding. It may be clinically feasible to adsorb PDGF-BB to anorganic bovine bone and that the combination of bone growth factor and matrix has the potential for clinical applications.
41	"Multiple Uses of Resorbable Tricalcium Phosphate" By: KW Judy Synopsis: This paper presents cases where the	B-TCP; 3 patients (each in a different case study)	N/A	Case 1: Treatment of a chronic sinus tract extending from the buccal plate to the sinus with involvement of the palatal plate. Following complete debridement of the tract (removal of soft tissue), porous TCP was used to fill the resultant bone void. Two years later, the radiograph shows complete healing. The	Principles for use of porous, resorbable TCP in general and complex restorative situations were presented. The cases presented showed that the use of porous TCP for various dental and craniofacial applications requiring bone fill was

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	local irritation, and genotoxicity.			suggestive of accelerated bone remodeling, (one of the therapeutic benefits to be achieved in Vitoss Plus) . These findings were not unexpected based on the pharmacologic activity of becaplermin. . Becaplermin had no effect on body weight. -Antibody Response Study: Antibodies were detected in some rat serum at weeks 3, 5, and 9. None of the 20 rats that demonstrated an antibody response produced neutralizing antibodies to becaplermin, but in a low concentration of becaplermin, 4 of 20 rats showed 30-60% neutralizing activity to becaplermin. Some changes in the subcutis of rats were seen ranging from mild mixed inflammatory cell infiltrate to fibroblast activation to minimal fibroplasia. There was no effect of becaplermin on body weight and no drug-related clinical findings. -Cardiovascular Effects: Only minimal effects, which were seen in control dogs, were observed. In other studies, no cardiographic abnormalities were seen. -Sensitization and irritation potential: Dermal sensitization results were not unexpected for a recombinant human-derived protein. In dermal irritation studies it did not cause irritation or systemic toxicity when applied topically. -Genotoxic Potential: The results in multiple models and at multiple doses indicated that becaplermin is not mutagenic. -Reproductive Toxicity and Carcinogenic Potential of Becaplermin: Since becaplermin is an endogenous protein, it lacks genotoxic potential. It is also unlikely to increase the risk of local carcinogenicity following topical application.	
43	"Autologous bone marrow stromal cells	4 ewes	Used, but number	-In vitro characterization of sheep BMSC: In contrast with the characteristic heterogeneous	This study suggests that the use of autologous bone marrow stromal cells

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	loaded onto porous hydroxyapatite ceramic accelerate bone repair in critical-size defects of sheep long bones" By: E. Kon, A. Muraglia, A. Corsi, P. Bianco, M. Marcacci, I. Martin, A. Boyde, I. Ruspantini, P. Chistolini, M. Rocca, R. Giardino, R. Cancedda, R. Quarto Synopsis: This study tested the ability of marrow-derived osteogenitor cells to promote repair of critical-size tibial gaps upon autologous transplantation on a hydroxyapatite ceramic carrier in a sheep model.		unspecified	morphology of human BMSC in culture, sheep BMSC colonies were composed of very homogeneously small, thin, and elongated fibroblast-like cells. Additionally, while human BMSC colonies are positive for alkaline phosphatase activity, sheep BMSC are not. - Repair of critical-size tibial defects by autologous sheep BMSC: Proper integration of the implant with bone at sites of resection was obvious in retrieved implants. Formation of new bone onto the outer surface was much more substantial in cell-loaded ceramic implants compared to control implants. Prominent bone formation also was observed in retrieved cell-loaded implants within the inner canal of the original HAC. Osteogenesis at the equivalent site was negligible in control implants. In retrievals from animals with cell loaded HAC cylinders, most of the inner pore spaces contained newly formed bone while most inner pores from bone retrieved from animals that received cell-free implants were free from bone. Growth of bone within micropores and fissures of the original HAC cylinder was obvious in cell-loaded implants but negligible in controls. Histomorphometry analysis showed a higher amount of newly formed bone was observed in the cell-loaded implants compared to controls. In both groups the new bone in direct apposition to HA appeared less well mineralized than the original tibial shaft. -Biomechanical testing: The characteristics of both implant types are an intermediate between bioceramic porous scaffold and bone tissue. Cell-loaded specimens presented higher stiffness and a lower standard deviation than cell loaded ones.	(BMSC) in conjunction with hydroxyapatite ceramic (HAC)-based carriers results in faster bone repair compared to HAC alone. This study indicates that these matrices, while providing a good osteoconductive scaffold need to be supplemented with other biological stimuli to achieve improved bone regeneration.
44	"Biosynthetic Bone Grafting"	Numerous studies	Numerous studies	-Osteoconductive Matrices: Ideally, an osteoconductive matrix should possess its own	The data compiled in this report supports the concept that although

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	By: J.M. Lane, E. Tomin, and M.P.G. Bostrom Synopsis: In this review, the authors discuss the three main components of an ideal bone graft: (1) osteoconductive matrix (2) osteoinductive growth factors and (3) osteoprogenitor cells, and how these three components may be put together in order to create a more effective biosynthetic bone graft.			beneficial conductive effect on bone growth as well as serve as a carrier for delivery of bone growth factors. Many biodegradable and ceramic carriers have been used as vehicles or carriers for GFs. Polylactic and polyglycolic acid porous microspheres, collagen and ceramic materials have all been used with varying degrees of success. Overall the development of osteoconductive matrices that can also act as carriers for bone growth factors has slowed down the development of clinically successful biosynthetic bone grafts. -Osteoinductive Bone Growth Factors: The TGF-B family of growth factors appears to regulate the proliferation and expression of differentiated phenotypes for many cell populations, including chondrocyte, osteoblast, and osteoclast precursors. Other growth factors involved in fracture healing, found in the callus, include PDGF, FGF, and IGF. -Bone Morphogenetic Proteins: BMPs appear to be one of a promising bone growth modulator. Native human BMP has been used successfully in spinal fusion procedures. Studies have shown that rhBMP-2 and rhBMP-7 used in pharmacological doses in various animal models are superior to autografts in achieving spinal fusion. -Transforming Growth Factor-Beta: This growth factor has been shown to have a stimulatory effect on bone formation, possibly by enhancing the osteoinductive activities of the BMP's. Its application appears to augment the healing of fractures, but not dramatically. -<u>Platelet-Derived Growth Factor</u>: PDGF is a mitogenic and chemotactic factor for bone cells. It is naturally produced during the early phases of fracture repair and also present in mature bone	products that contain only one of the three key components of an ideal bone graft (an osteoconductive matrix, osteoinductive growth factors, and osteoprogenitor cells) may regenerate bone successfully, composites of these three key components will be more successful clinically.

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				matrix. PDGF appears to stimulate bone and cartilage cells in normally healing human fractures. The A chain has been found in many cells during a long period of fracture healing while B chain expression was more notable during the remodeling stage.. -Insulin-like Growth Factor: This GF plays a role in bone formation, fracture repair, and may be important in endochondral bone formation. -Fibroblast Growth Factor: Acidic and basic FGF have been shown to induce and inhibit bone repair, depending on the applied dose. -Osteoprogenitor Stem Cells: Studies have shown that bone marrow can be used to successfully treat nonunions. Bone marrow appears to be the source that is most capable of providing these uncommitted cells. Experiments have also shown that various components of the bone matrix or osteoconductive materials along with bone marrow are synergistically superior to each alone and significantly enhance the healing of bone fractures.	
45	"Enhanced bone formation by controlled growth factor delivery from chitosan-based biomaterials" By; Jue-Yeon Lee, Sung-Heon Nam, Su-Yoen Im, Yoon-Jeong Park, Yong-Moo Lee, Yang-Jo Seol, Chong-Pyoung Chung, Seung-Jin Lee 2002 *J of Controlled Release*	Porous chitosan matrices, chitosan-poly(l-lactide) (PLLA). Release of Platelet derived growth factor-BB (PDGF-BB) from these matrices. Primary cultures of embryonic rat calvarial osteoblasts.	Release experiments into phosphate buffer with 0% PDGF-BB Osteoblast proliferation In vivo bone regeneration – Control untreated defect in 4 weeks post-surgery – filled	Initial release of 90 ng, PDGF-BB then consistent 4-5 ng /day with no significant leveling off from the matrix. Consistent stimulation by prolonged release of PDGF-BB was beneficial to enhance osteoblast migration & proliferation within porous chitosan matrix. Osteoblast proliferation in PDGF-BB loaded chitosan matrix showed a high level of cell attachment due to the chemotactic effect of the growth hormone. Osteoblast and osteoids around the matrix can freely migrate into the PDGF loaded chitosan matrix when applied in a bony defect. The chitosan coated matrix and the chitosan composite matrix showed markedly higher density of osteoblasts compared with that	Controlled release of PDGF-BB from PLLA-chitosan composite porous matrices significantly promoted bone healing and regeneration. These results suggest the feasibility of a combinative strategy of controlled drug release and tissue engineering in reconstructive therapy in the field of aerodonetics, orthopedics and plastic surgery.

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	Synopses: This study tests the usefulness of chitosan as drug releasing scaffolds and as modification tools for currently used biomaterials to enhance tissue regeneration efficacy. Release of platelet-derived growth factor-BB (PDGF-BB) from these matrices exerted significant osteoinductive effect.	In vivo studies-Sprague-Dawley rats. A craniotomy defect (8 mm in diameter) was formed by a trephine needle.	with connective tissue, only limited new bone formation.	of PLLA matrix. In vivo - Bone regeneration: PDGF-BB loaded chitosan matrix in a rat calvarial defect showed an increase of osteogenesis compared to untreated defects. Optimal therapeutic efficacy has not been achieved because of the short biological half-life; therefore the development of a delivery system to release PDGF-BB in a controlled manner is essential for clinical efficacy.	
46	"The Bone Regenerative Effect of Platelet-Derived Growth Factor (PDGF-BB) Delivered with a Chitosan/Tricalcium phosphate Sponge Carrier" By: Y-M Lee, Y-J Park, S-J Lee, et al. Synopsis: This study evaluated the bone regenerative effect of rhPDGF-BB delivered in a chitosan/tricalcium phosphate (TCP) sponge in a rat calvarial defect model.	rhPDGF-BB and TCP/chitosan sponge; 24 Sprague-Dawley rats	8	-Fabricated Chitosan-TCP Sponge: The sponge showed a 3D porous structure that was brittle when dehydrated, but soft and malleable and expanded when rehydrated with saline or blood. -Release Kinetics of rhPDGF-BB: After an initial burst release, PDGF-BB was consistently released at a rate of 6 ng/day up to 7 days. The rate then diminished for the next 14 days to 2-3 ng/day. -Bone Regeneration by the Sponges: No subjects showed inflammatory tissue reactions and all wounds showed a good healing response. At 2 weeks, the control wounds were invaded by loosely organized connective tissue while both the chitosan/TCP sponge and the PDGF-BB loaded chitosan/TCP sponge wounds showed bone regeneration. At 4 weeks, the control wounds showed a limited amount of new bone formation, the chitosan/TCP wounds showed increased bone regeneration and the rhPDGF-BB chitosan/TCP treated wounds showed the greatest regeneration. Degradation of the TCP/chitosan sponge progressed throughout the time of the study. The rhPDGF-BB significantly enhanced the osteogenic effect of the	The results of this study support the use of TCP/chitosan sponges as a delivery system for rhPDGF-BB and demonstrate that rhPDGF-BB loaded onto an osteoconductive TCP-containing scaffold produces osteogenseis leading to enhanced repair of craniofacial bone defects.

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				TCP/chitosan sponge carrier at both time points.	
47	"Biodegradable Ceramic in Periodontal Defects" By: M.P. Levin, L. Getter, D.E. Cutright, and S.N. Bhaskar Synopsis: This study was conducted in order to determine the response of periodontal tissues to the placement of biodegradable tricalcium phosphate (TCP)	5 beagle dogs	5 (one quadrant in each dog)	Over a 22 week course, results of the ceramic implants were accumulated. A round cell inflammatory response from the trauma of the operation (and not due to the ceramic) was noted for time points less than 3 weeks. Small, multi-nucleated, elliptical giant cells continuously removed ceramic from the site. Ceramic was not completely removed from the area at 19 weeks in some samples, but no ceramic was detected in any sample by the end of 22 weeks. Osteoid was formed around the vessels penetrating the ceramic and rapidly separated the ceramic into segments. Osteoblastic activity occurred at the very edge of the ceramic initially and later within the islands of connective tissue. Repair of injuries to the tooth occurred with a deposition of new cementum and growth of a new periodontal ligament. The ceramic was very well tolerated by the tissue. At 4 weeks, the controls had greater osteoblastic activity than experimental sites.	Biodegradable TCP implants resulted in regeneration of lost periodontium with excellent tissue tolerance to the material.
48	"HEALING OF PERIODONTAL DEFECTS WITH CERAMIC IMPLANTS" By: M.P. Levin, L. Getter, J. Adrian, D.E. Cutright J Clin Periodontol 1974	TCP; Macca rhesus monkeys. 2-3 mm x 0.5 mm defects createdin 4 quandrants in facial surfaces of premolars and molars. TCP packed intodefect of 3 quandrants and 1 quadrant left unfilled. Monkeys	Unfilled quadrant of each monkey.	At 1 week, implant well-vascularized with loose granulation tissue surrounding ceramic and moderate inflammatory cell infiltration; control area less vascularized with less granulation tissue and inflammation. At week 2, implants had more dense and mature connective tissue surrounding the ceramic, inflammation decreased and fibrous connective tissue infiltrating ceramic; controls exhibited increased inflammation and immature granulation tissue in the defect site with fibroblast activity evident around defect site. At week 3, implants exhibited mature granulation tissue, little chronic inflammation, and osteoid material found occasionally in ceramic; control	TCP is well-tolerated by the tissue and periodontium is regenerated. TCP may be a stimulator of bone formation.

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		sacrificed at 1,2,3,4,5, and 9 weeks. Tissues evaluated histologically.		sites had marked osteblastic activity, mature granulation tissue, and scattered chronic inflammatory cells. At week 4, implants vascularity and inflammation almost resolved, ceramic segmented with ingrowing connective tissue, and bone formation occurring at interface of connective tissue and ceramic; controls demonstrated that defect consisted mainly of numerous trabeculae of woven bone with vascular connective storma in spaces between trabeculae, inflammation absent, and early laminar pattern characteristic of maturing bone. At 5 weeks, ceramic was filled with connective tissue exhibiting a peripheral eosinophilic characteristic of osteoid and amount of collagen increasing; control similar to week 4 but regenerated bone had fewer osteocytes. At 9 weeks, implants still contained areas of ceramic in defects, considerable woven bone observed around and within ceramic, osteoid forming at the interface of ceramic and connective tissue; controls showed no inflammation or granulation tissue, bone more mature and less cellular than at 5 weeks, approximately 50% of new bone remodeled. At 4, 5 and 9 weeks, implant sites showed the presence of a band of cellular, eosinophilic cementoid material between the tooth and the healing periodontal ligament that was separated from the tooth. The 9-week controls showed same characteristics but the cementum formation was not as advanced.	
49	"AD MODIFIED GINGIVAL INDEX FOR USE IN CLINICAL TRIALS" By: R. Lobene, T. Weatherford, W. Ross, et	Formulation of a modified gingival index (MGI) and methods. MGI	N/A	Agreement between examiners was 67 to 68%. Examiners scored within 1 integer of the MGI from 95 to 97% of the time.	Correlation coefficients among the three examiners were high and statistically significant, demonstrating the reliability of the MGI. Advantages of MGI versus GI are that it provides

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	al. Clin Prev Dent 1986	evaluated in long-term clinical trial of antiseptic mouth rinse. A principal examiner compared against two additional examiners. Degree of correlation between examiners for measuring gingivitis calculated. Consensus based on re-examination was used to resolve any disagreements in scores.			greater sensitivity at the lower end of the rating scale and detects more subtle, earlier changes in progression or regression of gingivitis.
50	"A combination of platelet-derived and insulin-like growth factors enhances periodontal regeneration" By: S.E. Lynch, R.C. Williams, A.M. Polson, et al. Synopsis: This article reports on the findings of a study to determine whether the combination of PDGF and IGF-1	purified human PDGF and rhIGF-I; 3 beagle dogs with naturally occurring periodontal disease (five periodontal defects);	Surgery plus methylcellulose gel; (7 periodontal defects)	Two weeks post-surgical animals euthanized and block biopsies of teeth and surrounding bone were taken for histologic examination: -Control Specimens: Epithelium lined most of the instrumented root surfaces. In the area of connective tissue adjacent to the root surface, there was no new cementum formation. There was little overall evidence of resorptive or formative activity and little evidence of change on cemental or bone surfaces. -Experimental surfaces: Marked differences from the controls were seen in the supracrestal connective tissue region. In the experimental group, new bone formation extended from the	The results of this study show that in vivo application of the combination of PDGF and IGF-1 enhances regeneration of periodontal structures, including bone, cementum and periodontal ligament.

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results	Conclusions
	stimulates periodontal regeneration when applied onto the bone defect and tooth roots during periodontal surgery.			crest of the original alveolar bone and was adjacent to the notched root surface. A distinct deposit was also found on the root surface that resembled cementum and extended from the apical end of the notch in a coronal direction. Active bone formation was evident throughout the extent of the new bone, and it was more osteoid-like in the coronal region. New bone formation was also noted on the periosteal and periodontal ligament surfaces, with a 116 μ width compared to the 13 μ of the control specimens.	
51	"The effect of short term application of a combination of platelet-derived and insulin-like growth factors on periodontal wound healing" By: S.E. Lynch, G.R. Castilla, R.C. Williams, et al. Synopsis: This study tests the hypothesis that the combination of PDGF-B and IGF-1 can predictably enhance periodontal regeneration. Additionally, the authors seek to determine what length of time is required for the growth factors to exert their effect at the site.	rhPDGF-BB and rhIGF-I in methylcellulose gel; 13 beagle dogs with naturally occurring periodontal disease (26 osseous periodontal defects). Osseous defects in 4 additional animals received radiolabelled PDGF or IGF-I in the treated sites to determine clearance rates	Gel alone; 26 defects	-Local Clearance: The clearance studies revealed that the half-life of the proteins at the site was 3.0 hours for IGF-I and up to 4.2 hours for rhPDGF-BB. Greater than 96% of the radio-labeled proteins was cleared by 96 hours and there was no detectable radio-labeled protein 2 weeks after application. -Osseous Metabolic Activity: The rate of bone-seeking radiopharmaceutical uptake increased in both control and growth factor treated sites after surgery. At 2 and 4 weeks postoperatively greater increases were observed in the sites receiving rhPDGF-BB/IGF-1. At 2 and 4 weeks the increases were 2 times and 2.7 times the controls, respectively. -Histologic Analysis: A sequence of wound healing events was seen. In most control specimens, the epithelium migrated apically to the base of the instrumented root surface. There was no new cementum on the root surfaces lined by the epithelium. Dense collagen fibers occupied most of the area in the intrabony defects at 2 weeks. In the supracrestal area of the bone specimens, no new bone formation was observed. Near the base of intrabony defects, small islands of new bone were noted, but no cementogenic or	This study shows that the bolus administration of rhPDGF-BB/rhIGF-1 to a bony site can stimulate rapid osteogenesis. The single application of these growth factors at the time of surgery can stimulate regeneration of the periodontal attachment apparatus, including bone, cementum and ligament during the early phases of wound healing.

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results	Conclusions
				osteogenic activity was noted within existing periodontal ligament spaces. In the experimental specimens treated with rhPDGF-BB and rhIGF-I, new bone formation was evident in the horizontal and vertical alveolar bone defects. At 2 weeks a continuous layer of osteoblast-like cells surrounded almost all surfaces of the new bone. Numerous osteocytes were present within the new bone. This new bone became more immature in nature as its distance from the original bone increased. New bone was seen on the periosteal surfaces of the original alveolar bone. New cementum was also seen on the root surface. At 5 weeks the new bone was denser in both control and treated sites and contained numerous osteocytes. In the treated sites, large clusters of osteoblast-like cells were seen adjacent to the new bone. The new cementum was still lined by a nearly continuous layer of cells in the treated sites. Strikingly, a physiologic periodontal ligament space (occupied by dense connective tissue and vascular channels) was maintained between the new cementum and new bone.	
52	"Structural Evolution of Vitoss™ Scaffold to Bone in Canine Humeral Defect at 1 Year" By: J.G. Marx, E.M. Erbe, and C.V. Mesropian Synopsis: This study tests the hypothesis that Vitoss Scaffold synthetic bone void filler is resorbed and replaced by normal viable bone over time.	Vitoss (β-TCP) cylinders; Large adult mongrel dogs with surgically created cylindrical defects in the proximal humeral metaphysis.	Cancellous bone adjacent to the implant area acted as an intra-specimen control; unimplanted Vitoss served as an external control	Both X-Ray Diffraction (XRD) and Fourier Transform Infrared (FTIR) spectroscopy showed the evolution of the chemical structure of the implant into cancellous bone with time, occurring by the coupled process of bone formation and maturation, and resorption of the Vitoss scaffold. When analyzed by XRD the strongest peak for the 3 and 24 week samples was the β-TCP peak, indicative of a strong Vitoss Scaffold presence. The primary peak for the 52 week sample matched that of adjacent cancellous bone. FTIR analysis showed the same results since the Vitoss phosphate doublet gradually diminished by 52	This study demonstrates replacement, over time of the Vitoss (β-TCP) scaffold with host bone as evidenced by chemical, crystallographic and structural methods of analysis. Vitoss is now being used safely in a variety of clinical applications to treat skeletal defects.

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results	Conclusions
				weeks as the Vitoss was resorbed at defect site. Thus, all methods of analysis demonstrate an increasing match to cancellous bone over the 52-week period.	
53	"Platelet-rich plasma growth factor enhancement for bone grafts" By: R.E. Marx, E.R. Carlson, R.M. Eichstaedt, S.R. Schimmele, J.E. Strauss, and K.R. Georgeff Synopsis: This article presents data documenting that platelet rich plasma (PRP) increases platelet concentration when placed into grafts, showing the levels of at least 3 growth factors (PDGF, TGF-β1 and TGF-β2). It also explores the potential of PRP to increase the rate of bone formation and enhance new bone density and presents a model of bone graft bone regeneration and how PRP may enhance bone regeneration in this model.	Platelet-rich plasma (PRP) containing PDGF and TGF-β; 44 patients with 5mm or greater mandibular discontinuity defects.	Autograft; 44 patients with 5mm or greater mandibular discontinuity defects.	-PRP monoclonal antibody study: Platelets isolated by centrifugation showed uptake of PDGF and TGF-β monoclonal antibodies on all slides, confirming the presence and retention of these growth factors in the PRP preparation -Bone graft harvest material monoclonal antibody study: All slides had cells that tested positive for receptors to PDGF and TGF-β. Most of the cell groups were centered around blood vessels in a perivascular sheet. These results identified the presence of marrow stem cells and osteoprogenitor cells in human cancellous bone marrow capable of responding to the increased PDGF and TGF-b in the PRP preparation. -Platelet count study: Counts done on each patient yielded a mean platelet count value of 232,000. The PRP mean platelet count was 785,000. -Assignment of radiographic graft maturity: At 2 and 4 months, the grafts without growth factor additions were assessed at or just below their actual maturity. At 6 months they were assessed at or just ahead of the actual graft maturity. The grafts with PRP additions were consistently judged to be slightly more than twice their actual maturity. The ratios were 2.16 at 2 months, 1.88 at 4 months, and 1.62 at 6 months. -Six-month graft assessment with monoclonal antibodies: At 6 months, continued production of TGF-b was seen. Monoclonal antibodies identified TGF-b but not PDGF by marrow stem cells and endosteal osteoblasts. The TGF-b	This study shows that PRP is an enhanced source of PDGF and TGF-β. The cancellous cellular marrow graft was shown to contain cells responsive to these growth factors and addition of these bone growth factors to the graft, through the use of PRP, produced quantifiably enhanced bone maturation and density in comparison to grafts performed without the addition of PRP.

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results	Conclusions
				positive cells were noted to be concentrated on the trabecular bone endosteal surface, on the periosteal surface, and within active marrow stem cells. -Six-month graft assessment with histomorphometry: Bone grafts in general produced a trabecular bone area greater than that of native posterior mandible. Bone grafts with growth factors added by means of PRP demonstrated even greater trabecular bone density than did bone grafts without PRP. The results indicate that PRP addition accelerated the rate and degree of bone formation in bone grafts. It also showed that growth factors in platelets can be sequestered, concentrated, and added to bone grafts along with a fibrin network. Cancellous marrow grafts contain cells with PDGF and TGF-b receptors as the targets of PRP.	
54	"A SOURCE OF AUTOLOGOUS GROWTH FACTORS FOR BONE GRAFTS. IN TISSUE ENGINEERING: APPLICATIONS IN MAXILLOFACIAL SURGERY AND PERIODONTICS" By: R.E. Marx Quintessence Publishing Co., Inc. 1999	Text book chapter on the use of growth factors, specifically autologous PRP, to enhance wound healing. Review of preparation methods and applications of PRP for cancellous cellular marrow bone grafts, and the role of several important growth factors in PRP including PDGF, TGF-$ß_1$, and TGF-$ß_2$, and IGF-I. Review of the stimulation of cellular activity in bone grafts that are enhanced with addition of PRP. PRP-initiated bone regeneration is a magnification or enhancement of the natural bone regeneration pathway. PRP has a documented clinical track record and has been shown to increase the rate of clinical graft consolidation and to produce a more mature and dense bone graft than grafts without PRP.			
55	"TRICALCIUM PHOSPHATE CERAMIC AS IMMEDIATE ROOT IMPLANTS FOR THE MAINTENANCE OF ALVEOLAR BONE IN PARTIALLY	Assess TCP as an immediate root implant in maintenance of alveolar bone. Three (3)	Mirror image of 5 sockets on contralateral side of each patient.	Healing was uneventful and there was no evidence of inflammatory reaction or immunological rejection. In most implant sites an apparent prominence in height and width was observed. The control sockets showed a decrease in height of the aveolar bone compared	TCP implant inextraction sockets assisted in maintenance of height and width of alveolar bone. No immunological reaction or toxic effects were observed. No problems encountered in preparation, storage, or

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	ENDENULOUS MANDIBULAR JAWS. A CLINICAL STUDY." By: J.K. Mathai, S. Chandra, K.V. Nair, K.K. Nambair Aust Dent J 1989	patients with five TCP implants. Standard full-thickness, microperosteal flaps were raised, extractions were made, TCP implanted, and flaps repositioned and sutured. Patients assessed at 1, 12, 20, and 78 weeks post implantation.		to the implant. No conclusive evidence of bone regeneration at the implant sites. However, alveolar bone appeared to resorb at a faster rate at control sites compared to implant sites.	implantation of TCP encountered. No specialized skills required for TCP implantation. Long-term evaluation of TCP in larger population is justified.
55				-The Specificity of the Inhibition of DNA Synthesis by Bradykinin: Endothelin, vasopressin, and des-arg9 bradykinin had little inhibitory activity, suggesting that the inhibition of EGF-mediated DNA synthesis is specific for bradykinin through its B2 receptor.	
56	"INTRAINDIVIDUAL COMPARATIVE ANIMAL STUDY OF ALPHA- AND BETA-TRICALCIUM PHOSPHATE DEGRADATION IN CONJUNCTION WITH SIMULTANEOUS INSERTION OF DENTAL IMPLANTS" By: H.A. Merten, J. Wiltfang, U. Grohmann, J.F. Hoenig	Assess degradation characteristics of α- and β-TCP using adult Goettinger miniature pigs. Proximal tibial marrow defects. Left side of defect filled with β-TCP and right side filled with	Control defects were made in two other animals.	Both α- and β-TCP were osteoconductive. Centripetally oriented angiogene bone regeneration occurred at the margins of the circular defects. Ceramic degradation occurred both hydrolytically and within cells. Within 86 weeks, nearly 80% to 90% of the larger α-TCP granules, and nearly 90% to 95% of the β-TCP granules were degraded. Especially for the α-TCP, ceramic microparticles were found in the marrow either unbound or within polynuclear macrophages.	Intra- and transtrabecular microcracks could be detected but only in the α-TCP implants. β-TCP resulted in faster degradation and micro-osseous conduction, and exhibited better tissue response in comparison to α-TCP. Due to initial pronounced accumulation of macrophages, dental implants should not be inserted simultaneously with ceramic but after further progress of ceramic degradation.

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results	Conclusions
	J Craniofac Surg 2001	α-TCP. Dental screw implants were inserted in each ceramic. Reorganization and degree of bone regeneration, and degradation characteristics measured.			
57	"The functional interaction of EGF and PDGF with bradykinin in the proliferation of human gingival fibroblasts" By: B.S. McAllister, F. Leeb-Lundberg, J.T. Mellonig, and M.S. Olson Synopsis: This study explores the effects of bradykinin on growth factor-induced DNA synthesis in cells of the periodontium and provides proposed mechanisms involved in this interaction.	Gingival, bone, and periodontal ligament explants were taken form healthy human patients	N/A	-Arrest of DNA Synthesis in Confluent Cells: A 4-hour serum-free period reduced optimally cell signaling to basal levels. A total of 24 hour serum-free followed by a 4 hour pulse label resulted in very low levels of DNA synthesis. Twenty four hours after growth factor stimulation, for a total of 24 hours in a serum-free environment, a 4 hour pulse labeling was performed to mimic the physiological state of cells just prior to the initial phase of stimulation during wound healing. -Effects of Bradykinin on EGF- and PDGF-BB Induced DNA Synthesis: EGF- and PDGF-BB were both found to stimulate DAN synthesis in a concentration dependent manner. Bradykinin inhibited GF-induced DNA synthesis. This suggested that the inhibitory effect of bradykinin is a specific intracellular signaling event. The degree of inhibition by bradykinin was less in PDGF-BB stimulated cells. The extent of inhibition of EGF-mediated DNA synthesis by bradykinin varied, but was mostly 90-100%. The inhibition of PDGF-mediated DNA synthesis varied, with most at 50-60%. -PGE_2 Release and Involvement in the Bradykinin-Mediated Inhibition of EGF-Induced DNA Synthesis: Cells stimulated with bradykinin released PGE_2 and this release was potentiated	rhPDGF-BB and EGF both stimulate DNA synthesis in periodontal ligament and alveolar bone cells. EGF-induced proliferative responses, and to a lesser extent PDGF-induced proliferation, are inhibited by bradykinin via a cAMP dependent pathway. Supplemental application of PDGF may be needed to overcome the inhibitory effect of bradykinins in growth factor mediated tissue repair.

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				when bradykinin was added in combination with EGF. Pretreatment with indomethacin completely blocked the bradykinin-stimulated release of PGE_2. When indomethacin was examined as an inhibitor of the bradykinin effect on EGF-induced synthesis, indomethacin abolished the inhibitory effect of bradykinin further supporting a PGE_2 dependent mechanism of inhibition.	
57				-Histopathology: rhPDGF-BB administration increased osteoblast number and osteoblast perimeter without consistent changes in osteoclast estimates. The systemic administration of rhPDGF-BB also decreased body fat and resulted in extraskeletal collagen deposition.	
58	"Effect of Autogenous Marrow and Calcitonin on Reactions to a Ceramic" By: P.T. McDavid, M.E. Boone, A.H. Kafrawy, and D.F. Mitchell Synopsis: This study explored the hypothesis that autogenous marrow or calcitonin applied over the surface of a porous biodegradable ceramic would be associated with bone formation when the ceramic was implanted subcutaneously in rats.	β-TCP pellets coated with autogenous marrow or calcitonin; 25 male Wistar rats	25 (one control pellet implanted in each rat)	5 animal were euthanized at 2 days and 2, 4, 8 and 16 weeks post-implantation. The implants were excised and processed for histologic examination. At 2 days all implants were associated with moderate inflammatory reactions with PMNs, lymphocytes, and occasional plasma cells in the connective tissue that surrounded the implant. The interstices of the ceramic were infiltrated with fibrin and some leukoctyes. Other reactions were mild with scattered round cells. Both the uncoated and coated ceramic interstices were infiltrated with granulation tissue that matured into fibrous connective tissue with no evidence of bone formation. At 4 weeks and thereafter, bone was evident surrounding and infiltrating the pores of all marrow coated TCP implants. The new bone was initially woven and then matured into lamellar bone with evidence of remodelling. Degradation of the TCP was evident.	This study shows that the porous β-TCP used has a low irritational potential, but by itself, or when coated with calcitonin, it was not osteogenic. However, the TCP appeared to be an excellent vehicle for osteogenic marrow, with the combination of TCP and marrow resulting in extensive osteogenesis.

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results	Conclusions
59	"The effect of Systemically Administered rhPDGF-BB on the Rodent Skeleton" By: B.H. Mitlak, R.D. Finkelman, E.I. Hill, et al. Synopsis: This study evaluates the effect of rhPDGF-BB (administered systemically) on the skeletons of sexually mature, estrogen-deficient rats.	rhPDGF-BB; 33 female Sprague-Dawley rats rendered acutely estrogen – deficient by ovarectomy were randomized to treatment administered by tail vein injection, every other day for 6 weeks. Treatment groups included control (8) using PBS; rhPDGF-BB 2mg/dose (8); rhPDGF-BB 2mg/dose + alendronate 0.075mg/dose (9); and alendronate 0.075mg/dose (8)	8 vehicle (PBS alone)	-DXA: Spine BMD decreased by 5% after ovariectomy in vehicle animals. In the animals treated with PDGF or alendronate alone, spine BMD increased about 8 to 9 %. The combination treatment of both produced an 18% increase in spine BMD. The combination also produced a higher whole skeleton BMD increase than each alone. -QCT: Along the femoral diaphysis, PDGF treatment caused significantly greater bone mineral content (BMC) than in the vehicle animals. PDGF treatment alone resulted in higher BMC than alendronate alone. The combination resulted in higher BMC at all sites than alendronate alone or the vehicles, but not higher than PDGF alone. In the metaphyseal sites treatment with PDGF (with or without alendronate) significantly increased the diaphyseal cortical volume. -Proximal Tibiae and vertebrae structure: In vehicle treated animals, the growth plate was narrow and clearly defined. In animals treated with PDGF (with or without alendronate), the growth plate was mineralized by cancellous bone. In the vertebrae, the growth plate was narrowed and less cellular. In both tibiae and vertebrae of PDGF treated animals, increases in trabecular density in the secondary spongiosa were seen. In animals treated with alendronate alone, growth plates were well defined and some of the primary spongiosa was replaced by mineralized cartilage. In animals receiving the combination treatment, increased trabecular density and architecture were seen in the primary and secondary spongiosa -Biochemical tests: Serum calcium, albumin, glucose, and creatinine concentrations, alkaline phosphatase activity, and creatinine clearance	The systemic administration of rhPDGF-BB increased bone mass, density, strength and osteoblast cell numbers throughout the skeleton (in both long and flat bones). The effectiveness of rhPDGF-BB was generally equal to or greater than alendronate (Fosomax). The combined treatment of rhPDGF and alendronate resulted in a greater increase than either agent alone. Thus, rhPDGF-BB stimulated bone formation and strength throughout the skeleton.

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results	Conclusions
				were not different among any of the groups. Serum inorganic phosphate and osteocalcin were increased in PDGF-treated animals compared with vehicle animals. Alendronate treatment caused a decrease in mean serum osteocalcin level, and this was opposed by the coadministration of PDGF. -Biomechanical testing: rhPDGF-BB administration significantly increased vertebral body compressive strength and femoral shaft torsional stiffness and resulted in trends toward enhanced femoral head shearing strength. Coadministration of rhPDGF-BB and alendronate further enhanced these indices of bone strength.	
60	Mohan S, Baylink DJ. Therapeutic potential of TGF-ß, BMP, and FGF in the treatment of cone loss. *Principles of Bone Biology*, 1996; 1111-23.				
61	"Enhancement of Osteoblast Proliferation in vitro by Selective Enrichment of Demineralized Freeze-dried Bone Allograft with specific Growth factors" By; D.A. Mott, J. Mailhot, M.F. Cuenin, M. Sharawy, & J. Borke 2002 *Journal of Oral Implantol.* Synopses: The purpose of this study is to determine what effect, if any,	Primary cultured osteoblastic cells harvested from 1-day –old neonatal mice. The calvaria of these mouse pups were digested and frozen for use. 2% residual calcium-DFDBA and supplemented by one or combinations of Growth factors;	0 cells, A control group with 2% residual calcium DFDBA without additional growth factors Addition of growth factors to 1% and 10% fetal bovine serum	Cells were evaluated at different intervals and the proliferation assay with (Cy-Quant Cell Proliferation System). Analyses were done at days 0, 7, 14, 21 and 28. There was a statistically significant difference at day 7 with combination growth factors IGF-1/TGF-β, IGI-I/PDGF and PDGF/TGF-β compared with controls.	The osteoblasts exposed to 2% DFDBA and enhanced with a combination of growth factors including IGF, TGF-β, and PDGF showed a significant difference at day 7 compared to controls. The DFDBA as a carrier appear to be required for these growth factors to stimulate an increase in cell proliferation. Individual growth factors IGF-I, PDGF, and TGF-β all showed a noticeable effect and a significant increase in proliferation when adsorbed in DFDBA at day 14.

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results	Conclusions
	decalcified Freeze-Dried Bone Allografts (DFDBA) enriched with specific growth factors has on the in vitro proliferation of murine osteoblasts.	transforming growth factor-β (TGF-β), insulin-like growth factor-I (IGF-I), platelet-derived growth factor (PDGF), fibroblast growth factors basic (bFGF), or vascular endothelial growth factors (VEGF).			
62	"B-tricalcium phosphate as a bone substitute for dorsal spinal fusion in adolescent idiopathic scoliosis-preliminary results of a prospective clinical study" By: M. Muschik, R. Ludwig, S. Halbhübner, K. Bursche, and T. Stoll Synopsis: This study evaluates the performance of β-TCP in granular form in human dorsal spine fusion and assesses the quality of bone fusion obtained in the treatment of adolescent idiopathic scoliosis.	B-TCP mixed with autograft; 9 patients with idiopathic scoliosis	Autograft bone mixed with allograft bone; 19 patients	Significant correction of the spine was achieved by surgery in both groups. No significant differences were noted in blood loss during the operation, transfusion of blood, and operation time. CT scans used to measure the bone mineral density revealed that BMD was 430±111 mg/cm^3 in the TCP group and 337±134 mg/cm^3 in the allograft bone group. No pseudoarthrosis was observed. Resorption of the TCP was complete on radiographs after 8±2 months. No complications were noted that were related to use of β-TCP.	This study shows that β-tricalcium phosphate used in combination with autogenous bone for dorsal spinal fusion in adolescent idiopathic scoliosis is as effective as treatment using allograft bone in combination with autogenous bone.
63	"Effect of platelet-derived growth factor on tibial osteotomies in rabbits"	rhPDGF-BB; 7 rabbits in the experimental	Collagen vehicle; 7 rabbits in	The effect of rhPDGF-BB on bone healing was tested in unilaterial tibial osteotomy defects. Each osteotomy was injected with either collagen	Clinical, radiographic, mechanical and histological data show that local implantation of rhPDGF-BB to tibial

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results	Conclusions
	By: T.J. Nash, C.R. Howlett, C. Martin, I. Steele, K.A. Johnson, and D.J. Hicklin	group	control group (14 rabbits total)	or collagen containing 80 micrograms of rhPDGF-BB. Clinical observations, radiographs, strength tests and histology were use to assess the outcome at each site. At day 28, both tibiae from each rabbit were harvested and subjected to three point bending to failure. Results of the experimental side were compared to the unoperated contralateral tibiae. -Radiographically at 2 and 4 weeks there was a clear increase in callus density and volume around the PDGF-treated osteotomies compared to controls. -Biomechanical testing: control tibiae treated with the collagen vehicle alone were significantly weaker than their nonoperated contralateral tibiae, whereas tibiae treated with rhPDGF-BB had increased strength and were not significantly different from the normal contralateral tibiae. -Histologically, rhPDGF-BB treated tibiae had a more robust and advanced state of osteogenesis both endosteally and periosteally than the vehicle controls.	osteotomies stimulates osteogenesis and functional fracture repair.
64	"Bioceramic Implants in Surgically Produced Intrabony Defects" By: E.B. Nery, K.L. Lynch, W.M. Hirthe, and K.H. Mueller Synopsis: This study histologically evaluates the use of bioceramic material as a potential alternative to bone grafting.	TCP; 6 adult mongrel dogs	Sham surgery; 6 dogs on opposite side of maxilla from test sites	1 week: Nonspecific inflammation with numerous proliferating endothelial cells is seen. Bone resorption and regeneration are seen concurrently in many areas. There is no evidence of new cementum being laid down on the tooth surface. The control defects are filled with acute inflammatory cells. 2 weeks: Inflammatory responses have diminished, but budding capillaries and fibroblasts have proliferated. Some hemorrhages with a fibrin network border the implant. New reattachment is suggested. At the bottom of the defect, osteoblasts are seen. No ulceration is seen. In the control, giant cells seen earlier are fewer in number and intense osteoblastic activity is seen. 4 weeks: Chronic inflammation is noted in the	The results show that TCP is well tolerated by tissue and produces no toxic reactions. Bone ingrowth into the pores and repair of the periodontium are clearly demonstrated. No significant hematological changes were observed.

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results	Conclusions
				crevicular areas. Most of the pores of the ceramic implant are now replaced by fibrovascular connective tissue, and initial osteoid formation is seen in some areas. Giant cells are also seen in appostion with the implant. Bone remodelling is found at the alveolar crest. New reattachment is seen as evidenced by many connective tissue fibers being trapped in a newly formed cementum, which is being laid down on dentin or old cementum. The control defects are filled with fibrous connective tissue and new reattachment of periodontal fibers is seen (by new cemental deposits). 8 weeks: Tissues surrounding the implant, and those that grew into the pores of the interconnecting channels are free of inflammatory infiltrate. Circularly arranged bone seams and a central mass of bone surrounded by connective tissue are seen. The overlying epithelial tissue is more mature with a thicker keratinous layer. At the control site, new bone is found mainly at the base of the defect and has not reached alveolar height. 24 weeks: The implant retrieved shows increased bone deposition in the pores and interconnecting channels. The bone seams are matured with osteocytes entrapped in a thick layer of matrix. New cementum is thicker, giant cells bordering the material are fewer. In the control, the defect is filled with mature bone, making it identical to the old bone. Blood studies: The operation is well tolerated with no harmful effects, since no blood values changed.	
65	"Preliminary Clinical Studies of Bioceramic in Periodontal Osseous Defects" By: E.B. Nery, and K.L.	6 male adult patients	N/A	Postoperative healing was uneventful for all patients except one patient that was eliminated from the study because the ceramic implant exfoliated due to inadequate soft tissue coverage during wound closure.	This study shows that porous TCP is biocompatible and resulted in improved clinical and radiographic findings. It was concluded that restoration of the periodontium may be

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results
	Lynch Synopsis: This study investigates whether TCP will function the same way in naturally occurring periodontal osseous defects as it does in healthy animal models with surgically created or induced periodontal osseous defects. The study assesses gingival tissues at the implant site, the change in pocket depth measurement, and blood chemistries.			-Gingival Index: The tissue healed within a period of 2 months with no evidence of an inflammatory response. Normal postoperative inflammation as a result of surgical trauma subsided in 3 to 4 weeks. -Pocket Depth: Radiographically, a significant coronal increase was seen in bone height when measured from the base of the intrabony defec the base of the amalgam marker. There was a mean gain (fill) of 5.2 mm. The ceramic mate seems to blend with the surrounding bone, indicating that the radiographic density is the same for both the implant and the patients bon At the interface between the root surface and t ceramic, the periodontal ligament space was difficult to discern, suggesting that there was inadequate space for development of the entire width of the ligament. -Blood Chemistries: Pre- and post-operative blood chemistry studies showed no significant changes.
66	"Periodontal Regeneration in Humans Using Recombinant Human Platelet-Derived Growth Factor BB (rhPDGF-BB) and Allogeneic Bone" By: M. Nevins, M. Camelo, Marc Nevins, R. Schenk and S. Lynch 2003 *Int Journal of Perio & Restorative Dentistry* Synopsis: The objective of this human trial was to	rhPDGF-BB in three amounts 0.5 mg/ml, 1.0 mg/ml and 5.0 mg/ml; 9 patients- 11 osseous defects.	Historical controls	This study was designed to evaluate the safety and preliminary effectiveness of rhPDGF-BB administered in a bone matrix to periodontal osseous defects, including both mandibular mc Class II furcation defects and interproximal intrabony defects. There were no safety issues associated with use of rhPDGF-BB administer in a bone matrix. Efficacy data demonstrated a favorable outcome clinically, radiographically and histologically following application of the rhPDGF-BB product. Clinical measurements pocket depth (PD) and clinical attachment leve (CAL) were significantly improved. Comparison to a meta analysis of sixteen previous bone grafting clinical trials, suggestec that rhPDGF-BB may lead to improved clinica

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results
	evaluate the safety and effectiveness of rhPDGF-BB in an osteoconductive matrix for treatment of osseous periodontal defects.			outcomes compared to existing therapies. Radiographic analysis suggested bone fill in the defects and no abnormal changes in bone architecture or root configuration. Histologic analysis showed remarkable regeneration of bone, periodontal ligament and cementum in both furcation defects and interproximal angular defects, especially in the 1.0 mg/mL group.
67	"Mitogenic Effects of Growth Factors on Human Periodontal Ligament Cells In Vitro" By: T.W. Oates, C.A. Rouse, and D.L. Cochran Synopsis: The purpose of this study was to determine the effects of PDGF, IL-1 and TGF-β on human periodontal ligament (PDL) cell mitogenesis, and to identify the regulatory influences of TGF-β on the response to PDGF and IL-1.	Human periodontal ligament (PDL) cells dissected from the mid-root of 4 fully erupted premolar and molar teeth Culture medium containing either control (0.1%FBS) or test medium (PDGF-AA, PDGF-BB, IL-1β, and TGF-β) was added to triplicate cultures at various concentrations.	Culture medium with 0.1% FBS was used as a control	rhPDGF-BB and rhPDGF-AA were found to be potent mitogens for human PDL cells, maximally enhancing the mitogenic response 12- and 10-fold, respectively. The mitogenic response for both PDGF isoforms increased in a dose-dependent manner. The addition of exogenous IL-1B to human PDL cells produced no apparent mitogenic enhancement. High concentrations of IL-1B produced a statistically significant inhibition of the mitogenic response compared to the controls. No time dependence was observed during the 24-28 hour incubation period. TGF-B produced a significant increase in the mitogenic response relative to control levels. The concentration of 1.0 ng/ml produced the greatest response. These responses were weaker than those of the PDGF isoforms. Unlike the PDGF isoforms, the TGF-B response after 48 hours was greater than that measured after 24 hours of incubation. A maximal response was observed at 32 hours. Pre-incubation with both 1.0 ng/ml and 10.0 ng/ml for 8 to 24 hours prior to IL-1B incubation showed a statistically significant enhanced mitogenic response over the levels of response achieved with no or 1 hour of TGF-B pre-incubation. PDGF-AA and BB produced enhanced responses with pre-incubation of 1.0 ng/ml of TGF-B for 8 to 24 hours before PDGF incubation. The PDGF-BB response was slightly enhanced with 10 ng/ml of TGF-B pre-

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results
				incubation.
68	Parashis A, Andronikaki-Faldami A, Tsiklakis K. Comparison of 2 Regenerative Procedures – Guided Tissue Regeneration and Demineralized Freeze-Dried Bone Allograft – in the Treatment of Intrabony Defects: A Clinical and Radiographic Study.			The article is provided in Appendix 10.2.2.
69	"Periodontal Regeneration in Class III Furcation Defects of Beagle Dogs Using Guided Tissue Regenerative Therapy With Platelet-Derived Growth Factor" By: J.B. Park, M. Matsuura, K.Y. Han, O. Norderyd, W.L. Lin, R.J. Genco, and M.I. Cho Synopsis: This study compares rhPDGF-BB guided tissue regenerative therapy (P-GTR) and guided tissue regenerative therapy alone (GTR) for their ability to effectively repair and regenerate bone and periodontal ligament in large horizontal furcation defects in dog mandibles.	rhPDGF-BB and guided tissue regeneration (GTR) barrier membranes; 6 male beagle dogs (one quadrant in each dog)	GTR barrier membranes alone; 6 dogs (one quadrant in each dog)	-Morphometric Analysis: At 5 weeks, GTR and P-GTR (rhPDGF-BB plus GTR) groups did not show any significant differences in areas filled with epithelium, inflamed tissue, new fibrous connective tissue, bone and PDL. At 8 weeks, the rhPDGF-BB-GTR group showed almost complete healing. The furcations were filled with 80% new bone and 20% PDL, while the GTR group had 26% unoccupied space, 31% inflamed tissue and only 15% new bone. At 11 weeks, the rhPDGF-BB-GTR sites remained completely healed with 87% new bone and 13% PDL. The GTR group progressed over its results at 8 weeks but still had less fill than the rhPDGF-BB treated sites. Overall, the furcation defects of the P-GTR group advanced faster and more effectively than those of the GTR group. -Histological Observations: Repair of defects around fourth premolars -8 weeks: GTR defects showed the least repair among all the samples in the study. No bone formation or cementum was observed. The defects in P-GTR showed almost complete regeneration. No signs of ankylosis or root resorption were found.

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results
				-11 weeks: GTR therapy furcation defects still were not completely healed. Healthy connective tissue was seen between inflamed connective tissue and new alveolar bone. After P-GTR therapy, the furcation lesion was completely regenerated. Repair of defects around second premolars -5 weeks: GTR therapy produced more advanced healing than those of the fourth premolars at 5 weeks. A thin layer of fibrous connective tissue along the root surface extended coronally close to the epithelium. A small amount of new bone and cementum started to form. P-GTR therapy produced complete healing in the lesion, filling the furcation with new bone, new fibrous connective tissue, and PDL. Although mineralized cementum was not observed at this time, collagen was deposited on the root surface. -8 weeks: Healing of the GTR sites improved and was comparable to the 5 week P-GTR sites. The P-GTR lesion showed complete regeneration with new cementum on the root surface, alveolar bone, and PDL.
70	"Controlled release of platelet-derived growth factor from porous poly (L-lactide) membranes for guided tissue regeneration" By: Y.J. Park, Y. Ku, C.P. Chung, S.J. Lee August 1998 *J Control Release* Synopses: The aim of this study was to design a	*In vitro* activity studies: Gingival fibroblast suspension ($1x10^5$ cells/ml) in cultural media (DMEM) were incubated with released samples of PDGF-BB from PLLA membranes or standard PDGF-BB solution.	A-MEM control media for the *in vitro* studies. Rats with circular defects untreated with any regenerative material for the *in vivo* studies.	*In vitro:* PDGF-BB released from PLLA membrane showed almost the equivalent cell migration activity (98.3%) as compared to standard PDGF-BB solution. *In vivo*: In the control rats, connective tissue spanned host bone margin and defect was penetrated by connective tissue (CT). There was no evidence for new bone formation. In membrane treated defect, small amount of new bone was formed beneath membrane during 2 week implantation period, and fibrous tissue bridged the remaining defect gap. Indication of reunion of new bone was not observed in this

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results
	PDGF-BB releasing porous poly-L-lactide (PLLA) membrane for guided tissue regeneration (GTR).	*In vivo* studies: (Sprague-Dawley rats) PDGF-BB loaded PLLA membranes were placed in 5 mm circular defects of the rat calvarial disc. Rats were sacrificed in 1 and 2 weeks after implantation.		period. In PDGF-BB loaded PLLA, membranes treated defects, after 1 week, significantly high amount of osteoid tissue and new bone were formed beneath membrane and osseous bridge was developed. New bone formation continued up to 2 week implantation period and complete bony reunion was achieved.
71	"Controlled release of platelet-derived growth factor-BB from chondroitin sulfate-chitosan sponge for guided bone regeneration" By: Y.J. Park, Y.M. Lee, J.Y. Lee, Y.J. Seol, C.P. Chung, S.J. Lee 2000 *Journal of Controlled Release* Synopses: The aims of this study are to design a way of controlling growth factor delivery by fabrication of a PDGF-BB releasing porous chondroitin-4-sulfate (CS)-chitosan sponge. 1) To achieve steady	Primary cultures of rat calvarial cells were dissected from 21-day-old Sprague-Dawley rat fetus and then striped of periosteum and loosely adherent tissue. Aliquots of 20 μl of cell suspension were seeded on top fop rewetted CS-chitosan sponges (density of 10^5 cells/sponge) and incubated for 7 days.	Chitosan sponges devoid of CS.	Release of PDGF-BB was efficiently sustained as the content of CS in chitosan sponge increased. Initial bursts of PDGF-BB was reduced and the following release rate after initial burst was higher and more steady than that of chitosan sponge devoid of CS. After an initial release of 90 ng, PDGF-BB was consistently released from CS (20%)-chitosan at a rate of 4-5 ng/day. Significantly leveling off in PDGF-BB release was not observed. In vitro experiments revealed that seeded osteoblasts proliferated throughout the 14-day period. PDGF-BB-loaded CS-chitosan sponge markedly enhanced cell proliferation rate up to 14 days.

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results	Conclusions
	release of PDGF-BB via ionic interaction between PDGF-BB and CS. 2) To control porosity of the sponge due to ionic aoacervation between chitosan and CS.				
72	Persson GR, Falk H, Laurell L. A retrospective radiographic outcome assessment study of intra-bony defects treated by osseous surgery or by bone graft procedures. *J Clin Periodontol* 2000; 27:104-108.			The article is provided in Appendix 10.2.2.	
73	"Study of the Growth Factor Requirements of Human Bone-derived Cells: A Comparison with Human Fibroblasts" By: J.E. Piche, and D.T. Graves Synopsis: This study investigates the growth factor response of normal human bone-derived cells utilizing growth factors that may play a vital role in osseous wound healing. The results obtained were compared with those observed for normal human fibroblasts.	Tissue samples (sub-periosteal trabecular bone and dense gingival connective tissue) from healthy adults	(-) control: cells incubated in DMEM supplemented with platelet-poor plasma. (+) control: cells incubated in DMEM with 10% FBS	Bone-derived cells increased 5-fold over 9 days in 4% platelet-poor plasma. The rate of proliferation was increased by addition of PDGF and further increased by a combination of PDGF, TGF-β and EGF. When testing responses to combinations of growth factors, EGF and TGF-B only slightly enhanced the proliferation of bone derived cells over the negative control. The combination of PDGF and either EGF or TGF-B produced an intermediate response of cellular proliferation in bone-derived cells. PDGF, EGF, and TGF-B were added together and produced a response equal to 10% FBS. Radiographic studies showed that in cells incubated in 3% platelet-poor plasma only 1.2% of the normal human fibroblast nuclei were labeled. In the bone-derived cells, 8.8% of the nuclei were labeled. Thus, a much larger number of bone-derived cells synthesize DNA under conditions in which fibroblasts are inactive. Also, human fibroblast and bone-derived cells were incubated in insulin and transferrin, but both results were	The results of this study demonstrate that PDGF is an important mitogen for human bone cells (osteoblasts) as well as human fibroblasts. Co-administration of EGF and TGF increased proliferation of bone cells but not fibroblasts. These findings indicate that the factors that regulate osseous regeneration may differ from those involved in repair of soft tissue wounds, but that PDGF is likely to be important in the repair of both bone and soft tissue wounds.

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results	Conclusions
				insignificant. Thus, factors in platelet-poor plasma that are absent in insulin, IGF, and transferrin support DNA synthesis in bone-derived cells but not normal human fibroblasts. For the human fibroblasts, all combinations with PDGF produced a significant increase in cellular proliferation equal to that of 10% FBS. When EGF and TGF-B were incubated without PDGF, no increase in fibroblast proliferation above the negative control was seen. When growth factors were added to DMEM supplemented with BSA without plasma or serum factors, no single growth factor maximally stimulated DNA synthesis in bone-derived cells. The combination of EGF, TGF-B, and PDGF produced greater than a 400% increase in DNA synthesis, which was statistically equivalent to the 10% FBS stimulation. Addition of IGF to this combination produced a response equal to the 10% FBS positive control.	
74	"Platelet-derived growth factor and bone morphogenetic protein in the healing of mandibular fractures in rats" By: L. Rasubala, H. Yoshikawa, K. Nagata, T. Lijima, M. Ohishi 2003 *British Journal of Oral and Maxillofacial Surgery* Synopses: This study investigates the effects of platelet-derived growth factor-B (PDGF-B) and bone morphogenetic	Unilateral closed fractures were created in the mandibles of thirty Wistar rats (aged 12 weeks). Rats were sacrificed on days 3, 7, 14, and 21 after the operation and anti-BMP-2 antibody and rabbit polyclonal anti-PDGF-B antibody washed over the excised mandibles.	As negative controls, normal goat IgG, normal rabbit IgG and normal mouse IgG were used instead of the primary antibodies.	By day 3 after the fracture, PDGF-B can be detected in some of the macrophage-like cells in the haematoma adjacent to the fracture site, but this was not present in the periosteal cells. On the other hand, most of the proliferating periosteal cells, except for the cells around the periosteum, showed staining for BMP-2 in the cytoplasm. By day 7, PDGF-B and BMP-2 were common among the cells lining the observed newly formed woven bone. In the endochrondral ossification area, precursor cells of chrondrocytes showed the most intense staining for PDGF-B and BMP-2. By day 14, there was intense staining of PDGF-B and BMP-2 in the osteoblasts lining the calcified cartilage matrix and woven bone.	This study concludes that PDGF-B and BMP-2 have different regulatory functions during the healing of the mandibular fractures. PDGF-B was associated with the proliferation and migration of primitive mesenchymal cells and BMP-2 was related to the differentiation of mesenchymal cells into osteoblasts and chondroblasts.

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results	Conclusions
	protein-2 (BMP-2) during the healing of mandibular closed fractures in rats by immunohistochemical methods.			By day 21, PDGF-B was no longer detected. BMP-2 can still be detected in the osteoblasts lining trabecular bone.	
74	"Regulation and role of PDGF receptor alpha subunit expression during embryogenesis" By: G.C. Shatterman, K. Morrison-Graham, A. Van Koppen, J.A. Weston, and D.F. Bowen-Pope 1992 *Development*	PDGFR-alpha; Patch mice embryos	Normal mice embryos	The PDGF receptor alpha-subunit (PDGFR-alpha) can bind both the A and B chains of PDGF. By 9.5 days of development, embryos homozygous for the deletion of the PDGFR-alpha display obvious growth retardation and deficiencies in mesoderm structures and many die. Those that survive later develop a series of connective tissue defects. In situ hybridization and Northern blot analysis show that PDGFR-alpha is important for formation of the axial skeleton. Absence of the PDGFR leads to severe skeletal abnormalities.	This study demonstrates that PDGF plays a critical function in the normal development of the skeleton and that the absence of its activity as a result of a mutation of the PDGFR-alpha leads to severe skeletal defects.
75	Reynolds MA, Aichelmann-Reidy ME, Branch-Mays GL, Gunsolley JC. The Efficacy of Bone Replacement Grafts in the Treatment of Periodontal Osseous Defects. A Systematic Review. *Ann Periodontol.*			The article is provided in Appendix 10.2.2.	
76	"Polymeric system for dual growth factor delivery" By: Thomas P. Richardson, Martin C. Peter, Alessandra B. Ennett and David J. Mooney	*In vitro* studies: Release rates and bioassay using cell proliferation assays with smooth muscle cells and endothelial cells. *In vivo* tests:	Lewis rats with blank scaffolds (no growth factors) implanted into the sub-cutaneous tissue. Growth factors injected alone	By combining two approaches for the incorporation of growth factors, VEGF in vitro release rates were 1.7 pmol/day (79 ng/day) for the first seven days followed by a slower delivery rate. While the release rates of PDGF from scaffolds varied from 4.2 pmol/day to 0.10 pmol/day by altering the degradation rate of the polymer using various polymer formulations and molecular weights. The first approach involves mixing lyophilized VEGF with polymer particles	This study documents that dual delivery of growth factors involved in distinct aspects of vascular development is critical to the rapid formation of mature vasculature. Sustained, localized delivery of two growth factors both initiates formation of a significant number of blood vessels and induces their maturation.

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results	Conclusions
	2001 *Nature Biotechnology* Synopses: This study tests the hypothesis that dual delivery of VEGF and PDGF can direct the formation of a mature vasculature, as compared to the delivery of VEGF or PDGF delivered alone. The polymeric scaffold fabricated from poly(lactide-co-glycolide) (PLG) allowed dual delivery of vascular endothelial growth factor (VEGF)-165 and platelet-derived growth factor (PDGF)-BB resulting in the rapid formation of a mature vascular network.	Lewis rats – sub-cutaneous implantation. *In vivo* model of therapeutic angiogenesis Non-obese diabetic (NOD) mouse model subjected to femoral and vein ligation.	or together into blank scaffolds at the time of implantation leading to short-term presence of growth factors in the implant site.	before processing the polymer into a porous scaffold. The second approach involves pre-encapsulating a factor in PLG microspheres. In vivo results: Growth factors injected alone or together in the scaffold at implantation reveal relatively few mature blood vessels at 2 & 4 weeks. However, with sustained & localized release with an appropriate combination of VEGF & PDGF:-VEGF alone led to a sharp increase of angiogenesis, but resulting in small vessels with little matrix or mural cell association. Sustained PDGF alone did not significantly alter the density of blood vessels although the existing blood vessels appeared larger. In contrast, the dual-release scaffolds led to an elevated density and apparently more mature vessels. After 4 weeks, the differences were notable leading to a higher blood vessel density and more mature larger vessels. In vivo model of therapeutic angiogenesis: Measurement of induced collateral vascularization in a hind limb ischemia model in NOD mice with dual delivery of both factors resulted in a statistically significant increase in density of vessels positively stained for α smooth muscle actin (marker for pericytes & smooth muscle cells in larger mature vessels).	The polymeric scaffold allows controlled dual release of two distinct proteins.
77	"Platelet-derived and insulin-like growth factors stimulate regeneration of periodontal attachment in monkeys" By. R.B. Rutherford, C.E. Niekrash, J.E. Kenney, and M.F. Charette	rhPDGF and rhIGF-I; 3 cynomolgus monkeys- total of 8 defects per monkey	Surgical debridement of defect (5 defects) and implantation of carrier alone (5 defects)	Lesions treated with PDGF-BB/IGF-1 or PDGF-AA/IGF-1 showed new bone and cementum with attached fibers. New bone formed on the crest of alveolar septa. The rhPDGF/Igf-I treatment was significantly more effective in stimulating regeneration than either placebo or debridement-only controls. Areas of ankylosis were not detected, nor was inflammation present apical to the junctional epithelium in the growth factor	The therapeutic regimen of rhPDGF-BB or -AA with IGF-1 was effective for the treatment of osseous periodontal defects in primates. This growth factor treatment resulted in regeneration of nearly 50% of the lost attachment compared to 14% observed with control (vehicle) treatment alone. This finding suggests that predictable,

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results	Conclusions
	Synopsis: This study tests whether addition of rhPDGF and rhIGF-I in a carrier vehicle to debrided osseous lesions in monkeys can lead to regeneration of bone, cementum, and ligament, i.e., regeneration of a new attachment apparatus.			treated lesions. Lesions with vehicle or debridement did not form a detectable new cementum, and ligament fiber bundles were evident. The growth of new tissue was not related to the presence or absence of methylcellulose vehicle. In the control lesions where loose fibrous connective tissue formed, it was parallel to the root and not in the more functionally configured state as seen in the growth factor treated lesions. The new attachment observed with the combined treatment of PDGF-AA or –BB with IGF-1, extended coronally to regenerate an average of about 50% of the linear distance from the apical extension of the notch to the cementoenamel junction compared to 14% observed in the control lesions.	clinically significant gains in new bone and attachment may be possible through the implantation of rhPDGF and rhIGF, in debrided osseous lesions.
78	"Bone Formation in Tricalcium Phosphate-Filled Periodontal Intrabony Lesions. Histological Observations in Humans" By: J.L. Saffar, M.L. Colombier, and R. Detienville Synopsis: This study evaluates the capacity of a tricalcium phosphate (TCP) ceramic to promote bone formation after grafting intrabony periodontal defects in humans. The data were collected from patients having reentry surgery 16-40 months after original implantation.	5 biopsies from 4 patients treated with TCP to fill deep, wide intrabony periodontal defects.	N/A	-Case 1 (Biopsy at 16 Months): Bone and granules were easily distinguished, since they stained differently. The granules were surrounded by densely arranged bundles of fiber continuous with the framework of the already mineralized bone. Diffuse areas of mineralization were seen at the interface between the matrix and medullary spaces. One end of the biopsy was made of lamellar bone, with modified TCP in contact with it. -Case 2 (Sampling at 16 Months): The biopsy consisted of bone and endosteal spaces. The bone was thick with many parallel lamellae. The endosteal spaces were either vascular channels or spaces with large vessels and fibrous tissue. There was no clear-cut separation between the new and old bones. Remnants of graft particles were seen, but were cell-free and in intimate contact with bone. -Case 3 (Sampling at 18 Months): Most of the granules were of unchanged appearance and were in a highly cellular, fibrous, and poorly	The data obtained from this study indicate that TCP is biocompatible, provides a scaffold for new bone formation and is degraded over time. [Adjunction with biologic stimuli (e.g. growth factors) may be necessary to achieve more robust bone regeneration.]

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results	Conclusions
				-Deaths: 37 patients died during or after participation in these studies, but only 1 died during the treatment phase. Most of the deaths were due to the patients' underlying diseases and were a result of disorders commonly experienced in people with diabetes, such as myocardial infarction, cardiac arrest, and congestive heart failure. There was no correlation between deaths and treatment group. -Time to First Occurrence of Infection-Related Adverse Events: Patients treated with good ulcer care alone showed the shortest time before an infection related adverse event. The others showed similar times before the occurrences. -Changes in Clinical Laboratory Parameters: These values were generally comparable and not clinically meaningful. There was no correlation between changes in any laboratory parameter and treatment group. -Adverse Events Associated with the Cardiovascular System: There was no statistically significant difference in the incidence of cardiovascular adverse events for any of the becaplermin gel treatment groups when compared with control therapies.	
80	"CLOSED VERSUS OPEN DEBRIDEMENT OF FACIAL GRADE II MOLAR FURCATION" By: M. Schroer, t. Wahl, I. Hutchens, J. Moriarty J Clin Periodontol 1997	A study to compare clinical responses of facial grade II molar furcations to scaling and root planing with and without surgical flap access. 15 patients providing 25	N/A	No significant differences were found between the treatment groups for plaque, gingival or gingival fluid indices, changes in pocket depths or attachment levels.	In this study, both closed and open debridement therapies in furcations were found to be successful in controlling inflammation.

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results	Conclusions
		teeth with facial grade II furcations for study. All patients received initial periodontal therapy consisting of oral hygiene instructions, scaling and root planing with ultrasonic and hand instruments, occlusal adjustment, if necessary, caries control, extraction of teeth, if necessary, and fabrication of parial dentures if required for cosmetics or function. Additional plaque calus was removed form teeth of interest at 1 and 2 months post initial periodontal therapy. At 4 months, teeth			

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results	Conclusions
		were selectively assigned to closed or open debridement groups. Selection was done to ensure equal representation of the type of teeth, location and depth of pockets in each group. Patients were then followed at 7, 10, 13 and 16 months for evaluation of pocket depths, attachment levels, gingival index, fluid index, and bleeding.			
81	"Beta Tricalcium Phosphate (CEROS-82): The Indian Experience" By: J.C. Sharma, S.P. Gupta, and J. Sen Synopsis: This study evaluates the effects of beta tricalcium phosphate (β-TCP) used in combination with autogenous bone graft in 16 orthopedic cases requiring bone grafting	β-TCP; 16 patients undergoing orthopedic bone grafting procedures for treatment of various osseous defect types	N/A	The 2 cases of non-union in the femur were treated elsewhere with broad DCP fixation. Two cases of infected non-union were observed in the bones of the lower limb in the presence of bone defects. Both of these cases were treated with modified Phemister bone grafting and β -TCP. A patient with non-union of the humerus was treated with a 10-hole narrow DCP with autogenous bone graft and β-TCP. K-wire stabilization and a mixture of autogenous bone graft and β-TCP treated infected non-unions of 2 forearm bones. In 2 cases of comminuted trochanteric fracture, angle blade plate fixation was performed and a β-TCP bone graft mixture	The results of this study demonstrate that β-TCP is a biocompatible, biodegradable material that produces excellent results when used to augment autogenous bone graft.

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results	Conclusions
	treatment.			was placed at the fracture site. Two cases of fibrous dysplasia were treated with a bone graft and β-TCP mixture. Another patient with congenital pseudoarthrosis of the lower limb was treated with application of autogenous bone graft and β-TCP at the site. Posterior spinal fusion in another patient with congenital scoliosis was achieved with the same mixture. Three patients with bone cysts had procedures performed followed by the application of an autogenous bone graft and β-TCP mixture. Bone union and elimination of the defect were achieved in 12-20 weeks in all cases. There were no significant pre- or post-operative complications in any case related to β-TCP. There were no cases of non-union or refracture. Bony union was achieved in all of the cases by 12 to 20 weeks.	
82	Shatterman GC, Morrison-Graham K, Van Koppen Schroer M, Wahl T, Hutchens I, Moriarty J, Bergenholtz B. Closed versus open debridement of facial grade II molar furcation. *J Clin Periodontol*, 1997; 18:323-29.				
83	"Clinical Safety of Becaplermin (rhPDGF-BB) Gel" By: J.M. Smiell Synopsis: This article presents an overview of the safety of becaplermin (rhPDGF-BB) based on 6 controlled clinical studies conducted in patients with	Becaplermin (rhPDGF-BB) Numerous human clinical studies; 538 patients received rhPDGF-BB; 1,006 patients considered in total.	Used in all studies; 190 received good wound care and 278 received placebo gel	Absorption: Upon becaplermin gel application, only small changes that exhibited no relation to the treated surface area were noted. Thus, there is minimal potential for becaplermin gel to elicit adverse systemic reactions. Skin Irritancy and Sensitization Tests: No side effects or adverse effects were observed. One subject developed erythema at the vehicle site, while 4 others developed it at the saline site. In a chamber scarification test conducted, no	The incidence of cardiovascular, respiratory, musculoskeletal, and central and peripheral nervous system disorders were similar across all treatment groups. Mortality rates were also similar across all treatment groups. Patients did not develop neutralizing antibodies against rhPDGF-BB. Therefore becaplermin appears to be a safe therapy for treatment of lower

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results	Conclusions
	lower extremity diabetic ulcers.			adverse events were observed. A repeat insult patch test showed no side effects, no production of anti-PDGF-BB antibodies, or contact sensitization. Clinical Trials of Becaplermin Gel -Summary of Treatment Emergent Adverse Effects: In all studies, 357 of 538 patients treated with becaplermin reported at least one treatment-emergent adverse event. The overall incidence of at least one adverse event was higher for patients with good ulcer care alone (81%) than it was for those with treated with placebo gel (67%). The most common adverse events reported were infection, cellulitis, skin ulceration (at untreated sites), pain, and osteomyelitis. Infections were higher in the group receiving good ulcer care alone than with groups receiving becaplermin or placebo gel. Any dose of becaplermin gel resulted in 18% incidence of infection. Patients receiving good ulcer care alone had a higher incidence of pain, cellulitis, osteomyelitis, and skin ulceration than other treatment groups. -Ulcer Infection-Related Adverse Effects: The incidence of these events was highest in patients receiving good ulcer care only (33%) and lowest in patients treated with becaplermin at any dose (22%). A slightly higher percent of people with good ulcer care alone experienced resistance mechanism disorders than did patients with becaplermin or placebo gel. Patients treated with good ulcer care alone had a higher incidence of osteomyelitis and cellulitis than patients treated with placebo or becaplermin gel did. Erythematous rashes occurred in 2% of patients with suspected wound infections, but in none of the others. There was lower overall incidences of infection-related adverse events in the 30 μg/g becaplermin gel, and all becaplermin gel doses combined, but not in the 100 μg/g becaplermin	extremity chronic diabetic ulcers.

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results	Conclusions
				treatment group.	
84	"Alloplastic Implants of Tricalcium Phosphate Ceramic in Human Periodontal Osseous Defects" By: A.J. Snyder, M.P. Levin, and D.E. Cutright Synopsis: This study evaluates tricalcium phosphate ceramic's potential for the treatment of periodontal osseous defects in humans, due to its extremely desirable properties.	Tricalcium Phosphate (TCP); 17 patients	N/A	After 18 months, reentry procedures were done on 10 of the 17 patients and no residual ceramic was found. Of the 17 patients, 2 were failures since they involved extensive defects and originally had poor prognoses. The data of several patients was not obtained for various reasons. There was a mean decrease in pocket depth and a mean increase in attachment level in the patients who had 18 month reentry procedures performed on them. Use of the X-ray digital subtraction technique allowed definitive postoperative radiopaque changes to be seen in areas originally exhibiting massive radiolucencies. Their was a mean of 2.8 mm of new bone formation measured upon reentry surgery. In 4 of the 10 cases, osseous repair was greater than 3 mm with 2 cases exhibiting 5 mm or more.	Tricalcium phosphate (TCP) ceramic fill of periodontal defects was well tolerated and in many cases resulted in new bone formation. Although it was not found to be completely predictable in this study, it may become a useful graft material since it has the potential for osseous repair in addition to its availability, host acceptability, ease of manipulation, and storage advantages.
85	"Abnormal kidney development and hematological disorders in PDGF B-receptor mutant mice" By: P. Soriano Synopsis: To understand the role of the PDGF Beta receptor, this study introduces 2 mutations into the gene by homologous recombination in embryonic stem (ES) cells and derives mutant mice.	Mutant BR mice	N/A	High levels of B receptor expression were seen during day 8.5, in the cephalic mesenchyme but not in the heart. At days 9.5 and 10.5, expression was more restricted, particularly to the heart, but B-geo-positive cells were observed in the somites and mesenchyme of the limb buds. At day 12.5, high levels of expression were observed in the heart, choroid plexus, and vertebrae along the neural tube. -BR (PDGF B receptor) mutants die at birth: At day 16, purpura (an accumulation of blood under the surface of the dermis) was seen in 1/3 of the mutant embryos. The majority of the homozygous mutant embryos died perinatally. When studying 10 mutant pups without purpura through cesarean delivery, they died within a matter of minutes. Blot analysis of RNA and protein from the homozygous embryos failed to	PDGF-β-receptor deficient mice were hemorrhagic, thrombocytopenic, and severely anemic, exhibited defective kidneys and died shortly before birth. The PDGF-β receptor is critical for normal embryological development in mammals; its absence leads to severe developmental defects.

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results	Conclusions
				show the presence of immunoreactive PDGFBR. -Hemolytic Defects in BR mutants: Among the 5 mutants examined, all were significantly anemic. Their blood cells had a significantly elevated number of nucleated erythrocytes. Mutants were also significantly thrombocytopenic. Hematological disorders and hemorrhages play a major role in their perinatal lethality. -Anatomical Defects In Mutant embryos: At day 18, glomeruli of the embryos were filling up with blood cells. The glomeruli were present in normal numbers; thus, the defect probably lies in glomeruli maturation and not formation. There were few capillaries in the glomeruli as well.	
86	"Histological Evaluation of Human Intraosseous Healing Responses to the Placement of Tricalcium Phosphate Ceramic Implants" By: S.S. Stahl and S. Froum Synopsis: This study describes periodontal healing responses of 8 lesions to tricalcium phosphate implants, clinically and histologically.	Tricalcium phosphate; 4 patients, total of 8 periodontal sites	N/A	-Clinical Observations: Preoperative pocket depth at the eight sites varied from 7.1 to 10.2 mm while intraosseous depth varied from 3.0 to 5.6 mm. Following treatment, the sites showed pocket depths varying from 2.8 to 4.6 mm. All sites showed evidence of gingival recession that varied from 0.8 mm to 4.5 mm. Clinical gain in closure varied from 2.0 mm to 3.1 mm. -Histological Observations: All sites showed similar responses; thus, the healing pattern was deemed a composite. When the block was removed, gingival recession apical to the marginal notch had occurred in all specimens. Closure was observed via a long junctional epithelium, as evidenced by cementogenesis and inserted fibers at the site. Cementogenesis was confined to small distances incisal to the adjacent crest and did not seem to relate spatially to the presence of the implant particles. Implant particles were seen in the vertical lesions and were surrounded by a connective tissue capsule. No significant inflammation was observed, and there was no osteogenesis surrounding the implant particles. The alveolar crest showed	This study shows that tricalcium phosphate implants improve clinical closure, but the histological observations do not show that they improve new attachment or bone. The TCP particles acted as biocompatible fillers. [Thus TCP alone appears to require adjunction to enhance its biological effectiveness in bone and periodontal regeneration.]

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results	Conclusions
				remodeling with limited osteogenesis and osteoclasis taking place at the crestal bony surfaces. In each site, the most apical deposition of root accretions was within the apical notch. Collagen fibers arose from the dentinal wall and evidence of dentin resorption was present. This suggests that various repair processes were occurring within the same site after debridement of a human intrabony lesion. These processes include epithelial adhesion of the exposed site, partial blockage of epithelial migration by exposure of dentinal collagen, or limited new attachment. These processes were seen 8 months postoperatively.	
87	"Histologic and Clinical Responses to Porous Hydroxylapatite Implants in Human Periodontal Defects" By: S.S. Stahl and S.J. Froum Synopsis: This article describes clinical and histologic responses to porous hydroxylapatite implants in human periodontal defects over a 1-year observation period.	3 patients with a total of 12 periodontal lesions	N/A	-Clinical Observations: The preoperative pocket depth at the 12 sites ranged from 6.3 to 9.9 mm while the intraosseus depth ranged from 2.8 to 10.7 mm. When the blocks were removed, the sites showed pocket depth ranging from 3.0 to 5.0 mm. Gingival recession was present at all the sites, ranging from .8 to 2.3 mm. Clinical gain in closure ranged from 2.0 to 4.2 mm. Mobility pattern did not change 3 months after implantation, but was less at the 6 and 12 month intervals. -Histologic Observations: The 3 month blocks showed closure by long junctional epithelial adhesion. Inflammation was seen within the marginal gingival region. At the implant sites, the implant particles were surrounded by cellular connective tissue. There was no inflammatory infiltrate present in these areas. Bone formation was seen within the implant and at its periphery. Osteogenesis within and at the periphery of the particles was limited at this time. Crestal remodeling was present in all sections. The 6 month specimens showed similar responses to the 3 month specimens. After 1 year, the specimen	Treatment of advanced periodontal defects with surgical debridement and grafting with porous hydroxylapatite results in reduction in pocket depth consisting of bone recession and gain in clinical attachment. Histologically, the graft was well tolerated and new bone was observed within the implant pores and periphery. However, there was a long junctional epithelium and no new attachment. [This study suggests that adjunction of synthetic porous b one substitutes may be required to provide an optimum environment for periodontal regeneration./

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				showed closure by the epithelial adhesion, this time lining the apical notch. Adjacent to the notch, ossification of graft particles was evident. There was loose connective tissue with minimal inflammatory infiltrate between the notch and ossifying graft particles. Cellular repair cementum was seen at the level of the crest and slightly incisal at a site of previous root resorption. The root resorption/repair in this specimen coupled with the active root resorption seen in the 3 month specimen focuses on this resorptive process as a possible component of the repair/regeneration phenomenon observed in these sites.	
88	"Porous Tricalcium Phosphate and Transforming Growth Factor Used for Anterior Spine Surgery" By: T. Steffen, T. Stoll, T. Arvinte, and R.K. Schenk Synopsis: This report examines the results of two animal studies in which β-TCP bone substitutes were used for anterior spinal surgery in sheep and baboons.	β-TCP; 1) 60 female Dorset sheep 2) 9 male baboons	1) 18 sheep with an empty cage 2) 3 with unplugged defect	1) Instrumented anterior interbody spinal fusion: No anterior implant migration was seen. Large anterior bone bridges were seen at the instrumented levels, especially in the 16 week and more notably in the 32 week survival groups. The average Ranges Of Motion (ROM) significantly decreased for each consecutive survival interval. No significant differences were seen between treatment groups, except at 8 weeks when the group with autologous bone had a significantly smaller ROM than the sham and β-TCP groups. A higher histology grading indicated better fusion. At 8 weeks, grades for the autologous group were better than the sham and β-TCP groups, but at 6 weeks the β-TCP groups were almost as good as the autologous group. Between 16 and 32 weeks, the sham and autologous groups improved, but the β-TCP group remained unchanged. At 32 weeks, the autologous groups showed the best results. Also, the cages with β-TCP had significant filling defects, but wherever granules were remaining, bone ingrowth into the ceramic material was regularly seen. More than half the specimens had	The results of this study showed that β –TCP is a good osteoconductor ant that use of pre-filled titanium cages with porous TCP can improve the fusion outcome in anterior spinal fusion procedures. Additionally, β-TCP performed well as a carrier for the growth factor tested, with the combination of the osteoconductor (β-TCP) and growth factor leading to enhanced bone formation and faster remodeling of the graft material.

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				a fibrocartilaginous layer running horizontally through a large portion of the cage's inner portion, most likely an indication of micromotion associated with the cage implant. This was seen in all 3 treatment groups, but mostly at 16 and 32 weeks in the β-TCP and autologous groups. This lack of segmental stability of stand-alone anterior lumb are interbody fusion compared to circumferential fusion can be reproduced in human cadaveric studies and is supported by clinical outcomes, which are unfavorable for stand-alone anterior interbody fusion. Use of ceramic pre-filled titanium cages showed better histological fusion than the ones filled with granular material at the time of surgery. The filling defects noted above and the fibro-cartilagenous tissue layers running through the cage were avoided with use of the pre-filled cages. 2) Vertebral Body Block Filling: None of the vertebral bodies showed a fracture or had a radiologically documented height loss. The sham group regularly showed minimal bone growth into the defect starting from its posterior wall. Most of the defect was still filled with soft tissue. The β-TCP plugs seemed well integrated at 3 and 6 months. Throughout the 6 month period, however, the ceramic plug was clearly visible. At 3 months, CT scans of the ceramic plugs with TGF-B indicated a prominent peripheral resorption within the original graft boundaries. At 6 months this resorption zone was filled with dense bone The baboons treated with TGF-B plugs showed massive subperiostal new bone formation along the anterior aspect of the vertebral body, but no formation in the spinal canal or neuroforaminal regions. The β-TCP plugs had much ceramic still in place while the TGF-B plugs showed resorption of the ceramic	

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				replaced with remodeled cancellous bone.	
89	"Posterolateral and Anterior Interbody Spinal Fusion Models in the Sheep" By: T. Steffen, D. Marchesi, and M. Aebi Synopsis: This study describes posterolateral and anterior interbody fusion models in sheep. Both models used instrumented fusions, applying porous mineral scaffolds alone or mixed with bone.	Autograft, nonresorbable hydroxyapatite (HA) and resorbable HA; 52 Female Dorset sheep	Sham surgery group used	-Surgery: No intraoperative or postoperative complications occurred when the posterolateral fusion model was used. -Coralline Graft Substitutes: Nonresorbable coralline graft substitutes used alone or mixed with autologous bone were inferior to autologous bone alone in stabilizing a motion segment. Coralline substitutes were more effective than the sham group. Radiologic assessment was not useful in predicting the fusion outcome. Histologic assessment revealed cores of woven bone, often encompassed by lamellar bone, in contact with the coral surfaces. No inflammatory signs were observed, suggesting biocompatibility of the coralline ceramic materials. The coral surfaces showed no signs of resorption. The resorbable coralline graft material progressively degraded over 36 weeks. At 20 weeks, all but one segmental level had solid fusion with new bone cortex formation. There were no significant differences between the 50/50 volume mixture of Pro Osteon 500 R with autologous bone and autologous bone alone at 20 or 36 weeks. Neutral zones were fewer for the resorbable graft material at 20 weeks compared to 12 weeks. The porous resorbable ceramic material had a 90% average surface coverage at 20 weeks and nearly complete coverage at 36 weeks. The nonresorbable material had only 34% coverage at 20 weeks.	The results of this study indicate that resorbable porous mineral bone substitutes mixed with locally harvested autologous bone had fusion outcomes similar to pure autologous bone and may therefore alleviate the need for extensive autologous bone harvesting. Nonresorbable HA did not perform as well. The study results demonstrate that the porous resorbable material with a lower hydroxyapatite content results in better bone fill by undergoing substitution with host bone.
90	"Platelet-derived growth factor enhancement of a mineral-collagen bone substitute" By: E.B. Stephan, R.	Anorganic bovine bone matrix incubated with radiolabeled PDGF-BB	Anorganic bovine bone matrix incubated with water	Osteoblastic cells cultured on PDGF-BB-treated matrix showed significantly greater proliferation compared with cells grown on matrix incubated with water or bone mineral w/o collagen (both $P<0.05$).	This *in vitro* study showed that PDGF-BB is rapidly adsorbed then slowly released from anorganic bovine bone-collagen matrix. PDGF-BB adsorbed to this material is able to stimulate proliferation of attached osteoblastic

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	Renjen, S.E. Lynch, and R. Dziak December 2000 *J Periodontol* Synopses: This *in vitro* study examines the interaction of anorganic bovine bone-collagen matrix ("matrix") with platelet-derived growth factor BB isoform (PDGF-BB), and determines if the adsorption of PDGF-BB to the matrix stimulates proliferation of neonatal rat (Sprague-Dawley) osteoblastic cells.	To measure proliferation/attachment, neonatal rat osteoblastic cells were cultured with matrix (or bone mineral w/o collagen) incubated with PDGF-BB (2.5 x 10^6 M). To measure interaction dynamics, radiolabeled PDGF-BB (0.7 to 11.0 x 10^6 M) was incubated with matrix over a range of time periods (1 min to 10 days).		Osteoblastic cells attached to the matrix more rapidly than to the bone mineral w/o collagen. There was no significant difference in cell attachment to the PDGF-BB-treated versus water-treated matrix. PDGF-BB adsorbed to the matrix in a rapid (<1 minute), concentration-dependent fashion. The difference in the amount of PDGF-BB absorbed to the matrix at each concentration was significant ($P<0.05$) except between the 2.8 and 5.5 x 10^6 M concentrations. The absorption curve reached a plateau at the 2.8 x 10^6 M concentration. PDGF-BB was slowly released from the matrix, with approximately 30% liberated over 10 days.	cells. These data suggest that it may be clinically feasible to adsorb PDGF-BB to bone-collagen matrix and this combination has the potential for clinical applications.
91	"Growth factors regulate expression of osteoblast-associated genes" By: C.L. Strayhorn, J.S. Garrett, R.L. Dunn, J.J. Benedict, and M.J. Somerman November 1999 *J Periodontol* Synopses: This in *vitro* study determines the	To measure proliferation, MC3T3-E1 cells were exposed to mineralization media, 2% fetal bovine serum (FBS), plus varying doses of PDGF and/or IGF (1, 10, and 20 ng/mL each). To measure gene	MC3T3-E1 cells exposed to 2% FBS only (negative control); mineralization media plus 2% FBS (vehicle control); or mineralization media plus 10% FBS (positive control).	When compared with negative and vehicle controls at Day 8, PDGF 10 and 20 ng/mL significantly enhanced ($P\leq0.01$) MC3T3-E1 cell numbers; IGF produced no significant effect at any dose; BP 10 and 20 µg/mL significantly decreased ($P\leq0.01$) cell proliferation; and PDGF/IGF significantly increased ($P\leq0.01$) cell numbers, but this effect was not greater than for cells exposed to PDGF alone. Northern blot analysis revealed that PDGF blocked gene expression of osteopontin (OPN) and osteocalcin (OCN), a calcium binding	The results of this *in vitro* study indicated that BP, PDGF, and IGF influence cell activity differently. Both IGF and BP promoted osteoblast differentiation as measured by expression of BSP, OPN, and OCN, whereas PDGF promoted proliferation in MC3T3-E1 cells and blocked differentiation of osteoblasts as measured by decreased expression of OCN. These findings raise the possibility that combining growth factors in a time-

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	effects of a low molecular extract of bovine bone protein (BP) containing bone morphogenetic proteins (BMPs) 2, 3, 4, 6, 7, 12, and 13, alone, or in combination with platelet-derived growth factor (PDGF) and/or insulin-like growth factor (IGF) on osteoblast differentiation of MC3T3-E1 cells (mouse osteoprogenitor cell line).	expression, MC3T3-E1 cells were exposed to PDGF (10 ng/mL), IGF (10 ng/mL), or BP (10 µg/mL), or a combination of these factors.		protein, while BP and IGF promoted gene expression of bone sialoprotein (BSP), a glycoprotein selective to mineralized tissue, and OPN, a regulator of crystal growth and promoter of cell migration and attachment at sites of bone formation and/or resorption. The combination of BP and IGF enhanced expression of OPN beyond that of either BP or IGF alone. PDGF was able to block the effects of IGF on gene expression, but not those of BP. In pulse experiments, no differences in gene expression were noted between exposures of MC3T3-E1 cells to IGF/BP for 8 days compared with adding BP on Day 6. Similarly, exposing cells to PDGF/BP for 8 days gave comparable results to adding BP on Day 6. PDGF's inhibitory effect on OPN expression was noted in PDGF/IGF/BP-treated cells even after addition of BP on Day 6. BP and IGF did not affect the expression of Osf2, an osteoblast-specific transcription factor and regulator of osteoblast differentiation, while PDGF inhibited Osf2 expression when compared with vehicle control.	dependent and orchestrated manner can enhance cell proliferation and differentiation.
92	"Comparison of Tricalcium Phosphate and Frozen Allogenic Bone Implants in Man" By: J.R. Strub, T.W. Gaberthüel, and A.R. Firestone Synopsis: This study compares the reduction of pocket depth and amount of bone apposition when	Tricalcium phosphate and allergenic bone. 8 patients, with 47 periodontal pockets- 29 treated with TCP and 18 with allogenic bone	N/A	The average reduction of clinical pocket depth of tricalcium phosphate filled pockets was 1.8 mm and 38% of the pockets remained deeper than 3 mm. When comparing the pre and post surgical X-ray films with pins in place, the bottoms of the bony pockets were displaced about 1.05 mm coronally. The average amount of bone apposition was 1.2 mm. The average reduction of clinical depth o f allogenic bone filled pockets was 2.0 mm and 22% of the pockets remained deeper than 3 mm. X-ray film comparisons indicated the bottom of	Both TCP and allergenic bone were biocompatible. No significant differences between the groups were reported although allogenic bone implants seemed to lead a slightly greater bone apposition and reduction in pocket depth than TCP alone. TCP was much simpler to store and handle. [Thus, TCP may require supplemental biological materials to enhance its beneficial effect.]

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	tricalcium phosphate and frozen allogenic implants were used to treat intrabony periodontal pockets in man.			the bony pockets to be displaced about 0.9 mm coronally. The average amount of bone apposition was 1.5 mm. No foreign body reactions or other complications were encountered during the course of the experiment.	
93	"Effect of IGF-I and PDGF administered in vivo on the expression of osteoblast-related genes in old rats" By: H. Tanaka, A. Wakisaka, H. Ogasa, S. Kawai, and C.T. Liang 2002 *J Endocrinology* Synopses: This *in vivo* study tests the hypothesis that individual and combined effects of insulin-like growth factor-I (IGF-I) and/or platelet-derived growth factor (PDGF) can restore age-related impairment in bone formation activity in a rat bone marrow ablation model.	Direct infusion of PDGF and/or IGF-I into right femoral cavity for 5 days following marrow ablation of adult (6-months) and old (24-months) male Wister rats.	Left femoral cavity infused with vehicle only for 5 days following marrow ablation.	At Day 6 after marrow ablation, mRNA levels of osteoblast-related markers (collagenα1-I [COLI], alkaline phosphatase [AP], osteopontin [OP] and osteocalcin [OC]) were significantly lower (all $P<0.0001$) in old as compared with adult rats. Infusion of PDGF 25 and 50 ng/day significantly stimulated ($P<0.05$) the expression of OP mRNA in both old (33% and 30%) and adult rats, whereas COLI, AP and OC mRNA expression were not significantly affected. IGF-I infusion did not have a significant effect on mRNA expression of any osteoblasts-related marker in adult rats. In contrast, treatment with IGF-I significantly enhanced ($P<0.05$) the mRNA levels of COLI, AP and OP in old rats. In adult rats, combined treatment with PDGF and IGF-I caused a significant increase ($P<0.05$) in OP gene expression but no significant change in AP, OC or COLI genes. Although neither IGF-I nor PDGF alone was effective in stimulating the expression of OC, the combined treatment in old rats enhanced OC expression significantly ($P<0.05$). Since OC is known to be produced by mature osteoblasts and to regulate the calcification of bone tissue, it is possible that PDGF plus IGF-I may stimulate maturation and mineralization in bone formation. The expression of COLI, AP and OP genes in old rats was also significantly stimulated ($P<0.05$) by PDGF plus IGF-I, but the stimulation was no different from IGF-I alone.	The results of this *in vivo* study showed that PDGF and, to a greater extent, IGF-I may partially restore the deficit in the expression of osteoblast markers in old bones, and that the combination of both factors is better than IGF-I alone in stimulating OC expression.

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94	Toback GA, Brunsvold MA, Nummikoski PV, Masters LB, Mellonig JT, Cochran DL. The Accuracy of Radiographic Methods in Assessing the Outcome of Periodontal Regenerative Therapy. *Journal of Periodontology*, 1999; 70:669.			The article is provided in Appendix 10.2.2.	
95	"Distribution of fibroblast growth factors (FGFR-1 and -3) and platelet-derived growth factors receptors (PDGFR) in the rat mandibular condyle during growth By: V Visnapuu, T. Peltomaki, O Ronning, T. Vahlberg, H. Helenius August 2002 *Orthod Craniofacial Res* Synopses: The aims are to elucidate the role of the fibroblast growth factors 1 and 3 (FGFR-1,-3) and the platelet derived growth factor (PDGFR) in the growth of the mandibular condyle cartilage in the rat.	1- to 21-day-old Long-Evans/Turku rats (total 24 animals, 3 in each age group) were killed and their condylar area excised and FGFR-1, FGFR-3 monoclonal and PDGFR polyclonal antibodies were applied.	Positive controls for FGFR-1, -3 and PDGFR were tested in prostate carcinoma tissue by manufacturers Negative controls samples did not contain primary antibodies.	Staining of FGFR-1 monoclonal antibody was detected in the proliferative and upper hypertrophic cell layers of the mandibular condyle of 1- to 10- day old rats, in the proliferative and hypertophic cell layers and in the subchondral bone of the mandibular condyle of 14- to 21-day old rats. FGFR-3 polyclonal antibody staining was observed in the proliferative cell layer of the mandibular condyle of 1- to 10-day-old rats and in the proliferative and upper hypertrophic cell layers of 14- to 21-day old rats. PDGF polyclonal antibody staining was detected in the lower part of the proliferative cell layer of the mandibular condyle of 1- to 10-day-old rats whereas it was only found in the hypertrophic cell layers of 14- to 21-day old rats. Regression analysis revealed a significant age-related increase in the proportional depth of the cell layer labeled for FGF-1, -3 and PDGFRs in ever sagittal region of the condylar head ($P<0.001$) Coefficient determinations (R^2) ranged from 0.44 to 0.85 and showed that there is a strong	Observations show that the proportional depth of the cell layer labeled for FGFR-1, 03 and PDGFR in the mandibular condylar cartilage of rats increases significantly as a function of age and thus these factors have an important role in the growth regulation of the mandibular condylar cartilage during the most rapid post-natal growth period in the rat.

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results	Conclusions
				dependency between the proportional labeled cell layer in the mandibular condylar head and age in every region.	
96	"A Pilot Study of the Value of Ceramics for Bone Replacement" By: H. Waisbrod and H.U. Gerbershagen Synopsis: This study investigates the ability of hydroxyapatite (HA) and (tricalcium phosphate (β-TCP) used in combination with autogenous bone graft to facilitate bone ingrowth, leading to graft incorporation and successful union of the graft and host bone .	β-TCP; 8 patients undergoing arthrodesis of the sacroiliac joints (6) or posterolateral lumbar spine fusion (2)	3-HA implants 5-TCP implants The HA and TCP materials were of the same volume and pore size	No adverse host reactions occurred in this study. In the 3 patients with HA block implants, bone union was not achieved. At 17, 18, and 19 month follow ups, the HA ceramic plates were clearly discernible from the bone. No union was observed between the bony surfaces, the HA ceramic and bone, or the HA ceramic implants themselves. The density of the foreign blocks did not change. No signs of degradation or resorption were noted. In 4 of the 5 patients with TCP implants, bony union was achieved. There was clear continuity between the ceramic implant and cancellous bone. In 2 patients, union between the implants was observed, with the disappearance of ceramic boundaries. In the 4 patients with bony union, resorption was also evident since the blocks lost their original configuration. One case that did not unite involved technical problems as the ceramic was found to be implanted too proximal to and too distant from the cancellous bone surface.	The results of this study indicate that TCP ceramic implants are very successful in replacing bone grafts while HA is not a suitable ceramic implant material and does not promote union.
97	"The Effect of Platelet-Derived Growth Factor on the Cellular Response of the Periodontium: An Autoradiographic Study on Dogs" By: H.L. Wang, T.D. Pappert, W.A. Castelli, D.J. Chiego Jr., Y. Shyr, and B.A. Smith Synopsis: This investigation evaluated	6 adult mongrel dogs, with 16 treatment sites per dog	2 dogs with 8 control sites (1 of 4 treatments) were used for each of 3 treatment periods; a total of 24 controls sites were used	-Total Labeled Non-Inflammatory Cells: There was an increase in mean labeled cells overall at day 7 compared to days 1 or 3. When all time periods and non-inflammatory cell types were totaled both PDGF and ePTFE + PDGF showed increased cellular proliferation when compared with either the control or ePTFE treatments. -Total Labeled Cells (Including Inflammatory Cells): The inclusion of inflammatory cells obscured the trends at earlier time periods because of the predominance of inflammatory cells in most slide sections. As the mean number of inflammatory cells decreased from day 1 to 7,	This study demonstrated that PDGF-BB stimulates the proliferation of periodontal ligament fibroblasts, cementoblasts and osteoblasts and, when used in combination with a ePTFE membrane also perivascular and endothelial cells. ePTFE membranes by themselves had no effect on cell proliferation. There were fewer inflammatory cells in sites treated with PDGF-BB. Thus, the biological rationale for the use of rhPDGF-BB was established in vivo,

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	the effect of platelet-derived growth factor (PDGF-BB) on the pattern of proliferation and migration of cells residing within the periodontium, including periodontal ligament and bone cells, cementoblasts, perivascular cells and endothelial cells with and without guided tissue barrier membranes.			the trend of increased labeled cells for the PDGF groups was more evident. At day 7, the PDGF and ePTFE + PDGF treatments had more active cells than C or ePTFE groups. When considering the pattern of cellular labeling when the 5 inflammatory cells (fibroblasts, cementoblasts, osteoblasts, perivascular, and endothelial cells) were viewed by time, treatment, and area, there was a trend for more labeling for the PDGF and the ePTFE + PDGF groups. At day 7, both PDGF and ePTFE + PDGF treatments showed significantly increased labeling in comparison with C, while the PDGF group had more cellular labeling than ePTFE. -Total Labeled Cells by Time, Area, and Treatment: For C treatments, cells significantly decreased from day 1 to 7 for all three areas and were found mostly in the middle region. The ePTFE treatment cells were highest in the middle regions for all times, though not significantly. The PDGF treatment cells were more pronounced for the middle area but most activity was noted at day 7 compared to day 1 and 3. The PDGF treatment showed proliferation of the PDL fibroblasts into the defect with cellular labeling. The ePTFE + PDGF treatment cells showed a similar pattern of activity to the PDGF treatment group. -Labeled cells for all times and treatments by cell type: The general trend was moderate cellular activity at day 1, decreasing at day 3, and a dramatic increase at day 7. At day 1, significant differences existed between the PDGF and ePTFE groups, and between the ePTFE + PDGF and both the ePTFE and C groups. No significant differences were noted at day 3. By day 7, labeling in the PDGF group was much greater than either the C or ePTFE group. The e PTFE + PDGF treatment was slightly more effective at	i.e., stimulation of cells responsible for bone and periodontal regeneration.

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				enhancing cellular activity at day 7 than the C treatment. For cementoblasts, labeling was least at day 3 and greatest at day 7 for all but the C group. For the osteoblasts, labeling was greatest for the PDGF group at day 7, which was significantly better, compared to the C group. For the perivascular cells, day 7 showed the highest degree of cell activity for all but the C group. Both PDGF treatment groups were significantly greater than the C group at day 7. For the endothelial cells, the trend of highest activity for PDGF and ePTFE + PDGF groups at day 7 held. At day 7, statistical differences existed between the ePTFE + PDGF and C groups, with the PDGF + ePTFE group having the greatest proliferation of endothelial cells, suggesting that PDGF may increase angiogenesis, an important requisite for healing and osteogenesis.	
98	"Stiffness of Subchondral Bone Defects of the Tibial Plateau Supplemented with Autograft or Tricalcium Phosphate in a Caprine Model" By: D.L. Wheeler, P. Gallogly, E. Eschbach, B. Conrad, D. Lewis, and A.R. Cross Synopsis: This study evaluated the structural competency of TCP or autograft used to supplement a massive subchondral bone defect in a caprine model and compared these groups to	B-TCP; 16 adult goats	autograft	Gross examination of the harvested experimental tibiae showed 6 out of 7 in the AUTO group and 0 out of 8 in the TCP group had degenerative changes. One goat in the AUTO group died prematurely. Two of the 7 goats in the AUTO group had a large-scale collapse of the caudal tibial plateau. Two specimens in the Central Position for the AUTO group had stiffness values of zero. CTL specimens had significantly higher values for stiffness than the experimental specimens for both AUTO and TCP. The average stiffness for the TCP group was higher than for the AUTO group, but the difference was not statistically significant.	The results of this study indicate that TCP and autograft produce equivalent stiffness of regenerated bone. Tibiae implanted with the TCP did not develop degenerative joint disease or plateau collapse, as occurred in the autograft group. Thus, TCP may be a useful alternative to autograft in the repair of tibial plateau fractures.

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results	Conclusions
	the contralateral tibiae (CTL).				
99	"Effect of Serum and Platelet-Derived Growth Factor on Chondrocytes Grown in Collagen Gels" By: L. Weiser, M. Bhargava, E. Attia, P.A. Torzilli November 1999 *Tissue Engineering* Synopses: In this *in vitro* study, cell proliferation, viability and morphology; proteoglycan synthesis; and gel contraction were assessed over a 15-day period (days 3, 6, 9, 12, 15) for mature bovine chondrocytes cultured in collagen gels within varying concentrations of PDGF-BB.	Chondrocytes were isolated from mature bovine occipital cartilage (≥ 2 years of age; ≥10 animals) within 4 hours of animal death. Cells were then cultured in rat tail collagen gel loaded with varying concentrations of PDGF-BB and serum.	Collagen gels with 1%, serum and no PDGF	Increasing the serum concentration resulted in a significant increase in cell proliferation ($p<0.001$). Chondrocyte proliferation also increased with increasing PDGF concentration ($p<0.001$ between all groups). Cells in gels containing 1% serum and 10ng/ml of PDGF proliferated at approximately four times the rate of the control gels. Gels with 100ng/ml of PDGF proliferated at 11 times the rate of controls. There is no significant effect of PDGF concentration on PG content per gel within any time interval. ($p>0.5$)	In this study, the amounts of serum and PDGF were found to have no effect on cell viability. The increase in the amount of serum or PDGF in gels caused a significant increase in the rate of chondrocyte proliferation.
100	"Platelet-derived growth factor (PDGF)-AA: a self-imposed cytokine in the proliferation of human fetal osteoblasts" By: D. Yang, J. Chen, Z. Jing, and D. Jin August 2000 *Cytokine* Synopses: This *in vitro* study investigates how	To measure osteogenic potential, human fetal osteoblasts were cultured in mineralizing media. To measure proliferation and cell cycle, human fetal osteoblasts were	Human fetal osteoblasts were cultured with 199 medium, (supplemented with 1% FBS for cell proliferation only).	Treatment with PDGF-AA 40 ng/mL for 24 hours increased the replication index (percentage of cells increase [PCI]) by 48.2% ($P<0.05$ versus control); PCI decreased after the removal of PDGF-AA. At 24 hours, the percentage of osteoblasts in G_0/G_1 phase decreased and those in the S and G_2-M phase increased compared with control. The proliferation index (PI) (S% + G_2-M%) also increased after PDGF-AA treatment reflecting either the induction of quiescent cells in the G_0 phase into the proliferative portion of the cell	Results from this *in vitro* study in human fetal osteoblasts showed that PDGF-AA enhances cell replication by accelerating cell recycle and inducing quiescent cells into the proliferation portion of cell cycle. PDGF-AA is a self-imposed cytokine in human fetal osteoblast replication.

Reference No.	Title, Author, and Synopsis	Test Article (No. of Patients)	Control (No. of Patients)	Results	Conclusions
	platelet-derived growth factor AA isoform (PDGF-AA) affects human fetal osteoblast proliferation and cell cycle, and whether PDGF-AA and other growth factors affect secretion of PDGF-AA.	cultured with PDGF-AA (40 ng/mL) for 24 hours followed by 199 medium (supplemented with 1% fetal bovine serum [FBS] for cell proliferation only).		cycle, or acceleration of the cycle of cell growth. The expression of PDGF-AA mRNA was upregulated by PDGF-AA, PDGF-BB, transforming growth factor beta (TGF-β), and basic fibroblast growth factor (bFGF). Since the synthesis of protein depends on mRNA level, PDGF-AA enhances the PDGF-AA-osteoblasts autocrine loop. The upregulation of PDGF-AA by other cytokines supports the hypothesis that many factors form a network to modulate the growth of bone.	
101	"Platelet-derived growth factor (PDGF)-BB stimulates osteoclastic bone resorption directly: the role of receptor beta" By: Z. Zhang, J. Chen, and D. Jin October 1998 *Biochemical and Biophysical Research Comm* Synopses: This *in vitro* study analyzes platelet-derived growth factor receptors (PDGFR) on adult human osteoclasts and examines the effect of PDGF BB isoform (PDGF-BB) on osteoclastic bone resorption activity.	Adult human osteoclastic cells harvested from human iliac spongy bone were cultured with PDGF-BB, isolated, and placed on human dense cortical bone slices in PDGF-BB-containing medium (10 to 40 ng/mL).	Harvested human osteoclastic cells on human dense cortical bone slices in medium without PDGF-BB	PDGFR beta (PDGFR-β) was detected on osteoclast membranes through immunohistochemistry and immunomicroscopy. Exposure to PDGF-BB 20 to 40 ng/mL caused a significant dose-dependent increase ($P \leq 0.05$) in the volume and number of resorption pits on dense cortical bone slices. Following exposure to PDGF-BB 40 ng/mL, there was a significant increase in the activity of both tartrate-resistant acid phosphatase (TRAP) ($P=0.008$), which is a sensitive and specific marker for bone turnover, and total acid phosphatase (ACP) ($P=0.014$).	Results from this *in vitro* study showed that PDGF-BB promotes adult osteoclastic bone resorption directly through PDGFR-β. Because PDGF-BB is a potent mitogen that promotes cell proliferation and differentiation, the increase in resorption pits may reflect, in part, PDGF-BB-induced fusion of mononuclear cells into osteoclasts and promotion of osteoclast attachment to bone. These findings suggest that PDGF-BB and PDGFR-β play important roles in the bone healing process and reconstruction.