PHARMACOLOGY WRITTEN SUMMARY

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ABBREVIATIONS

ANOVA	One-way analysis of variance
β-TCP	Beta-tricalcium phosphate
CNAA	Complete new attachment apparatus
DFDBA	Demineralised freeze-dried bone allograft
DMSO	Dimethyl sulphoxide
HDPE	High density polyethylene
INN	International non-proprietary name
MEM	Minimum essential medium
PDGF	Platelet-derived growth factor
PETG	Glycol-modified polyethylene terephthalate
PII	Primary irritation index
PMA	Premarket Approval Application
rhPDGF-BB	Recombinant human platelet-derived growth factor BB homodimer
SBA	Summary basis of approval (FDA)
U.S. FDA	United States Food and Drug Administration

2.6 NONCLINICAL SUMMARY

2.6.2 Pharmacology Written Summary

2.6.2.1 Brief Summary

Augment is intended for use in the treatment of bone fusions of the foot and ankle in patients with chronic inflammation or tissue degeneration in these joints. A positive outcome for the patient requires bone formation stimulated by Augment across the decorticated junction of two or more bones in the foot and ankle. The preclinical studies presented in this section demonstrate the safety and efficacy of rhPDGF-BB in combination with β-TCP for bone fusion and accelerating fracture healing using models that mimic the osseous healing required following foot-and-ankle fusion surgery. The studies presented below utilize matrices containing chemically identical β-TCP products. Study reports are provided in Appendix 2.6.2.1-1.

The first and second studies presented in this section describe the results of fracture healing conducted to evaluate rhPDGF-BB combined with a β-TCP/collagen matrix in two rat models of delayed or impaired bone repair (aged, ovariectomised or diabetic). In both models, treatment with rhPDGF-BB combined with β-TCP/collagen resulted in acceleration of fracture healing at either the tibia or the femur, demonstrating also that rhPDGF-BB at a dose of 0.3 mg/mL is as efficacious as or better than at a dose of 1.0 mg/mL. The third study describes results from a canine partial arthrodesis model in which rhPDGF-BB combined with a β-TCP matrix equivalent to Augment's β-TCP or a β-TCP/collagen matrix leads to improved bone fusion compared to the matrices alone. In addition, bone fusion with rhPDGF-BB combined with β-TCP appeared to be equivalent to or better than autograft. The fourth study presented is an evaluation of rhPDGF-BB combined with β-TCP for treatment of rabbit tibial osteotomies. Although there were no differences in the amount of bone formation at osteotomies treated with or without rhPDGF-BB, the combination of rhPDGF-BB and β-TCP did not interfere with bone formation at the sites and led to increased bone remodelling after 4 weeks.

Finally, animal studies are presented that provide additional support for the safety and efficacy of rhPDGF-BB for bone healing, and involve combinations of the protein with matrices composed of β-TCP or other materials.

Overall, the preclinical data in these animal models support the safety and efficacy of rhPDGF-BB combined with β-TCP matrix for the healing of bone defects and fractures in a variety of animal backgrounds that model the bone healing taking place following a foot-and-ankle fusion procedure. The data support the use of Augment for the clinical treatment of foot-and-ankle fusions in which the product enhances the healing and formation of new bone at the fusion sites.

2.6.2.2 Primary Pharmacodynamics

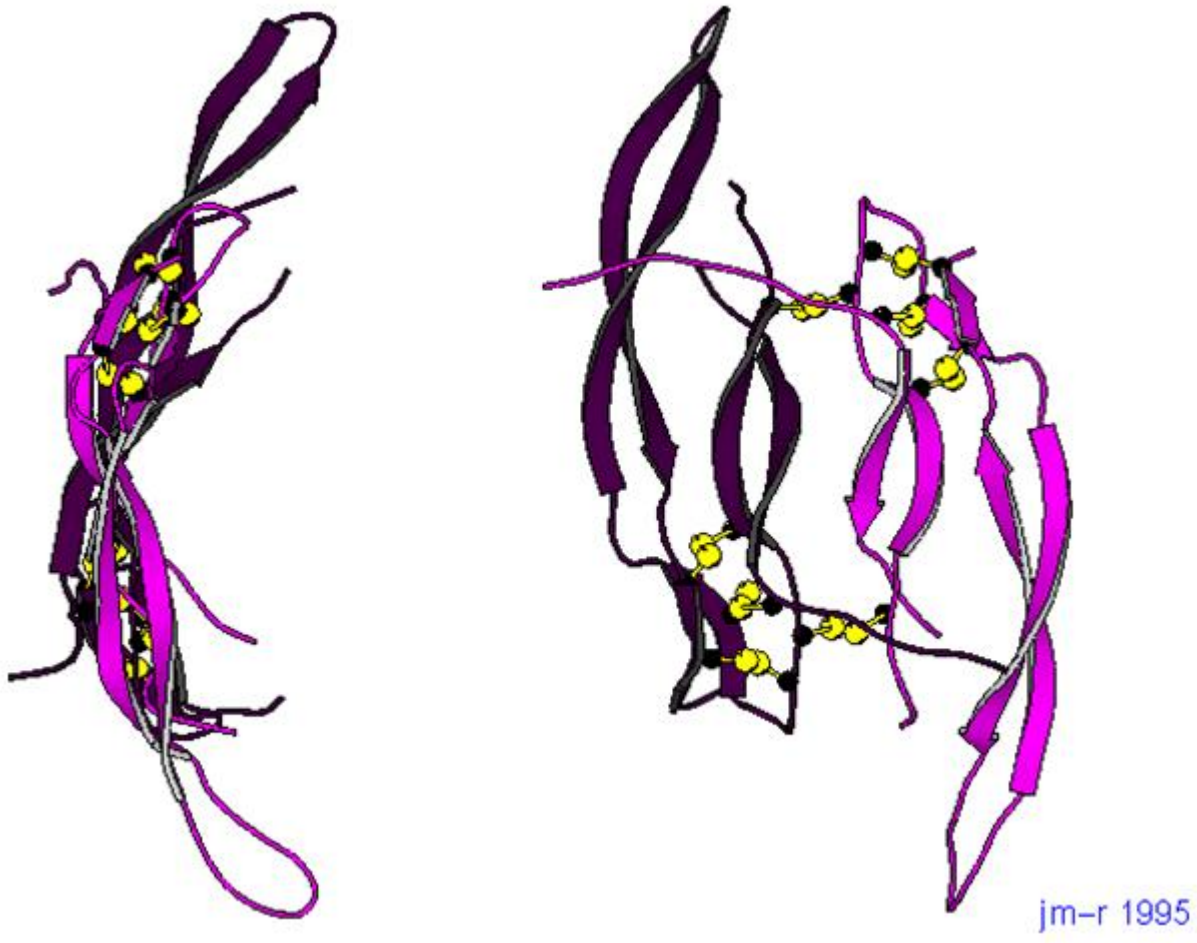
2.6.2.2.1 Pharmacodynamics of rhPDGF-BB (Becaplermin)

The pharmacological action of PDGF and PDGF-BB in particular has been extensively reported in the literature.

Platelet-Derived Growth Factor, or PDGF, is a family of growth factors which contains five members that are found naturally in the body. These include AA homodimer, AB heterodimer, BB homodimer, CC homodimer and DD homodimer. Augment contains a recombinant human version of the BB form of PDGF (rhPDGF-BB), which is a homodimer of two separate B-chains that are covalently linked through disulfide bonds. This is consistent with other members of the same protein structure super-family, the cysteine knot super-family of growth factors, most of which are also characterised by linkage of the dimer subunits by covalent disulphide bonds. In the BB homodimer, the disulphide links are formed between the second and fourth cysteine residues (Cys-43 and Cys-52 of the monomer polypeptide).

The rhPDGF-BB that is used in Augment is manufactured using recombinant DNA technology in a yeast expression system. The gene that codes for the human sequence of the PDGF B-chain is inserted into *Saccharomyces cerevisiae* cells. During fermentation the gene is activated to cause the expression of the PDGF B-chain that within the cell will crosslink and fold into the BB dimer. The protein is secreted from the yeast cell into its culture media, and subsequently purified from the media. The 3-Dimensional structure of rhPDGF-BB is depicted in Figure 2.6.2-1 that shows the anti-parallel structure of the B-chains and the positions of the disulfide bonds (in yellow).

Figure 2.6.2-1: 3-Dimensional Structure of the PDGF-BB Dimer



J Murray-Rust (Birkbeck College, London; unpublished structure)

PDGF receptors are protein tyrosine kinases and exist in two isoforms, α and β, which are expressed widely in various cell types, either individually or together. The binding of PDGF leads to dimerisation of the PDGF receptors, which then transduce various cellular responses. The α-receptor can bind both A and B subunits of PDGF with high affinity, while the β-receptor binds only the B subunit with high affinity. As a result, the BB homodimer can effectively engage both the α and β receptors, making it the preferable form for therapeutic purposes.

The ability of rhPDGF-BB to activate all PDGF receptor combinations is key to its therapeutic utility by stimulating activities on cells that express the α receptor, the β receptor, or both

receptors. PDGF receptor binding and activation induces a number of cellular responses, including the stimulation of chemotaxis, mitosis and actin reorganisation, all critical events for appropriate tissue repair. An overview of cell types bearing the two PDGF receptor types is given in Table 2.6.2-1.

Table 2.6.2-1: Cell Types Expressing the PDGF α- and β-receptors

Cell Type	α-receptor	β-receptor
Fibroblasts	+	+
Kidney mesangial cells	+	+
Leydig cells	+	+
Itoh cells (liver)	-	+
Liver sinusoidal endothelial cells	+	-
Myoblasts	-	+
Vascular smooth muscle cells	+	+
Capillary endothelial cells	-	+
Pericytes	-	+
Astrocytes	+	-
Neurons	+	+
Schwann cells	+	+
Mammary epithelial cells	-	+
Retinal pigment epithelial cells	+	+
Platelets/megakaryocytes	+	-
T cells	-	+
Myeloid haematopoietic cells	-	+
Macrophages	-	+

Table adapted from Heldin and Westermark (1999)

rhPDGF-BB is mitogenic for a range of cells including fibroblasts, osteoblasts, chondrocytes and other cell types expressing PDGF receptors. rhPDGF-BB is also chemotactic for fibroblasts, neutrophils and monocytes, cell types important for the early phases of tissue repair. As such, rhPDGF-BB is able to stimulate bone tissue formation and remodelling, which contributes toward defect repair in periodontal wounds.

A significant portion of the published studies on the function of PDGF in wound healing have focused on soft tissue and skin lesions. Nevertheless, a sizeable number of nonclinical studies have been published (Table 2.6.2-2) that demonstrate the *in vitro* and *in vivo* activity of PDGF for osteoblasts, osteoclasts, gingival fibroblasts and chondrocytes, supporting the role of rhPDGF-BB as a therapeutic treatment for bone regeneration.

Table 2.6.2-2: PDGF Published Nonclinical Studies: Wounds and Bone Repair	
***In vitro* studies**	
Primary mouse osteoblasts	Mott *et al*., 2002
Primary rat osteoblasts	Hsieh and Graves, 1998 Stephan *et al*., 2000 Bouletreau *et al*., 2002 Lee *et al*., 2002
Primary bovine chondrocytes	Weiser *et al*., 1999
Human gingivial fibroblasts	Piché and Graves, 1989 Bartold, 1993 Dennison *et al*., 1994 McAllister *et al*., 1995
Primary human bone cells	Piché and Graves, 1989
Human mesenchymal progenitor cells	Fiedler *et al*., 2002
Human periodontal ligament cells	Oates *et al*., 1993
Primary human foetal osteoblasts	Yang *et al*., 2000
Primary human osteoclasts	Zhang *et al*., 2000
Table 2.6.2-2 continues on the following page…	

Table 2.6.2-2: PDGF Published Nonclinical Studies: Wounds and Bone Repair (continued)	
***In vivo* studies**	
Mice	Fujii *et al*., 1999 Bowen-Pope *et al*., 1991 Soriano *et al*., 1994 Schatteman *et al*., 1992
Rats	Howes *et al*., 1988 Mitlak, *et al*., 1996 Strayhorn *et al*., 1999 Lee *et al*., 2000 Tanaka *et al*., 2002 Rasubala *et al*., 2003
Rabbits	Nash *et al*., 1994
Dogs	Lynch et al., 1989 Lynch et al., 1991 Lynch et al., 1994 Lynch et al., 1999 Wang et al., 1994 Cho et al., 1995 Park et al., 1995
Monkeys	Rutherford et al., 1992 Giannobile, et al., 1996

2.6.2.2.2 Fracture Healing of the Tibia in Geriatric-Osteoporotic Rats

A development study was completed in 2005 to determine the impact of rhPDGF-BB on fracture repair model using aged osteoporotic female rats. The geriatric osteoporotic rat model used in this study mimics delayed bone healing that is common in elderly osteoporotic individuals. The study design further allows for controlled and reproducible osseous defects. The use of geriatric and osteoporotic rats is well-established as a model of delayed healing due to the advanced age of the animals and decreased bone mass and bone mineral density that leads to inferior bone quality. Due to its specialized nature, this study was performed under procedures similar to GLP requirements, but was not conducted under strict GLP.

The study design compared two concentrations of rhPDGF-BB combined with β-TCP/collagen, β-TCP/collagen alone and an untreated control (fracture alone). The study showed both an increase in bone healing rate with increased strength in the β-TCP/collagen + rhPDGF-BB

treated groups compared to the β-TCP/collagen and untreated control groups. The treatment plan in this rodent study specifically allowed for the use of a dissolvable, soluble excipient in order to ensure that the material was physically maintained at the fracture site immediately following the surgical implantation. The excipient (a soluble collagen) is known to have no effects on bone regeneration. Thus, BioMimetic believes that the results of this study provide supporting data for the use of rhPDGF-BB treatment to stimulate fracture repair in an animal model with compromised capacity for fracture healing.

Study Procedure

Eighty (80) two year old female Sprague-Dawley rats weighing at least 200 gm each were used in the study. The rats were ovariectomised at Hilltop Lab Animals, Inc. (Scottsdale, PA) to make them estrogen deficient. The ovariectomised rats were housed at Hilltop in individual stainless steel cages for 4 months for stabilization after ovariectomy. The rats were put on a 30% calorie-reduced diet to ensure osteopenia over the 4-month course of recovery.

The ovariectomised rats were transferred to the testing site and remained on a restricted diet for 25 weeks prior to the osteotomy procedure. Following anaesthesia, the soft-tissue overlying the tibiae and knee joint were dissected. With the leg in extension, the patella ligament was lateralized, and under flexion, an access hole in the proximal metaphysis into the medullary canal was prepared using a surgical drill and physiological saline irrigation. A 0.7 mm K-wire was cut to the appropriate length to be sized to the tibiae. A transverse osteotomy was created in the upper 3rd of the right tibial diaphysis with a reciprocating saw, and a reduction of the fracture was achieved using the K-wire inserted through the distal portion of the tibia.

For the three groups that received the β-TCP/collagen matrix ± rhPDGF-BB, the matrix material was placed around the transverse osteotomy prior to closing the soft tissues. Unprotected weight-bearing was allowed, and the animals resumed normal activity after the surgeries. A total of 20 animals were included in each of the 4 treatment groups. The study design is described in Table 2.6.2-3.

At the time of sacrifice, either 3 weeks or 5 weeks post-surgery, the animals were humanely euthanized, and the tibiae, including contralateral uninjured tibiae, were harvested, the K-wires

were removed and radiographs of the fractures performed. Tibiae selected for micro-CT analysis were fixed in 10% neutral buffered formalin; following micro-CT analysis the specimens were processed for histology.

Table 2.6.2-3: Geriatric-Osteoporotic Rat Tibial Osteotomy Model - Study Design		
Treatment	**Number of Animals Evaluated with Radiographs and Biomechanical Testing***	**Number of Animals Evaluated with Micro-CT and Histology**
Week 3 Post-Surgery		
Untreated Fracture	8	2
β-TCP + sodium acetate	8	2
β-TCP + 0.3 mg/mL rhPDGF-BB	8	2
β-TCP + 1.0 mg/mL rhPDGF-BB	8	2
Week 5 Post-Surgery		
Untreated Fracture	8	2
β-TCP + sodium acetate	8	2
β-TCP + 0.3 mg/mL rhPDGF-BB	8	2
β-TCP + 1.0 mg/mL rhPDGF-BB	8	2
* Torsional biomechanical testing was conducted on both the fractured and contralateral unfractured bones by applying external rotational displacement at a rate of 0.25 degrees per second until failure.		

The 0.3 mg/mL rhPDGF-BB is the concentration of the growth factor contained in Augment. In order to accommodate the smaller defect size in the rodent, the particle size of the β-TCP used in the study described here was 250 - 1000 μm, which is smaller than the β-TCP particles being used in the foot and ankle clinical study (1000 - 2000 μm). Additionally, the β-TCP was from a different source than the material used in the clinical study. The β-TCP used in this study and the β-TCP used in the clinical study are, however, chemically identical and conform to ASTM Standard F-1088. It should be noted that the treatment plan in this rodent study specifically allowed for the use of a dissolvable, soluble excipient in order to ensure that the material was physically maintained at the fracture site immediately following the surgical implantation. The excipient (a soluble collagen) is known to have no effects on bone regeneration. Thus, BioMimetic believes that the results of this study are representative of the clinical formulation.

Results

Of the 80 animals in the study, 6 were excluded from the data analysis, 2 due to fracture complications resulting from inappropriate K-wire placement, 2 due to technical problems with biomechanical testing, and 2 due to death post-surgery. Neither death was associated with the study materials.

Due to the presence of β-TCP at the fracture site, the radiographs taken to confirm the location of the fractures and the reproducibility of injury between groups were found to be of limited value for assessment of fracture healing.

Histomorphometric Analysis: For the β-TCP control and untreated animals there was limited to no callus formation across the fracture site at 5 weeks. For both rhPDGF-BB groups, increased bone healing was seen across the fracture site with moderate to marked bridging callus present. There was no evidence of ectopic bone formation or of the formation of dense fibrous connective tissue at the treatment site for either of the rhPDGF-BB-treated groups.

Micro-CT Analysis: At both the 3 and 5 week time points there was evidence of the β-TCP particulate around the fracture sites, but no evidence of ectopic calcification in soft tissues. By week 5, the amount of β-TCP had decreased compared to week 3. For both rhPDGF-BB-treated groups, the process of bone fracture healing was normal and confined to the fracture site.

Biomechanical Testing: Analysis of biomechanical strength at 3 weeks after injury demonstrated that the fractured legs were statistically weaker than the uninjured contralateral legs for all four test groups as shown in Figure 2.6.2-2. At the 5 week time point, sufficient healing had occurred in the rhPDGF-BB treated groups such that the fractured legs were found to have equivalent torsional strength to the unfractured control legs. This is in contrast to the two control groups where the fractured legs were still significantly weaker than the opposite unfractured legs 5 weeks after surgery. Additionally, the rhPDGF-BB-treated groups demonstrated a statistically significant and time-dependent increase in torsional strength at the fracture site when comparing

3 and 5 week data. Comparison of the two rhPDGF-BB treatment groups demonstrated that the 0.3 mg/mL dose of rhPDGF-BB was as effective as the 1.0 mg/mL dose.

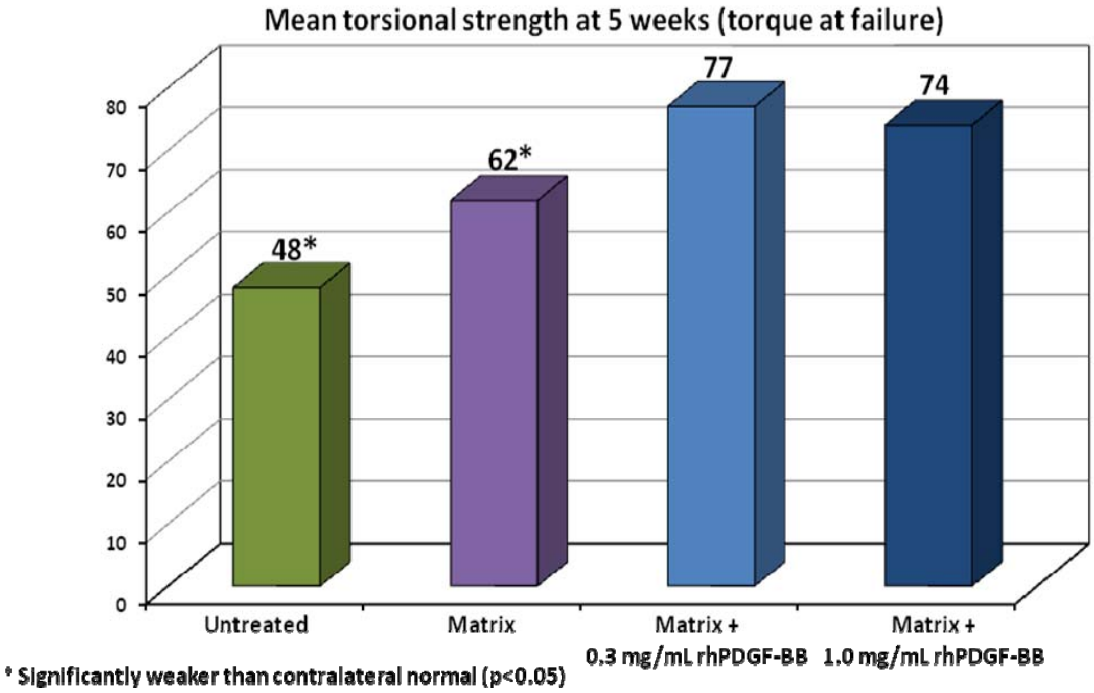


Figure 2.6.2-2: Torsion biomechanical testing of rat tibial fractures treated with or without rhPDGF-BB combined with β-TCP/collagen matrix

Conclusions

This study demonstrated that the rats treated with rhPDGF-BB combined with β-TCP/collagen matrix exhibited accelerated healing of the fracture with more mature bone than either the untreated or β-TCP/collagen control groups. In addition, mechanical testing showed that at five weeks, the β-TCP + rhPDGF-BB treatment had increased healing such that the mechanical strength of the treated tibias was not statistically different from the contralateral tibia of the same animals, while, in the control groups (non-rhPDGF-BB treated), treated tibias were statistically significantly weaker than the contralateral tibia. Finally, there were no untoward tissue responses (e.g., heterotopic bone formation) or adverse events found in any treatment group, and there was no evidence that the addition of rhPDGF-BB to the β-TCP product raises any safety concerns.

2.6.2.2.3 Diabetic Rat Fracture Model

A study was conducted to evaluate the use of rhPDGF-BB to enhance fracture repair in diabetic male rats. Diabetic-prone BB Wistar rats develop insulin-dependent diabetes mellitus in which the animals sustain an intense insulitis accompanied by selective destruction of beta cells. In the absence of insulin treatment, death usually results within 5 - 10 days after onset of glycosuria. This model represents a close homolog of human type I diabetes. These animals exhibit delayed fracture repair as a result of the onset of diabetes, similar to the situation in humans with type I diabetes. As a result, this model provides the opportunity to assess the ability of rhPDGF-BB to enhance fracture repair in a model of impaired healing. In this study, rhPDGF-BB was used in conjunction with a β-TCP matrix formulated with a soluble bovine type I collagen matrix. The treatment plan in this rodent study specifically allowed for the use of a dissolvable, soluble excipient in order to ensure that the material was physically maintained at the fracture site immediately following the surgical implantation. The excipient (a soluble collagen) is known to have no effects on bone regeneration. Thus, BioMimetic believes that the results of this study provide supporting data for the use of rhPDGF-BB treatment to stimulate fracture repair in an animal model with compromised capacity for fracture healing.

Study Procedure

The study investigated rhPDGF-BB treatment as a supplement to standard bone grafting to facilitate bone regeneration in a "worst case" or impaired healing model. The study evaluated early and late endpoints including: (1) cell proliferation (4 days post-treatment), (2) biomechanical strength (6 and 8 weeks post-treatment), and (3) histo-morphometric analysis (12 weeks post-treatment). Four test groups were included as outlined in Table 2.6.2-4; two doses of rhPDGF-BB combined with matrix, buffer combined with matrix, and untreated controls.

Table 2.6.2-4: Diabetic rat fracture model - study design

Group	Treatment
1	Diabetic (Negative Control - No treatment)
2	β-TCP/collagen + Buffer (Mock Control)
3	β-TCP/collagen + rhPDGF-BB (0.3 mg/mL)
4	β-TCP/collagen + rhPDGF-BB (1.0 mg/mL)

A K-wire was placed in the femur of the leg to be studied and a mid-diaphyseal femur fracture created using blunt trauma. After creation of the fracture, the fracture site was opened in a cranial lateral plane while maintaining periosteal integrity. Each rat was treated with one of the test materials (Groups 2 - 4), or left untreated (Group 1) as a negative control. Only animals that exhibited non-comminuted, transverse fractures, confirmed by radiographs, were used in the study. Radiographs were taken at 2 - 4 week intervals to assess the quality of the fracture, correct placement of the K-wire and pin migration.

Results

At Day 4, the cell proliferation results demonstrated that treatment with locally-delivered rhPDGF-BB led to an increase in gap callus cellular proliferation in a dose dependent manner with both doses of rhPDGF-BB having statistically greater number of proliferating cells per unit area compared to the no treatment and matrix + buffer control groups (Figure 2.6.2-3).

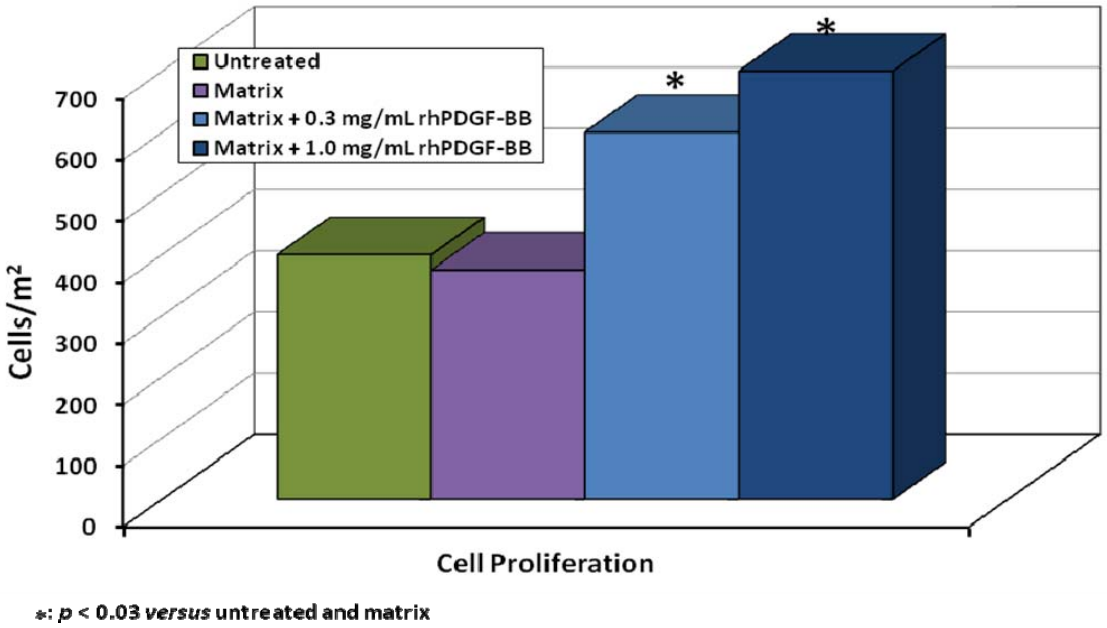


Figure 2.6.2-3: rhPDGF-BB increases cell proliferation in the Day 4 fracture callus. Cellular proliferation, as measured by BrdU staining, was significantly increased in rhPDGF-BB-treated groups compared to the No Treatment and Buffer (20 mM sodium acetate solution, pH 6.0) groups.

At 6 weeks post-fracture, biochemical testing results demonstrated that treatment with locally-delivered 0.3 mg/mL rhPDGF-BB trended towards an increase in percent (%) torque to failure and maximum torsional rigidity, when compared to the untreated fracture group. By week 8 post-fracture the percent torque to failure of the 0.3 mg/mL group was significantly higher compared to the untreated and buffer groups (Figure 2.6.2-4). At the 8 week time point the percent torsional rigidity was also significantly higher in the 0.3 mg/mL rhPDGF-BB group compared to the buffer group, and approached significance compared to both the untreated ($p < 0.08$) and the 1.0 mg/mL rhPDGF-BB ($p < 0.06$) groups. At both the 6 and 8 week time points, there were no differences in shear strength among the four groups.

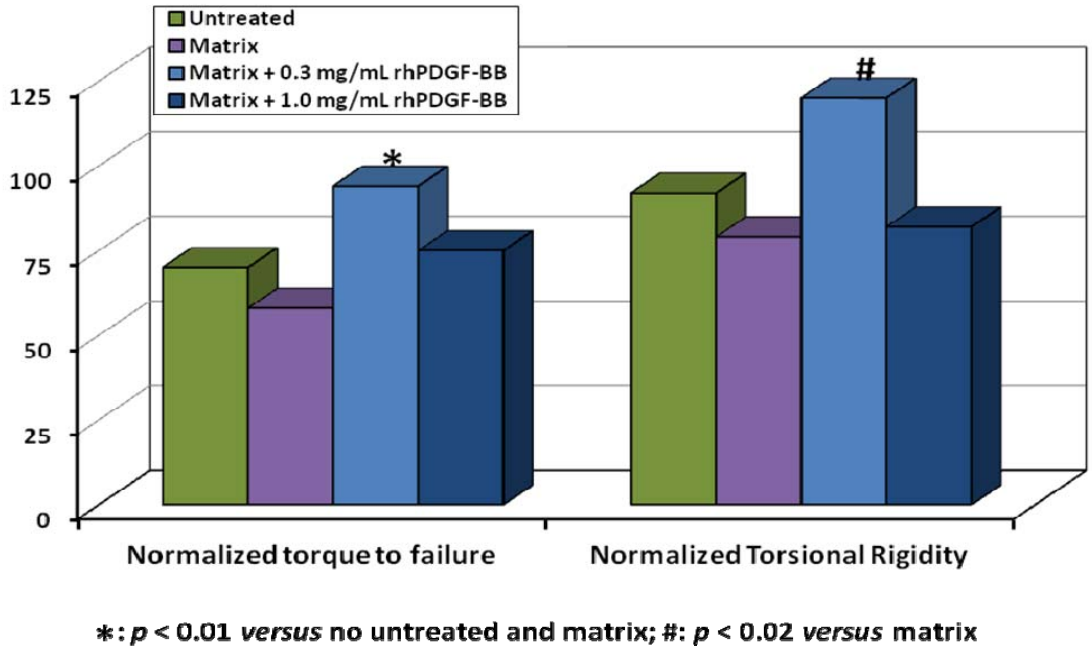


Figure 2.6.2-4: Treatment with low dose (0.3 mg/mL) rhPDGF-BB increases torsional mechanical strength 8-weeks post-fracture. Results for fractured legs were normalized to results from the contralateral unfractured leg for each animal to give percent values. *p < 0.05 versus No treatment, **p < 0.005 versus Buffer

At 12 weeks the histomorphometric results of the study demonstrated that treatment with locally-delivered rhPDGF-BB (0.3 and 1.0 mg/mL doses) led to bridging of the fracture callus cortices whereas the control groups (untreated fracture and buffer) showed incomplete bridging. The control groups also showed little evidence of remodelling which may have resulted in the continued presence of the fracture callus. The callus in the control groups also contained a

greater proportion of fibrous and cartilaginous tissue. No significant differences were observed in gap callus area among the four experimental groups, though there was a significant increase in the percent of mineralised bone area of the callus in the 1.0 mg/mL rhPDGF-BB group compared to the 0.3 mg/mL rhPDGF-BB, buffer-treated and untreated groups. There were no significant differences in percent of mineralised bone area among the 0.3 mg/mL rhPDGF-BB, buffer-treated or untreated groups.

The effect of rhPDGF-BB to stimulate fracture repair was observed at all time points analysed. The increase in cell proliferation at day 4 post-treatment suggests a critical role for rhPDGF-BB to enhance the early phase of tissue healing. A hypothesis of the study was that increased cell proliferation at an early time point should lead to an increase in mechanical strength and accelerated callus remodelling. Both activities were observed with rhPDGF-BB treatment at 8 and 12 weeks, respectively.

Amendment to Final Report

The final study report included the use of the Student's t-test and one-way Analysis of Variance (ANOVA) as part of the statistical analyses to determine differences among groups in the study for the outcomes measured. The statistical analyses have since been amended to use a statistical assessment more appropriate for comparing differences across more than two groups. In the revised analysis, only ANOVA was used for determining if any differences exist among the four treatment groups in the study, and in place of the Student's t-test, the Holm-Sidak *post-hoc* test was used to establish if any differences exist among any two treatment groups ($p \leq 0.05$).

It was originally reported that the biomechanical testing data showed a significant difference in the percent maximum torque to failure between the low dose treatment group (0.3 mg/mL rhPDGF-BB) and the buffer and untreated groups at six (6) weeks post-surgery. Following the revision to the statistical analyses of these data, this outcome changed such that no significant differences were found to exist between the low dose treatment group and the buffer or untreated fracture groups at 6 weeks post-surgery. All other statistical outcomes reported in the study were unaffected by the change described in this amendment, and remain as provided in the original final study report.

Overall, the study outcomes to evaluate the impact of rhPDGF-BB to enhance multiple parameters of healing in a diabetic rat fracture model remain consistent with the statements provided in the final report. The outcomes reported included: (1) increased early cell proliferation within the gap callus, (2) increase in percent torque to failure biomechanical properties at 8 weeks post treatment, and (3) accelerated remodelling of the fracture callus at the 12 week time point as demonstrated by an increase in the percent mineralised bone area of the callus.

Conclusions

Treatment with rhPDGF-BB was shown to be effective in augmenting a standard bone grafting matrix to enhance long bone healing in the diabetic rat, a model of impaired fracture repair. The results from this study demonstrate that rhPDGF-BB treatment leads to enhanced fracture healing as exhibited by increased cell proliferation at 4 days post-treatment, increased biomechanical strength at 8 weeks and increased bone content of the fracture callus at 12 weeks post-fracture. rhPDGF-BB treatment appears safe with no evidence of either abnormal or ectopic bone formation. The data supports the clinical evaluation of rhPDGF-BB in conjunction with β-TCP, especially in patients with a compromised capacity for fracture healing.

2.6.2.2.4 Partial Arthrodesis of the Carpus in Dogs

A study was completed in 2007 to determine the impact of rhPDGF-BB in a dog carpus bone fusion model. The model utilised represents a standard surgical procedure used in dogs requiring an arthrodesis procedure to the foot, generally as a result of trauma to the foot. Due to its specialised nature, this study was performed under procedures similar to GLP requirements, but was not conducted under strict GLP.

The study design compared various matrices including autograft (bone harvested from the individual dogs), the current standard of care for dogs that require foot arthrodesis, β-TCP, and a β-TCP/collagen matrix, and included test groups with and without rhPDGF-BB added to the test matrix. The study demonstrated that the addition of rhPDGF-BB to any of the matrices improved bone fusion when compared to the matrix alone, and that the combination of the

β-TCP and β-TCP/collagen matrices with rhPDGF-BB were equivalent to or better than autograft alone.

Study Procedure

Thirty (30) dogs were randomly assigned to treatment groups as described in Table 2.6.2-5.

Table 2.6.2-5: Partial arthrodesis of the carpus in dogs – study design

Group No.	Side A Treatment	Side B Treatment	No. Animals
1	Autograft	Autograft + rhPDGF-BB*	10
2	β-TCP	β-TCP + rhPDGF-BB*	10
3	β-TCP/collagen	β-TCP/collagen + rhPDGF-BB*	10

* in 20 mM sodium acetate, pH 6.0

The β-TCP particles used in treatment Group 2 ranged in size from 250 to 1000 µm in diameter. Although Augment contains β-TCP particles with a nominal size ranging from 1000 to 2000 µm, a smaller size of β-TCP particle was chosen to accommodate the smaller space available between the bones of the canine foot for implantation of the material during surgery. All β-TCP particles used are chemically identical. All dogs were in good health and underwent a 14-day quarantine period prior to study initiation. Following anaesthesia, the dogs' forelimbs were shaved of all hair and prepared for surgery.

If the animal was in the autograft group, a 10 x 10 inch area above the iliac crest was also shaved, a 5 cm incision was made over both hips and the fascia was incised down to the crest. Rongeurs were used to obtain 1.5 cc of cortico-cancellous bone from both iliac crests, and the surgical sites were closed. The autograft was morselised into 1 - 2 mm chips.

A partial arthrodesis of the radial carpal/3rd carpal and 3rd carpal/3rd metacarpal was done bilaterally in each dog. A 5 cm midline dorsal incision was made just below the cephalic and accessory cephalic veins and extended distally for 5 cm. The deep antebrachial fascia was incised midway between the extensor *carpi radialis* tendon and the common digital extensor and retracted with a self-retaining retractor. A 2 mm round high-speed burr was used to denude the joint surfaces which included the distal surface of the radial carpal, proximal and distal surfaces of the 3rd carpal and the proximal surface of the 3rd metacarpal.

Each dog received matrix only (autograft, β-TCP or β-TCP/collagen) in Side A, and the same matrix + rhPDGF-BB in Side B. Approximately 0.75 cc of graft was placed above and below the 3rd carpal bone in the joint space. A 2T3 plate was used to help stabilize the joint after removal of the cartilage tissues between the joints and the subsequent placement of the test articles. To secure the plate in place two 2.7 mm self tapping screws, 18 mm in length, were placed in the radial carpal and one screw placed in both the metacarpal and in the carpal. The surgical sites were closed, and each dog's forelimbs were immobilised in a short leg cast following surgery, except for the first dog in the study which had a short leg brace in use for the first week followed by placement of the leg cast (use of the leg brace did not have an impact on overall healing of the animal).

The dogs were humanely euthanized at either 5 or 12 weeks post surgery. Radiographs, manual palpation and histology were used to assess healing of the graft area.

Results

Thirty (30) dogs were entered into the study, with 28 used for analysis. Two dogs were prospectively omitted, one (autograft group) had a left soft tissue infection of the carpus, and one (β-TCP/collagen group) had surgical complications with the plate at time of surgery. The dogs tolerated the surgery and casting well, and regained normal activity by day 3 post-operative. There was minimal swelling in the limbs over the surgical site and no other complications were seen regardless of the test article.

The overall results at 12 weeks, including histology (Figure 2.6.2-5) and manual palpation (Table 2.6.2-6) suggest that autograft + rhPDGF-BB treatment stimulated the most complete and widest extent of fusion, followed by β-TCP + rhPDGF-BB treatment. The addition of PDGF-BB showed trends toward improving the amount of bridging bone or fusion when compared to the matrix or autograft alone.

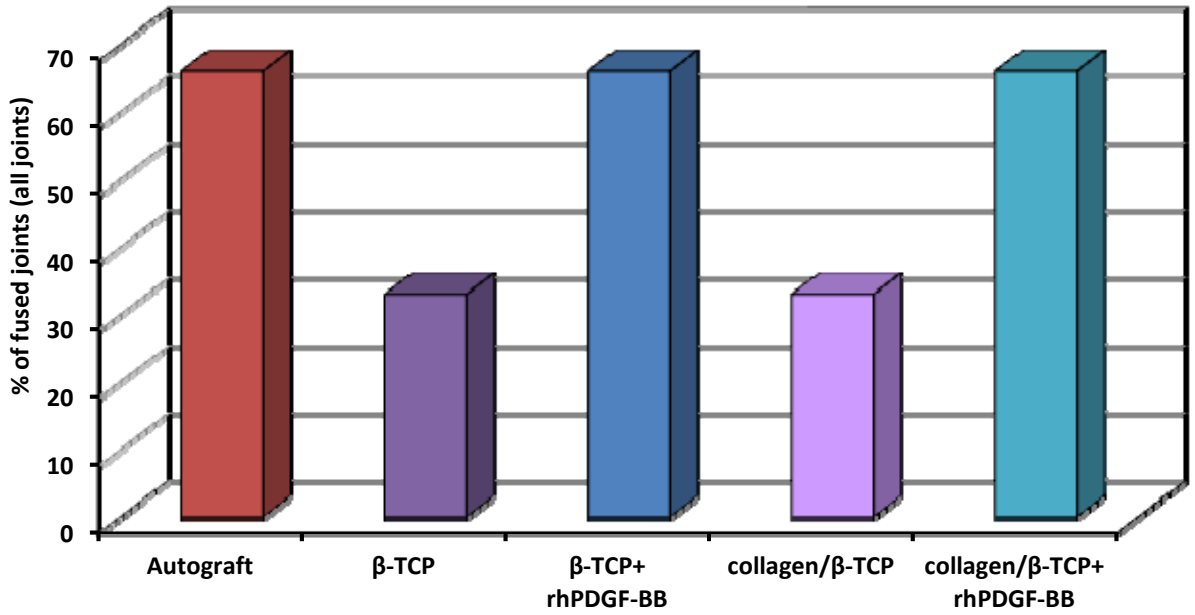


Figure 2.6.2-5: Treatment with rhPDGF-BB increases the number and extent of fused joints assessed by histology 12 weeks after surgery.

Table 2.6.2-6: Partial Arthrodesis of the Carpus In Dogs – Results for Fusion (%) Assessed by Manual Palpation

Treatment	Week 5 Results		Week 12 Results	
	radial carpal/ 3rd carpal joint	3rd carpal/ 3rd metacarpal joints	radial carpal/ 3rd carpal joint	3rd carpal/ 3rd metacarpal joints
Autograft	50%	17%	66%	66%
Autograft + rhPDGF-BB	66%	33%	100%	100%
β-TCP	14%	42%	66%	66%
β-TCP + rhPDGF-BB	57%	43%	66%	66%
β-TCP/collagen	50%	33%	66%	66%
β-TCP/collagen + rhPDGF-BB	50%	66%	100%	100%

At 5 and 12 weeks manual palpation results (Table 2.6.2-6) suggested that β-TCP + rhPDGF-BB and β-TCP/collagen + rhPDGF-BB were equivalent to autograft and autograft + rhPDGF-BB. At 12 weeks, the presence of woven bone in joints treated with rhPDGF-BB was a consistent

histological observation. Woven bone maturing to lamellar bone would be expected to result in joint fusion. The incomplete fusions observed at 12 weeks that contained woven bone promoted by rhPDGF-BB would likely mature to lamellar bone to achieve a stable joint fusion.

Conclusions

The rhPDGF-BB administered in this study tended to increase the number and extent of bridged/fused joints for each treatment group compared to non-rhPDGF-BB treated joints. The bone formed following rhPDGF-BB treatment was normal bone. In addition, there was no evidence of ectopic bone formation in soft tissues contiguous to the joints treated with rhPDGF-BB, nor was there evidence of acute or chronic toxicity from rhPDGF-BB containing test articles in bone or surrounding soft tissues. Qualitative assessments suggested a trend of more complete bone fusion in the rhPDGF-BB-containing treatments compared to the corresponding matrix alone treatments.

2.6.2.2.5 Biological Assessment of a Bone Repair Model (Rabbit Tibial Osteotomy)

A study was conducted to evaluate the impact of rhPDGF-BB treatment on remodelling in bone defects in 30 New Zealand White rabbits. The study was designed to evaluate the impact of rhPDGF-BB combined with β-TCP to improve remodelling within a bone defect as compared to animals treated with β-TCP alone, and to confirm that none of the test formulations would negatively impact bone remodelling at the implant sites. Due to its specialised nature, this study was performed under procedures similar to GLP requirements, but was not conducted under strict GLP.

Study Procedure

Unicortical, 5 mm osteotomies were created in the proximal-medial tibial metaphyses of 30 skeletally mature New Zealand White rabbits. The limbs were assigned to one of three groups: those treated with cylinders of β-TCP (control); those treated with β-TCP containing 25 µg rhPDGF-BB (low dose); and those treated with β-TCP containing 75 µg rhPDGF-BB (high dose). Five rabbits from each group were euthanized at four and eight weeks after surgery and evaluated histologically.

The test materials were provided as sterile packages and were mixed per standard aseptic operating room procedures. Prior to adding the rhPDGF-BB to the β-TCP cylinders, the cylinders were trimmed to 5.5 mm diameter by 8.5 mm to fill the osteotomies. The test articles and control materials are referenced in Table 2.6.2-7.

Table 2.6.2-7: Rabbit tibial osteotomy model - test articles and control materials

Identifier	Description
Formulation 1	β-TCP: Porous cylinder
Formulation 2	25 µg rhPDGF-BB*/ β-TCP: Porous cylinder
Formulation 3	75 µg rhPDGF-BB*/β-TCP: Porous cylinder
* in 20 mM sodium acetate, pH 7.4	

The desired total dose of rhPDGF-BB was 25 µg (Dose 1) and 75 µg (Dose 2) per cut cylinder. To obtain this, 0.2 mL of 0.125 mg/mL and 0.375 mg/mL solutions of rhPDGF-BB were added to the blocks to give the desired total doses. Dose 1 corresponded to a dose of 5 µg rhPDGF-BB/kg body weight and Dose 2 corresponded to a dose of 15 µg rhPDGF-BB/kg body weight. Solutions at these concentrations were made from a 10 mg/mL stock of rhPDGF-BB (lot# QA2217), diluted with sterile 20 mM sodium acetate, pH 7.4. Cylinders were placed individually in 60 mm sterile culture dishes for each dose, and 0.2 mL of the appropriate solution was slowly pipetted on top of the cylinder.

The 30 animals were weighed prior to surgery and randomly assigned to treatment groups. Following anaesthesia, the right hind leg was shaved and prepared for surgery. An incision was made over the proximal medial tibia and the medial aspect of the proximal tibia was exposed. Using a rotary surgical tool, a single 5.0 mm diameter hole was drilled into the tibia approximately 15 mm below the joint, half-way between the anterior and posterior borders. The designated test or control article was inserted medially to laterally, stopping against the lateral intact cortex, and the surgical site was closed. All surgeries were uneventful and all animals

recovered without incident. After four or eight weeks the rabbits were humanely euthanized and leg tissues were taken for histological processing and analysis.

Stained sections were assessed to evaluate bone formation across the drill hole, the appearance of the residual implant materials, and the biological remodelling response of the host bone to the implant materials. The area of bone bridging the gap in the medial cortical wall was measured in all specimens. Measured values were standardized to a field area percent. An ANOVA was used to evaluate the effect of treatment on the healing of the osteotomy at four and eight weeks and the biodegradation of the carrier at four weeks. Differences were considered significant at $p < 0.05$.

Results

The clinical and histological evaluations revealed that all three treatment groups responded well. All formulations were osteoconductive and bone remodelling appeared normal in response to all test formulations at both the four and eight week time periods. At four weeks post-surgery, all treatment groups showed evidence of a fully developed callus largely composed of bone. In all instances, the gap was bridged by woven bone mixed with residual β-TCP. The pattern within and among treatment groups at four weeks was highly consistent and completely within the normal range of expected healing. At eight weeks, each osteotomy was bridged by consolidating lamellar bone. Most of the β-TCP was resorbed and the marrow cavities were restored.

Qualitative assessment (Table 2.6.2-8) indicated that, at eight weeks, there was no statistical difference among the three treatment groups ($p = 0.98$) in the amount of bone spanning the gap on the medial cortical wall. There was also no statistical difference at four weeks among the three treatment groups in the amount of bone spanning the gap on the medial cortical wall ($p = 0.25$) or in the amount of residual β-TCP ($p = 0.38$).

Table 2.6.2-8: Rabbit tibial osteotomy model - measured value of new bone formed across the gap

Group	Week 4	Week 8
β-TCP	9.54 (±1.46 SD)	37.11 (±6.61 SD)
β-TCP + 25 µg rhPDGF-BB	14.5 (±2.92 SD)	37.79 (±9.36 SD)
β-TCP + 75 µg rhPDGF-BB	11.63 (±5.34 SD)	37.98 (±4.48 SD)

Conclusions

Over the eight week time course, all defects integrated with host bone through callus formation, and osseous contours were re-established. Residual β-TCP detected at four weeks was significantly reduced by eight weeks. Lymphocytic infiltrate was negligible at four and eight weeks. While histomorphometric data indicated there was no statistically significant difference in the area of bone in the defects treated with 0, 25 or 75 µg of rhPDGF-BB, the findings suggested that treatment with either 25µg or 75 µg of rhPDGF-BB led to a trend for increased remodelling of bone at the 4-week time point. The rabbit model used in this study appears to be useful for establishing the ability of bone grafting material to evoke normal bone remodelling, though it may not be sufficiently discriminating to discern statistical differences in accelerated bone remodelling for the rhPDGF-BB concentrations tested.

2.6.2.2.6 Evaluation of the Proliferative Response of Dermal Fibroblasts From Multiple Species to Recombinant Human Platelet-Derived Growth Factor-BB (rhPDGF-BB) Using the ^{3}H - Thymidine Uptake

BMTI has undertaken experiments to determine the receptor affinities of fibroblast cells of rat, rabbit, dog, and human origin for the yeast derived rhPDGF-BB. The effect of rhPDGF-BB on dermal fibroblasts from multiple species (human, rat, rabbit, goat, dog, sheep) was assessed by measuring the proliferative response of the fibroblasts to different doses of rhPDGF-BB. Proliferation was used as an indicator of activity because the mitogenic effect of PDGF-BB on multiple species is well documented. The EC_{50} was chosen as an indicator of the rhPDGF-BB activity. The EC_{50} is a parameter determined from a 4-parameter logistic curve fit of the ^{3}H uptake vs. concentration graph and indicates the concentration of rhPDGF-BB that elicits half of the maximum effect. A decrease in the EC_{50} indicates an increased effect of rhPDGF-BB on these cells. The EC_{50} was determined in a classic pulse-and chase ^{3}H-thymidine incorporation assay to determine the relative response of dermal fibroblasts to rhPDGF-BB.

The EC_{50} for human adult dermal fibroblasts is higher ($p < 0.05$) than for human neonatal, rat, goat, dog, and sheep dermal fibroblasts but not different than for rabbit dermal fibroblasts. The EC_{50} for the human neonatal dermal fibroblasts was equivalent to the EC_{50} for other species (Table 2.6.2-9; Figure 2.6.2-6).

Table 2.6.2-9: Comparison of the proliferative effect of rhPDGF-BB on Human Adult, Human Neonatal, Rat, Rabbit, Goat, Dog, and Sheep Dermal Fibroblasts Reported as the EC_{50} Determined using the ^{3}H-Thymidine Incorporation Assay.				
Species	**Average EC_{50} (nM)**	**CV (%)**	**Fold-Difference (compared to human adult)**	**Fold-Difference (compared to human neonatal)**
Human Adult	21.76 ± 6.67	30.7	1.0	+2.6
Human Neonatal	8.47 ± 2.10	24.9	-2.6	1.0
Rat	13.23 ± 1.96	14.8	-1.6	+1.6
Rabbit	15.15 ± 5.42	35.8	-1.4	+1.8
Goat	4.56 ± 1.43	31.4	-4.8	-1.9
Dog	9.99 ± 3.76	37.7	-2.2	+1.2
Sheep	10.94 ± 5.38	49.2	-2.0	+1.3

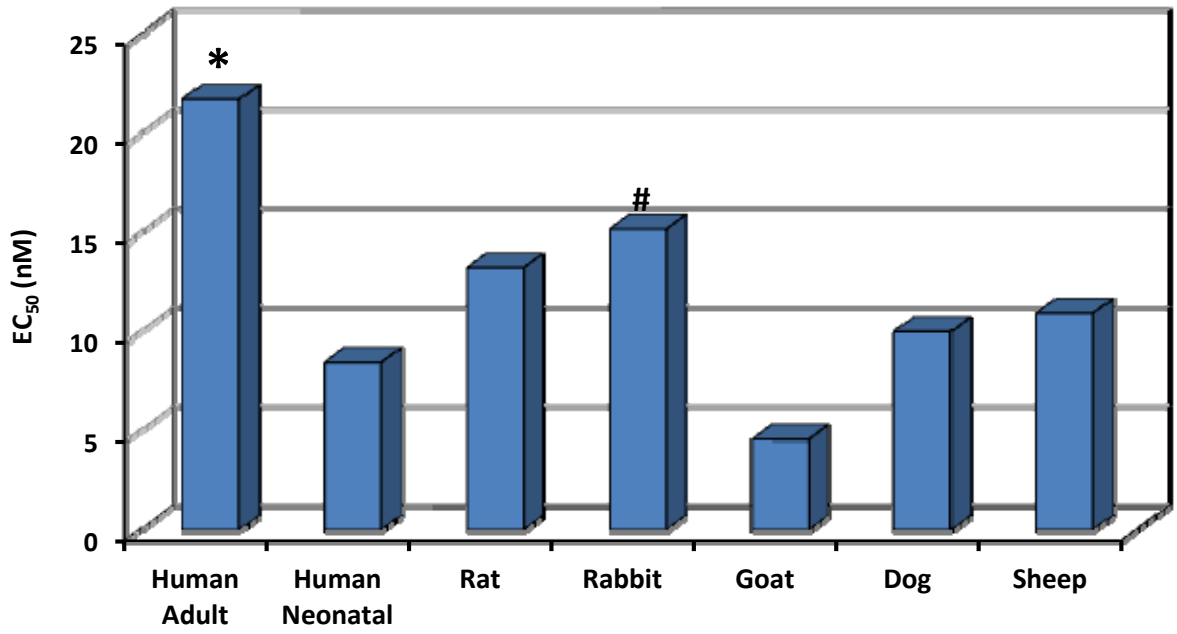


Figure 2.6.2-6: Mean and standard deviations for the EC_{50} across species as determined over 4 assays for rat, goat, dog, and sheep and 5 assays for human adult, human neonatal, and rabbit. *: $p < 0.05$ compared to human neonatal, rat, goat, dog, and sheep dermal fibroblasts. #: $p < 0.05$ compared to goat dermal fibroblasts.

These data (Figure 2.6.2-6) indicate that the effect of rhPDGF-BB, when exogenously applied to dermal fibroblasts of the species tested here, is at least as effective, as in humans. These results suggest that the potency of rhPDGF-BB across species is maintained, and support the use of these species in pre-clinical safety and efficacy studies.

2.6.2.3 Secondary Pharmacodynamics

2.6.2.4 Safety Pharmacology

There were no adverse pharmacodynamic or pathophysiological effects observed in toxicology testing or in the clinical studies for Augment. Therefore, additional safety pharmacology studies were not warranted to evaluate further pharmacology safety properties relevant to human safety.

2.6.2.5 Pharmacodynamic Drug Interactions

PDGF is a naturally occurring protein that is released from platelets at the site of an injury. As Augment is not administered systemically but is delivered locally, and is implanted in a single administration, the nonclinical programme did not include testing of rhPDGF-BB for drug interactions.

2.6.2.6 Discussion and Conclusions

The pharmacological action of PDGF and of PDGF-BB in particular, has been extensively reported in the literature. Published information provides strong evidence for the continued safe use of this growth factor, particularly in the present posology where a single dose, administered locally, will lead to minimal systemic exposure. The lack of systemic toxicity, together with its relatively weak immunogenic potential following parenteral injection, provides a strong assurance of safety.

Several different animal models of bone healing were used to demonstrate the effect of rhPDGF-BB in combination with β-TCP matrices. These included models of fracture and fusion healing, and each demonstrated the local pharmacological activity and safety of rhPDGF-BB. Additional *in vivo* studies to evaluate rhPDGF-BB combined with materials other than β-TCP also provide evidence of the local pharmacological activity and safety of rhPDGF-BB.