Office of Cellular, Tissue, and Gene Therapies
Center for Biologics Evaluation and Research
Food and Drug Administration

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FDA BRIEFING DOCUMENT

BLA 125400

Organogenesis, Inc.

Allogeneic Cultured Keratinocytes and Fibroblasts in Bovine Collagen, Apligraf

For the treatment of surgically created gingival and
alveolar mucosal surface defects in adults

Disclaimer*: The attached package contains background information prepared by the Food and Drug Administration (FDA) for the members of the advisory committee. The FDA background package often contains assessments and/or conclusions and recommendations written by individual FDA reviewers. Such conclusions and recommendations do not necessarily represent the final position of the individual reviewers, nor do they necessarily represent the final position of the Review Division or Office. We have brought the Apligraf® application to this Advisory Committee in order to gain the Committee's insights and opinions. The background package may not include all issues relevant to the final regulatory recommendation; instead, it is intended to focus on issues identified by the Agency for discussion by the advisory committee. The FDA will not issue a final determination on the issues at hand until input from the advisory committee process has been considered and all reviews have been finalized. The final determination may be affected by issues not discussed at the advisory committee meeting.*

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ABBREVIATIONS

AE	Adverse Events
ANG	Angiogenin
ANT	Angiostatin
BLA	Biologic License Application
CDRH	Center for Devices and Radiological Health
CWHS	Clinical Wound Healing Score
DAGID	Division of Anesthesiology, General Hospital, Infection Control, and Dental Devices
DE	Dermal Equivalent
DFU	Diabetic Foot Ulcers
ELISA	Enzyme Linked Immunosorbent Assay
EPI	Epidermal Layer
FDA	Food and Drug Administration
FGF-2	Fibroblast Growth Factor-2
FGG	Free Gingival Graft
GCF	Gingival Crevicular Fluid
GM-CSF	Granulocyte Macrophage Colony-Stimulating Factor
H&E	Hematoxyline and Eosin
ICH	International Conference on Harmonisation
IHC	Immunohistochemistry
IDE	Investigational Device Exemption
IL-8	Interleukin-8
IP-10	Interferon-inducible protein-10
KT	Keratinized Tissue
MAUDE	Manufacturer And User Facility Device Experience database
MCB	Master Cell Bank
MDR	Medical Device Report
mm	Millimeter
MTT	Mitochondrial Tetrazolium Test
OCP	Office of Combination Products
OCTGT	Office of Cellular, Tissue and Gene Therapies
PAL	Post Air-Lift
PDGF-BB	Platelet Derived Growth Factor-BB
PI	Patient Information (medication package insert)
PMA	Premarket Approval Application
RT-PCR	Reverse Transcription - Polymerase Chain Reaction
SCTG	Subepithelial Connective Tissue Grafts
TGF	Transforming Growth Factor
TIMP-1	Tissue Inhibitor of Metalloproteinases-1
TIMP-2	Tissue Inhibitor of Metalloproteinases-2
VEGF	Vascular Endothelial Growth Factor

ABBREVIATIONS (continued)

VLU	Venous Leg Ulcers
WCB	Working Cell Bank
WF	Wound Fluid

1. INTRODUCTION

The product, Apligraf, is a bi-layered tissue construct consisting of an upper layer that is made of human keratinocytes and a supporting lower layer that is constructed of bovine-derived collagen and human neonatal foreskin-derived dermal fibroblasts. The layers adhere as one unit to form the Apligraf product. The Apligraf product for the oral indication in this BLA is the same final product as the commercially available Apligraf® product, approved by the Center for Devices and Radiological Health (CDRH) for the treatment of venous leg and diabetic foot ulcers that have not healed with conventional therapies.

Organogenesis, Inc. (hereafter referred to as "Organogenesis" or the "applicant") has submitted a BLA to license Apligraf for the treatment of surgically created gingival and alveolar mucosal surface defects in adults. The application (BLA 125400) is being reviewed by the Office of Cellular, Tissue and Gene Therapies (OCTGT) within Center for Biologics Evaluation and Review (CBER), in collaboration with the Division of Anesthesiology, General Hospital, Infection Control, and Dental Devices (DAGID) and the Division of Surgical, Orthopedic and Restorative Devices (DSORD) within the Center for Devices and Radiological Health (CDRH) of FDA. This application is the subject of the advisory committee meeting of November 17, 2011.

This FDA Briefing Document for the BLA summarizes FDA's review of the BLA to this point, highlighting particular areas for which FDA is seeking this Advisory Committee's expertise and input.

2. EXECUTIVE SUMMARY

2.1 Product Overview

Apligraf is a bi-layered tissue construct consisting of allogeneic epidermal keratinocytes (--b(4)----------), fibroblasts (--b(4)----------) and bovine type I Collagen. The upper layer is formed from living human keratinocytes which are organized into a well-differentiated cornified layer similar to human skin. The supporting lower layer consists of human dermal fibroblasts suspended in a bovine collagen matrix. The upper and lower layers of the Apligraf product combine to form a bi-layered unit and make up approximately ------b(4)------ of the construct, respectively. Apligraf is supplied as a circular disk approximately 75.0 mm in diameter and 0.75 mm thick.

Product quality for Apligraf is ensured by a combination of testing performed at key manufacturing steps and for the final product. The human keratinocytes and fibroblasts are isolated from neonatal foreskin tissue, cultured, and cryopreserved in a two-tiered system of master and working cell banks. The banks have limited expansion potential, and therefore new banks from new donor tissue must be generated regularly. Testing conducted at the master and working cell bank level for both cell types is critical in ensuring that the different cell banks used in the manufacturing of different lots of Apligraf are comparable.

Product potency is another key characteristic and is determined for Apligraf using a histological analysis of biopsies obtained from the final bi-layered product. This assay is supported by in vitro and in vivo studies which correlate various histological potency parameters with the quality of the two layers and their resulting functions.

The FDA requests scientific discussion from the Advisory Committee regarding the testing strategy used to qualify the keratinocyte and fibroblast cell banks and the histological assay used to determine Apligraf potency.

2.2 Clinical Program Overview

The proposed indication is “for the treatment of surgically created gingival and alveolar mucosal surface defects in adults.”

The effectiveness data for this proposed indication come from two completed clinical trials: a pivotal study (06-PER-002-CTX, N=96) and the preceding pilot study (05-PER-001, N=25) on which the design of the pivotal study was based. In Studies 05-PER-001 and 06-PER-002-CTX, Apligraf was applied over the defect (non-submerged). An additional clinical trial (07-PER-004-CTX, N=15), was conducted for an oral indication; in this study, Apligraf was placed under a surgical flap (submerged) at the oral mucosal defect site. This BLA seeks approval of Apligraf for a non-submerged use. However,

Study 07-PER-004-CTX will be briefly discussed in this document because it also provides data from oral experience with the product.

In addition to safety data from the three studies in oral indications, there is extensive safety data and postmarket experience with Apligraf for the cutaneous venous leg ulcer and diabetic foot ulcer wound indications.

3. BACKGROUND

3.1 Mucogingival Defects

3.1.1 Overview of Disease

Mucogingival defects are soft tissue defects involving both the attached gingiva and alveolar mucosa at the mucogingival junction (See Figures 1 and 2) [Prato 1999]. They may be caused by anatomic, traumatic, or infection-related factors. These defects are generally associated with a loss of sufficient amounts of attached gingiva to cause soft tissue inflammation that is not resolved by oral hygiene procedures alone. Tooth root surfaces are frequently exposed, and gingival recession is usually present in varying amounts [Chambrone 2009; Oh 2009].

Figure 1. Diagrams of Gingival and Alveolar Mucosa [Johnson 2011]

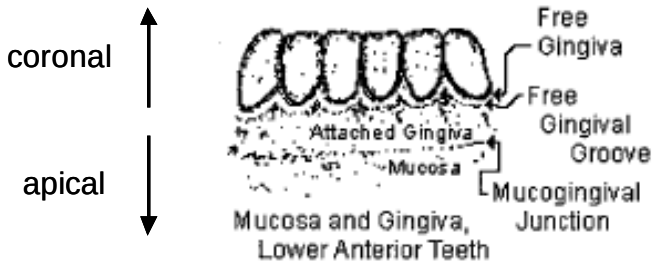
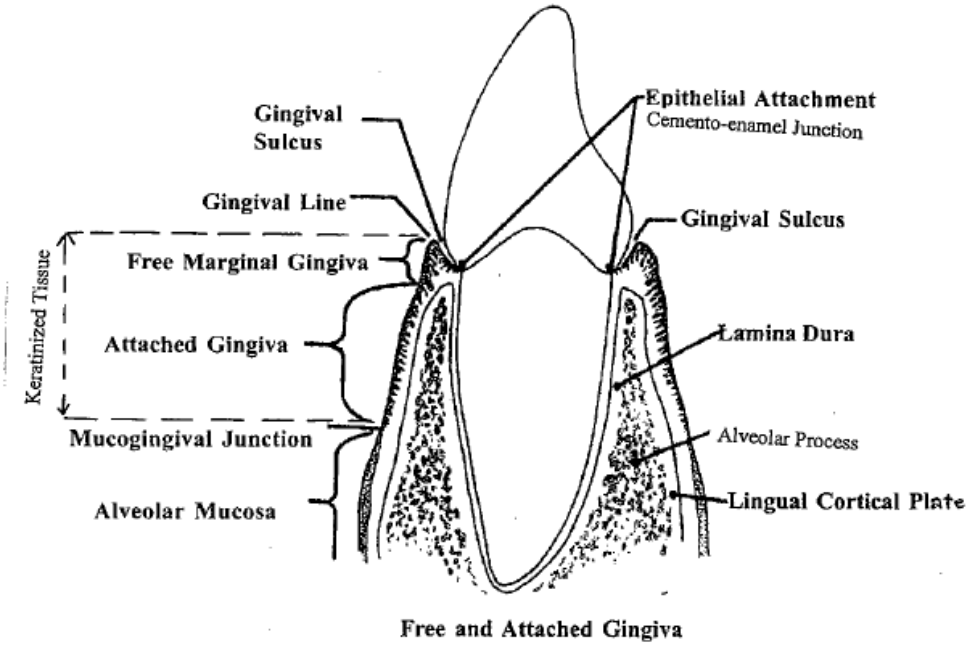


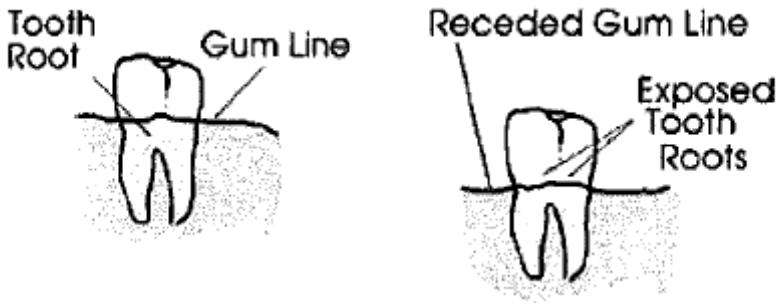
Figure 2. Diagram of the Investing Structure of the Tooth [Tooth Anatomy Drawing 2011]



Gingival recession (See Figure 3) is defined as the oral exposure of the root surface due to a displacement of the gingival margin apical to the cementoenamel junction and it is regularly linked to the deterioration of dental aesthetics. Recession defects have been attributed to a number of causes, such as traumatic toothbrushing, intraoral body piercings, anatomical conditions, and destructive periodontal disease. Gingival recession with exposure of the root surface has a number of potential implications, including root sensitivity, root caries, complications of home care procedures, and poor aesthetic appearance [Nicolucci 2011].

Gingival recession is typically diagnosed visually and recorded in patient records in millimeters (mm) using a periodontal probe. When the mucogingival junction is involved, surgical intervention is warranted. The main objectives of periodontal surgical therapies are to improve the prognosis of the teeth and/or their replacements and to improve aesthetics.

Figure 3. Depiction of Gingival Recession and Exposed Roots [SaveYourSmile 2011]



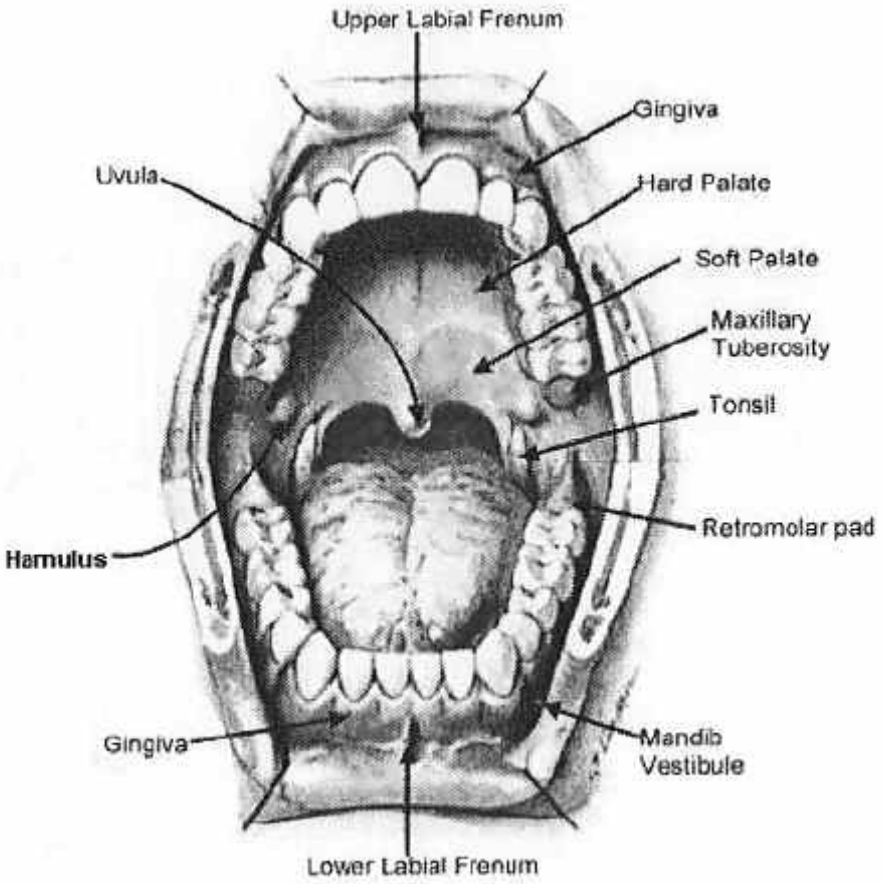
3.1.2 Currently Available Treatment(s)/Intervention(s) for Proposed Indication

Mucogingival surgical procedures are utilized to correct soft tissue defects associated with the mucogingival junction. Mucogingival surgical procedures may be used to treat:

1. Anatomic soft tissue defects such as:

    a. An insufficient zone of attached gingiva caused by an abnormally positioned tooth (naturally occurring or secondary to orthodontic treatment),
    b. Aberrant or high frenum placement, causing muscle pull against the gingival margin (See Figure 4), and
    c. Abnormally shallow vestibular (See Figure 4) anatomy defects.

Figure 4. Oral Anatomy [Spiller, 2011]



2. Mucogingival soft tissue defects causing chronic gingival inflammation, due to any of the following:
    a. Improper dentogingival contouring
    b. Improper tooth brushing technique
    c. Damage to oral tissues caused by intraoral piercings
    d. Drug abuse caused by direct intraoral placement of recreational drugs
    e. Periodontitis

Apligraf is proposed to be administered in the treatment of mucogingival problems such as the ones described above. Palatal soft tissue autografts, or free gingival grafts (FGG) or subepithelial connective tissue grafts (CTG) are considered the current standard of care for treatment of such mucogingival defects. Each of these standard procedures acts by increasing the zone of keratinized tissue around teeth. When root coverage is warranted, barrier membranes may also be used. In some cases, multiple and sometimes sequential procedures may be necessary [Nicolucci 2011; Oh 2009]. Other treatment modalities would include pedicle graft procedures and coronally positioned flap grafting procedures. Soft tissue autografts can be used in an initial step to increase the zone of attached gingiva in preparation for pedicle or coronally positioned graft procedures.

3.2 Regulatory History of Apligraf

This product is manufactured using the same process as the applicant's commercially available, FDA-approved medical device, Apligraf®. Apligraf® was approved by FDA for the treatment of Venous Leg Ulcers (VLU) on May 22, 1998 and for the treatment of Diabetic Foot Ulcers (DFU) on June 20, 2000. Apligraf was the subject of three IDE studies for oral indications prior to submission of BLA 125400 on May 13, 2011:

- G050122 – 05-PER-001: "A Pilot trial of Apligraf in establishing a functional zone of attached gingiva"
- G070012 – 06-PER-002: "A clinical trial to evaluate CelTx (Apligraf) as an alternative to tissue from the palate to enhance oral soft tissue regeneration and wound healing"
- G070178 – 07-PER-004-CTX: "A prospective, randomized, controlled pilot study of CelTx (Apligraf) as an alternative to tissue from the palate in the treatment of gingival recession requiring root coverage"

Assignment of the lead center for the review and regulation of a combination product is determined by its primary mode of action (PMOA), in accordance with section 503(g)(1) of the Federal Food, Drug, and Cosmetic Act and 21 CFR section 3.4. PMOA is defined at 21 CFR 3.2(m) as "the single mode of action of a combination product that provides the most important therapeutic action of the combination product. The most important therapeutic action is the mode of action expected to make the greatest contribution to the overall intended therapeutic effects of the combination product." In this case, the biological product component of the combination product was determined to provide the PMOA for the dental indication.

4. MANUFACTURING PROCESS AND PRODUCT TESTING

4.1 Manufacturing Process Overview

Apligraf is manufactured by combining viable allogeneic human fibroblasts and keratinocytes with a type I bovine collagen biomaterial. The product is manufactured using a 3-4 week process resulting in a bi-layered structure that resembles the dermal and epidermal structures found in skin. Apligraf, however, does not contain Langerhans cells, melanocytes, macrophages, lymphocytes, blood vessels or hair follicles.

The allogeneic fibroblasts and keratinocytes are derived from human neonatal foreskin tissue. The fibroblasts and keratinocytes are expanded in culture separately to generate master and working cell banks (MCB, WCB) that are cryopreserved, and cells from the WCB are thawed when needed for manufacture of a lot of the cell-scaffold construct.

Because expansion of the cell bank is limited, new banks from new donor tissue must be generated on a periodic basis.

The critical production steps involved in the manufacture of the final cell-scaffold construct are described below.

- ***Production of Dermal Equivalent (DE)***
  An acellular matrix layer of bovine collagen is -----b(4)----------. Fibroblasts are seeded onto this layer and grown for 6 days to create a dermal layer referred to as a dermal equivalent (DE).
- ***Production of Epidermal Layer (EPI)***
  Keratinocytes are seeded onto the DE and incubated for 2 days
- ***Differentiation***
  The construct is incubated further under differentiating conditions
- ***Cornification***
  The construct is placed on supports at this stage to lift the upper surface of the developing construct out of the cornification medium ("Air Lift"). This condition is maintained for 9 days in total, with media exchanges until a differentiated, cornified (i.e. mature) product results.

  The mature cell-scaffold product contains upper EPI and lower DE layers combined to form a bilayered unit and make up approximately –b(4)--- of the construct, respectively. This construct is stored under conditions to maintain product quality ("Maintenance") until it can be packaged and shipped to the clinical site within a 7-day window.

4.2 Quality Control Testing of Apligraf

Each lot of Apligraf is tested for sterility, purity, identity and potency through a combination of final product tests and in-process tests at key manufacturing steps. An overview of quality control testing is shown in Figure 5.

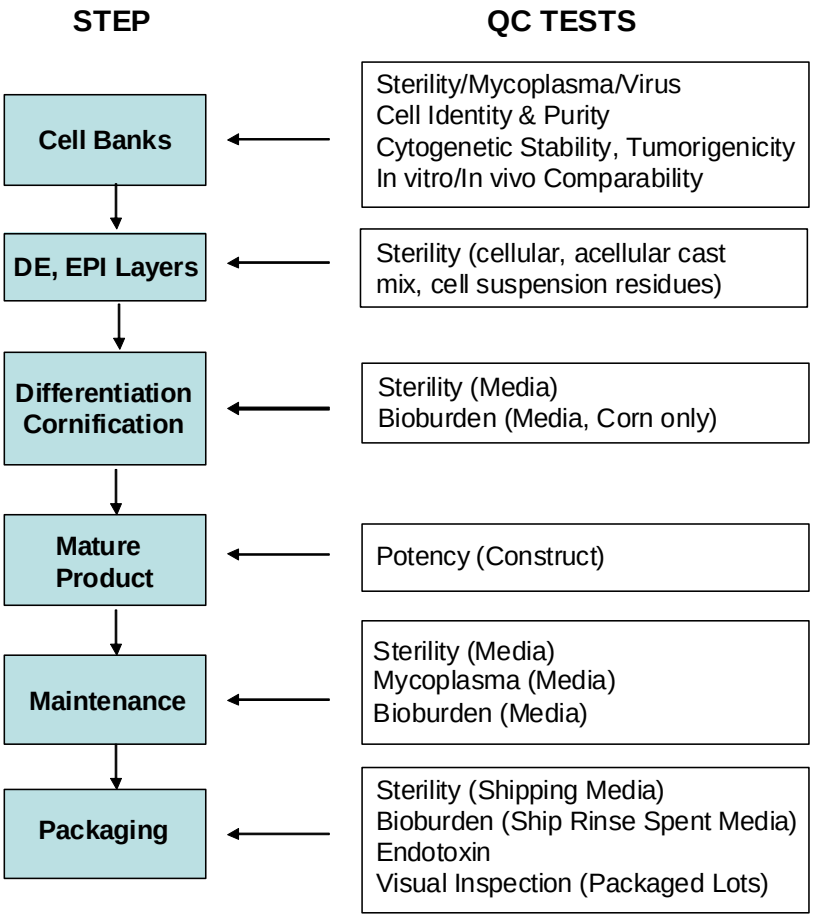


Figure 5. Overview of Quality Control Testing for Apligraf

4.3 Qualification and Release of Cell Banks for Allogeneic Keratinocytes and Fibroblasts

4.3.1 Cell Banking System

The allogeneic cells used for the production of Apligraf are derived from neonatal foreskin tissue. Donor testing and screening meet the requirements for Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/P) outlined in 21 CFR 1271.

Mechanical and enzymatic dissociation of the excised foreskin tissue releases keratinocytes and fibroblasts. The heterogeneous population of primary cells resulting from tissue dissociation is directed to produce a more homogeneous culture of keratinocytes and/or fibroblasts. This is achieved by means of cell selective and growth promoting media. For cell production, Organogenesis utilizes a two-tiered banking system consisting of master cell banks (MCBs) and working cell banks (WCBs) for both cell types. The keratinocytes and fibroblasts are sub-cultured from the dissociated tissue using defined culture conditions to create each respective MCB. The WCBs are derived via serial passage from one or more vials of a single MCB. The keratinocytes and fibroblasts are cryopreserved at the MCB and WCB stages. WCBs are further passaged to generate the keratinocyte and fibroblast seed pools which form the starting material for the constructs. There are defined, limited numbers of passages for each stage of the cell banking procedure.

Given the limited yield of primary cells from the dissociated foreskin tissue and the limited expansion potential of these diploid cells, there is an ongoing need for Organogenesis to generate and qualify new cell banks. Cell bank qualification data will be submitted to the FDA as supplements for approval before release of the cell banks. The applicant anticipates submitting qualification data for several banks annually to the FDA for approval.

4.3.2 Cell Bank Testing and Qualification

Qualification of cell banks includes several broad test categories including: adventitious agent testing, neoplastic safety testing, *in vitro* and *in vivo* comparability testing, identity testing, and cell performance characterization.

The number of cells obtained from the primary tissue is limited, resulting in a relatively small MCB. There are a low number of passages between the MCB and creation of the WCB. For these reasons, the testing scheme for qualification of the MCB and WCB is designed with overlap. To obtain sufficient cells for MCB testing, a portion of the MCB is passaged using the same conditions used to generate the WCB. The overall testing scheme includes tests that are performed on cells from this MCB passage, cells from the WCB, and on final cell-scaffold constructs made from MCB and WCB cells. This testing scheme is outlined below. Unless otherwise noted, the testing performed applies to both keratinocytes and fibroblasts.

<u>MCB Qualification Testing</u>

Testing performed on cells:

- *Adventitious agents*:
  - <u>Microbiological and viral safety testing</u>: Used to show the cells are free from contamination due to bacteria, fungi, mycoplasma, and viruses.
- *Neoplastic safety testing*:

- o Isoenzyme analysis: Used to positively identify the species of the cell line as human.
- o Karyology (cytogenetic analysis): Used to determine whether chromosomal aberrations have occurred over time during cell culture. The upper limits of acceptability for abnormalities are calculated using expected frequencies for diploid cell lines.
- o Senescence testing: Used to demonstrate that the diploid cells have a finite life span and will not indefinitely replicate. A negative growth index is used to determine when cells no longer have the ability to divide.
- o Tumorigenicity testing: Used to evaluate tumorigenic potential of the cells. Groups of nu/nu female mice are subcutaneously injected with one of the following: the cell bank to be tested, positive control –b(4)-- cells or serum-free medium. Mice are observed every day and followed for 3 months. At the end of three months the animals are euthanized and necropsied.

- *In vitro comparability*:
  - o Cell purity: Used to detect the level of cellular impurities. A flow cytometer is used to acquire and analyze the percent expression of cell surface markers present on control and test samples.

Testing performed on final cell-scaffold constructs made from MCB cells:

- *In vitro comparability*:
  - o Percutaneous absorption: Used to measure the barrier function of the final construct. Percutaneous absorption is determined as a percentage of applied tritiated water ---b(4)----------. Test units are compared with control units produced from qualified banks and must not exceed pre-determined absorbance rates.
  - o Cytokine profile assay: Used to determine presence of cytokines from keratinocytes and/or fibroblasts in the mature product. The RT-PCR assay includes three markers for keratinocytes and/or firboblasts (IL-1α, PDGFα, and TGFβ1), a positive control marker (-b(4)-), and a negative control marker (IL-4).
  - o Mitochondrial tetrazolium test (MTT): Used to evaluate metabolic activity and viability of cells in the final construct. This colorimetric assay is dependent on intact mitochondrial function. Test units are compared with control units produced from qualified banks and must meet pre-determined tetrazolium conversion rates.
  - o Histological analysis of final product: Used to evaluate if the cells of the MCB can produce a final construct which passes pre-determined specifications.
- *In vivo comparability in athymic mice*:
  - o *In vivo* comparability: Used to examine histological assessment, immunohistochemical staining for human involucrin, graft take and integration as well as % contraction of a graft made from the mature construct. The final assessment of *in vivo* comparability is determined by a trained pathologist based on comparability of test articles to control articles produced from qualified banks for each test parameter.

WCB Qualification Testing

Testing performed on cells:

- *Adventitious agents:*
  - Microbiological and viral safety testing

- *Identity and cell performance characterization*:
  - Cell growth: Used to evaluate if cells have a kinetic profile typical of normal doubling mammalian cells.
  - Cell viability: Used to determine if cells are alive. Cells must be –b(4)- viable post-thaw.
  - Isoenzyme analysis: Used to positively identify the species of the cell line as human.
  - Collagen biosynthesis: Used as an identity test for fibroblasts. A key function of fibroblasts is the ability to synthesize collagen. Spent culture media from test fibroblast and control lots are quantitated for collagen content using a –b(4)----------
  - Involucrin content: Used as an identity test for keratinocytes. Involucrin expression is a marker for keratinocytes and is detected in keratinocyte culture extracts by ELISA.

Testing performed on final cell-scaffold construct made from WCB cells:

- *Identity and cell performance characterization*:
  - VEGF quantification: Used as a measure of cell viability and bioactivity. The concentration of VEGF in spent maintenance media of the final product is determined using a -----b(4)----------
- *In vitro comparability*:
  - Histological analysis of final product: Used to evaluate if cells of WCB can produce a final construct which passes pre-determined specifications.

4.3.3 Stability of Cell Banks

Karotypic stability of the culture-expanded fibroblasts and keratinocytes was identified as a potential safety concern. Organogenesis has an extensive cell banking history to date which allows for the comparison of numerous cell strains. The testing results for cytogenetic analysis of all MCBs generated since 2000 were submitted to the BLA and the data showed a consistently low frequency of chromosomal aberrations.

4.3.4 Summary

New cell banks are introduced on a periodic basis for Apligraf manufacture. The quality and comparability of new cell banks is determined through conformance with pre-determined acceptance criteria at the donor, MCB, WCB, and final product stages.

The committee will be asked to discuss the sponsor's approach to qualify and demonstrate comparability of new cell banks.

4.4 Product Potency

4.4.1 Regulatory Requirements for Potency of Licensed Biological Products

Potency is defined as "the specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, to effect a given result." (21 CFR 600.3(s)). CBER/OCTGT issued a guidance document with recommendations on potency assay development for cellular and gene therapy products [CBER Potency Guidance 2011]. The applicant proposes to measure potency of the product using histological parameters. Histology is not a classical measure of biological activity and so the justification for this assay is described here in greater detail. The histology assay and specifications are identical to the test used for the approved medical device Apligraf®.

4.4.2 Overview of Proposed Potency Assay

The applicant proposes to measure product potency using a set of histological parameters which collectively assess the quality of the epidermal and dermal layers present in the final product. The parameters assessed are epidermal coverage, epidermal development, basal aspect, suprabasal aspect, dermal thickness, fibroblast density, and matrix aspect.

Description of Histology Assay on the Final Construct

The proposed histological potency measurement utilizes Hematoxylin and Eosin (H&E) staining to distinguish fibroblasts and collagen within the dermal matrix (lower layer), as well as keratinocytes within the epidermal layer (upper layer). The stains distinguish the basophilic and eosinophilic cellular structures within the tissue layers. Based on the experience with that product, the applicant describes the relevance of the morphological structures observed through the histological parameters of the potency assay to the function of the Apligraf® product as follows:

- The lower dermal layer is believed to provide both a structural matrix for the fibroblasts and a substrate for the development and maintenance of the upper layer.

- The upper epidermal layer is believed to impart important structural elements to the construct, which contributes in turn to its biomechanical strength, handling properties, and barrier properties.

Samples of the product for H&E staining are obtained from three biopsy punches taken from Apligraf units from each lot submitted for testing. The punches are fixed in formalin prior to slicing into 3 mm strips and further processing, embedding, sectioning and

staining according to established standard operating procedures. The slides are assessed via light microscopy for the histological parameters of the potency assay which are described below. Figure 6 shows a representative image.

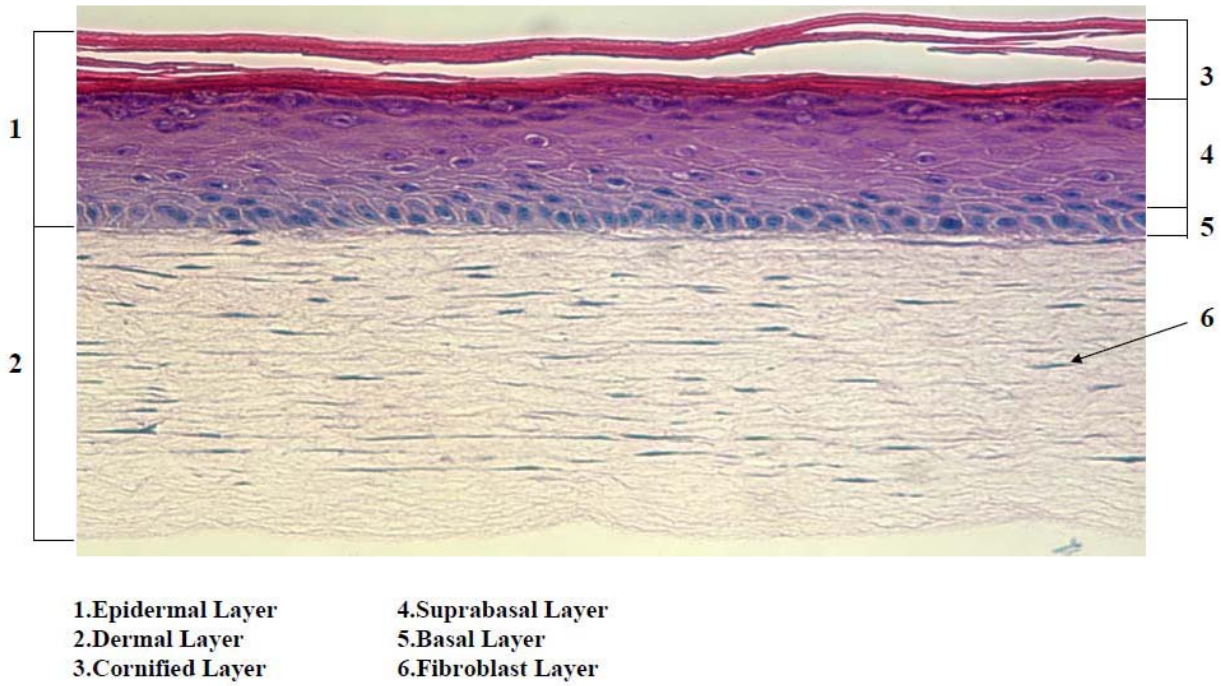


Figure 6. Representative histology image used to assess product potency (Image from BLA 125400 submission)

- *Epidermal Coverage:* Percentage of the surface of the dermal matrix that is covered by epidermis.

- *Epidermal Development:* Percentage of acceptable epidermal development which combines several aspects of functional development; a basal layer of keratinocytes of cuboidal-columnar shape, 5 or more stratified suprabasal layers and ≥1 cornified layer(s).

- *Basal Cell Layer Keratinocyte Viability:* Percentage of the epidermis containing basal keratinocytes with a basophilic cytoplasm, lacking severe vacuolization or necrosis. Vacuolization and necrosis are used by the applicant as metrics to determine basal keratinocytes viability.

- *Suprabasal Cell Layer Keratinocyte Viability:* Percentage of keratinocytes containing basophilic cytoplasm without vacuolization, necrosis or pyknosis (non-viable).

- *Dermal Thickness:* Mean thickness of the dermal matrix in 5 randomly selected fields across the length of the specimen.

- *Fibroblast Density:* Fibroblast density in 5 randomly selected fields of the dermal matrix. Pyknotic nuclei (non-viable) are not included in the count.

- *Matrix Aspect:* Percentage of dermal matrix collagen present on the slide which stains uniformly without large holes or inclusions is determined.

Table 1. Histology-Based Potency Testing of Apligraf Construct

LAYER	PARAMETER	SPECIFICATION
Epidermal	Epidermal Coverage	> 95%
	Epidermal Development	≥ 70%
	Basal Cell Layer Keratinocyte Viability	≥ 95%
	Suprabasal Cell Layer Keratinocyte Viability	≥ 80%
Dermal	Dermal Thickness	≥ 40 μm
	Fibroblast Density	≥ 4 fibroblast nuclei per field
	Matrix Aspect	≥ 95% uniform stain

Rationale for Establishment of Specifications for Histology Assay
The proof of concept and performance for the original Apligraf product was first demonstrated in the early nu/nu mouse studies (circa 1980). Due to the limited product shelf-life, the applicant conducted bridging studies in early 1990s to develop an in vitro assay to be used for lot release. These studies were used to correlate the performance of Apligraf in the mouse model to the histological parameters of the final construct prior to grafting. The applicant implemented the histological assay for PMA approval in 1998 and has not changed these specifications in the current BLA application.

4.4.3 Overview of Characterization Methods and Additional Studies on Biologic Activity

The applicant supports the histological potency assay used for product release by citing a set of supporting characterization methods and studies that have been utilized since the original development of the product to assess Apligraf's various biological attributes. These are listed in Table 2 and described in this section.

Table 2. Additional characterization methods and studies supporting the proposed potency assay

TEST	FUNCTION	RELATION TO POTENCY ASSAY
***In Vitro* Assays**		
Percutaneous water absorption (Final construct)	Measures extent of barrier function; assesses level of epidermal differentiation	Correlated to histology parameters: Epidermal coverage, development
Cytokine profile [expression of PDGF-α, TGF-β1, IL-4 and IL-1α] (Final construct)	Assesses presence of key wound healing cytokines and absence of inflammatory mediator	Supports product identity/potency
Cytokine production (VEGF detection in maintenance medium of Final construct)	Indicates appropriate epidermal development, which is indicative of the correct differentiation pattern of keratinocytes	Supports product identity/potency Correlates to histology parameters. Epidermal development.
MTT assay (Final construct)	Measures overall cell viability and function by assessing total mitochondrial activity	Correlates to histology parameters: Cell viability in the upper layer (basal and supra basal keratinocyte cell viability)
***In Vivo* Athymic Mouse Graft Model**		
Pre-graft morphology	Morphological analysis of Apligraf integration into athymic mouse skin	Showed comparability to control in: • Integrity, organization and viability of epidermal layer • Fibroblast morphology and viability • Fibroblast density • Lattice morphology
Graft take and integration	Morphological analysis of Apligraf integration into athymic mouse skin	Showed graft take and integration comparison between test and control articles
Graft contraction	Morphological analysis of Apligraf integration into athymic mouse skin	Showed graft contraction comparison between test and control articles

Graft morphology • Epidermal layer • Dermal layer • Graft integration and remodeling	Morphological analysis of Apligraf integration into athymic mouse skin	Showed comparability to control in: • Integrity • Organization • Viability of epidermal layer Showed comparability to control in: • Fibroblast viability • Fibroblast morphology • Fibroblast density Showed comparability to control in: • Edge integration • Center integration • Epidermal coverage • Damage due to dressing slippage • Vascularization • Degree of vascularization • Cellularity
Immunohistochemistry (IHC)	Anti-human involucrin staining	Showed expression of human involucrin confirms the epidermal cells are human

Functional testing methods used to support histological potency assay:

- *Percutaneous Water Absorption* – as described in cell bank section.

- *Mitochondrial Tetrazolium Testing (MTT) Assay* – as described in cell bank section.

- *VEGF concentration* – has been performed on the final construct as a part of working cell bank qualification to date and therefore has not been applied for Apligraf lot release. It has also been used by the applicant to monitor cell function during manufacturing processes as a part of development studies. The applicant believes that VEGF concentration is indicative of the relevant biological factors produced by the keratinocytes in the final construct.

Figure 7 shows data regarding VEGF concentration in the media during product manufacture from development studies conducted by the applicant (N=25). It is noted that cornification stages I, II and III, at which VEGF concentration is maximum and then subsequently decreases, are the stages at which the cell-scaffold construct develops into the final product. The applicant suggests that this assay may be of sufficient sensitivity for detecting manufacturing process deviations and potential effects on product potency.

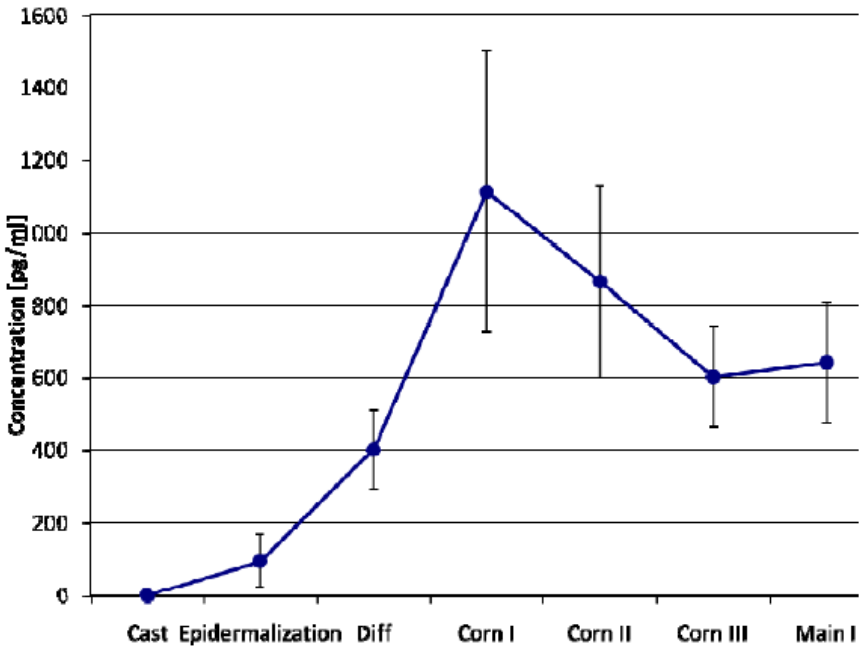


Figure 7. VEGF concentration of media at each stage of Apligraf manufacture (N=25). Corn I, II and III refer to culture steps at which cornification occur. Main I refers to the maintenance step prior to packaging and shipping. Data were provided in the BLA submission.

- *In vivo nu/nu Mouse Assay* - The competence of the barrier function *in vivo* had also been investigated in a nu/nu mouse model during the initial development of the related product, Apligraf. Parenteau et al. [Parenteau 1996] grafted Apligraf at various product maturation stages onto athymic mice and showed that 1) product persistence was dependent upon the length of the maturation stages during product manufacture and 2) there may be a minimum requirement for development of the barrier function and its persistence. These results were consistent with the results of the percutaneous water absorption studies used to assess Apligraf's barrier function. It is noted however that there may be some key mechanistic differences between the mouse model and human clinical use for Apligraf in that the product has been observed to engraft and heal by primary intention in the mouse model while it does not engraft in human clinical use. Please refer to sections 7.2 - 7.4 of this briefing document regarding studies of Apligraf function in clinical use.

4.4.4 Summary

For Apligraf, product potency is determined by a set of histological parameters which collectively assess the quality of the epidermal and dermal layers present in the product after maturation. These parameters include epidermal coverage, epidermal development, basal cell layer, suprabasal cell layer, dermal matrix thickness, fibroblast density, and matrix aspect. These parameters were shown to correlate with percutaneous water absorption, cell metabolic activity by MTT, VEGF secretion and an in vivo mouse assay.

It is noted that supporting information for the biologic activity of the keratinocytes and fibroblasts is provided by testing at the cell bank level.

FDA requests the scientific input from the advisory committee regarding the current understanding of how the product functions and the use of histology to address product potency.

5. EFFICACY ANALYSIS OF CLINICAL STUDIES

5.1 Efficacy Results for Pilot Study 05-PER-001 (Non-submerged)

Title
"A pilot Clinical Trial to Assess the Safety and Efficacy of Apligraf in Establishing A Functional Zone of Attached Gingiva"

Objectives
The objectives were to assess the safety and efficacy of Apligraf in establishing a functional zone of attached gingiva in patients with an insufficient zone of attached gingiva.

5.1.1 Study Design

This was a prospective, randomized, single-center, within-subject controlled (matched for teeth and gingival condition), pilot study of 25 subjects.

The first three subjects participated as training subjects to help determine surgical and material handling techniques and were not included in the analysis of efficacy. Subsequent subjects were enrolled when the first three had completed four weeks of follow-up. Treatment site and order of treatment were randomized. Following randomization, subjects received both a palatal graft and Apligraf. The primary efficacy evaluation occurred at Month 6, with interim visits at Week 1, Month 1, and Month 3.

The **primary efficacy endpoint** was the change in the amount of attached gingiva at Month 6 compared between treatments. The trial was designed to demonstrate non-inferiority between Apligraf treatment and free gingival graft (FGG) control in the amount of change in attached gingiva over six months.

The **secondary endpoints** were:
1. Inflammation
2. Color and texture match of the graft to the adjacent tissue
3. Resistance to oral muscle pull
4. Probing depth
5. Clinical attachment level

6. Subject preference or satisfaction (including pain experience)
7. Change in recession depth
8. Width of keratinized tissue

The trial protocol did not provide a detailed statistical analysis plan for secondary endpoints and no adjustments were made for multiplicity.

Enrollment criteria
Subjects were adult males and females with an insufficient zone of attached gingiva associated with at least two non-adjacent teeth; root coverage was not desired. Selected enrollment criteria are summarized below:

Inclusion Criteria
1. Age 18 – 70 years
2. At least two non-adjacent teeth with an insufficient zone of attached gingiva, which required soft tissue grafting. The two selected teeth needed to be located in contralateral quadrants. (In case of adjacent teeth requiring grafting, only one tooth at each site acted as test or control tooth, but both teeth received the same treatment.)
3. Root coverage was not desired or indicated at the time of grafting.

Exclusion Criteria
1. Teeth that had an insufficient zone of attached gingiva that would be best treated using soft tissue grafts to cover the denuded root surface
2. Any systemic conditions (i.e., diabetes mellitus, cancer, HIV, bone metabolic diseases) that could compromise wound healing and preclude periodontal surgery
3. Currently receiving, or had received within one week prior to study entry, systemic corticosteroids (including inhaled), immunosuppressive agents, topical antibiotics, topical cytotoxic agents, radiation therapy, and/or chemotherapy, which could compromise wound healing and preclude periodontal surgery
4. Presence of acute infectious lesions in the areas intended for surgery
5. Subjects who smoke
6. Teeth requiring treatment were molars
7. Teeth with axial mobility
8. Known hypersensitivity to bovine collagen
9. Subjects enrolled in medical, dental, or any investigational device study for any disease within the past four weeks before study assessment
10. Subjects who had received an investigational drug or biological treatment within the three months prior to study enrollment
11. Subjects previously treated with Apligraf, Dermagraft, or any other skin graft at the target site(s)
12. Subjects who, in the opinion of the investigator, for any reason other than those listed above, would not be able to complete the study per protocol

Treatment Procedure for Apligraf
Preparation of the oral mucosal defects for Apligraf placement in surgically created recipient sites involves current standard soft tissue preparation techniques. The recipient site is anesthetized using standard local anesthetic procedures. This is followed by placement of Apligraf that has been properly folded and adapted to fit the recipient site. Apligraf is then sutured into place. Additional sutures may be placed to tack down the alveolar mucosal flap.

In the pilot (and pivotal) studies with Apligraf for the oral indication, investigators were instructed to remove the mucosa and any underlying connective tissue from the facial aspect of the mucogingival deformity, as well as to remove any muscle fibers with scissors to create a clean periosteal bed prior to placing either Apligraf or the FGG control. Thus, an "oral mucosal defect" (i.e., surgical wound bed) was created prior to the non-submerged placement of Apligraf.

Apligraf was prepared in an "s-fold" to form a 3-layer construct (epidermal side out) and trimmed to the size needed according to the number of teeth treated and the subject's anatomy. Apligraf was applied to the base of the mucosal defect within 15 minutes of removing it from the storage container. The prepared Apligraf was placed in direct contact with the appropriate randomized study mucosal defect bed, centered on the study tooth (any remaining length could have been used to treat adjacent teeth), and sutured in place at the papilla with resorbable sutures. If possible, Apligraf was secured apically at the discretion of the surgeon. If technically feasible, a criss-cross (i.e., suspensory) suture was placed over Apligraf to enhance stability and help maintain direct contact with the base of the mucosal defect. The Apligraf application procedures are illustrated in Figures 8 and 9 below.

Figure 8. Deformity (prior to creation of the oral mucosal defect) (*left*) and prepared oral mucosal defect (*right)* (illustrations from Applicant's BLA)

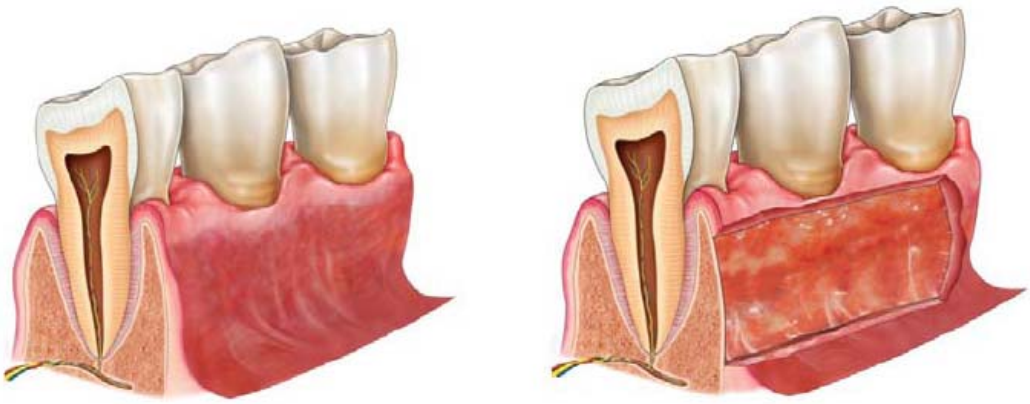
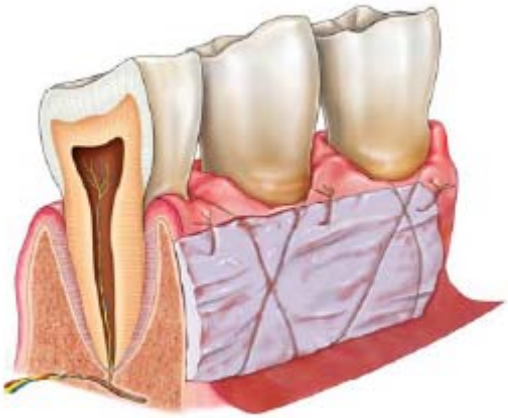


Figure 9. Placement of s-folded Apligraf over the oral mucosal defect (illustration from Applicant's BLA)



An additional layer of Apligraf that extended laterally beyond the wound margins was placed over the entire preparation. The additional layer was placed in close apposition to the base of the mucosal defect and sutured at the four corners, when possible. The lip or cheek adjacent to the mucosal defect was placed under tension to make certain that Apligraf was free of movement during muscle traction. Coe-Pak surgical dressing was placed over the treatment site. Coe-Pak was left in place until it fell off on its own or was removed by the investigator by the two-week follow-up visit. The investigators were permitted to re-apply a new piece of Apligraf if the original piece was dislodged within the first 48 hours after surgery.

One to three teeth could be treated. In case of adjacent teeth requiring grafting, only one tooth at each site acted as treatment or control tooth, but all teeth received the same treatment. Root coverage at the identified study teeth was not performed at the time of grafting.

Procedure for Palatal Graft /Control Site
The palatal site (also called harvest site or donor site in the submission) was harvested and grafted according to standard practices. The width of all palate grafts was 5 mm with the length dictated by the size of the mucosal defect. Preparation of the oral mucosal defects for the control site (also called control recipient site in the submission) followed current standard of care.

Concomitant Therapy
Medications listed in the exclusion criteria were not permitted. Only those agents specified in the protocol were to be used in the oral cavity. These were to be recorded in the case report forms at each visit. All patients were instructed to rinse with 0.12% chlorhexidine for 1 minute twice daily for the first four weeks to maintain plaque control in the surgically treated areas. Topical antibiotics were only allowed if a clinical infection was diagnosed after treatment.

Study Schedule/Monitoring
Following screening and randomization, subjects received both a palate graft and Apligraf, with primary endpoint evaluation at Month 6. A schedule of study procedures and monitoring time points is provided in Table 3.

Table 3. Study and Monitoring Schedule for Pilot Study 05-PER-001

	Baseline Screen	**Treatment**			**Follow-up**	**Final Study Visit**
Study Procedures:		Day 0 Surgery ± 2 days	Week 1 (Day 7) ± 2 days	Month 1 (Day 28) ± 2 days	Month 3 ± 7 days	Month 6 ± 14 days
Dental and Medical History	X					
Review Hygiene Procedures	X		X	X	X	X
Surgery		X†				
Plaque Score	X				X	X
Bleeding	X				X	X
Probing Depth		X†				X
Cementoenamel Junction	X	X†				X
Mucogingival Junction	X	X†				X
Recession Depth	X		X	X	X	X
Clinical Attachment	X					X
Resistance of Muscle Pull	X					X
Inflammation Score	X	X†	X	X	X	X
Keratinized Tissue	X	X†			X	X
Radiograph	X					X
Photographs	X	X†	X	X	X	X
Persistence						X
3 mm Biopsies						X
Subject Discomfort Questionnaire			X			
Subject Aesthetics Questionnaire						X
Medication	X	X	X	X	X	X
Adverse Events	X	X	X	X	X	X

† The Baseline and Surgery (Day 0) visit could be combined into a single visit.

Additional Studies
At Month 6, biopsy specimens were obtained from seven subjects' Apligraf and control graft sites for histologic evaluation to examine the cellular composition and tissue architecture of the surgical tissues. DNA persistence studies were also performed on biopsy specimens from two of these seven biopsies.

Outcome Assessment Methodology
The primary and secondary efficacy outcome variables are listed above.

The primary efficacy variable, the absolute change in the amount of attached gingiva at six months, was analyzed using a non-inferiority comparison of the mean within-subject difference in gingival attachment between Apligraf and control. The non-inferiority margin was a 1 mm difference in change in gingival attachment.

The amount of attached gingiva was evaluated using a calibrated periodontal probe. Measurements were made to the nearest 0.5 mm.

Secondary efficacy outcomes included inflammation score, tissue color, tissue texture, resistance to oral muscle pull, and subject satisfaction. Secondary endpoints were evaluated using superiority comparisons. No adjustments were made for multiplicity. The assessment methods for these endpoints are briefly described below. The time points for these assessments are summarized in Table 3 above.

- Inflammation was scored by an examiner on a scale of 0 (absence of inflammation) to 4. Data on inflammation were analyzed by comparing the changes from baseline in the Apligraf and control sites.

- Tissue color was compared to the surrounding tissue and rated by the examiner as either “More Red,” “Less Red,” or “Equally Red.”

- Tissue texture was compared to the surrounding tissue and rated by the examiner as either “Less Firm,” “Equally Firm,” or “More Firm.”

- Resistance to muscle pull was based on whether the free gingival margin of the tissue facial to the site moves when the adjacent cheek is retracted.

- Subject satisfaction was based on responses to two questionnaires:

    - The “Subject Aesthetics Questionnaire,” where subjects mark their responses on a line with “Disappointed” labeled at one end and “Fully Satisfied” at the other end.

    - The “Subject Discomfort Questionnaire” queried subjects for perceptions of the severity of pain, bleeding, swelling, and sensitivity for the Apligraf site, control site, and palate site. These were rated as None, Mild, Moderate, or Severe. The duration of these signs and symptoms was recorded.

- Recession depth was answered either “No” or “Yes”, with probing depth recorded in mm.

All evaluations except for the subject satisfaction questionnaire were performed by the investigator.

Safety Evaluations
Pre-treatment, Day 0, and post-treatment clinical assessments of subjects were performed to rule out infection and local or systemic reactions. Additional safety assessments consisted of monitoring and recording all adverse events, including serious adverse events (SAEs). The investigator queried the subjects on possible adverse events at each visit. (Safety results are provided separately in Section 6.2.1)

5.1.2 Study Results

Patient Disposition
Twenty-five subjects were enrolled in the study. All 25 subjects completed all visits.

Demographics
Baseline characteristics of the 25 subjects are provided in Table 4.

Table 4. Pilot Study 05-PER-001: Demographics of Study Populations

Demographics	Efficacy Analysis (N=22)* Statistics Mean (n%)	Safety Analysis (N=25)* Statistics Mean (n%)
Age – mean (range)	50.0 years (31-69)	49.1 years (31-69)
Gender		
Male	7 (31.8%)	8 (32.0%)
Female	15 (68.2%)	17 (68.0%)
Race/Ethnicity		
Caucasian, non-Hispanic	19 (86.4%)	22 (88.0%)
Hispanic	1 (4.5%)	1 (4.0%)
Asian/Pacific Islander	1 (4.5%)	1 (4.0%)
Middle Eastern	1 (4.5%)	1 (4.0%)

* Data from the three training subjects were not used for efficacy analysis; data from all 25 subjects were used for safety analysis. Fifty percent of the subjects were former smokers; no current smokers were enrolled in the study.

Efficacy

Primary efficacy endpoint
At six months, Apligraf sites in 14/22 (63.6%) subjects showed an increase in attached gingiva, while 21/22 (95%) of control sites showed an increase in attached gingiva. The average increase in the amount of attached gingiva at Month 6 was 0.85 mm (0.48, 1.21) for Apligraf and 2.43 mm (2.06, 2.79) for control. Thus Apligraf failed to demonstrate non-inferiority to control for the primary efficacy endpoint. The results are summarized in Table 5.

Table 5. Primary Endpoint Results for Study 05-PER-001 (N=22)

	Baseline Mean (95% CI)	**6 Months** Mean (95% CI)	**Change** Mean (95% CI)
Attached Gingiva			
Apligraf	0.30 (0.13, 0.46)	1.14 (0.77, 1.50)	0.85 (0.48, 1.21)
Control	0.27 (0.11, 0.44)	2.71 (2.34, 3.07)	2.43 (2.06, 2.79)

Secondary Endpoints
Width of keratinized tissue was a secondary endpoint. At 6 months, at least 2 mm of keratinized tissue width was established in 18/22 (81.8%) of Apligraf sites and in 22/22 (100%) of control sites. At 6 months, the mean increase in KT width was 1.37 mm (0.97, 1.77) at Apligraf sites and 3.33 mm (2.93, 3.74) at control sites. Thus, there was a larger change from baseline to 6 months in the width of keratinized tissue in the control sites compared to the Apligraf sites.

When assessing periodontal health around test and control teeth, there were no differences between Apligraf and control in the change from baseline to 6 months in probing depth, recession, and clinical attachment. There was also no important difference in resistance to muscle pull, inflammation, or bleeding on probing within the two groups. Table 6 shows the results for Apligraf and control sites for the secondary endpoints of probing depth, recession, clinical attachment, and keratinized tissue width. The applicant presents data for changes in the first three parameters as baseline minus 6 months.

Table 6. Change in Secondary Endpoint Clinical Variables from Baseline to 6 Months in Study 05-PER-001 (N=22)

	Baseline Mean (95% CI)	**6 Months** Mean (95% CI)	**Change (Baseline to 6 months)** Mean (95% CI)
Probing Depth			
Apligraf	1.37 (1.18, 1.56)	1.41 (1.24, 1.58)	-0.04 (-0.26, 0.18)
Control	1.36 (1.17, 1.55)	1.68 (1.51, 1.85)	-0.32 (-0.54, -0.11)
Recession			
Apligraf	2.42 (2.17, 2.66)	2.16 (1.89, 2.44)	0.25 (0.09, 0.42)
Control	2.36 (2.11, 2.60)	2.02 (1.74, 2.30)	0.34 (0.18, 0.50)
Clinical Attachment			
Apligraf	3.79 (3.49, 4.08)	3.58 (3.32, 3.83)	0.21 (-0.05, 0.47)
Control	3.71 (3.42, 4.01)	3.70 (3.45, 3.95)	0.01 (-0.25, 0.28)
Keratinized Tissue Width*			
Apligraf	1.13 (0.92, 1.33)	2.50 (2.18, 2.82)	1.37 (0.97, 1.77)
Control	1.24 (1.03, 1.44)	4.57 (4.25, 4.89)	3.33 (2.93, 3.74)

* The sponsor expressed changes in KT width by subtracting the baseline from the 6-month values. Changes for the three other parameters are expressed as baseline minus 6 months.

The Applicant found Apligraf to be superior to control for the following secondary endpoints: tissue color (more equally red compared to surrounding tissue), texture matching (more equally firm compared to surrounding tissue), and patient preference.

The perception of pain at Week 1 was one of the variables in the patient preference assessment, evaluated by the "Subject Discomfort Questionnaire." Using the four-point scale described above, there was no evidence of a decrease in reported pain between the Apligraf and control recipient or donor sites. At Week 1, more subjects reported severe pain at the Apligraf graft site than at either the control graft or donor site.

Results for pain at Week 1 in Study 05-PER-001 are shown in Table 7.

Table 7. Evaluation of Pain at Apligraf/Control Graft/Palate Sites at Week 1, Study 05-PER-001 (N=22)

	Pain at Specified Site			
Grading:	**Apligraf**	**Control (C)**	**Palate (P)**	**C/P***
None	3 (13.6%)	2 (9.1%)	8 (36.4%)	1 (4.5%)
Mild	5 (22.7%)	9 (40.9%)	9 (40.9%)	8 (36.4%)
Moderate	9 (40.9%)	10 (45.5%)	4 (18.2%)	11 (50.0%)
Severe	5 (22.7%)	1 (4.5%)	1 (4.5%)	2 (9.1%)

* C/P stands for Control (Graft Site)/Palate and represents the more severe of the pain scores reported by the patient for the palate and control graft sites.

Due to the lack of adjustment for multiple testing across secondary endpoints, statistical testing of these results is not included here.

In summary, Apligraf failed to demonstrate success in the non-inferiority primary endpoint of establishing a zone of attached gingiva comparable to that of a free autogenous palatal graft. There was some indication of success in three (tissue color matching, texture matching, and patient preference) of the eight secondary endpoints. The increase in the amount of KT noted in this pilot trial helped in the design of the Pivotal Study 06-PER-002-CTX.

5.2 Efficacy Analysis of Pivotal Study 06-PER-002-CTX (Non-submerged)

Title
"A Clinical Trial to Evaluate CelTx (Apligraf) as an Alternative to Tissue from the Palate to Enhance Oral Soft Tissue Regeneration and Wound Healing"

Objectives
The primary objective was to assess the ability of Apligraf to achieve a "clinically acceptable threshold for keratinized tissue (KT) at 6 months (> 2 mm KT)."

Five of the six secondary objectives (numbers 1, 2, 4-6 below) were to determine if Apligraf was superior to FGG for the following six effectiveness endpoints. The other secondary objective (number 3 below) was to measure superiority of Apligraf compared to a pre-specified 80% success standard of achieving KT ≥ 1mm. The secondary objectives, in order of statistical testing, were:

1. Color same as adjacent tissues after 6 months
2. Texture same as adjacent tissues after 6 months
3. KT ≥1 mm for Apligraf after 6 months (superiority vs. an 80% success standard)
4. Patient preference after 6 months
5. Surgical site sensitivity mild or absent after 1 week
6. Pain absent after 3 days

5.2.1 Study Design

Design
This was a prospective, randomized, within-subject controlled (matched for teeth and gingival condition), treatment comparison, pivotal, multicenter (four sites in US) trial of 96 subjects with insufficient zone (≤ 1 mm) of attached gingiva requiring soft tissue grafting.

The first two subjects treated per investigator participated as training subjects for surgical and material handling techniques and were excluded from the efficacy analysis. Treatment site and order of treatment were randomized. Study teeth in contralateral quadrants were selected by the investigator at the baseline visit; however, randomization of study teeth to receive either Apligraf or control treatment did not occur until immediately prior to surgery at Day 0. A single application of Apligraf was applied to an oral mucosal defect on one study tooth at Day 0. The control treatment consisted of a free gingival graft (FGG) harvested from the subject's palate and applied to an oral mucosal defect on a second, contralateral study tooth on Study Day 0. Due to the nature of the control treatment, neither the investigator nor the subject could be blinded to study tooth treatment assignment or order of surgical procedure. In addition, according to the

Applicant, given the distinct appearance of the FGG, it was not possible to ensure blinding of a possible third-party evaluator.

The primary efficacy evaluation occurred at Month 6, with interim visits at 48 and 72 hours, 1 and 2 weeks, Month 1, and Month 3. There were no follow-up visits or contacts for safety or efficacy beyond 6 months.

Enrollment Criteria
Adult males and females with an insufficient zone of attached gingiva associated with at least two non-adjacent teeth, and root coverage was not desired. Selected enrollment criteria are summarized below.

Inclusion Criteria
1. Age 18 - 70 years
2. Subject has at least two non-adjacent teeth in contralateral quadrants of the same jaw with an insufficient zone (≤1 mm) of attached gingiva that requires soft tissue grafting. One to three teeth may be treated. In case of adjacent teeth requiring grafting, only one tooth at each site will act as test or control tooth, but all teeth will receive the same treatment.
3. Root coverage is not desired at the time of grafting.

Exclusion Criteria
1. Class III recession in the presence of a shallow vestibule, or class IV recession
2. Vestibule depth of less than 7 mm from base of recession
3. Any systemic conditions that could compromise wound healing and preclude periodontal surgery (i.e., diabetes mellitus, cancer, HIV, bone metabolic diseases)
4. Currently receiving, or has received within two months prior to study entry, systemic corticosteroids, immunosuppressive agents, radiation therapy, and/or chemotherapy which could compromise wound healing and preclude periodontal surgery
5. Presence of acute infectious lesions in the areas intended for surgery
6. Subject who has used any tobacco product within 3 months
7. Subject who is taking intramuscular or intravenous bisphosphonates
8. Only molar teeth suitable for soft tissue grafting
9. Teeth that have Miller Grade 2 or higher mobility
10. Known hypersensitivity to bovine collagen and/or iodine (shellfish allergy)
11. Subject who has received an investigational drug or biological/bioactive treatment within 30 days prior to study enrollment
12. Subject who was previously treated with Apligraf, Dermagraft or any other skin graft at the target site(s) or immediately adjacent teeth
13. Subject, who in the opinion of the Investigator, for any reason other than those listed above, will not be able to complete the study per protocol

Treatment Procedure
The surgical treatment procedure performed in this study was essentially the same as that used in pilot Study 05-PER-001. In this study, the Apligraf product was “z-folded”, rather than “s-folded”, for treatment application. The minimum size was 5 mm wide by 10 mm

long and was sized to fit the defect. (See Section 6.1.1 for further detail on treatment procedure).

The palatal graft harvest followed standard procedures. The width of all grafts was 4mm, whereas graft widths were 5mm in Study 05-PER-001.

In addition, an adjunct biomarker laboratory study was conducted. The objective of this study was to compare the expression of angiogenic biomarkers involved in the wound healing process of the Apligraf- vs. FGG-treated sites. (See Section 7.4 for further details).

Study Schedule/Monitoring
Following screening and randomization, subjects received both a palatal graft and Apligraf.

The first follow-up visit occurred one week post-surgery. Any adverse events or changes in medications were documented. The subject's surgical procedure preference and determination of sensitivity were recorded and oral hygiene instructions reviewed. Additional follow-up evaluations occurred at 4 weeks, 3 months, and 6 months post-surgery. Changes in medications and adverse events were documented at each visit. Photographs of the test sites and clinical measurements were obtained and texture and color of the test areas were evaluated. At 4 weeks, pain and sensitivity were also assessed. An oral exam was performed at 4 weeks and 6 months. At 3 and 6 months post-surgery a dental cleaning was performed. At 6 months post-surgery, a Subject Preference Questionnaire was completed and radiographs of the study teeth obtained.

The study events schedule is shown in Table 8.

Table 8. Study Schedule for Pivotal Study 06-PER-002-CTX

	Screen	**Baseline**	**Treatment**	**Follow-up**						**Final Visit**
Study Procedure:		(16 days post screen)	**Day 0/Surgery** (14 days post baseline)	**48 & 72 hr.**	**1 Week**	**2 Weeks**		**4 Week**	**3 Month**	**6 Month**
						Visit	Phone Call			
Medical and Dental History	X									
Oral Hygiene Procedures	X	X	X		X	X		X	X	
Surgery			X*							
Telephone Well-Being Check				X			X			
Plaque Score		X							X	X
Bleeding on Probing		X							X	X
Probing Pocket Depth		X								X

Proximal Probing Depth		X								X
Recession Depth		X			X	X		X	X	X
Clinical Attachment		X								
Muscle Pull Resistance		X								
Inflammation Score		X			X	X		X	X	X
Keratinized Tissue Width		X							X	X
Radiographs		X								X
Photographs			X		X	X		X	X	X
Texture and Color Match								X	X	X
Sensitivity, Bleeding, Swelling					X	X		X		
Dental Cleaning		X							X	X
Subject Surgical Preference					X					
Subject Preference Questionnaire										X
Medication	X	X	X	X	X	X	X	X	X	X
Adverse Events		X	X	X	X	X	X	X	X	X

* Re-treatment with Apligraf could occur if original treatment was lost within 48 hrs. of surgery.

Concomitant Medications and Treatments

Medications listed in the exclusion criteria were not permitted. Only those agents specified in the protocol were to be used in the oral cavity. These were to be recorded in the case report forms at each visit. All patients were instructed to rinse with 0.12% chlorhexidine for 1 minute twice daily the first four weeks to maintain plaque control in the surgically treated areas. Topical antibiotics were only allowed if a clinical infection was diagnosed after treatment.

Methodology for Outcomes Evaluation

Subjects were given a paper diary to be completed daily through Day 14. Subjects were asked if the surgical dressing stayed on at each of the surgical sites and the palatal donation site. Subjects recorded pain associated with each surgical site and the palatal graft donation site by assessing the pain for each as none, mild, moderate and severe. Additionally, this diary was used to record any medications the subject had taken for mouth-related pain. In addition, study personnel placed telephone calls to subjects at 48 hours post-surgery, 72 hours post-surgery, and at 2-weeks post-surgery to perform a well-being check.

All clinical measurements were performed by investigators, who were specifically trained to make the specific assessments (training protocol 06-PER-003). Measurements included probing depth, recession depth, and identification of the mucogingival junction (as used to obtain keratinized and attached gingiva measures).

Keratinized tissue was measured by the roll technique with Schiller's Iodine Stain. Measurements were taken with a UNC-15 probe and rounded to the nearest 0.5 mm. Within each clinical site, attempts were made to have the same examiner complete the clinical measurements for all subjects and all visits.

Assessments of color and texture of treated (Apligraf or control) oral mucosal defects to adjacent, non-treated mucosa were performed at Months 1, 3, and 6 and were rated as either less (red or firm), the same, or more (red or firm) than adjacent mucosa.

At six months, Subject Preference was assessed by asking subjects to respond to the question "Taking into account all aspects of treatment (surgery, recovery, and appearance) which treatment is preferred?" Subject Surgical Preference was assessed at one week.

Sensitivity was assessed by elicitation with a three-second puff of air. The subject was asked to report the amount of sensitivity at each site (none, mild, moderate, or severe).

Criteria for Evaluation
The primary and six secondary efficacy outcome variables are listed above. The primary efficacy variable, the per cent of Apligraf sites with ≥ 2 mm KT at six months, was analyzed using a superiority comparison to the pre-defined standard (50% success).

Five of the six secondary endpoint evaluations were superiority comparisons between Apligraf and control, where the order of testing was pre-specified and conducted sequentially as described above. The other endpoint was a superiority comparison of Apligraf (oral) to pre-specified benchmark.

Safety Evaluations
Safety was assessed by monitoring and recording treatment-specific and systemic adverse events. (Safety results are provided separately in Section 6.2.2.)

5.2.2 Study Results

Subject Disposition
Of the 119 subjects screened, 23 did not meet the inclusion/exclusion criteria. Major reasons for screen failure were lack of teeth in contralateral quadrants with insufficient gingiva, previous treatment with graft at target or adjacent teeth, use of a prohibited medication, use of tobacco within 3 months, and subject unwillingness to follow study procedures and instructions.

Eleven of the 96 subjects enrolled were considered training subjects and were not included in the primary efficacy analyses; their results were analyzed separately and also pooled with pivotal subjects in supportive analyses. The remaining 85 subjects were considered 'pivotal' subjects for the primary efficacy analyses.
Of the 96 subjects enrolled, all completed the study.

Subjects were enrolled at four US study sites with a total of six investigators. However, one site treated only three subjects, all of whom participated as training subjects. Therefore, all of the 85 pivotal subjects were treated at three sites by four surgeons/investigators. Pivotal subject sample sizes were approximately evenly distributed across these three sites (30, 27, and 28).

Dropouts and/or Discontinuation
All 96 subjects completed the 6-month study and all required study visits (Weeks 1 and 4, Months 3 and 6).

Subject Demographics
Demographic characteristics of the efficacy population (n=85) and overall subjects (n=96) are shown in Table 9.

Table 9. Demographics for Non-Submerged Pivotal Study 06-PER-002-CTX

Parameter	Statistics	Efficacy	Training	All
Gender				
Female	n (%)	46 (54.1%)	6 (54.5%)	52 (54.2%)
Male	n (%)	39 (45.9%)	5 (45.5%)	44 (45.8%)
Age (years)	n	85	11	96
Min – Max		18.0 - 70.8	21.2 - 70.3	18.0 - 70.8
Mean (SD)		46.9 (12.7)	49.4 (16.67)	47.1 (13.13)
Median		48.3	53.3	48.8
Race				
White	n (%)	77 (90.6%)	10 (90.9%)	87 (90.6%)
Black/African American	n (%)	1 (1.2%)	0	1 (1.0%)
Native Hawaiian/Other Pacific Islander	n (%)	0	0	0
Asian	n (%)	4 (4.7%)	1 (9.1%)	5 (5.2%)
American Indian/Alaska Native	n (%)	0	0	0
Other	n (%)	3 (3.5%)	0	3 (3.1%)
Site				
Site 10	n (%)	30 (35.3%)	4 (36.4%)	34 (35.4%)
Site 15	n (%)	0	3 (27.3%)	3 (3.1%)
Site 16	n (%)	27 (31.8%)	2 (18.2%)	29 (30.2%)
Site 17	n (%)	28 (32.9%)	2 (18.2%)	30 (31.3%)
Previous Tobacco Use				
Yes	n (%)	34 (40.0%)	4 (36.4%)	38 (39.6%)
No	n (%)	51 (60.0%)	7 (63.6%)	58 (60.4%)

Efficacy Outcomes[1]

Primary
Eighty-one of the 85 subjects (95.3%) met success criteria of ≥ 2mm KT at the Apligraf site (exact binomial 95% CI 88.4%, 98.7%). All 11 subjects in the training cohort also met the primary endpoint at the Apligraf-treated site, and all 96 subjects met the primary endpoint at the control site.

Secondary Efficacy Outcomes
With respect to the six secondary efficacy endpoints, Apligraf was statistically significantly superior to control treatment for the first four: color matching, texture matching, and patient preference, and had a KT ≥ 1 mm success rate that was significantly greater than the 80% success standard. There was no significant difference between Apligraf and control in sensitivity (the fifth secondary endpoint). Because of the pre-planned fixed sequence testing of endpoints, no statistical significance testing could be performed to assess differences in pain between Apligraf and control sites, because Apligraf failed to show superiority for the fifth endpoint (sensitivity).

Results for the secondary endpoints are summarized in Table 10.

Table 10. Secondary Endpoint Efficacy Results for Pivotal Study 06-PER-002-CTX

Endpoints:	**Results**		
Color (N=85)	(Month 6)		
		Apligraf	
		Equally Red	Not Equally Red
	Control		
	Equally Red	23	0
	Not Equally Red	56	6
Texture (N=85)	(Month 6)		
		Apligraf	
		Equally Firm	Not Equally Firm
	Control		
	Equally Firm	46	0
	Not Equally Firm	35	4
KT ≥ 1mm (N=85)	(Month 6)		
		Apligraf	**Control**
		85 (100%)	85 (100%)
	(Month 6)		
		Apligraf	**Control**

[1] The original data analysis plan called for the primary efficacy analysis to be performed on a modified ITT population, consisting of all randomized subjects who received Apligraf and control, with support from the per-protocol analysis. However, patient disposition was such that the three populations (ITT, mITT, and PP) were identical.

Subject Preference (N=85)	**Control**		
	Overall	61 (71.8%)	24 (28.2%)
	Appearance only	65 (76.5%)	20 (23.5%)
Surgical Site Sensitivity (N=71)	(Week 1)		
		Apligraf	
		Not Sensitive	Sensitive
	Control		
	Not Sensitive	67	3
	Sensitive	1	0
Pain (N=84)	(Day 3)		
		Apligraf	
		No Pain	Pain
	Control		
	No Pain	54	7
	Pain	5	18

Representative photographs of graft sites at baseline and six months, to illustrate the anatomy and the nature of the cosmetic issues, are provided in the **Appendix.**

Other Effectiveness Endpoints
At FDA's request, the applicant also provided summaries and post-hoc statistical analyses of other effectiveness endpoints: Recession Depth, Recession (%), Probing Pocket Depth (including mesial and distal), Clinical Attachment Level (CAL), Keratinized Tissue Width (including mesial and distal), Attached Gingiva, Bleeding on Angulated Probing, Muscle Pull Resistance, Plaque scores (buccal and lingual), Inflammation Score, Bleeding, and Swelling.

The applicant assessed changes from baseline to Month 6 in each of the endpoints listed above. For the Apligraf-treated site, there were improvements from baseline to Month 6 in KT width, attached gingiva width and recession depth. No other endpoints yielded clinically important changes from baseline to Month 6. For the control site, there were improvements from baseline to Month 6 in KT width and attached gingival width. There was no clinically important improvement in recession depth at the control site.

The sponsor also compared the Apligraf and control sites on each of these endpoints at Month 6. The control site had greater KT width and attached gingival width at Month 6 than the Apligraf site. The mean (SD) KT width at Month 6 was 3.21 (1.14) mm for the Apligraf site compared to 4.57 (1.00) mm for the control site. The mean (SD) attached gingival width at Month 6 was 1.77 (1.32) mm for the Apligraf site compared to 3.17 (1.17) mm for the control site. There were no other differences between sites on 'other effectiveness endpoints' at Month 6.

Subpopulation Analyses
There was no examination of subgroups in this study.

In summary, the study met its primary endpoint, KT ≥ 2 mm at six months. Eighty-one of the 85 subjects (95.3%) met success criteria at the Apligraf site. The study also demonstrated superiority of Apligraf to FGG in four of the six secondary endpoints: color matching, texture matching, KT ≥ 1mm, and Patient Preference.

6. SAFETY ANALYSIS OF CLINICAL STUDIES WITH APLIGRAF

Safety data for Apligraf are derived from the three clinical studies of Apligraf plus pre- and post-marketing studies of Apligraf for the treatment of chronic cutaneous wounds.

6.1 Safety Database

The safety database for Apligraf is derived from the three clinical studies of Apligraf for the oral indication, plus pre-marketing studies and post-marketing data of Apligraf for the treatment of chronic cutaneous wounds.

6.2 Safety Analysis of Studies

The following is an analysis of safety data from the clinical studies described above, plus an additional small pilot safety study (07-PER-004-CTX, N=15) of the use of Apligraf for treatment of gingival recession requiring root coverage. Each study is described separately. An analysis of post-marketing safety information from use of Apligraf in the treatment of chronic cutaneous wounds follows this section.

It should be noted that the pivotal and two pilot studies were within-subject controlled trials, which may limit the ability to judge relatedness of systemic adverse events to the product.

6.2.1 Pilot Study 05-PER-001 (Non-submerged)

Study Design and Schedule
These are described in detail in Section 5.1.1.

Method of Assessment
The safety of Apligraf was assessed by recording adverse events, and by clinical assessment of the subject for local and systemic signs of infection and immune reaction.

In addition, at Month 6, biopsy specimens were obtained from seven subjects' control and Apligraf graft sites to examine the cellular composition and tissue architecture of the tissues. The objective of this additional histological study was to see if there were differences in the type, distribution, and arrangement of collagen fibers; changes in the extracellular matrix protein composition; and differences in vascularity, between the palatal graft and Apligraf-treated sites, and between the tissues present at 6 months and baseline.

DNA persistence studies were also performed on biopsy specimens from two subjects.

At each visit, subjects were evaluated for evidence of Apligraf rejection, infection, and bleeding, and were queried regarding occurrence of adverse events. Oral examinations were conducted at screen/baseline and at Month 1. Adverse events were assessed at all visits, i.e., at screen/baseline, Day 0/Surgery, Week 1, Month 1, Month 2, and Month 6.

Definitions of adverse events, including serious adverse events, were in accordance with standard terminology and FDA regulations. Adverse events were rated as mild, moderate, or severe, and were recorded on the case report form (CRF).

Other Clinical and Laboratory Testing
Vital signs and physical findings (i.e., physical exams) were not required per protocol and were not collected. However, oral exams (i.e., face, lymph nodes, lips, buccal mucosa, floor of the mouth, tongue, hard and soft palates, gingival and edentulous ridges and teeth) were performed. There were no laboratory tests as part of the protocol.

Study Agent Exposure
Each subject received a single application of Apligraf, which was applied over a surgically prepared vascular wound bed. The graft bed was prepared utilizing a partial thickness dissection to remove the keratinized gingival mucosa and nonkeratinized alveolar mucosa, creating a vascular gingival and alveolar mucosal surface defect. The amount of Apligraf used in one application (or one dose) depended on the size of the wound.

Overview of Adverse Events
Twenty-two adverse events were reported in 17/25 (68%) subjects. Of these events, 16/22 (73%) were rated as mild. Twenty-seven percent (6/22) were rated moderate. No severe events were reported. Six events were related to the Mouth and Throat. No event was determined by the investigator to be related to treatment. The investigators reported no signs of local or systemic reaction to Apligraf. The treatment-emergent adverse events are shown in Table 11.

Table 11. Treatment-Emergent Adverse Events for Study 05-PER-001

System	Event	No. of Subjects	No. of Events	Severity	Treatment Related (Investigator Assessment)
Head	Migraine headache	1	1	Mild	No
Nose and Sinuses	Sinusitis	3	4	Mild	No
	Allergic Rhinitis	3	3	Mild	No
Mouth and Throat	Toothache in maxillary arch	1	1	Mild	No
	Excessive bleeding from Site A	1	1	Mild	No
	Paresthesia in lower left lip since graft surgery	1	1	Mild	No
	Fractured tooth #18	1	1	Mild	No
	Soreness in maxillary left molars requiring bite guard adjustment	1	1	Mild	No
	TMJ discomfort	1	1	Mild	No
Skin/Breast	Mastitis left breast	1	1	Moderate	No
Gastrointestinal	"Upset stomach" secondary to medications	1	1	Mild	No
Metabolic	Hypercholesterolemia	1	1	Mild	No
Musculoskeletal	Injury left shoulder	1	1	Moderate	No
	Wrist tendonitis unresponsive to Celebrex	1	1	Moderate	No
	Wrist discomfort requiring tendon release surgery	1	1	Moderate	No
Respiratory	Community acquired pneumonia	1	1	Moderate	No
Reproductive	Spontaneous post-menopausal vaginal bleeding	1	1	Moderate	No

Histology Study and DNA Persistence Studies – (See Sections 7.2 and 7.3 for details.) The histology study showed no evidence of abnormal cells or abnormal tissue architecture at six months. In addition, the polymerase chain reaction (PCR) studies at 6 months showed no sign of Apligraf DNA persistence.

Summary of Safety Results for Pilot Study 05-PER-001
Twenty-two Adverse Events were reported among 17 subjects. All were judged unrelated to treatment. None were severe. There were no reports of infection at the wound site. In addition, there were no reports of clinical immune response to Apligraf (sensitization or rejection) at any time point.

There were no serious adverse events, deaths, or dropouts due to adverse events.

6.2.2 Pivotal Study 06-PER-002-CTX (Non-submerged)

Study Design (See Section 5.2.1 for further description)
Study 06-PER-002-CTX was a prospective, randomized, within-subject controlled trial, conducted at four US sites.

Method of Assessment
Safety was assessed by spontaneous adverse event reporting and by clinical assessment by protocol-mandated scheduled monitoring of treatment-related and systemic adverse events. Subject follow-up visits occurred at 48 and 72 hours post-surgery, Weeks 1 and 2, and Months 1, 3, and 6.

Oral examinations were conducted at visits for screening and at scheduled post-surgical Month 1 and Month 6 visits. The oral examination included evaluation of the face, lymph nodes, lips, buccal mucosa, floor of the mouth, tongue, hard and soft palates, gingiva, and edentulous ridges.

Complete details regarding scheduled assessments are provided in the description of the trial in the efficacy section (Section 5.2.1).

Study Agent Exposure
Treatment consisted of a single application of z-folded Apligraf to the oral mucosal defect on Study Day 0. Apligraf was applied over a surgically created vascular wound bed, prepared utilizing a partial-thickness dissection to remove the keratinized gingival mucosa and nonkeratinized alveolar mucosa. Apligraf was applied to an oral mucosal defect on one study tooth and a free gingival graft (FGG) was harvested from the subject's palate and applied to an oral mucosal defect on a second, contralateral study tooth. The amount of Apligraf used in one application, or one dose, depended on the size of the wound.

Subject Disposition
Details regarding patient disposition are provided in Section 6.2.2. Ninety-six subjects were enrolled in the trial. All were treated with Apligraf and FGG. All subjects completed the six-month study, including all interim assessments. There were no dropouts due to adverse events.

Overview of Adverse Events
Overall, 24/96 (25%) subjects experienced at least one adverse event (AE) during the study. A total of 43 events were reported in the study. Three subjects reported an SAE during the study.

Table 12 shows the reported adverse events by MedDRA System Organ Class (SOC).

Table 12. Non-Submerged Pivotal Study 06-PER-002-CTX: Incidence of Treatment-Emergent Adverse Events by MedDRA System Organ Class (Safety Population, N=96)

System Organ Class	Subject n (%)	Event n
Overall	24 (25.0%)	43
Infections and Infestations	8 (8.3%)	10
Gastrointestinal Disorders	8 (8.3%)	9
Skin and Subcutaneous Tissue Disorders	5 (5.2%)	5
Injury, Poisoning and Procedural Complications	4 (4.2%)	4
Musculoskeletal and Connective Tissue disorders	2 (2.1%)	3
Neoplasms Benign, Malignant and Unspecified (including Cysts and Polyps)	2 (2.1%)	2
Respiratory, Thoracic and Mediastinal Disorders	2 (2.1%)	2
Vascular Disorders	2 (2.1%)	2
Investigations	1 (1.0%)	2
Blood and Lymphatic System Disorders	1 (1.0%)	1
Cardiac Disorders	1 (1.0%)	1
General Disorders and Administration Site Conditions	1 (1.0%)	1
Immune System Disorders	1 (1.0%)	1

Serious Adverse Events
There were three SAEs reported during the study. A tabular summary of the three SAEs is provided in Table 13. The serious adverse events of pneumonia and chest pain were assessed as unrelated by investigators. The third serious adverse event, metastatic malignant fibrous histiocytioma, was judged as unlikely related. A narrative summary of this SAE follows the table.

Table 13. SAEs Reported in Study 06-PER-002-CTX

Subject ID	AE (Preferred Term)	SAE Start Date (relative to Day 0)	Relationship to Tx (by Investigator)
010-06/MLW	Pneumonia	Day 100	Not Related
010-44/I-D	Chest Pain	Day 7	Not Related
016-36/RWM	Metastatic Malignant Fibrous Histiocytoma	Day 154	Unlikely

Subject 016-36, a 61 year old Caucasian male, had Apligraf applied on June 26, 2008. Pertinent past medical history included a posterior mediastinal mass (May 9, 2003). On November 27, 2008 (Study Day 154), he was hospitalized for back and abdominal pain, underwent a series of

diagnostic tests, and was diagnosed with metastatic malignant fibrous histiocytoma, with extension of the large mass to other organs. The patient received medication and radiation therapy and was discharged home in stable condition on December 5, 2008 (Study Day 162). The event was ongoing at the time of study completion. This subject completed the final Month 6 Study Visit on December 8, 2008. The Principal Investigator assessed this event as “severe” and unlikely to be related to the product.

Incidence of Adverse Events by Location
Given that Apligraf was applied locally, adverse events were also summarized by event location (Apligraf, Control, Palatal Harvest, Mouth [intra-oral but not at treatment or harvest sites], and Other). Data were prospectively collected on the Adverse Event CRF.

Three subjects reported an adverse event at the Apligraf-treated site. Two subjects (---b(6)----------) reported gingival pain or injury subsequent to inadvertent placement of the Apligraf transwell membrane at the time of Apligraf application. These adverse events were rated as "mild" and related to treatment. The third subject (-b(6)-) reported ulceration, which was rated as "mild" and not related to treatment.

Three subjects reported an adverse event at a control procedure site, either the control-treated site or palatal harvest site. One subject (-b(6)-) reported gingivitis at the control treated site; the gingivitis was rated as "severe" and not related. At the palatal harvest site, there was one report of post-procedural hemorrhage (-b(6)-) and one report of local thrombosis (-b(6)-). The post-procedural hemorrhage was rated as "mild" and the thrombosis was "moderate"; both palatal harvest events were assessed as not related to treatment by the investigator.

Deaths
There were no deaths reported during the study.

Adverse Events of Special Interest
In addition to the adverse events described above, a single case of follicular thyroid neoplasm was reported during the trial:

Subject 016-06 a 66 year old Caucasian female, had Apligraf and control FGG applied on January 14, 2008. Pertinent medical and surgical history included hypothyroidism (1997, treatment and status unknown), arthritis (start date unknown), heart murmur (September 26, 1941), hypercholesterolemia (June 1, 2007), hypertension (1997), osteopenia (October 1997), pre-cancerous lesion removed from face (July 2006), and spinal surgery (November 3, 2003). On April 15, 2008 (Study Day 92) this subject underwent same-day surgery of the left thyroid for removal of a Hurthle Cell Lesion. The subject was discharged home and the event resolved on the same day as surgery (April 15, 2008). This subject completed the final Month 6 Study Visit on July 10, 2008. The Principal Investigator assessed the severity of this event as “moderate” and not related to the product. There were no further details regarding histology, workup for metastases, or subsequent treatment of the thyroid neoplasm.

Summary of Safety Results for Study 06-PER-002-CTX
A total of 119 patients were screened and 96 patients were enrolled and treated in the study. All 96 subjects were treated with Apligraf and the control free gingival graft (FGG). No subjects withdrew from the study: all subjects completed the required study visits (Weeks 1 and 4, Month 3, and Month 6). There were three SAEs and no deaths.

Overall, 25% of the subjects experienced an adverse event during the study, with a total of 43 adverse events reported.

Fifteen AEs reported by six subjects were assessed by the investigator to be related (unlikely, possible, probably, definite) to study treatment. Of these 15, two AEs reported by two subjects occurred at the Apligraf site (gingival injury and gingival pain) and were assessed as possibly or probably related, respectively.

Three SAEs were reported during the study and all three events were assessed by the investigator to be either not related (pneumonia and chest pain) or of unlikely relationship (metastatic malignant fibrous histiocytoma). An additional non-oral cavity malignancy was reported during the study, a follicular thyroid neoplasm, which the investigator assessed as not related.

Seven adverse events occurred at the three treatment locations (the Apligraf-treated site, control-treated site, and the palatal harvest site):

- Three subjects experienced AEs occurring at the Apligraf-treated site. Two subjects, both in the training cohort, had inadvertent placement of the Apligraf transwell membrane to the oral mucosal defect at the time of Apligraf placement which resulted in AEs of gingival injury and gingival pain. For both of these subjects the membrane was able to be removed without sequelae and ≥ 2 mm KT was regenerated at the treatment site. The Applicant addressed this safety issue by training the investigators, and these types of adverse events were not seen beyond the training cohort. The third subject experienced an AE of mouth ulceration.
- Two subjects experienced AEs occurring at the control-treated site (gingivitis and skin exfoliation).
- Two subjects experienced AEs occurring at the palatal harvest site (postprocedural hemorrhage and thrombosis). Palatal harvest morbidity is an expected risk with harvest of a FGG.

There were no immune-related adverse events.

6.2.3 Safety Results from Study 07-PER-004-CTX

Title
"A Prospective, Randomized, Controlled Pilot Study of CelTx™ (Apligraf®) as an Alternative to Tissue from the Palate in the Treatment of Gingival Recession Requiring Root Coverage"

Objectives
The primary study objective was to assess the safety of Apligraf as a material for the treatment of Miller Class I or II recession defects in which root coverage was required. The secondary objective was to collect preliminary effectiveness data to aid in the design of a subsequent pivotal study.

Design
This was a prospective, single-center, randomized, within-subject controlled treatment comparison six-month pilot study. Subjects were given both Apligraf and a palatal graft on Day 0. The originally-proposed study size was up to 26 subjects. The first two subjects treated by each surgeon (estimated at two surgeons for a total of four subjects) were to participate as training subjects and were to be included in the statistical analysis for safety only.

Main Criteria for Enrollment
Adult males and females aged 18 to 70 years, with at least two non-adjacent teeth in contralateral quadrants of the same jaw with Miller Class I or II buccal recession (≥3 mm) that required tissue grafting. Other than requirement for root coverage, the remaining criteria were similar to those of the two previous trials.

Treatment Procedure
A single submerged application of Apligraf or palatal graft control was applied to the oral mucosal defect at each of the two randomized sites on study Day 0.

Study schedule/monitoring
The monitoring schedule was essentially the same as in the two previous trials. Subjects were treated on Day 0 and attended scheduled visits at 48 hours, one week, two weeks, three weeks, one month, three months, and six months.

Evaluation of Outcomes
The primary outcome was safety of Apligraf in the treatment of gingival recession requiring root coverage. In addition, the secondary outcome was to obtain preliminary evidence of efficacy to be used to design a subsequent pivotal trial. Analysis of safety included recording of all adverse events throughout the study. In addition, the protocol specified assessments of safety through dental examinations including radiographs.

Results

Subject Disposition

Of the 19 subjects screened, 15 were treated. Fourteen completed the assessment schedule through Month 6. One subject dropped out (reasons not provided by Applicant) and was lost to follow-up.

There were 11 (73.3%) females, 12 (80%) Caucasians, and the mean age was 42.5 (SD 11.0) years.

Study Agent Exposure

All 15 subjects in this study received one treatment (one dose) of Apligraf at Day 0, placed under a surgical flap (submerged). The amount of Apligraf used in one application (or one dose) depended on the size of the wound.

Safety Assessment Methodology

Adverse events were recorded at each scheduled visit through Month 6.

The technique of Apligraf application was modified twice in the study. This resulted in three distinct groups of subjects. As a result of the study changes and the small number of subjects in each treatment group, the Applicant determined that the data could not provide information regarding efficacy.

Safety Results

As noted above, the Applicant determined that the analytical populations were too small to yield informative efficacy data. Accordingly, the Applicant terminated the study after 15 subjects were enrolled and treated.

Overview of Adverse Events

A total of 20 adverse events were reported for nine subjects in this study. Table 14 summarizes AEs by site: (Apligraf), Control, Palatal Graft Site, Mouth, and Other.

Two subjects experienced two AEs at the Apligraf-treated site; two subjects experienced two AEs at the control-treated site; and four subjects experienced seven AEs in areas of the mouth other than the Apligraf and control-treated sites. Five subjects experienced nine AEs in locations other than the mouth.

Table 14. 07-PER-004-CTX Submerged Study: Treatment-Emergent Adverse Events by MedDRA System Organ Class and Preferred Term and by Location (N=number of subjects; n=number of events)

SYSTEM ORGAN CLASS **PREFERRED TERM**	**Location of Adverse Event**			
	Apligraf	**Control**	**Mouth**	**Other**
	N (%) [n]	N (%) [n]	N (%) [n]	N (%) [n]
Gastrointestinal Disorders	-	-	**3 (20%) [4]**	**3 (20%) [4]**
Dental Caries	-	-	1 (7%) [1]	-
Irritable Bowel Syndrome	-	-	-	1 (7%) [1]
Mouth Ulceration	-	-	1 (7%) [1]	-
Nausea	-	-	-	2 (13%) [2]
Esophagitis	-	-	-	1 (7%) [1]
Oral Mucosa Erosion	-	-	1 (7%) [1]	-
Toothache	-	-	1 (7%) [1]	-
General Disorders and Administration Site Conditions	**1 (7%) [1]**	-	-	-
Impaired Healing	1 (7%) [1]	-	-	-
Infections and Infestations	-	-	**2 (13%) [3]**	**2 (13%) [3]**
Conjunctivitis	-	-	-	1 (7%) [2]
Pneumonia	-	-	-	1 (7%) [1]
Tooth Abscess	-	-	2 (13%) [3]	-
Injury, Poisoning and Procedural Complications	**1 (7%) [1]**	**2 (13%) [2]**	-	-
Gingival Injury	-	1 (7%) [1]	-	-
Suture Related Complication	1 (7%) [1]	1 (7%) [1]	-	-
Neoplasm Benign, Malignant and Unspecified (Incl Cysts and Polyps)	-	-	-	**1 (7%) [1]**
Pyogenic Granuloma	-	-	-	1 (7%) [1]
Nervous System Disorders	-	-	-	**1 (7%) [1]**
Headache	-	-	-	1 (7%) [1]

Relatedness: One AE was judged by the investigator to be related to treatment. Subject -b(6)------ had impaired healing of the right target tooth that was considered to be mild and probably related to treatment with Apligraf. The subject underwent treatment with Orabase and an additional gingival suture and the AE resolved.

Severity: All AEs were judged by the Investigator to be mild in severity, with the exception of esophagitis, which was judged to be of moderate severity. None of the AEs were considered to be severe.

There were no SAEs, deaths or other significant adverse events during this study. None of the 14 subjects dropped out due to an AE. The reason for discontinuation of one subject was not provided.

6.3 Safety Experience for Apligraf for Cutaneous Wounds

6.3.1 Premarket Experience

As previously stated, the Apligraf product is approved for the treatment of venous leg and diabetic foot ulcers in the US. The safety results from the pivotal studies that supported the approval of Apligraf for these two indications are summarized below.

Venous Leg Ulcers (VLU): Protocol 92-VSU-001
This was a randomized, controlled, multicenter, unblinded clinical trial. The study population included adult subjects with non-infected partial or full thickness ulcers of greater than one month duration, which had not adequately responded to conventional ulcer therapy.

The venous ulcers AEs were recorded as mild, moderate, severe, or life-threatening. Of the 297 subjects (161 Apligraf, 136 active control) enrolled, there were one life-threatening and three severe infections reported in the Apligraf group and none in the control arm. Two of the three severe infections were considered related to treatment; however, one occurred one month after the last application of Apligraf, and the other occurred following application on a pre-existing Pseudomonas infection.

The discontinuation rate for all subjects was 76/291 (26%) prior to the 6-month evaluation, and 105/291 (36%) at 12 months. Within the safety cohort, 59 Apligraf group (36.6%) and 50 (36.8%) control group subjects discontinued prior to the 12-month visit. Five (3.1%) Apligraf group subjects and 8 (5.9%) control group subjects withdrew from Study 92-VSU-001 due to adverse events or intercurrent illness.

Diabetic Foot Ulcer (DFU): Protocol 95-DUS-001
This was a randomized, controlled, multicenter trial to evaluate the safety and efficacy of Apligraf for the treatment of full-thickness neuropathic diabetic foot ulcers in adults. Major eligibility criteria included ulcers of greater than three weeks duration which had not adequately responded to conventional ulcer therapy and which extended through the dermis but without tendon, muscle, capsule or bone exposure. Subjects were required to be diagnosed with type I or type II diabetes, with a HbA1C between 6% and 12%. Subjects on renal dialysis or receiving immunosuppressive medications or therapies were excluded.

Two hundred and eight subjects were treated in the study; 112 received Apligraf and 96 received control therapy. Subjects received 12 weeks of treatment and three additional months of follow-up. Twenty-two subjects per group were discontinued prior to the Month 6 visit.

A total of 208 subjects (112 Apligraf, 96 control) were evaluated for safety. Adverse events were recorded as mild, moderate, severe, or life threatening. There were no life-threatening events. Ten severe infections occurred in the Apligraf group. There were

two reports of life-threatening AEs in the control arm. The severe infections in the Apligraf subjects included one wound infection, seven cases of cellulitis, and two cases of osteomyelitis. In the control arm, the severe infections were four wound infections, four cases of cellulitis, and ten cases of osteomyelitis. The life-threatening events (death and dyspnea) experienced by two control subjects were not related to control treatment. When all infections, regardless of severity were considered (wound infection, cellulitis, and osteomyelitis), six (5.4%) Apligraf and five (5.2%) control events were assessed as related to the ulcer treatment.

The discontinuation rate for all subjects prior to the 6-month evaluation was 44/208 (21%), including 22 (19.6%) Apligraf and 22 (22.9%) control group subjects. Fifteen subjects withdrew due to adverse events or intercurrent illness, six (5.4%) Apligraf and nine (9.4%) controls.

There were no differences in adverse events reported in this study except for the incidence of diarrhea and peripheral edema that occurred less frequently in the Apligraf group. Additional safety parameters (amputations or resections on the study limb, hospitalization, sepsis, life-threatening adverse events, and death) were comparable in the two groups.

There was no clinical evidence of Apligraf rejection in any subjects. In this study, immune response testing was also conducted. (See Section 7.5 for further detail). In tests of subjects' sera, there were no observations of antibody responses against bovine type I collagen, bovine serum protein or Class I HLA antigens on human dermal fibroblasts and human epidermal cells. T-cell specific responses were not observed against bovine type I collagen, human fibroblasts or human keratinocytes.

6.3.2 Postmarket Adverse Events Reported to FDA

Since approval in 1998, over 400,000 units of Apligraf have been shipped for patient treatment. There were a total of 839 complaints; 197 of these complaints were evaluated as potential adverse events. The most common complaints and adverse events included pre-treatment layer separation; high pH; and others, such as visual anomaly and not classified.

Since approval, nine medical device reports (MDRs)[2] have appeared in the FDA MAUDE database. All MDRs have been reviewed at FDA. A summary of the nine MDRs is shown in Table 15.

[2] Complaints include any comments/complaints that come to the sponsor. MDRs are submitted only when events are AEs meeting FDA's requirement for sending an MDR.

Table 15. Summary of Post-Marketing Adverse Events Reported to FDA (1998 to June 30, 2011)

Event Date	Event Type and Patient Outcome	Adverse Event	Relationship to Tx (as assessed by the Applicant)
27 Sept 1999	Injury, Requiring Intervention	Erosion Skin Inflammation Eschar	Related
13 Dec 2001	Injury; Other	Bacterial Infection	Related
27 July 2004	Injury, Requiring Intervention	Allergic Reaction	Related
26 May 2006	Injury; Hospitalization	Suspected Wound Infection	Related
22 May 2008	Injury, Requiring Intervention	Growth observed near periwound area. Biopsy; Squamous Cell Carcinoma	Unlikely
16 Sept 2010	Injury	Allergic Reaction; Acute Respiratory Distress Syndrome	Not Related
13 Jan 2011	Injury; Requiring Hospitalization	Pain, Chills, Increased Fibromyalgia	Related
23 Mar 2011	Injury; Requiring Intervention	Dramatic Blistering	Related
23 Mar 2011	Injury; Requiring Intervention	Dramatic Blistering	Related
Source: FDA MAUDE Database			

Review of the case report forms showed that the first case of allergic reaction consisted of local redness at the Apligraf site. The second allergic reaction occurred in a patient with a history of multiple allergic reactions, and any relation to Apligraf is remote. The two reports of dramatic blistering on March 23, 2011 were two separate patients but under the clinical care of the same physician. Both patients had identical reported adverse events on the same day. Both patients had blistering occurring on the plantar and dorsum aspects of the foot at the site of a treated wound where a contact cast had been placed. The relative contributions of Apligraf and the cast could not be determined.

A case of squamous cell carcinoma, assessed by the investigator as unlikely related, was reported in May 2008. The investigator stated that the lesion had been noted prior to application of the product. There have been no cases of clinical signs or symptoms of acute rejection or allergy to Apligraf reported in the commercial use of the product.

7. RELEVANT NON-CLINICAL, CLINICAL AND LABORATORY STUDIES/DATA

7.1 Animal Studies

The same final product of Apligraf has been commercially available and marketed for the treatment of venous leg and diabetic foot ulcers by FDA/CDRH/DGRND under PMA P950032. Besides the nonclinical studies that were submitted for the PMA (P950032), two additional studies were conducted and submitted for BLA review. The nonclinical studies submitted for the PMA showed that Apligraf was able to engraft into nude mice with full-thickness cutaneous wounds. A series of in vitro studies were conducted to assess the potential of Apligraf's cellular components (i.e., keratinocytes and dermal fibroblasts) to generate an alloimmune response. The results of the studies showed that keratinocytes and dermal fibroblasts were incapable of generating an alloimmune response by the direct pathway of allorecognition. In the nonclinical studies conducted for the BLA, Apligraf was shown to be compatible with periodontal dressings (Barricaid® and Coe-Pack™) and anti-microbial rinse (0.12% chlorhexidine solution) as assessed by the ability of Apligraf to engraft onto full-thickness cutaneous wounds of nude mice.

7.2 Histology Study for Study 05-PER-001

In the pilot study 05-PER-001, biopsy specimens were taken from seven subjects at baseline and at 6 months from the control and Apligraf-treated sites for histologic evaluation to examine the cellular composition and tissue architecture of the surgical tissues. The objective of this additional histological study was to see if there were differences in the type, distribution and arrangement of collagen fibers, changes in the extracellular matrix protein composition and vascularity between the palatal graft and Apligraf treated sites, and between the tissues present at 6 months and baseline. The specific aim was to see whether changes in histology were consistent with the formation of site-appropriate tissue (i.e., keratinized gingival tissue).

Three-millimeter biopsies were obtained at baseline during surgery and at 6 months, from the Apligraf and Control sites of seven (7) subjects. The tissue was processed by fixing one-half in 10% formalin. The other half of the surgical tissue and Month 6 biopsy tissue were flash frozen. Standard Hematoxylin & Eosin (H&E) staining was performed. The examiner evaluating the slides was blinded to the site of origin (Control or Apligraf) of the tissue specimen. All biopsy specimens showed intact stratified squamous epithelium of normal thickness and tissue architecture. The observed tissue types are listed in Table 16.

Table 16. Tissue Types in Biopsy Specimens, Study 05-PER-001

Subject ID	Apligraf Site		Control Site	
	Baseline	**6 months**	**Baseline**	**6 months**
---b(6)---	Gingival mucosa	Transition zone from gingival to alveolar mucosa	Alveolar mucosa	Transition zone from gingival to alveolar mucosa
---b(6)---	Alveolar mucosa	Alveolar mucosa	Alveolar mucosa	Alveolar mucosa
---b(6)---	Alveolar mucosa	Transition zone from gingival to alveolar mucosa	Alveolar mucosa	Transition zone from gingival to alveolar mucosa
---b(6)---	Transition zone from gingival to alveolar mucosa	Alveolar mucosa	Alveolar mucosa	Transition zone from gingival to alveolar mucosa
---b(6)---	Alveolar mucosa	Alveolar mucosa	Alveolar mucosa	Gingival mucosa
---b(6)---	Alveolar mucosa	Transition zone from gingival to alveolar mucosa	Alveolar mucosa	Alveolar mucosa
---b(6)---	Transition zone from gingival to alveolar mucosa	Transition zone from gingival to alveolar mucosa	Transition zone from gingival to alveolar mucosa	Transition zone from gingival to alveolar mucosa

Overall, the biopsies sampled at baseline were representative of normal gingival epithelium. The epithelia subtypes of these biopsies included both gingival and alveolar mucosal phenotypes that were representative of the sampled oral site. There were varied degrees of chronic periodontal inflammation which were within normal limits. From the table above, 6-month biopsies generally demonstrated the presence of both gingival and alveolar mucosal phenotypes with a transition between these two types at the biopsied site. The origins of these cell types were not determined in this study.

7.3 DNA Persistence of Apligraf

Testing for persistence of DNA from Apligraf was done on biopsy specimens from two of the seven subjects in Study 05-PER-001; tissue samples were provided at baseline and Month 6. Persistence of allograft cells at the Apligraf-treated site was evaluated by polymerase chain reaction (PCR) analyses of DNA. The frozen biopsy tissue of two (2) subjects was obtained at 6 months and subjected to DNA analysis by multiplex Polymerase Chain Reaction to detect persistence of allograft DNA at the Apligraf-treated site. Prior to biopsy, a buccal swab was obtained from each patient. Two samples each of Control and Apligraf biopsy site tissue were analyzed for each patient. An Apligraf specimen was also analyzed. The samples were amplified using Applied Biosystems AmpFISTR Identifiler™ for 15 Short Tandem Repeat loci, as well as for the Amelogenin locus.

In both patients, results from the Control site and the Apligraf site (Sites A and B) displayed the same Identifiler STR profile. In each case, the biopsy results were identical to the buccal swab results for the patient from whom the biopsy was obtained. All the patient samples were taken from female patients, and only showed the X amelogenin locus. In contrast, the Apligraf specimen also exhibited the Y version of this sex-linked locus. At six months, there was no evidence of DNA persistence at the Apligraf site; the Control sites were also negative for allograft DNA.

7.4 Biomarker Adjunct Study 06-PER-002-CTX

Study Initiation Date: 24 December 2007
Study Completion Date: 31 August 2009
Three clinical sites

Title: *"Expression of Angiogenic Biomarkers During Healing of Intra-oral Soft Tissue Engineered Grafts"*

The purpose of the study was to compare the expression of angiogenic biomarkers involved in the wound healing process of two different periodontal surgical approaches: 1. Apligraf; and 2. Free gingival grafts (FGG).

Method
Gingival crevicular fluid (GCF), a serum transudate found in the space between the free gingiva and the tooth, i.e. the gingival sulcus, was collected at baseline (pre-treatment) for 30 seconds using Periopaper from Apligraf- or FGG-treated tooth prior to preparation of the mucosal defect (graft bed). Wound fluid (WF) was collected from the 4 corners of the treated site (Apligraf or FGG) with Periopaper for 30 seconds at 1, 2, 3, and 4 weeks post-treatment. Wound fluid samples were also collected from the palatal donor site from the FGG group at the same time points.

The gingival crevicular fluid and wound fluid samples were analyzed with human angiogenesis array kit (RayBiotech, Inc.; Norcross, GA) for the following proteins: angiogenin (ANG), angiostatin (ANT), fibroblast growth factor-2 (FGF-2), platelet-derived growth factor-BB (PDGF-BB), interleukin-8 (IL-8), interferon-inducible protein-10 (IP-10), granulocyte macrophage colony-stimulating factor (GM-CSF), tissue inhibitor of metalloproteinases-1 and -2 (TIMP-1 and TIMP-2), and vascular endothelial growth factor (VEGF).

The expression levels of each of the 10 angiogenic biomarkers were measured at each post-treatment time point (at 1-, 2-, 3-, and 4-weeks) and compared using a paired t-test to evaluate treatment differences in the concentration of each biomarker. Post hoc exploratory analyses were conducted to investigate potential correlations between the levels of individual biomarkers and the clinical outcomes from Protocol 06-PER-002-CTX within each treatment.

Results
A total of 44 subjects completed the study. Samples from baseline were collected from 29 of 44 subjects (65.9%). Samples from Week 1 were collected from 36 of 44 subjects (82%). Samples from Weeks 2 and 3 were collected from 42 of 44 subjects (95.5%). Finally, Week 4 samples were collected from 40 subjects (91%). The angiogenic biomarkers in both treatment groups and the donor site generally had the highest expression at Week 1, and the expression decreased substantially by Week 2.

Conclusion
There were no biomarker correlations to the quality of healing results (i.e., color, texture, pain and inflammation) in either treatment group.

7.5 Immunogenicity

An acute rejection phenomenon or development of immune sensitization might be expected with the use of allogeneic cells. However, these reactions were not seen clinically when using Apligraf, presumably because of the lack of antigen-presenting cells in Apligraf.

In the pivotal study (Protocol 95-DUS-001) that supported the approval of Apligraf for cutaneous wounds (chronic diabetic foot ulcer), in tests of subjects' sera, there were no observations of antibody responses against bovine type I collagen, bovine serum protein or Class I HLA antigens on human dermal fibroblasts and human epidermal cells. T-cell-specific responses were not observed against bovine type I collagen, human fibroblasts, or human keratinocytes.

Vascularization of Apligraf does not occur clinically as suggested by the use of Apligraf for cutaneous wound (Griffiths et al. 2004). The lack of Apligraf vascularization presumably reduces immune cell migration from the host tissue to Apligraf and HLA-antigen transport from Apligraf to the host tissue. These factors may contribute to the absence of immune reaction.

7.6 Potential for Malignant Transformation

7.6.1 Laboratory

There have been no documented histological reports of tumor formation at the site of Apligraf applications.

7.6.2 Testing for the Potential for Malignant Transformation

Information regarding the tumorigenic potential of Apligraf is provided by testing conducted on the keratinocyte and fibroblast cell banks used to manufacture Apligraf, according to the ICH and 1993 cell substrate guidelines. The testing includes:

- Donor screening and quantitative PCR testing for potential oncogenic viruses (e.g. human papilloma viruses, bovine polyvoma virus)
- Senescence testing did not demonstrate neoplastic transformation of the cell lines
- In vivo testing in nude mice injected with the cells which did not show a tumorigenic result at 3 months with any of the cell strains used to date.

8. DRAFT QUESTIONS TO THE ADVISORY COMMITTEE

Product Quality - Cell Bank

Apligraf is manufactured using allogeneic keratinocytes and fibroblasts derived from neonatal foreskin of human donors. Because expansion of the cell bank is limited, new banks from new donor tissue must be generated on a periodic basis.

1. Discussion Question: Please discuss the applicant's approach to qualify and demonstrate comparability for new cell banks used for Apligraf manufacture.

Product Quality - Potency

For Apligraf, product potency is determined by a set of histological parameters which collectively assess the quality of the epidermal and dermal layers present in the product after maturation. These parameters include epidermal coverage, epidermal development, basal cell layer, suprabasal cell layer, dermal matrix thickness, fibroblast density and matrix aspect. These parameters were shown to correlate with percutaneous water absorption, cell metabolic activity by MTT, VEGF secretion and in vivo mouse assay.

2. Discussion Question: Please discuss the use of H&E staining as a product potency measure for Apligraf.

Effectiveness

In the pilot study, Apligraf failed to meet non-inferiority margins for amount of attached gingiva, compared to autogenous palate graft control. However, the sponsor found that the width of keratinized tissue (KT) at the Apligraf site was numerically increased over baseline and used this information to design their pivotal study.

In the pivotal study, 81/85 subjects (95.3%) met the primary endpoint of KT ≥ 2mm at the Apligraf-treated site at 6 months (88.4%-98.7%, 95% CI). All 85 subjects met the primary endpoint at the Control site. All 11 training subjects met this goal at both the Apligraf and Control sites.

With respect to the secondary endpoints of the pivotal trial, Apligraf was statistically significantly superior to control treatment for color matching, texture matching, and patient preference and had a KT ≥ 1 mm success rate that was significantly > 80% (the success standard for that secondary endpoint of the trial). There was no significant difference between Apligraf and control with regard to sensitivity.

Apligraf has a theoretical advantage over a free gingival autograft due to the requirement for harvesting a donor palatal site in the latter procedure. However, with respect to the secondary endpoint of pain at Day 3 in the pivotal trial, there was no evidence of a

difference between Apligraf and control, with 54/84 (64.3%) subjects reporting no pain at either site and 18/84 (21.4%) reporting pain at both sites. (The remaining 12 subjects were discordant in pain between the sites in roughly equal proportions.)

The proposed indication for Apligraf is for the "treatment of surgically created gingival and alveolar mucosal surface defects in adults."

3. Discussion Question: Please discuss the effectiveness of Apligraf for the proposed indication, particularly considering the study results for KT, appearance, texture, patient preference, and pain.

4. Voting Question: Based on the data provided, is Apligraf effective for the treatment of surgically created gingival surface defects in adults?

Patient population

Studies 006-PER-002 and 005-PER-001 evaluated the use of the product in subjects with an insufficient (≤ 1 mm) zone of attached gingiva, requiring soft tissue grafting, where root coverage was not desired. The proposed indication is for the "treatment of surgically created gingival and alveolar mucosal surface defects in adults." However, alveolar mucosal defects were not studied in the pilot or pivotal trials.

5. Discussion Question: Please discuss whether the results of these studies are applicable to the broader patient population as described in the proposed indication.

Safety

Apligraf has been studied for the oral indication in three clinical trials. The period of observation in these trials was six months. In addition, there are clinical safety data from post-market use in chronic cutaneous wounds. The sponsor has also provided histological data on seven clinical trial subjects, and DNA persistence data on two.

6. Discussion Question: Please discuss the safety of Apligraf for the proposed oral indication, considering the available nonclinical and clinical study results, including both premarketing and postmarketing experience.

7. Voting Question: Do the data presented demonstrate the safety of Apligraf for the proposed indication?

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APPENDIX

The following are selected slides depicting the appearance of graft sites at baseline and 6 months from a subject in the pivotal trial.

Figure A-1: Before and after photos of patient –b(6)--
A: Soft tissue autograft, baseline
B: Soft tissue autograft, at 6 months
C: Apligraf site, baseline
D: Apligraf site, at 6 months

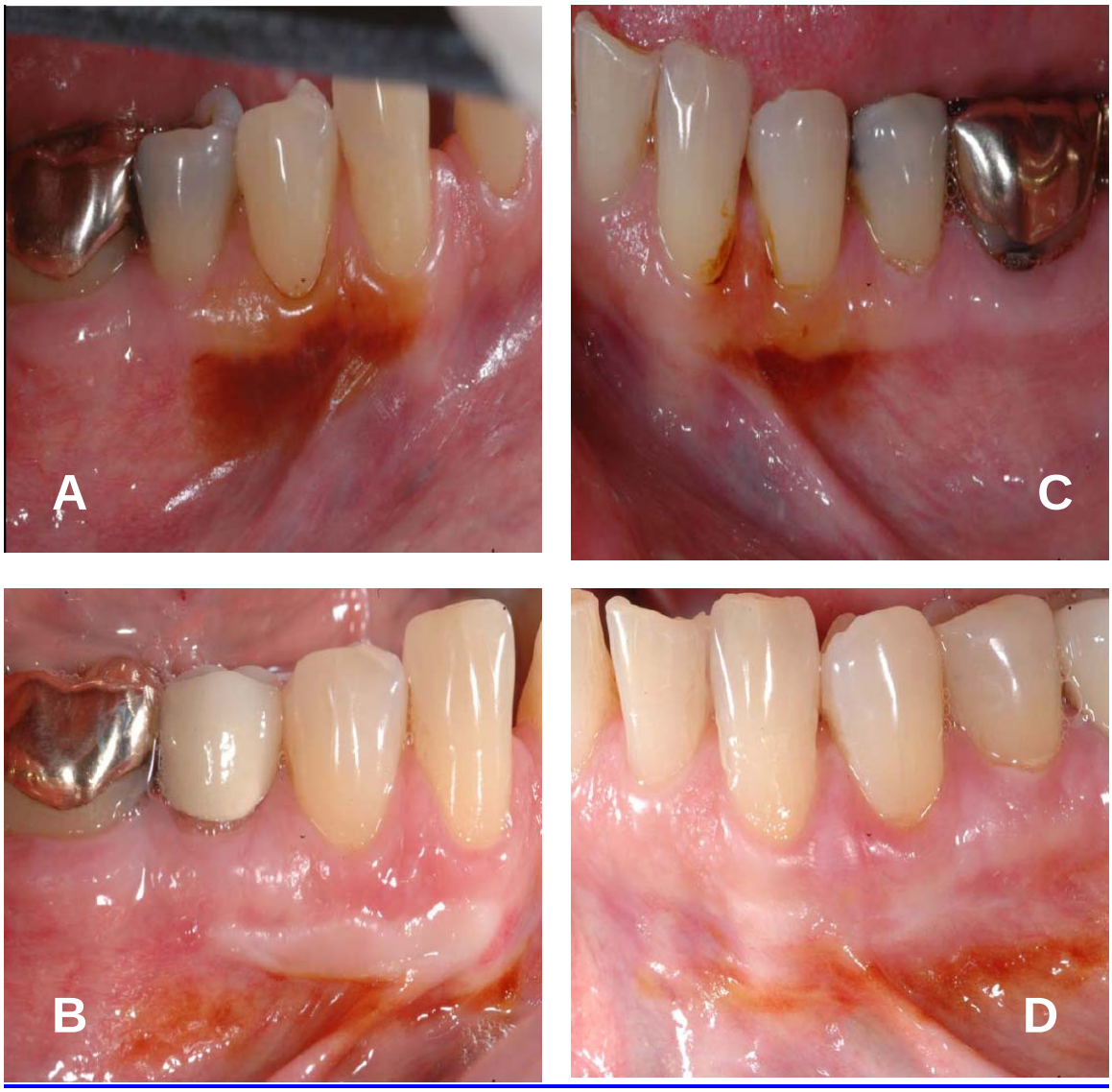
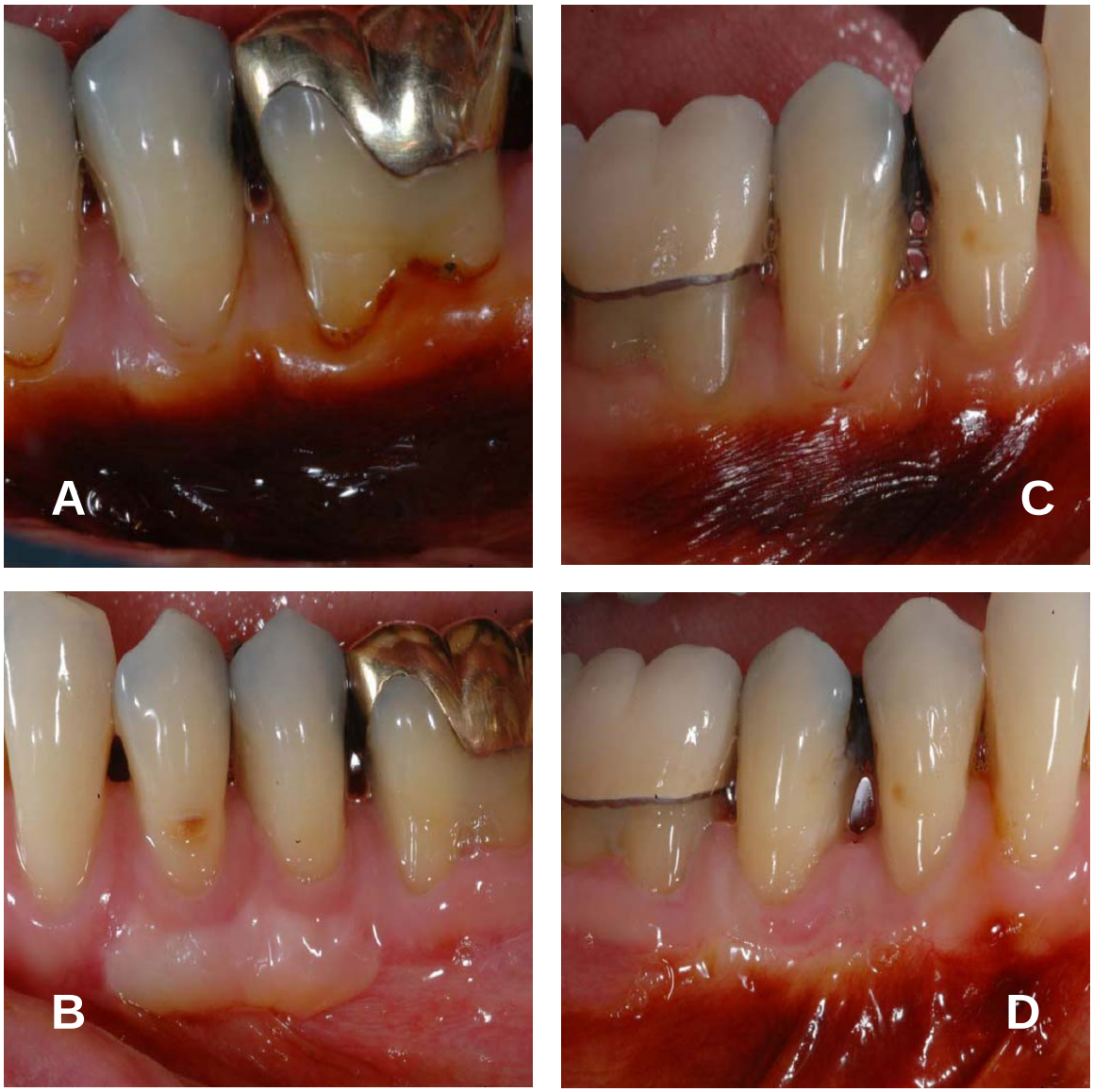
Figure A-2: Before and after photos of patient –b(6)--
A: Soft tissue autograft, baseline
B: Soft tissue autograft, at 6 months
C: Apligraf site, baseline. The amount (≤ 1mm) of keratinized gingiva is less obvious, but still present (no blanching of gingiva). The edge of the darkened (iodine stained) area is the mucogingival junction. Note the transition between keratinized and nonkeratinized tissues.
D: Apligraf site, at 6 months