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APPENDIX

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ABBREVIATIONS

ADA	American Dental Association
AE	Adverse event
AG	Attached gingiva
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
AM	Alveolar mucosa
BLA	Biologics License Application
BSE	Bovine spongiform encephalopathy
CAL	Clinical attachment level
CBER	Center for Biologics Evaluation and Research
CDRH	Center for Devices and Radiological Health
CO_2	Carbon dioxide
CSO	Cotton seed oil
cGMP	Current Good Manufacturing Practices
DAGID	Dental Products Branch and the Division of Anesthesiology, General Hospital, Infection Control, and Dental Devices
DE	Dermal equivalent
DFU	Diabetic foot ulcer
DGRND	Division of General, Restorative, and Neurological Devices
ECM	Extracellular matrix
EGF	Epidermal growth factor
ELISA	Enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
FGF	Fibroblast growth factor
FGG	Free gingival graft
GMS	Gomori methenamine silver
HCT/P	Human Cells, Tissues, and Cellular and Tissue-Based Products
HDF	Human dermal fibroblasts
H&E	Hematoxylin and eosin
HEP	Human epidermal progenitors (keratinocytes)
ICH	International Conference on Harmonization
IDE	Investigational Device Exemption
IHC	Immunohistochemistry
IGF	Insulin-like growth factor

IL	Interleukin
ISO	International Organization for Standardization
KG	Keratinized gingiva
KT	Keratinized tissue
MDR	Medical Device Report
MedDRA	Medical Dictionary for Regulatory Activities
MGJ	Mucogingival junction
MMP	Matrix metalloproteinase
MT	Masson's trichrome
MTG	Masson's trichrome-Goldner version
MTT	Mitochondrial tetrazolium testing
NaCl	Sodium chloride
OCTGT	Office of Cellular Tissue and Gene Therapies
PA	Percutaneous absorption
PBMC	Peripheral blood mononuclear cells
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PDGF	Platelet derived growth factor
PHA	Phytohaemagglutin
PMA	Premarket approval
PSR	Picrosirius red
QSR	Quality system regulation
RhD	Rh D-positive
SAEs	Serious adverse events
SCC	Squamous cell carcinoma
SOC	System organ class
TGF	Transforming growth factor
TEM	Transmission electron microscopy
TIMP	Tissue inhibitors of metalloproteinases
UADE	Unanticipated adverse device effect
US	United States
VEGF	Vascular endothelial growth factor
VLU	Venous leg ulcer
VVG	Verhoef van Gieson

1 EXECUTIVE SUMMARY

1.1 Introduction and Proposed Indication

1.1.1 Introduction

Organogenesis, Inc. ("Organogenesis"), a leading regenerative medicine company, seeks approval of a Biologics License Application (BLA) for a new indication for its existing cell-based product, currently marketed under the trade name Apligraf®. Specifically, Organogenesis has submitted BLA # 125400 for approval to market the product for the treatment of surgically created gingival and alveolar mucosal surface defects in adults. Apligraf has been previously approved by FDA for the treatment of venous leg ulcers (VLUs) and diabetic foot ulcers (DFUs).[a]

If approved, Apligraf will be the first cellular product available for treating oral soft tissue wounds. Apligraf has the ability to regenerate "site-appropriate" tissue (correct type, amount and distribution) by modulating and improving secondary intention wound healing. Efficacy and safety for this BLA are based on 2 Apligraf (oral) clinical studies, which are further supported by 13 years of Apligraf post-marketing safety experience in VLUs and DFUs.

1.1.2 Proposed Indication

The proposed new indication is "for the treatment of surgically created gingival and alveolar mucosal surface defects in adults. The product is applied over a vascular wound bed to regenerate site-appropriate oral mucosal tissues."

1.2 Disease Background

1.2.1 Normal Anatomy

The oral cavity contains two main types of soft tissue, each with its own specialized function: keratinized tissue (KT)[b] and alveolar mucosa (AM). KT is the tough, functional tissue which forms a protective barrier around teeth, dental implants and edentulous (no teeth) sites. AM is unattached and loosely moveable, and functions to provide strain relief and to allow for normal movement of the mouth. KT occupies the most coronal (towards

[a] Apligraf has historically been regulated by the Center for Devices and Radiological Health as a device, and accordingly, the VLU and DFU indications were approved by that Center pursuant to a Premarket Approval Application (PMA). Apligraf for the venous leg ulcers and diabetic foot ulcers was approved under PMA P950032 on 22 May 1998 and 20 June 2000.

[b] Here the more general term keratinized tissue (KT) is used for the wider audience and to reflect the primary endpoint of the pivotal clinical trial. It is recognized that KT, by definition, encompasses both palatal and gingival tissue. Here it is referring to keratinized gingiva and not palatal tissue.

the tooth) aspect and the AM apical (away from the tooth) to that. KT transitions into AM, demarcated by a visible, continuous line, called the mucogingival junction (MGJ). In their seminal work of 1972, Lang and Loe concluded that a minimum amount or width of 2 mm of keratinized gingiva (or keratinized tissue, KT) around teeth was adequate to resist inflammation and maintain gingival health (Lang, 1972, *J Periodontol*).

Figure 1: Normal Soft Tissue Anatomy

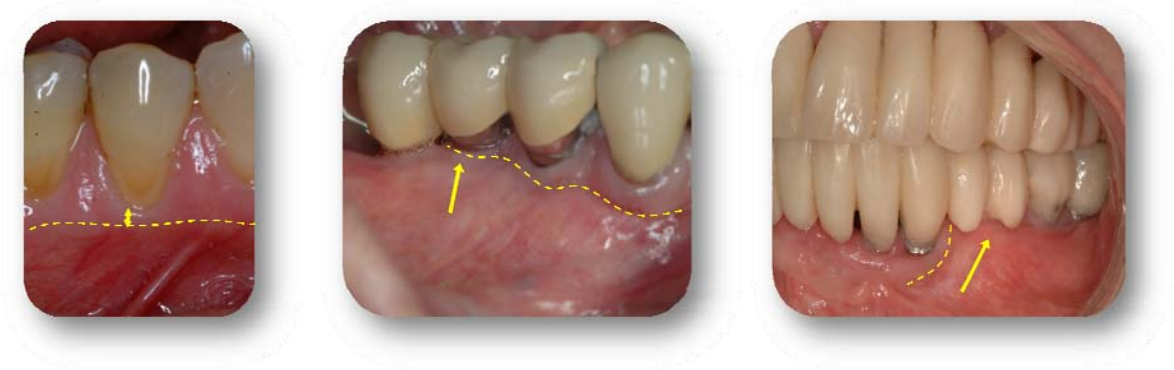
(a) **(b)**

Normal soft tissue anatomy represented (a) schematically and (b) clinically. KT (shown here in (a)) has segments that are both free (unattached at the sulcus) and attached gingiva. For the sake of simplicity, only the KT is shown here; this is what the primary endpoint of the pivotal clinical trial measured. Below the KT is the AM, demarcated by the MGJ. Figures adapted from Carranza's Clinical Periodontology 10th ed.

1.2.2 Abnormal Anatomy: Oral Mucosal Defects

To maintain health and function in the oral cavity, an adequate quantity and quality of functional soft tissue must be present. Abnormalities of the soft tissues (developmentally acquired or caused by trauma) compromise the health of the tissues. Without the proper tissue types around teeth or implants, there is evidence that plaque accumulation and chronic inflammation will occur (Lang, 1972, *J Periodontol*; Kennedy, 1985, *J Clin Periodontol*; Bouri, 2008, *IJOMI*). Persistence of periodontal/peri-implant inflammation leads to damage of the tooth attachment or loss of osseointegration of the implant increasing the probability of loss of the tooth or implant integrity (Hirschfeld 1978, *J Periodontol*; Wilson 1987 *J Periodontol*, Lang, 2009, *J Clin Periodontol*; Bouri, 2008, *IJOMI*; Roos-Jansaker, 2007, *Swed Dent J*; Schou, 2006, *Clin Oral Implant Res*). Because oral mucosal defects, do not self-resolve, they must be surgically corrected. **Figure 2** shows a range of oral mucosal defects around a) teeth; b) implants; c) edentulous ridge, and their lack of functional attached tissue (yellow arrow) and an abnormal MGJ (yellow line). The goal of surgery is to regenerate tissue that is site appropriate; that is, having the proper tissue type, amount of tissue and distribution of tissue compared to native tissue, thereby restoring the normal anatomy.

Figure 2: Oral Mucosal Defects of Inadequate Functional Tissue



(a) **(b)** **(c)**

1.2.3 Current Therapies

One surgical strategy in correcting these oral mucosal defects is to augment the amount of KT at the site in need, in order to buttress against further deterioration. The most widely used technique for augmenting KT (where tooth root coverage is not the goal) is through the free gingival graft (FGG). The FGG, which is harvested from the palate, has been used routinely since the 1960s, and it remains the standard of care to address deficiencies in functional gingival tissue (McGuire, 2008, *J Periodontol*). Briefly, an approximately 2 mm thick strip of tissue is taken from the palate and transplanted to a surgically created recipient wound bed at the site of the defect. As the graft takes, a large, stable band of KT protects the site from further deterioration. However, the transplanted palatal tissue remains paler in color and different in texture than the surrounding gingival tissue (see **Figure 3** below for an example). The donor site is left to heal on its own. Besides the FGG procedure, off-the-shelf alternative therapies (ie, collagen membranes) have recently been used clinically, but no predictable alternative supported by level 1 evidence exists.

1.2.4 Medical Need

While the FGG has a well-established record of creating a functional zone of KT, this repair strategy does so by using the improper tissue type (ie, palatal tissue), amount and distribution, all while incurring donor-site morbidity. In many patients there is a limited amount of donor tissue, leading to a triaging of the sites most in need of treatment and a subsequent under-treatment of the condition. The palatal donor site is associated with pain and – although infrequent – serious complications such as palatal bleeding. Because palatal tissue has a different color and texture than gingival tissue, grafting results in a poor esthetic match with the surrounding tissue. The amount of tissue grafted is typically

wider and thicker than the minimum needed and is noticeably discontinuous with the native KT and AM. **Figure 3** is a site repaired with an FGG, showing different type (palatal mucosa), amount (band of KT is greater than that around adjacent teeth) and distribution of tissue (discontinuous with adjacent KT and AM, MGJ denoted by yellow line) than normal architecture.

Figure 3: Site Repaired with FGG

In summary, there remains a need for an effective treatment that will regenerate site-appropriate oral soft tissue supported by a high level of clinical evidence. Additional information on the medical need is further discussed in **Section 5.4**.

1.3 Product Description and Mechanism of Action (MOA)

1.3.1 Product Description

Apligraf (oral) is a cell-based product that is effective in regenerating KT. Apligraf consists of 2 layers. The upper layer is comprised of living human keratinocytes and the supporting lower layer is constructed of living human dermal fibroblasts in a matrix of bovine-derived collagen and human extracellular matrix proteins (EMC) produced by the fibroblasts. The layers adhere as 1 unit to form the final construct.

Apligraf is supplied as a circular disk approximately 75 mm in diameter and 0.75 mm thick. The Apligraf construct is supported on a porous (polycarbonate) membrane within a culture insert; this culture insert is contained within a clear plastic processing tray (see **Figure 7** in **Section 3.1**). A schematic of the manufacturing process is provided in **Section 3.1** as **Figure 5**. Information about control of cell banks, release testing and potency assay is given in **Section 3.2** and **Section 3.3**.

1.3.2 Mechanism of Action (MOA)

While the mechanism of healing is multi-modal, Apligraf does not function as a skin or gingival graft. Despite having the appearance of skin, Apligraf does not vascularize or

integrate, nor do the Apligraf cells persist as occurs in a graft. It is believed that the production of cytokines and growth factors by the living cells, as well as providing a stratum corneum barrier upon application to the wound, are the key factors responsible for the ability of Apligraf to enhance healing by secondary intention.

Nonclinical studies have shown that the keratinocytes and fibroblasts present within Apligraf synergistically interact to produce many of the signaling molecules, such as platelet-derived growth factor (PDGF), fibroblast growth factors, epidermal growth factor (EGF) and other growth factors and cytokines that are involved with the various phases of wound healing (ie, hemostasis, inflammation, proliferation, and remodeling) (Brem, 2003, *Surg Technol Int*). Furthermore, Apligraf contains a number of matrix proteins that play key structural roles in skin and in healing, such as collagen type IV, tenascin, and fibronectin. Apligraf also contains tissue inhibitors of metalloproteinases (TIMPs) that can inhibit matrix metalloproteinase (MMP) activity and reduce the proteolytic burden in chronic wounds, to promote normal healing (Osborne, 2002, *Br J Dermatol*). The abundant collagen (Osborne, 2002, *Br J Dermatol*) in Apligraf may also function as a decoy for proteases, saturating active sites with excess collagen. Further information regarding the role of Apligraf in wound healing is provided in **Section 3.4**.

1.4 Nonclinical Studies

The overall safety of the product has been well-characterized in the cutaneous wounds. Nonclinical studies of Apligraf in the oral indication have not been performed because the nonclinical development program that was conducted to support the original product approvals provides adequate non-human safety data, as cutaneous wounds have more similarities than differences to wounds of the oral mucosa. The nonclinical body of data on Apligraf includes product characterization, cell persistence/migration, immunotoxicity, International Organization for Standardization (ISO) toxicity testing, and drug interaction with common periodontal dressings/anti-microbial rinses. The results of these studies do not suggest a potential for immunological, toxicological, or oncogenic risks. Additional information on the Apligraf nonclinical development program is provided in **Section 4** and **Appendix 1**.

1.5 Clinical Development Program

Organogenesis has completed 1 pilot and 1 pivotal oral clinical investigation with Apligraf under Investigational Device Exemptions (IDEs) in support of the proposed indication. The primary objective of the pivotal study was to determine the ability of Apligraf (oral) to achieve a clinically acceptable threshold (≥ 2 mm) for KT at 6 months.

The 2 mm threshold was chosen based on literature establishing that 2 mm of KT around teeth is adequate to resist inflammation and maintain gingival health (Lang, 1972, *J Periodontol*).

Safety data from a third small (pilot) study of the use of Apligraf to treat a different indication (root coverage) are provided for completeness in **Section 7.3.1**.

1.6 Treatment

The 2 oral clinical studies for the surgical defect indication were randomized within-patient controlled (ie, split-mouth), meaning that each patient received the Apligraf-treatment and an FGG on contralateral sides of either the mandible or maxilla at Day 0, with each patient thus serving as his or her own control. The study designs are discussed in detail **Sections 6.2.1** and **6.2.2** for the pilot and pivotal studies, respectively.

1.7 Summary of Clinical Studies

1.7.1 Pilot Study (05-PER-001): A Pilot Clinical Trial to Assess the Safety and Efficacy of Apligraf in establishing a Functional Zone of Attached Gingiva

There were 25 subjects total, all of whom were evaluated as part of the safety cohort; 22 of these were also evaluated for efficacy.[c] The study was carried out at a single clinical study center in the US and had a 6-month follow-up.

The primary objective of this pilot study was to assess the change in the amount of attached gingiva (AG) with Apligraf application compared to FGG at 6 months following initial treatment. At this early point of clinical development it was thought that Apligraf had the potential to behave as a graft and thus was compared directly to FGG. The change in AG from baseline to 6 months was a mean of 0.85 mm (95% CI 0.48, 1.21) at Apligraf-treated sites and a mean of 2.43 mm (2.06, 2.79) at FGG-treated sites; $p<0.001$.

While the primary objective of this pilot study was not met, it demonstrated the potential for Apligraf to regenerate site appropriate KT which became the primary endpoint for the pivotal trial. The results of the secondary endpoints are provided below:

- Apligraf treatment established at least 2 mm of KT width in 81.8% of the cases. The mean width of KT at 6 months was 2.50 mm (95% CI 2.18, 2.82).

[c] Three subjects pre-specified as training subjects were not included in the efficacy population.

- The changes from baseline to Month 6 were comparable between Apligraf and FGG in terms of periodontal health measures (probing depth, recession, clinical attachment, resistance to muscle pull, inflammation, and bleeding on probing).
- There was a considerably higher subject satisfaction/preference score for Apligraf treatment compared to FGG.
- Tissue color and tissue texture in the Apligraf-treated sites were more closely matched to adjacent tissue than in the FGG-treated sites.
- Duration of pain and sensitivity in the FGG-treated sites and palatal donor site was greater than in the Apligraf-treated sites.

Three millimeter biopsies were obtained at baseline during surgery and at 6 months, from the Apligraf and FGG sites of 7 patients. Using hematoxylin and eosin (H&E) staining, the cellular composition of the surgical tissues was examined. Qualitatively, all biopsy specimens showed intact stratified squamous epithelium of normal thickness and tissue architecture.

1.7.2 Pivotal Study (06-PER-002-CTX): A Clinical Trial to Evaluate CelTx[d] (Apligraf®) as an Alternative to Tissue from the Palate to Enhance Oral Soft Tissue Regeneration and Wound Healing

There were a total of 96 subjects in the pivotal trial, all of whom were evaluated as part of the safety cohort; 85 of these were also evaluated for efficacy.[e] The primary objective of the study was to determine the ability of Apligraf to achieve a clinically acceptable threshold (≥ 2 mm KT) for KT at 6 months. KT was measured by the calibrated examiner using a UNC 15 probe, aided by Schiller's iodine staining of mucosa. The percentage of subjects who achieved ≥ 2 mm KT at the Apligraf-treated site was compared to a 50% success standard with a one-sided binomial test.[f]

The primary outcome of at least 2 mm of KT was achieved in 95.3% (81 of 85) of Apligraf-treated sites at 6 months. This was statistically significant ($p<0.0001$) as compared with the 50% threshold. A mean width (SD) of 3.2 (1.0) mm of KT was regenerated at Apligraf-treated sites.

[d] CelTx™ is the proposed proprietary name for Apligraf® (oral), currently under review by the FDA.
[e] Eleven (11) subjects were pre-specified as training subjects, and were therefore not included in the efficacy population.
[f] The 50% success standard was established in consultation with the Dental Products Brach of CDRH, which was overseeing clinical investigations of this indication pursuant to an IDE.

As opposed to the regenerative healing process of Apligraf, FGG simply transfers tissue from one site in the oral cavity to another. Therefore the amount of KT post-procedure closely approximates the size (apical-coronal width) of the FGG grafted. Accordingly, a direct comparison of the amount of KT in the Apligraf-treated sites versus the FGG-treated sites would not be meaningful, although such comparisons were carried out with respect to the secondary endpoints. Predictably, all FGG treated sites (85 of 85) achieved at least 2 mm KT at 6 months and the mean KT width (SD) at 6 months was 4.6 (1.0) mm.

There were 6 secondary endpoints prospectively identified in the study. To maintain an overall Type I error rate of 0.05, the secondary hypotheses were tested using a closed testing strategy where the order of testing was pre-specified. The summary of the 6 secondary endpoints, results and p-values are presented in **Table 1.**

Table 1: Summary of Secondary Endpoints

SECONDARY ENDPOINT	RESULTS	P-VALUE
1. Color Match at 6 Months	Apligraf-treated sites, 79 (92.9%) were equally red to the adjacent tissue compared to only 23 (27.1%) of the FGG-treated sites. An examiner assessed color of the Apligraf and FGG treated sites compared with their respective adjacent, non-treated tissue. The assessment was recorded as “More Red,” “Equally Red,” or “Less Red” as compared to with adjacent, non-treated tissue. A match in color with the surrounding tissue is considered a positive -esthetic outcome.	$p < 0.0001$
2. Texture Match at 6 Months	Apligraf-treated sites, 81 (95.3%) were equally firm as the adjacent tissue compared to 46 (54.1%) of the FGG-treated sites.	$p < 0.0001$
3. At least 1 mm KT at 6 Months	Measured 85 (100%) Apligraf-treated sites regenerated ≥ 1 mm KT at 6 Months KT was measured by a calibrated examiner using a UNC-15 probe, aided by Schiller’s iodine staining of mucosa.	$p < 0.0001$
4. Patient Preference at 6 Months	Apligraf preferred by 61 (71.8%) of subjects	$p < 0.0001$
5. Surgical site sensitivity mild or absent after 1 week	Apligraf-treated sites, 68 of 71* (95.8%) had mild or no sensitivity at 1 week compared to 70 of 71* (98.6%) of the FGG- sites (donor and recipient).	$p = 0.3173$
6. Pain absent after 3 days	The majority of subjects (54 subjects) had mild or absent pain at all Apligraf-treated and FGG-sites (donor and recipient) at Day 3.	Not Tested
Abbreviations: FGG, free gingival graft; KT, keratinized tissue *Presence of Coe-Pak at Week 1 prevented assessment of sensitivity in 14 subjects.		

These findings support the use of Apligraf (oral) as an efficacious treatment to regenerate site-appropriate tissue of the proper type (KT), amount (≥ 2 mm of KT) and distribution

(color and texture match as compared to adjacent non-treated tissue). These results are achieved without harvesting tissue from the patient's palate, which is a significant benefit to the patient. A photograph of a study subject at 13 months post-treatment is provided in **Figure 4** to illustrate these results.

Figure 4: Panoramic Photograph Showing the Clinical Outcomes FGG (left) and Apligraf (right)*

Panoramic clinical photograph showing the repair outcome of the FGG (left, above area of yellow dotted bracket) compared to the regenerative outcome of Apligraf (right)
*13 month follow-up (post conclusion of 06-PER-002-CTX) provided courtesy of Rodrigo Neiva, DDS, MS.

1.7.3 Additional Analyses

Using data collected from these 2 clinical studies, an additional histological evaluation and independent blinded photographic assessment were conducted and these studies are further detailed in **Appendix 2** and **Appendix 3**.

1.7.3.1 Independent Photograph Assessment

As described in **Section 1.6.1**, it was not possible to blind the pivotal study. However, following completion of the pivotal study, photographs taken at the 6-month follow-up visit were independently evaluated by 3 dental examiners. The photographs were reviewed for:

(1) Color match with adjacent non-treated tissue (more red, equally red, less red)

(2) Soft tissue match (presence or absence of keloid-like scar formation)

(3) Mucogingival alignment (aligned or not aligned with adjacent teeth)

In all 3 parameters there was statistical significance in favor of Apligraf compared to FGG. Additional information on the Independent Photograph Assessment is presented in **Appendix 2**.

1.7.3.2 Additional Histological Evaluation

Pre-treatment and 6-month post-treatment biopsies were obtained and analyzed from a subset of 7 subjects from the pilot clinical study. The pre-treatment biopsy was obtained from the gingiva (primarily AM) prior to creation of the wound bed and the 6-month post-treatment biopsy was obtained from the MGJ of the treated teeth. These biopsies were originally evaluated with standard H&E staining to examine the cellular composition and tissue architecture. An additional histological analysis was conducted and is presented in **Appendix 3**.

In summary, both Apligraf and FGG produced KT with improvements in rete ridge formation that was histologically different from the tissue present at baseline (ie, AM). Compared with FGG treated sites, regenerated tissue at Apligraf-treated sites had less elastin and tenascin. Apligraf-treated sites also had a greater percentage of dense collagen and fewer collagen fibers that were parallel to the epithelium than the FGG-treated group. There was a decrease from baseline in reticulin and vascularity in both the Apligraf and FGG treated groups. Myofibroblasts were not seen in either baseline or study groups. There appeared to be a mild increase in inflammation in Apligraf-treated groups, but this change was well within baseline variability.

1.7.4 Integrated Summary of Safety

The safety of Apligraf (oral) has been well characterized from the clinical development program for oral mucosa wounds, supported by 20 years of experience with the use of Apligraf in hundreds of thousands of patients with VLUs and DFUs, with only 9 Medical Device Reports (MDRs). The collective safety data from the Apligraf development program, together with post-marketing experience for Apligraf (VLU and DFU), demonstrate a favorable safety product profile.

The primary safety data were collected from the clinical studies of the proposed oral indication with a total safety population of 121 subjects. Adverse events (AEs) were systematically collected throughout the duration of the study and are described below.

1.7.5 Common Adverse Events

A total of 65 AEs were reported for 41 subjects in the pilot (17 of 25 subjects) and pivotal (24 of 96 subjects) studies. There were 80 subjects who did not experience an AE during either study.

The largest number of AEs occurred in the categories of Gastrointestinal Disorders (12 subjects with 13 events) and Infections and Infestations (13 subjects with 16 events). In these 2 categories, only one type of event was reported more than twice: there were 5 subjects with 6 reports of sinusitis. The only other specific AE reported in more than 2 subjects was hypersensitivity (4 events in 4 subjects)[g]. Refer to **Table 19** in **Section 7.2.1.1** for tabular listing of the AEs for the pilot and pivotal studies.

1.7.6 Serious Adverse Events

No serious adverse events (SAEs) were reported in the pilot clinical study. There were 3 SAEs reported during the pivotal study. Two of the 3 SAEs, chest pain and pneumonia, were deemed not related to the Apligraf-treatment by the investigator and occurred at Day 100 and Day 74[h], respectively. The third SAE was metastatic malignant fibrous histiocytoma diagnosed on Day 154[h] in a patient with a pertinent past medical history; this event was deemed likely unrelated to the Apligraf-treatment.

1.7.7 Other Significant Adverse Events

No other significant AEs were reported in the pilot clinical study. However there were 3 AEs reported during the pivotal study that were not reported as SAEs but judged by the Sponsor's Medical Monitor to be clinically important.

In the pivotal study, inadvertent placement of the Apligraf polycarbonate membrane to the oral mucosal defect occurred in 2 subjects and resulted in 2 AEs (gingival injury and gingival pain). These AEs occurred only in the training subjects.

The third AE was a single case of follicular thyroid cancer, diagnosed on Day 92[h], in a patient with a prior history of hypothyroidism; this event was deemed by the investigator as not related to treatment.

[g] Three subjects in the pilot study were reported to have 1 AE each of allergies. These events were coded to the MedDRA preferred term "hypersensitivity." One subject in the pivotal study was reported to have an AE of "environmental allergies" and this was also coded to the MedDRA preferred term of "hypersensitivity." These events were all not related to Apligraf.
[h] Relative to Apligraf study treatment performed on Day 0.

1.7.8 Other Safety Information

1.7.8.1 Summary of Apligraf Immunology

Apligraf contains allogeneic cells derived from donated neonatal foreskin tissue. These cells are extensively tested to ensure safety, however the immunogenicity of applying allogeneic cells to patients is a potential concern. In the oral clinical development program, immunological testing was not required by FDA during the pilot or pivotal clinical studies and therefore was not conducted. Although immunological testing has not been carried out during clinical investigations of the oral indication, data from the acute cutaneous clinical studies are adequate to establish the immunological safety of Apligraf for this indication because both types of wounds are highly vascular.

The immunological clinical safety testing and results from the acute and chronic cutaneous clinical trials are further described in **Section 7.3.2**. In summary, there have been no confirmed reports of immunological reaction to Apligraf in these studies.

These findings are consistent with clinical observations in both cutaneous and mucosal (oral) wounds. No evidence of an immune response to Apligraf was reported in either the pilot or pivotal oral clinical studies, and there have been no reported cases of clinical signs or symptoms of acute allergy ie, or immunologically mediated rejection response in over 787 evaluable acute and chronic cutaneous clinical study subjects studies or in more than 13 years of commercial use (in patients with chronic wounds that may be treated with as many as 5 applications of Apligraf within the first 4 weeks of treatment).

1.7.8.2 Summary of Apligraf Persistence

Data on the persistence of Apligraf DNA are available from acute and chronic cutaneous applications. Clinical studies in acute wounds (3 studies, 7 biopsied treatment sites) and literature reports (40 subjects) from acute and chronic cutaneous Apligraf applications generally suggest that Apligraf DNA persists in acute and chronic cutaneous wounds for up to 6 weeks (Falabella, 2000, *Arch Dematol*; Fivenson, 2000, *Wounds*; Fivenson, 2003, *JAAD*; Griffiths, 2004, *Tissue Eng*; Jiang, 2002, *J Pediatr*; Serena, 2009, *Ostomy*; *Wound Manage*; Nevins, 2010, *Int J Periodontics Restorative Dent*).

There is limited information in the oral cavity regarding Apligraf DNA persistence. Clinically, Apligraf appears to degrade rapidly in the oral cavity likely due to the action of salivary enzymes and movement of oral tissues. By Day 7 Apligraf is typically remodeled to give the appearance of a fibrinous exuate. By 2 weeks there is no clinical evidence of Apligraf presence. Two subjects from the pilot study were tested for

persistence of DNA from the Apligraf allogeneic cells. At 6 months post-treatment, there was no persistence of Apligraf DNA in either subject (McGuire, 2008, *J Periodontol*). In a separate report, Nevins et al found that in all subjects tested (N=4), no Apligraf DNA was present in the Apligraf- treated sites at 20, 29, 35 and 45 days (1 subject per timepoint) (Nevins, 2010, *Int J Periodontics Restorative Dent*).

1.8 Post-Marketing Safety Surveillance and Risk Management

As requested by the FDA, Organogenesis submitted a Pharmacovigilance Plan (PVP) for Apligraf (oral) on 20 July 2011 that is currently under review with the FDA. The 2 potential risks identified by Organogenesis in the PVP are gingival injury and gingival pain.

1.9 Benefit-Risk Overview

The known and/or potential risks associated with Apligraf (oral) can be categorized into those inherent to the product and those related to the procedure.

Potential risks related to the product (ie, allergic reaction to the bovine collagen and disease transmission from the cells) are the same as those associated with the Apligraf (cutaneous) product. These risks are detailed in the proposed Package Insert, specifically in the contraindications, warnings and precautions sections.

Risks related to the procedure have been minimized by using a surgical technique (wound bed preparation) that is identical to the standard of care and therefore very familiar to dental surgeons. Use of the product in surgery carries some risk of inadvertent placement of the polycarbonate membrane, which may result in gingival injury and/or gingival pain. These known risks are also addressed in the proposed Package Insert along with the Sponsor's provision of medical and training resources when requested.

The demonstrated benefits of Apligraf (oral):

- Regeneration of a clinically significant amount of functional, site-appropriate tissue in terms of the type (KT), amount (≥ 2mm) and distribution (continuous, color, and texture match).
- Excellent cosmetic results and patient preference
- Favorable safety profile in oral clinical studies supported by 13 years post-marketing experience (420,000 applications) in cutaneous wound healing

In practice, additional benefits of Apligraf (oral) may include:

- No palate donor site required, eliminating morbidities
- Simple procedure and readily available material, thereby increasing the likelihood of having all defects treated

The long history of Apligraf use in acute and chronic cutaneous wounds further supports the safety and efficacy of the product in this indication. Overall, Apligraf (oral) is a unique advancement in the treatment of oral mucosal defects that is well-tolerated and has a favorable safety profile. It provides clinicians and patients with a new, truly regenerative treatment with clinically significant and esthetically pleasing benefits.

2 INTRODUCTION

A BLA has been submitted to support the safety and efficacy of Apligraf® (oral), a bilayered living cell-based product for the treatment of surgically created gingival and alveolar mucosal surface defects in adults. This is the third indication for Apligraf, which currently has FDA approval for the treatment of VLUs and DFUs.

Apligraf consists of two layers: an upper layer comprised of living human keratinocytes and a supporting lower layer consisting of bovine-derived collagen, ECM proteins, and living human fibroblasts. These layers adhere as one unit to form the Apligraf construct as it matures during the manufacturing process.

Organogenesis originally developed Apligraf as a human living skin substitute that would permanently replace and restore missing or damaged host skin (ie, act as an immunoprivileged skin graft and effect healing by primary intention). As the MOAs were elucidated, it became apparent that in addition to its biomechanical and barrier attributes, Apligraf acts as a stimulus to provide a wide array of human cytokines and growth factors to induce healing through secondary intention. In addition to having a better understanding of the product's MOA, the similarity of wound healing between cutaneous and gingival wounds, provoked exploration of oral soft tissue regeneration.

2.1 Proposed Label Indication and Treatment Regimen

The proposed label indication is: "Apligraf (oral) is indicated for the treatment of surgically created gingival and alveolar mucosal surface defects in adults. The product is applied over a vascular wound bed to regenerate site-appropriate oral mucosal tissues."

The recommended treatment regimen is a single application of Apligraf to a surgically prepared vascular wound bed to regenerate "site-appropriate" oral mucosal tissue. Site-appropriate refers to the appropriate functional type of tissue (ie, KT and AM) in the proper amount and distribution compared with the surrounding native tissue. A dose of Apligraf (oral) is defined as the amount of Z-folded product needed to cover the surgically prepared vascular wound bed. Repeated applications of Apligraf (oral) have not been clinically studied for the proposed oral indication.

2.2 Regulatory History

Apligraf was originally designated by FDA in 1986 as a medical device. A premarket approval (PMA) application for Apligraf was initially approved in 1998 by the Division of General, Restorative, and Neurological Devices (DGRND) within the Center for Devices and Radiological Health (CDRH) for the treatment of VLUs that have not healed by 4 weeks with conventional therapy. Since that time, CDRH has approved over 50 supplements including but not limited to the extension of indication to DFU in 2000, manufacturing changes, and cell bank releases.

Since 2005, Organogenesis has been developing of Apligraf for an oral soft tissue indication, with oversight primarily by CDRH. Clinical studies for this oral indication were carried out under 2 IDEs, G050122 and G070012. These IDEs were reviewed and approved by the CDRH Dental Products Branch in the Division of Anesthesiology, General Hospital, Infection Control, and Dental Devices (DAGID) in anticipation of a PMA submission. Based on consultation with CDRH, Organogenesis submitted a PMA for the treatment of oral soft tissue defects (P090027) on 09 December 2009. However, FDA's Office of Combination Products (OCP) subsequently determined that Apligraf (oral) was a combination product (device/biologic) under the jurisdiction of the Center for Biologics Evaluation and Research (CBER). At the first Type B meeting held on 02 September 2010, the FDA requested that Organogenesis convert PMA (P090027) to a BLA including current Good Manufacturing Practice (cGMP) for combination products. On 13 May 2011 the Apligraf BLA was submitted electronically to the Office of Cellular Tissue and Gene Therapies (OCTGT)/CBER/FDA and is currently under review at the FDA.

Apligraf (oral) has not been marketed in the US or any foreign country for the proposed label indication.

3 PRODUCT DESCRIPTION, CHARACTERIZATION, AND QUALTIY

Apligraf (oral) consists of 2 layers: an upper layer comprised of living human keratinocytes and a supporting lower layer constructed of bovine-derived collagen, human ECM proteins, and living human dermal fibroblasts. The layers adhere as one unit to form the final construct. The lower layer (matrix) contributes to the overall functional and biological attributes of the product, through the living fibroblast cells that secrete cytokines, growth factors and other proteins. The upper layer imparts important structural elements to the product that contribute to its biomechanical strength and handling, and barrier properties. The upper layer also contains living keratinocyte cells, which secrete biologically active proteins known to stimulate wound healing. It is important to note that both layers are essential to biological function (potency) of the final Apligraf product. Apligraf (oral) is the same product as Organogenesis' commercially available Apligraf product that is indicated for VLUs and DFUs.

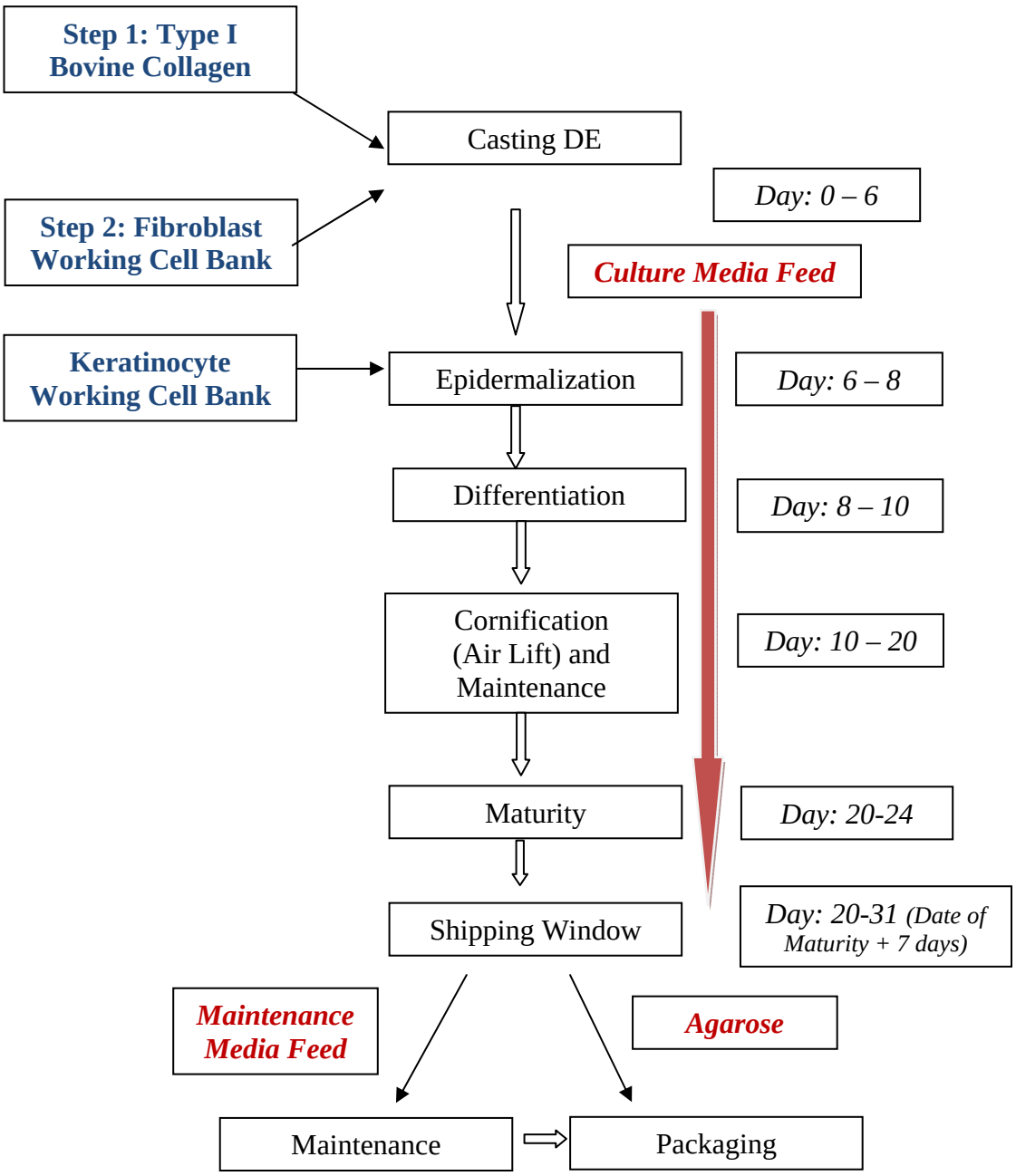
3.1 Product Description and Manufacture

Manufacturing of Apligraf is a continuous process that takes 20-31 days depending on product maturation and date of packaging. This process entails the following major steps:

1. Production of the Lower Layer
    a. Deposition of acellular matrix layer (pre-mix media and bovine collagen)
    b. Deposition of the cellular layer (pre-mix media, bovine collagen and fibroblasts on top of the acellular matrix layer)
    c. Incubation to promote contraction of the lower layer
2. Production of the Upper Layer
    a. Deposition of keratinocytes onto the surface of the lower layer
    b. Incubation to allow attachment, proliferation and migration of the keratinocytes to cover the lower layer
    c. Exposure to air-liquid interface and incubation to promote further differentiation and development of the stratum corneum
3. Maturation of Apligraf
    a. Maintenance feeds and incubation

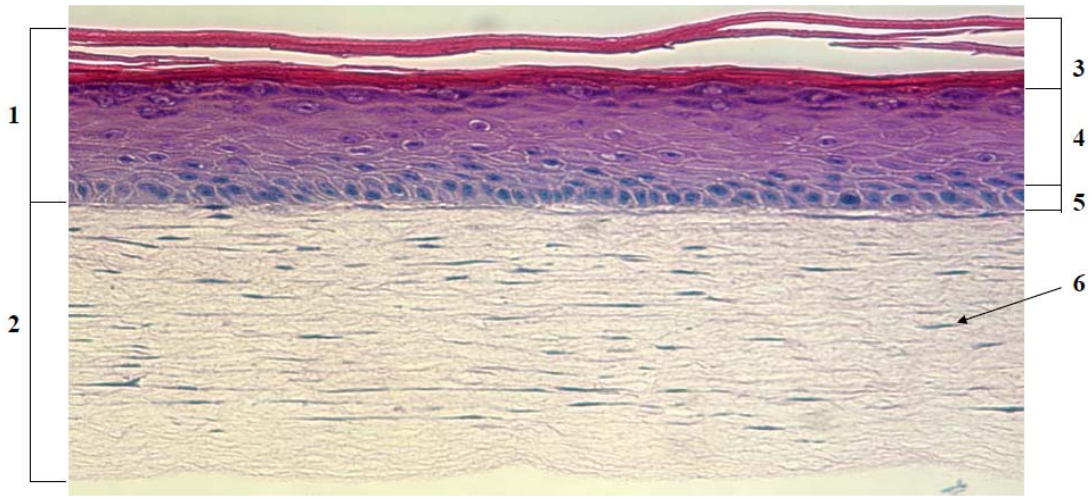
To maintain cell viability, the product is aseptically manufactured, but not terminally sterilized. Testing for absence of microbial contamination is performed throughout the production process. A manufacturing process schematic is provided as **Figure 5**.

Figure 5: Apligraf Manufacturing Process



The structure of the mature Apligraf as assessed by histology is shown in **Figure 6**.

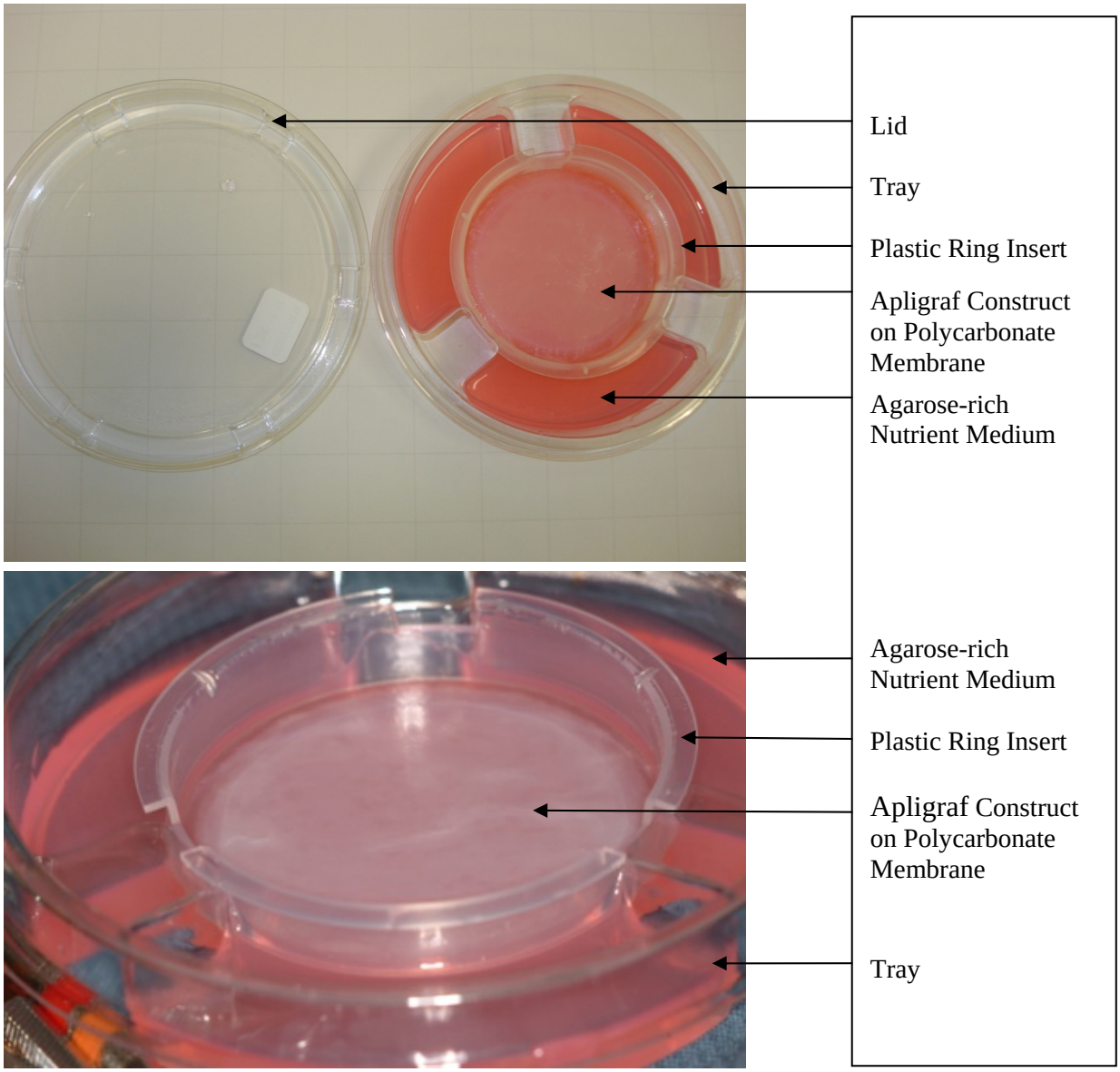
Figure 6: Histological Section of the Mature Apligraf Construct



Histology section of mature Apligraf construct showing the (1) Upper (Epidermal) Layer, (2) Lower (Dermal) Layer, (3) Cornified Layer, (4) Suprabasal Layer, (5) Basal Layer, and (6) Fibroblast.

Each construct is manufactured as a circular disk approximately 75 mm diameter and is 0.75 mm thick. The construct is supported on a porous polycarbonate membrane within a culture insert is contained within a clear plastic processing tray (see **Figure 12**).

Figure 7: Apligraf Primary Packaging



Once the construct reaches maturity (between Day 20 and Day 24 in culture) where potency (ie, morphological assessment) criteria have been met, the final packaging of Apligraf is initiated. Prior to packaging, the culture insert containing the mature construct is transferred to the final shipping tray. The final packaging is defined as the shipping tray (primary packaging) containing the Apligraf construct resting on a bed of agarose shipping medium, which together, is aseptically packaged into a heavy-gauge polyethylene transparent bag. During the heat sealing process, CO_2 is injected into the bag to achieve a final concentration of 10% CO_2 in air.

The final product is tested for potency, sterility, mycoplasma, and physical container integrity. Every unit is subjected to visual inspection, including observation for pH and absence of defects. Strict controls over the cell banking and Apligraf manufacturing process as well as final product testing for safety and potency ensure product consistency and reproducibility.

The manufacture of Apligraf includes reagents derived from animal materials such as bovine pituitary extract. All animal derived reagents are tested for viruses, retroviruses, bacteria, fungi, and mycoplasma prior to before use. Bovine materials are appropriately sourced and tested to minimize the risk of bovine spongiform encephalopathy (BSE).

The packaged Apligraf product has a 15-day shelf life from the day of packaging when maintained at 20-23°C. Apligraf is shipped following initiation of finished product microbiological testing to determine the absence of microbial growth. Final microbiological test results may not be available at the time of application due to the limited shelf-life. In the unlikely event of a positive test result after product distribution (<0.3% occurrence in 13 years of commercial distribution), a recall procedure has been initiated. In the event that recalled product is applied to patient(s), Medical Affairs with consult with the patient's Physician(s) to ensure that appropriate actions are taken.

Figure 8: Apligraf Final Packaging

3.2 Control of Cell Banks

Organogenesis utilizes a 2 tiered cell banking system to create master cell bank(s) and working cell banks. A master cell bank represents the resultant cells that have dissociated from the tissue and grown in culture for 1 or 2 passages for keratinocytes and fibroblast cells, respectively. The tissues are sourced from neonatal foreskin tissue obtained in accordance with established tissue donation guidelines and informed consent procedures. Donor eligibility is determined by screening and by testing for relevant communicable disease agents and diseases. All donor sites are qualified in accordance with internal procedures. The tissue donation protocol and informed consent procedures are approved by IRBs. The donor program complies with current regulations for HCT/Ps.

The working cell bank is derived from one or more vials of a single master cell bank and is serially passaged (ie, subcultured). The working cell banks are then further passaged to generate the fibroblast and keratinocyte seeds pools that are starting materials for the manufacture of Apligraf. **Table 2** and **Table 3** list the master and working cell banks testing. The characterization panel (**Table 2**) ensures that the cells are bioequivalent to previously qualified cell banks. These characterization assays are performed to assess the cell bank functionality which is a measure of suitability for manufacturing.

Table 2: Master Cell Bank Testing

SAFETY TESTING	CHARACTERIZATION PANEL
1. Sterility	**1. *In vitro*** - PA - Cytokine profile - MTT - Cell purity - Histology/morphology
2. Mycoplasma	
3. Viral - *In vitro* virus (by TEM and RT) - *In vivo* viral assay - Specific human viral testing (N=14) - Porcine viruses - Bovine viruses	**2. *In vivo*** - IHC - Graft take and integration - Graft contraction - Graft morphology
4. Neoplastic Safety - Karyology and isoenzyme - *In vivo* tumorigenicity - Senescence	
Abbreviations: IHC, immunohistochemitry; MTT, mitochondrial tetrazolium testing; PA, percutaneous absorption; RT, reverse transcriptase; TEM, transmission electron microscopy. Source: data on file at Organogenesis.	

Table 3: Working Cell Bank Testing

SAFETY TESTING	ANALYTICAL TESTING
1. Sterility	**1. Identity** - Involucrin (HEP) - Collagen biosynthesis (HDF)
2. Mycoplasma	
3. Viral - *In vitro* viral - *In vivo* viral	**2. Functionality** - Cell viability - Cell growth - Histology/morphology
Abbreviations: HDF, human dermal fibroblast; HEP, human epidermal progenitor. Source: data on file at Organogenesis.	

3.3 Apligraf (oral) Release Testing

Apligraf (oral) will be released for treatment based on a combination of in-process test results and final product testing results are available at the time of shipment. Data will be accumulated from multiple quality control tests performed at critical steps throughout the manufacturing process.

The following tests are conducted for product release:

1. Potency
2. Purity (endotoxin)
3. Visual inspection
4. Safety testing
    - In-process bioburden
    - In-process sterility
    - In-process mycoplasma
    - Final product bioburden
    - Final product sterility

Final sterility, bioburden and mycoplasma test results are reported post-distribution of the product, because the time it takes to complete then test, exceeds the 15-day shelf-life. To mitigate the risk of microbial contamination, in-process testing occurs throughout the manufacture of Apligraf by analyzing the fresh and spent media used in the manufacturing process. A maximum of 90 in-process samples taken at up to

15 manufacturing steps are tested throughout the manufacturing process. The testing of the final product is balanced against loss due to destructive testing.

3.3.1 Potency Assay (Morphological Assessment)

At product maturity, 2 units from a lot size of 200 units are assessed for potency. Three biopsy punches are taken from each of the 2 units and thus, a total of 6 samples are evaluated for potency. During product development of Apligraf, it was determined that histological assessment of the mature product was the most appropriate measure of product potency (discussed in further detail below). The potency of Apligraf includes the 9 parameters and is measured by a morphological (also known as 'histological') assessment of the mature product. The morphological assessment is performed to determine the microscopic anatomy of the final product's cells and tissue with regard to orientation and geometry. Apligraf is required to have a specific and well-defined structure in order to function properly in the clinic.

The structure and function of Apligraf are intimately linked and depend upon the correct development of the lower and upper layers during manufacture. The well-developed lower layer (dermal matrix) is necessary for correct development of the upper (epidermal) layer, which in turn is essential for the functionality of the product. The formation of the 3 aspects of Apligraf (lower layer, upper layer and mature product) as depicted in **Figure 5** are evaluated and confirmed by morphological assessment (ie, potency). The potency assay includes 9 parameters that are evaluated both quantitatively and qualitatively to specifically examine the characteristics indicative of correct manufacture and formation of a viable and functional Apligraf.

The potency assay utilizes H&E staining to differentiate between fibroblasts and collagen within the lower layer (dermal matrix), as well as between keratinocytes within the upper (epidermal) layer. The stains also clearly differentiate the basophilic and eosinophilic cellular structures within the tissue layers. The potency assay is based on the USP <Graftskin[i]> monograph and provides quantitative validated measures of histological parameters to ensure potency of Apligraf. Furthermore, analysts undergo an extensive training program of at least 6 months in duration, and must pass a rigorous exam prior to being qualified for scoring of morphological sections for Apligraf for release. The

[i] "Graftskin™" was the original name for Apligraf therefore earlier clinical development will refer to Graftskin instead of Apligraf. Furthermore Apligraf has been clinically studied for the treatment of acute oral mucosa defects under the trade name "CelTx™".

morphological assay has been validated in accordance with International Conference on Harmonization (ICH) guidelines for method validation.

After initial examination of the product sample slide, the process of scoring the slides for all 9 morphological aspects of the potency assay is initiated. The elements that are examined, scored and recorded for each of these aspects are summarized below relative to the respective histological representations derived from USP <Graftskin> monograph.

Epidermal Coverage:

The percentage of the surface of the dermal matrix present on the slide, which is covered by epidermis, is determined.

Epidermal Development:

The 20x objective is used to determine the percentage of acceptable epidermal development as define by a basal layer of keratinocytes of cuboidal-columnar shape, 5 or more stratified suprabasal layers and ≥ 1 cornified squamous cell layer on the apical surface.

Basal Aspect:

The 20x objective is used to determine the percentage of the epidermis present on the slide containing basal keratinocytes with a basophilic cytoplasm, lacking severe vacuolization or necrosis.

Suprabasal Aspect:

The slides are examined for the following:

- Pink cytoplasm
- Pink nucleus
- Severe vacuolization
- Necrosis

The percentage of the suprabasal keratinocytes containing basophilic cytoplasm without vacuolization, necrosis or pyknosis (non-viable) is determined.

Dermal Thickness:

The 20x objective is used to evaluate the thickness of the dermal matrix in 5 randomly selected fields across the length of the specimen is determined. The mean thickness of all 5 fields is calculated.

Fibroblast Density:

The 40x objective is used to calculate the mean fibroblast density in 5 randomly selected fields of the dermal matrix present on the slides. Pyknotic nuclei (non-viable) are not included in the count.

Matrix Aspect:

The percentage of dermal matrix collagen present on the slide that stains uniformly without large holes or inclusions is determined.

Table 4 lists the release specification for the potency assay.

Table 4: Potency Release Specifications for Apligraf Final Product

MATERIAL TESTED	SPECIFICATION
Mature Apligraf (at 20- 24 days from start of manufacturing)	Epidermal Coverage: ≥ 95% Epidermal Development*: ≥ 70% Basal Cell layer: ≥ 95% Suprabasal Cell layer: ≥ 80% Dermal Matrix Thickness: ≥ 40 µm Fibroblast Density**: ≥ 4 fibroblast nuclei per field Matrix Aspect: ≥ 95% uniform stain without holes or inclusions
*The epidermal development data element includes 2 independent aspects of functional development each of which have the following specific acceptance criteria: ≥ 5 stratified suprabasal layers and ≥ 1 cornified squamous cell layer. **The fibroblast density data element includes an assessment of viability by excluding pyknotic cells from the density determination. Source: data on file at Organogenesis.	

3.3.1.1 Characterization Panel and Additional Studies to Support Potency Assay Development and Justification

Underpinning the potency assay (ie, determined by morphological assessment) are characterization tests (see **Table 5**) that directly to support the essential biological functions of Apligraf, namely barrier function and viable cells that produce growth factors/cytokines. The characterization panel (**Table 5**) is also performed each time a new

master cell bank is introduced for the manufacturing process. These tests are designed to confirm that such changes do not impact functionality of the product and ensure the relevant biological properties of Apligraf are not altered by these changes. The functional tests in the characterization panel tests are linked to the structural characteristics evaluated during the potency assay (ie, morphology), and that same assay is performed as part of the characterization panel. As stated previously, the morphological assessment represents 9 parameters that are evaluated both quantitatively and qualitatively. These parameters relate to specific attributes that collectively ensure potency of the final product and are discussed herein.

This potency assay has been used for the past 13 years for the release of Apligraf for the approved cutaneous indications.

Table 5: Characterization Panel

TEST	FUNCTION	SPECIFICATION
***In vitro* Assays**		
Percutaneous water absorption	Measures extent of barrier function. Indicates level of epidermal differentiation	≤ 1.97% penetration/hour
Cytokine expression (expression of PDGF-α, TGF-β1, IL-4 and IL-1α	Determines presence of key wound healing cytokines and absence of inflammatory mediator	Presence/Absence of mRNA bands: PDGF-α Present TGF-β1 Present IL-1α Present IL-4 Absent
MTT assay	Determine overall viability cells of product by assessing total mitochondrial activity	Absorbance ≥0.237
Cytokine production (VEGF detection in maintenance medium Day 10 Post-Air Lift)	Indicates appropriate epidermal development, which is indicative of the correct differentiation pattern of keratinocytes	≥ 100 pg/ml
Table 5 continues to the following page…		

Table 5: Characterization Panel (continued)

TEST	FUNCTION	SPECIFICATION
***In vitro* Assays (continued)**		
Potency Assay via histological assessment*	Product morphology using lot release histological parameters for dermal and epidermal layers	Epidermal Layer: • Epidermal coverage ≥ 95% • Epidermal development ≥ 70% • Basal keratinocyte aspect ≥ 95% • Suprabasal keratinocyte aspect ≥ 80% Dermal Layer: • Dermal matrix thickness ≥ 40µm • Fibroblast density ≥ 4 nuclei/field • Matrix aspect ≥ 95% uniform stain
***In vivo* Athymic Mouse Graft Model**		
Pre-graft morphology	Morphological analysis of Apligraf (oral) integration into athymic mouse skin	Comparable 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	Graft take and integration comparison between test and control articles.
***In Vivo* Athymic Mouse Graft Model**		
Graft contraction	Morphological analysis of Apligraf integration into athymic mouse skin	Graft contraction comparison between test and control articles
Table 5 continues to the following page…		

Table 5: Characterization Panel (continued)

TEST	FUNCTION	SPECIFICATION
***In Vivo* Athymic Mouse Graft Model (continued)**		
Graft morphology • Epidermal layer • Dermal layer • Graft integration and remodeling	Morphological analysis of Apligraf (oral) integration into athymic mouse skin	Comparable to control in: • Integrity • Organization • Viability of epidermal layer Comparable to control in: • Fibroblast viability • Fibroblast morphology • Fibroblast density Comparable to control in: • Edge integration • Center integration • Epidermal coverage • Damage due to dressing slippage • Vascularization • Degree of vascularization • Cellularity
IHC	Anti-human involucrin staining	Expression of human involucrin confirms the epidermal cells are human

Abbreviations: IHC, immunohistochemistry; IL, interleukin; MTT, mitochondrial tetrazolium testing; PDGF, platelet-derived growth factor; TGF, transforming growth factor; VEGF, vascular endothelial growth factor.

*Keratinocyte and fibroblast viability assessment is included in functional assessments of the epidermal and dermal layer.

**Control group are FDA approved HDF and HEP cell strains used to create the Apligraf unit.

Source: data on file at Organogenesis.

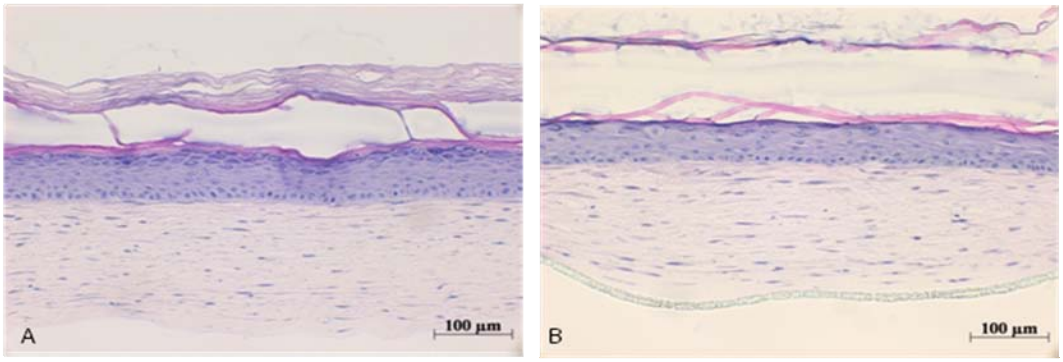
Percutaneous Water Absorption: Link to Stratum Corneum Development

A mature and complete cornified upper layer presents a barrier to transmission of water through the skin. Percutaneous absorption (PA) of water is considered to be a measure of the functionality of the stratum corneum and is therefore a measure of the maturity and completeness of the cornified upper layer. Two studies are presented below that provide data to support the relationship between Apligraf maturation barrier function as measured by PA and potency. In the first study (Parenteau, 1996, *Biotech and Bioeng)*, PA of Apligraf sampled at different time points during the cornification stage (ie, maturation of the upper layer). In order to determine how PA varied with increasing maturity of Apligraf. After the first 4 days in the development stage of the cornified layer, the barrier function had not yet developed. The barrier function changed rapidly over the next 5 and

8-9 days of maturation. After 9 days, the PA had dropped significantly and was seen to stabilize at 30 cm/h which is much higher than normal human skin [$1x10^{-3}$ cm/h (Gay. 1992, *Toxic In Vitro*)]. Further maturation of the cornified upper layer beyond Dady 9 did not lead to any significant improvement in the PA. This study established the relationship between time in culture and development of barrier function in Apligraf.

A subsequent study[j] was performed to demonstrate the relationship between PA and aspects of the potency assay related to development of the upper layer (epidermal coverage and development). Apligraf was manufactured from different keratinocyte working cell banks (HEP012B and HEP074C[k]) that were from two different master cell banks. Of note, the HEP012B cells were shown to generate poor quality Apligraf construct due to poor growth kinetics and differentiation capacity. Both the PA and potency assays were performed on Apligraf units generated from these 2 HEP cell banks to evaluate the extent of barrier function relative to epidermal coverage and development. The results showed that the HEP012B cell strain failed the PA assay specification as well as the epidermal development specification; while HEP074C passed the PA specification and epidermal development. Analysis of the histology of Apligraf generated from the HEP074C cell strain produced constructs that were comprised in all aspects important for barrier function, that is, a well-stratified epidermal layer (**Figure 9A**), while the HEP012B cell strain clearly lacks many of the required attributes indicative of a fully matured epidermal layer important in imparting barrier function, specifically, continuous basal cell alignment and well-stratified suprabasal layers (**Figure 9B**).

Figure 9: Representative Hematoxylin & Eosin (H&E) Images of Apligraf Units Generated from HEP074C (A) and HEP012B (B) Cell Strains



[j] Data on file at Organogenesis: RDR110124A_KD.
[k] HEP012 and HEP074 were FDA approved cell banks but working cell bank HEP012B was not released into production while HEP074 was released into product.

Collectively, these studies demonstrate the direct relationship between PA and epidermal coverage and the development aspect of the potency (morphological) assay for the mature product.

Cytokine Expression*: Link to Epidermal Development*

The expression of a range of cytokines is used to confirm that the fibroblast and keratinocyte cells used in the generation of Apligraf maintain their capacity to express key growth factors/cytokines (eg, IL-α, PDGFα and TGF-β1) that are secreted by healthy and metabolically active keratinocytes and fibroblasts grown in a bilayered tissue engineered configuration.

Table 6 provides summary of several released keratinocyte master cell banks that shows the relationship between the expression of 4 key cytokines and the epidermal development aspect of potency. The data demonstrate that each cell bank that met the acceptance criteria for cytokine expression also met the epidermal development aspect of potency for the mature product.

Table 6: Comparison of Cytokine Expression Results with Epidermal Development

LOT NO	PDGF-α	TGβ-1	IL-1α	IL-4	EPIDERMAL DEVELOPMENT
Specification	**present**	**present**	**present**	**absent**	**(≥70%)**
HEP066	present	present	present	absent	89 ± 6
HEP074	present	present	present	absent	97 ± 2
HEP072	present	present	present	absent	94 ± 1
HEP081	present	present	present	absent	97 ± 1

Abbreviations: IL, interleukin; PDGF, platelet-derived growth factor; TGF, transforming growth factor
Note: Representative HEP cell strains are included from recent bioequivalence studies (mean ± SD, N=4 units)
Source: data on file at Organogenesis : RDR110124A_KD

Mitochondrial Tetrazolium Testing (MTT) Assay: Link to Cell Viability

The viability of Apligraf is determined as part of the potency assay through evaluation of the basal and suprabasal layers within the upper layer (epidermis) of the unit to ensure that basophilic cytoplasm (histological hallmark of viable cells) is present without vacuolization or necrosis. The exclusion of pyknotic nuclei from the fibroblast count is also evaluated under the fibroblast parameter density in the potency assay.

The MTT assay is commonly used to measure the biochemical activity of viable cells and is an indicator that the cells are actively metabolizing; MTT is included as part of the characterization panel (see **Table 5**), to confirm the metabolic activity of Apligraf.

While the potency assay identifies cells within the layers of the construct that are necrotic and/or non-viable, the MTT assay measures the overall biochemical activity of the entire Apligraf unit. Study RDR101208A_ACBR evaluated the relationship between the cellular viability of Apligraf as measured by MTT and the potency assay. The relationship between the assays was established utilizing compromised packaged Apligraf beyond the usable shelf-life. Beyond expiration, the structural and functional attributes of packaged Apligraf are expected to be below the targeted specifications.

As expected, the results showed that expired units failed the morphological assessment for basal and suprabasal aspects. Furthermore, MTT results were consistent with the morphological results, which failed the established MTT acceptance criteria. The results provided in **Table 7** show the correlation between metabolic activity and cellular viability as measured by MTT and histology.

Table 7: Comparison of MTT Assay Results with Histological Attributes of Cell Viability in the Upper (Epidermal) Layer

TIME POINT	APLIGRAF LOT#	AVERAGE MTT RESULT Spec ≥ 0.237	AVERAGE BASAL ASPECT Spec ≥ 5%	AVERAGE SUPRABASAL ASPECT Spec ≥ 80%
16 days post-packaged	GS101028.02	0.223	87.8	26.7
16 days post-packaged	GS101028.03	0.180	84.7	23.2

Abbreviations: MTT, mitochondrial tetrazolium testing
Source (data on file at Organogenesis): RDR101208A_ACBR

VEGF Production: Link to Epidermal Development by Viable Keratinocytes

VEGF has been shown to be a highly specific mitogen for endotheial cells, and shows angiogenic properties *in vivo* (Leung, 1989, *Science*; Gospodarvicz, 1989, *PNAS*). *In vitro* studies have shown that quiescent keratinocytes express low levels of VEGF mRNA. However, during normal wound healing, studies have shown that there is a rapid induction of VEGF expression *in vivo* (Frank, 1995, *J Bio Chem*) with the exception of a few mononuclear cells, VEGF mRNA is detected primarily in keratinocytes at the wound edge and from those keratinocytes that have migrated to cover the wound surface

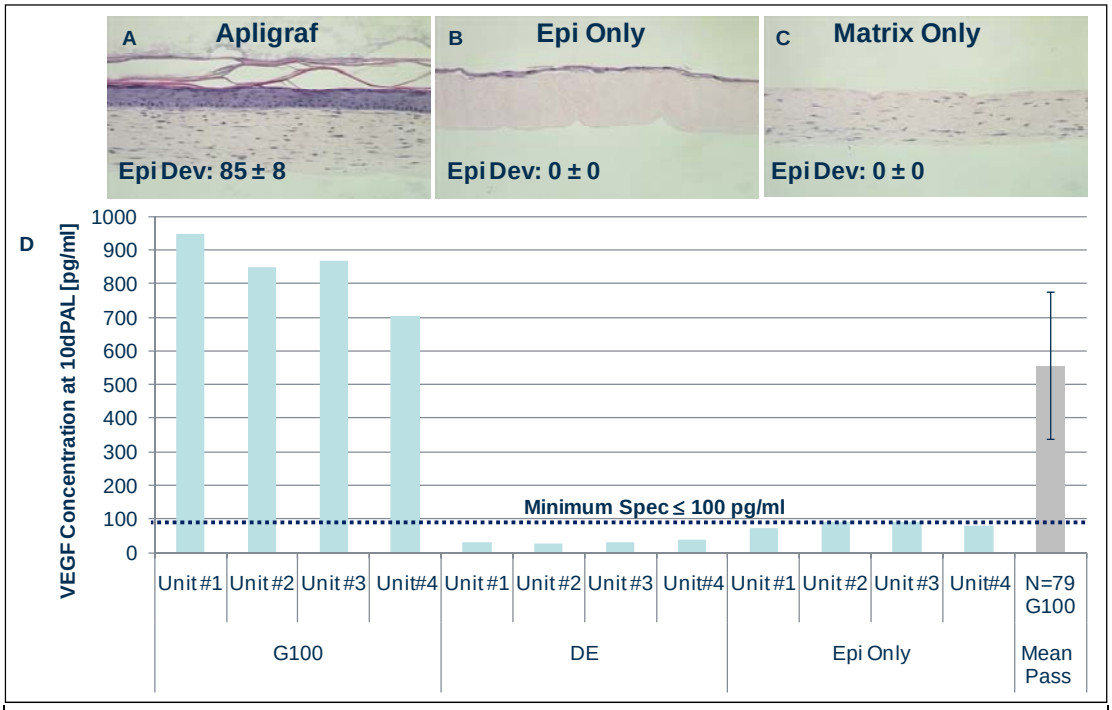
(Brown, 1992, *J Exp Med*). Taken together, keratinocytes represent a major source of VEGF in the wound and therefore, essential in wound angiogenesis. Furthermore, their ability to produce different levels of VEGF appears to be modulated by their functional state (quiescent vs. migratory, etc). As such, VEGF was chosen as a candidate cytokine biomarker to investigate during Apligraf manufacture to determine relevance to epidermal development by keratinocytes.

A development study[l] has shown that VEGF can be detected in the spent media collected at each medium change during the manufacturing process. VEGF production increases from epidermalization to differentiation and peaks during the maturation of the cornified layer. VEGF levels then decrease as the product reaches maturity. This VEGF production profile was shown to be consistent across multiple production lots (N=25) and appears to be linked to the biological state of the keratinocytes. This state is influenced by the different media compositions as well as interactions with the underlying fibroblasts embedded in the collagen matrix. These results show that the keratinocyte developmental state (migratory/proliferative vs. differentiated/quiescent) correlates to VEGF production of different levels. These findings are supported by *in vitro* studies showing that VEGF expression is modulated by keratinocyte functional state (Frank, 1995, *J Biol Chem*).

A subsequent study[m] has shown that Apligraf that passes the potency release assay produces an average VEGF concentration ≥ 100 pg/mL. In these studies, compromised product was created deliberately, such that either cell type was absent, leading to lack of a lower (dermal) layer or upper (epidermal) layer. In these compromised units the concentration of VEGF measured at maturity was significantly lower than those typically obtained with product that passes the potency (morphological) assay (**Figure 10**).

[l] Data on file at Organogenesis: Reference: RDR100720A_SZDB.
[m] Data on file at Organogenesis: Reference: RDR091117A_KDDB.

Figure 10: VEGF Production from Compromised and Standard Apligraf Units at Day 20 in Culture



Definitions: DE = matrix (fibroblasts only), Epi only = epidermal layer (keratinocytes only). Representative H&E stained images for Apligraf at 20 days in culture (A) Apligraf (control), (B) upper layer only and (C) lower layer only. Note that in (B) and (C) there is a lack of proper epidermal development, as compared with (A) control; the epidermal development specification of >70% is not met. (D) Graph showing the quantification of VEGF from N=4 units per group.

Source data on file at Organogenesis: RDR091117A_KDDB

The morphological appearance of these compromised units demonstrated a complete lack of epidermal development as scored by histological assessment. In this study, the VEGF concentration detected in the control Apligraf group was comparable to the average VEGF concentration (approximately 500 pg/mL) collected from 79 commercial Apligraf lots that passed the potency release assay.[n]

While the quantification of the VEGF content of the medium at product maturity provides a biological indication of the degree of development of the product, the potency assay based on the morphological assessment of the 9 parameters provides a higher degree of sensitivity when there are changes to the manufacturing process that may impact potency of the product. As such, histological assessment remains the most appropriate measure of potency for Apligraf.

[n] Data on file at Organogenesis: RDR100720A_SZDB.

Additional Characterization Study (Viability Assessment)

In order further validate the ability of the potency (morphological) assay to delineate live vs. dead cells; a direct measurement of cell viability (viable cell counts) was used in an experimental design in which the Apligraf units were challenged.[o] Stressed Apligraf units were created by devitalizing the cells within the unit and compared with control (ie, non-stressed) units. Biopsy samples were collected from each unit and cell viability was analyzed by the potency release assay and trypan blue exclusion. Results from this study showed that the control units passed all aspects of the potency (morphological) assay, whereas the stressed units failed 3 of the morphological aspects studied. Although the bilayered morphology of the overall matrix remained intact in the stressed units, failure in any aspect of the potency assay would have resulted in release failure. Cell viability test results of the upper and lower layers demonstrated that the stressed units exhibited at least 30% cell death relative to the control. Importantly, the basal and suprabasal layers and the fibroblast density aspects of the potency assay also revealed the state of cell viability in the stressed units.

Summary

In conclusion, the individual attributes of the potency assay are designed to confirm the correct assembly of the product as an inevitable consequence of the growth and maturation of the two cell types and their biological interactions. The functionality of the product is inextricably linked to its structure and viability as demonstrated by the studies described above. In the absence of correct proportions of fibroblasts and keratinocytes, the upper layer (epidermis) fails to develop correctly. Normal cellular activity, as denoted by VEGF production, is severely reduced by absence of the correct cell types and matrix formation.

The potency assay permits a 2-dimensional analysis of each of the key attributes important in substantiating the full maturation state of Apligraf. These attributes of potency have been used for product release for Apligraf (cutaneous) over the last 13 years for the treatment of DFUs and VLUs, and has proven to be essential for clinical efficacy. This includes appropriate and complete epidermal development, which leads to an intact stratum corneum, which in turn is important in imparting a proper barrier function.

The characterization panel described within this section provides evidence of the essential biological functions of the product relative to the potency assay (see **Table 8**).

[o] Data on file at Organogenesis: RDR110209A_KD.

As such, the morphological assay has been established as an appropriate measure of Apligraf potency and passing this test is required for product release.

Table 8: Biological Relevance of Potency Assay

PRODUCT MATURATION	MANUF. STEP	BIOLOGICAL PROCESS	BIOLOGICAL ATTRIBUTE	HISTOLOGICAL PARAMETER	FUNCTIONAL ASSAYS UNDERPINNING HISTOLOGICAL PARAMETER
Development of Upper (Epidermal) Layer	Development of stratum corneum	Formation of cornified squamous layer	Complete stratum corneum capable of providing barrier functionality	Epidermal Coverage (≥95%)	PA assay – determines barrier integrity/functionality
	Proliferation and differentiation of HEPs	Formation of viable epidermal layer	Basal and suprabasal layers within the epidermis of the unit contain basophilic cytoplasm (histological hallmark of viable cells) without vacuolization or necrosis.	Keratinocyte viability: (keratinocyte aspect) Basal cell layer: measured by vacuolization or necrosis (≥95%) Suprabasal: measured by vacuolization or necrosis (≥80%)	MTT assay
		Formation of epidermal layer with correct architecture	Basal layer with columnar-cuboidal morphology, clearly distinguished stratified suprabasal layer	Epidermal development: basal layer of keratinocytes with a cuboidal-columnar shape, with ≥5 stratified suprabasal cell layers and ≥1 cornified squamous cell layer	VEGF assay *in vivo* graft morphology assessment

Table 8 continues to the following page…

Table 8: Biological Relevance of Potency Assay (continued)

PRODUCT MATURATION	MANUF. STEP	BIOLOGICAL PROCESS	BIOLOGICAL ATTRIBUTE	HISTOLOGICAL PARAMETER	FUNCTIONAL ASSAYS UNDERPINNING HISTOLOGICAL PARAMETER
Development of Lower (Dermal) Matrix	HDFs contract collagen gel	Presence of viable HDFs induces re-organization and contraction of collagen gel. Production of adequate numbers of HDFs to allow appropriate cross-talk with HEPs	Viability of fibroblasts	Fibroblast viability (pyknotic cells excluded from density determination)	MTT assay
			Unit contains a sufficient number of viable fibroblasts	Density (mean number of nuclei) ≥4	*in vivo* graft morphology assessment
	Proliferation of HDFs to produce dermal matrix		Indicative of the ability of viable functional fibroblasts to contract the collagen gel and populate the gel matrix during the manufacture of the product.	Dermal layer thickness ≥40 µm	Growth index as determined by total viable cells at gel cast and final product stage*
			Ensures that dermal matrix is free of occlusions, rips, tears, or holes	Matrix aspect (≥95%)	*in vivo* graft morphology assessment

Abbreviations: HDF, human dermal fibroblast; HEP, human epidermal progenitor; MTT, mitochondrial tetrazolium testing; PA, percutaneous absorption; VEGF, vascular endothelial growth factor.

* Assay is not performed on a routine basis or as part of the characterization panel.

Source: data on file at Organogenesis.

3.3.2 Purity (Endotoxin)

Purity of Apligraf is assessed by measuring endotoxin per USP <85>. Endotoxin testing is performed on the final product samples prior to shipment and is a release test for Apligraf. Three Apligraf units (1 from the beginning, middle and end of the packaged lot) are tested for endotoxin content. The endotoxin test specification is ≤ 0.5 EU/mL. Endotoxin is defined as a purity assessment under 21 CFR 610.13

3.3.3 Visual Inspection

One hundred percent (100%) visual inspection is performed on all packaged units just prior to final labeling and release. First, the lot number and packaging data are verified as correct and legible. The in-process label is first verified to be correct and accurate relative to the lot number and packaging date. Packaged Apligraf is then visually examined for evidence of microbial growth, visual anomalies, holes, delamination and bubbles. Visual guidelines are utilized for assessing visual anomalies and holes. Delamination is assessed using a traceable exclusion tool to determine the minimum usable area of product which must be greater than 55 mm in diameter. Bubbles are assessed based on the established limits. Examination for foreign matter and syneresis (excessive liquid expelled from agarose shipping medium and/or condensation) is also performed. The pH of the agarose shipping medium is verified to be within the specified range, indicated by the reference pH chart. Seal integrity of the secondary packaging (polybag) is checked for gross defects and/or leaks. Any individual units failing to meet these criteria are rejected, segregated from the lot and discarded. If the number of individual rejects exceeds the maximum allowable limit, as a percentage of the packaging lot size, an out-of-specification investigation is initiated.

3.3.4 Bioburden

Bioburden safety testing is conducted per USP <61> with the exception that samples are held for 7 days of observation, rather than 5 days as required per USP <61>, to allow for the possibility of slow growing or injured microorganisms.

Bioburden Testing for In-Process Samples

Bioburden testing is performed on cornification spent media at Day 17 of the production process and again immediately prior to final packaging using ship rinse spent media.

Bioburden Testing of Final Packaged Product

Three (3) Apligraf units, 1 from the beginning, middle and end of the packaged lot, are submitted for bioburden testing. The bioburden test specification is 0 CFU/sample.

3.3.5 Sterility

Sterility safety testing is used to assess the microbial attributes of the product at critical stages of the manufacturing process. For sterility testing, the primary method of detection for in-process liquid samples (eg, cells, fresh and spent media) is BacT/ALERT 3D Rapid Microbial Detection System. Sterility of the final packaged (3 units/packing lot) product

is assessed according to current USP <71>, which can also be used as an alternative to the BacT/ALERT 3D Rapid Microbial Detection System for in-process samples. The BacT/ALERT 3D Rapid Microbial Detection System has been validated for a 7-day incubation period and implemented as an equivalent to USP <71>. The sterility test specification is Negative.

3.3.6 Mycoplasma

Mycoplasma safety testing is performed as an in-process test during Apligraf manufacturing and is used to release Apligraf. It is performed on Apligraf units that have passed morphological analysis to ensure that no adventitious mycoplasma was introduced during processing. Spent maintenance media is sampled from 3 units and to a shipped contract testing facility. The mycoplasma test specification is Negative.

3.3.7 In-Process Microbial Testing

Table 9 summarizes the microbial tests performed at each critical step of the Apligraf manufacturing process, in order to mitigate the risk of microbial contamination.

Table 9: Comprehensive Summary of Microbial Testing of Apligraf

MATERIAL TESTED	ASSAY	SPECIFICATION	DAY SAMPLED	FINAL RESULT AVAILABLE AT SHIPMENT*
Acellular Cast Mix	Sterility	Negative	0	Yes
Cellular Cast Mix			0	
HDF Cell Residue			0	
HEP Residue			6	
Differentiation Spent Media			10	
Cornification Spent Media II			15	Yes if shipment at Day 22-29.
Cornification Spent Media III			17	Yes if shipment at Day 24-29.
Cornification Spent Media III	Bioburden	0 CFU		
Maintenance Spent Media I	Mycoplasma	Negative	20	No
Maintenance Spent Media I Maintenance Spent Media II Maintenance Spent Media III Maintenance Spent Media IV	Sterility	Negative	20-29	No
Ship Rinse Spent Media	Bioburden	0 CFU	20-29	No

Abbreviations: HDF, human dermal fibroblast; HEP, human epidermal progenitor.
*Based on 7-day BacT/ALERT 3D sterility test results, which is the primary test method. Results available at the time of shipment may be reduced if 14-day USP <71> testing is performed.
Source: data on file at Organogenesis.

3.4 Mechanism of Action (MOA)

Although the exact mechanism of action of Apligraf is unknown, it appears that its wound healing properties result from both from providing a barrier and from the delivery of healthy viable cells. These cells secrete growth factors and cytokines that improve and modulate the process of secondary intention healing.

As applied to the surgically created site, Apligraf provides services as a wound covering to facilitate a moist wound healing and to protect from trauma. This barrier function, demonstrated *in vivo*, was investigated during the initial development of Apligraf by grafting product at various developmental stages onto athymic mice (Parenteau, 1996, *Biotechol Bioeng*) and monitoring the survival of the graft. The results indicated that the survival of the product was dependent upon the length of the maturation stage (days in culture), indicating that there is a minimum requirement for development of the barrier function. This early work showed that barrier function imparted by Apligraf is largely attributed to its structural organization, which is clearly assessed by histological technique.

Characterization studies have also shown that the HEPs and HDFs present within Apligraf synergistically interact to produce many of the signaling molecules that are involved with the various phases of wound healing (ie, hemostasis, inflammation, proliferation, and remodeling) (Brem, 2003, *Surg Technol Int*). For instance, IL6, IL8, GM-CSF, and TNFα are secreted by Apligraf.[p] Nonclinical (*in vitro*) data generated to-date confirms that the factors produced by Apligraf are bioactive as evidenced by *in vitro* assays of cell migration, angiogenesis.[q,r] Taken together the presence of viable cells produce factors that can stimulate biological function important in proper wound healing. The interaction with Apligraf in the oral cavity with the patients' cells has been demonstrated to produce physiologic levels of angiogenic cytokines (Morelli, 2010, *J Dent Res*).

Apligraf's ability to provide a barrier function and release cytokines and growth factors relevant to wound healing contribute to its multimodal mechanism of action. Apligraf does not persist and effects healing by modifying the behavior of the patient's own cells. The net effect is that Apligraf's attributes function to modulate and improves wound healing by secondary intention to regenerate site-appropriate tissue.

[p] Data on file at Organogenesis: RD100720A_SZDB.
[q] Data on file at Organogenesis: RD110214_KDSO.
[r] Data on file at Organogenesis: RD091117A_KDDB.

4 NONCLINICAL

4.1 Nonclinical Overview

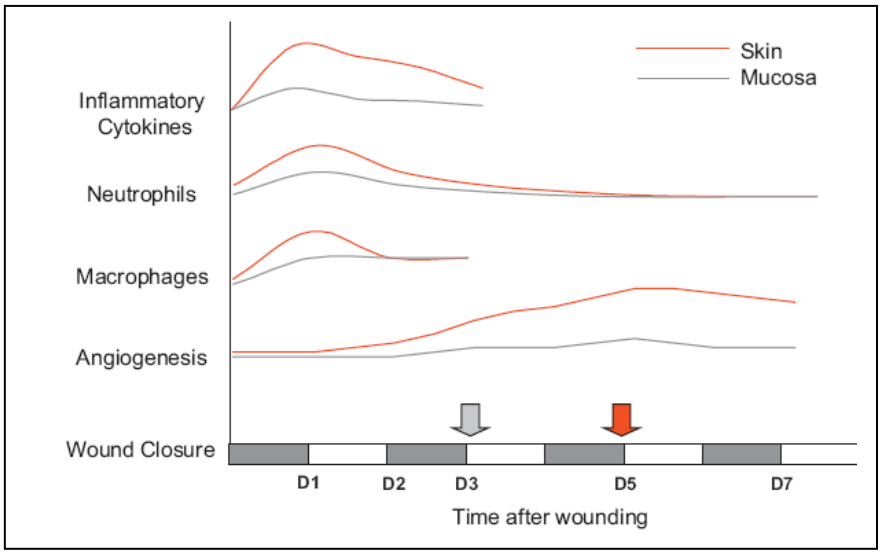
Apligraf was approved by the FDA/CDRH as a Class III medical device for the treatment of VLUs in 1998 and DFUs in 2000. Thus the nonclinical and clinical development programs for Apligraf (oral) were conducted under the IDE pathway, with the assumption that this product would also be marketed as a medical device. Nonclinical work was conducted to exclude major product risks (eg, cell bank purity and safety testing); these studies were comprised of product characterization, cell persistence/migration, immunotoxicity, ISO toxicity testing, and drug interaction with common periodontal dressings/anti-microbial rinses.

Nonclinical studies in the oral application have not been performed to date because the differences between oral mucosa and skin suggest that the data generated in the context of the cutaneous indications should be adequate to established safety for purpose of the oral indication. Both oral mucosa and skin consist of epithelial and connective tissue layers with the predominant cell types being epithelial cells and fibroblasts. Both oral mucosa and skin are physically and functionally similar: oral mucosa is composed of epithelium and lamina propria (connective tissue) resting on top of submucosa or periosteum; and skin is composed of an epithelium (ie, epidermis) and dermal layer resting on a subcutaneous layer. The lamina propria in the oral mucosa is physically analogous to the dermis of the skin and the submucosa of the oral mucosa is physically analogous to the subcutaneous tissue of the skin.

Wound healing in the oral mucosa and the skin also are very comparable: both tissues are subject to the same stages of healing, ie, hemostasis, inflammation, proliferation and remodeling and both require the same elements for the healing process to proceed (ECM, specific cell types and growth factors) (Enoch, 2008, *Oral Surgery*; Szpaderska, 2003, *J Dent Res*; Warburton, 2005, *Wound Rep Reg*). The different cell populations that migrate to the wound site are remarkably similar: platelets, neutrophils, macrophages, endothelial cells; and the growth factors and inflammatory cytokines that facilitate the process are basically the same (PDGF, VEGF, IL-6, IL-8, LTPB-1, fibromodulin, FN-ED, TNC) (Szpaderska, 2003, *J Dent Res*; Wong, 2009, *Wound Rep Reg).* Subtle differences in the kinetics and quantity of cytokine production and cell migration however, do provide the oral mucosa an advantage with regard to the speed of healing and relative absence of scarring. Szpaderzka *et al* for instance, showed in a mouse model of wound healing that although the kinetics of cytokine production in both types of wounds are basically

superimposable when comparing mRNA levels of IL-10, IL-6, KC (mouse version of IL-8), TGF-β, LTPB-1 and fibromodulin, the levels of IL-8, IL-6 and TGF-β were roughly half in the oral wounds (Szpaderska, 2003, *J Dent Res)*. Additionally, the presence of macrophages, T cells and neutrophils was shorter lived in the oral mucosa than in the skin. Other subtle differences have also been shown when comparing the amount of angiogenesis and the levels of VEGF, which are less abundant in oral wounds (Szpaderska, 2005, *J Dent Res)*. A study comparing gene expression in oral versus cutaneous wounds in an established mouse model suggests that the gene expression patterns are similar, but not identical (Chen, 2010, *BMC Genomics*). **Figure 11** illustrates the time after wounding in relation to inflammatory cytokines, neutrophils, macrophages, and angiogenesis for mucosa and skin. Overall, oral mucosa wounds, as illustrated in **Figure 11** heal more rapidly than skin. In general and as suggested in **Figure 11**, wound response for mucosa is more rapid than skin suggesting that mucosa heals in a less complex manner than skin wounds. Overall the similarities outweigh the differences and conceivably both wound types should benefit from the same therapeutic approach.

Figure 11: Time Line of Specific Aspects of Wound Healing in Mucosa and Skin



Source: Chen, 2010, *BMC Genomics*; Figure 7.

Tabular summaries of the Apligraf nonclinical program are provided in **Appendix 1**. In general nonclinical results do not suggest a potential for immunological, toxicological or oncogenic risks. An overview of the Apligraf nonclinical program is provided herein.

4.1.1 Pharmacology

Several different *in vitro* assays and *in vivo* models have been developed to evaluate the functionality and physical properties of Apligraf. The assays and models employed in these studies were selected for their ability to evaluate the development, characterization, immunological and microbiological aspects of Apligraf.

The majority of the *in vivo* pharmacological testing of Apligraf was primarily performed in the athymic mouse acute wound model. The athymic (nu/nu) mouse is a strain with a genetic mutation that causes a compromised or absent thymus resulting immunodeficiency characterized by a lack of mature T cells. Apligraf performs as a skin graft in this model. This means Apligraf heals wounds through primary intention in this animal model. In contrast, Apligraf's predominate role in the clinic is as a stimulus for healing by secondary intention. Vascularization, (via angiogenesis) as characterized in the athymic mouse model, relates to graft take (healing by primary intention) and not to granulation tissue formation, which would be observed in the context of healing by secondary intention. Characterization of angiogenesis in the athymic mouse model is nevertheless important as it helps to better understand the quality of the Apligraf. More specifically this athymic (nu/nu) mouse model evaluates the following:

- The ability of the upper layer of the Apligraf construct to maintain a well-stratified epithelium (ie, sustain the structure of upper layer), which is directly linked to the robustness of the keratinocyte cells used in Apligraf production.[s]
- The ability of the lower layer (fibroblasts within the collagen gel) to effectively sustain graft contraction. Graft contraction is indicative of the ability of the host cells to interact efficiently with the collagen gel layer and is a normal occurrence in wound healing.
- The ability of the host cell tissue to integrate fully with Apligraf at the wound (ie, graft-take), which is a direct consequence of the robustness of both the keratinocytes and fibroblasts used to generate the final product.

Extensive testing was conducted to characterize and understand the morphologic development and maturation of Apligraf *in vitro* during the different stages of the manufacture of Apligraf.[t] The epidermis undergoes a sequence of morphologic changes during development and maturation which consist of changes in basal cell proliferation,

[s] Data on file, Organogenesis. RDR11010124A_KD.
[t] Data on file, Organogenesis. Morphological Development and Maturation of Graftskin (Apligraf) *In Vitro*

barrier function development and morphological appearance. These characteristics are interrelated and develop in parallel. One of the most important functions of the epidermis is to serve as a permeability barrier. Once the Apligraf construct has matured (20-24 days in culture) no significant changes in the morphology or barrier function were noted up 31 days in culture. The resulting mature Apligraf product has morphology very similar to that of human skin. .[u]

The stratum corneum of Apligraf is an essential part of the product which provides the barrier function necessary to prevent wound desiccation and possibly microbial penetration from above. Results from the athymic mouse model indicate that the barrier function of Apligraf was comparable to human skin by 14 days post-graft and remained consistent during the extended graft period.[v] The development of a barrier function in Apligraf occurred rapidly between 14-19 days in culture. Although barrier function continues to improve as Apligraf matures in culture, the improvement is unnecessary for optimum performance upon grafting. Results indicate that there were no differences in Apligraf grafted after 21 days in culture. While a minimum of development may be required, further enhancement of the barrier did not provide any additional benefit *in vivo*. These results are important in supporting the release criteria (minimum days in culture) in the Apligraf manufacturing process.

The mRNA expression of cytokines in healthy gingiva has been reported and IL-1, IL-6 and TNF alpha are expressed albeit at lower levels compared to inflamed tissue. mRNA expression of growth factors has also been demonstrated in healthy gingiva (EGF, TGF-β, and PDGF) (Okada, 1998, *Crit Rev Oral Biol Med*). Studies were conducted to determine the cytokine mRNA and cytokine receptor mRNA expression profile of HEPs and HDFs, and fully differentiated Apligraf. RT-PCR was used to test samples for mRNA expression of 31 cytokines and 13 cytokine receptors.[w] The cytokine/receptor profile Apligraf at 20 days in culture was comparable to that of normal human skin (eg, mRNA expression of PDGF-A. TGF-α, TGF-β, IL-1a as detected, while hematopoietic cell derived cytokines (IL-2 and IL-4) which can be found in normal skin were not detected in Apligraf). This lack of IL-2 and ILC-4 mRNA expression is consistent with the use of pure HEP and HDF populations in the manufacture of Apligraf and the absence of cell types (eg, immune cells) which express these cytokines. This study also demonstrated that Apligraf and HEP/HDF cells express mRNAs for many of the

[u] Study entitled: Morphological Development and Maturation of Graftskin (Apligraf) *In Vitro*
[v] Data on file, Organogenesis. RDR-024.
[w] Data on file, Organogenesis. ITS97001.

cytokines that are necessary for wound healing. In addition, Apligraf has not been found to express mRNA for cytokines which are exclusively derived from immune cells. Complete profiling of genes expressed in oral and cutaneous wound healing has not been reported.

Apligraf is capable of responding to physical trauma by healing through normal mechanisms of re-epithelialization and differentiation, as demonstrated in an *in vitro* study.[x] Cytokine mRNA regulation in this wound healing model is consistent with wound healing in normal skin. Furthermore, the wounded Apligraf healed from the wound margins inward which is a similar to the physiology behind secondary intention healing.

Athymic mouse studies were conducted to also assess the ability of Apligraf to integrate with the host tissue and persist for 60 days compared to split-thickness human skin grafts.[y] Apligraf performed much like split-thickness skin grafts when applied to full-thickness wounds on athymic mice. Integration with the host, or graft take, was excellent with only 1 event of graft loss in the 48 mice. Evidence of basement membrane formation and maintenance of a differentiated epidermis was observed in the grafts and persistence of the human epidermis was confirmed throughout the study period.

An *in vivo* study in the athymic mouse model was performed to better understand: 1) Apligraf's ability to remodel and 2) the immunohistolocalization of the cellular/matrix components of the graft from 0 to 365 days.[z] In this study, at 365 days post graft, human cells were still present and biologically active at the wound site. No signs of abnormal skin development or hyper-inflammation were found. In addition, the presence of type IV and type V human collagens increased progressively during the first 6 months then decreased. Endothelial cells progressively colonized the graft to normal levels. Myofibroblasts increased during the first 2 weeks then disappeared by apoptosis, avoiding of a hypertrophic scar.

The interaction between Apligraf and host tissue of the Swiss nude mouse was studied using human involucrin staining to identify human differentiated keratinocytes in this animal model.[aa] At the mouse tissue-Apligraf junction the stained biopsies showed that the mouse keratinocytes had slightly migrated into Apligraf by 7 days post-grafting.

[x] Data on file, Organogensis, RR-0128.
[y] Data on file, Organogenesis. *Evaluation of Graftskin (Apligraf) Composite Grafts on Full-Thickness Wounds on Athymic Mice*
[z] Data on file, Organogenesis. *Remodeling of a Bioengineering Living Skin Construct Grafted onto Nude Mice*
[aa] Data on file, Organogenesis. PCLR_101219_IVV7_CFR_1.

Human keratinocytes from the Apligraf construct were limited to the construct itself and were not distributed in the surrounding mouse tissues. Vimentin-positive fibroblasts were present in the dermis of Apligraf pre-grafting and after 6 months post-grafting, but they could not be found in the mouse tissue surrounding the graft. To further define whether human cells migrate outside the graft, a DNA Alu probe (Alu is a family of repetitive DNA elements in the human genome) was used to track all human cells. This *in situ* hybridization test showed that Alu-positive human cells are present only within Apligraf up to 6 months post-grafting. Human cells were not detected within the immediate surrounding mouse tissue (approximately 1.2 ± 0.4 mm from all sides of the graft)

Persistence of Apligraf as a graft in the athymic mouse may reflect the inability of this animal to reject allogeneic cells. For this reason, engraftment and persistence of Apligraf in this mouse model should not be misinterpreted as a clinical property of Apligraf. Apligraf clinical studies in cutaneous wounds showed that the persistence of Apligraf DNA was up to a maximum of 6 weeks, however persistence of viable Apligraf cells is likely to be shorter than this (see **Section 7.3.3**). Because the Apligraf cells generally persist up to 6 weeks (and likely much shorter) in humans, and because the cell banks are subjected to a safety testing regimen (including tumorigenicity testing) that has been FDA approved, it is unlikely that the cell banks used in the manufacture of Apligraf would cause a tumorigenic effect when administered to a patient.

4.1.2 Immunology

Activation of T cells requires two (2) signals from APCs. The first signal is T cell receptor recognition of antigen (eg, alloantigen) presented by the APC. The second signal is a costimulatory signal that is antigen nonspecific and occurs between the T cell and the APC. One example of costimulation is the interaction between CD28 on the T cell and the family of B7 molecules on the APC (ie, CD80 and CD86). Results from these studies clearly show that the HEPs and HDFs in Apligraf are not professional APCs; they lack functional costimulatory molecules (eg, CD40 and B7).

Studies were conducted to address the ability of HEPs and HDFs to activate unprimed allogeneic T cells. HEPs and HDFs are not professional antigen presenting cells (APCs). In addition to lacking class II HLA antigens, they also lack the costimulatory molecules B7 and CD40 that are required as a second signal to fully activate T cells (Sibbald, 1998, *J Cutan Med Surg*; Theobald, 1993, *Transplantation*; Niederwieser, 1988, *J Immunol*; Gaspari, 1991, *J Immunol*). In the absence of costimulation, T cells are incapable of reacting to foreign antigen and instead become anergic (unresponsive) to those antigens.

The inability to deliver the requisite "second signal" is the primary reason that HEPs and HDFs are unable to activate allogeneic T cells. Because there are reports of "costimulatory" cytokines which are able to provide a form of the "second signal," further investigation occurred regarding the possible effect that this could have at the wound site.[bb] The study concluded that pro-inflammatory cytokines (IL-1α, IL-6 and IL-12) in combination with IFN-γ were unable to overcome the deficiency of HEP and HDF in terms of costimulation. These results suggest that direct antigen presentation by HEPs and HDFs is unlikely to occur. The cell types present in Apligraf are incapable of priming naive T cells due to the lack of MHC class II and costimulatory molecule expression.

Furthermore, other *in vitro* studies demonstrated that HEPs down-regulate the proliferation of T cells to alloantigen presented by other cells (eg, allogeneic leukocytes, recipient APCs).[cc] This mechanism may be important in preventing the stimulation of allogeneic responses through the indirect antigen presentation pathway; in this pathway, recipient T cells recognize donor-derived antigens that have been taken up and processed by recipient APCs. Severe combined immunocompromised (SCID) mice reconstituted with human lymphocytes (hu-SCID) (Briscoe, 1999, *Transplantation*), were used as an *in vivo* models to evaluate the response of human T cells to Apligraf compared with the response to human skin controls. Apligraf was not observed to undergo rejection during the time course of the study (> 60days). In contrast, most of the grafted human skin controls were rejected within 21 days. Induction of HLA-class II molecules on Apligraf cells by pre-treatment with IFN-γ prior to grafting did not appear to affect Apligraf survival in hu-SCID mice. In summary, it appears that Apligraf does not undergo graft rejection in hu-SCID mice since it is not immunogenic.

In addition, DNA encoding the Rh factor was identified by PCR analysis of DNA extracted from HEPs used in the manufacture of Apligraf.[dd] No detectable amounts of RhD blood group antigen on HEPs or HDFs in by flow cytometry.[ee] Weak, patchy staining of the B blood group antigen in the epidermal layer of Apligraf was detected by immunohistochemical analysis, whereas no staining of the A blood group antigen was detected.[ff]

[bb] Data on file, Organogenesis. ITS97003.
[cc] Data on file, Organogenesis. ITS97004, RR-0076.
[dd] Data on file, Organogenesis. RDR-029
[ee] Data on file, Organogenesis. RDR-030.
[ff] Data on file, Organogenesis, RDR-031.

4.1.3 Toxicity

Toxicological and biocompatibility studies were conducted for Apligraf as a medical device. The initial assessment of this testing was determined on the basis of the indication for Apligraf as a wound treatment of short-term contact with a breached or compromised surface. The following studies were performed (according to the Tripartite Guidance for Medical Devices, Sept. 1986); General Safety Test, Tissue Culture-Agar Diffusion Test, Hemolysis Test, General Safety Test, Primary Skin Irritation, (cytotoxicity), Kligman Maximization Study (sensitization assay), USP Class V, and Subcutaneous Implantation Test-Subchronic. In addition to the Tripartite Tests, the device was subjected to the standard General Safety Test (21 CFR Part 610.11) to assess additional toxicological parameters.

For the Tissue Culture-Agar Diffusion Test, after placing Apligraf on confluent monolayers of L929 mouse fibroblasts, there was no evidence of diffusion of cytotoxic substances from Apligraf cells.[gg] Hemolytic activity was evaluated in an *in vivo* study showing that Apligraf did not cause hemolysis of rabbit erythrocytes.[hh]

Acute systemic toxicity was evaluated on spent culture medium, supernatant from Apligraf and a high speed centrifuged pellet from homogenized Apligraf.[ii] These samples were injected intraperitoneally into 2 guinea pigs and 2 mice. No signs of systemic toxicity appeared over the following 7 days.

Primary skin irritation was evaluated in an *in vivo* study using rabbits.[jj] Apligraf was applied to intact and abraded skin on rabbits and irritancy was assessed at intervals over 72 hours. No signs of irritation were observed.

A Kligman Maximization study was performed on a saline extract and cotton seed oil extracts of Apligraf (Soxhlet extraction for 24 hours at 70°C).[kk] These extracts did not cause primary irritation of guinea pig skin. In the challenge phase of the Kligman Maximization study there was no additional evidence of contact sensitization.

Apligraf toxicity was also evaluated by Systemic Injection Test (ie, USP Class V).[ll] Saline, alcohol and polyethylene glycol extracts of Apligraf did not produce toxicity upon

[gg] Data on file: Organogenesis. 91G-1313.
[hh] Data on file, Organogenesis. 91G-1312.
[ii] Data on file, Organogenesis, General Safety Test.
[jj] Data on file, Organogenesis. 91G-1315.
[kk] Data on file, Organogenesis. 91G-1310.
[ll] Data on file, Organogenesis. 91G-1311.

intravenous or intraperitoneal injection in mice, nor did they produce local irritation upon subcutaneous injection in rabbits.

Subchronic toxicity was evaluated by administration of Apligraf fragments that were surgically implanted into rabbits' subcutaneous tissue.[mm] The implantation sites were clinically observed and were collected and processed for histopathology after 90 days. There was some chronic inflammatory cell infiltration at the implantation sites consistent with the anticipated immune reaction against xenogeneic cells.

Genotoxicity studies have not been performed in consideration of the testing regimen performed for cell banks and clinical trial experience for the product. Additionally, this testing is not a requirement for a cell therapy product.

Implantation to assess the local pathological effects of living tissue was not required due to clinical experience for the product and the potential for xenogeneic responses with the *in vivo* testing. However, Apligraf was implanted subcutaneously in normal and nude mice. The objective of the implantation test is to evaluate the local effects after implantation of biomaterials intended for use in medical devices. Evaluation of the biological response includes macroscopic observations, as well as tissue sample collection and histology analysis with a scoring system to evaluate the host response. Such nonclinical tests are not suitable to evaluate the local effects of Apligraf due to the nature of the test article, which would represent a xenogeneic transplant.

Chronic toxicity testing is performed to evaluate the effects of either single or multiple exposures to medical devices, materials and/or their extracts during at least 10% of the life-span of the test animal (eg, more than 90 days in rats). Long-term grafting onto a Swiss nude mouse acute would model demonstrated sufficient evidence for the lack of chronic toxicity of Apligraf.[aa] Standard chronic toxicity models in non-immunocompromised animal models were not performed because they would be confounded by the reaction of the host system to xenogeneic nature of the product's human cells.

Carcinogenicity testing was not performed in consideration of clinical trial experiences for the product and in light of the fact that Apligraf DNA is generally not detected at the application site more than 6 weeks post-application; this considerably minimizes any safety concerns related to carcinogenic potential.

[mm] Data on file, Organogenesis. 91G-1314.

An irritation test in the oral mucosa hamster cheek was not performed in consideration of previous primary skin irritation study in rabbits[jj] and clinical trial experiences for the product.

An oral toxicity test, which assesses the potential for the medical device to cause an adverse reaction, was not performed. Appropriate toxicity testing was described previously in this section **(Section 4.1.3)** and did not need to be duplicated due to limitations of the xenogeneic model, and in consideration of nonclinical toxicity testing and clinical trial experience for the product.

Additional toxicity testing that is normally conducted for cell-based products has been performed on the individual components of Apligraf (ie, cells and collagen) to demonstrate their safety prior to the initiation of the manufacturing process. The serially passaged HEP strains and HDF strains used in the manufacture of Apligraf are derived from neonatal foreskin tissue and expanded *in vitro* into working cell banks that are subsequently frozen. Immunocytochemical testing is conducted on each cell bank to confirm cell purity and the absence of MHC class II expression on HDFs and HEPs. Each cell bank is further cultured and tested for the absence of adventitious pathogens and tumorigenic potential according to the guidelines established in "Points to Consider in the Characterization of Cell Lines" from the FDA Office of Biologics, Nov. 1993. All cell strains used in the manufacture of Apligraf passed all of these tests.

Karyotype analyses of HEP and HDF cells used in the manufacture of Apligraf revealed that no structural aberrations of chromosomes 3, 11 and 17 at any passage (deletions of these chromosomes are considered to be associated with neoplastic transformation). The presence of a 7:12 translocation was not found in any of the analyzed metaphases. There were instances of absence or lack of recognition of chromosomes 11 or 17 in some cells of all tested cell banks. However other numerical chromosomal aberrations were observed with similar frequencies and there was no apparent accumulation of specific aneuploid cells at higher passages.[nn]

Extensive histological examination has been performed on Apligraf used in grafting experiments in nude mice (Swiss and athymic). All of the histological evaluations have shown the appearance of normal ‘skin’, which affords further supporting evidence that the donor selection and manufacturing process have not produced atypical or abnormal tissue constructs. However the total experience is limited in terms of the numbers of

[nn] BLA 125400: Section 3.2.S.2.3.3.

donors and of cell banks used. It is very unlikely that a complete set of mutations required for neoplasia would occur during the relatively brief period of culture required for them in manufacturing process and the short-term persistence of Apligraf DNA of Apligraf cells following clinical application.

For further information on the immunological testing performed in the clinical see **Section 7.3.2**.

4.1.4 Drug-Drug Interactions

Common periodontal dressings and anti-microbial rinses that were considered for the pivotal study were evaluated to gain a better understanding of any interactions with Apligraf.[oo] This study was conducted in the athymic (nu/nu) mouse model. It was determined that that Barricaid® dressing was most compatible with Apligraf. Apligraf treated with either Coe-Pak™ or 0.12% Chlorhexidine solution showed some variation in epithelial structure but overall the tissue was not compromised.

4.1.5 Conclusion

Overall, Apligraf produces a range of growth factors and extracellular matrix proteins, which are representative of those found in healing skin. These growth factors and matrix proteins are likely expressed in the wound at a level that is appropriate for modulating the growth and migration of host cells, and therefore play a role in stimulating secondary intention healing. Nonclinical studies have not shown Apligraf to cause unexpected inflammatory responses when applied to a wound bed.

Apligraf DNA persists up to at least 1 year in immunocompromised mouse models (ie, SCID and nude mice) but the Apligraf cells do not migrate from the site of application beyond the Apligraf construct.

The potential risks of Apligraf as a tissue engineered product containing 2 types of cultured human cells (HEPs and HDFs) and bovine collagen are:

1. Possible infection transmitted from the foreskin donor or acquired during the manufacturing process,
2. Genotypic abnormality in the donor leading to abnormal properties or performance of the cells,
3. Immunological reactions leading to rejection by the recipient or theoretically a graft-versus-host disease,

[oo] Data on file, Organogenesis. NCLR-0254-001.

4. Inadequate function in healing wounds,
5. Distribution of the cells to sites distal to the wound bed and induction of systemic toxicity, and
6. Tumorigenicity.

The effective exclusion of transmissible infectious agents in the HEPs, HDFs, culture media and collagen and has been exhaustively considered. Donor eligibility is determined per 21 CFR Part 1271 (subpart C); once a donor is eligible, extensive donor testing is performed prior to procurement of the tissue to mitigate transmission of infectious agents. Organogenesis has an established policy to ensure that purchased products and materials are received in accordance with approved specifications and requirements to mitigate the risk of obtaining compromised materials. Suppliers of critical materials and critical services are qualified and audited prior to their utilization. A risk assessment is also performed for the materials that will be utilized in the manufacturing process and includes, but is not limited to the criticality of the supplier, single or multi-sourced, animal vs. non-animal, country of origin and introduction into the manufacturing process (upstream vs. downstream). In particular, collagen is manufactured using a specific in-house process, which includes stringent raw material requirements and purification steps.

It is very unlikely that genotypic abnormality in the donor would lead to overt or even latent dysfunction of the product or in the wound bed. The mother (and father when available) cannot have a history suggestive of any disorder(s) which might be present in and/or transmissible by a tissue engineered product. The epidermal and dermal cells used in the manufacture of Apligraf are derived from neonatal donors, and it is unlikely that these cells would have acquired transforming mutations necessary to produce neoplasia during the few days between birth and collection of the foreskin. Also, all fibroblast and keratinocyte cell banks are tested for safety and performance prior to FDA approval and release into the Apligraf manufacturing process. The cell bank safety testing consists of sterility, mycoplasma, a panel of viruses, senescence, karyology, and *in vivo* tumorigenicity.

Nonclinical work has been done to exclude major product risks (eg, a bank purity and safety testing). Much of this safety data was collected using toxicology program designed for a medical device, since the Apligraf (DFU and VLU) was developed in this manner and it was assumed that Apligraf (oral) would follow the same regulatory path. The

results do not suggest any particular potential for immunological, toxicological or oncogenic risks.

Based on the results of *in vitro* and *in vivo* drug interaction studies that addressed Apligraf viability, and taking into account the oral environment, the following cytotoxic agents will be included in the proposed package insert for Apligraf (oral) under WARNINGS AND PRECAUTIONS: Povidone-iodine solution, chlorhexidine (greater than 0.12%), Polymyxin, and Nystatin. A tabular listing of the nonclinical drug-drug interaction studies performed for Apligraf is provided in **Appendix 1**.

Since FDA designated the Apligraf (oral) product as a combination product with its main mode of healing as a cell therapy, after the clinical program was completed, the nonclinical program for Apligraf is more limited than that of a typical cell therapy product. Nonclinical data for Apligraf includes toxicity studies, as well as extensive safety testing performed on the cell banks and animal derived materials. There are also significant clinical and post-marketing data supporting the safety of Apligraf. During the more than 13 years in which Apligraf has been commercially available for cutaneous applications, 420,000 units have been shipped for patient application with no confirmed reports of immunological reaction, tumorigenicity or malignant transformation, or disease transmission. The Sponsor believes the existing nonclinical data, coupled with clinical and post-marketing data, are sufficient to evaluate product safety for approval.

5 DISEASE BACKGROUND AND MEDICAL NEED

5.1 Normal Oral Mucosa Anatomy

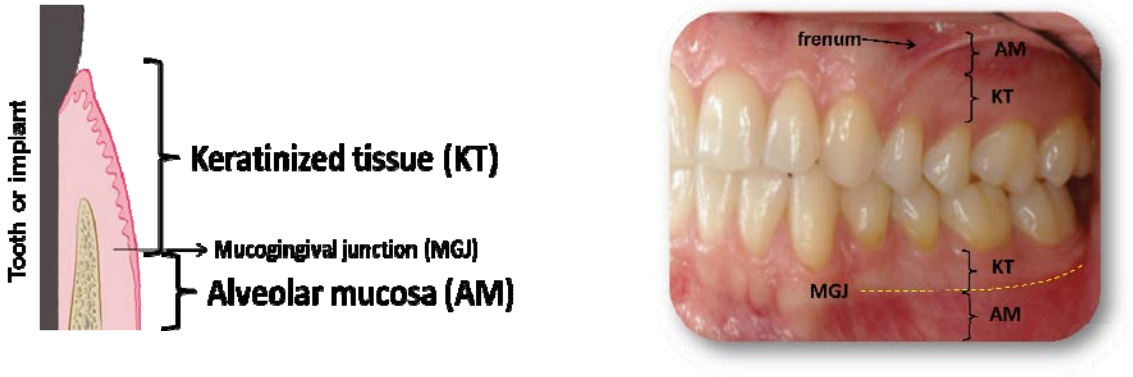
To maintain health and function in the oral cavity, an adequate quantity and quality of functionally appropriate soft tissue must be present. The 2 functional tissues of importance in the oral cavity are the masticatory mucosa and the lining mucosa. The masticatory mucosa is made up of the **keratinized gingiva** and the **alveolar mucosa** (AM). The keratinized gingiva is comprised of the free marginal and attached gingiva, which in this document we collectively refer to as "**keratinized tissue**" (KT). Normally, KT surrounds the necks of the teeth and functions as a tough, outer layer to protect the alveolar ridge supporting the teeth during mastication. KT transitions to the more loose and moveable AM and is demarcated by the "mucogingival junction" (MGJ). The AM provides strain relief and allows for normal movement of the mouth and cheeks. The distribution of these 2 tissue types is such that the KT occupies the most coronal (towards the tooth) aspect and is continuous around the arch of the maxilla or mandible, forming a "band" of KT. AM is apical (away from the tooth) to the KT, similarly continuous along the arch, and transitions into the lining mucosa of the cheek. While the amount or "width" of KT varies depending on location (Bowers, 1963, *J Periodontol*), a seminal article in 1972 concluded that a minimum **width of 2 mm of keratinized gingiva** was adequate around teeth to maintain good gingival health (Lang, 1972, *J Periodontol*). This conclusion was based on the observation that areas with less than 2 mm of keratinized gingiva (or KT) showed significantly greater signs of inflammation than those areas with more than 2 mm. Subsequent studies by others challenged this conclusion, but a key observation in many of the challenging studies was that areas with less than 2 mm of KT remained stable only in the mouths of well maintained patients, but *not* in patients for whom periodontal maintenance therapy had been inadequate and where inflammation was present. It is generally accepted that a minimal amount of this protective tissue is desired around dental implants as well (Bouri, 2008, *IJOMI*; Adibrad, 2009, *J Oral Implant*).

Figure 12 illustrates normal soft tissue anatomy represented (a) schematically and (b) clinically. In (a), the KT is shown as attached to the tooth/implant surface and underlying bone. AM, which is non-keratinized, is loose and moveable and extends through the vestibule and into the cheek. KT is comprised of both free (sulcus forming) and attached gingiva (not shown for clarity). KT is distinguished clinically (b) by its pink, bound appearance and stippled texture (akin to an orange peel), compared to the smooth-

textured, more red and moveable mucosa. The border between the two, the MGJ (shown as a yellow line), is a continuous line around the buccal aspect of the entire arch.

Figure 12: Normal Soft Tissue Architecture (a) and Clinical Observation (b)

(a) **(b)**



In summary, normal tissue is defined by:

- Physiologic TYPE: KT and AM
- Physiologic AMOUNT: minimum of 2 mm KT, variable for mucosa
- Physiologic DISTRIBUTION: consistent band of color and texture for both KT and AM, the transition between the 2 demarcated by a continuous MGJ

5.2 Abnormal Anatomy: Oral Mucosal Defects

A tooth, implant or edentulous site which lacks the proper quantity and quality of tissue is defined as an oral mucosal defect (also known as a "mucogingival deformity"[pp]). Without the proper tissue types around teeth or implants, there is evidence that plaque accumulation and chronic inflammation will occur (Loe, 1972, *J Periodontol*; Kennedy, 1985, *J Clin Periodontol*; Bouri, 2008, *IJOMI*). Persistence of periodontal/peri-implant inflammation leads to damage of the tooth attachment or loss of osseointegration of the implant, thereby increasing the probability of tooth loss or implant integrity (Hirschfeld, 1978, J *Periodontol*; Wilson, 1987, *J Periodontol*; Lang, 2009, *J Clin Periodontol*; Bouri, 2008, *IJOMI*; Roos-Jansaker, 2007, *Swed Dent J*; Schou, 2006, *Clin Oral Implant Res*). These defects may be developmental or acquired. Developmental causes can include high frenum attachments, muscle pulls, and buccal or lingual tooth eruptions. Acquired defects may arise from periodontal disease, trauma, surgery, loss of teeth, neoplasms, or overly

[pp] The American Academy of Periodontology defines a mucogingival deformity as a departure from the normal dimension and morphology of and/or interrelationship between gingiva and alveolar mucosa. (American Academy of Periodontology, 2000, *J Periodontol*)

aggressive tooth brushing. Abnormalities of the soft tissues of the oral cavity require surgery to correct these deformities since they will not self-resolve. The goal of surgery is to prevent deterioration by restoring the site with site-appropriate tissue (type, amount and distribution).

Figure 13 shows oral mucosal defects of inadequate KT around (a) teeth, (b) dental implants and (c) edentulous ridge. MGJ, shown as dotted line, demarcates transition from KT to AM while the arrow indicates the defect.

Figure 13: Oral Mucosal Defect of Inadequate KT

(a) **(b)** **(c)**

5.3 Current Therapies for Oral Mucosa Defects

Surgical intervention is necessary to correct these defects in order to improve function, comfort or esthetics. This includes traumatic injuries, prior to the placement of dental implants, initiation of orthodontic therapy, and placement of fixed/removable prosthetics and dental restorations.

In correcting these defects, the surgeon may aim to augment KT, cover root surfaces and/or add bulk/thickness to the sites. In this application, the scope is restricted to augmentation of KT (around teeth, dental implants and edentulous sites). When preparing to augment KT, surgeons first prepare a recipient wound bed. Following this, there are two basic strategies for closing the wound: primary and secondary intention healing. Primary intention healing involves replacement of tissue through the use of grafting or flap transposition. Secondary intention healing allows the wound to self-heal through granulation tissue formation and marginal re-epithelialization.

One of the original strategies in re-establishing a functional zone of KT was the “pushback” technique, a form of secondary intention healing. In this technique, the soft tissue, including the periosteum, was surgically excised from the site, resulting in

denuded bone. Despite generating KT at the site, the procedure was tremendously painful for the patient and also saw significant bone resorption (Bohannon, 1962, *J Periodontol*; Sullivan, 1969, *Dent Clin North Am*). As such, this procedure was abandoned in practicality, and primary intention strategies became favored.

Primary intention healing typically involves treatment by grafting from an autologous or xenogeneic source. Most commonly, surgeons use an FGG which involves transplanting a section of keratinized palatal mucosa. In the FGG procedure, a strip of tissue (epithelial and connective tissues) is taken from the roof of the patient's mouth (palate) and transplanted to a recipient wound bed at the site of the defect. As the graft takes, a large, stable band of KT protects the site from further deterioration. The donor site is left to heal on its own. In addition to autografts, xenografts (specifically collagen membranes) have emerged as potential alternatives. The collagen membranes are decellularized to minimize their immunogenicity and aim to serve as a mucosal graft, to be gradually replaced with host tissue over time. By definition, for a product to be considered a graft, it must persist long-term, vascularize and integrate with the surrounding tissues. Thus far, these collagen membranes have not been shown, through rigorous clinical study and high level evidence, to be a predictable alternative.

5.4 Medical Need

As previously discussed, the FGG has been used by clinicians routinely since the 1960s and remains the most common strategy to augment KT (where root coverage is not desired) (McGuire, 2008, *J Periodontol*). Patients who undergo this procedure have a remote surgical site created when donor tissue from the palate is harvested as shown in **Figure 14**.

Figure 14: FGG being Harvested from the Palate.

This palatal donor site is associated with morbidity (pain) and, while infrequent, complications such as palatal bleeding do occur constituting a serious, medical emergency. The pain and anxiety associated with palatal surgery often results in many patients to opting out of treatment. This "under-treatment" is exacerbated by many patients who simply do not have enough donor tissue of adequate quality available on their palate to cover all of the gingival areas that lack adequate KT, leading to a triaging of the sites most in need of treatment (Harris, 2001, *J Periodontol*; Griffin, 2006, *J Periodontol*; Kloostra, 2007, *J Periodontol*).

Moreover, if surgery is performed, the transplanted tissue may result in a poor esthetic match. Since palatal tissue type is not the same as gingiva, and has a different color and texture, the grafted tissue is usually visibly different than the surrounding tissue. The amount (width and thickness) of tissue grafted is typically in excess of the minimum needed and is noticeably discontinuous with the native KT and AM. **Figure 15** shows the different type (palatal mucosa), amount, and distribution (discontinuous with adjacent KT and AM) as compared to the normal architecture surrounding this area.

Figure 15: Site Repaired with an FGG, "Tire-patch" Appearance

In summary, there remains a need for an effective treatment that will regenerate site-appropriate oral soft tissue supported by a high level of clinical evidence.

5.5 Apligraf (oral) Opportunity

Apligraf aims to treat defects by regenerating site-appropriate oral mucosal tissues. As will be shown through the clinical program, Apligraf regenerates at least 2 mm of KT around teeth in 95% of the subjects treated. In addition, the color and texture match to adjacent tissue results in a continuous, consistent regenerative outcome. As new

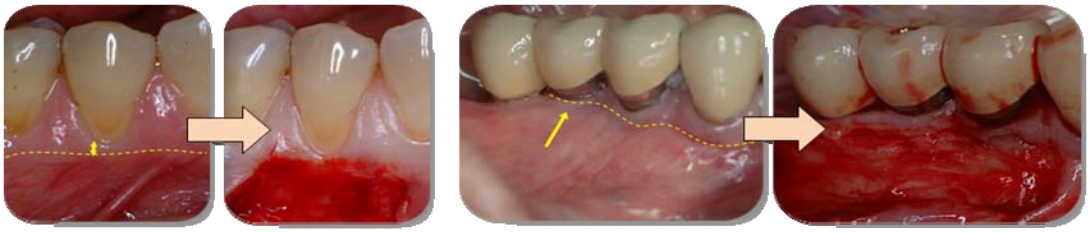
alternative for augmenting KT, Apligraf may be able to address the shortcomings associated with donor site morbidity and tissue limitations.

In addition to teeth, oral mucosal defects occur around dental implants and edentulous sites. Correction of these defects follows the same surgical principles as with teeth. The clinical scenario that was studied is representative of a class of defects for which Apligraf (oral) may be useful. The range of defects which is covered by the suggested "Indication For Use" label is summarized in **Table 10**. This table provides an overview of the types of oral mucosal defects lacking or having insufficient dimensions of oral mucosa. The justification for including these defects in the indication statement is based on the key commonalities for these defects and the conservation of the wound healing process in humans. These key commonalities are:

- The recipient wound bed is vascular (see **Figure 16**, below)
- The recipient wound bed consists of connective tissue and periosteum (see **Figure 16**, below)
- The types of tissue to be restored include KT and AM

This core set of surgical principles is important, as they represent the underlying principles of wound healing and are important for predicting the regenerative outcome. As an illustrative example, **Figure 16** shows that the wound bed preparation is the same for teeth (a) and implants (b). In both panels (a and b), a defect exists in the left hand picture (yellow arrow). To prepare the wound bed, non-functional tissue is excised, down to periosteum.

Figure 16: Oral Mucosal Defect Around Teeth and Implants



(a) **(b)**

As opposed to the scarring which may follow secondary intention healing, Apligraf positively modulates secondary intention healing to regenerate oral mucosal tissue.

In the oral clinical development program, a protocol, specifically around teeth, was designed which serves as a model for these other applications (eg, implants, edentulous ridge and vestibule). Through the oral clinical studies around teeth, Apligraf demonstrates regeneration of site appropriate tissue by modulating and improving secondary intention wound healing applicable to a broader array of similar indications.

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Table 10: Measurable Attributes of Oral Mucosal Defects

DEFECT ATTRIBUTES	TYPE OF DEFECT				
	LACK OF AND/OR INSUFFICIENT DIMENSION OF ORAL MUCOSA*				
	Around Teeth	**Around Implant(s)**	**Edentulous ridge**	**Vestibular/ oral sulcus defect**	**Alveolar Mucosa**
Tissue in wound bed	Connective tissue +/- periosteum	Connective tissue +/- periosteum	Connective tissue +/- periosteum	Connective tissue +/- periosteum	Connective tissue +/- periosteum
Vascularity of wound bed	Vascular	Vascular	Vascular	Vascular	Vascular
Typical Size of mucosal defect	10-40 mm^2	10-40 mm^2	20-60 mm^2	150-400 mm^2	20-60 mm^2
Mobility of mucosal bed	Fixed	Fixed	Fixed	Mobile	Mobile
Types of oral mucosa needing to be restored	Keratinized mucosa (ie, attached gingiva), non-keratinized mucosa (ie, oral mucosa)	Keratinized mucosa (ie, attached gingiva), non-keratinized mucosa (ie, oral mucosa)	Keratinized mucosa (ie, attached gingiva), non-keratinized mucosa (ie, oral mucosa)	Non-keratinized mucosa (ie, oral mucosa)	Non-keratinized mucosa (ie, oral mucosa)
*Includes traumatic or iatrogenic defects to buccal or palatal alveolar mucosa; exposed foreign body (eg, implant, PTFE) and exposed bone					

6 CLINICAL DEVELOPMENT PROGRAM

6.1 History of Development Program

There have been no physical or material changes to the Apligraf product from that of the current commercially available Apligraf product for DFU and VLU. The Apligraf product that was studied in the oral clinical investigations and reported in this section and **Section 7** represents the final product that is intended for commercialization following approval of this marketing application.

In support of the proposed oral indication, the Sponsor has conducted two clinical investigations with Apligraf (oral) under an IDE: a pilot (05-PER-001) and a pivotal study (06-PER-002-CTX) (IDE G050122 and G070012, respectively). Using data collected from these 2 clinical studies, an additional histological evaluation and independent blinded photographic assessment were conducted. The additional histological evaluation was performed to supplement the original findings from the histological evaluation performed as part of the pilot study. The independent blinded photographic evaluation of the pivotal study photographs provides corroborating evidence of site-appropriate tissue regeneration.

6.2 Pilot Study (05-PER-001): A Pilot Clinical Trial to Assess the Safety and Efficacy of Apligraf in establishing a Functional Zone of Attached Gingiva

6.2.1 Pilot Study Design

Study 05-PER-001 was a randomized, within subject controlled (matched for teeth and gingival condition), single center, pilot study to assess the safety and efficacy of Apligraf in establishing a functional zone of AG.

Twenty-five subjects were planned for enrollment at 1 study center in the US. Subjects were screened and eligible subjects were enrolled into the clinical study. The first 3 subjects were used to help determine surgical and material handling techniques, and were not included in the efficacy analyses. Subsequent subjects were enrolled after the first 3 subjects completed the first 4 weeks of follow up. Treatment site and order of treatment were randomized. Following screening and randomization, subjects received both a palatal graft and Apligraf, with primary endpoint evaluations at Month 6.

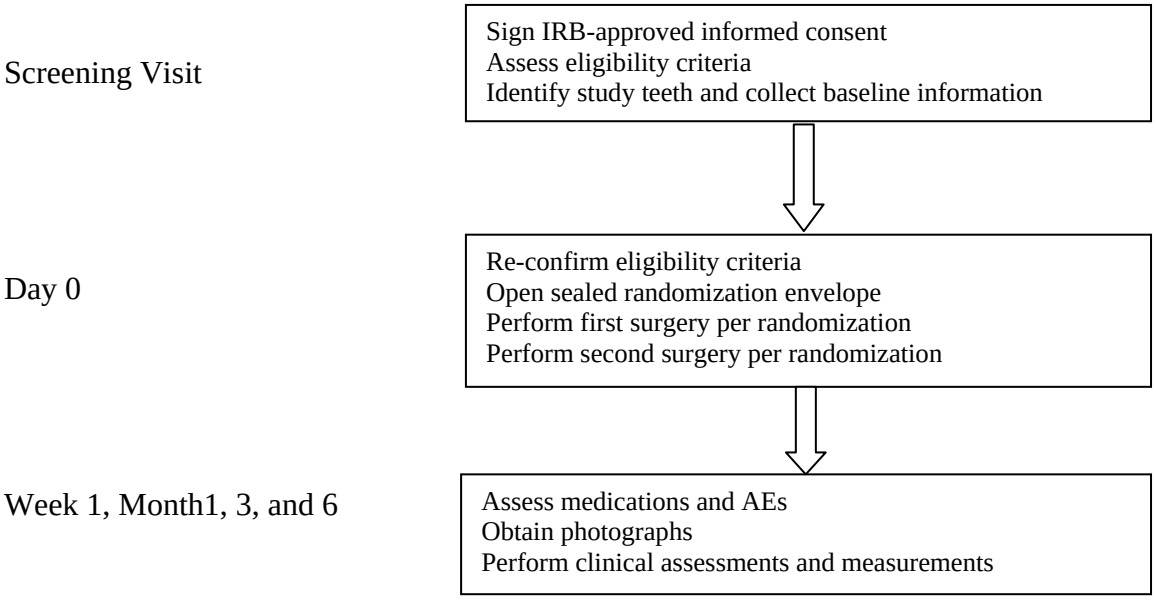
There were follow-up visits at Week 1, Month 1, Month 3 and Month 6.

At baseline and Month 6, biopsy specimens were obtained from 7 subjects' FGG- and Apligraf-treated sites for histologic evaluation. DNA persistence studies were performed on Apligraf-treated biopsy specimens from 2 of these 7 subjects.

6.2.1.1 Treatment Schema

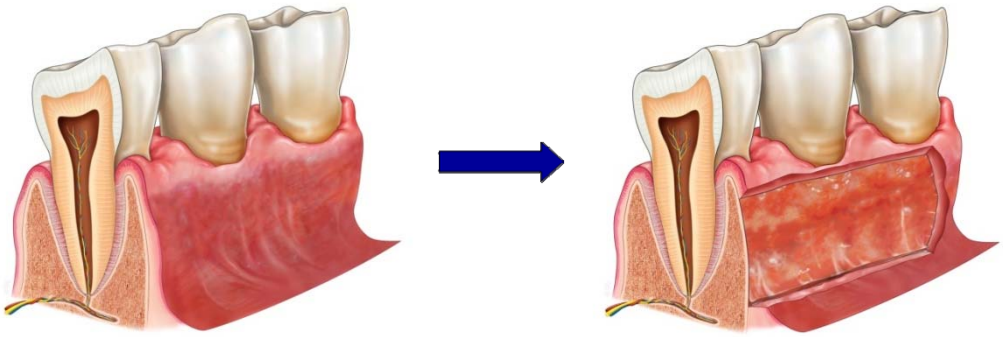
The pilot study used a within patient controlled (ie, split-mouth) design, meaning each patient received both Apligraf and a FGG on contralateral sides of either the mandible or maxilla at Day 0. The overall study design is depicted in **Figure 17**.

Figure 17: Treatment Scheme for the Pilot Study



The surgically created wound beds for both the Apligraf- and FGG-treated sites were created in accordance with standard surgical technique. **Figure 18** depicts the baseline defect and the resultant surgically created wound bed.

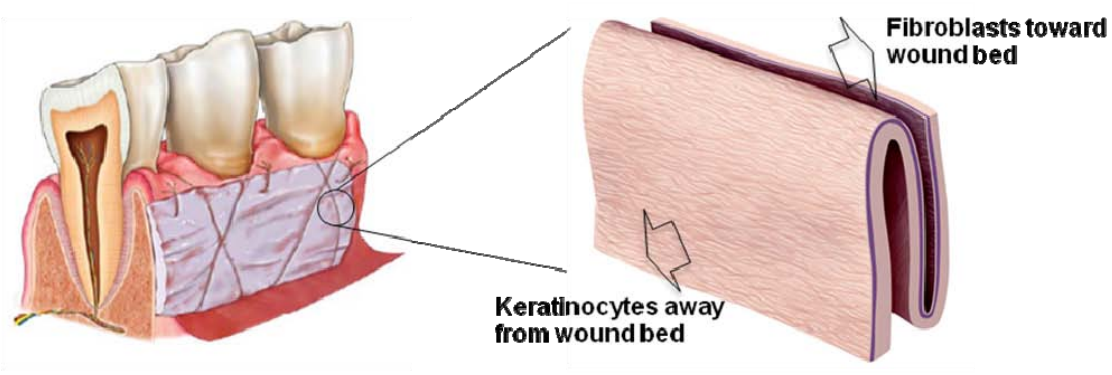
Figure 18: Baseline Defect (Left) and Surgically Created Wound Bed (Right)



Apligraf Treatment:

Subjects were treated with 1 application of Apligraf at Day 0, which was "Z-folded" prior to placement on the surgically created wound bed (**Figure 19**). The product was "Z-folded" to improve handling and durability characteristics. The width (apical-coronal) of the "Z-folded" construct was 5 mm. Apligraf was sutured in place with 5/0 gut suture and covered with Coe-Pak periodontal dressing for additional durability.

Figure 19: Apligraf Application (Z-folded)



Control Treatment - FGG:

The control treatment consisted of harvesting a 5 mm width (apical-coronal) FGG from the subject's palate and placing it on the surgically created wound bed. FGG was sutured in place with 5/0 gut suture and covered with Coe-Pak periodontal dressing.

FGG is the standard of care for augmenting KT when root coverage is not desired and thus can be considered as a positive control. It is a highly predictable procedure but it involves creation of a second surgical site on the subject's palate. Co-morbidities associated with FGG harvest from the palate include bleeding and pain.

6.2.1.2 Inclusion and Exclusion Criteria

The inclusion criteria for the study were:

- Subject is 18 years of age or older at the time of enrollment, and not older than seventy years.
- Subject has at least 2 non-adjacent teeth with an insufficient zone of attached gingiva which requires soft tissue grafting. The 2 selected teeth must be located in contralateral quadrants. (In case of adjacent teeth requiring grafting, only one tooth at each site will act as test or control tooth, but both teeth will get the same treatment).
- Root coverage is not desired or indicated at the time of grafting.
- Females of childbearing potential must have a documented negative urine or serum pregnancy test.
- Subjects must have read, understood and signed an institutional review board (IRB) approved informed consent form.
- Subjects must be able and willing to follow study procedures and instructions.

Subjects were not eligible if they met any of the following exclusion criteria:

- Teeth that have an insufficient zone of attached gingiva that would be best treated using soft tissue grafts, which would attempt to cover the denuded root surface.
- Subjects with any systemic conditions (ie, diabetes mellitus, cancer, HIV, bone metabolic diseases) which could compromise wound healing and preclude periodontal surgery.
- Subjects who are currently receiving or have received within one week prior to study entry, systemic corticosteroids (including inhaled), immunosuppressive agents, radiation therapy, and/or chemotherapy which could compromise wound healing and preclude periodontal surgery.

- Subjects with the presence of acute infectious lesions in the areas intended for surgery.
- Subjects who smoke.
- Teeth requiring treatment are molars.
- Teeth with axial mobility.
- Known hypersensitivity to bovine collagen.
- Subjects enrolled in medical, dental, or any investigational device study for any disease within the past four weeks.
- Subjects who have received an investigational drug or biological treatment within the three months prior to study enrollment (medical or dental).
- Subjects previously treated with Apligraf, Dermagraft or any other skin graft at the target site(s).
- Subjects, 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.

6.2.1.3 Statistical Methods

The sample size was calculated based on a paired analysis of the data. Calculations at 5% significance level showed that 20 evaluable subjects would be sufficient to detect non-inferiority with over 95% power when the margin of equivalence is a 1.0 mm change in the amount of AG and the true difference is 0 mm. These calculations are based on an assumed within-subject variation of 1.0 mm.

The trial was designed to demonstrate non-inferiority between Apligraf and FGG in the change in amount of AG over the 6 month observation period:

H_0: $D \leq -1$ mm

H_a: $D \leq -1$ mm

Where D=mean difference within subjects (Apligraf change over 6 Months – FGG change over 6 Months)

The secondary objectives of the study were to compare Apligraf to FGG for: 1) Inflammation score; 2) Color and texture match of the grafted tissue to the adjacent

tissue; 3) Resistance to oral muscle pull; 4) Probing depth; 5) Clinical attachment level (CAL); 6) Subject preference or satisfaction (including pain perception); 7) Change in recession depth; and 8) Width of keratinized tissue.

Measures of AG, recession depth, CAL, and probing depth over time were compiled for each subject. To test for differences in these variables over time between Apligraf and FGG, repeated measures analysis of covariance (ANCOVA) was conducted with adjustment for initial amount of AG included as a covariate. These repeated measures ANCOVA models also took into account the paired nature of the study design.

For change in KT width, recession, and CAL over time for sites with positive AG at baseline, repeated measures analysis of variance (ANOVA) was also used.

Friedman's test for related outcomes was utilized to test for differences in subject perceptions of duration of pain, bleeding, swelling, and sensitivity among surgical sites.

Wilcoxon signed rank test was used to compare other secondary variable scores at each time point postoperatively (inflammation scores, change in bleeding on probing, muscle pull, patient preference and color and texture match).

6.2.1.4 Study Endpoints

The primary objective of the pilot study was to determine if Apligraf could provide a functional zone of AG comparable to FGG. Accordingly, the primary efficacy variable was the change in the amount of AG at Month 6 compared between the 2 treatment groups. The amount of AG is calculated as the distance from the free gingival margin to the MGJ minus the probing depth. [See **Figure 12** in **Section 5.1**]

Secondary endpoints in the study included evaluation of the following variables as the change from baseline to the final evaluation at six months, Apligraf and FGG:

(1) Inflammation rated according to the following scale

- 0 Normal (absence of inflammation)
- 1 Mild inflammation of any portion of the marginal unit (eg, slight changes in color
- 2 Mild inflammation of the entire gingival unit (but no edema)
- 3 Moderate inflammation (moderate glazing, redness, edema and/or hypertrophy)

4 Severe inflammation (marked redness and edema/hypertrophy, spontaneous bleeding, or ulceration)

(2) Color and texture match of the treatment to the adjacent tissue

(3) Resistance to oral muscle pull

(4) Probing depth

(5) CAL

(6) Subject preference or satisfaction (including pain experience)

(7) Change in recession depth

(8) Width of KT

Safety of Apligraf was assessed by spontaneous AE reporting and by clinical assessment to detect local and systemic reactions. In addition, baseline and 6 month biopsy specimens were examined histologically (N=7) and for persistence of Apligraf DNA (N=2).

6.2.2 Pilot Study Efficacy Results

6.2.2.1 Demographic and Baseline Characteristics

The clinical study data were collected and analyzed according to the study protocol. A total of 25 subjects were screened and enrolled in the study: all 25 subjects were treated with Apligraf and FGG and all completed the study. The initial 3 subjects were considered training cases and were not included in the statistical analyses for efficacy.

Descriptive statistics for demographics (**Table 11**) of the study population (N=25) were calculated: 68.0% (17/25) of the subjects were women, the average age was 49.1 years old (range: 31 to 69 years), and 88.0% (22/25) of the subjects were non-Hispanic Caucasian.

Table 11: Demographics of Pilot Study Population (N=25)

DEMOGRAPHICS	STATISTICS MEAN (RANGE)
Age (range)	49.1 years (31-69)
	N (%)
Sex	
Male	8 (32.0%)
Female	17 (68.0%)
Race/Ethnicity	
Caucasian, non-Hispanic	22 (88.0%)
Hispanic	1 (4.0%)
Asian/Pacific Islander	1 (4.0%)
Middle Eastern	1 (4.0%)
Source: Section 3 Table 2 in Clinical Study Report (CSR), 05-PER-001	

No Apligraf failures, replacements, or patient complaints were reported.

Table 12: Summary Statistics for Baseline Clinical Variables by Treatment Site (N=22)

BASELINE CLINICAL VARIABLE	MEAN ± SD	MEDIAN	RANGE	*P**
Probing Depth (mm)				
Apligraf	1.36 ± 0.49	1.0	1 - 2	1.000
FGG	1.36 ± 0.49	1.0	1 - 2	
Recession (mm)				
Apligraf	2.41 ± 1.13	2.0	0 - 5	0.721
FGG	2.36 ± 1.07	2.5	0 - 4	
CAL (mm)				
Apligraf	3.77 ± 1.37	4.0	1 - 7	0.917
FGG	3.73 ± 0.99	4.0	2 - 6	
Attached Gingiva (mm)				
Apligraf	0.30 ± 0.48	0.0	0 - 1.5	1.000
FGG	0.27 ± 0.46	0.0	0 - 1	
KT Width (mm)				
Apligraf	1.14 ± 0.77	1.0	0 - 2.5	0.719
FGG	1.23 ± 0.75	1.0	0 - 2	
Alveolar Bone Level (mm)				
Apligraf	4.50 ± 1.22	5.0	2 - 7	1.000
FGG	4.50 ± 0.86	5.0	3 - 6	
Surgical Position (mm)				
Apligraf	2.00 ± 1.48	2.0	0 - 5	0.186
FGG	2.27 ± 1.28	2.0	0 - 5	

Table 12 continues to the following page…

Table 12: Summary Statistics for Baseline Clinical Variables by Treatment Site (N=22) (continued)

BASELINE CLINICAL VARIABLE	MEAN ± SD	MEDIAN	RANGE	*P**
Plaque Index**				
Apligraf	0.21 ± 0.30	0.0	0 - 1	0.102
FGG	0.30 ± 0.33	0.25	0 - 1	
Bleeding on Probing†				
Apligraf	0.00 ± 0.00	0.0	0	0.317
FGG	0.05 ± 0.21	0.0	0 - 1	
Inflammation Score‡				
Apligraf	0.32 ± 0.48	0.0	0 - 1	1.000
FGG	0.32 ± 0.48	0.0	0 - 1	

*P-values are based on Wilcoxon signed-rank test
**Plaque Index: 0-none, 1-plaque
†Bleeding on Probing: 0-no, 1-yes
‡Inflammation Score: 0 – normal, 1 = mild inflammation of any portion of the gingival unit, 2 = mild inflammation of the entire gingival unit, 3 = moderate inflammation, 4 = severe inflammation
Source: Clinical Study Report 05-PER-001, Section 3 Table 3.

6.2.2.2 Primary Efficacy Endpoint – Established Adequate Functional Zone of AG

The primary objective of this pilot study was to assess the change in the amount of AG with Apligraf application compared to FGG at 6 months following initial treatment (non-inferiority). While the primary endpoint of this pilot study was not met, Apligraf was effective in establishing an adequate functional zone of AG at Month 6 compared to baseline, 1.14 mm at 6 months, a mean increase over baseline of 0.85 mm. When Apligraf was compared to the FGG, FGG showed a greater increase from baseline in the amount of AG compared to Apligraf sites ($p < 0.001$). The results for AG are presented in **Table 13**.

Table 13: Attached Gingiva

	Apligraf Mean (95% CI)	FGG Mean (95% CI)	P-value
Baseline	0.30 mm (0.13,0.46)	0.27 mm (0.11,0.44)	
6 Months	1.14 mm (0.77,1.50)	2.71 mm (2.34,3.07)	
Change (baseline to 6 months)	0.85 mm (0.48, 1.21)	2.43 mm (2.06, 2.79)	< 0.0001

Source: Clinical Study Report 05-PER-001, Section 3 Table 4.

6.2.2.3 Secondary Efficacy Endpoints

Although, the primary efficacy endpoint for the pilot study was not met, the results of the secondary endpoints demonstrated the potential for Apligraf to regenerate site appropriate tissue and thus supported the subsequent pivotal study. Specifically, Apligraf treatment established at least 2 mm of KT width in 81.8 % of the cases. Apligraf sites exhibited an average increase in KT width from 1.13 mm (95% CI 0.92, 1.33) at baseline to 2.50 mm (2.18, 2.82) at 6 months. FGG treated sites had a mean KT width from 1.24 mm (1.03, 1.44) at baseline to 4.57 mm (4.25, 4.89) at 6 months. There was a larger change from baseline to 6 months in width of KT in the FGG site compared to the Apligraf site ($p<0.001$). This result was not unexpected. Because Apligraf employs a regenerative healing process while FGG simply transfers tissue from one site in the oral cavity to another, the amount of KT post-procedure closely approximates the size (apical-coronal width) of the FGG grafted. Accordingly, a direct comparison of the amount of KT in the Apligraf-treated sites versus the FGG-treated sites would not be meaningful.

When assessing periodontal health around Apligraf and FGG-treated sites, there were no differences between Apligraf and FGG in the change from baseline to Month 6 in probing depth, recession and CAL. There was also no difference in resistance to muscle pull, inflammation, or bleeding on probing between Apligraf and FGG.

Apligraf treatment showed a higher subject satisfaction/preference score and a strong difference between tissue color (more equally red compared to surrounding tissue) and in tissue texture (more equally firm compared to surrounding tissue).

Since the FGG treatment necessitates harvesting an autologous palatal graft, subject pain perception was compared between the combined FGG-treated and palatal donor site vs. the Apligraf-treated site. When comparing duration of pain in Apligraf-treated sites to composite measures of pain duration for FGG-treated and donor sites taken together, duration of pain was found to be greater in the FGG-treated and donor sites taken together, compared to the duration of pain in Apligraf sites ($p=0.021$). In addition, duration of sensitivity in FGG-treated and donor sites taken together was greater than duration of sensitivity in Apligraf site ($p=0.027$).

6.3 Pivotal Study (06-PER-002-CTX): A Clinical Trial to Evaluate CelTx (Apligraf®) as an Alternative to Tissue from the Palate to Enhance Oral Soft Tissue Regeneration and Wound Healing[qq]

6.3.1 Pivotal Study Design

The pivotal study was a randomized, within subject controlled (matched for teeth and gingival condition), multi-center, pivotal study to assess the safety and efficacy of Apligraf as an alternative to tissue from the palate to enhance oral soft tissue regeneration and wound healing.

Approximately 96 subjects were planned for enrollment at 4 study centers across the US. Subjects were screened and eligible patients were enrolled into the clinical study. Enrolled subjects were treated with a single application of Apligraf applied to a surgically created wound bed on one study tooth and an FGG was harvested from the subject's palate and applied to wound bed on a second, contralateral study tooth. The first 2 subjects treated by each surgeon were training cases for surgical and material handling techniques. Treatment site and order of treatment were randomized. Following screening and randomization, subjects received both an FGG and Apligraf, with the primary endpoint evaluation at Month 6.

Due to the use of FGG as the control in the study, neither the Investigator nor the subject was blinded to study treatment assignment or order of surgical procedure. Efforts were made to minimize the potential for bias by use of calibrated examiners, which performed all clinical distance measurements. Calibration of the examiners occurred prior to study initiation for measurements of probing depth, recession depth, and identification of the MGJ (as used to obtain KT and AG measures) against a standard examiner. The pivotal study protocol specified that the calibrated examiners were to be blinded to treatment assignment, but given the distinct appearance of the FGG it was not possible to ensure blinding of the calibrated examiners. Within each clinical site, attempts were made to have the same calibrated examiner complete the clinical measurements for all subjects and all visits.

Subject follow-up visits occurred at Week 1, Week 4, Month 3, and Month 6.

6.3.1.1 Study Design Rationale

In order to demonstrate the ability to generate site appropriate soft tissue in the oral cavity this pivotal study was designed with the following attributes:

[qq] CelTx™ is the proposed proprietary name for Apligraf (oral), currently under review by the FDA.

1) Availability of an ethically and clinically acceptable control standard of care

Full thickness palatal tissue grafts (FGG) are standard of care for increasing the zone of KT around teeth (AAP, 2000, *J Periodontol*; Newman, 2006, *Carranza's Clinical Periodontology 10th Ed.*; Miller, 1987, *J Periodontol*; Cohen, 2007, *Atlas of Cosmetic and Reconstructive Periodontal Surgery 3rd Ed.*). This is both an ethical and clinically acceptable control. A 4 mm width of FGG was applied to the surgically prepared wound bed in this study. The 4 mm width was used to minimize the poor esthetic outcome (color and texture match) commonly seen with FGG procedures.

The most direct way to assess the effect of Apligraf would be to utilize a control group in which the open wound is allowed to heal by secondary intention (ie, denudation procedure). However, the denudation procedure is no longer routinely performed and would not be considered ethical given the suboptimal functional and esthetic results and post-operative pain associated with this technique.

2) Have nearby anatomical landmarks that allow reproducible objective quantification of type and location of oral mucosa being regenerated

Teeth provided landmarks for assuring a standardized and reproducible method for measuring the amount of gingival mucosa. In addition to having anatomical landmarks, this multi-center study employed calibrated examiners to ensure reproducible, objective quantification of the efficacy assessments.

3) Use of a commonly performed procedure

Surgical correction of insufficient gingiva by harvesting of a palatal graft (FGG) and placement on top of wound bed is a commonly performed procedure. The 2005-2006 ADA Survey of Dental Services Rendered documented that 274,000 "free soft tissue grafts" (D4271) were performed by periodontists annually (American Dental Association (ADA), 2007, *2005-06 Survey of Dental Services*).

4) Objectively evaluate regeneration of two types of oral mucosa that can be easily differentiated clinically.

The anatomical region of gingival recessions treated in this study contains two types of mucosa (keratinized gingival mucosa and AM) that can be easily differentiated clinically and are located in well defined anatomical locations. Both gingiva and AM were excised to create the oral mucosal defects treated in this study.

5) Primary endpoint should include an accepted and objective definition of what constitutes site appropriate regenerated soft tissue in the oral cavity.

Failure to maintain gingival health often results in unstable gingival recession which may lead to tooth loss (Newman, 2006, *Carranza's Clinical Periodontology 10th Ed.)*. This study used Lang and Loe's objective standard of 2 mm of keratinized gingival tissue as a measure to quantify and substantiate site appropriate oral soft tissue regeneration (Lang, 1972, *J Periodontol*). The pre-defined primary efficacy endpoint, ability of Apligraf to generate at least 2 mm KT width at 6 months (superiority vs. 50% success standard) prospectively agreed upon with FDA prior to conduct of the pivotal study and was objectively and directly measured in the clinical study with the roll technique aided by Schiller's Iodine staining.

6.3.1.2 Treatment Schema

Apligraf Treatment:

The pivotal clinical study was within patient controlled (ie, split-mouth), meaning each patient received both Apligraf and FGG on contralateral sides of either the mandible or maxilla at Day 0, thus serving as their own control. The pivotal study design is the same as presented in **Figure17** in the pilot study.

Creation of the Surgically Prepared Wound Bed:

The surgically created wound bed was created in accordance with standard surgical technique. The wound bed for both the Apligraf- and FGG-treated sites were created in an identical manner. **Figure 18** depicts the baseline defect and the resultant surgically created wound bed, which were the same as in the pilot study.

Apligraf Treatment:

Subjects were treated with 1 application of Apligraf at Day 0 [minimum dimensions (10mm length x 5mm width) of the Z-folded configuration], which was "Z folded" prior to placement on the surgically created wound (see pilot study **Figure 19**). Apligraf was "Z folded" to improve handling and durability characteristics. Apligraf was sutured in place with 5/0 resorbable sutures. In the pivotal study an additional layer of Apligraf was sutured in place over the initial "Z-folded" Apligraf application for protection of the Apligraf. This protective layer was added in the pivotal study to serve as a protective barrier between the therapeutic "Z-folded" Apligraf and the Coe-Pak periodontal dressing, so that if Coe-Pak was traumatically removed from the treatment site the

"Z-folded" Apligraf would not be disturbed. This single layer was also sutured with 5/0 resorbable sutures and the entire treated area was covered with Coe-Pak periodontal dressing.

Control Treatment - FGG:

The control treatment (also referred to as "standard of care") consisted of harvesting a 4 mm width (apical-coronal) FGG from the subject's palate and placing it on the surgically created wound bed. FGG was sutured in place with 5/0 gut suture and covered with Coe-Pak periodontal dressing. The 4 mm width was used to minimize the poor esthetic outcome (color and texture match) commonly seen with FGG procedures, ie, "tire-patch" appearance. No minimum length was specified in the protocol.

6.3.1.3 Inclusion and Exclusion Criteria

The inclusion criteria for the study were:

1. Subject is at least 18 years of age but no more than 70 years of age.
2. Subject has at least two non-adjacent teeth in contralateral quadrants of the same jaw with an insufficient zone (≤ 1mm) of attached gingiva that requires soft tissue grafting. (1-3 teeth may be treated. In case of adjacent teeth requiring grafting, only <u>one</u> tooth at each site will act as test or control tooth, but all teeth will get the same treatment).
3. Root coverage is not desired at the time of grafting.
4. Females of childbearing potential must have a documented negative urine pregnancy test.
5. Subjects must have read, understood and signed an IRB approved informed consent form.
6. Subjects must be able and willing to follow study procedures and instructions.

Subjects were not eligible if they met any of the following exclusion criteria:

1. Subject with class III recession in the presence of a shallow vestibule or class IV recession.
2. Subject with vestibule depth of less than 7 mm from base of recession.

3. Subject with any systemic conditions that could compromise wound healing and preclude periodontal surgery (ie, diabetes mellitus, cancer, HIV, bone metabolic diseases).
4. Subject who is 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. Subject with the 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. Subject with only molar teeth suitable for soft tissue grafting.
9. Subject with teeth that have Miller Grade 2 or higher mobility.
10. Subject with 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 (medical or dental).
12. Subject who was previously treated with Apligraf (CelTx™), 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.

6.3.1.4 Statistical Methods

Power calculations were predicated upon achieving 85% power for the primary endpoint of the study, namely demonstrating superiority of Apligraf relative to a pre-defined standard (50% success) for a 2 mm KT threshold after 6 months. A one-sided exact binomial test with 5% Type I error was used to test this endpoint. For power calculations, it was assumed that the success rate under the alternative hypothesis was 66%. Note that the hypothesized Apligraf success level both (a) represents a clinically meaningful threshold endorsed by the Lead Investigator (Dr. Michael K. McGuire) and (b) accounts

for the expansion to multicenter setting, which included both private practice and academic centers.

Using the above parameters, a sample size of 74 patients was required to achieve 85% power. Accounting for training patients (N=14) and a 10% lost-to-follow-up rate (N=8), a total number of 96 patients were to be enrolled in the study.

Efficacy was analyzed on all subjects not identified as training cases (N=85). Safety was assessed on all subjects (N=96).

Continuous variables were summarized using descriptive statistics, specifically the mean, median, standard deviation, minimum, and maximum. Categorical variables were summarized using frequencies and percentages. Inferential tests were performed at the 5% level of significance. All p-values were rounded to 4 decimal places. If a rounded p-value was 0.0000 (ie, p-value <0.00005), then this was presented as a p-value of < 0.0001.

The superiority of Apligraf relative to a pre-defined standard (50% success) for a 2 mm KT threshold after 6 months was also tested with an exact binomial test, using a Type I error rate of 0.05. At the suggestion of the FDA, a minimally acceptable threshold was to be ruled out using a 95% one-sided confidence bound to rule out being non-inferior to a pre-defined standard. It was determined that the minimum threshold would be 50% (eg, 50% of Apligraf-treated sites achieve KT of at least 2 mm). To establish a 50% floor (according to a 95% lower confidence bound), it was determined that the study would need to achieve at least a 60% success rate. With a sample size of 96 subjects, a study result of at least ≥ 60% would demonstrate clinically acceptable rates of ≥ 2 mm of KT for the Apligraf-treated site.

While the study used a split-mouth design, the primary objective was not a direct comparison of Apligraf and FGG (ie, superiority trial). Use of a FGG for augmenting KT is a highly predictable clinical procedure, with the amount of KT post-procedure closely approximating the size (apical-coronal width) of the FGG grafted at Day 0. Apligraf is not intended to regenerate the same amount of KT compared to FGG. It is intended to generate an acceptable level of KT; thus, the primary endpoint chosen was not a direct comparison to FGG. Comparison of the percentage of Apligraf-treated sites that regenerated ≥ 2 mm KT to the 50% success standard ensures that it met at least a minimum level of KT performance that signifies a positive clinical outcome.

There were six secondary endpoints prospectively identified in the study. To maintain an overall Type I error rate of 0.05, the secondary hypotheses were tested using a closed testing strategy where the order of testing was pre-specified. The 6 secondary endpoints, their assessment methodology and statistical test methodology are presented in **Table 14**. The secondary endpoints where the treatment groups are directly compared (ie, color match, texture match, sensitivity and pain) are tested using a one-sided $\alpha = 0.025$ where the clinical interest was in assessing Apligraf's superiority to FGG. For the remaining 2 endpoints (ie, 1 mm KT and patient preference) one-sided $\alpha = 0.05$ was used.

Table 14: Summary of Secondary Endpoints

SECONDARY ENDPOINT	ASSESSMENT METHODOLOGY	STATISTICAL TEST
1. Color Match at 6 Months	An examiner assessed color of the Apligraf- and FGG treated sites compared with their respective adjacent, non-treated tissue. The assessment was recorded as "More Red," "Equally Red," or "Less Red" as compared to adjacent, non-treated tissue. A match in color with the surrounding tissue is considered a positive esthetic outcome.	The superiority of Apligraf compared to FGG as compared to adjacent non-treated tissues after 6 months was tested ("match" vs. "no match") with McNemar's marginal homogeneity test, using a one-sided $\alpha = 0.025$.
2. Texture Match at 6 Months	An examiner assessed the texture (firmness of the tissue) of the Apligraf- and FGG-treated sites compared with their respective adjacent, non-treated tissue. The assessment was recorded as "More Firm," "Equally Firm," or "Less Firm" as compared to adjacent, non-treated tissue. An equivalent firmness with the surrounding tissue is considered a positive esthetic and functional outcome.	The superiority of Apligraf compared to FGG in terms of texture compared to adjacent non-treated tissues after 6 months was tested ("match" vs. "no match") with McNemar's marginal homogeneity test, using a one-sided $\alpha = 0.025$.
3. At least 1 mm KT at 6 Months	Measured by the calibrated examiner using UNC-15 probe, aided by Schiller's iodine staining of mucosa.	The superiority of Apligraf relative to a pre-defined standard (80% success) for a 1 mm KT threshold after 6 months was tested with an exact binomial test, using a one sided $\alpha = 0.05$ Apligraf.
4. Patient Preference at 6 Months	Subjects were asked the question, "Taking into account all aspects of treatment (surgery, recovery, appearance) which treatment is preferred?"	The superiority of patient preference for Apligraf compared to FGG after 6 months was tested with an exact binomial test, using a one-sided $\alpha = 0.05$.

Table 14 continues to the following page…

Table 14: Summary of Secondary Endpoints (continued)

SECONDARY ENDPOINT	ASSESSMENT METHODOLOGY	STATISTICAL TEST
5. Surgical site sensitivity mild or absent after 1 week	The surgical site sensitivity of the FGG donor site and both treated sites was assessed by a 3 second puff of air and recorded as none, mild, moderate or severe.	The superiority of Apligraf compared to FGG (donor site and recipient site) in terms of mild or absent surgical site sensitivity after 1 week was tested with McNemar's marginal homogeneity test, using a one-sided $\alpha = 0.025$.
6. Pain absent after 3 days	Subjects made a daily pain assessment (none, mild, moderate, severe) of the FGG donor site and both treated sites in a study diary.	The superiority of Apligraf compared to FGG (donor site and recipient site) in terms of absence of pain after 3 days was tested with McNemar's marginal homogeneity test, using a one sided $\alpha = 0.025$.

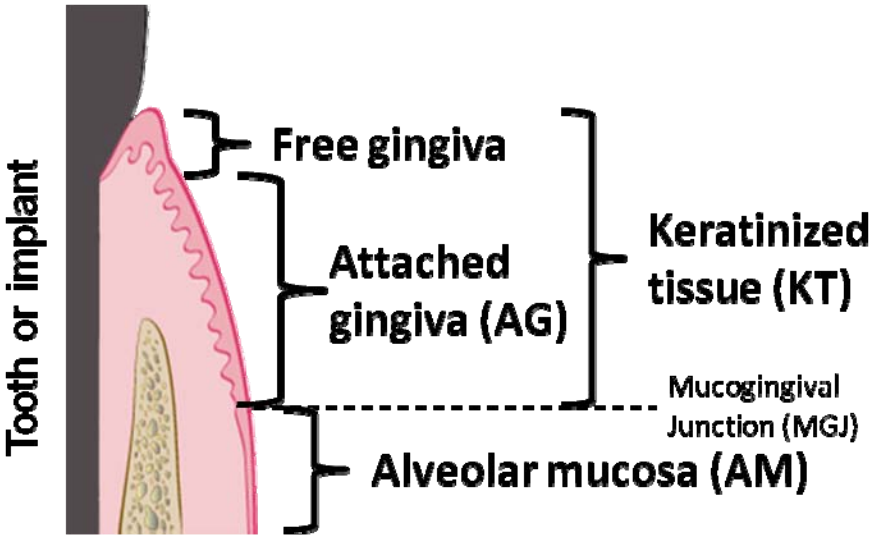
At the request of the FDA, two post hoc statistical analyses were performed. In response to a comment at the pre-PMA Meeting on 03 March 2009, regarding the importance of understanding the relationship of AG and KT, the Sponsor agreed to look at the relationship between AG and KT (referred to as "post hoc relationship between AG and KT"). In a follow-up statistical teleconference on 25 March 2009, the Sponsor agreed to perform inferential testing of other clinical efficacy endpoints (referred to as "post hoc inferential testing").

6.3.1.5 Study Endpoints

Width of KT was selected as the primary endpoint for this study for two important reasons. KT is able to be directly measured whereas AG is an indirect/derived measurement [AG=KT- probing depth, and as previously discussed in **Section 6.3.1**, the seminal work of Lang and Loe established 2 mm of keratinized gingiva (corresponding to 1 mm AG) around teeth as the adequate amount for maintenance of gingival health (Lang, 1972, *J Periodontol*). Without this, plaque accumulation and chronic inflammation may occur (Lang, 1972, *J Periodontol*; Kennedy, 1985, *J Clin Periodontol*; Bouri, 2008, *IJOMI*), increasing the probability of loss of the tooth or implant integrity (Hirschfeld 1978, *J Periodontol*; Wilson 1987 *J Periodontol*, Lang, 2009, *J Clin Periodontol*; Bouri, 2008, *IJOMI*; Roos-Jansaker, 2007, *Swed Dent J*; Schou, 2006, *Clin Oral Implant Res*).

Figure 20 is a schematic showing the functional soft tissue types surrounding a tooth, implant or edentulous site. Underneath the tough KT and flexible AM is periosteum lining the alveolar bone.

Figure 20: Anatomy of the Soft Tissue



Adapted from Newman, 2006, *Carranza's Clinical Periodontology 10th Ed.*

The primary objective of the study was to determine the ability of Apligraf to achieve a clinically acceptable threshold for KT at 6 months (≥ 2 mm KT).

There were 6 secondary endpoints prospectively identified in the study. These were previously described in detail in **Section 6.3.1.4**:

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

The primary and secondary endpoints were prospectively designed for the pivotal study to reflect the many factors clinicians consider when determining whether soft tissue augmentation procedures have been effective. These endpoints include a critical clinical measure of periodontal health (width of KT), esthetics (color and texture match to surrounding tissue), patient comfort (pain and sensitivity) and overall patient preference.

Additional efficacy assessments were also performed at 6 months: width of AG, width of KT, resistance to muscle pull, CAL, recession, inflammation, and bleeding on probing. These additional efficacy endpoints are measures of oral health and are routinely evaluated on an ongoing basis as standard of care.

Safety of Apligraf was assessed by monitoring treatment-specific and systemic AEs.

6.3.2 Pivotal Study Efficacy Results

6.3.2.1 Demographic and Baseline Characteristics

Of the 96 subjects, 54.2% were female and the mean age was 47.1 years with a range of 18.0 to 70.8 years. Subjects were enrolled at 4 participating clinical sites with relative balance in terms of number of subjects among 3 sites (Site 010: 34 subjects, Site 016: 29 subjects, Site 017: 30 subjects). The fourth site (Site 015) experienced delays in initiating subject enrollment and consequently enrolled only 3 subjects, see **Table 15**.

Table 15: **Pivotal Study: Selected Demographics**

PARAMETER	STATISTICS	PIVOTAL	TRAINING	ALL
	n	85	11	96
Sex				
Female	n (%)	46 (54.1%)	6 (54.5%)	52 (54.2%)
Male	n (%)	39 (45.9%)	5 (45.5%)	44 (45.8%)
Age (years)				
	Mean (SD)	46.9 (12.7)	49.4 (16.7)	47.1 (13.1)
	Median	48.3	53.3	48.8
	Min, Max	18.0, 70.8	21.2, 70.3	18.0, 70.8
Race				
White	n (%)	77 (90.6%)	10 (90.9%)	87 (90.6%)
Black of African American	n (%)	1 (1.2%)	0 (0.0%)	1 (1.0%)
Asian	n (%)	4 (4.7%)	1 (9.1%)	5 (5.2%)
Other	n (%)	3 (3.5%)	0 (0.0%)	3 (3.1%)

Table 15 continues to the following page…

Table 15: Pivotal Study: Selected Demographics (continued)

PARAMETER	STATISTICS	PIVOTAL	TRAINING	ALL
Site				
Site 10	n (%)	30 (35.3%)	4 (36.4%)	34 (35.4%)
Site 15	n (%)	0 (0.0%)	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%)
Source: 06-PER-002-CTX CSR Table 14.1.2 and 14.1.5				

The subjects enrolled in the study were generally healthy. Aside from abnormal gingiva, the results of the oral exam were not clinically remarkable.

The baseline characteristics of the treated oral mucosal defects for the subjects in the efficacy (N=85) population are provided in **Table 16**. The study inclusion criteria required ≤ 1 mm AG at study entry, but did not have a requirement with regard to KT.

Table 16: **Pivotal Study: Baseline Oral Mucosal Defect Characteristics**

PARAMETER	STATISTICS	APLIGRAF STUDY TOOTH	FGG STUDY TOOTH
KT Width (mm)			
	Mean (SD)	1.41 (0.72)	1.43 (0.69)
	Median	1.0	1.5
	Min, Max	0.0, 3.0	0.0, 3.0
AG Width (mm)*			
	Mean (SD)	0.02 (0.76)	0.08 (0.79)
	Median	0.0	0.0
	Min, Max	-2.0, 1.5	-2.0, 1.5
Recession Depth (mm)			
	Mean (SD)	2.13 (1.33)	1.92 (1.27)
	Median	2.0	2.0
	Min, Max	0.0, 5.0	0.0, 5.5
Table 16 continues to the following page…			

Table 16: Pivotal Study: Baseline Oral Mucosal Defect Characteristics (continued)

PARAMETER	STATISTICS	APLIGRAF STUDY TOOTH	FGG STUDY TOOTH
Inflammation Score**			
0	n (%)	71 (83.5%)	70 (82.4%)
1	n (%)	12 (14.1%)	14 (16.5%)
2	n (%)	2 (2.4%)	1 (1.2%)
3	n (%)	0 (0.0%)	0 (0.0%)
4	n (%)	0 (0.0%)	0 (0.0%)
Bleeding on Angulated Probing			
None	n (%)	62 (72.9%)	67 (78.8%)
Bleeding	n (%)	23 (27.1%)	18 (21.2%)
Plaque Scores, Buccal			
Absent	n (%)	67 (78.8%)	66 (77.6%)
Present	n (%)	18 (21.2%)	19 (22.4%)
Plaque Scores, Lingual			
Absent	n (%)	57 (67.1%)	55 (64.7%)
Present	n (%)	27 (31.8%)	29 (34.1%)

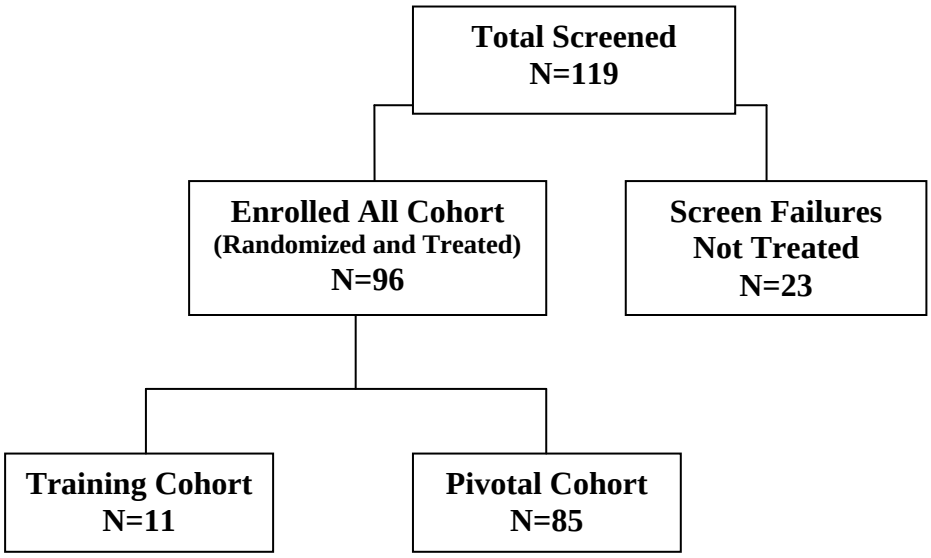
*AG is a calculated value, KT – probing pocket depth. Negative AG is a result of a probing pocket depth greater than the KT width.
**Inflammation scores ranged from 0 to 4, 0: Normal; 1: Mild of any portion of the marginal unit; 2: Mild of the entire gingival unit; 3: Moderate; 4: Severe.
Source: 06-PER-002-CTX CSR Table 14.2.10.1.1.

Protocol deviations occurred during the course of the study (primarily due to deviation in procedure or visit schedule), and were assessed by the Sponsor as minor and not being clinically significant. No subject was discontinued from the study due to a deviation.

6.3.2.2 Patient Disposition

Figure 21 presents disposition for subjects enrolled in this study, including cohort allocation. Subjects were screened into the study after signing informed consent. Randomization to determine which treatment (Apligraf or FGG) was applied to each defect surgically created wound bed and the order of treatment occurred immediately prior to surgery (Day 0).

Figure 21: Subject Disposition



Source: 06-PER-002-CTX CSR Table 14.1.1

Of the 119 subjects screened, 23 subjects did not receive treatment and are not included in any analyses. The most common reasons for withdrawal prior to surgery were failure to meet inclusion/exclusion criteria, specifically lack of teeth in contralateral quadrants with insufficient gingiva (N=6), use of a prohibited medication that would compromise wound healing/preclude surgery (N=6) and subject unwillingness to follow study procedures and instructions (N=5). The other reasons for withdrawal were previous treatment with any graft at target or adjacent teeth (N=2) and vestibule depth less than 7 mm from base of recession (N=1), use of tobacco within 3 months (N=1), only molar teeth suitable for grafting (N=1) and known hypersensitivity to bovine collagen and/or iodine (N=1).

The remaining 96 subjects were enrolled, randomized and treated. Per the protocol, the first 2 treated subjects per Investigator were designated as training subjects. All 96 subjects completed the 6 month study and all required study visits. For this study, the modified intent-to-treat (mITT), per protocol (PP), and safety populations were all identical (96 subjects).

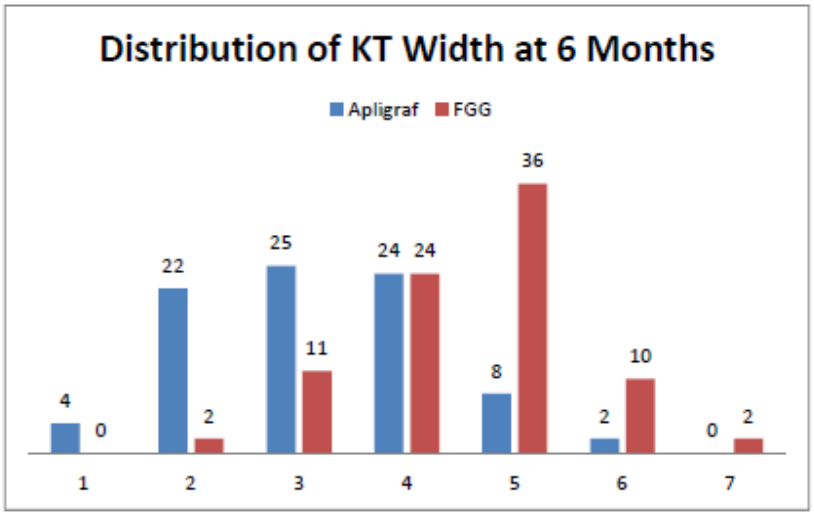
6.3.2.3 Primary Efficacy Endpoint: KT ≥ 2.0 mm at Month 6

There were 95.3% (81 of 85) of the Apligraf-treated sites that had at least 2 mm of KT at 6 months. As compared to the 50% success threshold this was statistically significant,

p < 0.0001. A mean width of 3.2 (SD 1.1) mm of KT was regenerated at Apligraf-treated sites.

As explained previously, it would be expected that the amount of KT generated by FGG would be closely related to the size of the graft; the results of this study confirm this. All FGG-treated sites (85 of 85) regenerated at least 2 mm KT at 6 months and the mean KT width at 6 months was 4.6 (1.0) mm. By contrast, the Apligraf-treated site must regenerate KT de novo. Accordingly, a direct comparison of the amount of KT in the Apligraf-treated sites versus the FFG-treated sites would not be meaningful. Comparison of the percentage of Apligraf-treated sites that regenerated at least ≥ 2 mm KT to the 50% success standard ensures that it met at least a minimum level of KT performance, signifying a positive clinical outcome. **Figure 22** displays the distribution of KT responses at 6 months for both the Apligraf- and FGG-treated sites.

Figure 22: Distribution of KT Widths at 6 Months, Apligraf- and FGG-treated Sites



Source: Derived from 06-PER-002-CTX CSR 16.2.15, KT measurements rounded down, ie, 2.5 mm displayed as 2.0 mm.

6.3.2.4 Secondary Efficacy Endpoints

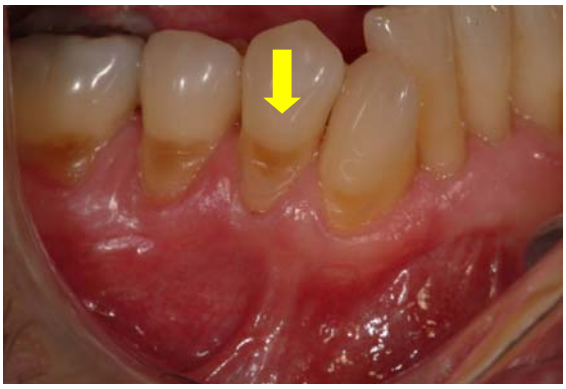
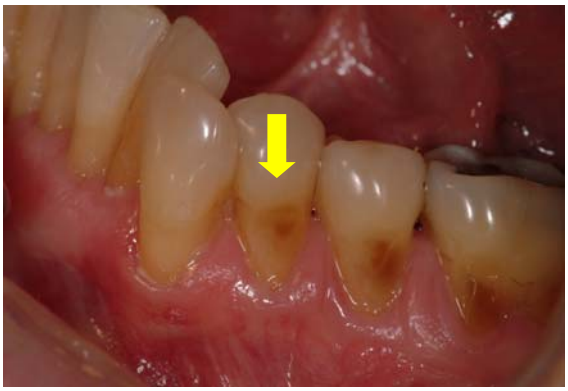
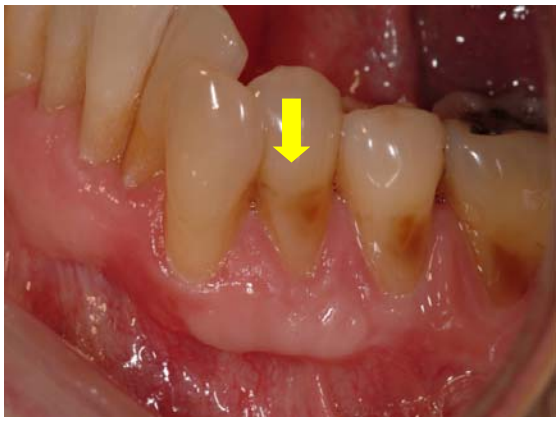
The study results supported the following 4 secondary efficacy superiority tests in favor of Apligraf:

Color at Month 6 was significantly different for Apligraf compared to FGG (p < 0.0001). Of the Apligraf-treated sites, 79 (92.9%) were equally red compared to the adjacent tissue versus only 23 (27.1%) of the FGG-treated sites.

Texture at Month 6 was significantly different for Apligraf compared to FGG ($p < 0.0001$). Of the Apligraf-treated sites, 81 (95.3%) were equally firm as the adjacent tissue compared to 46 (54.1%) of the FGG-treated sites. In addition, 39 (45.9%) of the FGG-treated sites were assessed as more firm than the adjacent tissue compared to 0 Apligraf-treated sites.

These side by side pictures of the baseline and 6-month photographs of the Apligraf- and FGG-treated teeth illustrate that while there was > 2 mm KT at each study tooth at 6 months, there is also an appreciable difference in terms of color and texture match between the treated teeth (**Figure 23**). Apligraf resulted in a higher percentage of color and texture matches compared to adjacent untreated tissue, without the creation of the palatal donor site.

Figure 23: Illustration of Color and Texture Results, Pivotal Study Subject

 Baseline Apligraf-treated site; KT = 2.0 mm	 Baseline FGG-treated site; KT = 2.0 mm
Month 6 Apligraf-treated site, KT = 4.0 mm	 Month 6 FGG-treated site, KT = 5.0 mm
Year 2 Apligraf-treated site*	Year 2 FGG-treated site*

*Year 2 follow-up (post conclusion of 06-PER-002-CTX) provided courtesy of Marc L. Nevins, DDS, MS. No KT measuresments available.

KT ≥ 1.0 mm at Month 6 was significantly greater for Apligraf compared to pre-defined 80% clinical success standard ($p < 0.0001$). Eight-five (100%) Apligraf-treated sites and 85 FGG-treated sites (100%) had KT ≥ 1.0 mm at Month 6.

Overall subject preference at Month 6 was significantly greater for Apligraf compared to FGG ($p < 0.0001$). Subject preference for Apligraf was reported by 71.8% of the subjects. The finding that Apligraf treatment was preferred over FGG treatment may be due to several things, including the ability of Apligraf to regenerate KT without the creation of a palatal donor site, and the comparability of Apligraf-treated tissue to adjacent untreated tissue in terms of color and texture.

Sensitivity and Pain. The secondary efficacy superiority hypotheses for sensitivity (at Week 1) and pain (at Day 3) were not supported by the study data. However, the sensitivity and pain results were comparable between the study procedures. It is important to note that the sensitivity and pain results for FGG included both the FGG-treated site and the palatal donor site at these time points; thus, pain specific to the palatal donor site is not specifically called out in this analysis. However, the Apligraf procedure, by virtue of its design, did not result in palatal graft harvest site pain on the day of surgery, in contrast to the FGG procedure. For sensitivity and pain results, the FGG treatment includes results reported for both the treated site and donor site.

6.3.2.5 Additional Efficacy Endpoints

Additional efficacy endpoints defined in the protocol included: width of AG, width of KT, resistance to muscle pull, CAL, recession, inflammation, and bleeding on probing. These endpoints are considered standard of care and are routinely performed as measure of periodontal health.

As shown in **Table 17**, subjects in the pivotal study maintained the same level of periodontal health throughout the study. The amount of AG generated at Month 6 for both the Apligraf- and FGG-treated sites was statistically significant compared to baseline ($p < 0.001$).

Table 17: Additional Efficacy Assessments (Efficacy, N=85)

PARAMETER	APLIGRAF-TREATED SITE MEAN (MIN, MAX)		FGG-TREATED SITE MEAN (MIN, MAX)	
	BASELINE	6 MONTHS	BASELINE	6 MONTHS
Recession depth (mm)	2.13 (0.0, 5.0)	2.01 (0.0, 5.0)	1.92 (0.0, 5.5)	1.82 (0.0, 6.0)
Recession (%)*	Not evaluated	3.08 (-100.0, 100.0)	Not evaluated	8.25 (-150.0, 100.0)
Probing Pocket Depth (mm)	1.35 (0.5, 2.0)	1.44 (1.0, 2.0)	1.35 (1.0, 3.0)	1.40 (1.0, 2.0)
Proximal Probing Depth, Mesial (mm)	2.30 (1.0, 4.0)	2.32 (1.0, 11.0)	2.39 (1.0, 4.5)	2.33 (1.0, 11.0)
Proximal Probing Depth, Distal	2.16 (0.5, 5.0)	2.24 (1.0, 10.0)	2.08 (1.0, 4.0)	2.22 (1.0, 11.0)
CAL (mm)	3.52 (1.0, 7.0)	3.45 (1.0, 7.0)	3.26 (1.0, 7.5)	3.22 (1.0, 8.0)
Attached Gingiva (mm)**	0.02 (-2.0, 1.5)	1.77 (-1.0, 5.0)	0.08 (-2.0, 1.5)	3.17 (0.0, 6.0)
	N (%)		N (%)	
No Bleeding on Angulated Probing (%)	62 (72.9%)	68 (80.0%)	67 (78.8%)	72 (84.7%)
No Muscle Pull Resistance† (%)	60 (70.6%)	73 (85.9%)	63 (74.1%)	82 (96.5%)
Inflammation, Score of 0 (Normal) (%)	71 (83.5%)	76 (89.4%)	70 (82.4%)	76 (89.4%)

*For subjects with a baseline recession depth equal to 0, the recession % is unable to be calculated. Numbers are shown for subjects with baseline recession depth greater than 0; Apligraf N=75, FGG N=72.

**AG is a calculated value, KT – probing pocket depth. Negative AG is a result of a probing pocket depth greater than the KT width

†The Protocol stated "Resistance to muscle pull will be based on whether the free gingival margin of the tissue facial to the site moves when the adjacent cheek is retracted", however additional instructions to clearly indicate what was meant by either a "Yes" or "No" response was not provided. During a post-study Investigators' Meeting (March 13-14, 2009) these results were reviewed and it was determined that the Muscle Pull Resistance data were not collected consistently

Source: 06-PER-002-CTX CSR Table 14.2.10.2.1.

6.3.2.6 Additional Analyses

Additional analyses were performed in terms of the number of teeth treated (ie, single teeth or multiple teeth at the treatment site). The results from these additional analyses generally demonstrated statistically significant effects of Apligraf ($p < 0.05$) regardless of whether the treatment site involved a single tooth or multiple teeth. At Month 6, 94.1%

(48/51) of Apligraf-treated sites in the efficacy cohort involving a single tooth had achieved ≥ 2 mm KT while 97.1% (33/34) involving multiple teeth had achieved ≥ 2 mm KT.

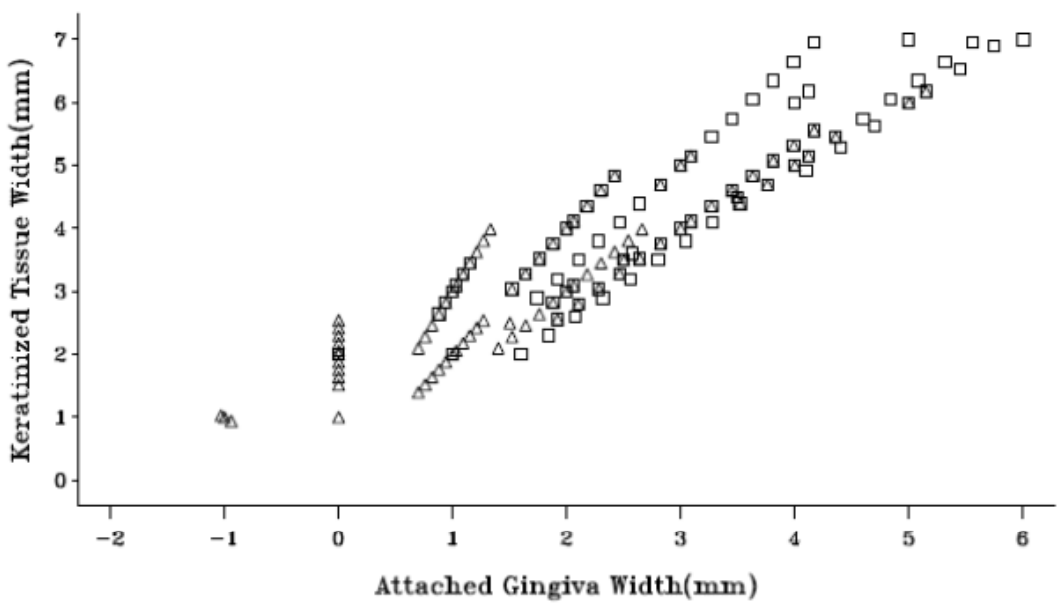
6.3.2.7 Additional Analyses Performed at the Request of the FDA

As stated previously, 2 additional analyses were performed at the request of the FDA: the post hoc relationship between AG and KT; and the post hoc inferential testing (ie, inferential testing of other clinical efficacy endpoints).

6.3.2.7.1 Post hoc relationship between AG and KT

Given the relationship between KT and AG, a post hoc analysis was performed to analyze this relationship. **Figure 24** presents a scatter plot where each treated site is represented on the plot. This post hoc analysis confirmed that generally the amount of AG is approximately 1-2 mm less than the amount of KT measured.

Figure 24: AG Width vs. KT Width at Month 6 (N=85)



Treatment Group **Δ Δ Δ Apligraf** **□ □□ FGG**

Source: 06-PER-002-CTX CSR Figure 14.5.1

6.3.2.7.2 Post hoc inferential testing of additional efficacy endpoints

Post hoc inferential testing of the additional efficacy endpoints tested change from baseline within a treatment group. In the Apligraf-treated sites, changes from baseline were noted in KT ($p < 0.0001$), AG ($p < 0.0001$), and resistance to muscle pull[rr] ($p = 0.017$). For the FGG-treated sites, changes from baseline were noted in KT ($p<0.0001$), change in AG ($p<0.0001$), change in resistance to muscle pull[i] ($p<0.0001$), and mesial proximal probing depth ($p = 0.022$). The result for mesial proximal probing depth in the Apligraf-treated sites was marginally different ($p = 0.055$).

Differences between treatment sites (Apligraf and FGG) were observed at 6 months for KT width ($p < 0.0001$) and AG ($p < 0.0001$) with more KT and AG at the FGG-treated sites.

6.4 Additional Clinical Evidence for Apligraf

This section includes a summary of additional analyses that were performed in conjunction with the pilot and pivotal clinical studies:

- An Independent Photograph Assessment was performed to corroborate the assessments of color and texture that were made by examiners at each of the pivotal study investigational sites;
- An additional histologic evaluation on the biopsy specimens taken at baseline and 6 months in the pilot study.

Detailed information on the Independent Photographer Assessment and Additional Histological Evaluation are provided in **Appendices 2** and **3**.

6.5 Efficacy Conclusions

Apligraf demonstrated clinically and statistically significant regeneration of at least 2 mm KT. Specifically, the pilot and pivotal studies showed the following:

- In the pilot study 82% of Apligraf-treated sites regenerated at least 2 mm KT with a mean width of 2.5 mm at 6 Months

[rr] The Protocol stated "Resistance to muscle pull will be based on whether the free gingival margin of the tissue facial to the site moves when the adjacent cheek is retracted", however additional instructions to clearly indicate what was meant by either a "Yes" or "No" response was not provided. During a post-study Investigators' Meeting these results were reviewed and it was determined that the Muscle Pull Resistance data were not collected consistently.

- In the pivotal study 95% of Apligraf-treated sites regenerated at least 2 mm KT with a mean width of 3.2 mm at 6 Months
- Both studies demonstrated that Apligraf-treated sites are better matched to the adjacent, non-treated tissue compared to FGG-treated sites with respect to both color and texture

In contrast to Apligraf, FGG relies on primary intention healing via engraftment of the FGG to the base of the mucosal defect and this tissue characteristically retains the appearance of palatal tissue. KT is formed but it is not site appropriate for gingiva: it is keratinized palatal mucosa (Ivancie, 1957, *J Periodontol*).

It is also important to note that Apligraf did not accelerate healing back to baseline conditions (eg, minimal KT) but actually altered the healing trajectory by inducing the formation of more KT relative to baseline. In contrast to the typical sequelae of approaches relying on healing by secondary intention alone, there was no prolonged healing (maximal KT width was established by 3 months), no scar formation, no return to baseline and no site inappropriate tissue (eg, AM).

The pivotal study, in its design, execution and results, fulfilled all of the criteria listed in **Section 6.3.1.1** and provides objective criteria for the efficacy of Apligraf. The results in the pivotal study demonstrated efficacy of Apligraf:

- The primary efficacy endpoint (Apligraf generates ≥ 2 mm KT) was met ($p < 0.0001$).
- Four of the secondary efficacy endpoints (color same as adjacent tissue, texture same as adjacent tissue, Apligraf generates ≥ 1 mm KT, and patient preference) were met ($p<0.0001$). The two other secondary efficacy endpoints (sensitivity and pain), although not statistically significant as tested, had similar results for both the Apligraf- and FGG-treated sites.
- With the exception of a statistically significant increase in KT and AG at 6 months, the additional efficacy endpoints did not indicate a change in periodontal health due to the study treatment. This demonstrates that augmenting the width of KT on both the Apligraf and FGG study teeth was effective in preventing further recession or worsening of baseline inflammation.

These data are also supportive of the potential utility of Apligraf in clinical situations not directly investigated in the pivotal study. Examples of this include situations in which

donor tissue cannot be harvested due to technical reasons, donor tissue is insufficient for the defect (Hall, 1993, *Dent Clin North Am*) or harvesting donor palatal tissue cannot be reasonably justified given the presence of a small defect. In all of these clinical scenarios it would still be appropriate to use Apligraf. The harvest of palatal tissue (FGG) exposes the subject to risks (Wagshall, 2002, *ASDC J Dent Child*) such as bleeding and clot formation as experienced by two subjects in the current pivotal study. These risks can be avoided with use of Apligraf.

Given the favorable profile with respect to the clinically relevant efficacy outcomes based on improvements in the rate and quality of healing by secondary intention, Apligraf represents a new treatment for gingival and alveolar mucosal surface defects when applied over a vascular wound bed to generate a functional zone (≥ 2 mm) of KT (including AG) as well as to support site appropriate regeneration of oral mucosa.

7 SAFETY

7.1 Exposure

A summary of overall exposure of Apligraf for the pilot and pivotal studies directly supporting the indication (05-PER-001 and 06-PER-002-CTX) is provided in **Table 18**. Safety results from the third oral clinical study (07-PER-004-CTX, N=15) are summarized separately in **Section 7.3.1**.

Across the studies included in this summary of clinical safety, a total of 121 subjects received 1 dose of Apligraf at Day 0 of the clinical study with a 6 month follow-up. No subjects in either trial were retreated with Apligraf during the 6 month study. The amount of Apligraf used in 1 application or 1 dose is dependent on the size of the wound being treated.

Table 18: Overall Exposure of Apligraf and Comparators

	APLIGRAF (*OR COMPARATOR*)		
	NO. OF SUBJECTS EXPOSED	MEAN TREATMENT DURATION, DAYS	ACTUAL DOSE
Non-submerged Oral Mucosal Defects			
Pilot Study			
Apligraf	25	1 treatment of Apligraf at Day 0	1 dose of Apligraf
FGG	*25*	*1 FGG procedure at Day 0*	*1 FGG procedure*
Pivotal Study			
Apligraf	96	1 treatment of Apligraf at Day 0	1 dose of Apligraf
FGG	*96*	*1 FGG procedure at Day 0*	*1 FGG procedure*
Source: 05-PER-001 Study Final Report and 06-PER-002-CTX Final Study Report.			

7.2 Adverse Events Overview

Adverse Events (AEs) included any adverse change in the subject's medical status when compared with the subjects' baseline condition, *whether or not the event is related to the study device or a study procedure*; or an exacerbation (either in frequency or severity) in the subject's pre-existing condition.

Anticipated post-surgical sequelae (eg, bleeding, swelling, pain) were not recorded as an AE unless (1) treatment/procedure was required (eg, emergency visit for uncontrolled bleeding) or (2) the severity or duration deviated from the expected course of healing, as determined by the Investigator.

SAEs were defined as any event which is or results in any of the following outcomes: (1) life-threatening event or death, (2) persistent or signification disability/incapacity, (3) requires or prolongs the subject hospitalization, and (4) important medical events, which may jeopardize the subject and may require medical or surgical intervention to prevent one of the above outcomes.

Unanticipated adverse device effects (UADEs) were defined per 21 CFR 812.3.

All AEs presented in this summary of safety were coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 10.0. Adverse events from the pilot study were not coded in MedDRA for the pilot clinical study report and were reported based on the verbatim term reported by the Investigator. In order to present an integrated summary of AEs for both studies, all AEs reported in the pilot study were subsequently coded and reviewed by the Sponsor's Chief Medical Officer to confirm that the coded events were consistent with the events as originally reported by the investigator.

7.2.1 Common Adverse Events

7.2.1.1 Common Adverse Events in Apligraf Oral Studies (05-PER-001 and 06-PER-002-CTX)

Table 23 presents the integrated summary of safety at both the subject level and the event level. At the subject level, multiple occurrences of an event of a subject were counted only once for a given SOC or a preferred term. At the event level, every occurrence is counted.

A total of 65 AEs were reported for 41 subjects in the pilot (17 of 25 subjects) and pivotal (24 of 96 subjects) studies. There were 80 subjects who did not experience an AE during either study.

In general, most events were reported with only 1 or 2 occurrences. In terms of SOC, the 2 organ classes with the most reported events were Infections and Infestations (13 subjects with 16 events) and Gastrointestinal Disorders (12 subjects with 13 events). Within these 2 SOCs, only 1 type of event was reported more than twice: there were 5 subjects with 6 reports of sinusitis.

The only other specific AE reported in more than 2 subjects was hypersensitivity (4 events in 4 subjects), refer **Table 19**. Three subjects in the pilot study were reported to have 1 AE each of "seasonal allergies." These events were re-coded to the preferred term hypersensitivity. One subject in the pivotal study was reported to have an AE of

"environmental allergies" and this was also coded to the preferred term of hypersensitivity.

Table 19: Adverse Events: Integrated Safety Summary (N=121 Subjects)

SYSTEM ORGAN CLASS PREFERRED TERM	SUBJECT N (%)	EVENT N
Overall	41 (33.9%)	65
INFECTIONS AND INFESTATIONS	13 (10.7%)	16
Mastitis	1 (0.8%)	1
Nasopharyngitis	2 (1.7%)	2
Oral Herpes	1 (0.8%)	1
Pneumonia	2 (1.7%)	2
Respiratory Tract Infection	2 (1.7%)	2
Sinusitis	5 (4.1%)	6
Upper Respiratory Tract Infection	2 (1.7%)	2
GASTROINTESTINAL DISORDERS	12 (9.9%)	13
Abdominal Pain	1 (0.8%)	1
Aphthous Stomatitis	2 (1.7%)	2
Dental Caries	2 (1.7%)	2
Gingival Pain	1 (0.8%)	1
Gingivitis	1 (0.8%)	1
Mouth Ulceration	1 (0.8%)	1
Oral Pain	2 (1.7%)	2
Paraesthesia Oral	1 (0.8%)	1
Stomach Discomfort	1 (0.8%)	1
Toothache	1 (0.8%)	1
INJURY, POISONING AND PROCEDURAL COMPLICATIONS	5 (4.1%)	5
Gingival Injury	1 (0.8%)	1
Joint Injury	1 (0.8%)	1
Mouth Injury	2 (1.7%)	2
Post Procedural Hemorrhage	1 (0.8%)	1

Table 19 continues to the following page…

Table 19: Adverse Events: Integrated Safety Summary (N=121 Subjects) (continued)

SYSTEM ORGAN CLASS PREFERRED TERM	SUBJECT N (%)	EVENT N
SKIN AND SUBCUTANEOUS TISSUE DISORDERS	5 (4.1%)	5
Hypoaesthesia Facial	2 (1.7%)	2
Psoriasis	1 (0.8%)	1
Skin Exfoliation	1 (0.8%)	1
Urticaria Papular	1 (0.8%)	1
IMMUNE SYSTEM DISORDERS	4 (3.3%)	4
Hypersensitivity	4 (3.3%)	4
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS	4 (3.3%)	5
Back Pain	1 (0.8%)	1
Bursitis	1 (0.8%)	1
Temporomandibular Joint Syndrome	1 (0.8%)	1
Tendonitis	2 (1.7%)	2
VASCULAR DISORDERS	3 (2.5%)	3
Hypertension	1 (0.8%)	1
Thrombosis	1 (0.8%)	1
Wound Hemorrhage	1 (0.8%)	1
INVESTIGATIONS	2 (1.7%)	3
Blood Cholesterol Increased	1 (0.8%)	1
Prostate Examination Abnormal	1 (0.8%)	1
Weight Decreased	1 (0.8%)	1
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCLUDING CYSTS AND POLYPS)	2 (1.7%)	2
Follicular Thyroid Cancer[ss]	1 (0.8%)	1
Malignant Fibrous Histiocytoma Metastatic[tt]	1 (0.8%)	1

Table 19 continues to the following page…

[ss] This AE was reported on Study Day 92. The subject had a medical history that included hypothyroidism and removal of a precancerous lesion on the face.
[tt] This AE was reported on Study Day 154. The subject's medical history included a mass that had been documented several years prior to entry into this study, but the subject had not sought diagnosis of and treatment for it.

Table 19: Adverse Events: Integrated Safety Summary (N=121 Subjects) (continued)

SYSTEM ORGAN CLASS PREFERRED TERM	SUBJECT N (%)	EVENT N
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	2 (1.7%)	2
Pharyngolaryngeal Pain	1 (0.8%)	1
Pleural Effusion	1 (0.8%)	1
SURGICAL AND MEDICAL PROCEDURES	2 (1.7%)	2
Tendon Operation	1 (0.8%)	1
Tooth Extraction	1 (0.8%)	1
BLOOD AND LYMPHATIC SYSTEM DISORDERS	1 (0.8%)	1
Anaemia	1 (0.8%)	1
CARDIAC DISORDERS	1 (0.8%)	1
Bifascicular Block	1 (0.8%)	1
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	1 (0.8%)	1
Chest Pain	1 (0.8%)	1
NERVOUS SYSTEM DISORDERS	1 (0.8%)	1
Migraine	1 (0.8%)	1
REPRODUCTIVE SYSTEM AND BREAST DISORDERS	1 (0.8%)	1
Vaginal Hemorrhage	1 (0.8%)	1
Source: 05-PER-01 Data on file, Organogenesis, and 06-PER-02-CTX Final Study Report (Listing 16.2.18.1).		

7.2.2 SAEs

No SAEs were reported in the pilot study. However there were 3 SAEs reported during the pivotal study. A brief tabular summary of the 3 SAEs are provided in **Table 20**.

Of note, subject b(6) had a history that included a posterior mediastinal mass and herpes zoster. On Day 154, the subject was hospitalized with a diagnosis of metastatic malignant fibrous histiocytoma. The Principal Investigator assessed the severity of this event as “severe” and unlikely to be related to the Apligraf treatment.

Table 20: Summary of SAEs (Pivotal Study)

SUBJECT ID	ADVERSE EVENT (PREFERRED TERM)	SAE START DATE (RELATIVE TO DAY 0)	RELATIONSHIP TO TREATMENT
b(6)	Pneumonia	Day 100	Not Related
	Chest Pain	Day 7	Not Related
	Metastatic Malignant Fibrous Histiocytoma	Day 154	Unlikely
Source: Listing 16.2.18.1.1, Listing 16.2.18.1.2			

7.2.2.1 Other Significant AEs

No other significant AEs were reported in the pilot study. However there were 3 AEs reported during the pivotal study that were not reported as SAEs but judged by the Sponsor's Medical Monitor to be clinically important. A brief tabular summary of these 3 AEs is provided in **Table 21**.

Inadvertent placement of the Apligraf polycarbonate membrane to the surgically created wound bed at the time of Apligraf placement occurred in 2 subjects and resulted in 2 AEs (gingival injury and gingival pain) in the pivotal study. These AEs occurred only in the training subjects and not in the efficacy cohort (ie, non-training subjects).

Follicular thyroid cancer was not reported as a SAE. The subject underwent same-day surgery for removal of a Hurthle cell lesion and this was assessed by the Investigator as not meeting the criteria for SAE reporting.

Table 21: Summary of Other Significant AEs (Pivotal)

SUBJECT ID	ADVERSE EVENT (PREFERRED TERM)	SAE START DATE (RELATIVE TO DAY 0)	RELATIONSHIP TO TREATMENT
b(6)	Gingival injury	Day 94	Possibly
	Gingival pain	Day 2	Probably
	Follicular thyroid cancer	Day 92	Not Related
* Training Subject Source: Listing 16.2.18.1.1, Listing 16.2.18.1.2			

7.2.3 Deaths

No deaths were reported in the pilot or pivotal study for the proposed oral indication.

7.3 Other Safety Information

7.3.1 Summary of Safety from 07-PER-004-CTX

Fifteen subjects were treated in pilot study 07-PER-004-CTX to evaluate the safety of Apligraf in the treatment of gingival recession requiring root coverage. All subjects had coronally advanced flaps for root coverage and a single application of Apligraf[uu] at 1 treated tooth and a sub-epithelial connective tissue graft (CTG) at a contralateral treated tooth at Day 0. Subjects were followed for up to 6 months.

Nine subjects experienced 20 AEs during the study. No deaths, SAEs, or UADEs occurred during the study.

Two subjects experienced an AE at the Apligraf-treated site (2 AEs total). One subject had impaired healing of the right target tooth; this AE was considered to be mild and probably related to treatment with Apligraf. Of note, the investigator used resorbable sutures at both surgical sites instead of nylon sutures, which constituted a protocol deviation. The subject underwent treatment with Orabase and placement of an additional gingival suture and the AE resolved. The second subject had gingival inflammation due to sutures reported at both the Apligraf- and CTG-treated sites. Both AEs were considered mild, not related to treatment with Apligraf, and resolved without intervention.

Two subjects experienced an AE (2 AEs total) at the CTG-treated site. One subject is described above, and the other subject experienced a gingival lesion at the CTG-treated site secondary to toothbrush trauma. This AE was also considered mild, not related to treatment with Apligraf, and resolved without intervention.

Four subjects experienced 7 AEs in areas of the mouth other than the Apligraf- and CTG-treated sites. Five subjects experienced 9 AEs in locations other than the mouth.

7.3.2 Summary of Apligraf Immunology Results from Cutaneous Apligraf Clinical Trial Experience

In the oral clinical development program, no immunological testing was conducted. Immunological testing was not required by CDRH during either the pilot or pivotal clinical studies.

There is significant immunological clinical safety experience with Apligraf in acute and chronic cutaneous wounds. Similar to the surgically created wound bed that is treated

[uu] The first 8 subjects had a double layer of Apligraf and the remaining 7 subjects had a single layer applied.

with Apligraf in the oral clinical program, acute cutaneous wounds are highly vascular; therefore, the immunological clinical safety data obtained in acute cutaneous wound clinical studies are relevant to the oral indication. **Table 22** summarizes the Organogenesis acute and chronic cutaneous wound clinical trials in which the humoral and cellular responses to Apligraf were assessed. A total of 589 Apligraf-treated subjects in 11 Organogenesis sponsored clinical trials (1990 – 2002) were evaluated for immunological responses to Apligraf.

Table 22: Summary of Organogenesis-Sponsored Clinical Studies Assessing Immunological Responses to Apligraf

STUDY NO.	STUDY POPULATION	NO. OF APLIGRAF PATIENTS	STUDY DURATION	APLIGRAF TREATMENT
Acute Cutaneous Wounds				
91-EXC-001	Acute surgical excision wounds	107	12 months	Single application
94-GDS-001	Acute partial thickness donor sites	20	2 months	Single application
96-GDS-002	Acute partial thickness donor sites in patients with burn wounds.	7	6 months	Single application
97-EXC-002	Acute full-thickness surgical excision wounds	88 [also 93 Control subjects]	6 months	Single application
97-GDS-003	Acute partial thickness donor sites	12	6-months	Single application
98-GDS-004	Acute partial thickness donor sites	10	3-months	Single application*
420	Second and third degree burn wounds	15	24 months	Single application
420-A	Second and third degree burn wounds	56	24 months	Single application
Chronic Cutaneous Wounds				
92-VSU-001	Chronic VLUs	161 [also 137 Control subjects]	12 months	Up to 5 applications within first 3 weeks of study
95-CUP-001	Recalcitrant chronic ulcer secondary to congenital hydrocele	1	15 months	Up to 5 applications over a 3 month period

Table 22 continues to the following page…

Table 22: Summary of Organogenesis-Sponsored Clinical Studies Assessing Immunological Responses to Apligraf (continued)

STUDY NO.	STUDY POPULATION	NO. OF APLIGRAF PATIENTS	STUDY DURATION	APLIGRAF TREATMENT
95-DUS-001	Chronic DFUs	112 [also 96 Control subjects]	6 months	Up to 5 applications in the first 4 weeks.
*Treatments included Apligraf with and without epidermis applied to separate donor sites (within-patient) Source: Data on file, Organogenesis Individual study protocols and CSRs for each study				

Subjects were monitored for the development of humoral and cellular immune responses to Apligraf. Briefly, the humoral response was monitored by testing subjects for circulating antibodies specific for bovine Type I collagen and the cellular components (fibroblasts and keratinocytes) of Apligraf. The cellular response was monitored by testing subjects for antigen specific T cells which recognize components of Apligraf.

In summary, immune responses were tested in patient populations with a wide spectrum of immunological conditions and the observations relating to humoral and cellular reactivity were consistent across these patient populations [ie, no detectable antibodies specific for Apligraf components (or no increase from baseline), absence of T cell proliferation in response to the cellular components of the construct and no obvious clinical signs of rejection].

Note that the results of the 420 study are unable to be integrated into this analysis because the assay protocols were different[vv]. Despite the variations in assay protocols, the same variables were assessed and the same general methods were used (ie, ELISA, complement dependent micro-lymphocytotoxicity assay, and proliferation assay). The immunological results from the Protocol 420 are consistent with the results from the other Apligraf studies.

The schedule of blood sampling for immunological evaluations is shown in **Table 23**.

[vv] Protocol 420 was the initial clinical trial with Apligraf, initiated in 1990. Full immunological assay protocols were not yet fully developed.

Table 23: Schedule by Study of Blood Sampling for Immunological Testing

STUDY	STUDY DAY OR MONTH					
	BASELINE	1 WEEK	4 WEEKS	2 MONTH	3 MONTH	6 MONTH
420-A	Yes	No	Yes	No	No	Yes
91-EXC-001	Yes	No	Yes	No	No	No
92-VSU-001	Yes	Yes	Yes	No	No	Yes
94-GDS-001	Yes	No	Yes	No	No	No
95-CUP-001	Yes	Yes	No	Yes	No	No
95-DUS-001	Yes	No	Yes	No	Yes	No
96-GDS-002	Yes	No	No	No	Yes	No
97-EXC-002	Yes	No	No	No	No	Yes*
97-GDS-003	Yes	No	Yes	Yes	No	Yes*
98-GDS-004	Yes	No	Yes	No	Yes*	No

*Blood sampling at Early Termination, if applicable.
Source (Data on file, Organogenesis): Study protocols for each individual study.

The humoral immune response to Apligraf was tested in various ways, including anti-bovine Type I collagen antibody testing [via enzyme-linked immunosorbent assay (ELISA)] and cytotoxic anti-HLA antibody testing (via complement dependent micro-lymphocytotoxicity assay[ww]). The cellular immune response to Apligraf was tested with an allospecific T cell proliferation assay. The results from each of these tests are summarized herein.

7.3.2.1 Results for Anti-Bovine Type I Collagen ELISA Testing

Test results from the anti-bovine Type I collagen antibody ELISA showed that Apligraf does not elicit an antibody response to collagen. There were no meaningful differences in the distribution of results between Apligraf and the control groups. Of all subjects who entered the studies, 99% were without pre-existing antibodies to bovine Type I collagen (titers of < 40) while 1% of the patient population entered with pre-existing antibodies (titers of ≥ 40). Subjects with pre-existing antibodies to collagen did not show significant increases in antibody titer after exposure to Apligraf.

All Apligraf subjects in studies 420-A, 91-EXC-001, 92-VSU-001, 95-CUP-001, 95-DUS-001, 96-GDS-002, and 98-GDS-004 whose baseline anti-bovine collagen Type I Ab titers were < 40 (negative) remained < 40 (negative) during the studies.

[ww] A commercial kit was used: frozen T cells trays from One Lambda (Canoga Park CA).

All (N=20) subjects in the 94-GDS-001 clinical trial had baseline anti-bovine collagen Type I antibody titers <40 (negative). One subject showed a change from negative to positive at Week 4; however, complete wound closure was observed, suggesting that the development of antibodies did not preclude clinical efficacy.

In 97-GDS-003 (N=12) all but one subject were negative at baseline for anti-bovine collagen type I antibodies. One subject was positive for anti-bovine collagen Type I antibodies at baseline, Week 4 and Month 6 but was negative at Week 8. This subject had complete closure of the Apligraf-treated wound at 3 weeks.

In 97-EXC-002, all subjects who tested positive in the Control treatment group (N=93) remained positive at Month 6. In the Apligraf treatment group (N=88) one subject tested positive at baseline and at Month 6, one subject tested positive at baseline and negative at Month 6, and 1 subject tested negative at baseline and positive at Month 6.

7.3.2.2 Anti-HLA Antibody Results

Results of testing for anti-HLA antibodies showed that all but 1 control subject and all but 4 Apligraf subjects tested negative for the presence of cytotoxic anti-human leukocyte antigen antibodies (specific for the human leukocyte antigen (HLA) class I antigens expressed on Apligraf) at baseline.

All Apligraf subjects in the 92-VSU-001, 91-EXC-001, 94-GDS-001, 96-GDS-002, 95-CUP-001 and 95-DUS-001 studies who entered the study negative remained negative during the study.

Two subjects in the 420-A study tested positive for antibodies to HLA-A2 during the study (1 subject at Week 1 and 1 subject at Week 4). Both subjects received blood transfusions during the time they were treated with Apligraf. It is likely that the antibody response is due to the transfusion. The switch from negative to positive was not considered clinically significant.

In the within-subject controlled study 97-GDS-003, one subject tested positive at baseline, Week 4 and Month 6 for the presence of cytotoxic anti-HLA antibodies. However, at Week 8 this subject had a negative result.

Three Apligraf subjects (1 from 94-GDS-001 and 2 from 95-DUS-001) and one control subject from 95-DUS-001 tested positive at baseline for the presence of cytotoxic anti-HLA antibodies and remained positive during the study. No clinical signs or symptoms of rejection were reported for any of these subjects.

These data show that subjects did not generate cytotoxic anti-HLA antibodies after exposure to Apligraf.

7.3.2.3 Allospecific T Cell Proliferation Assay

A proliferation assay was used to detect antigen specific activated T cells in peripheral blood. Peripheral blood mononuclear cells (PBMC) were isolated from each subject's heparinized blood and used as responder cells. PBMC pooled from healthy volunteers, were used as positive control stimulator cells to ensure the subject's responder cells could recognize allogeneic cells. To detect allo-specific T cells, responder cells were cultured with either test stimulator cells (HEPs and HDFs used in the manufacture of Apligraf) or positive control stimulator cells (pooled PBMC). The test stimulator cells were treated with gamma-interferon to insure surface expression of both class I and class II MHC antigens. Pooled PBMCs and HDFs were treated with Mitomycin-C prior to use in the assay, to ensure they did not proliferate. The maximum proliferative potential of responder cells was determined by culturing cells in the presence of the T cell mitogen phytohaemagglutin (PHA). Cultures were incubated for 5 days and pulse-labeled overnight with 1 μCi of ^{3}H-thymidine/well. Cultures were harvested and ^{3}H-thymidine incorporation measured on a LKB BetaPlate Reader (Wallac; Gaithersburg, MD).

In all of the clinical studies, T cell proliferation was seen in both the Apligraf and control groups in response to allogeneic pooled PBMC. These results demonstrate that the lymphocytes isolated from subjects were viable and capable of responding to allo-antigen (allo-Ag) presented by professional antigen presenting cells.

T cells from Apligraf- and Control-treated subjects also proliferated in response to the positive control, the T cell mitogen, PHA. These results demonstrate that the lymphocytes isolated from subjects were viable and capable of proliferating in response to the correct stimuli. Even though T cells from Apligraf-treated subjects proliferated in response to allo-Ag presented by pooled PBMCs, those same T cells did not proliferate to allo-Ag presented by the HEPs or HDFs used in the manufacture of Apligraf clinical product. Subjects were specifically tested for their T cell responses to cells from Apligraf that they had received during the study.

No meaningful differences were seen in the distributions of positive and negative T cell responses to HEPs, HDFs, or pooled PBMC for Control and Apligraf populations during the studies. In addition, no significant transition of positive and negative responses was seen from baseline to a specific time point for any of the stimuli.

These results show that HEPs and HDFs do not initiate the expansion of allospecific T cells. Results from cellular testing support the hypothesis that Apligraf does not elicit the expansion of allospecific T cells.

7.3.2.4 Summary of Immunological Data

The safety of Apligraf depends in part on the inability of the product to induce an immune response in the recipient. Overall, the evidence described above generally supports the hypothesis that Apligraf does not induce a humoral or cellular response. These findings are consistent with clinical observations, where there have been no reported cases of signs or symptoms of Apligraf rejection, even after Apligraf was re-applied to subjects exposed up to 4 weeks previously.

Furthermore, Eaglstein et al. stated that immunological tests for humoral and cellular responses to the components of Apligraf are consistent with the conclusion that if an immunological response occurs, it is not at a level that is easily detected or likely to cause acute adverse events (Eaglstein, 1999, *Dermatol Surg*). Possible explanations for these observations include the absence of Langerhans cells and vascular endothelial cells, absence of Class II HLA antigens on the surface of the fibroblasts and keratinocytes, absence of co-stimulatory molecules, an inability of keratinocytes and fibroblasts to present alloantigen to T cells (Eaglstein, 1999, *Dermatol Surg*), and a possible immunomodulatory effect as a result of secretion of immunomodulatory molecules such as TGF-β by keratinocytes (Laning, 1997, *Cell Immunol*).

Although the possibility of indirect antigen presentation taking place upon Apligraf application cannot be completely ruled out, it is conceivable that the possibility of indirect presentation is reduced because Apligraf does not vascularize. Therefore, cellular debris, including degraded HLA antigens, would have to be picked up at the vascular interface. Theoretically, T cell responses to indirect antigen presentation alone will be at most weak primary responses and a secondary response would be observed only after repeat application. As noted in the clinical trials, (Falanga, 1998, *Archives Dermatol*; Veves, 2001, *Diabetes Care*) we did not detect antibodies specific for Apligraf cellular components or bovine collagen, even after repeat application. Production of antibodies to specific antigens is a T cell-dependent B cell function that requires activated T helper cells to provide signals to B cells to proliferate, form germinal centers, and undergo isotype switching. Therefore the presence (or absence) of specific antibodies is an indirect measure of T cell activation. Based on the results of the clinical trials, we believe that any indirect presentation that might have taken place did not provide the necessary

signals for T cells to become fully activated and differentiate into helper T cells. Lastly, there was no report of Apligraf rejection in the clinical trials after repeat application. There have also been no reports of rejection after 13 years of clinical use with Apligraf, even after repeat applications.

These findings are consistent with clinical observations, where there have been no reported cases of clinical signs or symptoms of acute allergy or an immunologically mediated rejection in 787 evaluable subjects from 19 Organogenesis-sponsored clinical trials and in commercial use. This clinical trial experience includes subjects receiving a single Apligraf application in highly vascular acute wounds and subjects receiving multiple applications (up to 5 applications within the first 4 weeks of treatment) in chronic wounds. In addition, no evidence of a clinical immune response to Apligraf was reported in either the oral pilot (05-PER-001) clinical trial or pivotal (06-PER-002-CTX) clinical trial.

7.3.3 Summary of Apligraf Persistence Results from Organogenesis-Sponsored Clinical Trial Experience

Clinical pharmacology studies as such have not been conducted using Apligraf. However, the persistence of Apligraf DNA following Apligraf application has been evaluated in the following acute cutaneous studies, in addition to the previously reported oral pilot clinical trial, refer to **Table 24**.

Table 24: Organogenesis-Sponsored Clinical Studies – Persistence of Apligraf Cells

PROTOCOL NUMBER	PROTOCOL TITLE
05-PER-001	A Pilot Clinical Trial of Apligraf in Establishing a Functional Zone of Attached Gingiva
97-GDS-003	A Pilot, Within-Subject Controlled Study to Evaluate the Persistence of Graftskin in Split-Thickness Wounds
98-GDS-004	A Pilot Within-Patient Controlled Study to Evaluate Persistence of Graftskin Cells in Donor Site Wounds Comparing Graftskin, Graftskin without Epidermis, and Film Dressings

Apligraf is not metabolized; therefore no data exist on the pharmacokinetics and product metabolism in humans. There is no convincing or consistent evidence in man that Apligraf can become vascularized and engraft in a manner similar to an autologous skin graft. Apligraf is applied to the wound surface and may visibly remain on a wound in some subjects for a period of time before it breaks down. The persistence of the bovine collagen matrix of Apligraf remains unknown. However, it is unlikely that the matrix survives for long periods of time, as it is most likely degraded by collagenases in the

wound bed. It is for this reason that no safety issues with regards to the bovine collagen matrix are expected.

Information regarding the persistence of Apligraf DNA after application to the treatment site is discussed herein.

Subjects have been treated with Apligraf in acute cutaneous wounds (eg, burn surgery, donor sites, and surgical excision wounds). While clinical observations of "graft-take" have been reported, there is no definitive evidence of Apligraf persistence. The objective of the persistence studies was to assess the persistence of Apligraf DNA in acute wounds.

Table 25 lists the study design and subject enrollment by clinical protocol.

Table 25: Study Design and Biopsy Time Points by Apligraf Clinical Protocol

PROTOCOL	INDICATION/STUDY DESIGN	NUMBER OF SUBJECTS TREATED WITH APLIGRAF	NUMBER OF TREATMENT SITES BIOPSIED	POST-TREATMENT BIOPSY TIME POINTS
05-PER-001	Oral Mucosa Defects	25	2	6M
97-GDS-003	Acute wound - donor site: persistence; within-subject controlled	12	2	2W, 4W, 6W, 8W, 3M, 4M, 5M and 6M*
98-GDS-004	Acute wound - donor site: persistence; within-subject controlled	10	3	1W, 4W*, 3M* or early termination

*Biopsies to be taken until Apligraf DNA was not detected.
Source (Data on file, Organogenesis): 05-PER-001 CSR Section 2 Table 1, Section 3; 97-GDS-003 CSR Section 2 Table 1, Section 2.1 and 3.0; 98-GDS-004 CSR Section 2 Table 1, Section 3.0.

For the study 05-PER-001, persistence was determined by using female subjects who only had X-linked amelogenin loci. In contrast, because they are derived from ale tissue, Apligraf cells have in addition a Y chromosome-linked amelogenin locus. Frozen tissue biopsies were subjected to DNA analysis by multiplex PCR to detect persistence of Apligraf DNA at the Apligraf-treated site. The samples were amplified using Applied Biosystems AmpFlSTR Identifiler for 15 Short Tandem Repeat loci, as well as for the Amelogenin locus. Detection of a Y chromosome-linked locus in a female subject was considered a positive result for Apligraf DNA.

In 97-GDS-003 and 98-GDS-004, Apligraf cells were detected in wound biopsies using PCR specific for Apligraf HLA genes. In 97-GDS-003, a subject was considered negative upon negative biopsy results at two consecutive visits, at which point biopsy was

discontinued. In 98-GDS-004, a subject was no longer biopsied after a single negative biopsy result. For the purposes of comparison between studies, if a subject was determined to be negative at Week 1, he or she was listed as negative at all later visits. Persistence results from baseline to Week 4 per clinical study protocols 97-GDS-003 and 98-GDS-004 are provided in **Table 26**. Week 4 data has been presented for consistency between clinical study protocols. For 05-PER-001, persistence was measured only at 6 months; therefore a comparison cannot be made with studies 97-GDS-003 and 98-GDS-004. For 05-PER-001, all the subject samples were taken from female subjects, and only showed the X amelogenin locus. There was no evidence of Apligraf DNA in biopsies from the FGG- or Apligraf-treated sites at 6 months.

Table 26: Distribution of Persistence Results by Apligraf Clinical Protocol

	BASELINE		WEEK 4	
	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE
97-GDS-003	0	7	0*	7
98-GDS-004	NT	NT	1	6†

* This subject tested negative at Week 2, positive at Week 4, and negative at Week 6. Of note, a control-treated site tested positive for Apligraf DNA at Weeks 4 and 6. Potential hypotheses for these results are that cross-contamination occurred, either because of close proximity of the wounds, dressing materials that displaced cells, or specimens were mislabeled or processed incorrectly.

† Negative at previous visit (Week 1) or tested negative at Week 4; one additional subject tested positive at Week 1 but was not tested at Week 4 and their results are not included in this table.

Abbreviations: NT, not tested

Source (Data on file, Organogenesis): 97-GDS-003: PT Table 8, Listing 9; 98-GDS-004: PT Table 7, Listing 11, Listing 23.c

Studies reported in the literature have confirmed that Apligraf cells do not persist indefinitely, refer to **Table 27** for a summary of these literature cases.

Table 27: Literature Cases of Apligraf Persistence

LITERATURE REFERENCE	INDICATION	NO. OF PATIENTS BIOPSIED	FINDINGS
Falabella, 2000, *Arch Dermatol*	EB	12	Apligraf DNA persisted for up to 6 weeks in 2 patients biopsied.
Fivenson, 2000, *Wounds*	EB	2	Apligraf DNA persisted for up to 3 months in 1 patient biopsied.
Fivenson, 2003, *JAAD**	EB	7	Apligraf DNA persisted at various early timepoints and up to 20-28 weeks in 2 patients biopsied.
Griffiths, 2004,*Tissue Eng*	Acute Wounds	13 patients with 14 wounds	At Week 4, Apligraf DNA was detected in all of the wounds. One of 14 biopsies was positive at Week 6. At Week 8, all biopsies were negative.
Jiang, 2002, *J Pediatr*	EB	1	Apligraf DNA persisted for up to 9 weeks.
Serena, 2009, *Ostomy Wound Manage*	Ecthyma Gangrenosum	1	Apligraf DNA persisted for up to 10 months in 1 patient.
Nevins, 2010, *Int J Periodontics Restorative Dent*	Oral Regeneration	4	No Apligraf DNA detected at any up to 45 days.
*2 of 3 biopsies taken at weeks 20-28 were positive for HLA markers but none of the women were positive for the Y chromosome as assessed by short tandem repeat methods.			

These clinical results and literature reports generally suggest that Apligraf DNA persist in acute and chronic wounds for up to 4 to 6 weeks, the timing of which may vary with the location and type of wound. Additionally, the presence of DNA does not necessarily support cellular viability. Given the lack of vascularization, it is assumed that Apligraf cellular viability is considerably shorter than this; indeed the work of Griffiths et al (Griffiths, 2004, *Tissue Eng*) suggests little cellular viability beyond 1 week.

7.3.4 Post-Marketing Experience

Over 420,000 units of Apligraf have been shipped for patient application during a period of more than 13 years. Review of the MAUDE database and published literature reveal a single report of squamous cell carcinoma (SCC) occurring in conjunction with Apligraf use. Extensive analysis of this case both internally and by an independent external examiner concluded that it was highly unlikely that this SCC was related to the use of Apligraf, because the treating physician stated that the suspected lesion was present at the time of the Apligraf application.

From 22 May 1998 to 09 June 2011 a total of 197 AEs were reported to Organogenesis. This includes 9 AEs that met the definition of serious injury, requiring a MDR submission to the FDA. **Table 28** lists all post-marketing AEs reported to FDA during this reporting period.

Table 28: Summary of Post-Marketing Adverse Events Reported to FDA by the Manufacturer (22 May 1998 to 09 June 2011)

EVENT DATE	EVENT TYPE AND PATIENT OUTCOME	ADVERSE EVENT	RELATIONSHIP TO TREATMENT
27 Sep 1999	Injury, Requiring Intervention	Skin Inflammation Eschar	Related
13 Dec 2001	Injury; antibiotic therapy	Bacterial Infection	Possibly Related
27 July 2004	Injury, Requiring Intervention	Allergic Reaction	Related
25 May 2006	Injury; Hospitalization	Suspected Wound Infection Cellulitis	Related
22 May 2008	Injury, Requiring Intervention	Verrucous lesion in the healing wound Biopsy: Squamous Cell Carcinoma	Unlikely Related
16 Sep 2010	Injury; requiring hospitalization	Allergic reaction (ARDS)	Related
20 Jan 2011*	Other, unanticipated AE	Pain, Chills, fibromyalgia symptoms worsened	Unlikely Related
23 Mar 2011	Injury, requiring intervention	Blistering	Possibly Related
23 Mar 2011	Injury, requiring intervention	Dramatic Blistering	Possibly Related

Source: FDA MAUDE Database; Data on File. Organogenesis
* 20 Jan 2011 is actual event date, however in MAUDE Database this event date is 18 Feb 2011.

In addition, there have been no reported cases of clinical signs or symptoms of acute rejection to Apligraf in commercial use of the product.

7.4 Safety Summary and Conclusions

The pilot and pivotal clinical studies assessed safety and efficacy of Apligraf as compared to a FGG. In terms of safety (events reported or observed), a total of 65 AEs were reported for 41 subjects in the pilot and pivotal studies. There were 80 subjects who did not experience an AE during either study. In general, most events were reported with only 1 or 2 occurrences. In terms of SOC, the 2 SOCs with the most reported events were

Infections and Infestations (13 subjects with 16 events) and Gastrointestinal Disorders (12 subjects with 13 events). Within these 2 system organ classes, only one type of event was reported more than twice: there were 5 subjects with 6 reports of sinusitis. The only other specific AE reported in more than 2 subjects was hypersensitivity (4 events in 4 subjects), which were all recorded by the Investigator as seasonal (3) or environment (1) allergies but coded in MedDRA to hypersensitivity. In addition, PCR studies in 2 subjects at 6 months showed no sign of DNA persistence in the Apligraf-treated sites. Apligraf has been shown to be safe for the application over a vascular wound bed to generate site appropriate keratinized gingiva and alveolar mucosal as evident in the two complete clinical studies.

Over 420,000 units of Apligraf have been shipped for patient. Apligraf has been approved for over 13 years for the treatment of VLU and DFU indications, and has shown a favorable safety profile with 9 Organogenesis-reported MDRs since 1998.

8 BENEFIT-RISK EVALUATION

Grafting procedures, specifically FGGs, are considered the standard of care because of the predictability in augmenting a functional zone of KT (where root coverage is not desired). Autografting procedures minimize risk as they use the patient's own tissue, thereby eliminating risk of disease transmission or an allergic response to a foreign material. The amount of KT added to the recipient site is typically the width of the harvested palatal tissue (less shrinkage) and is very often significantly more than the adequate amount of 2 mm. This excessive addition of tissue results in an esthetically unnatural look as evidenced by a discontinuous MGJ and color/texture mismatch with adjacent tissue. Risks and disadvantages associated with grafts include the lack of adequate quality or quantity of donor tissue, poor esthetic outcomes (ie, the "tire patch" appearance), failure of graft take, a second surgical site to procure the donor tissue, and potential for a second (revision) procedure to reshape the grafted tissue. Donor site morbidity such as tissue trauma and bleeding from injury to the greater palatine artery in the case of FGG is also a potential risk. Because of the many treatment disadvantages, FGG may be inappropriate for some patients, and others may choose to avoid the procedure altogether.

Because of the disadvantages associated with the current standard of care, alternatives which obviate the need for grafting procedures, and instead use an alternate method to regenerate site appropriate tissue, have been explored.

Alternatives include soft tissue allo- or xenografts (collagen membranes); however, they have the potential for unpredictable results and scar formation. Soft tissue xenografts also pose the risk of disease transmission (risk of disease from an animal-sourced material), as well as an allergic reaction to the material components (eg, collagen). The benefit associated with each of these materials is that they offer the patient an alternative to a grafting procedure and the associated morbidities. However, collagen membranes have yet to show through rigorous, high level evidence that they can predictably augment an adequate zone of functional KT.

8.1 Apligraf Potential Risks

The risks associated with the use of Apligraf include possible allergic reaction to the collagen in the construct and the risk of disease transmission from the donor cells. Apligraf will be manufactured in compliance with the Quality System Regulation (QSR)/cGMP requirements per combination product (biologic-device) and other appropriate regulations for human cell and tissue products. During the procurement of

materials used in the manufacture of Apligraf, and throughout the manufacture and release of the product, testing is performed and release steps are in place to ensure that the risk of any disease transmission has been minimized. For those potential risks related to the product, possible warnings, precautions, and contraindications are detailed in the Package Insert.

While there are known risks, they are believed to be very minor in light of the established safety record with Apligraf. With over 13 years of post-marketing experience and over 420,000 units shipped for patient application, there have been no confirmed reports of disease transmission, tumorigenicity, or malignant transformation.

In the oral environment, the known risks of Apligraf in the treatment of oral mucosal defects have been well-characterized in 121 subjects enrolled into 2 clinical trials. The most frequent AEs were sinusitis and hypersensitivity which were reported in 4% and 3% of the subjects, respectively; all were mild in severity and were deemed not related to the Apligraf treatment. In the pivotal study, the inadvertent placement of the Apligraf polycarbonate membrane to the oral mucosal defect at the time of Apligraf placement resulted in 2 AEs (gingival injury and gingival pain). These AEs occurred only in the training subjects and not in the efficacy cohort.

There were no deaths. Three SAEs occurred in the pivotal study and were assessed by the Investigator to be either not related or of unlikely relationship to treatment. No SAEs occurred in the pilot study.

Between the well-characterized and refined manufacturing process, established safety record in cutaneous wound healing, and randomized controlled trials in the oral environment, the risks associated with Apligraf are minimal.

8.2 Benefits

The benefits of Apligraf therapy in the treatment of oral mucosal surface defects are:

- Clinically significant regeneration of functional, site-appropriate tissue (type, amount and distribution) that is similar to the native surrounding tissue;
- Favorable safety profile;
- Long history of Apligraf use, attesting to the safety of the product and demonstrating efficacy in the treatment of acute and chronic cutaneous wounds.

- An alternative to the palatal grafting procedure, eliminating any co-morbidities associated with the graft site and surgery
- No “tire patch” appearance, unlike grafting outcomes
- Unlimited supply and therefore increased likelihood of having all defect sites treated

Figure 25: Panoramic Photograph Showing the 13 Month Clinical Outcome for FGG (left) and Apligraf (right)*

Panoramic clinical photograph showing the repair outcome of the FGG (left, above area of yellow dotted bracket) compared to the regenerative outcome of Apligraf (right)
*13 month follow-up (post conclusion of 06-PER-002-CTX) provided courtesy of Rodrigo Neiva, DDS, MS.

Apligraf may also be used in situations where FGG is not feasible. For example, there may be clinical situations in which donor tissue cannot be harvested for technical reasons, such as insufficient tissue for the defect, or harvesting palatal tissue for a graft is not justified due to small defect size. Furthermore, there may be clinical situations in which grafting is not indicated due to a low likelihood of graft take. Finally, there may be instances in which the patient is unwilling to undergo palatal harvest. In all of these clinical scenarios, it would be appropriate to use Apligraf.

8.3 Risk and Benefit Conclusion

Apligraf provides a unique advancement in the treatment of oral mucosal surface defects, providing clinicians and patients with a new, well-tolerated and esthetically pleasing treatment option.

9 CONCLUSIONS

Apligraf is a safe and effective treatment of oral mucosal defects. In the oral pivotal study, Apligraf demonstrated statistically significant regeneration of site-appropriate of KT and clear improvement in the quality of healing. Apligraf was well-tolerated and had a favorable safety profile. The pilot study further supported the amount of KT and quality of healing of Apligraf (oral) with a similar safety profile to that of the pivotal study.

Apligraf is an attractive alternative to the FGG in that it eliminates the need for the palate grafting procedure and its attendant morbidities. The submitted BLA supports the safety and efficacy of Apligraf for the treatment of surgically created gingival and alveolar mucosal defects in adults.

Apligraf has been approved for over 13 years for the treatment of VLU and DFU indications, and has shown a favorable safety profile with 9 Organogenesis-reported MDRs since 1998. A wide-range of nonclinical testing has been conducted using Apligraf and its component cells (ie, HEPs and HDFs) which comprise product characterization, cell persistence/migration, immunotoxicity, and ISO toxicity testing.

Immunological data from clinical studies to date have demonstrated an absence of humoral and cellular immune responses to Apligraf. Clinical studies and literature reports generally suggest that Apligraf DNA persist in acute and chronic cutaneous wounds for up to 6 weeks. The pilot study showed that at 6 months post-treatment, DNA from Apligraf allogeneic cells did not persist (N=2) (McGuire, 2008, *J Periodontol*). Nevins et al found that in all subjects (N=4) no Apligraf DNA was present in the healing sites at 20, 29, 35 and 45 days post-Apligraf treatment (Nevins, 2010, *Int J Periodontics Restorative Dent*).

Overall, Apligraf in the treatment of oral mucosal defects has been shown to regenerate site appropriate tissue with an improved quality of healing via secondary intention healing. Apligraf has been demonstrated to be safe and well-tolerated as an alternative to the current standard procedures (ie, FGG).

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APPENDIX

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Appendix 1: Tabular Summaries of the Apligraf Nonclinical Program

Table 29: Preclinical Apligraf Safety Pharmacology Studies

STUDY TITLE	ORGAN SYSTEM EVALUATED	SPECIES/STRAINS	METHOD OF ADMIN.	DOSES	GENDER AND NO. PER GROUP	SUMMARY
Neutral allograft study	Not Applicable	Allogeneic Stimulator Cells: HDFs; HEPs; HUVEC (pos. control)	Not Applicable	Not Applicable	Not Applicable	Fibroblasts and keratinocytes expressing Class II antigens do not stimulate proliferation of allogeneic PBLs.
Expression of AB blood group antigens on Apligraf (RDR-031)	Not Applicable	Apligraf; positive and negative control skin from biopsies of known blood type and cadaver skin	Not Applicable	Not Applicable	Not Applicable	The data confirmed that Apligraf can express blood group antigens (which may not match the host), and are weakly expressed and limited to the upper layers of the epidermis.
The Molecular Typing of Apligraf Cells for Rh Blood Group Anitgens (RDR-029)	Not Applicable	HDFs	Not Applicable	Not Applicable	Not Applicable	All Apligraf cell donors tested were found to be positive-RhD using molecular typing technique.
In vitro Allograft Tolerance Studies	Not Applicable	HEPs	Not Applicable	Not Applicable	Not Applicable	Keratinocytes are capable of indirectly suppressing proliferation of T cells though the production of soluble factors.
In vitro analysis of the relative roles of costimulatory receptor-ligand interactions in the T cell alloantigen response to Graftskin (Apligraf)™ cellular components	Not Applicable	HDFs; HEPs; Tumor APC	Not Applicable	Not Applicable	Not Applicable	The results indicate that lack of costimulatory molecules in keratinocytes and fibroblasts contribute to the immuno-privileged profile of Apligraf.

Table 29 continues on the following page

Table 29: Preclinical Apligraf Safety Pharmacology Studies (continued)

STUDY TITLE	ORGAN SYSTEM EVALUATED	SPECIES/STRAINS	METHOD OF ADMIN.	DOSES	GENDER AND NO. PER GROUP	SUMMARY
Analysis of the role of inflammatory/costimulatory cytokines on the alloantigenicity of the cellular components of Graftskin (Apligraf)™	Not Applicable	HDFs; HEPs; HUVEC; B cell tumor APC	Not Applicable	Not Applicable	Not Applicable	The immuno-privileged profile of HEP and HDF in Apligraf was not altered by the addition of the immunostimulatory cytokines IL-1α, IL-6 or IL-12.
Characterization of primary and secondary allogeneic T cell responses after priming with HLA-matched professional and non-professional APC (RR-0076)	Not Applicable	Dendritic cells; HDFs; HEPs	Not Applicable	Not Applicable	Not Applicable	Keratinocytes and fibroblasts do not prime allogeneic T cells nor do they activate T cells primed by HLA matched dendritic cells.
Apligraf: Determination of cell purity in HEP and HDF cell banks by flow cytometry	Not Applicable	HDFs; HEPs	Not Applicable	Not Applicable	Not Applicable	Flow cytometric analysis indicated that keratinocyte and fibroblast cell banks lack detectable numbers of professional APCs, even at low passages.
Determination of residual bovine serum proteins in Apligraf (NCLR-0105-001)	Not Applicable	Apligraf construct; DE	Not Applicable	Not Applicable	Not Applicable	The data indicated that approximately 2.6 ± 0.07% of the total protein content of 1 Apligraf unit is bovine serum proteins incorporated through the various feeding steps in the production of Apligraf.

Abbreviations: APCs, antigen-presenting cells; DE, dermal equivalent; HDF, human dermal fibroblast; HEP, human epidermal progenitor; HLA, human leukocyte antigen; HUVEC, human umbilical vein endothelial cells; IL, interleukin; PBL, peripheral blood lymphocyte; Rh, Rhesus; RhD, Rh D-positive.

Source (data on file at Organogenesis):

Table 30: Preclinical Apligraf Pharmacokinetics Distribution Studies

STUDY TITLE	TEST SYSTEM/ TEST ARTICLE	METHOD OF ADMIN.	STUDY DESIGN	SUMMARY
Evaluation of Graftskin (Apligraf) Composite Grafts on Full-Thickness Wounds on Athymic Mice	Apligraf; Control: Human Skin/Athymic (nu/nu) mice; N=48 (6/group)	Cutaneous	A 2 x 2 cm full-thickness skin section was excised from the dorsum of each mouse. Twenty-four (24) animals had Apligraf pieces applied to the wound bed and 24 animals had split-thickness HCS applied to the wound bed. Applications were sutured in place and covered with Vaseline gauze, dry gauze, and adhesive bandages. Six (6) mice each from the test and control groups were euthanized at 6, 15, 30, and 60 days post-surgery. At each time point, grafts were photographed and macroscopically examined for evidence of wound contraction, areas of graft loss, and presence of vascularization (blanching with pressure) and photographed. Tissue biopsies were collected and processed for light and transmission electron microscopy and immunocytochemisty analysis. IHC was used to detect the presence of human epidermis using an antibody specific involucrin, a keratinocyte envelope protein and antibodies to human laminin, a component in the basement membrane.	Study results showed persistence of the human epidermis, comprised of Apligraf cells, through the study period of 60 days by TEM analysis and IHC staining
Tissue Remodelling and Cellular Persistence in Skin Construct Implants	Apligraf/ Athymic (nu/nu) mice	Cutaneous	Approximately 3 cm^2 full-thickness excision wounds were created on the central dorsum of 6 to 8 week-old athymic mice and a piece of Apligraf was cut to size an applied to each wound. The graft was dressed with Vaseline-impregnated gauze and 2 adhesive bandages. The bandages were removed 7-10 days later and left off for the remainder of the study. At each time point, grafts were collected and processed for light microscopy and TEM according to standard operating procedures. Formalin fixed paraffin embedded sections were used for H&E staining, trichrome staining and IHC to assess morphology, matrix remodelling and cellular persistence. Antibodies specific for human involucrin and human vimentin proteins were used to detect Apligraf keratinocytes and fibroblasts respectively. Sections fixed and processed for TEM analysis were used to assess BMmaturation. The barrier function was determined using a PA.	Results showed that Apligraf keratinocytes and fibroblasts persisted for at least 1 year after grafting Matrix remodeling was a slow and steady process similar to uninjured skin. Rapid maturation of normal barrier function and basement membrane was demonstrated.

Table 30 continues to the following page…

Table 30: Preclinical Apligraf Pharmacokinetics Distribution Studies (continued)

STUDY TITLE	TEST SYSTEM/ TEST ARTICLE	METHOD OF ADMIN.	STUDY DESIGN	SUMMARY
Remodeling of a Bioengineering Living Skin Construct Grafted onto Nude Mice	Apligraf/ Athymic (nu/nu) mice	Cutaneous	Approximately 3 cm^2 full-thickness excision wounds were created on the central dorsum of 6 to 8 week-old athymic mice and a piece of Apligraf cut to fit was applied to each wound. The graft was dressed with Vaseline-impregnated gauze and 2 adhesive bandages. The bandages were removed 7-10 days later and left off for the remainder of the study. Grafts were harvested from 7, 14, 28, 56, 100, 180, 232, and 365 days after grafting. Tissue samples were fixed and processed for histology and electron microscopy analyses. Species-specific antibodies (murine, bovine, human) were used to label extracellular matrix proteins or cell markers. The proteins and markers of interest were: (1) extracellular matrix proteins: type IV and V collagens and (2) cell markers: human involucrin (keratinocytes), human vimentin (fibroblasts), CD31 (endothelial cells), α-smooth muscle actin (myofibroblasts).	At 365 days post-grafting, human cells from Apligraf were determined to be present and biologically active at the wound. Based on an evaluation of total cell number, there was evidence that the cells were progressively disappearing from the grafting site.
Preclinical evaluation of Apligraf over short (7-28 days) and long (3-6 months) full thickness wounds in Swiss Nude mice (PCLR_101219_IVV7_CFR_1)	Apligraf/Swiss Nude mice, 4 mice/group at 7D, 14D. 28D and 3M 7 mice/group at 6M (N=23)	Cutaneous	Apligraf was applied on full-thickness wounds created on the dorsum of each Swiss female nude mouse. Four (4) animals were euthanized for each of the following time points: 7d, 14d, 28d, and 3m; 7 mice were used at the 6-month time point. Photographs were taken of the implant site and surrounding tissues and any notable adhesions or adverse tissue reactions were also recorded. Biopsies from the graft site were fixed and processed for paraffin embedding. Five (5) µm sections of tissues were stained with HES and MTG for histological and cellular evaluation. IHC using specific antibodies for cellular and extracellular markers was performed to characterize the constructs in vitro and in vivo. Tissue samples were also processed for TEM analysis. Samples were processed to be analyzed by in situ hybridization to evaluate the expression of genes of interest. In addition to specific IHC, human cell persistence was analyzed using in situ hybridization targeting the ALU repetitive sequences in human DNA, using DIG-AP Rembrandt® Universal DISH & Detection Kit and Digoxigenin labeled Positive Control Oligo Probe (ALU).	A DNA ALU probe was used to determine if the human cells migrated outside the graft site. *In situ* hybridization showed that human cells remained present in the Apligraf graft for up to 6 month post-graft. Human cells of any type were not detected in the surrounding tissue (approximately 1.2 ± 0.4 mm for all sides of the graft).

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Table 31: Preclinical Apligraf Toxicity Studies

Test Article: Apligraf

SPECIES AND STRAIN	METHOD OF ADMINISTRATION	DURATION OF DOSING	DOSES	GENDER AND NO. PER GROUP	SUMMARY	STUDY TITLE/ STUDY NO.
Albino mice, guinea pigs	Injected peritoneally for mice and guinea pigs	1 injection, animal, observed for 7 days after injection	Test materials diluted 1:20 with PBS	Not Provided	All animal survived the test period, did not exhibit signs of toxicity and weigh no less at the end of the test period.	General Safety Test
Mouse fibroblast L929 cell line	*In vitro* directly into the test system	Mouse cells exposed once to the test article or control, observed at 0, 24 and 48 hours	Not Applicable	Not Applicable	No biological reactivity was observed in the L929 mammalian cells by 48 hours, post exposure to the test article.	Tissue Culture-Agar Diffusion Test /91G-1313
Guinea pig	Intradermal and topical	1 injection at Day 0; topical application at Day 7; Challenge application at Day 21; Skin Readings at Day 22, 23, & 24	0.1 mL of test article extracts at 25, 50, 75 or 100%	2 experimental groups with 5M/5F 2 neg. control groups with 1M/1F 2 pos. control groups with 1M/1F 2 primary irritation groups with 0M/1F	All animals gained body weight. No overt sings of toxicity were exhibited in treatment and control groups. None of the treated (NaCl or CSO extracts) or negative control animals exhibited any reaction to the challenge (0% sensitized). The positive control animals exhibited skin reactions of 2 or 3 at each observation point (100% sensitized).	Kligman Maximization Study/91G-1310
New Zealand White Rabbits	Topically to 1 intact and 1 abraded site.	1 application; observed 24 and 72 hours	A 7cm^2 disk of test article	1 group with 3 M/3 F	All animal gained body weight. No overt signs of toxicity were evident in any of the animals during the course of the study. No signs of erthyema or edema were present at 24 or 72 hours.	Primary Skin Irritation Test and Intracutaneous Test/91G-1315

Table 31 continues on the following page

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Table 31: Preclinical Apligraf Toxicity Studies (continued)

SPECIES AND STRAIN	METHOD OF ADMINISTRATION	DURATION OF DOSING	DOSES	GENDER AND NO. PER GROUP	SUMMARY	STUDY TITLE/ STUDY NO.
Albino Swiss mice, New Zealand White Rabbits	Systemic Injection for Mice, Intracutaneous injection for Rabbits	1 injection; observed at 0, 1, 2, and 3 days.	50 mL/kg or 10g/kg	Mice: all female with 4 mice/group with 4 groups.Rabbits: 0.9% NaCl & CSO - 2M/2F and EtOH & PEG 400 – 2M/2F	All animal gained body weight. No signs of erthyema or edema were observed at any of the test or control sites. No overt signs of toxicity were evident in any of the animals	Class V Test (Systemic Injection Test)/91G-1311
New Zealand White Rabbits	Subcutaneous tissue	1 injection; observed at 0 and 90 days.	Test article strip prepared per USP XXII	1 M/1F with 1 group	Both animals gained body weight. Neither of the animals exhibited any signs of toxicity. Significant histopathologic differences were noted between the control and test sites; test article is considered toxic In discussions between the Sponsor and the Study Director, it was noted that this protocol was designed for plastics or relatively inert materials and therefore validity of the test was compromised by the nature of the test article, which represented a xenogeneic transplant. Because of the study design, the immune response cannot be discerned from a toxic response that might be seen with inert materials. This test was considered invalid measurement of toxicity for Apligraf.	Subcutaneous Injection Test/91G-1314
New Zealand White Rabbits blood	*In vitro* directly into the test system	Rabbit blood exposed once to the test article or control, observed at 0, 24 and 48 hours	60 cm^2/ 10mL NaCl	Not provided, blood samples were drawn from the animal and experiment was performed *in vitro*.	The test article showed hemolysis of 0.23%. The test article was considered non-hemolytic based upon the criteria of the study protocol.	Hemolysis Test/91G-1312
Abbreviations: M, male; F, female; CSO, cotton seed oil; NaCl, sodium chloride; PBS, phosphate buffered saline; MTG, Masson's trichrome-Goldner version Source (data on file at Organogenesis):						

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Table 32: Apligraf *In Vitro* and *In Vivo* Interaction Studies

TYPE OF STUDY	STUDY TITLE	TEST SYSTEM/ TESTARTICLE	METHOD OF ADMINISTRATION	STUDY FINDINGS
PHARMACODYNAMIC DRUG INTERACTION	Apligraf Periodontal Preclinical Dressing Evaluation (NCLR-0254-001)	Mice/ Untreated Apligraf, Apligraf-treated with either Barricaid®, Coe-Pak®, 0.12% chlorhexidine gluconate	Cutaneous	Common periodontal dressings and anti-microbial rinses that were considered for the 06-PER-CTX-002 clinical study were evaluated to gain a better understanding of any interactions with Apligraf. This study was conducted in the nude mouse model. It was determined that that Barricaid® dressing was most compatible with Apligraf. Both Coe-Pak™, and 0.12% chlorhexidine solution showed some variation in epithelial structure but overall the tissue was not compromised
	Graftskin (Apligraf) Compatibility with Antimicrobial Agents *In Vitro* Studies	*In Vitro*/ Untreated Apligraf; Treated Apligraf with either Tobraymycin, Neomycin, Furacin, Ceftazadime, Neosporin, Polysporin, Bactroban, Bacitracin Zn, or Dakin's solution	Not Applicable	Study results concluded that with respect to the Apligraf cell construct Ceftzadime was the most tolerated antibiotic at a concentration of 25µg/mL. Neosporin and Polysporin showed deterioration in histological scores. Bactroban appeared well-tolerated when applied topically while Bacitracin Zn showed moderate toxicity to the zinc formulation. The Dakin's solution was clearly toxic. Sulfamylon also appeared to be toxic. Silvadene was inconclusive since the Apligraf that was used was outside the approved 5 day shipping window.

Table 32 continues to the following page…

Table 32: Apligraf *In Vitro* and *In Vivo* Interaction Studies (continued)

TYPE OF STUDY	STUDY TITLE	TEST SYSTEM/ TESTARTICLE	METHOD OF ADMINISTRATION	STUDY FINDINGS
PHARMACODYNAMIC DRUG INTERACTION	Investigation of *in vitro* tolerability by the human skin equivalent Apligraf® of exposure to antibiotics/antiseptics for 24 hours (001974)	*In Vitro*/ Untreated Apligraf; Treated Apligraf with either Amoxicilin/Clavulanate, Ceftazidime, Fusidic acid or Chlorhexidine gluconate	Not Applicable	After 24 hours of exposure Amoxicilin/Clavulanate, Ceftazidime, and Fusidic acid were all well tolerated by Apligraf. However, concentrations of Chlorhexidine gluconate (topical application) above 2% induced a marked decrease in cell viability (observed at the biochemical and morphological levels) and detachment of epidermis from dermis. These effects were concentration dependent
	Graftskin (Apligraf) Compatibility with Antimicrobial Agents *In vivo* Animal Study	Mice/Untreated Apligraf; Treated Apligraf with either Silver sulfadiazine, Mafenide acetate, Sliver nitrate, GU Irrigant, Dakin's solution, polymyzin-nystatin, Gentamicin	Cutaneous	Using the athymic mouse model, it was concluded that Mafenide acetate (Sulfamylon™) was the most damaging agent to the Apligraf construct and agents such as Dakin's solution and Polymixin/Nystatin showed some level of negative side effects on Apligraf. Comparisons of the 7- and 14-Day timepoints showed that GU Irrigant had a transitory effect from which Apligraf was able to recover in vivo. Gentamicin solution had no significant effect on Apligraf histology or host integration

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Appendix 2: Independent Photograph Assessment

After the pivotal study was completed, an objective, unbiased assessment of photographs collected at the final (Month 6) follow-up visit was conducted to confirm gingival tissue color assessments performed by the investigators or designees (examiners) at each clinical study site and to provide additional esthetic data on the effects of Apligraf on site appropriate oral mucosal tissue. In addition to assessing gingival tissue color as per the pivotal study, soft tissue texture, marginal tissue contour and mucogingival alignment were assessed as previously identified as relevant esthetic parameters in the literature (Cairo, 2009, *J Periodontol*).

Background

Per pivotal study protocol, clinical photographs of each treated site were taken prior to surgery, on the day of the procedure (after wound bed preparation, placement of Apligraf and the FGG and placement of the Coe-Pak dressing) and at each subsequent visit (Weeks 1, 2[xx], and 4; Months 3 and 6). Photographs of the target sites were to include: (1) the incisal edge of the target tooth and the entire FGG or Apligraf treatment area (test); (2) tissue 2 mm apical to the MGJ; and (3) at least one tooth adjacent to the target tooth on either side. Consistent magnification, 1:1 (global photographs) and 1:1.5 (close-up photographs), was to be used. The color and texture of the gingival tissue at each treated site was assessed during the subject's clinic visit by an examiner who compared the surrounding (untreated) tissue in the same quadrant to the treated site. The results of the examiner's assessment recorded in the study case report forms.

Since the per-protocol assessment was performed by an examiner at each investigational site, an additional analysis of the photographs taken during the pivotal study was performed by independent reviewers. Even though tissue texture (ie, firmness of gingival tissue) was assessed tactilely by participating examiners at investigational sites during the conduct of the pivotal study, a similar parameter, the soft tissue texture (ie, presence or absence of scar formation or keloid-like appearance) was assessed during this independent review as defined by Cairo et al (Cairo, 2009, *J Periodontol*).

Methods

Three independent reviewers qualified by education and experience and who had no past or present affiliation with the Sponsor or the pivotal clinical study, were chosen to assess (in a

[xx] The Week 2 visit was required for only those subjects who had Coe-Pak in place on either test site or palatal harvest site at Week 1.

blinded fashion) the Month 6 photographs. Two of the independent reviewers were general dentists (DDS_1 and DDS_2), and one reviewer was a periodontist (PERIO).

Two global Month 6 photographs from each subject, one of the Apligraf site and one of the FGG site, were presented for review. Global photographs that included at least one tooth adjacent to the target tooth on either side were selected for this review to allow for assessment of the treatment site in comparison to surrounding tissue. The photographs were randomly ordered to eliminate bias resulting from sequential ordering and no treatment identifiers (Apligraf or FGG) were included on the photographs. Digital photographs provided by the clinical sites were presented electronically (PowerPoint software). The photographs were identified by randomization number and presented to the reviewers sequentially.

The 4 parameters were assessed as follows:

- Color match - gingiva of the treated tooth more red, equally red or less red than adjacent untreated tissue (identical assessment to pivotal study)
- Soft tissue texture - presence or absence of scar formation or keloid-like appearance
- Marginal tissue contour – follows/does not follow the cemento-enamel junction (CEJ)
- MGJ alignment – aligned/not aligned with the MGJ of adjacent teeth

Kappa statistics were used to test the agreement of the assessed gingival tissue color match between the following:

• Reviewer 1 Assessment versus Clinical Site Examiner Assessment (from clinical database)
• Reviewer 2 Assessment versus Clinical Site Examiner Assessment (from clinical database)
• Reviewer 3 Assessment versus Clinical Site Examiner Assessment (from clinical database)
• Reviewer 1 Assessment versus Reviewer 2 Assessment
• Reviewer 1 Assessment versus Reviewer 3 Assessment
• Reviewer 2 Assessment versus Reviewer 3 Assessment

In addition, kappa statistics were used to test agreement of the marginal tissue contour, soft tissue texture and MCJ alignment assessments between the following:

• Reviewer 1 Assessment versus Reviewer 2 Assessment
• Reviewer 1 Assessment versus Reviewer 3 Assessment
• Reviewer 2 Assessment versus Reviewer 3 Assessment

As a post-hoc analysis, the percentage of exact agreement was also analyzed, since the reviewers' responses were not well-distributed among potential responses, resulting in small marginal totals that can make kappa statistics somewhat unstable.

Results

The kappa statistics and percent agreement for color match were analyzed for inter-examiner variability and are presented in **Table 33**.

Table 33: Color Agreement between Reviewers and the Clinical Site (All subjects)

Reviewer	**DDS_1**		**DDS_2**		**Clinical Site**	
	Kappa Statistic	Exact Agreement (%)	Kappa Statistic	Exact Agreement (%)	Kappa Statistic	Exact Agreement (%)
PERIO	0.685	85.3% (163/191)	0.558	75.9% (145/191)	0.702	85.3% (163/191)
Clinical Site	0.491	76.0% (146/192)	0.401	67.2% (129/192)		
DDS_2	0.487	72.9% (140/192)				

Kappa statistics demonstrated moderate agreement between reviewers and against the clinical trial data; a much stronger agreement was demonstrated between reviewers and the clinical trial data for percent agreement. The strongest correlations between reviewers were associated with the Periodontist participating. The variation in agreement between reviewers may be attributed to the subjective nature of the color assessment (color of the treated site compared to surrounding tissue). Additionally, the variation between reviewers and the clinical trial data would suggest there are limitations (photograph quality, dimension, visualization of treatment margins) associated with a retrospective review of photographs as compared to direct assessment of the subject's treated sites.

Of the 4 parameters examined, 3 (color, soft tissue texture, and MCJ alignment) showed strong differences ($p \leq 0.0001$, McNemar's paired comparison test) in favor of Apligraf for all 3 reviewers. The number of subjects in which the Apligraf-treated site achieved color and texture comparable to surrounding tissue was greater than the number of subjects in which the FGG-treated site achieved color comparable to surrounding tissue. In addition, the number of subjects in which the MGJ maintained alignment comparable to surrounding tissue at the Apligraf-treated site was greater than the number of subjects in which the MGJ maintained alignment comparable to surrounding tissue at the FGG-treated site. The results of the fourth variable, marginal tissue contour, did not discern a difference between treatment groups.

Discussion

The Independent Photograph Assessment supports the results of assessments made by examiners at each clinical site as demonstrated in **Table 34**.

Table 34: Independent Photograph and Clinical Sites Assessments of Color at 6 Months

COLOR	INDEPENDENT PHOTOGRAPH ASSESSMENT*	CLINICAL SITES
Apligraf-treated sites	95.3%, 69.4%, and 92.9% Assessed as "equally red" as adjacent non-treated tissue	92.9% Assessed as "equally red" as adjacent non-treated tissue
FGG-treated sites	49.4%, 36.5%, and 25.9% Assessed as "equally red" as adjacent non-treated tissue	27.1% Assessed as "equally red" as adjacent non-treated tissue
* The values are reported individually for each independent examiner, DDS_1, DDS_2 and PERIO respectively Source: 06-PER-002-CTX Independent Photograph Assessment Report Table 4		

The color results analyzed by the same methodology used for the secondary efficacy endpoint in the pivotal study (ie, discordance testing) and as interpreted by any of the examiners are strongly in favor of Apligraf ($p < 0.0001$).

The assessment of color in the Independent Photographic Analysis presented additional complications than in the pivotal study: the independent examiners used two-dimensional photographs rather than visually inspecting the tissue of the treated sites. However, utilizing the additional criteria recommended by Cairo, et al for esthetic evaluation (ie, soft tissue texture, marginal tissue contour, and MGJ) provides a more robust approach to the evidence that site appropriate tissues have been regenerated in the correct anatomical locations (Cairo, 2009, *J Periodontol*).

The results from this additional analysis provide support for the proposed indication for use. This independent assessment of color demonstrated that Apligraf regenerates site appropriate tissue. The results of color, texture, and MGJ results as interpreted by any of the reviewers were strongly in favor of Apligraf ($p \leq 0.0001$).

Conclusions

Color assessment was compared between reviewers as well as against the results of the clinical trial examiner. The variation in agreement between reviewers may be attributed to the subjective nature of the color assessment. Additionally, the variation between reviewers and the clinical trial data would suggest there are limitations (photograph quality, dimension, visualization of treatment margins) associated with a photographic review as compared to direct assessment of the subject's treated sites.

In the clinical trial data and in the Independent Photographic Analysis, results for color agreement were strongly in favor of Apligraf ($p \leq 0.0001$; McNemar's paired comparison test). Thus, the results demonstrate Apligraf is producing natural appearing tissue regardless of reviewer variability in assessing color and provide strong evidence of the validity of the clinical trial results.

Consistent with Apligraf generating site appropriate tissue, the esthetic measures of soft tissue texture ($p \leq 0.0001$) and MGJ alignment ($p \leq 0.0001$) showed strong results in favor of Apligraf for all reviewers. No difference between Apligraf and FGG could be discerned for marginal tissue contour, which may be attributed to some of the mucosal defects being created apically to the free gingival margin of the treated tooth.

Appendix 3: Additional Histological Evaluation

Histological evaluations were performed as part of the pilot study. This evaluation permitted comparison of the type and quality of tissue established with the FGG- and Apligraf-treated tissue. Standard H&E staining was performed to examine the cellular composition and tissue architecture of the surgical tissues.

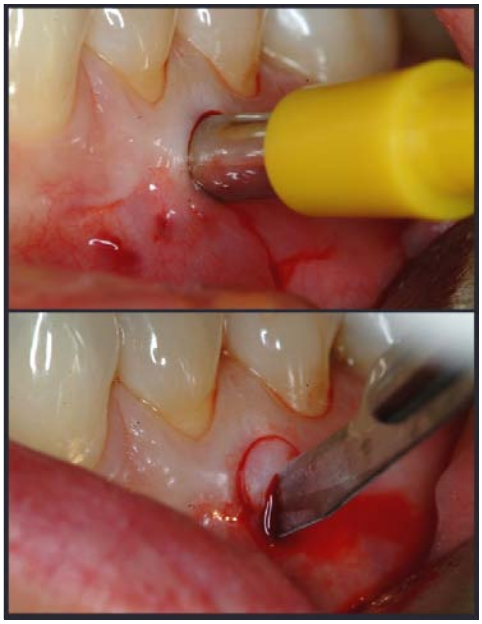
Punch biopsies (3 mm) were obtained at baseline during surgery and at six (6) months, from the Apligraf and FGG sites of 7 subjects. The tissue was processed by fixing one-half in 10% formalin. The remaining half of the tissue was flash frozen and stored. The examiner evaluating the slides was blinded to the site of origin (FGG or Apligraf) of the tissue specimen. The composition of the surgical tissues was examined and evaluation of all biopsy specimens at 6 months showed intact stratified squamous epithelium of normal thickness and tissue architecture.

This additional histologic evaluation utilized the H&E stained slides from the initial analyses and the remaining paraffin-embedded biopsy specimens. 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 ECM protein composition and vascularity between the FGG- and Apligraf-treated sites, and between the tissues present at 6 months and baseline. The specific aim was to see whether changes in histology and extracellular protein expression were consistent with the formation of site appropriate tissue (ie, gingiva).

Materials and Methods

At baseline, 7 biopsies each were taken from the gingiva (Apligraf- and FGG-treated site) prior to preparation of the wound bed. At 6 months, 7 biopsies each were taken in the same subjects from the Apligraf- and FGG-treated sites. **Figure 26** shows the biopsy procedure for the Apligraf- and FGG-treated sites.

Figure 26: Biopsy Procedure for the Apligraf- and FGG-treated sites



The upper photo shows placement of the punch across the mucogingival junction. The lower photo demonstrates vertical notching of the specimen in-situ to assure the orientation of KT and AM portions were captured in each section.

Biopsy specimens were prepared and then analyzed to assess epithelium, vascularity (endothelial cells), myofibroblasts, collagen, reticulin, elastin, and other ECM proteins (ie, tenascin and fibronectin). **Table 35** provides an overview of how each parameter was assessed.

Table 35: Additional Histologic Evaluation: Parameters Examined

PARAMETER	ANTIBODY / STAINING PROCEDURE	EVALUATION OF SPECIMENS	ASSESSMENT SCALE
Epithelium	H&E stain	Presence of rete ridges	Presence of rete ridges graded as follows: 0 absent; 1+ poorly formed; 2+ well-formed.
		Presence of chronic inflammation	Presence of chronic inflammation graded as follows: 0 absent; 1+ isolated foci of lymphocytes and/or plasma cells <100 cells; 2+ isolated foci of lymphocytes and/or plasma cells >100 cells; 3+ diffuse band of lymphocytes and/or plasma cells.
Collagen	Masson trichrome (MT) stain	Percentage of dense collagen	Percentage of dense collagen compared to total collagen on the slide using the MT stain.
	Picrosirius red (PSR) stain	Orientation of fibers	Orientation of fibers expressed as the percentage of fibers running parallel to the base of the epithelium using the PSR stain.
		Overall thickness of fibers	Overall thickness of fibers expressed as a percentage of fibers that are stained orange-red (as compared to greenish-yellow) using the PSR stain.
Reticulin	Gomori methenamine silver (GMS) stain	Quantity of reticulin fibers	Each slide was evaluated for the quantity of the reticulin fibers and graded 0, 1+, 2+ and 3+.
Elastin	Verhoef van Gieson (VVG) stain	Quantity of elastin fibers	Each slide was evaluated for the quantity of elastin fibers and graded 0, 1+, 2+ and 3+.
ECM proteins	Tenascin-C Fibronectin	Degree of staining	Each slide was evaluated for the degree of staining and graded 0, 1+, 2+ and 3+.
Vascularity (endothelial cells)	CD31	Number of vessels	Each slide was evaluated for the number of vessels per 4 high-power fields.
Myofibroblasts	αSMA	Number of cells	Each slide was evaluated for the number of cells per 4 high-power fields.

Statistical Analysis

Data came from paired observations (ie, samples taken at baseline and at 6 months). Since all categorical variables were ordered, the change from baseline to Month 6 was presented by 3 categories: decreased since baseline, remained the same as baseline, and increased since baseline.

At pre-treatment and 6 months, categorical data was presented as cross tabulations and numerical data was presented by summary statistics. Due to the small sample size, no p values were calculated.

Results

Table 36: Summary of Histology Results

Tissue Component	Apligraf	FGG	Paired Difference
Collagen parallel to epithelium, % (SD)	-34.2 (38.3)	-39.3 (36.3)	-1.7 (29.4)
Dense collagen (MT stain), % (SD)	28.6 (24.1)	0.0 (24.5)	26.0 (39.1)
Thick collagen fibers, % (SD)	4.2 (29.1)	-8.6 (42.5)	24.2 (40.1)
Vascularity, CD31, n (SD)	-14.2 (9.7)	-18.0 (16.2)	5.0 (14.6)
Myofibroblasts, αSMA; n (SD)	-14.7 (5.8)	-12.8 (15.9)	0.0 (15.7)

A summary of the results are provided in **Table 37**. The inflammation noted in the Apligraf-treated sites was within baseline variability. Both Apligraf- and FGG-treated sites showed an increase in the percentage of dense collagen (MT stain). However, there was a slightly greater increase in dense collagen in the Apligraf-treated sites. Staining of denser collagen (PSR stain with full polarization, not shown) was increased compared to baseline for the Apligraf-treated sites, whereas the FGG-treated sites showed slightly decreased staining.

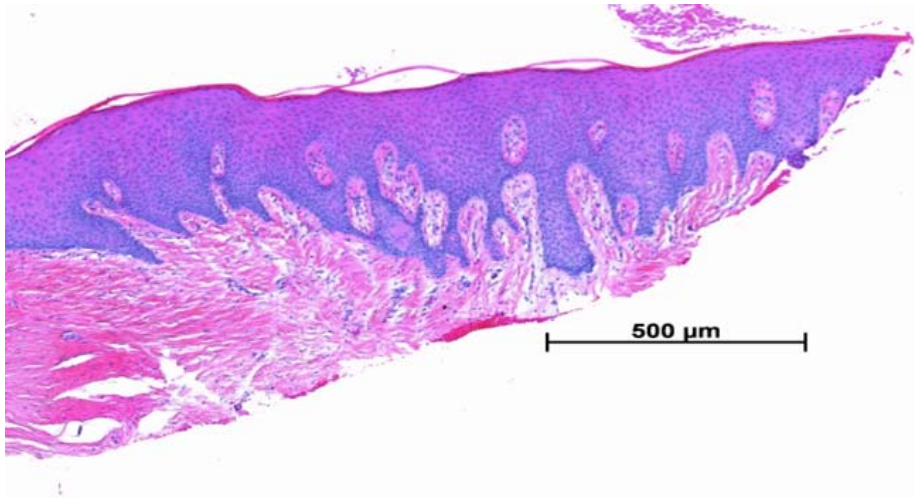
Representative histological images from baseline and Apligraf- and FGG-treated sites are provided in **Figure 27** **to** **Figure 31**.

Table 37: Additional Histology Evaluation: Summary of Results

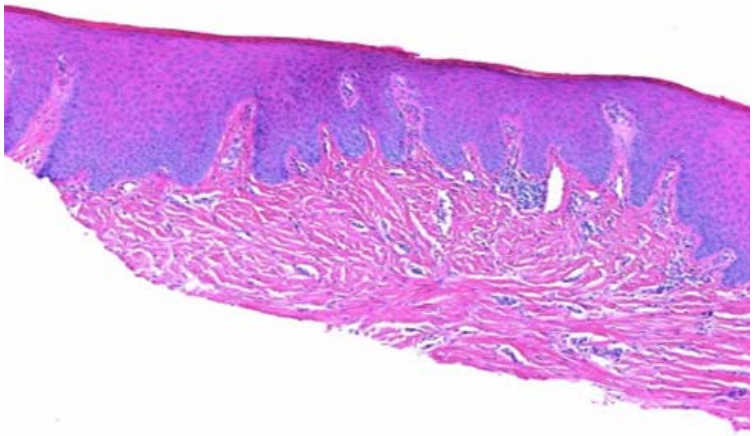
PARAMETER	SUMMARY OF RESULTS			GINGIVA CHARACTERISTICS	ALVEOLAR MUCOSA CHARACTERISTICS
		APLIGRAF-TREATED SITES	FGG-TREATED SITES		
Epithelium	Similarities	Thin parakeratinization Good rete ridge formulation		Well defined, thicker keratin layer Long, thin papillae and ridges (Gingiva) shorter more slender papillae and ridges (Palate)	Cell nuclei visible in superficial layers Wide short ridges and papillae
	Differences	↑ epithelial thickness from baseline. Mild ↑ in inflammation (but within baseline variability)			
Collagen	Similarities	Less parallel collagen (to the basement membrane) than baseline but similar % of parallel collagen present ↑ in % of dense collagen compared to total collagen (MT stain)		Straight, dense, less parallel bundle structure	Wavy, parallel collagen in a mixed dense and loose arrangement
	Differences	Slightly greater ↑ in dense collagen in Apligraf group (MT stain and PSR stain).	Slight ↓ staining of dense collagen (PSR stain).		
Reticulin	Similarities	No significant presence in connective tissue		No significant presence except in development and wound healing	No significant presence except in development and wound healing
	Differences	None	None		
Elastin	Similarities	↓ elastin from baseline		Present at lower level than AM	Abundant
	Differences	Marginally greater ↓ in elastin			
ECM proteins	Similarities	No obvious changes in fibronectin		Fibronectin present in ground substance between collagen fibrils Low levels tenascin concentrated near basement membrane	Fibronectin present in ground substance between collagen fibrils Low levels tenascin concentrated near basement membrane
	Differences	No change in tenascin expression	↑ in tenascin compared to Apligraf and baseline		
Vascularity (endothelial cells)	Similarities	Tendency to ↓ vascularity compared to baseline (CD31 and αSMA)		Moderate vascularity Lower level of CD31 and αSMA expression	Highly vascular Increased level of CD31 and αSMA expression
	Differences	None	None		
Myofibroblasts	Similarities	Not present at baseline or 6 months (αSMA)		Not present	Not present
	Differences	None	None		

Figure 27: Epithelium (Rete Ridges and Inflammation), H&E Stain

Baseline (Magnification 5X) Lacks distinct keratinized layer of cells at mucosal surface.

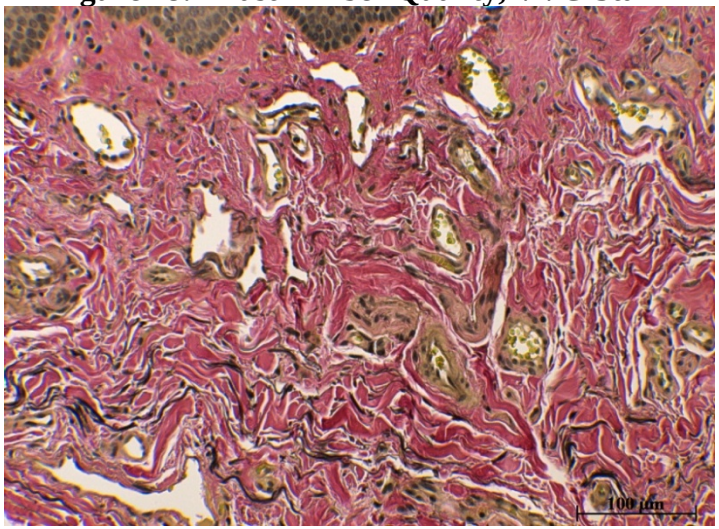


FGG-treated site at 6 Months
(Magnification 5X)

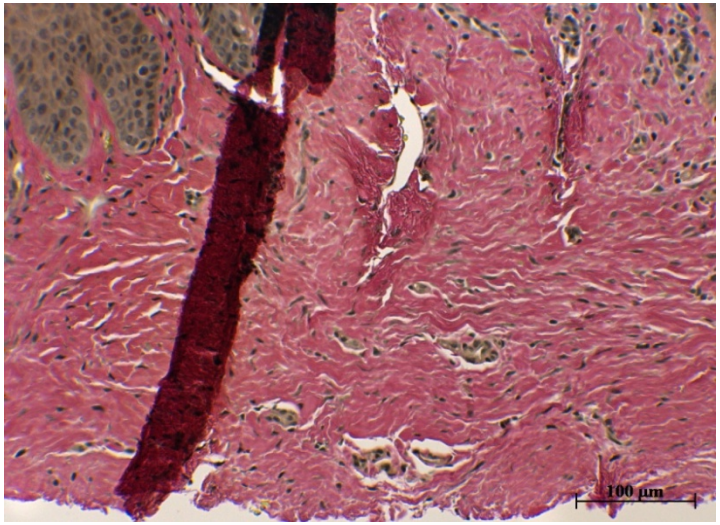


Apligraf-treated site at 6 Months
(Magnification 5X)

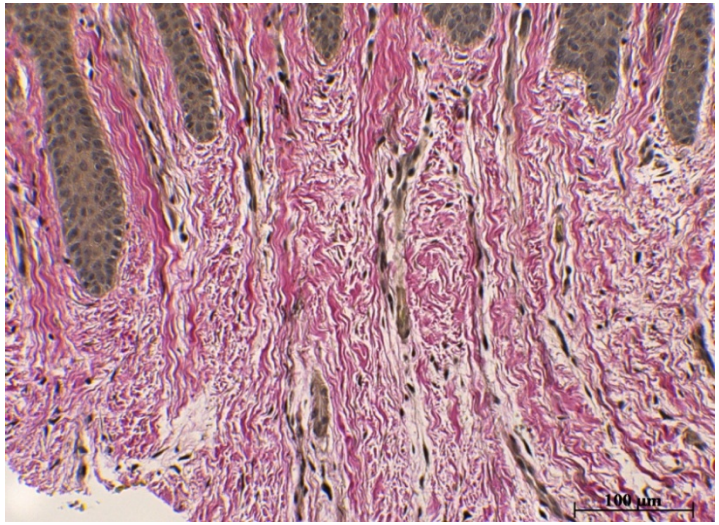
Figure 28: Elastin Fiber Quality, VVG Stain



Baseline (Magnification 200 X) Well demarcated black fibrils of elastin are seen.

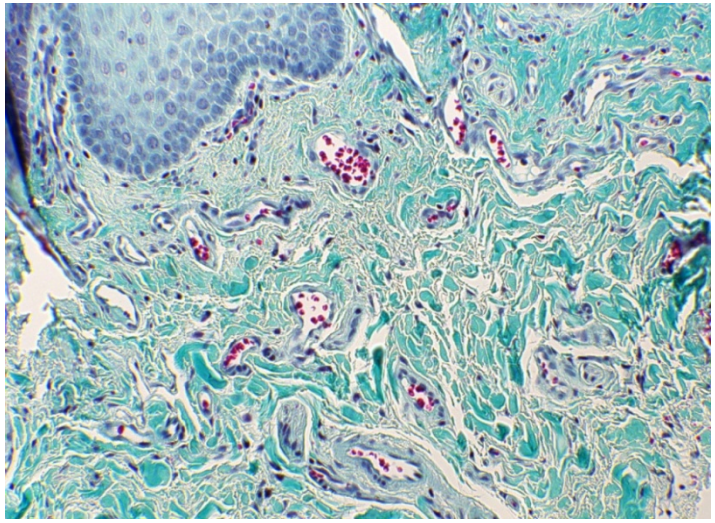


Apligraf-treated site at 6 months has less elastin than at baseline. (Magnification 200 X)

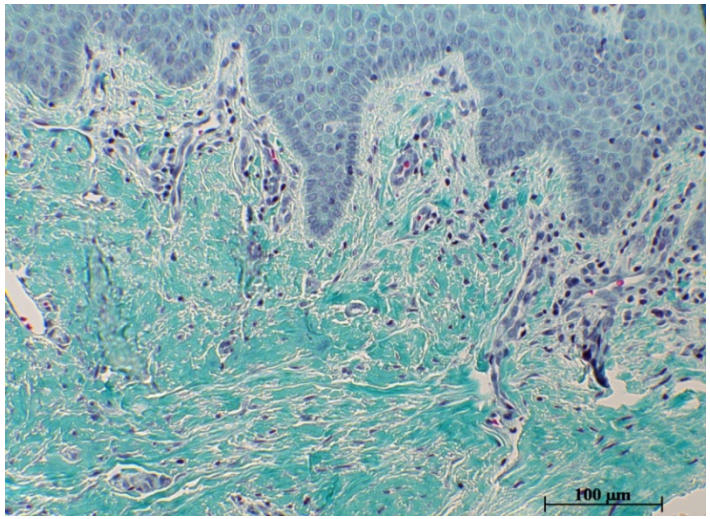


FGG-treated site at 6 months has less elastin than at baseline. (Magnification 200 X)

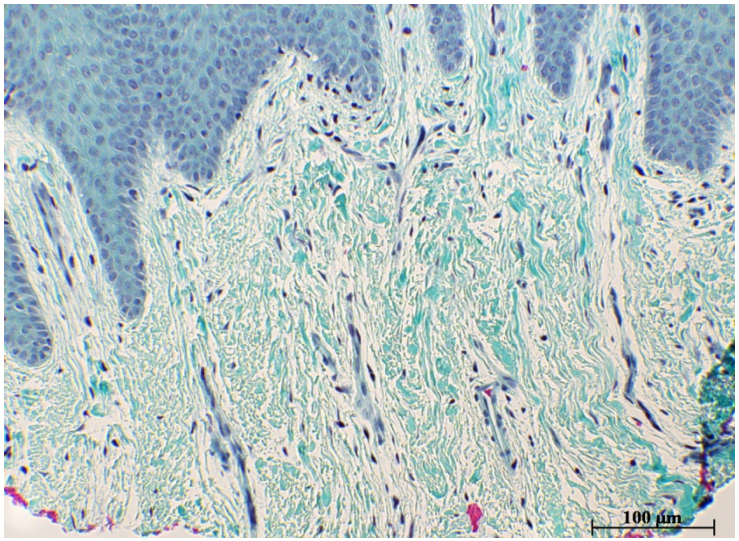
Figure 29: Percentage of Dense Collagen, MT Stain



Baseline (Magnification 200 X) Darker azure structures are dense collagen bundles.

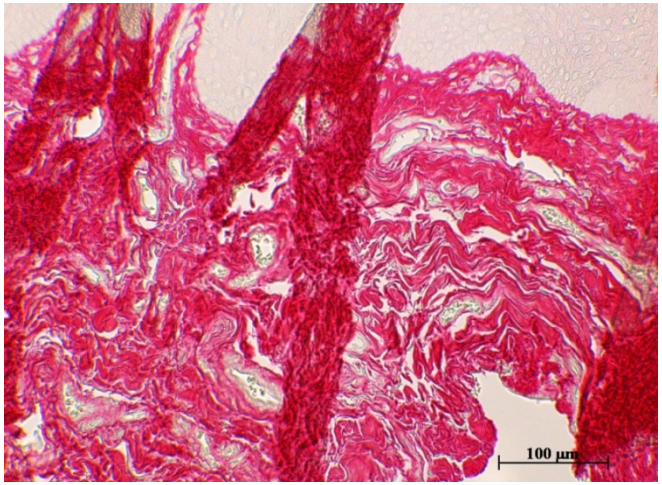


Apligraf-treated site at 6 months with a similar percentage of dense collagen as baseline. (Magnification 200 X)



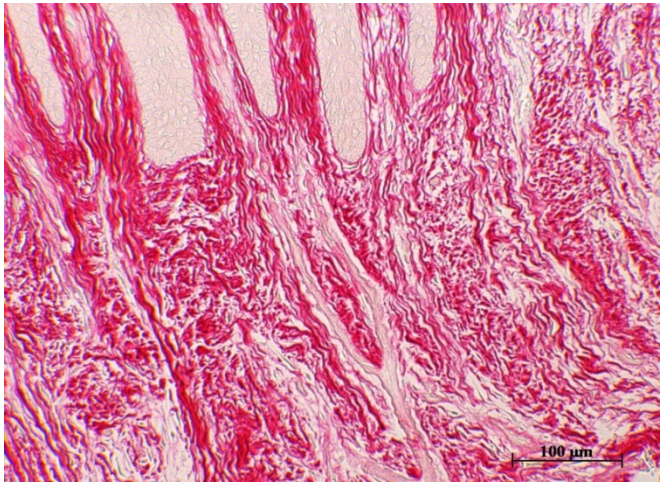
FGG-treated site at 6 months with a similar percentage of dense collagen as baseline. (Magnification 200 X)

Figure 30: Collagen Fiber Thickness, Orientation and Density, PSR Stain



Baseline (Magnification 200 X) Collagen fibers are ordered in a wavy linear pattern showing moderate parallelism.

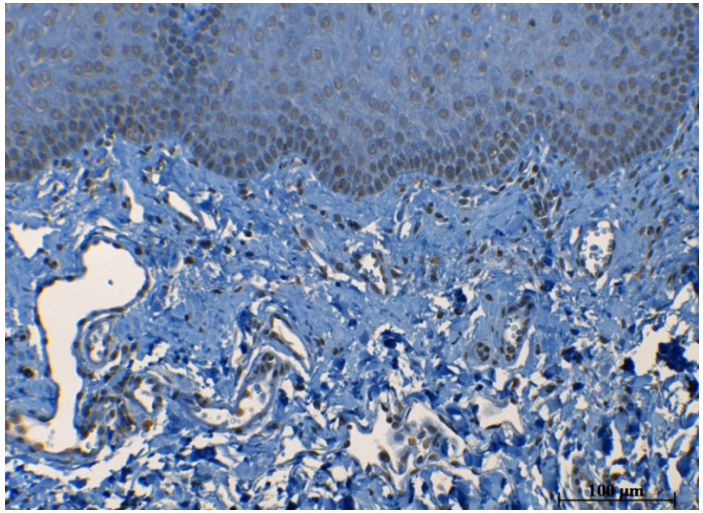
Apligraf-treated site at 6 months with less parallel collagen than baseline. (Magnification 200 X)



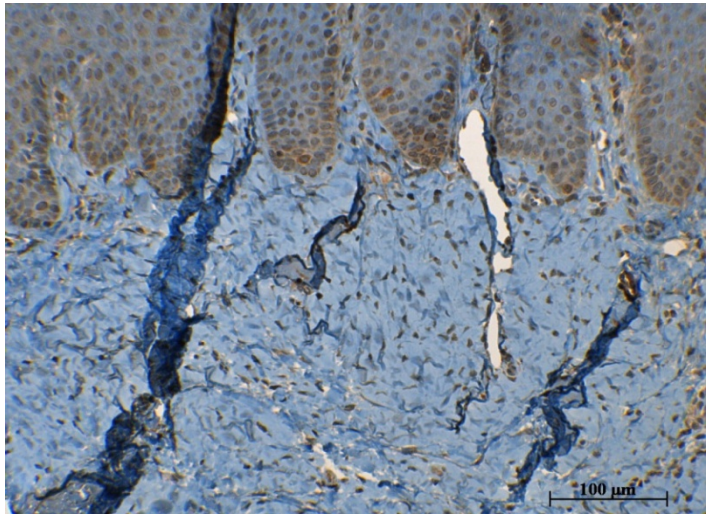
FGG-treated site at 6 months with less parallel collagen than baseline. (Magnification 200 X)

*Note: Viewed under a polarizing lens, these pictures would have orange/red and yellow/green staining. The percentage of thick fibers was estimated when viewed under a polarizing lens.

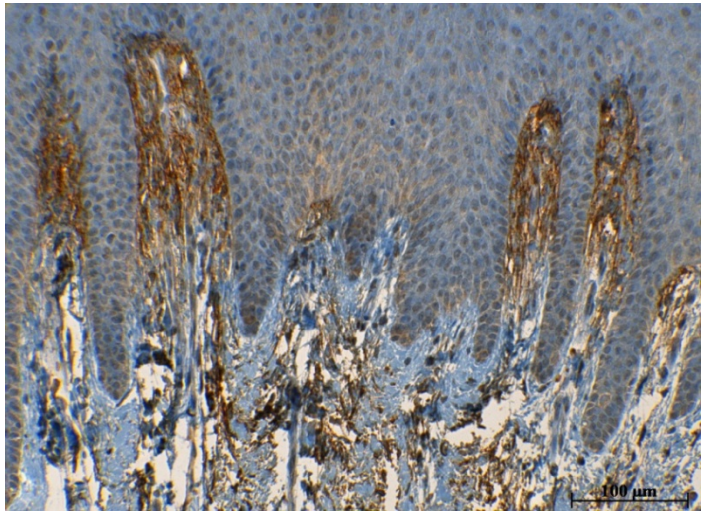
Figure 31: ECM Proteins, Tenascin-C Expression



Baseline (Magnification 200 X) Lack of Tenascin-C is indicated by lack of distinct brown staining.



Apligraf-treated site at 6 months with no Tenascin-C present.
(Magnification 200 X)



FGG-treated site at 6 months with Tenascin-C present
(Magnification 200 X)

Discussion

Gingival epithelium is characterized by long and slender rete ridges whereas those of the palatal mucosa are shorter and more slender. In contrast, the rete ridges of AM are wide and short. The connective tissue of AM is characterized by more widely spaced collagen fibers, elastin, blood vessels (with accompanying reticulin fibers) and myofibroblasts. In contrast, the connective tissue of the gingiva and palatal mucosa is devoid of elastic fibers in most cases (Wei, 2002, *J Periodontol)* and has more densely packed collagen without the fibers running parallel near the junction with the epithelium as is seen with AM (Karring, 1971, *J Periodontol Res*). In addition, there is less cellularity and far fewer blood vessels in both gingival and palatal mucosa than in AM.

The changes in both groups appeared to be similar and included improvements in rete ridge formation (ie, more like gingiva and less like AM). Furthermore, these results help support the proposed indication statement that Apligraf (oral) regenerates site appropriate oral mucosal tissues. Consistent with the notion of site appropriate tissue formation (ie, gingiva), Apligraf-treated sites had greater increases in the density of collagen together with a non-parallel distribution. There was a decrease in elastin (hallmark of AM) was also greater in the Apligraf group. While there is less elastin in gingiva than in AM, elastic fibers have been described in the connective tissue of palatal mucosa (Karring, 1971, *J Periodontol Res*). A similar observation was made in this study. There was a decrease in vascularity in both the Apligraf- and FGG-treated groups. There appeared to be a mild increase in inflammation in Apligraf-treated groups but this change was well within baseline variability. Apligraf and FGG diverged with respect to tenascin staining with Apligraf showing similar levels as baseline and FGG showing an increase. A recent publication suggests that tenascin expression may be a characteristic of palatal mucosa (Wong, 2009, *Wound Repair Regen*).

Thus, the histological differences observed between biopsy specimens taken from the Apligraf- and FGG-treated sites may help to explain the clear differences that were seen in the clinical appearance between the two treatment sites; Apligraf appeared to generate site appropriate oral mucosal tissue while FGG retained the characteristics of its palatal tissue origin, the later commonly referred to as a “tire patch” appearance of FGG.

Histology Conclusions

Overall, the study is underpowered to detect statistical differences. However, both treatments tended to produce tissue that was histologically different from baseline (ie, AM). The differences in clinical appearance of the Apligraf group may be related to subtle differences in the presence

of elastin (less in Apligraf), collagen distribution (denser in Apligraf) and tenascin (less in Apligraf). These differences are consistent with the baseline histological differences between gingiva and palatal mucosa. Overall, these results are consistent with Apligraf inducing site appropriate gingival differentiation and FGG retaining many of its characteristics of origin (ie, palatal mucosa).