Organogenesis inc.
Advancing Healing

Apligraf®

Caution: Federal law restricts this device to sale by or on the order of a physician (or properly licensed practitioner).

1. DEVICE DESCRIPTION

FDA has approved Apligraf as a Class III medical device via premarket approval [PMA]. In addition, Apligraf meets applicable requirements for a Human cell, tissue, and cellular and tissue-based product [HCT/P] in accordance with 21 CFR Parts 1270 and 1271.

Apligraf is supplied as a living, bi-layered skin substitute: the epidermal layer is formed by human keratinocytes and has a well-differentiated stratum corneum; the dermal layer is composed of human fibroblasts in a bovine Type I collagen lattice. While matrix proteins and cytokines found in human skin are present in Apligraf, Apligraf does not contain Langerhans cells, melanocytes, macrophages, lymphocytes, blood vessels or hair follicles. In a 10 patient venous leg ulcer study to determine the longevity of Apligraf cells, 2 of 8 patients evaluated at 4 weeks demonstrated Apligraf DNA. Neither of these patients showed Apligraf DNA at 8 weeks.

Cells used in the manufacture of Apligraf are processed under aseptic conditions. The cells are originally derived from donated human neonatal male foreskin tissue. The foreskin donor's mother is tested and found negative for human viruses, including antibodies to human immunodeficiency virus types 1 and 2 (HIV-1 and HIV-2), human immunodeficiency virus type 1 (HIV-1), human T-lymphotropic virus types 1 and 2 (HTLV-1 and HTLV-2), hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis B surface antigen (HbsAg), hepatitis C virus (HCV), west nile virus (WNV), epstein barr virus (EBV), cytomegalovirus (CMV), and syphilis. Maternal donor testing is performed by a laboratory registered with FDA to perform donor testing and certified in accordance with the Clinical Laboratory Improvement Amendments of 1988 (CLIA) and 42 CFR part 493. Based on the results of screening and testing the donor was found to be suitable by Organogenesis Inc. The fibroblast and keratinocyte cell banks which are the source of the cells from which Apligraf is derived are tested for human and animal viruses, retroviruses, bacteria, fungi, yeast, mycoplasma, karyology, cell identity, and tumorigenicity. The final product is tested for morphology, cell viability, epidermal coverage, sterility, mycoplasma, endotoxin, and physical container integrity. Product manufacture also includes reagents derived from animal materials including bovine pituitary extract. All animal derived reagents are tested for viruses, retroviruses, bacteria, fungi, yeast, and mycoplasma before use. Bovine materials are sourced to minimize bovine spongiform encephalopathy (BSE).

2. INTENDED USE / INDICATIONS

Apligraf is indicated for use with standard therapeutic compression for the treatment of non-infected partial and full-thickness skin ulcers due to venous insufficiency of greater than 1 month duration and which have not adequately responded to conventional ulcer therapy.

Apligraf is also indicated for use with standard diabetic foot ulcer care for the treatment of full-thickness neuropathic diabetic foot ulcers of greater than three weeks duration which have not adequately responded to conventional ulcer therapy and which extend through the dermis but without tendon, muscle, capsule or bone exposure.

3. CONTRAINDICATIONS

- Apligraf is contraindicated for use on clinically infected wounds.
- Apligraf is contraindicated in patients with known allergies to bovine collagen.
- Apligraf is contraindicated in patients with a known hypersensitivity to the components of the Apligraf agarose shipping medium (Section 8).

4. WARNINGS

Warning: DO NOT OPEN AND DO NOT USE Apligraf after the expiration date or if the pH is not within the acceptable range (6.8-7.7) as determined by the provided pH color chart (Section 9).

Warning: Allergic reactions to the components in the Apligraf agarose shipping medium (Section 8) and bovine collagen, (a component of Apligraf), have been reported. Discontinue product use if a patient shows evidence of an immunologic reaction. Patients should notify their physician of any symptoms of an allergic reaction. In clinical studies evaluating over 1000 patients, no allergic reactions to Apligraf were reported.

5. PRECAUTIONS

Caution: Do not use Apligraf if there is evidence of container damage or product contamination.

Caution: Apligraf should not be reused, frozen or sterilized after opening.

Caution: Apligraf should be kept in its tray on the shipping medium in the sealed bag under controlled temperature 68°F-73°F (20°C-23°C) until ready for use.

Caution: Apligraf should be handled using sterile technique and placed on a prepared wound bed within 15 minutes of opening the package.

Caution: Do not use cytotoxic agents, including Dakin's solution, Mafenide Acetate, Scarlet Red Dressing, Tincoban, Zinc Sulfate, Povidone-iodine solution, or Chlorhexidine with Apligraf. In *in vitro* and *in vivo* histology studies, exposure to these agents degraded Apligraf. Device exposure to Mafenide Acetate, Polymyxin/Nystatin or Dakin's Solution also reduced Apligraf cell viability.

Caution: Diagnosis of wound infection may be complicated by the white or yellow appearance of Apligraf after it becomes hydrated with wound fluid. Apligraf-treated wounds with respect to signs of suspected infection, including a change from baseline at the ulcer site for pain, edema, erythema, drainage, odor, warmth and/or unexplained fever, should be evaluated and treated according to standard practice for infection.

Caution: The persistence of Apligraf cells on the wound and the safety of this device in venous ulcer patients beyond one year and in diabetic foot ulcer patients beyond 6 months has not been evaluated. Testing to date has not revealed a tumorigenic potential of the cells contained in the device. However, the long term potential of skin cancers from these cells is unknown.

Caution: Although the cells and Apligraf have been tested for multiple pathogens and are processed under aseptic conditions, all living tissue may transmit infectious agents.

Caution: The safety and the effectiveness of Apligraf have not been established for patients receiving greater than 5 device applications.

6. ADVERSE EVENTS

A. Venous Leg Ulcers (VLU)

All reported adverse events, which occurred in the Apligraf cohort in the study evaluating Apligraf for the treatment of venous leg ulcers at an incidence of 1% or greater are listed in Table 1. The adverse events are listed in descending order according to frequency. This table lists all adverse events reported in the VLU study including those attributed and not attributed to treatment.

Table 1
Adverse Events Reported in Greater than 1.0% of Apligraf Patients in the Venous Leg Ulcer Study

	Apligraf (n = 161)	Control (n= 136)
	Total	Total
Suspected wound infection[1] (study site)	47 (29.2%)	19 (14.0%)
Suspected wound infection[1] (non-study site[2])	16 (9.9%)	15 (11.0%)
Cellulitis[3] (study site)	13 (8.1%)	11 (8.1%)
Cellulitis[3] (non-study site)	12 (7.5%)	7 (5.1%)
Dermatitis (non-study site)	10 (6.2%)	10 (7.4%)
Exudate (study site)	9 (5.6%)	0 (0.0%)
Peripheral edema	8 (5.0%)	7 (5.1%)
Pain (study site)	7 (4.3%)	7 (5.1%)
Death	6 (3.7%)	6 (4.4%)
Skin ulcer (non-study site)	6 (3.7%)	5 (3.7%)
Pain (non-study site)	5 (3.1%)	4 (2.9%)
Pruritus (non-study site)	5 (3.1%)	2 (1.5%)
Skin Ulcer (study site)	5 (3.1%)	3 (2.2%)
Infection (non-wound)	4 (2.5%)	1 (0.7%)
Positive wound culture[4] (study site)	4 (2.5%)	3 (2.2%)
Rhinitis	4 (2.5%)	1 (0.7%)
Dermatitis (study site)	4 (2.5%)	2 (1.5%)
Pain (overall body)	3 (1.8%)	2 (1.5%)
Congestive heart failure	3 (1.8%)	0 (0.0%)
Accidental injury (musculoskeletal)	3 (1.8%)	0 (0.0%)
Dyspnea	3 (1.8%)	1 (0.7%)
Pharyngitis	3 (1.8%)	0 (0.0%)
Rash (study site)	3 (1.8%)	2 (1.5%)
Accidental injury (overall body)	2 (1.3%)	1 (0.7%)
Asthenia	2 (1.3%)	0 (0.0%)
Arrhythmia	2 (1.3%)	0 (0.0%)
Abscess (non-study site)	2 (1.3%)	0 (0.0%)
Arthralgia	2 (1.3%)	2 (1.5%)
Cough increased	2 (1.3%)	0 (0.0%)
Rash (non-study site)	2 (1.3%)	5 (3.7%)
Erythema (study site)	2 (1.3%)	1 (0.7%)
Kidney failure	2 (1.3%)	0 (0.0%)
Urinary tract infection	2 (1.3%)	5 (3.7%)

Adverse events were recorded as mild, moderate, severe or life-threatening. In the venous leg ulcer study, there were 1 life-threatening and 3 severe infections reported in the Apligraf group and none in the control arm. Of the four events, two severe infections were considered related to treatment: however one occurred one month after the last application of Apligraf and the other occurred following application on a pre-existing *Pseudomonas* infection.

B. Diabetic Foot Ulcers (DFU)

All reported adverse events, which occurred in the study evaluating Apligraf for the treatment of diabetic foot ulcers at an incidence of 1% or greater in the Apligraf group are listed in Table 2. This table lists all adverse events reported in the DFU study including those attributed and not attributed to treatment.

Table 2
Adverse Events Reported in Greater than 1.0% of Apligraf Patients in the Diabetic Foot Ulcer Study

	Apligraf (n = 112)	Control (n= 96)
	Total	Total
Neuropathic ulcer (non-study site[2])	19 (17.0%)	9 (9.4%)
Suspected wound infection[1] (non-study site)	15 (13.4%)	7 (7.3%)
Non-neuropathic skin alteration (non-study site)	13 (11.6%)	11 (11.5%)
Suspected wound infection[1] (study site)	12 (10.7%)	13 (13.5%)
Cellulitis[3] (non-study site)	11 (9.8%)	4 (4.2%)
Cellulitis[3] (study site)	10 (8.9%)	8 (8.3%)
Osteomyelitis (non-study site)	10 (8.9%)	3 (3.1%)
Vesicular bullous rash (non-study site)	9 (8.0%)	5 (5.2%)
Pain (overall body)	8 (7.1%)	4 (4.2%)
Fungal infection (non-study site)	7 (6.3%)	9 (9.4%)
Hypoglycemia	7 (6.3%)	3 (3.1%)
Infection (overall body)	6 (5.4%)	4 (4.2%)
Hematoma (non-study site)	6 (5.4%)	2 (2.1%)
Deteriorating ulceration (study site)	5 (4.5%)	6 (6.3%)
Rash (non-study site)	5 (4.5%)	4 (4.2%)
Non-neuropathic skin alteration (study site)	5 (4.5%)	2 (2.1%)
Pain (non-study site)	5 (4.5%)	1 (1.0%)
Bone dislocation (non-study site)	5 (4.5%)	1 (1.0%)
Peripheral edema	4 (3.6%)	11 (11.5%)
Accidental Injury (overall body)	4 (3.6%)	8 (8.3%)
Accidental Injury (non-study site)	4 (3.6%)	5 (5.2%)
Fever (overall body)	4 (3.6%)	5 (5.2%)
Hyperglycemia	4 (3.6%)	4 (4.2%)
Dry skin (non-study site)	4 (3.6%)	2 (2.1%)
Chest pain	4 (3.6%)	1 (1.0%)
Bronchitis	4 (3.6%)	0 (0.0%)
Osteomyelitis (study site)	3 (2.7%)	10 (10.4%)
Nausea	3 (2.7%)	6 (6.3%)
Pharyngitis	3 (2.7%)	6 (6.3%)
Anemia	3 (2.7%)	5 (5.2%)
Right Heart failure	3 (2.7%)	3 (3.1%)
Abscess (study site)	3 (2.7%)	3 (3.1%)
Urinary tract infection	3 (2.7%)	2 (2.1%)
Deteriorating ulceration (non-study site)	3 (2.7%)	2 (2.1%)
Gastroenteritis	3 (2.7%)	2 (2.1%)
Cataract	3 (2.7%)	2 (2.1%)
Abscess (overall body)	3 (2.7%)	0 (0.0%)
Gastritis	3 (2.7%)	0 (0.0%)
Spontaneous bone fracture	3 (2.7%)	0 (0.0%)
Diarrhea	2 (1.8%)	8 (8.3%)
Positive Wound Culture[4] (study site)	2 (1.8%)	3 (3.1%)
Arthrosis (non-study site)	2 (1.8%)	3 (3.1%)
Malaise	2 (1.8%)	2 (2.1%)
Rash (study site)	2 (1.8%)	2 (2.1%)
Hematoma (study site)	2 (1.8%)	2 (2.1%)
Gangrene (non-study site)	2 (1.8%)	2 (2.1%)
Dyspepsia	2 (1.8%)	1 (1.0%)
Accidental Injury (study site)	2 (1.8%)	1 (1.0%)
Infection (non-study site)	2 (1.8%)	0 (0.0%)
Gangrene (study site)	2 (1.8%)	0 (0.0%)
Spontaneous bone fracture (non-study site)	2 (1.8%)	0 (0.0%)
Viral infection	2 (1.8%)	0 (0.0%)
Back pain	2 (1.8%)	0 (0.0%)
Angina pectoris	2 (1.8%)	0 (0.0%)
Arteriosclerosis	2 (1.8%)	0 (0.0%)
Cardiomegaly	2 (1.8%)	0 (0.0%)
Gastrointestinal carcinoma	2 (1.8%)	0 (0.0%)
Colitis	2 (1.8%)	0 (0.0%)
Rhinitis	2 (1.8%)	0 (0.0%)
Arthritis	2 (1.8%)	0 (0.0%)
Confusion	2 (1.8%)	0 (0.0%)

In the clinical trials the following definitions were used:

[1] *Suspected wound infection:* a wound with at least some clinical signs and symptoms of infection such as increased exudate, odor, redness, swelling, heat, pain, tenderness to the touch, and purulent discharge; quantitative culture was not required.

[2] *Non-study site event* – An adverse event occurring on either extremity, but not located at or involving the study ulcer.

[3] *Cellulitis:* a non-suppurative inflammation of the subcutaneous tissues extending along connective tissue planes and across intercellular spaces; widespread swelling, redness and pain without definite localization.

[4] *Positive wound culture:* reported as an adverse event, but not reported as a wound infection.

Table 3 lists all DFU infectious adverse events (i.e., wound infection, cellulitis, osteomyelitis, gangrene, abscess, and fungal infection) as well as resections and amputations occurring on the study limb by first occurrence.

Table 3
Infectious Adverse Events and Amputations Occurring on the Study Limb in Diabetic Foot Ulcers by Number of Apligraf Applications

# Applications	# Patients n=112	# Closed	Mean Days to Closure (range)	# First Infections on Study Limb	# Amputations and Resections on Study Limb
1	10 (8.9%)	9/10 (90.0%)	15 (7-57)	1	0
2	11 (9.8%)	8/11 (72.7%)	15 (8-36)	2	0
3	15 (13.4%)	10/15 (66.7%)	22 (22-29)	5	0
4	17 (15.2%)	9/17 (52.9%)	36 (29-78)	6	1
5	59 (52.7%)	27/59 (45.8%)	51 (36-88)	24	6
Total Apligraf	112	63 (56%)	36 (7-88)	38/112 (34%)	7
Control	96	36 (38%)	50 (15-92)	36/39 (38%)	15

7. CLINICAL STUDIES

A. Venous Leg Ulcers (VLU)

Study Design

A prospective, randomized, controlled, multi-center, multi-specialty, unmasked study was conducted to evaluate the safety and effectiveness of Apligraf and compression therapy in comparison to an active treatment concurrent control of zinc paste gauze and compression therapy. The study population included consenting patients who were 18-89 years old, available for one year follow-up, with venous insufficiency confirmed by plethysmography (venous reflux < 20 sec.); associated with non-infected partial and / or full thickness skin loss ulcer (IAET Stage 2 or 3) of greater than one month duration and which had not adequately responded to conventional ulcer therapy. Patients were excluded for ankle brachial index < 0.65, severe rheumatoid arthritis, collagen vascular disease, pregnancy/lactation, cellulitis, osteomyelitis, ulcer with necrotic, avascular or bone/tendon/fascia exposed-bed, clinically significant wound healing impairment due to uncontrolled diabetes, or renal, hepatic, hematologic, neurologic or immune insufficiency or due to immunosuppressive agents such as corticosteroids (> 15 mg/day), radiation therapy or chemotherapy; or enrollment in studies within the past 30 days for investigational devices or within the past three months for investigational drugs related to wound healing.

Extremities with multiple ulcers were enrolled; however, only one ulcer per extremity was studied. Non-study ulcer care was not specifically defined. Study ulcer care was defined for the treatment (Apligraf and compression therapy) and control (zinc paste gauze and compression therapy), treatment groups in two phases:

1) Active Phase (0-8 weeks): All patients received: i) a non-adherent, ii) a non-occlusive, and iii) a therapeutic compression dressing on day 0, mid-week during the first week (day 3-5), and at weeks 1-8. Control treated patients also received zinc impregnated gauze at each visit. All Apligraf patients received Apligraf on day 0. At the day 3-5 and weeks 1, 2, and 3 visits, if less than 50% Apligraf take was observed, then patients received an additional application of Apligraf. Patients were not allowed to receive more than 5 Apligraf applications total.

2) Maintenance Phase (8-52 weeks): Closed-ulcer extremities received non-specified elastic compression stockings. Open-ulcer extremities continued with dressing changes.

Wound closure was defined as 100% epithelialization without drainage and assessed by clinical observation at visits on day 0, day 3-5, weekly from weeks 1-8, months 3 and 6 after initial treatment application or until wound closure was achieved. Additional follow-up visits were 9 and 12 months after initial treatment.

VLU Study Results

The incidence of VLU wound closure at set visits up to 6 months is presented below as the raw data results (Figure 1) and the results after adjustment for pooled center, baseline ulcer duration, and baseline area (Figure 2).

Figure 1
VLU Efficacy Cohort (n=240)
Raw Frequency of Complete Wound Closure as a Function of Time

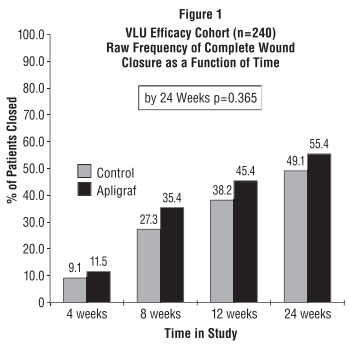
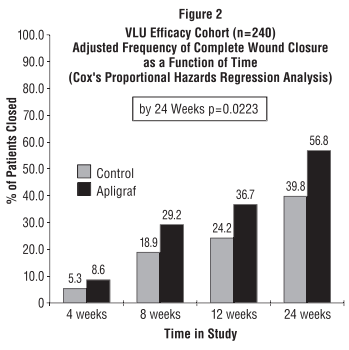


Figure 2
VLU Efficacy Cohort (n=240)
Adjusted Frequency of Complete Wound Closure as a Function of Time
(Cox's Proportional Hazards Regression Analysis)



VLU Ulcer recurrence

At six months, the incidence of VLU recurrence was 8.3% (6/72) for Apligraf- and 7.4% (4/54) for control-treated patients. The incidence of VLU recurrence by 12 months was 18.1% (13/72) in the Apligraf group and 22.2% (12/54) in the control group.

VLU Suspected wound infection

In the VLU effectiveness cohort, there were 33/130 (25.4%) Apligraf-treated and 15/110 (13.6%) control-treated ulcers with suspected wound infection. While the overall incidence of wound infection was higher in the Apligraf arm, the incidence of wound closure (Figures 1 and 2) was also higher for Apligraf-treated patients.

VLU Baseline status impact on wound closure

The impact of VLU patient baseline status on wound closure was evaluated for the patient populations above and below the median values for ulcer duration and ulcer size as well as for baseline IAET Ulcer Stage, the presence of diabetes and a patient's Ankle Brachial Index. The results of these analyses are displayed in Table 4.

Table 4
Pre-Treatment Status and Wound Closure
VLU Effectiveness Cohort (n=240 patients)

	Pre-Treatment Status		Number and Percent of Wound Closure by 6 months	
Patient Condition	**No. and (%) Apligraf Pts.**	**No. and (%) Control Pts.**	**Apligraf**	**Control**
Total	130 Patients	110 Patients	72 (55.4%)	54 (49.1%)
Ulcer Duration				
≤ 1 year	58 (44.6%)	62 (56.3%)	38/58 (65.5%)	45/62 (72.6%)
> 1 year	72 (55.4%)	48 (43.6%)	34/72 (47.2%)	9/48 (18.8%)
***Ulcer Area**				
< 500 mm^2	65 (50.0%)	60 (54.5%)	45/65 (69.2%)	35/60 (58.3%)
> 500 mm^2	63 (48.5%)	50 (45.5%)	26/63 (41.3%)	19/50 (38.0%)
IAET Staging				
Stage II	63 (48.5%)	56 (50.9%)	34/63 (54.0%)	32/56 (57.1%)
Stage III	67 (51.5%)	54 (49.1%)	38/67 (56.7%)	22/54 (40.7%)
Diabetes				
Yes[1]	25 (19.2%)	11 (10.0%)	12/25 (48.0%)	4/11 (36.4%)
No	105 (80.8%)	99 (90.0%)	60/105 (57.1%)	50/99 (50.5%)
****Ankle Brachial Index data (ABI)**				
> 0.65 ≤ 0.8	9 (6.9%)	10 (9.1%)	4/9 (44.4%)	4/10 (40.0%)
>0.8 <1.0	43 (33.1%)	50 (45.5%)	26/43 (60.5%)	27/50 (54.0%)
>1.0	75 (57.7%)	49 (44.5%)	40/75 (53.3%)	22/49 (44.9%)

* Baseline ulcer area missing for two patients in the Apligraf® group.
** ABI data are missing for 3 Apligraf and 1 control patient.
[1]This category includes both insulin-dependent and non-insulin dependent diabetes patients, because the insulin-dependence of patients was not determined in this clinical trial.

VLU Secondary Endpoints
Clinical assessment (scale 1-4) of wound depth (IAET staging), erythema, edema, wound pain, fibrin, exudate, granulation tissue, and overall assessment by changes in mean score and analysis of variance from baseline to the 6 month visit indicated no differences between VLU treatment groups at 6 months.

VLU Immune response
In tests of VLU patients' sera there were no observations of antibody responses against bovine Type I collagen, bovine serum proteins or the Class I HLA antigens on human dermal fibroblasts, and human epidermal cells. T-cell specific responses were also not observed against bovine Type I collagen, human fibroblasts or human keratinocytes. There was also no clinical evidence of Apligraf rejection by any patient.

B. Diabetic Foot Ulcers (DFU)

Study Design
A prospective, randomized, controlled, multi-center unmasked study was conducted to evaluate the safety and efficacy of Apligraf in comparison to Control treatment, saline moistened gauze ,in the treatment of diabetic neuropathic foot ulcers. The study population included consenting patients who were between 18 and 80 years old, with a 0.4 cm^2 – 16.3 cm^2 full-thickness foot ulcer of neuropathic etiology of at least 2 weeks duration, located on the plantar, medial or lateral surface of the foot at least 2 cm away from any other ulcers on the same extremity. The study participants were required to be diagnosed diabetics with type 1 or type 2 diabetes, a HbA1C between 6% and 12% and available for six-month follow-up. Patients were excluded for ulcers with tracts or tunnels, a clinical infection at the study ulcer site, ABI < 0.65, active Charcot's arthropathy at the study extremity, a study ulcer that healed > 30% from post-debridement at Study Day –7 to Day 0, renal dialysis, history of alcohol or substance abuse within one year, acute or chronic hepatitis, receiving corticosteroids, immunosuppressive agents, radiation therapy or chemotherapy one month prior to study enrollment, or enrollment in clinical studies evaluating a device within the past 30 days or within the past 3 months for pharmaceuticals or biologics.

Two-hundred-seventy-seven patients were entered into the screening phase of the study. Sixty-nine patients did not meet inclusion/exclusion criteria. After randomization and screening, 208 patients were treated in the study, i.e., 112 received Apligraf and 96 received Control therapy. Patients received 12 weeks of treatment and 3 additional months of follow-up. Complete wound closure was evaluated by or on 12 weeks. Patients were evaluated weekly for the first 12 weeks with mid-week visits for dressing changes from Day 0 through Week 5 and follow-up visits at Months 4, 5 and 6.

Both treatment groups received good ulcer care consisting of sharp debridement, saline moistened dressings and a non-weight bearing regimen. All patients in the Apligraf treatment group received Apligraf at Day 0. At Study Weeks 1, 2, 3 and 4, if Apligraf coverage was less than 100% and the wound was not progressing to healing then an additional Apligraf unit was applied. A maximum of 5 Apligraf applications was allowed. The Apligraf was dressed with saline-moistened non-adherent dressing, tape, dry gauze, petrolatum gauze and gauze wrap. The Control treated patients received saline-moistened non-adherent dressing, tape, saline moistened gauze, dry gauze, petrolatum gauze and gauze wrap from Day 0 through Study Week 4.

Patients in both treatment groups who did not heal by Study Week 5 were treated with saline-moistened gauze, dry gauze, petrolatum gauze and gauze wrap from Study Week 5 through Study Week 12. The patients were instructed to change this dressing two times per day.

Patients were instructed to avoid weight bearing on the affected foot throughout the duration of the study. During the first 6 weeks patients were instructed to use crutches or a wheelchair. Each patient was fitted with a customized tri-density sandal. These sandals were to be worn throughout the entire study.

In keeping with good medical practice, early detection and treatment of ulcer infection using standard procedures was advised.

DFU Study Results
The incidence of DFU wound closure at set visits is presented below (Figure 3).

Figure 3
Frequency of Complete DFU Wound Closure as a Function of Time (Kaplan-Meier) (Treated Population n=208)

DFU Ulcer recurrence
The incidence of DFU recurrence as a function of device applications is presented in Table 5.

Table 5
Ulcer Recurrence in Diabetic Foot Ulcers by Number of Applications

# Applications	# Patients n = 112	# Closed	# Re-opened	# Re-closed by 6 months
1	10 (8.9%)	9	4/9 (44%)	4/4
2	11 (9.8%)	8	3/8 (38%)	2/3
3	15 (13.4%)	10	4/10 (40%)	4/4
4	17 (15.2%)	9	2/9 (22%)	0/2
5	59 (52.7%)	27	12/27 (44%)	10/12
Apligraf Total*	112	63	25/63 (40%)	20/25 (80%)
Control*	96	36	16/36 (44%)	10/16 (63%)

* Three patients in each group re-opened > 4 weeks after attaining complete wound closure.

DFU Baseline status impact on wound closure
The impact of DFU patient baseline status on wound closure was evaluated for patient gender, age, Charcot's status, diabetes type, number and location of ulcers on study foot as well as the patient populations above and below the median values for ulcer duration, size and HbA_{1c} level (%). The results of these analyses are displayed in Table 6.

Table 6
Pre-Treatment Status and Wound Closure
DFU Treated Population (n=208 patients)

	Pre-Treatment Status		Number and Percent of Wound Closure by 12 Weeks	
Patient Condition	**No. and (%) Apligraf**	**No. and (%) Control**	**No. and (%) Apligraf**	**No. and (%) Control**
Total	112 Patients	96 Patients	63 (56.3%)	36 (37.5%)
Charcot Joint Deformity				
Absent	95 (84.8%)	74 (77.1%)	60/95 (63.2%)	28/74 (37.8%)
Inactive	17 (15.2%)	22 (22.9%)	3/17 (17.6%)	8/22 (36.4%)
Diabetes*				
Type 1 (IDDM)	41 (36.6%)	26 (27.1%)	20/41 (48.8%)	6/26 (23.1%)
Type 2 (NIDDM)	69 (61.6%)	70 (72.9%)	42/69 (60.9%)	30/70 (42.9%)
Ulcer Location**				
Toes	22 (19.6%)	13 (13.5%)	14/22 (63.6%)	8/13 (61.5%)
Metatarsal heads	58 (51.8%)	49 (51.0%)	36/58 (62.1%)	20/49 (40.8%)
Midfoot	30 (26.8%)	34 (35.4%)	12/30 (40.0%)	8/34 (23.5%)
Age				
18 – 70 years	98 (87.5%)	91 (94.8%)	55/98 (56.1%)	34/91 (37.4%)
71 – 80 years	14 (12.5%)	5 (5.2%)	8/14 (57.1%)	2/5 (40.0%)
Gender				
Male	88 (78.6%)	74 (77.1%)	46/88 (52.3%)	30/74 (40.5%)
Female	24 (21.4%)	22 (22.9%)	17/24 (70.8%)	6/22 (27.2%)
Ulcer Area†				
≤ 177 (mm^2)	59 (52.7%)	45 (46.9%)	39/59 (66.1%)	20/45 (44.4%)
> 177 (mm^2)	52 (46.4%)	51 (53.1%)	23/52 (44.2%)	16/51 (31.4%)
Ulcer Duration				
≤ 6 months	61 (54.5%)	51 (53.1%)	38/61 (62.3%)	22/51 (43.1%)
> 6 months	51 (45.5%)	45 (46.9%)	25/51 (49.0%)	14/45 (31.1%)
Number of Ulcers on Study Foot				
Single	100 (89.3%)	90 (93.8%)	57/100 (57.0%)	33/90 (36.7%)
Multiple	12 (10.7%)	6 (6.2%)	6/12 (50.0%)	3/6 (50.0%)
HbA1c				
≤ 8.40	63 (56.3%)	42 (43.8%)	34/63 (54.0%)	12/42 (28.6%)
> 8.40	49 (43.8%)	54 (56.3%)	29/49 (59.2%)	24/54 (44.4%)

* Two patients in the Apligraf group did not have type of diabetes specified.
** Two patients in the Apligraf group had ulcers located not at the toes, metatarsal heads, or midfoot.
†One patient in the Apligraf group did not have a baseline ulcer tracing available.

DFU Secondary endpoints
Between Study Day 0 and Study Week 12, both DFU Apligraf and Control groups showed statistically significant improvement in undermining, maceration, exudate, granulation, eschar and fibrin slough. At Study Week 12, Apligraf showed statistically significant improvements when compared to Control in maceration (p=0.0233), exudate (p=0.0290) and eschar (p=0.0293).

DFU Immune response
In tests of DFU patients' sera there were no observations of antibody responses against bovine collagen Type I collagen, bovine serum proteins or Class I HLA antigens on human dermal fibroblasts, and human epidermal cells. T-cell specific responses were not observed against bovine Type I collagen, human dermal fibroblasts or human keratinocytes. In addition, there was no clinical evidence of Apligraf rejection by any patient.

8. HOW SUPPLIED
Apligraf is supplied sealed in a heavy gauge polyethylene bag with a 10% CO_2/air atmosphere and agarose nutrient medium. Each Apligraf is supplied ready for use and intended for application on a single patient. To maintain cell viability, Apligraf should be kept in the sealed bag at 68°F -73°F (20°C - 23°C) until use. Apligraf is supplied as a circular disk approximately 75 mm in diameter and 0.75 mm thick. The agarose shipping medium contains agarose, L-glutamine, hydrocortisone,human recombinant insulin, ethanolamine, O-phosphorylethanolamine, adenine, selenious acid, DMEM powder, HAM's F-12 powder, sodium bicarbonate, calcium chloride, and water for injection.

To maintain cell viability, the product is aseptically manufactured, and cannot be terminally sterilized. Testing for absence of microbial contamination is performed throughout the production process. Apligraf is shipped following initiation of the finished product microbiological testing to determine the absence of microbial growth. Final microbiological test results may not be available at the time of application.

9. DIRECTIONS FOR USE
Apligraf is indicated for use with standard therapeutic compression for the treatment of non-infected partial and full-thickness skin ulcers due to venous insufficiency of greater than 1 month duration and which have not adequately responded to conventional ulcer therapy. Apligraf is also indicated for use in the treatment of full-thickness diabetic foot ulcers of neuropathic etiology of at least three weeks duration which have not adequately responded to conventional ulcer therapy and which extend through the dermis but without tendon, muscle, capsule or bone exposure, and are located on the plantar, medial or lateral area of the foot, excluding the heel. Apligraf consists of living cells which must be kept sealed in its nutrient medium and 10% CO_2/air atmosphere under controlled temperature 68°F-73°F (20°C-23°C) and used within 15 minutes of opening.

Preparation of the Wound Bed Prior to Apligraf Application

1. Wound Infection:
Apligraf should not be applied over infected or deteriorating wounds until the underlying condition has been resolved.

2. Bacterial Containment:
Antimicrobial agents may be used during the week prior to Apligraf application to reduce the risk of infection. Dakin's solution, Mafenide Acetate, Scarlet Red Dressing, Tincoban, Zinc Sulfate, Povidone-iodine solution, and Chlorhexidine have been determined to be cytotoxic to Apligraf.

3. Wound Bed Preparation:

Venous Leg Ulcers
Apligraf should be applied to a clean, debrided wound after thoroughly irrigating the wound with a non-cytotoxic solution. Oozing or bleeding resulting from debridement should be stopped through the use of gentle pressure. Previous ulcer treatments other than standard therapeutic compression should be discontinued.

Diabetic Foot Ulcers
Apligraf should be applied to a clean wound base. Debridement should extend to healthy, viable, bleeding tissue. Prior to Apligraf application, hemostasis must be achieved. Prior to debridement thoroughly cleanse the wound with sterile saline to remove all loose debris and necrotic tissue. Using tissue nippers, a surgical blade or curette remove all hyperkeratotic and/or necrotic tissue and debris from the wound surface. Ulcer margins should be debrided to have a saucer effect. After debridement, cleanse the wound thoroughly with sterile saline solution and gently dry with gauze. Oozing or bleeding resulting from debridement may be stopped through the use of gentle pressure.

4. Control of Heavy Exudation:
Heavy exudation may displace Apligraf and reduce adherence. Exudation should be minimized by appropriate clinical treatment. If exudation persists, Apligraf should be made permeable to exudate by perforating the Apligraf to allow for drainage.

Suggested Technique for the Application of Apligraf to the Wound

1. Check expiration date. If expired, do not open or use.
2. Check product pH. If not 6.8-7.7 by the provided pH chart, do not open or use.
3. Apligraf should be kept in its polyethylene bag at controlled temperature 68°F-73°F (20°C -23°C) until immediately prior to use.
4. Cut open the sealed polyethylene bag and transfer the plastic tray to the sterile field with aseptic technique. Apligraf should always be handled aseptically.
5. Lift off the tray lid and note epidermal and dermal layer orientation: Apligraf is packaged with the epidermal (dull, matte finish) layer facing up and the dermal (glossy) layer facing down, resting on the polycarbonate membrane.
6. Using a sterile atraumatic instrument, gently dislodge approximately 0.5 inch of Apligraf away from the wall of the tray.
7. There should be no evidence of contamination, visible particulates or pungent odor.
8. When lifting Apligraf, be careful not to perforate or lift the polycarbonate membrane beneath Apligraf. The polycarbonate membrane should remain in the tray.
9. With sterile gloved hands, insert one index finger under the released section of Apligraf. Use the other index finger to grasp the Apligraf in a second spot along the edge of the device. Holding the Apligraf in two places lift the entire Apligraf out of the tray using a smooth, even motion. This easy motion should prevent Apligraf from bending and folding over onto itself. To minimize Apligraf damage, avoid Apligraf contact with foreign bodies and minimize handling Apligraf except by its margins.
10. Do not allow Apligraf to fold or wrinkle on itself. If excessive folding occurs, Apligraf can be floated (epidermal surface up) onto warm sterile saline solution in a sterile tray.
11. Apligraf should be placed so that the dermal layer (the glossy layer closest to the medium) is in direct contact with the wound surface. Using a saline moistened cotton applicator, smooth Apligraf onto the wound bed so there are no pockets or wrinkled edges. If there is excessive Apligraf, which is not in contact with the wound bed, it should be trimmed away or it may adhere to the dressing.
12. Dressings:

Venous Leg Ulcers
Secure Apligraf with a three-layer dressing so as to assure contact to wound bed:

- Apply a non-adherent dressing over the ulcer and Apligraf, extending 0.5 inch beyond the ulcer perimeter and inflamed skin margins.
- Apply a non-occlusive dressing such as fine mesh gauze. This may be folded or rolled as a bolster.
- Apply a self adherent elastic wrap from metatarsals to tibial plateau so that therapeutic compression is applied to the ulcer site.

Diabetic Foot Ulcers
Apligraf should be dressed with non-adherent saline moistened dressing, a layer of dry gauze, a layer of petrolatum gauze and gauze wrap.

Frequency of Dressing Changes and Apligraf Applications

Venous Leg Ulcers
1. The wound should be inspected and the dressing changed at least once a week during the immediate post application period. More frequent changes may be required on highly exudative wounds.
2. Additional applications of Apligraf may be necessary. Prior to additional applications, non-adherent remnants of Apligraf should be gently removed. Healing tissue or adherent Apligraf should not be disrupted. The wound bed should be cleansed with a non-cytotoxic solution prior to additional applications of Apligraf. Additional applications of Apligraf should not be applied over areas where Apligraf is adherent.
3. The wound site should continue to be dressed with a non-adherent dressing, pressure bolster and elastic overwrap as described above.
4. Upon complete wound closure, patients should be continued with compression therapy such as support stockings.
5. The safety and the effectiveness of Apligraf have not been established for patients receiving greater than 5 device applications.

Diabetic Foot Ulcers
1. The wound should be inspected and the dressing in contact with Apligraf should be changed once a week. Outer dressings may be changed more frequently (daily).
2. Additional applications of Apligraf may be necessary. Prior to additional applications, non-adherent remnants of Apligraf should be gently removed. Additional saucerized debridement may be needed to remove non-viable tissue. Healing tissue or adherent Apligraf should not be disrupted. The wound bed should be cleansed with a non-cytotoxic solution prior to additional applications of Apligraf. Additional applications of Apligraf should not be applied over areas where Apligraf is adherent.
3. The wound site should continue to be dressed as described.
4. After healing patients should continue to wear appropriate pressure relieving footwear and utilize other ulcer preventive footcare practices.
5. The safety and the effectiveness of Apligraf have not been established for patients receiving greater than 5 device applications.

10. PATIENT'S MANUAL
Venous Leg Ulcers
A brochure is available to:
1. Provide basic information about chronic wounds.
2. Address standard patient care while receiving Apligraf treatment.
3. Educate patients on Apligraf-related healing process.

Diabetic Foot Ulcers
A brochure is available to:
1. Provide basic information about diabetic foot ulcers.
2. Address standard patient care while receiving Apligraf treatment.
3. Educate patients on Apligraf -related healing process

11. PEEL-OFF LABEL
Remove the peel-off label from the lower right corner of the Apligraf package label and place it in the patient's chart. This label bears a unique lot number and expiration date of the Apligraf.

Apligraf®
Essential Prescribing Information
Numbers in parentheses ( ) refer to sections in the main part of the product labeling.

Device Description
Apligraf is supplied as a living, bi-layered skin substitute manufactured from cells processed under aseptic conditions using neonatal foreskin-derived keratinocytes and fibroblasts with bovine Type I collagen. **(1)**

Intended Use/Indications
Apligraf is indicated for use with standard therapeutic compression in the treatment of uninfected partial and/or full-thickness skin loss ulcers due to venous insufficiency of greater than 1 month duration and which have not adequately responded to conventional ulcer therapy. **(2)**

Apligraf is indicated for use with standard diabetic foot ulcer care for the treatment of full-thickness foot ulcers of neuropathic etiology of at least three weeks duration, which have not adequately responded to conventional ulcer therapy and extend through the dermis but without tendon, muscle, capsule or bone exposure. **(2)**

Contraindications
Apligraf is contraindicated for use on clinically infected wounds and in patients with known allergies to bovine collagen or hypersensitivity to the components of the shipping medium. **(3, 4, 5, 8)**

Warnings and Precautions
If the expiration date or product pH (6.8-7.7) is not within the acceptable range DO NOT OPEN AND DO NOT USE the product. A clinical determination of wound infection should be made based on all of the signs and symptoms of infection. **(4, 5)**

Adverse Events
All reported adverse events, which occurred at an incidence of greater than 1% in the clinical studies are listed in Table 1, Table 2 and Table 3. These tables list adverse events both attributed and not attributed to treatment. **(6)**

Maintaining Device Effectiveness
Apligraf has been processed under aseptic conditions and should be handled observing sterile technique. It should be kept in its tray on the medium in the sealed bag under controlled temperature 68°F -73°F (20°C-23°C) until ready for use. Apligraf should be placed on the wound bed within 15 minutes of opening the package. Handling before application to the wound site should be minimal. If there is any question that Apligraf may be contaminated or compromised, it should not be used. Apligraf should not be used beyond the listed expiration date. **(9)**

Use in Specific Populations
The safety and effectiveness of Apligraf have not been established in pregnant women, acute wounds, burns and ulcers caused by pressure.

Patient Counseling Information
VLU patients should be counseled regarding the importance of complying with compression therapy or other treatment, which may be prescribed in conjunction with Apligraf.

DFU patients should be counseled that Apligraf is used in combination with good ulcer care including a non-weight bearing regimen and optimal metabolic control and nutrition. Once an ulcer has healed, ulcer prevention practices should be implemented including regular visits to appropriate medical providers.

Treatment of Diabetes
Apligraf does not address the underlying pathophysiology of neuropathic diabetic foot ulcers. Management of the patient's diabetes should be according to standard medical practice.

How Supplied
Apligraf is supplied sealed in a heavy gauge polyethylene bag with a 10% CO_2/air atmosphere and agarose nutrient medium. Each Apligraf is supplied ready for use and intended for application on a single patient. To maintain cell viability, Apligraf should be kept in the sealed bag at 68°F-73°F (20°C-23°C) until use. Apligraf is supplied as a circular disk approximately 75 mm in diameter and 0.75 mm thick. **(8)**

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