A PROSPECTIVE, MULTI-CENTER, RANDOMIZED, PARALLEL-GROUP STUDY COMPARING AMNIOEXCEL® PLUS PLACENTAL ALLOGRAFT MEMBRANE TO APLIGRAF® BI-LAYERED SKIN SUBSTITUTE AND STANDARD OF CARE PROCEDURES IN THE MANAGEMENT OF DIABETIC FOOT ULCERS

SHORT TITLE: AMNIOEXCEL® Plus vs Apligraf® vs SOC in the Management of Diabetic Foot Ulcers

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TABLE OF CONTENTS

FIGURES

TABLES

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STATEMENT OF COMPLIANCE

By signing this document, I, the Investigator, certify that this trial will be carried out in accordance with International Conference on Harmonization Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 11, 21 CFR Part 50, and 21 CFR Part 56)

Additionally, I and any clinical trial site staff who are responsible for the conduct, management, or oversight of this trial will complete Human Subjects Protection/ICH GCP Training.

The protocol, informed consent form(s), any recruitment materials, and/or all subject materials associated with this trial will be submitted to an Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any subject is screened.

Finally, I understand that any amendments to the protocol or consent forms will require review and approval by the Sponsor and IRB before the changes are implemented to the study.

Investigator Name

Investigator Signature Date

1 PROTOCOL SUMMARY

Table 1: Protocol Synopsis

Title:	A Prospective, Multi-Center, Randomized, Parallel-group Study Comparing AMNIOEXCEL Plus Placental Allograft Membrane to Apligraf Bi-layered Skin Substitute and Standard of Care Procedures in the Management of Diabetic Foot Ulcers
Short Title:	AMNIOEXCEL Plus vs Apligraf vs Standard of Care (SOC) in the Management of Diabetic Foot Ulcers
Study Description:	This is a multicenter, randomized, parallel-group study comparing the outcomes associated with the use of AMNIOEXCEL Plus Placental Allograft Membrane, Apligraf bi-layered skin substitute and SOC alone in the management of diabetic foot ulcers (DFUs).
Pre/Post-Market:	Post-Market
Study Design:	This study will have three (3) phases: (1) a Screening Phase, (2) a Treatment Phase, and (3) a Follow-up Phase. Subjects will be seen weekly in the Screening and Treatment Phases. Subjects who achieve wound closure during the Treatment Phase will be entered into the Follow-up Phase and will return to the site for up two (2) additional weeks for confirmation visits. An interim analysis will occur after 50% of subjects have reached the primary endpoint.
Primary Objective:	The primary objective of this study is to compare outcomes associated with the use of AMNIOEXCEL Plus Placental Allograft Membrane, Apligraf living, bi-layered skin substitute and SOC in the management of DFUs.
Secondary Objective(s):	The secondary objectives of this study are to: 1. Determine the proportion of subjects in each study arm with complete closure of the study ulcer at or before 12 weeks of treatment 2. Determine the time to complete wound closure in each study arm 3. Determine the rate of wound closure in each study arm 4. Assess medical resource utilization outcomes in each study arm
Safety Objective:	The safety objective of this study is to assess adverse events associated with any of the investigational products and/or study procedures in the management of DFUs.
Exploratory Objective(s):	The exploratory objectives of this study are to: 1. Determine the proportion of subjects in each cohort whose wounds are closed at 4 weeks of treatment. 2. Determine the effect of any covariate on the primary endpoint.
Primary Endpoint:	The primary endpoint of this study is the incidence of complete wound closure, as assessed by the Investigator at or before Week 12 of the Treatment Phase, which is confirmed closed two weeks later. Complete wound closure is defined as complete skin re-epithelialization that is without drainage or dressing requirements
Secondary Endpoint(s):	1. The proportion of subjects in each group with complete closure of the study ulcer at or before Week 12 as assessed by computerized planimetry.

	2. The proportion of subjects in each group with complete wound closure of the study ulcer at or before Week 12 as assessed by independent, blinded, assessment of photographs. 3. The time to complete wound closure, as assessed by the Investigator. 4. The time to complete wound closure for all closed wounds in each group, as assessed by computerized planimetry. 5. For those wounds that have closed, the rate of wound closure for each group, as assessed by computerized planimetry. 6. Medical resource utilization associated with management of DFU and related complications as determined by a comparison of estimated costs from the presumed billing codes which would have been used for each patient.
Safety Endpoint:	Incidence of all investigational product, procedure, and/or wound-related adverse events or serious adverse events.
Exploratory Endpoint(s):	1. The proportion of closed wounds in each group at Week 4 of the Treatment Phase 2. Co-variate analyses of the primary endpoint using those listed in section 9.3.
Description of Study Intervention(s):	Intervention 1: SOC therapy consisting of the use of moist wound dressings, debridement, infection surveillance and management and consistent and appropriate use of an offloading boot. Intervention 2: AMNIOEXCEL Plus used in conjunction with SOC therapy. Intervention 3: Apligraf used in conjunction with SOC therapy.
Indications for Use:	AMNIOEXCEL Plus is a human placental-based tissue consisting of dehydrated, tri-layer Placental (Amnion/Chorion/Amnion) Allograft Membrane (T-PAM) layers. T-PAM is a minimally manipulated placental membrane product made from tissues donated by pre-screened mothers during planned C-sections and is intended for use as a wound covering. This product is an allograft tissue intended for homologous use for the repair, reconstruction and replacement of skin at the direction of a physician. AMNIOEXCEL Plus contains Human Cellular and Tissue Based Products (HCT/P) as defined by US FDA 21 CFR Part 1271. All donor recoveries are performed by BioDlogics, LLC, and adhere to the regulations regarding HCT/P recovery and the screening and testing of the tissue donor as verified through supplier audits. Apligraf is 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. It 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 DFU care for the treatment of full-thickness neuropathic DFUs 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.

Study Population:	114 subjects, at least 21 years of age with a DFU will be randomized into this trial in the United States.
Inclusion Criteria	*All of the following criteria must be met* by a potential subject to be enrolled (i.e., randomized) in the study. The subject must: 1. Have participated in the informed consent process and signed a study-specific informed consent document. 2. Be able and willing to comply with study procedures, including study visits, study dressing regimens, and compliance with study required offloading device. 3. Be ≥ 21 years of age. 4. Have Type I or Type II diabetes mellitus with Investigator-confirmed glycosylated hemoglobin (HbA1c) of ≤ 12% within 3 months prior to screening visit. 5. Have at least one diabetic foot ulcer that meets ALL of the following criteria: • Ulcer has been in existence for a minimum of four weeks prior to signing the Informed Consent Form for trial participation but no more than 12 months • Ulcer is a partial or full thickness diabetic foot ulcer without capsule/tendon/bone exposure (Wagner Grade 1 or superficial 2). • Ulcer is located on the foot or ankle (with no portion above the top of the malleolus, i.e., proximal to the malleolus). • If the subject has more than one ulcer that meets the eligibility criteria, the ulcer designated as the study ulcer will be at the discretion of the Investigator. • There is a minimum 1 cm margin between the qualifying study ulcer and any other ulcer on that same foot, post-debridement. 6. Ulcer size (i.e., area) is ≥ 1 cm^2 and ≤ 12 cm^2 post-debridement at Screening Visit 1 and Randomization (Day 0). 7. Have adequate vascular perfusion of the affected limb as defined by **at least one** of the following (in order of preference): • Ankle-Brachial Index (ABI) ≥ 0.65 and ≤1.2, performed *within 3 months* of screening visit 1 • Toe pressure (plethysmography) >50 mmHg *at time* of screening visit 1, • $TcPO_2$ >40 mmHg *at time* of screening visit 1 8. Be willing and able (or have a family member/friend willing and able) to apply required applicable dressing changes as well as study-required offloading/protective device if and when applicable.
Exclusion Criteria	Potential subjects will not be enrolled in the study if any of the following criteria are met: 1. The subject was previously randomized *and* treated under this clinical study protocol. 2. The study ulcer has: a. Unexplored tunneling,

	b. Undermining and/or c. Sinus tracts that necessitates surgical operating-room debridement and/or penetrates to capsule/tendon/bone. 3. The subject has a known sensitivity to ethanol. 4. The subject has a known allergy to bovine collagen. 5. The subject has a known hypersensitivity to any of the components of the Apligraf agarose shipping medium listed below: o agarose, o L-glutamine, o hydrocortisone, o human recombinant insulin, o ethanolamine, o O-phosphorylethanolamine, o adenine, o selenious acid, o DMEM powder, o HAM's F-12 powder, o sodium bicarbonate, o calcium chloride 6. The subject has a known sensitivity to any of the SOC materials which come in contact with the skin: o Coban™ o Conforming gauze o Optifoam® non-adhesive dressing o Normal Saline (liquid or gel) o Cotton Gauze o Normal saline (liquid & gel) o Non-adhering dressings o Steristrips 7. The subject is unable to safely ambulate with the use of a study required offloading boot. 8. The subject has suspected or confirmed gangrene or wound infection of the study ulcer, as evidenced by tissue necrosis, redness, pain, and/or purulent drainage and/or receiving systemic antibiotics for the treatment of such. 9. The subject has suspected or confirmed osteomyelitis of the foot with the study ulcer. 10. The subject has participated in another clinical trial involving a device or an investigational study drug/treatment within 28 days of randomization. 11. In the opinion of the Investigator, the subject has received, within 28 days of signing the Informed Consent Form, or is scheduled to receive during study participation, a medication or treatment which is known to interfere with or affect the rate and quality of wound healing (e.g., systemic steroids, immunosuppressive therapy, autoimmune disease therapy, dialysis, radiation therapy to the foot, vascular surgery, angioplasty, or thrombolysis).

	12. The subject has a history of bone cancer or metastatic disease of the affected limb, radiation therapy to the foot, or has had chemotherapy within the 12 months prior to signing the Informed Consent Form for trial participation. 13. The subject is currently pregnant or is actively trying to conceive. (Patient statement confirming lack of pregnancy is sufficient.) 14. In the opinion of the Investigator, the subject is unable to comply with the treatment regimen (i.e., return for clinic visits, perform required dressing changes and use study-designated offloading device(s)). 15. In the opinion of the Investigator, the subject has a history of, or is currently diagnosed with, any illness or condition, other than diabetes, that could interfere with wound healing (e.g., end-stage renal disease, severe malnutrition, liver disease, aplastic anemia, Raynaud's Syndrome, connective tissue disorder, acquired immune deficiency syndrome, HIV positive, or sickle cell anemia). 16. In the opinion of the Investigator, the subject has unstable Charcot foot or Charcot with bony prominence that could inhibit wound healing. 17. The subject has ulcers secondary to a disease other than diabetes (e.g., vasculitis, neoplasms, or hematological disorders). 18. In the opinion of the Investigator, the subject has excessive lymphedema that could interfere with offloading and/or wound healing. 19. The study ulcer has received wound dressings that include growth factors, engineered tissues, or skin substitutes within 28 days of randomization or is scheduled to receive treatment during the study (e.g., PriMatrix, AMNIOEXCEL (first generation), Regranex, Dermagraft, EpiFix, GraftJacket, OASIS, Omnigraft, or Integra BMWD). 20. The study ulcer closed more than 30% in area, *post-debridement*, between Screening Visit 1 and Randomization (Day 0, as measured by planimetry).
Description of Sites/Facilities Enrolling Subjects:	Up to 15 sites will participate in this study. All sites will be located in the United States. Each site may enroll up to 38 subjects.
Study Duration:	This study is anticipated to have an enrollment period of up to 2 years. Total duration of the study is expected to be up to 2 years.
Subject Duration:	All subjects will have a SOC screening period of up to 2 weeks ± 3 days prior to randomization (Day 0). Investigational product with SOC treatments *or* SOC alone treatments will begin on Day 0 (randomization) and will be applied weekly for up to 11 weeks ± 3 days. If the wound closes on or before week 11, follow-up will begin and will last 2 weeks ± 3 days after the date of closure. If the wound does not close by week 12, the subject will be immediately exited from the study and no further follow-up will occur as part of the study. The total duration for a single subject may last up to 16 weeks ± 3 days.
Statistical Methods & Analyses:	This is a prospective, multicenter, parallel-group, randomized, three-arm trial designed to assess the proportion of subjects achieving complete wound closure of their DFUs, in a population with adequate arterial circulation, following up to 11 weeks of treatment receiving either AMNIOEXCEL Plus Placental Allograft Membrane with SOC, Apligraf bi-layered skin substitute with SOC, or SOC alone. Subjects will be randomized 1:1:1, to receive either

	AMNIOEXCEL Plus Placental Allograft Membrane with SOC, Apligraf bi-layered skin substitute with SOC, or SOC alone. **Sample Size** A total of 114 subjects, 38 per cohort, will be randomized, inclusive of a 20% in-study attrition rate. It is assumed that the maximum wound closure rate is 90% with no difference between the study cohorts. Based on the 38 subject per cohort sample size, the half width of the 95% confidence interval for the wound closure rate is 13.5%. However, the study is not adequate for hypothesis testing. **Data Evaluation** The Intent-to-Treat (ITT) population, defined as all randomized subjects, will be the primary population for the analysis of primary and secondary efficacy endpoints. **Analysis Population** The Per Protocol (PP) population, defined as all subjects who complete the study without a major protocol violation, will be used as the primary analysis dataset. **Primary Efficacy Analysis** For the primary efficacy analysis, ITT population will be used. The proportion of subjects with complete wound closure, will be compared using the Cochran–Mantel–Haenszel (CMH) test. For the primary efficacy analysis, missing values will be imputed using last observation carried forward (LOCF). The differences in complete wound closure rates between treatment arms will be summarized and 95% confidence intervals calculated. **Safety Assessment** Adverse Events related to the investigational products, study ulcer and/or study procedures and all Serious Adverse Events will be recorded. All safety parameters will be summarized descriptively by treatment assignment. No inferential statistics are planned. **Exploratory Analyses** Exploratory analyses will be performed to examine wound closure status at Week 4 of Treatment and co-variate impact on the primary endpoint.

Figure 1: Study Schema

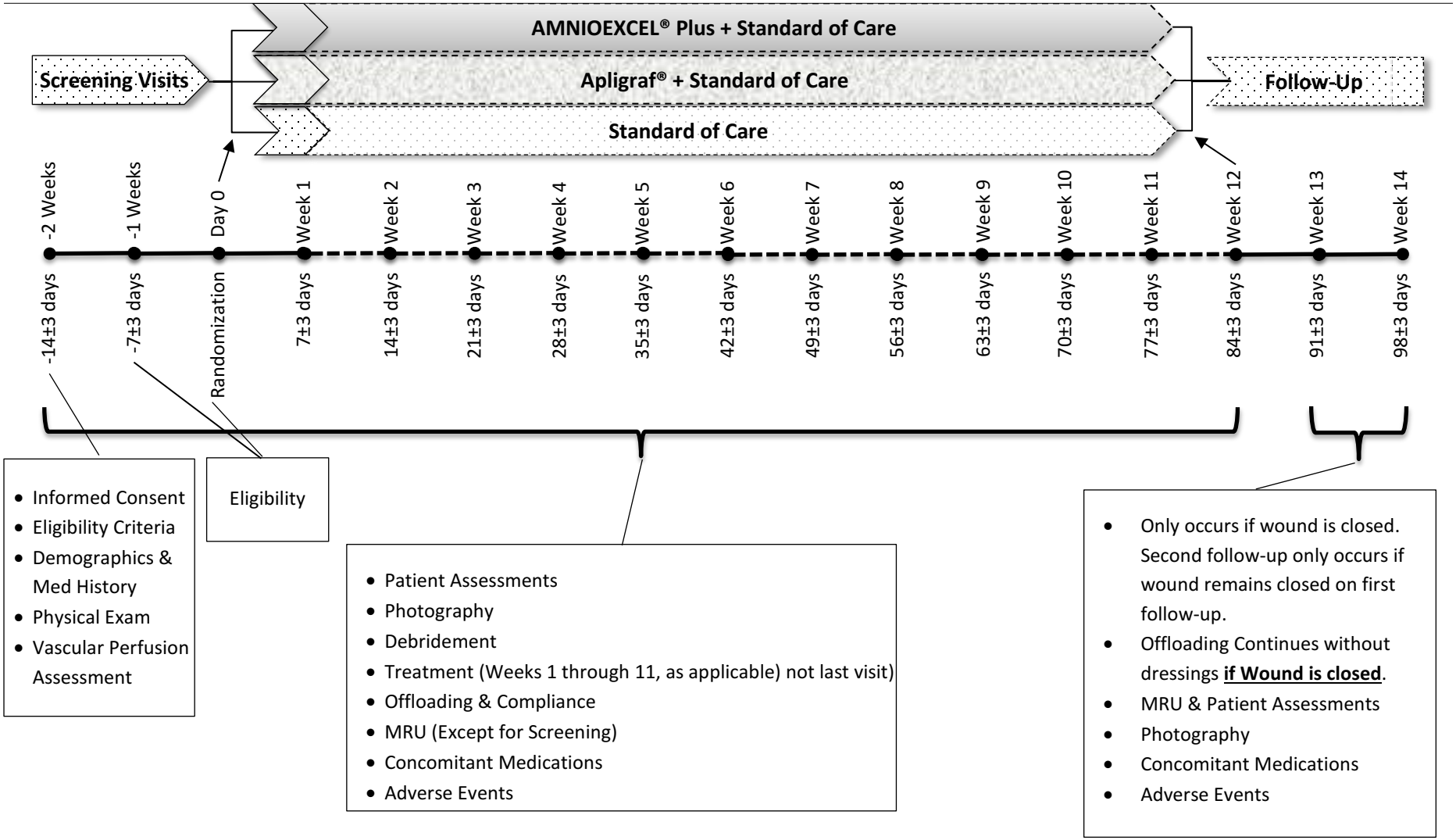

Table 2: Schedule of Activities

	Screening		Treatment													Follow-Up Visits		
Procedures (See Section 8 for details)	Visit 1 -2 Weeks -14±3 days	Visit 2 -1 Week -7±3 days	Random-ization Day 0	Week 1 7±3 days	Week 2[a] 14±3 day	Week 3[a] 21±3 days	Week 4[a] 28±3 days	Week 5[a] 35±3 days	Week 6[a] 42±3 days	Week 7[a] 49±3 days	Week 8[a] 56±3 days	Week 9[a] 63±3 days	Week 10[a] 70±3 days	Week 11[a] 77±3 days	Week 12[a] 84±3 days	Visit 1[b] Week 13 91±3 days	Visit 2[b] Week 14 98±3 days	Unsched. Visit
Inf. Consent & HIPAA	X																	
Eligibility Criteria	X	X[c]	X															
Demographics & Medical History	X																	
Physical Examination	X																	
Vascular Perfusion Assessment	X																	
Treatment Compliance Review	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X
Debridement[d]	X	X	X	X	X	X	X	X	X	X	X	X	X	X				X
Photography	X	X	X	X[e]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Ulcer Assessments[f]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Standard of Care[g]	X	X		X	X	X	X	X	X	X	X	X	X	X				
Boot Application[h]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Randomization			X															
Investigational Prod.[i]			X	X[j]	X	X	X	X	X	X	X	X	X	X				X
MRU Assessment				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ConMeds & Procedures	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Events		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

[a] Optional Visit – Conducted if wound is not closed

[b] Optional Visit – Complete ulcer is closed by Week 12. Follow-up visit 2 only occurs if wound remains closed on Follow-up visit 1. Otherwise subject is exited.

[c] Exclusion based on Ulcer Area not applicable at this visit

[d] If Investigator deems it necessary

[e] If subject has been randomized to Apligraf, imaging may not occur at this timepoint. See sections 8.1.2 or 8.2 for detailed instructions.

[f] Study Ulcer Assessments at 1st visit include: 1) location and 2) duration. At each subsequent visit, ulcer area, area reduction, level and type of exudate, presence of signs/symptoms of infection will be made

[g] Weekly *and* daily dressing changes during Screening; Daily continues *only* if randomized to SOC cohort.

[h] Offloading boot required for ulcers on lateral or bottom part of the foot. Boot may be used as a protective device for those subjects with ulcers on the dorsum of the foot

[i] Investigational Product = AMNIOEXCEL Plus or Apligraf is added to SOC Treatment if applicable per randomization, see also footnote *e*.

[j] If Apligraf being used, non-adherent dressing should be left in place on day 7. See Section 6.1 for more information.

2 INTRODUCTION

2.1 STUDY RATIONALE

AMNIOEXCEL® (Integra LifeSciences Corporation, Plainsboro, NJ) is a commercially available dehydrated amniotic membrane allograft (DAMA) used in advanced wound care. The product is provided in multiple geometric configurations to be applied directly to clean, debrided wounds where bacterial burden and offloading have been addressed.[1] Other advanced wound care therapies, including biological dressings such as Apligraf® (Organogenesis, Canton, MA, USA) and EpiFix® (MiMedx Group Inc., Marietta, GA), have been used in various types of ulcers including the treatment of DFUs.[2,3]

The composition of these advanced wound care allografts varies. Whereas AMNIOEXCEL is a dehydrated amniotic membrane allograft, Apligraf is 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.[4] Another allograft, EpiFix®, is similar to AMNIOEXCEL, but includes the chorion layer in addition to the amnion layer [a dehydrated human amnion/chorion membrane(dHACM)].

Recently, Integra LifeSciences has developed ***AMNIOEXCEL® Plus Placental Allograft Membrane*** (AMNIOEXCEL Plus) which, unlike its predecessor, AMNIOEXCEL, retains the chorion layer of tissue and goes further to add another layer of amnion creating an amnion-chorion-amnion sandwich. The study detailed in this protocol has been designed to assess the use of AMNIOEXCEL Plus in the clinic against a comparator product (Apligraf) and SOC procedures.

2.2 BACKGROUND

Diabetic foot ulcers (DFUs) are a major health complication that will affect up to 15% of individuals with diabetes mellitus over their lifetime. The treatment of DFUs is an extremely challenging scenario, as these ulcers may be recalcitrant to SOC treatments, thus increasing the risk of infection and sequelae such as amputations. It is estimated that approximately 15% of all DFUs will result in a lower extremity amputation and develop concomitant medical complications that are associated with increased mortality rates.[5–8]

DFUs not only have a detrimental effect on a patient's quality of life,[9,10] but also pose a significant burden on healthcare facilities and the public and/or private payers who support these facilities. Waycaster et al. noted a recent economic evaluation of Medicare beneficiaries which concluded that the United States spent a conservative estimate of $32B USD in 2014 on 8.2 million patients.[11] This equates to approximately $4,000 USD per patient.

Human amniotic membrane has been used in the treatment of wounds since the early 20th century.[12] Numerous potential applications of this tissue have been investigated since then. One noted application is for use in patients with DFUs. A recent systematic review and meta-analysis by Laurent et al. which aimed to "identify all relevant studies [using] search terms [such as] 'diabetic foot ulcers' OR 'diabetic foot' AND 'amniotic membrane' OR 'amnion' […] AND 'standard therapy' or 'standard of care'" identified seven (7) randomized controlled trials involving these products.[13] This analysis demonstrated that "[h]uman amnion/chorion membrane + standard of care treatment heals DFUs significantly faster than standard of care alone." Prior to this review, other studies demonstrated that amniotic membranes have anti-inflammatory effects,[12] are antimicrobial,[14] demonstrate anti-scarring, maintain an anti-adhesive

activity,[12,15] are non-immunogenic with low antigenicity,[14] have analgesic properties,[16] and promote re-epithelialization.[14,15,17]

AMNIOEXCEL (AMNIOEXCEL, Integra LifeSciences Corporation, Plainsboro, NJ) is a commercially available amniotic membrane allograft. The product is provided in multiple geometric configurations to be applied directly to clean, debrided wounds where bacterial burden and offloading have been addressed. As one might assume, adequate vascular status and perfusion must also exist for DFUs to heal in a timely and orderly fashion.

In recent months, Integra LifeSciences has begun a limited marketing release of its AMNIOEXCEL Plus allograft which, unlike its predecessor, AMNIOEXCEL, retains the chorion layer of tissue and goes further to add another layer of amnion, thus creating an amnion-chorion-amnion sandwich.

AMNIOEXCEL Plus is processed in compliance with US CFR Title 21 Part 1271 and Section 361 of the Public Health Service Act and regulated as a human cell and tissue products (HCT/P). The base material for these products is collected from live, healthy, planned cesarean section births of appropriately screened women, per American Association of Tissue Banks requirements. The collected placental tissue is then washed, dehydrated, cut, and packaged for commercial distribution.

Another allograft used in the treatment of DFUs is Apligraf (Organogenesis, Canton, MA).[3,18] The Apligraf allograft is a "living cell based product for chronic venous leg ulcers and DFUs. Apligraf is supplied as a living, bi-layered skin substitute."[4] Unlike the AMNIOEXCEL Plus products, the Apligraf product is not derived from amniotic tissue. Apligraf is also a HCT/P; however, it is registered with the FDA as a medical device.

Per the manufacturer's website, "Apligraf consists of living cells and structural proteins. A lower dermal layer combines bovine type 1 collagen and human fibroblasts (dermal cells), which produce additional matrix proteins. The upper epidermal layer is formed by promoting human keratinocytes (epidermal cells) first to multiply and then to differentiate to replicate the architecture of the human epidermis. Apligraf does not contain melanocytes, Langerhans' cells, macrophages, and lymphocytes, or other structures such as blood vessels, hair follicles or sweat glands. Apligraf is supplied as a circular disc approximately 75 mm in diameter and 0.75 mm thick." [4]

This study is designed to compare clinically-relevant outcomes of subjects with DFUs who have been randomized to one of the following treatment cohorts: (1) AMNIOEXCEL Plus in conjunction with SOC procedures; (2) Apligraf in conjunction with SOC procedures; or (3) SOC procedures alone.

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2.3 RISK/BENEFIT ASSESSMENT

The presence of a DFU incurs certain risks. Compared to people with diabetes alone, those with diabetes and a foot ulcer have a 9-fold greater risk for developing an infection, a 7-fold greater risk of amputation, a 3.5-fold greater risk of a fracture and are more than twice as likely to fall.[19] The longer the ulcer is open, the greater the chance for infection and amputation.[20] Thus, the focus of care of the DFU is to close the wound as quickly as possible.

2.3.1 KNOWN POTENTIAL RISKS

AMNIOEXCEL Plus

AMNIOEXCEL Plus is intended for use as a wound covering. This product is an allograft tissue intended for homologous use for the repair, reconstruction, and replacement of skin at the direction of a physician.

AMNIOEXCEL Plus contains trace amounts of ethanol. It should be not used in patients with known sensitivity to ethanol.

In order to reduce the risk of complications, AMNIOEXCEL Plus should not be used in the presence of active infection.

Although donor tissue is evaluated and processed following strict FDA guidelines, the donor screening methods are limited and may not detect all diseases. As with any allograft, complications at the graft site may occur post-operatively (i.e., post-application) that are not readily apparent. These include, but are not limited to:

- Transmission of communicable diseases, including those of unknown etiology
- Transmission of infectious agents such as viruses, bacteria, and fungi
- Immune reaction of, or allergic reaction to, implanted HCT/P

Apligraf

Apligraf is indicated for use with standard DFU care for the treatment of full-thickness neuropathic DFUs 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.

The following are adverse events (AEs) reported in greater than 1.0% of Apligraf patients in a DFU study conducted by the manufacturer.

10-20% of Subjects

- Neuropathic ulcer (non-study site)
- Suspected wound infection (non-study site)
- Non-neuropathic skin alteration (non-study site)
- Suspected wound infection (study site)

5-10% of Subjects

- Cellulitis (non-study site)
- Cellulitis (study site)
- Osteomyelitis (non-study site)
- Vesicular bullous rash (non-study site)
- Pain (overall body
- Fungal infection (non-study site)
- Hypoglycemia
- Infection (overall body
- Hematoma (non-study site)

<5% of Subjects

- Deteriorating ulceration (study site)
- Rash (non-study site)
- Non-neuropathic skin alteration (study site)
- Pain (non-study site)
- Bone dislocation (non-study site)
- Peripheral edema
- Accidental Injury (overall body
- Accidental Injury (non-study site)
- Fever (overall body
- Hyperglycemia
- Dry skin (non-study site)
- Chest pain
- Bronchitis
- Osteomyelitis (study site)
- Nausea
- Pharyngitis
- Anemia
- Right Heart failure
- Abscess (study site)
- Urinary tract infection
  Deteriorating ulceration (non-study site)
- Gastroenteritis
- Cataract
- Abscess (overall body
- Gastritis
- Spontaneous bone fracture
- Diarrhea
- Positive Wound Culture (study site)
- Arthrosis (non-study site)
- Malaise
- Rash (study site)
- Hematoma (study site)
- Gangrene (non-study site)
- Dyspepsia
- Accidental Injury (study site)
- Infection (non-study site)
- Gangrene (study site)
- Spontaneous bone fracture (non-study site)
- Viral infection
- Back pain
- Angina pectoris
- Arteriosclerosis
- Cardiomegaly
- Gastrointestinal carcinoma
- Colitis
- Rhinitis
- Arthritis
- Confusion

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 listed below:

- agarose,
- L-glutamine,
- hydrocortisone,
- human recombinant insulin,
- ethanolamine,
- O-phosphorylethanolamine,
- adenine,
- selenious acid,
- DMEM powder,
- HAM's F-12 powder,
- sodium bicarbonate,
- calcium chloride

Standard of Care (SOC) Treatment

SOC consists of: 1) moist wound dressings, 2) debridement of necrotic/non-viable tissue, 3) infection surveillance and management and 4) offloading of weight bearing from vicinity of the ulcer.

In this study, the moist wound dressings include normal saline gel which is then covered with a non-adhesive foam and secured with conforming roller gauze and dressed with Coban.

The risks of SOC procedures are as follows:

Use of Wrap and Bandages

Tightness - Some subjects may experience a sense of tightness after the study doctor or staff apply the bandages over the treatment area.

Contact Dermatitis - Some subjects may experience a localized skin reaction to the bandages

Use of the offloading boot

Imbalance – Some subjects may have initial or long-term difficulty walking with the offloading boot.

Restriction of activities/movement – Some subjects may experience an inability or reduction in ability to do things that they were once able to do (i.e., wear shoes on the foot, ambulate, etc.)

Debridement Procedure

The risks associated with the debridement procedure are as follows:

- Pain - Most patients will experience pain either during or after the debridement procedure.
- Bleeding – Most patients will experience bleeding of the ulcer after debridement
- Infection - Few patients will develop an infection after debridement
- Delayed Healing - Few patients will develop delayed healing of the ulcer after debridement
- Loss of Healthy Tissue – Few patients will experience removal of healthy tissue in addition to removal of dead tissue.

Reaction to topical or local anesthesia (if used) – Few patients will experience a reaction to topical or local numbing agents used to decrease pain prior to debridement.

Hemoglobin A1c Test (Finger stick or Blood Draw)

The risks associated with the hbA1c procedure are as follows:

- pain
- bruising
- dizziness
- infection

Vascular Perfusion Tests

Risks of Measuring the Ankle-Brachial Index

- Pressure/Discomfort around the arm and/or the leg – Most subjects who have this test performed will experience pressure and discomfort from the blood pressure cuff that is applied to either the leg or arm.
- Bruising – Some subjects may experience bruising from the blood pressure cuff

Risks of Taking Toe Pressure

- Pressure/Discomfort around the big toe – Some subjects who have this test performed will experience pressure and discomfort from the cuff.

Risks of Measuring Transcutaneous Oximetry

- Contact Dermatitis - Some subjects may experience a localized skin reaction to the gel used with the probes that will be applied to the skin for this test.

2.3.2 KNOWN POTENTIAL BENEFITS

A recent meta-analysis noted that some patients who use tissue-based allografts heal sooner than those who undergo SOC procedures alone.[13] However, because this is the first clinical trial using AMNIOEXCEL Plus in the management of DFUs, improved outcome for subjects in this group cannot be guaranteed.

Subjects in this study may undergo more rigorous and/or more frequent treatments compared to their non-study counterparts. This may assist in the entire study population's prognosis, although it cannot be guaranteed.

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3 OBJECTIVES AND ENDPOINTS

Table 3: Objectives and Endpoints

OBJECTIVES	ENDPOINTS
Primary	
The primary objective of this study is to compare outcomes associated with the use of AMNIOEXCEL Plus Placental Allograft Membrane, Apligraf living, bi-layered skin substitute and SOC in the management of DFUs.	The primary endpoint of this study is the incidence of complete wound closure, as assessed by the Investigator at or before Week 12 of the Treatment Phase and confirmed 2 weeks later. Confirmed complete wound closure is defined as complete skin re-epithelialization that is without drainage or dressing requirements.
Secondary	
1. Determine the proportion of subjects in each study arm with complete closure of the study ulcer at or before 12 weeks of treatment	1. Proportion of subjects with complete closure of the study ulcer at or before Week 12 and confirmed 2 weeks later as assessed by computerized planimetry. 2. Proportion of subjects with complete wound closure of the study ulcer at or before Week 12 and confirmed 2 weeks later as assessed by independent, blinded, assessment of photographs.
2. Determine the time to complete wound closure in each study arm	1. Time to complete wound closure, as assessed by the Investigator. 2. Time to complete wound closure, as assessed by computerized planimetry.
3. Determine the rate of wound closure in each study arm	1. Rate of wound closure, as assessed by computerized planimetry.
4. Assess medical resource utilization outcomes in each study arm	1. Medical resource utilization associated with treatment of DFU and related complications as determined by a comparison of estimated costs from the presumed billing codes which would have been used for each patient.
Exploratory	
1. The proportion of subjects in each cohort whose wounds are closed at 4 weeks of treatment.	1. Wound closure status at Week 4 of Treatment
2. The effect of any covariate on the primary endpoint.	1. Co-variate analyses of the primary endpoint using those listed in section 9.3.
Safety Endpoints	
The safety objective of this study is to assess adverse events associated with either of the study treatments and/or all study procedures in the management of DFUs.	Incidence of all treatment, procedure, and/or wound-related adverse events or serious adverse events in any of the study cohorts.

4 STUDY DESIGN

4.1 OVERALL DESIGN

This is a prospective, multicenter, parallel-group, randomized, three-arm trial designed to assess the proportion of ulcers achieving complete wound closure and confirmed 2 weeks later in a population with adequate arterial circulation, following up to 11 weeks of either AMNIOEXCEL Plus Placental Allograft Membrane with SOC, Apligraf bi-layered skin substitute with SOC, or SOC alone.

Subjects will be randomized 1:1:1 to receive one of the following:

- AMNIOEXCEL Plus Placental Allograft Membrane with SOC
- Apligraf bi-layered skin substitute with SOC
- SOC alone

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

Prospective, multicenter, randomized studies provide the most objective methods for analyzing the outcomes of multiple treatment groups. The use of a three-cohort study allows simultaneous comparison of the treatment in question utilizing an active comparator and current SOC, the true baseline.

4.3 JUSTIFICATION FOR INVESTIGATIONAL PRODUCTS/TREATMENTS

While there may be generalizable evidence that all tissue-based allografts outperform SOC therapies in the management of DFUs, the performance of specific products varies. Although there is evidence that AMNIOEXCEL Amniotic Allograft Membrane outperforms SOC procedures[1,21–23], AMNIOEXCEL Plus Placental Allograft Membrane has not been assessed as an advanced wound therapy for DFUs.

The comparator treatment, Apligraf, has been investigated as an advanced treatment for patients with DFUs and has been compared to SOC procedures.[3,18,24]

The use of an offloading boot is a known and widely used SOC adjunctive therapy. Clinical studies have shown that offloading with rigid boots provide a more aggressive healing rate as compared to shoes, custom insoles, wheelchairs, bedrest, and/or crutches.[25] The use of a boot in this clinical trial, in addition to moist wound dressings, will provide the most robust baseline to compare the active treatments (i.e., Investigational products) against.

4.4 END OF STUDY DEFINITION

The study is considered completed after all of the screened and enrolled (i.e., randomized) subjects have completed all of their study treatments and been signed off by the investigative sites in the electronic data capture (EDC) system.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

All of the following criteria must be met by a potential subject to be enrolled in the study. The subject must:

1. Have participated in the informed consent process and signed a study-specific informed consent document.
2. Be able and willing to comply with study procedures, including study visits, study dressing regimens, and compliance with study required offloading device.
3. Be ≥ 21 years of age.
4. Have Type I or Type II diabetes mellitus with Investigator-confirmed glycosylated hemoglobin (HbA1c) of ≤ 12% within 3 months prior to screening visit.
5. Have at least one diabetic foot ulcer that meets ALL of the following criteria:
   - Ulcer has been in existence for a minimum of four weeks prior to signing the Informed Consent Form for trial participation but no more than 12 months
   - Ulcer is a partial or full thickness diabetic foot ulcer without capsule/tendon/bone exposure (Wagner Grade 1 or superficial 2).
   - Ulcer is located on the foot or ankle (with no portion above the top of the malleolus, i.e., proximal to the malleolus).
   - If the subject has more than one ulcer that meets the eligibility criteria, the ulcer designated as the study ulcer will be at the discretion of the Investigator.
   - There is a minimum 1 cm margin between the qualifying study ulcer and any other ulcer on that same foot, post-debridement.
6. Ulcer size (i.e., area) is ≥ 1 cm^2 and ≤ 12 cm^2 post-debridement at Screening Visit 1 and Randomization (Day 0).
7. Have adequate vascular perfusion of the affected limb as defined by **at least one** of the following:
   - Ankle-Brachial Index (ABI) ≥ 0.65 or ≤1.2, performed *within 3 months* of screening visit 1
   - Toe pressure (plethysmography) >50 mmHg *at time* of screening visit 1,
   - $TcPO_2$ >40 mmHg *at time* of screening visit 1
8. Be willing and able (or have a family member/friend willing and able) to apply required applicable dressing changes as well as study-required offloading/protective device if and when applicable.

5.2 EXCLUSION CRITERIA

Potential subjects will not be enrolled in the study *if any of the following criteria are met*:

1. The subject was previously randomized *and* treated under this clinical study protocol.
2. The study ulcer has:
   a. Unexplored tunneling,
   b. Undermining and/or
   c. Sinus tracts

that necessitates surgical operating-room debridement and/or penetrates to capsule/tendon/bone.

3. The subject has a known sensitivity to ethanol.
4. The subject has a known allergy to bovine collagen.
5. The subject has a known hypersensitivity to any of the components of the Apligraf agarose shipping medium listed below:
   - agarose,
   - L-glutamine,
   - hydrocortisone,
   - human recombinant insulin,
   - ethanolamine,
   - O-phosphorylethanolamine,
   - adenine,
   - selenious acid,
   - DMEM powder,
   - HAM's F-12 powder,
   - sodium bicarbonate,
   - calcium chloride
6. The subject has a known sensitivity to any of the SOC materials which come in contact with the skin:
   - Coban™
   - Conforming gauze
   - Optifoam® non-adhesive dressing
   - Normal Saline (liquid or gel)
   - Cotton Gauze
   - Normal saline (liquid & gel)
   - Non-adhering dressings
   - Steristrips
7. The subject is unable to safely ambulate with the use of a study required offloading boot.
8. The subject has suspected or confirmed gangrene or wound infection of the study ulcer, as evidenced by tissue necrosis, redness, pain, and/or purulent drainage and/or receiving systemic antibiotics for the treatment of such.
9. The subject has suspected or confirmed osteomyelitis of the foot with the study ulcer.
10. The subject has participated in another clinical trial involving a device or an investigational study drug/treatment within 28 days of randomization.
11. In the opinion of the Investigator, the subject has received, within 28 days of signing the Informed Consent Form, or is scheduled to receive during study participation, a medication or treatment which is known to interfere with or affect the rate and quality of wound healing (e.g., systemic steroids, immunosuppressive therapy, autoimmune disease therapy, dialysis, radiation therapy to the foot, vascular surgery, angioplasty, or thrombolysis).
12. The subject has a history of bone cancer or metastatic disease of the affected limb, radiation therapy to the foot, or has had chemotherapy within the 12 months prior to signing the Informed Consent Form for trial participation.
13. The subject is currently pregnant or is actively trying to conceive. (Patient statement confirming lack of pregnancy is sufficient.)

14. In the opinion of the Investigator, the subject is unable to comply with the treatment regimen (i.e., return for clinic visits, perform required dressing changes and use study-designated offloading device[s]).
15. In the opinion of the Investigator, the subject has a history of, or is currently diagnosed with, any illness or condition, other than diabetes, that could interfere with wound healing (e.g., end-stage renal disease, severe malnutrition, liver disease, aplastic anemia, Raynaud's Syndrome, connective tissue disorder, acquired immune deficiency syndrome, HIV positive, or sickle cell anemia).
16. In the opinion of the Investigator, the subject has unstable Charcot foot or Charcot with bony prominence that could inhibit wound healing.
17. The subject has ulcers secondary to a disease other than diabetes (e.g., vasculitis, neoplasms, or hematological disorders).
18. In the opinion of the Investigator, the subject has excessive lymphedema that could interfere with offloading and/or wound healing.
19. The study ulcer has received wound dressings that include growth factors, engineered tissues, or skin substitutes within 28 days of randomization or is scheduled to receive treatment during the study (e.g., PriMatrix, AMNIOEXCEL (first generation), Regranex, Dermagraft, EpiFix, GraftJacket, OASIS, Omnigraft, or Integra BMWD).
20. The study ulcer closed more than 30% in area, *post-debridement*, between Screening Visit 1 and Randomization (Day 0, as measured by planimetry).

5.3 LIFESTYLE CONSIDERATIONS

Similar to patients experiencing SOC procedures outside of this study, subjects in this study will have to:

- Wear an offloading boot. This may cause subjects to ambulate with a limp or may reduce the subject's ability to perform activities they are used to doing (e.g., walk upstairs, go outside, etc.)
- Change dressings on a daily basis when in the Screening Period and if randomized to the SOC cohort. This may add time to a subject's daily grooming regimen.

All of these activities are similar to treatments/procedures that occur outside of this study and would occur whether or not this subject was included in the study.

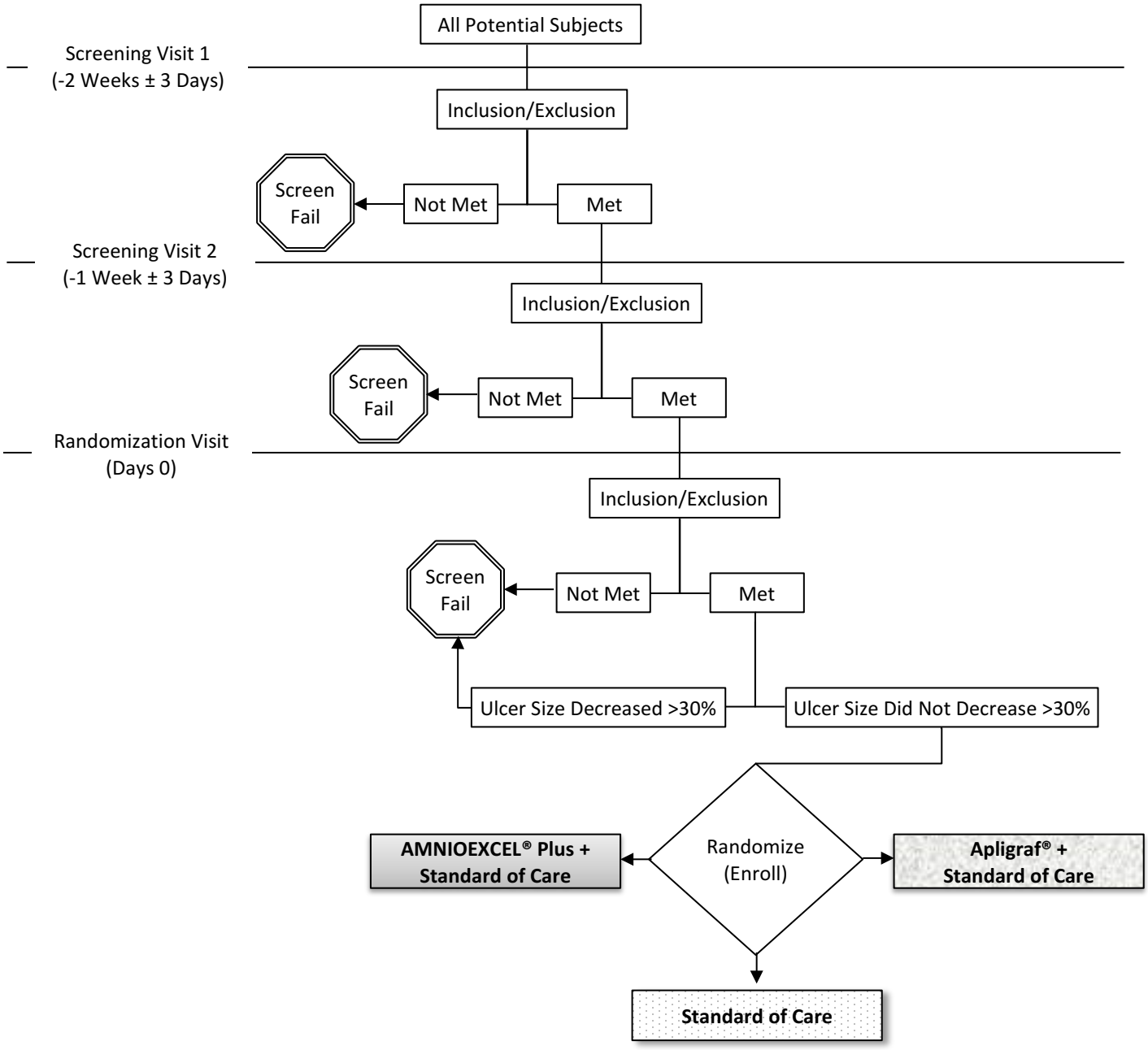
5.4 SCREEN FAILURES AND RESCREENING

Screen failures are defined as subjects who consent to participate in the clinical trial but are not subsequently randomized to a study intervention. A minimal set of information is required to ensure transparent reporting of screen failure subjects, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse events (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) because of inability to attend second screening visit, recent participation in a former clinical trial, or remission of exclusionary cancer status as described in the exclusion criteria may be rescreened once. Rescreened subjects will be assigned a different subject number from the one designated at the initial screening.

The enrollment and screening flow chart of activities can be seen in figure 2. NOTE: Patients who enter the Screening Period will also be designated as a screen fail if they withdraw consent.

Figure 2: Screening Flow of Activities



5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

RECRUITMENT

Pre-screening activities for this study should include identification of potential subjects that meet the following general criteria.

- Age ≥ 21 years,
- a diagnosis of diabetes and
- presence of DFU.

Note: pre-screening activities should never include the collection of study-related information or protected health information (PHI).

Advertisements are not prescribed for this study, per se; however, Integra may approve site-specific advertisements if warranted. Any site-specific advertisements must be approved by both the Sponsor and the site's Institutional Review Board prior to distribution to outside parties.

RETENTION

Investigators and site staff should make every effort to retain enrolled subjects and reduce loss-to-follow-up. The following methods should be employed in order to prevent subject loss:

- Ensure complete understanding of the roles and responsibilities of the study subject. This can be achieved with a thorough informed consent process.
- Make follow-up appointments far in advance
- Develop a strong rapport with study subjects, offer flexible appointment times, and ensure strong PI involvement

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6 STUDY INTERVENTION

6.1 STUDY INTERVENTIONS ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTIONS

6.1.1.1 INVESTIGATIONAL PRODUCTS (to be used with SOC products)

AMNIOEXCEL Plus

AMNIOEXCEL Plus tri-layer Placental Allograft Membrane (T-PAM) is a minimally manipulated placental membrane product made from tissues donated by pre-screened mothers during planned C-sections. AMNIOEXCEL Plus is intended for use as a wound covering. This product is an allograft tissue intended for homologous use for the repair, reconstruction, and replacement of skin at the direction of a physician.

More information can be found in the supplied Instructions for Use document that is included with the AMNIOEXCEL Plus product.

In this study, AMNIOEXCEL Plus will be applied weekly, in conjunction with SOC, as long as the ulcer remains open, or up to and including Week 11, whichever comes first. Note: In order to reduce the risk of complications, AMNIOEXCEL Plus should not be used in the presence of active infection. See Section 7.1 for instructions on treating infections as part of this trial.

Apligraf

Apligraf is 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.

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. 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, isoenzymes, and tumorigenicity. The final product is tested for morphology, cell viability, epidermal coverage, sterility, mycoplasma, 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).

Apligraf is also indicated for use with standard DFU care for the treatment of full-thickness neuropathic DFUs 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.

More information can be found in the supplied Prescribing Information that is included with the Apligraf product.

In this study, Apligraf will be applied weekly, in conjunction with SOC, as long as the ulcer remains open, or up to and including Week 11, whichever comes first. Note: In order to reduce the risk of complications, Apligraf should not be used in the presence of active infection. See Section 7.1 for instructions on treating infections as part of this trial.

CONTROL PRODUCTS (i.e., SOC)

The control treatment reflects SOC appliances and materials:

- Coban™
- Conforming gauze
- Optifoam® non-adhesive dressing
- Cotton Gauze
- Normal saline (liquid or gel)
- Non-adhering dressings
- Steristrips
- An offloading boot (Pneumatic Short Leg Walker), as appropriate

6.1.2 UTILIZATION AND ADMINISTRATION

IMPORTANT: These recommendations are designed only to serve as a general guideline. They are not intended to supersede institutional protocol or professional clinical judgement concerning patient care.

6.1.2.1 RECOMMENDED INSTRUCTIONS FOR USE OF THE PNEUMATIC SHORT LEG WALKERS (BOOT) & SUPPLIES (SOC DRESSINGS)

During the Screening Phase of the study, all subjects will have SOC products applied by the investigative site at the weekly visits. They will be then instructed on how to change their dressings on a daily basis. After randomization, only subjects who are randomized to the SOC cohort will continue daily dressing changes. Those subjects in either the AMNIOEXCEL Plus or Apligraf cohorts will only have their dressings changed weekly at follow-up visits until the investigator determines that their wound has closed. If the wound closes on or before week

12, offloading (if previously utilized for that subject) will continue *without* dressings during the follow-up period.

The off-loading boot/protective device and SOC dressings to be used in this study will be provided by the Sponsor through a specific distributor. The off-loading boot/protective device will only be used by subjects who require offloading throughout the Screening and Treatment (i.e., those subjects with study ulcers located on the plantar or lateral surfaces of the foot or any location experiencing weight-bearing or shear forces *as determined by the Investigator*). These subjects will wear the boot up to their exit from the study. The boot will be applied to the subject's foot on top of the dressings.

Application of the dressings & boot by the **Investigator**:

1. Gently remove any dressing previously applied to the wound and clean the area with a gentle cleanser.
2. If appropriate, debridement and hemostasis will be performed. (Note: in this study only sharp debridement will be used)
3. During Screening and for SOC-randomized subjects: Instruct the subject on the proper way to change dressing
   a. Inspect the wound and perform study assessments as directed elsewhere
4. Apply normal saline gel to the thickness of a coin.
5. Cover the gel with a foam dressing.
6. Secure the foam dressing with conforming roller gauze.
7. Cover the roller gauze with Coban.
8. For subjects who have ulcers that require offloading, apply the boot.

Daily Changing of the Dressings by the **Subject**:

1. Remove boot, if applicable
2. Gently remove dressings
3. Clean the wound with tap water and pat the area dry
4. Inspect the wound
   a. Subject will contact the site by phone if they believe there is anything wrong with the wound.
5. Apply normal saline gel to the thickness of a coin.
6. Cover the gel with a foam dressing.
7. Secure the dressings with conforming roller gauze.
8. Cover the roller gauze with Coban.
9. For subjects who have ulcers that require offloading, apply the boot.

Note: Subjects who use the offloading boot will be instructed that it is to be worn **at all times** except when bathing or sleeping.

6.1.2.2 RECOMMENDED INSTRUCTIONS FOR USE OF AMNIOEXCEL PLUS

Preparation Instructions (also found in the product's Instructions for Use supplied with the product):

1. Open carton or box containing AMNIOEXCEL Plus and remove the peel-pack.
2. Peel open the outer package and remove the inner foil pouch using aseptic technique. *Note: The inner foil pouch and its contents are sterile and may be placed directly into the sterile field.*
3. Peel the inner pouch open and place the AMNIOEXCEL Plus into the sterile field. *Note: Application of AMNIOEXCEL Plus will be easier if the surface to be covered is relatively dry*.
4. Either side of the membrane may be applied to the wound surface.
5. It is sometimes necessary to gently "roll" the AMNIOEXCEL Plus at the edges to smooth out wrinkles and folds that can occur during placement.
6. Secure the AMNIOEXCEL Plus, when necessary, using the physician's choice of fixation (Steri-strips are provided).
7. Cover the AMNIOEXCEL Plus product with the non-adherent dressing
8. Optional: A saline-moistened bolster dressing is applied to ensure full contact with the wound bed.
9. Proceed with SOC dressings as described earlier.

Additional training will be supplied by an Integra team member either via onsite training or video conference. *Important note: Subject records must be maintained for the purpose of tracking tissue post-transplant per The Joint Commission on Accreditation of Healthcare Organizations and FDA requirements. The AMNIOEXCEL Plus ID number must be recorded in the recipient's medical record. Patient labels which include tissue numbers are contained in the AMNIOEXCEL Plus package to aid in the tracking process.*

In this study, the AMNIOEXCEL Plus will be covered with a non-adherent dressing and a saline moistened bolster applied, when appropriate, to ensure that the AMNIOEXCEL Plus is maintained with intimate contact to the wound bed. A foam dressing will be applied to cover the bolster and the dressings secured with conforming roller gauze. Finally, a cover dressing of Coban will be applied.

The dressing should be kept dry and will be changed once weekly during study visits. The subject will be instructed on the signs that indicate a need to seek medical attention.

6.1.2.3 RECOMMENDED INSTRUCTIONS FOR USE OF APLIGRAF[26]

More information about application of the product can be found on manufacturer's website: http://www.apligraf.com/professional/what_is_apligraf/how_is_it_applied/index.html

1. Wound Bed Preparation:
   - When applying Apligraf there should be no signs of infection (see Section 7.1 for subjects who become infected during the course of the study).
   - The wound bed should be prepared with appropriate method of debridement
2. Placement of the Apligraf®

- If desired, fenestrate Apligraf® with a knife blade or mesh (1.5-1 ratio). Apply Apligraf to wound and affix with method of preference i.e., Steri-Strips™, wound glue at periphery, or sutures

3. Primary Dressing
   - Cover Apligraf® with primary non-adherent dressing
4. Secondary Dressing
   - Apply appropriate dressing determined by wound type. A non-cytotoxic antimicrobial may also be applied at this time.
   - Provide Offloading for DFU
   - Keep dressing dry until first follow-up appointment.
5. First Follow-up
   - At Week 1 follow up, remove secondary dressing only for wound inspection and evaluation. Primary dressing (non-adherent) should be left undisturbed to facilitate integration of Apligraf®.
6. Application of Secondary Dressing
   - Reapply appropriate secondary dressings
7. Subsequent Follow-ups
   - On second follow up (14 days, Week 2), remove secondary and primary dressing for wound inspection and evaluation. Do not disturb or debride the wound bed. Perform light normal saline wash
8. Application of Primary and Secondary Dressings
   - Apply new primary (shown) and secondary dressings
   - On subsequent weekly follow-ups, continue to change primary and secondary dressings to monitor wound healing.
   - Apligraf may be reapplied if needed until wound healing is complete, as shown.

   Additionally, the site should refer to Apligraf's Instructions for Use documents that are supplied with the product and/or consult an Apligraf representative for appropriate training.

6.2 HANDLING/STORAGE

AMNIOEXCEL PLUS

AMNIOEXCEL Plus Placental Allograft Membrane is provided in prescribed multiple geometric configurations. AMNIOEXCEL Plus Membrane is dehydrated during processing and should be visibly dry when the package is opened. The inner peel pouch and tissue product are terminally sterilized via E-beam irradiation and may be placed directly into the sterile field. Included in the packaging along with this insert are a Tracing Record and a set of patient labels.

- AMNIOEXCEL Plus is sterile and packaged for single patient, one time use only.
- Once opened, AMNIOEXCEL Plus must be used immediately or discarded.

AMNIOEXCEL Plus is to be stored in a clean, dry location at room temperature.

APLIGRAF

The site should refer to Apligraf's Instructions for Use documents that are supplied with the product and/or consult an Apligraf representative for information on appropriate handling and storage of the product.

CONTROL/STANDARD OF CARE SUPPLIES

All supplies for the SOC procedures can be kept at room temperature. General adherence to product expiration dates should be undertaken.

6.2.1 ACQUISITION AND ACCOUNTABILITY

Sites will be supplied by Integra with AMNIOEXCEL Plus and the SOC materials (i.e., boot, gauze, Coban, etc.) to be used with each of the investigational products. Specifically, sites will be provided with:

- Daily dressing kits used at home by all subjects during the Screening Phase of the study and if the subject is subsequently randomized to the SOC cohort.
- Weekly dressing kits used by the investigator on all subjects during the Treatment Phase of the study.

Important note: Subject records must be maintained for the purpose of tracking tissue post-transplant per The Joint Commission on Accreditation of Healthcare Organizations and FDA requirements. The AMNIOEXCEL Plus ID number must be recorded in the recipient's medical record. Patient labels which include tissue numbers are contained in the AMNIOEXCEL Plus package to aid in the tracking process.

Sites will purchase Apligraf from the manufacturer and order for arrival the day prior to the projected randomization date for every subject in the event that the subject is randomized to the Apligraf product. This purchase will be reimbursed by Integra.

Sites will document the use of all study products in the subject's medical records, in the required electronic case report forms (eCRFs), any applicable study logs.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

This study will use randomization to minimize bias in this open label study. Randomization will occur in a 1:1:1 ratio and use a mixed block methodology. No blinding is possible. As well, no analytical blinding will occur during data review and/or analysis.

6.4 STUDY INTERVENTION COMPLIANCE

Subject compliance will be assessed by the site via standard didactic and interview methods. An initial training on dressing changes and application of the boot with the subject will occur on Screening Visit 1 (-12 weeks ± 3 days). Compliance and subsequent (i.e., refresher) training will occur on each follow-up visit until the patient completes the study. This is shown in detail in the Table 2: Schedule of Activities.

6.5 CONCOMITANT THERAPY

Local Anesthesia (as required)

Debridement will be performed when appropriate on any study visit, at the discretion of the investigator. Debridement involves the use of a scalpel or other sharp implement to remove all dead and dying tissue on and around the ulcer. Debridement may involve the use of local anesthetics to reduce the possibility of pain the subject may feel during this procedure. The use of topical anesthetics is forbidden in this study.

All anesthetics should be documented on the Concomitant Medications eCRF.

NO other therapies such as devices, grafts, or medications which are **intended to improve wound healing** are permitted to be used on enrolled subjects.

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7 STUDY INTERVENTION DISCONTINUATION AND SUBJECT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

For Subjects Who Develop an Infection:

Subjects who, during the course of the Treatment Phase, develop clinical signs and/or symptoms of infection (e.g., erythema, edema, pain, inflammation, increased drainage etc.) and in the opinion of the Investigator, require oral antibiotic therapy may receive a single course of oral antibiotics during the Treatment Phase of the study. **Should a second consecutive course be required/desired, the subject will be exited from the study.** This antibiotic therapy will be noted on the Concomitant Medications eCRF.

In addition to the above, for those subjects receiving Apligraf or AMNIOEXCEL Plus and develop signs/symptoms of infection, application of those allografts will be withheld on that visit. Application of allografts may be withheld for no more than 1 week at a time and no more than twice (non-consecutive visits) during the 12-week Treatment Phase. If signs and symptoms of infection requiring antibiotic therapy persist for more than one visit, or appear on more than 2 non-consecutive visits during the Treatment Phase, the subject will be discontinued from study participation.

For Subjects Whose Wound Closes:

Discontinuation from treatment in any of the study cohorts does not mean discontinuation from the study. There is one scenario in which a subject may continue in the study after intervention is final:

> After treatment with AMNIOEXCEL Plus, Apligraf, and/or SOC **AND** if the subject's wound is documented as closed at or before week 12, the subject will continue to be monitored for two weeks to confirm wound closure. This is noted as week 13 and 14 in the Schedule of Activities, above, but may occur earlier.

If the subject's wound has NOT closed by week 12, the subject will be considered a study failure and will not have any further study visits.

7.2 SUBJECT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Subjects are free to withdraw from participation in the study at any time upon request. As well, an investigator may discontinue or withdraw a subject from the study for the following reasons:

- If the subject's wound is open at follow-up week 1, the subject will be exited. They will be noted as having completed their study requirements.
- Significant study intervention non-compliance
- If any adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the subject

- Anytime continued participation in the study is not in the best interest of the subject
- Disease progression which requires discontinuation of study participation
- Signs/symptoms of infection which require antibiotic therapy that persist more than 1 visit at a time or more than twice (non-consecutive visits) during the 12-week Treatment Phase.
- If the subject meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation
- If the subject's site withdraws from the study
- If the Sponsor decides to stop the study

The reason for subject discontinuation or withdrawal from the study will be recorded on the appropriate eCRF. Subjects who sign the informed consent form ***and*** are randomized but do not receive the study intervention may be replaced.

7.3 LOST TO FOLLOW-UP

If a subject does not return for a study-related visit, the subject is not automatically considered lost-to-follow-up (LTFU). The site should proceed with the following prior to designating a subject as LTFU:

1. Contact the subject via phone
    a. Use the primary phone; if the subject's phone has been disconnected utilize any other phone numbers ever provided.
    b. Contact the subject's Primary Healthcare Physician for any contact information
2. Contact the subject via certified mail
    a. Send a certified letter to the subject's most recent address.

Only after the subject fails to respond to the certified letter may the subject be considered LTFU.

Subjects who are discontinued, lost-to-follow-up or withdrawn and who have not achieved complete wound closure by week 12 of the Treatment Phase of the study will not be replaced.

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8 STUDY ASSESSMENTS AND PROCEDURES

8.1 STUDY PROCEDURE OVERVIEW

The below list is an overview of procedures or information to document at each visit. Where added, information in parentheses relate to the procedures listed in Table 2: Schedule of Activities at the beginning of the protocol. The detail about these procedures is found in the following section.

8.1.1 SCREENING VISIT 1 (-2 WEEKS [14 DAYS] ± 3 DAYS)

1. Conduct Informed Consent Process (Informed Consent & HIPAA)
2. Review Eligibility Criteria (Inclusion/Exclusion)
3. Collect Demographics & Medical History from Patient
4. Perform Physical Examination
5. Perform Vascular Perfusion Assessment (if most recent ABI is greater than 3 months old or no assessment is present in subject's medical records)
   a. ABI, Toe Pressure, **or** $TcPO_2$ (ABI is preferred)
6. Discuss Requirements for Study Compliance (Treatment Compliance Review)
7. Perform Sharp Debridement on Study Ulcer, if needed (Debridement)
8. Collect Images of the DFU (Photography)
9. Perform Ulcer Assessments
10. Treat Subject Using SOC Procedures (Standard of Care)
11. Train subject on wound cleansing and dressing changes
12. Apply Boot, if applicable (Boot Application)
13. Document Concomitant Medications & Procedures (ConMeds and Other Procedures)

8.1.2 SCREENING VISIT 2 (-1 WEEKS [7 DAYS] ± 3 DAYS)

1. Review Eligibility Criteria (Inclusion/Exclusion)
2. Review Study Compliance with the Subject (Treatment Compliance Review)
3. Perform Sharp Debridement on Study Ulcer, if needed (Debridement)
4. Collect Images of the DFU (Photography)
5. Perform Ulcer Assessments
6. Treat Subject Using SOC Procedures (Standard of Care)
7. Apply Boot, if applicable (Boot Application)
8. Document Adverse Events
9. Document Concomitant Medications & Procedures (ConMeds and Other Procedures)

8.1.3 RANDOMIZATION VISIT (DAY 0) – POINT OF ENROLLMENT

1. Review Eligibility Criteria (Inclusion/Exclusion)
2. Review Study Compliance with the Subject (Treatment Compliance Review)
3. Perform Sharp Debridement on Study Ulcer, if needed (Debridement)
4. Collect Images of the DFU (Photography)
5. Perform Ulcer Assessments
6. Randomize Subject (Randomization)

7. Treat per Randomization Group (SOC + Treatment)
    a. AMNIOEXCEL Plus Cohort (Discontinue *Daily* Dressing Regimen)
    b. Apligraf Cohort (Discontinue *Daily* Dressing Regimen)
    c. Standard of Care Cohort
8. Apply Boot, if applicable
9. Train subject on maintenance of dressing and when to call/return to the study site
10. Document Adverse Events
11. Document Concomitant Medications & Procedures (ConMeds and Other Procedures)

8.1.4 WEEK 1 (7 ± 3 DAYS) THROUGH WEEK 11 (77 ± 3 DAYS); UNSCHEDULED VISITS

1. Review Study Compliance with the Subject (Treatment Compliance Review)
2. Collect MRU Data for the Previous Week (MRU Assessment)
3. Perform Sharp Debridement on Study Ulcer, if needed (Debridement)
4. Collect Images of the DFU (Photography)
    a. Important: If the subject has been randomized to the Apligraf cohort, the non-adherent layer should not be removed at the Week 1 timepoint (See section 6.1.2.3 RECOMMENDED INSTRUCTIONS FOR USE OF APLIGRAF). Sites do not need to image the wound at Week 1 if the subject is in the Apligraf cohort; however, if the non-adherent layer disengages from the wound <u>of its own volition,</u> imaging may occur. In this latter case, care should be taken to not disturb the Apligraf.
5. Perform Ulcer Assessments
6. Treat per Randomization Group (SOC + Treatment)
    a. AMNIOEXCEL Plus Cohort (Weekly Dressing Changes, Only)
    b. Apligraf Cohort (Weekly Dressing Changes, Only)
    c. Standard of Care Cohort
7. Apply Boot, if applicable
8. Document Adverse Events
9. Document Concomitant Medications & Procedures (ConMeds and Other Procedures)

8.1.5 WEEK 12 (84 ± 3 DAYS)

1. Review Study Compliance with the Subject (Treatment Compliance Review)
2. Collect MRU Data for the Previous Week (MRU Assessment)
3. Collect Images of the DFU (Photography)
4. Perform Ulcer Assessments
5. Apply Boot, if applicable
6. Document Adverse Events
7. Document Concomitant Medications & Procedures (ConMeds and Other Procedures)

8.1.6 FOLLOW-UP VISITS - WEEKS 13 & 14 (91 & 98 ± 3 DAYS)

1. Collect MRU Data for the Previous Week (MRU Assessment)
2. Collect Images of the DFU (Photography)
3. Perform Ulcer Assessments
4. Apply Boot, if applicable
5. Document Adverse Events

6. Document Concomitant Medications & Procedures (ConMeds and Other Procedures)

IMPORTANT: If the subject's wound is open at Follow-up visit 1 (e.g., week 13), the subject is exited from the study and is marked as having completed their study requirements. The site will note why they believe the wound is still open.

8.2 ASSESSMENTS

The following assessments will occur for each subject consented into the study according to the Study Procedure Overview above:

- **Informed Consent (Screening Visit 1) – ALWAYS THE FIRST ACTIVITY**
  Please see the section dedicated to the informed consent process in section 10.1.1 of this document.
- **Eligibility (i.e., Inclusion/Exclusion; Screening Visits 1 and 2 and Randomization [Day 0])**
  The Investigator will review medical records and query the subject as needed to determine if the subject meets the inclusion and exclusion criteria for the study. Inclusion/exclusion review will be complete on Randomization (Day 0) after the Investigator reviews the amount of wound closure after debridement. The site will enter whether the subject meets each applicable criterion into the EDC system.
- **Demographics & Medical History (Screening Visit 1)**
  The site will collect the subject's date of birth, gender, ethnicity, race, and will assess nicotine use. Diabetic history will be noted. Diabetes type, date of diagnosis, date of most recent HbA1c test, and %HbA1c will be documented. A separate HbA1c test does not need to occur unless the subject's most recent test is greater than 3-months (90 days) from the date of Screening Visit 1. Documentation of other medical conditions will be made based on body systems (e.g., cardiovascular, dermatologic, endocrine, gastrointestinal, etc.).
- **Physical examination (Screening Visit 1)**
  Height and weight, along with blood pressure, will be documented. Sites will document any abnormalities in any of the following: extremities, neurologic, cardiovascular, musculoskeletal, dermatological and/or other systems. A vascular perfusion assessment (either the ABI, systolic pressure of the great toe, or $TcPO_2$) will be noted. Sites will document whether or not the subject has multiple DFUs.
  - **Vascular Perfusion Assessment (Screening Visit 1, if necessary)**
    Perform one of the following:
    - **ABI (Preferred Assessment)**
      The ABI is the ratio of the brachial pressure (i.e., blood pressure in the arm) to the blood pressure in the ankle. Briefly, the blood pressure is obtained in the arm (brachial) and then obtained in a similar manner in the ankle. To obtain the ratio (i.e., index), divide the systolic pressure from the ankle by that obtained in the arm.

$$ABI = \frac{Systolic\ Pressure_{ankle}}{Systolic\ Pressure_{arm}}$$

      Subjects enrolled in this study must have an ABI of between 0.65 and 1.2. NOTE: An ABI result obtained within 3 months of the first screening visit may be used to qualify the potential subject for participation in this study.

- **Toe Pressure**
  The blood pressure within the toe is measured in much the same way as obtaining blood pressure in the arm. Briefly, use a Doppler probe to determine that a pulse is present at the base of the great toe. Apply the correct sized cuff to the great toe and place the Doppler probe distal to the cuff (the pulse should be audible). Inflate the cuff sufficiently to occlude the pulse and slowly release the cuff. Note the pressure, in mmHg, when the pulse returns. To participate in this study, the potential subject must have a toe pressure greater than 50mmHg in the foot with the study ulcer.
- **Transcutaneous Oxygen Level** ($TcPO_2$)
  $TcPO_2$ is a measure of oxygen delivery to a local area. A special machine must be used to assess $TcPO_2$. Briefly, electrodes for the sensor will be placed onto the intact skin close to the study ulcer. The oxygen sensor then can read the oxygen content of the tissue and reports this as mmHg pressure. Each site should refer to the equipment's manual for specific and correct instructions for use. For this study, potential study candidates must have a $TcPO_2$ greater than 40 mmHg.

- **Treatment Compliance Review (Screening Visit 1 & 2, Randomization, Weeks 1 - 12)**
  Screening Visits 1 &2: The site will instruct the subject on how to perform daily dressing changes and apply the offloading boot, if offloading is used.

  Randomization Visit: For subjects randomized to the SOC cohort, the site will instruct the subject on how to perform daily dressing changes and apply the offloading boot, if offloading is used. For those subjects randomized to the AMNIOEXCEL Plus or Apligraf cohorts, only the offloading boot will be discussed, if applicable. Subjects in these arms do not change their dressings at home.

  On subsequent visits, the site will ask the subject whether they are performing the dressing changes and/or offloading, as applicable. To appropriately assess compliance, the site will ask the subject if they are wearing their boot at all times except when bathing or sleeping. Further, when the offloading boot is removed, the site will examine the exterior of the boot for signs of wear and the interior for evidence of depressions in the boot liner. The site will document their assessment of compliance on the appropriate eCRF. *Note: The site should replace worn liners as necessary.*

- **Debridement (Screening Visit 1 & 2, Randomization, Weeks 1 - 11)**
  Debridement is the removal of dead, damaged, or devitalized tissue to improve the healing potential of the remaining healthy tissue. Removal may be sharp, mechanical, chemical, or autolytic (self-digestion); however, in this study only sharp debridement will be used.

  Following debridement, hemostasis should be obtained with pressure. The use of silver nitrate sticks, styptic pencils or electro-cautery should be avoided as these coagulation methods induce further tissue damage.

- **Photography (All Study Visits)**
  For this study, imaging software created by Tissue Analytics, Inc. will be used to collect images of the subjects' ulcers.

Using the app provided by Tissue Analytics, Inc., sites will take an image of the study ulcer. These images will be used to: 1) document the status of the wound and 2) use planimetry to calculate the area of the open wound. Photographs of the wound will be taken after the dressings are removed, the wound is cleansed, debrided, and hemostasis has been achieved, if debridement is performed. The app will automatically measure the wound area; the site does not need to enter this information into the EDC system.

The site will use the imaging methodology set forth in the current imaging vendor's procedure manual. Imaging should be conducted in such a manner as to eliminate (to the best of the site's ability) any identifying information such as tattoos, birthmarks, etc.

Important: If the subject has been randomized to the Apligraf cohort, the non-adherent layer should not be removed at Week 1 (See section 6.1.2.3 RECOMMENDED INSTRUCTIONS FOR USE OF APLIGRAF). Sites do not need to image the wound at Week 1 if the subject is in the Apligraf cohort; however, if the non-adherent layer disengages from the wound of its own volition, imaging may occur. In this latter case, care should be taken to not disturb the Apligraf.

- **Ulcer Assessments**
  - **Screening Visits 1 &2**
    The location of the ulcer will be noted. Extent of ulceration (i.e., tunneling, tendon exposure, etc.), exudate assessment, and infection assessment. **Ulcer area will always be assessed using the post-debridement (or pre-debridement if no debridement was performed) photographs.**

  - **All Study Visits following Screening Visits 1 & 2**
    Exudate assessment, infection assessment, and investigator assessment of wound closure will be collected.

  - **Completion of Study Assessment (Follow Up Visits)**
    Sites will assess completion status of the subject and will make a determination about the status of the wound (closed or not).

- **Standard of Care, Offloading (Screening Visits 1 & 2, Randomization, Weeks 1-11 as applicable)**
  Sites will apply SOC materials and boots according to the procedures laid out in section 6.1.2 and will document in the EDC system what materials were used. The site will document that a weekly kit was used and that they provided materials and training to the subject as applicable.

- **Randomization (Day 0)**
  Sites will review eligibility criteria a final time and document that the subject has met these criteria in the EDC system. The EDC system will provide the site with the appropriate cohort based on a pre-determined randomization scheme. The site will proceed with applying the appropriate treatment products based on the mandated cohort for that subject.

- **Investigational Product, Offloading (Randomization, Weeks 1-11, as applicable)**
  Sites will apply investigational product and SOC materials and boots, if applicable, according to the procedures laid out in section 6.1.2 and will document in the EDC system what materials were

used. Sites will document the product used (AMNIOEXCEL Plus or Apligraf), the product's lot number, and method of securing. The site will also document that a weekly kit was used, that they provided materials and training to the subject as applicable.

- **MRU Assessment (Weeks 1 – 12, Follow-Up Weeks 1 & 2)**
  As applicable, the site will document the following for the subject's previous visit week:
  - Any and all DRG Codes, using ICD-10, that were or would be applied to the subject
  - The associated CPT codes and modifiers for those CPT codes

- **Concomitant Medications (All Study Visits)**
  Sites will query the subjects at each visit about any medications they are currently taking. Only medications that (1) affect the wound area, (2) affect the limb, (3) are related to the treatment of the wound, (4) are being used to treat any AEs that develop during this study, will be documented. The name, indication/reason for use, dose, route, frequency, start/stop date, and whether the medication is being used to treat any adverse events will be noted.

- **Assessment of Adverse Events (All Study Visits after Screening Visit 1)**
  After the initial screening visit, sites will report full information on those AEs possibly, probably, or definitely-related to the investigational product or study procedure. Other AEs that are not related or unlikely related will only have basic information collected (Stop and Start Date). If these AEs change in relationship (e.g., an ongoing unrelated AE becomes possibly related) full information will be collected.

  Sites will collect the AE onset date and a description of the event. The sites will then make a determination as to whether the AE is Serious or not and will grade its severity marking it as either (1) mild, (2) moderate, or (3) severe. For any treatment or procedure-related SAEs that are graded as (1) possibly, (2) probably or (3) definitely-related, the site will also indicate whether the event is anticipated or unanticipated. Definitions of these terms can be found in the following section of the protocol.

  Sites will also note the action taken (none, medication given, hospitalization required, surgical intervention, or other), and the outcome of the AE (resolved with or without sequelae, death, ongoing, or other). If available, an adverse event resolution date will be noted. The site will also make a determination as to whether the AE caused the subject to discontinue participation in the study.

  See Section 8.3 below for more information on AE definitions and procedures.

8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.3.1 DEFINITION OF ADVERSE EVENT (AE)

For the purposes of this research protocol, an **adverse event** means any untoward medical occurrence or adverse reaction (AR) associated with the use of an intervention in humans, that is considered possibly, probably, or definitely-related to the investigational product or study procedure. All events starting or worsening after the first randomized treatment through study exit will also be collected.

8.3.2 DEFINITION OF INVESTIGATIONAL PRODUCT

Investigational Product is defined as either the AMNIOEXCEL Plus or Apligraf allograft.

8.3.3 DEFINITION OF STUDY PROCEDURE

Study Procedure is defined as the application of any of the SOC products used in the care of the wound and/or sharp debridement.

8.3.4 DEFINITION OF ADVERSE REACTION

An Adverse Reaction is defined by the FDA as any noxious or unintended response for which there is a reasonable possibility that the HCT/P caused the reaction. This includes, but is not limited to, the transmission of communicable diseases or infectious agents such as viruses, bacteria, or fungi, or allergic reaction. In addition to your normal adverse event reporting procedures in this study, adverse reactions should be reported within 2 business days to a Sponsor representative. This person can either be the Clinical Research Manager, the Clinical Research Associate assigned to the site, or the Medical Monitor. In this study, only those subjects randomized to receive AMNIOEXCEL Plus may experience an adverse reaction as it is the only HCT/P used in this study.

8.3.5 DEFINITION OF SERIOUS ADVERSE EVENT (SAE)

An adverse event is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death,
- a life-threatening adverse event,
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- a congenital anomaly/birth defect.

8.3.5.1 SUB-DEFINITIONS OF SERIOUS ADVERSE EVENT COMPONENTS

- **Death** - If it is suspected that the death was an outcome of the adverse event
- **Life-threatening** - If it is suspected that the subject was at substantial risk of dying at the time of the adverse event, or use / continued use of the investigational (study) device or study procedure might have resulted in the death of the subject.
- **Hospitalization (initial or prolonged)** - If subject admission to the hospital or prolongation of hospitalization was a result of the adverse event. Emergency room visits that do not result in admission to the hospital should be evaluated for one of the other serious outcomes (e.g., life-threatening; required intervention to prevent permanent impairment or damage; other serious medically important event).
- **Disability or Permanent Damage** - If the adverse event resulted in a substantial disruption of a person's ability to conduct normal life functions, i.e., the adverse event resulted in a significant,

persistent or permanent change, impairment, damage or disruption in the subject's body function/structure, physical activities and/or quality of life.

- **Congenital Anomaly/Birth Defect** - If it is suspected that exposure to the investigational (study) device or study procedure prior to conception or during pregnancy may have resulted in an adverse outcome in the child.
- **Required Intervention to Prevent Permanent Impairment or Damage** - If it is suspected that medical or surgical intervention was necessary to preclude permanent impairment of a body function, or prevent permanent damage to a body structure, either situation suspected to be due to the use of the study intervention or study procedure
- **Other Serious Important Medical Events** - When the event does not fit the other outcomes, but the event may jeopardize the subject and may require medical or surgical intervention (treatment) to prevent one of the other outcomes. Examples include allergic bronchospasm (a serious problem with breathing) requiring treatment in an emergency room, serious blood dyscrasias (blood disorders) or seizures/convulsions that do not result in hospitalization. The development of drug dependence or drug abuse would also be examples of important medical events.

Note: Planned hospitalization for a pre-existing condition, or a procedure required by the clinical study protocol, without serious deterioration in health, is not considered a serious adverse event.

8.3.6 CLASSIFICATION OF AN ADVERSE EVENT

8.3.6.1 SEVERITY OF EVENT

For adverse events (AEs) not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- **Mild** – Transient or mild discomfort; no limitation in activity; no medical intervention or therapy required. The subject may be aware of the sign or symptom, but tolerates it reasonably well.
- **Moderate** – A moderate limitation in activity, minimal medical intervention and/or therapy is required to provide relief and/or resolution.
- **Severe** – A marked limitation in activity; advanced medical intervention and/or therapy, possibly including hospitalization was required to provide relief and/or resolution.

8.3.6.2 RELATIONSHIP TO STUDY INTERVENTION

Relationship of all AEs to the investigational product, the comparator, or study procedure may be classified according to the following classifications/definitions.

- **Not Related**: The AE is due to an underlying or concurrent illness or the effect of another device, another drug or another intervention and is not related to the study device and/or study procedure (i.e. clearly not related).
- **Unlikely**: The relationship with the use of the investigational product or procedures seems not relevant and/or the event can be reasonably explained by another cause, but additional information may be obtained.

- **Possibly Related**: The AE occurred in a reasonable time period relative to use of the investigational product and/or study procedure, which makes a causal relationship possible, but an alternative etiology is equally or less likely compared to the potential relationship to the investigational product and/or study procedure (i.e., may be related).
- **Probably Related**: The AE occurred in a reasonable time period relative to use of the investigational product and/or study procedure, and another etiology is unlikely or significantly less likely, which makes a causal relationship probable (i.e. likely related).
- **Related**: The AE occurred in a reasonable time period relative to use of the investigational product and/or study procedure and has a known relationship to the study device and/or study procedure, or is the only etiology available, making the causal relationship certain (i.e., clearly related).

8.3.6.3 EXPECTEDNESS

Study Investigators will be responsible for determining whether a product or procedure-related SAE is "anticipated" or "unanticipated". A product or procedure-related SAE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

In determining expectedness of a SAE, the Investigator should consult the following:

- Previous experience with the product or procedure
- Literature provided with any treatments (AMNIOEXCEL Plus; Apligraf product inserts)

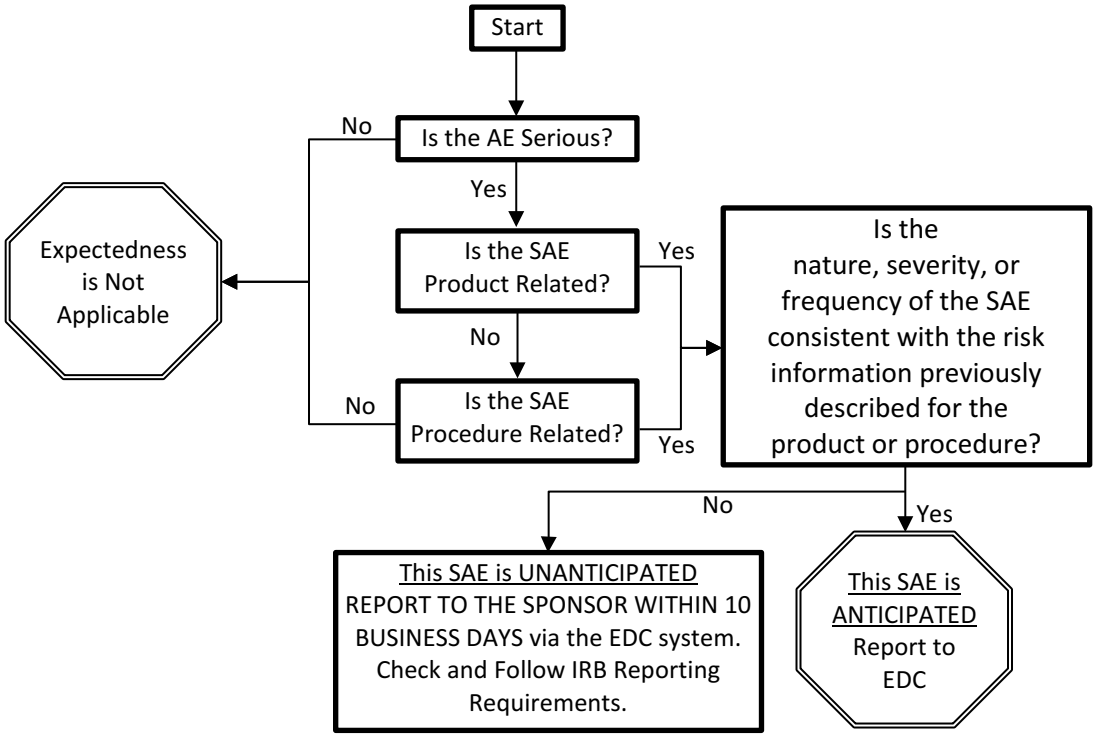
Although every adverse event occurring in an individual may be considered unanticipated, for the purposes of this study, anticipated events are those which are known to commonly occur in individuals with diabetes and DFUs. Although every adverse event occurring in an individual may be considered unanticipated, for the purposes of this study, anticipated events are those which are known to commonly occur in individuals with diabetes and DFUs. Those events include but are not limited to:

- Ulcer enlargement, tunneling and/or undermining
- Malodor
- Erythema
- Edema
- Maceration
- Excessive drainage
- Development of a new ulcer,
- Ulcer infection or abscess,
- Osteomyelitis
- Trauma/falls and/or
- Amputation

Although these remain adverse events, they are considered "anticipated". The following diagram (Figure 3) is to assist in the expectedness determination:

[See Next Page for Diagram]

Figure 3: Expectedness Determination of Investigational Product or Procedure-Related SAEs



8.3.7 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study subject presenting for medical care, or upon review by a study monitor. All AEs will be captured on the appropriate eCRF as noted above in section 8.2.

Any medical condition that is present at the time that the subject is initially screened will be considered as pre-existing and not reported as an AE. However, if the study subject's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The study Investigator will record all reportable events with start dates occurring any time after informed consent is obtained the last day of study participation (i.e., Follow-Up Visit 2). At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

If an AE is not resolved by the end of the study, that event will be marked as such by the Investigator.

8.3.8 ADVERSE EVENT REPORTING

Adverse events will be reported to the Sponsor using the eCRFs in the EDC as the Investigator becomes aware of them. The Investigator is responsible for reporting any and all adverse events to their federal, state, and local oversight committees (e.g., IRBs) as required by that committee.

Adverse reactions to the AMNIOEXCEL Plus must be reported within 2 business days to the Sponsor (Integra) via the AE eCRF in the EDC system. *Important note: Subject records must be maintained for the purpose of tracing tissue post-transplant per The Joint Commission on Accreditation of Healthcare Organizations and FDA requirements. The AMNIOEXCEL Plus ID number must be recorded in the recipient's medical record. Patient labels which include tissue numbers are contained in the AMNIOEXCEL Plus package to aid in the tracking process.*

8.3.9 SERIOUS ADVERSE EVENT REPORTING

Investigators are instructed to report SAEs to Integra within 10 business days of the Investigator becoming aware of the SAE.

SAEs must be reported by the Investigator to Integra by completing the SAE eCRF. All SAEs and deaths that are possibly related, probably related, or definitely related to the investigational product or study procedures must be reported. The information collected will include a minimum of the following: subject number, a narrative description of the event, lot number of device used on subject, and an assessment by the Investigator as to the intensity of the event and relatedness to study device. Follow-up information on the SAE may be requested by the Sponsor.

Any subjects reporting SAEs that have not resolved by their last treatment visit will be followed via phone or clinic visit through resolution or 14 days post final visit, whichever comes first.

8.3.10 UNANTICIPATED SERIOUS ADVERSE EVENT REPORTING

Investigators are instructed to report UNANTICIPATED SADEs to Integra *AND* to the IRB, if required, within 10 business days of the Investigator becoming aware of the SAE. *NOTE: Local IRBs may require different timelines and/or reporting requirements. Each Investigator approved to conduct research under the authority of a local IRB is responsible for knowing these requirements and for abiding by them.*

9 STATISTICAL CONSIDERATIONS

This section presents general information about statistical methodologies and concepts for this study. Further technical details (e.g., statistical analysis methods and data conventions) will be provided in the Statistical Analysis Plan (SAP).

9.1 STATISTICAL ENDPOINTS

- Primary Efficacy Endpoint(s):

The primary endpoint of this study is the incidence of complete wound closure, as assessed by the Investigator at or before Week 12 of the Treatment Phase and confirmed closed 2 weeks later. Confirmed complete wound closure is defined as complete skin re-epithelialization that is without drainage or dressing requirements two weeks later.

A subject is considered to have a closed wound (i.e., a closure success) if the subject's treated wound closes between week 1 and 12 and is noted as closed on both follow-up visits.

A subject is considered to not have a closed wound (i.e., a closure failure) if they meet any of the following:

- the subject's treated wound does not close by week 12
- the subject's treated wound closes between week 1 and 12 and is noted as open on either or both of the follow-up visits.

A subject is considered to have an undetermined wound closure if the subject's treated wound closes between week 1 and 12 and the subject misses either or both of the follow-up visits. These subjects may become part of extended analyses.

- Secondary Efficacy Endpoint(s):

1. Proportion of subjects with complete closure of the study ulcer at or before Week 12 as assessed by computerized planimetry.
2. Proportion of subjects with complete wound closure of the study ulcer at or before Week 12 as assessed by independent, blinded, assessment of photographs.
3. Time to complete wound closure, as assessed by the Investigator.
4. Time to complete wound closure, as assessed by computerized planimetry.
5. Rate of wound closure, as assessed by computerized planimetry.
6. Medical resource utilization associated with treatment of DFU and related complications as determined by a comparison of estimated costs from the presumed billing codes which would have been used for each patient.

9.2 SAMPLE SIZE DETERMINATION

A total of 114 subjects, 38 per cohort, will be enrolled, inclusive of a 20% in-study attrition rate.

It is assumed that the maximum wound closure rate is 90% with no difference between the study cohorts. Based on the 38 subject per cohort sample size, the half width of the 95% confidence interval for the wound closure rate is 13.5%. However, the study is not adequate for hypothesis testing.

9.3 POPULATIONS FOR ANALYSES

The following populations are defined for the analysis of the data to be collected as part of this study. All decisions on eligibility for inclusion in these populations will be made prior to the end of the study.

The Intent-to-Treat (ITT) population, defined as all randomized subjects who received at least one application of study intervention, will be the primary population for the analysis of primary and secondary efficacy endpoints.

The Per Protocol (PP) population, defined as all subjects in the ITT population for who there were no major protocol violation, will be used in supportive as the primary analysis dataset analyses of the primary and secondary endpoints. In efficacy or exploratory analyses, covariate analyses will be conducted to assess the impact of various prognostic factors on closure and also to demonstrate the robustness of the primary analysis. Prognostic factors (i.e., covariates) will be included if they are found to be contributing factors (i.e., the individual covariate p-value is less than 0.10). Potential prognostic factors to be considered include:

- Ulcer area
- Gender
- Ulcer Location
- Race
- Baseline BMI
- Duration of ulcer
- HbA1c
- Diabetes type
- Nicotine Use

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

All study data collected in the eCRF will be presented in subject data listings. Statistical analyses will be performed using SAS® for Windows, version 9.4 or later. Descriptive statistics (n, mean, standard deviation, median, minimum and maximum) will be calculated by treatment group for continuous variables. Frequencies and percentages will be presented by treatment group for categorical variables.

Unless otherwise specified, all testing will be performed using two-sided test at the 0.05 level of significance.

9.4.2 SUBJECT DISPOSITION

The disposition of all subjects who sign an ICF will be provided. The numbers of subjects screened, randomized, completed, and discontinued during the study, as well as the reasons for all post-randomization discontinuations will be summarized by treatment group, for all sites combined and each site separately. Disposition and reason for study discontinuation will also be provided as a by-subject listing.

9.4.3 PROTOCOL DEVIATIONS

Protocol deviations will be identified and listed by study sites based on the definitions and constraints set forth in section 10.1.9 of the protocol, below.

9.4.4 BASELINE DESCRIPTIVE STATISTICS

Demographic data including but not limited to age, race, gender and ethnicity and other baseline characteristics, but not limited to medical and surgical history will be tabulated by treatment and

overall. Summarized demographic data will include mean, standard deviation, minimum and maximum; as well as analogous summary statistics for presenting diagnosis and other relevant patient characteristic factors.

9.4.5 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

The primary objective of this study is to assess the proportion of subjects who achieve complete wound closure by 12 weeks and confirmed 2 weeks later, for the Active Treatment Groups (AMNIOEXCEL Plus Placental Allograft Membrane with SOC, Apligraf bi-layered skin substitute with SOC) and Control Treatment Group (or SOC alone).

The primary analysis will be conducted on the ITT population. The proportion of subjects with confirmed closed study wound, as assessed by the investigator, will be compared between AMNIOEXCEL Plus and Apligraf, and AMNIOEXCEL Plus and SOC. The proportion of subjects with confirmed complete wound closure will be compared using the Cochran–Mantel–Haenszel (CMH) test.

For the primary efficacy analysis, missing values will be imputed using last observation carried forward (LOCF). The differences in confirmed complete wound closure rate between treatment arms will be summarized along with 95% confidence intervals.

9.4.6 ANALYSIS OF THE SECONDARY ENDPOINT(S)

The following are for exploratory analysis purposes.

Proportion of ulcers with complete wound closure during the treatment phase as assessed by planimetry will be compared using Logistic Regression with selected baseline covariates in the model.

Time to complete wound closure will be analyzed using log rank test or Cox proportional regression model. Kaplan-Meier methods will be used to present median time to complete wound closure.

Summary statistics will be provided for the rate of wound closure for the two treatment groups.

9.4.7 SAFETY ANALYSES

AEs/SAEs related to the investigational product, study ulcer and/or study procedures and will be recorded.

AEs will be coded according to the most recent version of the Medical Dictionary for Regulatory Activities (MedDRA) AE coding dictionary, and grouped by body system. The number and percentage of subjects experiencing at least one AE of any type, AEs within each body system and AEs within each preferred term will be presented for each treatment group. Separate summaries will be provided for all AEs, device related AEs, SAEs and AEs leading to withdrawal.

All safety parameters will be summarized descriptively by treatment assignment. No inferential statistics are planned.

Listings of complications will be presented, include complication type, relation to study device, relation to procedure, action taken, and clinical outcome. Separate listings will be constructed for complications with possible, probable, or definite relationship to the device, serious complications, and for all complications. The listing for all complications will be sorted by complication type.

9.4.8 PLANNED INTERIM ANALYSES

An interim analysis will occur after 50% of subjects have satisfied the primary endpoint of the study. Descriptive statistics will be provided. Primary and secondary endpoints will be evaluated at the time of interim analysis.

9.4.9 SUB-GROUP ANALYSES

Sub-group analysis is not planned for this study.

9.4.10 TABULATION OF INDIVIDUAL SUBJECT DATA

All collected study data will be presented in subject data listings. Details will be provided in SAP.

9.4.11 MULTIPLICITY ADJUSTMENT

There will be no multiplicity adjustment for primary and secondary endpoint analysis for comparing three treatment groups nor any adjustment for the planned interim analysis.

9.4.12 EXPLORATORY ANALYSES

Exploratory analyses will be performed to examine wound closure status at Week 4 of Treatment and wound closure status at the 2-week follow-up visit

Proportion of subjects with confirmed complete wound closure of the study ulcer at week 4 Treatment Phase, and at the 2-week follow-up visit as assessed by computerized planimetry for each of the treatment groups will be summarized. A point estimate will be presented along with the exact 95% confidence interval based on the binomial distribution.

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10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO SUBJECTS

Consent forms describing in detail the study intervention, study procedures, and risks will be given to the subject and written documentation of informed consent (i.e., a signed consent form) is required prior to performing any study-related procedures.

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. The subject must agree to participate in the study and sign the informed consent document prior to any procedures being done specifically for the study**.** Consent forms will be IRB-approved and the subject will be asked to read and review the document. A verbal explanation will be provided in terms suited to the subject's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research subjects. Subjects will have the opportunity to carefully review the written consent form, ask questions and discuss the study with their family/friends/surrogates prior to agreeing to participate. **Subjects must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice.** A copy of the signed informed consent document will be given to the subjects for their records. The informed consent process will be documented in the subject's medical record and source document, the rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the study suspension or termination, will be provided by the suspending or terminating party to investigator and oversight authorities (e.g., IRB). If the study is prematurely terminated or suspended by the Sponsor, the Investigators will promptly inform study subjects, the IRB. If an oversight authority (e.g., IRB) suspends or terminates a study at a site, the Investigator should notify the Sponsor within 24 hours. Study subjects will be contacted, as applicable, and be informed of changes to their visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to subjects
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

The study may resume if resolution of the suspending/terminating factor occurs.

10.1.3 CONFIDENTIALITY AND PRIVACY

Subject confidentiality and privacy is strictly held in trust by the participating Investigators, their staff, oversight authorities (e.g., IRBs), the Sponsor, and third party affiliates. This confidentiality is extended to cover all data collected within the confines of this protocol. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the Sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the IRB, regulatory agencies or pharmaceutical company supplying study product may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. The clinical study site will permit access to such records.

The study subject's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study subject research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to the sponsor via an EDC system (Clindex®, Fortress Medical Systems). This will not include the subject's contact or identifying information. Rather, individual subjects and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by the Sponsor's research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at the Sponsor's headquarters.

10.1.4 FUTURE USE OF DATA

Data collected for this study will be analyzed and stored at the Sponsor's headquarters. After the study is completed, the de-identified, archived data will be retained by the Sponsor and will not be used by other researchers outside of the study. Deidentified images obtained in the course of study conduct, will also be stored with other study data. All stored data may be used as part of future post-hoc analyses by the Sponsor.

10.1.5 SAFETY OVERSIGHT

Safety oversight will be under the direction of the Medical Monitor. The Medical Monitor will review SAEs related to the study and provide guidance on actions, if any, which need to be taken by the study team.

10.1.6 CLINICAL MONITORING

Monitoring of the clinical data collected for a study is performed to ensure that the rights and well-being of trial subjects are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with ICH GCP, and with applicable regulatory requirement(s).

- Monitoring for this study will be performed by the Sponsor or authorized Sponsor representative.
- Monitoring will incorporate risk-based (e.g., centralized) and on-site review of data. The frequency of monitoring visits will vary based on factors such as information seen during risk-based monitoring activities, frequency of enrollment, and other pertinent constraints (i.e., audit preparation, etc.). The extent of the monitoring will be to achieve comprehensive, 100% source data verification.
- The schedule of monitoring activities (i.e., timing of confirmation letters, reports, follow up letters, etc.) will follow the Sponsor's SOPs and the Clinical Monitoring Plan (CMP).

10.1.7 QUALITY ASSURANCE AND QUALITY CONTROL

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, ICH GCP, and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

10.1.8 DATA HANDLING AND RECORD KEEPING

10.1.8.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site Investigator. The Investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Hardcopies of the study visit worksheets may be provided for use as source document worksheets for recording data for each subject enrolled in the study. Data recorded in the eCRF derived from any source documents should be consistent with the data recorded on those source documents.

Clinical data (including AEs), concomitant medications, and expected adverse reactions data, and randomization) and clinical laboratory data will be entered into Clindex® (Fortress Medical Systems, Inc.), a 21 CFR Part 11-compliant data capture system provided by the Sponsor. The data system includes password protection and internal quality checks, such as automatic range

checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

A randomization code will be developed by the Sponsor's Statistical and Data Management teams and will be entered into the EDC mentioned above. Only these teams will know or have access to these codes and/or the software systems used by the study's investigators. Randomization via the EDC system will only occur on Day 0 of the study after a subject meets all of the inclusion and exclusion criteria set forth in this protocol.

10.1.8.2 STUDY RECORDS RETENTION

Study documents should be retained until at least 2 years after the formal closure of the clinical trial at the study site. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the Sponsor to inform the investigator when these documents no longer need to be retained. If the documents are to be moved from the original Investigator's site (i.e., for storage purposes), a formal letter must be sent to the Sponsor informing them of the move date, the new location, and, if there is a change in oversight of the documents, the person who will be retaining oversight. Letters can be sent to the Director of Global Clinical Operations at Integra LifeSciences. The Sponsor should be contacted for the current and appropriate address.

10.1.9 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, ICH GCP, or any supplementary protocols provided by third party vendors which have bearing on the collection of clinical trial data.

Major protocol deviations are a subset of protocol deviations that might significantly affect the completeness, accuracy, and/or reliability of the study data or that might significantly affect a subject's rights, safety, or well-being.

The noncompliance may be either on the part of the subject, the Investigator, or the study site staff. As a result of major deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site Investigator to use continuous vigilance to identify and report deviations within 10 working days of identification of the protocol deviation, or within 5 working days of the scheduled protocol-required activity. All deviations must be addressed in study source documents, reported to the Sponsor. Protocol deviations must be sent to the reviewing IRB per their policies. The site Investigator is responsible for knowing and adhering to the reviewing IRB requirements. Protocol deviations and their impact on the data analysis will be addressed in the study's statistical analysis plan

10.1.10 PUBLICATION AND DATA SHARING POLICY

The results of the study may be published in scientific literature and may also be used in submissions to regulatory authorities. It is the intent of the Sponsor to publish or present the study results together with study results from the other sites, unless specific written permission is obtained in advance from the Sponsor to publish separate results. The Investigator will ensure that any site and/or Sponsor personnel making a significant contribution to the study or development of a manuscript are recognized as co-authors in any publication according to guidelines set forth by the International Committee of Medical Journal Editors (ICJME).

All information received by the Investigator concerning the Sponsor's business operations (such as patent applications, product design, formulae, manufacturing processes, basic scientific data, or characterization or formulation information supplied to the Investigator and not previously published) is considered confidential by the Sponsor and shall remain the sole property of the Sponsor. The Investigator agrees not to use it for any purpose other than conduct of the study without the prior written consent of Sponsor.

It is understood by the Investigator that the Sponsor will use the information developed in this clinical trial ("Study Data") in connection with the development of the AMNIOEXCEL Plus Placental Membrane. Therefore, Study Data may be disclosed to other Investigators or appropriate regulatory authorities. By agreeing to participate in this clinical trial, the Investigator understands that he/she has an obligation to provide the Sponsor with complete and accurate Study Data.

Publication and Disclosure: Because this is a multi-center trial, Investigators and/or their academic Institutions shall not independently publish, publicly disclose, present or discuss any Study Data or other information pertaining to their respective activities conducted under this study protocol until a multi-center publication is released under Sponsor's direction, unless otherwise agreed upon in the study agreement.

10.1.11 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial should be disclosed and managed. Furthermore, persons who have a perceived conflict of interest should have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial.

10.1.12 FINANCING AND INSURANCE

This study is being financed by the Sponsor, Integra LifeSciences, Inc. The Sponsor holds a certificate of insurance for this study.

10.2 ABBREVIATIONS AND DEFINITIONS

Table 4: Abbreviations/Definitions

Abbreviation/Term	Clarification/Definition
ABI	Ankle-Brachial Index
AE	Adverse Event
AMNIOEXCEL Plus	AMNIOEXCEL® Plus Placental Allograft Membrane; the investigational product
Apligraf	a living, bi-layered skin substitute; the comparator investigational product
AR	Adverse Reaction
CFR	Code of Federal Regulations
CMH	Cochran–Mantel–Haenszel
CMP	Clinical Monitoring Plan
CONSORT	Consolidated Standards of Reporting Trials
DAMA	Dehydrated Amniotic Membrane Allograft
DFU(s)	Diabetic foot ulcer(s)
dHACM	dehydrated Human Amnion/Chorion Membrane
eCRF	Electronic Case Report Forms
EDC	Electronic Data Capture
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HbA1c	Glycosylated Hemoglobin (A.K.A., Hemoglobin A1C)
HCT/P	Human Cellular and Tissue Based Products
HIPAA	Health Insurance Portability and Accountability Act
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IRB	Institutional Review Board
ITT	Intention-To-Treat
LOCF	Last Observation Carried Forward
LTFU	Lost-to-Follow-Up
PP	Per-Protocol
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SOC	Standard of Care
SOP	Standard Operating Procedure
T-AEPDFU-001	Protocol Number
T-PAM	Tri-layer Placental (Amnion/Chorion/Amnion) Allograft Membrane (T-PAM)
$TcPO_2$	Transcutaneous Oxygen Level
US	United States

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10.3 PROTOCOL AMENDMENT HISTORY

Table 5: Protocol Amendment History

Version	Date	Description of Changes	Brief Rationale
1.0	14 Feb 2018	N/A, Original Version	N/A, Original Version
2.0	01 Aug 2018	1. Removing Medical Monitor Information 2. Table 2 and Sections 8.1.4 and 8.2 were modified to allow for no imaging in the Apligraf cohort at Week 1. 3. Added numbering to sections in 6.1.2.	1. Change in staffing; if sites require medical assistance, they will be provided with current appropriate staff member's information. 2. Per the manufacturer's instructions, the non-adherent layer should not be removed to allow for full integration of the product into the surface of the wound. See Section 6.1.2 Utilization and Administration, point 5 of the Apligraf Instructions for Use. 3. To assist with and clarify edits above.
3.0	05 Nov 2018	1. Modified language in Study Design the Summary section 2. Added the following language to section 7.2: "If the subject's wound is open at follow-up week 1, the subject will be exited. They will be noted as having completed their study requirements." 3. The following language was added to section 8.1.6: "IMPORTANT: If the subject's wound is open at Follow-up visit 1 (e.g., week 13), the subject is exited from the study and is marked as having completed their study requirements. The site will note why they believe the wound is still open." 4. Figure 1 and Table 2 have been updated	1. To clarify and be in line with the other changes in this document. 2. To clarify what should happen to subject's whose wounds re-open at follow-up visit 1. 3. To clarify what should happen to subject's whose wounds re-open at follow-up visit 1. 4. These changes were made to be in line with the changes previously mentioned.

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