A Randomized Controlled Clinical Trial Evaluating The Efficacy Of A Unique Advanced Bioengineered Skin Substitute with Standard of Care Versus An Active Comparator with Standard of Care In The Treatment Of Non-Healing Diabetic Foot Ulcers

ENCOLL
Enhancing life through collagen

Protocol Number: ENC-HEL-DFU-02
Version: 1.0
Date: June 7, 2024

CONFIDENTIAL

INVESTIGATOR'S SIGNATURE PAGE

INVESTIGATOR'S SIGNATURE

I have read the protocol specified above and agree to participate in and comply with the procedures, as outlined herein for the conduct of this clinical trial. I also agree to comply with the applicable US Food and Drug Administration (FDA) regulations and Investigational Review Board (IRB) requirements for testing on human subjects. I agree to ensure that the requirements for obtaining informed consent are met.

Investigator's Signature	Date
Print Name	
Site Number	

Contents

TABLE 1: CONTACT INFORMATION

Role	Contact Information
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TABLE 2: VERSION HISTORY

Version Number	Release Date	Change(s)	Reason for Change
1.0	June 7, 2024	NA	NA

LIST OF ABBREVIATIONS

TERM	DEFINITION
ABI/ABPI	Ankle/Brachial Index/Ankle Brachial Pressure Index
AE	Adverse Event
CFR	Code of Federal Regulations
CRF	Case Report Form
DFU	Diabetic foot ulcer
EOS	End of study
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HCV	Healing Confirmation Visit
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency virus
IRB	Institutional Review Board
ITT	Intent-to-treat
PAR	Percentage area reduction
SAE	Serious adverse event
SAP	Statistical analysis plan
SOC	Standard of care
SOP	Standard operating procedure
SPP	Skin perfusion pressure
SV	Screening visit
TCOM	Transcutaneous oxygen measurement
TV	Treatment Visit
US	United States of America

PROTOCOL SYNOPSIS: ENC-HEL-DFU-02

Objective	The purpose of this clinical evaluation is to collect patient outcome data on a commercially available 510K FDA cleared advanced skin substitute. The commercially available product is Helicoll® Advanced Skin Substitute. In this trial two groups of subjects with Wagner 1 diabetic foot ulcers (DFUs), will receive standard of care (SOC) treatment for their condition. Half of the patients will have their SOC treatment with Epifix® or Grafix® and the other half will receive a 510K FDA cleared Helicoll® Advanced Skin Substitute as the primary treatment. The primary endpoint is the percentage wound area reduction of the target ulcer. Secondary endpoints include the proportion of subjects that obtain complete closure over the 5-week treatment period, the time to achieve complete wound closure of the target ulcer by the end of 5 weeks, and mean number of IP applications.
Intervention Groups	Group 1: SOC primary dressing with Helicoll® Group 2: SOC primary dressing with Epifix® or Grafix®
Study Design	Multi-center, open label, randomized controlled trial
Sample Size	For this RCT, N=24 subjects completed
Centers	Up to 5 centers within the United States, Open/competitive enrollment
Endpoints	**Primary endpoint:** - Percentage wound area reduction from TV1 to TV5 measured manually with digital photography **Secondary endpoints:** - The time to achieve complete wound closure of the target ulcer by the end of 5 weeks - The proportion of subjects that obtain complete closure over the 5-week treatment period - Mean number of repeated applications of the Advanced Skin Substitute used to obtain wound closure **Exploratory Endpoint:** - The appearance, structural stability, and fragility of the new skin formed documented at each visit. Any recurrence of the wound also will be monitored.

	Safety Analysis: - The number and type of Treatment Emergent Adverse and Serious Adverse Events
Safety	There is no formal safety objective for this study as Helicoll®, Epifix® and Grafix® are commercially available, 510K FDA cleared products for application on diabetic foot wounds. However, there is the possibility of disease transmission due to the risk of viral infection upon application of an allograft-tissue-derived skin substitute. Therefore, in accordance with FDA regulations, adverse reactions to the product will be recorded and provided to the study Sponsor for required FDA reporting and will be concurrently reported to all Investigators and IRBs. Adverse events associated with the application of standard of care dressing will be collected during the course of the study. Collection and reporting of any adverse reaction data, in accordance with FDA regulations and adverse events associated with product application will ensure study subject safety.
Surveillance Schedule	Subjects will undergo a screening visit to determine eligibility. Eligible subjects will then undergo up to a 5-week treatment phase involving weekly evaluations over 5 weeks and after 5 weeks, will exit the study. Additional weekly visits will occur during the 2nd and 3rd week.
Study Duration	It is estimated that about 3 months will be required to complete the study with data collection and reporting.

Inclusion Criteria	Potential subjects are required to meet all the following criteria for randomization into the study. 1. Subjects must be at least 18 years of age or older. 2. Subjects must have a diagnosis of type 1 or 2 Diabetes mellitus. 3. At randomization subjects must have a target diabetic foot ulcer with a minimum surface area of 1.0 cm^2 and a maximum surface area of 10.0 cm^2 measured post debridement using a ruler to measure wound area. 4. The target ulcer must have been present for a minimum of 4 weeks and a maximum of 52 weeks of standard of care prior to the initial screening visit. 5. The target ulcer must be located on the foot with at least 50% of the ulcer below the malleolus. 6. The target ulcer must be full thickness on the foot or ankle that does not probe to bone. 7. Adequate circulation to the affected foot as documented by any of the following methods performed within 3 months of the first screening visit: a. TCOM ≥30 mmHg b. ABI between 0.7 and 1.3 c. PVR: Biphasic d. TBI >0.6 e. As an alternative, arterial Doppler ultrasound can be performed evaluating for biphasic dorsalis pedis and posterior tibial vessels at the level of the ankle of the target extremity. 8. If the subject has two or more ulcers, they must be separated by at least 2 cm. The largest ulcer satisfying the inclusion and exclusion criteria will be designated as the target ulcer. 9. The subject must consent to using the prescribed off-loading method for the duration of the study. 10. The subject must agree to attend the twice-weekly/weekly study visits required by the protocol. 11. The subject must be willing and able to participate in the informed consent process. 12. Patients must have read and signed the IRB approved ICF before screening procedures are undertaken.

Exclusion Criteria	**Exclusion Criteria:** Potential subjects meeting any of the following criteria will be excluded from randomization**.** 1. A subject known to have a life expectancy of <6 months. 2. If the target ulcer is infected or if there is cellulitis in the surrounding skin. 3. Presence of osteomyelitis or exposed bone, probes to bone or joint capsule on investigator's exam or radiographic evidence. 4. A subject that has an infection in the target ulcer that requires systemic antibiotic therapy. 5. A subject receiving immunosuppressants (including systemic corticosteroids at doses greater than 10 mg of Prednisone per day or equivalent) or cytotoxic chemotherapy. 6. Topical application of steroids to the ulcer surface within one month of initial screening. 7. A subject with a previous partial amputation on the affected foot is excluded if the resulting deformity impedes proper offloading of the target ulcer. 8. A subject with a glycated hemoglobin (HbA1c) greater than or equal to 13% taken at or within 3 months of the initial screening visit. 9. A subject with a serum creatinine ≥ 3.0mg/dL within 6 months of the initial screening visit. 10. A subject with an acute Charcot foot, or an inactive Charcot foot, that impedes proper offloading of the target ulcer. 11. Women who are pregnant or considering becoming pregnant within the next 6 months. 12. A subject with end stage renal disease requiring dialysis. 13. A subject who participated in a clinical trial involving treatment with an investigational product within the previous 30 days. 14. A subject who, in the opinion of the Investigator, has a medical or psychological condition that may interfere with study assessments. 15. A subject treated with hyperbaric oxygen therapy or a Cellular and/or Tissue Product (CTP) in the 30 days prior to the initial screening visit. 16. A subject who has a sensitivity to bovine (cattle) or ovine (sheep) material. 17. A subject that is allergic to aminoglycoside antibiotics (gentamycin, tobramycin, etc.)

1 INTRODUCTION

1.1 Costs of Non-healing Diabetic Foot Ulcers

The economic burden of DFUs costs the United States over $50 billion each year.[1] The cost to treat one patient with DFUs ranges from $11,700 to $16,883.[2] Although approximately 70% of DFUs are shown to heal with good SOC, at least 30% become chronic wounds.[3] These non-healing wounds are at greater risk for infection and lower extremity amputation.[4] Consequently, improved treatment options are important for patients with chronic DFUs to improve patient outcomes, lower treatment costs and reduce the risk of complications.

1.2 Helicoll® Advanced Skin Substitute

Helicoll® Semi-Occlusive, Self-Adhering and Sterilized Type-I Collagen Sheet for Wound Treatments and Chronic Ulcers.

DESCRIPTION OF THE DEVICE

Helicoll® is a translucent, off-white, semi-occlusive, self-adhering and pre-sterilized Type-I Collagen Sheet for use as a bioactive membrane. Helicoll® is flexible with moderate tackiness. Helicoll® is a reconstituted collagen sheet free of contaminants like lipids, elastin, and other immunogenic proteins (US Patented).

Helicoll® is 510K cleared (K040314) for use in diabetic foot ulcers and a range of other acute and chronic wounds.

2 RATIONALE FOR STUDY

Helicoll® is an advanced skin substitute device that is 510K cleared for application on diabetic foot wounds and has been shown in case studies and clinical practice to assist in wound healing. Therefore, based on this early promising data, a 24 subject RCT is necessary to further validate these case studies and identify the likelihood of wound healing with weekly or as needed application. For consistency, one type of wound, Wagner 1, will be studied in this trial and DFUs have been chosen as they are some of the most common wounds seen in wound clinics.

3 STUDY ENDPOINTS

The purpose of this clinical evaluation is to collect patient outcome data on 2 commercially available standard of care dressings in partial/full-thickness diabetic foot ulcer subjects. Patient outcomes will be compared at 5 weeks.

The **primary endpoint** is the percentage wound area reduction from TV1 to TV5 measured manually with digital photography.

Secondary endpoints include a comparison between groups of the following clinical measures:

1. The time to achieve complete wound closure of the target ulcer by the end of 5 weeks.
2. The proportion of subjects that obtain complete closure over the 5-week treatment period.
3. Mean number of repeated applications of the Advanced Skin Substitute used to obtain wound closure.

Exploratory Endpoint:

1. The appearance, structural stability, and fragility of the new skin formed documented at each visit. Any recurrence of the wound also will be monitored.

Safety Analysis:

1. The number and type of Treatment Emergent Adverse and Serious Adverse Events.

4 STUDY DESIGN

This study is a prospective, multi-center, RCT designed to collect patient outcome data on 2 commercially available SOC dressings for the treatment of DFUs.

There are two standard of care arms in the study:

Arm 1: The SOC therapy in this study is offloading of the DFU (CAM boots or total contact casting [TCC] if the subject's foot is too large for a CAM boot), appropriate sharp or surgical debridement, infection management (systemic antibiotics only in conjunction with debridement) and wound care covering with Helicoll® applied weekly or as needed followed by a padded 3-layer dressing comprised of first layer - non-adherent and porous, second layer – absorbent 4x4 gauze pads & third layer - soft roll and compressive wrap (Dynaflex™ or equivalent).

Arm 2: The SOC therapy in this study is offloading of the DFU (CAM boots or total contact casting [TCC] if the subject's foot is too large for a CAM boot), appropriate sharp or surgical debridement, infection management (systemic antibiotics only in conjunction with debridement) and wound care covering with Epifix® or Grafix® followed by a padded 3-layer dressing comprised of first layer - non-adherent and porous, second layer – absorbent 4x4 gauze pads & third layer - soft roll and compressive wrap (Dynaflex™ or equivalent).

The study involves two phases: Screening and Treatment.

4.1 Phase 1: Screening

The Screening Phase is designed to determine whether subjects are eligible to proceed to the Treatment Phase of the study and consists of a series of screening assessments designed to determine eligibility.

At the Screening Phase Visit (SV1), written informed consent (ICF) from the subject will be obtained by the Investigator or suitably qualified designee before any protected health information is obtained and prior to the performance of any protocol-specific procedures.

After obtaining ICF, the Investigator will select the study target ulcer. Each subject will have only one DFU selected as the target ulcer. If the subject has more than one DFU at the SV1 visit, the Investigator will select the largest DFU that meets the eligibility criteria of the protocol as the target ulcer. If the subject meets all eligibility criteria at the Screening Visit, then the Screening Visit and first Treatment visit may occur on the same day.

4.2 Phase 2: Treatment

The Treatment Phase (up to 5 weeks) begins with a series of assessments designed to confirm the subject's continued eligibility. Investigators will debride the target ulcer, in accordance with SOC if required. Subjects whose ulcers continue to meet eligibility criteria will then be randomized to one of two groups: (1) SOC with Helicoll® primary dressing applied weekly or as needed per investigator discretion or (2) SOC with Epifix® or Grafix® applied weekly or as needed per investigator discretion.

4.3 Subject Treatment Assessments

4.3.1 DFU Assessments

During the Treatment Phase, subjects will be evaluated on a weekly basis, with the exception of week 2 and 3 where the patient will be seen twice weekly. Weekly/Twice-Weekly patient outcome evaluations include the Investigator's assessment of target ulcer healing and measurements of ulcer size using a ruler to measure wound area and provide photos of wounds, including photos of healed wounds.

Note: All procedures required during Screening are included in TV1 prior to the first treatment visit if the subject fulfills eligibility criteria.

Other evaluations during the Treatment Phase are detailed in the procedures section.

Subjects will be seen weekly (± 2 days) until the ulcer is healed or they meet protocol criteria to exit the study with the exception of the 2nd and 3rd week of treatment where the subject will be seen twice. For both groups, assigned treatment will be applied only once during the 2nd and 3rd week unless the investigator feels an additional application is necessary. If the additional application is not necessary, the additional visit will involve a dressing change and wound evaluation.

4.3.2 Wound Healing Assessment

Initial and confirmation evaluations of wound healing will be subject to oversight by the study chair who will review all photographs of healed wounds and confirm wound healing.

4.4 Study Subjects

This prospective randomized trial will randomize 24 subjects in up to 5 centers located in the USA.

4.4.1 Inclusion Criteria

Potential patients are required to meet all the following criteria for randomization into the study:

1. Subjects must be at least 18 years of age or older.
2. Subjects must have a diagnosis of type 1 or 2 Diabetes mellitus.
3. At randomization, subjects must have a target diabetic foot ulcer with a minimum surface area of 1.0 cm^2 and a maximum surface area of 10.0 cm^2 measured post debridement using a ruler to measure wound area.
4. The target ulcer must have been present for a minimum of 4 weeks and a maximum of 52 weeks of standard of care prior to the initial screening visit.
5. The target ulcer must be located on the foot with at least 50% of the ulcer below the malleolus.
6. The target ulcer must be full thickness on the foot or ankle that does not probe to bone.
7. Adequate circulation to the affected foot as documented by any of the following methods performed within 3 months of the first screening visit:
    a. TCOM ≥30 mmHg
    b. ABI between 0.7 and 1.3

   c. PVR: Biphasic
   d. TBI >0.6
   e. As an alternative, arterial Doppler ultrasound can be performed evaluating for biphasic dorsalis pedis and posterior tibial vessels at the level of the ankle of the target extremity.
8. If the subject has two or more ulcers, they must be separated by at least 2 cm. The largest ulcer satisfying the inclusion and exclusion criteria will be designated as the target ulcer.
9. The subject must consent to using the prescribed off-loading method for the duration of the study.
10. The subject must agree to attend the twice-weekly/weekly study visits required by the protocol.
11. The subject must be willing and able to participate in the informed consent process.
12. Patients must have read and signed the IRB approved ICF before screening procedures are undertaken.

4.4.2 Exclusion Criteria

Potential subjects meeting any of the following criteria will be excluded from randomization:

1. A subject known to have a life expectancy of < 6 months.
2. If the target ulcer is infected or if there is cellulitis in the surrounding skin.
3. Presence of osteomyelitis or exposed bone, probes to bone or joint capsule on investigator's exam or radiographic evidence.
4. A subject has an infection in the target ulcer or in a remote location that requires systemic antibiotic therapy.
5. A subject receiving immunosuppressants (including systemic corticosteroids at doses greater than 10 mg of Prednisone per day or equivalent) or cytotoxic chemotherapy.
6. Topical application of steroids to the ulcer surface within one month of initial screening.
7. A subject with a previous partial amputation on the affected foot is excluded if the resulting deformity impedes proper offloading of the target ulcer.
8. A subject with a glycated hemoglobin (HbA1c) greater than or equal to 13% taken at or within 3 months of the initial screening visit.
9. A subject with a serum creatinine ≥ 3.0mg/dL within 6 months of the initial screening visit.
10. A subject with an acute Charcot foot, or an inactive Charcot foot, that impedes proper offloading of the target ulcer.
11. Women who are pregnant or considering becoming pregnant within the next 6 months.
12. A subject with end stage renal disease requiring dialysis.
13. A subject who participated in a clinical trial involving treatment with an investigational product within the previous 30 days.

14. A subject who, in the opinion of the Investigator, has a medical or psychological condition that may interfere with study assessments.
15. A subject treated with hyperbaric oxygen therapy or a Cellular and/or Tissue Product (CTP) in the 30 days prior to the initial screening visit.
16. A subject who has a sensitivity to bovine (cattle) or ovine (sheep) material.
17. A subject that is allergic to aminoglycoside antibiotics (gentamycin, tobramycin, etc.)

4.5 Study Schedule

The study is divided into two phases: Screening and Treatment Phases. The schedules for the protocol-specified assessments and procedures in each phase are detailed in the following sections. Note that there is an end of study visit (EOS1) scheduled on Week 5 or earlier if the wound heals.

4.5.1 Visit Windows

Subject visit dates must be scheduled within the visit windows detailed in the Schedule of Study Visits table. When determining visit dates, the reference should always be seven days (+/- 2 days), except for TV1 which can be +2 days from SV1 or occur on the same day as SV1. Every attempt should be made to maintain subjects on their original treatment schedule.

TABLE 1: SCHEDULE OF VISITS. LIGHT GREY (SCREENING); LIGHT YELLOW (TREATMENT); LIGHT BLUE (END OF TREATMENT VISIT)

Visit	SV1	Wk 1 TV1		Wk 2 TV2		Wk 3 TV3		Wk 4 TV4	Wk 5 [C] EOS 1
		Pre** Rand	Post Rand						
Weeks from Treatment Date	0	0		1	1.5	2	2.5	3	4
Window Period		+2 days		±2 days	+2 days	±2 days	+2 days	±2 days	±2 days
Assessment of eligibility	X	X							
Informed consent	X								
Ulcer history	X								
Demographics	X								
Medical History or changes	X	X		X		X		X	X
Prohibited Therapies assessment	X	X		X		X		X	X
Concomitant Medication assessment	X	X		X		X		X	X
ABI/SPP/TCOM/ TBI or Arterial Doppler study	X			X	X	X			
Randomization			X						
Ulcer assessments	X	X		X	X	X	X	X	X
Infection assessment of target ulcer	X	X		X	X	X	X	X	X [B]
Physical exam or changes	X	X		X	X	X	X	X	X
Vital signs	X	X		X	X	X	X	X	X
Assess for Adverse Effects and Adverse Events			X	X	X	X	X	X	X
Urine or blood pregnancy test control (females of childbearing potential)	X								

Visit	SV1	Wk 1 TV1		Wk 2 TV2		Wk 3 TV3		Wk 4 TV4	Wk 5 [C] EOS 1
		Pre** Rand	Post Rand						
Weeks from Treatment Date	0	0		1	1.5	2	2.5	3	4
Assurance of effective birth control (females of childbearing potential)	X								
Pain assessment	X	X		X	X	X	X	X	X
Monofilament Test (10-point)	X	X		X	X	X	X	X	X
Serum Creatinine	X								
Blood Glucose	X	X		X	X	X	X	X	X
HbA1c Lab	X								X
Take X-ray to rule out osteomyelitis or bone infection	X								
Target ulcer photographs	X	X		X	X	X	X	X	X
Target ulcer measurements	X	X		X	X	X	X	X	X
Target ulcer cleaning	X	X		X		X		X	X [B]
Target ulcer debridement	X [A]	X [A]		X [A]		X [A]		X [A]	X [A] [B]
Target ulcer closure assessment		X		X	X	X	X	X	X
Assessment of offloading		X		X	X	X	X	X	X
Apply Helicoll® or Epifix® or Grafix®			X	X		X		X	
Initiate offloading of the target ulcer	X								
Apply outer dressings	X		X	X	X	X	X	X	X [B]

[A]: If required per guidelines; [B] Only if subject is not healed; [C]: Will be earlier if wound heals before this date or subject is withdrawn from the study

**Pre-randomization assessments are only performed if treatment is not done on the same day as screening. Otherwise, Screening and Randomization (first treatment) can occur on the same day, screening procedures are performed, then followed by randomization and completion of post randomization schema of events.

4.5.2 Screening Phase

The subject will sign and date the ICF and HIPAA authorization (according to site practices) prior to collection of any protected health information, as well as any study-related procedures. A subject ID will be assigned to each subject in successive order of entering the study after signing the ICF, beginning with 001 at each site.

SV1 can occur up to 2 days prior to randomization. However, subjects who meet all eligibility criteria can enter the Treatment Phase immediately once all of the eligibility criteria are reviewed and the subject meets all inclusion and no exclusion criteria.

4.5.3 SV1: Procedures to be performed include:

- Informed consent will be obtained prior to the collection of any protected health information as well as any study related procedures and the subject will sign a written ICF
- Assess current wound therapies for prohibited therapies within 30 days of randomization
- Demographics
- Medical history
- Physical examination
- Assess concomitant medications
- Vital signs
- Pain assessment (see section 8.5)
- Labs: HbA1c (if no documented results within 90 Days of the first screening visit are available), assess blood glucose, and serum creatinine (if no documented results within 6 months of the first screening visit are available)
- Pregnancy test (blood or urine) if female subject is of childbearing potential. If female is not of childbearing potential the reason for inclusion must be included in the study record
- Assurance of effective birth control if female subject is of childbearing potential
- Ulcer history
- Target Ulcer assessment
- Assess signs and symptoms for clinical infection of the target ulcer (see section 8.6.3)
- Target ulcer foot neuropathy Semmes Weinstein 10-point test; (see section 8.9)
- Clean the target ulcer
- Debridement of the target ulcer, if applicable (see Appendix E)
- Digital imaging of the target ulcer
- Record target ulcer measurements
- Obtain X-ray to rule out osteomyelitis or bone infection of the affected foot
- ABI, SPP, TCOM, TBI, PVR measurement or Arterial Doppler Study will be obtained (see Appendices B-D)
- Initiate offloading of the target ulcer

- Assessment of subject's eligibility to continue in the study
- Apply appropriate SOC to target ulcer and outer dressing if patient does not enter into the treatment phase immediately following SV1

4.5.4 Treatment Phase

When determining the visit dates, the reference should always be seven days (±2 days). Every attempt should be made to maintain subjects on their original treatment schedule.

4.5.4.1 TV1: Pre-Randomization

The Treatment Phase begins with a series of assessments designed to confirm the subject's continued eligibility (Pre-randomization, Table 1). The following assessments and activities are performed at this visit (unless randomization is performed at the same visit as screening):

- Medical history changes from previous visit
- Physical examination changes from previous visit
- Assess concomitant medications, changes from previous visit and prohibited therapies
- Vital Signs
- Pain assessment (see section 8.5)
- Assess blood glucose
- Target ulcer Assessments
- Assess signs and symptoms for clinical infection of the target ulcer (see section 8.6.3)
- Target ulcer foot neuropathy Semmes Weinstein 10-point test; (see section 8.9)
- Clean the target ulcer
- Debridement of the target ulcer if applicable (see Appendix E)
- Digital imaging of the target ulcer
- Record target ulcer measurements
- Assessment of offloading
- Target ulcer closure assessment will be determined by a site Investigator
    - If the subject is seen on a subsequent visit to enter the treatment phase and the target ulcer is 100% re-epithelialized, the subject will be considered a screen failure.
- Assessment of subject's eligibility to continue in the study
- If the subject is eligible, they will enter the treatment phase
- If the subject is not eligible, discharge the subject from the study as a screen failure

4.5.4.2 TV1: Randomization

The following assessments and activities are performed at this visit:

- Randomize the subject
- Apply appropriate treatment to target ulcer:
    - Apply SOC therapy with Helicoll® - Group 1
    - Apply SOC therapy with Epifix® or Grafix® - Group 2
- Apply outer layer dressings, including first layer - non-adherent and porous, second layer – absorbent 4x4 gauze pads & third layer - soft roll and compressive wrap (Dynaflex™ or equivalent).
- Check for any changes in the subject's health and assess them for any adverse effects and adverse events
- Ensure offloading of the wound is satisfactory
- Schedule the next study visit for one week later (±2 days) , except TV2 and TV3 where the patient will be seen twice weekly.

4.5.4.3 Treatment Phase Visits: Weeks 2-4

The following assessments and activities are performed at these visits:

- Medical history changes from previous visit
- Physical examination changes from previous visit
- Assess concomitant medications, changes from previous visit and prohibited therapies
- ABI, SPP, TCOM, TBI, PVR measurement or Arterial Doppler Study will be obtained (see Appendices B-D)
- Vital Signs
- Pain assessment (see section 8.5)
- Assess blood glucose
- Assessment of offloading
- Target ulcer foot neuropathy Semmes Weinstein 10-point test; (see section 8.9)
- Check for any changes in the subject's health and assess them for any adverse effects and adverse events
- Target ulcer closure assessment will be determined by a site Investigator
    - If the target ulcer is 100% re-epithelialized, the subject will exit study at the conclusion of the visit (the visit will be considered EOS1). Please perform all applicable assessments and study procedures associated with EOS1.
- Target ulcer Assessments
- Check for signs of clinical infection. If a clinical diagnosis of infection has been made, then the subject can be treated with oral antibiotics, but topical antibiotics or antimicrobial dressings CANNOT be used on the target ulcer (see section 8.6.3)
- Clean the target ulcer

- Debridement of the target ulcer if applicable (see Appendix E)
- Digital imaging of the target ulcer
- Record target ulcer measurements
- Apply appropriate treatment to the index ulcer:
    - Apply SOC therapy with Helicoll® - Group 1
    - Apply SOC therapy with Epifix® or Grafix® - Group 2
- For the TV2 and TV3 **additional visit** where application of a new Helicoll® does not occur, the primary dressing will be lifted from the wound site along with the Helicoll® Advanced Skin Substitute or Epifix® or Grafix®. If the Helicoll® Advanced Skin Substitute is adherent to the wound, moisten gently with saline before attempting to remove. Cleaning of the target ulcer as noted above is suggested, however debridement should be limited, and it is suggested that only a very gentle removal of any slough be performed at the investigator discretion. Digital Photography is then taken and the Helicoll® Advanced Skin Substitute or Epifix® or Grafix® and primary dressing should be placed back on the wound. If the primary dressing is soiled or damaged a new primary dressing can be applied. Additional assessments will be performed as referenced in the Schedule of Visits (Section 4.5, Table 1).
- Ensure offloading of the Target ulcer is satisfactory.
- Apply outer layer dressings, including first layer - non-adherent and porous, second layer – absorbent 4x4 gauze pads & third layer - soft roll and compressive wrap (Dynaflex™ or equivalent).
- The next visit will be scheduled for one week (+/- 2 days)

4.5.5 End of Study (EOS1) Visit

The following assessments and activities are performed at this visit:

- Medical history changes from previous visit
- Physical examination changes from previous visit
- Assess concomitant medications, changes from previous visit and prohibited therapies
- Vital Signs
- Pain assessment (see section 8.5)
- Assess Blood glucose
- HbA1c
- Assessment of offloading
- Target ulcer foot neuropathy Semmes Weinstein 10-point test; (see section 8.9)
- Check for any changes in the subject's health and assess them for any adverse effects and adverse events
- Target ulcer closure assessment will be determined by a site Investigator
- Target Ulcer Assessments

- Check for signs of clinical infection. If a clinical diagnosis of infection has been made, then the subject can be treated with oral antibiotics, but topical antibiotics or antimicrobial dressings CANNOT be used on the study ulcer
- Clean the target ulcer
- Debridement of the target ulcer if applicable (see Appendix E)
- Digital imaging of the target ulcer
- Record target ulcer measurements
- Ensure outer dressings are applied and if applicable, make an appointment for the subject with their desired clinician for follow-up care

4.5.6 Unscheduled Visits

Unscheduled visits may be required in addition to the visits detailed above. Additional visits are at the discretion of the Investigator. An example of an unscheduled visit is when a change in dressings is required between scheduled visits. The details of these unscheduled visits with subjects will be recorded in the medical records/source documents and on the Unscheduled Visit CRF.

4.5.7 Missed Visits

If a subject misses a visit, the site is to make every effort to have the subject return as soon as possible to make up the visit. Once the subject is seen, he/she is to return to his/her original weekly visit schedule. For example, if a subject was seen regularly on Mondays but missed a scheduled Monday visit and came in on Wednesday, he/she should return the next Monday to maintain his/her weekly Monday visit schedule.

5 SUBJECT COMPLETION, SUCCESS/FAILURE, WITHDRAWAL AND SCREEN FAILURE

5.1 Subject Completion

- A subject whose target ulcer has closed will be considered as having completed the study
- A subject who completes the Treatment Phase but still has an unhealed target ulcer will be considered as having completed the study
- A subject who does not complete the Treatment Phase and still has an unhealed target ulcer will be considered as not completing the study

5.2 Success and Failure

- A subject whose ulcer heals sooner than 5 weeks with periodic assessment on a twice weekly/weekly basis will be considered a treatment success. The relative success of healing in percentage will be measured for each subject using the following calculation:

Percentage of Healing = (Actual days to heal / 28) X 100

- A subject whose ulcer has failed to heal by 5 weeks will be considered a treatment failure
- A subject who experiences an amputation of the affected ulcer area is also considered a treatment failure

5.3 Premature Withdrawal from the Study

A subject who is randomized into the Treatment Period of the study but who does not complete the study has prematurely discontinued.

All subjects have the right to withdraw at any point during treatment without prejudice. It will be documented whether or not each subject completes the study. If for any subject, study treatment or observations were discontinued, the reason(s) will be recorded.

The Investigator can discontinue a subject at any time if it is considered medically necessary. In addition, the subject will be withdrawn from the study, if any of the following events occur:

- A subject is significantly non-compliant with the requirements of the protocol
- A subject becomes pregnant or does not confirm use of birth control. (Note: the pregnancy will be followed up to term for safety. Relevant safety information collected after the study has been completed will be reported as supplemental information.)
- A subject has revascularization surgery on the lower extremity on which the target ulcer is located
- The subject's target ulcer deteriorates and is infected to the point where there is exposed bone
- An infection episode lasting more than two weeks with no response to allowable treatments

Premature withdrawal from the study may occur if:

- A subject is treated with a prohibited medication. This decision will be made by the Investigator as to whether premature withdrawal is warranted.

Every attempt should be made to collect follow-up information. The reason for treatment discontinuation or withdrawal from the study will be recorded in the source documents and on

the appropriate CRF.

Before a subject is identified as lost-to-follow up, the site should make all reasonable efforts to contact the subject. Three follow-up attempts are required. These attempts must be documented and should include two phone calls and one certified letter.

In the event that a subject is prematurely discontinued from the study at any time due to an adverse effect or adverse event, the procedures in Section 10 of this protocol must be followed.

5.4 Screen Failures

A subject who has signed a consent form, has been assigned a screening number, but is not entered into the treatment phase of the study is classified as a screen failure. Subject number, demography and reason for screen failure will be collected. Subjects who fail their first screening attempt may be re- screened again (i.e., up to two screenings total) and may be enrolled if they are found to meet all inclusion and none of the exclusion criteria. Rescreening follows a subject, not a specific wound, so subjects cannot be screened > 2 times for different wounds. Anytime a subject screen fails and later rescreens, they must be re-consented for the study.

6 STUDY TREATMENT

6.1 Method for Assigning Eligible Subjects to Treatment

Eligible subjects will be assigned to one of the two treatment groups based on a randomization schedule. Details of the randomization are provided int the statistical analysis section.

6.2 Helicoll® Product Information (provided from IFU)

INSTRUCTIONS FOR USE

Helicoll®

Semi-Occlusive, Self-Adhering and Sterilized Type-I Collagen Sheet for Wound Treatments, Second Degree Burns, and Chronic Ulcers.

Description Of The Device

Helicoll® is a translucent, off-white, semi-occlusive, self-adhering and pre-sterilized Type-I Collagen Sheet for use as a bioactive membrane. Helicoll® is flexible with moderate tackiness. Helicoll® is a reconstituted collagen sheet free of contaminants like lipids, elastin and other immunogenic proteins (US Patented). Helicoll® maintains a physiologically moist microenvironment at the wound surface.

Intended Uses

Helicoll® is intended for the topical wound management that includes:

- Partial and full-thickness wounds.
- Pressure ulcers.
- Venous ulcers.
- Chronic vascular ulcers.
- Diabetic ulcers.
- Trauma wounds (abrasions, lacerations, second-degree burns, skin tears).
- Surgical wounds (donor sites/grafts, post-Mohs' surgery, post-laser surgery, podiatric, wound dehiscence).

Advantages Of Helicoll® Membrane

- High purity type-I Collagen: Helicoll® is a patented reconstituted bioactive collagen sheet, free of immunogenic proteins, lipids, and elastin.
- Faster Healing: Collagen phosphorylation attracts cells, regenerates tissue, and stimulates blood capillaries/granulation within 4 to 5 days.
- Innovative Technology: Better than intact tissue-based membranes like amnion, intestinal wall, urinary bladder, etc. which contain 15% elastin.
- Easy Application: No washing needed prior to use.
- Pain Control: Effectively reduces pain.
- Various Sizes: Choose from standard or customized dimensions.
- Cost-Effective: Accelerated wound healing and tissue remodeling with minimal applications.
- Long Shelf Life: Remains clinically usable for 3 years when stored in room temperature conditions.

Directions For Use

Helicoll® comes in a sterile double packaging as a transparent pliable sheet. It has a back and a top protection cover sheet of medical grade synthetic polymer.

Upon opening the sterile package, the top sheet of polymer can be removed carefully and the remaining can be soaked in sterile water or normal saline solution for 5 to 10 minutes to easily remove the backing sheet.

Prepare wound area using standard methods to ensure wound is free of debris and necrotic tissue. An initial surgical debridement of the wound may be necessary to ensure the wound edges contain viable tissue.

Do not apply ointment or any greasy cream on the site prior to Helicoll®. Do not try to over stretch the membrane.

Helicoll® can be applied on either of its surfaces and it adheres to the wound instantly. In case of dry wounds, sprinkle sterile saline solution on the surface and apply
If there is a need to retain the skin-substitute in place, the perimetry can be taped, sutured or stapled as preferred by the doctor. If a secondary dressing is required, a non-adherent gauze with or without antibiotic can be placed to prevent unwanted adherence of the bandage to Helicoll®.

Repeated application on every 2nd or 3rd day like a typical wound dressing is not required, unless the wound is infected or accumulates excessive exudate underneath, which can be drained by making slit openings in the Helicoll® product.

Depending on the treatment modality, sometimes Helicoll® may remain intact and gets peeled off as the wound heals, which may carefully be removed by moistening with saline soaked gauze for a few minutes. However, in some cases, Helicoll® may get incorporated into the wound bed in about 4 to 5 days resulting in complete absorption of Helicoll®.

For donor site application, after surgical removal of donor tissue, arrest bleeding by conventional methods, clean the site and apply Helicoll®.

Oral or systemic antibiotics may be given as prescribed in infected cases and in non-infected cases as a preventive measure for better and faster results.

CAUTION: Always handle Helicoll® using aseptic techniques. Helicoll® should not be applied until excessive exudate, bleeding, acute swelling, and infection is controlled. If air pockets appear beneath the applied Helicoll®, it can be gently pressed and removed using sterile methods. In case of localized bulging due to fluid accumulation beneath Helicoll®, a small incision can be made to exude fluid. This incision can be patched with a small piece of Helicoll® adhering to the original applied Helicoll® sheet. After application, use an appropriate, non-adherent, secondary dressing to maintain a moist wound environment. Frequency of secondary dressing change will depend on the volume of exudate produced and the type of dressing used. Do not forcibly remove sections of Helicoll® that may adhere to the wound. Helicoll® may form a caramel-colored gel, which can be rinsed away with gentle irrigation.

CONTRA-INDICATIONS: Helicoll® is derived from a bovine or ovine source and should not be used in patients with known sensitivity to such material. This device is not indicated for use in third degree burns.

PRECAUTIONS: Helicoll® is sterile if the package is dry, unopened, and undamaged. Do not use if the package seal is broken. The device must be used prior to the expiration date. Discard all open and unused portions of Helicoll®.

Do not re-sterilize the products and this device is intended for one time use only. Helicoll® is available by medical prescription only.

STORAGE: Helicoll® should be stored in a clean, dry location at room temperature under normal storage conditions, Do Not Store Above 32℃ (90℉). Helicoll® has a minimum of 3 years shelf-life.

STERILIZATION:
Helicoll® has been sterilized with ethylene oxide.

AVAILABLE SIZES (inches & centimeters):

0.5 in dia disc (1.27 cm dia disc) 1 sq cm	1.0 in dia disc (2.54 cm dia disc) 5 sq cm	0.8 in x 1.6 in (2 cm x 4 cm) 8 sq cm
1.2 in x 1.6 in (3 cm x 4 cm) 12 sq cm	1.6 in x 1.6 in (4 cm x 4 cm) 16 sq cm	2 in x 2 in (5 cm x 5 cm) 25 sq cm
2 in x 4 in (5 cm x 10 cm) 50 sq cm	other custom sizes available. (Each individually sterile packaged)	

Manufactured and Marketed by:
ENCOLL Corp.
4576 Enterprise St.,
Fremont, CA 94538, USA
Tel. 510-659-1466 Fx. 510-795-7571
website:www.helicoll.com
e-mail: info@encoll.com

6.3 Grafix® Product Information

Construction
GRAFIX* cryopreserved placental membrane is supplied frozen in sheet form and packaged in a sterile cryobag contained within a heat-sealed pouch contained within a tertiary box. This packaging configuration allows for the introduction of GRAFIX* into the sterile field. GRAFIX PL* is supplied in sheet form between two (2) mesh applicators and packaged within a heat-sealed pouch contained within a tertiary box. This packaging configuration allows for the introduction of GRAFIX PL* into the sterile field.

Mode of Use/Application
GRAFIX* Membrane Products can be applied in an office, hospital outpatient setting or in an operating room. GRAFIX* Membrane Products naturally conform to complex anatomies and may be used over exposed bone, tendon, joint capsule, muscle, and hardware. GRAFIX* Membrane Products may be used to repair acute and chronic wounds, encompassing both upper extremity and lower extremity, including but not limited to: diabetic foot ulcers, venous leg ulcers, pressure injuries, dehisced surgical wounds, burns, acute surgical wounds, pyoderma gangrenosum, and epidermolysis bullosa.

Removal & Change Frequency
Reapply weekly at the discretion of the responsible physician or health care professional for the duration of treatment.

Additional Recommended Dressings
Non-adherent dressing and outer dressings.

Benefits
• Can be used for acute and chronic wounds such as, diabetic ulcers, pressure injuries, surgical wounds, burns, and venous ulcers
• Flexible, conforming cover that naturally adheres to complex anatomies and may be used over exposed structures such as bone, tendon, joint capsule, muscle, and hardware
• Designed for application directly to acute and chronic wounds
• Immune neutral
• No need for sutures or Steri-Strips®
• Available in multiple sizes to reduce wastage

Recommended Use
Acute Wounds
Arterial Ulcers
Burns
Chronic Wounds
Deep Wounds
Dehisced Wounds
Diabetic Foot
Granulating/Epithelializing Wounds
Pressure Ulcers
Pyoderma Gangrenosum
Superficial Wounds
Surgical Dehiscence
Surgical Incisions
Trauma
Venous Leg Ulcers

Contraindications
There are no known contraindications for these products
.

Warnings and Precautions
Intended for use in one patient, on a single occasion only.
Do not use if package integrity has been compromised. Once the user breaks the seal on the pouch,

GRAFIX* Membrane Products must be transplanted or discarded.
GRAFIX* Membrane Products may not be sterilized.
GRAFIX* Membrane Products are intended for use by qualified health care specialists such as physicians, podiatrists, or other appropriate health care professionals.
The same medical/surgical conditions or complications that apply to any medical/surgical procedure may occur during or following application.
The health care professional is responsible for informing the patient of the risks associated with his/her treatment and the possibility of complications or adverse reactions.
Caution should be exercised for patients with known sensitivities for reagents used for processing, disinfection, and storage which may remain on the product.

Adverse Effects/Reactions
Donor screening methods are limited; therefore, certain diseases may not be detected. The following complications of tissue transplantation may occur:
• Transmission of infectious agents or diseases of known or unknown etiology including, but not limited to fungi, bacteria, or viruses;
• Immune rejection of implanted GRAFIX* Membrane Products; or
• Loss of function and/or integrity of GRAFIX* Membrane Products due to resorption, fragmentation, and/or disintegration.

Storage Requirements
GRAFIX* Cryopreserved Placental Membrane has a 3 year shelf life and and should be stored frozen at -75°C to -85°C (-103°F to -121°F). GRAFIX* PL Lyopreserved Placental Membrane Tissue has a 2 year shelf life and should be stored at room temperature.

How Supplied/Sizing
1.5cmx2cm, 2cmx3cm, 3cmx4cm, 5cmx5cm. Round: 16mm.

6.4 Epifix® Product Information

Description

Human amniotic membrane is a thin, collagenous membrane derived from the placenta, the area in which the human fetus grows and develops within the mother's uterus. Human amniotic membrane consists of multiple layers.

EpiFix is a minimally manipulated, dehydrated, non-viable cellular amniotic membrane allograft that contains multiple extracellular matrix proteins, growth factors, cytokines and other specialty proteins present in amniotic tissue to provide a barrier membrane that enhances healing.

EpiFix allografts are human tissue products and appearance may vary between donors. Variations in color (tan to light brown), opacity, and thickness are normal due to the nature of human tissue.

Tissue Uses
EpiFix Amniotic Membrane Allograft is intended for homologous use in the treatment of acute and chronic wounds to reduce scar tissue formation, modulate inflammation, provide a barrier, and enhance healing.

Contraindications

EpiFix should not be used on (1) areas with active or latent infection and/or (2) a patient with a disorder that would create an unacceptable risk of post-operative complications.

Precautions/Warnings

• EpiFix allografts remain suitable for transplantation in an unopened, undamaged package, under proper storage conditions.
• Please inspect the integrity of the package upon receipt. If package and contents appear defective or damaged in any way, immediately contact the distributor.
• This allograft is intended for single-patient use only. Discard all unused material.
• The procedure should be performed by an authorized medical professional.
• Strict donor screening and laboratory testing, along with dedicated processing and sterilization methods, are employed to reduce the risk of any disease transmission. However, as with all biological implants, an absolute guarantee of tissue safety is not possible. This allograft has the potential to transmit infectious disease to the recipient.
• The reaction of the body to any biological implant is not completely understood.
• Caution should be used when treating patients with a known sensitivity to aminoglycoside antibiotics.
• Discard all damaged, mishandled or potentially contaminated tissue.
• This product has not been tested in combination with other products.
• DO NOT RE-STERILIZE.

Preparation, Reconstitution and Use

Prior to implantation, carefully follow the EpiFix allograft preparation steps below using aseptic technique:

Wound Bed Preparation

• Ensure the wound is free from clinical signs of infection.
• Prepare wound bed as needed.

Removing EpiFix from Packaging

• The outer peel pouch is NOT sterile. The inner pouch that contains EpiFix is sterile (unless the pouches are damaged or compromised).
• Carefully open the peelable corner of the outer pouch and extract the inner pouch using aseptic technique. Ensure the inner pouch does not come in contact with any portions of non-sterile surface of the outer pouch.
• Using aseptic technique, SLOWLY peel a corner of the inner peel pouch and grasp EpiFix with fingers or non-toothed, sterile forceps.
• Use EpiFix promptly after opening the inner, sterile pouch.

PLEASE TAKE GREAT CARE WHEN REMOVING EpiFix FROM THE INTERNAL POUCH. EpiFix IS THIN AND EXTREMELY LIGHTWEIGHT.

EpiFix Preparation

1. In a dry state, use sterile dry scissors to cut EpiFix to fit within the wound margins. It is acceptable to overlap the wound margins with EpiFix by 1mm.
2. EpiFix can be applied wet or dry.
3. EpiFix can be hydrated while on the wound site with sterile saline solution. Simply apply several drops of sterile solution to EpiFix. During and following hydration, the embossment on EpiFix will begin to fade.

EpiFix Orientation & Application

EpiFix should be placed on the wound site, using the orientation of the embossment lettering as a guide. Proper orientation of EpiFix can be noted when the embossment nomenclature reads correctly from left to right. If the mesh process has diminished the ability to clearly read the embossment, the following two alternate methods may be used for placement:

1. An elongated, horizontal perforation is located in the top left area of the graft connecting two adjacent perforations. Proper orientation can be noted when the graft is placed on the wound site such that this horizontal perforation remains visually in the top left area of the graft.
2. The clear side of the inner pouch is indicative of the UP side of the graft orientation. Proper placement can be achieved by removing the graft from the pouch with the clear side up and moving to the wound site with that same orientation.

Absorbable, non-absorbable suture material and/or tissue adhesives can be used to fixate EpiFix to the wound site, if desired.

Primary Dressing

• EpiFix should be covered with a non-adherent contact layer.
• EpiFix should NOT be disturbed, if possible, for several days or before the next application, if needed.
• If an infection occurs at the graft site, treat infection per institution's protocol. Secondary Dressing
• EpiFix requires a moist wound environment. Use appropriate moisture management dressings for the wound type and treatment ideology.

Support Therapies

• EpiFix is compatible with offloading/compression/negative pressure therapies.
• EpiFix can be used in conjunction with hyperbaric oxygen therapy.

Re-application of EpiFix

• It is recommended that EpiFix grafts are applied weekly until wound epithelialization is achieved. However, clinician discretion should be used based on patient and wound condition/progress. It is clinically acceptable to apply EpiFix on a biweekly basis if desired.

Adverse Effects & Reporting

• As with any procedure, the possibility of infection exists.
• Proprietary processing and validated sterilization methods are employed to eliminate potential deleterious components of the allograft. However, as with all biological implants, the possibility of rejection exists.
• Any adverse reactions, including the suspected transmission of disease attributable to this allograft, should be reported immediately to MiMedx®.

Acceptable Storage

EpiFix allografts should be stored in a clean, dry environment at ambient conditions. EpiFix allografts have a 5 year shelf life. Check the label for the expiration date.

7 STANDARD OF CARE, CONCOMITANT MEDICATIONS, EXCLUDED/ALLOWED THERAPIES/MEDICATIONS AND ALLOWED DRESSINGS

Beginning at the screening visit, ALL subjects must have their target ulcer managed using the SOC procedures noted below.

7.1 SOC Procedures

7.1.1 Cleaning the Target Ulcer

Remove all dressings. The leg should be elevated for as much time as possible during this process. Wash the foot with sterile water or saline solutions. Gently irrigate the target ulcer prior to each dressing change with the same solution. Wound cleansers containing antiseptics (examples: Hypochlorous Acid, Vashe, ExSept, PrepIt, etc.) are prohibited for use during the duration of this study.

During the Treatment Phase, the washing and cleaning of the DFU must be done prior to application of SOC primary dressing.

For the TV2 and TV3 additional visit where application of a new Helicoll® does not occur, the primary dressing will be lifted from the wound site along with the Helicoll® Advanced Skin Substitute or Epifix® or Grafix®. If the Helicoll® Advanced Skin Substitute is adherent to the wound, moisten gently with saline before attempting to remove. Cleaning of the target ulcer as noted above is suggested, however debridement should be limited, and it is suggested that only a very gentle removal of any slough be performed at the investigator discretion. Digital Photography is then taken and the Helicoll® Advanced Skin Substitute or Epifix® or Grafix® and primary dressing should be placed back on the wound. If the primary dressing is soiled or damaged a new primary dressing can be applied.

7.1.2 Debridement of the Target Ulcer

Debridement is an essential technique and SOC in the treatment of DFU. It is important to remove all non-viable and necrotic material from the target ulcer prior to enrolling the patient. Debridement is allowed during the treatment phase at the treating Investigator's discretion. For detailed guidelines please see Appendix E as well as recommendations for debridement during the 2nd visit in week 2 and 3 in which a new Helicoll® is not placed.

7.1.3 Offloading

Offloading is essential if the wound is to heal. A diabetic offloading CAM boot will be given to each subject, and this may be augmented with felt or foam to offload the ulcer or if the subject cannot be accommodated into a CAM boot, a TCC will be provided.

Subjects will be educated on the importance of using the device to offload their DFU and instructed on keeping dressings dry and to call or visit the study site if the dressing becomes soiled, wet or is removed. In addition, subjects should be educated on wound infection and if they observe infection, to call or visit the study site.

7.2 Concomitant Medications

The subject may be administered any necessary medications, at the discretion of the Investigator; provided such medications are not applied topically to the target ulcer surface (topical medication can be applied to other surfaces around the ulcer or other non-reference ulcers).

All medications and therapies administered or taken by the subject beginning 30 days prior to signing the ICF and throughout the study will be recorded in the source documents and for randomized subjects, on the appropriate CRF.

7.3 Prohibited Medications and Therapies

The following treatments and medications are prohibited 30-days prior to first screening visit and throughout the trial:

- Any cellular and/or tissue-based products or wound dressings that include growth factors (e.g., Regranex, Dermagraft, Apligraf, GraftJacket, OASIS, Primatrix, Matristem) placed on the target ulcer
- Revascularization surgery on the lower extremity with the target ulcer
- Radiation therapy to the foot

7.3.1 Excluded starting at SV1

- Topical antibiotics* or other topical agents, including or containing silver, honey, hydrofera blue, etc. (with the exception of anesthetics used during debridement)
- Systemic steroids/oral corticosteroids in excess of 10mg daily dose. (Note: inhaled steroids are acceptable)
- Other immunosuppressive agents and chemotherapy
- Cytotoxic therapies
- Negative pressure wound therapies
- Hyperbaric oxygen
- Any other investigational treatment/medications in the Investigator's opinion are likely to seriously impact wound healing
- Heat lamps
- UV lights
- Whirlpool baths
- Water Piks™
- Jet water streams (other than gentle water irrigation)
- Wound cleansers that contain active ingredients and are not just normal saline (e.g., Hypochlorous Acid, Vashe, ExSept, PrepIt, etc.)
- Selective COX-2 inhibitors (such as Celecoxib)

*Topical antibiotics must be stopped at the first screening visit.

7.4 Allowable Medications and Therapies

The following medications and therapies are allowed during the study, if in the opinion of the Investigator, they are required for proper care of the study subject:

- Use of anesthetics for debridement
- Treatment with systemic antibiotics for acute or chronic infection, however, prophylactic use of systemic antibiotics is not allowed

- Other medications/therapies that are not otherwise prohibited and in the opinion of the Investigator, are required for proper medical care

7.5 Approved Dressings

The following is a list of approved dressings suitable for study DFUs receiving SOC treatment:

SOC Helicoll® Group

- Helicoll® – Advanced Skin Substitute

Helicoll Sizes intended for this study	Grafts per box
0.8 in x 1.6 in 2 cm x 4 cm (8 sq cm)	2
1.6 in x 1.6 in 4 cm x 4 cm (16 sq cm)	2

- Plain foam (e.g., Allevyn gentle, Mepilex) or Mepitel/Adaptic Touch
- Three-layer dressing of first layer - non-adherent and porous, second layer – absorbent 4x4 gauze pads & third layer - soft cast roll and ace bandage/Coban

SOC Epifix® or Grafix® Group

- Epifix® or Grafix®
- Plain foam (e.g., Allevyn gentle, Mepilex) or Mepitel/Adaptic Touch
- Three-layer dressing of first layer - non-adherent and porous, second layer – absorbent 4x4 gauze pads & third layer - soft cast roll and ace bandage/Coban

8 DESCRIPTION OF PROTOCOL ASSESSMENTS AND PROCEDURES

8.1 Informed Consent

Written informed consent will be obtained for this study by the Investigator or suitably qualified designee from all subjects before the collection of any protected health information and performance of any protocol-specific procedure.

In obtaining and documenting informed consent, the Investigator must comply with applicable regulatory requirements and must adhere to Good Clinical Practice (GCP). The Investigator, or designee, must fully inform subjects of all pertinent aspects of the study. Before informed consent may be obtained, the Investigator, or a person designated by the Investigator, must provide the subject ample time and opportunity to inquire about details of the study and to decide whether or not to participate in the study. All questions about the study must be answered to the satisfaction of the subject. Prior to the subject's participation in the study, the ICF must be signed and personally dated by the subject and by the person who conducted the informed consent discussion. For screen failure subjects who re-screen at a later date, re-consenting is required.

8.2 Assessment of Eligibility

During the Screening Visit/Period, the Investigator must assess a subject's continued suitability and eligibility for the study, especially with regard to the Inclusion and Exclusion criteria. If the subject is no longer suitable or eligible for the study, the subject will be considered a screen failure. Screen failure subjects may be re-entered into the study at a later time and re-screened.

8.3 Subject Demographics, Medical History and Ulcer History

8.3.1 Demographics

For the purposes of this study, demographic information will include:

- Date of ICF signature
- Date of birth
- Gender at birth
- Race (American Indian/Alaskan Native, Asian, Black/African American, Native Hawaiian/Pacific Islander, Caucasian or other)
- Ethnicity (Hispanic/Latino or Not Hispanic/Latino)
- Use of tobacco products

8.3.2 Medical History

A medical history will be recorded during the Screening Period and will include:

- All ongoing medical conditions
- All previously resolved medical conditions related to diabetes or foot ulceration or which are relevant in the opinion of the Investigator

Events that emerge prior to the randomization visit will be recorded in the medical history and not as Adverse Events. Aside from being used to determine subject eligibility, this information will permit the Investigator to record the nature, duration and severity of any ongoing baseline medical conditions that occur prior to randomization.

Medical histories will be recorded using the body system categories outlined below:

- Cardiovascular
- Respiratory
- Gastrointestinal
- Renal
- Hepatic
- Neurological
- Endocrine
- Lymphatic
- Hematologic
- Immunologic
- Dermatologic
- Psychiatric
- Genitourinary
- Other
- DFU History

For each relevant history, the following will be documented:

- Disease/disorder/condition
- Date of diagnosis
- History status (resolved or ongoing)

8.3.3 Foot Ulcer History

- Duration of the current DFU

  Note: "Duration" is defined as the length of time that the target ulcer has been open at this location since the last time it was fully closed.

- Current offloading system used for the DFU (if any) and length of time that this has been used
- Prior treatments that have been used on the DFU for up to 1 year
- Age when the subject developed his/her first DFU
- Total number of previous DFUs
- Location of the current DFU

  Note: "Target ulcer location" is defined by the ulcer being on the left or right foot, by the location of the ulcer on the foot, dorsal or plantar, toe (which toe), forefoot, midfoot, hind foot, ankle heel and by the positioning of the ulcer as lateral, medial, dorsal, or plantar located.

- Number of additional DFUs and location of each present at the screening visits
- History of DFU recurrence

  Note: "Recurrence" is defined as the re-opening of the target ulcer after complete healing.

- History of any amputations in the study foot and contralateral foot or leg
- History of any significant foot deformities, dermatological abnormalities, fungal lesions, or other findings.

8.3.4 Physical Exam

The physical examination will include routine examinations for the following:

- Head, ears, eyes, nose, and throat
- Abnormalities of the extremities

- Neurologic abnormalities
- Heart/cardiovascular abnormalities
- Musculoskeletal abnormalities
- Dermatologic abnormalities
- Any other body system for which an abnormality is noted and which, in the opinion of the Investigator, is relevant to the safety of the subject or could impact safety or efficacy results for the subject, i.e., the abnormality is clinically significant

Each abnormality will be recorded, and the Investigator will record an assessment of its clinical significance.

8.3.5 Vital Signs

The following vital signs will be collected:

- Height
- Weight
- Seated blood pressure (take after the subject has been seated for at least 5 minutes)
- Pulse
- Temperature

8.3.6 Pregnancy test

Females who are of childbearing potential must have a urine or blood pregnancy test at SV1.

8.3.7 Laboratory tests

The following laboratory values will be collected per the schedule of visits (Table 1):

- Serum Creatinine
- HbA1c
- Blood Glucose

8.4 Peripheral Perfusion Assessment

One of the following assessments will be completed to confirm sufficient tissue perfusion for trial participation.

8.4.1 ABI or Arterial Doppler Ultrasound

ABI, also known as the Winsor Index and the Ankle Brachial Pressure Index, is the ratio of blood pressure measured at the ankle to that measured at the arm. Details of the procedure can be found in Appendix B. An ABI < 0.8 indicates that there is a high probability that arterial insufficiency is present (positive predictive value 95% in a general practice population).

It should be noted that incompressible, calcified arteries may occur in diabetes causing a falsely elevated ABI, so if the subject has other signs or symptoms that could suggest peripheral arterial disease, further investigations to determine vascular status may be warranted.

A documented record of an ABI test performed using the target ulcer leg, within one month of SV1 is acceptable for the purposes of this study, otherwise this must be completed in the Screening Period.

As an alternative, arterial Doppler ultrasound can be performed evaluating for biphasic dorsalis pedis and posterior tibial vessels at the level of the ankle.

ABI test to be included in week 1, 1.5 and 2 to monitor neo-vascularization.

8.4.2 SPP

SPP provides another noninvasive method for measuring microcirculatory pressure of the artery at the skin level. It measures the pressure at which perfusion first returns to the cutaneous microcirculation following a controlled release of occlusion. SPP is useful in assessing the ischemic severity of lower limb and predicting ulcer healing in chronic critical limb ischemia with a value of <30 mmHg. See Appendix C for the appropriate technique of assessment.

8.4.3 TCOM

A TCOM assesses the partial pressure of oxygen molecules dissolved in the blood plasma. The amount of oxygen detected by the sensor is a balance of the oxygen delivery and local physiologic demands and reflects the metabolic status of the skin; it indicates the level of oxygen available at the ulcer site to assist in the ulcer healing process. A TCOM less than 30 mmHg of oxygen indicates impaired ulcer healing and ischemic disease. An appropriate technique for a TCOM assessment is provided in Appendix D.

8.5 Pain Assessment: NPRS

Pain intensity of the reference DFU is to be assessed before any dressing changes or other ulcer manipulations at all screening and treatment visits.

The subject will be asked to indicate a numerical value that best represents the current pain intensity at the target ulcer site on a scale of 0 to 10, anchored by word descriptors at each end, as "no pain" on the left side and "worst possible pain" on the right side of the number line. The number 0 represents “no pain,” the number 5 represents “moderate pain”, and the number 10 represents the “worst possible pain.” The subject indicates the level of pain intensity by selecting a number on the line shown in Figure 3 below that represents their perception of their current state.

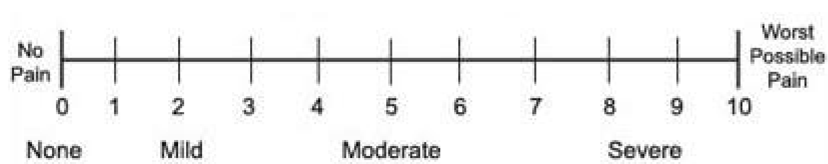


FIGURE 3: SUBJECT PAIN SCALE (NPRS)

8.6 Ulcer Assessments

A number of different target ulcer assessments are required, including appearance, exudate characteristics, infection, and peri-wound characteristics.

8.6.1 Target Ulcer Appearance Assessment (Pre-debridement)

Target Ulcer Appearance Assessment (pre-debridement) categories includes:

- % Granulating *(red/brown bumpy tissue that bleeds upon light debridement)*
- % Non-viable tissue
- % Epithelialized Skin

Note: the categories should add to 100%.

8.6.2 Target Ulcer Exudate Assessments

The Investigator will determine the amount and type, if any, of target ulcer exudate. In determining the amount of target ulcer exudate, the Investigator must take into account the amount of exudate absorbed into the target ulcer dressing. Table 2 below details the categories will be used to quantify the amount and describe the type of target ulcer exudate:

TABLE 2: TARGET ULCER EXUDATE ASSESSMENT

Target Ulcer Exudate Assessment	
Volume	• No exudate • Minimal amount • Light (scant) or small amount • Moderate amount • Heavy/large/copious amounts
Type	• Not applicable: no exudate present • Serous: clear or light-yellow watery plasma • Serosanguinous: pink to light-red watery plasma • Sanguineous: red with fresh bleeding • Purulent: thick and opaque exudate, of creamy yellow, green, white, or tan color

8.6.3 Target Ulcer Infection Assessments

The presence/absence of the following signs of infection at the target ulcer site will be documented at each visit. Infection of the target ulcer will be assessed using the STONEES method developed by Woo and Sibbald.[19] Table 3 below lists the criteria for considering an target ulcer infected:

TABLE 3: TARGET ULCER INFECTION ASSESSMENT

Target Ulcer Infection Assessment
<u>Three</u> or more of the following signs or symptoms are present: • Increased surface area • Increased peri-wound margin temperature by more than 3^0F difference between two mirror image sites • Exposed bone or can be probed to the bone

- New areas of breakdown or satellite lesions
- Presence of swelling or reddened skin in peri-wound area
- Increased wound drainage
- Unpleasant, sweet, or sickening odor present

NOTE: An ulcer that is deemed to be infected with three or more of the following signs will undergo a wound culture to confirm the presence of infection and identify the pathogenic organism, so the study subject receives the most appropriate antibiotic treatment

Infection at Target Ulcer site prior to Randomization

If the infection occurs prior to randomization i.e., prior to TV1, then the subject will be ineligible to be randomized.

Infection at Target Ulcer site after Randomization

If infection of the target ulcer site occurs after randomization i.e., after Randomization visit record the infection as an adverse event and treat it as appropriate with oral antibiotics at the discretion of the Investigator.

Note: application of topical antibiotics and anti-microbial dressings to the target ulcer site are prohibited.

A subject with an infected target ulcer that is being treated by the Investigator will remain in the study unless the situation requires an alternative methodology that violates the protocol. Antibiotic interventions will be recorded on the Concomitant Medications CRF, and the event will be categorized as an Adverse Event, serious if it meets the definition of that category. All subjects who show evidence of an ulcer infection must have it reported on an Adverse Event CRF.

All subjects will be instructed to contact the Investigator if signs or symptoms of infection develop prior to their next scheduled visit.

During an episode of infection, the Investigator should not continue TCC, if it is being used, until the infection is resolved, and dressings should be changed at least every 72 hours.

Evaluate the skin surrounding the target ulcer for presence of signs such as erythema, edema, and cellulitis.

8.6.4 Investigator Assessment of Target Ulcer Closure

"**Complete healing**" of the target ulcer is defined as 100% re-epithelialization without drainage. At each visit, the Investigator will assess the ulcer by answering the following questions:

- Is target ulcer 100% re-epithelialized?
- Is drainage absent?

Both questions must be answered "yes" for the target ulcer to be considering having reached "complete closure."

The date of complete healing is defined as the date of the first assessment of 100% re-epithelialization.

Target Ulcer Photographs and Measurements

The target ulcer will be digitally imaged with a digital camera that is at least 20 (twenty) megapixels, with the photo taken at a focal distance of approximately 18 inches and a two-dimensional calibration scale with two photos taken at a

distance of approximately 18 inches and one taken close up in good focus. The Investigator will also take a ruler and measure length and width of the wound and record appropriately. The area will be calculated using a length by width calculation.

8.7 Randomization

Subjects who are eligible to participate in the study will be randomized to one treatment group at the time of completion of screening.

8.8 Assessment of Offloading

The following questions will be asked to assess the performance of the offloading during the Screening and Treatment Phase visits and at selected follow-up visits:

- Did the subject have the offloading device with him or her or is the TCC cast still present? (Yes/No)
- What percentage of the time is the subject using the offloading device while not sleeping? (Note: heel ulcers might still need to be offloaded while sleeping.)

8.9 Semmes Weinstein Monofilament Test for Peripheral Neuropathy

This is a non-invasive topical evaluation of neuropathy with a monofilament wire to the foot with the target ulcer. The test will be performed in a standard manner with 10 points being evaluated on each visit. At each point, the Investigator asks the subject whether they can feel the filament (yes or no). Note: If a subject is missing a body part called out in the Semmes Weinstein test, the most skin in the area where the missing body part is located is used for the test.

9 DATA AND STATISTICAL ANALYSES

9.1 Data Analysis

This study consists of primary, secondary and exploratory endpoints. Data analysis of study endpoints includes use of validated DFU wound healing measures, quantitative and qualitative analyses, as well as exploratory methodologies. Table 4 below details each study endpoint, corresponding data, and planned analyses.

TABLE 4: DFU ENCOLL ENDPOINT DATA ANALYSIS

Endpoint	Endpoint Description	Supporting Study Data	Endpoint Analysis
Primary	Percentage wound area reduction from TV1-TV5 measured manually with digital photography	Digital imaging	Mathematical wound area change
Secondary	Mean number of repeated applications of the Advanced Skin Substitute used to obtain wound closure	Data on IP application at each visit	Data Management will determine during review/analysis
	The proportion of subjects that obtain complete closure over the 5-week treatment period	Investigator assessment of periodic healing (up to 100% epithelization w/no drainage), measurements of ulcer size using digital photos	Study Chair will determine using photographs of the DFU
	The time to achieve complete wound closure of the target ulcer by the end of 5 weeks	Data on the progress of wound healing measured at different time periods	Mathematical time based on healing The relative success of healing in percentage will be measured for each subject using the following calculation: Percentage of Healing = (Actual days to heal / 28) X 100
Exploratory	The appearance, structural stability, and fragility of the new skin formed documented at each visit. Any recurrence of the wound also will be monitored.	Observation	Complete listing of TEAEs, categorized by treatment group and system organ class

	The number and type of Treatment Emergent Adverse Events		

9.2 Statistical Analysis

Descriptive statistical methods will be used to summarize the data from this study with no hypothesis testing performed for the endpoints.

A formal Statistical Analysis Plan (SAP) will be created prior to database lock. The primary endpoint analysis will be at 5 weeks and a series of secondary and exploratory endpoints will be analyzed at 5 weeks (PAR will be analyzed at all timepoints). The SAP will provide a more technical and detailed description of the proposed data analysis methods and procedures. Any deviation from the analyses outlined in the protocol will be described in the most current version of the SAP. All statistical analyses will be conducted using PASW 28.

No power analysis was conducted as the numbers of subjects enrolled in this trial are too small.

The populations defined for analysis will include the intent-to-treat (ITT) and per protocol (PP). Additional analysis populations may be defined to evaluate study results. Any additional analysis populations will be defined in the SAP.

Primary analysis: The percentage wound area reduction from TV1 to TV5 measured manually with digital photography

Secondary analysis: The time to achieve complete wound closure of the target ulcer by the end of 5 weeks

The proportion of subjects that obtain complete closure over the 5-week treatment period

Mean number of repeated applications of the Advanced Skin Substitute used to obtain wound closure

Endpoint analysis: The appearance, structural stability, and fragility of the new skin formed are documented at each visit. Any recurrence of the wound also will be monitored.

Safety analysis: Tabular listings of all TEAEs per treatment group will be created using system organ class. Other analysis will be described in the SAP.

Every effort will be made to obtain required data at each scheduled evaluation from all subjects who have been enrolled and treated. Subjects who are withdrawn or lost to follow-up will be included in the ITT analysis of primary and secondary endpoints using data imputation methods detailed in the SAP. Sensitivity analysis will also be conducted for the primary endpoint using different imputation algorithms.

10 ADVERSE EFFECTS AND EVENTS (DEFINITIONS AND REPORTING)

The Investigator is responsible for the detection and documentation of any events meeting the criteria and definition of an adverse effect or adverse event (AE), as described in this protocol. During the study when there is a safety evaluation, the Investigator or site staff will be responsible for detecting, documenting, and reporting any adverse effect or adverse event, as detailed in this section of the protocol.

10.1 SOC Adverse Events

Since this a post market study involving commercially available SOC dressings of FDA cleared and approved medical devices; adverse events associated with their use may also require reporting to FDA along with the possible reason for the failure of the treatment which may include product handling and other details.

An AE is defined as any unfavorable or unintended sign, symptom or disease that occurs or is reported by the subject to have occurred, or a worsening of a pre-existing condition related to the procedure or medical treatment (device/drug) used. All AEs related to the study procedure or medical treatment, including intercurrent illnesses, must be recorded in the subject's medical records and on the CRF. CTCAE v5.0 will be used for AE reporting.

AEs will be defined as those events that occur after the first study treatment is applied at TV1, through the final study visit. A description of the AE along with the onset date, end date, severity, action taken, treatment, outcome, likely cause and relationship to the study procedure or products will be recorded in the CRF.

AEs associated with each treatment arm will be tabulated and compared at the end of the study. AEs will be elicited through direct questioning, subject reports, and physical examination.

An abnormal laboratory test result is not by itself considered to be an AE unless the Investigator considers the finding of clinical significance that should be reported in such a manner**.**

The Investigator is responsible for assessing the relationship of the AE to the procedure or medical treatments used and the seriousness and expectedness of the AE at the time of occurrence. A medically qualified person appointed by the Sponsor will also assess this once the Sponsor has been notified of an AE. For enrolled subjects, all AEs that occur during the trial will be documented on the AE CRF.

AEs reported during the study should be followed to resolution of the AE or within thirty days from the end of the study. A final assessment of the outcome will be made at that time.

Each AE related to the study procedure or medical treatment used will be categorized as "serious" or "not serious," based on the definition of a serious adverse event (SAE). A SAE is defined as an AE resulting in at least one of the outcomes described in Section 10.1.3.

10.1.1 AE Severity Assessments

The guidelines outlined in CTCAE v4.0 will be used for severity assessments. <u>Note</u>: the term "severe" is a measure of intensity and that a severe AE is not necessarily serious. Table 5 below provides guidance on determining the severity of an AE.

TABLE 5: AE SEVERITY GRADING SCALE

Severity Grade	Description
Mild (1)	Awareness of sign, symptom, or event, but easily tolerated; does not interfere with usual daily activities or tasks.
Moderate (2)	Discomfort is enough to cause interference with usual daily activity. It may warrant therapeutic intervention.
Severe (3)	Incapacitating: inability to perform usual activities and daily tasks; significantly affects clinical status; requires therapeutic intervention.
Life-threatening (4)	Emergency treatment required, life-threatening, death.

A grade of 1-4 is assigned by the Investigator to each AE.

10.1.2 AE Causality Assessments

AEs will be assigned a relationship (causality) to the study procedure or medical treatments. The Investigator will be responsible for determining the relationship between an AE and the study procedure or medical treatment. The type of event, organ system affected and timing of onset of the event will be factors in assessing the likelihood that an AE is related to the study procedure or medical treatment. The relationship of AEs to study procedures or medical treatments will be classified as follows:

- Not Related: No relationship exists between the AE and the study procedure or medical treatment. The event is attributed to a pre-existing medical condition or an inter-current event unrelated to the study procedure or medical treatments.
- Possibly Related: Follows the study procedure or medical treatment but may have developed as a result of an underlying clinical condition or treatments/interventions unrelated to the study.
- Probably Related: Follows the study procedure or medical treatment but is unlikely to have developed as a result of the subject's underlying clinical condition or other treatment or other interventions.
- Definitely Related: Follows the study procedure or medical treatment and physical evidence shows a convincing relationship to the study procedure or medical treatment.
- Unknown: Follows the study product or medical treatment, but unable to determine the relationship to the study procedure or medical treatment.

10.1.3 Serious Adverse Events

A SAE is any untoward medical incident that occurs during the course of the study beginning after informed consent has been executed.

A SAE is defined as any AE that:

- Results in death
- Is life threatening (the subject is at immediate risk of dying from the adverse experience)
- Requires subject hospitalization or prolongs existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect

Note: Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a SAE when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

10.1.3.1 Reporting of Serious Adverse Events

The Investigator or delegated site personnel is required to report all SAEs that occur during the study. Once the Investigator or delegated site personnel becomes aware of a SAE, he/she must report the SAE to the Sponsor and copy the CRO within 24 hours.

The study sponsor contact is: Subra Guna, PhD

Phone: 510-396-8581

Email: guna@encoll.com

A written SAE report must follow and must include a full description of the event and all supporting documentation available at the time (e.g., lab reports, culture reports, etc.). Additional follow-up information as it becomes available must be reported to the CRO and/or Sponsor.

The Investigator or delegated site personnel is also responsible for reporting all SAEs to the IRB in accordance with IRB reporting guidelines, local laws and regulations. The Investigator is responsible for maintaining documentation in the study file that indicates the IRB has been properly notified.

10.1.3.2 SAE Follow-up

All SAEs will be monitored for a minimum of 30 days until they are resolved, have stabilized, or are clearly determined to be due to a subject's stable or chronic condition or intercurrent illness (es).

11 DIRECT ACCESS TO SOURCE DATA/DOCUMENTATION

Subjects will be identified on CRFs by a unique subject identification number and on source documents by name and date of birth. No personal identifier will be used in any publication or communication used to support this research study. The subject identification number will be used if it becomes necessary to identify data specific to a single subject.

The monitors, auditors, personnel authorized by the Sponsor, the local IRB and applicable regulatory bodies are eligible to review medical and research records related to this study as a part of their responsibility to protect human subjects in clinical research and will be given direct access to source data and documentation (e.g., medical charts/records, printouts, etc.) for source data verification, provided that subject confidentiality is maintained in accordance with local requirements. Access to electronic medical records may be governed by institution policy and each site will be required to ensure access while remaining compliant with institutional requirements.

12 QUALITY CONTROL AND QUALITY ASSURANCE

12.1 Acceptability of CRF

CRFs must be completed for each subject who has signed an ICF. For subjects who are screen failures, this would be limited to Screening or other applicable forms. All source documents and CRFs will be completed as soon as possible after the subject's visit.

12.2 Modification of Protocol

The Investigator will not modify or alter this protocol without first obtaining the concurrence of the Sponsor. Approval by the Investigator's IRB must also be obtained prior to implementation of the change, with two exceptions:

- When necessary to eliminate apparent immediate hazard to the subject, or
- When the modification does not involve the subject's participation in the trial.

An amendment may also require modification of the ICF. The Investigator will provide an approval letter for the amendment and revised ICF, if applicable, to the Sponsor. An amendment must be made in writing, and it must be dated by both the Sponsor and the Investigator. All material must be approved by the IRB.

12.3 Reporting Protocol Deviations

The Investigator is obligated to follow the protocol without departure from the requirements written in the protocol. Any time there is deviation from any protocol requirements, a protocol deviation is required to be documented within the subject's source documentation. If the deviation may impact subject participation, the Sponsor should be notified and will make the determination as to whether the subject will continue in the study. The Sponsor also has the right to discontinue the subject for protocol violations. The IRB may also have to be contacted if safety to the subject or if the scientific soundness of the study is involved. All protocol deviations must be documented in the CRFs. Protocol deviations will be tracked at the site level and (by the Sponsor) at the study level for the duration of the trial.

13 ETHICS AND REGULATORY REQUIREMENTS

This study is to be conducted in accordance with the requirements of this protocol and in accordance with principles consistent GCP, ICH E6, HIPAA regulations (45 CFR Part 164) and the Belmont Principles of respect for persons, beneficence, and justice. No protocol changes will be implemented without prior review and approval of the IRB, except where it may be necessary to eliminate an immediate hazard to a study subject. In such a case, the change will be reported to the IRB as soon as possible, according to IRB requirements. Additionally, all products used in this study are manufactured, handled, and stored in accordance with their FDA labeling.

13.1 IRB

The Investigator at each center will provide the IRB with study materials, including but not limited to the clinical study protocol, ICF and any advertising materials. The study will not be initiated until the IRB provides written approval of the aforementioned documents and until approval documents have been obtained by the Principal Investigator and Sponsor or Sponsor designee. The site Investigator will not participate in the decision. If the Investigator is an IRB member, documentation must be provided indicating recusal from the approval process. Appropriate reports on the progress of this study by the Investigator will be made to the IRB as required by local and applicable government regulations and in agreement with policy established by the Sponsor. The Investigator is required to maintain an accurate and complete record of all written correspondence to and received from the IRB and must agree to share all such documents and reports with the Sponsor.

No changes from the final approved protocol will be initiated without IRB prior written approval or favorable opinion of a written amendment, except when necessary to eliminate immediate hazards to subjects or when the change involves only logistics or administration.

13.2 Investigator's Responsibilities

The Investigators are responsible for performing the study in full accordance with the protocol and the current revision of the Declaration of Helsinki, the ICH GCP Consolidated Guideline and any applicable national and local laws and regulations. Information regarding any study centers participating in this study that cannot comply with these standards will be documented.

13.3 Subject Informed Consent Requirements

Written and oral information about the study in a language understandable by the patient will be given to all patients by the Investigator and/or designee. Written informed consent will be obtained from each patient before any procedures or assessments that would not otherwise be required for the care of the patient are done and after the aims, methods, anticipated benefits, potential hazards, and insurance arrangements in force are explained, and the patient has been given sufficient time to ask questions and consider participation in the study. It will also be explained to the patients that they are free to refuse entry into the study and free to withdraw from the study at any time without prejudice to future treatment. It is permissible for a third person (e.g., a family member) to be present during the explanation of the study.

The written ICF is to be in compliance with 21 CFR § 50.27 and GCP requirements. The Sponsor and/or designated CRO will approve the ICF and all amendments to the ICF prior to submission to the IRB. A copy of the ICF to be used will be submitted by the Investigator to the IRB for review and approval prior to the start of the study. Each study site must provide

the Sponsor with an unsigned copy of the IRB-approved ICF along with applicable documentation to support this approval. The original signed ICF is retained in the subject's study records and a copy are provided to the subject. A second copy may be filed in the subject’s medical record, if allowed by institutional policy.

14 DATA HANDLING AND RECORD KEEPING

14.1 Recording and Collection of Data

The primary source document for this study will be the subject's medical record. If separate research records are maintained by Investigators, the medical record and the research records will be considered the source documents for the purposes of auditing the study.

Applicable source data will be manually transcribed to the approved CRF. The Investigator is ultimately responsible for the accuracy of the data transcribed on the forms. All source documents and CRFs will be completed as soon as possible after the subject's visit.

The Investigator will review CRFs to indicate that, to his/her knowledge, they are complete and accurate. If further changes are made after this, the Investigator will need to again sign the Investigator signature page. Designated source documents will be signed and dated by the appropriate study personnel. The Investigator must agree to complete and maintain source documents and CRFs for each subject participating in the study.

All research data will be entered, either electronically or manually, into a computerized database, designed in accordance with the clinical data manager.

14.2 Clinical Data Management and Monitoring

The CRO will be responsible for the processing and quality control of the data. The study will be monitored by the CRO. Monitoring may consist of either on site or remote review of any and all applicable study records. All study CRFs and background source documents will be made available for monitoring. The study monitor will ensure that the investigation is conducted according to the protocol design and regulatory requirements.

14.3 Archiving

All study documentation at the Investigator site will be archived in accordance with the study Clinical Trial Agreement and this protocol.

Study records are subject to inspection by applicable health and regulatory agencies at any time.

Records to be retained by the Investigator include, but are not restricted to:

- Source data and the primary records upon which they are based (e.g., subject's progress notes, AE data, test results and any other diagnostic procedures required to evaluate the progress of the study)
- Signed protocols and protocol amendments
- Laboratory results, ranges, and certifications
- Product (SOC dressing supplies) and accountability records
- Study personnel signature log
- Correspondence to and from the Sponsor, designee, and IRB
- Investigator and sub-Investigator CVs
- Signed ICF and HIPAA consent forms
- Subject screening and enrollment log
- AE or SAE event reports

- IRB approval, re-approval letters and reports
- Any documents pertaining to the conduct of the study

These documents must be maintained and kept on file by the Investigator so that the conduct of the study can be fully documented and monitored.

15 PUBLICATION PLAN

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 and the Principal Investigator to publish or present the study results together with the other sites unless specific permission is obtained in advance from the Sponsor to publish separate results. Co-authorship with any of the Sponsor's personnel will be discussed and mutually agreed upon submission of a manuscript for publication.

All information concerning the Sponsor's operations (such as patent applications, formulae, manufacturing processes, basic scientific data 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 other purposes without written consent.

Publication and Disclosure: Because this is a multi-center study, sites and Investigators shall not independently publish, publicly disclose, present or discuss any results or information pertaining to site's and Investigator's activities conducted under this agreement, until such a multi-center publication is released under Sponsor's direction; provided, however, that if a publication is not released within eighteen (18) months after completion of analysis of all study data from all sites within the multi-center study, site and Investigator shall have the right to publish the results of and information pertaining to site's and Investigator's activities conducted under this protocol and the clinical trial agreement with sponsor permission. Site and Investigator agree to submit any proposed manuscript, presentation, or other public disclosure regarding the study to Sponsor for review at least thirty (30) days prior to submitting such proposed manuscript to a publisher or delivering or making such presentation or other public disclosure to any third party. Within thirty (30) days of its receipt, Sponsor shall advise site and/or Investigator, as the case may be, in writing of any information contained therein that is confidential information (other than research results included in a proposed manuscript) or that may impair Sponsor's ability to obtain patent protection. Sponsor shall have the right to require site and/or Investigator, as applicable, to remove specifically identified confidential information (but may not require removal of research results from a proposed manuscript. However, the Sponsor retains all the rights to use their own technical explanation to explain the research results which cannot be avoided by the Investigator) and/or to delay the proposed submission or delivery of the proposed manuscript or presentation or other public disclosure, for an additional sixty (60) days to enable Sponsor to seek patent protection. Site and Investigator shall not publish, publicly disclose, present, or discuss any results of or information pertaining to sites and Investigator's activities prior to completion of the trial, even if the study is terminated before its completion and the final clinical study report is signed off or with respect to any endpoints or analyses, other than those specified in this protocol.

16 REFERENCES

1. Driver VR, Fabbi M, Lavery LA, et al. The costs of diabetic foot: the economic case for the limb salvage team. J Am Podiatr Med Assoc 2010;100:335-41.
2. Rice JB, Desai U, Cummings AKG, et al. Burden of diabetic foot ulcers for Medicare and private insurers. Diabetes Care 2014;37:651-8.
3. Margolis DJ, Kantor J, Berlin JA. Healing of diabetic neuropathic foot ulcers receiving standard treatment. A meta-analysis Diabetes Care 1999;22:692-54.
4. Mauricio D, Piaggesi A, Ragnarson Tennvall G, et al. Predictors of lower-extremity amputation in patients with an infected diabetic foot ulcer. Diabetes Care 2015;38:852-7.

17. SIGNATURES

The protocol has been reviewed in respect to compliance with ISO 14155: 2020, applicable national regulations and with Encoll Corp's SOPs.

Subramanian Gunasekaran *Electronically signed by: Subramanian Gunasekaran Reason: I am an Approver of this document. Date: Jun 7, 2024 21:17 PDT* 07-Jun-2024

Dr. Subra Guna, PhD Date
Phone: 510-396-8581
Email: guna@encoll.com

APPENDICES

Appendix A: Wagner Grades

Grade 1: Superficial diabetic ulcer Grade 2: Ulcer extension

- Involves ligament, tendon, joint capsule, or fascia
- No abscess or osteomyelitis

Grade 3: Deep ulcer with abscess or osteomyelitis

Grade 4: Gangrene to portion of forefoot

Grade 5: Extensive gangrene of foot

Appendix B: ABI or Arterial Doppler Ultrasound

ABI Technique

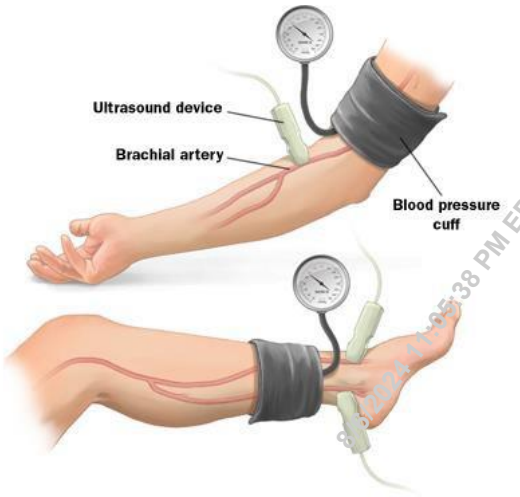
Place the subject in the supine position, with the arms and legs at the same level as the heart, for a minimum of 10 minutes before measurement,

Select an appropriately sized blood pressure cuff for both the ankle and the arms (see figure below), the cuff width should be, at a minimum, 20% greater than the diameter of the extremity. The ankle cuff should be placed on the leg between the malleolus and the calf. Enough room should be left below both cuffs to permit placement of the ultrasound gel, so that the Doppler device can adequately detect the brachial, dorsalis pedis and posterior tibial arteries.

Obtain the brachial systolic pressures of both arms. Use the higher of the arm pressures in the ABI calculation. Obtain the pressure in the dorsalis pedis and posterior tibial arteries for the extremity with the target ulcer. Use the highest pressure for the ABI calculation.

Ankle-Brachial Index = Highest ankle pressure/ Highest brachial pressure.

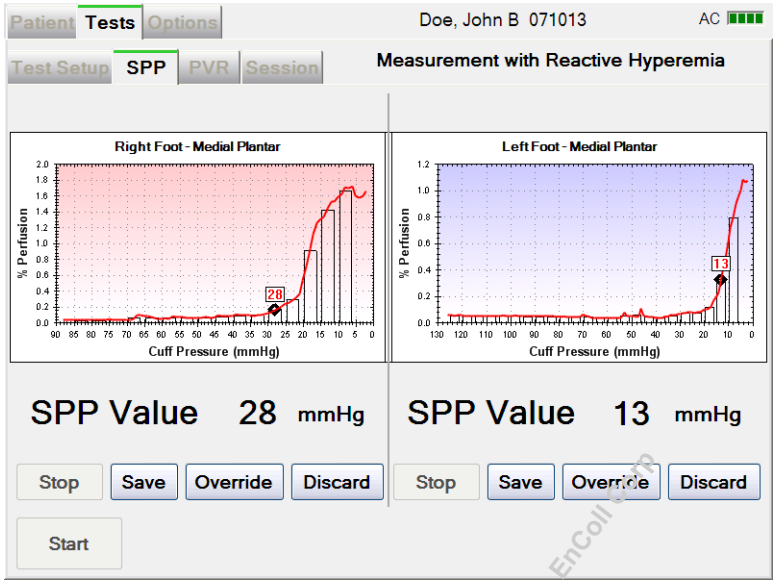
Care should be taken to cover the index ulcer during the ABI measurement. In addition, subjects should be informed that they may experience discomfort during the test, secondary to the pressure exerted by the cuff in the area of skin breakdown.



Arterial Doppler Ultrasound Technique

Place the subject in the supine position, with the arms and legs at the same level as the heart. Ultrasound gel will be applied at the ankle level to the anatomic position of the dorsalis pedis and posterior tibial vessels. The Doppler will then be applied to each vessel until a waveform is established, a printer will be used to document the Doppler waveform and at least five waveforms will be printed for each vessel. The clinician will verify biphasic or triphasic waveform.

Appendix C: SPP



SPP can be employed to evaluate the subject's vascular status. SPP is obtained using a laser Doppler.

Technique

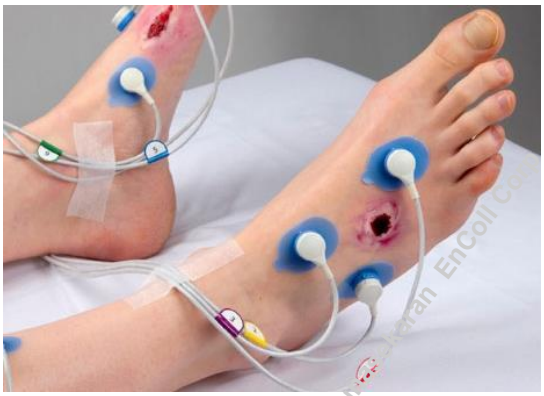
1. Secure the laser Doppler flow sensor within the bladder of a blood pressure cuff equipped with a transparent polyvinyl chloride window for measuring microcirculatory perfusion during cuff inflation and deflation.
2. Place the subject in supine position and keep still for five minutes.
3. Apply the cuff to the proximal margin of the ulcer and inflate to 20 mmHg above the brachial systolic pressure. A stable laser Doppler output value near zero (< 0.1 volume %) should be reached before deflating.
4. Deflate the cuff, first in 10 mmHg-stepwise decrements every five seconds to a pressure of 50 mmHg, and then in 5-mmHg decrements every 15 seconds until the laser Doppler output increased for two consecutive pressure values.
5. The pressure at which this first occurred is considered the SPP value.

Subjects with SPP less than 30 mmHg have vascular insufficiency and are not candidates for enrollment.

Appendix D: TCOM

Technique

Place the subject in the supine position, with the arms and legs at the same level as the heart. Electrodes must be in contact with the tissue through the contact liquid. If there is air between the tissue and an electrode, TCOM values will be questionable. Erroneous readings may also occur if electrodes are placed directly over a bone or there is severe edema around the wound. For the best results, tests should be conducted at ambient temperature (21-23^0) and the subject should not have smoked, nor had caffeine for several hours prior.



1. Calibrate the TCOM electrode—this takes about 15-20 minutes.
2. Clean the selected measuring site with alcohol or other skin-preparation solution.
3. Dry the site well with a gauze pad.
4. Take a standard fixation ring.
5. Remove the fixation ring from the protective film.
6. Apply the fixation ring to the measuring site as follows:
   - Press the center of the fixation ring onto the measuring site with a finger.
   - Run a finger around the rim circumference.
   - Press firmly to prevent leaks.
7. Fill the hole in the fixation ring with 3-5 drops of the contact liquid.
8. Affix the electrode into the fixation ring as follows:
   - Align the arrow on the electrode with one of the marks on the fixation ring.
   - Turn the electrode 90° clockwise to fasten it in the fixation ring.
9. Repeat steps 1 to 8 if more electrodes are to be applied; note: several electrodes can be calibrated at the same time.

It is sometimes advantageous to simultaneously use several electrodes placed strategically around the wound and calculate mean values from individual readings.

The normal sequence of events for TCOM is measurement in air, the leg elevation test (optional) and the oxygen challenge. Shah et al[20] determined that the optimal times for these events in terms of measurement time are 20, 5 and 10 minutes, respectively.

Appendix E: Debridement

Many chronic wounds contain necrotic tissue that has a black or dark gray appearance. Wound eschar is usually full-thickness, dry devitalized tissue arising from prolonged ischemia and slough is an adherent fibrous material commonly creamy yellow in appearance. Chronic wounds can also have colonized bacteria in the form of biofilm or cells, particularly along the margins of a wound, which have stopped dividing - a process known as senescence. Finally, devitalized tissue and biofilms can harbor high levels of cytokines or cellular remnants that maintain the wound in the inflammatory or proliferative stages of healing, through cellular trafficking processes. Any of these factors can disrupt the normal stages of wound healing.

Debridement is the process by which these elements are removed to permit healing, in effect changing the stalled chronic wound into an acute wound. In this clinical study, we have restricted the type of debridement to sharp debridement.

Technique

Sharp debridement comprises the following elements:

1. The target ulcer and the surrounding skin are prepped with water or Saline.
2. Anesthesia, topical or injected, is applied to the ulcer as necessary to reduce subject discomfort.
3. Using a sterile technique, all non-viable tissue in the wound bed is excised using a scalpel and scissors.
4. Excessive bleeding is controlled by using direct pressure, but cautery may be employed if necessary.

If extensive surgical debridement is necessary during the run-in period (e.g., general anesthesia is required), the patient is not a candidate for this trial. Likewise, if other forms of debridement (e.g., enzymatic) are required during run-in, based on the opinion of the treating clinician, the subject should be screen failed. During the Treatment Phase of the trial, other forms of debridement are NOT permitted. If this should happen, the type of debridement should be noted in the CRF, using other forms of debridement besides sharp debridement more than once during the treatment phase will result in the subject being withdrawn from the trial.

Although there is increasing evidence that more frequent debridement results in faster healing on average, the frequency and level of debridement in this trial is left up to the treating physician's judgment. Excessive debridement can be as deleterious to healing as too little debridement.

For wound bed preparation, follow the TIME principles: Tissue management (primarily debridement in this trial), control of Infection and Inflammation (management of infection, control of edema, management of exudate, etc.), Moisture imbalance (ensuring that the wound is at all times in a moist healing environment, as well as dealing with excessive wound exudate), and advancement of the epithelial Edge of the wound (addressing hypoxia, infection, desiccation, dressing trauma, hyperkeratosis and calluses, and cell senescence at the wound margin). (Dowsett C, Newton H. Wounds UK 2005; 1:58-70)