

PROTOCOL SUMMARY

Title: A prospective, randomized, controlled trial comparing Cytal[®] Wound Matrix 1-Layer to Standard of Care (SOC) in the management of Diabetic Foot Ulcers (DFUs)

Protocol Number: CR2017-006

Study Design: Multi-site, prospective, randomized study comparing Cytal[®] Wound Matrix 1-Layer (Group 1) to Standard of Care (Group 2) in the management of diabetic foot ulcers.

Subjects who meet the inclusion and exclusion criteria for the study will be randomized (2:1) to Group 1 or Group 2 using a permuted block randomization scheme.

Study DFUs will be treated up to 10 times in a 12 week period and must be located on a single foot.

Study Objectives:

Primary Objective

1. To compare the incidence of complete wound closure (defined as 100% epithelialization) by 12 weeks between randomized groups.

Secondary Objective(s)

1. To compare the rate of wound healing over time in cm²/week between randomized groups.
2. To compare the time to complete wound closure between randomized groups.
3. To compare DFU recurrence after healing between randomized groups.
4. To compare subject reported outcomes between randomized groups.
5. To compare the intervention-emergent adverse event (AE) safety profile between randomized groups.

Health Economic Objectives

1. Perform a health economics and cost effectiveness analysis between randomized groups.

Eligible subjects will be recruited and enrolled, under the responsibility of the participating Investigators. The actual overall study duration or subject recruitment period may vary.

4.2.1 PRIMARY ENDPOINT

Measure incidence of complete wound closure (defined as 100% epithelialization) by 12 weeks between randomized groups.

4.2.2 SECONDARY ENDPOINTS

The secondary endpoints of the study, comparing measurements between randomized groups, include:

1. Measure changes in wound size, measured in cm² per week between randomized groups by 12 weeks.
2. Measure time to complete wound closure, in days between randomized groups by 12 weeks.
3. Measure wound recurrence after healing is complete at 26 weeks and 52 weeks (6 & 12 months post intervention initiation).
4. Changes in subject reported outcomes including quality of life and pain (quality of life surveys & visual analogue scale), pre- intervention to post- intervention over time and by 12 weeks.
5. Measure frequency of adverse events related to DFU including unexpected adverse device events or serious adverse experiences throughout the duration of the study.

4.2.3 HEALTH ECONOMIC OUTCOMES

The health economic outcomes of the study include:

1. Any changes in the prescription of narcotics related to the DFU(s) by subject and between randomized groups.
2. Any changes in ambulatory status (i.e. bed, wheel chair, walk w/ assistance, or walk independent) by subject and between randomized groups.
3. Any changes in “return to work/reported work status”, activities of daily living, or disability status by subject and between randomized groups.
4. Health economics analysis, as measured by direct costs as measured by total cost per subject stratified by healed vs. non healed DFU.

5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 PARTICIPANT INCLUSION CRITERIA

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Provision of signed and dated informed consent form by subject or legally authorized representative.
2. Stated willingness to comply with all study procedures and availability for the duration of the study. Subject is able and willing to tolerate non-removable offloading device for the duration of the run-in and intervention phases of the study.
3. Subject is male or female and ≥ 22 years of age.
4. Subject has a clinical diagnosis of type 1 or type 2 diabetes.
5. Subject's current foot ulcer(s) has been present for ≥ 30 days and ≤ 365 days.
6. Subject's current foot ulcer(s), post-debridement is/are predominantly below the malleoli and on the plantar surface of the foot.
7. Subject's foot ulcer(s) must be Wagner Grade 1 or 2.
8. Post debridement, subject's ulcer(s) are free of necrotic debris and appear to be comprised of healthy, vascularized tissue.
9. All qualifying ulcers ≥ 5 cm away from any other ulcer on the same foot.
10. Subject's ulcer(s) surface area is $\geq 1\text{cm}^2$ and $\leq 20\text{cm}^2$ at randomization.
11. Subject's HbA1C reading is $\leq 10\%$.
12. Subject's Serum Creatinine $\leq 3.0\text{mg/dL}$.
13. Subject has adequate circulation to the foot as measured by Ankle-Brachial Index (ABI) ≥ 0.7 .
14. Negative pregnancy test at randomization for women of childbearing potential.

5.2 PARTICIPANT EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Subject is pregnant, breastfeeding, or unwilling to practice birth control during participation in the study, if applicable.
2. Allergy or hypersensitivity to materials in porcine-based study products (per subject report) or personal preference.
3. Subject report of concurrent participation in another clinical trial that involves a drug or device.
4. The subject has any condition that, in the Investigator's opinion, would warrant exclusion from the study or prevent the subject from completing the study.

tunneling, sinus tracts, slough, and/or adverse odor; Appearance of Exudate: serous, serosanguinous, purulent, and bloody.

11. Record Appearance of Wound Bed : Beefy Red, Red, Pink, White, or Yellow. If debridement is performed, record appearance of wound bed post-debridement.
12. Use the Silhouette system to capture at least one image per wound **pre-debridement** at the first run-in visit only (this is a reference picture and measurements will not be recorded).
 - Images should be captured in a manner where no identifiable information is contained in the photograph (i.e. tattoos, full body, etc.). Refer to the SilhouetteConnect User Guide and the SilhouetteStar Quick Reference Guide for detailed instructions on how to use the Silhouette system.
13. Use the Silhouette system to capture at least one image per wound **post-debridement** at the first and second run-in visit and record the length, width, and depth of the diabetic foot ulcer(s) in centimeters (surface area, volume, and percent changes from baseline will be auto-calculated). If debridement is not performed, at least one image per wound must be captured and measured as noted above.
14. Treat the DFU(s) with SOC to include debridement (if necessary), moist wound therapy, the appropriate secondary dressing, and off-loading (per SOC) for 2 consecutive weeks prior to randomization. This 2 week run-in period is required to determine continued eligibility in the study. No advanced wound dressings (i.e. Fibracol™) should be used prior to the initiation of the treatment phase as SOC treatment.

7.1.2 BASELINE/RANDOMIZATION (STUDY VISIT 1)

Baseline/Randomization visit should occur 1 week following second run-in visit (+/- 2 days) for all subjects.

1. Verify and document inclusion/exclusion criteria continue to be met. Subjects whose DFU(s) surface area decreases in size by > 30% or increases by > 50% from the initial run-in visit will not be eligible for study intervention and will be considered a screen failure. Qualifying subjects will be randomized to either Group 1 or Group 2.
2. Record subject's employment status, disability status, ambulatory status, medical history (including duration of diabetes disease, past diabetes complications, and DFUs), and surgical history per available medical reports and/or patient self-report.
3. Record social information including alcohol and tobacco use.
4. Confirm concomitant medication use (type, dose, frequency, duration, indication) for the following (including but not limited to): pain medication related to DFU, any anti-hypertensives, medication or supplements to control diabetes, nutritional supplements, and antibiotics.
5. Perform urinary pregnancy test for women of childbearing potential.

5. Confirm concomitant medication use for the following (including but not limited to): pain medication use relating the DFU(s), any anti-hypertensives, medications or supplements to control diabetes, nutritional supplements, and antibiotics, if applicable.
6. Administer VAS for pain-overall body pain and DFU foot pain prior to intervention weekly, as appropriate.
7. Record weight (pounds) and BMI at wound healing.
8. Collect blood samples for Hemoglobin HbA1C at **Visit 13** and Albumin at **Visit 3, Visit 6, Visit 9 and Visit 13**. For confirmation visits, collect blood sample at the third and final confirmation visit only.
9. Record ABI to confirm appropriate perfusion PRN for non-healing wounds.
10. Administer DFS-SF at the **last closure confirmation visit**.
11. Administer SF-20 Health Survey and the Katz ADL prior to intervention at **Visit 7 & 13** and at the third and final confirmation visit if the wound heals prior to **Visit 13**.
12. Record the following information for all treated wound(s) pre-debridement, if debridement at this visit is deemed necessary by the Investigator. Wound appearance and characteristics including, but not limited to: Presence of epibole, eschar, undermining, tunneling, sinus tracts, slough and/or adverse odor; Appearance of Exudate: Serous, serosanguinous, purulent, and/or bloody; Appearance of Wound Bed: Beefy Red, Red, Pink, White, or Yellow.
13. Record the following information for all treated wound(s) post-debridement, if debridement at this visit is deemed necessary by the Investigator:
 - a. Use the SilhouetteStar and SilhouetteConnect system to capture at least one wound image and record measurements including length, width, and depth of the diabetic foot ulcer(s) in centimeters (surface area, volume, and percent changes from baseline will be auto-calculated). Capture images in a manner where no identifiable information is contained in the photograph (i.e. tattoos, full body, etc.). If debridement is not performed, capture at least one wound image and record measurements as noted.
 - b. If wound(s) is healed, Use the SilhouetteStar and SilhouetteConnect system to capture at least one wound image of the healed wound area.
 - c. Additional images beyond the requirements stated above may be taken however are not required under this protocol. Additional images should not be measured within ARANZ.
14. Proceed with treatment procedures as outlined below (Note, a maximum of 10 treatments within the 12 week period or until wound has completely closed, whichever occurs first, is allowed. These applications may be made per the discretion of the Investigator.)
 - a. **Group 1 Procedures (ACell Products)**

- a. Wound appearance and characteristics including, but not limited to:
Presence of epibole, eschar, undermining, tunneling, sinus tracts, slough and/or adverse odor; Appearance of Exudate: Serous, serosanguinous, purulent, and/or bloody; Appearance of Wound Bed: Beefy Red, Red, Pink, White, or Yellow
- b. Using the SilhouetteStar and SilhouetteCentral, obtain at least one wound image and measurements including length, width, and depth of the diabetic foot ulcer(s) in centimeters (surface area, volume, and percent changes will be auto-calculated). Capture images in a manner where no identifiable information is contained in the photograph (i.e. tattoos, full body, etc.). If debridement is performed, images must be post-debridement.
- c. If wound(s) remains healed, use the SilhouetteStar and SilhouetteConnect system to capture at least one wound image of the healed wound area.
- d. Additional images beyond the requirements stated above may be taken however are not required under this protocol. Additional images should not be measured within ARANZ.

7.1.5 UNSCHEDULED VISIT

Unscheduled visits may occur at any time between Study Visit 1 and Visit 13, in the event of an adverse event (i.e. recurrence, infections, etc.) related to the original, qualifying DFU(s) up to week 52; or in the event that product is applied at Visit 13. Reasons for an Unscheduled Visit include, but are not limited to: diabetic foot ulcer events, assessment or follow-up of adverse events, etc.

1. Document reason for the unscheduled visit.
2. Review and record any changes to subject's employment status, disability status, ambulatory status, medical history, and surgical history, if applicable.
3. Record changes to concomitant medication use for the following (including, but not limited to): pain medication use relating the DFU(s), any anti-hypertensives, medications or supplements to control diabetes, nutritional supplements, and antibiotics, if applicable.
4. Record diabetic and wound-related AEs and all SAEs as reported by participant or observed by Investigator.
5. As applicable:
 - a. Confirm closure or record if there has been a recurrence at the original anatomic location of the diabetic foot ulcer(s).
 - b. Record wound appearance and characteristics including but not limited to:
Presence of epibole, eschar, undermining, tunneling, sinus tracts, slough, and/or adverse odor; Appearance of Exudate: Serous, serosanguinous,

purulent, and bloody; Appearance of Wound Bed: Beefy Red, Red, Pink, White, or Yellow.

- c. Using the SilhouetteStar and SilhouetteCentral, obtain at least one wound image and record measurements including length, width, and depth of the diabetic foot ulcer(s) in centimeters (surface area, volume, and percent changes will be auto-calculated). Capture images in a manner where no identifiable information is contained in the photograph (i.e. tattoos, full body, etc.). If debridement is performed, images must be post-debridement.
 - d. Record ABI to confirm perfusion PRN for non-healing wounds.
6. Record actions taken to treat the wound(s), if applicable.

7.1.6 MISSED VISIT

If a subject misses a visit, the site is to make every effort to have the subject return as soon as possible for the missed visit. Once the subject is seen, he/she is to return to his/her original weekly visit schedule (window +/- 2 days). 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.
- If the same subject was not able to come in until Friday, the subject would still come in the next Monday to maintain the weekly schedule.

7.1.7 DESCRIPTION OF SYSTEMS/OUTCOME MEASUREMENTS

Wagner Ulcer Classification System

GRADE	LESION
0	No open lesions; may have deformity or cellulitis
1	Superficial diabetic ulcer (partial or full thickness)
2	Ulcer extension to ligament, tendon, joint capsule, or deep fascia without abscess or osteomyelitis
3	Deep ulcer with abscess, osteomyelitis, or joint sepsis
4	Gangrene localized to portion of forefoot or heel
5	Extensive gangrenous involvement of the entire foot

The SF-20[®] Health Survey (SF-20)

The SF-20 is a 20-item questionnaire used to assess generic health outcomes from the subject's perspective. Generic subject-reported outcome (PRO) measures like the SF-

20 assess general health and well-being [or health-related quality of life (HRQOL)], including the impact of any and all illnesses on a broad range of functional domains. The SF-20 consists of a subset of 20 items from the SF-36[®] Health Survey (SF-36), covering the same eight domains of health outcomes, including physical functioning, role functioning, mental health, current health perceptions, and pain.

Diabetic Foot Ulcer Scale- Short Form (DFS-SF)

The DFS-SF is a 29-item questionnaire is a specific instrument designed to assess the impact of diabetic foot ulcers and their intervention on quality of life in people with diabetes. The short form is based on the original Diabetic Foot Ulcer Scale; this questionnaire reduces the burden of a lengthy survey on subjects and has been shown to have good internal consistency, reliability, construct validity, and demonstrated sensitivity to ulcer healing¹⁴.

Visual Analogue Scale for Pain (VAS)

The visual analogue scale or visual analog scale (VAS) is a psychometric response scale which can be used in patient-based outcome questionnaires. It is a measurement instrument for subjective characteristics or attitudes that cannot be directly/objectively measured. The VAS is anchored by two verbal descriptors, one for each symptom extreme measured from the zero mark, indicating “no pain” (score of 0) to the mark of 10 on the 10-cm line, indicating “pain as bad as it could be” or “worst imaginable pain.” Respondents are asked to report current pain intensity or pain intensity in the last 24 hours.

When responding to a VAS item, respondents specify their level of agreement to a statement by placing a vertical line perpendicular to the VAS line at the point that represents their pain intensity. After the patient has marked, using a ruler, the score is determined by measuring the distance (mm) on the 10-cm line between the “no pain” anchor and the patient’s mark.

Katz Index of Independence in Activities of Daily Living (KATZ ADL)

The Katz Index of Activities of Daily Living, formerly referred to as the Katz Activities of Daily Living Scale¹⁵, is a validated measure used to quantify independence in activities of daily living. These activities include bathing, dressing, toileting, transfers, feeding, and continence. The Katz ADL is a 6-item questionnaire where each item is dichotomized as either having a score of zero (i.e. dependence) or one (i.e. independence). A score of 2 or less indicates a dependent subject, 3-5 for partially dependent and 6 for independent.

ARANZ Medical Silhouette

- Non-characteristic wound odor not associated with product degradation (odor or suspected infection of wound)
- Pus drainage from the treated wound
- Serosanguinous drainage from the wound- excessive or exacerbated
- Bleeding- excessive or exacerbated
- Hematoma
- Seroma
- Diabetes related, specify
- Diabetic foot ulcer – newly developed or worsening
- Necrotic Tissue-excessive or exacerbated
- Gangrene
- Wound dehiscence
- Osteomyelitis
- Cellulitis
- Periwound maceration
- Wound recurrence (after closure)
- Hypergranulation of treated wound(s)
- Amputation
- Death

8.1.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An AE or suspected adverse reaction is considered "serious" if, in the view of either the Investigator or Sponsor, it results in any of the following outcomes:

- Death
- A life-threatening adverse event
- In-patient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject or may require medical or surgical intervention to prevent one of the outcomes listed in this definition. All SAEs are required to be collected following randomization through the final study visit for this study.

8.1.3 DEFINITION OF UNANTICIPATED ADVERSE DEVICE EFFECT (UADE)

Unanticipated adverse device effect is any serious adverse effect on health or safety, any life-threatening problem or death caused by, or associated with a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the application; or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

8.2 CLASSIFICATION OF AN ADVERSE EVENT

8.2.1 SEVERITY OF EVENT

The Investigator will be asked to assess the severity of the AE using the following categories:

- Mild:** Events require minimal or no intervention and do not interfere with the participant's daily activities.
- Moderate:** Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- Severe:** Events interrupt a participant's usual daily activity and may require systemic drug therapy or other intervention. Severe events are usually potentially life-threatening or incapacitating.

8.2.2 RELATIONSHIP TO STUDY AGENT

For all collected AEs, the clinician who examines and evaluates the participant will determine the AE's causality based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below.

- Definitely:** The relationship of the AE and the study device or the study procedure can definitely be established.
- Probably:** While a clear relationship to the study device or to the study procedure cannot be established, the AE is associated with an expected AE or there is no other medical condition or intervention, which could explain the occurrence of such an event.
- Possibly:** There is no clear relationship between the AE and the study device or study procedure; however, one cannot definitely conclude that there is

no relationship.

Unrelated: There is no relationship between the AE and the study device or study procedure. This may include but is not limited to the incident being an expected outcome of a previously existing or concurrent disease, concomitant medication or procedure the subject experienced.

8.2.3 EXPECTEDNESS

The Investigator will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study products.

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

The occurrence of an AE or SAE may come to the attention of study personnel during study visits or upon review by a study monitor. All AEs (per Investigator discretion) that are deemed 'Possibly', 'Probably' or 'Definitely' related to the development or healing of the DFU(s) and those including local and systemic reactions not meeting the criteria for an SAE, will be captured on the appropriate CRF. Information to be collected includes, but is not limited to, event description, date of onset, clinician's assessment of severity, relationship to study products, and date of resolution/stabilization of the event. All AEs will be followed to adequate resolution.

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

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

The PI will record all reportable events with start dates occurring any time following initial treatment application through final study visit. At each study visit, the Investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

8.4 REPORTING PROCEDURES

8.4.1 ADVERSE EVENT REPORTING

Adverse Events will be documented on the appropriate Case Report Form (CRF) as the Investigator learns of the event. All AEs, not serious in nature, will be reviewed by the Sponsor during scheduled Interim Monitoring Visits (IMVs). The Investigator will follow all AEs until adequate resolution is achieved or until AE returns to baseline status, at which point the AE would be considered resolved. The IRB should be notified of all AEs according to their notification policies.

All AEs will be reported to ACell Quality Assurance and reported under the appropriate regulatory consideration.

8.4.2 SERIOUS ADVERSE EVENT (SAE) REPORTING

In the case of an SAE, the Investigator must immediately notify (within 1 working day of becoming aware of the event) the study Sponsor (contact information is provided in **Section 1, Key Roles**). The IRB must also be notified according to their notification policies.

All SAEs will be followed until satisfactory resolution or until the site Investigator deems the event to be chronic or the appearance to be stable. Other supporting documentation of the event may be requested by the study Sponsor and should be provided as soon as possible. The study Sponsor will be responsible for notifying the FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than 7 calendar days after the Sponsor's initial receipt of the information.

8.4.3 UNANTICIPATED ADVERSE DEVICE EFFECT (UADE) REPORTING

The study Investigator shall submit to the Sponsor and to the reviewing IRB as soon as possible, but in no event later than 10 working days after the Investigator first learns of the effect. The Sponsor contact information is provided in **Section 1, Key Roles**. The study Sponsor is responsible for conducting an evaluation of an UADE and shall report the results of such evaluation to the FDA, all reviewing IRB(s) and Investigator(s) within 10 working days after the Sponsor first receives notice of the effect.

This study is not being conducted under an IDE; therefore, Sponsor reporting timelines may differ from those defined above.

8.4.4 REPORTING OF PREGNANCY

If a female subject becomes pregnant during the trial, she must be followed until the outcome of the pregnancy is known.

If pregnancy occurs, the Investigator must contact the Sponsor immediately for further instruction. Both the detection and the outcome of the pregnancy must be reported to the Sponsor.

9 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of human subjects are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with Good Clinical Practice (GCP), and with applicable regulatory requirement(s).

A Clinical Monitoring Plan will describe in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports.

10 STATISTICAL CONSIDERATIONS

10.1 STATISTICAL AND ANALYTICAL PLANS

A Statistical analysis Plan (SAP) will be written and signed-off prior to the first subject being enrolled in the study. The SAP will include a comprehensive description of the statistical methods and analyses to be included in the final study report. Any change to the analysis methods described in the protocol will require an amendment only if it changes a principal feature of the protocol. Any other change to the analysis methods described in the protocol, and the justification for making the change, will be described in the SAP.

Tabulations of summary statistics, graphical presentations, and statistical analyses will be performed using SAS software version 9.4. The statistical analyses will be based on data pooled across subject and wound characteristics and presented in aggregate; a separate series of analyses will retain specific subject and wound characteristics found to be significant covariates in response. All subset summary tables and data listings will be sorted by subject. The pre-procedure observations will be used as the baseline value for calculating post-procedural changes from baseline.

Statistical significance will be declared if the two-sided probability value is <0.05 ; tests for interaction will be performed at the 0.1 alpha level. Asymptotic confidence limits will be presented based on the normal approximation to the binomial distribution for differences in proportions. Exact confidence intervals will be used for all other presentations. All computations will be performed using SAS® (Version 9.4 or higher).

model specifying the distribution of the dependent variable as continuous. The time to complete wound closure will be calculated in days from the date of the initial study intervention and compared between the intervention and standard of care groups using a log-rank test. Subjects who fail to achieve complete wound closure will be censored using the last date of examination in the study. Wound recurrence after healing is complete at 26 weeks and 52 weeks will be tabulated by group and the proportions will be compared between intervention and standard of care groups using a 2-tailed Fisher's exact test. The time to wound recurrence will also be compared between the intervention and standard of care groups. Changes in subject reported outcomes will be summarized and compared based on the distribution of the data. A random-effects generalized linear model, specifying the distribution of the dependent variable based on the type of data, will be used to compare the results over time between the intervention and standard of care groups.

The incidence of adverse events related to DFU recorded during the course of the study will be totaled by intervention group and compared between randomized groups using a generalized linear model and specifying the distribution as binomial. A separate tabulation will be prepared comparing the overall incidence of all adverse events, and restricted to serious adverse events.

The first health economic outcome parameter to be summarized is the change from baseline in the prescription of narcotics related to the DFU(s) by subject and between randomized groups. The summary statistics will be calculated using the daily dosage converted into morphine equivalent units, averaged over successive 7-day intervals from the time of study enrollment until week 52. The average 7-day changes will be summarized by group assignment.

The second health economic outcome parameter is the intra-subject change in ambulatory status (i.e. bed, wheel chair, walk w/ assistance, or walk independent). The distribution of subjects by ambulatory status (improved, no change, worse) at 12, 26 and 52 weeks will be summarized by group. The 2-sided 95% exact binomial confidence intervals will be summarized by ambulatory status category and treatment group. The time to a change in status (improved status and worsening status) will also be summarized using K-M estimates and compared between the intervention and standard of care groups using a log-rank test.

The third health economic outcome includes work status, disability status, and activities of daily living. The change in "return to work/reported work status" or disability status as defined by the investigator's evaluation of the subject's ability to return to work related to their DFU status will be derived for each subject and compared between randomized groups. It is expected a measureable proportion of the subjects may not be employed at the time of enrollment for reasons not related to

value, initialed and dated, and the corrected documentation should be verified again by the monitor.

The handling of the data by the Sponsor after review of the eCRFs may generate additional requests to which the Investigator is obliged to respond by confirming or modifying the data questioned.

Participating site(s) will maintain appropriate medical and research records for this trial, in compliance with ICH E6 and regulatory and institutional requirements for the protection of confidentiality of participants. Each site will permit authorized representatives of the Sponsor or designee, and regulatory agencies to examine (and when permitted by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety, progress, and data validity.

Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Examples of these original documents and data records include, but are not limited to: hospital records, clinical and office charts, laboratory notes, memoranda, participants' memory aids or evaluation checklists, pharmacy records, recorded data from automated instruments, , and participant files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial.

The SF-20 Health Survey, DFS-SF, and VAS for Pain will be considered source documents and also act as the original CRFs.

12 QUALITY ASSURANCE AND QUALITY CONTROL

Quality Control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database. Any missing data or data anomalies will be communicated to the site for clarification/resolution.

Following written Standard Operating Procedures, the monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol and GCP.

The site will provide direct access to all trial related materials, including source data/documents, electronic medical records (if applicable), CRFs, and reports for the purpose of monitoring and auditing by the Sponsor and/or the IRB.

13 ETHICS/PROTECTION OF HUMAN SUBJECTS

13.1 ETHICAL STANDARD

APPENDIX II: STATEMENT OF COMPLIANCE

The trial will be conducted in accordance with the ICH E6, the United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and 21 CFR Part 812).

The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the Sponsor and documented approval from the Institutional Review Board, except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection Training.

I agree to ensure that all staff members involved in the conduct of this study are informed about their obligations in meeting the above commitments.

Signature of Principal Investigator	Date (mm/dd/yy)
Printed Name	
Name of Institution	

Endpoints:**Primary Endpoint**

1. Measure incidence of complete wound closure (defined as 100% epithelialization) by 12 weeks between randomized groups.

Secondary Endpoint(s)

1. Measure changes in wound size, measured in cm² per week between randomized groups by 12 weeks.
2. Measure time to complete wound closure, in days, between randomized groups by 12 weeks.
3. Measure wound recurrence after healing is complete at 26 weeks and 52 weeks (6 & 12 months post intervention initiation) between randomized groups.
4. Changes in subject reported outcomes including quality of life and pain (quality of life surveys & visual analogue scale), pre-intervention to post-intervention between randomized groups over time and by 12 weeks.
5. Measure frequency of adverse events related to DFU including unexpected adverse device events or serious adverse experiences throughout the duration of the study and evaluate between randomized groups.

Health Economic Outcomes

1. Any changes from baseline in the prescription of narcotics related to the DFU(s) by subject and between randomized groups.
2. Any changes in ambulatory status (i.e. bed, wheel chair, walk w/ assistance, or walk independent) by subject and between randomized groups.
3. Any changes in "return to work/reported work status", activities of daily living, or disability status by subject and between randomized groups.
4. Health economics analysis, as measured by direct costs as measured by total cost per subject stratified by healed vs. non healed DFU.

Population:

A maximum of 150 subjects who present to the participating Investigator(s) in the in-patient or out-patient facility with at least a single diabetic foot ulcer that is characterized as Wagner Grade 1 or Grade 2 will be enrolled and randomized. An interim assessment will be performed after 60 subjects complete the 12

5. Subject has clinical evidence of gangrene on any part of the affected foot.
6. The subject's ulcer(s) is/are definitively due to a non-diabetic etiology, ulcers of arterial, venous stasis, pressure, radiation, traumatic, rheumatoid, vasculitis, collagen vascular disease, or other non-diabetic etiologies in the opinion of the Investigator.
7. Subject has unstable Charcot foot, Charcot foot with a bony prominence(s) or Charcot amputation.
8. Qualifying wound(s) is connected to another ulcer via a fistula.
9. Subject has one or more medical condition(s) including renal, hepatic, hematological, neurologic, or immune disease that in the opinion of the Investigator would make the subject an inappropriate candidate for this wound healing study.
10. Subject has or has had a malignant disease (other than basal cell carcinoma) that has not been in remission for at least five years.
11. Subject is receiving oral or parenteral corticosteroids, immunosuppressive or cytotoxic agents, or is anticipated to require such during the course of the study.
12. Subject has acute osteomyelitis of the affected foot.
13. Subject's ulcer(s) is accompanied by active cellulitis.
14. Subject has received growth factor or enzymatic therapy in the area of the wound, within 2 weeks of consent.
15. Subject has ever received radiation or radiologic implants in the area of the wound OR chemotherapy within the past 5 years.
16. Subject is allergic to any of the primary or secondary dressing materials, including occlusive dressings and the adhesives on such dressings.
17. Subject's ulcer(s) surface area has decreased in size by > 30% during the run-in phase.
18. Subject's ulcer(s) surface area has increased in size by > 50% during the run-in phase.
19. Subject's ulcer(s) has tunnels or sinus tracts that cannot be completely debrided.
20. Subject has severe malnutrition as evidenced by albumin <2.0 g/dL.
21. Subject has a bleeding disorder that would make the subject unsuitable for serial bedside sharps or chemical cautery debridement.
22. Subject is on dialysis.
23. Any DFU(s) is actively infected and has not been treated for any clinically suspected infection prior to application of any product.

5.3 STRATEGIES FOR RECRUITMENT AND RETENTION

Eligible subjects will be recruited and enrolled, under the responsibility of the participating Investigators.

6. Administer the SF-20 Health Survey, DFS-SF, Visual Analogue Scale for Pain (VAS), and KATZ ADL prior to intervention.
7. Record the following information for the eligible wound(s) (record wound characteristics pre-debridement, if debridement at this visit is deemed necessary by the investigator). Wound appearance and characteristics including but not limited to: Presence of epibole, eschar, undermining, tunneling, sinus tracts, slough, and/or adverse odor; Appearance of Exudate: Serous, serosanguinous, purulent, and bloody; Appearance of Wound Bed: Beefy Red, Red, Pink, White, or Yellow.
8. Record the following information for each of the eligible ulcer(s) (record size and classification post-debridement, if the ulcer(s) was debrided at this visit):
 - *If debridement is performed:* Use the SilhouetteStar and SilhouetteConnect system to capture at least one wound image post debridement and prior to product application. Capture images in a manner where no identifiable information is contained in the photograph (i.e. tattoos, full body, etc.). Use SilhouetteConnect to trace one image in order to calculate and record the length, width, depth, surface area and volume.
 - *If debridement is **not** performed:* Use the SilhouetteStar and SilhouetteConnect system to capture at least one wound image prior to product application. Capture images in a manner where no identifiable information is contained in the photograph (i.e. tattoos, full body, etc.). Use SilhouetteConnect to trace one image in order to calculate and record the length, width, depth, surface area and volume.
 - Additional images beyond the requirements stated above may be taken however are not required under this protocol. Additional images should not be measured within ARANZ SilhouetteConnect.
 - Categorize the wound using the Wagner Classification System
9. If all eligibility criteria continue to be met, perform randomization and proceed with intervention procedures as outlined below, depending on randomization assignment. If there is more than one qualifying wound, randomize by subject so all wounds are in the same intervention group..

Group 1 (ACell products) Procedures

- i. Wash the wound using saline prior to debridement, as needed.
- ii. Sharps debride the eligible diabetic foot ulcer(s) to remove all non-viable tissue, callus, epibole at wound edges, slough, and debris (at the physician's discretion). The wound margins should be excised to healthy bleeding tissue.
- iii. Wash the wound(s) once more with saline to remove remaining debris and confirm hemostasis is achieved, as needed.
- iv. Refer to section 7.1.2 Number 8 above for wound image and measurement instructions for the eligible wound(s) post-debridement.

- i. Do not remove or disrupt any residual ACell product (Group 1) when preparing the wound bed for reapplication.
- ii. Minimal “spot” sharps debrides and gentle saline washes may be performed at subsequent treatment visits as determined by the investigator.
- iii. Apply weekly applications of Cytal® Wound Matrix 1-Layer until wound closure or up to, and including, week 12, whichever occurs first. Record appropriate wound care time inclusive of product preparation, debridement and dressing if additional study devices are applied.
- iv. Secure product with a non-adherent, primary contact layer.
- v. Apply a water-based surgical lubricant of physician choice as necessary to maintain a moist wound environment.
- vi. Apply the appropriate secondary dressing based on wound characteristics and physicians discretion.
- vii. Apply the OPTIMA® Diab boot with the appropriate insole kit based on subject weight and wound size and make the boot non-removable by using the locking system.
- viii. In cases of AEs/SAEs in the **ACell arm**, the PI may treat subjects with any other primary wound contact layer as per the Investigator’s discretion **except** for any Fibracol™ devices, as to not confound study arms and results.

b. Group 2 Procedures (SOC Only)

- i. Minimal “spot” sharps debrides and gentle saline washes may be performed at subsequent intervention visits as determined by the investigator.
- ii. Place an appropriately sized Fibracol™ sheet (per the manufacturer’s Instructions for Use) into the wound bed Record appropriate wound care time inclusive of product preparation, debridement and dressing as appropriate. Apply a water-based surgical lubricant of physician choice as necessary to maintain a moist wound environment.
- iii. Apply the appropriate secondary dressing based on wound characteristics and physicians discretion.
- iv. Apply the OPTIMA® Diab boot with the appropriate insole kit based on subject weight and wound size and make the boot non-removable by using the locking system.
- v. In cases of AEs/SAEs in the **SOC arm**, the PI may treat subjects with any other primary wound contact layer per the Investigator’s

Silhouette® is an easy to use wound imaging, 3D measurement, and documentation system using non-invasive laser technology. As an FDA approved medical device, Silhouette has been proven to provide accurate, precise, and repeatable wound assessments for all phases of clinical research in more than 70 studies globally.

- SilhouetteStar™ + SilhouetteConnect™: The SilhouetteStar camera is used with the SilhouetteConnect application software for fast and easy imaging, 3D wound measurement and clinical notes capture. SilhouetteStar connects via USB to a computer with SilhouetteConnect installed.
- SilhouetteCentral™ (central server) is an integrated electronic wound information management system that enables trial managers to review, report, securely share, and analyze data collected by Silhouette point-of-care devices.

7.2 CONCOMITANT MEDICATIONS

At the discretion of the Investigator, the subject may be administered any necessary medications, provided such medications are not listed under the study exclusion criteria. Concomitant medications that will be documented in the subject's source documents and CRFs at all of the intervention and follow-up visits as well as recorded on the Medications Log include but not limited to: pain medication use relating the DFU(s), any anti-hypertensives, medications or supplements to control diabetes, nutritional supplements, and antibiotics, if applicable.

8 ASSESSMENT OF SAFETY

8.1 SPECIFICATION OF SAFETY PARAMETERS

8.1.1 DEFINITION OF ADVERSE EVENTS (AE)

Adverse Event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related.

Adverse events of interest under this protocol are those to be considered related to diabetes or the DFU. They include but are not limited to:

- Infection
- Increased chronic inflammation
- Allergic reaction
- Unexplained fever or chills
- Excessive redness
- Pain – excessive or exacerbated
- Swelling - excessive or exacerbated

10.2 STATISTICAL HYPOTHESES

Primary Efficacy Endpoint(s):

The primary endpoint of the study is the incidence of complete wound closure by 12 weeks between Group 1 and Group 2. The comparison between the randomized groups will be based on the overall incidence of complete closure.

Ho: Incidence of complete wound closure by 12 weeks in Group 1 = Incidence of complete wound closure by 12 weeks in Group 2

Ha: Incidence of complete wound closure by 12 weeks in Group 1 \neq Incidence of complete wound closure by 12 weeks in Group 2

Secondary Efficacy Endpoint(s):

The first secondary endpoint is the change in wound size, measured in cm² per week. The null and alternative hypotheses are presented below.

Ho: Change in the wound surface area in Group 1 = Change in the wound surface area in Group 2

Ha: Change in the wound surface area in Group 1 \neq Change in the wound surface area in Group 2

The second secondary endpoint is the time to complete wound closure, in days, between Group 1 and Group 2. The null and alternative hypotheses are presented below.

Ho: Median time to complete closure in Group 1 = Median time to complete closure in Group 2

Ha: Median time to complete closure in Group 1 \neq Median time to complete closure in Group 2

The third secondary endpoint is the incidence of wound recurrence after healing is complete at 26 weeks and 52 weeks (6 & 12 months post intervention initiation). The null and alternative hypotheses are presented below.

Ho: Incidence of wound recurrence after healing at 26 weeks in Group 1 = Incidence of wound recurrence after healing at 26 weeks in Group 2

Ha: Incidence of wound recurrence after healing at 26 weeks in Group 1 \neq Incidence of wound recurrence after healing at 26 weeks in Group 2

their DFU. Shift tables will be prepared by intervention and standard of care only assignment based on sequential 30 day intervals to categorize the proportion of subjects who transition from not working because of the DFU to working following study intervention for the DFU. Separately, the time to return to work will be summarized using descriptive statistics and Kaplan-Meier estimates. Finally, the investigator will be asked to provide the date of the examination where the subject is cleared to return to work, independent of the subject's actual work status. Using this date, the number of days from the date of enrollment will be summarized by intervention assignment using descriptive statistics and summarized by intervention group using Kaplan-Meier estimates. This tabulation will include all subjects, independent of the subject being employed prior to enrollment into the study.

The scores from the Activities of Daily Living questionnaire will be summarized each time the questionnaire is administered. The actual scores, and the changes from scores recorded at baseline, i.e., prior to study intervention, will be summarized using descriptive statistics and compared between the intervention and standard of care only groups using an analysis of variance test.

The fourth health economic outcome is the total direct cost per DFU by intervention and standard of care only group assessed during the treatment period. The total intra-subject direct cost will include the wound care time (physician time, nursing time, procedural time, application of products dressing time, application of off-loading device, etc.) and will be modeled over sequential 30 day intervals culminating at the end of the intervention phase; additional factors that are known predictors of healing, along with specific data about the size, depth, and location of the ulcer will be added to the model to derive an estimated direct intervention cost.

10.4.4 SAFETY ANALYSES

Safety will be assessed by physical examinations, clinical laboratory tests and collection of AEs as outlined in the Schedule of Events. All summaries of AEs will be based on intervention-emergent adverse events, which will be coded according to the MedDRA system dictionary. The number and percentage of subjects experiencing adverse events will be summarized by body system organ class and description. Subject counts will be tabulated for all intervention- emergent adverse events for the ITT population. Adverse events will also be tabulated for events that occurred following initial treatment application through final study visit. Summaries by maximum severity and relationship to the study intervention will also be provided. SAEs and AEs leading to discontinuation from the study will be presented by system organ class and preferred term. Tabulated subject counts will be presented as a proportion of the ITT population with 95% binomial confidence intervals.

The Investigator will ensure that this study is conducted in full conformity with Regulations for the Protection of Human Subjects of Research codified in 45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and/or the ICH E6.

13.2 LAWS AND REGULATIONS

This clinical study will be conducted in compliance with all national laws and regulations of the countries in which the clinical trial is performed, as well as any applicable guidelines. The trial will be registered on www.clintrials.gov and on other sites, as appropriate.

13.3 INSTITUTIONAL REVIEW BOARD

The protocol, informed consent form(s), and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether previously consented participants need to be re-consented.

13.4 INFORMED CONSENT PROCESS

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of participation will be provided to the participants and their families. Consent forms will be IRB approved and the participant will be asked to read and review the document. The Investigator will explain the research study to the participant and answer any questions that may arise. All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. The participants may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

If a consent document is revised due to changes in study procedures, subjects who were enrolled prior to the change, but are affected by the change, will be informed of the

APPENDIX III: OPTIMA DIAB BOOTS USER'S GUIDE

See attachment.