

Use of Cytal[®] Wound Matrix and MicroMatrix[®] for the Management of Wounds

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Use of Cytal® Wound Matrix and MicroMatrix® for the Management of Wounds

CR2017-012

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LIST OF ABBREVIATIONS

AE	Adverse Event
BMI	Body Mass Index
CFR	Code of Federal Regulations
CMS	Centers for Medicare and Medicaid Services
CRF	Case Report Form
eCRF	Electronic Case Report Form
GCP	Good Clinical Practice
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IRB	Institutional Review Board
FSV	Final Study Visit
LTFU	Last Follow-up
QC	Quality Control
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAS	Statistical Analysis System
SID	Subject Identification Number
SOC	Standard of Care
UADE	Unanticipated Adverse Device Effect
VAS	Visual Analog Pain Scale
W-QoL	Wound Quality of Life

PROTOCOL SUMMARY	
Title:	Use of Cytal® Wound Matrix and MicroMatrix® for the Management of Wounds
Protocol Number:	CR2017-012
Study Design:	<p>This study is a single-site, prospective, observational clinical study of Cytal® Wound Matrix alone or in combination with MicroMatrix® for the management of wounds.</p> <p>In this study, Cytal® Wound Matrix 1-Layer will be applied topically to study wounds, with MicroMatrix® applied to fill the base of deep wounds (exceeding 3mm in depth) according to product use instructions. Patients with diverse wound types will be observed in the study, including patients with venous ulcers, diabetic foot ulcers, pressure ulcers, trauma wounds, external surgical wounds, and wound dehiscence/surgical site infection.</p> <p>Patients may undergo weekly treatment for their wounds and can be treated with up to 10 applications of Cytal® (with MicroMatrix® when wound depth exceeds 3mm) within the first 12 weeks, including unscheduled visits (within this 12 week period). If the Subject has multiple wounds, the most-appropriate wound that meets inclusion criteria and has at least two cm of healthy tissue between itself and other wounds (as determined by the investigator) will be selected as the Study Wound. A protocolized standard of care will be followed for Study Wounds in addition to the application of Cytal® and MicroMatrix® (i.e. surgical debridement, offloading etc. as appropriate for the particular wound type and severity).</p>
Objectives:	<p>Primary Objective</p> <p>The primary objective is to assess the effect of Cytal® with or without MicroMatrix® on primary measures of wound healing efficacy.</p> <p>Secondary Objective(s)</p> <ol style="list-style-type: none"> 1. Assess the effect of Cytal® with or without MicroMatrix® on secondary measures of wound healing efficacy. 2. Assess effect of treatment on patient-reported quality of life at baseline and at time of healing or at 12 weeks if the wound remains unhealed. 3. Assess complete wound management. 4. Wound-related adverse events. <p>Tertiary Objective(s)</p> <ol style="list-style-type: none"> 1. Assess costs of treatment and patient work status.

	<p>2. Assess wound pathology.</p>
<p>Endpoints:</p>	<p>Primary Endpoint</p> <p>1. Effect of Cytal® with or without MicroMatrix® on primary measures of wound healing efficacy will be assessed by determining:</p> <ul style="list-style-type: none"> a. Number and percentage of wounds with area reduced by greater than or equal to 40% by week 4, 60% by week 8, and 80% by week 12 as determined by wound image planimetry for each wound type. b. Number and percentage of completely healed wounds (100% epithelialization) by 12 weeks for each wound type. This will be verified by investigators and through a blinded review of wound photos by independent wound physicians. <p>Secondary Endpoints</p> <p>1. Secondary measures of wound healing efficacy will be assessed through week 12 by determining:</p> <ul style="list-style-type: none"> a. Time to complete wound closure as determined by wound image planimetry using the Silhouette Star camera system by Aranz®. b. Wound characteristics including granulation tissue quality and presence/quantity of exudate as determined by standardized clinician evaluation. c. Incidence of bridging to definitive closure such as graft, flap (when bridging is the goal of treatment), or transition to cellular therapy as determined by investigator. <p>2. Effect of treatment on patient-reported quality of life will be assessed by Visual Analog Scale (VAS), Wound Quality of Life (W-QOL), and Katz ADL health-related quality of life surveys at healing or at 12 weeks if the wound remains unhealed.</p> <p>3. Complete wound management will be assessed through week 12 by recording the following on a standardized questionnaire:</p> <ul style="list-style-type: none"> a. Method of application of Cytal®/MicroMatrix® and how the product is secured (e.g. sutures, steri-strips). b. Adjunctive treatments and dressings (e.g.

	<p>debridement, offloading, negative pressure wound therapy, hyperbaric oxygen).</p> <p>4. Number and type of wound-related adverse events through week 12 as recorded on adverse event case report forms.</p> <p>Tertiary Endpoints</p> <p>1. Costs of treatment and patient work status through week 12 will be assessed by the following:</p> <ul style="list-style-type: none"> a. Direct wound-related costs will be calculated and stratified by product and care cost. b. Current employment status and return to work status will be recorded and frequency of changes will be assessed. c. Measure frequency of wound specific AEs, and SAEs. <p>2. Wound Pathology will be assessed through week 12 by the following:</p> <ul style="list-style-type: none"> a. Effect of treatment with Cytal® with or without MicroMatrix® on pro-inflammatory and anti-inflammatory macrophage biomarkers, vascularization, and fibrosis in wound tissue biopsies will be assessed through quantitative image analysis for the following stains: <ul style="list-style-type: none"> i. CD86 and CD163 immunostains to quantify pro-inflammatory M1 and anti-inflammatory M2 macrophage phenotypes. ii. CD31 immunostain to quantify vascularization. iii. Masson’s trichrome to quantify fibrosis. b. Pathologists’ findings over the course of treatment will be assessed by quantification of standardized pathologists’ findings in wound pathology reports based on Hematoxylin and Eosin staining.
<p>Number of Subjects:</p>	<p>Estimated 80 subjects for treatment. (Maximum of 100). Subjects who are screened and meet all eligibility are enrolled in the study.</p>
<p>Study Criteria:</p>	<p>Inclusion Criteria: <i>(All inclusion criteria must be met for enrollment)</i></p> <ul style="list-style-type: none"> 1. Subject has at least one wound that the treating physician determines may be treated with Cytal® with or without MicroMatrix®. 2. Subject is ≥ 22 years of age.

	<ol style="list-style-type: none"> 3. Subject is willing and able to adhere to protocol requirements and agrees to participate in the study program and comply with the study follow-up regimen. 4. Subject or legal representative is willing to provide informed consent. 5. For females of reproductive potential, confirmed negative pregnancy test at enrollment. <p>Exclusion Criteria: <i>(No exclusion criteria may meet for enrollment)</i></p> <ol style="list-style-type: none"> 1. Allergy or hypersensitivity to materials in porcine-based study products (per subject report) or personal preference. 2. Subject report of concurrent participation in another clinical trial that involves an investigational drug or device that would interfere with this study. 3. The subject has any physical or psychiatric condition that, in the Investigator's opinion, would warrant exclusion from the study or prevent the subject from completing the study. 4. The subject's wound shows evidence of infection as determined by the Principal Investigator (which may be indicated by presence of: elevated WBC, pus, moderate or greater discharge, abnormal odor, or acute osteomyelitis). 5. Wound with exposed organs or hardware. 6. Wound with burn etiology.
Number of Sites enrolling participants:	1 (NYU Winthrop Hospital)
ACell Study Products:	<ol style="list-style-type: none"> 1. Cytal®™ Wound Matrix 1-Layer 2. MicroMatrix®
Participant & Study Duration:	<p>80 subjects are expected to complete this trial (maximum of 100 may be enrolled). The duration of participation for each subject will be up to a maximum of 55 weeks (1 week screening/treatment; 12 week follow-up; 26 week confirmation of wound closure/recurrence; 52 week final study visit). All visits have protocol stipulated windows.</p> <p>Recruitment and enrollment efforts are expected to last for approximately 12 months, starting in June 2018. The study will end in approximately May 2019, with Last Subject Last Visit expected in May 2020.</p> <p>The actual overall study duration or subject recruitment period may vary.</p>
Statistical Methodology:	The objective of this study is to define the trajectory of healing using either 1 or 2 test products; the intra-patient change will be the focus

	<p>of the analyses, not the comparison of response between 1 vs. 2 product application.</p> <p>All statistical tests to examine the change from baseline (study pre-treatment) will be conducted using a type 1 error rate of 5%. Simultaneous testing for the 2 primary endpoints will be conducted based on individual a priori thresholds. The analyses of the secondary endpoints will be conducted without adjustment for multiple testing.</p> <p>The analysis of the primary endpoints will be based on a generalized linear model specifying the distribution as binomial. If a patient withdraws prior to completing the study, however the last recorded observation revealed the wound had closed, the patient will be counted in the primary analysis as having achieved complete wound epithelization. If a patient withdraws prior to the 12-week wound evaluation visit and the last recorded observation revealed the wound had not closed, the patient will be counted in the primary analysis as not having achieved complete wound epithelization. This procedure will be also be followed for the examination at the patients by the various percent reduction thresholds. The analyses of all non-primary endpoints will be based on the distribution of data. For continuous variables, descriptive statistics will be used and the change from baseline will be tested against a difference of zero. For binomial and multinomial variables, results will be presented using counts, percentages, and shift tables. The analysis of the time of the initial observation of complete wound epithelization will be reported using Kaplan-Meier estimates.</p>
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1. KEY ROLES

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2. INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 BACKGROUND INFORMATION AND RATIONALE

Treatment for acute and chronic wounds is evolving, and will likely continue to change as new therapies for the condition are developed and incorporated into standard of care. Micromatrix® and Cytal® received FDA 510(k) clearance (K152721 and K060888) based on required biocompatibility and performance data to manage chronic and acute wounds, including partial- and full-thickness wounds, pressure ulcers, venous ulcers, diabetic ulcers, chronic vascular ulcers, tunneled/undermined wounds, surgical wounds, trauma wounds, and draining wounds. This study is a prospective observational study to illustrate how Micromatrix® and Cytal® perform when used as a treatment system for multiple chronic wound types in clinical settings.

2.2 POTENTIAL RISKS AND BENEFITS

2.2.1 RISKS

Risks involved with this treatment are similar to risks of other standard treatments of wounds of this nature. Possible complications include infection, chronic inflammation (inflammation other than that which may occur immediately following application of product), allergic reaction, excessive redness, pain, swelling, or blistering. If any of these conditions occur, the dressing should be removed.

2.2.2 BENEFITS

This treatment may result in significant and/or complete healing of patients' wounds. If there is no improvement in healing, it is expected that the information gained in this study will be beneficial to wound care patients in the future.

3. OBJECTIVES AND PURPOSE

3.1 PRIMARY OBJECTIVES

The primary objective is to assess the effect of Cytal® with or without MicroMatrix® on primary measures of wound healing efficacy.

3.2 SECONDARY OBJECTIVES

1. Assess the effect of Cytal® with or without MicroMatrix® on secondary measures of wound healing efficacy.

2. Assess effect of treatment on patient-reported quality of life at baseline and at time of healing or at 12 weeks if the wound remains unhealed.
3. Assess complete wound management.
4. Wound-related adverse events.

3.3 TERTIARY OBJECTIVES

1. Assess costs of treatment and patient work status.
2. Assess wound pathology.

4. STUDY DESIGN AND ENDPOINTS

4.1 DESCRIPTION OF THE STUDY DESIGN

This study is a single-site, prospective, observational study of Cytal® Wound Matrix alone or in combination with MicroMatrix® for management of wounds. Cytal® and MicroMatrix® both consist of decellularized porcine urinary bladder matrix (UBM). The processed UBM retains native extracellular matrix structure with intact epithelial basement membrane and numerous collagens that may promote improved wound healing. Cytal® consists of single- or multilayer sheets of UBM, while MicroMatrix® is a micronized powder form of UBM. Both products are FDA 510k cleared (K152721 and K060888) for use in the management of wounds.

In this study, Cytal® Wound Matrix 1-Layer will be applied to study wounds, with MicroMatrix® applied topically to fill the base of deep wounds (exceeding 3mm in depth) according to product use instructions. Patients with diverse wound types will be observed in the study, including but not limited to patients with venous ulcers, diabetic foot ulcers, pressure ulcers, trauma wounds, external surgical wounds, and wound dehiscence/surgical site infection.

Patients may undergo weekly treatment for their wounds and can be treated with up to 10 applications of Cytal® (with MicroMatrix® when wound depth exceeds 3mm) within the first 12 weeks. If the Subject has multiple wounds, the most-appropriate wound that meets inclusion criteria and has at least two cm of healthy tissue between itself and other wounds (as determined by the investigator) will be selected as the Study Wound. A protocolized standard of care will be followed for Study Wounds in addition to the application of Cytal® and MicroMatrix® (i.e. surgical debridement, offloading etc. as appropriate for the particular wound type and severity).

4.1.1 PRIMARY ENDPOINT

1. Effect of Cytal® with or without MicroMatrix® on primary measures of wound healing efficacy will be assessed by determining:

- a. Number and percentage of wounds with area reduced by greater than or equal to 40% by week 4, 60% by week 8, and 80% by week 12 as determined by wound image planimetry for each wound type.
- b. Number and percentage of completely healed wounds (100% epithelialization) by 12 weeks for each wound type. This will be verified by investigators and through a blinded review of wound photos by independent wound physicians.

4.1.2 SECONDARY ENDPOINTS

1. Secondary measures of wound healing efficacy will be assessed through week 12 by determining:
 - a. Time to complete wound closure as determined by wound image planimetry using the Silhouette Star camera system by Aranz®.
 - b. Wound characteristics including granulation tissue quality and presence/quantity of exudate as determined by standardized clinician evaluation.
 - c. Incidence of bridging to definitive closure such as graft, flap (when bridging is the goal of treatment), or transition to cellular therapy as determined by investigator.
2. Effect of treatment on patient-reported quality of life will be assessed by Visual Analog Scale (VAS), Wound Quality of Life (W-QOL), and Katz ADL health-related quality of life surveys at healing or at 12 weeks if the wound remains unhealed.
3. Complete wound management will be assessed through week 12 by recording the following on a standardized questionnaire:
 - a. Method of application of Cytal®/MicroMatrix® and how the product is secured (e.g. sutures, steri-strips).
 - b. Adjunctive treatments and dressings (e.g. debridement, offloading, negative pressure wound therapy, hyperbaric oxygen).
4. Number and type of wound-related adverse events through week 12 as recorded on adverse event case report forms.

4.1.3 TERTIARY ENDPOINTS

1. Costs of treatment and patient work status will be assessed through week 12 by the following:
 - a. Direct wound-related costs will be calculated and stratified by product and care cost.
 - b. Current employment status and return to work status will be recorded and frequency of changes will be assessed.
 - c. Measure frequency of wound specific AEs and SAEs.
2. Wound Pathology will be assessed through week 12 by the following:
 - a. Effect of treatment with Cytal® with or without MicroMatrix® on pro-inflammatory and anti-inflammatory macrophage biomarkers, vascularization, and fibrosis in wound tissue biopsies will be assessed through quantitative image analysis for the following stains:

- i. CD86 and CD163 immunostains to quantify pro-inflammatory M1 and anti-inflammatory M2 macrophage phenotypes.
 - ii. CD31 immunostain to quantify vascularization.
 - iii. Masson's trichrome to quantify fibrosis.
- b. Pathologists' findings over the course of treatment will be assessed by quantification of standardized pathologists' findings in wound pathology reports based on Hematoxylin and Eosin staining.

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. Subject has at least one wound that the treating physician determines may be treated with Cytal® with or without MicroMatrix®.
2. Subject is ≥ 22 years of age.
3. Subject is willing and able to adhere to protocol requirements and agrees to participate in the study program and comply with the study follow-up regimen.
4. Subject or legal representative is willing to provide informed consent.
5. For females of reproductive potential, confirmed negative pregnancy test at enrollment.

5.2 PARTICIPANT EXCLUSION CRITERIA

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

1. Allergy or hypersensitivity to materials in porcine-based study products (per subject report) or personal preference.
2. Subject report of concurrent participation in another clinical trial that involves an investigational drug or device that would interfere with this study.
3. The subject has any physical or psychiatric condition that, in the Investigator's opinion, would warrant exclusion from the study or prevent the subject from completing the study.
4. The subject's wound shows evidence of infection as determined by the Principal Investigator (which may be indicated by presence of: elevated WBC, pus, moderate or greater discharge, abnormal odor, or acute osteomyelitis).
5. Wound with exposed organs or hardware.
6. Wound with burn etiology.

5.3 STRATEGIES FOR RECRUITMENT AND RETENTION

80 subjects (maximum of 100 enrolled) are expected to complete the trial after an approximate 20% dropout/withdrawal rate. The enrollment process will take place over a 12 month period

between June 2018 and May 2019, with all subjects expected to have completed the study by May 2020. All eligible patients will be selected for participation from within NYU Winthrop Hospital, including the outpatient Wound Healing Center clinic.

Patients will be compensated for participation in the trial. Patients will receive one \$25 gift card for each study visit (1 – 12), as well as a \$50 gift card for both the Long-Term Follow-Up visit at Week 26 and the Final Study visit at Week 52.

5.4 PARTICIPANT WITHDRAWAL OR TERMINATION

5.4.1 REASONS FOR WITHDRAWAL OR TERMINATION

A subject may be discontinued from the study at any time if the subject, the Investigator, or the Sponsor feels that it is not in the subject's best interest to continue. The following is a list of *possible* reasons for study discontinuation:

- Screen Failure
- Subject withdrawal of consent
- Subject is not compliant with study procedures
- Adverse Event that, in the opinion of the Investigator, would be in the best interest of the subject to discontinue study participation and/or study intervention
- Protocol violation requiring discontinuation
- Lost to follow-up
- Sponsor request for early termination of study
- Subject death

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without repercussion.

Reasonable attempts will be made by the Investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents and the Case Report Form (CRF).

If a subject is withdrawn from treatment due to an AE, the subject will be followed and treated by the Investigator until the abnormal parameter or symptom has resolved or stabilized.

The Investigator must make every effort to contact subjects who are lost to follow-up. Three (3) attempted contacts should be documented by key research personnel in order to consider the participant lost to follow-up. Attempts to contact such subjects must be documented in the patients' records (e.g., times and dates of attempted telephone contact, etc.).

5.4.2 HANDLING OF PARTICIPANT WITHDRAWALS OR TERMINATION

Although subjects may withdraw from the study at any time and for any reason, (or may be withdrawn at the Investigator's discretion), subject withdrawal should be avoided as much as reasonably possible. However, data already accumulated on the patient will be utilized in the final analysis.

Subjects who prematurely discontinue are not to be replaced. For subjects considered lost to follow-up, the CRFs must be completed up to the last visit performed.

5.5 PREMATURE TERMINATION OR SUSPENSION OF STUDY

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to the Investigator, the Sponsor and the Institutional Review Board (IRB), as appropriate. If the study is prematurely terminated or suspended, the Investigator will promptly inform the IRB and will provide the reason(s) for the termination or suspension.

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

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and/or data quality are addressed and satisfy the Sponsor and/or the IRB.

6. STUDY AGENT

6.1 STUDY DEVICE DESCRIPTION

MicroMatrix®

MicroMatrix® is composed of a porcine-derived extracellular matrix known as urinary bladder matrix and is intended for the management of topical wounds including: partial and full-thickness wounds, pressure ulcers, venous ulcers, diabetic ulcers, chronic vascular ulcers, tunneled/undermined wounds, surgical wounds (donor sites/grafts, post-Mohs surgery, post-laser surgery, podiatric, wound dehiscence), trauma wounds (abrasions, lacerations, second-degree burns, skin tears) and draining wounds. The device is intended for one-time use.

The devices are supplied in a particle form in masses up to 1000mg, and packaged in a glass vial and a peel-open pouch.

Refer to Instructions for Use for further details.

Cytal® Wound Matrix 1-Layer

Cytal® Wound Matrix 1-Layer is composed of a porcine-derived extracellular matrix also known as urinary bladder matrix. The device is intended for the management of wounds including: partial and full-thickness wounds, pressure ulcers, venous ulcers, diabetic ulcers, chronic vascular ulcers, tunneled/undermined wounds, surgical wounds (donor sites/grafts, post-Mohs surgery, post-laser surgery, podiatric, wound dehiscence), trauma wounds (abrasions, lacerations, second-degree burns, skin tears) and draining wounds. The device is intended for one time use.

The devices are supplied in a sheet configuration in sizes up to 10 cm x 15 cm and packaged in double peel-open pouches.

Refer to Instructions for Use for further details.

6.1.1 ACQUISITION AND ACCOUNTABILITY

The Sponsor (or designee) will ship Cytal® Wound Matrix 1-Layer and MicroMatrix® to the investigational site. The initial study product shipment will be shipped after site activation (i.e., when all required regulatory documentation has been received by the Sponsor and a contract has been executed). Subsequent study product shipments will be made after the site requests for resupply.

Upon receipt of the Cytal® Wound Matrix 1-Layer and MicroMatrix®, an inventory check must be performed against the device receipt log, filled out and signed by the person accepting the shipment. It is important that the designated study staff counts and verifies that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study devices in a given shipment will be documented in the study files. The Investigator must notify the study Sponsor of any damaged or unusable study devices that were supplied to the Investigator's site.

Regular study device reconciliation will be performed to document device assignment and use. This reconciliation will be logged on the Device Accountability Log and signed and dated by the PI.

6.1.2 PRODUCT STORAGE

MicroMatrix® and Cytal® Wound Matrix 1-Layer should be stored at room temperature in a clean, dry environment, in the unopened and undamaged package. The products should be protected from freezing temperatures, excessive heat, and high humidity.

6.1.3 RETURN OR DESTRUCTION OF STUDY DEVICE

All unused Cytal® Wound Matrix 1-Layer and MicroMatrix® will be returned to the Sponsor at the time of the study Close Out Visit (COV). A detailed Device Accountability Log of the returned study product(s) will be provided to the Sponsor at the end of the study.

7. STUDY PROCEDURES AND SCHEDULE

7.1 STANDARD OF CARE

7.1.1 STANDARD OF CARE DEFINITION

1. No concurrent use of tissue engineered materials (e.g. Apligraf®, Dermagraft®) or other scaffold materials (e.g. Oasis®) at initial treatment or during the 12 week treatment phase.
2. Compression therapy for venous patients, unless contraindicated.
3. Offloading of pressure ulcers and diabetic ulcers as indicated.
4. Negative pressure wound therapy for deep wounds as indicated.
5. Hyperbaric oxygen therapy as indicated.
6. Visual Analog Pain Scale (SOC at each study visit).
7. If signs and symptoms indicate a likely infection, the study product will not be placed, and the wound will be managed per investigator discretion for resolution of infection.
 - a. This includes but is not limited to: topical antimicrobials, systemic antibiotics and debridement in the operating room.

7.2 STUDY SCHEDULE

7.2.1 SCREENING VISIT

(MUST OCCUR WITHIN 7 DAYS OF THE TREATMENT VISIT)

The Screening Visit may be combined with the Treatment Visit, as long as all eligibility criteria have been confirmed prior to treatment.

1. Evaluation for potential eligibility for study inclusion.
2. Obtain informed consent from the potential subject or Legally Authorized Representative.
3. Assign a unique subject identification number (SID). SIDs will be assigned in consecutive order and will not be re-used in the case of Screen Failures.

4. Perform physical examination, wound SOC activities, and review medical, surgical, and medication histories.
5. Perform a pregnancy test for females of reproductive potential.
6. Collect demographic data including: date of birth, gender, race, ethnicity, alcohol use and tobacco use.
7. Collect employment and ambulatory status.
8. Quality of Life Assessments (Katz ADL, W-QoL, VAS).
9. Full wound evaluation and wound management as appropriate.
 - a. Wound photos to include one zoomed out photo with at least one anatomical landmark, at least one zoomed in photo before debridement, at least one zoomed in photo after debridement and at least one zoomed in photo after product application(if applied). All photos taken in accordance with standardized wound care photography protocols.

7.2.2 TREATMENT VISIT (STUDY DAY #0)

1. Ensure subject remains eligible prior to study device application.
2. Document laboratory results and ABI (as required) for lower extremity wounds. Required labs include Hgb A1C (for diabetic subjects), pre-albumin and albumin.
3. Confirm negative pregnancy test for females of reproductive potential prior to initial device application.
4. Document and update changes to concomitant medication log and medical history.
5. Record AEs as reported by participant or observed by Investigator.
6. Perform wound SOC and application of product(s).
7. Document updates and/or changes to Employment and/or Ambulatory status.
8. Wound biopsy prior to product application for pathological evaluation.
9. Full wound evaluation and wound management as appropriate.
10. Wound photos to include one zoomed in photo before debridement, at least one zoomed in photo after debridement and at least one zoomed in photo after product application(if applied). All photos taken in accordance with standardized wound care photography protocols.

7.2.3 REGULAR STUDY VISITS #1-12 AS INDICATED (VISITS ARE SEQUENTIAL AND CONTIGUOUS, BASED OFF OF TREATMENT VISIT)

For All Follow-Up Visits:

1. Full wound evaluation and wound management as appropriate.
 - a. Wound photos to include one zoomed in photo before debridement, at least one zoomed in photo after debridement and at least one zoomed in photo after product application(if applied). All photos taken in accordance with standardized wound care photography protocols.

2. Study device application (up to a maximum of 10 applications between Weekly Follow-up Visit 1 - 12, unscheduled visits and the Initial Device Application at the Treatment Visit) as needed.
3. Assessment of target wound related Adverse Events.
4. Document and update changes to concomitant medication log and medical history
5. Document updates and/or changes to Employment and/or Ambulatory status.
6. Perform delayed wound healing labs & procedures (PRN, as determined by the Investigator).
7. Perform closure and recurrence assessment.

For Follow-Up Visit 6 & 12 ONLY (Wound SOC, FUV activities above, PLUS the following):

1. Document updates and/or changes to Employment and/or Ambulatory status.
2. Document laboratory results and ABI (as required) for lower extremity wounds. Required labs include Hgb A1C (for diabetic subjects), pre-albumin and albumin.
3. Quality of Life Assessments (Katz ADL, W-QoL, VAS).

Biopsy and Pathology (Follow-Up Visits):

For normally healing wounds there will be wound biopsies taken at the following intervals. Each will include IHC staining for macrophage phenotype and quantitative analysis.

1. Biopsy at Initial treatment visit before product application
2. Visit 1 (approximately 7 days post-initial treatment) biopsy required, before debridement and product application, if either is performed
3. A third and final biopsy, when normal healing progress is being made to characterize the normal healing trajectory, but prior to complete epithelization. Timing of biopsy is at the treating Physician's discretion but must be recorded in the database.

For wounds which, in the view of the Investigator, appear to not be progressing to full wound healing, or are stalled in progress, there will be additional wound biopsies taken at the following intervals. Each will include IHC staining for macrophage phenotype and quantitative analysis.

1. Biopsy at Initial treatment visit before product application
2. Visit 1 (approximately 7 days post-initial treatment) biopsy required, before debridement and product application, if either is performed
3. Visit 4 biopsy required, before debridement and product application, if either is performed.
4. A fourth and final biopsy at ~ 70-90% wound closure.

7.2.4 UNSCHEDULED VISIT (RECORD DATE OF VISIT)

Unscheduled visits may occur at any time after the informed consent form is signed until the Final Study Visit occurs. All activities listed below must be conducted on the same calendar day as applicable for that visit, and the visit must be related to the targeted study wound.

1. Document reason for the unscheduled visit.
2. Document and update changes to concomitant medication log and medical history.
3. Perform a physical examination and wound SOC.
4. Record AEs as reported by participant or observed by Investigator.
5. Perform closure and recurrence assessment.
6. Full wound evaluation and wound management as appropriate.
 - a. Wound photos to include one zoomed in photo before debridement, at least one zoomed in photo after debridement. All photos taken in accordance with standardized wound care photography protocols.
7. Perform delayed wound healing labs & procedures. Labs include Hgb A1C (for diabetic subjects), pre-albumin and albumin, as applicable.

7.2.5 LONG-TERM FOLLOW UP VISIT & FINAL STUDY VISIT

(LTFU AT 26 WEEKS [182 DAYS] AND FSV AT 52 WEEKS [365 DAYS] FROM TREATMENT VISIT, ± 14 DAYS)

The following activities are required for the Long-Term Follow-Up Study Visit and the Final Study Visit:

1. Record any changes to employment and ambulatory status.
2. Document and update changes to concomitant medication log and medical history.
3. Record AEs as reported by participant or observed by Investigator.
4. Quality of Life Assessments (Katz ADL, W-QoL, VAS).
5. Perform a physical examination and wound SOC.
6. Perform delayed wound healing labs & procedures (PRN, as determined by the Investigator).
7. Collect updates to tobacco use.
8. Document laboratory results and ABI (as required) for lower extremity wounds (PRN at these visits, not required).
9. Perform closure and recurrence assessment.
10. Full wound evaluation and wound management.
 - a. Wound photos to include one zoomed in photo before debridement, at least one zoomed in photo after debridement (if performed). All photos taken in accordance with standardized wound care photography protocols.

7.3 CONCOMITANT MEDICATIONS, TREATMENTS, AND PROCEDURES

Concomitant prescription medications taken during study participation will be recorded on the case report forms (CRFs). For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the CRF are those related to the study condition as well as any steroids, antibiotics, anti-hypertensives, pain medications, or nutritional supplements (including TPN).

8. ASSESSMENT OF SAFETY

8.1 SPECIFICATION OF SAFETY PARAMETERS

8.1.1 DEFINITION OF ADVERSE EVENTS (AE)

Target wound related Adverse Events (AEs) will be captured from the time of initial treatment. An AE is defined as follows:

- Any adverse change in the subject's medical status when compared with the subject's baseline condition, whether or not the event is related to the device or a procedure; or
- An exacerbation (either in frequency or severity) in a subject's pre-existing condition.

Known sequelae, judged by the Investigator to be associated with target wound procedures (e.g., pain or bleeding associated with debridement) and/or the course of normal wound healing (e.g., slough or mild exudate) should not be recorded as an AE unless: 1) an additional treatment/procedure is required (e.g., use of a concomitant medication); or 2) the frequency, severity and/or duration deviates from the expected course. These include but are not limited to:

- Infection/sepsis
- Worsening of wound bed/failure to heal
- Increased chronic inflammation
- Allergic reaction
- Unexplained fever or chills
- Death
- Neurologic symptoms such as light headedness, headache
- Gastrointestinal symptoms including nausea and vomiting
- Pain

In some cases, an increase in wound size may be documented as an AE. This determination will be made using the following thresholds for overall change in wound size (**from baseline post-debridement measurement**):

- 0-25% - Does not require AE reporting
- 25-50% - Physician discretion – potential opportunity for further investigation of size changes.
- 50%+ - Must be reported as an AE.

The AE will be noted as “resolved” upon $\geq 10\%$ reduction (from largest wound size) maintained for 2 consecutive weeks.

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
- Inpatient 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 patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

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 treatment 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 treatment. Severe events are usually potentially life-threatening or incapacitating.

8.2.2 RELATIONSHIP TO STUDY DEVICE AND/OR PROCEDURE

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 possibly impactful to the development or healing of the target wound will be captured on the appropriate CRF. Information to be collected includes event description, date of onset, clinician's assessment of severity, relationship to study products (assessed only by those with the training and authority to make a diagnosis), and time 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 device application through the last day of study participation. 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. 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 REPORTING

In the case of a 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 adherence to be stable. Other supporting documentation of the event may be requested by the DCC/study sponsor and should be provided as soon as possible. The study Sponsor will be responsible for notifying 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 REPORTING

An Investigator shall submit to the Sponsor and to the reviewing IRB a report of any UADE occurring during an investigation as soon as possible, but in no event later than 10 working days after the investigator first learns of the effect [21 CFR 812.150(a)(1)]. A Sponsor who conducts an evaluation of an UADE under 812.46(b) shall report the results of such evaluation to FDA and to all reviewing IRBs and participating investigators within 10 working days after the Sponsor first receives notice of the effect. Thereafter, the Sponsor shall submit such additional reports concerning the effect as FDA requests [21 CFR 812.150(b)(1)].

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 becomes pregnant during this trial, she must be followed until the outcome of the pregnancy is known. Should she become pregnant during the follow-up visit period, no additional study devices will be placed.

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 DESCRIPTION OF STATISTICAL METHODS

Wound area will be analyzed for size, percentage closure on weekly basis. The mean, median and standard deviations will be calculated and scatter plots developed to determine healing rates by application number, and days in treatment. The mean number of application of product will be tabulated.

All statistical tests to examine the change from baseline (study pre-treatment) will be conducted using a type 1 error rate of 5%. Simultaneous testing for the 2 primary endpoints will be conducted based on individual a priori thresholds. The analyses of the secondary endpoints will be conducted without adjustment for multiple testing.

The analysis of the primary endpoints will be based on a generalized linear model specifying the distribution as binomial. If a patient withdraws prior to completing the study, however the last recorded observation revealed the wound had closed, the patient will be counted in the primary analysis as having achieved complete wound epithelization. If a patient withdraws prior to the 12-week wound evaluation visit and the last recorded observation revealed the wound had not closed, the patient will be counted in the primary analysis as not having achieved complete wound epithelization. This procedure will also be followed for the examination of the patients by the various percent reduction thresholds. The analyses of all non-primary endpoints will be based on the distribution of data. For continuous variables, descriptive statistics will be used and the change from baseline will be tested against a difference of zero. For binomial and multinomial variables, results will be presented using counts, percentages, and shift tables. The analysis of the time of the initial observation of complete wound epithelization will be reported using Kaplan-Meier estimates. The sample size for this clinical investigation was derived based on establishing sufficient precision among the different endpoints to be able to reliably estimate the expected effect of the 2 product used either alone or in combination. For proportions, the length of the 2-sided 95% confidence interval from the proportion to the limit with 80 patients is between 9% and 10% (ref. table presented below).

Scenario	Lower 95% Exact Confidence Limit (%)	Percentage of Patients Who Respond	Upper 95% Exact Confidence Limit (%)
1	15.99	25.00	35.94
2	26.92	37.50	49.04
3	30.33	40.00	50.28
4	50.96	62.50	73.08
5	64.06	75.00	84.01

For mean changes from baseline, if the standard deviation of the difference from baseline for a parameter is 10 units, the confidence level will exceed 90% with a distance from the mean to the limit as small as 2 units.

For data derived from a binomial distribution and a continuous distribution, 80 patients should be sufficient to detect a clinically-meaningful change in this clinical investigation.

In summary, the sample size for this clinical investigation is sufficient to detect a 34% difference in the proportion of responders between the 2 treatments (power: 81.9%, alpha: 5%, 40 patients per treatment arm with 30% response and 64% response, respectively). If the observed proportion of response between the 2 treatment arms is 15% and 46% respectively, with 40 patients per treatment arm, the power will be 81.1%. If there is an imbalance between the 2 treatment arms (30 patients and 50 patients, respectively), and observed proportion of response between the 2 treatment arms is 15% and 47%, the power will be 80.3%.

10.1.1 SAFETY ANALYSES

Safety will be assessed by wound examination, clinical laboratory tests and collection of AEs as outlined in the Schedule of Events. All summaries of AEs will be based on treatment-emergent AEs and presented using the incidence and a tabulation of the number of events. The number and percentage of subjects experiencing AEs will be summarized by system organ class and description. Summaries by maximum severity and relationship to the study treatment will also be provided. SAEs and AEs leading to discontinuation from the study will be presented by system organ class and preferred term.

10.1.2 PLANNED INTERIM ANALYSES

There are no planned interim analyses for this clinical investigation.

10.2 SAMPLE SIZE

Estimated 80 subjects will be treated (maximum of 100) in this prospective cohort evaluation of all wounds that will be managed with ACell, Inc. Urinary Bladder Matrix products.

11. SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

Participating site 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. The 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, photographic negatives, and participant files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial.

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

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 INSTITUTIONAL REVIEW BOARD

The protocol, informed consent form(s), and all participant materials will be submitted to the NYU Winthrop Hospital 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.

13.3 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 will 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 signed 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 changes and will sign the amended consent document. If a consent document is revised due to changes in the risks or safety of the study, all active participants must sign the revised consent and a signed copy will be given to them.

13.4 PARTICIPANT AND DATA CONFIDENTIALITY

Participant confidentiality is strictly held in trust by the participating Investigator(s), their staff, the Sponsor and their agents. The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the Sponsor.

The study monitor, other authorized representatives of the Sponsor, and/or representatives of the IRB may inspect all documents and records required to be maintained by the Investigator for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the local IRB and Institutional regulations.

14. DATA HANDLING AND RECORD KEEPING

14.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

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

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Black ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. Do not erase, overwrite, or use correction fluid or tape on the original.

Copies of the electronic CRF (eCRF) will be provided for use as source documents and maintained for recording data for each participant enrolled in the study. Data reported in the eCRF derived from source documents should be consistent with the source documents or the discrepancies should be explained and captured in a progress note and maintained in the participant's official electronic study record.

Clinical data (including AEs, concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into ACell's EDC system, a 21 CFR Part 11-compliant data capture system. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

14.2 STUDY RECORDS RETENTION

All study documents (patient files, signed informed consent forms, Study Regulatory Binder, etc.) must be kept secured for a period of two years following completion of the study. There may be other circumstances for which the Sponsor is required to maintain study records and, therefore, the Sponsor should be contacted prior to removing study records for any reason. It is the responsibility of the Sponsor to inform the Investigator when these documents no longer need to be retained.

14.3 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol or GCP requirements. The noncompliance may be either on the part of the participant, the Investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

All protocol deviations/violations should be documented using the Protocol Deviations CRF and submitted to the IRB according to their reporting guidelines.

14.4 PUBLICATION AND DATA SHARING POLICY

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement among the study Sponsor and participating institutions. The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

15. CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the device industry, is critical. Therefore any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the trial. The study leadership has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

16. APPENDIX I: SCHEDULE OF EVENTS

Evaluation	Screening *	Treatment Visit	WEEKLY FOLLOW-UP VISITS												Week 26 (± 14 days) LTFU Visit	Week 52 (± 14 days) Final Study Visit	Unscheduled
			Week 1 (Visit 1)	Week 2 (Visit 2)	Week 3 (Visit 3)	Week 4 (Visit 4)	Week 5 (Visit 5)	Week 6 (Visit 6)	Week 7 (Visit 7)	Week 8 (Visit 8)	Week 9 (Visit 9)	Week 10 (Visit 10)	Week 11 (Visit 11)	Week 12 (Visit 12)			
Informed Consent	X																
Eligibility Assessment ¹	X	X															
Significant Medical, Surgical, Medication History	X																
Laboratory results & ABI (for lower extremity wounds) ²		X							X					X			X
Pregnancy test ³	X	X															
Employment & Ambulatory Status	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Demographics, Social Info	X														X	X	
Physical Examination	X														X	X	X
Surveys (W-QoL, VAS, Katz)	X								X					X	X	X	
Independent 3 rd party wound assessment ⁴		X				X				X				X			
Wound photographs & planimetry ⁵	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X
Delayed wound healing labs & procedures ⁶																	
Wound SOC activities	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Wound evaluation ⁷	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X
Wound management	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X
Closure & Recurrence assessment			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Cyral®/MicroMatrix® Applications ⁸		X	X	X	X	X	X	X	X	X	X	X	X	X			X
Study Wound related Adverse Events		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Review Concomitant medications and Medical History		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Biopsy and quantitative histopathology ⁹		X	X														

*--Screening Visit must occur within 7 days of Treatment Visit. The Screening Visit may be combined with the Treatment Visit, as long as all eligibility criteria have been confirmed prior to treatment.

1 – If Screening and Treatment Visits are not combined, verify that subject remains eligible prior to study device application (no need to repeat activities).

2 – ABI is PRN, as determined by the Investigator.

3 – Confirm a negative pregnancy test prior to study device application (test does not need to be repeated twice).

4 – Independent 3rd party expert will complete wound assessment as photographs are available. Additional assessments to be completed as appropriate.

5 – This is done through the use of the Silhouette Star camera system by Aranz®. To be completed at LTFU/FSV if applicable.

6 – PRN activity at all visits.

7 – Wound evaluations to be completed at LTFU/FSV if applicable.

8 – Study device may be applied no more than 10 times total. This includes one (1) application at the Treatment Visit, and up to nine (9) additional applications throughout FUV 1-12 and unscheduled visits.

- 9 - Pathology on biopsy – For Normally healing wounds: Biopsies to be completed before debridement and product application at Initial Treatment Visit, Visit 1, and a third and final biopsy to be obtained at timepoint determined by the Investigator. For delayed healing wounds: Biopsies to be completed before debridement and product application at Initial Treatment, Visit 1, Visit 4, and a fourth and final biopsy at ~70-90% wound closure.
- 10 – Surveys will also be completed at time of healing.

17. 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	