A Randomised Controlled Multicentre Trial, Examining the Effect of Natrox® Oxygen Wound Therapy on the Healing Rate of Chronic Diabetic Foot Ulcers (NOW.T-001)

Version 2.0 / 01 November 2019

Regulatory Sponsor: Inotec AMD Ltd.
200 1st Avenue NW, Suite 408
Hickory, NC 28601-6113

Study Product: Natrox® Oxygen Therapy Kit®

Trial Registration #: NCT03905863

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Statement of Compliance

This document is a protocol for a human research study. This study will be conducted according to US and International standards of Good Clinical Practice, applicable government regulations and Institutional research policies and procedures.

All individuals responsible for the design and conduct of this study have completed Human Subjects Protection Training and are qualified to be conducting this research prior to the enrollment of any subjects.

As Principal Investigator, I agree to conduct this clinical study in accordance with the design and specific provisions of this protocol. Modifications to the study are acceptable only with an approved protocol amendment. I agree to obtain approval from the IRB and/or regulatory bodies of competent jurisdiction, for the protocol and informed consent before initiating the study, to obtain consent from subjects prior to their enrollment in the study, to collect and record data as required by this protocol and case report forms, to prepare adverse event and study reports as required by this protocol and to maintain study documentation for the period of time required.

Thomas E. Serena MD FACS

Name

15 April 2019

Date

Version History

<table>
<thead>
<tr>
<th>Version #</th>
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<th>Significant Changes from Previous Version</th>
</tr>
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<tbody>
<tr>
<td>Version 1.0</td>
<td>15 April 2019</td>
<td>Original Protocol Version</td>
</tr>
<tr>
<td>Version 2.0</td>
<td>01 November 2019</td>
<td>Amendment</td>
</tr>
</tbody>
</table>
# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Statement of Compliance</td>
<td>2</td>
</tr>
<tr>
<td>Version History</td>
<td>2</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>3</td>
</tr>
<tr>
<td>List of Tables</td>
<td>6</td>
</tr>
<tr>
<td>List of Figures</td>
<td>6</td>
</tr>
<tr>
<td>Study Synopsis</td>
<td>7</td>
</tr>
<tr>
<td>Abbreviations</td>
<td>10</td>
</tr>
<tr>
<td>1 Study Contact Information</td>
<td>11</td>
</tr>
<tr>
<td>1.1 Sponsor Contact Information</td>
<td>11</td>
</tr>
<tr>
<td>1.2 Principal Investigator Contact Information</td>
<td>11</td>
</tr>
<tr>
<td>1.3 Medical Monitor</td>
<td>11</td>
</tr>
<tr>
<td>2 Introduction / Background and Rationale</td>
<td>12</td>
</tr>
<tr>
<td>3 Device Description</td>
<td>12</td>
</tr>
<tr>
<td>3.1 Natrox® Oxygen Wound Therapy System</td>
<td>12</td>
</tr>
<tr>
<td>3.2 Natrox® Oxygen Delivery System</td>
<td>13</td>
</tr>
<tr>
<td>4 Device Accountability</td>
<td>13</td>
</tr>
<tr>
<td>4.1 Device Receipt</td>
<td>13</td>
</tr>
<tr>
<td>4.2 Device Storage</td>
<td>14</td>
</tr>
<tr>
<td>4.3 Device Dispensing</td>
<td>14</td>
</tr>
<tr>
<td>4.4 Device Disposition</td>
<td>14</td>
</tr>
<tr>
<td>4.5 Return or Destruction of Unused Devices</td>
<td>14</td>
</tr>
<tr>
<td>5 Study Objectives</td>
<td>14</td>
</tr>
<tr>
<td>5.1 Primary Objective</td>
<td>14</td>
</tr>
<tr>
<td>5.2 Secondary Objectives</td>
<td>14</td>
</tr>
<tr>
<td>6 Study Design</td>
<td>15</td>
</tr>
<tr>
<td>6.1 Overview of Study Design</td>
<td>15</td>
</tr>
<tr>
<td>6.2 Anticipated Duration of the Clinical Investigation</td>
<td>15</td>
</tr>
<tr>
<td>6.3 Evaluation Criteria / Effectiveness and Safety</td>
<td>15</td>
</tr>
<tr>
<td>6.3.1 Primary Clinical Endpoint</td>
<td>15</td>
</tr>
<tr>
<td>6.3.2 Secondary Clinical Endpoint(s)</td>
<td>15</td>
</tr>
</tbody>
</table>
6.4 Study Population .................................................................................................................. 15
  6.4.1 Sample Size ......................................................................................................................... 16
  6.4.2 Subject Recruitment .......................................................................................................... 16
  6.4.3 Subject Screening (~4 weeks) .......................................................................................... 16
  6.4.4 Prior and Concomitant Therapy or Medications ................................................................. 18
  6.4.5 Inclusion Criteria .............................................................................................................. 19
  6.4.6 Exclusion Criteria ............................................................................................................. 20
  6.4.7 Exit / Discontinuation Criteria .......................................................................................... 21

7 Study Procedures .................................................................................................................... 21
  7.1 Informed Consent ................................................................................................................. 21
  7.2 Vulnerable Populations ....................................................................................................... 21
  7.3 Randomization Scheme ...................................................................................................... 21
  7.4 Laboratory Testing Procedures ............................................................................................ 21
  7.5 Clinical Procedures ............................................................................................................. 22
    7.5.1 Screening Visit (~2 weeks) ............................................................................................. 22
    7.5.1b Visit 1(Day 0 ± 3) ......................................................................................................... 23
    7.5.2 Visit 2 (Day 7 ± 3) ......................................................................................................... 24
    7.5.3 Visit 3 (Day 14 ± 3) ....................................................................................................... 24
    7.5.4 Visit 4 (Day 21 ± 3) ...................................................................................................... 25
    7.5.5 Visit 5 (Day 28 ± 3) ...................................................................................................... 25
    7.5.6 Visit 6 (Day 35 ± 3) ...................................................................................................... 26
    7.5.7 Visit 7 (Day 42 ± 3) ...................................................................................................... 26
    7.5.8 Visit 8 (Day 49 ± 3) ...................................................................................................... 26
    7.5.9 Visit 9 (Day 56 ± 3) ...................................................................................................... 27
    7.5.10 Visit 10 (Day 63 ± 3) ................................................................................................. 27
    7.5.11 Visit 11 (Day 70 ± 3) ................................................................................................. 28
    7.5.12 Visit 12 (Day 77 ± 3) ................................................................................................. 28
    7.5.13 Visit 13 – End of Study Visit (Day 84 ± 3) .................................................................. 28
    7.5.14 Unscheduled Visit ...................................................................................................... 29
  7.6 Follow-Up Procedures and Therapy Transitions ................................................................. 29
  7.7 Study Timetable / Schedule of Events .............................................................................. 29
  7.8 Study Protocol Compliance / Treatment Adherence ......................................................... 32
7.9 Deviations from the Clinical Protocol ................................................................. 32
7.10 Subject Withdrawal ....................................................................................... 32
  7.10.1 How to Withdraw Subjects .................................................................. 32
  7.10.2 Data Collection and Follow-Up for Withdrawn Subjects .................. 32
7.11 Subject Compensation ................................................................................. 32
8  Data Collection and Analysis ......................................................................... 33
  8.1 Subject Population(s) for Analysis ............................................................. 33
  8.2 Statistical Methods .................................................................................... 33
9  Safety and Adverse Events ............................................................................ 33
  9.1 Definitions .................................................................................................... 33
  9.2 Safety Monitoring Plan .............................................................................. 36
    9.2.1 Anticipated Risks / Risk Mitigation ...................................................... 36
    9.2.2 Medical Monitoring for Participant Safety ........................................ 36
  9.3 Anticipated Adverse Events ................................................................. 36
  9.4 Adverse Event Reporting ...................................................................... 37
    9.4.1 Adverse Events .................................................................................. 37
    9.4.2 Serious Adverse Events ................................................................. 38
    9.4.3 Unanticipated Adverse Device Effects (UADE) ............................ 39
10 Data Handling and Record Keeping ............................................................. 40
  10.1 Confidentiality .......................................................................................... 40
  10.2 Source Documents .................................................................................. 40
  10.3 Case Report Forms ................................................................................. 40
  10.4 Clinical Reports ...................................................................................... 41
  10.5 Records Retention .................................................................................. 41
11 Study Monitoring, Auditing, and Inspecting ............................................. 41
  11.1 Study Monitoring Plan ......................................................................... 41
  11.2 Quality Assurance Procedures ............................................................. 41
  11.3 Auditing and Inspection ....................................................................... 41
12 Administrative Study Information ............................................................ 42
  12.1 Technical Support .................................................................................. 42
  12.2 Pre-Study Site Qualification ................................................................. 42
  12.3 Protocol Amendments After Study Initiation ........................................ 42
12.4 Materials / Services Provided by Sponsor and Coordinating Center ........................................... 42
13 Ethical Considerations .................................................................................................................... 42
14 Study Finances ............................................................................................................................ 43
  14.1 Funding Source ..................................................................................................................... 43
  14.2 Conflicts of Interest ............................................................................................................. 43
15 Publication Plan ............................................................................................................................ 43
16 Definition of Standard of Care .................................................................................................... 43
  16.1 Cleaning the Study Ulcer ..................................................................................................... 43
  16.2 Debridement of the Study Ulcer ......................................................................................... 43
  16.3 Offloading ......................................................................................................................... 44
  16.4 Moisture balance .............................................................................................................. 44
  16.5 Subject Instructions ............................................................................................................ 44
17 Ulcer Infection During the Trial ................................................................................................. 45
18 References .................................................................................................................................... 45
19. Appendices and Attachments .................................................................................................. 47
   Appendix A: Photographic Measurement ................................................................................. 47
   Appendix B: Application Guide for NATROX® ......................................................................... 49
   Appendix C: Debridement Technique ....................................................................................... 50
   Appendix D: Screening for Peripheral Arterial Disease. .......................................................... 51
     Transcutaneous Oximetry (TCOM) ......................................................................................... 53

List of Tables

Table 1. IDSA Assessment/Grading of Diabetic Ulcer Infection .................................................. 18
Table 2. SINBAD Score ................................................................................................................ 18
Table 3. Schedule of Events ......................................................................................................... 30
Table 4. Statistical Analyses ......................................................................................................... 33
Table 5. Anticipated Risks .......................................................................................................... 36

List of Figures

Figure 1. Natrox® Oxygen Generator and Batteries ................................................................. 13
Figure 2. Natrox® Oxygen Delivery System ............................................................................. 13
**Study Synopsis**

<table>
<thead>
<tr>
<th><strong>Title</strong></th>
<th>A Randomised Controlled Multicentre Trial, Examining the Effect of Natrox® Oxygen Wound Therapy on the Healing Rate of Chronic Diabetic Foot Ulcers</th>
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<tbody>
<tr>
<td><strong>Short Title</strong></td>
<td>Natrox® Oxygen Therapy Kit</td>
</tr>
<tr>
<td><strong>Trial Registration Numbers</strong></td>
<td>NCT03905863</td>
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| **Study Sponsor** | Inotec AMD, Ltd.  
200 1st Avenue NW. Suite 408  
Hickory, NC 28601-6113 |
| **Principal Investigator** | Thomas E. Serena MD FACS |
| **Study Design** | Multi-center, open label, randomized controlled trial |
| **Study Duration** | Study is estimated to require 9 months from first subject enrolled to last subject visit. |
| **Study Center(s)** | Up to 40 SerenaGroup or affiliated centers |

**Objectives**

**Primary Objective:** To demonstrate increased healing rates of chronic wounds (diabetic foot ulcers) which have been unresponsive to standard therapy with the addition of Natrox® oxygen therapy when compared to standard of therapy alone.

**Secondary Objective:** To demonstrate the ease of implementation of the addition of Natrox® to standard therapy regimens.

**Number of Subjects**

120 evaluable subjects will be required for this study. To achieve this number 132 will be enrolled.

**Main Inclusion / Exclusion Criteria**

**Inclusion Criteria**

1. Subjects are male or female, 18 years of age or older. At least 50% of the enrolled population must be ≥ 65 years of age.
2. Subjects with one of the following wounds:
   A. Diabetic foot ulcer present for greater than 4 weeks (documented in the medical record) but less than 12 months duration if being treated with active SOC.
   B. Minor amputation wound sites
3. Subject has clinical documentation of no visible wound improvement after 4 weeks of standard of care. (Objectively, less than 40% reduction in surface area in the four weeks prior to the first treatment visit.)
4. Study ulcer is a minimum of 0.5 cm² and a maximum of 25 cm² at first treatment visit
5. Subject’s wound score on IDSA tool is Grade 1 or 2.
6. The subject is able and willing to follow the protocol requirements
7. Subject has signed informed consent
8. Adequate circulation to the affected foot as demonstrated by a dorsum transcutaneous oxygen measurement (TCOM) or a skin perfusion pressure (SPP) measurement of ≥ 30 mmHg; an ABI between 0.7 and ≤ 1.3, or TBI of >6 within 3 months of the first Screening Visit.
9. Females of childbearing potential must be willing to use acceptable methods of contraception (birth control pills, barriers, or abstinence) starting at Screening and continuing through the duration of their study participation
10. The target ulcer has been offloaded for at least 14 days prior to randomization, if applicable.

Exclusion Criteria

1. Subject has a known life expectancy of < 1 year
2. Subject or caregiver is unable to manage the Natrox® device (charge and change batteries daily)
3. Subject has ulcers that are completely necrotic or if the clinician feels it is clinically necessary to cover the wound surface in gel or creams that would prevent the transmission of oxygen to the wound surface.
4. Subject has major uncontrolled medical disorders such as serious cardiovascular, renal, liver or pulmonary disease, lupus, palliative care or sickle cell anemia.
5. Subject currently being treated for an active malignant disease or subjects with history of malignancy within the wound.
6. The Subject has other concurrent conditions that in the opinion of the Investigator may compromise subject safety
7. Known contraindications for the Natrox system
8. Known allergies to any of the Natrox system components
9. Concurrent participation in another clinical trial that involves an investigational drug or device that would interfere with this study.
10. Subject with known HbA1C > 12.0%
11. Subject is pregnant or breast feeding or planning to become pregnant or breastfeed during the trial
12. Subjects with a history of more than two weeks treatment with immunosuppressants (including systemic corticosteroids >10mg daily dose), cytotoxic chemotherapy, or application of topical steroids to the ulcer surface within one month prior to first Screening Visit, or who receive such medications during the screening period, or who are anticipated to require such medications during the course of the study.

13. Index ulcer has been previously treated with tissue engineered materials (e.g. Apligraf® or Dermagraft®) or other scaffold materials (e.g. Oasis, Matristem) within the last 30 days preceding the first treatment visit.

14. Affected extremity requiring hyperbaric oxygen during the trial or within 2 weeks of treatment visit 1.

15. An ulcer that has healed by more than 20% in the 2 weeks prior to the first screening visit (“historical” run-in period) while receiving standard of care treatment

16. Index ulcer has reduced in area by 20% or more after 2 weeks of standard of care treatment from the first screening visit (S1) to the TV1/Randomization visit, (“in-clinic” run-in period)

17. Subject has End Stage Renal Disease

<table>
<thead>
<tr>
<th>Study Device</th>
<th>Natrox® Oxygen Therapy Kit</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Duration of Device Exposure</strong></td>
<td>Device exposure is estimated to be a maximum of 12 weeks.</td>
</tr>
<tr>
<td>Reference Therapy</td>
<td>Standard of Care: Debridement, offloading, and proper moisture balance</td>
</tr>
<tr>
<td><strong>Endpoints</strong></td>
<td><strong>Primary endpoint:</strong></td>
</tr>
<tr>
<td></td>
<td>- Complete wound closure within the 12-week study</td>
</tr>
<tr>
<td></td>
<td>- The percentage change in ulcer size at 12 weeks relative to baseline measurement – this parameter will be performed using a standardised method of wound area measurement</td>
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<tr>
<td></td>
<td><strong>Secondary endpoint:</strong></td>
</tr>
<tr>
<td></td>
<td>- Assessment of the pain level linked to the wound, using a specific pain scale, visual analog scale (VAS)</td>
</tr>
<tr>
<td></td>
<td>- Evaluate average percentage rate of wound closure</td>
</tr>
<tr>
<td></td>
<td>- The number of adverse events between the two arms</td>
</tr>
<tr>
<td></td>
<td>- Number of dressing changes carried out during study</td>
</tr>
<tr>
<td></td>
<td>- Evaluate the number of new wound infections experienced</td>
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</tbody>
</table>
- Evaluate device usability

| Statistical Methods | Statistical analyses will consist of descriptive statistics only. Continuous variables will be assessed by mean, standard deviation, and range. Group comparisons will be assessed at a significance level of $p < 0.05$. |

**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AE</td>
<td>Adverse Event</td>
</tr>
<tr>
<td>CFR</td>
<td>Code of Federal Regulations</td>
</tr>
<tr>
<td>CRF</td>
<td>Case Report Form</td>
</tr>
<tr>
<td>DSMB</td>
<td>Data and Safety Monitoring Board</td>
</tr>
<tr>
<td>DSMP</td>
<td>Data and Safety Monitoring Plan</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>GCP</td>
<td>Good Clinical Practice</td>
</tr>
<tr>
<td>HIPAA</td>
<td>Health Insurance Portability and Accountability Act of 1996</td>
</tr>
<tr>
<td>ICH</td>
<td>International Conference on Harmonization</td>
</tr>
<tr>
<td>IRB</td>
<td>Institutional Review Board</td>
</tr>
<tr>
<td>PHI</td>
<td>Protected Health Information</td>
</tr>
<tr>
<td>SAE</td>
<td>Serious Adverse Event</td>
</tr>
<tr>
<td>UADE</td>
<td>Unanticipated Adverse Device Effect</td>
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</table>
1 Study Contact Information

1.1 Sponsor Contact Information

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1.4 Vice President of Clinical Research

Sarah Moore
Phone: 786-371-6184
E-mail: smoore@serenagroups.com
2 Introduction / Background and Rationale

There is a wealth of evidence to support the benefits of oxygen therapy on wound healing\(^1\) - \(^7\). Oxygen is required for all major processes of wound healing and wound hypoxia is common. Skin wounds can receive oxygen from the blood stream via perfusion and from oxygen uptake through the skin. The increased energy demands of healing tissues lead to dramatic increases in oxidative (oxygen-dependent) metabolism\(^8\),\(^9\) increasing oxygen demands of wound tissues while generating Adenosine Triphosphate (ATP). Yet, both wound perfusion and blood oxygen levels are frequently insufficient in patients with chronic wounds due to poor circulation, vascular disruption, and vasoconstriction, thereby reducing the wound’s capacity to heal.

All diabetic foot ulcers (DFUs) are complex/chronic wounds from onset, as they are a marker of serious disease and comorbidities. They have a major long-term health impact on patients’ morbidity and mortality\(^10\). Diabetic foot ulceration also represents a significant quality of life issue for patients, for example through reduced mobility, loss of independence, and multiple clinic visits. Many patients with DFUs report pain levels that affect daily activities and disrupt sleep\(^12\).

The risk of another recurrence is high: a patient with a healed DFU has a 17–60% risk of another DFU occurring within the following 3 years\(^13\). Diabetic foot wounds are costly to treat and frequently involve risk of increased complications, such as infection, delayed healing and amputation. Around 50% of DFUs become infected, and in approximately 20% of these patients, infection will lead to amputation\(^13\). Half of patients with a DFU who undergo amputation will die within the following 5 years\(^14\).

3 Device Description

The Natrox® Oxygen Wound Therapy System consists of two main elements, the Natrox® Oxygen Generator and the Natrox® Oxygen Delivery System.

3.1 Natrox® Oxygen Wound Therapy System

The Natrox® Oxygen Generator (Figure 1) is a battery-operated device which delivers 98% pure humidified oxygen to the wound bed through water electrolysis. It is supplied with two rechargeable batteries and a charging kit, thus allowing the user complete freedom of movement. Each battery will last for a minimum of 30 hours; however, users should be advised to keep one on charge whilst using the other and changing them over daily.
3.2 Natrox® Oxygen Delivery System

The NATROX® Oxygen Delivery System (Figure 2) is a sterile, single-use wound interface. Its “web” like design allows free passage of wound exudate into the secondary dressing whilst optimising the flow and diffusion of oxygen across the wound bed. It has a 1 m long thin flexible tube which connects directly to the Natrox® Oxygen Generator. The Natrox® Oxygen Delivery System should be changed at each dressing change in accordance with good clinical practice. However, it can remain in place for up to 7 days if clinically appropriate.

4 Device Accountability

4.1 Device Receipt

Inotec will ship devices directly to the clinical sites. Device receipt and inventory procedures will be determined by the Sponsor.
4.2 Device Storage

The Natrox® devices will be stored in secured rooms under ambient conditions, as there are no special storage requirements. Device access will be controlled by the Site Investigator or designee.

4.3 Device Dispensing

The Natrox® Devices will be dispensed to be applied to individual subjects as per the randomization scheme. Device serial numbers will be recorded on the subject’s CRF. Device records will be reconciled at each monitoring visit. This reconciliation will be logged on the device accountability form, and signed and dated by the study team.

4.4 Device Disposition

At the conclusion of the study, subjects are to return all study devices. Investigators will collect the devices from subjects at the termination visit. Device return will be recorded and reconciled with device assignment and accountability records.

4.5 Return or Destruction of Unused Devices

All used and unused devices will be returned to Inotec at the conclusion of the study for full product reconciliation, unless requested earlier by Inotec. Devices will be returned to Inotec at the address below.

Inotec
200 1st Avenue NW. Suite 408
Hickory, NC 28601-6113

5 Study Objectives

5.1 Primary Objective

To demonstrate increased healing rates of chronic wounds (diabetic foot ulcers), which have been unresponsive to standard therapy, when Natrox® O2 therapy is added to standard therapy.

5.2 Secondary Objectives

To demonstrate the ease of implementing Natrox® as an addition to standard therapy regimens.
6 Study Design

6.1 Overview of Study Design

This study is a multi-center, open label, randomized clinical trial. Clinical trial data will not be utilized to make clinical or therapeutic decisions during the trial.

After signing the informed consent, and successfully completing the Screening period, the patient will be randomized to standard of care (SOC) or topical oxygen therapy and SOC.

6.2 Anticipated Duration of the Clinical Investigation

The study is anticipated to require nine months to complete. A minimum anticipated recruitment rate of 1.5 subjects per site per week will complete enrollment within 6 months.

6.3 Evaluation Criteria / Effectiveness and Safety

6.3.1 Primary Clinical Endpoint

There are two primary clinical endpoints:

- Complete wound closure within the 12-week study.
- The percentage of change in ulcer size at 12 weeks relative to baseline measurement – to be performed using a standardized method of wound area measurements.

6.3.2 Secondary Clinical Endpoint(s)

Secondary endpoints include:

- Evaluate the average percentage rate of wound closure
- Evaluate the number of new wound infections experienced
- Number of dressing changes carried out during the study
- The number of adverse events between the two arms
- Assessment of the pain level linked to the wound, using a specific pain scale, visual analog scale (VAS)
- Evaluate Device usability

6.4 Study Population

The study population will be drawn from diabetic subjects attending wound clinics for treatment of diabetic foot ulcers or minor amputation wound sites. These subjects will be drawn from the general population.
6.4.1 **Sample Size**

The study is intended to provide data to support reimbursement. A statistical justification for the sample size was not performed. A dropout rate of 10%-20% is anticipated; therefore, 132 subjects will be enrolled in order to generate an evaluable subject population of 120 subjects.

6.4.2 **Subject Recruitment**

Subjects will be recruited from the investigators’ clinical practices within the participating wound care clinics, and from the general public.

6.4.3 **Subject Screening (-4 weeks).**

Subject screening will be conducted to ensure that subjects meet the study inclusion and exclusion criteria. Documentation will be required to confirm the following:

- Onset of diabetic foot ulcer and “Historical” Run-In measurements: The Investigator will obtain from the medical record the size of the ulcer 14 days +/- 3 days prior to SV1: “historical” run in period. If the ulcer has failed to heal by 20% or greater from that date, and the patient has received standard of care therapy during that time, then the subject can enter screening. If a subject does not have the required measurements, or did not receive SOC treatment, the Investigator can take a manual measurement, treat with SOC and follow the patient for 2 weeks. Subjects must not be consented prior to the completion of the historical run-in period.

- Ulcers that have received standard of care in the wound clinic setting for more than one year are excluded.

- Wound size – as determined by digital photography

- Infection status – as determined by IDSA tool\(^{15}\) (See Table 1)

- Healing status of minor amputations in 4 weeks prior to enrollment

- Acuity (SINBAD) Score\(^{16}\) at time of enrollment (See Table 2).

- Adequate circulation to the affected foot as demonstrated by a dorsum transcutaneous oxygen measurement (TCOM) or a skin perfusion pressure (SPP) measurement of ≥ 30 mmHg; an ABI between 0.7 and ≤ 1.3, or TBI of >6 within 3 months of the first Screening Visit

- Pain Assessment
• Females who are of childbearing potential must have a urine or blood pregnancy test at screening.
Table 1. IDSA Assessment/Grading of Diabetic Ulcer Infection

<table>
<thead>
<tr>
<th>Grade / Severity</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1 / Uninfect</td>
<td>No clinical signs of infection</td>
</tr>
</tbody>
</table>
| Grade 2 / Mild | Superficial tissue lesion with at least two of the following signs:  
- Local warmth  
- Erythema > 0.5 – 2 cm around the ulcer  
- Local tenderness / pain  
- Local swelling / induration  
- Purulent discharge  
Other causes of inflammation of the skin must be excluded |
| Grade 3 / Moderate | Erythema > 2 cm and one of the findings above or:  
- Infection involving structures beneath the skin/subcutaneous tissues (e.g., deep abscess, lymphangitis, osteomyelitis, septic arthritis or fasciitis)  
- No systemic inflammatory response (see Grade 4) |
| Grade 4 / Severe | Presence of systematic signs with at least two of the following:  
- Temperature > 39°C or < 36°C  
- Pulse > 90 bpm  
- Respiratory Rate > 20/min  
- PaCO₂ < 32 mmHg  
- White Cell count > 12,000 / mm³ or < 4,000 / mm³  
- 10% immature leukocytes |

Validated by the IWGDF and the Infectious Disease Society of America¹⁵

Table 2. SINBAD Score

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Score</th>
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<tbody>
<tr>
<td>Site</td>
<td>Forefoot</td>
<td>0</td>
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<tr>
<td></td>
<td>Hindfoot</td>
<td>1</td>
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<tr>
<td>Ischaemia</td>
<td>At least one Pedal Pulse</td>
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<td>Clinical Evidence of reduced blood supply</td>
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</tr>
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<td>Not Intact 8/10 and less</td>
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<td>Bacterial Load</td>
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<td></td>
<td>Present</td>
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</tr>
<tr>
<td>Area</td>
<td>Ulcer &lt; 1 cm²</td>
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<tr>
<td></td>
<td>Ulcer &gt; 1 cm²</td>
<td>1</td>
</tr>
<tr>
<td>Depth</td>
<td>Texas 0 or 1</td>
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<tr>
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<td>Texas 2 or 3</td>
<td>1</td>
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<table>
<thead>
<tr>
<th>SINBAD Score Total</th>
<th>Estimated Time to Heal</th>
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<tr>
<td>0-2 Moderate</td>
<td>Up to 77 days</td>
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<tr>
<td>3-6 Severe</td>
<td>Range 126 – 577 days</td>
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</tbody>
</table>

6.4.4 Prior and Concomitant Therapy or Medications

6.4.4.1 Prior and Concomitant Therapies

In the month prior to enrollment, the ulcer cannot have been treated with Cellular or Tissue-Based Products for Wound Care (CTP). Ulcer cannot be treated with Hyperbaric Oxygen therapy 2-weeks prior to randomization or throughout the trial.
6.4.4.2 Prior and Concomitant Medications

Patients on greater than 10mg of Prednisone daily are excluded. In addition, if the patient must take medications that the PI believes will interfere with wound healing (e.g. chemotherapy, biologics) then the patient should be excluded from the trial.

6.4.4.3 Rescue Medications

There are no rescue medications for this study.

6.4.5 Inclusion Criteria

Subjects will be eligible to participate in the study if all of the following conditions exist:

1. Subjects are male or female, 18 years of age or older. At least 50% of the enrolled population must be ≥ 65 years of age.
2. Subjects with one of the following wounds:
   A. Diabetic foot ulcer present for greater than 4 weeks by patient report or documented in the medical record but less than 12 months duration if being treated with active SOC.
   B. Minor amputation wound sites
3. Subject has clinical documentation of no visible wound improvement after 4 weeks of standard of care. (Objectively, less than 40% reduction in surface area in the four weeks prior to the first treatment visit.
4. Study ulcer is a minimum of 0.5 cm² and a maximum of 25 cm² at first treatment visit
5. Subjects’ wound score on IDSA tool is Grade 1 or 2.
6. The subject is able and willing to follow the protocol requirements
7. Subject has signed informed consent.
8. Adequate circulation to the affected foot as demonstrated by a dorsum transcutaneous oxygen measurement (TCOM) or a skin perfusion pressure (SPP) measurement of ≥ 30 mmHg, an ABI between 0.7 and ≤ 1.3, or TBI of >6 within 3 months of the first Screening Visit.
9. Females of childbearing potential must be willing to use acceptable methods of contraception (birth control pills, barriers, or abstinence) starting at Screening and continuing through the duration of their study participation.
10. The target ulcer has been offloaded for at least 14 days prior to randomization, if applicable, i.e. plantar wounds.

Infection status will be determined by the IDSA grading tool (Table 1).
6.4.6 Exclusion Criteria

Subjects will be excluded from participation in the study if any of the following conditions exist:

1. Subject has a known life expectancy of < 1 year
2. Subject or caregiver is unable to manage the Natrox® device (charge and change batteries daily)
3. Subject has ulcers that are completely necrotic or if the clinician feels it is clinically necessary to cover the wound surface in gel or creams that would prevent the transmission of oxygen to the wound surface.
4. Subject has major uncontrolled medical disorders such as serious cardiovascular, renal, liver or pulmonary disease, lupus, palliative care or sickle cell anemia.
5. Subject currently being treated for an active malignant disease or subjects with history of malignancy within the wound.
6. The Subject has other concurrent conditions that in the opinion of the Investigator may compromise subject safety
7. Known contraindications for the Natrox system
8. Known allergies to any of the Natrox system components
9. Concurrent participation in another clinical trial that involves an investigational drug or device that would interfere with this study.
10. Subject with known HbA1C > 12.0%.
11. Subject is pregnant or breast feeding or planning to become pregnant or breastfeed during the trial.
12. Subjects with a history of more than two weeks treatment with immunosuppressants (including systemic corticosteroids >10mg daily dose), cytotoxic chemotherapy, or application of topical steroids to the ulcer surface within one month prior to first Screening Visit, or who receive such medications during the screening period, or who are anticipated to require such medications during the course of the study.
13. Index ulcer has been previously treated with tissue engineered materials (e.g. Apligraf® or Dermagraft®) or other scaffold materials (e.g. Oasis, Matristem) within the last 30 days preceding the first treatment visit.
14. Affected extremity requiring hyperbaric oxygen during the trial or within 2 weeks of treatment visit 1.
15. An ulcer that has healed by more than 20% in the 2 weeks prior to the first screening visit (“historical” run-in period) while receiving standard of care treatment
16. Index ulcer has reduced in area by 20% or more after 2 weeks of standard of care treatment from the first screening visit (S1) to the TV1/Randomization visit, (“in-clinic” run-in period)
17. Subject has End Stage Renal Disease
6.4.7 Exit / Discontinuation Criteria

Subjects will exit the study if any of the following conditions exist:

1. Subject voluntarily withdraws from the study.
2. Subject death.
3. Subject acquires any of the listed exclusion criteria.
4. Subject completes the protocol
5. Subject is non-compliant with the protocol (as defined in Section 7.8)
6. Subject’s well-being, in the opinion of the Investigator, would be compromised by study continuation.
7. Subject reaches the clinical endpoint of total wound closure (Days of wound closure will be counted from day of closure to Day 84)
8. Subject experiences a protocol deviation that in the Investigator’s opinion will compromise the subject’s continuation in the study.

7 Study Procedures

7.1 Informed Consent

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. The consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the IRB-approved consent form, must be obtained before that subject is submitted to any study procedure. This consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent. A blank copy of the IRB-approved form must be kept on-site and by the investigator.

7.2 Vulnerable Populations

While vulnerable subjects will not specifically be recruited for this study, vulnerable subjects may be present in the potential subject pool. Additional procedures will not be required to ensure the human subjects protections for these subjects.

7.3 Randomization Scheme

Subject randomization will be performed by envelope across all sites.

7.4 Laboratory Testing Procedures

There are no associated laboratory procedures for this study.
7.5 Clinical Procedures

Clinical procedures are described for each clinic visit. Subjects will be seen at weekly intervals (± 3 days) for the 12 week follow up period. If additional dressing changes are required between the scheduled visits, the occurrence of these visits will be recorded; however, the subject assessment will be abbreviated for those visits.

Re-Screening

If a subject initially fails to meet inclusion/exclusion criteria and is later reconsidered for participation, the subject will be re-consented and assigned a new screening number at the time of re-screening. Subjects who fail their first screening attempt may be re-screened again (i.e., up to two screenings total) and may be enrolled if they are found to meet all inclusion and no exclusion criteria at the second screening visit.

Pain Assessment

Current pain intensity of the reference DFU is to be assessed before any dressing changes or other ulcer manipulations at all screening and treatment visits. Subject will be asked to indicate a numerical value that best represents the pain intensity at ulcer site on a scale of 0 to 10 anchored by word descriptors at each end, as "no pain" on the left side and "worst possible pain" on the right side of the number line. The number 0 represent “no pain”, the number 5 represents “moderate pain” and the number 10 represents the “worst possible pain”. The subject indicates the level of pain intensity by selecting a number on the line that represents their perception of their current state.

7.5.1 Screening Period (-2 weeks)

The Screening Period starts with SV1 (-2 weeks), continues with SV2 (-1 week), and concludes on Day 0/TV1. All protocol required Screening procedures must be completed before Randomization on Day 0/TV1.
If a subject requires offloading and has never worn a TCC before, they must return 3 days after SV1 for a cast change; this visit will be treated as an USV and all USV data will be collected.

At SV1 (-2 weeks) the study will be described in detail to potential subjects, and informed consent obtained.

The ICF should not be signed before measuring the wound and determining if the target ulcer has healed by greater than 20% in the past two weeks. (Comparison to historical manual measurement from patient’s medical record, Exclusion #15)

The following procedures will occur during the Screening Period:

- Manual measurement to determine if the ulcer has healed by greater than 20% in the past two weeks. (Comparison to historical manual measurement from patient’s medical record)
- Signing of Informed Consent
- Collection of Medical History and Demographics, including foot ulcer history and any additional wounds
- Physical Exam, including vital signs
- Review of concomitant medications and therapies
- Baseline pain assessment – VAS
- Urine or blood pregnancy test (females of childbearing potential)
- HbA1c test (fingerstick) if no test results within the past 90 days
- Study ulcer history and assessment (pre-debridement)
- Assessment of infection status (see Table 1)
- SINBAD Score (see Table 2)
- Screening for peripheral arterial disease: ABI, TCOM, SPP – as defined in inclusion criteria.
- Review of potential adverse events
- Review of Subject Eligibility (Inclusion/Exclusion)
- Wound Debridement, if clinically indicated
- Baseline post-debridement, digital wound measurements / photographs (see Appendix A) (SV1 only)
- Placement of absorbent dressings of hydrofiber/alginate
- Offloading of plantar wounds with a Total Contact Cast. (Exceptions must be approved by the Medical Monitor)
- Assessment of offloading

7.5.1b Visit 1 (Day 0 ±3)

- Update Medical History
- Review of potential adverse events
- Review of concomitant medications and therapies
- Assessment of offloading
• Pain assessment – VAS
• Study ulcer assessment (pre-debridement)
• Assessment of infection status (see Table 1)
• SINBAD Score (see Table 2)
• Wound Debridement, if clinically indicated
• Post-debridement, digital wound measurements / photographs (see Appendix A) (Document of Standard of Care/Wound Status (4 weeks prior to TV)
• Review of Subject Eligibility (Inclusion/Exclusion) prior to randomization.
• Randomization
• Placement of Natrox® Device (if applicable: randomized to treatment arm)*
• Placement of absorbent dressings of hydrofiber/alginate provided for the study.
  Offloading of plantar wounds with a Total Contact Cast. Exceptions must be approved by the Medical Monitor
*The placement of the Natrox® Device (Oxygen Delivery System) will be as per the Natrox® Application Guide (See Appendix B)

7.5.2 Visit 2 (Day 7 ± 3)

At Visit 2 the following procedures will be conducted:

• Review of potential adverse events
• Review of concomitant medications and therapies
• Assessment of pain status
• Study ulcer assessment (pre-debridement)
• Assessment of infection status (see Table 1)
• Wound Debridement (if applicable)
• Assessment of offloading
• Post-debridement digital wound measurement / photographs (See Appendix Error! Reference source not found.)
• Placement of Natrox® Device (if applicable)
• Placement of dressing
• Offloading of plantar wounds with a Total Contact Cast. Exceptions must be approved by the Medical Monitor

7.5.3 Visit 3 (Day 14 ± 3)

At Visit 3 the following procedures will be conducted:

• Review of potential adverse events
• Review of concomitant medications and therapies
• Assessment of pain status
• Study ulcer assessment (pre-debridement)
• Assessment of infection status (see Table 1)
• Wound Debridement (if applicable)
• Assessment of offloading
• Post-debridement digital wound measurement / photographs (See Appendix Error! Reference source not found.)
• Placement of Natrox® Device (if applicable)
• Placement of dressing
• Offloading of plantar wounds with a Total Contact Cast. Exceptions must be approved by the Medical Monitor

7.5.4  Visit 4 (Day 21 ± 3)
At Visit 4 the following procedures will be conducted:

• Review of potential adverse events
• Review of concomitant medications and therapies
• Assessment of pain status
• Study ulcer assessment (pre-debridement)
• Assessment of infection status (See Table 1)
• Wound Debridement (if applicable)
• Assessment of offloading
• Post-debridement digital wound measurement / photographs (See Appendix A)
• Placement of Natrox® Device (if applicable)
• Placement of dressing
• Offloading of plantar wounds with a Total Contact Cast. Exceptions must be approved by the Medical Monitor

7.5.5  Visit 5 (Day 28 ± 3)
At Visit 5 the following procedures will be conducted:

• Review of potential adverse events
• Review of concomitant medications and therapies
• Assessment of pain status
• Study ulcer assessment (pre-debridement)
• Assessment of infection status (see Table 1)
• Wound Debridement (if applicable)
• Assessment of offloading
• Post-debridement digital wound measurement / photographs (See Appendix A)
• Placement of Natrox® Device (if applicable)
• Placement of dressing
• Offloading of plantar wounds with a Total Contact Cast. Exceptions must be approved
by the Medical Monitor

7.5.6  Visit 6 (Day 35 ± 3)

At Visit 6 the following procedures will be conducted:

- Review of potential adverse events
- Review of concomitant medications and therapies
- Assessment of pain status
- Study ulcer assessment (pre-debridement)
- Assessment of infection status (see Table 1)
- Wound Debridement (if applicable)
- Assessment of offloading
- Post-debridement digital wound measurement / photographs (See Appendix A)
- Placement of Natrox® Device (if applicable)
- Placement of dressing
- Offloading of plantar wounds with a Total Contact Cast. Exceptions must be approved by the Medical Monitor

7.5.7  Visit 7 (Day 42 ± 3)

At Visit 7 the following procedures will be conducted:

- Review of potential adverse events
- Review of concomitant medications and therapies
- Assessment of pain status
- Study ulcer assessment (pre-debridement)
- Assessment of infection status (see Table 1)
- Wound Debridement (if applicable)
- Assessment of offloading
- Post-debridement digital wound measurement / photographs (See Appendix A)
- Placement of Natrox® Device (if applicable)
- Placement of dressing
- Offloading of plantar wounds with a Total Contact Cast. Exceptions must be approved by the Medical Monitor

7.5.8  Visit 8 (Day 49 ± 3)

At Visit 8 the following procedures will be conducted:

- Review of potential adverse events
- Review of concomitant medications and therapies
• Assessment of pain status
• Study ulcer assessment (pre-debridement)
• Assessment of infection status (see Table 1)
• Wound Debridement (if applicable)
• Assessment of offloading
• Post-debridement digital wound measurement / photographs (See Appendix A)
• Placement of Natrox® Device (if applicable)
• Placement of dressing
• Offloading of plantar wounds with a Total Contact Cast. Exceptions must be approved by the Medical Monitor

7.5.9 Visit 9 (Day 56 ± 3)

At Visit 9 the following procedures will be conducted:

• Review of potential adverse events
• Review of concomitant medications and therapies
• Assessment of pain status
• Study ulcer assessment (pre-debridement)
• Assessment of infection status (see Table 1)
• Wound Debridement (if applicable)
• Assessment of offloading
• Post-debridement digital wound measurement / photographs (See Appendix A)
• Placement of Natrox® Device (if applicable)
• Placement of dressing
• Offloading of plantar wounds with a Total Contact Cast. Exceptions must be approved by the Medical Monitor

7.5.10 Visit 10 (Day 63 ± 3)

At Visit 10 the following procedures will be conducted:

• Review of potential adverse events
• Review of concomitant medications and therapies
• Assessment of pain status
• Study ulcer assessment (pre-debridement)
• Assessment of infection status (see Table 1)
• Wound Debridement (if applicable)
• Assessment of offloading
• Post-debridement digital wound measurement / photographs (See Appendix A)
• Placement of Natrox® Device (if applicable)
• Placement of dressing
• Offloading of plantar wounds with a Total Contact Cast. Exceptions must be approved by the Medical Monitor

7.5.11  Visit 11 (Day 70 ± 3)

At Visit 11 the following procedures will be conducted:

• Review of potential adverse events
• Review of concomitant medications and therapies
• Assessment of pain status
• Study ulcer assessment (pre-debridement)
• Assessment of infection status (see Table 1)
• Wound Debridement (if applicable)
• Assessment of offloading
• Post-debridement digital wound / photographs (See Appendix A)
• Placement of Natrox® Device (if applicable)
• Placement of dressing
• Offloading of plantar wounds with a Total Contact Cast. Exceptions must be approved by the Medical Monitor

7.5.12  Visit 12 (Day 77 ± 3)

At Visit 12 the following procedures will be conducted:

• Review of potential adverse events
• Review of concomitant medications and therapies
• Assessment of pain status
• Study ulcer assessment (pre-debridement)
• Assessment of infection status (see Table 1)
• Wound Debridement (if applicable)
• Assessment of offloading
• Post-debridement digital wound measurement / photographs (See Appendix A)
• Placement of Natrox® Device (if applicable)
• Placement of dressing
• Offloading of plantar wounds with a Total Contact Cast. Exceptions must be approved by the Medical Monitor

7.5.13  Visit 13 – End of Study Visit (Day 84 ± 3)

At Visit 13 the following procedures will be conducted:

• Review of potential adverse events
• Review of concomitant medications and therapies
• Assessment of pain status
• Study ulcer assessment (pre-debridement)
• Assessment of infection status (see Table 1)
• Wound Debridement (if applicable)
• Assessment of offloading
• Post-debridement digital wound measurement / photographs (See Appendix A)
• Placement of dressing
• Completion of Study Exit Form*

* Study Exit Form will be completed at any visit in which the subject heals, or study participation terminates early

7.5.14 Unscheduled Visit
At an Unscheduled Visit the following procedures will be conducted:

• Review of potential adverse events
• Review of concomitant medications and therapies
• Assessment of pain status
• Wound Debridement (if applicable)
• Assessment of offloading
• Placement of Natrox® Device (if applicable)
• Placement of dressing
• Offloading of plantar wounds with a Total Contact Cast. Exceptions must be approved by the Medical Monitor

7.6 Follow-Up Procedures and Therapy Transitions
At the exit from the study, if the wound has not healed, the subject will return to standard of care treatments as prescribed by their physician. No additional follow-up procedures or transitions are required

7.7 Study Timetable / Schedule of Events
After a 2-week historical run-in period and a 2-week screening period, eligible subjects will be followed for 12 weeks, with weekly clinic visits as described in “Section 7.5 Clinical Procedures.” A Schedule of Events is shown in Table 3.
### Table 3. Schedule of Events

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<th>Window Period Day # +/- 3</th>
<th>Screen Visits</th>
<th>Visit 1</th>
<th>Visit 2</th>
<th>Visit 3</th>
<th>Visit 4</th>
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<th>Visit 7</th>
<th>Visit 8</th>
<th>Visit 9</th>
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<tr>
<td>Assessment of Offloading</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>Natrox Placement, if applicable</td>
<td>✓</td>
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<tr>
<td>Dressing Placement</td>
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<tr>
<td>Offloading, if applicable</td>
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<tr>
<td>Assessment of Pain Status</td>
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<tr>
<td>Study Exit Form</td>
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</table>
7.8 Study Protocol Compliance / Treatment Adherence

Subject compliance to the treatment regimen and continuation in the study if there is evidence of non-compliance will be assessed jointly on a case-by-case basis by the Investigator and Sponsor. Completion of 8 or more of the scheduled visits will be considered complete.

7.9 Deviations from the Clinical Protocol

When a deviation from the protocol is necessary for an individual subject, the investigator must contact the sponsor (or its acting representative) prior to the deviation (unless the deviation is safety related). The subject may continue in the study by mutual agreement of the sponsor and the investigator. Deviations occurring without prior approval must be assessed by the Investigator and reviewed by the Sponsor. Subject continuation must be indicated on the Protocol Deviation Form. A description of the deviation from the protocol and justification must be recorded on the Protocol Deviation Form.

7.10 Subject Withdrawal

7.10.1 How to Withdraw Subjects

Subjects withdrawn or terminated early may return to receiving Standard of Care treatment from their pre-study physicians. The termination of study participation is not anticipated to affect subject safety. Subject withdrawals/terminations in excess of the anticipated 5% attrition will be replaced pending appropriate protocol amendment and IRB approval.

7.10.2 Data Collection and Follow-Up for Withdrawn Subjects

If a subject withdraws consent to participate in the study, or is withdrawn by the Investigator, attempts must be made to obtain permission to record at least survival data up to the protocol-described end of subject follow-up period. Reasons for withdrawal or early termination will be obtained whenever possible.

A subject will be considered lost to follow-up if they cannot be contacted after 5 phone calls and 3 letters, at least one of which must be sent certified.

7.11 Subject Compensation

Subject compensation will be $100/visit as described in the Informed Consent form.
8 Data Collection and Analysis

8.1 Subject Population(s) for Analysis

The following subject populations are subject to study analysis.

- All-randomized population: Any subject randomized into the study, regardless of whether they received study device or treatment
- All-treated population: Any subject randomized into the study that received at least one exposure to study device
- Protocol-compliant population: Any subject who was randomized and received the protocol required study device exposure.
- Intent to Treat population: Any subject who is randomized according to the randomization assignment.

8.2 Statistical Methods

Statistical methods will be restricted to descriptive statistics only. Safety data (adverse events) will be compiled and compared between groups. Wound area measurements will be determined and compared over time as a change from baseline between groups. Pain assessments will be compiled and compared over time as a change from baseline between groups. Days of wound closure will be compared between groups.

Missing data will not be imputed, and no attempt will be made to provide values for missing data. The sponsor holds the right to analyze data at any time.

Table 4. Statistical Analyses

<table>
<thead>
<tr>
<th>Data Collected</th>
<th>Analysis to be Performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics data</td>
<td>Descriptive statistics only; comparison to general population significance at p &lt; 0.05</td>
</tr>
<tr>
<td>Pain Assessments (VAS)</td>
<td>Descriptive statistics only; Change from baseline over time in control and interventional groups; significance at p &lt; 0.05</td>
</tr>
<tr>
<td>Wound Measurements (Area)</td>
<td>Descriptive statistics only; Area measurements change from baseline over time; comparison between groups, significance at p &lt; 0.05</td>
</tr>
<tr>
<td>Adverse Events</td>
<td>Descriptive statistics only; Total number of events over time; comparison between groups; significance at p &lt; 0.05</td>
</tr>
<tr>
<td>Days of wound closure</td>
<td>Descriptive statistics only; days of wound closure (out of 84 day study period)</td>
</tr>
</tbody>
</table>

9 Safety and Adverse Events

9.1 Definitions

Adverse Event (AE)

An adverse event (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries will
be regarded as adverse events. Abnormal results of laboratory or diagnostic procedures are considered to be adverse events if the abnormality:

- Results in study withdrawal
- Is associated with a serious adverse event
- Is associated with clinical signs or symptoms
- Leads to additional treatment or to further diagnostic tests
- Is considered by the Investigator to be of clinical significance.

**Serious Adverse Event (SAE)**

A serious adverse event (SAE) is any adverse event that is:

- Fatal
- Life-threatening
- Requires or prolongs a hospital stay
- Results in persistent or significant disability or incapacity
- A congenital anomaly or birth defect

Important medical events are events that may not be immediately life-threatening, but are clearly of major clinical significance and may be SAEs. They may jeopardize the subject, and may require intervention to prevent one or the other serious outcomes noted above.

**Hospitalization**

Hospitalization shall include any initial admission (even if less than 24 hours) to a healthcare facility as a result of a precipitating clinical adverse effect; to include transfer within the hospital to an intensive care unit. Hospitalization or prolongation of hospitalization in the absence of a precipitating, clinical adverse effect (e.g., for a preexisting condition not associated with a new adverse effect or with a worsening of the preexisting condition; admission for a protocol-specified procedure) is not, in itself, a serious adverse effect.

**Unanticipated Adverse Device Effect (UADE)**

An unanticipated adverse device effect is any serious adverse effect on health or safety or 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 investigational plan, or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

**Adverse Event Relationships to the Study Device**

The relationships between adverse events and the study device will be characterized by the definitions below.
• **Unrelated:** This category applies to those adverse events which, after careful consideration, are clearly and incontrovertibly due to extraneous causes (disease, environment, etc.)

• **Possibly Related:** This category applies to those adverse events for which, after careful medical consideration at the time they are evaluated, a connection with the study device administration appears unlikely but cannot be ruled out with certainty. An adverse experience may be considered possibly related if or when (at least two of the following):
  - It follows a reasonable temporal sequence from administration of the study device.
  - It could not readily have been produced by the subject’s clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
  - It follows a known pattern of response to the study device.

• **Probably Related:** This category applies to those adverse events which, after careful medical consideration at the time they are evaluated, are felt with a high degree of certainty to be related to the study device. An adverse experience may be considered probably related if or when (at least three of the following):
  - It follows a reasonable temporal sequence from administration of the study device.
  - It could not be reasonably explained by the known characteristics of the subject’s clinical state, environmental or toxic factors or other modes of therapy administered to the subject.
  - It disappears or decreases on cessation or reduction in device exposure. There are important exceptions when an adverse event does not disappear upon discontinuation of the device, yet device-relatedness clearly exists.
  - It follows a known pattern of response to the study device.

• **Definitely Related:** An adverse event may be considered definitely related if or when
  - The event is a known effect of the device, or procedure
  - The event follows an obvious sequence of time, from the device’s implantation or activation, or procedure, for which the event is directly attributed to the administration, implantation, activation, or procedure.
  - The event ceases with discontinuation of the device, or procedure (and reoccurs on restarting).

**Device Malfunction/Failure – Device Specific Events**
A device specific event (DSE) is any malfunction of the device, related or not to the device, resulting or not in the patient undergoing undesirable or harmful experience, that occurs in relation with the conduct of the study.
Device malfunction means the failure of a device to meet its performance specifications or otherwise perform as intended. Performance specifications include all claims made in the labeling for the device. The intended performance of a device refers to the intended use for which the device is labeled or marketed.

Device malfunction may or may not result in the subject experiencing a harmful effect. All AEs/SAEs associated with a device failure are by definition device related.

9.2 Safety Monitoring Plan

Safety monitoring activities are described in the following sections. The emergency medical safety contact for this study is listed below. Each clinical site will designate an individual who will serve as the Emergency Medical Safety Contact person (usually the Investigator of Record).

9.2.1 Anticipated Risks / Risk Mitigation

Anticipated risks associated with study procedures are listed below along with the applicable risk mitigation.

Table 5. Anticipated Risks

<table>
<thead>
<tr>
<th>Study Procedure</th>
<th>Anticipated Risks</th>
<th>Risk Mitigation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wound Debridement</td>
<td>Pain</td>
<td>Procedures to be performed by trained clinical staff</td>
</tr>
<tr>
<td>Wound Measurements</td>
<td>None anticipated</td>
<td>N/A</td>
</tr>
<tr>
<td>Pain Assessments</td>
<td>None anticipated</td>
<td>N/A</td>
</tr>
<tr>
<td>Wound Photos</td>
<td>None anticipated</td>
<td>N/A</td>
</tr>
<tr>
<td>Natrox Placement</td>
<td>Potential allergic reaction/ skin irritation</td>
<td>Device components composed of medical grade materials with known sensitivity profiles.</td>
</tr>
<tr>
<td>Dressing Placement</td>
<td>None anticipated</td>
<td>N/A</td>
</tr>
<tr>
<td>TCC/Fixed Walker Boot</td>
<td>None anticipated</td>
<td>N/A</td>
</tr>
<tr>
<td>Ankle-Brachial Index (ABI)</td>
<td>Discomfort in area of skin breakdown secondary to pressure from cuff</td>
<td>Topical lidocaine</td>
</tr>
</tbody>
</table>

9.2.2 Medical Monitoring for Participant Safety

The Principal Investigator will oversee the safety of the study, including careful assessment and appropriate reporting of adverse events as outlined in Section 9.4 Adverse Event Reporting. Medical monitoring will include a regular assessment of the number and type of serious adverse events. The Sponsor will designate a Medical Monitor.

9.3 Anticipated Adverse Events

Device related adverse events are not anticipated in this study. Adverse events consistent with a diabetic population will be expected and reported as per the sections below.
9.4 Adverse Event Reporting

All Adverse Events occurring during the study period must be recorded. The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that study treatment or participation is not the cause.

The Investigator will promptly review documented adverse effects and abnormal test findings to determine:

1) if the abnormal test finding should be classified as an adverse effect;
2) if there is a reasonable possibility that the adverse effect was caused by the investigational device or, if applicable, other study treatment or diagnostic product(s); and
3) if the adverse effect meets the criteria for a serious adverse effect.

If the Investigator’s final determination of causality is “unknown and of questionable relationship to the investigational device or, if applicable, other study treatment or diagnostic product(s)”, the adverse effect will be classified as associated with the use of the investigational device or study treatment or diagnostic drug product(s) for reporting purposes. If the investigator’s final determination of causality is “unknown but not related to the investigational device or, if applicable, other study treatment or diagnostic product(s)”, this determination and the rationale for the determination will be documented in the respective subject’s case history.

9.4.1 Adverse Events

All observed or volunteered adverse effects and abnormal test findings, regardless of treatment group, if applicable, or suspected causal relationship to the investigational device or, if applicable, other study treatment or diagnostic product(s) will be recorded in the subjects’ case histories. For all adverse effects, sufficient information will be pursued and/or obtained so as to permit:

1) an adequate determination of the outcome of the effect (i.e., ongoing at this time, recovered, recovered with sequelae, not recovered, death, lost to follow-up) and;
2) an assessment of the causal relationship between the adverse effect and the investigational device or, if applicable, the other study treatment or diagnostic product(s).

Adverse effects or abnormal test findings felt to be associated with the investigational device or, if applicable, other study treatment or diagnostic product(s) will be followed until the effect (or its sequelae) or the abnormal test finding resolves or stabilizes at an acceptable level.

Adverse Events, including Device Specific Events,
that do not qualify as Serious Adverse Events or as Unanticipated Adverse Device Effects will be reported to Serena Group at weekly intervals.

Adverse Events that do not qualify as Serious Adverse Events or as Unanticipated Adverse Device Effects will be reported to the IRB with the continuing review progress report or at intervals designated by the IRB.

9.4.2 Serious Adverse Events

Investigators must report product related or possibly related serious adverse events to the Study Sponsor within 24 hours of learning of the event. A serious adverse event form must be completed by the Investigator and emailed to the Study Sponsor within 24 hours. Study Sponsor contact information for Serious Adverse Event Notification:

Kathy Hohenberger
+1.843.714.8947 (office)

kathy.hohenberger@natroxwoundcare.com

Investigators must report unrelated serious adverse events to the medical monitor within 24 hours of learning of the event.

Thomas E. Serena, MD, FACS
814-688-4000 (24 hours)

serena@serenagroups.com

At the time of the initial report, the following information should be provided:

<table>
<thead>
<tr>
<th>Study Identifier</th>
<th>Whether study treatment was discontinued</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study Center</td>
<td>Reason the event is classified as serious</td>
</tr>
<tr>
<td>Subject Number</td>
<td>Investigator assessment of association</td>
</tr>
<tr>
<td>Event Description</td>
<td>between event and study device</td>
</tr>
<tr>
<td>Date of Onset</td>
<td></td>
</tr>
<tr>
<td>Current Status</td>
<td></td>
</tr>
</tbody>
</table>

Serious Adverse Events that are at least possibly related must be reported by the site to the IRB within 10 working days, and by the Sponsor to the FDA within 10 calendar days.

The clinical course of each serious adverse event, regardless of attribution, will be followed until resolution, stabilization, or thirty (30) days after study exit.
9.4.3 Unanticipated Adverse Device Effects (UADE)

Investigators are required to submit a report of a UADE to the Sponsor and the reviewing IRB as soon as possible, but in no event later than 10 working days after the Investigator first learns of the event.

Sponsors must immediately conduct an evaluation of a UADE and must report the results of the evaluation to FDA, all reviewing IRBs, and participating Investigators within 10 working days after the Sponsor first receives notice of the effect.

If the Adverse Event is Serious, Unanticipated, Device Related, and determined by the Sponsor to present an unreasonable risk to subjects, the Sponsor must terminate the study within 5 working days of that determination.
10 Data Handling and Record Keeping

10.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e., that the subject is alive) at the end of their scheduled study period.

10.2 Source Documents

Source Data are the clinical findings and observations, laboratory and test data, and other information contained in Source Documents. Source Documents are the original records (and certified copies of original records); including, but not limited to, hospital medical records, physician or office charts, physician or nursing notes, subject diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, x-rays, etc. When applicable, information recorded on the CRF shall match the Source Data recorded on the Source Documents.

10.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, indicate “N/D”. If the item is not applicable to the individual case, indicate “N/A”. Case Report Forms for this study will be electronic.

Case Report Forms will be completed for each subject enrolled into the clinical study. The investigator will review, approve, and digitally sign/date each completed CRF; the investigator’s signature serving as attestation of the investigator’s responsibility for ensuring that all clinical and laboratory data entered on the CRF are complete, accurate and authentic.
10.4 Clinical Reports

The clinical study reports that are required for this study include: annual and or final progress reports to IRB, and a final Clinical Study Report to the Sponsor. Serena Group will prepare the final Clinical Study Report for submission to the governing IRB(s).

10.5 Records Retention

The investigator will retain the specified records and reports for up to 2 years after the completion of the study. Investigators will notify the Sponsor at least 30 days prior to any scheduled destruction of records. Written notification will be provided to the Sponsor via mail or email at the addresses below:

Inotec
200 1st Avenue NW, Suite 408
Hickory, NC 28601-6113

Kathy.hohenberger@natroxwoundcare.com

11 Study Monitoring, Auditing, and Inspecting

This study will be monitored according to FDA/GCP guidelines. The Investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all study-related documents and study related facilities (e.g., pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

11.1 Study Monitoring Plan

Independent monitoring of the clinical study for clinical protocol compliance will be conducted periodically (i.e., at a minimum of annually) by qualified staff from SerenaGroup®.

11.2 Quality Assurance Procedures

Data management and processing integrity will be maintained as per Standard Operating Procedures.

11.3 Auditing and Inspection

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor or sponsor designee, government regulatory bodies, and institutional compliance, and quality assurance groups of all study related documents (e.g., source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities...
Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable institutional compliance and quality assurance offices.

12 Administrative Study Information

12.1 Technical Support

Technical support questions should be forwarded to:

Inotec
200 1st Avenue NW. Suite 408
Hickory, NC  28601-6113
Tel: +1 888 354 9772

12.2 Pre-Study Site Qualification

Potential study sites will be pre-qualified by site audit/questionnaire to determine appropriate resources, facilities, and subject populations.

12.3 Protocol Amendments After Study Initiation

Should changes in the study plan or protocol become necessary in the course of the clinical trial, those specific changes will be discussed and agreed upon by the Sponsor, its acting representative if appropriate, Investigator, and appropriate IRB approval obtained before the changes are implemented. All changes must be documented as protocol amendments.

12.4 Materials / Services Provided by Sponsor and Coordinating Center

The following materials will be provided by the Sponsor:

- iPhone with pre-loaded Tissue Analytics application for wound measurements and Electronic Data Capture (EDC)
- Natrox® Oxygen Generators and Oxygen Delivery Systems
- Primary dressings, e.g. foam, calcium alginate
- Urine pregnancy tests and cups
- Total Contact Casts and Fixed Ankle Walkers

13 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical
Practice, applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study.

14 Study Finances

14.1 Funding Source

This study is sponsored by Inotec AMD, Ltd

14.2 Conflicts of Interest

Potential conflicts of interest will be subject to the processes and procedures of the institution where the potential conflict exists.

15 Publication Plan

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the study sponsor. Any investigator involved with this study is obligated to provide the sponsor with complete test results and all data derived from the study.

16 Definition of Standard of Care

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

16.1 Cleaning the Study Ulcer

Remove all dressings and wash the foot. The leg should be elevated for as much time as possible during this process. Wash the foot with sterile water or saline solutions. Gently irrigate the study ulcer prior to each dressing change with warm tap water. Strict aseptic technique is not needed.

16.2 Debridement of the Study Ulcer

Debridement is an essential technique and standard of care in the treatment of diabetic foot ulcerations. It is important to remove all non-viable and necrotic material from the target ulcer prior to enrolling the subject. Debridement is allowed during the treatment
phase at the treating physician’s discretion. For detailed guidelines please see Appendix C.

16.3 Offloading

Offloading for plantar surface wounds is essential if the wound is to heal. The choice of offloading device is a total contact casting (TCC). Subjects who have never worn a TCC must return in 3 days for a cast change and assessment of offloading, and also 7 days from their screening visit for the same. Thereafter, TCC changes will be weekly at the subject’s scheduled treatment visit.

If the wound is infected or there is a high likelihood of infection occurring TCC should not be used.

Exceptions to the use of TCC can be granted by the Medical Monitor. In that case the subject should receive a fixed ankle walker boot or similar device. Other methods, such as shoe inserts are not acceptable.

16.4 Moisture balance

One of the key elements to optimal wound healing is maintaining a moisture balance. Too much moisture and the wound becomes macerated; too little moisture and the essential healing processes cannot function. Hence, absorbent dressings of hydrofiber/alginate will be utilized under the TCC.

16.5 Subject Instructions

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

For wounds on the plantar surface subjects will need to be offloaded in a total contact cast (TCC). If they’ve never worn a TCC before they will need to return in 3 days for a cast change and offloading assessment. They will also need to return 7 days from their first screening visit for a cast change. Thereafter, cast changes will be weekly at the subject’s scheduled treatment visit.
17 Ulcer Infection During the Trial

Infection at Study Ulcer site prior to Randomization:

If the infection occurs prior to randomization i.e., prior to the first Treatment Phase visit, then the subject will be ineligible to be randomized.

Infection at Study Ulcer site after Randomization:

If infection of the study ulcer site occurs after randomization i.e., after the Randomization visit, record the infection as an adverse event, and treat as appropriate with topical antimicrobial (e.g., topical silver antimicrobial) and/or oral antibiotics at the discretion of the investigator.

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

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

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

18 References

173, 515-519.
19. Appendices and Attachments

Appendix A: Photographic Measurement

Tissue Analytics eClinical Software Specifications September 2019
Wound Analysis and Automated Documentation

Tissue Analytics is a cloud-based HIPAA and CFR part 11 compliant eClinical platform. Tissue Analytics main feature is automated wound/lesion imaging that provides area and volume measurements (total surface area, length, width, perimeter, maximum depth, and total volume) through its proprietary technology which leverages machine learning algorithms (ML) and artificial intelligence (AI).

Users access the Tissue Analytics platform through a mobile device (iOS & Android) by launching a secured mobile application. Once the user has been identified by their unique credentials, the user can document assessment data by collecting images, videos, and text during the visit. All information is encrypted and data is purged from the device compliant with the National Institute of Standards and Technology’s guidelines for media sanitization. After data is acquired at the point of care, it is automatically transmitted to Tissue Analytics’ secure cloud hosted on Amazon Web Services (AWS) for analysis.

Tissue Analytics User-Facing Web Portal Data transferred to Tissue Analytics’ cloud is subsequently accessed by Tissue Analytics’ web portal where it can be viewed by users (clinicians, researchers, monitors, etc.) overseeing their subjects/patients. The primary features of the web portal include:

- Display of images and any other data collected at the point of care
- Ability to view analysis performed by Tissue Analytics’ cloud in a centralized location
- Ability to view longitudinal progress in patients’ wound healing via figures hosted on the web portal
- Master table of all of the clinician’s patients including treatments being used and wound etiology

Figure 1. 3D rendering examples for analysis
In addition to best in class imaging solutions, Tissue Analytics eClinical also provides Electronic Data Capture (EDC) capabilities to facilitate the collection of trial data for each visit maintaining a unified record, both compliant and auditable.

Users can document Visit Based Documentation building the electronic case report form requirements (eCRF) by selecting the specific visit or form needed. Each user has the ability to independently document in an asynchronous environment (Mobile/Desktop) the necessary documentation needed to build the eCRF. Users can also lock records by electronically signing the file ensuring the data remains protected for monitoring purposes.

Trial auditors/monitors can remotely access each site and patient record through a secured desktop portal allowing them to view and audit the data collected by each user. In the event a query needs to be triggered, auditors/monitors can submit a query to the user and the record can then be un-locked for a response or resolution purposes.

**The EDC system is built following QMS and CSV guidelines ensuring the integrity of the data fulfills protocol requirement.**
Appendix B: Application Guide for NATROX®

CLINICIAN USE – APPLICATION

1. Read manual.
2. Apply dressing.
3. Secure dressing.
4. Connect to oxygen source.
5. Monitor patient.
6. Check dressing.
7. Check oxygen levels.
8. Apply NATROX®.
10. Dispose of waste.

PATIENT USE – BATTERY CHANGE & CHARGE

1. Unplug battery.
2. Replace battery.
3.Charge device.
4. Use device.
5. Repeat cycle.
Appendix C: Debridement Technique

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

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

Technique: Sharp debridement comprises the following elements: The target ulcer and the surrounding skin are prepped with water or Saline. Anesthesia, topical or injected, is applied to the ulcer as necessary to reduce subject discomfort. Using a sterile technique, all non-viable tissue in the wound bed is excised using a scalpel and scissors. Excessive bleeding is controlled by using direct pressure but cautery may be employed if necessary. If extensive surgical debridement is necessary during the run-in period (i.e., general anesthesia is required), the subject is not a candidate for this trial. Likewise if other forms of debridement (e.g., enzymatic) are required during run-in based on the opinion of the treating clinician, the patient should be screen failed. During the Treatment Phase of the trial, other forms of debridement are NOT permitted. If this should happen, the type of debridement should be noted in the CRF; using other forms of debridement besides sharp debridement more than once during the treatment phase will result in the patient being withdrawn from the trial.

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

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

Ankle-Brachial Index

Technique: Place the patient in the supine position, with the arms and legs at the same level as the heart, for a minimum of 10 minutes before measurement. Select an appropriately sized blood pressure cuff for both the ankle and the arms (figure 1); the cuff width should be, at a minimum, 20% greater than the diameter of the extremity. The ankle cuff should be placed on the leg between the malleolus and the calf. Enough room should be left below both cuffs to permit placement of the ultrasound gel, so that the Doppler device can adequately detect the brachial, dorsalis pedis, and posterior tibial arteries. Obtain the brachial systolic pressures of both arms. Use the higher of the arm pressures in the ABI calculation. Obtain the pressure in the dorsalis pedis and posterior tibial arteries for the extremity with the target ulcer. Use the highest pressure for the ABI calculation. Ankle-Brachial Index = Highest ankle pressure/ Highest brachial pressure. Care should be taken to cover the ulcer during the ABI measurement. In addition, patients should be informed that they may experience discomfort during the test secondary to the pressure exerted by the cuff in the area of skin breakdown.
Skin Perfusion Pressure (SPP)

Skin perfusion pressure (SPP) can be employed to evaluate the subject’s vascular status. SPP is obtained using a laser Doppler.

**Technique**

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

Subjects with SPP less than 30 mmHg have vascular insufficiency and are not candidates for enrollment.
Transcutaneous Oximetry (TCOM)

Technique
Place the patient in the supine position, with the arms and legs at the same level as the heart. Electrodes must be in contact with the tissue through the contact liquid. If there is air between the tissue and an electrode, TCOM values will be questionable. Erroneous readings may also occur if electrodes are placed directly over a bone or there is severe edema around the wound. For best results, tests should be conducted at ambient temperature (21-23⁰), and the patients should not have smoked nor had caffeine for several hours prior.

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

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

The normal sequence of events for TCOM is measurement in air, the leg elevation test (optional), and the oxygen challenge. Shah et al\textsuperscript{18} determined that the optimal times for these events in terms of measurement time are, 20, 5, and 10 minutes, respectively.