Study Title: Safety and Efficacy of the Noxsano Wound Care Bandage: A First-in-Human Study

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OhioHealth

STUDY TITLE:	Safety and Efficacy of the Noxsano Wound Care Bandage: A First-in-Human Study
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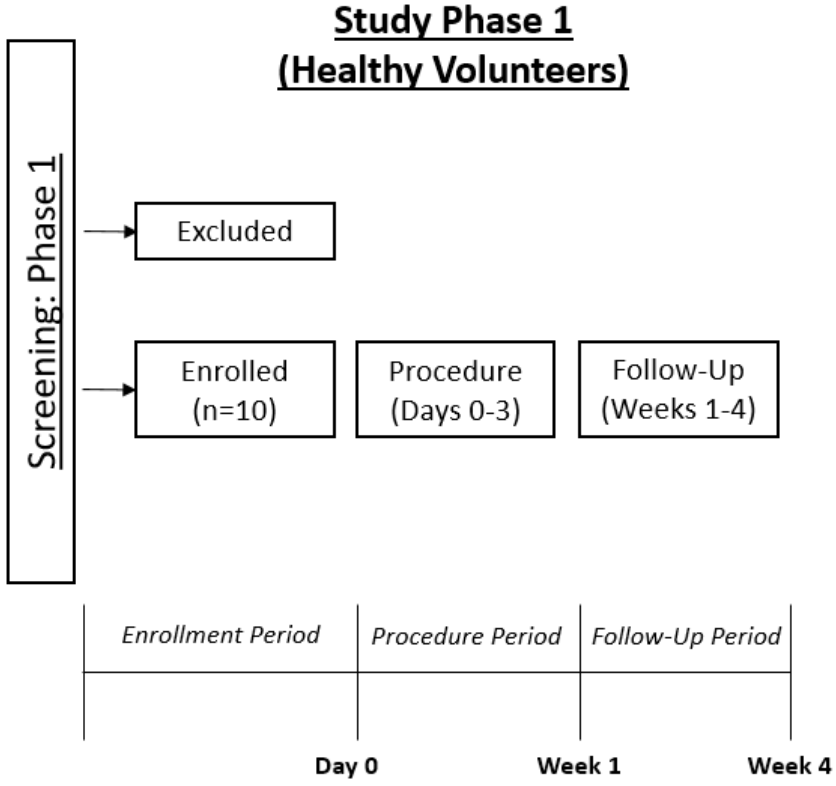
SPONSOR:	Mitch Silver, DO, FACC, FSVM, RPVI
SUPPORT/FUNDING:	Noxsano, Inc.
SUPPLIED DEVICE:	Noxsano Bandage
VERSION NUMBER:	6.0
VERSION DATE:	February 23, 2021
IRB NUMBER:	1331496

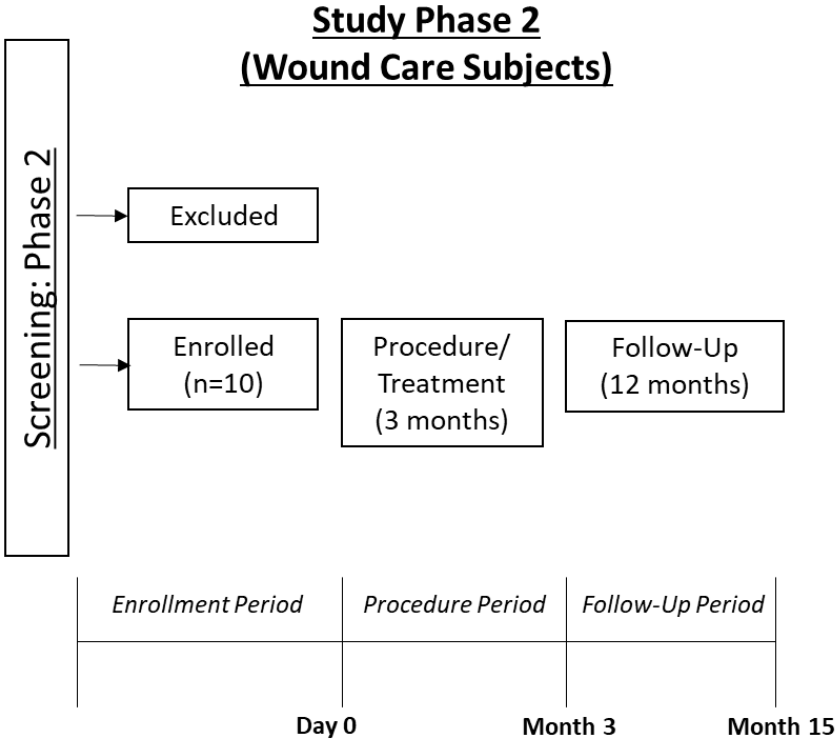
SUMMARY OF CHANGES

Protocol Date	Section	Change
		Initial IRB approval
07/11/2019	1.3.5, 3.3	Spelling and grammatical errors corrected
07/11/2019	5.5	Specified that any member of study staff can shave posterior leg area
0711/2019	5.6	Removed reference to Dr. Mehl and specified that the principal investigator or sub-investigator may apply and remove the study device for Healthy Volunteers.
7/19/19	4.1.2	Inclusion of OhioHealth associates in the healthy volunteer study population
7/19/2019	5.12	Removal of mechanism of payment from compensation language
9/24/19	5.1	Location change for the baseline visit for Healthy Volunteers
9/24/19	7.3, 8.7	Updated IRB reporting requirements
12/17/19	5.1, 5.7	Updated Visit Requirements
12/17/2019	5.6	Removed reference to Dr. Mehl and specified that principal investigator or sub-investigator may apply or remove the study device for wound care patients
12/17/19	4.1.1	Location change for the baseline visit for Healthy Volunteers
12/17/19	5.3, 5.6	Removed reference to Dr. Mehl taking all measurements for consistency and specified that current procedures used by the Critical Limb Heart and Vascular Clinic nursing staff will be used to ensure consistency of all wound measurements.
12/17/19	5.3	Defined minimum standard wound measurements completed by nursing staff per Critical Limb Heart and Vascular Clinic protocol
02/23/2021	Protocol Summary, 2.2, 3.3, 5.1, 5.8,	Changed the study visit schedule for Group 2 from once weekly visits during the first four weeks of the treatment period to two times per week for up to 4 weeks.
02/23/2021	4.4, 4.5	Clarification to Group 2 inclusion and exclusion criteria was made to specify which criteria are specific to the wound to be treated with the Noxsano bandage.
02/23/2021	4.5	Separated Group 2 exclusion criteria for patients with arterial insufficiency and diabetes
02/23/2021	5.0	Renumbered sub-headings to align with the order presented in the schedule of events in section 5.1
02/23/2021	5.3	Clarification that wound photographs are also taken during the treatment period
02/23/2021	5.4	Edited to add that wound measurements will be collected in the REDCap data collection tool. Removed reference to other measurements and descriptors performed per standard of care that will not be collected in the REDCap data collection tool.

Protocol Date	Section	Change
02/23/2021	5.1, 5.5, 5.8, 5.9, 5.10	Edited to add study assessment of percent granulation tissue
02/23/2021	5.7	Edited to add that shape of the wound can also be used to determine the size device to be used
02/23/2021	5.8, 5.10	Edited to add after hours contact number for Dr. Anderson
02/23/2021	5.11	Clarified language that wound healing is evidenced by a 50% decrease in wound surface area.
02/23/2021	5.11	Edited to add that missing two consecutive follow-up visits for Group 2 subjects will result in efforts to contact the subject and establish if they are lost to follow-up.
02/23/2021	5.11	Edited to add that subjects may be offered the option to have data collected on wound progression after they withdraw from the study.
02/23/2021	10.0	An addendum was added to the protocol to describe two adverse events related to the Noxsano study device and outcomes from the OhioHealth Institutional Board review of these events. Information included about bandage updated to have softer edges.

STUDY SCHEMA





PROTOCOL SUMMARY

STUDY TITLE	Safety and Efficacy of the Noxsano Wound Care Bandage: A First-in-Human Study
STUDY PHASE	Safety and efficacy
DEVICE	Noxsano Bandage
OBJECTIVES	*Group 1 (Safety): Healthy Volunteers.* The initial phase of the study is designed to determine the safety of the study device in healthy volunteers without wounds. If there are no issues with tolerance, side effects, and/or adverse reactions in healthy volunteers, the second phase of the study will proceed with active wound care subjects. *Group 2 (Efficacy): Wound Care Subjects.* The second phase of the study is designed to determine the effectiveness of the study device on wound healing in subjects with active wounds (see Primary Endpoint below), and to determine if the study device is equivalent to standard and established techniques (standard techniques demonstrate 50% wound healing at 2 months).
STUDY DESIGN	This study is a prospective, interventional, non-randomized, sequential phase study designed to assess the safety and efficacy of the Noxsano Bandage (study device) in subjects with a diabetic lower extremity ulceration and/or arterial insufficiency lower extremity ulceration.
SUBJECT POPULATION	*Group 1:* Initial subjects will be limited to healthy volunteers without wounds. Five (5) white subjects and 5 black or African American subjects will be enrolled to evaluate skin differences. *Group 2:* Ten (10) wound care subjects with lower extremity ulceration attributed to diabetes and/or arterial insufficiency.
NUMBER OF SUBJECTS	20 subjects will be included in the analysis (10 healthy volunteers for Group 1, and 10 active wound care subjects for Group 2).
NUMBER OF SITES	One site located at Riverside Methodist Hospital.
PRIMARY ENDPOINT	The primary endpoint for this study is wound healing (Group 2), as defined by the percent change in wound surface area (surface area is calculated in cm^2) from baseline through the active treatment period.
SUBJECT FOLLOW-UP	*Group 1*: The initial healthy volunteer safety study will last a total of 4.5 weeks. Healthy volunteers will have the study device applied for 3 consecutive days (up to 72 hours), followed by weekly visits for 4 weeks of observation for tolerance, side effects, and adverse reactions. *Group 2*: The wound care subject treatment period will consist of up to 3 consecutive months of study device application to a specific ulceration. The study device will be changed two times per week for up to 4 weeks from the start of treatment and then

	weekly for the remainder of the study treatment period. At the conclusion of the treatment period, subjects will be followed every 3 months for 12 consecutive months for observation of late side effects and/or adverse reactions (up to 3 months of active treatment, 12 months of follow-up observation, 15 months total participation).
TIMELINE	Anticipated Enrollment Timeframe: 6 months Anticipated Study Timeframe: 21 months

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1.0 INTRODUCTION

1.1 Background

Wound repair is a very complex and highly coordinated pathway that includes a series of overlapping phases: inflammation, cell proliferation, matrix deposition, and tissue remodeling. This pathway includes a complex, changing series of events including clotting, inflammation, granulation tissue formation, epithelialization, neovascularization, collagen synthesis, and wound contraction [1]. Impairment of this pathway of wound healing often leads to severe disabilities. Accordingly, chronic, non-healing wound conditions represent a situation of major clinical importance, and a large burden to the health care system. There are several well-described disease states that ultimately cascade into impaired wound healing [2]. Among those, the most prominent chronic wound impairments include decubitus or pressure ulcers, venous ulcers, diabetic ulcers, and ischemic ulcers from concomitant peripheral arterial disease (PAD). The advent of molecular and cellular biology and the use of different modeling systems, most notably genetically engineered animals, have greatly extended our knowledge of wound repair. Inflammation, re-epithelialization, and granulation tissue formation are driven in part by a complex mixture of growth factors and cytokines, which are released coordinately into the wounds [1, 2]. Besides these protein-type factors and mitogens, emerging evidence suggests the importance of small diffusible molecules, such as nitric oxide (NO) in wound repair [3].

As a testimony to the rapidly expanding knowledge about its multiple biological roles, NO, after its discovery in 1987, was named molecule of the year in 1992 [4]. There is increasing evidence for a functional role of NO in wound healing. Inhibition of the inducible isoform of NO, iNOS, by competitive inhibitors decreases collagen deposition and breaking strength of incisional wounds and impairs the healing of other wound models [5-7]. The use of NO donors has also been shown to improve incisional and excisional wound healing in rats [8-10]. Inhibition of iNOS by competitive inhibitors, either applied to the wound surface [11] or given systemically [6], decreases collagen deposition and breaking strength of incisional wounds and impairs the healing. Finally, there are strong correlations between reduced cutaneous NO levels and impaired wound healing under disease conditions such as diabetes [6, 12-14], malnutrition [15], and chronic steroid treatment [11].

Angiogenesis, the process of forming new micro-vessels, is an important component of normal wound repair. NO plays a central role in this process [16] as it increases angiogenesis in ischemic murine tissues [17]. NO is also vital to the activity of pro-angiogenic cytokines. Vascular endothelial growth factor (VEGF) is a potent angiogenic factor which involves the modulation of NO generation [18].

1.2 Study Rationale

Wound dressings and devices form an important segment of the medical and pharmaceutical wound care market worldwide. The last two decades have witnessed the introduction of many dressings, with new ones becoming available each year. These modern dressings are based on the concept of creating an optimum environment to allow

epithelial cells to move unimpeded, for the treatment of wounds. Such optimum conditions include a moist environment around the wound, effective oxygen circulation to aid regenerating cells and tissues, and a low bacterial load. The active ingredients used in wound management have evolved alongside the pharmaceutical agents and dressings used to deliver them. The use of topical pharmaceutical agents in the form of solutions, creams and ointments to wound sites has been the mainstay of wound dressings for the past decade.

Controlled delivery dressings can provide an excellent means of delivering drugs to wound sites in a consistent and sustained fashion over long periods of time without the need for frequent dressing change [19]. This form of a local drug delivery dressing is potentially useful in the treatment of wounds where it may be beneficial to have increased local concentrations of the active drug while avoiding high systemic doses, and thus minimizing systemic side effects. Improvement of patient compliance is another advantage with local drug delivery dressings especially in chronic wound management where patients usually undergo long treatments and frequent changing of dressings that can lead to noncompliance. A dressing that will deliver an active substance to a wound site in a controlled fashion for a sustained period could help solve or minimize this problem.

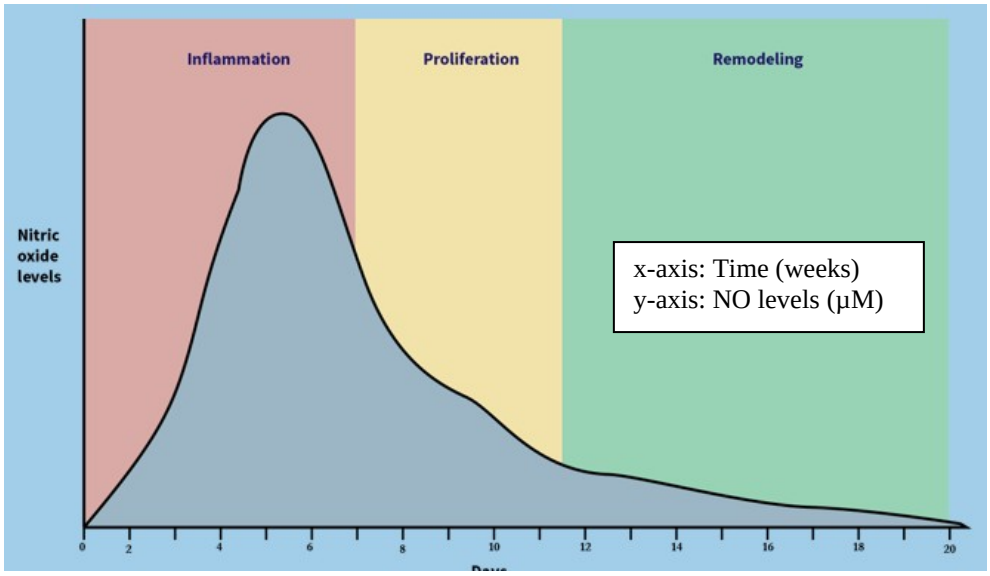
1.3 Device Description

1.3.1 Preclinical Data

Nitric Oxide and Wound Healing. The process of wound healing is complex and involves a multitude of signaling pathways, effector molecules, and response phases. In certain circumstances such as diabetes, disability, old age, and obesity, wound healing can be delayed, and result in a non-healing (chronic) wound. Infection is a significant risk in chronic wounds, and can be difficult to treat as chronic wounds are associated with restricted blood flow (ischemia), which limits efficacy of systemic antibiotics. If an infection cannot be controlled, amputation of the affected limb is required to prevent death from sepsis. The mortality rate in people who must receive an amputation is startlingly high, greater than the mortality rate of several major cancers combined. [20]

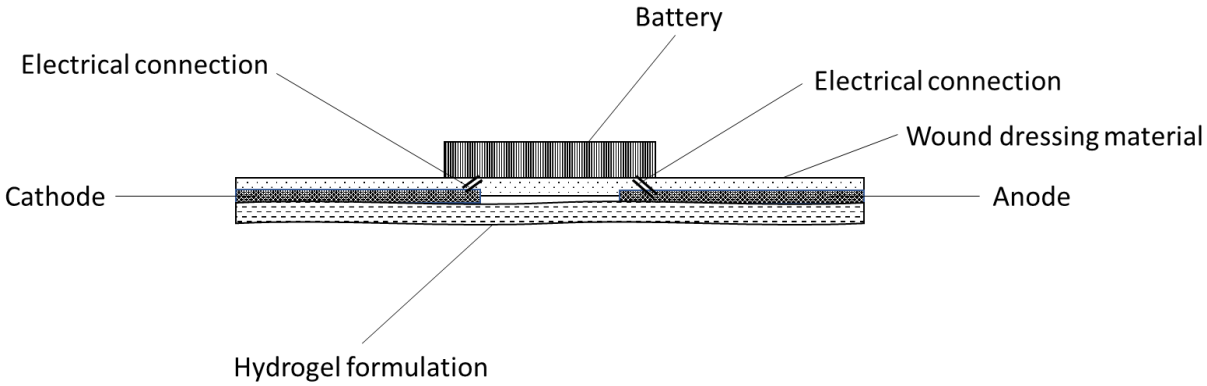
NO is the key molecule controlling many signaling cascades in wound healing and insufficient production is a primary cause of chronic wounds [21]. NO is present throughout healing (weeks) and its level varies as healing progresses (Fig. 1).

Figure 1. Nitric Oxide Levels during the Healing Process.



Noxsano Bandage Device. The Noxsano Bandage (study device) is designed to exogenously replace the deficient NO in wounds and restore normal signaling required for wounds to heal. The Noxsano Bandage uses an electrochemical reaction to generate highly controlled doses of NO for wound healing. The electrochemical reaction reduces sodium nitrite (meat tenderizer) to NO. To achieve this efficiently, an electrochemical mediator is used that 'shuttles' electrons from the anode to the nitrite. The dressing (Fig. 2) has a battery, two electrodes, electrical connectors, and a hydrogel formulation containing the sodium nitrite and electrochemical mediator. Each component is described in greater detail below.

Figure 2. Diagram of the Noxano Bandage Dressing.



- ◊ *Specifications* – The device sizes available are small (4 x 4 cm), medium (6 x 6 cm), and large (6 x 8 cm), and are all 1-2 cm in thickness (1 cm at the ends and 2 cm in the center, i.e., the location of the battery). The size used will depend on the size of the wound in the wound care subject population (the device must cover the entire area of the wound) and could vary over time, while the healthy volunteers will all receive the small (4 x 4 cm) device size.
- ◊ *Electrodes* – Non-woven conductive carbon fiber cloth. The carbon fiber allows efficient reaction with the mediator and prevents galvanic corrosion of the electrode that would occur with a metal electrode.
- ◊ *Connectors* – 3M non-metallic conductive tape.
- ◊ *Battery* – Three volt (3V) lithium ion battery. The battery is similar to standard coin cell watch batteries such as the CR2032, but has a higher operating temperature allowing for steam sterilization.
- ◊ *Hydrogel formulation* – Medical grade cross-linked poly-(acrylic acid), sodium nitrite, buffer (pH 7.5-8.0), and an electrochemical mediator. The formulation is 'activated' by soaking the dressing in sterile water. The final concentration of sodium nitrite in the wet hydrogel is 1-4%, depending on the NO level being delivered. The mediator is a derivative of benzophenone (sunscreen) and is used at 100 ppm in the wet hydrogel.

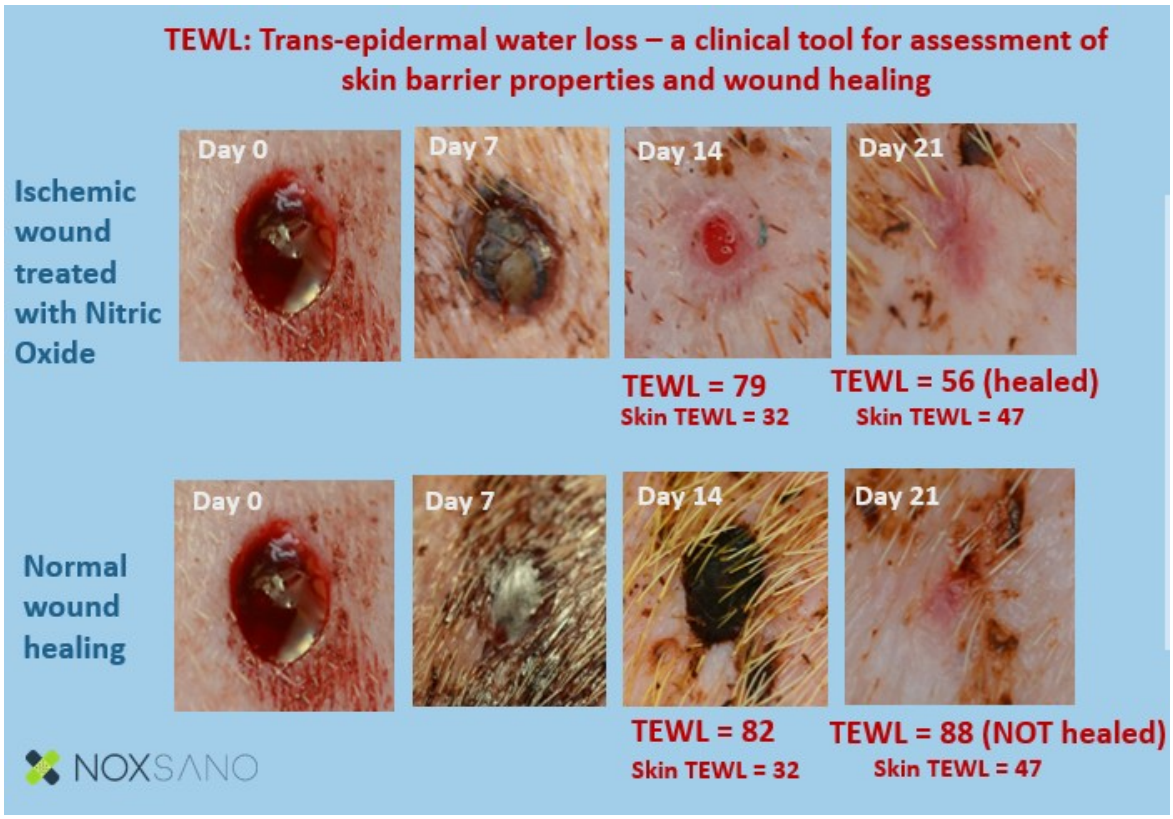
The generation of NO from the Bandage has been quantified in vitro using a gel (gelatin) containing a colorimetric sensor, 1,2-diaminoanthroquinone, (DAQ) which selectively reacts with NO to form a colorless product. The flux of NO (moles per minute) was calculated by following the loss of the DAQ from the gel. A formulation/device combination generating the appropriate NO flux to promote granulation tissue development ($0.5\text{-}1.5 \times 10^{-7}$ moles per minute = 50-150 nM per minute) during healing was identified and used in porcine wound healing studies [22].

Ischemic Wound Healing. Noxsano is collaborating with Prof. Valerie Bergdall (Ohio State University Laboratory Animal Resource, ULAR). Prof. Bergdall has extensive experience [23-27] in the development and application of wound models for the evaluation of wound healing technologies. Based on Prof. Bergdall's advice, Noxsano undertook the Bandage evaluation using a porcine ischemic wound model, a good model for wound healing in chronic wounds [31]. The ischemic wound model involves creating a 12.5 cm x 5.0 cm flap with reduced blood flow (ischemia). In the center of the flap an 8 mm full thickness wound is created, and this wound is used to evaluate ischemic wound healing. The first ischemic wound study sought to validate our hypothesis that restoring approximately 1×10^{-7} moles per minute of NO delivered to an ischemic wound would promote granulation and therefore speed healing time.

Wound healing was evaluated visually and via histology. Results indicated that investigators could indeed heal an ischemic wound significantly faster than a similar wound treated with conventional care (Tegaderm™). The ultimate objective of the Noxsano Bandage is to restore 'normal healing' to an ischemic (chronic) wound. This was demonstrated in the porcine wound model by comparing the healing of an ischemic

wound on the pig to that of a normal wound; an 8 mm full-thickness wound without the 'flap.' The healing was evaluated visually and using transepidermal water loss (TEWL), which measures the movement of water through the skin. If the skin is compromised, the movement of water is significantly increased; TEWL therefore provides a very sensitive measure of when a wound has healed (skin integrity restored). The control (normal) wound was treated with a simple Tegaderm™ dressing. The ischemic wound was treated with a Noxsano dressing delivering between 0.8 and 1.2 x 10^{-7} moles per minute NO. The dressing was changed every 3-4 days and delivered NO continuously between Bandage changes. At day 21 the TEWL assessment of the ischemic wound indicated it was healed. The TEWL was similar or equivalent to normal skin indicating normal barrier function (Fig. 3). In contrast, the normal wound was still an 'open wound.' The TEWL was significantly higher than normal skin indicating that the barrier function had not been restored and the wound was still prone to infection (Fig. 3).

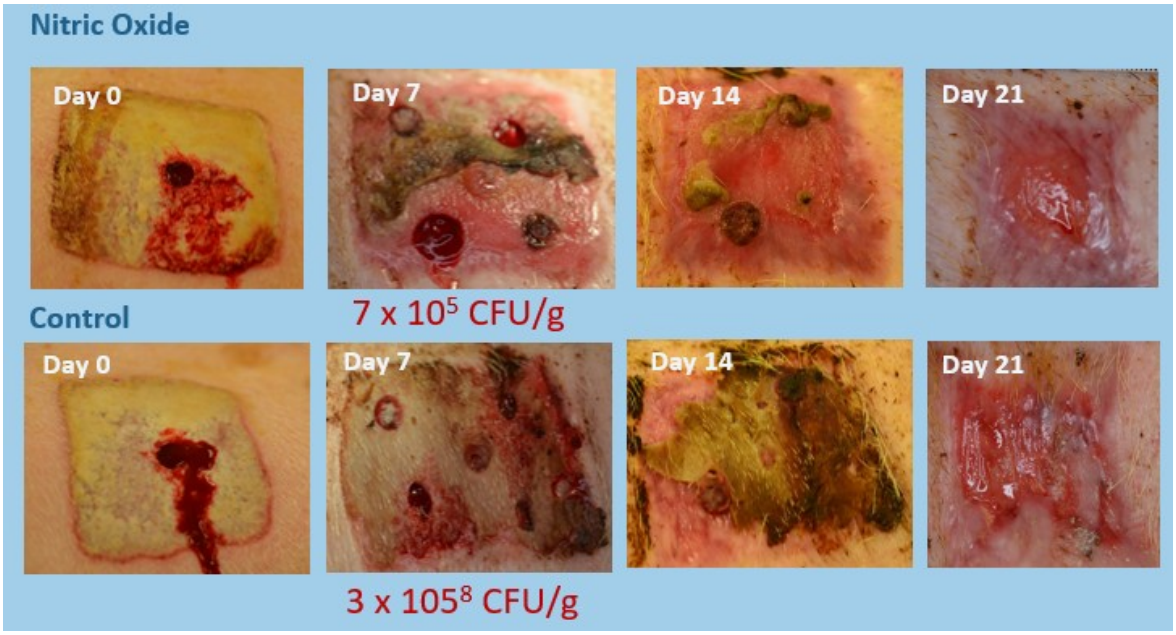
Figure 3. Transepidermal Water Loss (TEWL) as an Assessment for Wound Healing [proprietary data].



Biofilm Control. A third porcine study was used to evaluate the Noxsano Bandage's control of a wound biofilm. In order to establish a stable biofilm, a second-degree burn was applied and the burn infected with a non-pathogenic, biofilm-forming bacteria (*P. aeruginosa*). Once the biofilm had stabilized (3 days) an 8 mm full-thickness punch wound was created in the center of the burn wound. The infected burn wound was treated with either Tegaderm™ (control) or a Noxsano dressing delivering approximately 1 x 10^{-7} moles per minute of NO. As with the previous tests, the dressing was changed every 3-4

days. The biofilm was evaluated by taking punch biopsies. After 7 days, the control biofilm remained intact. In comparison, the biofilm had been reduced by ~3 orders of magnitude to a bio-burden with the Noxsano Bandage. The test was continued and showed accelerated healing of both the burn and punch wounds (original and punch biopsies) (Fig. 4).

Figure 4. Wound Healing between the Noxsano Bandage and Control (Tegaderm™) [proprietary data].



1.3.2 Clinical Data

The proposed study will be the first clinical study for the Noxsano Bandage.

1.3.3 Clinical Pharmacokinetics

As mentioned, NO is involved in the wound healing process at all stages and its mechanism of action is not yet completely understood. The following outline of the role of NO is summarized further from a review by Luo et al [28].

Nitric Oxide and Angiogenesis. Angiogenesis, the process of forming new micro vessels, is an important component of normal wound repair. NO is vital to the activity of pro-angiogenic cytokines. Vascular endothelial growth factor (VEGF) is a potent angiogenic factor controlled by NO. VEGF depends on NO for control of VEGF-induced endothelial cell proliferation and mitogen-activated protein (MAP) kinase. In addition, NO plays a role in controlling monocyte-induced angiogenesis, substance P, and transforming growth factor (TGF)-β1. Taken together, NO clearly plays a crucial role in post-wound angiogenesis.

Nitric Oxide and Inflammation. NO has been shown to modulate chemoattractant cytokines that initiate post-wound inflammation, including interleukin (IL)-8, TGF-β1, monocytes, and neutrophils. Because IL-1 is a potent chemoattractant for keratinocytes,

the modulation of IL-1 by NO may usher keratinocyte recruitment, proliferation, and differentiation. Taken together, NO modulation of inflammation-associated cytokines may affect the inflammatory phase of wound healing.

Nitric Oxide and Cell Proliferation, Differentiation, and Apoptosis. NO affects proliferation, differentiation, and apoptosis in key cell types involved in wound healing. Low levels of NO increase keratinocyte proliferation. NO has been shown to stimulate the proliferation of endothelial cells, protect endothelial cells from apoptosis, and mediate VEGF production. Together these effects suggest NO is critical during the proliferative phase of healing.

Nitric Oxide and Matrix Deposition and Remodeling. The final phases of healing require increased collagen synthesis and deposition. NO increases collagen formation in fibroblasts derived from both normal and wound skin. Conversely, inhibition of NO synthesis has been shown to decrease collagen formation and deposition.

All the effects described above are dependent on delivery of the correct concentration. At the extreme, very high levels of NO become cytotoxic. To create the benefits described above a low, carefully controlled level of NO must be delivered continuously to the wound. The Noxsano Bandage achieves this as it generates a controlled flux of NO during use, restoring the normal biological processes required for wound healing.

1.3.4 Device Regulatory Pathway

Under 21 CFR 812.3(m), a significant risk device means an investigational device that:

1. Is intended as an implant and presents a potential for serious risk to the health, safety, or welfare of a subject,
2. Is purported or represented to be for use supporting or sustaining human life and presents a potential for serious risk to the health, safety, or welfare of a subject,
3. Is for a use of substantial importance in diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health and presents a potential for serious risk to the health, safety, or welfare of a subject, and/or
4. Otherwise presents a potential for serious risk to the health, safety, or welfare of a subject.

The Noxsano Bandage is not an implantable device nor is it designed to sustain life; in this regard it is clearly not a significant risk device as defined by the FDA. The Noxsano Bandage also does not treat a disease, but does treat wounds which certainly could be considered an impairment of human health. The Bandage does not, in the manufacturer's opinion however, represent a serious risk to the health, safety, or welfare of the study subjects. The study subjects already have non-healing wounds that impair their health. The objective of the study will be to show improved healing relative to established techniques. Subjects will be monitored and returned to established techniques if the Noxsano Bandage fails to improve their healing. No preclinical evidence of significant risk has been demonstrated, and therefore significant risk is not anticipated or expected:

- ◊ NO is a naturally-occurring species produced by the body during healing as a bio-signal, but is absent in chronic wounds.

- ◊ Exogenous delivery replaces the missing NO at levels equivalent to those generated naturally, restoring the wound to its 'natural state.'
- ◊ NO is short lived (1-2 seconds) and therefore does not become systemic. Biological activity is limited to the wound.
- ◊ The level of NO is highly controlled by the electrochemical reaction, thus preventing any risk of overdose.
- ◊ The level delivered to the wound is fixed by the battery voltage and the formulation and cannot be altered by the patient.
- ◊ The dressing is powered by a 3V battery commonly found in a wide range of consumer electronics. It presents no risk for electric field exposure, electrical shock, or electrocution.
- ◊ The dressing contains a safe, stable precursor to NO, sodium nitrite. Sodium nitrite is commonly used to tenderize and cure meats, and can safely be consumed.
- ◊ The mediator is a benzophenone derivative present at very low levels (100 ppm), and is similar in structure to a common sunscreen ingredient. It has no known adverse toxicological effects.
- ◊ The hydrogel is a medical grade supersorber, essentially the same material used in baby diapers. It has no known adverse toxicological effects.
- ◊ The hydrogel formulation does not directly contact the patient's skin. The formulation is contained in a medical grade nonwoven dressing material.

The factors described above lead Noxsano to the view that the proposed study device is 'non-significant risk' (NSR) as defined by the FDA in 21 CFR 812.3(m). In addition, the protocol has been designed to ensure that the subjects' health and safety is not imperiled.

1.3.5 Group 1 (Healthy Volunteers) Rationale

Group 1 healthy volunteers are not dysfunctional in the generation of NO due to chronic wound healing, and therefore their endothelium will contain 'normal levels.' As such, use of the Bandage will represent an increase above physiologically normal levels in the endothelium of the healthy volunteers. In contrast, Group 2 subjects have wounds that are known to be dysregulated in NO. In this instance, the treatment returns their endothelium to physiologically normal levels of NO. Although the porcine study demonstrated efficacy, it could not indicate if irritation occurred as a side effect. As a result, a healthy volunteer group was included in this study to assess irritation from the use of the Bandage. As NO is a biologically-active species involved in a number of pathways in the endothelium, the manufacturer recommends a short 'acute exposure' of 72 hours. Longer-term overexposure could lead to artifacts as the body reacts to elevated levels of this biosignal.

The founders of Noxsano have worn devices for up to 72 hours with no apparent negative effects. The excess NO did appear to cause melanogenesis (skin darkening) and hyperemia (increased local blood flow). A period of 72 hours for Group 1 is therefore believed to be sufficient to determine if irritation occurs but short enough to avoid any potential artifacts from NO above physiological levels. Longer-term exposure to the Group 2 subject population is, in the opinion of the manufacturer, justified by the

preclinical data from porcine studies. These studies demonstrate that long-term (weeks) NO exposure to chronic wounds leads to improved wound healing without any identifiable negative physiological effects (upon observation or histology).

2.0 STUDY OBJECTIVES AND ENDPOINTS

2.1 Primary Objective

Group 1 (Safety): Healthy Volunteers. The initial phase of the study is designed to determine the safety of the study device in healthy volunteers without wounds. If there are no issues with tolerance, side effects, or adverse reactions in healthy volunteers, the second phase of the study will proceed with active wound care subjects.

For the purposes of this study, if two subjects experience an unacceptable adverse reaction warranting discontinuation of treatment in the opinion of the Investigators during the initial phase of the study, the study will be halted and the second phase (and the remainder of the initial phase, if applicable) will not be completed. Allergic reactions and infections at the bandage site will be considered unacceptable at any clinical severity; other adverse reactions will be deemed "unacceptable" by the Investigators on a case-by-case basis dependent upon clinical severity (App. A).

Group 2 (Efficacy): Wound Care Subjects. The second phase of the study is designed to determine the effectiveness of the study device in wound healing in subjects with active wounds (see Primary & Secondary Endpoints below).

2.2 Primary Endpoint

The primary endpoint for this study is wound healing (Group 2), as defined by the percent change in wound surface area (surface area is calculated in cm^2) from baseline through the active treatment period. Specifically, wound care subjects will have the study device applied at baseline then re-applied two times per week for up to 4 weeks from the start of the active treatment period. Device reapplication will change to weekly when the principal or sub-investigator determines it is permissible or when a maximum of 4 weeks of treatment is reached. Device re-application will continue weekly until the wound is judged to be healed by the wound care practitioner or up to 3 months, whichever comes first. If after 2 consecutive months of treatment with the study device the wound does not exhibit healing, evidenced by a decrease in surface area (cm^2), the subject will be returned to standard and established techniques of treatment consistent with the wound etiology.

2.3 Secondary Objective *(Group 2 only)*

Group 2 (Efficacy): Wound Care Subjects. The secondary endpoint of the study is to determine if the study device is equivalent to standard and established techniques (standard techniques demonstrate 50% wound healing at 2 months) in subjects with active wounds.

2.4 Secondary Endpoint *(Group 2 only)*

Standard wound healing techniques generally demonstrate 50% wound healing at 2 months. Therefore, the secondary endpoint for this study is the proportion of wound care patients that have at least 50% improvement in surface area within 2 months of treatment. This information will be used to describe if the study device is equivalent to standard and established techniques.

3.0 STUDY DESIGN

3.1 Study Design Overview

This study is a prospective, interventional, non-randomized, sequential phase study designed to assess the safety and efficacy of the Noxsano Bandage (study device) in subjects with a diabetic lower extremity ulceration and/or arterial insufficiency lower extremity ulceration.

3.2 Subject Population and Sites

Group 1: Initial subjects will be limited to healthy volunteers with intact skin on their lower extremities. Five (5) white subjects and 5 black or African American subjects will be enrolled to evaluate skin differences. Group 1 (healthy volunteers without wounds) will have all follow-up completed and the results assessed before the investigators initiate prior to proceeding to Group 2 (wound care subjects).

Group 2: The subsequent wound care subject study will include 10 subjects from the patient population treated at the OhioHealth Riverside Methodist Hospital Critical Limb Care Center for lower extremity ulceration attributed to diabetes and/or arterial insufficiency.

3.3 Expected Study Duration and Subject Participation

Group 1: The initial healthy volunteer safety study will last a total of 4.5 weeks. Healthy volunteers will wear the study device for 3 consecutive days (up to 72 hours), followed by weekly visits for 4 weeks of observation for tolerance, side effects, and/or adverse reactions.

For the purposes of this study, if 2 subjects experience an unacceptable adverse reaction warranting discontinuation of treatment in the opinion of the Investigators during the initial healthy volunteer phase, the study will be halted and Group 2 (and the remainder of the initial phase, if applicable) will not be completed. Allergic reactions and infections at the study device application site will be considered unacceptable at any clinical severity; other adverse reactions will be deemed “unacceptable” by the Investigators on a case-by-case basis dependent upon clinical severity (App. A).

Group 2: The wound care subjects will have twice per week study device applications to a specific ulceration for up to the first 4 weeks of treatment and then weekly re-application thereafter. For subjects that exhibit any reduction in wound surface area, this application will occur until the wound is healed, or for up to 3 months (whichever occurs first). For subjects that do not exhibit any reduction in wound size at 2 months, application will stop and standard treatment protocols will be pursued. At the conclusion of the treatment window (up to 3 months), subjects will be followed every 3 months for 12 consecutive months for observation of late side effects or adverse reactions (3 months of active treatment, 12 months of follow-up observation, 15 months total).

4.0 SUBJECT SELECTION

4.1 Subject Identification and Recruitment

4.1.1 Research Match

ResearchMatch.org is a national electronic, web-based recruitment tool that was created through the Clinical & Translational Science Awards Consortium in 2009 and is maintained at Vanderbilt University Medical Center. There is no cost for researchers at participating institutions in the ResearchMatch Network to use ResearchMatch for the purposes of conducting recruitment feasibility analysis or participant recruitment. The Vanderbilt University Medical Center IRB provides oversight for ResearchMatch as a recruitment tool). However, individual requests to use ResearchMatch as a recruitment tool are required to be approved by the participating institution's IRB. OhioHealth is an approved ResearchMatch institution.

ResearchMatch© consists of a registry of volunteers that have signed up to receive notifications about potential research studies. ResearchMatch© provides standard notification language that will be received by all ResearchMatch© volunteers who may be eligible for a given study. When a volunteer is sent a notification about a study, they will reply ("yes" or "no") using the appropriate notification quick links. When volunteers select "yes" to affirm their interest in participating, ResearchMatch© will release their contact information through the online portal to the Principal Investigator (PI) or research team. All information will be managed per strict ResearchMatch© Guidelines. Research staff will then call volunteers to prescreen for medical history pertaining to the study and determine eligibility. If the volunteer is interested, the consent will be sent via email for review, and the Baseline visit will be scheduled at the OhioHealth Research Institute.

4.1.2 Identification and Recruitment

Group 1: Healthy volunteers will be identified from the community using recruitment flyers posted at OhioHealth hospital campuses (App. B) and notifications in ResearchMatch© (App. C). ResearchMatch© notifications will be limited to registry participants located within a 25 mile radius of OhioHealth Riverside Methodist Hospital. OhioHealth associates can be included in the healthy volunteer cohort.

Group 2: Patients presenting to the OhioHealth Riverside Methodist Hospital Critical Limb Care Center with a diabetic lower extremity ulceration and/or arterial insufficiency lower extremity ulceration will be pre-screened for possible inclusion in the study. If determined preliminarily eligible, a research staff member will either approach the patient at their visit to the Critical Limb Care Center, or contact them by phone following their visit, if necessary.

4.2 Informed Consent

Written informed consent using the most current Institutional Review Board (IRB)-approved informed consent form (ICF) will be obtained from all subjects prior to any study-specific tests or procedures performed (App. D & E). This does not include those procedures or tests that are obtained in the normal course of the subject's routine care.

4.3 Enrollment

Subjects will be considered enrolled once signed informed consent has been obtained. Subjects that do not present for the initial Noxsano Bandage application appointment will be withdrawn from the study and replaced.

All subjects who have been consented will be registered in the Research Electronic Data Capture (REDCap™) database and will be assigned a unique subject study number.

The screening and enrollment log will also be completed to document the outcome of all subjects who underwent screening (App. F). The screening and enrollment log will contain the enrollment and study number of subjects who were enrolled, or the reason for non-enrollment of subjects who did not meet inclusion/exclusion criteria.

Group 1 and Group 2 subjects will be screened according to the protocol inclusion and exclusion criteria. Each of the criteria in sections 4.4 and 4.5 must be met in order for a subject to be considered eligible for this study.

4.4 Inclusion Criteria

Group 1: Subjects must meet **all** of the following criteria to be eligible for enrollment:

1. Subject is ≥ 18 and < 80 years of age.
2. Subject is white, black or African American
3. Subject has provided written informed consent.
4. Subject is willing to comply with study follow-up requirements.
5. Subject has intact skin on lower extremities.

Group 2: Subjects must meet **all** of the following criteria to be eligible for enrollment:

1. Subject is ≥ 18 and < 80 years of age.
2. The wound to be treated with the Noxsano bandage has a baseline wound surface area of < 25 cm^2.
3. Subject has provided written informed consent.
4. Subject is willing to comply with study follow-up requirements.
5. Subject with at least **one** of the following:
    a. Diabetic lower extremity ulceration with a hemoglobin A1c (HgbA1c) value ≤ 9.0, drawn within 3 months prior to study participation[1], and/or
    b. Arterial insufficiency lower extremity ulceration with a post-revascularization ankle-brachial index (ABI) value of ≥ 0.40 and ≤ 0.80 on the involved extremity, performed within 3 months prior to study participation[1], and/or

[1] Baseline measures of HgbA1c or ABI (within 3 months prior to consent) must be available for inclusion.

c. Diabetic and/or arterial insufficiency lower extremity ulceration deemed ineligible for revascularization with 3 months prior to study participation

4.5 Exclusion Criteria

Group 1: Subjects will be excluded from the trial if **any** of the following criteria are met:

1. Subject is < 18 or ≥ 80 years of age.
2. Subject has a history of diabetes, arterial insufficiency, or osteomyelitis.
3. Subject has a known hypersensitivity to adhesives.
4. Subject is on any prescription medications, including contraceptives. Due to the short duration of the procedure period (3 days), subjects who initiate prescription medications during study participation will continue in the study.
5. Subject is pregnant, plans to become pregnant during the study period, or is breastfeeding.
6. Subject is non-English speaking or reading.
7. Subject is unable to give informed consent.

Group 2: Subjects will be excluded from the trial if **any** of the following criteria are met:

1. Subject is < 18 or ≥ 80 years of age.
2. The wound to be treated with the Noxsano bandage has a baseline wound surface area of ≥ 25 cm^2.
3. The wound to be treated with the Noxsano bandage is a plantar wound.
4. Subject with diabetes with an HgbA1c value of > 9.0, drawn within 3 months prior to study participation[2].
5. Subject with arterial insufficiency with an ABI value of < 0.40 or > 0.80, performed within 3 months prior to study participation[3].
6. The wound to be treated with the Noxsano bandage has osteomyelitis contiguous with the treatment site.
7. Subject with peripherally-inserted central catheter (PICC) line antibiotic treatment within the previous 6 months.

[2] Hemoglobin A1c is known to be significantly associated with wound healing rates, making it an important marker in the prediction of wound healing in diabetic patients [29]. A cutoff of 9.0% was chosen to exclude those with uncontrolled diabetes at highest risk for poor healing outcomes, as well as surgical complications including wound infections [30].

[3] Patients with a normal-to-borderline ABI (> 0.80) are not characterized or diagnosed as having arterial insufficiency and are not referred to a vascular specialist; on the opposite end of the spectrum, those with severe arterial disease (< 0.40) typically proceed directly to hyperbaric treatment.

8. Subject requiring any type of amputation on the treatment limb within 3 months prior to study participation.
9. Subject with a known hypersensitivity to adhesives.
10. Subject is on active steroid therapy (does not include inhaled steroids).
11. Subject is pregnant, plans to become pregnant during the study period, or is breastfeeding.
12. Subject is non-English speaking or reading.
13. Subject is unable to give informed consent.
14. Subject is currently enrolled in another interventional study.

5.0 STUDY PROCEDURES AND ASSESSMENTS

5.1 Schedule of Events

Group 1 (Healthy Volunteers):

The initial healthy volunteer safety study will last a total of 4.5 weeks. Healthy volunteers will have the study device applied for 3 consecutive days (up to 72 hours), followed by weekly visits for 4 weeks of observation for tolerance, side effects, and adverse reactions. Weekly visits will occur every 7 ± 2 days after the Day 3 visit.

Assessment	**Screening** *(via phone)*	**Baseline**	**tion: Day 0Study Device**	**3 ± 0 days Treatment:**	**y 7 ± 2 days[4] Follow-Up:**	**Unscheduled Visit**
Informed Consent		x				
Medical history	x					
Concomitant medications	x	x	x	x	x	x
OhioHealth Research Institute Clinic Visit (Research Only)		x				
Critical Limb Care Center (Riverside Methodist Hospital Wound Center) visit (research-only)			x	x	x	
Shaving of left posterior calf		x				
Photograph			x	x[5]	x	
Noxsano Bandage application			x			
SAR Questionnaire			x	x	x	

Group 2 (Wound Care Subjects):

The wound care subject treatment period will consist of twice per week study device applications to a specific ulceration for up to the first 4 weeks of treatment and then weekly re-application thereafter. When deemed permissible by the clinical judgment of the principal or sub-investigator or when 4 weeks is reached, re-application of the study device will change to weekly. This weekly re-application will occur until the wound is healed, or for up to 12 weeks (whichever occurs first). For subjects that do not exhibit any reduction in wound size at 2 months, application will stop and standard treatment protocols will be pursued.

At the conclusion of the treatment period (maximum of 3 months), subjects will be followed every 3 months for 12 consecutive months for observation of late side effects or adverse reactions (up to 3 months of active treatment, 12 months of follow-up observation, 15 months maximum).

[4] Day 10, 17, 24, and 31

[5] Photograph and compensation are given on Day 3 only during Treatment Period

Assessment	Screening / Baseline	Day 0Initial Study Device	ery 3 ± 2 days[6]Treatment:	ery 7 ± 2 days[7] Treatment:	mo ± 14 days[8] Follow-Up:	Unscheduled Visit
Informed Consent	x					
Routine medical history / physical exam	x					x
Concomitant medications	x	x	x	x	x	x
HgbA1c *(subjects with diabetes)*	x					
ABI *(subjects with arterial insufficiency)*	x					
Critical Limb Care Center visit (Riverside Methodist Hospital Wound Center)	x	x	x	x	x	x
Photograph (i.e., wound image)	x	x	x	x	x	x
Wound measurements	x	x	x	x	x	x
Percent granulation tissue	x	x	x	x	x	x
Noxsano Bandage application		x	x	x		
SAR Questionnaire		x	x	x	x	x

5.2 Concomitant Medications

A list of all prescription and over the counter medications, including topical treatments, will be recorded for all subjects at each visit.

Although subjects are excluded from Group 1 if they are on any prescription medications at the time of study initiation, due to the short duration of the procedure period (3 days), subjects who initiate prescription and/or over the counter medications during study participation will continue in the study.

5.3 Photographs

Photographs of the study device application site and wounds (Group 2) will be taken using a digital camera, an FDA Class 1 510(k) exempt medical device. Digital photographs are routinely taken in the OhioHealth Riverside Methodist Hospital Critical Limb Care Center, therefore, their equipment will be utilized for the study.

Group 1(Healthy Volunteers): A photograph will be taken on Day 0 of the shaved area on the left posterior calf prior to study device placement. A photograph of the site where the study device was applied will also be taken on Day 3, following removal of the study device, and at each follow-up visit (Days 10, 17, 24, and 31). All photographs will be

[6] Weeks 1-4, 2 times per week study device changes or until change to weekly is permitted by investigator.
[7] Weeks 5-12, weekly study device changes until wound has healed or 3 months is reached.
[8] 3, 6, 9, and 12 months post-treatment

uploaded into the study electronic case report forms (eCRFs) by a member of the study staff.

Group 2 (Wound Care Subjects): Photographs of the wound with a conventional ruler in place will be obtained at baseline, Day 0 (Initial Device Application), treatment, and follow-up visits. Photographs will be taken by a wound care nurse and uploaded in the study eCRFs by a member of the study staff.

5.4 Wound Measurements

The standard measurement per the current protocol at the Center for Critical Limb Care is a vertical and horizontal measurement taken in centimeters using a conventional ruler. These measurements are used to calculate the wound area (cm^2). All wound measurements for this study will be performed according to The OhioHealth Heart and Vascular Wound Care Center protocol to ensure consistency visit to visit. Current protocol involves trained nursing wound care staff obtaining photographic images with standard conventional ruler. Wound length, width, and surface area will be reported in the REDCap data collection tool. Sub-investigators will review the photographs to ensure in agreement with correct measurements.

5.5 Granulation Tissue

Percentage of granulation tissue present in the wound being treated with Noxsano bandage will be recorded in the REDCap data collection tool at each study visit.

5.6 Baseline Procedures

Group 1(Healthy Volunteers): If the subject meets inclusion/exclusion criteria and the informed consent is signed, a 4 x 4 cm segment of healthy, intact skin on the left posterior calf will be shaved by study staff. Study device application will occur within 1-3 days after the Baseline visit in the event that shaving causes irritation.

Group 2 (Wound Care Subjects): Baseline data will be collected, including a routine medical history and physical examination pertaining to study eligibility. The most recent documented baseline HgBa1c and/or ABI values will be collected from the subject's medical record. A baseline photograph and the standard wound measurement using a conventional ruler will be obtained as described in section 5.3. Baseline photographs will be taken with the conventional ruler in place. When possible subjects may proceed immediately to the initial study device application once baseline assessments have been completed.

5.7 Initial Study Device Application Visit

The device sizes available are small (4 x 4 cm), medium (6 x 6 cm), and large (6 x 8 cm), and are all 1-2 cm in thickness (1 cm at the ends and 2 cm in the center, i.e., the location of the battery). The size used will depend on the size and shape of the wound to be treated (the device must cover the entire area of the wound) and could vary over time. Healthy volunteers will all receive the small (4 x 4 cm) device size.

All subjects will be given a Noxsano Bandage Information Sheet (App. G), which will reiterate conversations from the Informed Consent process, including, but not limited to the following:

- ◊ "Troubleshooting" the Noxsano Bandage, including device malfunctions and frequently asked questions;
- ◊ Reporting safety issues, side effects, and adverse reactions.

Group 1 (Healthy Volunteers): A photograph will be taken on Day 0 of the shaved area where the device will be place prior to study device application. The study device will be applied by the Principal Investigator or delegated Sub-Investigator to the shaved area of skin on the left posterior calf. The study device will not be changed over the 3-day treatment period in order to complete an uninterrupted application of 3 total days (up to 72 hours). A photograph of the site where the study device was applied will also be taken on Day 3, following removal of the study device, and at each follow-up visit.

The SAR questionnaire will be administered by study staff immediately following application of the study device to assess tolerance, side effects, and/or adverse reactions (App. A). For the purposes of this study, if 2 subjects experience an unacceptable adverse reaction warranting discontinuation of treatment in the opinion of the Investigators during the initial phase of the study, the study will be halted and the second phase (and the remainder of the initial phase, if applicable) will not be completed.. Allergic reactions and infections at the device application site will be considered unacceptable at any clinical severity; other adverse reactions will be deemed "unacceptable" by the Investigators on a case-by-case basis dependent upon clinical severity (App. A).

Group 2 (Wound Care Subjects): The initial device application visit (Day 0) may be combined with the screening/baseline visit. Group 2 subjects will be limited to a maximum wound surface area of < 25 cm^2, to allow for complete coverage with the largest device size of 6 x 8 cm. Those with wounds ≥ 25 cm^2, comprising less than 5% of the wound care population, will be excluded. Conversely, we are not limiting minimum wound size, as some of the most complicated wounds are actually under 1 cm^2.

The standard wound measurement in centimeters using a conventional ruler will be obtained according the The OhioHealth Heart and Vascular Wound Care Center standard protocol prior to the initial device application at Day 0. The standard measurement per the current protocol at the Center for Critical Limb Care will be implemented and a photograph with the conventional ruler in place will be taken.

The study device will be applied to the lower extremity wound site by a trained investigator or sub-investigator. Routine wound care techniques such as debridement will be performed in the usual standard fashion prior to application of the Noxsano Bandage. The SAR questionnaire will be administered by study staff immediately following application of the Noxsano Bandage to assess tolerance, side effects, and/or adverse reactions (App. A).

5.8 Treatment Visits and Assessments

Group 1 (Healthy Volunteers): Healthy volunteers will be seen in the Critical Limb Care Center on Day 0 for Application (± 0 days) and Day 3 for Removal (± 0 days) during the study treatment period. Subjects will be instructed to call the Critical Limb Care Center (614-566-2682) during regular business hours (0700-1600), and the Study Investigators will be available after-hours (614-738-8938 for Dr. Mehl, 614-404-7512 for Dr. Silver, 937-599-4852 for Dr. Anderson)for concerns regarding adverse reactions or other general questions. Contact numbers for the study investigators will be provided in the informed consent document. The following evaluations and processes will be completed and data recorded in the REDCap eCRF:

- ◊ SAR Questionnaire (App. A)
- ◊ Concomitant medications
- ◊ Photograph (Day 3 only after the removal of the study device)

Group 2 (Wound Care Subjects): Subjects will be seen in the Critical Limb Care Center twice a week (every 3 days ± 2 days) for up to 4 weeks. Clinical judgment of the principal or investigator will permit a subject to discontinue twice weekly bandage changes prior to the end of 4 weeks. After the first 4 weeks of treatment or when deemed permissible by the investigator, subjects will be seen in the Critical Limb Care Center weekly (7 days ± 2 days) for a total treatment period of up to 3 months. The following evaluations will be completed and data recorded in the REDCap eCRF:

- ◊ Study device application
- ◊ Photograph of the study device application site (on Day 0 prior to study device application; following the removal of the old device and prior to application of the new device on subsequent treatment visits)
- ◊ Wound measurements
- ◊ Percent granulation tissue
- ◊ SAR Questionnaire (App. A)
- ◊ Concomitant medications

5.9 Required Follow-Up Visits and Assessments

Group 1 (Healthy Volunteers): Healthy volunteers will be seen in the Critical Limb Care Center once a week (every 7 days ± 2 days) for a total of 4 weeks of follow-up. The following evaluations will be completed and data recorded in the REDCap eCRF:

- ◊ Photograph of the study device application site
- ◊ SAR Questionnaire (App. A)

Group 2 (Wound Care Subjects): After completion of treatment (completed when wound has healed or 3 months is reached), subjects will be seen by the principal or sub-investigator in the Critical Limb Care Center every 3 months ± 14 days for 12 months to evaluate for any late-term issues of tolerance, side effects, and/or adverse reactions. The following evaluations will be completed and data recorded in the REDCap eCRF:

- ◊ Photograph of the study device application site
- ◊ Wound measurements
- ◊ Percent granulation tissue
- ◊ SAR Questionnaire (App. A)
- ◊ Concomitant medications

5.10 Unscheduled Visits

A critical limb wound care podiatrist will be on call at all times.

Subjects will be instructed to call the Critical Limb Care Center (614-566-2682) during regular business hours (0700-1600), and the Study Investigators will be available after-hours (614-738-8938 for Dr. Mehl, 614-404-7512 for Dr. Silver, 937-599-4852 for Dr. Anderson) for concerns regarding adverse reactions or other general questions. Subjects will be seen on an urgent basis as-needed based on podiatrist recommendation during regular business hours at the Critical Limb Care Center.

Unscheduled visits are additional visits that occur at times other than the protocol pre-determined required visits during study participation. If an unscheduled visit occurs, the following will be collected from the subject's medical record and entered in the REDCap eCRF:

- ◊ Routine physical examination by a podiatrist, including assessment of the lower extremities
- ◊ Concomitant medications
- ◊ Photograph of the study device application site
- ◊ Wound Measurements
- ◊ Percent granulation tissue
- ◊ Bandage re-application if necessary
- ◊ SAR Questionnaire (App. A)

5.11 Subject Withdrawal or Discontinuation

Subjects have the right to withdraw from the study at any time and for any reason without penalty or loss of benefits to which the subject is otherwise entitled. If a subject decides to withdraw, all study assessments, tests, and procedures will be stopped. An exit interview will be requested to understand why the subject chose to withdraw. The subject will also be offered the option to have data collection from the electronic medical record to continue in order to track wound progression. The Investigators may withdraw the subject at any time to protect the health, safety, or welfare of the subject. At the last point of contact, the date and reason for discontinuation will be documented, and every effort should be made to follow-up the status of any ongoing adverse events prior to withdrawal.

Subjects that do not present for the initial Noxsano Bandage application appointment will be withdrawn from the study and replaced.

Study participation may continue per the Schedule of Events (section 5.1) until one of the following criteria applies:

Group 1:

- ◊ *Unacceptable adverse reactions warranting discontinuation of treatment in the opinion of the Investigators:* Subjects experiencing any allergic reaction or infection at the device application site will be removed from study treatment.

Group 2:

- ◊ *Unacceptable adverse reactions warranting discontinuation of treatment in the opinion of the Investigators*: Subjects experiencing any allergic reaction or infection at the device application site will be removed from study treatment.
- ◊ *Missed weekly treatment visits and discontinuation from study treatment*: Weekly treatment visits will be indicated as "missed" if the deviation from the planned date is > 2 days (i.e., 3 or 7 days ± 2 days, during the 3 month study period). If a subject misses 2 consecutive treatment visits, they will be removed from study and not replaced.
- ◊ *Missed study follow-up visits:* Subjects who miss two consecutive follow-up visits will be contacted as described in section 5.12 in order to determine whether they wish to withdraw for the study or re-establish regular study follow-up visits. If subjects are able to re-establish study follow-up visits they may remain on the study. If the subject is unable to be contacted through the procedures outlined in section 5.12 data collection from the electronic medical record may continue, but the subject will be considered "lost to follow-up".
- ◊ *Wound healing and discontinuation from study treatment*: Clinical benefit may be determined during the treatment process by documentation of a healed ulceration, at which time the study device application will be discontinued. The post-treatment follow-up period will then begin for observation of late side effects and/ or adverse reactions.
- ◊ *No evidence of wound healing at 2 months*. If a subject has no evidence of decreased wound surface area at 2 months (8 week treatment visit), the patient will be returned to standard and established techniques consistent with the wound etiology, and study follow-up will be discontinued.
- ◊ *Wound < 50% healed at the completion of the study treatment period and discontinuation from study follow-up*: If after 3 consecutive months (12 weeks) of treatment with the study device the wound does not exhibit at least a 50% decrease in surface area (cm^2), the subject will be returned to standard and established techniques consistent with the wound etiology, and study follow-up will be discontinued. Clinical benefit experienced after the three-month treatment period, but not during the treatment period is not anticipated

In the absence of discontinuation due to adverse reactions (for both Groups 1 and 2) or wound healing outcomes (for Group 2 only) as indicated above, treatment may continue per the Schedule of Events (section 5.1) until one of the following criteria applies:

- ◊ Subject decision to withdraw from treatment (partial consent) or from the study (full consent),
- ◊ Death, or
- ◊ Funder reserves the right to temporarily suspend or prematurely discontinue this study.

5.12 Lost to Follow-Up

The subject may be lost to follow-up after 3 documented phone calls and a certified letter has been mailed to the subject in order to obtain compliance with study requirements. These subjects will not be replaced.

5.13 Compensation

Participant compensation will be done using a method approved by OhioHealth Research Institute (OHRI) Research Business Services. OhioHealth Associates enrolled in the healthy cohort will not be compensated.

Group 1: Compensation for healthy volunteers will be disbursed for the Baseline visit, the completion of the treatment period on Day 3, and the weekly 4 week follow-up period resulting in up to $150 in total compensation (see compensation milestones below).

Time Point	Compensation Amount
Baseline	$25
Completion of treatment	$25
Week 1 follow-up	$25
Week 2 follow-up	$25
Week 3 follow-up	$25
Week 4 follow-up	$25

Subjects who discontinue study participation in Group 1 or Group 2 due to unacceptable adverse reactions per section 7.0 will receive a one-time discontinuation payment of $25. Those subjects that discontinue, withdraw, or are lost to follow-up will not receive any further compensation.

Group 2: Compensation for wound care subjects will be disbursed for the completion of the treatment period (completed when wound has healed, subject is transitioned to standard of care techniques, or 3 months is reached, whichever comes first), and disbursed for each completed follow-up visit during the 12 month follow-up period resulting in up to $125 in total compensation (see compensation milestones below).

Time Point	Compensation Amount
Completion of treatment	$25
3-month follow-up	$25
6-month follow-up	$25
9-month follow-up	$25
12-month follow-up	$25

Subjects who discontinue study participation due to unacceptable adverse reactions per section 7.0 will receive a one-time discontinuation payment of $25. Those subjects that discontinue, withdraw, or are lost to follow-up will not receive any further compensation

6.0 PRODUCT DESCRIPTION

6.1 General

The Noxsano Bandage (study device) uses an electrochemical reaction to generate highly controlled doses of NO for wound healing. The electrochemical reaction reduces sodium nitrite (meat tenderizer) to NO. To achieve this efficiently, an electrochemical mediator is used that 'shuttles' electrons from the anode to the nitrite. The dressing has a battery, two electrodes, electrical connectors, and a hydrogel formulation containing the sodium nitrite and electrochemical mediator. Each component is described in Figure 2 (section 1.3.1) above.

6.2 Manufacturer

The Noxsano Bandage is manufactured by:

Noxsano, Inc.
1275 Kinnear Rd.
Columbus, OH 43212

6.3 Packaging

The Noxsano Bandage is assembled by Noxsano, Inc., autoclave sterilized and supplied to OhioHealth Research Institute (OHRI). The hydrogel formulation in the dressing is 'activated' by soaking the device in 25-50 ml (depending on dressing size) of sterile water for 5 minutes. This process 'connects' the electrodes and initiates the electrochemical generation of NO. NO delivery continues until the dressing is removed (3-5 days). The dressing delivers 0.8-1.2 x 10^{-7} moles of NO per minute to the wound continuously during use. This level of NO mimics the level the body would generate during normal wound healing. The dressing will be packaged in a sterile container and delivered in-person by the manufacturer (staff from Noxsano, Inc.) to OHRI.

6.4 Intended Population

The Noxsano Bandage is intended for use in patients with chronic and non-healing wounds.

6.5 Product Training Requirements

Investigators, wound care practitioners, and research staff will be trained by Noxsano, Inc. staff in an onsite in-service at the Critical Limb Care Center at OhioHealth Riverside Methodist Hospital on the usage of the Noxsano Bandage, including, but not limited to the following:

- ◊ Clinical aspects of packaging, handling, application, and mechanism of action of the Noxsano Bandage;
- ◊ "Troubleshooting" the Noxsano Bandage, including device malfunctions and frequently asked questions;
- ◊ Reporting and advising on patient safety issues, side effects, and adverse reactions.

6.6 Product Receipt and Tracking

The study device will be delivered in-person by the manufacturer (staff from Noxsano, Inc.) and stored in a secure area at the offices of OHRI or in a locked storage room accessible only to OHRI staff at the OhioHealth Riverside Methodist Hospital Critical Limb Care Center.

Study device receipt will be tracked by research staff at each delivery using Device Tracking Logs (App H).

6.7 Product Storage and Accountability

The device will be labeled and stored in a secure area at the offices of OHRI or in a locked storage room accessible only to OHRI staff at the OhioHealth Riverside Methodist Hospital Critical Limb Care Center. Device storage and accountability will be controlled only by the assigned, trained research staff at the site. Each device will be labeled with the following:

- ◊ Company name
- ◊ Company contact information
- ◊ Device identification number
- ◊ Date of sterilization
- ◊ Shelf life (6 months)

Study device usage and administration will be tracked by research staff at each subject visit using Device Tracking Logs (App H). Per the manufacturer, there are no special storage requirements (shelf-stable at room temperature).

6.8 Product Return or Destruction

Any unused or expired product will be retrieved in-person by the manufacturer (staff from Noxsano, Inc.) upon study closure.

Study device return will be tracked by research staff at the conclusion of study enrollment using Device Tracking Logs (App. H).

7.0 SAFETY REPORTING

7.1 Definitions

7.1.1 Adverse Event

An **adverse event** (AE) is any unfavorable or unintended event, physical or psychological, associated with a research study, which causes harm or injury to a research participant as a result of the participant's involvement in a research study. The event can include abnormal laboratory findings, symptoms, or disease associated with the research study. The event does not necessarily have to have a causal relationship with the research, any risk associated with the research, the research intervention, or the research assessments.

Adverse events may be the result of the interventions and interactions used in the research; the collection of identifiable private information in the research; an underlying disease, disorder, or condition of the subject; and/or other circumstances unrelated to the research or any underlying disease, disorder, or condition of the subject.

Subjects will be followed in terms of tolerance/side effects/adverse reactions:

- ◊ **Tolerance**: pruritus (itchy skin), dermatitis (dry skin), pain
- ◊ **Side effects**: erythema (red skin), edema (swollen skin), pressure ulcer (skin breakdown), melanogenesis (skin darkening), hyperemia (increased local blood flow)
- ◊ **Adverse reactions**: any systemic side effect, including (but not limited to) fever, nausea, vomiting, or hypotension (low blood pressure)

Group 1 healthy volunteers are not dysfunctional in the generation of NO due to chronic wound healing, and therefore their endothelium will contain 'normal levels.' As such, use of the Bandage will represent an increase above physiologically normal levels in the endothelium of the healthy volunteers. In contrast, Group 2 subjects have chronic wounds that are known to be dysregulated in NO. In this instance, the treatment returns their endothelium to physiologically normal levels of NO (see section 1.3.5).

Therefore, Group 1 healthy volunteers are more likely to experience adverse events related to hypersensitivity and skin reactions, and are thus being limited to 3 days (up to 72) hours of Bandage wear. It is anticipated that symptoms will manifest within 24-72 hours of application and resolve within 7-10 days of removal, but could take as long as 30 days or more depending on individual skin regeneration. Regarding Group 2 wound care subjects, we expect manifestation and resolution of symptoms on the same timeline, but cannot state decisively as this is the first study in this population. Finally, as itching is a normal consequence of wound healing, we expect this to occur in the majority of Group 2 subjects. No preclinical evidence of significant risk has been demonstrated with the Noxsano Bandage, and therefore is not anticipated or expected during this study.

7.1.2 Adverse Device Effect

An adverse device effect (ADE) is defined as any AE that is related to the use of the investigational medical device.

7.1.3 Device Deficiency, Malfunction, and User Error

Investigators are instructed to report all possible device deficiencies, malfunctions or user errors during the course of the trial. These incidents will be documented in the eCRF as follows:

- ◊ **Device Deficiency** (ISO 14155:2011): Inadequacy of a medical device with respect to its identity, quality, durability, reliability, safety, or performance
- ◊ **Device Malfunction**: Failure of a device to meet its performance specifications or otherwise perform as intended [21 CFR 803.3].
- ◊ **User Error**: Device-related error or mistake made by the person using the device.

7.1.4 Serious Adverse Events

A **serious adverse event** (SAE) is any adverse experience occurring at any dose that results in any of the following outcomes:

- ◊ Results in **death.**
- ◊ Is a **life-threatening** adverse experience. The term "life-threatening" in the definition of "serious" refers to an adverse event in which the subject was at risk of death at the time of the event. It does not refer to an adverse event which hypothetically might have caused death if it were more severe.
- ◊ Requires **inpatient hospitalization or prolongation of existing hospitalization**. Any adverse event leading to hospitalization or prolongation of hospitalization will be considered as Serious, UNLESS at least one of the following expectations is met:
    - o The admission results in a hospital stay of less than 24 hours; OR
    - o The admission is pre-planned (e.g., elective or scheduled surgery arranged prior to the start of the study); OR
    - o The admission is not associated with an adverse event (e.g., social hospitalization for purposes of respite care.

  However it should be noted that invasive treatment during any hospitalization may fulfill the criteria of "medically important" and as such may be reportable as a serious adverse event dependent on clinical judgment. In addition, where local regulatory authorities specifically require a more stringent definition, the local regulation takes precedent.
- ◊ Results in **persistent or significant disability/incapacity**. The definition of disability is a substantial disruption of a person's ability to conduct normal life's functions.
- ◊ Is a **congenital anomaly/birth defect**.
- ◊ Is an **important medical event**. Important medical events that may not result death, be life-threatening, or require hospitalization may be considered a serious adverse experience when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical

intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood disease or disorders, or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse. The development of a new cancer is always considered an important medical event.

7.1.5 Serious Adverse Device Effect

A serious adverse device effect (SADE) is any ADE that has resulted in any of the consequences characteristic of an SAE.

7.1.6 Unanticipated Adverse Device Effect

An unanticipated device effect (UADE) is defined in 21 CFR 812.3 as any serious AE 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 protocol, or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of a subject.

7.1.7 Unexpected

Any AE that is not consistent in specificity or severity with the current protocol, including all amendments, is considered unexpected.

7.2 Documentation

Adverse events will be documented on the appropriate eCRF. All AEs will be characterized by the following criteria:

- ◊ Seriousness
- ◊ Intensity or severity
- ◊ Relatedness to the device and procedure
- ◊ Outcome
- ◊ Treatment or action taken
- ◊ Determination to continue study participation

7.2.1 Intensity or Severity

Intensity or severity will be recorded as mild, moderate, or severe using the following definitions:

- ◊ **Mild:** Minor signs/symptoms; no specific medical intervention required; asymptomatic laboratory findings only; marginal clinical relevance Subject is aware of symptoms, but symptoms are easily tolerated.
- ◊ **Moderate:** Requiring minimal, local, or noninvasive intervention only. Symptoms interfere with normal daily activities.
- ◊ **Severe:** Significant symptoms requiring hospitalization or invasive intervention. Symptoms are incapacitating.

7.2.2 Relatedness

Relatedness to the device and procedure will be recorded as unrelated, possibly related, or related.

7.2.3 Outcome

Outcome will be recorded as ongoing, resolved with sequelae, or resolved without sequelae (with the date resolved).

7.3 Reporting of Adverse Events

The Principal Investigator or designee is responsible for ensuring that all AEs (both serious and non-serious) observed by the clinical team or reported by the subject which occur after the subject has signed the informed consent are fully recorded in the subject's medical record. Source documentation must be available to support all adverse events.

A critical limb wound care podiatrist is on call 24/7/365. Subjects will be instructed to call the Critical Limb Care Center, and will be seen on an urgent basis as needed based on podiatrist recommendation. The adverse event will be immediately reported to the study Principal Investigator.

7.3.1 Reporting of Serious Adverse Events

Reporting of SAEs includes reporting of any SAE, any medical device deficiency that might have led to an SAE, and any new finding / update in relation to already reported events.

It is the responsibility of the Principal Investigator or designee to report all SAEs to Noxsano, Inc. (the study device manufacturer) within **2 business days** of discovery or notification of the event.

It is the responsibility of the Principal Investigator or designee to report all SAEs according to the IRB of record's policies and procedures.

7.3.2 Reporting of Unanticipated Adverse Device Effects

If a complication occurs that the Principal Investigator believes may be a potential UADE, the site should immediately contact the device supplier to determine reporting requirements.

It is the responsibility of the Principal Investigator or designee to report all UADEs according to the IRB of record's policies and procedures.

8.0 STUDY ADMINISTRATION

8.1 Data Management

The Principal Investigator is required to prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the investigation.

The Research Electronic Data Capture (REDCap™) system will be utilized, as required by OHRI, for data collection for both accrual entry and trial data management. REDCap is an electronic data capture (EDC) system housed on secure servers maintained at OhioHealth. Access to data through REDCap is restricted by user accounts and assigned roles. Once logged into the REDCap system with a user ID and password, REDCap defines roles for each user which limits access to appropriate data. User information and passwords can be obtained by contacting the REDCap Administrator.

REDCap is designed with the capability for study setup, activation, tracking, reporting, data monitoring and review, and eligibility verification. This study will utilize electronic case report form (eCRF) completion in the REDCap database. A calendar of events and required eCRFs are available in REDCap.

8.2 Clinical and Safety Monitoring

Qualified monitors from CSSi LifeSciences representing the Funder (Noxsano, Inc.) will conduct on-site monitoring visits to ensure that all Investigators conduct the study in compliance with the protocol.

8.2.1 Background

The purpose of the Clinical Monitoring Plan for the Safety and Efficacy of the Noxsano Wound Care Bandage: A First-in Human Study, protocol number 1331496, is to ensure compliance with the Principles of International Conference on Harmonization - Good Clinical Practice (ICH-GCP).

8.2.2 Objectives

The main objectives of the Monitoring Plan are to:

- ◊ Ensure that the rights and well-being of human subjects are protected.
- ◊ Ensure that key reported trial data are accurate, complete, and verifiable from source documentation.
- ◊ Assure the study site is familiar with and follows the protocol.
- ◊ Assure the site is GCP compliant and compliant with applicable regulatory requirements.

“Safety and Efficacy of the Noxsano Wound Care Bandage: A First-in Human Study” is designed to determine the safety and effectiveness of the Noxsano device. The initial safety assessment will be conducted in healthy volunteers as part of an IRB-approved study. Once safety has been established, the efficacy and safety of the Noxsano device will be evaluated in subjects with an active wound as part of an IRB-approved study.

8.2.3 Monitoring Plan

A site initiation visit will be conducted. At this visit, the regulatory binder will be reviewed to ensure that it contains all necessary documents. The monitor will ascertain that the Principal Investigator and study staff are knowledgeable about the protocol and proper use of the Noxsano device. The initial monitoring visit will be conducted within the first month of patient enrollment. Thereafter, interim visits will be conducted every 6-8 weeks. Additional visits will be scheduled to address unanticipated issues which require training, remediation, or site assistance.

During the monitoring visits, the Clinical Research Associate (CRA) will ensure that the trial is conducted and documented properly. The CRA will perform the monitoring tasks in accordance with the protocol specific requirements, ICH/GCP Guidelines, and other applicable regulatory requirements. The CRA will verify that written informed consent was obtained before each subject participated in the trial. The CRA will verify the accuracy and completeness of the Case Report Forms (CRFs) and source documents for every subject enrolled in the trial. During the course of monitoring visits, the CRA will ensure that the regulatory binder is complete and up to date.

8.3 Regulatory and Ethical Considerations

The study will be conducted in compliance with International Conference on Harmonization (ICH) guidelines and with all applicable federal (including 21 CFR parts 56 & 50), state, or local laws.

8.3.1 Role of the Principal Investigator

The Principal Investigator has the overall responsibility for the conduct of the study, including assurance that the study meets and is conducted within the regulatory requirements specified by each reviewing regulatory authority. The Principal Investigator is responsible for ensuring informed consent is obtained, proper quality monitoring is performed, and quality data is collected and reported.

Provision of written informed consent must be obtained prior to any study-related procedures. The Principal Investigator will ensure that the subject is given full and adequate oral and written information about the nature, purpose, possible risks, and possible benefits of the study as well as the subject's financial responsibility. Subjects must also be notified that they are free to discontinue/withdraw from the study at any time. The subject should be given the opportunity to ask questions and be allowed time to consider the information provided.

8.4 Record Retention

The Principal Investigator supervises the retention of all study documentation and will maintain study records and reports for a minimum of 3 years after the study is terminated or completed. Electronic files will be stored on password-protected computers, and paper files will be stored in a secure facility with limited access at the offices of OHRI.

8.5 Audits and Inspections

Authorized representatives of the sponsor, OhioHealth Research Compliance (ORC), or a regulatory authority may visit the site to perform audits or inspections, including source data verification. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analysed, and accurately reported according to the protocol, Good Clinical Practice (GCP) guidelines, ICH guidelines, and any applicable regulatory requirements.

8.6 Subject Confidentiality

Subject confidentiality will be maintained throughout the clinical study to ensure that data can always be tracked to the source. For this purpose, a unique subject study number will be used that allows identification of all data reported for each subject.

Protected Health Information (PHI) will be treated and maintained in compliance with the Healthy Insurance Portability and Accountability Act of 1996 (HIPAA) Privacy Rule on the protection of individuals with regard to the processing of personal data.

8.7 Protocol Deviations

The Investigators and research staff will not deviate from the protocol without prior written approval of the Institutional Review Board except in medical emergencies.

It is the responsibility of the Principal Investigator or designee to report all protocol deviations according to the IRB of record’s policies and procedures.

9.0 STATISTICAL CONSIDERATIONS

Primary Objective.
Group 1 (Safety): Healthy Volunteers. The initial phase of the study is designed to determine the safety of the study device in normal healthy volunteers without wounds. If there are no issues with tolerance, side effects, and/or adverse reactions in normal healthy volunteers, the second phase of the study will proceed with active wound care subjects.

Group 2 (Efficacy): Wound Care Subjects. The second phase of the study is designed to determine the effectiveness of the study device in wound healing in subjects with active wounds (see Primary Endpoint below), and to determine if the study device is equivalent to standard and established techniques (standard techniques demonstrate 50% wound healing at 2 months).

Primary Endpoint.
The primary endpoint for this study is wound healing (Group 2), as defined by the percent change in wound surface area (surface area is calculated in cm^2) from baseline through the active treatment period. Wound care subjects will have the study device applied (and re-applied weekly) until the wound is judged to be healed by the wound care practitioner, for up to 3 months total duration. If after 2 consecutive months of treatment with the study device the wound does not exhibit at least 50% improvement in surface area (cm^2), the subject will be returned to standard and established techniques consistent with the wound etiology. We will also determine if the study device is equivalent to standard and established techniques (standard techniques demonstrate 50% wound healing at 2 months).

Statistical Analysis Plan.
Descriptive statistics will comprise the bulk of the statistical analysis. Continuous variables will be reported as means and standard deviations, along with median and minimum/maximum values and compared between baseline and follow-up times using paired t-tests and/ or repeated measures ANOVA. Dichotomous and categorical variables will be reported as frequencies with percentages and compared between follow-up times using Chi-square tests or Kruskal Wallis tests. Imputation techniques will be used to account for missing data. Statistical significance will be set at $p<0.05$ for all the tests.

10.0 ADDENDUM

The first two subjects enrolled in the Group 2, wound care subjects cohort experienced adverse events which were promptly reported to the OhioHealth Institutional Review Board.

One subject experienced skin erosion around the site of the Noxsano bandage application. The skin around the borders of the bandage had redness and breakdown which was attributed to the stiffness of the bandage. This event was determined by the OhioHealth Institutional Review Board to be unexpected and related. The Board acknowledged the event as a minor adverse event that did not increase risk to the subject or others.

One subject experienced an increase in wound size at the site of the Noxsano bandage application. This event was determined by the OhioHealth Institutional Review Board to be expected, related, and did not suggest that participants or others were at greater risk of harm.

To address the aforementioned adverse events, the informed consent will be edited to include information regarding the potential for increased pain consistent with treatment of these types of wounds and the wound may appear worse before looking better. Additionally, a continuing review report will be submitted to the OhioHealth Institutional Review Board 3 months after study enrollment activities resume. The makers of the Noxsano bandage have also updated the design of the device to reduce stiffness of the bandage edges. The study schedule has also been updated so that Noxsano bandage changes will be done twice a week up to the first 4 weeks of treatment to assess for adverse events and wound healing.

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