CLINICAL PROTOCOL

VASCUSTIM WOUND HEALING

Background

Non-healing wounds, whether due to venous stasis, diabetes, peripheral arterial disease (PAD), or other causes, is a major cause of morbidity and mortality, and associated with a large and increasing cost of care. Recent data has shown that it has become the leading cause of hospital readmission, and non-healing ulcers result in over 20,000 amputations each year of the foot or leg. Peripheral artery disease is a condition that causes a significant reduction in the inflow of blood into the lower extremity and typically pain referred to as claudication with exertion such as ambulation due to an imbalance in blood supply and demand. The most common cause of PAD is Diabetes, which can cause conduit vessel disease, but more commonly results in occlusion of the microvasculature, and is not very responsive to current surgical or catheter-based revascularization therapies. Diabetes care alone in the U.S. currently costs $115 billion dollars, and one third of that total, or $38 billion, is due to wound care. Other estimates include costing 2% of the entire health care budget for the United Kingdom.

There are many electrical fields in the body, examples of which include the electrocardiogram (EKG) and electroencephalogram (EEG). Bioelectric stimulation (BES) involves the delivery of microcurrents to target tissues to precisely amplify or upregulate expression of local target pro-regenerative proteins such as SDF, VEGF, and IGF, as shown by quantitative PCR. The role of SDF is to stimulate stem cell homing from local tissues and from niches such as the bone marrow in the body, while VEGF is arguably the most potent pro-angiogenic protein known, and IGF needed for cellular repair. There is a large body of literature describing the safety of BES in a wide variety of applications, including as an anti-cancer therapy. It has also been shown that these proteins are largely depleted in non-healing wounds and that the resting electrical current present in healthy tissue is also not present. Bioelectric stimulation can be adjusted to both address the polarity of the electrical field and current needed to restore healthy tissue.

The goal of using BES in this protocol will be to upregulate the expression of these important pro-regenerative proteins in non-healing wounds of the foot and ankle with the goal of inducing total wound healing. The BES will be delivered by both a 510K approved bench top stimulator and a wearable stimulator to use at home at night.

Skin breakdown and ulcers due to venous congestion associated with advanced heart failure also often fail to heal despite the use of several current treatment options including aggressive medical management as well as hyperbaric oxygen and various dressings. These data provide the stimulus for identification of new cost-effective treatment options. Two of the most promising are use of low current Bioelectric Stimulation (BES) and biologics such as Amniotic Fluid or PRF

derived biologic Membranes for wound coverage, and to provide much needed growth factors to stimulate wound healing.
Non-healing wounds present a portal for infection as well as seepage of fluids. We will also deliver adjunctive therapy with Platelet Derived Fibrin, which is an unaltered fraction of native plasma delivered by microneedles, and topical coverage of the wound with unaltered FDA approved amniotic membranes, which have also been shown to have a significant beneficial effect on wound healing.

Study Design	Prospective, non-randomized, open label, consecutive series pilot study.
Number of Patients	50
Number of Study Sites	Up to 10
Number of Treatments	12
Duration of Study	8 weeks
Frequency of Treatments	2 X's/week for 4 weeks, then weekly x 4
Duration Each Treatment	30 minutes

Goal

The goal of this Registry is to determine the initial safety and efficacy of a series of treatments with precise bioelectric stimulation signals for controlled upregulation in the local tissue expression of specific pro-regenerative proteins in the treatment of non-healing skin ulcers of leg or foot to reduce the size and depth of non-healing ulcers from any cause including venous stasis, chronic edema, Diabetes, PAD, or other causes.
The study, including the protocol and consent form, will have been approved without stipulations by a local or national certified Institutional Review Board as meeting safe and good clinical practice before any subject will be enrolled.

Inclusion Criteria

1. Age 20-70 yrs of age
2. Non-healing wound of leg or foot > 2 x 2 cm in diameter

3. Able to tolerate up to 30 minutes of BES on the non-involved leg or foot to prove tolerance to the treatment
4. Able and willing to make the required study visits.
5. Able and willing to give informed consent and follow study instructions.
6. Agree to allow photographs to be taken of the wound before and at the end of the treatment period to document change.

Our Chief Medical Officer, Dr. Leslie Miller, former Chief of Cardiovascular Medicine at the University of Minnesota, has been involved in over a dozen stem cell therapy cardiovascular related clinical trials. He is co-editor of the textbook Stem Cell and Gene Therapies for Cardiovascular Disease. Our Scientific Advisory Board is comprised of over 30 leading clinicians and scientists with unprecedented experience in the field.

Exclusion Criteria

1. Use of concomitant treatments to improve wound healing, including topical medications, oral and/or IV medications including antibiotics, hyperbaric oxygen therapy, non-ablative fractional or low-level laser treatment, or PRP injection, within the preceding month.
2. Allergic to lidocaine or epinepherine
3. Planned revascularization procedure in next 2 months
4. Individuals with diminished decision-making capacity
5. Renal replacement therapy
6. Pregnancy or lactating period for females

Screening

Any subject that has a non-healing wound of > 2 x 2 cm in diameter, who meet all Inclusion and none of the Exclusion criteria, will be eligible for participation. Each potential subject will have a brief history and examination performed by the Investigator, and if acceptable, will be provided with an overview of the study including the protocol, and then will be provided with a copy of the Consent Form. If they choose to participate, and sign the Consent form, they will be enrolled in the study.

Baseline Evaluation

All potential study candidates will be evaluated by a wound care specialist, vascular surgeon, or cardiologist, who stipulates that the patient has a significant wound of the foot or leg that has not been responsive to conventional treatments.

Baseline Photo of the wound

Before the procedure, a baseline photo will be taken of the planned treatment wound to

compare to the measurements after 4 weeks of treatment, and at the end of treatment period of 8 weeks. It will be repeated at the end of the treatment.

Treatment Schedule

- Treatment Frequency: Twice during the first 4 weeks, then once /week
- Treatment Duration: 30 minutes each
- Total Treatment Duration: 8 consecutive weeks
- Total Number of Treatments: 12
- Location of Treatment: Only in the clinic of an approved investigator
- Stimulator to be used: FDA 510K and CE Mark approved model 240
- Home Care: Prizm 510K approved unit for overnight added bioelectric stimulation

Primary Outcome Measure

The primary end point will be the degree of healing of the treated wound at the end of the 6-week study period. This quantitative assessment will be based on the photos taken of the wound and will be made by a blinded observed trained in the analysis of wound depth and healing from baseline to study completion. The goal of this study is complete healing of the wound by the end of the treatment period.

Secondary Outcome Measures

1. Any adverse events reported including pain or irritation at the site of the electrodes or the wound thought possibly due to the electrical stimulation.
2. Incidence of need to terminate the study for pain or other cause
3. Mechanical/technical failure of the Bioelectric Stimulators to function appropriately

Bioelectric Stimulation: Screening Assessment

All participants will have two standard gel patch conductive skin electrodes applied to the sides of the leg opposite the side with the non-healing wound, and connected to a 510 K approved Mettler bioelectric stimulator, which has 510K approval in the US, as well as a CE Mark for improved blood supply and relief of pain.
The low -level current that will be used in the study will be applied first to the uninvolved leg or foot at increasing levels for up to 15 minutes to reach the level to be used in the study. If there are no complaints of pain or other ill effects, the subject will be eligible to participate in the Registry.

Treatment Protocol

Simple skin electrodes will be applied to within 3 inches on either side of the target wound, and

connected to the bioelectric stimulator provided. The Mettler stimulator will run through a 30-minute cycle targeting 6 precise regenerative protein signals to stimulate increased local tissue expression of a number of growth factors that include SDF-1, VEGF, and IGF, as well as an important anti-inflammatory protein called Klotho.

Wound Care

Care of for wound may include the following options:

Dressings

A non-adhesive sterile gauze dressing will be applied over the wound and Amniotic Membrane, if used, at the completion of each treatment. Based on the degree of wound healing, a patient may receive application of a new Amniotic Membrane every 7-10 days for the first 3-4 weeks of the follow up period. The physician may elect to leave the wound uncovered after 10 days from the last use of an amniotic membrane, however a fresh non-adhesive dressing will be maintained over the wound for a minimum of 10 days after placement of each Amniotic Membrane to optimize response. Other dressings may be used at the discretion of the treating physician.

Amniotic Fluid or PRF-derived Biologic Membrane

Although not a required part of the protocol, the preferred option for initial wound care would be a single covering of the wound, especially if due to Diabetes or PAD, with an appropriate sized Amniotic Membrane, which will be provided by Axolotl Biologix and VASCUSTIM, after being reconstituted with several cc's of Amniotic Fluid, and laid directly onto the wound. If used, repeat applications of an amniotic membranes are typically required at 7-10 day intervals for the first 3-4 weeks of treatment.

Home Care: Prizm stocking

Each patient will be provided with a pair of stocking or socks depending on the wound location, to be worn at home throughout the night on a daily basis to provide additional bioelectric stimulation and accelerated wound healing. The socks contain a set of electrical coils embedded in the sock material which are then connected to an external stimulator that is placed above the sock by a self-adhesive band. The light-weight stimulator is turned on once the sock is in place and turned off each morning and the sock removed. The sock needs to be worn every night during the study treatment period to provide additional bioelectric stimulation and accelerated wound healing. This device also has 510 K approval.

Follow Up Evaluations

At the end of 4 and 8 weeks, a photo will be taken by a camera capable of documenting the depth and margins of the wound being treated to document and quantitate the response to treatment. Each subject will also have a brief interview inquiring about any adverse effects noted by the subject at 1, 3, and 6 weeks after enrolling in the study, and have an examination by the Principal Investigator for any adverse effects of the therapy including damage or injury.

Stopping Rules

The Registry will be paused if a total of 3 of the study subjects experience a treatment-related side effect of at least moderate severity that prompts cessation of the treatment. It will be restarted when additional investigation yields a clear cause, and effective remediation action plan has been implemented.

Data Analysis

Data will be collected for each patient and analyzed when the last enrolled subject has reached the end of the 6 week treatment time point. The study may be stopped if the data demonstrates a > 67 % complete wound healing at the end of the 3 week treatment period in. Additional subjects may be enrolled into the study if approved by the IRB and Sponsor.