Effect of Oasis® Wound Matrix on Stage III and IV Trunk Pressure Wounds Treated with Negative Pressure Wound Therapy (NPWT)

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1. Background/Significance

Pressure ulcers, also known as bedsores, are one of the dire problems in the modern medical system. They are referred to as chronic wounds. Chronic wounds cost more than an estimated $3 billion per year to the hospital system. They are also extremely expensive for the well-being of the patients involved. Those affected experience severe discomfort at the wound site and intense emotional distress as a result of mounting hospital bills from extended treatments. . Pressure ulcers may take more than six months to fully heal and require constant attention from medical care providers. The elderly are at the highest risk of developing pressure ulcers; individuals over 65 make up nearly 85% of those afflicted[1]. With our aging population, the amount of patients at risk to develop bedsores is expected to rise exponentially in the next few years; thus a need arises for the development of novel therapeutic regimens to rapidly treat chronic wounds (CW).

Patients with diabetes, immunosuppression, and infection are at high risk for chronic wounding. Chronic wounds are characterized by acute inflammation in an affected area that persists for more than three months and the appearance of necrotic tissue on the lesion. Wounds exhibit a minimized ability to follow the normal wound healing process; senescent fibroblasts in the region express a lessened capacity to react to growth factors and cytokines that facilitate angiogenesis and induce full re-epithelization. Pressure wounds in particular occur in bedridden patients with incontinence and other medical co-morbidities.[1, 6]

AHCPR (Agency for Health Care Policy and Research) reports wound care for pressure ulcers is a $20 billion-a-year industry. The estimated cost to heal a single pressure ulcer ranges from $2,500 to $50,000. The standard of care includes wound debridement, application of negative pressure wound therapy, pressure reduction surface, patients repositioning, and nutritional support. In selected patients flap closure is indicated. The majority of patients are being treated conservatively using advanced modalities.[2]

Three major growth factors involved in wound healing will be assessed in this study. These include vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and transforming growth factor beta-1 (TGF-B1). Each of these play a significant role in the angiogenic process during wound healing. VEGF and FGF are important factors in the generation of endothelium. TGF-B1 is a master regulator of fibroblasts mitogenesis, cell growth, and apoptosis[9]. The cytokines interleukin-8 (IL8) and interleukin-6 (IL6) will also be measured in order to gauge the immune response incited by the two proposed wound treatment regimens.

IL8 is a major marker of the innate immune response and IL6 is a ubiquitous indicator of the overall inflammatory response produced by tissue damage [3].

The Oasis product used in this study is OASIS® Ultra, a tri-layer matrix; hereafter referred to as Oasis® Wound Matrix.

Oasis® Wound Matrix is a porcine acellular small intestine submucosa material compatible with human tissue. In contrast to other collagen-based products, Oasis® Wound Matrix is a complex scaffold that provides optimal environment for restoration of tissue structure. It guides tissue growth and traps growth factors. Oasis® Wound Matrix indications include partial and full thickness wounds and skin loss injuries as well as second-degree burns [4, 7, 8].

Our goal is to heal these wounds in a timely fashion to improve patient's quality of life, end suffering and reduce cost.

2. Specific Aims

The aim of this study is to determine the therapeutic effect of adding Oasis® Wound Matrix (Healthpoint Biotherapeutics, Fort Worth, Texas) to stage III and IV pressure wounds during negative pressure wound therapy (NPWT) treatment. We hypothesize that the addition of Oasis® Wound Matrix to NPWT will increase the closure rate of non-healing wounds. This will be correlated to increased growth factors and interleukins collected from the wound in the canister.

3. Subject Selection

Potential participants will be evaluated by the study principal investigator for Inclusion/Exclusion criteria. The majority of the subjects will be enrolled at Sycamore Medical Center's Wound Center, but subjects may also be enrolled at any participating site.

Inclusion criteria for this study include:

1. Adults aged 18-89 who exhibit stage III or IV trunk pressure wounds with no signs of infection.
2. HbA1C < 8 (if patient is diabetic)
3. Adequate nutrition including albumin ≥ 2.0 and prealbumin ≥ 12.5.

Exclusion criteria for this study include:

1. Wounds that cannot have a NPWT device properly applied due to location (too close to anus), diarrhea, periwound skin issues.

2. Patients with Infected wounds.
3. Patients with HbA1C >8, uncontrolled diabetes.
4. Malnourished patients.
5. Patients that are immunodeficient or immunocompromised.
6. Patients that have a religious or ethical aversion to porcine products.
7. Patients that have any allergy to porcine products.
8. Patients who are at High Risk of bleeding including:
    - Patients who have weakened or friable blood vessels or organs in or around the wound as a result of, but not limited to:
        - Suturing of the blood vessel (native anastomoses or grafts) / organ
        - Infection
        - Trauma
        - Radiation
    - Patients without adequate wound hemostasis
    - Patients who have been administered anticoagulants or platelet aggregation inhibitors
    - Patients who do not have adequate tissue coverage over vascular structures
9. Patients who are DNR/DNI

The patient's medical record will be referenced for all Inclusion and Exclusion criteria. Lab results for albumin and prealbumin up to 30 days prior to enrollment and HbA1C results up to 100 days prior to enrollment will be considered in determining eligibility.

4. Subject Enrollment

This study will begin around April 2014 (or after IRB approval date) and continue for three months after last subject enrollment. Study duration from approval to last subject out is anticipated to be less than 2 years.

Potential subjects will be given the IRB approved consent to review. They will be given an opportunity to discuss participation in the research study with a member of the study team, especially with regard to the implications of consent, study design, procedures, risks, benefits, and costs. The patient will be given a period of time to think of any concerns he/she may have with the research including the recording and retaining of records pertaining to patient chart, laboratory data, long-term specimen storage, and consent to photography. The subject will be considered enrolled once the consent has been signed.

If a patient is known to have diminished decision-making capacity, the study and consent will be discussed with the patient's legally authorized representative (LAR). The LAR will be allowed to provide consent on behalf of the patient. In cases where the patient may be physically unable to sign, an LAR will be allowed to sign documents for the patient.

Target enrollment is 12 subjects from each group to complete the study. Subjects expulsed from the study for infection or other reasons will be replaced. Since the dropout rate is unpredictable, enrollment will continue until 12 subjects in each arm have completed all study visits.

Subjects' Protected Health Information (PHI) will be safeguarded to the greatest extent possible. Subjects will be asked to sign a Medical Records Release allowing information from other health facilities to be shared with the study team. Subjects will also be asked to sign a release allowing research data and photographs to be shared outside of the study locations. In all cases, subject PHI will be removed from records and photographs prior to release.

Consenting patients will allow for the following data to be collecting for an initial assessment:

-Albumin, pre-albumin

-Serum HbA1C

-Wound photographs, measurements

Randomization

Subjects will be randomized in a 1:1 ratio to one of two arms.

- Studied arm: Subjects will receive Oasis® Wound Matrix along with standard of care including NPWT.
- Control arm: Subjects will receive standard of care including NPWT.

Randomization will be regulated by an envelope system. Sequentially numbered envelopes, each containing a study-arm designation card, will be kept at Dr. Simman's Sycamore Medical Center Wound Center office. Once a subject is assigned to a study arm, the subject's initials will be recorded on the designation card. The card with subject's initials will then be returned to the envelope and kept as the study-arm assignment record. The envelope number will also be used as the subject ID number.

5. Study Procedures

Because the target population may require long-term skilled care, follow up visits may take-place at a variety of facilities including hospitals, extended care facilities, or private homes. Permission to conduct the study will be sought from each facility or home care organization prior

to starting the research procedures. If the facility does not allow the research to take place on site, the subject will be withdrawn from the study and will be followed with standard wound care.

Data Collection

At each visit, a chart review and personal interview will be performed with all patients (or LAR) documenting the following: (see excel sheet attached)

-Age

-Race

-Gender

-Prescriptions

-Prior surgeries

-Comorbidities

-Alcohol, caffeine, use

-Smoking history

-BMI/height, weight

-Albumin, pre-albumin

-HbA1C in diabetic patients

Study data at enrollment and assessment visits will be recorded on a data collection form.

Wound Health Assessment

Wounds will be verified to be free of necrotic tissue and slough, as per the qualification that a patient's wound remain uninfected throughout the study. If an infection is suspected, cultures will be taken as part of routine care. If an infection is confirmed, the subject will be expulsed from the study. The patient will continue to receive standard wound care.

Oasis® Wound Matrix Application

In the studied group, Oasis® Wound Matrix will be applied over the entire wound bed. A non-adherent dressing that is compatible with NPWT will be applied over the Oasis® Wound Matrix. Finally, a NPWT device will be activated and continuously operated at 125mmHg suction. KCI and Genadyne devices will be the preferred devices used as NPWT in this study.

Application of Standard NPWT Wound Covering

Dressing changes and NPWT for the group not receiving Oasis® Wound Matrix, will follow standard of care. Once the wound is inspected and ready for dressing application, standard of care dressings will be placed over the wound. Finally, a NPWT device will be applied and operated continuously at 125mmHg suction

Visits

Assessment visits will occur approximately weekly over a period of twelve weeks. Wounds will be inspected to verify progress and health. Photographs of the wounds will be taken at a standard distance (approximately 30 cm) with a ruler in the photo to measure rate of healing as the change in percentage area healed. Wounds measurements will be obtained approximately weekly to evaluate healing rate. Wound cultures will be performed if infection is suspected to rule out infection.

At approximately months 1, 2, and 3, wound specimen canisters will be collected and taken to the laboratory at Wright State University (WSU) department of Pharmacology and Toxicology for analysis of the drained fluids from all wounds. Specimen canisters will be secured in biohazard bags and transported to WSU by a member of the study team within 24 hrs of collection.

To insure detectable concentrations of the studied materials, the first 6 samples will be analyzed at 210 Health Sciences Laboratory at WSU for the content of drainage including studied growth factors and Interleukins to insure detectable concentrations of the studied materials. Multiplex 30 Assays for Protein Biomarkers will be used. If detectable values are confirmed, then the remaining samples will be analyzed according to the protocol. Prior to obtaining aliquots from the canisters, a metalloprotease inhibitor will be added to the canisters fluids 30 minutes prior to removal.

For all wounds fluid samples, 30 ml of the canisters fluids will be obtained in a sterile fashion. 10 ml will be used for the study and the other 20 ml will be stored in liquid nitrogen at- 80C for potential later use, possibly unrelated to this study. If a subject refuses to allow their samples to be used outside of this study, that subject's extra 20ml fluid sample will be destroyed.

To maintain blinding for the assessment lab personnel, specimen canisters and stored specimens will be identified by a coded label.

Pressure reduction beds and patient repositioning will be employed throughout the study as a standard of care for those patients.

Fluid Analysis

The following is the proposed method of fluid analysis. Test methods may vary due to equipment change and improved methods, but the information sought in the analysis will remain consistent with the stated goals.

ELISA Materials and Methods

Based on established methods of wound fluid analysis [3, 5, 6], clinical samples may be used directly or preserved with protease inhibitors for later use.

Materials Required:

1. Human IL-8β ELISA kits with Human IL-8 β standard
2. Human IL-6 ELISA kits with Human IL-6 standard
3. Human EGF ELISA kits with Human EGF standard
4. Human TGF β ELISA kits with Human TGF α standard
5. Human VEGF ELISA kits with Human TGF α standard
6. Human FGF ELISA kits with Human TGF α standard
7. Standard laboratory pipettes
8. 1.5mL eppendorf tubes
9. Deionized, distilled water.

Reagent preparation before starting experiment

1. Bring all reagents to room temperature (18 – 25°C) before use.
2. Standards :
    a. Reconstitute 1 vial lyophilized Standard with ELISA dilution buffer to prepare a 125 pg/ml stock standard.
    b. Add 300µl ELISA dilution buffer to 6 tubes. Label as 62.5 pg/ml, 31.3 pg/ml, 15.6 pg/ml, 7.8 pg/ml, 3.9 pg/ml, and 1.95pg/ml.
    c. Perform serial dilutions by adding 300 µl of each standard to the next tube and
    d. Vortex between each transfer.
3. Dilute the 5x Assay wash buffer to 1x buffer: 40ml 5x Assay wash buffer + 160ml ddH2O.
4. Use serum-free conditioned media or original or 10-fold diluted sera. with 1 X Diluent buffer
5. Dilute 50X of biotin labeled antibody mixture with 1X Diluent buffer.

6. Dilute 200X of streptavidin-HRP with 1X Diluent buffer.

Assay procedures:

1. Add 100µl of standard, control or sample per well of the plate. The mixture will be incubated for 1 hour at room temperature with gentle shaking. Each sample will be analyzed for 3 times.
2. Aspirate each well and wash by adding 200µl of 1X wash buffer. Repeat the wash process three times. (Note: Complete removal of liquid at each wash. After the last wash, remove any remaining liquid by inverting the plate against clean paper towels.)
3. Add 100µl of diluted biotin-labeled antibody mixture to each well and incubate for 1 hour at room temperature with gentle shaking.
4. Repeat the aspiration and wash by adding 200µl of 1X Assay wash buffer. Repeat the wash process three times.
5. Add 100µl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gentle shaking.
6. Repeat the aspiration and wash by adding 200µl of 1X wash buffer. Repeat the process three times for a total of three washes.
7. Add 100µl substrate to each well and incubate for 5-30 minutes.
8. Add 50µl of Stop solution to each well. The color in the wells should change from blue to yellow.
9. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.

Costs

Smith and Nephew will provide the Oasis® Wound Matrix. They will also pay for the research laboratory assays and statistical analysis. Costs for all other wound care procedures and lab tests are standard of care and will be the responsibility of the patient or his/her representative insurance carrier.

6. Statistical Analysis:

Biostatistical analysis will be performed using all available measurements by a technician and verified by a statistician. Significance will set at $p<0.05$. Standard t-test and ANOVA will be

used depending on sample size. Chi-square or Tukey analysis will be applied as necessary. Quantitation will be performed using GraphPad PRiSM statistics software.

7. Risks and Discomforts:

- The risks of using NPWT include infection, pain, bleeding, periwound skin maceration.
- Risks involved in the use of Oasis® Wound Matrix include infection, allergy.

Adverse Events (AEs and SAEs):

The events and effects noted above as documented Risks will not be reported unless the event carries a level of severity that is unexpected.

Hospitalization due to subject co-morbidities unrelated to Wound Treatment in this at-risk population will not be considered an SAE.

Injury Compensation:

The sponsor will pay the reasonable and necessary medical expenses to treat illness or injury caused as a direct result of participating in this study. The sponsor will not pay for injuries due to the subject's conduct outside of the study, for lost wages, discomfort, or costs due to worsening of the subject's underlying condition. It is up the subject or subject's insurance company to pay costs to treat any medical condition not caused by this study.

Devices Risk Evaluation (SR vs. NSR)

Individually, neither Oasis® Wound Matrix nor NPWT meets the 21 CFR 812.3(m) criteria of a Significant Risk device in that they don't present a potential for serious risk to the health, safety, or welfare of a subject. Nor is there an expectation that when used in combination, the two devices would meet the Significant Risk criteria.

The NPWT Powered suction pump is a Class II device per 21 CFR Sec. 878.4780 (Subpart E--Surgical Devices). Oasis® Wound Matrix is unclassified.

Both devices are FDA approved for treatment of pressure wounds, the intended target of this investigation.

8. Potential Benefits: Benefits of combining the two devices may promote faster healing which may help reduce pain, discomfort and cost.

9. References:

1: Ann Epidemiol. 2002 Jul;12(5):321-5.

2: "Fact Sheets." Agency for Healthcare Research and Quality. Agency for Healthcare Research and Quality, n.d. Web. 1 Oct 2013.

3: J Trauma. 2009 Mar; 66(3):749-57.

4: Mostow EN, H. G., Dalsing M, Hodde JP, King D,*Effectiveness of an extracellular matrix graft (OASIS Wound Matrix) in the treatment of chronic leg ulcers: a randomized clinical trial.* J Vasc Surg, 2005. 41(5): p. 837-843.

5: ZentralblChir. 2006 Apr; 131Suppl 1:S62-7.

6: Trabuco EC, K.C., Gebhart JB, *Xenograftuse in reconstructive pelvic surgery: a review of the literatire.* Int Urogynecol J Pelvic Floor Dysfuact, 2007. 18: p. 555-563.

7: Niezgoda JA, v.G.C., FrykbergRG, Hodde JP, *Randomized clinical trial comparing OASIS Wound Matrix to Regranex Gel for diabetic ulcers*. Adv skin Wound Care, 2005. 18(5): p. 258-266.

8: Romanelli M, D.V. Berone M et al *OASIS Wound matrix versus OHyaloskin in the treatment of difficult-to-heal wounds in mixed arterial/venous aetiology*. In wound J, 2007. 4(1): p. 3-7.

9: Prog Mol Biol Transl Sci. 2010;93:179-212. doi: 10.1016/S1877-1173(10)93009-3.

Protocol Signature Page

I agree to adhere to the trial protocol and to all documents referenced in the trial protocol.

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Richard Simman, M.D. date
Principal Investigator