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Official IRB Approved Revised Study Title: **RCT: Larval Debridement Therapy versus Bedside Sharp Debridement to Remove Biofilm from Chronic Lower Extremity Ulcers or Diabetic Foot Ulcers**

NCT Study Title: **Larval Debridement Therapy Versus Sharp Debridement to Remove Biofilm**

University of Florida IRB #: **201400590**

NCT number: **NCT02294175**

Last Approved Revised Protocol (approved 8-3-2018)

1. **Project Title:**
   RCT: Larval Debridement Therapy versus Sharp Debridement to Remove Biofilm from Chronic Lower Extremity or Diabetic Foot Ulcers

2. **Investigator(s):**
   (***Protocol revised only for change in PI of Record since original PI transferred stations***)
   Matthew Morrow, Pharm D (PI of Record August 1, 2018 – 12/31/2018)
   Linda Cowan, PhD, ARNP, CWS (Co-I)
   Gregory Schultz, PhD (Consultant)

3. **Abstract:**

   Drug resistant organisms and bacterial biofilm pose an increasing threat to the health of millions of individuals world-wide. These organisms are being identified with an alarming prevalence among persons with chronic wounds. The presence of necrotic tissue has been associated with the deterioration of open wounds and serves as a breeding ground and nutrient source for bacteria. The removal of necrotic tissue is widely accepted as required for optimal wound healing.

   The primary purpose of this study is to assess the efficacy of larval debridement therapy (LDT) with bagged, sterilized, live, medicinal blow fly (*Lucilia sericata*) larvae (or " BioBags") versus bedside sharp debridement in removing harmful bacteria, biofilm and necrotic tissue from chronic wounds to promote wound healing. Characteristics associated with chronic wound environments will be evaluated through analysis of samples of tissue taken from wound beds before and after both types of debridement. One hundred and forty patients ≥ 21 years of age with an open, full thickness wound which is healing by secondary intention (of greater than 8 weeks duration and requires debridement) will be invited to participate. This recruitment number accounts for estimated 10% attrition rate, so final sample number is anticipated to be 128 subjects (64 in each arm). Samples of wound bed tissue and slough tissue (if present) will be collected on Days 0, 4 and 8 or prior to and after each larval debridement intervention or sharp debridement (control). Photos will be taken of the wound bed on Days 0, 4 and 8 or just prior to and after each debridement method. A randomized sampling procedure will place individuals into one of two groups: The intervention group will receive larval debridement therapy once every 4 days for 2 applications (with saline moistened gauze as cover dressing changed daily) and the control group will receive sharp debridement therapy every 7 days for 2 debridements (with wound gel dressing changed daily).

   The wound and wound environment will be characterized by assessing wound Matrix Metalloproteinases (MMPs), inflammatory cytokines and the presence of total bacteria, planktonic (free floating) bacteria, and biofilm (sessile) bacteria. Clinicians will be asked to grade the amount of non-viable tissue apparent in the wound bed, appearance of any signs of infection and overall visual appearance of the wound at each visit. Wound size will also be assessed. Subjects will be asked to rate their wound related pain level at each visit before and after the debridement method is applied. Subjects, caregivers and clinicians will be asked to complete a short survey to self-report their perceptions of each method of debridement. Data will be analyzed using bivariate and multivariate methods.

4. **Background:**
   Chronic wounds are a significant health problem in the U.S.. Chronic wounds are defined as wounds that have “have failed to proceed through an orderly and timely reparative process to produce anatomic and functional integrity." Health care costs related to the management and treatment of chronic wounds in the U.S. exceeds $20 billion annually. For many health care providers, the treatment and management of non-healing wounds is challenging. Traditionally, basic wound care has consisted of surgical debridement, manual irrigation, moisture retentive dressings, and topical and/or systemic antimicrobial therapy. Although there has been tremendous progress in the science of

wound healing, the prevalence and incidence of chronic wounds and their associated complications continues to escalate.

The presence and complexity of bacterial biofilms in chronic wounds has recently been recognized as a key aspect of non-healing wounds. Bacterial biofilms are sessile colonies of polymicrobial organisms (bacterial, fungal, and possibly, viral) which are often symbiotic. These biofilm colonies produce a protective coating to protect the colonies from host defenses. The character of this protective substance which is unique to biofilms is dynamic and production of its components seems to be triggered by hostile environments in the wound bed (such as the presence of topical antibiotics). Biofilms have been shown to have survival and defense mechanisms that: 1) inhibit the healing aspects of inflammatory cells; 2) resist antibiotics (topical and systemic) and other therapies; and 3) initiate cell to cell communication pathways (quorum sensing) which facilitate new biofilm growth, resulting in recalcitrant non-healing wounds.

Wound larval debridement therapy (LDT) has been shown to have promise in healing chronic wounds by eradicating biofilms. Recently (2012), the PI was part of a team of investigators who collaborated with the University of Florida Wound Research Laboratory and conducted several in vitro experiments to demonstrate the efficacy of larval exposure to biofilm (Cowan et al., 2013). These studies demonstrated bacterial biofilm (*Pseudomonas aeruginosa* (PA01) bacteria and *Staphylococcus aureus* (SA35556) bacteria) grown on pigskin explants were completely eradicated within 48 hours of exposure to loose medicinal maggots from Monarch Labs (larvae).

Maggot or larval debridement therapy (MDT or LDT) has been utilized for medical purposes for hundreds, if not thousands of years. Mayan Native Americans and other ancient cultures have documented reports of maggots being used in certain medical treatments. It was Napoleon's general surgeon, Baron Dominique-Jean Larrey (1766-1842), who documented observations that larvae of certain fly species, such as *Phaenicia* (*Lucilia*) *sericata* (blow fly), removed only dead tissue while promoting healthy tissue in the wound bed, helping wounds heal faster.

Dr. John Forney Zacharias (1837-1901), a Confederate American Civil War surgeon, is recognized as the first healthcare provider in the United States (U.S.) who intentionally applied maggots for wound care/debridement purposes. He noted that "maggots could clean a wound better in one day" than any other agent they had at their disposal. He also accredited maggots with saving many soldier's lives. In World War I, an orthopedic surgeon named Dr. William S. Baer recognized the efficacy of maggots on the battlefield to "clean up" compound fractures and large flesh wounds, even crediting maggots with preventing sepsis in two battlefield cases. He was impressed with the application of maggots as a medical treatment, so he conducted research at Johns Hopkins in 1929 using maggots that he found in the neighborhood or grew on a windowsill. Two patients contracted tetanus from contaminated maggots (one died), so he developed sterile maggot growing procedures. He used maggot therapy in 21 patients with chronic osteomyelitis which did not respond to other treatments. He demonstrated rapid wound debridement of necrotic tissue, a return of the wound bed to an alkaline pH environment, the reduction of bacteria, reduced odor levels, and complete healing of the osteomyelitis infections within six weeks.

With the development of antibiotics in the 1940's and various skin and wound antiseptics, the use of LDT declined. Arguably, one of the biggest reasons LDT may have lost favor in clinician's eyes was not ineffectiveness but was the "yuck factor"; patients, their caregivers and clinicians found it distasteful to apply small squirming worms that could crawl out of a wound. In fact, as much as Dr. Baer promoted the use of medicinal maggots, he himself said, "The sight [of maggots in an open wound] was very disgusting and measures were taken hurriedly to wash out these abominable looking creatures."

With the advent of antibiotic resistant organisms and increasing drug sensitivities, there was a renewed interest in Maggot therapy in the 1980's. The Food and Drug Administration (FDA) approved the use of medicinal maggots (*Phaenicia* (*Lucilia*) *sericata*) for debriding non-healing necrotic skin and soft tissue wounds including lower extremity or diabetic foot ulcers, pressure ulcers, non-healing surgical or

traumatic wounds and venous stasis ulcers. In the U.S., larval therapy with maggots is classified as a medical device. However, in Europe, Canada and Japan maggots are classified as medicinal drugs.

Although anecdotal clinical evidence suggests that larvae therapy is successful in removing necrotic tissue and bacterial biofilm, there are limited data from randomized clinical trials to support this claim. A new (to the United States) method of larval debridement therapy delivery is a prepackaged mesh bag (similar to a tea bag) which contains a premeasured amount of sterilized, live, medicinal blow fly (*Lucilia sericata*) larvae. This bag is self-contained and increases the ease of use, at the same time, reassures patients that all the larvae are contained within the dressing and will not "escape" during therapy. This proposed study seeks to test this dressing (called a "Biobag") to investigate its effectiveness as a debridement therapy to remove necrotic tissue and biofilm, in comparison with a standard debridement technique (bedside sharp debridement).

Further studies validate findings that green bottle fly larvae or maggots (*Lucilia sericata*) used for wound debridement do not ingest healthy tissue of certain species such as humans, cows, horses, or dogs because of a genetic marker in these species as opposed to healthy tissue of sheep, rabbits, or hedgehogs, which the larvae will ingest because they are missing this specific DNA marker. This is in contrast to other fly maggots NOT used for wound care such as the New World Arm Screw Worm Fly larvae, which have been known to ingest healthy human tissue. (REFs http://www.dailymail.co.uk/health/article-2365158/Horrified-woman-27-discovers-headaches-scratching-sounds-inside-head-FLESH-EATING-MAGGOTS.html).

Maggots used in the U.S. for larval debridement therapy are all processed under controlled laboratory conditions and are sterile (both free of disease as well as unable to reproduce). Larval debridement of non-viable tissue within chronic wounds results partly from the proteolytic digestive enzymes liquefying the necrotic tissue, which the larvae then "suck up" (along with bacteria and biofilm) and remove from the wound bed. As such, they are a most efficient way to debride a wound without the pain, bleeding, or inflammatory response associated with sharp debridement.

Unfortunately, current larval debridement methods available in the U.S. have not really addressed the "yuck factor" of loose or free-roaming maggots in open wounds. This may explain why, despite the clinically proven effectiveness of larval therapy to aid in the healing process, many U.S. clinicians do not select this method of wound treatment. Many nurses, doctors, caregivers, and patients alike have voiced an aversion to handling maggots or having to "count the number that go into a wound or come out of a wound" (Cowan et al., 2013). Having to count them is complicated by the fact that when the wound is sufficiently debrided, larger maggots may turn and eat smaller maggots.

Fortunately, there is a brilliant new alternative. A European company, BioMonde, has successfully addressed this issue by manufacturing sterile maggots in a mesh bag called a BioBag. This allows for an aesthetically and psychologically acceptable method of introducing the larval therapy to the wound and removing them all in "one neat package." BioMonde has recently obtained FDA approval for this medical device in the U.S. In addition, several studies validate the comparative effectiveness of these maggots in BioBags versus currently "free range" or loose maggots (Monarch Labs) (Blake et al., 2007). Now that this research has been completed, a randomized controlled trial (RCT) testing larval debridement therapy, also known as “biosurgery” using BioBags versus traditional bedside sharp debridement methods in human subjects with chronic wounds is warranted.

Purpose Statement

Our proposal includes the testing of the BioBag device in a randomized control trial (RCT) in a sample of subjects with chronic open, non-healing lower extremity or diabetic foot ulcers. While this would be the first clinical test in the U.S., the product has been widely tested elsewhere and is successfully used in over 40,000 British and European human wound treatments per year. However, there has been no quantitative measure of the comparative effectiveness of this product versus sharp debridement in regards to bacterial biofilm measures. The **purpose of this proposed RCT research study** is to

investigate the efficacy and acceptability of larval debridement therapy using "BioBags" versus bedside sharp debridement therapy in humans with chronic wounds.

Significance
This study has an enormous opportunity to contribute to wound care practice within the VA as well as all of the United States. The potential for improved quality of life for Veterans (due to avoidance of amputation and healing of chronic wounds) is priceless. This research is the first step in a program of research aimed at improving effectiveness of wound care at the bedside. This proposed research aims to provide clinicians with more effective tools in wound debridement and in particular, in the fight against biofilm (such as the removal of biofilm in wounds by larval therapies). Using the knowledge gained in this study, we will also be able to answer questions related to the comparative effectiveness of antibiofilm strategies such as larval debridement therapy versus sharp debridement therapy – and apply this knowledge to clinical practice. For instance, if larval therapy is demonstrated to be more effective at removing biofilm, and may also be appropriately applied by a nurse or informal caregiver (in the patient's own home) rather than a physician/ARNP/PA in a clinical setting, this could reduce health care costs and improve access to care. Furthermore, decreasing wound infection and improving wound healing has the potential for decreasing complications and improving the quality of life for Veterans, which is priceless.

5. **Specific Aims:**

Specific Aim 1

To compare the clinical effectiveness of a self-contained bagged larval debridement therapy (intervention) (BioBag) with bedside sharp debridement (control). The following comparisons will be made: 1) The visual reduction of non-viable tissue in the wound bed of chronic full-thickness lower extremity or diabetic foot ulcers, and 2) The reduction in colony forming units (CFU) of bacterial biofilm in samples taken from the wound bed.

Specific Aim 2

To compare wound characteristics (e.g., inflammatory markers, wound size) in the intervention group (BioBag therapy) and control group (Sharp debridement) over time.

Specific Aim 3

To investigate patient, caregiver and clinician perceptions of larval therapy dressings and bedside sharp debridement using measures of self-reported aesthetics, ease of use, and wound pain.

6. **Research Plan:**

Research design and methods
Design: A repeated measures, randomized control trial experimental design will be used for this study. Subjects will serve as their own control, in addition to a control group. Initial wound samples will be followed by two subsequent repeated wound samplings approximately four days apart. Informed consent will be obtained prior to all procedures, photo consents will also be obtained for wound photos.

Veteran Site: North Florida South Georgia Veterans Health System
Setting, Sample: A target goal final sample size of one hundred twenty eight adults from the North Florida South Georgia VHS will be recruited (total recruited number anticipated to be ~140 allowing for 10% attrition). Including target of 128 Caregivers and 6 Providers, and accounting for 12 caregivers lost to follow up or 10 subjects who fail screening, the total study recruitment population is 296. Wound providers and clinicians who care for lower extremity or diabetic foot ulcers will be provided with a "Provider Study Flyer" which gives a brief description of the study with inclusion/exclusion criteria listed (to remind the Provider to approach potentially eligible patients with Research Team Contact

information). The clinicians will also be asked to make a Recruitment Brochure available to potentially eligible patients (those with lower extremity or diabetic foot ulcers over 8 weeks duration). In addition, study flyers will be sent to select VA wound and podiatry offices in the NF/SG VHS. Providers will be asked to contact study team if any patient is willing to hear about the study. If the patient is willing to hear about the study, they may contact a study team member from the contact number on the recruitment brochure or the provider can let the patient know when the research team member is scheduled to be in the provider clinic office and the patient may talk directly to the research study team member for more information. If patients call a research team member, they will have their questions answered but will not be screened over the phone. They will be scheduled to come into the provider office to discuss the study with a research team member and review informed consent. Members of the research study team will review the informed consent with the patient and enroll the subject if they agree to participate. Wound clinicians will not be asked to consent subjects, or to provide a list of patients or appointment times to research team. However, the subject's wound providers (clinicians) will be the ones to perform the sample collection and apply the LDT or bedside sharp debridement. The sample also includes one available caregiver of the patient (non-professional) to answer the caregiver surveys if willing, and the wound care providers, who will be asked to answer the clinician survey regarding perceptions of debridement methods.

Sample Size Power Analysis: Power analysis is based on the patient sample and the primary study aim of comparing debridement methods on clinical effectiveness. The POWER procedure in SAS version 9.2 (Cary, N.C.) was used to conduct the power analysis. Assuming a Type I error rate of .05 and two-sided testing, a sample of 64 is needed in each group to detect a medium effect size of .50 with at least 80% power. This magnitude of effect is considered clinically significant.

Patient characteristics to be recorded: Age (in years), gender (Male/Female), presence of diabetes diagnosis (any "no" would be excluded from study), smoking behavior (Yes/No), presence or absence of pedal pulses (Yes/No) and Ankle Brachial Index (ABI), relevant laboratory values, culture reports and other pertinent medical history (co-morbid conditions). Additionally, telephone numbers will be obtained, with the participants consent, in order to call and remind the participant the day before there next upcoming visit.

Inclusion criteria: (1) Veterans over 21 years of age; (2) with chronic lower extremity or diabetic foot ulcers (wound duration over 8 weeks); (3) who at the clinician's judgment requires wound debridement (25% or more of wound bed covered with non-viable tissue); (4) wound size 1.5 cm (roughly the size of a quarter) or larger in diameter.

Exclusion criteria: (1) Cognitive impairment that would interfere with patient signing own Informed Consent; (2) Veterans on active anticoagulant therapy with most recent (within last week) PT/INR (international normalized ratio of prothrombin time) > 3.0, or other significant bleeding risk; (3) Active immune suppression just prior to or during study (on systemic corticosteroids* within 7 days prior, or chemotherapy for cancer or RA treatment within 4 weeks prior to study, or with diagnosis of HIV/AIDS) - *Nasal steroid sprays will not be excluded; (4) Active systemic antibiotics is an exclusion; (5) Absent dorsalis pedis pulses and Ankle Brachial Index (ABI) < 0.5 is an exclusion (indicates critical limb ischemia). Other possible reasons participants could be removed from this study include: transfers to other non-VA facilities, participant is unable to tolerate tissue sampling even with local anesthesia, at the discretion of the provider for clinical reasons, and/or inability to comply with scheduled research visits. Furthermore, if the participant has significant wound healing so that sampling is not possible after the initial sampling, they will be removed from the study.

Human Subjects Involvement and Characteristics: One hundred and forty consented veterans and their (non-professional) caregivers will be randomly assigned to one of two study arms: (1) Larval Debridement Therapy (LDT) or (2) Bedside Sharp Debridement (control). The intervention group, or LDT participants, will undergo LDT every 4 days for 2 applications as the main method of debridement strategy. They will have wound samples collected before the initial larvae are applied after 4 days of larval therapy and after 8 days of larval therapy. They will also both be asked to complete a short paper

survey on Days 0, 4 and 8. The subject's wound clinician will also be asked to complete a similar survey (only one round of surveys per consented wound clinician) at Day 0 and Day 8 and 3 months later or at completion of study regarding perceptions of wound debridement therapy.

The control group (bedside sharp debridement) will receive sharp debridement of their wounds 7 days apart x 2 sharp debridements and also have wound samples collected before the initial sharp debridement (Day 0), 4 days after the initial sharp debridement (Day 4), then eight days after initial sharp debridement, which will be 24 hours after the second sharp debridement (Day 8).

Both groups will also receive standard care and therapies ordered by their VA health care providers for their chronic wound care (excluding systemic antibiotics and topical wound antimicrobials), and standard emergency contact numbers. The study duration for the participants is 8 days,

IRB, Safety Committee, Information Security Officer (ISO) and VA R&D committee approval will be obtained prior to beginning this research study. The sample size is anticipated to be 140 total subjects (desired n=128 + 10% attrition). The desired sample size for data analysis is 128 to achieve adequate sample size for statistical power.

Data collection from human subject's electronic health record will include characteristics such as wound etiology, wound location and duration, wound treatments, wound photos, wound progress notes, age, gender, telephone number, comorbid conditions, pertinent laboratory values and medical history. This data will be conducted only by trained research team members designated in the IRB approved study protocol over the duration of the study. Data collection will only occur in compliance with VA Information Security requirements. The Veterans selected for this study will be adults and may involve any age over 18, race/ethnicity, gender, and medical comorbid conditions. No special classes of subjects will be sought out.

Recruitment and informed consent: Wound care providers such as in podiatry and lower extremity or diabetic limb preservation program (DPM) and Wound Team Clinicians will be approached and the study will be described. They will be asked to notify study investigators of potential subjects who are likely to have chronic lower extremity or diabetic foot ulcers (> 8 weeks duration) of 1.5cm size or greater -and who may be interested in participating in the study. If Veteran says they are willing to participate, wound provider will notify investigator and one of research team will meet Veteran and wound provider in clinic. Study will be explained to Veteran and the Veteran will sign informed consent if they agree.

Non-Veteran site: adding North Florida Regional Medical Center as a second site to meet recruitment

Setting, Sample: A target goal final sample size of forty adults (plus willing caregivers) from the North Florida Regional Medical Center will be recruited. Including the initial total target of 128 Caregivers and 6 Providers, and accounting for 12 caregivers lost to follow up or 10 subjects who fail screening, the total study recruitment population is still 296 for all recruitment sites. Wound providers and clinicians who care for lower extremity or diabetic foot ulcers will be provided with a "Provider Study Flyer" which gives a brief description of the study with inclusion/exclusion criteria listed (to remind the Provider to approach potentially eligible patients with Research Team Contact information). The clinicians will also be asked to give a Recruitment Brochure to potentially eligible patients (those with lower extremity or diabetic foot ulcers over 8 weeks duration). In addition, study flyers will be sent to select NFRMC wound and podiatry clinics. Providers will be asked to contact study team if any patient is willing to hear about the study. If the patient is willing to hear about the study, they may contact a study team member from the contact number on the recruitment brochure or the provider can let the patient know when the research team member is scheduled to be in the provider clinic office and the patient may talk directly to the research study team member for more information. If patients call a research team member, they will have their questions answered but will not be screened or consented over the phone. They will be scheduled to come into the provider office to discuss the study with a research team member and review informed consent. Members of the research study team will review the informed consent with

the patient and enroll the subject if they agree to participate. Wound clinicians will not be asked to consent subjects, or to provide a list of patients (PHI) to the research team. The subject's wound providers (clinicians) will be the ones to perform the sample collection and apply the LDT or bedside sharp debridement. The study sample also includes one available caregiver of the patient (non-professional) to answer the caregiver surveys if they are willing, and any willing wound care providers, who will be asked to answer the clinician survey regarding perceptions of debridement methods.

Sample Size Power Analysis: Power analysis for the entire study is based on the patient sample and the primary study aim of comparing debridement methods on clinical effectiveness. The POWER procedure in SAS version 9.2 (Cary, N.C.) was used to conduct the power analysis. Assuming a Type I error rate of .05 and two-sided testing, a sample of 64 is needed in each group to detect a medium effect size of .50 with at least 80% power. This magnitude of effect is considered clinically significant.

Patient characteristics to be recorded: Age (in years), gender (Male/Female), presence of diabetes diagnosis (any "no" would be excluded from study), smoking behavior (Yes/No), presence or absence of pedal pulses (Yes/No) and Ankle Brachial Index (ABI), relevant laboratory values, culture reports and other pertinent medical history (co-morbid conditions). Additionally, telephone numbers will be obtained, with the participants consent, in order to call and remind the participant the day before there next upcoming visit.

Inclusion criteria: (1) Individuals over 21 years of age; (2) with chronic lower extremity or diabetic foot ulcers (wound duration over 8 weeks); (3) who at the clinician's judgment requires wound debridement (25% or more of wound bed covered with non-viable tissue); (4) wound size 1.5 cm (roughly the size of a quarter) or larger in diameter.

Exclusion criteria: (1) Cognitive impairment that would interfere with patient signing their own Informed Consent; (2) Individuals on active anticoagulant therapy with most recent (within last week) PT/INR (international normalized ratio of prothrombin time) > 3.0, or other significant bleeding risk; (3) Active immune suppression just prior to or during study (on systemic corticosteroids* within 7 days prior, or chemotherapy for cancer or RA treatment within 4 weeks prior to study, or with diagnosis of HIV/AIDS) - *Nasal steroid sprays will not be excluded; (4) Active systemic antibiotics is an exclusion; (5) Absent dorsalis pedis pulses and Ankle Brachial Index (ABI) < 0.5 is an exclusion (indicates critical limb ischemia). Other possible reasons participants could be removed from this study include: transfers to other non-study participating facilities, participant is unable to tolerate tissue sampling even with local anesthesia, at the discretion of the provider for clinical reasons, and/or inability to comply with scheduled research visits. Furthermore, if the participant has significant wound healing so that sampling is not possible after the initial sampling, they will be removed from the study.

Human Subjects Involvement and Characteristics: After signing informed consent, participants and their (non-professional) caregivers will be randomly assigned to one of two study arms: (1) Larval Debridement Therapy (LDT) or (2) Bedside Sharp Debridement (control). The intervention group, or LDT participants, will undergo LDT every 4 days for 2 applications as the main method of debridement strategy. They will have wound samples collected before the initial larvae are applied after 4 days of larval therapy and after 8 days of larval therapy. They will also both be asked to complete a short paper survey on Days 0, 4 and 8. The subject's wound clinician will also be asked to complete (if willing) a similar survey (only one round of surveys per consented wound clinician) at Day 0 and Day 8 and 3 months later or at completion of study regarding perceptions of wound debridement therapy.

The control group (bedside sharp debridement) will receive sharp debridement of their wounds 7 days apart x 2 sharp debridements and also have wound samples collected before the initial sharp debridement (Day 0), 4 days after the initial sharp debridement (Day 4), then eight days after initial sharp debridement, which will be 24 hours after the second sharp debridement (Day 8).

Both groups will also receive standard care and therapies ordered by their health care providers for their chronic wound care (excluding systemic antibiotics and topical wound antimicrobials), and standard emergency contact numbers. The study duration for the participants is 8 days.

UF IRB and designated NFRMC and VA (as parent study site) approval(s) will be obtained as needed prior to beginning this research study. The total sample size (for all combined sites) is anticipated to be 140 total subjects (desired n=128 + 10% attrition).

Data collection from human subject's electronic health record will include characteristics such as wound etiology, wound location and duration, wound treatments, wound photos, wound progress notes, age, gender, telephone number, comorbid conditions, pertinent laboratory values and medical history. This data will be conducted only by trained research team members designated in the IRB approved study protocol over the duration of the study. Data collection will only occur in compliance with UF IRB and study parent site (VA) Information Security requirements. The participants selected for this study will be adults and may involve any age over 21, race/ethnicity, gender, and medical comorbid conditions. No special classes of subjects will be sought out.

Recruitment and informed consent: Wound care providers such as in podiatry, and wound and hyperbaric clinics will be approached and the study will be described. They will be asked to notify study investigators of potential subjects who are likely to have chronic lower extremity or diabetic foot ulcers (> 8 weeks duration) of 1.5cm size or greater -and who may be interested in participating in the study. If patients say they are willing to participate, their wound provider will notify the study investigator(s) and one of the research team members will meet with the patient and wound provider in clinic. The study will be explained to the patient and if they agree to participate, the participants will sign informed consent.

Both Research Recruitment Sites:

Confidentiality: All information collected from participants will be kept strictly confidential and used for research purposes only. All samples will be marked with de-identified information (an assigned subject number for each patient, date of collection, visit number, location of wound and duration of wound). Information regarding personal identifiers will be kept by the PI/project manager and stored separately in a locked cabinet. Participants will be assured that only the research study team and the Human Subjects Review Committees will have access to the study materials. All the study materials will be maintained in a locked/protected file within the VA Research Office until such time as the VA Research Compliance Officer gives the directive to destroy the files.

Study procedures (Sample Collection)

Larval Debridement Therapy (intervention) group: Briefly, wounds may be rinsed with saline and blotted with sterile gauze prior to collecting baseline wound samples at Day 0. Wound measurements and wound photos will be taken at baseline and Day 8. Wound samples will be collected on Day 0, Day 4, and Day 8. One BioBag (larval debridement therapy) will be applied to wound on Day 0 after baseline wound samples are obtained, and this will be removed on Day 4, at which time 2rd wound sample is obtained. Immediately after 2nd wound sample obtained, the 2nd BioBag will be applied. The dead larvae from the Biobags after removal from the subjects in this study will be placed in a sterile container and labeled with study subject number and "1st" or "2nd application." These specimens will be frozen in our VA research laboratory for a future secondary analysis. This will be the subject of a new grant proposal being submitted by the current investigators in 2015 with the aim of examining the digestive track and body of the larvae for the presence of bacteria and correlating this with the bacteria identified in the bacterial analysis of the subject wounds. Size of the Biobag is determined by the size of the wound (typically 5 to 8 larvae per cm^2 or 50 to 400 larvae per single Biobag). Only cover dressing (saline moistened gauze + dry gauze/secondary cover) will be changed over BioBag on days 1,2,3,5,6, and 7. Last wound sampling (3rd) will be done on Day 8, after 2nd BioBag is removed. Clinicians as well as Veteran participants and non-Veteran participants (at NFRMC) and their caregivers will be given copies of LDT instructions for use (Appendix A/Appendix B). Wound samples will be obtained at each

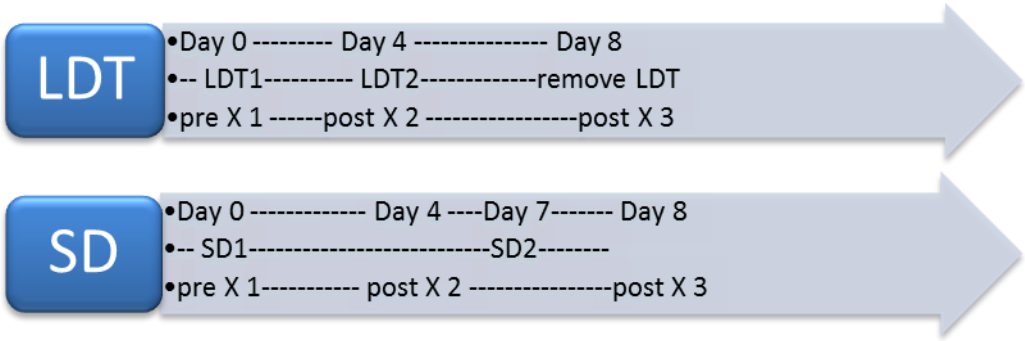
sampling date by sterile swab(s) and 3-4mm curette scraping of wound bed using a sterile curette sweeping z track method across wound bed. Sterile filter paper or micropipette/capillary tube may also be used to collect wound fluid. Wound samples will be collected at wound provider's office/clinic and placed in appropriate receptacle (sterile transport tubes with caps or vials with caps) for transport. The sample receptacles will be labeled with assigned subject number, number of visit (1, 2, 3, etc.), date of sample collection, duration and location of wound. Labeled samples will be placed in sealed biohazard bag, then in a biohazard marked cooler with a cold pack, and this container will be placed in a larger transport container for transport to the VA laboratory. Clinicians and clinic staff will be asked to complete sample collection training and biohazard shipping (if shipping is deemed necessary) training before collecting or shipping any wound samples to standardize the procedures and be in compliance with research, safety and shipping regulations. Clinicians will be asked to view a LDT training video before performing application of Larvae in order to further standardize the application.

Sharp Debridement Therapy (control) group: Briefly, wounds may be rinsed with saline and blotted with sterile gauze prior to collecting baseline wound samples at Day 0. Wound measurements and wound photos will be taken on Day 0, Day 4, Day 7 (before and after sharp debridement) and on Day 8. Wound samples will be obtained on Day 0, Day 4, and Day 8. Sharp debridement will be performed by patient's wound clinician using standard technique on day 0 after baseline wound measures are obtained and again on day 7. Wound samples will be obtained by sterile swab(s) and 3-4mm curette scraping of wound bed using a sterile curette sweeping z track method across wound bed. Sterile filter paper or micropipette/capillary tube may also be used to collect wound fluid. Wound samples will be placed in appropriate receptacle for transport as described above. Wound gel dressing (with gauze as secondary cover) will be applied to wound after debridement and changed daily as a control dressing. The following depicts the anticipated sampling timeframe for each participant.

<u>LDT group (X denotes sampling procedure)</u>
Day 0 - **X** pre-measures (**sample 1**); surveys; photos
Apply larvae bag 1
Day 4 - Remove larvae bag 1
X post-measure (**sample 2**); Pain survey; photos
Apply larvae bag 2
Day 8 - **X** final measures (**sample 3**); surveys; photos

<u>SD group</u>
Day 0 – **X** pre-measures (**sample 1**); surveys; photos
Sharp Debridement 1
Day 4 - **X** post measures (**sample 2**); pain survey; Wound photos
Day 7 - Sharp debridement 2 & photos

Day 8 - **X** final measures (**sample 3**); surveys; photos



Data Collection & Data Management
Measurement of bacterial colonization. A microbiological assessment of the wound samples will be performed. Samples will be measured for both planktonic (single) bacteria and biofilm bacteria by standard clinical microbiology techniques with serial dilution and plating on complete agar medium

plates. Colony forming units (CFUs) will be measured after approximately 24 hours of culture under standard laboratory conditions. Levels of planktonic bacteria and biofilm bacteria will be compared within each sample and chronologically within each patient. Photos will be reviewed by blinded wound clinicians and rated on amount of necrotic tissue present visually in wound bed at each sampling time (reported on scale 0 – 10 based on 0%, 1 = 10% or less, etc.). Data collected from human subject's electronic health record will include characteristics such as wound etiology, wound location/duration, wound treatments, wound photos, wound progress notes, age, gender, comorbid conditions, pertinent laboratory values and medical history. This data will be conducted only by trained research team members designated in the IRB approved study protocol over the duration of the study.

Only de-identified pertinent information per subject sample such as age of subject, visit number (Day 0 ,4, 8), location of wound, duration of wound, co-morbid conditions, medications and current wound treatments will be shared with the investigational laboratories or their personnel. Only the PI, site PI, Co-Investigators, and study coordinator(s) will have access to the subject PHI and study folders. UF lab has agreed to destroy any remaining samples at the conclusion of the current study.

Measurement of MMPs. Using techniques such as ELISA or florescence resonance energy transfer (FRET) assay methodology, the levels of active MMPs (or measures of MMP activity) will be calculated and expressed as pg/ml of wound fluid and pg/mg protein.

Measurement of IL6. Using techniques such as ELISA and florescence resonance energy transfer (FRET) assay methodology, the levels of active IL6 will be calculated and reported.

Methods for Data Collection & Data Management

Variable	**Significance**	**Sampling time**	**Source**	**Specific Aim**
Age	Demographic	Baseline	EHR	n/a
ABI	Exclusion criterion	Baseline - enrollment	ABI done after ICF	n/a
% nonviable wound tissue	Appearance of wound bed	Baseline Day 0 and Day 8	Provider assessment	Aim 1
Quantitative Cultures (planktonic and biofilm)	Microbial identification	Day 0 (pre-test), + Day 4 and Day 8	Wound samples	Aim 1
Wound Size in cm^2 (volume: L x W x D)	Descriptive	Baseline Day 0 and Day 8	Wound measurements	Aim 2
Interleukin 6	Marker of inflammation	Day 0 (pre-test), + Day 4 and Day 8	Wound sample (fluid and/or tissue)	Aim 2
Matrix metalloproteinases (MMP-2 and 9 equivalent)	Elevated MMPs associated with non-healing wounds	Day 0 (pre-test), + Day 4 and Day 8	Wound sample (fluid and/or tissue)	Aim 2
Perceptions of therapy	Perceptions of debridement	Day 0 (pre-test) + Day 8	Patient, Caregiver & Clinician Surveys	Aim 3

Survey Methods

Subjects will be asked to complete a paper survey at Day 0, Day 4 and Day 8 of the study, querying the patient's perceptions of: wound pain, satisfaction with most recent/current wound care method, ease of

care, effectiveness of most recent wound treatment, aesthetics of current wound treatment and toward sharp debridement and maggots for wound care.

Caregivers and wound clinical providers will be asked to complete a paper survey at Day 0 and Day 8 of the study (if wound clinician is caring for more than one study subject, they will be asked to complete a survey at day 0 of first subject, day 8 of first subject and 3 months later or at completion of the study for each type of debridement), querying them about their perceptions of what the patient's experience was regarding the most recent wound debridement methods as well as satisfaction with most recent/current wound care method, ease of care, effectiveness of most recent wound treatment, aesthetics of most current wound treatment and toward sharp debridement and maggots for wound care.

Data Analysis
Data will be summarized with descriptive statistics. Data will be checked for implausible values, missingness, and distributional form. Data transformations will be made as necessary to achieve normality. To address Specific Aim 1 (comparing the clinical effectiveness of a self-contained larval debridement therapy with a bedside sharp debridement therapy), the numeric outcome measures of changes (post minus pre) in percent of non-viable tissue and bacterial biofilm will be analyzed using independent samples t-tests. To address Specific Aim 2 (comparing wound characteristics of larval debridement and bedside sharp debridement therapies) over time, longitudinal generalized mixed models (GLMMs) will be used with the dependent repeated measures being wound characteristics (e.g., inflammatory markers, wound size) and the independent variable of group assignment (larval versus sharp debridement). To address Specific Aim 3 (investigating patient, caregiver, and clinician perceptions of larval therapy dressings and bedside sharp debridement using self-reported assessments, independent samples t-tests will be used to analyze data measured at the interval level, Wilcoxon rank sum tests will be used to analyze data measured at the ordinal level, and chi-square tests (or Fisher exact tests in the case of data sparseness) will be used to analyze categorical data. All hypotheses testing will be two-sided using a level of significance of .05. Data will be analyzed as "intent to treat." SAS version 9.2 (Cary, N.C.) will be used for all analysis.

Data and Safety Monitoring Plan
The main study PI will review the collected data monthly during sample collection to identify any possible breach of the study protocol, including incorrect consent procedure or data storage. This study will be monitored by the University of Florida IRB and the NF/SG VHS R&D Committee as well as the Research Compliance Officer, who have institutional responsibility to oversee the progress of research studies and the safety of study participants. The PI has the responsibility to promptly report any adverse event or breach of the study protocol. Should any adverse event or breach of protocol occur, they will be reported in accordance with the IRB standard operating procedures enforced by the IRBs at University of Florida, North Florida Regional Medical center, and the NF/SG VHS research Offices. All of the research team members will be required to pass the mandatory training programs for human subject research and be certified by the IRB for conducting this study.

*Inclusion of women, minorities and/or children***:** Efforts will be made to recruit women and minority patients.

7. **Possible Discomforts and Risks:**

Adequacy of Protection from Risk
Potential Risks: The risks to human subjects associated with this study are minimal related to the collection of wound samples, since these samples will be collected by the PI or patient's wound care clinicians. The risks related to the debridement techniques are also minimal since bedside sharp debridement is typically a standard of care in these patients with lower extremity or diabetic foot ulcers and the risk of larval debridement therapy is considered to be less than that of sharp debridement. Potential risks related to sharp debridement, and to a lesser extent, to larval debridement, may include: bleeding, pain, increased wound draining, infection, increased wound odor, and in the case of larval

debridement, a “crawling” sensation in wound. Potential risks related to subject, caregiver and wound clinician participation with the study survey component are minimal, due to the fact that no sensitive information is being collected.

Prior to study, it will be approved by the VA Subcommittee on Clinical Investigation (Research and Development committee) and the University of Florida Health Science Center Institutional Review Board (UF IRB). The UF IRB is the official review and approval process for all VA research studies at NF/SG VHS, and is the IRB of record for the additional North Florida Regional Medical Center Site. Despite the low risk of this study, we will take measures to safeguard all of the study participants (including caregiver and clinician) from inconvenience, unnecessary discomfort and Personal Health Information risk. First, we will meet with patient, caregiver and clinician at a convenient time for the participant, caregiver, and wound care provider/clinician. The surveys will be brief and will not ask for personally identifying or sensitive information (see Appendixes). We will take measures to safeguard any data collected and keep all data collected in a secure locked file on the VA server and in locked file cabinet (copies of regulatory documents and ICFs pertinent to NFRMC will be kept on site at NFRMC by site PI, Dr. Ellis). We will ensure that the participants are fully informed about the procedures, risks and objectives of the wound sample collection. The informed consents and all data collected at the clinician’s office or secondary study site(s) will be transported in a locked file box by the research team member and then stored in the Research office in a locked file cabinet. For VA participants, the original is scanned into patient’s electronic health record/CPRS per VA regulatory protocol; for NFRMC participants, the original is copied and kept onsite at NFRMC in a locked file cabinet with the study binder.

Potential Financial Risks: There are no anticipated financial risks to study subjects. The larval BioBags and lab tests performed on the wound samples collected from human participants for the sole purpose of this study will be the responsibility of the principal investigator.

8. **Possible Benefits:**

Benefits to Subjects

Potential Health Benefits: The human subjects in both arms of this study may benefit from the debridement therapies, as this has been demonstrated to improve wound healing trajectories. Information gained from this research may benefit future patients who suffer from chronic wounds.

9. **Conflict of Interest:**

Conflict of Interest Statements have been completed by all VA Investigators and are maintained on file in regulatory documentation. There is no conflict of interest identified with any of the Investigators. The Funding source/study Sponsor is Industry (Biomonde) and that is readily disclosed, but there are no financial obligations between this company and any of the investigators other than for study purposes as stated in the signed Cooperative Research and Development Agreement (CRADA) on file with the VA’s affiliated not-for profit entity, the North Florida Foundation for Research and Education (NFFRE).

Literature Cited

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