NPWT PRO vs KCI Ulta® NPWT and to Compare NPWT PRO vs NPWT PRO With Simultaneous Irrigation on Wound Healing

NCT02519621

06.05.2020

Clinical Research Protocol

A Pilot Study to Compare the Efficacy of NPWT PRO versus KCI Ulta® NPWT and to Compare NPWT PRO versus NPWT PRO with Simultaneous Irrigation on Wound Healing

Protocol Number:	CAHQ003A
Short title	Optimizing Negative Pressure Wound Therapy in DFUs
Version Date:	03.14.2019
Investigational Product:	Quantum NPWT/Black foam dressings
Clinicaltrials.gov number	NCTXXXXX
Development Phase:	4 – Post Market
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Significance:

Purpose

Wound healing, as a normal biological process in the human body, involves three precise and highly programmed phases: inflammation, new tissue formation, and remodeling. For a wound to heal successfully, all three phases must occur in the proper sequence and time frame. Many physiological or external factors can interfere with one or more phases of this process, thus causing improper or impaired wound healing and development of a chronic wound. Chronic wounds, often categorized as venous (and arterial) ulcers, diabetic ulcers, or pressure ulcers, all result from the wound healing process remaining in one of the aforementioned phases for too long (often the inflammatory phase) due to various physiological problems. Chronic wounds, if untreated or mismanaged, have various detrimental effects including reduced patient quality of life (pain, reduced ambulatory activity, etc), increased morbidity and mortality, and significant healthcare costs. It is estimated that chronic wounds affect 6.5 million patients in the United States alone and the cost to the health care system in enormous, with estimates exceeding $50 billion per year. The wound care management therapies available today are limited and many patients fail to heal or close their wound despite being provided numerous therapy options.

Endpoints

1. **To compare clinical outcomes in Wounds treated with Negative Pressure Wound Therapy PRO device versus KCI's Ulta device and Negative Pressure Wound therapy with and without Simultaneous Irrigation**
    a. Comparison of reduction in wound volume between wounds treated with Cardinal's PRO device and NPWT KCI's Ulta device
    b. Comparison of reduction in wound volume between wounds treated with NPWT and NPWT with simultaneous irrigation
    c. Comparison of rate of wound healing in wounds treated with NPWT and NPWT with simultaneous irrigation
    d. Comparison of the following variables: granulation tissue integrity including hypergranulation state, time to close, flap/graft take, dehiscence, and reoccurrence.
2. **To compare bacterial load, quantitative cultures, and clinical infections in wounds treated with NPWT with irrigation compared to patients treated with NPWT without continuous irrigation.**
    a. Comparison of reduction in bacterial load in wounds treated with NPWT and NPWT with simultaneous irrigation.
    b. Comparison of incidence of infection in wounds treated with NPWT and NPWT with simultaneous irrigation.
    c. Infection-induced necrosis
    d. Infection reoccurrence post-closure
3. **Assess changes in molecular markers associated with wound healing and regeneration of healthy tissue.**
    a. To compare changes in genes associated with wound healing between wounds treated with NPWT and NPWT with simultaneous irrigation.

b. To compare changes over time in genes associated with wound healing between wounds treated with NPWT and NPWT with simultaneous irrigation.

4. **Quality of Life and Patient Satisfaction Analyses**
   a. To compare Patient Satisfaction between patients with wounds treated with NPWT and NPWT with simultaneous irrigation.

Background:

Wound Repair

The repair of wounds is one of the most complex biological processes that occur during human life. The wound healing processes of normal healthy tissue involves 3 overlapping but distinct phases: inflammation, new tissue formation, and remodeling (reviewed in Gurtner, et al. [1]). The first stage of wound repair — inflammation — occurs immediately after tissue damage, and components of the coagulation cascade, inflammatory pathways and immune system are needed to prevent ongoing blood and fluid losses, to remove dead and devitalized (dying) tissues and to prevent infection. The inflammatory phase involves the upregulation of growth factors stimulate chemotaxis of neutrophils, monocytes, and fibroblasts to the area of injury [2]. After 2–3 days, monocytes appear in the wound and differentiate into macrophages. Macrophages are thought to be crucial for coordinating later events in the response to injury [3, 4].

The second stage of wound repair — new tissue formation — occurs 2–10 days after injury and is characterized by cellular proliferation and migration of different cell types. The first event is the migration of keratinocytes over the injured dermis (the inner layer of the skin). New blood vessels then form (a process known as angiogenesis), and the sprouts of capillaries associated with fibroblasts and macrophages replace the fibrin matrix with granulation tissue, which forms a new substrate for keratinocyte migration at later stages of the repair process. The keratinocytes that are behind the leading edge proliferate and mature and, finally, restore the barrier function of the epithelium. For the first 6 weeks, new collagen production dominates the wound healing process, deposited randomly in acute wound granulation tissue.

The third stage of wound repair — remodeling — begins 2–3 weeks after injury and lasts for a year or more. Most of the endothelial cells, macrophages and myofibroblasts undergo apoptosis or exit from the wound, leaving a mass that contains few cells and consists mostly of collagen and other extracellular-matrix proteins. Over the next 6–12 months, the acellular matrix is actively remodeled from a mainly type III collagen backbone to one predominantly composed of type I collagen [5]. This process is carried out by matrix metalloproteinases that are secreted by fibroblasts, macrophages and endothelial cells, and it strengthens the repaired tissue. However, the tissue never regains the properties of uninjured skin [6]. Tensile strength plateaus at 80% of the original strength approximately 1 year post-injury [7-9].

Chronic wounds are those that have failed to proceed through an orderly and timely reparative process to produce anatomic and functional integrity of the injured site [10]. Chronic wounds seem to be detained in one or more of the phases of wound healing. For example, chronic wounds often remain in the inflammatory stage for too long [11, 12]. These wounds may never heal or may take years to do so. Chronic wounds are often categorized into one of 3 groups, venous (and arterial) ulcers, diabetic ulcers, and pressure ulcers. Various physiologic and mechanical factors may impair the healing response such as local infection, hypoxia, trauma, foreign bodies, or systemic problems such as diabetes mellitus, malnutrition, immunodeficiency, or medications

are most frequently responsible. All wounds are contaminated, but most successfully resist invasive infection. When the concentration exceeds 100,000 (10^5) organisms per gram of tissue or the immune system becomes compromised, infection frequently ensues [13]. Cellulitis prolongs the inflammatory phase by maintaining high levels of proinflammatory cytokines and tissue proteases, which degrade granulation tissue and tissue growth factors, and by delaying collagen deposition [14, 15]. These wounds cause patients severe emotional and physical stress and create a significant financial burden on patients and the whole healthcare system [16].

Chronic wounds and healthcare costs

Often disguised as a comorbid condition, chronic wounds represent a silent epidemic that affects a large fraction of the world population and poses a major and gathering threat to the public health and economy of the United States. In developed countries, it has been estimated that 1 to 2% of the population will experience a chronic wound during their lifetime [17, 18] and in the United States alone, chronic wounds affect 6.5 million patients [19, 20]. A conservative estimate of the staggering cost of caring for these wounds exceeds $50 billion per year [21-24]. This is 10 times more than the annual budget of the World Health Organization. Despite the fact that the prevalence rate of chronic wounds is similar to that of heart failure [25], unlike heart failure, little is known regarding the outcome of these patients or the comparative effectiveness of the treatments they receive. According to data from the Department of Health and Human Services' Agency for Healthcare Research and Quality (AHRQ), the number of hospital patients with pressure sores rose by 63% during the period 1993–2003. The price of managing a single full-thickness pressure ulcer is as much as $70,000, and US expenditures for treating pressure ulcers have been estimated at $11 billion per year [21, 24].

Diabetes, chronic wounds and amputations

Over 29 million people or 9.3% of the U. S. population suffer from diabetes. While 21 million have been diagnosed, 8.1 million are unaware that they have the disease [26]. These numbers demonstrate an increase from 8.3% in 2012 [27]. Diabetics are at higher risk of developing a non-healing wound due to several factors, including hyperglycemia, poor circulation, sedentary lifestyle, neuropathy and compromised immunity [28]. It is estimated that up to 25% of all diabetics will develop a diabetic foot ulcer [29]. Clinical epidemiologic studies suggest that foot ulcers precede ~85% of non-traumatic lower extremity amputations in individuals with diabetes [30]. In fact, the main causes of limb loss are vascular disease (54%) – including diabetes and peripheral arterial disease – trauma (45%) and cancer (less than 2%) [31]. About 73,000 non-traumatic lower-limb amputations were performed in people with diabetes in 2010 [26]. The age-adjusted lower-extremity amputation rate for people with diabetes (5.5 per 1,000 people with diabetes) was 28 times that of people without diabetes (0.2 per 1,000 people). In 2009, hospital costs associated with amputation totaled more than $8.3 billion [32]. Amputation has long been considered an end-of-life event. Several studies have evaluated mortality rates after amputation in different eras [33-46], however most studies have demonstrated close to 50% mortality within the first year after operation, with cardiovascular disease a leading cause of death [33]. This is higher than the five year mortality rates for breast cancer, colon cancer, and prostate cancer [47]. Of persons with diabetes who have a lower extremity amputation, up to 55% will require amputation of the second leg within 2-3 years [48].

Morbidity and mortality of non-healing wounds

Diabetes is the most common underlying cause of non-traumatic amputation in the U.S. and Europe [49-52]. Of the 120,000 amputations performed in the U.S. every year, 40– 70% are in individuals with diabetes. Among

individuals with end-stage renal disease receiving dialysis, the incidence of amputation is about 10 times higher than in the general diabetic population [53]. Most of the existing work reports in-hospital or 30-day survival after amputation. In a study of 8,169 hospitalizations in California for lower-extremity amputation, we found that in-hospital mortality in individuals with diabetes was higher as amputation level increased (foot 1.5%, leg 4%, and thigh 7%) [54]. Mayfield et al. [37] reported 3-year survival results demonstrating the same trend (foot 54%, leg 44%, and thigh 30%). Ulcers and other foot complications are responsible for 20% of the nearly 3 million hospitalizations every year related to diabetes. Nearly 85% of lower-extremity amputations are preceded by a foot ulcer [55-57]. Once amputation occurs, 50% of patients will develop an ulcer in the contralateral limb within 5 years [58]. Unlike persons undergoing trauma or cancer-related amputations, persons with PVD and diabetes can experience a progression of the underlying disease process that results in amputation of the opposite lower limb [59]. In addition, failure of the primary surgical wound from a lower-limb amputation to heal or development of further vascular compromise can lead to a higher-level amputation on the same limb [60]. Having a lower limb amputation is associated with a somewhat high risk of not surviving within the first year from surgery, with perioperative mortality ranging from 9 to 16% [38, 61-64], and 1-year survival rates ranging from 86 to 53% [38, 58, 61-68]. The majority of non-traumatic amputations are most often caused by a vascular disease, followed by diabetes or a combination of both [58, 61, 63, 64, 66, 67], whereas worse survival rates have been associated with factors such as older age, diabetes, more than one co-morbidity, above knee amputations (AKAs), type of rehabilitation setting and the post-amputation physical independence grade [38, 61, 65, 66, 69, 70].

The Future

The prevalence of diabetes in the United States is projected to nearly double by the year 2030 solely because of changes in the demographic composition of the population. Estimates of limb loss reflect, and indeed magnify, these trends. Even assuming that age-, sex-, and race-specific rates of both diabetes and diabetes-related amputations remain unchanged, the number of people with diabetes who are living with the loss of a limb will nearly triple by the year 2050 [31]. Overall, the prevalence of limb loss will more than double from 1.6 to 3.6 million people. Given the increase in the prevalence of obesity and the known relationship between obesity and diabetes, a projected increase in the incidence of amputations secondary to dysvascular conditions is likely [71-75]. As the number and proportion of Americans with diabetes continue to grow, so will the pressure on the health care system to develop more cost-effective approaches to meet the medical needs of persons at risk for amputations (and re-amputations).

Negative Pressure Wound Therapy

Negative Pressure Wound Therapy **(**NPWT) has dramatically changed the care of complex wounds, especially in the diabetic foot. Compared to standard wound care, patients treated with NPWT are 5.9 times more likely to heal and 4.4 times less likely to require amputation [76, 77]. NPWT involves the delivery of sub-atmospheric pressure through a vacuum pump connected to a specialized dressing to maintain a closed environment. NPWT increases perfusion to the wound, accelerates granulation tissue formation, reduces edema, reduces bio-burden, increases healing and reduces the risk of lower extremity amputation [78].

Infection is highly common in chronic diabetic foot ulcers. The Infectious Disease Society of America and the International Working Group on the Diabetic Foot advocate that the diagnosis of diabetic foot infections (DFI's) should rely on clinical criteria and not routine bacterial cultures [79]. However, the same group suggests that

the classical signs of infection in diabetes are blunted because of leukocyte dysfunction, peripheral neuropathy, and peripheral arterial disease (PAD) [80]. Evolving technology such as quantitative PCR, provides an opportunity to accurately identify and quantify bacterial pathogens and perhaps even to redefine "infection" based on objective measurements. Infections $>10^5$ organisms per gram of tissue are associated with infection and delayed wound healing in chronic wounds [81, 82]. In a study by Xu et al., the rate of healing had a strong inverse relationship with log colony forming units (CFUs). For every log order of CFUs there was a 44% delay in wound healing [83].

To date, there are no head-to-head comparisons of fully powered, electrical NPWT devices to determine either superiority or noninferiority. KCI is the market leader in this category and therefore a study to determine whether Cardinal's PRO NPWT device is noninferiority is necessary. In addition, there are no studies that report a comparison of the economic parameters – both direct and indirect costs – between two NPWT devices.

NPWT with Irrigation

To date, there are minimal data examining the efficacy or effectiveness of NWPT with irrigation for the healing of acute or chronic wounds. Further, they are mostly limited to small case series, retrospective reviews and uncontrolled studies [84-94]. Studies demonstrate that NPWT decreases infections in pig models [95, 96] and in chronic wounds [97, 98] and adding irrigation with antiseptic solutions is also beneficial [99]. Other studies demonstrate efficacy of NPWT with irrigation however they compare NPWT with irrigation to 'standard' wound care (not NPWT alone) [90, 93], thus making it difficult to separate the effects of NPWT and the addition of irrigation. While several studies demonstrate the effectiveness of NPWT with irrigation, no there are no consistent data on irrigation rate [89-91, 93, 100], volumes of irrigation that are most effective [90, 93], or the duration of therapy that should be used [84, 93]. Further, there remains little data comparing the effectiveness of different irrigation solutions. In fact, the effectiveness of saline, polyhexanide, dakins, silver nitrate and mixed antibiotic solutions have all been reported [87, 88, 90, 101-104]. Polyhexanide, marketed under the name Prontosan, is a strong base and interacts with acidic phospholipids in the bacterial cell membrane, leading to increased permeability and cell death [105]. Polyhexanide promotes wound healing likely through biofilm reduction in wounds [101, 106-113] and is effective against pathogens in vitro [114] but better with gram positive vs gram negative bacteria [115]. Several studies have used polyhexanide irrigation with NPWT [93, 116-118].

Preliminary Studies

Study 1

Simultaneous irrigation and NPWT enhances wound healing and reduces wound bioburden in a porcine model (Published in the Journal of Wound Repair and Regeneration, 2013, Ref [119])

The aims of this study were two fold. First, this study was designed to test the effectiveness of NPWT with irrigation on wound healing and bioburden reduction. In addition, this study compared the wound healing and bioburden outcomes between saline or polyhexanide biguanide (PHMB) at low or high flow rates. A porcine model with dorsal full thickness excisional wounds was use for this study. The wounds were inoculated with *Pseudomonas aeruginosa* for 3 days prior to therapy initiation. Wounds were treated for 21 days of therapy with either NPWT, NPWT with simultaneous irrigation therapy using normal saline (Sal) or PHMB at low or high (Hi) flow rates, or control non-treated (Cont) wounds with dressings equivalent to the other groups. Data demonstrate that NPWT with either irrigation condition improved wound healing rates over control treated wounds, however did not differ from NPWT alone (Figure 1). When change in bioburden was compared to therapy day 0, NPWT improved bioburden in wounds over control therapy (Figure 2). The addition of saline or PHMB irrigation to NPWT further reduced bioburden over control and NPWT treated wounds. Both high and low flow rates resulted in similar decreases in wound bioburden. Together this is the first published controlled study demonstrating that NPWT with simultaneous irrigation therapy with either normal saline or PHMB has a positive effect on bioburden in a porcine model, which may translate clinically to improved wound healing outcomes.

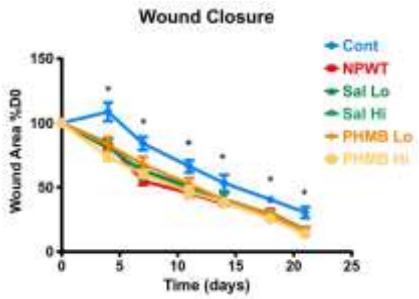

Figure 1. Average change in wound area for each condition over time.

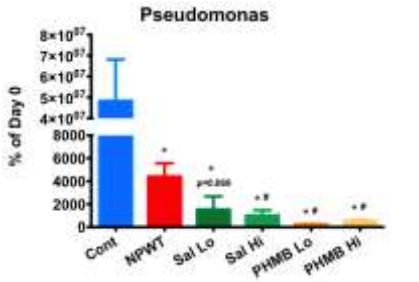

Figure 2. Average change in Pseudomonas levels for each condition over time.

Study 2

The Impact of Negative Pressure Wound Therapy with Instillation Compared to Negative Pressure Wound Therapy: A Retrospective Historical Cohort Controlled Study (Published in the Journal of Plastic and Reconstructive Surgery, 2013, Ref [94])

This retrospective, historical, cohort-control study examined the impact of negative pressure wound therapy with and without instillation. One hundred forty-two patients (negative-pressure wound therapy, n=74; therapy with instillation, 6-minute dwell time, n=34; and therapy with instillation, 20-minute dwell time, n=34) were included in the analysis. The number of operative visits was significantly lower for the 6- and 20-minute dwell time groups compared with the non instillation group. Hospital stay was significantly shorter for the 20-minute dwell time group compared with the no-instillation group. Time to final surgical procedure was significantly shorter for the 6- and 20-minute dwell time groups compared with the no-instillation group. Percentage of wounds closed before discharge and culture improvement for Gram-positive bacteria was significantly higher for the 6-minute dwell time group (94 and 90 percent, respectively) compared with the no-instillation group. These results suggest that negative-pressure wound therapy with instillation (6- or 20-minute dwell time) is more beneficial than standard negative pressure wound therapy for the adjunctive treatment of acutely and chronically infected wounds that require hospital admission.

Innovation

This is the first prospective study to compare parameters of clinical efficacy of two different NPWT devices while also collecting information on direct and indirect treatment costs.

This is also the first clinical study that compares the clinical outcomes of NPWT and NPWT with simultaneous irrigation. Results will help clinicians develop treatment protocols to prescribe NPWT so treatment is optimized. This is also the first human study to measure the effect of NPWT and irrigation on bio-burden and wound healing. Our plan is to establish a treatment algorithm with saline irrigation solution based on bioburden reduction and acceleration of wound healing. This study will help determine how long it takes to eradicate bacteria with the goal of determining how long wounds typically need to be treated to "sterilize" the wound bed. Other objectives include determining whether eradication of wound bioburden enhances wound-healing rates with additional focus on the effects NPWT and NPWT with irrigation on a number of molecular and inflammatory markers.

Study Objectives and Design:

Study Groups and Design:

This study is designed to assess the efficacy and economics of two NPWT branded devices of wound healing outcomes. It is also designed to assess the effectiveness of negative pressure and negative pressure with continuous irrigation on multiple parameters of wound healing. It is a single-center, open-label, active controlled, parallel-group trial that aims to determine the efficacy of Quantum with simultaneous irrigation in the treatment of diabetic foot ulcers. Specifically, eligible participants will be randomized to receive either PRO negative press therapy with simultaneous irrigation; or PRO negative pressure therapy without irrigation; or KCI Ulta negative pressure therapy. Primary outcomes will include rates of wound healing, time to closure by secondary intention, and recurrence rate.

Study Objectives and Endpoints

Objective 1

A) To compare clinical outcomes in Wounds treated with Negative Pressure Wound therapy with and without Simultaneous Irrigation.

H1: The wound healing outcomes of patients treated with NPWT Pro will be non-inferior to the wound healing outcomes of patients treated with NPWT-KCI Ulta

Primary Endpoint:

- Comparison of reduction in wound volume between wounds treated with NPWT PRO and NPWT-KCI Ulta

Secondary Endpoints:

- Comparison of rate of wound healing in wounds treated with NPWT PRO and NPWT-KCI Ulta.

- Comparison of the following variables between the groups: granulation tissue integrity including hypergranulation state, time to close, flap/graft take, dehiscence, and reoccurrence.

B) To compare clinical outcomes in Wounds treated with NPWT PRO with and without Simultaneous Irrigation.

H1: Patients treated with NPWT with irrigation will have higher proportions of wounds that heal and faster wound healing trajectories compare to patients treated with NPWT without irrigation.
H2: Patients treated with Negative Pressure Wound therapy with Irrigation will have fewer wound complications such as infections, amputations, and foot-related hospital admissions.

Primary Endpoints:

- Primary Endpoint: Comparison of reduction in wound volume between wounds treated with NPWT PRO and NPWT PRO with simultaneous irrigation

Secondary Endpoints:

- Comparison of rate of wound healing in wounds treated with NPWT and NPWT with simultaneous irrigation
- Comparison of the following variables between the groups: granulation tissue integrity including hypergranulation state, time to close, flap/graft take, dehiscence, and reoccurrence.

Objective 2

To compare bacterial load, quantitative cultures, and clinical infections in wounds treated with NPWT PRO compared to wounds treated with NPWT PRO with simultaneous irrigation.

H1: Bacterial load measured with quantitative polymerase chain reaction (qPCR) will be progressively lower with continuous irrigation.

H2: Patients treated with simultaneous irrigation and NPWT PRO will have fewer infections defined by qPCR and clinical criteria.

Primary Endpoint:

- Comparison of reduction in bacterial load in wounds treated with NPWT and NPWT with simultaneous irrigation.

Secondary Endpoints:

- Comparison of incidence of infection in wounds treated with NPWT and NPWT with simultaneous irrigation.
- Infection-induced necrosis
- Infection reoccurrence post-closure

Objective 3

Assess changes in molecular markers associated with wound healing and regeneration of healthy tissue.

H1: NPWT PRO with simultaneous Irrigation will improve the levels of growth factors, cytokines, and other molecular markers over NPWT PRO alone**.**
H2: Negative pressure wound therapy will drive molecular changes in growth factors and other cytokines associated with regeneration of healthy tissue.

Primary Endpoint:

- To compare changes in genes and other molecular markers associated with wound healing between wounds treated with NPWT and NPWT with simultaneous irrigation.

Secondary Endpoint:

- To compare the addition of simultaneous irrigation on gene expression and expression of molecular markers from baseline.

Objective 4

Quality of Life and Patient Satisfaction Analyses

H1: Patients treated with the NPWT PRO device will show greater patient satisfaction with treatment than those treated with KCI Ulta NPWT therapy.

Primary Endpoints:

- To compare Quality of Life and Patient Satisfaction between patients treated with NPWT PRO versus patients treated with KCI Ulta NPWT therapy.
- To compare Quality of Life and Patient Satisfaction between patients with wounds treated with NPWT PRO versus NPWT PRO + SI.
- Evaluate functional status, using the Short From 36 (SF36). These will be administered at baseline and at the last study visit.

Subjects and Recruitment

We will enroll 90 patients from University of Texas Southwestern Medical Center and Parkland Hospital. The clinics that are part of the Parkland and University of Texas medical center include a racially and ethnically diverse patient population and are representative of those who suffer from type 2 diabetes, foot ulceration and risk of amputation.

Subject Stipend

$35 per visit will be provided for research related visits (Up to a total of $350).

Randomization

Subjects will be taken to the operating room for the initial debridement procedure of the wound. At the end of the procedure, subjects who continue to meet all inclusion and no exclusion criteria will be randomized in a 1:1:1 ratio to be treated with either PRO, PRO with simultaneous irrigation (PROI), or KCI Ulta NPWT. Prior to study initiation sealed prenumbered randomization envelopes will be provided to the research staff and used to obtain randomization assignment. Opening of the randomization envelop will occur intraoperatively at the conclusion of the initial surgical debridement of the wound and conformation of all eligibility requirements. Study staff will use the randomization number labels contained in the envelop. The number will become the subject ID. The assignment will be subjects randomized to PRO, PRO with simultaneous irrigation, or KCI Ulta NPWT.

The research staff will note treatment assignments on the intra operative randomization CRF and instruct the investigator. Treatment therapy wound dressings will be applied in the operating room or in the patient's room immediately after surgery per the investigators discretion, according to the manufacturer's recommendations. In order to ensure consistent study treatment, subjects will receive assigned treatment therapies within their study arm after the initial and any subsequent surgical debridements until the wound is deemed ready for closure or coverage by the Investigator. Subjects randomized to the PRO with simultaneous irrigation Treatment arm are the only subjects that will receive irrigation therapy at any time during the study treatment period. If irrigation therapy is discontinued, subjects in the Treatment arm will transition to PRO NPWT without irrigation. Subjects randomized to the PRO Control arm will receive NPWT only from the PRO therapy unit.

Eligibility Criteria

Inclusion Criteria

To be eligible for study enrollment, a subject must satisfy each of the following criteria:

- Presents with an existing chronic or traumatic wound, sub-acute or dehisced wound, partial-thickness burn, ulcer (such as a diabetic or pressure ulcer), flap or graft as diagnosed by a qualified and certified medical practitioner (M.D., D.O., or D.P.M)
- Wound presents with full thickness loss of epidermis and dermis
- The presentation of a wound that in the opinion of the investigators will require surgical debridement, and the wound is expected to be a good candidate for NPWT.
- ABI≥0.5 or toe pressures >30 PVR/mmHg
- Subject is willing and able to abstain from partaking in any other form of treatment for his or her wound during the active treatment phase of this study , other than the study procedures described herein.
- 18 years of age or older

Exclusion Criteria

Subjects that satisfy any one or more of the following exclusive conditions criteria outlined below will be excluded from participation in the clinical study.

- Does not present with an existing chronic or traumatic wound, sub-acute or dehisced wound, partial-thickness burn, ulcer (such as a diabetic or pressure ulcer), flap or graft, or a definitive diagnosis cannot be made, as diagnosed by a qualified and certified medical practitioner (M.D., D.O., or D.P.M)
- Wound does not present with full thickness loss of epidermis and dermis

- ABI<0.5 or toe pressures <30 PVR/mmHg
- Subject is not willing or is not able or it is not medically prudent for the subject to abstain from partaking in any other form of treatment for his or her wound during the active treatment phase of this study, other than the study procedures described.
- Subject is unwilling or unable to use the NPWT device at home
- Active Charcot arthropathy
- Collagen vascular disease
- Sceleroderma
- Non-enteric and unexplored fistula
- Necrotic tissue with eschar present after debridement
- General skin disorder in the area of the wound such as psoriasis or penicilitis
- Malnutrition (Defined as BMI < 19)
- Hypercoagulable state based on documentation in their medical record
- Acute deep vein thrombosis
- Current active malignancy in the wound
- Current melanoma or history of melanoma at the wound
- Current active or history of invasive squamous cell carcinomas at the wound
- Sepsis (defined as positive blood culture with leukocytosis) and temperature >101.5 at the time of screening
- Significant hematologic disorders EXCLUDING anemia

- HIV
- Fever at screening > 101.5
- Deep X-ray therapy
- Untreated bone or soft tissue infection Any concomitant illness(es) or medical condition(s) that in the opinion of the investigator would render the subject not suited for study participation
- Subject is taking a regimen of any medication(s) in a significant enough dosage that may affect chronic wound healing, including corticosteroid, chemotherapeutic and non-steroidal anti-inflammatory (NSAID) medications
- Less than 18 years of age
- Developmental disability/significant psychological disorder that in the opinion of the investigator could impair the subject's ability to provide informed consent, participate in the study protocol or record study measures, including untreated schizophrenia, bipolar disorder and psychiatric hospitalization within the last 2 years.
- Females currently pregnant or planning pregnancy during the course of intended participation in the study
- Active alcohol or substance abuse in the opinion of the investigator that could impair the subjects ability to provide informed consent, participate in the study protocol or record study measures.

Study Devices:

The Cardinal Health NPWT PRO system (K143016) continuous/intermittent vacuum-assisted drainage with simultaneous delivery of topical wound treatment solutions and suspensions over the wound bed. The systems are AC-powered, portable suction devices with battery backup that provide localized negative pressure when

used with the Cardinal NPWT Dressing to remove fluid and infectious materials from the wound. The systems are designed for patients who would benefit from a suction device, particularly as the device may promote wound healing, including patients who would benefit from vacuum assisted drainage and removal of infectious material or other fluids from wounds under the influence of continuous and/or alternating suction pressure. It is intended for use on patients with chronic, acute, traumatic, sub-acute and dehisced wounds, diabetic ulcers, pressure ulcers, flaps, grafts, and partial thickness burns. The Cardinal Health NPWT PRO systems provide care in the acute and extended clinical and home care settings.

The Cardinal Health NPWT PRO systems consist of powered suction pump components. The Cardinal Health NPWT PRO systems include a built-in placement holder for the 300cc and 500cc collection canisters. They have a pushbutton ON/OFF operation with five user-selectable pressure settings. The system produces optional pressure settings of -50mmHg, -75mmHg, -l00mmHg, -l25mmHg, and –l50mmHg. It has alarms for Low Pressure/Leak, Full Canister, Low Battery and Therapy Timer. These alarms include both audible and visual indications. The system incorporates an IV pole hanger and bed hanger to make the product compatible with most clinical settings.

System Components

SpeedConnect Tubing

SpeedConnect is a single-lumen tubing set with adhesive flanges. The tube is kink-resistant and is 8 feet in length

Dressings

Black Foam Dressing
Hydrophobic, open-pore, reticulated foam with a high tensile strength is cut to fit the wound. The polyurethane foam dressings are provided in Small, Medium, Large, and XL sizes.

White Foam Dressing
Hydrophilic, high density foam dressing that is flexible when wet or dry.

Polyurethane Drape
Polyurethane drapes are provided in an 8” x 12” size and cover the wound and surrounding tissue.

Irrigation Tubing Set
Irrigation is supplied via a single-lumen tubing set with adhesive flanges and on end and an IV bag spike on the other end. The tube is kink-resistant and is 8 feet in length.

Canisters

The 300 cc and 500 cc single-patient use canisters collect exudate from the wound site. The canisters contain a porous polymer hydrophobic depth filter to prevent fluid ingress and gel packs to solidify wound exudate (300cc available with or without gel packs.) A red cap is included to prevent spillage upon disposal.Only 300 cc canisters are used in this study.

NPWT Operating Characteristics of Quantum NPWT Device	
Power Source	Electric/Battery
Mean Pressure	125mm Hg with continuous irrigation, normal saline 15 cc/hr
Mode	Continuous
Interface	Polyurethane Foam
Frequency of Interface Change	3x weekly

Control Devices: KCI USA, Inc. V.A.C. Ulta™ NPWT System & KCI USA, Inc. V.A.C. VIA™ NPWT System

Subjects in the KCI arm of this clinical study will receive NPWT alone (standard of care) until wound closure with the following two KCI devices that are both FDA cleared as Class II devices under Product Code OMP for the same indication and intended for the control subject group in this clinical study. In the acute care setting, patients will be treated with the KCI VAC Ulta NPWT device. In the home environment, patients will be treated with the KCI VAC Via NPWT system, paid for by the study at no cost to the subjects.

KCI USA, Inc. V.A.C.Ulta™ Negative Pressure Wound Therapy System (K100657). It includes a NPWT powered suction pump and a sterile dressing system applied to the wound, which is connected via tubing to a therapy unit that generates negative pressure at the wound. A sterile, disposable canister collects wound exudates removed via the negative pressure. The dressing utilizes an open-cell polymer foam dressing that conforms to the wound bed. When sealed and placed under negative pressure, the system creates an environment that has been shown to promote the wound healing process, reduce edema, prepare the wound bed for closure, promote the formation of granulation tissue and remove infectious materials. The V.A.C. Ulta NPWT System is indicated for patients with chronic, acute, traumatic, sub-acute and dehisced wounds, partial-thickness burns, ulcers (such as diabetic, pressure and venous insufficiency), flaps and grafts.

Subjects in the KCI arm of this clinical study will receive NPWT alone with the KCI USA, Inc. V.A.C.Via™ NPWT System (K132741) while residing at home. The V.A.C.Via NPWT System is a NPWT powered suction pump that consists of the following components: a sterile dressing system applied to the wound and connected via tubing to a therapy unit that generates negative pressure at the wound; and a sterile, disposable canister that collects wound exudates removed via the negative pressure. The V.A.C.Via™ NPWT System is intended for use as an integrated wound management system in acute, extended and home care settings. It is intended to create an environment that promotes wound healing by secondary or tertiary (delayed primary) intention by preparing the wound bed for closure, reducing edema, promoting granulation tissue formation and perfusion, and by removing exudates and infectious material. It is indicated for patients with chronic, acute, traumatic, subacute and dehisced wounds, partial-thickness burns, ulcers (such as diabetic, pressure or venous insufficiency), flaps and grafts.

Screening and Baseline Procedures

The following is a listing of all of the assessment and safety tools and evaluations to be used in this clinical study. For each study phase, the precise tools to be employed will be specified.

Ulcer History

- Type of wound: chronic or traumatic wound, sub-acute or dehisced wound, partial-thickness burn, ulcer (diabetic, pressure, etc.), flap, graft, other
- Duration since wound onset
- Wound location
- Previous wound history/amputation, if any

Medical Status

- The New York Heart Association (NYHA) Functional Classification System will be used to classify stage of heart failure.
    - The New York Heart Association (NYHA) functional classification system is used to classify the stage of heart failure from Class I to IV (as shown in the table below). This system relates symptoms to everyday activities and the patient's quality of life.

Class	**Patient Symptoms**
□ None	No history of limitations
Class I (Mild)	No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, or dyspnea (shortness of breath).
Class II (Mild)	Slight limitation of physical activity. Comfortable at rest, but ordinary physical activity results in fatigue, palpitation, or dyspnea.
Class III (Moderate)	Marked limitation of physical activity. Comfortable at rest, but less than ordinary activity causes fatigue, palpitation, or dyspnea.
Class IV (Severe)	Unable to carry out any physical activity without discomfort. Symptoms of cardiac insufficiency at rest. If any physical activity is undertaken, discomfort is increased.

- Body Mass Index (BMI): BMI is calculated as the ratio of body weight in kilograms (kgs) to height in meters squared (m^2).

Medical History

History or present existence of the following will be noted:

- lower extremity bypass
- lower extremity angioplasty
- coronary artery bypass surgery
- cardiac angioplasty
- arthritis

- liver disease
- osteoporosis
- malignancy
- bone tumors
- skin pathology
- vital signs (weight, height, HR, BP)
- diabetes history
    - duration
    - type
    - medication(antibiotics only)
- date and type of surgery
- vascular status
- neuropathy

Lab Values

- BUN
- Creatinine
- Estimated GFR (glomular filtration rate)
- Hemoglobin A1c
- Blood Glucose
- Prealbumin (transthyretin)
- Albumin
- CRP (C-reactive protein)
- ESR (Erythrocyte Sedimentation Rate)
- White blood cell countRBC
- Platelet Count
- Hemoglobin
- Hematocrit
- RDW
- Pregnancy test if subject is child bearing potential

Concomitant Antibiotic Therapy Use

All antibiotics and therapies engaged in by the subject will be recorded at screening visits and follow up visits.

Social Factors

The following social factors will be recorded:

- marital status
- years of education
- tobacco use history: number of years of smoking, current or previous smoker, use of chewing tobacco, average daily number of cigarettes for current smokers

- alcohol and drug use history: current and/or previous history, types of alcohol/drugs consumed in the past and/or present; frequency and amount of alcohol/drugs consumed in the past and/or present

Demographic Variables

The following demographics will be recorded for each subject:

- Gender: male or female.
- Age
- Language Spoken: English,Spanish, English and Spanish, other(specify)
- Ethnicity: Caucasian, Hispanic, African American, American Indian, Asian/Pacific Islander.

Wound Assessment

Digital Photos & Wound Measurement

The wound will be measured using the eKare InSight. The eKare Insight photo/measurment system is a wound imaging device that quickly, precisely and consistently measures the area, depth and volume of wounds and tracks their healing progress. This technique has been shown to be highly reproducible [120, 121]. The Insight measurement system functions in a similar fashion as an Aranz Unit and within our own group we have published studies on the equivalency of theseis two devices to accurately measure wound size [122].

Granulation Tissue Estimation

Granulation tissue will be estimated at each study visit and recorded as a percentage of wound bed coverage and change in volume measurements.

Human Wound Healing PCR Array

The Human Wound Healing RT² Profiler™ PCR Array profiles the expression of 84 key genes central to the wound healing response. Wound healing progresses via three overlapping phases: inflammation, granulation and tissue remodeling. After cutaneous injury, a blood clot forms, and inflammatory cells infiltrate the wound, secreting cytokines and growth factors to promote the inflammation phase. During the granulation phase, fibroblasts and other cells differentiate into myofibroblasts, which deposit extracellular matrix (ECM) proteins. Simultaneously, angiogenesis occurs, and keratinocytes proliferate and migrate to close the wound. In the final tissue remodeling phase, apoptosis eliminates myofibroblasts and extraneous blood vessels, and the ECM is remodeled to resemble the original tissue. Dysregulation of this last tissue remodeling phase leads to fibrosis. This array contains genes important for each of the three phases of wound healing, including ECM remodeling factors, inflammatory cytokines and chemokines, as well as growth factors and major signaling molecules. Using real-time PCR, you can easily and reliably analyze the expression of a focused panel of genes involved in wound healing, tissue injury and repair with this array. The tissue will be sent to Research and Testing Laboratory in Lubbock, Texas or UTSW core services for evaluation of the following:

Extracellular Matrix and Cell Adhesion

- ECM Components: COL14A1, COL1A1, COL1A2, COL3A1, COL4A1, COL4A3, COL5A1, COL5A2, COL5A3, VTN.
- Remodeling Enzymes: CTSG, CTSK, CTSL2, F13A1, F3 (Tissue Factor), FGA (Fibrinogen), MMP1, MMP2, MMP7, MMP9, PLAT (tPA), PLAU (uPA), PLAUR (uPAR), PLG, SERPINE1 (PAI-1), TIMP1.

  - Cellular Adhesion: CDH1 (E-cadherin), ITGA1, ITGA2, ITGA3, ITGA4, ITGA5, ITGA6, ITGAV, ITGB1, ITGB3, ITGB5, ITGB6.
  - Cytoskeleton: ACTA2 (a-SMA), ACTC1, RAC1, RHOA, TAGLN.
- Inflammatory Cytokines and Chemokines: CCL2 (MCP-1), CCL7 (MCP-3), CD40LG (TNFSF5), CXCL1, CXCL11 (ITAC/IP-9), CXCL2, CXCL5 (ENA-78/LIX), IFNG, IL10, IL1B, IL2, IL4, IL6.
- Growth Factors: ANGPT1, CSF2 (GM-CSF), CSF3 (GCSF), CTGF, EGF, FGF10, FGF2, FGF7, HBEGF (DTR), HGF, IGF1, MIF, PDGFA, TGFA, TGFB1, TNF, VEGFA.
- Signaling Transduction
  - TGFß: TGFB1, TGFBR3, STAT3.
  - WNT: CTNNB1, WISP1, WNT5A.
  - Phosphorylation: MAPK1 (ERK2), MAPK3 (ERK1), PTEN.
  - Receptors: EGFR, IL6ST (GP130).
  - Other: PTGS2.

Vascular Assessment

- Ankle Brachial Index (ABI)
- SensiLase System: The SensiLase System (Väsamed, Eden Prairie, MN) uses laser Doppler technology to assess Skin Perfusion Pressure (SPP), which is a measurement of the pressure at which perfusion first returns to the cutaneous microcirculation following a controlled release of occlusion. After application near the site of interest, a pressure cuff is inflated to stop capillary perfusion and deflated at a controlled rate as the laser sensor under the cuff detects the return of flow. The system calculates the cuff pressure at which capillary flow returns. The system is fully automated, so results are not operator dependent. Unlike most other instruments to evaluate perfusion, there are few site restrictions, so measurements can be taken on the toes and on the sole of the foot. Results are not altered by Monkenberg's arterial sclerosis. This technique has high sensitivity and high positive and negative predictive value to predict amputation [123, 124]. Measurements will be taken at the medial and lateral margins of the wound, 2 cm from the wound edge.
- Hyper spectral Imaging (HSI): HSI provides a novel diagnostic tool that quantifies tissue oxygenation and presents it in an anatomically relevant map. HSI quantifies tissue oxyhemoglobin and deoxyhemoglobin. In a small cohort study of 21 diabetic patients with foot ulcers, Khaodhiar et al reported that baseline hyperspectral index correctly predicted healing in 13 of 14 patients that healed, and it predicted non-healing in 6 of 7 ulcers with low values that did not heal. The sensitivity, specificity, and positive and negative predictive values of the hyperspectral index to predict healing were 93, 86, 93, and 86%. In a larger study of 66 diabetic patients with 73 foot ulcers, Nouvong et al developed a healing index using HSI data and reported a positive predictive value of 90% and a specificity and sensitivity of 74% and 80% (17). These compelling preliminary data show that HSI may represent a novel method to quantify wound perfusion. This may ultimately prove to be a critical

decision-making aid to predicting to help Vascular and Podiatric Surgeons determine whether further revascularization procedures would be necessary.

Neurological Assessment

- Monofilament Sensory Test:
- Vibration Threshold Perception Testing (VPT):
  Neuropathy Disability Score in Patients with Diabetes

Table 1. Neuropathy Disability Score in Patients with Diabetes.*

Sensation	Score
Vibration threshold (apply 128-Hz tuning fork to apex of great toe)	
Normal (can distinguish between presence and absence of vibration)	0
Abnormal	1
Temperature (to dorsum of foot, apply a tuning fork placed in a beaker of ice water or warm water)	
Normal (can distinguish between hot and cold)	0
Abnormal	1
Pinprick (apply pin proximal to great toenail to barely depress skin)	
Normal (can distinguish sharpness or lack of sharpness)	0
Abnormal	1
Achilles' reflex	
Present	0
Present with reinforcement	1
Absent	2
Total for one foot	0–5

* A score (for both feet) of 6 or greater is predictive of foot ulceration. The annual risk of ulceration is 1.1 percent if the score is less than 6 and 6.3 percent if it is greater than or equal to 6.

Safety Measures

Each of the following measures will be rated on the following 5-point Likert scale, as well as a descriptive evaluation being recorded, as applicable, for the study wound: 1) no incidence, 2) mild, 3) moderate, 4) severe, 5) extremely severe

- Erythema
- Discharge/drainage
- Malodor
- Tissue necrosis

Degree of Pain Rating

The subject will record his or her current degree/level of pain for the study wound using the following 10-cm (100 mm) long Visual Analog Scale (VAS) labeled from '0': no pain to '100': worst pain imaginable

No pain 0_____100 Worst Pain Imaginable

The VAS is the most commonly used scale for assessing pain [125]. The VAS is a simple scale that consists of a line anchored at one end by a label that indicates total absence of the measure being evaluated and at the other end by a label that indicates the worse imaginable presence of the measure being evaluated. The subject marks on the line the spot for the intensity of the measure, which is then measured using a ruler (in mm units).

In designing the VAS scale for this study, the standard guidelines for effective use of the VAS were followed, as listed below:

- The line should be 10, 15 or 20 cm long, as other lengths are less reliable.
- There should be a small vertical mark at each end, with numbers 0 and 100, and a verbal description.
- The verbal description must be in absolute terms (e.g. worst pain imaginable);
- The line itself should be clear of any markings and should be horizontal rather than vertical, for more reliable measurements.

Scales developed and used in the above way have been shown to be a proper ratio scale. Like a thermometer, this means that its two ends are rooted, and a doubling of the score does accurately reflect a doubling of the variable. Consequently, sensitive t-tests and ANOVA methods can be used in the analysis, so that significant differences can be identified with relatively small sample sizes or small differences between groups.

Infection Evaluation

Clinical Indicators

The following clinical indicators of significant bacterial infection will be recorded as present or absent for the study wound. 'Significant Bacterial Infection Clinical Indicators' are defined in this study as those presenting as excessive beyond which would typically be expected given the clinical condition and nature of the wound type.

- local heat
- spreading redness
- swelling
- increased purulent exudate (draining pus)
- odor
- cellulitis
- loss of function
- fevers
- chills

Swab Culture:

If evaluation of the clinical indicators suggest the presence of significant bacterial infection of the wound, swab culture, utilizing swab technique [126] will be performed to confirm or reject the presence of significant bacterial infection severe enough to warrant the subject terminated from the study. The swab culture will be performed using a laboratory-provided swab that is pressed down over a 1 cm^2 area of the wound that is then delivered to the lab for analysis to determine Colony-Forming Units (CFUs) per gram of viable wound tissue and beta-hemolytic Streptococcus per gram of tissue. The findings of a positive or negative swab culture utilizing Levine's technique will be interpreted according to the following criteria:

- Positive culture indicating presence of bacterial infection: >= 1,000,000 Colony-Forming Units (CFUs) per gram of viable wound tissue OR containing beta-hemolytic Streptococcus per gram of tissue at any level.
- Negative culture indicating absence of bacterial infection: < 1,000,000 Colony-Forming Units (CFUs) per gram of viable wound tissue OR no beta-hemolytic Streptococcus per gram of tissue at any level.

Tissue specimens will be obtained from the chronic wounds using 1) wound exudate, 2) broad Z-technique, and 3) Levine technique [126]. Acquisition of swab specimens was followed with a wound tissue biopsy. The diagnostic validity of each technique was determined by associating the quantitative culture findings of each swab specimen with the culture findings of concurrent tissue specimen (gold standard). Levine's technique performed best of the three swab techniques in terms of all four validity parameters: sensitivity was, specificity, positive predictive value, and accuracy.

Bio-burden

Bio-burden will be assessed using qPCR to quantify bacterial load from serial tissue specimens. Specific organisms that will be quantified using qPCR are listed in the table below. Bone, soft tissuewill be sent to Research and Testing Laboratory (Lubbock, TX) for analysis.

Specific organisms (and two resistance factors) to be quantified using qPCR.			
E. faecalis	E. faecium	S. epidermidis	M. morganii
K. pneumonia	S. aureus	C. striatum	S. haemolyticus
S. agalactiae	S. marcescens	F. magna	S. mutans
S. pyogenes	H. influenzae	P. mirabilis	Vancomycin res.
C. albicans	M. catarrhalis	C. parapsilosis	Methicillin res.
P. aeruginosa	S. pneumoniae	A. baumanii	Total Bioburden

Osteomyelitis

Osteomyelitis will be diagnosed when radiographic and clinical signs are consistent with bone infection. This diagnosis will be confirmed with imaging, bone biopsy, culture or histopathology as most applicable to the individual subject. The bacterial pathogens and sensitivities from bone and soft tissue cultures will be recorded and direct antibiotic therapy applied, if necessary [79].

Study Procedures

Procedures and Evaluations During the Research

The time period from Day 0 to up to 12 weeks will encompass the study therapy administration and evaluation phase. The precise therapy employed will be determined according to subject randomized group allocation, and the duration of the study therapy administration phase will be determined according to individual subject wound healing and status progression and safety evaluations. The study therapy administration phase will comprise the

following progressive activities, as applicable, and will be initiated in the inpatient clinic setting: therapy administration, , and weekly or standard of care wound healing and safety evaluation. The study therapies are delivered in continuous mode throughout the individual subject's entire study therapy administration phase; with the only consistent interruption being for the three times a week wound dressing changes. There will likely be other minimal daily interruptions such as to allow for subject showering and bathing. Therapy administration phase will continue until study wound is ready for closure by secondary intention, after which time the therapy will be discontinued.

Therapy Administration

- Therapy phase to be continued until wound is ready for closure by secondary intention
    - During this therapy administration phase, all subjects will receive NPWT therapy for 4 weeks or until the wound is deemed ready for surgical closure, whichever occurs first. This time point will serve as the individual subject's study evaluation endpoint.
    - Study wound readiness for surgical closure in this study is defined as the time point (evaluation visit) at which the following three factors pertaining to the status of the study wound are determined to co-exist by the study investigator:
        - deep structures (bone, tendon, joint) are covered with granulation tissue
        - the wound depth is < 2 mm
        - the wound bed is ready for skin graft, delayed primary closure or rotational flap to cover the defect.
    - It is anticipated that most wounds will be determined ready for surgical wound closure by 4 weeks of therapy administration; however this may occur as early as after 2 weeks or subjects may be followed up to the full 12 weeks, and closure may occur anywhere inbetween (to be determined by investigator). It is also possible for some subjects that the wound may not be ready for closure even after 12 weeks. These subjects will be discharged from the study at that time and continue appropriate care for their wound with their physician.
    - The surgical wound closure procedure is performed in the operating room, using the most applicable of the following common mechanisms for closure, as is optimal for the individual subject and wound: delayed primary closure, rotational flap to close the wound, split thickness skin graft.
- Post Wound Closure Evlauation Phase
    - Following surgical closure of the wound, the subject will enter the post wound closure evaluation phase. This phase will last up to week 12 following study enrollment and therapy administration start, such that the duration of the post wound closure evaluation phase will vary for each subject according to the time point at which their wound was deemed ready for surgical closure. For example, a subject whose wound is surgically closed after week 4 of the therapy administration phase will enter a post wound closure evaluation phase that spans from week 5 through week 12, at which time the subject will exit the study. A subject whose wound does not close sufficiently for surgical repair by week 12 will exit the study at that time without entering the post wound closure evaluation phase.
    - The post wound closure evaluation phase will comprise weekly or standard of care evaluations in the outpatient clinic setting. If the wound remains closed with no sign of re-opening or infection, no further efficacy or safety evaluations will be taken at that visit. At post therapy

administration phase completion (as applicable to the individual subject), a standard of care blood draw will again be taken as at the baseline evaluation to assess lab markers.

- If at any of the post wound closure evaluation visits, the wound is found to have re-opened and/or shows signs of infection, the following will occur at the discretion of the physician:
  - Measurements of dehiscence will be taken
  - Surgical debridement of the wound will be done
  - NPWT only will be reapplied per standard of care
  - The subject will re-enter the therapy administration phase including the weekly or standard of care efficacy and safety evaluation test site visits and procedures.

Home Health Visits

The study therapy will only be given while the subject is in the hospital. If the subject's wound is not ready for closure during the hospital stay, the subject will continue NPWT at home. NPWT at home will be without irrigation. If the subject continues to receive NPWT after hospital discharge, the subject will be seen twice weekly by a home health nurse for dressing changes. The home health nurse will collect sitting blood pressure and pulse rate. Amount, type and character of wound drainage will be documented, as well as any adverse events and changes to concomitant medications. Offloading will be reapplied after dressing changes. At the same time, subject will continue to be followed by the study doctor during regular post-operative visits at outpatient clinic. If the wound closes ,End of study visit will be performed and she/he will see the study doctor at outpatient clinic to have the closed wound checked.

Safety and Efficacy Evaluation

During the study therapy administration and evaluation phase, the subject will attend once weekly or standard of care test site visits, commencing at the end of Week 1 of therapy administration and ending at the weekly or standard of care visit where the wound is determined ready for surgical closure and the therapy administration process is complete.

- Weekly or standard of care Efficacy Evaluations
  - Digital Photography of the Study Wound
  - Wound Measurement (using a ruler and the eKare Insight System)
  - Granulation Tissue Estimation
  - Additionally, at the final week of efficacy evaluation test site evaluation, Subject Satisfaction with Study Procedure Outcome Evaluation will be performed.
- Weekly or standard of care Safety Evaluations
  - Safety Measures
  - Debridement Indication
  - Degree of Pain Rating
  - Infection Evaluation: encompassing swab culture and osteomyelitis assessment, if indicated.
- Inpatient Tissue Samples
  - Tissue samples will be taken and kept, for research, at inpatient study visits prior to wound closure for the following analyses.
    - For bacterial analyses
    - For gene expression analyses
  - After wound closure, no samples will be taken unless wound reoccurrence occurs.
  - Samples will be approximately 0.25-0.5 cm^3 in size

- Bio-burden**:** qPCR evaluation
- Other Activities
  - Changing of the wound dressing
  - Wound Debridement, if indicated

Post Procedure Administration Activities

- Following surgical closure of the wound, the subject will enter the post wound closure evaluation phase. This phase will last up to week 12 following study enrollment and therapy administration start, such that the duration of the post wound closure evaluation phase will vary for each subject according to the time point at which their wound was deemed ready for surgical closure. For example, a subject whose wound is surgically closed after week 4 of the therapy administration phase will enter a post wound closure evaluation phase that spans from week 5 through week 12, at which time the subject will exit the study. A subject whose wound does not close sufficiently for surgical repair by week 12 will exit the study at that time without entering the post wound closure evaluation phase.
- The post wound closure evaluation phase will comprise weekly or standard of care evaluations in the outpatient clinic setting. If the wound remains closed with no sign of re-opening or infection, no further efficacy or safety evaluations will be taken at that visit. At post therapy administration phase completion (as applicable to the individual subject), standard of care blood draw(s) will again be taken as at the baseline evaluation to assess lab markers.
- If at any of the post wound closure evaluation visits, the wound is found to have re-opened and/or shows signs of infection, the following will occur:
  - Measurements of dehiscence will be taken
  - Surgical debridement of the wound will be done
  - NPWT only will be reapplied
  - The subject will re-enter the therapy administration phase including the weekly or standard of care efficacy and safety evaluation test site visits and procedures.

Standard of Care Regimen

Standard of care procedures are intended to optimize conditions for wound healing and to ensure that a subject's safety is not compromised by taking part in this clinical study.

- <u>During the Procedure Administration Phase</u>: Negative Pressure Wound Therapy is considered standard of care for the wound types to be treated and evaluated in this clinical study. All subjects in this clinical study will receive active NPWT for his or her wound during the procedure administration phase regardless of whether he or she is randomized to the treatment or to the control procedure group.
- <u>Throughout the Procedure Administration and Post-Procedure Follow-Up Phases</u>: The following additional standard of care procedures will be applied to all subjects in this clinical study regardless of procedure group allocation across the entire duration of his or her participation in the clinical study.
  - *Wound Dressings*: Wound dressings will be changed three times each week at the test site. Wound dressings for each patient will be selected based on the therapy group to which the patient is assigned and applied per manufacturers and clinicians recommendations.
  - *Off-loading*: Off-loading, where applicable, will include use of healing sandals, removable cast boots or total contact casts, selected according to the site of the wound, postural stability of the patient, obesity status, and the subject's living condition and driving needs.

- *Surgical Wound Debridement*: Subject wounds will be continually evaluated for devitalized tissue in the wound bed and the consequent need for debridement. When the need is identified, surgical debridement will be performed on the wound. Surgical debridement involves the excision/removal of necrosis and fibrin coatings with the aid of a scalpel, scissors, sharp curette or laser, under surgical conditions or as an outpatient depending on the wound condition. It is regarded as the fastest and most effective debridement method because necrotic and infectious material is removed out of the wound "abruptly". It is considered mandatory for controlling severe infections.
  - Debridement will *only be performed* on an ulcer if indicated. Such indications include the presence upon physical examination of the ulcer of necrotic tissue, sinus tracts, exudation or transudation, and infection.
  - Debridement will *not be performed* on any ulcer for which the need for the procedure is not indicated or is contraindicated.

Schedule of Events

Protocol Activity	Screening	Baseline Day 0 – Day of Surgery	Week 1 Inpatient (may continue into Week 2)	Home Health Visits Weeks 2-4	Weeks 2-11 Foot Wound Clinic	End of Study-Week 12[2]	Notes
Informed Consent	X						
Demographics and Medical/Surgical History	X						
Sitting blood pressure & pulse rate	X						
Physical Exam	X					X (brief)	
Inclusion/Exclusion Criteria	X						
Weight/Height	X						
Lab Values	X				X*	X*	At screening and then * at (SOC)
Vascular/Neurological Evaluation	X						ABI,VPT and Monofilament Test at screening; VasoMed to occur prior to surgery
Wound Assessment/ Debridement	X	X	X		X		
Tissue /bone[1]/ wound culture samples for qPCR cultures & bioburden analysis		X	X (Subsequent surgery				

			while Inpatient)				
NPWT/NWPT (i) randomization/dressing application/changes		X	X	X (Weeks 2-4)	X (Weeks 2-4)		NWPT for maximum 4 weeks after baseline(Day0 surgery) if wound is not closed in the hospital
SF-36 Questionnaire	X					X	
Hyperspectral Imaging Analysis	X						To occur prior to surgery
Discharge Instructions			X		X		
Offloading			X	X	X		At hospital discharge & continuing to Week 12
Adverse Events		X	X	X	X	X	
Current/concomitant antibiotics	X	X	X	X	X	X	
Wound imaging/measurement		X	X		X	X	While active study wound is present
Neuropathy Disability Score in Patients with Diabetes	X						

[1] Only if performed as a necessary part of standard of care.
[2] Subjects who heal before week 12 will exit the study and complete EOS visit.

Biostatistics

Sample Size Justification:

There are no published data with the Cardinal PRO negative pressure wound therapy (NPWT) device with or without irrigation in order to determine an estimate of the sample size for this study. One of the purposes of this project is to obtain data, so we can power a larger multicenter study. Even though the KCI NPWT device with irrigation has been commercially available, there is little data about volume change or wound healing in the population we are studying.

The previous sample size justification was based on a comparison of the proportion of wounds expected to achieve surgical closure. The proportion of subjects with closed wound was 62% using traditional KCI NPWT and 94% using NPWT with periodic irrigations with Prontosan. For the sample size, we estimated 85-90% wound closure with NPWT with irrigation and 60% wound closure with NPWT without irrigation. We assume the NPWT devices (KCI and Cardinal) without irrigation would have similar results.

Equations

The following formula was used to estimate the required sample size, *n*, for the relative difference in proportions P_1 and P_2.

$$n = \frac{\left(Z_{\alpha/2}\sqrt{2P(1-P)} + Z_{\beta}\sqrt{P_1(1-P_1)+P_2(1-P_2)}\right)^2}{(P_1-P_2)^2}$$

where

$$P = \frac{P_1 + P_2}{2}$$

Using the formula above and assuming 90% wound closure with NPWT with irrigation and 60% wound closure with NPWT without irrigation and a type I error of 0.1, the required sample size is about 25. If a Type I error of 0.05 is assumed, then the required sample size is about 31.
As the relative difference gets smaller, the required sample size increases. The two tables below show estimates for sample size for values of alpha of 5% and 10% for five values of P_2.

P_1	P_2	rel. difference	α /2	β	power	n
0.6	0.9	-0.75	.05	0.200	0.800	25
0.6	0.89	-0.73	.05	0.200	0.800	27
0.6	0.88	-0.70	.05	0.200	0.800	29
0.6	0.86	-0.65	.05	0.200	0.800	35
0.6	**0.85**	-0.63	.05	0.200	0.800	41

P_1	P_2	rel. difference	α/2	β	power	n
0.6	0.9	-0.75	.025	0.200	0.800	31
0.6	0.89	-0.73	.025	0.200	0.800	34
0.6	0.88	-0.70	.025	0.200	0.800	37
0.6	0.86	-0.65	.025	0.200	0.800	45
0.6	**0.85**	-0.63	.025	0.200	0.800	51

PRELIMINARY DATA: Kim PJ, Attinger CE, Steinberg JS, Evans KK, Powers KA, Hung RW, Smith JR, Rocha ZM, Lavery L. The impact of negative-pressure wound therapy with instillation compared with standard negative-pressure wound therapy: a retrospective, historical, cohort, controlled study. Plast Reconstr Surg. 2014 Mar;133(3):709-16.

This study shows that NPWT with two different doses of Prontosan irrigation solution decreases the number of surgeries, length of hospitalization, and increases the proportion of wounds that are closed compared to "traditional NPWT'. We conducted a retrospective study of hospitalized patients with infected lower extremity wounds that received NPWT without irrigation (n=74), NPWT with irrigation with polyhexanide biguanide (PHMB) with a 6 minutes dwell time (n=34),and NPWT with irrigation with polyhexanide biguanide (PHMB) with a 20 minute dwell time (n=34). The proportion of wounds that were surgically closed was significantly higher, and the number of surgeries was significantly less in patients that received NPWT with 6 minutes of irrigation compared to standard NPWT without irrigation. Similar trends were seen with NPWT with 20 minutes of irrigation.

Table 1	NPWT n=74	NPWT 6 minute irrigation n=34		NPWT 20 minute irrigation n=34	
Number of Surgeries	3.0 ± 0.9	2.4 ± 0.9	p=0.04	2.6 ± 0.9	p=0.003
Length of Stay (days)	14.9± 9.2	11.9 ± 7.8	p=0.10	11.4 ± 5.1	p=0.03
Time to Final Surgical Procedure	9.23 ± 5.2	7.8 ± 5.2	p=0.04	7.5 ± 3.1	p=0.002
Percent Closed	62%	94%	p<0.001	80%	p=0.08

The inpatient stay and post-operative visits are covered under a 90-day global payment which is inclusive of all procedures, devices, testing and ancillaries associated with the surgical procedure, including NPWT. Subjects' insurance will not be charged any additional amount for the NPWT devices used in the study. The KCI devices are standard at Parkland; Cardinal is providing their devices. But again, in neither situation would a subject or their third party pay or be charged anything additional.

Survival Analyses Models

The primary analytical method presented in this report is based on a time-to-event strategy using Kaplan-Meier estimates, followed by a log-rank test. This statistical procedure provides a comparison of the distribution of events between the 2 treatment groups. In addition to the event rates calculated for the post-baseline time points, the median time to 100% closure, 75% closure, and 76-100% wound bed granulation will also be calculated. This same methodology will also be used to compare the overall duration of treatment, and within the individual patient subsets defined by their outcome status.

A Cox proportional hazard model will be used to relate time to specific events to various risk factors. The main modeling strategies will look at the time to adverse events with time being measured from the time of surgery or the time of NPWT application until either an adverse event occurs, the patient is considered healed, or the end of the study. Cox proportional hazard model allows the independent variables to be continuous covariates or categorical or binary factors. The type of NPWT treatment will be the main independent variable. We will use multivariate proportional hazard regression analysis methods to investigate what factors are significant for non-healing patients or patients that have complications. Similar to the logistic regression, maximum likelihood estimates of model parameters along with tail probabilities (p-values) and confidence intervals for parameters

will be derived based on asymptotic inferences. For the proportional hazard model the ratio of the hazards for two subjects one with and one without diabetes, given that the subjects have the same values for other parameters can be estimated from the model coefficients. Repeated measures ANOVA and ANOVA will be used to evaluate changes in quantitative cultures and laser Doppler measurements over time and between treatment groups.

Continuous demographic parameters, such as the patient's age at the time of enrollment, will be summarized for the population using descriptive statistics (N, mean, median, standard deviation, minimum and maximum value, and 95% 2-sided confidence limits) and compared between groups using a 2-sample t-test. Categorical demographic parameters, such as gender, were summarized as a proportion of the intent-to-treat (ITT) population and compared using a 2-tailed Fisher's exact test. Co-morbid risk factors will be summarized for the ITT population by treatment assignment and according to the type of variable (categorical, continuous) and compared between groups. Relative risk will be evaluated using a logistic regression model.

For direct comparisons of the NPWT PRO and KCI Ulta systems, a traditional non-inferiority statistical procedure will be performed. It will be assumed that the efficacy of the NPWT PRO device in wound outcomes is not inferior to the KCI device if it performs within $\pm$10% of the performance of the KCI Ulta system.

Microbial Diversity

Results from the qPCR assessment of bio-burden will be analyzed using standard multivariate approaches. Multivariate differences in bacterial composition among groups will be evaluated using distance based redundancy analysis (dbRDA) [128]. For the dbRDA, distances among samples first will be calculated using unweighted UniFrac distances. UniFrac distances are a robust phylogenetic measure that is useful for comparing microbial communities [129]. An ANOVA-like simulation will then be used to test for group differences.

Facilities

The University of Texas Southwestern Medical Center's Musculoskeletal and Vascular Effectiveness Research Collaboration (MAVERIC) group has 1,200 square feet of dedicated research and clinic space. We have office space for four investigators and seven research coordinators and two nurse coordinators. We have arterial Doppler systems, vibration perception threshold testing equipment and computer workstations. Research patients are seen at the NIH sponsored research center at the University of Texas (5 treatment rooms, laboratory) and the Ambulatory Surgery Center at Parkland Hospital (6 treatment rooms).

- *Human Subjects Research*: The proposed involvement of human subjects, characteristics of the subject population, inclusion/exclusion criteria, and recruitment and consent procedures are described in the Experimental Design and Methods section of this proposal.
- *Institutional Review Board*: Our Research protocol has been approved by the Institutional Review Board (DHHS IRB Registration No. 00001230). We have not request a waiver of the elements of consent.
- *Informed Consent*: Once a potential study subject is identified, one of the investigators or research staff will discuss the study design, duration of the study, and risks with the patient. We will then provide the subject with a consent form to read. The investigator or study coordinator will be available to answer questions or provide more explanation as requested by the potential study subject and their family. The

emergency department and diabetic foot clinics will be the primary sources for recruitment and screening.

- *Inclusion of Children*: We will not include children in the study because NPWT is not indicated for use in the pediatric population.

Subject Safety and Data Monitoring

All devices used in this comparative effectiveness trial are FDA approved, thus we will not include a formal data safety monitoring board in this study. However, to assure the safety of subjects in this study, an independent review of both aggregate and by treatment group in a blinded fashion each six months, or more often as needed, will be performed by an independent safety monitor, George Liu, DPM.

Adverse Events

All observed or volunteered adverse events regardless of treatment group or suspected causal relationship to the investigational product(s) will be reported as described in the following sections.

For all adverse events, the investigator must pursue and obtain information adequate both to determine the outcome of the adverse event and to assess whether it meets the criteria for classification as a serious adverse event requiring immediate notification to the Cardinal Health contact. For all adverse events, sufficient information should be obtained by the investigator to determine the causality of the adverse event. The investigator is required to assess causality. For adverse events with a causal relationship to the device, follow-up by the investigator is required until the event or its sequelae resolve or stabilize at a level acceptable to the investigator, and the Cardinal Health contact concurs with that assessment.

Reporting Period

For serious adverse events, the reporting period to Cardinal Health or its designated representative begins from the time that the subject provides informed consent, which is obtained prior to the subject's participation in the study, i.e., prior to undergoing any study-related procedure and/or receiving investigational product, through and including 28 calendar days after the last administration of the investigational product. Any serious adverse event occurring any time after the reporting period must be promptly reported if a causal relationship to investigational product is suspected.

Adverse events (serious and non-serious) should be recorded on the CRF from the time the subject has undergone one treatment through last subject visit.

Definition of an Adverse Event (AE)

An adverse event is any untoward medical occurrence in a clinical investigation subject administered a product or medical device; the event need not necessarily have a causal relationship with the treatment or usage. Examples of adverse events include but are not limited to:

- Abnormal test findings;
- Clinically significant symptoms and signs;
- Changes in physical examination findings;
- Hypersensitivity;

- Progression/worsening of wound.

Definition of Serious Adverse Event (SAE)

A Serious Adverse Event (SAE) is any serious unfavorable and unintended sign, symptom, or disease temporally associated with the use of the devices, whether or not considered related, including those that:

- results in death
- is life-threatening
- requires inpatient hospitalization or causes prolongation of existing hospitalization
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect
- requires intervention to prevent permanent impairment or damage

Medical and scientific judgment should be exercised in determining whether an event is an important medical event. An important medical event may not be immediately life-threatening and/or result in death or hospitalization. However, the important medical event should be reported as serious, if it is determined that the event may jeopardize the subject and/or may require intervention to prevent one of the other adverse event outcomes.

Causality Assessment of Adverse Events

The investigator's assessment of causality must be provided for all adverse events (serious and non-serious); the investigator must record the causal relationship in the CRF, as appropriate, and report such an assessment in accordance with the serious adverse reporting requirements if applicable. An investigator's causality assessment is the determination of whether there exists a reasonable possibility that the cleared medical device caused or contributed to an adverse event. If the investigator does not know whether or not medical device caused the event, then the event will be handled as "related to medical device" for reporting purposes. (see Section on Reporting Requirements). If the investigator's causality assessment is "unknown but not related to investigational product", this should be clearly documented on study records.

In addition, if the investigator determines a serious adverse event is associated with study procedures, the investigator must record this causal relationship in the source documents and CRF, as appropriate, and report such an assessment in accordance with the serious adverse event reporting requirements, if applicable.

Adverse Event Severity Assessment

The Investigator will provide an assessment of the severity of each adverse reaction by recording a severity rating on the appropriate SAE reporting page of the subject's file. Severity, which is a description of the intensity of manifestation of the SAE, is distinct from seriousness, which implies a patient outcome or SAE-required treatment measure associated with a threat to life or functionality. Severity will be assessed according to the following scale.

If required on the adverse event case report forms, the investigator will use the adjectives MILD, MODERATE, or SEVERE to describe the maximum intensity of the adverse event. For purposes of consistency, these intensity grades are defined as follows:	
MILD	Does not interfere with subject's usual function.
MODERATE	Interferes to some extent with subject's usual function.
SEVERE	Interferes significantly with subject's usual function.

Note the distinction between the severity and the seriousness of an adverse event. A severe event is not necessarily a serious event. For example, a headache may be severe (interferes significantly with subject's usual function) but would not be classified as serious unless it met one of the criteria for serious adverse events, listed above.

Withdrawal Due to Adverse Events (See Also Section on Subject Withdrawal)

Withdrawal due to adverse event should be distinguished from withdrawal due to insufficient response, according to the definition of adverse event noted earlier, and recorded on the appropriate adverse event CRF page.

When a subject withdraws due to a serious adverse event, the serious adverse event must be reported in accordance with the reporting requirements defined below.

Eliciting Adverse Event Information

The investigator is to report all directly observed adverse events and all adverse events spontaneously reported by the study subject. In addition, each study subject will be questioned about adverse events.

Reporting Requirements

Each adverse event is to be assessed to determine if it meets the criteria for serious adverse events. If a serious adverse event occurs, expedited reporting will follow local and international regulations, as appropriate.

Serious Adverse Event Reporting Requirements

If a serious adverse event occurs, Cardinal Health is to be notified within 24 hours of awareness of the event by the investigator. In particular, if the serious adverse event is fatal or life-threatening, notification to Cardinal Health must be made immediately, irrespective of the extent of available adverse event information. This timeframe also applies to additional new information (follow-up) on previously forwarded serious adverse event reports as well as to the initial and follow-up reporting of Exposure during pregnancy cases.

In the rare event that the investigator does not become aware of the occurrence of a serious adverse event immediately (e.g., if an outpatient study subject initially seeks treatment elsewhere), the investigator is to report the event within 24 hours after learning of it and document the time of his/her first awareness of the adverse event.

For all serious adverse events, the investigator is obligated to pursue and provide information to Cardinal Health in accordance with the timeframes for reporting specified above. In addition, an investigator may be requested by Cardinal Health to obtain specific additional follow-up information in an expedited fashion. This information may be more detailed than that captured on the adverse event case report form. In general, this will include a description of the adverse event in sufficient detail to allow for a complete medical assessment of the case and independent determination of possible causality. Information on other possible causes of the event, such as concomitant medications and illnesses must be provided. In the case of a subject death, a summary of available autopsy findings must be submitted as soon as possible to Cardinal Health or its designated representative.

Non-Serious Adverse Event Reporting Requirements

All adverse events will be reported on the adverse event page(s) of the CRF. It should be noted that the form for collection of serious adverse event information is not the same as the adverse event CRF. Where the same data are collected, the forms must be completed in a consistent manner. For example, the same adverse event term should be used on both forms. Adverse events should be reported using concise medical terminology on the CRFs as well as on the form for collection of serious adverse event information.

Reporting Requirements to Regulatory Authorities

Adverse events reporting, including suspected serious unexpected adverse reactions, will be carried out in accordance with applicable local regulations.

Potential Risks of Study Participation

Participation in this clinical investigation presents low risks to subjects. Some risks are generalized and are listed in Tables below.

Potential Risks Associated with the Investigational Product

All risks known or anticipated based on prior experience, risk analysis or reasonable grounds are listed in the Instructions for Use. This protocol document will be updated in the presence of new information of relevance to the safety of the subject or the conduct of the study.

Risks	Disorders/Conditions
Skin and Subcutaneous Tissue Reaction/Allergy	• Desiccation/injury • Skin rash, irritation, blistering • Pruritus/itching • Skin excoriation/breakdown • Skin stripping • Skin scarring if significant skin irritation were to occur • Maceration • Skin hyper/hypo-pigmentation at and/or around dressing application area • Erythema/redness, edema, inflammation, or swelling at and/or around dressing application area
Mild Pain or Discomfort	• Tenderness/minor ache at and/or around dressing application area

	• Perspiration associated with wearing dressing • Auditory irritation (due to mild buzzing sound of negative pressure unit) • Decreased sleep or sleep quality • Paresthesia (numbness, tingling, prickling, creeping sensation)
Other	• Bleeding • Cardiac compromise (vagal response, bradycardia) • Pulmonary compromise • Accidental instillation in a body cavity • Localized infection • Autonomic dysreflexia (in subjects with spinal cord injury) • Retained foreign debris (e.g.foam) in the wound • Impairment of mobility/activity (limitation secondary to weight and attachment of therapy unit) • Possible tubing entanglement/trip or slip hazard leading to fracture, tissue damage • Incorrect therapy unit settings resulting in incorrect frequency/dosing • Tunneling • Stalled healing/non-progression of healing • Deterioration of the wound • Systemic reaction (due to allergic reaction to dressing materials) • Burn secondary to therapy unit or power cord malfunction

Risk Related to Participation in this Study

Study participants will be required to have timed blood draws during inpatient care.

Risks	**Disorders/Conditions**
Skin and Subcutaneous Tissue Reaction	Erythema/redness, swelling, rash/hives Skin irritation Pruritus/skin itching Skin excoriation/breakdown Skin blistering Skin scarring if significant skin irritation were to occur Bruising/Petechiae
Mild Pain or Discomfort	Burning sensation Pinching sensation

	Cool sensation Tenderness/minor ache at the puncture site Paresthesia (numbness, tingling, prickling)
Other	Bleeding Infection Fainting Nerve damage Seizure

Confidentiality of Protected Health Information (PHI)

Protected health information (PHI) of clinical investigation Subjects are kept as confidential as possible in accordance with the Health Insurance Portability and Accountability Act of 1996 (HIPAA). However, confidentiality cannot be assured.

Data Collection, Retention and Monitoring

Data Collection Instruments

The Investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each subject treated with the study drug.

Study personnel at each site will enter data from source documents corresponding to a subject's visit into the protocol-specific electronic Case Report Form (eCRF) OR paper CRF when the information corresponding to that visit is available. Subjects will not be identified by name in the study database or on any study documents to be reviewed by the Cardinal Health contact, but will be identified by a subject number and initials.

For eCRFs: If a correction is required for an eCRF, the time and date stamps track the person entering or updating eCRF data and creates an electronic audit trail. *For paper CRFs:* If a correction is made on a CRF, the study staff member will line through the incorrect data, write in the correct data and initial and date the change.

The Investigator is responsible for all information collected on subjects enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the Investigator. A copy of the CRF will remain at the Investigator's site at the completion of the study.

Data Management Procedures

The data will be entered into the validated database,SPSS. All procedures for the handling and analysis of data will be conducted using good computing practices meeting FDA guidelines for the handling and analysis of data for clinical trials.

Archival of Data

The database is safeguarded against unauthorized access by established security procedures; appropriate backup copies of the database and related software files will be maintained. Databases are backed up by the database administrator in conjunction with any updates or changes to the database.

At critical junctures of the protocol (e.g., production of interim reports and final reports), data for analysis is locked and cleaned per established procedures.

Availability and Retention of Investigational Records

The Investigator must make study data accessible to a study monitor or other authorized representative of Cardinal, IRB/IEC, and Regulatory Agency (e.g., FDA) inspectors upon request. A file for each subject must be maintained that includes the signed Informed Consent, HIPAA Authorization and Assent Form and copies of all source documentation related to that subject. The Investigator must ensure the reliability and availability of source documents from which the information on the CRF was derived.

Waiver of HIPAA Authorization for Subjects not Recruited for Study

A partial HIPAA waiver will be utilized for patients during initial screening and will cover the collection of data to determine eligibility and/or recruit potential research subjects. Authorization by the subject will be obtained at the time of consent.

The types of PHI that will be collected are as follows:

- Past Medical History
- Antibiotic List
- Demographic Information
- Imaging Results
- Laboratory Results
- Physical exams

The essential documents for patients who are screened but not eligibility for study enrollment will be maintained securely for 6 months. The documents will be shredded and disposed of for individuals not enrolled in the study in 6 months. The essential documents for participants who are screened and then enrolled in the study will be filed in the individual's research file. The HIPAA Authorization covers all PHI collected through the course of the participation in the study in addition to that which is covered through the screening process.

The PHI will be protected to prevent subject identifiers from improper use and disclosure via the following:

- All electronic study data will be password protected
- Passwords will be changed on a regular basis
- Access to study data will be restricted to authorized study personnel only
- All paper study records will be kept in locked file cabinets and access liited to authorized study personnel only.

Monitoring

Monitoring visits will be conducted by representatives of Cardinal Health according to the U.S. CFR Title 21 Parts 50, 56, and 312 and ICH Guidelines for GCP (E6). By signing this protocol, the Investigator grants permission to

the Cardinal Health contact and appropriate regulatory authorities to conduct on-site monitoring and/or auditing of all appropriate study documentation.

Subject Confidentiality

In order to maintain subject confidentiality, only a site number, subject number and subject initials will identify all study subjects on CRFs and other documentation reviewed or submitted to the Cardinal Health contact. Additional subject confidentiality issues (if applicable) are covered in the Clinical Study Agreement.

Administrative, Ethical, and Regulatory Considerations

The study will be conducted according to the Declaration of Helsinki, Protection of Human Volunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312).

To maintain confidentiality, all laboratory specimens, evaluation forms, reports and other records will be identified by a coded number and initials only. All study records will be kept in a locked file cabinet and code sheets linking a patient's name to a patient identification number will be stored separately in another locked file cabinet. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the FDA. The Investigator must also comply with all applicable privacy regulations (e.g., Health Insurance Portability and Accountability Act of 1996, EU Data Protection Directive 95/46/EC).

Protocol Amendments

Any amendment to the protocol will be written by the investigator and Cardinal Health contact. Protocol amendments cannot be implemented without prior written IRB/IEC approval except as necessary to eliminate immediate safety hazards to patients. A protocol amendment intended to eliminate an apparent immediate hazard to patients may be implemented immediately, provided the IRBs are notified within five working days.

Institutional Review Board and Independent Ethics Committees

The protocol and consent form will be reviewed and approved by the IRB/IEC of each participating center prior to study initiation. Serious adverse experiences regardless of causality will be reported to the IRB/IEC in accordance with the standard operating procedures and policies of the IRB/IEC, and the Investigator will keep the IRB/IEC informed as to the progress of the study. The Investigator will obtain assurance of IRB/IEC compliance with regulations.

Any documents that the IRB/IEC may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning patient recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB/IEC. The IRB/IECs written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated. The IRB/IECs unconditional approval statement will be transmitted by the Investigator to Cardinal Health contact prior to the shipment of study supplies to the site. This approval must refer to the study by exact protocol title and number and should identify the documents reviewed and the date of review.

Protocol and/or informed consent modifications or changes may not be initiated without prior written IRB/IEC approval except when necessary to eliminate immediate hazards to the patients or when the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the IRB/IEC and written verification that the modification was submitted and subsequently approved should be obtained.

The IRB/IEC must be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the patients of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

Informed Consent Form

Informed consent will be obtained in accordance with the Declaration of Helsinki, ICH GCP, US Code of Federal Regulations for Protection of Human Subjects (21 CFR 50.25[a,b], CFR 50.27, and CFR Part 56, Subpart A), the Health Insurance Portability and Accountability Act (HIPAA, if applicable), and local regulations.

The Investigator will prepare the informed consent form, assent and HIPAA authorization and provide the documents to the Cardinal Health contact for approval prior to submission to the IRB/IEC. The consent form generated by the Investigator must be acceptable to the Cardinal Health contact and be approved by the IRB/IEC. The written consent document will embody the elements of informed consent as described in the International Conference on Harmonisation and will also comply with local regulations. The Investigator will send an IRB/IEC-approved copy of the Informed Consent Form to the Cardinal Health contact for the study file.

A properly executed, written, informed consent will be obtained from each subject prior to entering the subject into the trial. Information should be given in both oral and written form and subjects (or their legal representatives) must be given ample opportunity to inquire about details of the study. If appropriate and required by the local IRB/IEC, assent from the subject will also be obtained. If a subject is unable to sign the informed consent form (ICF) and the HIPAA authorization, a legal representative may sign for the subject. A copy of the signed consent form (and assent) will be given to the subject or legal representative of the subject and the original will be maintained with the subject's records.

Publications

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement among the investigator and the Cardinal Health contact. The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

Investigator Responsibilities

By signing the Agreement of Investigator form, the Investigator agrees to:

1. Conduct the study in accordance with the protocol and only make changes after notifying the Cardinal Health contact, except when to protect the safety, rights or welfare of subjects.
2. Personally conduct or supervise the study (or investigation).
3. Ensure that the requirements relating to obtaining informed consent and IRB review and approval meet federal guidelines, as stated in § 21 CFR, parts 50 and 56.
4. Report to the Cardinal Health contact any AEs that occur in the course of the study, in accordance with §21 CFR 312.64.
5. Ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.

6. Maintain adequate and accurate records in accordance with §21 CFR 312.62 and to make those records available for inspection by the Cardinal Health contact.
7. Ensure that an IRB that complies with the requirements of §21 CFR part 56 will be responsible for initial and continuing review and approval of the clinical study.
8. Promptly report to the IRB and the Cardinal Health contact all changes in the research activity and all unanticipated problems involving risks to subjects or others (to include amendments and IND safety reports).
9. Seek IRB approval before any changes are made in the research study, except when necessary to eliminate hazards to the patients/subjects.
10. Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in § 21 CFR part 312.

12 Month Extension

After the end of the defined study period, the patients will be followed observationally by their electronic medical records for 12 months as it would not be feasible to ask them to return for an in-person evaluation as: patients have moved, no longer have correct contact information, as well as it would create a financial hardship on the patients to return for further visits. In order to more appropriately determine long term results from participation, a longer period of time of follow up is necessary. Related outcomes that we would like to follow over a 12-month duration are incidence of new foot ulcerations, re-ulceration of a previously healed foot wound, healing and time to healing of patients who took longer than the current study allows, duration of antibiotics received for foot infections, re-admissions to the hospital, need for subsequent surgery, amputation, loss of limb, and death. This expanded follow up period would provide valuable information to the long-term outcomes and complications of this high risk population and will help direct how future efforts may be better focused to reduce complications and improve outcomes.

Sending Samples and Data for Analysis

For analysis purposes, de-identified tissue samples and de-identified data will be shipped to an institution in Australia that has the ability to perform the appropriate analysis on the samples and correlate it to the data. As all samples and data will be de-identified, subjects will not need to be re-consented. Ingham Institute of Applied Medical Research, 1 Campbell Street, Liverpool, Sydney, Australia.

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