A Prospective, Randomized Controlled Trial Evaluating the Effectiveness of the Dolphin Fluid Immersion Simulation® System versus Air Fluidized Systems in the Acute Post-Operative Management of Surgically Closed Pressure Ulcers

Purpose: To examine the effectiveness of the Dolphin Fluid Immersion Simulation® System compared to air fluidized systems in the acute post-operative management of pressure ulcers

Product: Dolphin Fluid Immersion Simulation® System

Protocol Number: 01.5

Date: 3-1-16

Protocol Version: 1.9

Sponsor: Joerns Healthcare, LLC

Contact Information

Sponsor Approval

Study Synopsis

Sponsor: Joerns Healthcare, LLC

Protocol Number: 01.5

Product: Dolphin Fluid Immersion Simulation® System

Regulatory Class: II

Development Phase: Commercialization

Title: A Prospective, Randomized Controlled Trial Evaluating the Effectiveness of the Dolphin Fluid Immersion Simulation® System versus Air Fluidized Systems in the Acute Post-Operative Management of Pressure Ulcers

Study Design: This study protocol outlines a prospective, randomized controlled post-market human subject trial.

Primary Objective: The primary objective is to compare the efficacy of the Dolphin Fluid Immersion Simulation® System on closure and complication rates as compared to air fluidized support systems after operative debridement and closure.

Secondary Objectives:

1.) Comparisons of the inflammatory and bacterial microbiomes of pressure ulcers following surgical therapy.
2.) Comparisons of baseline patient characteristics and their relationship to both early and late pressure ulcer recurrence, and complication rates.
3.) Assessment of outcomes and complications associated with flap technique and surgeon guided descriptions of the operative closure.
4.) Compare the absolute costs associated with the Dolphin FIS System against air fluidized systems.
5.) Compare the acceptance of each system by subjects and nursing staff, including the parameter of patient comfort.
6.) Assessing the incidence of complications and additional treatments needed following the two week study period

Number of Subjects: 100-200

Study Population: Adults, 18 years of age to 85 years of age, with 2 or fewer pressure ulcers and a history of fewer than 3 closures.

Control Therapy: Standard air fluidized systems

Duration of Study Participation: 1 year

Structure: Parallel Group **Duration of Treatment:** 2 weeks **Duration of Assessment:** 1 year

Blinding: Double-Blind

Randomization: Yes **Group Assignment:** 1:1

End Point Criteria Measurements:

Primary: Success of closure and incidence of wound related dehiscences or flap complications within the 2 weeks following operative closure

Secondary:

1. Bacterial speciation and antibiotic resistance taken just prior to and following the first surgical debridement and their subsequent relation to complications and time to closure in each group.
2. Differences in bacterial speciation and antibiotic resistance, total bacterial counts, and pressure ulcer microbiome. Bacterial speciation and antibiotic resistance will be determined by quantitative cultures and PCR analysis. Differences in total bacterial counts will be determined by quantitative PCR analysis. Pressure ulcer microbiome will be assayed via PCR assays conducted by PathoGenius Laboratory™. Comparisons will be made between biopsies taken prior to and following the initial operative debridement, following any subsequent debridements, prior to operative closure, and two weeks following definitive closure.
3. Qualitative and quantitative markers of inflammation in the pressure ulcer utilizing protein and PCR assays. Comparisons will be made between tissue biopsies taken prior to and following the initial operative debridement, following any subsequent debridements, prior to operative closure, and two weeks following definitive closure.
4. Evaluation of patient characteristics (age, comorbidities, nutritional parameters, spasticity, medications, ulcer history) and their relation to early and late recurrences as well as complications.
5. Comparison of outcomes and complication rates based on flap technique and surgeon guided descriptions of the operative closure.
6. Total costs incurred including treatment costs, the costs of associated complications, cost of the hospital stay, and any other unforeseen costs during the two week study period following operative closure.
7. Differences in quantitative patient and nurse survey responses regarding acceptability and tolerance of each therapeutic modality, including patient comfort.
8. Regular follow-up via phone interviews to assess for additional complications and need for additional therapies or surgical procedures up to 1 year after surgical closure.

Safety: Adverse events

Adverse Events: Both volunteered and elicited

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1. Introduction

1.1 Background

Pressure ulcers are defined by the National Pressure Ulcer Advisory Panel (NPUAP) as "localized injury to the skin and/or underlying tissue usually over a bony prominence, as a result of pressure, or pressure in combination with shear."[1] The epidemiology of pressure ulcers varies significantly by clinical care setting, with incidence rates ranging from 0.4% to 38% in acute care, 2.2% to 23.9% in long-term care, and 0% to 17% in home care.[2] In US acute care facilities alone, an estimated 2.5 million pressure ulcers are treated each year.[3] Coupled with the high incidence of pressure ulcers in the US population are the staggering health care costs associated with their management. The cost of managing a single full-thickness pressure ulcer is as much as $70,000 per event, and US expenditures for treating pressure ulcers have been estimated at $11 billion per year.[4, 5] The recent decision in 2009 by the Centers for Medicare & Medicaid Services deeming pressure ulcers reasonably preventable and halting additional reimbursement for the treatment of hospital-acquired pressure ulcers, even if clinicians deemed them unavoidable, has compounded the fiscal burden associated with pressure ulcer management.[6] The development of pressure ulcers also carries significant legal repercussions. Pressure ulcers litigation against long-term care providers has resulted in settlements favoring the plaintiffs in up to 87% of cases.[7]

The risk factors associated with increased risk of pressure ulcer development are broad and include older age, black race, lower body weight, physical or cognitive impairment, poor nutritional status, incontinence, and specific medical comorbidities that affect circulation such as diabetes or peripheral vascular disease.[8-12] Pressure ulcers are often associated with pain, can contribute to functional impairment with delayed returns to function, can lead to complications such as infection, and may significantly prolong hospitalizations.[13, 14] In addition, the

presence of pressure ulcers themselves are associated with a poorer overall prognosis and may also contribute to mortality risk.[14]

The consequences of pressure ulcer development and management highlight the value in devising novel, efficacious, and cost-effective preventative and management strategies.

1.2 Preventative Therapy

Recommended prevention strategies for pressure ulcers generally involve use of risk assessment tools to identify people at higher risk for ulcer formation in conjunction with interventions designed to avert ulcer formation.[15, 16] A number of instruments have been developed to assess the risk of pressure ulcer development. The three most widely used are the Braden scale, the Norton scale, and the Waterlow scale.[17-19] Categories of preventative interventions include support surfaces such as mattresses and integrated bed systems, repositioning, skin care, and nutritional support with each category encompassing a wide variety of diverse interventions.[15, 16] Prior trials have been limited in that none have evaluated the effectiveness of preventative interventions by clinical care setting and patient characteristics. Rather, the majority of trials have been conducted based on *a priori* risk assessments.

Support surface systems include static mattresses, overlays, or bed systems; or alternating air mattresses, overlays, and bed systems. These devices serve to redistribute pressure in order to manage tissue loads, micro-climate, and/or other therapeutic functions.[20] Results of studies investigating these systems have been equivocal overall. Five trials have compared static mattresses or overlays with standard operating room mattresses with two trials demonstrating increased risk and two trials demonstrating decreased risk.[21-25] Similarly, two trials have compared alternating support surfaces with static, usual care surfaces with no statistically

significant differences in pressure ulcer incidence, although results tended to favor alternating systems.[26, 27] Additionally, a variety of repositioning regimens have been studied including changes in body position every two hours[28, 29], repositioning at a 30 degree tilt[30], alternating semi-Fowler position (30 degree elevation of the head and feet) and a lateral position[31], and repositioning based on nurse clinical judgment.[32] Results were variable with only two trials demonstrating statistically significant reductions in risk with the initiation of repositioning protocols.[28, 30] All trials were hampered by methodological shortcomings including inadequate descriptions of randomization or allocation concealment methods and lack of blinding of outcome assessors.

Strategies for nutritional support include the supplementation of high-calorie oral liquid nutritional supplements[33-36] or supplementation of high fat, low-carbohydrate enteral formulas via tube feeding[37, 38]. Five of the six trials investigating nutritional supplementation found no difference between nutritional supplementation compared with standard hospital diet in risk of developing pressure ulcers. A single trial of 672 patients found that high-calorie oral liquid nutritional supplementation in addition to a standard hospital diet was associated with a slightly lower risk of pressure ulcers at 15 days compared with standard hospital diet alone in elderly patients during the acute phase of a critical illness.[33]

Finally, the category of preventative skin care products includes the application of creams, lotions, cleansers, dressings, and pads. Studies of topical creams, lotions, and cleansers have primarily focused on commercially available products. Studies of a hyperoxygenated fatty acid cream (Mepentol)[39] and Conotrane cream (benzalkonium chloride [an antiseptic] plus dimeticone [a silicone fluid which repels water])[40] demonstrated decreased risk of pressure ulcer development. Moreover, a trial found use of Clinisan cleanser associated with a lower risk

of ulcer development compared to standard soap and water in patients with incontinence.[41] In comparison, other commercial studies have not demonstrated the advantages associated with the addition of topical agents.[42, 43] Three trials have evaluated dressings or pads for the prevention of pressure ulcers with only one trial demonstrating a statistically significant reduction in pressure ulcer incidence.[44, 45]

Despite the wide variety of clinically available products for pressure ulcer prevention, it is readily apparent that further research is required and that currently available products, despite representing the standard of care, are woefully inadequate at attenuating the risk of pressure ulcer development. Further trials should employ a clearly delineated protocol for the use of preventative interventions based on validated risk-assessment scores. More research is needed to understand the effectiveness of preventative interventions with adherence to stringent methodological standards including the appropriate use of blinding with clear descriptions of usual care.

1.3 Management of Pressure Ulcers

Given the previously delineated negative sequelae of pressure ulcers on health status, patient quality of life, and health care costs, treatments that promote healing, shorten healing time, and minimize the risk of complications are urgently needed. Pressure ulcer management, like pressure ulcer prevention, requires a variety of different treatment modalities, including interventions to treat the conditions that give rise to pressure ulcers, interventions to protect and promote healing of the ulcer, and surgical repair of the ulcer.[46, 47] Most ulcers are treated using a combination of these approaches. The standard of care for pressure ulcer treatment is typically guided by clinical practice guidelines, such as those developed by NPUAP, but are also

informed by patient-related factors such as comorbidities and nutritional status, local practice patterns, and the stage and features of the wound.[48]

Support surfaces and nutritional supplementation are unique in the management of pressure ulcers as they represent interventions that address the underlying conditions that give rise to pressure ulcers. As such, these strategies are utilized in both preventative and therapeutic interventions. Examples of support surfaces include low-air-loss, alternating pressure, or air fluidized systems. Air fluidized beds are made up of a lining mattress filled with small beads; air is forced through to create a fluid-like surface that redistributes pressure. Five studies have compared air fluidized beds to other support surfaces and have consistently reported a positive effect compared to alternatives in promoting the reduction in size of pressure ulcers.[49-53] Alternating pressure systems have cells or sections that inflate and deflate to change the distribution of pressure. When compared against air, fluid, or standard beds, alternating pressure beds have generally yielded similar results.[54-56] Low-air-loss systems use power to provide a flow of air that helps regulate heat and humidity and may also adjust pressure. Studies comparing low-air-loss beds to foam surfaces showed similar wound improvement.[57-59]

Signs of poor nutrition, such as low levels of prealbumin, vitamin C, or zinc, are all associated with an increased incidence of pressure ulcers.[60, 61] Studies have identified that when used in addition to other measures for treating pressure ulcers, protein-containing nutritional supplementation results in wound improvement.[62-65] Two trials have examined the effect of micronutrient supplementation with either zinc sulfate or vitamin C with insufficient evidence for either intervention.[63, 66] As such, guidelines formulated by international experts, including the National Pressure Ulcer Advisory Panel and the European Pressure Ulcer Advisory

Panel, include recommendations for providing high-protein, mixed nutritional supplementation to all patients at risk for pressure ulcer development.[48]

Non-surgical interventions targeted at protecting and promoting ulcer healing include wound dressings, topical applications, and various adjunctive therapies, such as negative pressure wound therapy and hyperbaric oxygen therapy. Wound dressings represent a cornerstone of pressure ulcer treatment and serve multiple functions, including padding and protection of the ulcer from pressure and friction, the provision of a moist wound environment and protection against dessication, serving as a barrier in patients with incontinence or other sources of wound contamination, wound exudate absorption, and promoting autolytic debridement of necrotic tissue and slough. Studies have demonstrated that wound improvement is superior with hydrocolloid and foam dressings compared to gauze dressings.[67-73] Current evidence is insufficient to advocate the use of hydrogels, transparent films, silicone, and alginate dressings.[72, 74-78] Topical ointments and other therapies such as fibrinolytic enzymes and antimicrobial agents are also used in pressure ulcer management to provide moisture, promote tissue debridement, and eliminate or prevent infection. Evidence regarding the effectiveness of collagenase and other debriding enzymes is currently inconclusive [79-81], while several studies have shown superior results compared to placebo with the application of platelet-derived growth factor in Stage III and IV pressure ulcers. [82-85] Although topical antimicrobials are commonly used in pressure ulcer treatment, few studies have compared antimicrobials to placebos or other interventions and current evidence is insufficient to draw any conclusions regarding their effectiveness.[86, 87]

Finally, for chronic pressure ulcers that have progressed to an advanced stage and are unable to reliably heal with conservative measures, surgical debridement and vascularized soft

tissue reconstruction may serve to provide definitive closure. Surgical interventions range from local debridement of necrotic and nonviable tissue in the wound bed to direct closure, skin grafting, and closure with soft tissue flaps using either skin (cutaneous), fascia (fasciocutaneous), or muscle (myocutaneous) from unaffected parts of the body. Techniques for wound closure depend on the location, size, and depth of the pressure ulcer as well as the specific nature of the patient's medical comorbidities and previous surgical interventions.[88] Primary closure is typically contraindicated due to the high risk of failure from increased tension at the closure site.[89] Similarly, skin grafting is generally reserved for shallow nonhealing ulcers with a well-vascularized wound bed and is rarely used due to the high risk of failure from mechanical shear.[89] Current evidence is insufficient to determine if one approach to flap-based closure is superior to another due to overall poor study quality, study population heterogeneity, and differences in surgical technique.[90-95] In the face of a limited evidence base, surgeons typically resort to general reconstructive surgical principles and prior experience to guide flap selection. Fasciocutaneous flaps represent durable, well-vascularized flaps that spare the patient significant functional deformity and may provide good bony prominence coverage, but their limited bulk may prove insufficient for large or deep wounds.[88] Conversely, musculocutaneous flaps provide more depth of coverage at the cost of functional deformity, but are typically preferred in infected wounds due to the benefits imparted by a superior local blood supply including improved tissue oxygenation, antibiotic delivery, and enhanced lymphocytic function.[88] The relatively recent development of perforator-based flaps have gained popularity since their introduction by Koshima and colleagues in 1993.[96] Advantages of a perforator-based flap includes preservation of the blood supply and muscles for possible future reconstructions.[97] Despite the advantages associated with each reconstructive modality,

significant debate surrounds the selection of the optimal regional flap for the repair of pressure ulcers.[98-101] Moreover, predictors for the ultimate success of flap reconstruction are poorly delineated, but include bacterial inoculation[102, 103], ulcer location[94, 104], nutritional status[105], postoperative pressure relief[105, 106], length of hospital stay,[104] spinal cord injury[104], and presence of joint contractures.[105] The predictive effect of bacterial inoculation requires special attention. Previous investigations have demonstrated up to 100-fold increases in bacterial count in inoculated wounds subjected to pressure and have established a threshold of 10^5 organisms per gram of tissue as a significant predictor of flap failure.[102, 103, 107, 108] Despite these findings, the current evidence base is woefully inadequate due to the preponderance of case series and experimental animal models, necessitating the development of a well-powered clinical study of the bacterial microbiome in inoculated pressure ulcers to develop mechanistic and predictive models for flap failure. Recidivism rates due to pressure ulcer recurrence or flap failure is currently unacceptably high with overall rates as high as 39 percent.[109] These rates are even higher in spinal cord injured patients with a recurrence rate of 70 percent in one study.[95] Given these high rates of recurrence and the significant financial burden on the health care system associated with pressure ulcers, emphasis must be placed on the proper selection of patients for surgical reconstruction and the need to develop improved prevention strategies.

Current treatment strategies for pressure ulcers are guided by product availability, local practice patterns, anecdotal evidence, and individualized decision making based on specific patient and wound factors. Although there is general agreement on the standards of care with respect to the types of treatment employed, evidence comparing interventions within a given intervention strategy are sparse, limiting the ability for clinicians to judiciously select the optimal

interventions for their patients. Therefore, further studies should focus upon the comparative effectiveness of different management approaches.

1.4 Description of Study Products

Patients presenting for operative management of stage III or IV pressure ulcers will be assessed for study inclusion using study inclusion and exclusion criteria. Consenting subjects will be randomized to receive therapy with either the treatment support surface (Dolphin Fluid Immersion Simulation® System) or the control support surface (Clinitron® Rite Hite® Air Fluidized Bed).

The Dolphin Fluid Immersion Simulation® system represents a novel technology that was initially developed by the U.S. Navy in response to the need to transport specially trained marine mammals over long distances outside of water. The system was designed in order to minimize the harsh vertical shear forces of gravity, which ultimately cause internal organ trauma and circulatory distress. The Dolphin Fluid Immersion Simulation® system leverages an advanced 3D immersion technology in order to automatically simulate a fluid environment, thus maintaining near normal blood flow and optimizing tissue oxygenation. The system is fully autonomous with monitoring of the support surface over 100 times per second, facilitating constant adjustments based on patient repositioning. A recent study by Kohanzadeh et al. at the University of California San Diego has demonstrated statistically significant improvements in tissue blood flow compared to standard bed and gurney systems with 87% retention of perfusion on the Dolphin Fluid Immersion System® mattress vs. 16% on standard mattresses.[110] Moreover, Sparrow Specialty Hospital, a 36-bed long term acute care hospital, replaced a 6 week post myocutaneous flap protocol with an air-fluidized bed system with a 6 week protocol using

the Dolphin Fluid Immersion Simulation® system based protocol. After intervention, patients achieved equivalent flap outcomes with a $26,249 reduction in costs over the study time period.[111]

Dolphin Fluid Immersion Simulation® System

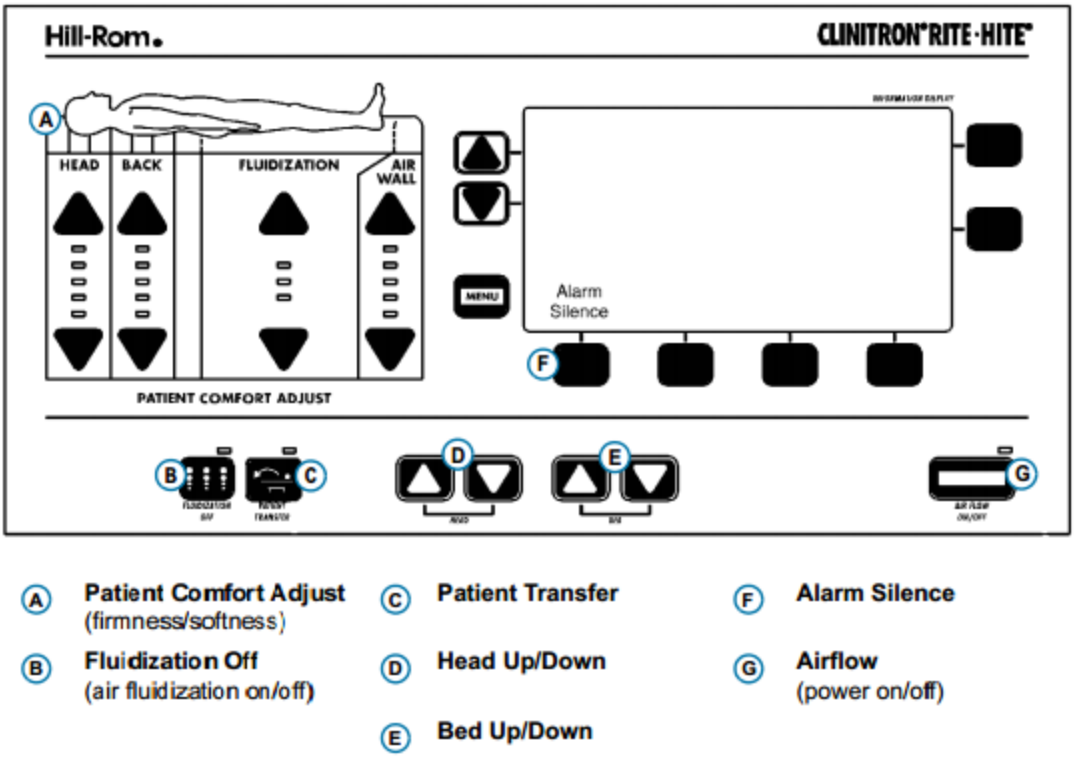
Like other air-fluidized systems, the Clinitron® Rite Hite® air fluidized therapy bed is made up of small beads with air forced through to create a fluid-like surface that redistributes pressure. Three randomized clinical trials, have specifically examined the Clinitron® system to alternatives. A study by Allman et al. comparing an air-fluidized system to an alternating pressure system showed a statistically significant decrease in total wound surface area and pain with a 5.6 relative odds of improvement compared to the alternating pressure system over the median 13 day follow-up period.[50] Furthermore, Monro and investigators compared 20

patients on air-fluidized beds to 20 people on standard hospital beds with mean ulcer area declining on the air-fluidized bed and increased on standardized beds over the course of each patient's hospital stay.[53] Finally, Jackson et al. compared 15 patients on an air-fluidized system to 20 individuals on several different standard care support surfaces with statistically significant reductions in wound area.[51]

Clinitron® Rite Hite® Air Fluidized Therapy Bed

Potential benefits touted by the Dolphin Fluid Immersion Simulation® System include a completely automated autonomic system with minimal required training as the system requires no programming or manual data input. This is in contrast to the Clinitron® Rite Hite® Air Fluidized Therapy Bed, which requires various adjustments by health care staff. The Dolphin Fluid Immersion Simulation® System fits into existing bed frames permitting potential

integration across the clinical care continuum and reducing the threshold for adoption of the therapeutic support surface. Current fluidization technologies are affected by environmental factors such as room temperature and humidity, potentially requiring health care staff to employ a variety of strategies such as lowering the room temperature, closing windows, and readjusting blankets on the unit that may negatively impact patient comfort while compromising health care resource utilization. In addition, patient transfers into and out of air-fluidized beds is technically difficult, requiring two providers, and may present an obstacle to care in the home environment where adequate staffing may not be available. Although Joerns makes no claims to the ease of patient transfers in the technical specifications of the Dolphin Fluid Immersion Simulation®, the facilitation of patient transfers could significantly reduce staffing needs while facilitating care in an outpatient environment. Finally, the previous study by Allman et al. demonstrated significant benefits with respect to wound healing and pain compared to conventional surfaces, even with a repositioning regimen of every 4 hours rather than every 2 hours in the conventional surface group.[50] The Dolphin Fluid Immersion Simulation® System claims to require no need for caregiver or staff intervention due to the continuous monitoring of the patient's weight, three-dimensional surface area, and movements in order to effectively manage the distribution of the patient's weight, potentially obviating the need for frequent repositioning regimens.



Clinitron® Rite Hite® Air Fluidized Therapy Bed Input System

1.5 Study Rationale

Although valuable, previous clinical studies investigating the Dolphin Fluid Immersion Simulation® system have not adequately compared the system to other standard of care support surfaces. Studies thus far have been limited to observational investigations of tissue perfusion and post-intervention investigations without an active comparator group. Furthermore, previous trials comparing air-fluidized beds to other systems have been limited by relatively small sample sizes with inadequate analysis of outcomes that focused primarily on wound surface area and complete tissue closure.[50, 51, 53] Thus, the proposed study is designed to examine the potential benefits of the Dolphin Fluid Immersion Simulation® system following operative closure of pressure ulcers compared to a representative air-fluidized system, the Clinitron® Rite

Hite® air-fluidized therapy bed. Currently, there is a paucity of data for clinicians to advocate for the usage of novel fluid immersion simulation systems compared to current standard of care support surfaces. The effects of the Dolphin Fluid Immersion Simulation® system will be studied and compared to the Clinitron® Rite Hite® air-fluidized therapy bed in a controlled manner after surgical closure of subjects' wounds.

2. Study Objectives

The primary objective is to compare the effects of the Dolphin Fluid Immersion Simulation® System to an air-fluidized system after definitive operative closure of pressure ulcers. This post-market, on-label study will evaluate the effectiveness of the Dolphin Fluid Immersion Simulation® System when compared to a standard of care support system.

2.1. Endpoint Criteria Measurements

2.1.1. Primary Endpoint

1. Success of closure and incidence of wound related dehiscences or flap complications within the two weeks following surgical closure of the pressure ulcer.

2.1.2. Secondary Endpoints

1. Bacterial speciation and antibiotic resistance taken just prior to and following the first surgical debridement and their subsequent relation to complications and time to closure in each group.
2. Differences in bacterial speciation and antibiotic resistance, total bacterial counts, and pressure ulcer microbiome. Bacterial speciation and antibiotic resistance will be determined by quantitative cultures and PCR analysis. Differences in total bacterial

counts will be determined by quantitative PCR analysis. Pressure ulcer microbiome will be assayed via PCR assays conducted by PathoGenius Laboratory™. Comparisons will be made between biopsies taken prior to and following the initial operative debridement, following any subsequent debridements, and prior to prior to operative closure.

3. Qualitative and quantitative markers of inflammation in the pressure ulcer utilizing protein and PCR assays. Comparisons will be made between tissue biopsies taken prior to and following the initial operative debridement, following any subsequent debridements, and prior to operative closure.
4. Evaluation of patient characteristics (age, comorbidities, nutritional parameters, spasticity, medications, ulcer history) and their relation to early and late recurrences as well as complications.
5. Comparison of outcomes and complication rates based off of flap technique and surgeon guided descriptions of the operative closure.
6. Total costs incurred including treatment costs, the costs of associated complications, cost of the hospital stay, and any other unforeseen costs during the two week study period following operative closure.
7. Differences in quantitative patient and nurse survey responses regarding acceptability and tolerance of each therapeutic modality, including pain management.
8. Regular follow-up via phone interviews to assess for additional complications and need for additional therapies or surgical procedures up to 1 year after surgical closure.

2.1.3. Safety Endpoints

1. Difference in number of Adverse Events

3. Study Design

3.1. Overview

This protocol outlines a prospective, randomized, single-center, human subject trial comparing the Dolphin Fluid Immersion Simulation® system to a representative air-fluidized support system, the Clinitron® Rite Hite® air-fluidized therapy bed. Following operative debridement, patients will be randomized to receive therapy with either the Dolphin Fluid Immersion Simulation® system (treatment) or the Clinitron® Rite Hite® air-fluidized therapy bed (control). Subjects will receive assigned study therapy following definitive pressure ulcer closure and extending two weeks beyond closure, reflecting current standard practice for pressure ulcer management. Investigators will follow, evaluate, and make clinical decisions regarding wound therapy, further debridement, or readiness for closure. The primary endpoint will not be followed during this period. Data regarding secondary endpoints other than the tissue biopsies delineated in Section 2.1.2 will also not be collected during this period. All other therapies will otherwise represent the standard of care for pressure ulcer management as delineated by the joint National Pressure Ulcer Advisory Panel and European Pressure Ulcer Advisory Panel guidelines.[48] The two week study period will begin following the definitive surgical procedure for pressure ulcer closure, at which time data regarding the primary endpoint and the secondary endpoints laid out in Section 2.1.2 will be collected.

The determination of a wound's appropriateness for definitive closure is currently viewed as a clinically subjective event. Readiness for closure is established when the Investigator deems that the wound has been adequately debrided, cleaned, and a definitive procedure can be performed.

The treatment period for Dolphin Fluid Immersion Simulation® system Treatment therapy or Clinitron® Rite Hite® air-fluidized therapy bed Control therapy will begin following the closure visit and will continue until two weeks following the definitive operative procedure. Both support surfaces will only be used in the inpatient setting. Subjects will remain hospitalized for at least two weeks following their definitive surgical procedure. After two weeks of inpatient hospitalization, Control or Treatment therapy may be continued although the only endpoint that will continue to be measured will be the incidence of additional complications, need for additional surgeries, or need for other therapeutic interventions.

Subjects will continue to be followed for one year after the two week study period in order to evaluate the incidence of complications and the need for additional therapeutic interventions. Assessments will include monthly telephone interviews.

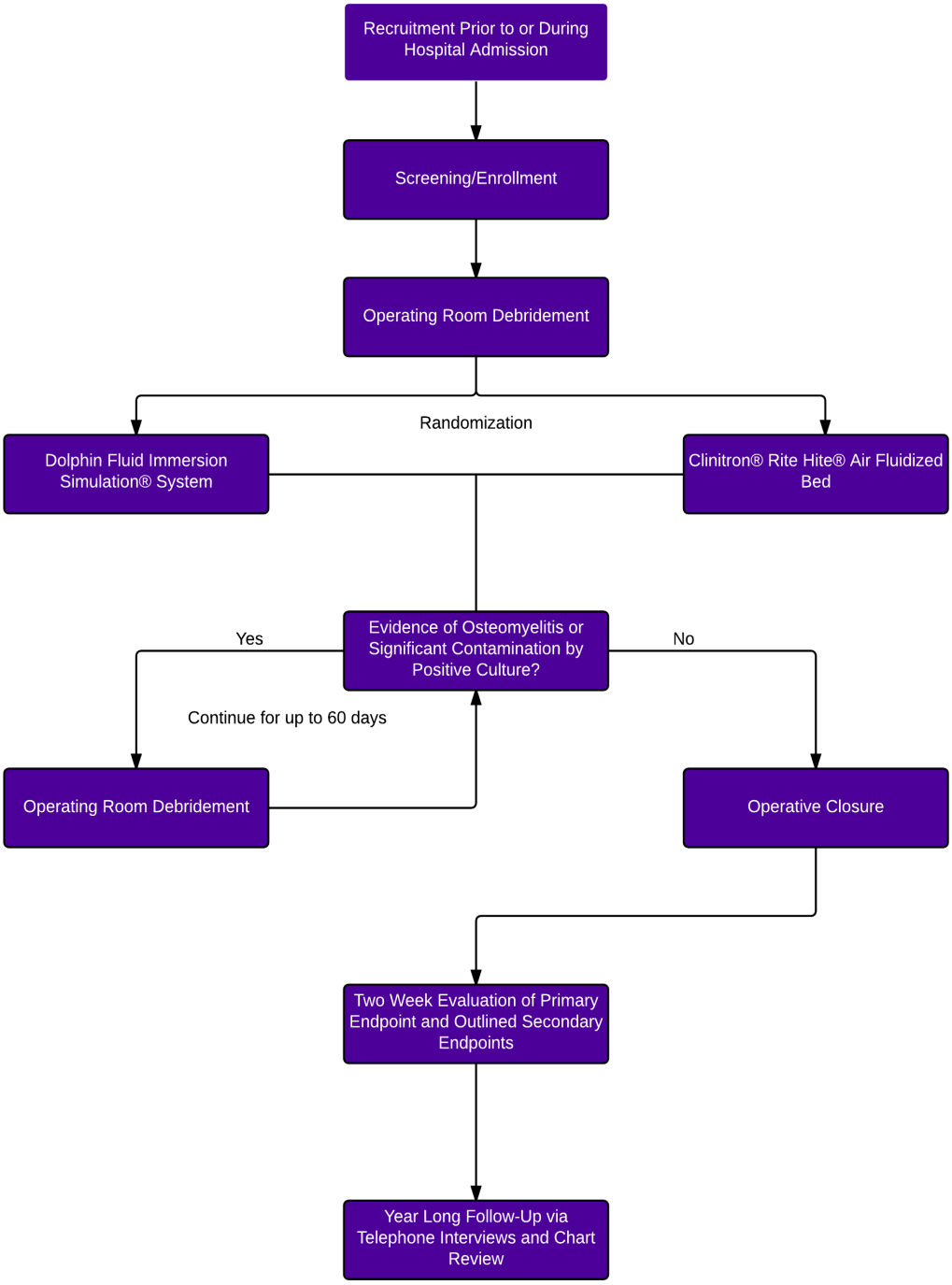
An interim analysis of the Intent-to-Treat (ITT) population will be performed when 50 subjects in each arm have satisfied the ITT criteria (see Section 7.4). An Independent Data Monitoring Committee (IDMC) comprised of a biostatistician and clinicians will convene to review the data from the interim analysis and evaluate the assumptions for the original sample size calculation. The IDMC will determine if the sample size will be adjusted based upon results from the interim analysis.

3.2. Duration of Clinical Investigation Participation

Screening: -90 days through day 0 (Initial OR Visit)
Treatment period following randomization: Up to 60 days
Treatment period evaluating primary endpoint: 14 days
Follow-up period: 365 (±20 days) after two week prior endpoint evaluation period
Total duration of participation per Subject: Up to 549 days

Duration of Study Enrollment Period: Up to 18 months

Figure 1. Clinical Decision Study Flowchart



4. Selection of Subjects

4.1. Recruitment and Screening

Patients who present to the investigator's institution (through clinic admission, direct transfer from another facility, or through the emergency room) and are, or will be, admitted as an inpatient for operative management of a stage III or IV pressure ulcer may be recruited to participate in the study. Additionally, direct marketing for subject recruitment will be performed by mailing letters to Dr. Robert Galiano's Rehabilitation Institute of Chicago patients and via television, radio, and online advertisements.

Patients approached for study participation will be at least 18 years of age and less than 85 years of age at the time of consent, will undergo wound debridement surgery and subsequent operative closure, and will meet all eligibility requirements. Those meeting eligibility criteria for the study will have the study explained to them by the Investigator. An Informed Consent Form will be provided to sign according to section 5.1 prior to undergoing any study procedures. Patients will be encouraged to ask questions of the investigators. It will be made clear to the patient that not participating in the study will in no way influence the treatment plan or the relationship with the physician.

There is no cap on the number of subjects that can be screened. Additional subjects will continue to be recruited as needed to reach the minimum number of 100 treated per protocol, per arm.

4.1.1. Subject Stipend

No reimbursement or compensation will be provided for participation in this study. Costs related to the treatment and control arms, including the support surfaces and staff training, will be covered by the Sponsor.

4.1.2. Wound Selection

Potential subjects who consent to study participation will be assigned a unique screening number. Only one wound per subject will be included in the study. Subjects with multiple wounds will have each wound measured for volume. The wound with the largest volume meeting all pre-operative eligibility requirements will be chosen for inclusion in the study. The location of the wound, and if it is a new or recurring wound, will be documented. Only stage III or IV pressure ulcers will be eligible for inclusion in this study.

At the conclusion of the initial surgical debridement, if all inclusion and no exclusion criteria continue to be met, the subject will be randomized into a study group and assigned a unique Randomization number. If the subject does not continue to meet all inclusion and no exclusion criteria at the end of surgical debridement, they will not be randomized and will be documented as a screen failure. The reason for exclusion will be noted on the Screening Log.

At the time of surgical closure, if all inclusion and exclusion criteria continue to be met, the Randomization number will become a unique Subject ID number. If the subject does not continue to meet all inclusion and no exclusion criteria number prior to and up to the point of operative closure, they will be documented as a randomization failure. The reason for exclusion will be noted on the Randomization log.

4.2. Inclusion Criteria

The Subject:

1. will be admitted as an inpatient
2. is ≥ 18 years of age and ≤ 85 years of age at time of consent
3. is able to provide his/her own informed consent
4. is deemed by the investigators to be reasonably compliant
5. has a pressure ulcer meeting criteria for stage III or IV
6. has not participated in a clinical trial within the past 30 days
7. has a 30 day wound history available if the wound has been previously treated

4.3. Exclusion Criteria

The Subject:

1. has a life expectancy of < 12 months
2. is not healthy enough to undergo surgery for any reason
3. has a history of radiation therapy
4. is, in the opinion of the investigator, noncompliant
5. has a history of > 3 closures of pressure ulcers in the same site
6. has a history of a bleeding disorder
7. has severe fecal incontinence

5. Study Procedures and Treatment

5.1. Informed Consent

The Investigator will discuss the purpose of this clinical investigation with potential subjects. Each potential subject will review the IRB approved Informed Consent Form (ICF). The subject and the Investigator (or designated medically licensed sub-investigator), must sign and date the ICF before the subject can undergo any clinical investigation-related procedures. The subject's informed consent will be obtained under these conditions:

- Subjects must be made aware of the purpose of the clinical investigation and the potential risks and benefits known or that can be reasonably predicted or expected
- Subjects must be given the opportunity to ask the Investigator questions and must be provided time to consider participation in the clinical investigation
- ICFs will be written in language that is non-technical and understandable to the subject
- Subjects will not be led to believe that they are waiving their legal rights to release the Investigator, Joerns®, Clinitron®, the Institution, or any of their agents from liability or negligence
- Subjects will be asked to sign and date the ICF indicating their informed consent to be enrolled in the clinical investigation
- The Investigator's responsibilities during the ICF process include:
    a. Screening out potential subjects who may not be able or willing to comply with the clinical investigation protocol
    b. Ensuring that subjects have signed the ICF prior to undergoing any clinical investigation related procedure
    c. Ensuring that subjects are provided a copy of the signed ICF

5.2. Screening

Patients who are scheduled for hospital admission or are already admitted with a pressure ulcer or multiple pressure ulcers will be identified, consented, and screened for the study according to Section 4. Potential subjects presenting with multiple pressure ulcers requiring debridement will have each wound assessed pre-operatively. The largest pressure ulcer meeting eligibility requirements listed in Section 4 will be screened for inclusion in the study. For subjects with multiple pressure ulcers, any pressure ulcers not selected as the study wound will not be followed but will otherwise receive institutional standard wound care at the direction of the treating clinician.

Upon signing the ICF, subjects will be assigned a unique Screening Number. The Screening number will start at 001 and increase sequentially with each subject screened. Screened subjects will be entered onto a screening log. Once a number is assigned it cannot be re-assigned to another subject. In the instance a subject is considered a screen failure, they will keep the screening number assigned to them and the screen failure log will be completed noting the reason(s) for screen failure.

Screened subjects consenting to participate in the study will not have screening laboratory tests collected. However, prior laboratory testing results will be documented, including WBC with differential, HgbA1c, glucose, BUN, Creatinine, Total Protein, Albumin, and Pre-Albumin. A focused medical and surgical history, physical exam, and wound history will be conducted and recorded. The subject's current medications will be documented.

5.3. Randomization

Subjects will be taken to the operating room for the initial debridement procedure of their 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 ration to be treated with either the Dolphin Fluid Immersion Simulation® System or the Clinitron® Rite Hite® Air-fluidized bed. Since there may be differences in pressure ulcer types, a stratified randomization will be used for this study to prevent imbalance between treatment arms. Permuted blocks will be used to achieve equal number of Subjects assigned to the Dolphin Fluid Immersion Simulation® System or the Clinitron® Rite Hite® Air-fluidized bed to generate a randomization schedule including Subject numbers and treatment assignments. Envelopes will be prepared corresponding to each row in the randomization schedule and each Subject number and treatment group will be printed on labels.

Prior to study initiation, sealed pre-numbered randomization envelopes will be provided to the research staff and will be used to obtain a randomization assignment. Opening of the randomization envelope will occur intra-operatively at the conclusion of the initial surgical debridement of the wound and confirmation that all inclusion and no exclusion criteria were encountered. Study staff will use the Randomization Number labels contained in the envelope. This number will become the Subject ID for patients who undergo operative closure. The assignment will be:

- Subject is randomized to Dolphin Fluid Immersion Simulation® System (Treatment arm)
- Subject is randomized to Clinitron® Rite Hite®Air-fluidized bed (Control arm)

The research staff will note treatment assignments and instruct the investigator. Control or Treatment therapy support surfaces will be initiated following operative closure, according to the manufacturer's recommendations.

In order to ensure consistent study treatment, subjects will receive assigned Control or Treatment therapy within their study arm after the wound is deemed ready for closure or coverage by the Investigator.

Support surface therapy crossover prior to and during the study treatment period is not permitted. Subjects randomized to the Dolphin Fluid Immersion Simulation® System Treatment arm are the only subjects that will receive this therapy at any time during the study period. If Dolphin Fluid Immersion Simulation® System is discontinued, subjects in the treatment arm will transition to an institutional standardized support surface. Subjects randomized to the Clinitron® Rite Hite® Air-fluidized bed will receive only this therapy prior to and during the study treatment period.

If the assigned Control or Treatment therapy must be discontinued prior to deeming the wound ready for closure or coverage, subjects will transition to a standard institutional support surface. These subjects will remain in the study and continue to be followed but will not be included in the per protocol analysis group.

5.4. Initial OR Debridement of Wound

Prior to start of the initial debridement procedure in the operating room, the wound will be assessed and the results documented. A pre-debridement digital photograph of the wound will be taken. Prior to the surgical prep, a pre-debridement wound swab will be obtained for aerobic and anaerobic culture and a pre-debridement wound scraping will be obtained for PCR and protein analysis.

The wound will then be debrided. At the conclusion of the debridement, the wound will be irrigated with at 5L of normal saline unless contraindicated in the judgment of the

Investigator. Antibiotic solutions are permitted for irrigation based on the judgment of the Investigator and standard of care practices.

If the wound is determined to be ready for immediate closure, the closure procedure can be performed. Closure at the time of the initial surgical debridement will be considered as the initiation of the study period.

If all inclusion criteria and no exclusion criteria are met at the end of the surgical procedure, the subject will be randomized according to Section 5.2. After randomization, a post-debridement wound assessment will be performed and documented. A post-debridement digital photograph of the wound will be taken. A post-debridement wound swab will be obtained for aerobic and anaerobic culture and a post-debridement wound scraping will be obtained for PCR analysis.

5.5. Wound Care Prior to Closure

During the subject's hospital admission, interventions other than the support surface utilized will be based on institutional standard of care practices. These include wound dressings, topical applications, and the use of adjunctive therapies such as vacuum-assisted closure and hyperbaric oxygen therapy. The type of dressing used will be documented.

5.6. Additional OR Wound Debridement

The Investigator will determine if additional surgical debridement is required after the Initial OR Visit. This decision is typically based on ulcer appearance and periodic post-debridement culture results. To ensure consistent treatment, subjects returning to the operating

room for additional debridement of their wound should resume their assigned study therapy after the procedure unless contraindicated in the judgment of the Investigator.

Prior to the start of the debridement procedure, the wound will be assessed and the results documented. The wound will be debrided according to the discretion of the Investigator. At the conclusion of the debridement the wound will be irrigated with at 5L of normal saline unless contraindicated. After wound irrigation, a post-debridement assessment will be performed and documented. Wound swabs will be taken both prior to and following all additional debridement procedures. Tissue scrapings will be taken following all additional debridement procedures.

5.7. Wound Closure or Coverage

When the Investigator determines that the wound is ready for closure or coverage, the study period will be initiated. A wound swab will be obtained for aerobic and anaerobic culture and a wound scraping will be obtained for PCR and protein analysis, a wound assessment will be performed, and the date of closure determination will be documented. Definitive wound closure for this study is defined as complete approximation of the wound edges, complete coverage of the wound via tissue transfer or skin graft, or any combination of these definitive techniques that results in complete elimination of the wound bed. Debridement may be performed prior to surgical closure or coverage. All operative procedures including any debridement must be documented. The use of a cutaneous, fasciocutaneous, myocutaneous, or perforator-based flap will be documented. The amount of tension and the potential dead space remaining following closure will be documented by the surgeon performing the procedure. The adequacy of these assessments will be assessed by a blinded surgeon reviewer. If a flap fails during the immediate postoperative period, the subject will be removed from the study and

transitioned to a standard institutional support surface. These studies will continue to be followed, but will not be included in the per protocol analysis group.

5.8. Two Week Study Period

Subjects will be followed for two weeks following primary closure for the primary endpoint of wound dehiscences and flap complications. Data regarding cost of hospital stay will also be compiled during the two week study period. Nurses caring for patients during this time period will receive surveys in order to assess the ease of use and acceptability of the Control or Treatment Arms. Patients will receive a survey after one week and two weeks in order to evaluate their comfort and acceptability of the Control or Treatment Therapies.

5.9. Follow-Up After Two Week Study Period

Subjects may be discharged from inpatient care when deemed appropriate by the Investigator following the two week study period. Monthly phone interviews will be conducted in order to assess for additional wound complications or necessary therapies related to the wound studied.

6. Study Assessments/Measurement Tools

6.1. Quantitative PCR Analysis

Specimens will be collected through tissue scrapings of the wound bed before and after the initial debridement, after each additional debridement and when the wound is deemed ready for closure. The samples will be analyzed for bacterial speciation and quantification through PCR techniques. Samples will be sent to the PathoGenius Laboratory® for measurements of the

tissue microbiome. Additionally, PCR assays for inflammatory markers will be conducted on the samples by the Principal Investigator's lab.

6.2. Qualitative Aerobic and Anaerobic Cultures

Culture specimens will be collected through swab cultures at the deepest point in the wound before and after the initial debridement, before and after each additional debridement, and when the wound is deemed ready for closure, according to current techniques. Culture swabs will be placed in an appropriate transport media and sent to the institutional laboratory for qualitative aerobic and anerobic analysis. Due to the turn-around-time of qPCR results, only the qualitative aerobic and anaerobic culture results will be used as the indicator of wound infection post debridement for the purposes of guiding treatment.

6.3. Quantitative Protein Analysis

Specimens will be collected through tissue scrapings of the wound bed before and after the initial debridement, after each additional debridement, when the wound is deemed ready for closure, and two weeks following closure. Protein-based assays of inflammatory markers will be conducted on the samples.

6.4. Laboratory Tests

Laboratory testing will not be performed for the study. If available, results of the following laboratory tests will be collected from the subject's medical history and/or from standard of care testing that is required per institutional standards. The Investigator will determine if abnormal test results are clinically significant. All clinically significant laboratory results will be recorded as AEs.

1. White Blood Cell Count with differential (CBC with differential)
    a. Pre-operatively, if available
    b. When wound is deemed ready for closure, if available
    c. Following two week study period, if available
2. HbA1c
    a. Pre-operatively, if available
3. Prealbumin/Albumin/Total Protein
    a. Preoperatively, if available
4. Glucose
    a. Pre-operatively, if available
5. BUN/Creatinine
    a. Pre-operatively, if available
6. C Reactive Protein
    a. Pre-operatively, if available
7. Erythrocyte Sedimentation Rate
    a. Pre-operatively, if available
8. Transferrin
    a. Pre-operatively, if available
9. Alkaline Phosphatase
    a. Pre-operatively, if available
10. Glomerular Filtration Rate
    a. Pre-operatively, if available
11. Aspartate Aminotransferase Test

a. Pre-operatively, if available

12. Alanine Aminotransferase Test

a. Pre-operatively, if available

6.5. Clinical Wound Assessments

Wound assessments are performed by the Investigator in order to capture key features of study wounds. Wounds will be measured to determine the volume. Wound volume will be recorded in cubic centimeters by standardized measurement of length, width, and depth using a ruler. Wound length will be measured along the surface of the body in the longest dimension of the wound. Width will be determined from the widest point of the wound that is perpendicular to the length in the same plane of measurement. Depth will be measured from the surface of the skin to the deepest part of the wound bed perpendicular to the plane of measurement for the length and width.

Wound characteristics and appearance will be recorded using the following scale:

Wound Depth	1 = Blanchable erythema on intact skin 2 = Partial thickness skin loss involving epidermis and/or dermis 3 = Full thickness skin loss involving damage or necrosis of subcutaneous tissue; may extend down to but not through underlying fascia; and or mixed partial and full thickness and or tissue layers obscured by granulation tissue 4 = Obscured by necrosis 5 = Full thickness skin loss with extensive destruction, tissue necrosis, or damage to muscle, bone, or supporting structures

6.6. Concomitant Medications and Antibiotic Use

This protocol does not specifically prohibit any concomitant medications. The study will collect and document the frequency, dose, indication, and classification of all antibiotic medications.

Antibiotic use will be at the discretion of the Investigator. Antibiotic name, category, dose, frequency, and duration for therapy must be documented. Rationale for antibiotic therapy must be documented including whether the antibiotic therapy is prophylactic, empiric, or culture directed and its relationship to the wound.

6.7. Wound History

The Subject's wound will be assessed at screening and a wound history will be captured. The history will include prior wound related surgeries and treatments including but not limited to hyperbaric therapy, use of biologics, debridements, closure, revascularization procedures, prior infection, previous use of negative pressure wound therapy, advanced moist wound therapy, which have occurred within the past 30 days. The history will also document onset and chronicity of the wound and anatomic location.

For Subjects who present with multiple wounds, all wounds will be assessed at screening. The wound with greatest volume will be selected for the study and a wound history will be recorded.

6.8. Operative Documentation

1. Pre-operative wound assessment and measurement of volume
2. Procedure Type:
    a. Debridement

b. Closure (type of closure—flap, autologous skin graft, delayed primary)

I. Type of Flap

i. Cutaneous

ii. Fasciocutaneous

iii. Myocutaneous

iv. Perforator-Based

c. Other (non-debridement, non-closure procedure)

d. Irrigation solution and volume used

3. Wound related operative complications
4. Post-operative wound assessment and measurement of volume (if applicable)
5. Treatment or control support surface
6. Surgeon guided description of operative closure will be evaluated using the following scale:

Tension	1 = No Tension 2 = Minimal Tension 3 = Moderate Tension 4 = High Tension 5 = Extremely high tension
Potential Dead Space Assessment	1 = No Dead Space 2 = Minimal Amount of Dead Space 3 = Moderate Amount of Dead Space 4 = High Amount of Dead Space 5 = Extremely High Amount of Dead Space

6.9. Digital Wound Photographs

Standard digital photographs will be captured for each study wound. Two dimensional wound images will be captured using a standard digital camera.

Digital photos will be taken of the wound for all subjects at the following times:

- Initial OR Debridement (pre- and post-debridement)
- Additional OR wound debridements (pre- and post-debridement)
- Surgical Closure (pre- and post-closure)
- Following two week study period

6.10. Clinical Acceptance of Treatment Arms

Nurses and patients will be asked to complete a quantitative survey evaluating ease of use, acceptability, and patient comfort. Nurses using each system will be asked to complete a survey at one week of treatment and following the trial. Patients will be asked to complete a survey at one week of treatment and following the end of the study period.

Nurse acceptability will be evaluated using the following scale:

Ease of Use	1 = No difficulty 2 = Minimal difficulty 3 = Moderate difficulty 4 = High difficulty 5 = Extreme difficulty
Amount of Training Required	1 = No training 2 = Minimal training 3 = Some training 4 = High amount of training 5 = Very high amount of training
Time Required for Troubleshooting or Otherwise Occupied by Device	1 = No time 2 = < 5 minutes a day 3 = 5 to 10 minutes a day 4 = 10 to 30 minutes a day 5 = > 30 minutes a day

Patient acceptability will be evaluated using the following scale:

Comfort	1 = Very comfortable 2 = Comfortable 3 = Neither comfortable or uncomfortable 4 = Uncomfortable 5 = Very uncomfortable
Difficulty with Mobilization	1 = No difficulty 2 = Minimal difficulty

	3 = Moderate difficulty 4 = High difficulty 5 = Extreme difficulty
Pain at Surgical Site	1 = No pain 2 = Minimal pain 3 = Moderate Pain 4 = High pain 5 = Extreme pain

7. Statistical Design

The primary objective is to measure and compare the effects of the Dolphin Fluid Immersion Simulation® System to air-fluidized beds in pressure ulcers undergoing operative closure.

Clinical outcomes of interest for this study are defined as the incidence of wound related dehiscences or flap complications within the two weeks following surgical closure of the pressure ulcer; the number of operating room debridements required after initial debridement to when the wound is deemed appropriate for closure or coverage; the difference in bacterial count from the time of the initial debridement to subsequent debridements and the time of operative closure; costs incurred during the two week period following surgical closure; and nurse and patient acceptability of the Treatment and Control arms. These outcomes will be analyzed and compared between the Dolphin Fluid Immersion Simulation® System and the Clinitron® Rite Hite® Air-fluidized bed.

7.1. Statistical Methods Planned

Unless specified otherwise, SPSS® Version 22 or higher will be utilized to perform the statistical analyses of efficacy and safety measures.

Categorical variables will be summarized in general using frequencies and percentages, whereas continuous variables will be summarized in general using descriptive statistics of

number of observations (n), mean, standard deviation (SD), minimum (Min), median, and maximum (Max).

Hypothesis testing, unless otherwise indicated, will be performed at the 5% significance level. All *p*-values will be rounded to four decimal places; *p*-values less than 0.0001 will be presented as '<0.0001' in all tables. All group comparisons from analysis of variance (ANOVA) and/or analysis of covariance (ANCOVA) models will be based on Type III sums of squares. All confidence intervals will be two-sided with 95% coverage.

7.2. Determination of Sample Size

An adaptive design will be employed to monitor the study to determine the target number of subjects required to achieve significance at the alpha = 0.05 level.

A previous systematic review of complications following flap based surgery for pressure ulcers demonstrated a mean complication rate of 19.6% with a standard deviation of approximately 3% following the usage of perforator based flaps.[106] We have determined that a difference in proportion of responders of at least 10% would be regarded as clinically meaningful.

Assuming a 10% delta in proportion between support surfaces and a "confirmed" complication rate of approximately 20% with a standard deviation of 5%, a sample size of 98 per group will provide a statistical power of 80% at the significance level of 0.05 (2-sided).

Therefore, a total of 200 subjects randomized with an equal allocation ratio (1:1) of the Treatment arm versus Control arm will be needed in the present study.

7.3. Interim Analysis

The study data will be evaluated when approximately 50 subjects in each treatment arm have completed the study. Data from the interim analysis will be used to verify the assumptions for sample size calculation or to re-estimate the sample size as well as to confirm the safety of the study. The interim analysis will be based off of the available data for the ITT population.

To ensure data integrity and unbiased clinical decisions, the planned interim analysis will be conducted under the auspices of the Independent Data Management Committee (IDMC) assigned to this study. The IDMC will disseminate interim results in a manner that will protect the scientific and ethical aspects of the study. The IDMC will review the study data at interim analysis to re-estimate the final target sample size using 80% power for the primary endpoint of number of wound dehiscences or flap complications following operative closure using the approach described by Chen et al.[112]

7.4. Analysis Populations

The following Subject populations will be considered for this clinical study:

- Per-Protocol Population (PP): All subjects who are randomized and received either Control or Treatment therapy for two weeks after wound closure, completed the protocol's required evaluations, and did not have any major protocol deviations. The primary and secondary effectiveness analyses for this study will be based on this population.
- Intention-to-Treat (ITT): All subjects who are randomized and received either Control or Treatment therapy regardless of its duration, and without a major medical event after

enrollment unrelated to the study treatment that significantly alters the treatment course or would affect the subject's ability to participate in the study.

- Safety Population: The Safety population will consist of all subjects who are randomized and treated with the Control or Treatment therapy regardless of its duration. All safety results will be presented based on the Safety Population

7.4. Efficacy Analyses

7.4.1. Primary Efficacy (Number of successful closures and wound related dehiscences or flap complications)

The primary effectiveness endpoint for this study is the number of successful closures and the total number of wound related dehiscences or flap complications during the two weeks following operative closure. The number of debridements as defined above will be calculated and displayed by treatment group and overall and then will be modeled using a two-sample t-test. The null and alternative hypotheses are below.

H_0: The Dolphin Fluid Immersion Simulation® System and Air-fluidized treatment groups have equal closure rates and numbers of wound related dehiscences and flap complications during the two weeks following operative closure.

H_1: The Dolphin Fluid Immersion Simulation® System and Air-fluidized treatment groups have unequal closure rates or different numbers of wound related dehiscences and flap complications during the two weeks following operative closure.

7.4.2. Secondary Efficacy

7.4.2.1 The difference in bacterial speciation and antibiotic resistance taken just prior to and following the first surgical debridement and their subsequent relation to complications and time to closure in each group. The differences will be calculated and displayed by treatment group and bacterial speciation and will be compared using an ANOVA analysis.

The null and alternative hypotheses are below.

H_0: Bacterial speciation, antibiotic resistance, and treatment surface have no relation to the overall incidence of complications or time to operative closure.

H_1: Bacterial speciation, antibiotic resistance, and treatment surface are related to the overall incidence of complications or time to operative closure.

7.4.2.2 The difference in total bacterial counts as determined by quantitative PCR analysis after the initial operative debridement, following initial debridement, following subsequent debridements, and prior to operative closure.

The differences will be calculated and displayed by treatment group and overall and then will be compared using a two-sample t-test.

The null and alternative hypotheses are below.

H_0: The Dolphin Fluid Immersion Simulation® System and Air-fluidized treatment groups have equal differences in total bacterial counts.

H_1: The Dolphin Fluid Immersion Simulation® System and Air-fluidized treatment groups have unequal differences in total bacterial counts.

7.4.2.3 Differences in pressure ulcer microbiome measured by quantitative PCR assays conducted by PathoGenius Laboratory™ just prior to and following initial debridement, following subsequent debridements, and prior to operative closure.

Differences will be calculated and displayed by treatment group and overall and then will be compared using a two-sample t-test.

The null and alternative hypotheses are below.

H_0: The Dolphin Fluid Immersion Simulation® System and Air-fluidized treatment groups have equal differences in pressure ulcer microbiome.

H_1: The Dolphin Fluid Immersion Simulation® System and Air-fluidized treatment groups have unequal differences in pressure ulcer microbiome.

7.4.2.4 Differences in qualitative and quantitative markers of inflammation in the pressure ulcer utilizing PCR and protein assays between tissue biopsies taken just prior to and following initial debridement, following any subsequent necessary debridements, and prior to operative closure.

Differences will be calculated and displayed by treatment group and overall and then will be compared using a two-sample t-test.

The null and alternative hypotheses are below.

H_0: The Dolphin Fluid Immersion Simulation® System and Air-fluidized treatment groups have equal differences in inflammatory markers.

H_1: The Dolphin Fluid Immersion Simulation® System and Air-fluidized treatment groups have unequal differences in inflammatory markers.

7.4.2.5 Differences in baseline patient characteristics including age, comorbidities, nutritional parameters, spasticity, medications, and ulcer history as well as the treatment group will be summarized and compared amongst patients who experience early and late pressure ulcer recurrences or wound-related complications. Differences will be calculated and displayed using an aggregate of these complications or by individual complication, and then will be compared using Chi-Square or Fisher's exact test for categorical variables and using a two-sample t-test for continuous variables. Multivariate logistic regression analyses will be performed to identify statistically significant independent predictors of recurrence and wound-related complications.

The null and alternative hypotheses are below.

H_0: Neither baseline patient characteristics nor the treatment group is associated with early or late pressure ulcer recurrence or the occurrence of a wound-related complication.

H_1: Baseline patient characteristics or the treatment group is associated with an increased or decreased risk of an early or late pressure ulcer recurrence or the occurrence of a wound-related complication.

7.4.2.6 Differences in flap technique and surgeon guided descriptions of the tension of closure and potential dead space will be assessed and compared according to clinical outcome and presence of a complication. Differences will be calculated and displayed by individual outcome and by the presence of a complication, and then will be compared using Chi-Square or Fisher's exact test for categorical variables and using a two-sample t-test for continuous variables. Multivariate logistic regression analyses will be performed to identify statistically significant independent predictors positive and negative outcomes as well as complications.

The null and alternative hypotheses are below.

H_0: Flap technique, tension of the operative closure, or potential dead space is not associated with clinical outcomes or the presence of a complication.

H_1: Either flap technique, tension of the operative closure, or potential dead space are associated with clinical outcomes or the presence of a complication.

7.4.2.7 Total costs including treatment costs, the costs of associated complications, the cost of hospital stay, and any other unforeseen costs during the two week study period following operative closure will be tabulated and compared between the Treatment and Control groups.

Differences will be calculated and displayed by treatment group and overall and then will be compared using a two-sample t-test.

The null and alternative hypotheses are below.

H_0: The Dolphin Fluid Immersion Simulation® System and Air-fluidized treatment groups result in equal costs.

H_1: The Dolphin Fluid Immersion Simulation® System and Air-fluidized treatment groups result in unequal costs.

7.4.2.8 Differences in quantitative measures of patient and nurse acceptability of the treatment and control arms will be tabulated and compared between the Treatment and Control groups.

Differences will be calculated and displayed by treatment group and overall and then will be compared using a two-sample t-test.

The null and alternative hypotheses are below.

H_0: The Dolphin Fluid Immersion Simulation® System and Air-fluidized treatment groups result in equal acceptability among patients and nurses.

H_1: The Dolphin Fluid Immersion Simulation® System and Air-fluidized treatment groups result in unequal acceptability between patients and nurses.

7.4.2.9 The number of complications or additional therapeutic interventions will be assessed by patient phone interviews and be displayed by treatment group and overall and then will be modeled using a two-sample t-test.

The null and alternative hypotheses are below.

H_0: The Dolphin Fluid Immersion Simulation® System and Air-fluidized treatment groups have equal numbers of complications and additional therapeutic interventions during the year following the two week study period.

H_1: The Dolphin Fluid Immersion Simulation® System and Air-fluidized treatment groups have unequal numbers of complications and additional therapeutic interventions during the year following the two week study period.

7.5. Safety Analyses

Safety analyses will be carried out using the Safety population to allow a benefit/risk assessment within the same study population.

All AEs recorded during the course of the study will be coded according to the MedDRA (version 13.0) classification system. An event will be considered as treatment emergent if the time of onset is on or after the initial debridement through Day 74. Any event with an onsent on

the day of initial operative debridement where the time of onset is missing will be assumed to be treatment emergent.

Number and percentage of treated subjects who experienced at least one adverse event in each system organ class by preferred term will be used to summarize all serious and non-serious adverse events. Summary tables will be provided of incidences for all treatment emergent adverse events, treatment-related adverse events, adverse events by maximum severity, serious adverse events, and adverse events that led to study discontinuation. In any given category (e.g. system organ class or preferred term) a subject will be counted only once. If a subject has the same adverse event on multiple occasions, only the one with maximum severity will be presented. The denominator for the calculation of percentages will be the number of subjects in the sub-arm of subjects being tabulated and not the number of events.

7.6 Handling of Missing and Incomplete Data

There will be no imputations made to accommodate missing data. All data recorded will be included in data listings.

8. Study Discontinuation and Withdrawal of Study Subjects

8.1. Premature Discontinuation of the Study

If the clinical investigation is terminated prematurely or suspended, the IRB will be informed promptly and provided with the reason(s) for the termination or suspension by the Sponsor or by the clinical investigator. If applicable, regulatory authorities and the personal physicians of the subjects will be informed by the clinical investigator.

<u>8.1.1 By Sponsor (Joerns)</u>

Joerns reserves the right to discontinue any clinical investigation for business or ethical reasons at any time, such as but not limited to:

- The clinical investigation is not conducted in accordance with the approved protocol
- Information on the investigational product causes doubt as to the benefit/risk ratio
- Changes in medical practice limit utility of the data obtained from the study
- Incidence or severity of AEs indicates a potential health hazard or poses an unreasonable risk to the study participants

8.1.2 By IRB

The IRB may choose to discontinue the clinical investigation if the:

- Clinical investigation is not conducted in accordance with the IRB's requirements
- Clinical investigation is associated with unexpected serious harm to subjects

8.2. Premature Discontinuation of the Subject

Subjects may withdraw from the study at any time at their own request, or they may be withdrawn at any time at the discretion of the PI for safety, behavioral, or administrative reasons. If a subject does not respond to study telephone surveys, every effort should be made to contact the subject, per institutional protocol.

In any circumstance, every effort should be made to document subject outcome. The Investigator should inquire about the reason for withdrawal and follow up with the subject regarding any unresolved AEs. Any related SAE occurring within 14 days following subject discontinuation must be reported to Joerns® and be followed up until stabilization or resolution.

If the subject withdraws from the study, and also withdraws consent for disclosure of future information, no further study evaluations will be performed, and no additional data will be collected. The PI may retain and continue to use any data collected before such withdrawal of consent.

8.2.1 By Investigator

The Investigator may choose to discontinue a Subject from the study with or without their consent for any of the following:

- Adverse Events
- Non-compliance
- Safety
- Complications
- Unforeseen events
- Or for any reason that may, in the opinion of the Investigator, affect negatively the well being of the subject

If for any reason the subject is withdrawn from the clinical investigation, the Investigator will inform the subject accordingly.

9. Potential Risks of Study Participation

9.1. Potential Risks Associated with the Investigational Product

All risks known or anticipated based on prior experience, risk analysis or reasonable grounds are listed in each product's 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.

10. Monitoring for Safety

The Investigator will perform safety monitoring in the following manner. AEs will be captured at each study visit during treatment regardless of causality or severity. All SAEs will be followed by the Investigator until resolution/stabilization.

10.1. Adverse Events

The intent of Joerns is to protect the safety of all subjects enrolled in this clinical investigation. Broadly defined an AE is any undesirable clinical occurrence in a subject. AEs will be 100% source-verified by the Joerns representative during each monitoring visit.

The intensity of an AE will be classified as one of the following:

Definition of Intensity of Adverse Events

Intensity	Definition
Mild	Transient, does not interfere with Subject's daily activities, does not require special treatment
Moderate	Interferes with Subject's daily activities, requires simple therapeutic measures
Severe	Intolerable experiences, interruption of Subject's daily activities, requires substantial therapy including medical or surgical intervention or hospitalization

The Investigator is responsible for completing the causality assessment. The relationship of an AE to the device, comparator, or surgical incision will be categorized as one of the following:

Relationship of Adverse Events to the Device

Relationship	Definition

Not Related	No relationship which makes a causal relationship incontrovertible (i.e. not related to the device)
Related	Definite temporal association exists, clinical experience makes the relationship definite and certain

10.2. Serious Adverse Events

A SAE is an AE that meets one or more of the following criteria:

- fatal
- life-threatening
- results in an unanticipated or prolonged hospitalization
- results in a significant and persistent disability/incapacity

10.3. Anticipated Events

Anticipated AEs for this study include but are not limited to periwound skin maceration, wound recurrence after definitive closure, hematoma, seroma, and surgical site infection. These events may result in therapeutic interventions, including surgical drainage, surgical incision packing, debridement, and reoperation. Although anticipated, these occurrences are considered AEs and should be documented appropriately.

10.4. Investigator Reporting Requirements

The Investigator has the primary responsibility for safety of subjects under his or her care. In this function, he/she should routinely monitor each subject for the occurrence of any AE. For every AE that occurs, the causal relationship will be determined. If the device, comparator, or study treatment relatedness is found, then a full description of the event should be recorded including the data of onset, time course, severity, action taken regarding the study treatment, outcome, and any treatment applied.

The Investigator should list the specific diagnosis, disease, or syndrome rather than associated signs and symptoms. However, if the Investigator does not consider an observed or reported sign or symptom a component of a specific disease or syndrome, it should be recorded as a separate AE. Clinical syndromes associated with the findings of laboratory abnormalities are to be recorded appropriately (e.g. bacterial infection of the surgical incision instead of increase in bacterial load) as AEs.

10.5. Device Malfunction

Device malfunction means the failure of a device to meet its performance specifications or otherwise perform as intended. Performance specifications include all claims made in the labelling for the device. The intended performance of a device refers to the intended use for which the device is labelled or marketed.

Device malfunction may or may not result in the subject experiencing harmful effect. All adverse device effects (ADEs)/unanticipated adverse device effects (UADEs) associated with a device failure are by definition device related and will be recorded and followed by an Investigator until stabilization or resolution. Device malfunctions that are not related to an adverse event will be reported to Joerns within 2 business days.

10.6. Adverse Event Related Withdrawal

Subject withdrawal due to an AE should be distinguished from withdrawal due to other reasons by appropriate documentation.

10.7. Follow-up of Subjects with Adverse Events

For subjects who are experiencing ongoing unresolved adverse events at the time of their study completion or early discontinuation from the study, the Investigator should schedule one or more follow-up visits as appropriate to follow the subject until the adverse event is resolved, or determined to be chronic.

11. Quality Control and Quality Assurance

11.1 Training on Study Products and Procedures

The Investigator and staff will be trained on the study protocol, the investigational device(s), and any specialized procedures prior to and/or during the initiation visit. Sponsor will provide clinical support to site staff for any questions or concerns related to treatment plans; however, the Sponsor will not have influence on subject clinical care.

11.2 Sponsor

The Sponsor will be responsible for implementing and maintaining quality assurance and a quality control system to ensure that the data generated are recorded and reported in accordance with established procedures.

Data from case report forms (CRFs) and other external data (i.e. laboratory data) will be entered into a clinical database. Quality control and data validation procedures will be applied to ensure the validity and accuracy of the clinical database.

The clinical database will be reviewed and checked for omissions, apparent errors, and values requiring further clarification. Data queries requiring clarification will be documented and returned to the site for resolution. Only authorized personnel will make corrections to the clinical database, and all corrections will be documented in an audit trail.

All agreements made by the Sponsor and Investigator will be reduced to a written document and serve as a separate contract and/or as an amendment to the protocol.

During the course of the clinical investigation, an amendment to the clinical investigation protocol may be necessary. Only the Sponsor is allowed to amend this protocol. Any amendments or modifications must be approved by the IRB prior to clinical investigation staff implementation, unless the modifications are implemented to eliminate immediate hazard to the subject. The research site will receive the following for their IRB submission:

- A memorandum outlining the changes and justification for modifications
- An updated protocol
- Changes to ICF template (if necessary)

11.3. Investigator

The Investigator assumes full responsibility for performance of the clinical investigation in accordance with the Clinical Trial Agreement, this protocol, IRB requirements, and applicable requirements to the jurisdiction in which the clinical investigation is being conducted.

12. Ethics

12.1 Institutional Review Board (IRB)

The Investigator must submit this protocol and all applicable supporting forms and documents to the IRB, and is required to forward to the Sponsor a copy of the written and dated approval/favorable opinion signed by the Chairman of the IRB committee. The study (study number, protocol title, and version number), the documents reviewed (e.g. protocol and ICF),

and the date of the review should be clearly stated on the written IRB approval/favorable opinion.

The ICF (plus additional forms, as applicable) used by the Investigator for obtaining the Subject's informed consent should be reviewed and approved by the Sponsor prior to submission to the IRB for approval/favorable opinion.

A progress report is sent by the Investigator to the IRB annually or as specified by the IRB and a summary of the clinical investigation outcome(s) is reported at the end of the clinical investigation.

13. Data Handling and Recording

13.1. Data Quality Assurance

Steps to be taken to ensure the accuracy and reliability of data include review of protocol procedures with the Investigator and associated personnel before the study commences and periodic on-site monitoring visits by the Sponsor. Guidance for CRF completion will be provided and reviewed with the study personnel before the start of the study. The Sponsor will review CRFs for completeness during on-site monitoring visits and after their return to the Sponsor; any discrepancies will be resolved with the Investigator or designee, as appropriate. The data will be entered into the clinical study database and verified for accuracy

13.2. Data Collection

The study CRF is the primary data collection instrument for this study. All data requested on the CRF must be recorded. All missing data must be explained.

13.3. Data Handling

The Investigator is responsible for compilation and verification of the study data, retention of the clinical study database, performance of statistical analysis, and preparation of the data reports.

13.4. Record Storage and Retention

13.4.1 Investigator

In accordance with the Clinical Trial Agreement, the Investigator shall maintain all study documentation in its possession and/or control and institute measures to prevent accidental or premature destruction of any data and/or documents related to the study.

After formal discontinuation of the clinical investigation, the Investigator shall retain the clinical trial documentation produced for a minimum of three (3) years or in accordance with the regulations in effect for the jurisdiction.

The investigator shall allow Sponsor and/or Sponsor's representatives to have access to the clinical investigation documents as defined in the Clinical Trial Agreement.

13.4.2 Sponsor

The Sponsor shall maintain all study documentation in its possession and/or control and institute measures to prevent accidental or premature destruction of any data and/or documents related to the study.

The Sponsor shall retain the study documentation according to Title 21 CFR 812.140 6(d):

> An investigator or sponsor shall maintain the records required by this subpart during the investigation and for a period of 2 years after the latter of the following two dates: The date on which the investigation is terminated or completed, or the date that the records are no longer required for purposes of supporting a premarket approval application or a notice of completion of a product development protocol.

14. Confidentiality of the Study

Pursuant to the terms and conditions set forth in the Clinical Trial Agreement, the Investigator and staff involved in the Study shall consider all information, results, discoveries, records accumulated, acquired, or deduced in the course of the study, other than that information to be disclosed by law, as confidential and shall not disclose any such results, discoveries, records to any third party without informing the Sponsor. Refer to the Clinical Trial Agreement for more specific terms and conditions.

15. Publication Policy

All publications and/or presentations are subject to the discretion of the Investigator. The Investigator will maintain complete control of the data and illustrations to be utilized in publications. The Sponsor will be informed prior to data publication.

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