

Protocol Title: Epidermal Coverage of Traumatic Wound Injuries via Use of Autologous Skin Cell Harvesting Device in Combination with Widened Meshed Autograft Applied Over Bilayered Wound Matrix

NCT02469168

Study Protocol including statistical plan

Approved V4 09-04-15

**WALTER REED NATIONAL MILITARY MEDICAL CENTER (WRNMMC)
BETHESDA, MD**

1. GENERAL INFORMATION

1.1 Protocol Title: Epidermal Coverage of Traumatic Wound Injuries via Use of Autologous Skin Cell Harvesting Device in Combination with Widened Meshed Autograft Applied Over Bilayered Wound Matrix

1.2 Principal Investigator (PI)

LTC Leon J. Nesti, MC, USA

Associate Professor, USUHS

Chief, Clinical and Experimental Orthopaedics

Uniformed Services University of the Health Sciences (USUHS)

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Hand and Upper Extremity Reconstructive Surgeon

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Directorate: Surgery

Responsibilities: Will be responsible for protocol development, adherence and execution, passage of the protocol through the local IRB, overall data integrity, applying for and receiving approval for any modifications to the protocol or informed consent form, training of associate investigators, ensuring safety of the volunteers, reporting of any AEs or protocol deviations, full accountability and proper storage of the investigational device, interpretation of study data results and presentation and preparation of manuscripts. The PI assures overall coordination of the study and the filing of a final clinical study report with the Sponsor.

1.3 Associate Investigator(s)

COL Barry Martin, MC, USA

Chief of Plastic and Reconstructive Surgery Service

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Directorate: Surgery

Responsibilities: Identification and enrollment of study subjects; will provide and/or assist with surgical intervention including use of the investigational device, provide input and expertise; clinical evaluator of surgical outcomes as needed; will assist PI and Sponsor with interpretation of study data results and presentation and preparation of manuscripts.

DATES OF INVOLVEMENT: Sept 2013 to study completion

LtCol Kerry Latham, MC, USAF

Staff Plastic Surgeon

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Directorate: Surgery

Responsibilities: Identification and enrollment of study subjects; will provide and/or assist with surgical intervention including use of the investigational device, provide input and expertise; clinical evaluator of surgical outcomes as needed; will assist PI and Sponsor with interpretation of study data results and presentation and preparation of manuscripts.

DATES OF INVOLVEMENT: Sept 2015 to study completion

LTC Jon H. Meyerle, MC, USA

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Responsibilities: Provide input and expertise; clinical evaluator of surgical outcomes. Will not be directly involved in treating the subject; will perform assessments for healing/scar evaluation via Vancouver Scar Scale (VSS) and the Patient and Observer Scar Assessment Scale (POSAS) (will be blinded to treatment provided at the target wound site).

DATES OF INVOLVEMENT: Sept 2013 to study completion

LTC Jason D. Marquart, MC, USA

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Walter Reed National Military Medical Center (WRNMMC)
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Directorate: Medicine

Responsibilities: Provide input and expertise; clinical evaluator of surgical outcomes. Will not be directly involved in treating the subject; will perform assessments for healing/scar evaluation via Vancouver Scar Scale (VSS) and the Patient and Observer Scar Assessment Scale (POSAS) (will be blinded to treatment provided at the target wound site).

DATES OF INVOLVEMENT: Sept 2013 to study completion

Nancy E. Lee, BA

Contractor: Geneva Foundation, Research Associate/Coordinator
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Directorate: Surgery

Responsibilities: Study coordinator, regulatory submission and communication with IRB and Sponsor, recruitment, obtain consent, perform/review screening evaluations, implementation of study protocol, data collection and analysis, query resolution, monitor subject for adverse event occurrences, assists with development of study reports (initial, continuing review and final)

DATES OF INVOLVEMENT: March 2013 to study completion

1.4 Collaborators/Consultants

James Holmes, IV, MD

Medical Advisor

Wake Forest School of Medicine

Institute for Regenerative Medicine

Richard H. Dean Biomedical

Building 391 Technology Way

Winston-Salem, NC 27101

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Directorate: Not Applicable

Responsibilities: Subject matter expert in regenerative medicine and the use of the ReCell device for the treatment of burns

DATES OF INVOLVEMENT: Feb 2013 to study completion

Kimberly Strohkirch, MSEE

Regulatory Consultant

Memphis Regulatory Consulting, LLC (MRC)

3786 Russell Hurst Drive W

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Directorate: Not Applicable

Responsibilities: Regulatory affairs consultant. Assist with FDA IDE preparation as needed.

DATES OF INVOLVEMENT: Feb 2013 to study completion

Sandy Maddock, RN, BSN, MSHS, CCRA

President

IMARC Research, Inc.

22560 Lunn Road

Strongsville, OH 44149

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Directorate: Not Applicable

Responsibilities: Contract Research Organization (CRO) which will be providing clinical

monitoring and auditing oversight. Collective monitoring activities will be provided to assist study site in compliance with the protocol, the signed agreement, IRB requirements, and applicable FDA regulations in an effort to ensure human subject protection.

DATES OF INVOLVEMENT: July 2013 to study completion

J. Peter Rubin, MD

Director, Center for Innovation in Restorative Medicine (CIRM)

University of Pittsburgh School of Medicine

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Pittsburgh, PA 15261

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Directorate: Not Applicable

Responsibilities: Sponsor of the IDE (will hold IDE), will retain overall responsibility for the progress of the clinical trial and will ensure that it is conducted, recorded and reported in accordance with the protocol, SOPs, GCP and regulatory requirements to assure the protection of human subjects and data integrity. Will submit IDE application to FDA as well as prompt reporting of safety reports to the FDA.

Additional Responsibilities: Data Coordinating Center (housing of data), will be accountable for maintaining oversight of all data management activities to ensure data quality and integrity with accurate reporting, interpretation and verification, and will ensure systems and procedures are in place for the protection and safeguarding of confidentiality of records.

DATES OF INVOLVEMENT: Feb 2013 to study completion

Patsy Simon, RN, BSN, CCRC

Director, Regulatory and Clinical Affairs UPMC Center for

Innovation in Restorative Medicine (CIRM) Department of

Plastic Surgery University of Pittsburgh

University of Pittsburgh School of Medicine

225 University Center

120 Lytton Avenue

Pittsburgh, PA 15213

Phone: 412-864-2580

Email: simonpa@upmc.edu

Directorate: Not Applicable

Responsibilities: Will provide direct management and operational aspects for the FDA IDE and local Institutional Review Board regulatory process and data management of the clinical trial. This includes assisting with development of clinical practice, SOP generation and clinical procedural process specific to the site while adhering to all levels of regulatory regulation and guidance, clinical trial conduct and data compliance with primary focus on good clinical practice.

DATES OF INVOLVEMENT: Fall 2012 to study completion

Patrick Cantini

OSD Program Manager

Director, Scientific Collaborations
McGowan Institute for Regenerative Medicine
450 Technology Drive, Suite 300
Pittsburgh, PA 15219
Phone: 412-624-5209

Email: cantinip@upmc.edu

Directorate: Not Applicable

Responsibilities: Will be responsible for all aspects of project planning, direction, and development. Additionally, will serve as the primary administrative liaison for this project and will coordinate the myriad of activities to support a seamless interface with WRNMMC, Geneva, ReCell (Avita) and other collaborators.

DATES OF INVOLVEMENT: Fall 2012 to study completion

Dale Glaser, Ph.D

Glaser Consulting
3115 4th Avenue
San Diego, CA 92103
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Website: www.glaserconsult.com

Directorate: Not Applicable

Responsibilities: Biostatistical support

DATES OF INVOLVEMENT: Oct 2013 to
study completion

Andrew Quick, MS

Avita Medical Americas, LLC
9221 Corbin Ave, Suite 220
Northridge, CA 91324-2494
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Directorate: Not Applicable

Responsibilities: Vendor agreement for the procurement of the ReCell Autologous Cell Harvesting Device that will be used for the proposed intervention. Will also provide training on device use for study team members

DATES OF INVOLVEMENT: Feb 2013 to study completion

1.5 Medical Research Monitor

Carlton Q. Brown, M.D.

Attending Anesthesiologist, Anesthesiology Service
Department of Surgery
Walter Reed National Military Medical Center (WRNMMC)
8901 Wisconsin Ave Bethesda, MD 20889
Phone: (301) 295-4455 x166

Email: Carlton.Q.Brown.ctr@mail.mil

Directorate: Surgery

RESPONSIBILITIES: To provide safety monitoring of research subjects for

conditions that may arise during the conduct of the study including the authority to remove human subjects from the study and take any other actions necessary to protect the subjects of the study. Duties may include discussion of the protocol with the investigators, review study monitoring plans, data collection and analysis, interview human subjects, and consult with others outside the protocol about the research and make recommendations on changes to the informed consent process based on the review of study events. All adverse events will be reviewed and signed off on by the research monitor. Any observations and findings considered unanticipated problems involving risk to subjects will be promptly reported to the Institutional Review Board (IRB), the Human Research Protection Office (HRPO), the Human Protections Administrator (HPA), or the Institutional Official as deemed appropriate. Additionally, will review and sign substantial submissions to the IRB. If necessary, shall have authority to stop this clinical trial, remove subjects and take whatever steps needed to protect the safety and well-being of human subjects until the IRB can assess the monitor's report.

DATES OF INVOLVEMENT: June 2013 to study completion

1.6 Outside Laboratory Facility

Annapath, Inc.

Sachin Shetty, President

4801 Telsa Drive, Suite H

Bowie, MD 20715

Phone: 301-352-6100 or 410-370-0836

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Email: sachinshetty_us@yahoo.com

Directorate: Not Applicable

Responsibilities: Processing of biopsy specimens to include grossing, processing (staining), embedding into paraffin blocks and slide preparation (Note: Histology assessments will be performed by a dermatopathologist at WRNMMC)

DATES OF INVOLVEMENT: Fall 2013 to study completion

Study Team Responsibilities	Principal Investigator	Associate Investigator	Collaborator
▪ Study design and general coordination of research	X	X	
▪ Provide access to subjects	X	X	
▪ Recruit eligible subjects	X	X	
▪ Screen eligible subjects	X	X	
▪ Obtain informed consent	X	X	
▪ Obtain Health Insurance Portability and Accountability Act (HIPAA) Authorizations from participants	X	X	
▪ Conduct research procedures involving direct interaction with subjects	X	X	
▪ Clinical laboratory tests or support	X	X	
▪ Research laboratory assays, tests, or support	X	X	
▪ Pharmacy support (i.e. storage, inventory, and dispensing of all investigational drugs used in the research)	X		
▪ Data entry (eCRFs at WRNMMC; database UPitt/UPMC)	X	X	X
▪ Data analysis	X	X	X
▪ Retain/share research data	X	X	X
▪ Retention of research protocol documents (including consent/information sheets)	X	X	
▪ Retention of HIPAA documentation, including HIPAA authorization or other HIPAA templates with required representations from PI	X	X	
▪ Retain/share specimens for current research	X	X	X
▪ Reporting to appropriate IRB(s), institutional officials, and /or sponsor - unanticipated problems involving risks to subjects or others, adverse events, and serious or continuing non-compliance	X	X	X
▪ Establishing procedures to ensure subject privacy and confidentiality of research data (DoD 6025.18R; 45 CFR 160 and 164).	X	X	
▪ Submission of Continuing Review reports for IRB review	X	X	
▪ Establish a plan for the destruction of research data containing identifiers or for the retention of the identifiers for public health or research purposes or as otherwise required by law	X	X	
▪ Post-approval monitoring of the conduct of the research	X	X	X
▪ Data monitoring plan – coordination and communication	X	X	X
▪ Clearance of research-related publications or presentations	X	X	
▪ Training of ReCell device, provide investigational ReCell device kits			X
▪ Sponsor for IDE			X

ABSTRACT

2.1 Purpose

The purpose of this investigation is to evaluate the safety, tolerability, preliminary and long-term effectiveness of utilizing the ReCell Autologous Cell Harvesting Device (ReCell) combined with widened split-thickness skin graft (STSG) mesh onto the dermal regenerate INTEGRA™ Meshed Bilayer Wound Matrix (MBWM) for healing of full-thickness wounds.

2.2 Research Design

The proposed study is a prospective, single-center, randomized within-patient controlled feasibility study to evaluate the safety, tolerability, preliminary and long-term effectiveness of the ReCell Device for re-epithelialization (healing) of full-thickness wounds following previously successful treatment with INTEGRA™ MBWM.

2.3 Methodology /Technical Approach

In the proposed study, each patient will serve as his/her own control. Due to the nature of the study, only patients whose wounds have been previously successfully treated with INTEGRA™ MBWM as part of their standard of care will qualify for participation.

Therefore, as part of enrollment eligibility criteria for this study, the identified study wound will first be treated with INTEGRA™ MBWM. The wound will then be allowed to heal for approximately two to four weeks, at which time a viable granulation layer will have developed thus allowing for next stage STSG grafting. The timing of the STSG application will be determined by clinician judgment based on the state of the granulation process, which varies between patients, but typically takes 2 to 4 weeks.

Approximately two to four weeks after INTEGRA™ MBWM treatment, the studied wound will be divided into a ReCell-treated area (over 1:5 meshed STSG) and a control area treated with 1:1.5 meshed STSG (no ReCell). In all cases, the ReCell region may be up to 320cm² in size; the upper limit of application area for one ReCell kit, with a similarly sized control region. If the wound is larger than the combined ReCell and control regions (over 640cm²), the areas outside the study regions will be designated as non-study areas and treated according to standard of care. The same primary and secondary dressings will be used on ReCell-treated areas, control areas and donor sites. Once the study and control wounds are determined to have healed, standard local clinical practice will be followed.

Within-subject comparisons of the ReCell region and control region, in order to evaluate improvements associated with use of ReCell, a battery of measurements and evaluations will be made. These measurements are divided into three categories: safety and tolerability, preliminary effectiveness (acute healing process) and long-term effectiveness.

The safety of research participants is foremost. Therefore, efforts will be made to control risks to participants throughout the duration of their study participation. Wound healing time, donor site morbidity and histology will be assessed during an acute 6

week phase, with follow-up visits at Weeks 1, 2, 3, 4 and 6 post-treatment.

Subjects will continue in the study for long-term follow-up with clinic visits at Week 12 and 24 post-treatment. Healing of treated wound sites (A region and B region) and donor sites (STSG 1:5, STSG 1:1.5 and ReCell) will be assessed at each visit. Treated wounds will be considered healed when 95% or greater of the study area has re-epithelialized by Week 6 post-treatment. Donor sites will be considered healed when $\geq 95\%$ of the donor site has re-epithelialized by Week 4 post-treatment. Aesthetic and functional outcomes of the treated areas will be assessed and documented. Subject satisfaction will also be assessed and documented at these two time points.

3.OBJECTIVES AND SPECIFIC AIMS

The goal of the study described herein is to determine the safety, tolerability, preliminary and long-term effectiveness of the use of the Recell device over a widened STSG mesh. It is hypothesized the ReCell cell suspension, in combination with INTEGRA™ MBWM, will improve upon the current standard of care. The potential for ReCell's promotion of healing in the interstices of the STSG mesh may close gaps that are potential points of failure during subsequent rehabilitation and return to physical activity. Within the current study, each participant will serve as his/her own control, allowing for comparison of ReCell treated (experimental) and non-ReCell treated (control) regions of the grafted wound. The specific aims of this pilot clinical study are delineated below.

Specific Aim 1: Evaluate **safety and tolerability** of ReCell treatment of full-thickness wounds treated STSG over INTEGRA™ MBWM compared to control site. The safety and tolerability of ReCell treatment relative to standard of care (the control site) will be evaluated during the first 12 weeks after treatment, when the wound is most vulnerable for potential graft failure. Safety-related issues will continue to be monitored through 24 weeks after treatment.

Participants will be assessed for the following safety issues at each visit:

- Delayed healing/non-healing of wound and donor site
- Graft loss
- Heterotopic ossification
- Infection
- Scar contracture
- Durability (i.e. abrasions/injuries at graft site due to graft fragility)
- Allergic response to trypsin (the enzyme used in the ReCell process)
- Subject Complaint (i.e. pain and itching)
- Vital Signs
- Blood chemistries and hematology
- Other Treatment-related adverse events requiring surgical intervention prior to Week 12 post-treatment and all serious adverse event (SAE) occurrences throughout the study.

We predict non-inferiority in safety measures between ReCell-treated areas and control

areas of the wound.

Specific Aim 2: Evaluate the **preliminary effectiveness** of ReCell treatment of full-thickness wounds treated with STSG over INTEGRA™ MBWM compared to a control site. Preliminary effectiveness will be assessed during an acute healing phase (Week 1–6) and will focus on healing of both wound and donor sites. Evaluations to be performed include:

- Wound epithelialization
- Histology
- Patient pain rating

We predict that, at each time point, more ReCell-treated areas will be healed to an extent defined as non-inferior compared to the control areas of the wound.

Specific Aim 3: Evaluate the **long-term effectiveness** of ReCell treatment of full-thickness wounds treated with INTEGRA™ MBWM compared to a control site. Long-term effectiveness will be assessed at Weeks 12 and 24 post-ReCell treatment. Evaluations will focus on the integrity and durability of regenerated tissue as well as physical characteristics such as pliability and cosmesis. Patient satisfaction will also be evaluated. Evaluations to be performed include:

- Scar assessment scale using the Patient and Observer Scar Assessment Scale (POSAS) and Vancouver Scar Scale
- Functional outcome rating
- Patient satisfaction
- Histology
- Radiographic evaluation for assessment of heterotopic ossification

We predict that scar/functional outcomes and patient satisfaction metrics will be non-inferior for ReCell treated areas.

INTRODUCTION TO STUDY CONDUCT

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (International Conference on Harmonization ICH E6), the Code of Federal Regulations Title 21 parts 803 and 812, and other applicable government regulations and Institutional research policies and procedures. No amendment to the protocol or deviation from the protocol will be implemented without the prior review and approval of the Institutional Review Board (IRB) except where it may be necessary to eliminate an immediate hazard to a research participant. In such a case, the deviation will be reported to the IRB as soon as possible.

4. BACKGROUND AND SIGNIFICANCE

4.1 Literature Review.

As survival rates of our war wounded continue to improve through significant advances

in our military's forward medical care, the number of complex soft tissue and orthopedic extremity injuries present difficult ongoing challenges. Extremity injuries secondary to war conflict, especially those caused by improvised explosive devices (IEDs), can result in massive soft tissue losses secondary to foreign body contamination, infection-related soft tissue necrosis, development of compartment syndromes, and secondary requirements for serial debridement and surgical procedures to aid in controlling the local wound environments. Often, these extremity injuries lead to significant utilization of health care resources not only within the post-trauma inpatient settings, but also through the course of the wounded service members extensive rehabilitation requirements.

In addition to the direct injury patterns and soft tissue losses experienced within certain wounded service members, donor site burdens for their reconstructions can also be rather significant. Many of the wounded service members who suffer from traumatic extremity injuries will require advanced reconstructions. These reconstructions typically involve complex soft tissue procedures often coupled with orthopedic surgeries to eventually cover vital structures and/or the soft tissue defects. Soft tissue injuries require various immediate as well as staged reconstructions including but not limited to need for eventual skin grafts, local or regional flaps, to advanced techniques such as microsurgical free tissue transfers.

Wounds that penetrate deep into the skin (full-thickness) remain a major clinical challenge to patients and reconstructive surgeons. Application of split or full-thickness skin grafts is associated with significant donor site morbidity (e.g., bleeding and scar formation) and often in the severely wounded patient, harvest sites are limited. For this reason, there has been much interest in the application of artificial substrates that can be used to enhance healing of the full-thickness wound. One such substrate is the INTEGRA™ MBWM (Integra Life Sciences Corporation, USA), a bilayer membrane designed to provide immediate coverage of full-thickness wounds. This wound care device is comprised of a porous matrix of cross-linked bovine tendon collagen and glycosaminoglycans and a semi-permeable polysiloxane (silicone layer). The semi-permeable silicone membrane controls water vapor loss, provides a flexible adherent covering for the wound surface and adds increased tear and suture retention strength to the device. The collagen-glycosaminoglycan biodegradable matrix provides a scaffold for dermal cellular invasion and capillary growth. During use, the INTEGRA™ MBWM is placed on a surgically debrided or excised wound, providing the needed framework for the blood vessels and dermal skin cells to remodel the damaged site. As skin cells migrate into the matrix, the collagen is slowly absorbed and replaced with collagen produced from the patient's own cells. In approximately 14 to 28 days, the scaffold is eventually remodeled as the patient's cells rebuild the damaged site. Once the dermal cells have integrated with the porous matrix, the silicone layer is removed and typically replaced with a thin meshed STSG. Application of the meshed STSG over INTEGRA™ MBWM is currently the standard care for patients with full-thickness wounds treated at Walter Reed National Military Medical Center (WRNMMC).

However, individuals who have sustained a traumatic injury, particularly wounded warriors, frequently possess limited donor site availability due to the nature and extent of associated injuries.

Therefore, harvest sites for the STSG are often limited. The ReCell Autologous Cell Harvesting Device system allows autologous transplant of the patient's own skin cells, including epidermal progenitor cells using a small thin biopsy site (typically no larger than 2 cm by 2 cm).

Our primary hypothesis is that when used in combination with a meshed STSG over INTEGRA™ MBWM, ReCell will produce non-inferior safety and performance using an expanded meshing ratio, thereby decreasing the required donor site and a secondary hypothesis of potential superiority to promote faster healing, minimization of mesh patterned scar and overall improved patient quality of life.

4.2 Preliminary Data and/or Findings

The safety of ReCell has been demonstrated with over 5,000 treatment procedures conducted worldwide. ReCell has CE Marking in Europe, TGA clearance in Australia, SFDA clearance in China and Health Canada approval as well as approval in other markets (e.g., Taiwan, Hong Kong, Mexico, Brazil and Venezuela). Case series studies include an 81-patient Australian study (PSR-01 and PSR-02) of epidermal replacement due to acute superficial skin injury, unhealed donor site, raised scars, or correction of hypopigmentation caused by a previous injury or skin resurfacing procedure. These studies included both pediatric and adult patients. Effectiveness assessments included: percent re-epithelialization through 6 weeks post-treatment, clinical assessment of wound appearance at recipient site (e.g., healed, not healed), assessment of skin quality by a blinded assessor (photography assessment) in terms of color match with the surrounding skin, pigment and texture at recipient site, and appearance of scar at donor site in terms of color, pigment and texture and degree of scarring. In both studies, minimum follow-up was 26 weeks post-treatment. Safety was assessed based on adverse events, vital signs and concomitant medication. All wounds achieved healing ($\geq 95\%$ re-epithelialization) by Week 6. At the final assessment (Week 52 for PSR-01 and Week 56 for PSR-02), no wounds were determined to be grossly mismatched to the surrounding area with respect to color, pigment, or texture. Within these studies, use of the ReCell device in epidermal restructuring was well tolerated and did not show any systemic effect. There was no association with any alteration in vital signs. Overall the safety profile of ReCell kit observed in these studies demonstrated that the ReCell device can be used in patients requiring epidermal restructuring without causing any systemic toxicity.

Furthermore, several recent publications report on positive clinical results with ReCell in a variety of indications, including deep partial thickness burns [Gravante G, et al., 2007], vitiligo [Mulekar SV, et al., 2007] and hypopigmented scarring [Goodman GJ, 2008, Cervelli V, et al., 2009, Draaijers LJ, et al., 2004].

To date, there is no preliminary data applicable for this indication. However, ReCell is currently being evaluated in the United States as a primary treatment of partial-thickness burns compared with standard split thickness meshed skin grafts in a multi-center clinical trial and at Wake Forest School of Medicine, Institute for Regenerative Medicine under the direction of Dr. James Holmes, Medical Advisor for this proposed clinical trial. Safety monitoring of adverse events reveals application of the ReCell device has been well-tolerated and there have been no adverse events which are inconsistent or unexpected for the subject population.

4.3 Scientific Justification and Military Relevance

Significant soft tissue losses due to blast injuries are becoming more common in modern warfare. The efforts to manage these wounds are becoming more challenging. More than half of the injuries in modern combat are due to explosions; including mortars, rockets, and IEDs. The majority of these injuries are to the extremities, resulting in significant soft tissue loss and the need for available autologous skin sources. Given the complex and massive soft tissue injuries that traumatic blast injuries cause in a number of our wounded warriors, improved availability of skin regenerative techniques and methods to not only expand autologous skin donor sources but also to aid in lessening donor site morbidity is of great interest and importance. The ReCell Device is a stand-alone, battery operated regenerative skin cell separation device that enables preparation of a cell suspension from a small, thin, split-thickness skin biopsy site, thus, providing for great expansion of a limited autologous skin donor site.

Additionally, because the autologous epidermal cell suspension is available for immediate delivery onto a prepared skin surface at the time of skin donor harvest, the need for further cell expansion within the laboratory setting is avoided. The performance of ReCell over INTEGRA™ MBWM in combination with 1:5 meshed split-thickness skin graft (STSG) will be compared to standard practice control (i.e., 1:1.5 meshed STSG over INTEGRA™ MBWM), with the ultimate potential benefit of earlier re-epithelialization, acceptable durability, and reduction in the risk of infection and scarring for those wounds treated with ReCell.

Based on preclinical and early clinical results, the short-term benefits of this study will include potential viable and readily available regeneration of necessary autologous skin to place on a dermal regenerate in massive soft tissue injuries. This clinical model would be the first to potentially show the benefit of such treatments in traumatic wounds, outside of burn patients. It would also have immense benefits - both for those military healthcare beneficiaries suffering from soft tissue injuries as well as traumatic civilian injuries that likewise pose significant issues with available autologous skin coverage (e.g. necrotizing fasciitis, motor vehicle accidents or other trauma with associated soft tissue avulsion injuries, etc.). Important data on the mechanism of action, refinements of technique, expansion of the device and technique to future applications, and most importantly, technology transfer throughout WRNMMC, associated military treatment facilities (MTF), and civilian or academic centers is of great interest.

We expect all ReCell treated and control areas of the wounds to heal adequately. However, we hypothesize areas treated with ReCell will re-epithelialize more quickly than control areas, which has the potential to reduce the risk of infection and scarring in ReCell treated areas compared to control areas. Healing will be defined as: 95% or greater re-epithelialization has occurred at area treated by Week 6 post-treatment.

4.4 Human Subjects Justification.

The proposed prospective, non-randomized, within-patient controlled feasibility study will enroll 20 military healthcare beneficiaries, both male and female, currently

receiving treatment for a traumatic wound injury at WRNMMC. The goal of this study is to evaluate the safety, tolerability, preliminary and long-term effectiveness of the ReCell device for re-epithelialization of full-thickness wounds treated with INTEGRA™ MBWM.

Data collected in the completion of the proposed study will support or refute the hypothesis that the use of the ReCell device over a widened STSG mesh in combination with INTEGRA™ MBWM will improve upon the current standard of care for treating full-thickness wounds within the traumatic injury population. We believe that ReCell will promote improved healing within the interstices of finely meshed STSG, thus improving durability among STSG gaps that are potential points of failure during later physical activity.

Furthermore, ReCell will reduce the burden of donor skin graft sites by decreasing the amount of autologous skin grafting harvest necessary to cover massive soft tissue. In effect, we propose by using the existing standard for dermal regenerate in INTEGRA™ MBWM, full thickness wounds will be converted into partial thickness wounds, which can then be covered with finely meshed STSG and ReCell regenerative skin expansion system to provide more durable stable skin coverage while also reducing donor site morbidity and pain.

The impact of scar complications (i.e. pain, itching and fragility), may have impact on the quality of life. For the patient, the presence of pain and itching is often more disturbing than the actual appearance, and therefore these symptoms have a major impact on the quality of life. Additionally, scar formation can result in disabilities that impact activity levels for some.

Leveraging from prior experiences and successes of using ReCell within burn patients, this model will be the first to explore and allow for treating massive soft tissue injuries in a true traumatic setting. The results of the study will be presented at national meetings geared toward audiences that provide care to similarly injured individuals.

5. PLAN

5.1 Study Design

This is a prospective, single-center, randomized within-patient controlled feasibility study to evaluate safety, tolerability, preliminary and long-term effectiveness of the ReCell device when used in combination with STSG mesh for healing of full-thickness wounds previously treated successfully with INTEGRA™ MBWM. Due to the nature of the study, only patients whose wounds have been treated with INTEGRA MBWM as part of their standard of care will qualify for study participation. The local standard of care is considered the application of INTEGRA MBWM after the wound is amenable. Amenable being no evidence of evolving necrosis, clean and devoid of infection. The INTEGRA is allowed to mature until it takes (adheres) to the underlying tissue, which takes 2 to 4 weeks until the wound is amenable for split thickness skin grafting. Therefore, before consenting and enrollment into the study, the wound area to be studied will be treated first with INTEGRA™ MBWM then be allowed to heal for approximately two to four weeks (consistent with standard of care), at which time an assessment will be made to

determine if a viable granulation layer suitable for second stage grafting is present.
(Note: At this time the patient will complete the consent process as described below in Section 5.5.2).

The basic study design has the following characteristics:

- Each subject will serve as his/her own control
- After INTEGRA™ MBWM treatment (and once viable granulation is present), the study wound area will be divided into two regions within itself. Study treatment to each of the two regions will be assigned by randomization. Subjects will be blinded to treatment area assignments to avoid bias
- Each subject will be treated with meshed STSG over both the control and ReCell regions of the Integra-treated wound. The control area will have standard 1:1.5 meshing (only) STSG, and the experimental area will have 1:5 meshing STSG plus application of ReCell (ReCell will be applied over the 1:5 meshed STSG).

Once harvested, a STSG may be meshed by placing the graft on a carrier and passing it through a meshing device. A skin graft may be meshed to provide coverage of a greater surface area at the recipient site, with expansion ratios generally ranging from 1:1 to 6:1. This also allows for expansion of the donor skin to be grafted/transferred and permits egress of serous or sanguineous fluid from under the graft; however, it results in a pebbled appearance upon healing that may ultimately be of poorer cosmesis.

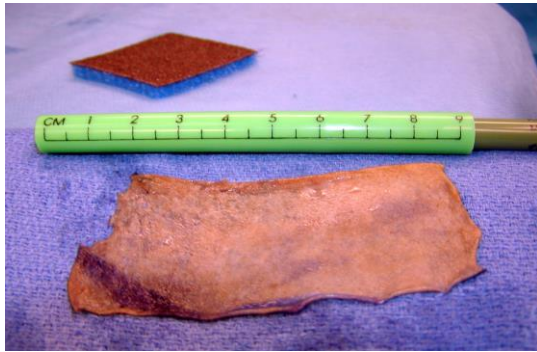
The carrier has a grooved side that must be directed superiorly and upon which the graft should be laid out. This technique allows expansion of the graft surface area up to 9 times the donor site surface area. Expansion slits allow wound fluid to escape through the graft rather than accumulating beneath the graft and preventing adherence. To create a meshed graft, a “mashing” apparatus (Zimmer, Padgett, and/or Bryan meshing systems) is used to put open spaces in the STSG in an organized fashion. This expands the graft so it can cover a larger surface area. The difference in ratios determines the expansion of the skin graft. For example, a 1:1.5 ratio has open spaces that are one and a half as large as the skin and a 1:5 ratio has open spaces that are five times as large as the skin. The larger the meshing ratio, the larger the surface area that can be covered by the graft.



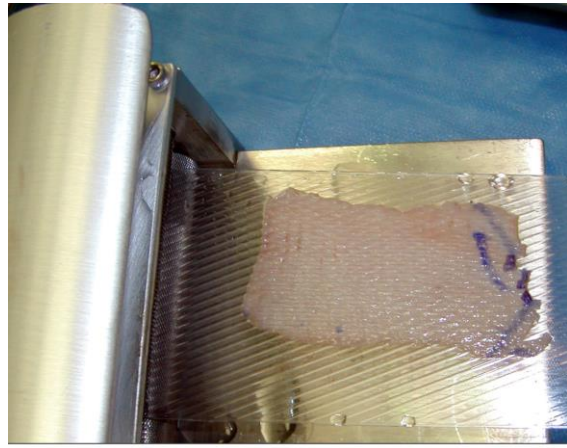
a.



b.



c.



d.



e.

- a. Zimmer Dermatome utilize to harvest the split thickness skin graft. The thickness and width of the harvested graft can be adjusted.
- b. Dermatome blade width to determine the width of the harvested graft.
- c. Harvested split thickness skin graft
- d. Graft mesher
- e. Recipient site grafted.

Treatment Summary Table:

ReCell Region (experimental)	Control Region (standard of care)
ReCell + 1:5 Meshed STSG	1:1.5 Meshed STSG (no ReCell)
INTEGRA™ MBWM	INTEGRA™ MBWM
Wound Bed	Wound Bed

Allocation of treatments (Recell vs Control) to the INTEGRA™ MBWM treated wound sites will be assigned at random using a computer generated randomization table assignment to the two defined wound regions. Wound regions will initially be labeled A region and B region by the surgeon, and then the subject assigned randomization envelope will be opened to determine which of the two treatments will be used at A region and which treatment will be used at B region.

The wound area to be followed in the study will be between 200cm² and 640cm². One ReCell kit is intended to treat up to 320cm² (which will cover half of the total “maximum” 640cm² wound area), allowing for comparison with a similar size control area of up to 320cm² that is treated according to the standard of care.

In all cases, the ReCell region may be up to 320cm² in size, the upper limit of application area for one ReCell kit, with a similarly sized control region. If the wound is larger than the combined control and ReCell regions (over 640 cm²), areas outside the study regions will be designated non-study areas and treated according to the standard of care. Additionally, areas with incomplete granulation, defined as a contiguous vascularized granulation layer at the INTEGRA™ MBWM application site, will be excluded from the study wound area.

The same primary and secondary dressings will be used on ReCell-treated areas, control areas and donor sites. Once the wounds are determined to be healed, standard local clinical practice will be followed.

In order to evaluate improvements associated with use of ReCell, within-subject comparisons across a battery of measurements and evaluations will be made. These measurements are divided into three categories:

- Safety and tolerability measures
- Preliminary effectiveness (acute healing phase)
- Long-term effectiveness

5.2 Anticipated Requirements

Study procedures will be performed at WRNMMC within the following areas: In a private room within the Orthopaedic Surgery and/or Plastic Surgery Service clinic (screening and most post-operative visits), WRNMMC laboratory, WRNMMC Radiology, WRNMMC Dermopathology, WRNMMC Biomedical Research Laboratory (BRL) (obtainment of liquid nitrogen), WRNMMC Peri-Operative Services and surgical ward (Note, as per standard of care for surgical procedure and recovery) and utilization of the WRNMMC Investigational Pharmacy Services and the WRNMMC Information Technology (IT) Services (CHCS, Essentris and AHLTA) for conducting medical record review. Study procedures being performed at/by non-WRNMMC include: The collective monitoring activities intended to assess the investigator’s compliance with the protocol, the signed agreement, IRB requirements, and applicable FDA regulations in an effort to ensure human subject protection will be performed by IMARC, Inc. Under the supervision of Dr. Peter Rubin (Sponsor and holder of the IDE), study data will be housed at UPMC and the Center for Innovation in Restorative Medicine (CIRM) will serve as the data management center. Dr. Rubin will maintain oversight regarding regulatory requirements and compliance of clinical data and data analysis. In collaboration with Dr. Rubin, coded data will be shared with Glaser Consulting who will perform all statistical analysis of data. Examination of cellular properties of the biopsy tissue material obtained from each subject by a member of the study team to determine skin composition relative to normal skin will be sent to and processed by an outside laboratory facility, Annapath, Inc. They in turn will ship slides back to WRNMMC to be interpreted by WRNMMC’s dermatopathologist, Dr. Abel Jarell.

The study design consists of screening pre-treatment/baseline assessments, an acute

phase and a long term follow-up phase. The acute phase duration is 6 weeks subsequent to treatment with INTEGRA™ MBWM and post treatment with the ReCell device. Subjects will continue to be followed for up to 24 weeks to capture long-term outcomes. The total duration of the subject's participation in this research study across all visits, including screening and follow-up surveillance is expected to be up to 26-28 weeks.

The time required to complete the research (including data analysis)

Anticipated Start Date: May 2014

Expected Completion Date: Oct 2017

Budget and Source of Funds

Please see Appendix K for copy of budget

Funding will be provided by the United States Army Medical Research Acquisition Activity (USAMRAA) under Grant # W81XWH1320004

5.3 Subject Population

The research population to be studied will include male or female military healthcare beneficiary of any race or ethnicity, aged 18 years or older, who is being treated for a traumatic wound at WRNMMC. With a target enrollment of 20 evaluable subjects, this investigation will include individuals treated for traumatic wounds at WRNMMC with full-thickness wounds, with loss of the dermis extending to deeper tissue layers and disrupting dermal blood vessels, and deep partial-thickness wounds, with injury limited to the epidermis and superficial dermis with no damage to the dermal blood vessels that were successfully treated with INTEGRA™ MBWM. Successful INTEGRA™ MBWM engrafting is defined as the presence of a contiguous vascularized granulation layer.

It is planned to enroll a sufficient number of subjects at WRNMMC in order to ensure a total number of 20 evaluable subjects. Therefore, up to 30 subjects will be consented and enrolled then screened to achieve a maximum of 20 evaluable subjects. Subjects who do not receive treatment with the assigned study device (ReCell) will be considered non-evaluable and therefore a screen failure for study participation.

WRNMMC will be the coordinating center for this clinical trial and will be the only site to enroll subjects for participation. The University of Pittsburgh, UPMC Center for Innovation in Restorative Medicine (CIRM), under the direction of Dr. J. Peter Rubin will be the Sponsor of the IDE and will also function as the Data Coordinating Center (DCC) accountable for maintaining oversight of all data management activities to ensure quality and integrity with accurate reporting, interpretation and verification, and will ensure systems and procedures are in place for the protection and safeguarding of the confidentiality of all data records.

It is anticipated the availability of the study population will be derived from the investigators' clinical practices, individuals evaluated and/or referred by healthcare providers aware of the study within the WRNMMC Orthopaedic Surgery, Plastic Surgery and General Surgery Clinics. Additionally, study participants will be recruited by use of a posting on the research registry at www.clinicaltrials.gov. Those interested

in study participation will be instructed to contact a study team member. This study will not use any advertisements, flyers or brochures.

5.4 Subject Inclusion and Exclusion Criteria

a. Inclusion Criteria

Subjects will be eligible for study participation if they meet all of the following criteria:

- The subject is a male or female military healthcare beneficiary of any race or ethnicity, aged 18 years or older, who is being treated for a traumatic wound at WRNMMC
- The subject has soft tissue loss resulting from a traumatic mechanism such as an explosive blast (i.e. mortar, rocket, IED), high-velocity shells (i.e. missile), an avulsion injury, gunshot wound motor vehicle accident and/or burn secondary to blast
- The subject's full-thickness or deep partial-thickness traumatic wound injury has been treated with INTEGRA™ MBWM as part of their standard of care
- The wound area is at least 200 cm²
- All areas of the study wound area are covered with INTEGRA™ MBWM and has fully engrafted – engrafting defined as the presence of a contiguous vascularized granulation layer indicated by the formation of a viable granulation layer (**Note:** there may be some areas of incomplete granulation at the INTEGRA™ MBWM application site, these areas will be excluded from the study wound area).
- The subject will comply with protocol requirements
- The subject will provide voluntary written informed consent and Health Insurance Portability and Accountability Act (HIPAA) authorization

b. Exclusion Criteria

Subjects will not be eligible for study participation if they meet any of the following criteria:

- The subject is pregnant and/or lactating (self-reported)
- The subject has evidence of the following lab value results:
 - a. Hematocrit \leq 20%
 - b. INR $>$ 1.8 second
 - c. Creatinine (serum) $>$ 2.0 mg/dL
 - d. Bilirubin Total (serum) within upper limit of normal (normal range 0.3-1.9 mg/dL)
 - e. Liver function test (AST/ALT) greater than 2 times upper limit of normal as defined by the clinical laboratory defined normal ranges
 - f. Albumin (serum) $<$ 2.0 g/dL
 - g. Platelets $<$ 70 K/ μ L
- The subject's targeted traumatic wound injury is a craniofacial wound

- The subject's targeted traumatic wound injury is on a weight-bearing surface
- The subject's targeted traumatic wound is a full-thickness burn injury with visible eschar present (Note: Subjects with a traumatic wound of a burn nature secondary to an explosive blast injury resulting in significant soft tissue loss **will NOT** be excluded)
- The subject has active infection processes, that in the opinion of the investigator may compromise safety or study objectives
- The subject is known to have a pre-existing condition that may interfere with wound healing, e.g. malignancy, diabetes or autoimmune disease, immunocompromised blood borne diseases, has AIDS, is HIV or Hepatitis-A positive, or currently has a severe dermatological disorder (e.g. severe psoriasis, epidermolysis bullosa, pyoderma gangrenosum)
- The subject has other concurrent conditions that in the opinion of the investigator may compromise safety or study objectives
- The subject has a known hypersensitivity to Trypsin and/or Compound Sodium Lactate for Irrigation (Hartmann's) solution
- The subject cannot be compliant with study procedures and that, in the investigator's opinion, would interfere with the study objectives

5.5 Study Methodology/Procedures

5.5.1 Describe when, where and how the study subjects will be identified and recruited. Study participants will be recruited from a population of patients being treated for a traumatic wound at Walter Reed National Medical Center (WRNMMC). In this investigation, patients whose full-thickness wounds have been treated with INTEGRA™ MBWM as part of their standard of care will be considered for potential participation when a viable granulation layer has formed to allow for second stage grafting (approximately 2 to 4 weeks after Integra treatment). It is anticipated availability of the population to be studied will be derived from investigators' clinical practices, individuals evaluated and/or referred by healthcare providers aware of the study within the WRNMMC Orthopaedic Surgery, Plastic Surgery or General Surgery clinics and services. Additionally, study participants will be recruited by use of a posting on the research registry at www.clinicaltrials.gov. Those interested in study participation will be instructed to contact a study team member to receive an explanation of the study purpose, requirements and risks. This study will not use any advertisements, flyers or brochures.

5.5.2 Consent Process

WRNMMC will be the only site to enroll subjects for study participation. Informed consent/HIPAA authorization will be obtained in a private room within the Orthopaedic Surgery, Plastic Surgery, General Surgery service clinic or at the potential subject's bedside if he/she is an in-patient, by a member of the research team after educating the subject on the study specifics to include: the nature of the research study, study design schema, the risks and benefits of participation, what will be expected of them should they choose to participate, their rights as a research subject, any cost and payments. The potential subject will be allowed

ample time to review all information, exchange information and to ask questions. Should the potential participant wish to take the consent and review it outside of the clinic setting or discuss with other family members or medical personnel, he/she will be able to leave the clinic to do so and return at a later date.

After reviewing all components of an IRB-approved informed consent/HIPAA authorization document and all questions regarding study participation have been answered to the potential participant's satisfaction, the study investigator or his designee will obtain written informed consent/HIPAA authorization prior to beginning any research activities. Written consent will be obtained and documented in accordance with the principles of Good Clinical Practice (GCP) and International Conference on Harmonisation (ICH). Source documentation of the consenting process will include, but not limited to, confirmation of the participants willingness to participate in the study after having been informed of all aspects of the clinical trial that are relevant to the volunteer's decision to participate and that written informed consent was obtained prior to conducting any study specific procedures. A copy of the signed informed consent/HIPAA authorization document will be provided to the subject for his/her records in order to provide continuing references for contacts and a copy will be placed in the subject's research chart. It will be documented in the source record that the subject was given a copy of the signed and dated fully executed consent/HIPAA authorization document.

The original signed consent document will be placed and maintained in the study site's regulatory files. The subject will then be assigned a unique 3-digit study ID number that will be used throughout the study to code all protected health information (PHI).

Additional protections are in place to minimize undue influence of DoD personnel. These additional protections include the following: 1) Officers will be not be in uniform (will be in scrub attire) when obtaining consent and no visible indication of rank; 2) Officers cannot influence the decision of their subordinates to participate in the research; 3) Officers and senior non-commissioned officers cannot be present at the time of recruitment into the research; 4) Officers and senior non-commissioned officers have a separate opportunity to participate in the research.

No research related tests or procedures, including but not limited to the screening procedures or the review of medical records will be performed before the informed consent/HIPAA authorization form has been obtained.

Efforts will be made to promote the subject's understanding of the consent. At all times, the use of explanatory terms that are in accordance with the subject's education level will be utilized; the information provided to the participant will be in language understandable to him/her. No subject should grant consent until questions have been answered to his/her satisfaction. Additionally, after obtaining the subject's voluntary consent, the Investigator and members of the study team will be obligated to continue to provide information as the subject's needs, or the clinical situation, dictate. The subject should understand that the study product is an investigational device and is not licensed by the FDA for commercial use, but is permitted to be used in this clinical research study. Informed consent includes the principle that it is critical that the subject be informed

about the principal potential risks and benefits. This information will allow the subject to make a personal risk versus benefit decision and understand the following:

- Participation is entirely voluntary,
- Subjects may withdraw from participation at any time,
- Refusal to participate involves no penalty, and
- The individual is free to ask any questions that will allow him/her to understand the nature of the protocol

If there are any modifications made to the original protocol that may be relevant to the subject's original consent (e.g. change in study procedures, new PI assigned, significant safety or new information becomes available about the study device), the consent document must be revised to reflect the changes to the protocol. If a previously enrolled subject is directly affected by the change, the subject will receive a copy of the revised IRB approved informed consent document. Subjects still participating in the study will be re-consented by repeating the process of reading, signing and dating the revised consent/HIPAA authorization form to acknowledge protocol changes.

5.5.3 Compensation for Participation

Participants will not be compensated for participating in this study. Should the participant be injured as a direct result of participating in this study, they will be provided medical care, at no cost, for that injury. They will not receive any injury compensation, only medical care.

5.5.4 Research Interventions

NOTE: All activities and procedures described below are being performed solely for the purpose of research unless otherwise specifically denoted as standard of care.

Participants recruited for study participation consideration will be derived from investigators' clinical practices, individuals evaluated and/or referred by healthcare providers aware of the study within the WRNMMC Orthopaedic Surgery, Plastic Surgery or General Surgery clinics and services. Additionally, study participants will be recruited by use of a posting on the research registry at www.clinicaltrials.gov. Those interested in study participation will be instructed to contact a study team member.

In this investigation, subjects whose full-thickness wounds have been treated with INTEGRA™ MBWM as part of their standard of care will qualify for participation. Approximately two to four weeks after the patient has undergone INTEGRA™ MBWM treatment, the wound will be assessed by a clinician to verify that the INTEGRA™ MBWM treated wound has produced a viable granulation layer suitable for meshed STSG and ReCell treatment. If there is incomplete take of the initial INTEGRA™ MBWM, an additional treatment with INTEGRA™ MBWM may be performed. The patient will be treated according to standard of care until a viable granulation layer is present (approximately two to four weeks). Standard clinical care for this type of wound injury includes treatment with negative pressure wound therapy (NPWT) such as a wound vac device over the INTEGRA™ MBWM. Therefore, potential study participants will be allowed to continue

receiving NPWT up until the time of the STSG procedure. After two to four weeks of incorporation of the INTEGRA™ MBWM product, coverage of the new dermal regenerate will then be the next step in staged reconstruction of these wounds. Assuming a viable granulation layer is present, the patient may be approached by an Investigator for their consent to participate.

Following discussion of the protocol, including a thorough explanation of the experimental procedures, written informed consent and Health Insurance Portability and Accountability Act (HIPAA) authorization will be obtained from all subjects prior to performing any study-related tests or procedures. The subject will then be enrolled into the study and assigned a unique 3-digit study ID number that will be used throughout the study to code all protected health information (PHI). The investigator will maintain a Subject Enrollment Log for all participants consented and enrolled into the study. The master Subject Enrollment Log will be kept in the study regulatory files in a locked file cabinet located in the Orthopaedic Surgery Clinic at WRNMMC.

After the subject has been consented, he/she will undergo thorough screening procedures to assess eligibility to participate in the study. The specific screening procedures are outlined below.

Screening Pre-Treatment/Baseline Assessments:

Screening procedures will be performed by an investigator (PI and/or AI) up to 30 days prior to receiving treatment with the investigational device.

A member of the study team will obtain a medical history including current medication use and allergies derived from both subject reporting and information obtained from a review of their medical record with a focus on pre-existing conditions that may interfere with wound healing. The surgical report from the INTEGRA™ MBWM treatment procedure will be included as part of the medical history and relevant data will be captured on the Baseline Medical History eCRF. Demographic information (date of birth, gender, race/ethnicity) will be recorded on the eCRF.

As part of the inclusion eligibility criteria, the subject will undergo a physical examination to include obtainment of the subject's height, weight, temperature and vital signs (heart rate, respiratory rate and blood pressure). Findings will be recorded on the eCRF. Any conditions or symptoms reported after the subject receives investigational product will be recorded as an adverse event (AE) on the AE eCRF. This includes new events after study device administration, conditions that become more severe or increase in frequency, any disease-related signs or symptoms present at the Screening Pre-Treatment visit that have worsened in severity or frequency, as well as any event or finding that the Investigator feels is clinically significant.

An evaluation of the traumatic wound area treated with INTEGRA™ MBWM will be made and recorded on the eCRF. The total wound area will be measured. The wound area will be inspected to ensure all areas of the study wound has fully engrafted as indicated by the formation of a viable granulation layer as this will allow for grafting. Both of these findings will be recorded on the eCRF.

A baseline assessment for heterotopic ossification will be established using radiographic imaging. Anterior/posterior and lateral films of the wound area targeted for treatment will be obtained. If the subject's medical record contains an x-ray taken of the wound area targeted for treatment within 30 days prior to enrollment indicating the presence or absence of heterotopic ossification, these radiograph images (x-rays) may be used to avoid unnecessary exposure to radiation.

A baseline assessment for pain and pruritus at the traumatic wound site will be obtained for comparison after receiving study treatment with ReCell. Subjects will be asked to rate their pain using a 0-10 point verbal numerical rating scale where "0" equals no pain and "10" equals worst pain possible. Additionally, the subject will be questioned if experiencing pruritus at the targeted wound site. If so, he/she will be asked to rate the intensity using the following options: mild, moderate and severe. Responses from both assessments will be recorded on the eCRF.

A blood sample (15 mls) will be drawn for hematological analysis including a complete blood count (CBC) with differential panel, coagulation studies to include prothrombin time (PT) and international normalized ratio (INR) and comprehensive metabolic panel (CMP). Results will be reviewed by a physician investigator and documented on the eCRF.

The total time of participant commitment for this portion of the screening pre-treatment baseline assessments including the consenting process will take approximately 1.5 to 2 hours.

Each subject must meet all inclusion and no exclusion criteria in order to qualify for receiving treatment using the ReCell device. The PI or designee will make the final determination of subject eligibility. Subjects who sign the informed consent/HIPAA authorization document, but do not meet eligibility criteria will be defined as a screen failure. The investigator will maintain a screening log that documents the subject ID number and reason(s) for screen failure.

Once the surgery plan has been determined, the following procedures **will occur as part of standard of care:**

Scheduling Surgery

An OR scheduler will select the first appropriate date and time for surgery according to OR time availability.

Scheduling Pre-Operative Clinic Visit

It is anticipated many of the subjects enrolled will be of an in-patient status. If not, the subject will be scheduled for a Pre-Operative clinic visit when the surgery date has been determined. The subject will be informed he/she will be required to undergo a pre-operative evaluation to ensure suitability for surgery as per standard of care. Per WRNMMC procedures and guidelines for pre-operative patients, individuals will undergo the following activities prior to surgery: obtainment of a medical history including current medication use, a physical examination, weight, vital signs (heart rate,

breathing rate, and blood pressure), temperature, laboratory work and possibly, a standard chest x-ray and electrocardiogram (EKG), if deemed necessary. All females will be required to provide a urine sample for the purpose of pregnancy testing that must be negative in order to participate in this study as pregnancy is an exclusion criterion. No data will be collected for the pre-operative procedures performed.

The total time of participant commitment for completing the Pre-Operative assessments will take approximately 1.5 to 2 hours.

Upon confirmation of the subject's eligibility and scheduled surgery date, an order for the ReCell kit will be written by the Investigator and sent to the WRNMMC investigational pharmacy to request the treatment ReCell device kit for availability on the scheduled day of surgery for the subject enrolled.

The manufacturer of the ReCell Autologous Cell Harvesting Device, Avita Medical Americas, LLC, will supply the study site with ReCell kits containing the processing unit and items for cell harvesting. Arrangements will be made to have the investigational device kits shipped from the manufacturer directly to the Investigational Pharmacy Services at WRNMMC, to the ATTN: Dr. Parvenah Moussavian, Departmental Chief, where they will be housed until ordered by the investigator for use.

Please refer to Section 5.6.2 for IDENTIFICATION AND DESCRIPTION OF INVESTIGATIONAL PRODUCT

A Pharmacy Manual will be provided by the Sponsor to maintain records of investigational device disposition. The Research Pharmacist will follow the procedures in this manual for maintaining adequate records for documentation purposes of shipment and receipt of the investigational device, device dispensing records and maintaining device accountability log and final disposition of the investigational devices to include return of unused devices.

The ReCell device will be administered only to those subjects enrolled in the clinical study and under investigator or designee's supervision (as noted on the Enrollment/Randomization Log and Delegation of Responsibility Log maintained in the study regulatory files).

Re-verification of subject meeting all inclusion and exclusion criteria will be confirmed by the investigator before obtaining the ReCell device kit from the investigational pharmacy on the day of surgery.

Treatments with the ReCell device will be staggered for the initial three subjects. A treatment pause period of 1 week (5-7 days) will be implemented for the first and second subject, as well as the second and third subject within this study.

On the day of surgery, a member of the study team will pick up 2 kits; one will be dispensed for use and the other will be obtained for the purpose of having "on-hand" in the event of a device malfunction while in the operating room. The investigational pharmacist will record dispensing of both device kits. One kit will be denoted as

“intended for use” and the second kit will be denoted as the “back-up device” in the device accountability log. At the end of surgery, the second device deemed as the “back up device” will be returned to the investigational pharmacy by the study coordinator and logged back in on the device accountability log to be dispensed for future use with the next subject enrolled. Stringent record keeping of this process will be maintained for device accountability.

Anesthesia procedures will follow the same clinical standards as all operative procedures performed at WRNMMC. Subjects will be maintained NPO after midnight the morning of surgery. With the ReCell treatment, the subject will be given general anesthesia during the entire procedure. No data will be collected for the anesthetic regimen provided.

Surgical Procedure and Methods:

Subjects will be anesthetized and the wound will be prepared according to hospital standard of care practices. It is anticipated that the total surgical time will be up to 3 hours. The operative procedure will be performed by the PI and/or the Co-PI in the operating room at WRNMMC. The INTEGRA™ MBWM treated wound will be sectioned into two **similarly sized** study regions up to 320cm² each, the upper limit of application area for one ReCell kit. The surgeon will label these two regions “A” and “B” with a surgical pen. A pre-generated randomization envelope will then be opened to determine which area will be the control area and which area will be treated with ReCell. Any wound area outside the study regions will be treated according to standard of care.

In this study, serial histology assessments of the wound site will be made. Baseline punch biopsies of 3 mm diameter tissue samples from the INTEGRA™ MBWM treated wound site and adjacent normal skin will be obtained in the operating room prior to start of the surgical procedure. This baseline histological analysis will examine the vasculogenesis and cytology of the regenerate before ReCell treatment. The biopsy procedure will be recorded in the eCRF.

A digital photograph will be taken of the control and ReCell sites (“A” region and “B” region) and noted on the eCRF. Next, the wound bed will be gently scrubbed and “prepared” then the thin silicone layer is removed, the wound bed will be prepped and prepared again for receipt of proposed STSG or STSG in conjunction with ReCell regenerative skin treatment system.

Harvesting of autograft will be the same thickness for both control and investigational site; at a depth of 0.012 inch to 0.016 inch which is alignment with standard of care practice. Donor tissue for the STSG for the ReCell treatment area may be harvested from the same area as for the control STSG or separate area. To assess healing of the donor sites for ReCell, the site has to be at a discrete location, but can be collocated, i.e. same thigh, but different area.

A digital photograph will be taken of donor sites following tissue removal and will be noted in the eCRF. These and all subsequent photographs at each study visit will be taken in ambient light, with a consistent background and distance between camera and subject.

Donor tissue for the control assigned area will be meshed at 1:1.5 with a meshing system of the surgeon's choice as per standard of care surgical practice. Application of the prepared STSG meshed at 1:1.5 will be secured in place with staples to the randomized control region.

Donor tissue for the ReCell treatment assigned area will have a 1:5 meshed STSG using the same meshing system as the control area. Application of the prepared STSG meshed at 1:5 will be secured in place with staples to the randomized investigational region followed by the use of the ReCell device treatment over this widened STSG mesh as outlined below.

ReCell

Below is a picture of the investigational product, the Autologous Cell Harvesting Device kit (ReCell):

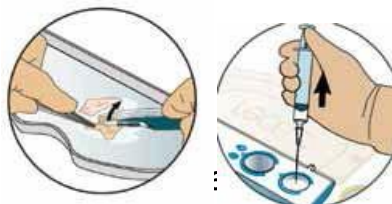


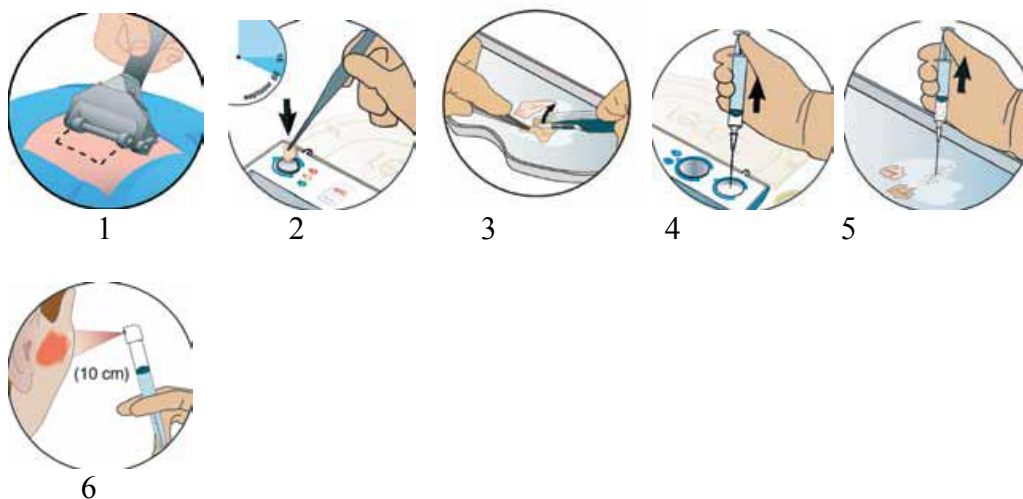
The ReCell assigned area will be treated as described in the ReCell Instructions For Use (IFU) document and the “Set Up Card” included with each ReCell device kit (see **Appendix A1 and 2**).

A representative from Avita Medical Americas, LLC will provide training to the PI and study team members for use of the ReCell device kit. Initial training will be conducted at the site initiation visit. Training will include review of the Instructions for Use (IFU) brochure which contains background and product safety information pertaining to the autologous cell harvesting device kit, materials and instruments needed during the ReCell procedure, step-by-step instructions for preparation and set up of the ReCell kit prior to use, ReCell skin processing, cell suspension preparation and cell suspension administration to the targeted ReCell wound region, aftercare and troubleshooting tips.

Subsequent, periodic training on the investigational product over course of study will be carried out by the Avita representative and/or PI as needed (i.e. addition of new study team members). All training sessions for study personnel will be documented on the Study Personnel Training Log and will be placed in the study regulatory files.

ReCell Procedure:





1. A thin, split thickness shave biopsy, 0.15 - 0.2 mm in depth, is taken of site matched skin
2. Skin sample is incubated in the ReCell enzyme solution for approximately 15 minutes
3. Skin sample is removed from incubator, mechanically agitated to separate cells
4. Cells are then rinsed and collected using ReCell buffer solution
5. Cells are filtered and suspension is drawn up
6. ReCell suspension is then sprayed or dripped on wound followed by covering with recommended dressing

The surgeon will follow manufacturer instructions for determining the appropriate skin biopsy size to cover the ReCell treatment area. The ReCell donor site will be approximately 2cm x 2cm in area and harvested using a dermatome instrument of surgeon's choice set at a depth of 0.006 inch to 0.008 inch (equivalent to 0.15 - 0.2 mm) (Note: the thickness for traditional skin grafts are thicker at a depth of 0.012 inch to 0.016 inch). Selection of a site-specific donor site is important for promotion of the same functional and cosmetic characteristics as the area to be treated with ReCell; e.g., a donor site is chosen with similar pigment and texture to the treatment site. Site-specific characteristics may include the thickness of keratinocyte layers in glabrous tissues (soles of the feet and palms of the hands), melanocyte distribution and secretory functions of the epithelium. It is essential the donor site is clean, of appropriate depth, and shows no evidence of surrounding cellulitis or infection. Biopsy size, location and depth will be recorded in the eCRF.

For optimum cell viability, the skin sample should be processed immediately after harvesting. If a skin sample is harvested and processed according to the IFU, it should only require between 15 and 30 minutes of contact with the enzyme. The total time of incubation in enzyme solution will be recorded on the eCRF. Contact in excess of 60 minutes is not recommended by the manufacturer; if this should occur, a protocol deviation will be reported to the IRB and Medical Research Monitor.

The volume of suspension prepared is dictated by the size of the treatment area as identified in the ReCell IFU as indicated in the table below. The volume of cell suspension produced will be recorded on the eCRF.

Treatment Area	200 cm ² - 260 cm ²	>261 cm ² – 320 cm ²
Buffer Solution Volume	3 mL	4.5 mL

The cell suspension spray created using the ReCell device will be applied over the prepared STSG wound site meshed at 1:5 as detailed below. The PI and/or Co-PI (only) will apply the investigational product to the pre-determined wound region.

- For wound areas 200cm² - 260cm²: The 18 gauge blunt cannula supplied with the ReCell device will be attached to the syringe containing the cell suspension. The syringe will be inverted several times prior to application to ensure an even suspension. Telfa™ Clear wound dressing (Covidien, USA) will be applied to the inferior margin of the wound and the cell suspension will be manually dripped along the superior edge of the wound.
- For wound areas >261cm² - 320cm²: Telfa™ Clear wound dressing (Covidien, USA) will be applied to the inferior margin of the wound. The spray nozzle supplied with the ReCell device kit will be attached to the syringe containing the cell suspension using firm pressure. The syringe will be inverted several times prior to application to ensure an even suspension. The spray applicator will be held approximately 10 cm from the most elevated point of the wound surface and moderate pressure will be applied to the plunger of the syringe. Spraying begins at the most elevated part of the wound so that any run-off helps to cover the more dependent areas of the wound. One application of a fine mist of cells will be delivered to the entire wound surface. To cover the area, the spray applicator will be carefully moved in one continuous motion from one side of the wound to the other while spraying. Special care will be taken by the surgeon when applying the ReCell spray to ensure the cell suspension is only applied to the designated treatment area; the dressing will serve as a barrier to avoid applying the spray skin to the control area.

The volume of cell suspension applied to the ReCell treated wound and method of application will be recorded on the eCRF.

The ReCell suspension will be applied to the designated randomized treatment region only; donor sites will not receive application of any Recell suspension. Any deviations from this will be captured in the eCRF.

The subject will not be made aware of treatment area assignments (will be blinded) until study participation completion to avoid bias when completing questionnaires provided at each follow-up visit.

Dressings:

Upon completion of the surgical procedure, the treatment area and donor sites will be dressed in the following manner:

The primary dressing will be Telfa™ Clear wound dressing (Covidien, USA). Post-treatment photographs will be taken after placement of the primary dressing. After

placement of the primary dressing, a secondary dressing, Xeroform™ Petrolatum Gauze (Covidien, USA) will be applied to keep wounds moist then area will be wrapped with bulk dressing (e.g. gauze) to protect the wound surface, with or without an adhesive retention dressing as clinically indicated. The primary and secondary dressings should remain in situ for a minimum of 6 to 8 days and are not to be manipulated until the first post-operative visit unless medically necessary.

The donor area will be dressed in the following manner: The primary dressing will be Xeroform™ Petrolatum Gauze (Covidien, USA). The area will then be wrapped with bulk dressing (e.g. gauze) plus or minus a compressive wrap (ace-wrap) based on the area of harvest (extremity vs torso). The donor area bulk dressing will be removed at 48 hours. The primary dressing (Xeroform™ Petrolatum Gauze) will remain in place and be gradually peeled off over several days.

The primary dressing can be trimmed and removed as the Telfa™ Clear lifts from the wound bed. Under no circumstances will the primary dressing be forcibly removed; the Telfa™ Clear should be allowed to fall off on its own accord. If the Telfa™ Clear dressing hasn't fallen off in 3 weeks, only at this time point can it be moisten to peel off. It is essential that any dressing not easily removed be soaked in aqueous or oil-based solutions to prevent trauma upon removal. Once the primary dressing has been removed, a dressing of the investigator's choice will be applied to protect the wound surface, with or without an adhesive retention dressing as clinically indicated. All dressing regimens, dressing changes and dressing types will be recorded on the eCRF. Once the wounds are determined to be healed, therapy will be applied that is consistent with the standard of care at the clinical site.

Any dressing used other than Telfa™ Clear as primary dressing and Xeroform™ Petrolatum Gauze as secondary dressing at the treatment site areas prior to wound healing will be considered a protocol deviation.

Negative pressure wound therapy (NPWT) (i.e. wound vac) will not be reapplied after treatment with ReCell as this may compromise the treated area by removing the applied cells.

Post-Operative Care:

Following surgery, subjects will be transferred to the Post Anesthesia Care Unit (PACU) and then to the In-Patient ward. Post-Operative recovery in terms of hospitalization and length of stay will be as per standard of care for this type of procedure.

It has been identified from previous use, the loss of formed skin at the ReCell site appears to be primarily associated with secondary trauma due to inadvertent inadequate care and/or protection of the newly epithelialized area. As a corrective measure, the wound will be appropriately protected while the skin is maturing. These measures include use of appropriate protective dressings after initial wound closure to give the epithelium adequate time to mature and cornify, ensuring the primary dressing removal is atraumatic and not before post-treatment Day 6 and avoidance of potentially damaging cleansing regimens and cytotoxic creams (i.e., silver sulfadiazine). Additionally, for the duration of their care, subjects will be informed on the following post-treatment ReCell site care

guidelines recommended by the manufacturer:

- Prevent the treated area from getting wet while the wound is still open
- Refrain from strenuous activity
- Protect the healed area; don't bump, wear loose clothing over treated area
- Once the area has healed, massage and keep area moisturized (using a gentle moisturizer) at least twice a day
- Avoid direct sun exposure at least 4 weeks following treatment and use sunscreen

Post-Treatment Study Visits

Subjects will be re-evaluated by the Orthopaedic Surgery Service, General Surgery Service and/or Plastic Surgery Service clinic at WRNMMC or on the in-patient wards. At all post-treatment study visits (Week 1 \pm 3 days, Weeks 2, 3, 4, 6 \pm 2 days, Weeks 12 and 24 \pm 14 days post-treatment), the PI and/or AI will perform the following assessments as outlined below:

- **Wound and Donor Site Healing Assessments:** The ReCell treated area and control site will be assessed by an Investigator for signs of atypical healing, including delayed healing (not healed 95% or greater by Week 6 post-treatment), inflammation, heterotopic ossification, scar contracture and graft loss. Inflammation in the absence of infection will be assessed as a possible allergic reaction to Trypsin (see below). Any signs of atypical healing will be detailed in the eCRF. Each of the donor sites will be assessed for signs of infection and healing (healed \geq 95% or not healed by Week 4 post-treatment). Findings will be recorded on the eCRF.
- **Infection:** The presence of infection for the ReCell treated areas and the control site will be evaluated at each postoperative visit. Infection will be evaluated in accordance with the Center for Disease Control (CDC) guidelines for nosocomial infections using standard clinical measures such as visual examination of the treatment sites for delayed healing, redness, inflammation and surrounding cellulitis. In the presence of symptoms (i.e., purulent exudate, changes in wound appearance such as hyperemia, and erythema in the uninjured skin surrounding the wound), infection will be confirmed using microbiological testing procedures and treatments initiated according to the institutions' infection management protocols, which will be recorded on the eCRF. Infection will be managed according to the standard protocols of the clinical site. For example, treatment of infection will involve the daily cleaning and dressing of wound sites until such time that the infection is clear. Treatment with broad spectrum antibiotics until microbiology sensitivities return from the testing laboratory is recommended. Upon return of sensitivities, antibiotic therapy may either continue as is, or be changed at the discretion of the investigator. All treatment regimens applied in the management of infection will be recorded in the eCRF.

- **Allergic response to Enzyme (Trypsin):** The allergic response to Trypsin, the enzyme used in the ReCell Device for disaggregation of the biopsy, will be evaluated. An allergic response to Trypsin is most likely to present as contact dermatitis (defined as an altered state of skin reaction induced by exposure to an external agent). Substances that produce this condition after single or multiple exposures may be irritating or allergic in nature and induce an inflammatory response. The most common clinical expression of this induced inflammation is dermatitis (eczema). The evaluation tools for assessment of the allergic response to Trypsin have been developed in accordance with guidelines published in the Journal of the American Academy of Dermatology [Drake, LA, et al., 1995]. Any allergic response(s) to trypsin will be recorded in the eCRF and depending on severity, will have additional reporting to the IRB and Research Monitor.
- **Durability:** Abrasions or injuries at the graft site due to graft fragility will be noted and described in the eCRF.
- **Digital Photography:** The healing process at the ReCell donor site and at the treated and control wound sites will be documented pictorially. Photographs obtained will be documented in the eCRF.
- **Vital Signs:** Vital to be assessed include oral temperature, heart rate, respiratory rate and blood pressure. Results will be recorded in the eCRF.
- **Subject complaint:** Subject complaints will be rated on a 10-point scale by the subject utilizing the “patient” portion on the POSAS (*see description below*) for the treated wound sites and documented in the eCRF.
- **Adverse Events:** The research team will assess if the subject has experienced any adverse effects post-treatment with the investigational device. The subject will be asked if he/she has any problems, issues and/or concerns since the last encounter visit that could be potentially related to study participation. Other treatment-related adverse events requiring surgical intervention prior to Week 12 post-treatment and all serious adverse event (SAE) occurrences will be treated and recorded in the eCRF. For all adverse events, the Investigator will provide an assessment of the event, its treatment resolution, and relationship to the investigational device.
- **Concomitant Medication Use:** Concomitant medication use (excluding anesthetic regimen) and all treatment regimens applied in the management of infection during the acute healing phase will be recorded in the eCRF. Concomitant medication used for the treatment of an adverse event occurring from time of receiving investigational product through Week 24 post-treatment will also be recorded in the eCRF.

In additional to above assessments, additional “visit specific” assessments will be made as outlined below:

At Week 1 post–treatment:

- **Bloodwork:** A blood sample (15 mls) will be drawn for hematological analysis including a complete blood count (CBC) with differential panel, coagulation studies to include prothrombin time (PT) and international normalized ratio (INR) and comprehensive metabolic panel (CMP), which is consistent with standard of care practices. The sample will be labeled and processed by WRNMMC laboratory medicine per institutional policy. Due to the nature of complexity and multi- system involvement resulting from trauma in the targeted subject population, the occurrence of a deviation from the institutional normal reference range(s) for laboratory assay results is anticipated. Therefore, laboratory levels falling outside of the “normal” reference range and/or with a change in test result of 20% from baseline value will be evaluated for clinical significance by the Principal Investigator and/or AI physician. Any laboratory value deemed clinically significant will be captured on the adverse event eCRF. Additional laboratory testing will be performed only as clinically indicated.

At Week 2, 4 and 12 post-treatment:

- **Histology: 3-mm** punch biopsies will be obtained by a physician investigator. A sample collection of 3mm diameter will be taken from both the control and ReCell treated sites and documented in the eCRF. Biopsies will be obtained from a centralized region of each wound. Biopsies will be taken for histologic analysis and analyzed to determine skin composition relative to normal skin.

At Week 12 and 24 post-treatment:

- **Scar Assessment Scales:** The entire POSAS (both patient and observer scales) will be utilized (*see Appendix B*). Scar appearance will be assessed by the patient and by two independent observers (WRNMMC dermatologist subject matter expert investigator) who will be blinded to treatment provided at the wound site. Each scar (control and ReCell-treated sites, denoted on assessment forms as A Region and B Region due to “blinded” component) will be evaluated once by each observer. The evaluations will be made blind to each other. Findings will be documented in the eCRF.

In addition, the Vancouver Scar Scale (VSS) (*see Appendix C*) will be used at each of these visits. Using word descriptions, the VSS assesses four variables: scores pigmentation, vascularity, pliability and scar height/thickness. The four categories are given a score between 0 and 3 or 0 and 5, depending on the value, leading to a total score between 0 and 13 points. Evaluations of the control and ReCell-treated sites (denoted on assessment forms as A Region and B Region due to “blinded to treatment” component) will be performed utilizing this scale by the two independent observers (WRNMMC dermatologist subject matter expert investigator) who will be blinded to treatment provided at the wound site and will be recorded in the eCRF.

- **Functional Outcome Rating Questionnaire:** The Functional Outcome Rating Questionnaire consists of a 10-point visual analog scale (*see Appendix D*) used to

rate scar pain. Subjects will be asked to evaluate the amount of scar pain experienced at the control and ReCell-treated sites (denoted on assessment forms as A Region and B Region due to the subject being “blinded” to treatment). Findings will be documented in the eCRF.

- **Patient Satisfaction:** A 2-question (per study area) non-standardized Patient Satisfaction Questionnaire regarding treatment (denoted on assessment forms as A Region and B Region due to “blinded to treatment” component) (*see Appendix E*) will be completed by the subject and documented in the eCRF.

At Week 24 post-treatment:

- Radiographic imaging (anterior/posterior and lateral films) for assessment of presence/absence of heterotopic ossification.

The total duration of participant time commitment for each of the post-treatment study visits is approximately 1 hour.

Subject Discontinuation and/or Withdrawal

A subject may withdraw consent and discontinue participation in the study at any time and for any reason without penalty, if he/she wishes to do so. The investigator may also withdraw a subject if continuing participation is believed to be harmful to the subject’s well-being or in the event of protocol violations, non-compliance (i.e. loss to follow-up), the need to initiate care contraindicated to study procedures and/or requirements, pregnancy, serious inter-current illness and if the study is cancelled due to other administrative reasons and/or unanticipated circumstances by the Sponsor of this study or the FDA. Additionally, the subject’s participation may be stopped without their consent if the military mission requires it or if the individual loses his/her DoD healthcare beneficiary status.

If the subject’s participation is stopped, the investigator will notify the subject and provide reason for doing so in person, by mail, email and/or telephone.

In the event that the subject withdraws and/or is withdrawn from the study before the completion of the Week 24 post-treatment assessment, he/she will be asked to come to the clinic for a final assessment of safety evaluations. If a subject withdraws before study completion, the reason for discontinuation will be captured on the appropriate eCRF page. Additionally, any data collected up until the time of subject withdrawal will be included in the final study analysis. (*Please see section 5.5.7 for additional information pertaining to biological specimens/withdrawal*)

Subjects who are withdrawn will not be replaced if they have received study treatment.

Additional Information:

Patient and Observer Scar Assessment Scale (POSAS)

The standard and comprehensive Patient and Observer Scar Assessment Scale (POSAS) [Draaijers LJ, et al., 2004] will be employed in this study, as a standard tool for capturing both the subject’s and two independent observer’s assessment of the scar post-treatment

[Idriss N, 2009]. Although many methods of rating scars exist, little consensus exists to dictate which method is optimal [Fearmonti R, et al., 2010, Durani P, et al., 2009]. Objective measures may be useful for evaluating a particular characteristic (e.g. color or elasticity), but these do not satisfactorily convey overall, clinically-relevant information about the scar as a whole [Brusselaers N, et al., 2010].

The POSAS, consists of two multi-item numeric rating scales, an observer scale and a patient scale. The Observer Scale is devised with items based on literature review and the authors' clinical experience: 'vascularization', 'pigmentation', 'thickness', 'relief', 'pliability' and 'vascularization' rated on a 1 - 10-point numeric scale, with 'normal skin' and 'worst scar imaginable' used as end-anchor labels. The items on the Patient scale directly correspond to these except with regard to scar color. Individual items for both scales are summed with higher scores representing poorer scars and lower scores representing scars more closely resembling normal skin. Both scales have demonstrated acceptable internal consistency (Cronbach's alpha 0.76 (patient) and 0.69 (observer scale)), suggesting that individual items for each scale can be reliably summed to generate a total score. The POSAS has the added benefit of capturing the subjects' ratings for scar pain and itching and expands on the objective data captured in the VSS.

Vancouver Scar Scale (VSS)

The VSS is a quantitative objective scale which seeks to provide a standard for analyzing scar tissue. This assessment tool is widely used in clinical practice and research as it allows for a means to document changes in scar appearance. The scale is based on four values: pliability, height, vascularity and pigmentation. The four categories are given a score between 0 and 3 or 0 and 5, depending on the value, leading to a total score between 0 and 13 points. A low score indicates an appearance closer to normal skin.

Malfunction of ReCell Device

In the event of a malfunction occurrence of the ReCell device or any of the kit components, the "back-up" ReCell device dispensed by the investigational pharmacy will be utilized. In addition, if a ReCell kit or any of its components malfunctions and is replaced or results in an adverse event occurrence, the information will be documented in source documents and captured in the eCRF.

Procedure If Malfunctioning of Investigational Device Occurs:

If any device fails to operate, the failed ReCell device (kit) system will be returned to Avita Medical for evaluation via use of their established Return Material Authorization (RMA) process. Per manufacturer directives, the study site will notify manufacturer of need to return malfunctioning investigational device kit. They in turn will generate and send site an Investigational Device Return Form along with a return FedEx air bill to cover return shipment fees. The devices will be returned to the address below:

Return to:

Avita Medical
c/o Parker Hannifin Corp
ATTN: Customer Service
3007 Bunsen Avenue, Suite L
Ventura CA 93003

Histological methods:

Histological methods will be similar to those used in the Moiemmen and colleagues article [2006] who described histological analysis of punch biopsies taken from patients who had received INTEGRA™ MBWM treatment. That study identified four stages of tissue development after application of Integra. The first three phases, imbibition, fibroblast migration and neovascularization should be well established by the proposed study's first histological assessment four to six weeks after INTEGRA™ MBWM application (two weeks after ReCell treatment). Histological assessment of the proposed study will therefore focus on the last phase, remodeling and maturation, which begins four weeks after INTEGRA™ MBWM application, coinciding in time with our first scheduled punch biopsy. During this remodeling and maturation phase, fibroblasts imbue the matrix and fill the interstices with host collagen. Matrix collagen is progressively replaced by this new host collagen which appears as normal dermis. The neodermis is initially thicker than normal dermis, but becomes thinner and more pliable as it matures over a period of months. In the Moiemmen study, an autograft was applied to the wound four weeks after INTEGRA™ MBWM treatment. The autograft was seen to adhere to the surface of the neodermis during this final phase, and rete ridges were seen along the dermal-epidermal junction. Given the similarities between the proposed study and the methods used by Moiemmen and colleagues, we expect both ReCell-treated and control areas will show histological similarities to the results seen during the remodeling and maturation phase described in that study.

Punch biopsies of 3mm diameter will be obtained pre-skin graft from the INTEGRA™ MBWM treated wound site and adjacent normal skin and from ReCell and control treated sites at Weeks 2, 4 and 12 post ReCell treatment. The investigator will discuss the procedure with the subject in detail and will obtain a separate consent form as per standard of care. Samples will be analyzed to confirm the thickness of new tissue and assess the normality of structures present in the newly regenerated skin.

With the exception of the baseline INTEGRA™ MBWM treated wound site and adjacent normal skin punch biopsies, the procedure will take place in a private room within the Orthopaedic Surgery, General Surgery Service and/or Plastic Surgery Service clinic at WRNMMC. Utilizing sterile technique, the site will be prepped and draped as per standard of care. Approximately 3-5cc's of 1% lidocaine (with epinephrine) solution will be instilled as a local analgesic to the treated wound area (Note: No local analgesic will be used for pre-STSG sample as subject will be anesthetized for surgical procedure). The specimen will be obtained by using a standard punch biopsy instrument consisting of a circular blade, which is rotated down through the epidermis and dermis, and into the subcutaneous fat. The PI and/or AI, who are surgeons, will collect a 3mm diameter cylindrical core of tissue sample from a centralized region of both the control and ReCell treated wound sites.

All tissue samples will be coded and labeled with the subjects' 3-digit ID number, date and time of harvest. Tissue samples will be cut in half with a sterile scalpel; one half of the sample will be preserved in 10% formalin by a member of the study team then delivered to Annapath Inc via courier pick-up for tissue processing, embedding (paraffin

blocks), microtomy and slide preparation as per Annapath, Inc Technical Guidelines and Annapath, Inc Procedure Protocol (*see Appendix F and Appendix G*). The other half of tissue sample will be flash frozen with liquid nitrogen then stored in a -80° freezer with alarm system located within the Department of Orthopaedics Biomedical Lab Room 2010, Building 19 (America).

Following the methods detailed by Moimen and colleagues, hematoxylin and eosin stains will be used to detect blood cells and adnexal structures, vimentin stain will be used to detect esenchyme-derived cellular elements including fibroblasts, and S100 will be used to stain neuroectodermal tissue. Vascularization will be assessed by immunohistochemical analysis using antisera to endothelial cell markers CD31 and CD34. In addition, Verhoeff-Van Gieson (VVG) stain will be used for identifying elastic fibers and Masson's Trichrome stain will be used for the detection of collagen fibers.

Sections will also be assessed visually with an electron microscope to aid morphological evaluation of structures by WRNMMC's dermatopathologist. The dermatopathologist will provide a written report summarizing their findings. This information will be captured in the eCRF and entered into the database.

The Research Coordinator will call the subject within 72 hours after the biopsy procedure to obtain a subject self-report assessment of signs/symptoms of an adverse event post-biopsy (bleeding or signs/symptoms of infection) as standard of care follow-up to a surgical procedure.

Digital Photography Method:

When followed by different physicians, a uniform assessment of changes in the healing process needs to be established. Therefore, to facilitate uniform assessment/rating and improve reproducibility of the results, digital photography for clinical evaluation and follow-up of subjects will be utilized to minimize variations between different assessors. The definition for successful healing of the target wound via digital photography will be defined as: The target wound will be deemed as "successful healing" when 95% or greater re-epithelialization is noted upon visualization of the treated wound within the photograph taken.

In this study, clinical photographs will be obtained through the Biomedical Photography Department located at WRNMMC.

The Biomedical Photography Department is responsible for all medical photography taken and stored at WRNMMC. Photos taken through Biomedical Photography Department are obtained as standard of care and are stored and maintained as a part of the patient's medical record following institutional policies. Digital photographs will be taken in ambient light with a consistent background and distance between camera and subject. Physical features identifying the subject (i.e. tattoo or birthmark), if present, will be hidden in photographs.

A photograph of the wound will be taken pre-treatment and then serial photographs will be taken of the ReCell donor site and at the treated and control wound sites at each follow-up visit. Additional photographs may be obtained outside of the

stated study time points for evaluation purposes if medically indicated (i.e. incomplete healing and/or AE occurrence).

A copy of the digital photographs will be obtained from the Biomedical Photography Department to be placed in the study files. Photographs will be coded by using the unique 3-digit study ID number and labeled each with the date the photograph was obtained, the corresponding visit (e.g. pre-treatment and/or post-treatment week) and site location (e.g. treatment site (ReCell or control) or donor site (ReCell donor site or STSG) then placed on a disc to be stored with the main study database at completion of the study.

5.5.5 Data Collection

Data management activities and statistical analytical support will be performed by the UPMC Center for Innovation in Restorative Medicine (CIRM) and its consultant, Glaser Consultants. With oversight provided by J. Peter Rubin, MD, CIRM will prepare a Data Management Plan (*see Appendix H*) and serve as the Data Coordinating Center (DCC) for all data management activities. The DCC will have no direct interaction or intervention with study subjects. The DCC will manage design, implementation and maintenance of the database, data entry, editing, ongoing quality control of the data, generation of reports, staff training, interval database access for investigative personnel, back-up and data security.

For this clinical trial, data will be collected at WRNMMC only then transcribed onto an electronic case report form (eCRF). No database is anticipated of data collected at WRNMMC; all data maintenance and analysis will occur only at CIRM.

Subject data collected for this research project will be entered into the Medrio electronic data entry system via use of a web browser. Medrio is commercially available software that provides an integrated eClinical Software as a Service (SaaS) platform with a fully hosted Electronic Data Capture (EDC) system. The Medrio system is a secure, regulatory compliant, web-based, electronic data capture tool designed to support research studies that requires an individualized password protected login in order to have access to the application.

The DCC/UPMC will secure the required educational license agreement (the University of Pittsburgh will serve as the educational institution of record) that is necessary, free of charge, for procurement of the Medrio system software tools to allow for database development. The DCC/UPMC will have sole ownership of the database. No parts of the database will be downloaded to the DoD network or devices.

Data will be collected from source documents and data collection forms designed in tandem with the protocol. Key datasets derived from the study protocol (*see Appendix L, Sample eCRF*) will be mirrored for database development by the DCC.

De-identified (coded) data collection entries into the Medrio system will occur on an ongoing basis during subject participation and will be performed by WRNMMC research team members only. Subjects will not be entering study specific questionnaire responses into the data entry system; they will be asked to complete a paper version handout.

Working from source documents and the data collection forms, the Research Coordinator will update the Medrio data management system within one week after each scheduled study visit for the subject.

All data collection activities will be monitored by IMARC Research, Inc.; qualified monitors, based on training and experience, will be assigned to this study as per Clinical Monitoring Plan (*see Appendix I*). Periodic onsite monitoring visits will be made to WRNMMC by the IMARC Research, Inc. clinical trial monitors to inspect the conduct of the study to determine if it is being executed according to the protocol and report their findings to the Sponsor. The monitors will assure that data is accurately captured on the eCRF and in agreement with source documentation; verify that investigational product is properly stored and accounted for, verify that subjects' consent for study participation has been properly obtained and documented, confirm that research subjects entered into the study meet inclusion and exclusion criteria, and assure that all essential documentation required by Good Clinical Practice (GCP) guidelines are appropriately filed.

In collaboration with Glaser Consultants, CIRM will perform all statistical analysis of intermittent data and upon completion of all subject visits.

The Investigator is responsible for maintaining any source documentation related to the study, including digital photographs of the donor sites and wound site treated with the investigational product, clinical laboratory data, radiographic imaging (x-ray results) for assessment of heterotopic ossification, histology analysis, informed consent forms and device accountability records.

a. Method of Collection from Study Participants:

Please refer to above Section 5.5.4

Please refer to CRF's included with this submission

The method for collection of clinical data from study participants by members of the study site personnel will be derived from interviews and assessments performed by the study investigator and/or coordinator during study visits. Study materials, information, photographs, radiographic imaging (x-ray results) for assessment of heterotopic ossification and biopsy samples obtained for the purpose of this clinical trial will be coded with the subjects assigned 3-digit study ID number. Listed below is data that will be collected at the following time points:

Screening/Pre-Treatment: Data collected will be information obtained from the subject's medical history including current medication use, allergies, information related to the traumatic injury, treatment with INTEGRA™ MBWM, demographic information, physical examination including height, weight and vital signs, pre-treatment photograph of treatment sites and donor sites, bloodwork for hematology/chemistry analysis, baseline pain and pruritus assessments at traumatic wound site using the POSAS tool, baseline radiographic imaging (x-ray results) for assessment of heterotopic ossification (if not previously obtained within 30 days of study enrollment), histological assessment and identification of randomized treatment

areas.

Surgical Procedure: Data collected will be: Wounds dimensions, the ReCell device lot number and expiration date, lot numbers (from inner and outer package box) for the enzyme and expiration date, information regarding device kit preparation for ReCell suspension and application, intervention photographs before treatment and after tissue removal, initial wound dressings, biopsy procedure information, and any device and/or surgical related problems or concerns, if any.

Acute Healing (Week 1, 2, 3, 4, and 6 post-treatment): Data collected will be atypical epithelialization (healing) characteristics at the wound and donor sites, wound dressing/treatment regimens, photography at the treatment and donor sites, biopsy for histology, scar pain/itching rating using the POSAS, results from routine laboratory tests obtained at Week 1 post-treatment, concomitant medication use and any adverse event occurrences; both subject reported and medical record review.

Long-term Outcome (Week 12 and 24 post-treatment): Data collected will be atypical epithelialization (healing) characteristics at the wound and donor sites, photography at the treatment and donor sites, radiographic imaging (x-ray results) for assessment of heterotopic ossification, biopsy for histology, scar pain/itching rating using the full POSAS (both Subject and Observer), scar assessment using the Vancouver Scar Scale, functional outcome rating questionnaire, patient satisfaction questionnaire results and any adverse event occurrences; both subject reported and medical record review.

- b. Source and Type of Data Collected from Existing Data Sources: Answer the questions below after considering the minimum necessary data required for the research study.
- i. Have you received a data consultation with a data expert to determine the data elements to be extracted or the data system to access? (Consulting with a data expert often saves time later in the compliance process because the data expert can advise on the data available in the numerous MHS data systems, the quality of that data and the methods for encrypting and collapsing data. You may contact a data expert at the following email address: TMADDataDetermination@tma.osd.mil)
- ☐ Yes, then complete the questions in this subparagraph b according to the information received from the data consult
- X No, then complete the questions in this subparagraph b according to the best of your knowledge (**NOTE:** It is highly recommended to get a data consult).
- ii. Indicate whether you will receive a data extract from the MHS or will access a data system to create a data set. A data extract is when the MHS or a contractor provides the data set directly to the researcher. When receiving a data set through data extract, the researcher may indicate whether the data

elements should be provided as is, encrypted or collapsed. In contrast to a data extract, access to a data system means that the researcher may access a data system and create a data set for the research study.

☐ Data Extract X Access

- iii. Do you intend to use only a de-identified data set in your research study?

A de-identified data set is a data set that does not include any of the identifiers listed in the table in 5.5.5 (b)(vi). In addition, the researcher does not have actual knowledge of another way the data can be used alone or in combination with other known information to identify an individual. De-identified data is also data that a person, with appropriate knowledge of and experience with generally accepted statistical and scientific principles and methods for rendering information not individually identifiable, determines is not individually identifiable in accordance with the conditions outlined in 45 CFR 164.514 (b)(1).

☐ Yes X No

- iv. Will you need MHS data with health information?

Data with health information means any information that is created or received by the MHS that relates to the past, present or future physical or mental health or condition of an individual, the provision of health care to an individual or the past, present or future payment for the provision of health care to an individual. Examples of MHS data with health information includes data maintained on AHLTA, CHCS and ESSENTRIS.

X Yes ☐ No

- v. Do you intend to access a data base to obtain personally identifiable information that is not health information (PII)?

X Yes, will access data base for (PII)

☐ No, will not access data base for PII

- vi. Include the following table in your protocol and put an “x” in the MHS column next to the categories of data that you are requesting from the MHS. If you are planning to receive a data extract of MHS data that includes a data element that will be deidentified by the MHS, then you do not need to put an “X” in the column corresponding to the category of data for that data element.

	Study Participant	MHS	Other
1. Names	X		
2. Postal address with only town, city, State and zip code	X		

3. All geographic subdivisions smaller than a State, including street address, city, county, precinct, zip code and their equivalent geocodes, except for the initial three digits of a zip code if, according to the current publicly available data from the Bureau of Census: 1) the geographic unit formed by combining all zip codes with the same three initial digits contains more than 20,000 people; and 2) the initial three digits of a zip code for all such geographic units containing 20,000 or fewer people is changed to 000.			
4. All elements of dates (except year) for dates directly related to an individual, including birth date, admission date, discharge date, date of death; and all ages over 89 and all elements of dates (including year) indicative of such age, except that such ages and elements may be aggregated into a single category of age 90 or older	X	X	
5. Telephone numbers	X	X	
6. Fax numbers			
7. Electronic mail addresses	X		
8. Social security numbers (In accordance with the DoD Social Security Number Reduction Plan, please justify the reason for collecting the social security number of study participants in the space after xi.)	X	X	
9. Medical record numbers			
10. Health plan beneficiary numbers			
11. Account numbers			
12. Certificate/license numbers			
13. Vehicle identifiers and serial numbers, including license plate numbers			
14. Device identifiers and serial numbers		X	
15. Web Universal Resource Locators (URLS)			
16. Internet Protocol (IP) address numbers			
17. Biometric identifiers, including finger and voice prints			
18. Full face photographic images and any comparable images			
19. Any other unique identifying number, characteristic, or code (DEERs ID, EDIPN, Rank)			

vii. If you are requesting access to an MHS data system, put an “x” in the Request from MHS column next to the MHS system(s) from which you are

requesting data. If you do not know what system contains the elements you intend to request, please refer to the *Guide for DoD Researchers on Using MHS Data* or seek guidance from a data expert.

PHI Systems

MHS Data Systems	Request from MHS
AHLTA	X
CDM (from the MDR)	
CHCS	X
ESSENTRIS	X
PDTS	
TRAC2ES	
M2	
MDR	
PDHRA	
PDHA	
TMDS	
PEPR	
JTTR	

PII Systems Only

MHS Data Systems	Request from MHS
DMDC	N/A
MHS Learn	N/A
DMHRIS	N/A

De-Identified Data System

MHS Data Systems	Request from MHS
EAS	N/A

Other Systems

List Other system(s): N/A

viii. Do you intend to merge or otherwise associate requested data with data from any other sources outside of MHS, including other DoD sources that are not part of the MHS?

☐ Yes, will merge data X No, will not merge data

ix. Using the tables in 5.5.5 (b) (vi), put an “x” in the Other column next to the categories of data or data elements that include the data elements you intend to merge. *Not Applicable*

- x. Is there any possibility that the data will become identifiable because of triangulation, a small cell size, or any unique data element(s)?

Triangulation means using different data elements that are not themselves identifiable but that in combination can be used to determine the identity of an individual. For example, triangulation would be using rank and race together to determine the identity of an individual with a particular health condition.

Small cell size means that the categories contain a small number of eligible individuals that satisfy the category description. Guidance for acceptable cell size is available from the Centers for Medicare and Medicaid Services. For example, the rank category of five star generals with a particular diagnosis may be less than 30 so the data category may need to include lower ranks too.

A unique data element includes any unique features that are not explicitly enumerated in the categories of data in Table vi above, but that could be used to identify an individual. Examples of unique data elements include 1) a unique number, such as a medical record number or EDIPN; 2) a unique code, such as a diagnosis code or a bar code on electronic health record; and 3) any unique characteristic, such as a high rank like general or admiral or a unique race or gender with another unique characteristic.

☐ Yes, there is a reasonable possibility the data will become identifiable.

X No, there is no reasonable possibility the data will become identifiable.

- xi. Please justify the reason for collecting the social security number of study participants.

We will collect the last four digits of the participants social security number for the purpose of accessing the medical record for chart review and verification of study data collected.

5.5.6 Collection of Human Biological Specimens

Human biological samples will be collected for this study; blood and tissue.

Bloodwork: A blood sample (15 mls) will be drawn for hematological analysis including a CBC with differential panel, coagulation studies to include prothrombin time (PT) and international normalized ratio (INR) and comprehensive metabolic panel (CMP) at screening pre-treatment and at Week 1 post-ReCell treatment, which is consistent with standard of care practices. Samples will be labeled and processed by WRNMMC laboratory medicine per institutional policy. Therefore, these samples will be labeled with subject identifiers. Additional laboratory testing will be performed only as clinically indicated. Any leftover blood from sample will be discarded per WRNMMC policy.

Punch biopsy: A tissue sample size of 3mm diameter will be obtained pre-treatment (baseline) from the INTEGRA™ MBWM treated wound site and adjacent normal skin and from the control and ReCell treated sites at at Week 2, 4 and 12 post-ReCell treatment. All tissue samples will be coded and labeled with the subject's 3-digit ID number, date and time of harvest. Tissue samples will be cut in half with a sterile scalpel; one half of the sample will be preserved in 10% formalin by a member

of the study team then delivered to Annapath Inc. via courier pick-up for tissue processing, embedding (paraffin blocks), microtomy and slide preparation as per Annapath, Inc Technical Guidelines and Annapath, Inc Procedure Protocol (see Appendix F and Appendix G). The other half of tissue sample will be flash frozen with liquid nitrogen then stored in a -80° freezer with alarm system located within the Department of Orthopaedics Biomedical Lab Room 2010, Building 19 (America).

As previously mentioned, one half of the tissue sample obtained for histology analysis will be frozen. The rationale for doing this is to preserve the ability to return to the frozen sample, if need be, in the event we do not get immunohistochemical analysis staining needed from the formalin fixed slides. Oftentimes formalin fixed tissue does not perform as expected therefore, necessitating the need to freeze part of the sample to preserve options. The frozen samples will only be stored for evaluation within the period of funding for this research project then destroyed as per institutional policy standards.

5.5.7 Banking of Human Biological Specimens

This clinical trial does not involve the long-term storage (banking) of biological specimens.

- **Where and how specimens will be stored** (including shipping procedures, storage plan, etc.): One half of the tissue specimen obtained from the surgical punch biopsies of the treated wound (performed at pre-treatment, Week 2, 4 and 12) and of normal adjacent skin will initially be sent to Annapath, Inc. for processing (slide preparation and paraffin block embedding) as per Annapath, Inc Technical Guidelines and Annapath, Inc Procedure Protocol (*see Appendix F and Appendix G*). Annapath, Inc. will coordinate and dispatch its own courier for tissue specimen pick-up at WRNMMC. Annapath, Inc. will return all slides and paraffin blocks to WRNMMC for analysis and storage. The prepared slides and paraffin blocks will be housed in a locked cabinet within the Department of Orthopaedics Biomedical Lab Room 2010, Building 19 (America). The other half of the tissue specimen will be flash frozen with liquid nitrogen obtained from the WRNMMC Biomedical Research Laboratory (BRL) then stored in a -80° freezer with alarm system located within the Department of Orthopaedics Biomedical Lab Room 2010, Building 19 (America).
- **How specimens will be labeled** (Specimens should be coded without any personal identifiers): No personal identifiers will be used in the coding of human specimens. All tissue samples will be coded and labeled with the subject's 3-digit ID number.

Who will have the access to the specimens, the clinical information, and the linkage: Annapath, Inc. laboratory staff will have access to coded tissue specimens up until the time they are processed and returned to WRNMMC. The study investigators will have access to the stored frozen samples, prepared slides and paraffin blocks. The information linking with the subject's 3-digit ID number to the name of the participant will be kept by the Principal Investigator or designee in the study regulatory files in a locked cabinet.

- **How specimens will be used (general and/or specific use):** The baseline histological analysis will examine the vasculogenesis and cytology of the regenerate before ReCell treatment. Serial samples will be analyzed to confirm

- the thickness of new tissue and assess the normality of structures present in the newly regenerated skin.
- **Specify the length of time that specimens will be stored:** The frozen samples and paraffin blocks will be housed in the Department of Orthopaedics Biomechanical Lab, Room 2010, Building 19 (America) and will be destroyed at the end of study completion. No specimens will be stored for future research. Slides will be stored according to institutional policy.
 - **How the confidentiality will be protected at the storage and at the time of distribution:** Please see section on confidentiality.
 - **Whether subjects will be contacted and consented for future uses:** At this time, we do not anticipate contacting subjects in the future for other uses of their stored tissue samples.
Approval process for future studies: At this time, we do not have plans for future studies. Should this change, the PI will seek sub-protocol IRB approval for any future related studies.
 - **How subjects may withdraw their specimens from storage:** Research participants may request withdrawal/destruction of their tissue samples up until the time that they are used for histology analysis by asking a study investigator. Should any research participants withdraw from study participation, they will be specifically asked if their collected samples may be studied per protocol.

This study will not obtain specimens from an existing bank/ repository, nor will there be any genetic research/testing.

5.5.8 Study Time Line

Please refer to Appendix J

5.6 Investigational Drugs/ Investigational Devices

5.6.1 Approval Status of Study Drugs

Not applicable.

5.6.2 Approval Status of Study Devices

The ReCell device used in this protocol is investigational meaning that it is not approved by the Food and Drug Administration (FDA) for use in the United States. This research project is being conducted under an Investigational Device Exemption (IDE) application and the ReCell device is categorized as a Category B, Class III under the FDA regulations. The operative procedure for this study is considered a research procedure.

The safety of ReCell has been demonstrated with over 5,000 treatment procedures conducted worldwide. ReCell has CE Marking in Europe, TGA clearance in Australia, SFDA clearance in China and Health Canada approval as well as approval in other markets (e.g., Taiwan, Hong Kong, Mexico, Brazil and Venezuela). There have been no reports of adverse events associated with the use of ReCell that have met the requirements for vigilance reporting to the respective regulatory authorities.

Investigational Product: Autologous Cell Harvesting Device (ReCell)

Dosing Instructions and Schedule: ReCell is supplied as a single use device. The contents of each kit are sufficient to prepare a cell suspension from a maximum biopsy size of 2cm x 2cm to cover a wound area up to and including 320cm² (*Note: each square cm of skin sample will treat 80 sq cm of treatment area*).

Proposed Indication: ReCell is intended to be used at point-of-care for the safe and rapid preparation of epidermal and dermal cells as well as the cells at the epidermal-dermal junction from a small sample of the patient's own skin. Under the provision of a healthcare professional, the cell suspension produced by the ReCell system is suitable for the treatment of partial-thickness wounds to promote re-epithelialization and for epidermal grafting over the INTEGRA™ MBWM.

Investigational Device Description: The investigational ReCell Autologous Cell Harvesting Device has been developed to provide a simple, safe technique for the harvesting of epidermal cells for epidermal repair. ReCell is a single-use, stand-alone, battery operated, autologous cell harvesting device kit that contains enzymatic and delivery solutions, sterile surgical instruments, and actuators. The contents of each kit are sufficient to prepare a cell suspension to cover a wound area up to and including 320cm².

This investigational device is used to disaggregate cells from a thin, shaved split-thickness skin biopsy sample obtained from the patient to be processed to produce a viable cell suspension for reintroduction to the patient.

After the skin sample is taken, the ReCell suspension is available in under 30 minutes for immediate use onto a prepared wound surface and can cover a wound 80 times the size of the skin sample (a thin, split-thickness shave biopsy of 0.15 mm - 0.2 mm depth delivers a 1:80 expansion ratio; meaning the cell suspension produced from each square cm of skin sample will treat 80 sq cm of treatment area). The delivered cell suspension enables quick epithelialization of the wound, usually in 5 to 7 days a site-matched skin sample allows reintroduction of the subject's own skin cells that will develop natural texture, vascularity (pinkness) and pigment.

The technology takes advantage of the regenerative capability of skin. Working with a small site-matched sample of the patient's healthy skin, ReCell produces a cell suspension that stimulates skin growth. The cell suspension, which is sprayed onto an affected area to be treated, contains the appropriate mixture of healthy cells to promote healing (*keratinocytes*), skin structure (*fibroblasts*), vascularity (*colour*), pigmentation (*melanocytes*) and texture.

The investigational product must be stored, handled and administered in accordance with this protocol, the manufacturer's IFU, as well as all applicable laws, regulations and institution requirements.

The Investigator will ensure control of the investigational device. The investigational devices will be administered only to those subjects enrolled in this study under the supervision of the investigator and under the terms of the clinical

protocol and Investigator's Agreement. All clinical supplies and study devices intended for use in the clinical study cannot be used, under any circumstances, for any purpose other than described in the protocol. The investigator will also ensure that the device components are maintained under secure storage and that device accountability records are maintained.

Investigational Device Accountability:

The Avita Medical Americas, LLC will supply the investigator with an adequate number of investigational devices for completion of the study. The investigator is responsible for ensuring all investigational product accountability. Any discrepancies must be described in writing. Throughout the study, device accountability records will be reviewed by the Sponsor's appointed clinical monitor. Records for dispensing study drug must be available for inspection by the clinical study monitor throughout the study. The investigator is responsible for ensuring that the device accountability records are complete and up to date at all times. Regular study device reconciliation will be performed to document device assigned to subject enrollment number by the clinical monitor.

The maintenance of study-related device accountability records does not preclude the Principal Investigator's responsibilities to maintain additional records in accordance with all national and/or local institutional requirements for investigational device use.

Receipt of Study Device:

Arrangements will be made to have the investigational device kits shipped from the manufacturer directly to the Investigational Pharmacy Services at WRNMMC, to the ATTN: Dr. Parvenah Moussavian, Departmental Chief, where they will be housed until ordered by the investigator for use.

The Principal Investigator, or a responsible party designated by the investigator, must maintain an inventory record to document the receipt and dispensation of each ReCell device kit.

The investigational pharmacist will be required to keep records pertaining to the total number of ReCell device kits received from the manufacturer, date of receipt, condition of kits upon receipt, location and conditions of storage for both ReCell device kit and enzyme, until use in the operating room will be recorded on forms provided in the Pharmacy Manual. Additionally, the investigational pharmacist will be required to record the lot number and expiration date for the Recell device kit and enzyme received. Any discrepancies must be described in writing.

Upon receipt of the of the study device supplies, an inventory must be performed and a device receipt log filled out and signed by the person accepting the shipment. It is important that the designated study staff counts and verifies that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study devices in a given shipment will be documented in the study files. The investigator must notify the study sponsor of any damaged or unusable study devices that were supplied to the investigator's site.

Investigational Device Packaging:

The ReCell device kit contains the following items provided in sterile packaging:

ReCell processing unit with built-in heating mechanism for warming the enzyme solution to optimum working temperature (+37°C)

Removable insert to act as a sterile Petri dish for use when preparing and scraping the skin biopsy

ReCell Enzyme

ReCell Buffer

Water

Syringes

Needles

Nozzles

Scalpel

Kits will be sealed with tamper resistant tape

ReCell Device Kit Labeling:

In accordance with The Code of Federal Regulations (CFR) 812.5 for investigational device labeling, the immediate package (outer box) of the ReCell kit bears the following information:

- The name, address, telephone number, facsimile number and website address of the manufacturer, Avita Medical Americas, LLC
- A separate label with the statement “**CAUTION – Investigational Device** Limited by Federal (or United States) Law to Investigational Use Only LBL090”
- Listing of package/kit contents
- Storage requirements to ensure stability of the investigational device and enzyme solution
- Label with various symbols identifying relevant contraindications, hazards, warnings, and precautions.
- Labels identifying investigational device kit date of manufacture, lot number and expiratory date of the product
- Label stating lot number and expiration date for enzyme solution

The ReCell kit and enzyme solution lot number and expiration date will be recorded in the eCRF.

Storage and Handling (refer to IFU, Appendix A):

The investigational device kits, including the enzyme (trypsin) will be maintained under controlled conditions.

Upon receiving ReCell, the pharmacist should examine the packaging for external signs of damage or tampering. If the external kit packaging or the packaging for any of the

individual components appears damaged, the kit should not be used. The pharmacist will be instructed to contact the investigator who in turn must notify the study sponsor of any damaged or unusable study devices that were supplied to the investigator's site.

To ensure stability of the device, storage requirements are as follows:

Once the contents of the kits have been inspected, the investigational pharmacist will remove the enzyme (trypsin) from each kit and immediately refrigerate at 2-8°C (35.6-46.4°F). The investigational pharmacist will be required to maintain a daily temperature log to record the temperature of the refrigerator where the enzyme is being stored. Any discrepancies must be described in writing.

All other components are to be stored at room temperature.

Dispensing of Investigational Device:

The investigational product (ReCell device) will not be applied to any person who is not a qualified study participant under this protocol. The ReCell device will be dispensed only by study site personnel designated by the Principal Investigator on the study site Delegation Log.

An order for the ReCell kit will be written by the Investigator and sent to the WRNMMC investigational pharmacy to request the treatment ReCell device kit for availability on the scheduled day of surgery for the subject enrolled.

When needed, the pharmacist will dispense 2 kits to a member of the research team. One ReCell kit will be dispensed for use and the other will be obtained for the purpose of having "on-hand" in the event of a device malfunction while in the operating room. The investigational pharmacist will record dispensing of both device kits. One kit will be denoted as "intended for use" and the second kit will be denoted as the "back-up device" in the device accountability log. If the second device deemed as the "back up device" was not needed, it will be returned to the investigational pharmacy by the study coordinator and logged back in on the device accountability log to be dispensed for future use with the next subject enrolled. Stringent record keeping of this dispensing process will be maintained for investigational device accountability.

The clinical site will utilize an investigational product accountability log provided by the manufacturer. The following information will be recorded on the log by the designated individual dispensing the investigational product: date investigational device(s) were dispensed and initials of device dispenser, subject number and lot number for device kits dispensed and documentation regarding date and lot number of returned "back-up device", if applicable.

Return or Destruction of Investigational Device:

At the completion of the study, there will be a final reconciliation of study devices shipped, devices consumed, and devices remaining. This reconciliation will be logged on the device reconciliation form, signed and dated by the Principal Investigator and the Investigational Pharmacist. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused study devices.

ReCell and all individual components included in each kit are intended for single use only. Used ReCell devices and kit components will be discarded into appropriate biohazard receptacles after single use. Devices destroyed on site will be done in accordance with manufacturer recommendations, institutional policies and procedures and documented in the study regulatory files. Unused (including expired) investigational devices are to be returned to the manufacturer, Avita Medical Americas, LLC via use of their established Return Material Authorization (RMA) process. Per manufacturer directives, to return all unused investigational device kits, the study site will notify the manufacturer. They in turn will generate and send site an Investigational Device Return Form along with a return FedEx air bill to cover return shipment fees. The devices will be returned to the address below:

Return to:

Avita Medical
c/o Parker Hannifin Corp
ATTN: Customer Service
3007 Bunsen Avenue, Suite L
Ventura CA 93003
Telephone: 818) 356-9419

5.7 Statistical Considerations

5.7.1 Study Endpoints

Primary Outcomes: Safety and Tolerability

The safety and tolerability endpoints for this feasibility clinical trial includes:

- Delayed healing/non-healing of wound and donor site
- Graft loss
- Heterotopic ossification
- Infection
- Scar contracture
- Durability (i.e. abrasions/injuries at graft site due to graft fragility)
- Allergic response to trypsin (the enzyme used in the ReCell process)
- Subject Complaint (i.e. pain and itching)
- Vital Signs
- Blood chemistries and hematology
- Treatment-related adverse events requiring surgical intervention prior to Week 12 post-treatment and all serious adverse event (SAE) occurrences throughout the study

Secondary Outcomes: Preliminary and Long-Term Efficacy

The preliminary efficacy (Week 1-6 post-treatment) endpoints for this feasibility clinical trial includes:

- Wound epithelialization 95% or greater re-epithelialization at treated wound site and donor sites
- Histology
- Patient pain rating

The long-term efficacy (Week 12 and 24 post-treatment) endpoints for this feasibility clinical trial includes:

- Scar assessment scale using the Patient and Observer Scar Assessment Scale (POSAS) and Vancouver Scar Scale
- Functional outcome rating
- Patient satisfaction
- Histology
- Radiographic evaluation for assessment of heterotopic ossification

5.7.2 Sample Size Estimation

No formal sample size estimation has been performed since this is a feasibility study. A sample size of 20 subjects was selected based upon the amount of subject exposure considered necessary in order for clinical assessments of safety, tolerability, preliminary and long-term effectiveness of the ReCell device when used for healing of full-thickness wounds treated with 1:5 meshed STSG and INTEGRA MBWM. This study does not evaluate formal hypotheses and is not prospectively powered to detect potentially clinically significant improvements in study endpoints. The results of this feasibility study may be analyzed to refine the study design for a pivotal trial.

5.7.3 Data and Statistical Analysis Plan

Due to the feasibility nature of this clinical trial, no formal statistical analysis nor interim analysis will be performed. Data collected in this study will be documented using summary tables. Quantitative variables will be summarized using descriptive statistics, including mean (and/or median), standard deviation (SD), and when appropriate minimum and maximum values. Categorical variables will be summarized by frequencies and percentages. Where appropriate, 95% confidence intervals either around the sample means or mean differences will be furnished. All data collected will be presented in subject data listings.

Power Analysis

Post hoc power analysis was conducted using G*Power 3.1 (Faul, Lang, & Buchner, 2007) for this 2 x 7 mixed design (i.e., two treatment groups x 7 waves of measurements). Given the constraints on sample size ($n = 20$), a power analysis was conducted with the intent to detect the obtained power ($1 - \beta$) given: (1) level of significance (set at $\alpha = .05$), effect size (standardized F statistic varied at .15, .2, and .25) and (3) rate of autocorrelation (average correlations of repeated measures varied at .3, .5, and .7). Even though ascertaining what constitutes a small/medium/large effect size is, in part, context and discipline-dependent, using Cohen's (2008) taxonomy of small ($F = .10$) and medium ($F = .25$) we see below that sufficient power (.80) to obtain significance for the 2 x 7 first order interaction will be obtained for a medium effect size when the autocorrelation is .5 or higher and for a slightly smaller effect size ($F = .2$) when autocorrelation approximates .7. However if the effect size approximates .15 sufficient

power will not be obtained, regardless of magnitude of autocorrelation.

AutoCorr	Effect Size	Power
0.3	0.25	0.729
0.5	0.25	0.881
0.7	0.25	0.989
0.3	0.2	0.503
0.5	0.2	0.672
0.7	0.2	0.904
0.3	0.15	0.286
0.5	0.15	0.399
0.7	0.15	0.639

Faul, F., Erdfelder, E., Lang, A.-G., & Buchner, A. (2007). G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods*, 39, 175-191.
Cohen (1988). *Statistical power analysis for the behavioral sciences*. (2nd Ed.). Hillsdale, NJ: Lawrence Erlbaum.

Proposed Analysis

For this 2 x 7 mixed design (i.e., two treatment groups x 7 waves of measurements) with a continuous level outcome, a two-way (completely within) repeated measures ANOVA will be performed with the overarching objective being the test of significance for the two-way treatment x time interaction (using $\alpha = .05$). If the two-way interaction is significant, simple effects analysis will be performed so as to assess at which time point significant differences for the treatment variable are obtained. Moreover, if the first order interaction is significant, trend analysis will be conducted in an effort to uncover any nonlinear patterns across time (up to polynomial $t - 1$, where $t = \text{time}$). Graphic displays will be provided so as to illuminate the nature of the findings and the between treatment trajectories.

All assumptions will be examined, including sphericity and homogeneity of variance/covariance matrices and any data anomalies (e.g., outliers) will be examined and treated accordingly. It should be kept in mind that given the small sample size ($n = 20$), there may still be substantive effects despite non-significant results, hence univariate and multivariate effect sizes will be reviewed (e.g., partial η^2).

There are many techniques beyond a mixed ANOVA approach that can more dynamically model the longitudinal nature of this design, such as multilevel modeling, generalized estimating equations, and/or latent growth curve modeling. However, those techniques will need to be deferred until a larger sample size is obtained. All analysis will be conducted using SPSS 21.0 or Stata 11.0.

Analysis Sets of ReCell Device Treatment vs. Control Comparison:

Safety Analysis Set:

The safety analysis population will include all enrolled subjects. Data will be analyzed based on treatment received. The number and percentage of subject who discontinued the study due to adverse event will be provided. Also, descriptive statistics, as

appropriate, will be provided for the following, by treatment:

- Delayed healing/non-healing of wound and donor site
- Graft loss
- Heterotopic ossification
- Infection
- Scar contracture
- Durability (i.e. abrasions/injuries at graft site due to graft fragility)
- Allergic response to trypsin
- Subject Complaint (pain and itching)
- Vital Signs
- Blood chemistries and hematology
- Safety-related issues occurring through 24 weeks after treatment, other treatment-related adverse events requiring surgical intervention prior to 12 weeks post-treatment and all serious adverse event (SAE) occurrences.

For Adverse Events (AEs), a tabulation of incidence, number of subjects and percent of subjects experiencing AEs will be provided for:

- Serious AEs
- Severity
- Expected or Unexpected
- Relationship to Study Treatment
- Body System and preferred term

A listing of all AEs along with severity, relation to treatment, date and outcome will also be provided.

Effectiveness Analysis Set:

Preliminary and long-term effectiveness data will be summarized descriptively for each of the assessment intervals by presenting the number and proportion of treatment and donor sites that achieved healing. The treated wound site will be considered healed if 95% or greater re-epithelialization has occurred by Week 6 post-treatment. Donor sites (STSG 1:5, STSG 1:1.5 and ReCell) will be considered healed when $\geq 95\%$ of the donor site has re-epithelialized by Week 4 post treatment. Both Observer and Patient Assessment scales of the POSAS, and total POSAS scores will be summarized descriptively (mean, median, standard deviation, range) at Weeks 12 and 24. The Vancouver Scar Scale (VSS) assessment tool and total VSS scores will also be summarized descriptively (mean, median, standard deviation, range) at Weeks 12 and 24. Patient satisfaction and functional outcome ratings data will be summarized in terms of counts, percentages and other descriptive statistics.

Histology Analysis

Histological analyses will be performed on punch biopsies of ReCell-treated and control sites for Week 2, 4 and 12 and also of the pre-treatment biopsies taken from the INTEGRA™ MBWM treated wound site and adjacent normal skin. This biopsy of

normal skin serves as the control control for all other biopsies. At each assessment interval, histological parameters from both ReCell-treated and control biopsies will be compared to the same parameters as measured in normal skin. Data will be summarized descriptively.

Cell Suspension Preparation/Application Parameters Analysis

Data concerning biopsy size and thickness, wound size, and ReCell cell suspension application (e.g., volume and details for application) will be documented on the eCRF. Data will be summarized using descriptive statistics.

Demographic Data Analysis

Tabulation of demographic data will be tabulated by age, gender and race/ethnicity.

Study Termination Analysis

The following analysis will be provided pertaining to Study Termination:

- Number and percent of subjects who completed the study
- Frequency of premature termination reasons
- A listing of all subjects who were prematurely terminated from the study along with the reason and date of early termination

The following will be considered as major protocol deviations:

- Major inclusionary/exclusionary deviations
- Missed visits/assessments such that a determination of healing status at both wound sites and ReCell donor site cannot be determined
- Significant protocol non-compliance that may confound evaluation of healing (e.g. use of inappropriate primary dressing)

Any significant deviations to the statistical analysis plan will be addressed in the final report for this study.

Randomization:

Allocation of treatments to the 2 defined wound regions at the INTEGRA™ MBWM treated wound sites will be done at random, using a computer generated randomization table provided by Glaser Consulting. Wound regions will initially be labeled “A” region and “B” region by the surgeon, and then an envelope will be opened which will indicate which treatment (ReCell or control) to assign to “A” region and which treatment to assign to “B” region. Randomization envelopes containing treatment region allocation will be opened in chronological order corresponding to subject enrollment.

Example Randomization Table

SUBJECT #	TREATMENT ASSIGNMENT
DERM- 001	A Region (ReCell) B Region (Control)
DERM- 002	A Region (ReCell) B Region (Control)

6. HUMAN SUBJECT CONSIDERATIONS

6.1 Anticipated Benefits

ReCell is designed to provide an environment to enhance skin regeneration, and to facilitate rapid wound healing, providing the potential to eliminate or minimize scar formation. By introducing an autologous epidermal cell suspension onto a wound surface, the ReCell technology takes advantage of, and enhances, the body's natural regenerative response to heal itself.

Wounded service members frequently possess limited donor site availability due to the nature and extent of associated injuries and therefore harvest sites for the split-thickness skin grafting is often limited. The ReCell Autologous Cell Harvesting System (ReCell device) allows autologous transplant of the patient's own skin cells, including epidermal progenitor cells using a small thin biopsy site (typically no larger than 2 cm by 2 cm). Used in combination with a meshed split-thickness skin graft (STSG) over INTEGRA™ MBWM, ReCell should allow for an expanded meshing ratio, thereby decreasing the required donor site. With the direct application of autologous epidermal progenitor cells the technology also offers the potential to promote faster healing, minimization of mesh patterned scar and improved patient quality of life.

ReCell has been demonstrated to deliver viable keratinocytes, melanocytes, fibroblasts and Langerhans cells to the wound surface. Preliminary clinical data demonstrate that wounds treated with ReCell rapidly re-epithelialize with reduction of systemic effects, wound infection or adverse reactions, yield improved scar texture, reduce contracture, heal free of erythema and achieve re-pigmentation at the treatment site.

Subjects participating in this clinical research trial may or may not derive a direct benefit from study participation. Although we cannot guarantee a positive outcome from this research project, validating the safety and preliminary efficacy of this treatment could provide a new approach in regenerative medicine. Increased knowledge and technological advancement may result from the conduct of this research study and potentially help a large number of individuals in the future.

6.2 Risks and Discomforts

Subjects will be monitored for safety from time of enrollment to conclusion of study visits. All adverse events will be collected by direct subject communication with investigator/study coordinator and review of source documents.

Any conditions or symptoms reported after the subject receives investigational product will be recorded as an adverse event (AE) on the AE eCRF. This includes new events after study device administration, conditions that become more severe or increase in frequency, any disease-related signs or symptoms present at the Screening Pre-Treatment visit that have worsen in severity or frequency, as well as any event or finding that the Investigator feels is clinically significant.

At each post-treatment visit, subjects will be asked by the PI and/or AI about any AE's

they have experienced since the previous visit. Any adverse condition or symptom reported will be reported and recorded as an AE in the subject's source documents and on the AE eCRF page. The subject will be instructed to contact the study site in the event they experience any significant AE's. At the discretion of the Investigator, the subject may be asked to come to the study site for an unscheduled visit.

There are no known risks of the ReCell device based on results from over 5,000-treatment procedures conducted worldwide. Potential risks to the volunteer that will be monitored may include: delayed healing/non-healing of wound and donor site, graft loss or rejection, heterotopic ossification, infection, scar contracture, durability (i.e. abrasions/injuries at graft site due to graft fragility), allergic response to trypsin, and other treatment-related adverse events requiring surgical intervention prior to 12 weeks post-treatment.

- Rare but serious (Event Rate < 1%)
 - Death, side effects and complications from anesthesia, allergic response to trypsin, allergic response to lidocaine used prior to the biopsy procedure and radiation exposure for radiographic imaging for assessment of heterotopic ossification
- Less Likely (1% ≤ Event Rate < 5%)
 - Keloid formation, infection at wound site or donor sites, reduced or loss of skin sensation or increased sensitivity, scarring, skin discoloration, uneven skin surface
- Likely (5% ≤ Event Rate < 10%)
 - Discomfort, bruising, bleeding with phlebotomy at site of blood draw and/or punch biopsy, scar contractures that impact limb function, total graft loss
- More likely (Event Rate ≥ 10%)
 - Heterotopic ossification, partial graft loss. Greater than 65% of combat casualties develop heterotopic ossification as a result of their injuries. A number of wounds may develop HO as a result of the initial trauma and not directly caused by the application of STSG or ReCell spray skin.

Risk Analysis

Anticipated Risks

The potential risks to the subject arising from the biopsy collection and grafting procedures, and from exposure to the ReCell Device, are analyzed in the tables below.

Risks and Mitigation Strategies for ReCell® Autologous Cell Harvesting Device

Potential Risks	Mitigation Strategies
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Allergic reaction to anesthesia, medications, or device	<ul style="list-style-type: none"> Enrollment will be restricted to subjects with no known allergies to study materials (device, anesthesia, and/or medications) Post-operative assessment of allergic response performed throughout the study period
Viral transfer from animal enzyme	<ul style="list-style-type: none"> Trypsin has been extensively tested for viruses and undergone viral elimination processes which have been validated
Potential Risks	Mitigation Strategies
<i>Risks related to tissue response at Donor and Treatment Sites</i>	
Infection	<ul style="list-style-type: none"> Instructions for use (IFU) detail wound bed preparation procedures Patients with active infection are excluded from the study Sterile study equipment Study procedures are performed according to aseptic principles required during surgery and post-operative dressing changes IFU allows for the use of prophylactic, perioperative antibiotics as part of the surgical management process
Rejection / loss of graft	<ul style="list-style-type: none"> Cell material is autologous IFU reinforces use of aseptic principles Patient selection (i.e., exclusion of patients known to have a pre-existing condition that may interfere with wound healing)
Excessive bleeding at donor site	<ul style="list-style-type: none"> IFU details biopsy harvesting processes (including infiltration with adrenaline)
Lack of take of graft	<ul style="list-style-type: none"> IFU details wound site preparation requirements IFU contraindicates infection in wound site
Cross contamination	<ul style="list-style-type: none"> ReCell Device is single use and is marked as used at 5 minutes post incubation commencement preventing further restarts Only one vial of Trypsin is included with each device IFU details that ReCell is for autologous use only
Worsening Scar	<ul style="list-style-type: none"> IFU/protocol details instructions for proper dressing/management of treatment and donor wounds.
<i>Risks related to Biopsy Processing</i>	
Biopsy separation not possible	<ul style="list-style-type: none"> Trypsin extensively tested and released following assessment of activity Incubation of biopsy takes place at ~36.5°C which is within the optimal temperature for Trypsin activity IFU details troubleshooting strategies for difficult to separate biopsies IFU details procedures for harvesting suitable biopsies IFU details that Trypsin is to be reconstituted with Water For Injection (WFI) and warn that reconstitution with Compound Sodium Lactate for Irrigation (CSLI) may impact enzyme activity

Cell viability low	<ul style="list-style-type: none"> • IFU details that biopsy should not be exposed to Trypsin for longer than 60 minutes • IFU describes the cell scraping process to maximize cell quantity isolation • Conical well has been designed into processing unit to allow for maximal cell draw-up post filtration • Biopsy incubation occurs at ~36.5°C which is within the optimal temperature for Trypsin activity. Device is fitted with alerts if this temperature is exceeded
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Potential Risks	Mitigation Strategies
No or minimal epithelialization due to improper processing, cell suspension application, inadequate cell suspension volume or inadequate healing	<ul style="list-style-type: none"> • IFU detailing biopsy size requirements • IFU detailing biopsy processing steps and troubleshooting measures • IFU detailing correct cell dilution • IFU detailing maximum time frame for biopsy exposure to Trypsin • IFU detailing contraindications for biopsy sites such as infection • Patient selection excludes patients with pre-existing infections. • IFU detailing correct dressing materials to be used and time frames and processes for replacing dressings so as not to damage healing wounds on dressing removal • Patient selection (i.e., exclusion of patients known to have a pre-existing condition that may interfere with wound healing)
<i>Risks related to cell application</i>	
Nozzle blockage	<ul style="list-style-type: none"> • Nozzles designed with an aperture which allows for cells to pass through easily • 100 micron filtration occurs prior to spraying of cells • Spare nozzle supplied with each device
Nozzle aperture position	<ul style="list-style-type: none"> • IFU recommend test spray • IFU describes aperture positioning

The potential risks to the subject and adverse events associated with use of the ReCell Device discussed in the tables above are minor and easily controlled.

Adverse Event Evaluations and Reporting

Subjects will be monitored for safety from time of enrollment to conclusion of study visits. All adverse events will be collected by direct subject communication with investigator/study coordinator and review of source documents.

At each post-treatment visit, the research team will assess if the subject has experienced any adverse effects post-treatment with the investigational device. The subject will be asked if he/she has any problems, issues and/or concerns since the last encounter visit that could be potentially related to study participation. Any conditions or symptoms reported after the subject receives investigational product will be recorded as an adverse event (AE) on the AE eCRF. This includes new events after study device administration, conditions

that become more severe or increase in frequency, any disease-related signs or symptoms present at the Screening Pre-Treatment visit that have worsen in severity or frequency, as well as any event or finding that the Investigator feels is clinically significant.

Other treatment-related adverse events requiring surgical intervention prior to Week 12 post-treatment and all serious adverse event (SAE) occurrences will be treated and recorded in the eCRF. For all adverse events, the Investigator will provide an assessment of the event, its treatment resolution, and relationship to the investigational device. Subjects who have an ongoing AE related to the study device at Week 24 post-treatment will be followed for 30 days until resolution and/or stabilization of the event at a level acceptable to the Investigator.

Adverse Event Definitions:

Identification of Adverse Events (AEs), Adverse Device Effects (ADEs) An adverse event is defined as any new medical problem or exacerbation of an existing problem, experienced by a subject while enrolled in the study after receiving investigational product, whether or not it is considered related to the investigational device by the Investigator.

Treatment-Related Adverse Event

A treatment-related adverse event is an adverse event that is judged to be related to the investigational device, study therapy and study-related procedures.

Unexpected Adverse Event

An unexpected adverse event is any adverse effect which the frequency, specificity or severity is not consistent with the risk information described in the investigational plan.

Anticipated (Expected) Adverse Event

Potential adverse event that a subject may experience following the use of the ReCell device as described in this section above and using the potential risks listed in the above tables.

Definitions of Specific Major Treatment –Related Adverse Events:

Infection: The presence of infection for the ReCell treated areas and control site will be evaluated at each post-treatment visit. Infection will be evaluated in accordance with the Center for Disease Control (CDC) guidelines for nosocomial infections using standard clinical measures such as visual examination of the treatment sites for delayed healing, redness, inflammation and surrounding cellulitis. In the presence of symptoms (i.e., purulent exudate, changes in wound appearance such as hyperemia, and erythema in the uninjured skin surrounding the wound), infection will be confirmed using microbiological testing procedures and treatments initiated according to the institutions' infection management protocols, which will be recorded on the eCRF and Adverse Event form. Infection will be managed according to the standard protocols of the clinical site. For example, treatment of infection will involve the daily cleaning and dressing of wound sites until such time that the infection is clear. Treatment with broad spectrum antibiotics until microbiology sensitivities return from the testing laboratory is recommended. Upon return of sensitivities, antibiotic therapy may either continue as is, or be changed at the discretion of the investigator. All treatment regimens applied in the management of infection will be recorded on the eCRF.

Allergic Response to Trypsin: The allergic response to Trypsin, the enzyme used in the ReCell Device for disaggregation of the biopsy, will be evaluated. An allergic response to Trypsin is most likely to present as contact dermatitis (defined as an altered state of skin reaction induced by exposure to an external agent). Substances that produce this condition after single or multiple exposures may be irritating or allergic in nature and induce an inflammatory response. The most common clinical expression of this induced inflammation is dermatitis (eczema).

The evaluation tools for assessment of the allergic response to Trypsin have been developed in accordance with guidelines published in the Journal of the American Academy of Dermatology. The subject will be evaluated at each post-treatment visit for any occurrences of an allergic reaction (e.g. eczema/dermatitis). Any allergic response(s) to trypsin will be recorded in the eCRF and depending on severity, will have an additional reporting to the IRB, Research Monitor and study report.

The incidence of adverse response to Trypsin is expected to be low.

Other Treatment-Related Adverse Event Requiring Subsequent Surgical Intervention

Any treatment-related adverse event resulting in subsequent surgical intervention (e.g., “graft loss” requiring retreatment) will also be considered a major treatment-related adverse event.

The incidence of other treatment-related adverse events requiring subsequent surgical intervention is expected to be low.

Clinical investigators and ultimately the Principal Investigator will be responsible for AE identification, documentation, grading and assignment of attribution to the investigational device.

All observed or volunteered adverse effects (serious or non-serious) and abnormal test findings deemed clinically significant by the investigator, regardless of treatment group, if applicable, or suspected causal relationship to the investigational device or, if applicable, other study treatment or diagnostic product(s) will be recorded in the subjects’ eCRF. Criteria for grading of adverse events will be as defined by the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 (<http://www.upToDate.com/contents/common-terminology-criteria-for-adverse-events>). For all adverse effects, sufficient information will be pursued and/or obtained so as to permit 1) an adequate determination of the outcome of the effect (i.e., whether the effect should be classified as a *serious adverse effect*) and; 2) an assessment of the causal relationship between the adverse effect and the investigational device or, if applicable, the other study treatment or diagnostic product(s).

Severity

Each adverse event will be assessed for its severity, or the intensity of an event experienced by the subject, using the Common Terminology Criteria for Adverse Events (CTCAE), v4.0. The CTCAE grading (severity) scale will be utilized for each AE based on the following:

Grade 1 = Mild: Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated, discomfort noticed, but no disruption to daily activity

Grade 2 = Moderate: Minimal, local or noninvasive intervention indicated, discomfort sufficient to reduce or affect normal daily activity

Grade 3 = Severe: Medically significant but not immediately life-threatening; hospitalization or prolonged hospitalization indicated; disabling, inability to work or perform normal daily activity

Grade 4 = Life-threatening: Urgent intervention indicated

Grade 5= Death: Death related to an adverse event

Relationship of Adverse Events to the ReCell Investigational Device

The Investigator should assess the relationship of the adverse event to the investigational device. The relationship should be assessed using the following categories:

- **Definitely Related:**
A direct cause and effect relationship between the investigational device/treatment and the adverse event exists.
- **Likely Related:**
A direct cause and effect relationship between the investigational device/treatment and the adverse event has not been clearly demonstrated, but is likely or very likely.
- **Unlikely Related:**
A direct cause and effect relationship between the investigational device/treatment and the adverse event is improbable, but not impossible.
- **Unrelated:**
The adverse event is definitely not associated with the investigational device/treatment.

Outcome

The Investigator will categorize the outcome of the adverse event using the following definitions:

Resolved: The subject recovered from the adverse event.

Resolved with sequelae: The subject recovered, but with an after effect possibly due to disease, injury, treatment, or procedure.

Ongoing: At the time of the last assessment, the event is ongoing, with an undetermined outcome.

Death: The adverse event directly caused death.

Unknown: There is an inability to access the subject or the subject's records to determine the outcome (i.e., subject withdrew consent or is lost to follow-up)

Unanticipated Adverse Device Effects (UADEs)

An unanticipated adverse device effect is defined as “any serious adverse effect on

health or safety, or any life-threatening problem, or death caused by, or associated with, a device; if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan, or application (including supplementary application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.”

If an unanticipated adverse device effect occurs, the investigator must report the event promptly to the Sponsor, the IRB and Medical Research Monitor via email or telephone within 24 hours of first learning of the event. The Sponsor will conduct an evaluation of the unanticipated adverse device effect and will provide a written report the results to FDA and to all reviewing IRBs and participating investigators within 5 working days.

Such reports will also be promptly reported by telephone (301.619.2165), by email (usarmy.detrick.medcom-usamrmc.other.hrpo@mail.mil), or by facsimile (301.619.7803) to the Human Research Protection Office (HRPO). A complete written report should follow the initial notification (*Please refer to Section 6.4 below*)

Serious Adverse Events (SAEs)

Each adverse event should be assessed for its seriousness using the criteria outlined below. The term serious adverse event is not synonymous with a “severe” adverse event, which may be used to describe the intensity of an event experienced by the subject. An adverse event should be classified as an SAE if it meets any of the following criteria:

- Results in, or contributes to, a death
- Life-threatening (i.e., the subject was, in the opinion of the investigator, at risk of death at the time of the event, but it does not include an event that, had it occurred in a more severe form, might have caused death)
- Results in permanent disability or incapacity (i.e., permanent impairment of a body function or permanent damage to a body structure)
- Requires in-subject hospitalization or prolongs hospitalization
- Necessitates medical or surgical intervention to preclude a permanent disability or incapacity
- Results in a congenital anomaly or birth defect

Non-serious adverse events are all events that do not meet the criteria for a “serious” adverse event.

Deaths

The Principal Investigator must notify the IRB (via IRBNet) and the sponsor of the IDE within 24 hours of first learning of a subject’s death, regardless of whether the death is related or unrelated to the investigational device. The Investigator should attempt to determine, as conclusively as possible, whether the death is related to the device. The cause of death and the Investigator’s discussion regarding whether or not the death was device-related should be described in a written report. Serious adverse events must be reported even if the PI believes that the adverse event is unrelated to the protocol.

6.3 Actions to Minimize Risks

a. Safety Monitoring Plan

Based on trials conducted with the ReCell device, no significant participant risks are anticipated and no interim analysis is planned; therefore, there are no pre-specified stopping rule procedures planned for this clinical trial. However, if any serious safety issues do arise, medical judgment will be used and the Investigator will, in consultation with the Medical Research Monitor, the Sponsor, appropriate regulatory authorities, and/or IRB, take steps necessary to modify or discontinue the trial.

- b. Safety Analysis Plan:** The IRB, medical research monitor, and principal investigator will review any safety concern. A data safety monitoring board (DSMB) is not required for this study. Safety concerns will be analyzed by the PI and medical research monitor to avoid unnecessary exposure of subjects to ineffective or dangerous interventions. Patients may be discontinued from participation in the study in the presence of exclusion criteria or at the recommendation of the Principal Investigator or by the study medical research monitor (Carlton Brown, MD).

The medical research monitor will function as an independent safety advocate for subjects enrolled into this clinical trial. The medical research monitor is required to review all unanticipated problems involving risk to subjects or others, SAEs, and all subject deaths associated with the protocol and provide an unbiased written report of the event. At a minimum the medical research monitor should comment on the outcomes of the event or problem and, in the case of a SAE or death, comment on the relationship to participation in the study. The medical research monitor should also indicate whether he concurs with the details of the report provided by the study investigator. Reports for events determined by either the investigator or medical research monitor to be possibly or definitely related to participation and reports of events resulting in death should be promptly forwarded to the IRB and/or ORP and USAMRMC CSSD PSSB (sponsor safety office).

c. Confidentiality Protection

Any personal information that could identify the subject as an individual will be removed from all study forms. Upon enrollment, the subject will be assigned a 3-digit study ID number that is not a part of their social security number. All information collected for the purpose of this study will be coded using the subject's 3-digit study ID number and will be used on all study data collection forms and eCRF's. The link between subject's name and their study ID number and initials will be kept confidential to the greatest extent provided by law. The master list linking the subject's 3-digit study ID number and their identifying information will be kept by the Principal Investigator (or his designee) in a locked file cabinet located in the Orthopaedic Surgery clinic at WRNMMC.

Study records and digital photographs will be maintained in a locked file cabinet in a locked room located in the Orthopaedic Surgery clinic at WRNMMC accessible only to personnel involved with this study. Precautions are in place to ensure all electronic records will be stored in password-protected encrypted files. Access to this information will be limited to research team members and to those

health care professionals who are providing clinical services as part of this research study.

Biopsy samples picked up via courier and taken to Annapath Inc for processing will be sent coded.

Transmission of eCRF's from WRNMMC to the Data Coordinating Center (DCC) located at the UPMC Center for Innovation in Restorative Medicine (CIRM) will be accomplished via use of a web browser to access the Medrio data entry system. The integrity of the data is paramount. The Medrio system is fully 21 CFR Part 11 compliant with the FDA and meets all HIPAA Privacy Rule requirements. To ensure data integrity is maintained, the database will be held on a secure server, access will be restricted by study role and every data entry person will have individualized password access to protected files for online data entry, audit trails will be available, all data will be encrypted and regularly backed up and secured.

Transmission of coded digital photographs to the DCC will be accomplished by mailing the computer disc overnight to the UPMC Center for Innovation in Restorative Medicine (CIRM), Department of Plastic Surgery at the University of Pittsburgh. . Transfer of the computer disc containing coded digital photographs will occur at the end of the study. The coded digital photographs will be stored indefinitely with the study Master Files on the disc on which they were received.

The research team in the Department of Orthopaedics and Rehabilitation will retain subjects coded PHI and coded photos for up to seven years (as mandated by the Sponsor) past completion of the study. Upon completion of the seven year storage of documents requirement, all documents will be shredded per WRNMMC policy regarding destruction of confidential documents and all digital/electronic files will be erased. The master code list which links the subjects name and 3-digit study ID number will be destroyed as soon as all data collection for this study is completed. Maintenance of data, coded biopsy results, coded digital photographs will be stored indefinitely at the discretion of the Principal Investigator for potential educational purposes as well as review and analysis.

All research staff at WRNMMC will provide evidence to the sponsor of training in research conduct and compliance via completion of all required research modules, and education in Good Clinical Practice in research. Copies of the CITI certificates will be utilized for compliance with this aspect of the protocol.

The Laboratory technicians will be well-versed in the risks of their practice and thoroughly address the subject prior to each exam and inclusive of the risks of venipuncture procedure. All exams will be terminated should the subject expresses any concerns, issues or discomfort.

All aspects of the study will be performed in a private room, including the

consenting process, exams and in assisting subject with completing questionnaires, laboratory blood draw and the ReCell device surgical procedure. The collection of sensitive information about subjects is limited to the amount necessary to achieve the aims of the research, so that no unneeded sensitive information is being collected; drapes or other barriers will be used for subjects who are required to disrobe.

d. Certificate of Confidentiality

Not applicable

6.4 Reporting Adverse Events and Unanticipated Problems

It is the responsibility of the Investigators to supply the medical documentation needed to support the expedited AE reports in a timely manner. Failure to provide the requested information may result in the termination of the study.

Expected adverse events which are not serious are reported on the Continuing Review (CR) Progress Report. The CR is generally performed on a 12-month cycle. More frequent Progress Reports may be required at the discretion of the IRB.

Serious Adverse Events: The PI, within 24 hours of first learning of the event, must report all related or possibly-related AND serious adverse events (SAE) occurring in subjects enrolled at WRNMMC. This is accomplished by submitting an adverse event report to the IRB via IRBNet. For protocols involving investigational drugs or devices, the investigator must also report a serious adverse event to the sponsor of the IND or IDE immediately (within 24 hours) of learning of the event. Serious adverse events must be reported even if the PI believes that the adverse events are unrelated to the protocol.

Unexpected (but not serious) adverse events occurring in subjects enrolled at WRNMMC which, in the opinion of the PI, are possibly related to participation AND places subjects or others at a greater risk of harm that was previously known or recognized in the protocol must be reported by the PI within 24 hours of discovery by email or phone to the IRB and the Medical Research Monitor. A follow-up written report within 5 business days to the IRB and the Medical Research Monitor through IRBNet is required.

Unanticipated problems involving risks to subjects or others (UPIRTSOs) must be reported to the IRB and Medical Research Monitor via email or telephone within 24 hours of discovery and a written follow up report within 5 business days.

When a protocol deviation and/or violation occurs, the investigator shall report the occurrence to the IRB. The investigator is required to make the determination whether the deviation and/or violation meets the criteria for an unanticipated problem involving risks to subjects or others. The IRB Chair or IRB staff member shall also make the determination if the protocol deviation meets the definition of an unanticipated problem involving risks to participants or others. If the IRB Chair or IRB Staff member determines and documents that the deviation is an unanticipated problem involving risks to subjects or others or the deviation resulted from serious or continuing noncompliance, the IRB staff member shall place the deviation on the agenda of the next

available IRB meeting for review. If the IRB Chair or IRB Staff member determines and documents that the deviation is not an unanticipated problem involving risks to subjects or others, the IRB Chair or staff member shall acknowledge the submission and complete the review through an administrative review procedure.

As a reminder, according to DoDI 3216.12 (November 8, 2011), the IRB shall approve an independent medical research monitor by name for all DoD-conducted research involving human subjects, determined by the IRB to involve more than minimal risk to human subjects. Additionally, the medical research monitor may be identified by an investigator or appointed by an IRB or IO for research involving human subjects determined to involve minimal risk.

The medical research monitor may perform oversight functions and will report their observations to the IRB or a designated official. The medical research monitor may discuss the research protocol with the investigators, interview human subjects, and consult with others outside of the study about the research. The medical research monitor shall have the authority to stop a research protocol in progress, remove individual subjects from a research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB can assess the monitor's report. Medical research monitors shall have the responsibility to promptly report their observations and findings to the IRB or other designated official. The medical research monitors shall have expertise consonant with the nature of risk(s) identified within the research protocol, and they shall be independent of the team conducting the research involving human subjects.

Reporting Requirements and Responsibilities for the Principal Investigator to the US Army Medical Research and Materiel Command (USAMRMC) Office of Research Protections (ORP) Human Research Protection Office (HRPO)

The standard reporting requirements to HRPO are outlined as follows:

- a. The protocol will not be initiated until written notification of approval of the research project is issued by the HRPO.
- b. For protocols involving multiple research sites and/or multi-institutional collaborations on a single study, the HRPO must review and approve site specific documentation prior to participation in human research activities.
- c. The Principal Investigator must comply with the following minimum reporting requirements. Specific reporting requirements for the protocol will be included in the HRPO Approval Memorandum. Failure to comply could result in suspension of funding.

(1) Substantive modifications to the research protocol and any modifications that could potentially increase risk to subjects must be submitted to the HRPO for approval prior to implementation. The USAMRMC ORP HRPO defines a substantive modification as a change in Principal Investigator, change or addition of an institution, elimination or alteration of the consent process, change to the study population that has regulatory implications (e.g. adding children, adding active duty

population, etc), significant change in study design (i.e. would prompt additional scientific review) or a change that could potentially increase risks to subjects.

(2) Any changes of the IRB used to review and approve the research will be promptly reported to the USAMRMC ORP HRPO.

(3) All unanticipated problems involving risk to subjects or others must be promptly reported by telephone (301-619-2165), by facsimile (301-619-7803) or by email (usarmy.detrick.medcom-usamrmc.other.hrpo@mail.mil), to the HRPO. A complete written report will follow the initial notification. In addition to the methods above, the complete report can be sent to the US Army Medical Research and Materiel Command, ATTN: MCMR-RP, 810 Schreider Street, Fort Detrick, Maryland 21702-5000.

(4) Suspensions, clinical holds (voluntary or involuntary), or terminations of this research by the Institutional Review Board (IRB), the institution, the Sponsor, or regulatory agencies will be promptly reported to the USAMRMC ORP HRPO.

(5) A copy of the continuing review approval notification by the IRB of Record must be submitted to the HRPO as soon as possible after receipt. For greater than minimal risk research, a copy of the continuing review report approved by the IRB must also be provided. Please note that the HRPO also conducts random audits at the time of continuing review. Additional information and documentation may be requested at that time.

(6) The final study report, including any acknowledgement documentation and supporting documents, must be submitted to the HRPO when available.

(7) The knowledge of any pending compliance inspection/visit by the FDA, DHHS Office of Human Research Protections (OHRP), or other government agency concerning this research, the issuance of Inspection Reports, FDA Form 483, warning letters or actions taken by any regulatory agencies including legal or medical actions and any instances of serious or continuing noncompliance with the regulations or requirements, must be promptly reported to the HRPO.

The USAMRMC ORP HRPO conducts site visits as part of its responsibility for compliance oversight. Accurate and complete study records must be maintained and made available to representatives of the USAMRMC as a part of their responsibility to protect human subjects in research. Research records must be stored in a confidential manner so as to protect the confidentiality of subject information.

7. INVESTIGATOR AGREEMENT

By submitting this protocol and providing an electronic signature in IRBNet, or an ink signature below, I agree to the following statements:

General Assurance: I agree to conduct the study as outlined herein. I certify that all procedures involving human subjects have been described in full.

Starting the Study: I understand I cannot begin the study until I have received an approval letter documenting approval by the WRNMMC IRB and HRPO.

Consent: I am responsible for assuring the quality of each subject's consent in accordance with current federal regulations. This includes ensuring that any "designee" that obtains consent on my behalf is completely familiar with the protocol and is qualified to perform this responsibility.

Adverse Events: I understand that I must report research related or possibly research related serious adverse events within 24 hours of discovery to the IRB. If the IRB has required a medical research monitor, the medical research monitor will also review the relatedness and the serious nature of the adverse event. I will report unexpected (but not serious) adverse events that may possibly be related to participation in the protocol within 5 working days of their discovery to the IRB using the same procedure.

Training: I verify that the personnel performing these procedures described in this protocol are technically competent, have been properly trained, and are appropriately qualified.

Compensation: I am aware that members of the research team are not authorized to accept any form of personal compensation for our efforts in conducting this research.

Modifications: I am aware that all changes to the protocol must be approved by the IRB before implementation. Examples of changes to protocols that require IRB approval include, but are not limited to, change of on-site PI, addition of personnel on study, increased sample size, addition of other data points, sources of outside funding, addition of data collection sites, and changes in the data requested or the purpose of the data use.

Deviations to the Protocol: I am aware that any protocol deviations discovered by either the PI or auditing official will be immediately reported to the IRB. All corrective actions will be documented and become a part of the master study file, along with the report.

Duplication of Effort: I have made a reasonable good faith effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

Reports: I agree to provide a Continuing Review Progress Report 30 days prior to the anniversary of the protocol's initial approval or as stipulated by the IRB. I agree to submit a final report within 30 days following closure, completion or termination of the study.

Maintain Study Files: I agree to maintain a Study File that must be kept for three years from the date the study is closed (32 CFR 219.115(b) consent and that HIPAA authorizations will be retained for 6 years after the study is closed and provided to WRNMMC upon request. If IND medication or IDE appliances are used, the file must be kept for 2 years after FDA approval and can then be destroyed; or if no application is

filed or approved, until 2 years after the study is discontinued and the FDA notified (21CFR 312.62(c)). However, per Sponsor request, study files, eCRF's, consent and HIPAA authorizations will be retained for 7 years after study completion. I acknowledge that research data are the property of the Command and will not be removed without prior approval. When I am scheduled to permanent change of station (PCS) or end of time in service (ETS), study records will be given to a new PI, the Department Chief.

This file may be inspected at any time by Department of Research Programs, DoD oversight entities, the FDA, and/or other applicable regulatory agencies responsible for the oversight of research. This file will include:

- A. The approved protocol and applicable amendments.
- B. The IRB minutes granting approval to initiate the study.
- C. IRB approval letter.
- D. Each Consent Form and HIPAA Authorization signed by the subject or Legally Authorized Representative (LAR).
- E. HIPAA documentation required to meet regulatory compliance,
- F. Continuing Review Progress Reports and Reports related to modifications.
- G. Reports of adverse effects.
- H. Reports of any significant new findings found during the course of the study.
- I. All study documents generated from study date.
- J. Publications, abstracts, and reprints resulting from study data.
- K. All information pertaining to an investigational drug or device.

Publications: I am aware that advertisements, abstracts, presentations or publications resulting from research protocols must have their products cleared by the Public Affairs Office, undergo Operation Security (OPSEC) review, undergo review for release of actionable medical information, and publication clearance.

HIPAA Compliance: I will provide each research participant with a copy of their signed and dated HIPAA Authorization and will immediately notify the IRB Privacy Board when a research participant revokes his/her signed Authorization, and I will no longer seek to obtain PHI pertaining to that individual for this research project, or any other purpose absent a separate authorization or appropriate waiver.

Applicable Regulations: I am familiar with applicable regulations governing research, and will adhere to all of the requirements outlined in the DoD Assurance for the WRNMMC.

I understand that if I fail to comply with any of these responsibilities, all projects for which I am an investigator may be suspended.

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8. LEADERSHIP ACKNOWLEDGEMENT

I concur with the submission of this proposal to the Department of Research Programs for review and approval.

DEPARTMENT CHIEF

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9. REFERENCES

Belmont, P.J., A.J. Schoenfeld, and G. Goodman, *Epidemiology of combat wounds in Operation Iraqi Freedom and Operation Enduring Freedom: orthopaedic burden of disease*. Journal of surgical orthopaedic advances, 2010. 19(1): p. 2-7.

Bumbasirevic, M., et al., Treatment of blast injuries of the extremity. J Am Acad OrthopSurg, 2006. 14(10 Spec No.): p. S77-81.

Covey, D.C., et al., *Orthopaedic war injuries: from combat casualty care to definitive treatment: a current review of clinical advances, basic science, and research opportunities*. Instructional course lectures, 2008. 57: p. 65-86.

Cernak, I., et al., Blast injury from explosive munitions. The Journal of trauma, 1999. 47(1): p. 96-103; discussion 103-4.

Conti E, Wood F, Leclerc-Chalvet M. ReCell®: Indications, Clinical practice and Expected outcome. *Journal of Wound Technology*. April 2009(4):59-62.

Deitch EA, Wheelahan TM, Rose MP, Clothier J, Cotter J. Hypertrophic burn scars: analysis of variables. *J Trauma*. Oct 1983;23(10):895-898.

DoD Personnel & Procurement Reports and Data Files: Military Casualty Information. 2011; Available from: <http://siadapp.dmdc.osd.mil/personnel/CASUALTY/castop.htm>.

Draaijers LJ, Tempelman FR, Botman YA, et al. The patient and observer scar assessment scale: a reliable and feasible tool for scar evaluation. *Plastic and Reconstructive Surgery*. Jun 2004;113(7):1960-1965; discussion 1966-1967.

Drake LA, Dorner W, Goltz RW, et al. Guidelines of care for contact dermatitis.

Committee on Guidelines of Care. *J Am Acad Derm.* Jan 1995;32(1):109-113.

Durani P, McGrouther DA, Ferguson MW. Current scales for assessing human scarring: a review. *Journal of Plastic, Reconstructive & Aesthetic Surgery.* Jun 2009;62(6):713-720.

Idriss N, Maibach HI. Scar assessment scales: a dermatologic overview. *Skin Res Technol.* Feb 2009;15(1):1-5.

Mabry, R.L., et al., *United States Army Rangers in Somalia: an analysis of combat casualties on an urban battlefield.* The Journal of trauma, 2000. 49(3): p. 515-28; discussion 528-9.

Masini, B.D., et al., Resource utilization and disability outcome assessment of combat casualties from Operation Iraqi Freedom and Operation Enduring Freedom. *Journal of orthopaedic trauma*, 2009. 23(4): p. 261-6.

Mazurek, M.T. and J.R. Ficke, *The scope of wounds encountered in casualties from the global war on terrorism: from the battlefield to the tertiary treatment facility.* J Am AcadOrthop Surg, 2006. 14(10 Spec No.): p. S18-23.

Moiemen NS, Vlachou E, Staiano JJ, Thawy Y, Frame JD. Reconstructive surgery with Integra dermal regeneration template: histologic study, clinical evaluation, and current practice. *Plastic and Reconstructive Surgery.* Jun 2006;117(7):160S-174S.

Noe, A., Extremity injury in war: a brief history. *J Am Acad Orthop Surg*, 2006. 14(10Spec No.): p. S1-6.

Owens, B.D., et al., *Characterization of extremity wounds in Operation Iraqi Freedom and Operation Enduring Freedom.* *Journal of orthopaedic trauma*, 2007. 21(4): p. 254-7.

Starnes, B.W., et al., *Extremity vascular injuries on the battlefield: tips for surgeons deploying to war.* *The Journal of trauma*, 2006. 60(2): p. 432-42.

U.S. Department of Defense Website. DefenseLINK US Casualty Status. 2011; Available from: <http://www.defense.gov/news/casualty.pdf>.

Wood FM, Giles N, Stevenson A, Rea S, Fear M. Characterisation of the cell suspension harvested from the dermal epidermal junction using a ReCell kit. *Burns.* 2011;in press.

Zouris, J.M., et al., *Wounding patterns for U.S. Marines and sailors during Operation Iraqi Freedom, major combat phase.* *Military Medicine*, 2006. 171(3): p. 246-52.

10.BUDGET

Will any outside organization provide funding or other resources? Yes (x) No ()

Please refer to Appendix K

APPENDICES

Appendix A1 and 2 - Manufacturer's Instructions for Use (IFU) for the Autologous Cell

Harvesting (ReCell) device and kit Set Up Card

Appendix B - Patient Observer Scar Assessment Scale (POSAS) Questionnaire and Copyright

Appendix C - Vancouver Scar Scale

Appendix D - Functional Outcome Rating: Scar Pain Questionnaire

Appendix E - Subject Satisfaction Questionnaire

Appendix F - Annapath, Inc Technical Guidelines

Appendix G - Annapath, Inc Procedure Protocol

Appendix H - Data Management Plan, including Statistical Management Plan

Appendix I - Clinical Monitoring Plan

Appendix J - Schematic

Appendix K - Budget

Appendix L – Sample electronic Case Report Forms (eCRF)

Procedure	Screening/ Pre-Treatment	OR	Assessments for Safety Tolerability and Preliminary Effectiveness					Assessments for Safety, Aesthetic, & Functional Outcomes	
			Week 1	Week 2	Week 3	Week 4	Week 6	Week 12	Week 24
Visit Window Interval	-30 Days		±3 days	±2 days	±2 days	±2 days	±2 days	±14 days	±14 days
Informed Consent/Enrollment	X								
Medical History ^a	X								
Demographics ^b	X								
Physical Examination	X								
Vital Signs ^c	X		X	X	X	X	X	X	X
Injury Assessment of area treated with INTEGRA TM MBWM ^d	X								
Pain and Pruritus (baseline eval)	X								
Hematology/Chemistry ^e	X		X						
Radiographic Imaging ^f	X								X
Verification of Eligibility Criteria ^g	X								
Randomization ^h		X							
Histology ⁱ		X		X		X		X	
Digital Photography ^j		X	X	X	X	X	X	X	X
Donor Site Tissue Harvesting ^k		X							
Application of Investigational Product (ReCell Procedure)		X							
Wound & Donor Site Healing Assessments ^l			X	X	X	X	X	X	
POSAS ^m			X	X	X	X	X	X	X

Vancouver Scar Scale (VSS)								X	X
Functional Outcome Rating								X	X
Patient Satisfaction								X	X
Concomitant Medicationsⁿ	X		X	X	X	X	X	X	X
Adverse Events^o	X	X	X	X	X	X	X	X	X

- a) Current medication use and allergies
- b) Date of birth, gender, race/ethnicity
- c) Oral temperature, heart rate, respiration rate and blood pressure
- d) Assessment to determine if traumatic wound is fully engrafted as indicated by formation of a viable granulation layer
- e) Complete blood count (CBC) with differential panel coagulation studies to include prothrombin time (PT) and international normalized ratio (INR) and comprehensive metabolic panel (CMP)
- f) Radiographic imaging (anterior/posterior and lateral films) for assessment of heterotopic ossification (presence/absence) pre-treatment/baseline if not previously obtained within 30 days of study enrollment and at Week 24 post-treatment
- g) Verification of eligibility will be performed at Screening and Re-verification of eligibility just prior to picking up investigational device from pharmacy
- h) Wound site will be labeled as “A” region and “B” region PRIOR to opening randomization envelope
- i) Pre-treatment (baseline) biopsies at Integra area and of adjacent normal skin; then at Weeks 2, 4, 12 post-treatment, biopsies will be taken from the ReCell and Control treated sites.
- j) Photos will be taken at following time points: PRE-TREATMENT- Control and ReCell sites (“A” region and “B” region); Donor sites (ReCell, 1:1.5 and 1:5 meshing) following tissue removal, at treatment wound area prior to primary dressing placement and of the ReCell donor site, control area and ReCell treated area at each post-treatment visit for assessment of healing process
- k) ReCell donor tissue harvested for cell suspension; donor tissue harvested for STSG Control area (meshed at 1:1.5) and donor tissue for the ReCell treatment area (meshed at 1:5 mesh)
- l) Assessments made for: Atypical healing and/or delayed healing/non-healing, graft loss, heterotopic ossification, infection scar contracture, durability (i.e. abrasions/injuries at graft site due to fragility), allergic response to trypsin
- m) Subject to complete “patient” part of POSAS at all post-treatment study visits. Full POSAS (including patient and two independent observers) completed at Week 12 and 24 post-treatment
- n) Concomitant medication use (excluding anesthetic regimen) and all treatment regimens applied in the management of infection during the acute healing phase
- o) AEs assessed from time of enrollment through study completion (Week 24) including AEs related to treatment requiring surgical intervention prior to Week 12 post-treatment and all serious adverse event occurrences