Regenerative Medicine at Early Echelons: Changing Medical Care & Outcomes

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ABSTRACT

This paper focuses on the potential applications of regenerative medicine to include cell and biomaterial-based therapies for treating combat casualties at early echelons. The intent is to explore and evaluate the potential improvements, such as faster healing and/or greater functional outcome, that can be gained from applications of these medical technologies at the early echelons to include Levels I, II, and III through an evaluation on the current state-of-the-art on wound healing and tissue regeneration technologies.

1.0   INTRODUCTION

Recent advances in both cell and biomaterial-based therapies warrant a revisit to evaluate these technologies for consideration as advanced medical applications at the early echelons of care given the significant improvements in reducing combat-related mortality for the current conflicts [1-5]. Although advances in medical evacuation typically takes about 4 to 7 days (range 3 to 28 days from point of injury on the battlefield to stateside; mean 8 days) to transport the injured U.S. service members from theatre to the States, the rehabilitation process for the injured Warfighters can be gruesome and takes anywhere from months to years [6-9]. The outcomes could be anywhere from good to limited healing and functions, pending the severity of the injuries and medical treatments available.

Bringing cell and biomaterial-based therapies that affect tissue healing and regeneration to the early echelons could be a paradigm shift to the casualty care and rehabilitation process and could shorten healing time and result in improved overall outcomes, including faster and greater number of return to duty personnel to the Armed Forces. The additional 4 to 7 days in transport could delay optimal healing and functional outcomes. Further, it is known that inflammation plays an immediate role following injuries [10-12]. Thus, it is important to apply cell and/or biomaterial-based therapies at point of injury or within hours of injury to affect the inflammation and healing process such that it could trigger the system to move in a path that will result in healing versus scarring [10, 13].

This paper examines and discusses a number of advanced cell and biomaterial-based therapies and technologies that could make a significant impact on wound healing and functional outcome if applied at the early echelons. In addition, these technologies will be limited to those that would be implementable in theatre (i.e. taking into consideration the environment and medical footprint) and include current research and development in: cellular by-products such as cytokines that can be applied topically to burn injuries; controlled cell spray gun for greater than 60% total surface area burns or other tissue injuries; controlled temporal therapeutic delivery devices such as novel wound bed dressing; bioreactor for amputated limbs to

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14. ABSTRACT

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regenerate additional tissue and bone for better prosthetic fit (short term goal); cell/biomaterials to regenerate organized complex tissue for addressing injuries due to compartment syndrome; and biomaterials and/or bioreactor to control inflammation and drive the process towards healing. In addition, farther out technologies to be developed such as combined in vivo bioreactor and extraction of autologous cells device will be discussed. It is the author's intention to generate a discussion from the audience on the balance of the possible benefits of bringing these regenerative medicine applications to the early echelons while weighing the associated cost and footprint.

2.0 TISSUE REPAIR PROCESS

Studies on wound healing, in particular for skin, have been well investigated for more than a century [14]. For simplicity in providing some background material for this paper, only a brief summary on the wound healing process is mentioned since there is an extensive body of research articles and review papers, which the interested reader can learn in greater details about this complex wound repair process [14-16].

When injury to the tissue causes some of the cells to break and die, a wound is created [17]. The resulting injury sends a signal to the body, which then triggers a cascade of biochemical, cellular, molecular, and biomechanical events to initiate wound repair. The repair process follows a healing pattern that has synchronized, overlapping phases: hemostasis, inflammation, proliferation, and remodeling (see Figure 1) [16, 18].
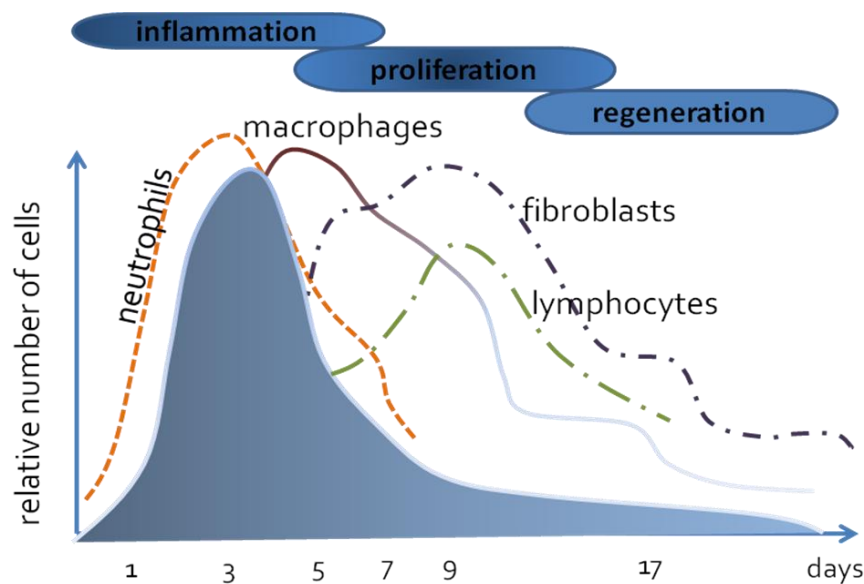

Figure 1: Wound healing pattern shown along with various inflammatory cells.

Within minutes of injury, epinephrine is released into the injury site to minimize bleeding by constricting blood vessels. Platelets are also released and aggregate to form a fibrin clot to arrest bleeding. Thrombin induces platelet degranulation, which causes the release of cytokines and growth factors such as platelet derived growth factor (PDGF), transforming growth factor-alpha (TGF-α), transforming growth factor-beta (TGF-β), and epidermal growth factor (EGF) [10, 15, 16]. Platelet degranulation also serves as a matrix for recruiting inflammatory cells and promotes migration of both keratinocytes and fibroblasts [10, 11, 15].

During the inflammation phase, which starts one to five days post-injury, neutrophils appear first, followed by macrophages, and then mast cells to remove bacteria, foreign debris, and damaged tissue via phagocytosis, release of free radicals, and secretion of proteases that break down damaged tissue [11, 19-23]. This is nature's way of debriding the injury site to make a clean wound bed in order for tissue to begin healing. The macrophages also release more PDGF and TGF-beta, and neutrophils also are a source of pro-inflammatory cytokines that may play a role in some of the earliest signals to activate local fibroblasts and keratinocytes [11]. Neutrophils primarily work to decontaminate the wound through phagocytosis of bacteria and foreign debris as well as through the release of free radicals [22]. Macrophages continue the phagocytosis of pathogens, matrix, and cellular debris including expended neutrophils, and are responsible for stimulating lymphocytes and other immune cells to act on the pathogen [12, 20]. Macrophages also initiate the transition from the inflammation phase to the proliferation phase, where granulation, contraction, and epithelialization occur during skin repair [15, 24].

The proliferation phase occurs from day 3 to 12 post injury and is responsible for re-establishing the integrity of the epidermis and dermis at the wound site. The major activities include angiogenesis, collagen deposition, granulation tissue formation, wound contraction, and epithelialization [10, 25]. Fibroblasts, macrophages, endothelial cells, and collagen migrate to the wound bed to form granulation tissue. As the wound site is filled with granulation tissue, its tissue margin also begins to contract through the action of myofibroblasts using a mechanism similar to that in smooth muscle cells [15]. The extent of contraction depends on the mobility of the surrounding tissue. Fibroplasia is formed during fibroblast proliferation and collagen deposition and is considered an early stage of matrix remodeling. This new, provisional extracellular matrix (ECM) is formed when fibroblasts excrete collagen and fibronectin [10]. In parallel, re-epithelialization of the epidermis begins when cells migrate from the wound edge, divide, proliferate and move across the top of the wound bed, and interface one another to seal the wound [11, 26]. Contraction of the wound helps bring the wound margins toward one another and thereby makes for the process of re-epithelialization easier [11]. Re-epithelialization provides cover for the new tissue and occurs only in the presence of viable tissue (i.e. new cells will not migrate across necrotic tissue) [27]. Resident stem cells from the epidermis and the bulge region of hair follicles also participate in this re-epithelialization event [28, 29]. When the wound is near closure, the excess cells undergo apoptosis [21].

Maturation and remodeling is 21 days post injury and up to 2 years later. Fibroblasts, matrix metalloproteinases (MMPs), and growth factors are critical during this phase. In the final phase of healing, the collagen fibers in the scar are reorganized to improve tensile strength. In normal healing if there are no complications such as infection, by day 21 accumulations of collagen decreases and the balance between the deposition and resorption generally leads to complete tissue healing [16, 18].

3.0   U.S. MILITARY TRAUMA CARE SYSTEM FOR COMBAT CASUALTIES

The U.S. military has developed a sophisticated, integrated trauma care system for management and evacuation of casualties starting with care at the point of injury on the battlefield and progressing through increasingly sophisticated levels of care as the casualty is evacuated from the battlefield to the continental United States (CONUS) [30]. This integrated trauma care system consists of five levels, or echelons, of care, in increasing sophistication as the level progresses towards CONUS. While each branch of the military has the same echelons of care, the care facility name or unit are generally different. For simplicity, the level of care summarized here is with respect to the Army [30].

The primary goal at Level I care is to provide immediate first aid and lifesaving measures at the point of injury on the battlefield. This care is often performed by a trained non-medical person, buddy aid, or trained

combat lifesaver. First aid includes tourniquet application, fracture stabilization with splints, and application of sterile dressings to treat open wounds [30]. Surgical capability is limited and the holding capacity is only a few hours. For casualty who is not able to return to duty within 24 hours, evacuation to the next level of care takes place. Generally, evacuation proceeds from the battlefield to the battalion aid station (BAS) for Army or a shock trauma platoon for Marine Corps, where a physician or physician assistant could initiate resuscitation and advanced trauma life support. In some cases when the casualty needs surgical resuscitation, evacuation would be directly to Level II, bypassing the BAS.

At Level II, where the earliest surgical resuscitation can be performed in the military trauma care system, highly mobile Army forward surgical team (FST) is assigned to directly support combatant units in the field. Hence, each unit must be 100% mobile. At this level, there is a basic lab with radiography capability. The holding capacity is limited to 72 hours and 30 medical operations without resupply. Total staff is a 20 person team that includes an orthopaedic surgeon, three general surgeons, two nurses, and additional nursing staff.

Level III care is the first large medical facility in theatre. The Army combat support hospital (CSH) offers the highest level of medical, surgical, and trauma care available within the combat zone. CSH, which takes some time to be fully operational, offers 248 beds, lab, radiography, blood bank, physical therapy, 24 intensive care unit, and 2 operating tables. Trained medical staff includes general surgeons, orthopaedic surgeons, thoracic surgeons, vascular surgeons, obstetrician/gynaecologist, urologic surgeons, nurses, and physical therapist.

At Level IV, the first echelon where definitive surgical care is offered outside the combat zone can be provided by a CSH or a fixed medical facility pending the situation and the evacuation route. For the current conflict, Level IV takes place at the Landstuhl Regional Medical Center (LRMC) in Germany. Patients are held no longer than 72 hours before evacuation to Level V care facility in CONUS. At LRMC, injuries are furthered evaluated, irrigated, and debrided. Adjustments to the external fixators are made as needed. In general, definitive surgical care is only performed for simple closed injuries.

Level V is the final stop in the medical evacuation chain to one of the major military centers in CONUS (e.g. Walter Reed Army Medical Center in Washington, DC, Brooke Army Medical Center (BAMC) at Fort Sam Houston, San Antonio, TX, or the Naval Medical Center in San Diego, CA) where definitive stabilization, reconstruction, or amputation of an injured extremity is performed. All burn patients are evacuated to BAMC, the designated Department of Defense burn center.

4.0 BATTLEFIELD INJURIES, MANAGEMENT, AND TREATMENTS

4.1 Battlefield Injuries

Management and treatment of battlefield injuries resulting from the current conflicts will no doubt challenge surgeons, clinical researchers, and biomedical engineers, and change both military and civilian trauma management and treatments. In the current conflicts, several factors lead to traumatic war injuries not seen in previous conflicts. One is the enemy combatants' use of rocket-propelled grenade (RPG), mortars, rockets, land mines, and in particular improvised explosive devices (IED) [31, 32]. Second is the advances in armor technology including body armor and "up-armored vehicles;" while decreasing mortality from most blast injuries and gunshot wounds that were not survivable in past wars, the armor technology exposes the extremities (e.g. face, neck, and limbs) to devastating deadly forces that often result in increasing incidence of augmented severity, complexity, and poly-trauma injuries [7, 33- 41]. These include burns, vascular injuries, fractures, long bone defects, large-fragment wound to the extremities, and mangled extremities that in some cases are beyond recognition, see Figures 2 and 3 [6, 7, 34, 40-42]. The other factors include forward surgical

team, which enables surgical resuscitation to be performed within the combat zone, rapid medical evacuation, and intense training of combat personnel [30, 38, 43].

As of January 22, 2010 the total number of Warfighters wounded in action (WIA) that have not returned to duty in Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF) are 13,910 (~44% of WIA) and 2,811 (~58% of WIA) respectively [33]. Most injuries resulting from gunshot wounds, blunt trauma, and explosives accounting for the majority, are to the extremities and involve the musculoskeletal system [7, 31, 32, 35, 37, 38, 40, 41, 44-47]. The severity and complexity of war injuries are significantly different than most civilian trauma, and in particular war injuries are often dirty (e.g. contamination with fragments dispersed in tissue and multiply resistant environmental bacteria) [42].

**Figure 2: Left, a common type of injury associated with roadside improvised explosive
device run over by a Humvee. Right, typical large-fragment wound of the leg. [34].**

Figure 3: Radiograph of mangled leg from blast injury [34].

4.2 Battlefield Injuries Management

Current battlefield injuries management for the U.S. military is to first resuscitate, stabilize, and evacuate the wounded from the combat zone [30, 45]. Definitive care is generally delayed until the casualty reaches Level V facility since the U.S. integrated trauma management system is designed to rapidly transport the casualty from point of injury through several advancing levels of medical care [6, 30]. This system also allows for a smaller medical footprint within the combat zone due to the rapid medical evacuation, which generally takes about 4 to 7days to transport the casualty from point of injury on the battlefield to CONUS [6-9, 40]. Hence, treatments at Levels I to III are limited to stabilization and preservation of life and limb, with the earliest echelon to provide definitive care is at Level IV outside the combat zone. Level IV is the first echelon where definitive surgical management may be performed (typically only on simple closed injuries); in general injuries are only furthered assessed, irrigated, and debrided [30]. At Level V, the final stage of evacuation, surgeons perform definitive stabilization, reconstruction, or amputation of the very complex extremity wound when it is determined that it is no longer salvageable. Therefore, the wounds of the transported casualty until they reach Level V are left opened and usually dressed with only a dry absorbent, non-occlusive dressing [30, 48]. While en route to CONUS, the wounds changes over time and requires re-evaluation at each level. Since definitive care is generally performed in CONUS, most medical and surgical procedures performed up until Level 5 are primarily executed to keep the wound clean (e.g. irrigation, debridement, and broad-spectrum antibiotics) following resuscitation, damage-control, and stabilization [6, 8, 9, 38, 41, 44, 47, 48].

While this has been the established medical care practice for treating wounded warriors with great results in increasing survivability compared to previous conflicts, the amplified severity and complexity of the wounds necessitate new treatment modalities since current reconstructive medicine does not restore the injured tissues and extremities to full function or with aesthetically acceptable appearance.

In recognizing this new military medical challenge to return dedicated service members to fully or near pre-injured state, the U.S. Congress recently issued a legislative request to the Surgeon General of the Army to fund studies on new methods for wound healing and scar reduction following battlefield injuries with the goal to develop new, innovative treatments (December 16, 2009, Department of Defense Appropriations Act 2010). Advances in regenerative medicine and tissue engineering offer the opportunities to develop novel and innovative technologies to treat traumatic war injuries for which current treatment modalities cannot reconstruct the warrior to full or near-full functional form and with aesthetically pleasing results. Thus, developments in regenerative medicine and tissue engineering applications could be the game changer.

4.3 Regenerative Medicine Technologies for Treatment of Traumatic War Injuries

Tissue engineers, surgeons, and clinicians have been working to develop innovative medical solutions such as gene therapy, cell therapy, and engineered tissues to treat injuries, disorders, diseases, and other types of ailments including replacement of damaged organs with a biologically engineered for some time [49-52]. One might say the earliest tissue engineering started as early as 1668 when surgeons utilized non-engineered biomaterials such as ivory, wood, and bamboo as transplants to stabilize factures or replace lost bone tissue following injuries [1, 53, 54]. In the U.S., a historical search on tissue engineering applications through the U.S. Patent Office reveals patents filed in the late 1970s by Drs. Yannas and Bell, who later created Organogenesis.

Recognizing that most of these tissue engineering efforts have been geared towards civilian trauma, whose wounds differ significantly from war injuries, the Department of Defense in particular the U.S. Army Medical Research and Materiel Command (USAMRMC) including its subordinate element the Telemedicine and Advanced Technology Research Center (TATRC) have been directing its investigators and research partners

to develop tissue engineering and regenerative medicine applications specific to treat war injuries, and in particular to the extremity since 2005 [1, 2, 46]. In addition, USAMRMC has increased funding for research in this area through establishing the Orthopedic Trauma Research Program (OTRP) in 2006, the Armed Forces Institute of Regenerative Medicine (AFIRM) in 2008, and more recently the Defense Medical Research and Development Program (DMRDP) in 2009. Almost all of these research programs focus on applications geared towards Level V care.

Regenerative medicine should also be developed for the acute phase of the injury (e.g. during early echelons of care). Even with the rapid medical evacuation of casualty from battlefield to CONUS, treatment is currently limited to keeping the wound clean. While this is important, it could also be delaying healing and prolonging inflammation (Figure 4). Further, each debridement essentially is re-injuring the tissue since some amount of healthy tissue is removed along with necrotic tissue during the process. Other surgical procedures performed on the wound affect it on some mechanical level to the tissue in some form of load. Prolonged inflammation resulting from bacterial infection or mechanical stress on wound has been found to cause unfavourable affect on wound healing; including delayed healing and tissue scarring [10, 55-58]. In addition to the limited treatment at early echelons and possible mechanical stress placed on the wound, full treatment of wounds beyond debridement is further delayed for an additional 4 to 7 days (range in time typically taken for casualty en route for medical evacuation from point of injury on the battlefield to a large, fixed medical facility in CONUS). Taking all of these issues together, the delayed treatment, even if it is only an additional 4 to 7 days (while not increasing mortality), could impact optimal healing, functional outcomes, and aesthetic appearance. Therefore, inclusion of novel, innovative regenerative medicine applied at early echelons to trigger the body immune system to start the healing process may be one possibility in improving wound repair and circumventing the additional debridement as the casualty goes through each successive level of care while en route to CONUS.

Development of innovative regenerative medicine applications geared towards use at early echelons of care needs to take into consideration the current medical footprint, including infrastructure and trained medical staff as well as the logistics in bringing supplies from CONUS to the battlefield. Evaluation of the current echelons of care and its capabilities indicates the earliest echelons of care for consideration would be at Level II. Products for use at Level II care must demonstrate substantial benefits only when it is applied within 24 to 72 hours of injury, have a small medical footprint (Figure 5), and withstand environmental conditions (e.g. variable temperature and humidity of outdoor conditions). This would be a significant engineering challenge. A more reasonable possibility would be regenerative medicine applied at Level III care, where there is greater capabilities and better infrastructure, including established logistics and refrigeration.

Some possible regenerative medicine applications, currently in research and development stage, which could be applied at Level III care and change the current trauma management from wound stabilization to accelerating wound healing, are briefly highlighted here.
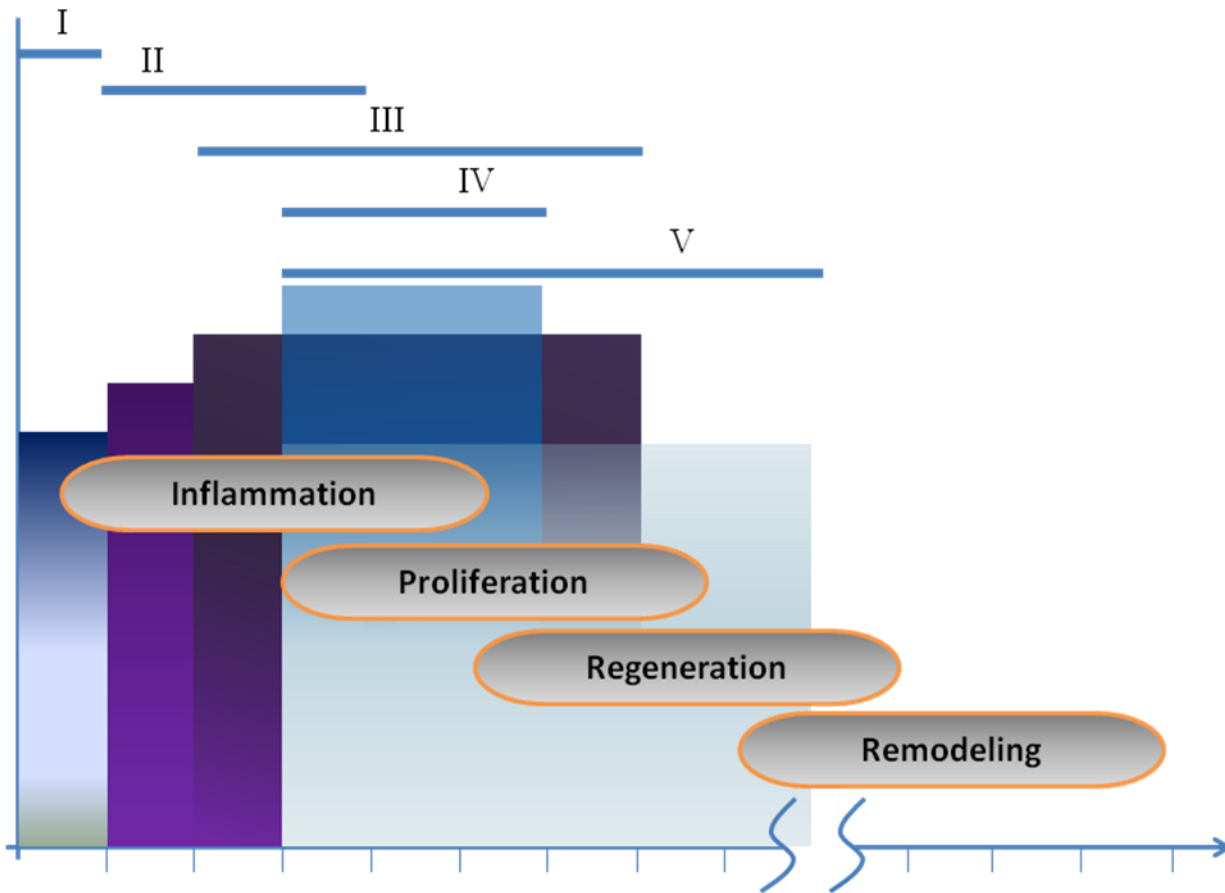
**Figure 4: Illustration showing Level I though V care and wound healing response over time.
Length of bar for Level care indicate range of time a casualty may spend at that echelon of
care. Note normal inflammation phase is about half way completed if casualty arrives
at Level V within 3 days. However, inflammation phase could be prolonged and
could last longer than what is shown above for typical wound healing pattern.**

**Figure 5: General surgery backpack and anaesthesia backpack, two of the five packs containing
the rapid-response surgical system [34]. Products must fit within this bag
for Level II and be stored without controlled environment.**

4.3.1 Therapies Derived from Proteins, Growth Factors, and/or Cells

Therapies that could change and control how inflammation and tissue repair during wound healing by redirecting the body's innate responses towards functional healing would have the greatest impact [12]. One possibility could be derived from studies on understanding the signalling mechanisms involved in regulating inflammation and fibrotic processes. For example, study on the matricellular protein HEVIN and its role in inflammation and wound repair could help understand the factors that regulate inflammation processes and use this information to guide the development of novel therapeutic treatments aimed at manipulating cellular trafficking to direct healing responses toward defined positive outcomes [59]. As a case in point, a unique population of T cells was found that respond to acute inflammation and then elaborate chemokines to attract further inflammation-suppressing cells to migrate to the site, a natural mechanism for the resolution of inflammation that might be exploited therapeutic use [60].

Novel stem cell based therapies using amnion-derived multipotent progenitor cells, including its by-products such as cytokines, could improve healing of burn, spinal cord, and traumatic brain injuries [61]. Amnion-derived multipotent progenitor cells are formed very early in embryonic development from the same embryonic cell layer that forms the fetus. Thus, these cells have strikingly similar characteristics to embryonic stem cells, but possess important characteristics that distinguish them from embryonic stem cells and adult stem cells. One of these characteristics includes defined cell growth, meaning they do not grow and multiply indefinitely, and thus are not considered immortal. This characteristic substantially reduces the risk for transplanted amnion-derived cells from forming spontaneous tumors following implantation. Also, these cells are readily available and easily harvested without any ethical issues associated with embryonic stem cells. Further, amnion-derived multipotent progenitor cells exhibit a unique combination of properties known to be associated with wound healing including extensive cell proliferation potential, differentiation into multiple cell types, and secretion of cytokines and many growth factors. Current research indicates that mechanism of protection is possibly through delivery or sustained secretion of neurotrophic factors [61].

Recent discovery of very small embryonic-like stem cells (VSELs) is being investigated as novel cell-based therapeutics to treat war injuries [62-66]. These VSELs have been found to be elevated following injury such as acute myocardial infarction and stroke [67-69]. The appearance of these VSELs in greater circulation following acute injury might be part of the body's self-repairing mechanism and recent studies appear to show a direct correlation between clinical outcomes to increasing circulating VSELs [68, 69]. Couple of advantages for employing this technology include its pluripotency without drawbacks of embryonic stem cells, its derivation from peripheral blood (enables easy access without having to extract from the bone marrow or destruction of embryos), and its potential for a wide range of therapeutics (not limited to certain types of cells like other adult stem cells) [70-73]. Most importantly, it appears that small numbers of VSELs should provide adequate cell doses for therapeutic effect. Thus, this type of cell-based therapy might have the most effect if apply early following injury, especially since it appears as part of the innate wound healing process.

4.3.2   Novel Cell-based Delivery Technologies

There are a couple of novel ways to deliver cells to improve wound healing and provide coverage for burns, in particular for partial or full-thickness skin injuries or greater than 60% total surface area. These technologies include the SkinGun, WoundCap, and skin printer.

The SkinGun, a controlled cell spray delivery system, when combined with an active wound dressing such as the WoundCap allows for even distribution of cells and for cell growth and support. The SkinGun is basically a device for depositing skin cell in a controlled manner, where the relevant cells suspended in solution can be evenly distributed via an innovative device for spraying a cell suspension under controlled conditions onto the wound. Such an approach could overcome some of the limitations found in current methods for treating skin wounds from skin grafting (i.e. split-thickness and full-thickness grafts, micro-grafting, and mesh graft) to cultured epithelial autografts, and to other mechanical hand driven spray techniques.

The SkinGun research now focuses on developing a methodology that allows for high yield autologous skin cell isolation in the intra-operative setting, and to deliver an expanded population of skin cells to wound sites in a controlled spray pattern. The controlled spraying allows for a more even distribution of cells to be deposited in the wound and enables a larger surface to be treated compared to current treatments such as cell sheets for a given therapeutic cell number. This provides an advantage for the spray deposition concept over cell sheets, which is avoidance of blister formation that results in irregular scar formation and insufficient take rates. These sprayed cells could be supported by an active, on-demand biodegradable wound dressing device (e.g. WoundCap – a biodegradable, porous hollow fiber delivery device) that also provides temporal delivery of therapeutics such as growth factors and/or antibiotics for enhancing healing and/or preventing bacterial infection. The concept of an active, on-demand biodegradable wound dressing device is to not disturb the healed layers of tissue. Such concepts, in particular the WoundCap if applied at Level III could mean an earlier start on tissue repair.

A portable skin printing system would be another novel delivery system for repairing burn injuries quickly and evenly *in situ* [74-76]. This system would enable bioprinting of both partial and full-thickness skin using human skin cells and dermal matrices. Different cells, suspended in solution, are placed in cartridges much like an inkjet printer. Thus, multiple cell types could be delivered to targeted sites. The process will be high throughout, automated, and cost-effective. Current research and development of a bench prototype found two different skin cell types can be directly delivered onto a wound in a murine model. The skin cells delivered through the printer nozzles remain viable and survive following printing. The printed skin cells found to form skin structures and integrate with the surrounding skin.

The goal of these technologies is to provide coverage and initiate wound healing. Another technology to advance cell-based therapy, recently solicited by TATRC through the Small Business Innovation Research (SBIR) funding, is development of an implantable cell bioreactor and extractor device designed to use the body as the natural incubator and bioreactor to capture and expand targeted cells in vivo for use in cell-based therapy. Current cell-based therapy requires ex vivo expansion of cells (isolated from the patient). This expansion process, would occur at Level V when patient arrives at CONUS, adds additional time before treatment could start for autologous cell source therapy. An implantable, miniaturized cell bioreactor and extractor that can be inserted into the patient's arm via a needle at early echelons could mean an earlier start in expansion of cells that be readily extracted at sufficient cell population for therapy once patient arrives at Level V.

5.0 SUMMARY

Regenerative medicine could be the game changer in improving functional and aesthetic outcomes of wounded warriors, whose traumatic war injuries are challenging, severe, and complex. The U.S. Army is leading the way in funding regenerative medicine research to develop innovative medical treatments specific to traumatic war injuries. One step further would be to develop regenerative medicine that could be applied at the earlier echelon such as Level III care. This would be a paradigm shift from the current trauma care management system where final definitive care takes place at Level V facility in CONUS. Current trauma management, while sophisticated and advanced, is mostly limited to keeping the wound clean following resuscitation and stabilization of the injured warrior at early echelons of care (Level I through IV). Although transport of casualty from battlefield to CONUS is rapid and typically takes 4 to 7 days, the delayed in definitive care could negatively impact the course of wound healing such as prolonged inflammation. Therefore, appropriate regenerative medicine applications could alter the course of wound healing towards tissue repair and regeneration when applied early in the injury phase. An evaluation on the current state-of-the-art regenerative medicine applications and technologies for use at Level III was performed. Several possible candidates are presented in this paper. The selection criteria included its medical footprint, operational requirements (e.g. infrastructure and trained staff), and potential benefits, in particular when result is time sensitive. Finally, advances in innovative military medicine will also positively impact civilian trauma care.

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