Plastic Surgery Challenges in War Wounded II: Regenerative Medicine

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Submitted for publication March 31, 2015.
Accepted in revised form July 3, 2015.

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Background: A large volume of service members have sustained complex injuries during Operations Iraqi Freedom (OIF) and Enduring Freedom (OEF). These injuries are complicated by contamination with particulate and foreign materials, have high rates of bacterial and/or fungal infections, are often composite-type defects with massive soft tissue wounds, and usually have multi-system involvement. While traditional treatment modalities remain a mainstay for optimal wound care, traditional reconstruction approaches alone may be inadequate to fully address the scope and magnitude of such massive complex wounds. As a result of these difficult clinical problems, the use of regenerative medicine therapies, such as autologous adipose tissue grafting, stem cell therapies, nerve allografts, and dermal regenerate templates/extracellular matrix scaffolds, is increased as adjuncts to traditional reconstructive measures.

Basic and Clinical Science Advances: The beneficial applications of regenerative medicine therapies have been well characterized in both *in vitro* studies and *in vivo* animal studies. The use of these regenerative medicine techniques in the treatment of combat casualty injuries has been increasing throughout the recent war conflicts.

Clinical Care Relevance: Military medicine has shown positive results when utilizing certain regenerative medicine modalities in treating complex war wounds. As a result, multi-institution clinical trials are underway to further evaluate these observations and reconstruction measures.

Conclusion: Successful combat casualty wound care often requires a combination of traditional aspects of the reconstructive ladder/elevator with adoption of various regenerative medicine therapies. Due to the recent OIF/OEF conflicts, a high volume of combat casualties have benefited from adoption of regenerative medicine therapies and increased access to innovative clinical trials. Furthermore, many of these patients have had long-term follow-up to report on clinical outcomes that substantiate current treatment paradigms and concepts within regenerative medicine, reconstructive, and rehabilitation care. These results are applicable to not only combat casualty care but also to nonmilitary patients.

Keywords: regenerative medicine, war trauma, limb salvage, complex wound care

BACKGROUND

NUMEROUS SYSTEMATIC advancements in forward surgical care, protective service gear, and rapid transport of critical war wounded from

the theater to stateside facilities have directly contributed to tremendous improvements in combat casualty survival rates.¹⁻⁴ As a result, an increasing number of service members

with severe craniofacial, extremity, and polytraumatic injuries and associated wounds have presented to stateside military treatment facilities for definitive reconstruction.^{1–5} These massive soft and composite-type tissue defects are often complicated by large zones of injury, have extensive areas of devitalization and gross contamination with foreign material, as well as have high rates of resistant bacterial and fungal infections.² Moreover, all the above concerning clinical issues occur commonly within the presence of polyextremity injuries, other concomitant injuries, and have an associated multisystem involvement (Fig. 1a, b).²

Reconstruction of these wounds begins at the point of injury, with acute care algorithms and management occurring throughout the international military trauma transport system.⁶ Definitive management, recovery, and rehabilitation care are primarily based at our stateside military tertiary and quaternary care facilities. Early and aggressive serial debridement of all devitalized tissue and control of contamination remain the mainstay of early treatment and en route care. However, blast-related trauma coupled with this necessary serial tissue debridement can lead to volumetric soft tissue deficits and potential avascular surfaces or structures such as denuded bone and tendon devoid of peritenon. Because neovascularization by capillary ingrowth from the vascular periphery is the foundation of successfully treating these wounds,⁷ avascular structures traditionally require the transfer of vascularized tis-

sue for definitive coverage. However, tissue transfer can produce significant donor site morbidity, prolonged operative and hospital courses, and a high rate of complications in unstable, critically ill blast casualty patients.⁸ Furthermore, polylimb amputations and trauma in combination with massive soft tissue losses further complicate wound management as traditional donor sites for reconstruction may not be available or compromised secondary to the initial combat injury event.

In recent years, surgical teams have utilized a “hybrid reconstructive ladder/elevator” paradigm, in which regenerative medicine therapies are used in conjunction with traditional reconstruction approaches (Fig. 2).^{4,9} This novel treatment paradigm has led to improved definitive closure of large wounds having extensive soft tissue losses. The objective of this chapter is to provide an overview of the use of regenerative medicine therapies, namely autologous adipose tissue grafting, stem cell therapies, and dermal regenerate template (DRT)/extracellular matrix (ECM) scaffolds, in conjunction with traditional reconstruction approaches for care of combat casualty injuries.

BASIC SCIENCE AND CLINICAL ADVANCES

Autologous adipose tissue grafting

Autologous adipose tissue grafting is being increasingly utilized to restore the subcutaneous adipose tissue layer and improve functional outcomes associated with traumatic and surgical soft tissue contour deformities and tethering of scars. The Coleman technique for fat grafting employs a minimally invasive harvesting procedure in an effort to maintain the structural integrity of the lipoaspirate.^{10,11} The components of the lipoaspirate include adipocytes, preadipocytes, and surrounding stroma. Immunohistochemical studies show that these preadipocytes are adipose tissue-derived stem cells capable of differentiating not only into adipocytes that help maintain volume over time but also can differentiate into other types of tissue, including muscle, nerve, and bone.^{12–15} Lipoaspirate that includes high concentrations of adipose-derived stem cells have more microvascular ingrowth than transfers with physiologic levels of these cells.^{16–18} However, confirmation of this association in human studies is still pending.¹⁹

The majority of clinical experiences with autologous fat grafting has been for aesthetic and certain reconstructive applications within the craniofacial, torso/breast, and extremity regions for such issues as aging, atrophy associated with HIV treatment,^{11,20} acquired deformities,²¹ and oncologic deformities.^{22–24}

Figure 1. (a, b) Clinical example of massive soft tissue and polyextremity injuries commonly seen in combat casualties secondary to blast exposure. (a) Shows left upper extremity (LUE) severe composite and soft tissue injury; (b) illustrates bilateral lower extremity injuries, including a left lower extremity traumatic amputation. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

Figure 2. The hybrid reconstructive ladder that integrates regenerative medicine therapies to traditional reconstruction measures. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

The application of autologous adipose tissue grafting is currently being translated to various military combat-related injuries as well. Examples include use of fat grafts for the functional and aesthetic reconstruction of craniofacial deformities, the release and interposition of fat in tethered and/or atrophic scars of the craniofacial, truncal, and extremities, as well as treating contour defects and providing additional tissue in residual limbs. Fat grafting to the extremities has been used to aid in prosthetic socket fit and residual limb interface strategies, as well as to potentially aid in lessening neuroma pain. Furthermore, it has also improved the healing of certain recalcitrant wounds and appearance of poor scars in certain combat injuries.

While a large body of evidence exists demonstrating the ability of autologous fat grafting to improve short-term cosmetic results, the clinical effectiveness (*i.e.*, graft volume retention) of the grafts over time has been debated.^{25,26} In recent years, much attention has been focused on enriching the autologous adipose tissue graft with the stromal vascular fraction (SVF) of adipose tissue. The SVF is a complex milieu consisting of several growth factors and cell types, including stem/progenitor cells. The SVF, for example, has been shown to contain factors such as insulin-like growth factor 1 that has been attributed to graft retention

properties. Indeed, numerous preclinical studies, as well as an emerging body of clinical data, suggest that enriching adipose tissue grafts with stem/progenitor cells from the SVF improves volume retention over time.^{25,27–29} It has been suggested that the adipose tissue serves as a scaffold upon which more concentrated stem/progenitor cells can organize and differentiate, thus promoting the secretion of soluble factors that enhance angiogenesis, decrease apoptosis, and/or modulate the immune response.^{25,27–29}

Stem/progenitor cells

Autologous and allogenic stem cell therapies retain a great potential for regenerative medicine applications as they have the ability to divide in an undifferentiated state to produce additional precursor cells, possess the potential to self-renew, as well as can differentiate into various specialized cell types. The ability to isolate stem cells from adult tissues avoids the controversy surrounding embryonic stem cells and certain tumor-based stem cell lines. For example, mesenchymal stem cells (MSCs) have high proliferative potential and can differentiate into specialized cell types derived from mesodermal germ layers at the site of injury.^{30,31} MSCs have a beneficial effect on wound care not only by replacing injured soft tissue, bone,

cartilage, tendon, and muscle cells but also have a significant anti-inflammatory effect through the release of paracrine mediators.³² By this mechanism, MSCs further promote wound healing by promoting cell migration and angiogenesis.³³ In animal studies, MSCs can turn a chronic nonhealing wound with impaired neovascularization to a wound with characteristics similar to an acute wound that becomes primed for vascular ingrowth.^{34–37} In addition, epidermal stem cells are thought to be responsible for repopulation of the epidermis following injury and have been used through spray skin technologies to restore epithelial continuity in certain burn and combat wounds.³⁸

MSCs can be harvested in relatively large quantities from multiple tissue sources at low risk to the patient. They can be harvested from an autologous source, including bone marrow, monocytes, muscle, and adipose tissue sources. MSCs typically require a scaffold for application; potential investigated scaffolds have included fibrin, collagen, urinary bladder matrix (UBM), and dermal regenerate matrices. The use of MSCs has found a number of therapeutic benefits in wound care as the transplantation of autologous MSCs has been shown to increase the rate of wound healing in both acute surgical and chronic wounds.³⁹

While vascularized bone and nonvascularized bone autografts remain the primary methods to address combat-related segmental bone defects of the extremities and craniofacial regions, increasing interest and application of human MSCs within combat casualty care have occurred.⁴⁰ Allograft MSCs are used in isolation, or more typically combined with autologous bone grafts, to aid in bone regeneration and filling bone gaps especially in patients with limited availability of traditional vascularized bone flaps (e.g., bilateral amputees who lack free fibula flaps). These MSCs are derived from allogenic adult bone marrow sources, have viable multipotent stem cells capable of differentiating along various lineage pathways, and can have demineralized bone grafts/scaffolds for structural support.⁴¹ These cancellous bone allograft matrices have MSCs that do not express MHC/HLA class II antigens and the costimulatory molecules, which are required to cause T-cell proliferation. Thus, they are immune naïve or nonimmunogenic.^{9,42}

For the common orthogenic MSCs employed in combat casualty care cases, these cellular-based products undergo proper donor screening and careful processing methods to preserve cellular viability and retain biologic properties. They have also completed proper microbial treatment for sterility purposes. Furthermore, these products have

undergone preclinical immunogenicity and biocompatibility testing for use as allogenic cellular bone matrices. MSC-based allografts (1) act as osteoconductive scaffolds, (2) have osteoinductive potential, and (3) possess osteogenic cells to aid in bone regeneration and formation while lacking donor-site morbidity.⁴³

There are several potential pitfalls to stem cell therapies worth noting. Stem cells removed from their natural environmental niche may change irreversibly in an *in vitro* cell culture environment. Exogenous stem cells may also divide and differentiate in a nonsite-specific manner and, thus, may not effectively reconstruct the damaged tissue of interest. In addition, stem cells derived from allogenic donor sources may not be fully immunocompatible with the recipient. Last, there are concerns that stem cell therapies utilized in certain injury mechanisms may contribute to the upregulation of abnormal physiological processes such as heterotopic ossification or bone tissue formation within soft tissue and nonskeletal structures.

An alternative to directly injecting or transplanting exogenous stem cells into injured tissue is to recruit endogenous host stem cells to the site of injury. Currently, one of the best ways to recruit endogenous adult stem cells is through the use of biologic scaffolds derived from decellularized ECMs.

ECM scaffold and DRT materials

Biologic scaffolds composed of ECMs and DRTs represent regenerative medicine approaches where these materials act as three-dimensional bioactive scaffolds. These scaffolds consist of structural and functional proteins along with growth factors. Currently, there exists more than 60 ECM scaffold products that are FDA approved, and companies continue to commercialize new ECM scaffold materials annually. These scaffolds are typically allogeneic or xenogeneic in origin and are derived from a wide variety of tissues (e.g., urinary bladder and small intestine).

ECM scaffolds and DRT materials, when prepared by appropriate methods, are capable of promoting constructive tissue remodeling (*i.e.*, limited reconstitution of functional, innervated, and vascularized tissue) rather than scar tissue formation in a number of tissues and organs within both preclinical animal models and human clinical studies.^{9,44,45} Unlike synthetic scaffolds, ECM scaffolds degrade over time *in vivo*, which is critical to creating an optimal microenvironment that enhances constructive tissue remodeling. As the ECM is broken down by host cells, growth factors and cytokines that were contained within the ECM are released and cryptic

peptides, portions of larger signaling proteins that are biologically active in chemotaxis, mitogenesis, and bacteriostasis, are generated.⁴⁵ These cryptic peptides, growth factors, and cytokines facilitate a microenvironmental milieu that supports the migration, proliferation, and differentiation of stem/

progenitor cells and modulates the host microenvironment toward an anti-inflammatory proremodeling response.^{9,44} DRTs, on the other hand, typically do not degrade and serve as a scaffold to restore lost or missing soft tissue while recreating or restoring a neodermis.

Blast trauma often results in large soft tissue deficits that are often devoid of a functional vascular bed required for subsequent reconstruction. As ECM scaffolds and DRTs have been shown to support constructive tissue remodeling, including neovascularization, they have been used as a supplement to conventional reconstruction, particularly in cases where prior attempts at facilitating wound healing had failed. Specifically, ECM scaffolds and DRTs have been applied to soft tissue defects before coverage with xenografts, skin grafts, and/or flaps to facilitate a healthy wound bed that could sustain further reconstruction efforts (Figs. 3a–c, 4a, b, and 5a–d). To date, these efforts have yielded positive results resulting in reduced morbidity to the patient.

Figure 3. (a) LUE blast injury illustrating large soft tissue injury of nearly entire volar forearm extending proximally to the brachial region. (b) Same LUE injury after lib salvage with free ALT flap coverage proximally and a dermal regenerate template (DRT)/extracellular matrix (ECM) placement to distal forearm. (c) LUE appearance 2 years postreconstruction and after liposuction contouring of proximal ALT flap. Note appearance of distal forearm DRT/ECM and skin graft 2 years after initial operative events. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

Figure 4. (a) Lower extremity combat casualty injury before hybrid reconstruction. (b, c) Same lower extremity injury after hybrid reconstruction with flap coverage of tibia fracture and open knee joint, DRT/ECM placement, and secondary skin grafting 2 years after reconstruction. (b) Left Lower Extremity in extension; (c) LLE in flexion. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

Figure 5. (a) Right Upper Extremity radiograph showing extensive orthopedic injuries. (b) RUE massive soft tissue injuries in face of severe orthopedic injuries with DRT in place for regeneration of dermal substitute in preparation of second stage Split Thickness Skin Graft. (c) RUE hybrid reconstruction with STSG after DRT incorporation. (d) RUE hybrid reconstruction and function illustrating patient throwing first pitch at professional baseball game 2 years postreconstruction. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

CLINICAL CARE RELEVANCE

Traditional therapies for the treatment of complex wounds include debridement of necrotic or infected tissue, clearance of bacterial load, pressure offloading, negative pressure wound therapy, and the ladder of soft tissue reconstruction employing techniques from primary closure to free tissue transfer to achieve an eventual closure.^{4,9,46} Our patients undergo early radical debridement to control infection and prepare the wound bed for closure or coverage with well-vascularized tissue in conjunction with regenerative medicine therapies. Treatment options are evaluated by a multidisciplinary team outside the deployed environment, taking into account the necessity to adequately and reliably cover vital structures while preserving the function. Patients often require multiple irrigation and debridement procedures, as well as orthopedic stabilization procedures, before definitive wound treatment.

In the subacute setting, bone and nerve autografts or allografts are used to reconstruct segmental deficits. These grafts function directly to produce tissue, stimulate the host system to produce the desired tissue, and serve as a nonviable scaffold for tissue ingrowth. Human and xenograft-derived ECMs are used to aid in soft tissue regeneration. Our institution uses a UBM in this capacity to contribute to increased vascularity within wound beds where peritenon and periosteum have been lost or damaged. UBM has similar structural and functional components of native ECM, thus undergoing the degradative processes that can aid in the develop-

ment of granulation and perivascular tissue. A combination of ECM and an acellular bilayer matrix prepares the wound bed for skin grafting. This technique provides a vascularized bed over exposed structures and allows a thinner split-thickness skin graft procedure to obtain adequate, stable coverage, reestablishment of the barrier function of skin, as well as restores skin continuity.

FUTURE DEVELOPMENTS

Autologous fat grafting, stem cells, and ECM scaffolds have found an increasing role in wound care in our war wounded. Our institution is involved in a number of multi-institution clinical trials to determine whether these and other regenerative medicine therapies can augment or, in some cases, replace traditional methods of reconstruction and wound care. Positive results may obviate the need for traditional soft tissue transfer, decreasing potential major operative intervention and donor site morbidity in critically ill patients. Early results show these therapies invaluable in wound healing while maintaining form and function.

ACKNOWLEDGMENTS AND FUNDING SOURCES

The authors wish to thank our fellow current or former service members from Walter Reed Bethesda as well as from other Department of Defense Military Treatment Facilities for their remarkable and noteworthy support in wounded warrior care during the recent war efforts.

AUTHOR DISCLOSURE AND GHOSTWRITING

The authors have no affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the article. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. The views expressed in this presentation are those of the authors and do not necessarily reflect the official policy or position of the Department of Army, Department of Defense, or the U.S. Government.

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Ian L. Valerio, MD, MS, MBA, FACS, served as Commander (CDR) in the U.S. Navy and Plastic and Reconstructive Surgeon at the Walter Reed National Military Medical Center (WRNMMC) (2010–2014). In 2014, CDR Valerio entered the U.S. Navy active reserve component while transitioning to the Ohio State University Wexner Medical Center (OSUMC) where he assumed the position of Division Chief of Trauma, Burn, and Wound and Associate Professor, Departments of Plastic and Reconstructive Surgery, General Surgery, and Orthopedic Surgery. Dr. Valerio maintains academic positions as Associate Professor at the Uniformed Services University of the Health Sciences and The University of Pittsburgh. He has been forward deployed acting as the Department Head for Trauma, General, and Plastic Surgery, at the NATO Role III Multinational Medical Unit (MMU), in Kandahar, Afghanistan (2012–2013). While at WRNMMC, he served as the WRNMMC/Johns Hopkins/University of Maryland Plastic Surgery Residency Program Director, Director of Plastic Surgery Research Programs, and was an Adjunct Assistant Professor of Plastic and Reconstructive Surgery at the Johns Hopkins University School of Medicine (2010–2014). His interests include traumatic, burn, wound, and oncologic reconstruction, microsurgery, craniofacial and head and neck reconstruction, breast reconstruction, general plastic reconstruction, and aesthetic surgery. He is actively involved in clinical and preclinical research on adipose-derived stem cell therapies (ADSCs), mesenchymal stem cells,

advanced regenerative medicine applications to combat casualty and trauma/burn/wound care, vascularized composite allotransplantation (VCA), targeted muscle reinnervation (TMR), peripheral nerve repair/restoration techniques, amputee care and prosthetic interfaces, and other traumatic, oncologic, and congenital reconstruction strategies. **Christopher L. Dearth, PhD**, concurrently serves as the Facility Research Director for the Extremity Trauma & Amputation Center of Excellence (EACE), Director of Research for the Department of Rehabilitation at Walter Reed National Military Medical Center (WRNMMC), and the Founding Director of the Regenerative Rehabilitation Laboratory at the Uniformed Services University of the Health Sciences (USUHS). In these roles, Dr. Dearth leads a multidisciplinary team of clinicians and researchers whose collective focus is on the mitigation, treatment and rehabilitation of traumatic extremity injuries and amputations by implementation of clinically relevant research aimed at optimizing the quality of life of service members and veterans. Dr. Dearth's preclinical and clinical research interests include: development of innovative regenerative medicine & tissue engineering constructs, utilization of next generation imaging modalities for extremity trauma applications, evaluation of advanced (i.e., powered and intuitively controlled) prosthetics for service members and veterans with limb loss, and championing a synergy of efforts between the fields of rehabilitative and regenerative medicine. **MAJ Jennifer Sabino, MD**, is a U.S. Army medical corps officer who has served for 14 years. She is a graduate of the United States Military Academy at West Point and of the Yale University School of Medicine. After graduating from Yale University SOM, she started and completed her general surgery residency at Walter Reed National Military Medical Center where her primary responsibilities included general surgery training and caring of those wounded warriors injured during the recent Iraq and Afghanistan Wars (Operation Iraqi Freedom and Operation Enduring Freedom). She is currently an independent plastic surgery resident at the Johns Hopkins University School of Medicine. Upon completion of training, she will be a plastic surgeon at an Army medical treatment facility.

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