Review Article

Emerging Implications for Extracellular Matrix-Based Technologies in Vascularized Composite Allotransplantation

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Received 15 July 2015; Accepted 5 October 2015

Academic Editor: Pavla Jendelova

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Despite recent progress in vascularized composite allotransplantation (VCA), limitations including complex, high dose immunosuppression regimens, lifelong risk of toxicity from immunosuppressants, acute and most critically chronic graft rejection, and suboptimal nerve regeneration remain particularly challenging obstacles restricting clinical progress. When properly configured, customized, and implemented, biomaterials derived from the extracellular matrix (ECM) retain bioactive molecules and immunomodulatory properties that can promote stem cell migration, proliferation and differentiation, and constructive functional tissue remodeling. The present paper reviews the emerging implications of ECM-based technologies in VCA, including local immunomodulation, tissue repair, nerve regeneration, minimally invasive graft targeted drug delivery, stem cell transplantation, and other donor graft manipulation.

1. Vascularized Composite Allotransplantation

The field of reconstructive surgery has made significant progress in the last few decades. Despite advancements in technique and instrumentation, severe trauma and/or congenital abnormalities necessitating complicated and extensive tissue reconstruction remain challenging clinical problems. Well established strategies to address these conditions include the use of complex techniques such as bone and muscle grafts, partial/full thickness dermal flaps, and composite tissue flaps. Nevertheless, these techniques are hampered by nontrivial complications such as morbidity at the donor site, limited availability of autologous tissues, and complications associated with extensive surgery [1–3]. Such problems are compounded by the high costs associated with multiple surgical procedures, extended hospital stay, and strenuous rehabilitation.

Novel strategies to circumvent these issues have recently emerged. Vascularized composite allotransplantation (VCA) is a promising field that investigates the transplantation of composite anatomic homologous structures from immunologically and aesthetically compatible donors. Using this approach, close to 200 VCA procedures have been successfully performed worldwide in the last decade, including more than 110 hand transplants and 35 facial transplants [4]. Overall, VCA has achieved encouraging graft survival rates and functional outcomes. With few exceptions, patients who have complied with their treatment regimens have experienced satisfactory restoration of significant tissue deficits, improved functional and aesthetic outcomes, and reduced complications associated with these procedures [5–7].

Despite promising results enabling independence with activities of daily living and social or professional reintegration of patients, a number of important obstacles and limitations persist with VCA. These limitations include (1) multidrug immunosuppression regimens [8, 9], (2) serious systemic side effects and toxicity from immunosuppressants including the risk of life threatening, life shortening, or
quality of life reducing complications [6, 10], (3) acute and chronic allograft rejection [6], and (4) suboptimal nerve regeneration negatively impacting overall motor or sensory functional outcomes [11–14] (Figure 1 and Table 1).

2. Novel Strategies and Implications in VCA

2.1. Drug Delivery Applications. A number of technologies are currently under investigation to address the limitations associated with VCA. Local delivery of immunosuppressive agents directly into the graft is a promising alternative to oral medication intake [15]. VCA, unlike other solid organs, are accessible for targeted interventional therapies and visible for clinical and histologic monitoring of grafts. Most systemic immunosuppressive agents are associated with high toxicity and/or narrow therapeutic windows of efficacy. Hence, the main purpose of graft targeted drug delivery strategies is to mitigate systemic exposure and adverse drug related side effects by localizing the delivery of therapeutic agents to the treatment site. Additional advantages of such an approach would include the minimization of overall dosing, further reducing complications associated side effects and toxicity, minimization of frequency of dosing, further reducing complications associated with patient noncompliance, and ease of removal, should an allergic or adverse reaction be experienced by the patient (Figure 2).

The concept of drug eluting biomaterials as implantable delivery systems is not novel. Originally conceived in the 1930s by Deanesly and Parks, this concept was further expanded in the 1960s by Folkman and Long with the investigation of implantable formulations with drug release rates controlled by a rate controlling membrane [16, 17]. Significant progress has been made since implantable drug delivery systems were first conceived. However, commercially available technologies for clinical use are still limited to drug eluting stents for cardiovascular applications [18, 19], intrauterine devices for contraception and treatment of diseases [20, 21], and intraocular inserts for the treatment of glaucoma and cytomegalovirus retinitis [22].

Technologies under development include a broader spectrum of biomaterials and applications. Latest generation technologies such as smart materials that can respond to environmental cues including temperature-responsive [23–27], pH-responsive [28–32], and solvent-responsive [33, 34] polymer-based drug delivery systems [35] offer greater control over their pharmacokinetic properties and drug release profiles. Highly complex bioresponsive materials include hydrogel-based on-demand drug delivery systems such as
Table 1: Summary of upper extremity transplantation experience.

| Country      | Center                                      | Total number of limbs | Number of limbs lost | Mortalities |
|--------------|---------------------------------------------|-----------------------|----------------------|-------------|
| Australia    | Melbourne                                   | 1                     |                      |             |
| Austria      | Innsbruck                                   | 9                     |                      |             |
| Belgium      | Brussels                                    | 1                     |                      |             |
| China        | Six centers                                 | 15                    | 7                    |             |
| France       | Lyon                                        | 11                    | 1                    | 1*          |
|              | Paris                                       | 2                     | 2*                   | 1*          |
| Germany      | Munich                                      | 2                     |                      |             |
| India        | Kochi                                       | 4                     |                      |             |
| Iran         | Tehran                                      | 1                     |                      |             |
| Italy        | Milan                                       | 3                     |                      |             |
|              | Monza                                       | 2                     |                      |             |
| Malaysia     | Selayang                                    | 1                     |                      |             |
| Mexico       | Mexico City                                 | 4                     | 2                    | 1*          |
| Poland       | Wroclaw                                     | 7                     | 1                    |             |
| Spain        | Madrid                                      | 2                     |                      |             |
|              | Valencia                                    | 6                     |                      |             |
| Turkey       | Ankara                                      | 2                     | 2*                   | 1*          |
|              | Antalya                                     | 6                     | 2*                   | 1*          |
| United Kingdom | Leeds                                     | 1                     |                      |             |
| United States | Brigham and Women's Hospital, MA             | 4                     | 2*                   |             |
|              | Emory, GA                                   | 3                     | 2                    |             |
|              | University of Pittsburgh/Johns Hopkins University | 11                 | 2                    |             |
|              | Massachusetts General Hospital, MA          | 1                     |                      |             |
|              | University of Louisville, KY                | 9                     | 2                    | 1*          |
|              | UCLA                                        | 1                     |                      |             |
|              | University of Pennsylvania                 | 4                     |                      |             |
|              | Wilford Hall, Medical Center, TX            | 1                     |                      |             |

Totals: 114, 26, 5

* Simultaneous hand and other body regions transplantation (face or leg). Table adapted from published and presented updates.

polysaccharide-based hydrogels that can release matrix metalloprotease (MMP) inhibitors in response to MMP activity [36], and reloadable constructs designed to circumvent serial implantations upon active ingredient depletion [37].

Despite promising results, limitations associated with implantable drug delivery systems that are particularly relevant to VCA remain to be addressed. The foreign body reaction and proinflammatory microenvironment associated with synthetically derived biomaterials [38, 39], as well as the residual effects associated with these technologies, particularly following consecutive implantations, are among the most important limitations.

2.2. Stem Cell-Based Therapies. Cell-based attempts to modulate the inflammatory response thereby enhancing graft survival following allotransplantation rely heavily upon the privileged immunological properties of stem cells. The objective of this approach is to harness the immunomodulatory properties of stem cells to induce long term tolerance and graft survival by delivering stem cells into the host.

The immunomodulatory properties of stem cells have been attributed to a low immunostimulatory profile as well as to active immunomodulatory processes both locally as well as a paracrine phenomenon. The low immunostimulatory profile is attributed to decreased antigenicity due to low levels of MHC-I molecule expression and lack of MHC-II expression in both embryonic stem cells (ESC) [40–45] and induced pluripotent stem cells (iPSC) [46–49]. On the other hand, active immunomodulation [42, 44, 50–52] has been shown to be the result of both ligand-mediated [42, 50] and soluble factor-mediated [53, 54] processes. Of these factors, prostaglandin-E2 (PGE2) has been identified as a potent soluble mediator of ESC-mediated immune suppression [55].

Initial in vivo studies reported partial success of ESC engraftment and avoidance of immune system activation. However, these experiments were carried out in immunodeficient animals [44, 53, 56, 57], and further work performed...
in wild type animals in more recent studies contradicted these initial results [58–60]. Although ESCs are still not considered a definite and reliable therapeutic agent for long term graft tolerance induction [61], bone marrow-derived and adipose-derived mesenchymal stem cells (MSCs) have recently emerged as potential immunomodulatory agents [62, 63] that have been shown to promote hematopoietic stem cell engraftment [64, 65] in solid organ transplantation [66, 67] and graft-versus-host disease [68] and to prolong graft survival in a preclinical model of VCA [69].

2.3. Tolerance Approaches. In contrast to other therapies, the advantage of establishing immune tolerance is to ideally prevent/control both acute and chronic rejection without maintenance therapy therefore reducing the overall burden of immunosuppression [9]. Although the mechanisms of immune tolerance are not entirely understood, spontaneous tolerance has been reported in liver and kidney transplants [70, 71], and associated with transient chimerism in renal transplant patients [72–74]. However, there appears to be an immunogenic hierarchy among different organs. While a significant number of liver transplant patients may spontaneously develop tolerance, and transient chimerism might be sufficient for tolerance development in renal transplant patients, VCA have reported to be particularly immunogenic and as a result, the establishment of mixed, stable chimerism seems to be the most promising option [75, 76]. In fact, while simultaneous transplantation of stem cells and VCA has been shown to achieve tolerance in experimental studies [9, 69], infusion of donor bone marrow has been shown to be immunomodulatory and potential facilitator for the eventual transition to maintenance immunosuppression with single-agent tacrolimus [48].

In summary, although adjuvant therapies under development are promising and address some of the issues associated with VCA, several nontrivial obstacles remain to be addressed including the well-described proinflammatory host response upon implantation of synthetic materials used in drug and cell delivery, immune system modulation, suboptimal nerve regeneration, and the necessity of at least partial systemic immunosuppression.

3. Extracellular Matrix-Derived Biomaterials and Reconstructive Surgery

3.1. The Extracellular Matrix. The ECM is a complex milieu of both structural and functional molecules that is secreted by the resident cell population of every tissue and organ. Every tissue is unique in its exact composition and 3-dimensional ultrastructural organization of the ECM. Collagen type I is the main component of the ECM in most tissues, comprising more than 90% of its mass [77, 78]. Other molecules such as laminin, fibronectin, glycosaminoglycans, and other types of collagen are also present in the ECM in various proportions depending upon the specific tissue type to which they belong.
For example, while tissues with a basement membrane have a higher proportion of collagen type IV [79], tendons and ligaments need a higher proportion of type I collagen to withstand the mechanical loads to which they are subjected [80]. Vascular tissues need to be flexible and elastic and hence have a higher proportion of laminin and elastin [81]. The ECM is responsible, at least in part, for the diverse mechanical properties found across tissues and organs, and therefore, its composition reflects the mechanical and physiologic demands of every tissue [39].

In addition to providing structural support, the ECM provides biochemical and mechanical cues to the cells of every tissue. The ECM participates in signal transduction directly by interacting with cell receptors, and/or indirectly by facilitating connectivity between adjacent cells [82, 83]. The ECM binds, sequesters, and stores signaling molecules that are released or exposed when needed. Among the diverse physiologic properties influenced by the ECM are stem/progenitor cell migration and chemotaxis, and modulation of the innate and acquired immune systems. In addition to serving as a reservoir for signaling molecules [84–86], the ECM can also communicate physical stimuli to local cell populations by being physically attached to cell receptors that transduce these cues to the cytoskeleton [87]. Hence, the ECM exists in a state of dynamic reciprocity with the microenvironment. While local cell populations secrete the ECM, the ECM itself can in turn influence cell signaling and behavior and modify gene expression profiles [88]. For this reason, the ECM is an important mediator of homeostasis both in health and disease.

3.2. Extracellular Matrix-Derived Biomaterials. ECM-derived biomaterials are typically manufactured by means of tissue decellularization. Although the exact composition and structure of the ECM varies from one tissue source to another, the main components of the ECM are generally conserved across different tissues and even across different species. This principle is the basis for the successful clinical implementation of regenerative medicine strategies using biomaterials originating from xenogenic sources (Figure 3).

The objective of the decellularization process is to remove immunogenic and proinflammatory material (i.e., cellular components) that could elicit an adverse immune response [89, 90] while conserving the structure and composition of...
Commercially available ECM-derived materials are manufactured from a variety of tissue sources and species and exist in a variety of formats including hydrogels, scaffolds, and cross-linked scaffolds. The ECM, both of which have beneficial effect in the tissue repair process. Specific details concerning the decellularization process have been reviewed elsewhere [91].

To date, a variety of source tissues have been successfully decellularized and products composed of mammalian ECM are commercially available and routinely used in surgical applications (Table 2). ECM-derived biomaterials have been used clinically in multiple reconstructive surgical applications including breast reconstruction [92], ventral hernia repair [93], facial reconstruction [94], and skeletal muscle repair [95], among others (Table 3). The clinical success of these therapies depends on a number of factors including both host-related factors and biomaterial-related factors. Host-related factors include age, comorbidities such as diabetes and obesity, and immunocompetence status, among others. The ECM-related factors include the composition and ultrastructure of the biomaterial, degradability, mechanical properties such as elasticity and compliance, and surface topography. The species and tissues from which the ECM material is procured [96], the decellularization and manufacturing process [91], and postprocessing modifications such as cross-linking [97], solubilization [98–101], and terminal sterilization [39, 102] are also important factors in clinical outcome. As a result, the clinical success of an ECM bioscaffold approach varies widely.

### Table 2: Commercially available ECM-derived products for surgical applications.

| Product            | Source tissue       | Manufacturer            | Postprocessing | Form            |
|--------------------|---------------------|-------------------------|----------------|-----------------|
| AlloDerm           | Human skin          | LifeCell                | Natural        | Dry scaffold    |
| Bard Dermal Allograft | Human dermis       | Bard                    | Natural        | Dry scaffold    |
| CuffPatch          | Porcine SIS         | Biomet Sports Medicine  | Cross-linked   | Hydrated scaffold |
| Dura-Guard         | Bovine pericardium  | Synovis Surgical Innovations | Cross-linked   | Hydrated scaffold |
| Durasis            | Porcine SIS         | SIS Cook                | Natural        | Dry scaffold    |
| Durepair           | Bovine fetal skin   | TEI Biosciences         | Natural        | Dry scaffold    |
| FasLata             | Human fascia lata   | CR Bard                 | Natural        | Dry scaffold    |
| GraftJacket        | Human Skin          | Wright Medical Tech     | Natural        | Dry scaffold    |
| MatriStem          | Porcine UBM         | ACell                   | Natural        | Dry scaffold    |
| Oasis              | Porcine SIS         | Cook Biotech/Healthpoint| Natural        | Dry scaffold    |
| Pelvicol           | Porcine dermis      | CR Bard                 | Cross-linked   | Hydrated scaffold |
| Peri-Guard         | Bovine pericardium  | Synovis Surgical Innovations | Cross-linked   | Dry scaffold    |
| Permacol           | Porcine skin        | Tissue Science Laboratories | Cross-linked   | Hydrated scaffold |
| PriMatrix          | Bovine fetal skin   | TEI Biosciences         | Natural        | Dry scaffold    |
| Restore            | Porcine SIS         | DePuy                   | Natural        | Dry scaffold    |
| Strataxis          | Porcine SIS         | Cook Biomedical         | Natural        | Dry scaffold    |
| SurgiMend          | Bovine fetal skin   | TEI Biosciences         | Natural        | Dry scaffold    |
| Surgis             | Porcine SIS         | Cook Biomedical         | Natural        | Dry scaffold    |
| Suspend            | Human fascia lata   | Mentor                  | Natural        | Dry scaffold    |
| TissueMend         | Bovine fetal skin   | TEI Biosciences         | Natural        | Dry scaffold    |
| Vascu-Guard        | Bovine pericardium  | Synovis Surgical Innovations | Cross-linked   | Dry scaffold    |
| VentiGel           | Porcine myocardium  | Ventrix                 | Gelation       | Hydrogel        |
| Veritas            | Bovine pericardium  | Synovis Surgical Innovations | Cross-linked   | Hydrated scaffold |
| Xenform            | Bovine fetal skin   | TEI Biosciences         | Natural        | Dry scaffold    |
| Zimmer Collagen Patch | Porcine dermis    | Tissue Science Laboratories | Cross-linked   | Hydrated scaffold |

Commercially available ECM-derived materials are manufactured from a variety of tissue sources and species and exist in a variety of formats including hydrogels, scaffolds, and cross-linked scaffolds.

### Table 3: Reports on clinical use of ECM-derived products.

| Clinical application | Report                        |
|----------------------|-------------------------------|
| Breast               | Butterfield [92]              |
| Dental               | Gholami et al. [103]          |
| Diabetic ulcers      | Lecheminant and Field [104]   |
| Gastrointestinal     | Badylak et al. [105]          |
| Maxillofacial        | Leventhal and Pribitkin [94]  |
| Skeletal muscle      | Sicari et al. [95]            |
| Urologic             | Alpert et al. [106]           |
| Ventricle hernia     | Kissing and Itani [93]        |
| Vascular             | J. M. Ladowski and I. S. Ladowski [107] |
| Colectrectal         | Cintron et al. [108]          |
| Thoracic             | Scholl et al. [109]           |

ECM-derived products are routinely used in various anatomic locations for tissue repair.

Studies have shown that constructive tissue remodeling, a term that implies the synthesis of site-appropriate, functional tissue, consistently occurs when (1) the materials are appropriately decellularized [89, 90], (2) chemical cross-linking is avoided [39, 97], (3) the materials are free of endotoxin and bacterial contamination [110], (4) the material is placed...
Figure 4: Biomaterial-mediated tissue repair: The biomaterial-host interaction is a complex process composed of multiple stages. Following implantation, the surface of the material is covered with blood and plasma protein through a process known as the Vroman effect. In conjunction with hemostasis, the Vroman effect facilitates the formation of a temporary fibrin-rich matrix that mediates the interaction between native tissues and the implanted construct. This temporary matrix also facilitates cellular access into the material. The innate immune system is activated and a neutrophil accumulation at the periphery of the implant becomes histologically apparent within minutes to hours of implantation. In the following days, this neutrophil accumulation is gradually replaced by a macrophage infiltrate that facilitates scaffold degradation and matricryptic peptide release. The macrophage infiltrate then transitions from an M1 proinflammatory phenotype into an M2 proremodeling phenotype. Signaling molecules produced by the innate immune system and scaffold degradation products act synergistically to recruit stem/progenitor cells from nearby tissues and the bone marrow. Together this heterogeneous cell population known as the constructive cell infiltrate is responsible for further scaffold degradation, neomatrix deposition, and constructive functional tissue remodeling.

in contact with healthy and vascularized surrounding tissue [96], and (5) the implant site is subjected to appropriate physiologic and mechanical loads and stimuli [95, 111].

3.3. Biomaterial-Mediated Tissue Repair. ECM biomaterials have the ability to change the default postnatal wound healing response from inflammation and scar tissue formation to site-appropriate constructive tissue remodeling (Figure 4). The properties that enable ECM-derived biomaterials to facilitate such a dramatic switch are inherent to the composition and structure of the ECM itself. The ECM has a complex structure and composition that are created by the local cell population of every tissue, and hence, it is thought to be an ideal and highly biocompatible substrate for regenerative medicine applications.

The host response to implanted biomaterials begins with activation of the innate immune system. Within minutes to hours, the implanted construct adsorbs blood and plasma proteins to its surface through a phenomenon known as the Vroman effect [112]. In combination with hemostasis and clot formation, the Vroman effect leads to the formation of a temporary fibrin-rich matrix that surrounds the implanted construct. This temporary matrix serves to facilitate cellular access to the material. Within hours of implantation, a neutrophil infiltrate accumulates at the surface interface of the implant. Neutrophils play important roles in the host biomaterial interaction including secretion of proteolytic enzymes and secretion of cytokines and chemokines which initiate and modulate subsequent phases of the host response. The neutrophil response is rapidly replaced by a macrophage infiltrate that becomes dominant as early as 2-3 days after implantation and can last for several months. Macrophages secrete additional proteolytic enzymes that degrade the implanted ECM construct, release bioactive cryptic peptides from the parent ECM molecules, and facilitate the deposition of new host-derived tissues.

Signaling molecules released from biologic scaffolds promote a phenotypic change in the macrophage infiltrate from an M1 proinflammatory phenotype to an M2 immunomodulatory phenotype. Biologic materials that can promote an M2 immunomodulatory macrophage phenotype are consistently associated with downstream constructive remodeling events, whereas biologic materials that promote an M1 proinflammatory phenotype are consistently associated with less favorable clinical outcomes including scar formation, encapsulation, foreign body reaction, and, in some cases, seroma formation.

The rate of scaffold degradation depends on a number of factors including the tissue source from which they are derived, and the manufacturing process. For example, while the dense structure of dermal ECM scaffolds prolongs in vivo degradation, ECM scaffolds derived from less dense tissues such as the small intestinal submucosa or the urinary bladder are rapidly degraded in vivo [113, 114]. Inhibition of ECM scaffold degradation by either chemically crosslinking methods or in vivo macrophage depletion prevents the release or exposure of matricryptic peptides and bioactive molecules
from the parent molecules such as collagen and laminin within the ECM.

Matricryptic peptides are molecular subunits of larger parent molecules that have biologic activity and can influence cell behavior. Many such oligopeptides have been identified and a more extensive description can be found elsewhere [115, 116]. These cryptic peptides can affect cell migration, proliferation, and differentiation, all of which are important processes for wound healing and tissue repair, and which can provide key components for functional tissue reconstruction in plastic and reconstructive surgery. Matricryptic peptides are thought to have evolved as a source of signals for tissue repair following natural ECM degradation following tissue injury.

4. Emerging ECM-Based Technologies

As stated previously, some of the major obstacles encountered in the field of VCA include high immunogenicity of transplanted tissues, patient noncompliance with increasingly complex immunosuppressive regimens, severe side effects from systemic immunosuppression, and suboptimal nerve regeneration. ECM-derived technologies that are currently under development are potential strategies to address these issues.

4.1. ECM-Based Drug Delivery Technologies. A proposed alternative for reducing the amount of oral immunosuppressive agents taken by patients and hence reducing noncompliance and systemic side effects and toxicity is the development of implantable drug eluting biomaterials. However, as previously discussed, most existing drug eluting biomaterials are synthetically derived, and such biomaterials are typically associated with an aggressive inflammatory response and/or foreign body reaction [38, 39]. Seroma, scar tissue formation, and fibrotic encapsulation are all processes that have been associated with implantable synthetic biomaterials. These unfavorable outcomes may not only interfere with release rates and pharmacokinetic profiles of drug eluting materials by creating a physical barrier between the implant and target host tissues, but can also promote a proinflammatory state that can trigger graft rejection.

Injectable hydrogels derived from synthetic polymers with tunable structural, chemical, and mechanical properties have traditionally been investigated for drug delivery applications [23, 117, 118]. Similarly, injectable hydrogels derived from naturally occurring biologic materials with superior biocompatibility and bioactivity compared to their synthetic counterparts are currently under development and their immunomodulatory properties are particularly relevant for VCA applications [98–100, 119–122]. The most common components of biologic hydrogels include type I collagen, hyaluronic acid, and other structural proteins such as laminin [122]. ECM-derived hydrogels provide an environment of naturally occurring molecules that allow for cellular infiltration and neovascularization in ischemic regions [123]. ECM-derived hydrogels exist as solutions or suspensions at room temperature 25°C and self-assemble to a gel state at body temperature 37°C [122, 124]. These favorable properties permit ECM-derived hydrogels to be manufactured and stored in liquid phase for minimally invasive deployment via injection and to self-assemble in situ, once they equilibrate with body temperature at 37°C (Figure 5). Furthermore, ECM hydrogels retain the properties of traditional ECM-derived biomaterials and therefore are completely degradable with no residual collateral tissue sequelae, are immunomodulatory, and can be replaced by site-appropriate, functional tissue.

4.2. ECM-Based Technologies for Cell Delivery. The potential of stem cell transplantation for immunomodulation as adjuvant treatment to immunosuppression has been demonstrated in solid organ transplantation and in preclinical models of VCA [69, 125]. Despite uncontrolled differentiation, low survival, and minimal integration upon implantation, stem cells have been shown to home to sites of injury and inflammation [126, 127], and transplanted tissue [125] and to exert their immunomodulatory effects via paracrine mechanisms [128, 129]. Cell transplantation directly into tissue deficits constitutes a viable alternative for the use of stem cells in VCA. However, the key to cell survival is their ability to integrate with adjacent tissues, and the ideal vehicle for stem cell delivery remains to be determined.

Biomaterials that promote improved cell survival could serve as delivery vehicles for VCA applications. Deployment of stem cells through minimally invasive hydrogel-based technologies offers many advantages such as minimization of iatrogenic injury during implantation, and the presence of a supportive microenvironment to promote cell engraftment.
A number of studies have suggested that utilizing ECM-derived biomaterials in anatomically homologous structures might offer site-specific advantages when compared to biomaterials derived from heterologous structures [145–148]. Consequently, biologic scaffolds composed of central nervous system (CNS) ECM have been developed and their properties have been studied in vitro. Results from these experiments suggest that CNS ECM provides tissue-specific advantages including the ability to stimulate migration of PC12 cells to a greater degree than non-CNS ECMs [149] and the ability to increase neurite length when compared to other non-CNS ECMs [99]. It should be noted, however, that heterologous tissue sources of ECM have also shown robust remodeling effects [93–95, 105].

Biomaterials derived from decellularized neural tissue have also been used clinically to bridge gaps in sensory, motor, and mixed nerves. This technology is limited to the repair of short gaps in peripheral nerves [150] and its application in VCA remains to be investigated. However, in nonneural tissue applications, nerve regeneration has been observed following implantation of ECM-derived scaffolds, and it has been identified as an early predictor of constructive tissue remodeling [140, 151, 152].

Together, these findings suggest that although the process of biomaterial-mediated tissue repair is inherently different from true tissue regeneration, the use ECM-based technologies in VCA can potentially modulate and positively contribute to the process of neural repair.

4.4. Donor Graft Manipulation. The objective of the decellularization process through which ECM-derived biomaterials are manufactured is to thoroughly remove proinflammatory cell-associated antigens while at the same time preserving the ultrastructure and composition of the ECM. Properly decellularized biomaterials can function as cell-guiding templates that contain the adequate three-dimensional architecture and biochemical cues to promote cell infiltration and mediate tissue repair upon implantation [39, 91, 96, 142]. Commercially available options (Table 2) typically exist in single sheet form and are normally used to repair tissue defects and approximate wound edges across large tissue gaps. However, more complex ECM-derived scaffolds such as decellularized myocutaneous flaps and composite tissue allografts are currently being developed. Comminuted forms of ECM are also available [153, 154].

Donor graft manipulation relies heavily upon perfusion decellularization, a technique originally developed for whole organ decellularization [155–159]. Using this technique, a number of composite allografts have been decellularized and characterized including fasciocutaneous flaps [160], tendon-bone composite grafts [161], and musculofascial grafts [162], among others.

In a recent study by Jank et al. [163], a bioartificial graft composed of allogeneic ECM and seeded cells was produced via whole limb decellularization. The authors report preservation of the passive musculoskeletal apparatus, nerve sheets, and an intact vasculature through which subsequent cell seeding can be performed. The authors also note that unlike solid organ transplants, anatomic structures transplanted in
VCA are not fully functional at the time of transplantation due to a lack of innervation. Functionalization of the resulting ECM construct requires that nerves must first regrow into the transplanted grafts and in the case of bioartificial grafts preserved nerve sheets may act as guiding templates for penetrating axons as they reinnervate sensory and motor organs within the skin [164]. When properly decellularized, ECM-derived materials have inherent immunomodulatory properties that promote constructive tissue remodeling. Bioartificial composite tissue grafts derived from decellularized tissues may be transplanted with or without a cellular component at an early maturation stage and allowed to regenerate and mature in vivo but vascularization, reinnervation and functionalization remain the key obstacles.

4.5. Current Challenges Associated with ECM-Based Applications for VCA.

A number of important obstacles remain before ECM-based technologies are successfully implemented as adjuvant therapy for VCA and become widespread in the clinical setting. Despite promising results obtained through decades of research in the fields of tissue engineering and regenerative medicine, the potential applications of these technologies in VCA are still hypothetical.

ECM-derived hydrogels have been manufactured from multiple tissue sources and their properties have been extensively characterized [98, 99, 119, 120, 165]. However, studies describing ECM-derived hydrogels as minimally invasive drug delivery vehicles are scarce [123]. Although the advantage of minimally invasive implantation of ECM hydrogels is evident, the ability and degree to which these technologies can carry and deliver pharmacological and cellular agents is less clear. Therefore, progress on the use of ECM-derived hydrogels for drug delivery applications will depend upon a number of milestones, including a description of the pharmacokinetic profiles of these materials, including their ability to carry and release specific pharmacological agents (i.e., hydrophilic, hydrophobic, and small and large molecules), and the effect of the degradation process and tissue integration upon these properties [113]. A phase I clinical study with porcine cardiac ECM hydrogel for treatment of damaged myocardium is currently in progress (Ventrix, Inc. Clinical trial identifier: NCT02305602).

In addition, the immunomodulatory properties of ECM-derived biomaterials have been well described only in the setting of tissue engineering and regenerative medicine. In these areas, ECM-derived biomaterials have been shown to promote a microenvironment that is conducive to constructive tissue remodeling via a complex process involving matricryptic peptide release and modulation of the innate immune system. However, the extent to which ECM-derived biomaterials can modulate the immune system and contribute to the promotion of a favorable microenvironment to prevent allograft rejection remains to be determined [96, 166, 167].

Lastly, the optimal anatomic placement of these materials to facilitate allograft survival has not yet been established. Localized drug delivery applications might merely require the material containing the pharmacologic agent to be placed near the periphery of the transplanted graft [15]. The pharmacologic effects of the therapy would thus be localized to the anatomic vicinity of the allograft and systemic exposure would be avoided. Interestingly, studies suggest that localization to regional lymphatic organs, particularly in the case of stem cell delivery, might be an efficacious strategy [69, 168]. In contrast, ECM-derived biomaterials intended for VCA applications unrelated to drug delivery, as in the case of promotion of constructive tissue remodeling and modulation of the immune system via intrinsic properties of the ECM, might need to be implanted at the interphase between the native tissue and the transplanted graft or other locations, depending on the specific application.

5. Discussion

Progress in field of regenerative medicine has resulted in emerging implications for VCA. The overlap between these two fields is still nascent and early independent results show great promise in drug delivery, stem cell delivery, tissue regeneration, immunomodulation, and combination of these approaches. The ECM is secreted by the cells of every tissue in an organ and, as such, constitutes the ideal substrate to promote cell proliferation and growth. Furthermore, the immunomodulatory properties of ECM-derived biomaterials that enable them to promote constructive tissue remodeling present clear advantages over synthetically derived biomaterials in VCA applications. However, unlike synthetically derived biomaterials, ECM-derived materials are subject to natural variability depending on the tissue source and species from which they are derived, their methods of manufacture, and their biomechanical properties (i.e., degradation rate, fiber diameter, pore size) are not as controllable and reliably replicated.

It is evident, however, that controlling the microenvironment within each transplant and adjacent regions will be key to successful engraftment and regeneration. These niche conditions include critical variables such as oxygen concentration, cytokine gradients, pH, nutrients availability, microarchitecture, and based composition, all of which are in a state of dynamic equilibrium in temporal and spatial patterns with the host and graft tissues [167].

Despite recent progress in VCA, clinical failures (many remain unpublished and unpublicized) have resulted from existing limitations including but not limited to adverse effects of chronic high dose immunosuppression regimens, chronic rejection or uncontrolled acute rejection due to medication nonadherence (which may be underreported in VCA) or other etiologies, and, importantly, suboptimal nerve regeneration with limited functional outcomes in many patients. A number of strategies are currently under development including novel minimally invasive drug delivery systems, tolerance-inducing protocols, and methods to mitigate ischemic reperfusion injury or reduce the antigenic load of transplanted grafts. ECM-derived biomaterials offer unique opportunities to define and control the microenvironment, promote nerve regeneration, and provide a stable substrate for minimally invasive drug and stem cell delivery systems. Despite these advantages, a single technology is unlikely to be effective in all situations. Instead, each tissue and each
pathologic condition will likely require a different strategy to obtain optimal results. The viability and sustainability of VCA as a field will rely heavily on strategies that reliably and reproducibly accomplish not only graft survival but also functional outcomes with minimal need or no reliance on systemic immunosuppression with its specter of long term risks.

Conflict of Interests
The authors declare no conflict of interests regarding the publication of this paper.

References

[1] F. Bodin, C. A. Dissaux, T. Dupret-Bories, T. Scholn, C. Fiquet, and C. Bruant-Rodier, “The transverse musculo-cutaneous gracilis flap for breast reconstruction: how to avoid complications,” Microsurgery, 2015.

[2] E. F. Duraes, G. Schwarz, P. Durand et al., “Complications following abdominal-based free flap breast reconstruction: is a 30 days complication rate representative?” Aesthetic Plastic Surgery, vol. 39, no. 5, pp. 694–699, 2015.

[3] J. W. Swanson, J. L. Johnston, B. T. Mitchell, K. Alcorn, and J. A. Taylor, “Perioperative complications in posterior pharyngeal flap surgery: review of the national surgical quality improvement program pediatric (NSqip-Peds) database,” The Cleft Palate-Craniofacial Journal, 2015.

[4] J. T. Shores, G. Brandacher, and W. P. A. Lee, “Hand and upper extremity transplantation: an update of outcomes in the worldwide experience,” Plastic and Reconstructive Surgery, vol. 135, no. 2, pp. 351e–360e, 2015.

[5] H. J. Klein, U. Schanz, M. Hivelin et al., “Sensitization and desensitization of burn patients as potential candidates for vascularized composite allotransplantation,” Burns, 2015.

[6] P. Petruzzo, M. Lanzetta, J.-M. Dubernard et al., “The international registry on hand and composite tissue transplantation,” Transplantation, vol. 90, no. 12, pp. 1590–1594, 2010.

[7] M. Siemionow, B. B. Gharb, and A. Rampazzo, “Successes and lessons learned after more than a decade of upper extremity and face transplantation,” Current Opinion in Organ Transplantation, vol. 18, no. 6, pp. 633–639, 2013.

[8] S. Fischer, C. G. Lian, M. Kueckelhaus et al., “Acute rejection in vascularized composite allotransplantation,” Current Opinion in Organ Transplantation, vol. 19, no. 6, pp. 531–544, 2014.

[9] D. A. Leonard, J. M. Kurtz, and C. L. Cetrulo, “Achieving immune tolerance in hand and face transplantation: a realistic prospect?” Immunotherapy, vol. 6, no. 5, pp. 499–502, 2014.

[10] S. Schneeberger, S. Luchina, M. Lanzetta et al., “Cytomegalo-virus-related complications in human hand transplantation,” Transplantation, vol. 80, no. 4, pp. 441–447, 2005.

[11] B. L. Cottrell, G. Perez-Abadia, S. M. Onifer et al., “Neuroregeneration in composite tissue allografts: effect of low-dose FK506 and mycophenolate mofetil immunotherapy,” Plastic and Reconstructive Surgery, vol. 118, no. 3, pp. 615–625, 2006.

[12] V. S. Gorantla and A. J. Demetris, “Acute and chronic rejection in upper extremity transplantation: what have we learned?” Hand Clinics, vol. 27, no. 4, pp. 481–493, 2011.

[13] B. Pmahac, R. M. Gobble, and S. Schneeberger, “Facial and hand allotransplantation,” Cold Spring Harbor Perspectives in Medicine, vol. 4, no. 3, Article ID a015651, 2014.

[14] R. Starzl, G. Brandacher, W. P. A. Lee et al., “Review of the early diagnoses and assessment of rejection in vascularized composite allotransplantation,” Clinical and Developmental Immunology, vol. 2013, Article ID 402980, 9 pages, 2013.

[15] J. T. Schnider, M. Weinstock, J. A. Plock et al., “Site-specific immunosuppression in vascularized composite allotransplantation: prospects and potential,” Clinical and Developmental Immunology, vol. 2013, Article ID 495212, 7 pages, 2013.

[16] J. Folkman and D. M. Long, “The use of silicone rubber as a carrier for prolonged drug therapy,” Journal of Surgical Research, vol. 4, no. 3, pp. 139–142, 1964.

[17] J. Folkman, D. M. Long Jr., and R. Rosenbaum, “Silicone rubber: a new diffusion property useful for general anesthesia,” Science, vol. 154, no. 3745, pp. 148–149, 1966.

[18] R. K. Aggarwal, D. C. Ireland, M. A. Azrin, M. D. Ezekowitz, D. P. de Bono, and A. H. Gershlick, “Antithrombotic potential of polymer-coated stents eluting platelet glycoprotein IIb/IIIa receptor antibody,” Circulation, vol. 94, no. 12, pp. 3311–3317, 1996.

[19] Y. Ozaki, A. G. Violaris, and P. W. Serruys, “New stent technologies,” Progress in Cardiovascular Diseases, vol. 39, no. 2, pp. 129–140, 1996.

[20] Y. Fu and Z. Zhuang, “Long-term effects of levonorgestrel-releasing intrauterine system on tamoxifen-treated breast cancer patients: a meta-analysis,” International Journal of Clinical and Experimental Pathology, vol. 7, no. 10, pp. 6419–6429, 2014.

[21] P. A. Royer and K. P. Jones, “Progestins for contraception: modern delivery systems and novel formulations,” Clinical Obstetrics and Gynecology, vol. 57, no. 4, pp. 644–658, 2014.

[22] T. Yasukawa and Y. Ogura, “Medical devices for the treatment of eye diseases,” Handbook of Experimental Pharmacology, vol. 197, pp. 469–489, 2010.

[23] M. Bikram, A. M. Gobin, R. E. Whitmire, and J. L. West, “Temperature-sensitive hydrogels with SiO2–Au nanoshells for controlled drug delivery,” Journal of Controlled Release, vol. 123, no. 3, pp. 219–227, 2007.

[24] H. J. Moon, D. Y. Ko, M. H. Park, M. K. Joo, and B. Jeong, “Temperature-responsive compounds as in situ gelling biomedical materials,” Chemical Society Reviews, vol. 41, no. 14, pp. 4860–4883, 2012.

[25] M. R. Matanović, I. Grabnar, M. Gosenca, and P. A. Grabnar, “Prolonged subcutaneous delivery of low molecular weight heparin based on thermoresponsive hydrogels with chitosan nanocomplexes: design, in vitro evaluation, and cytotoxicity studies,” International Journal of Pharmaceutics, vol. 488, no. 1-2, pp. 127–135, 2015.

[26] X. Sun, H. Gao, G. Wu, Y. Wang, Y. Fan, and J. Ma, “Biodegradable and temperature-responsive polyurethanes for adriamycin delivery,” International Journal of Pharmaceutics, vol. 412, no. 1-2, pp. 52–58, 2011.

[27] S. Y. Wang, M. C. Liu, and K. A. Kang, “Magnetic nanoparticles and thermally responsive polymer for targeted hyperthermia and sustained anti-cancer drug delivery,” in Oxygen Transport to Tissue XXXIV, vol. 765 of Advances in Experimental Medicine and Biology, pp. 315–321, Springer, New York, NY, USA, 2013.

[28] L. Fu, C. Sun, and L. Yan, “Galactose targeted pH-responsive copolymer conjugated with near infrared fluorescence probe for imaging of intelligent drug delivery,” ACS Applied Materials and Interfaces, vol. 7, no. 3, pp. 2014–2115, 2014.
[29] S. Kumar, R. Acharya, U. Chatterji, and P. De, "Side-chain amino-acid-based pH-responsive self-assembled block copolymers for drug delivery and gene transfer," Langmuir, vol. 29, no. 49, pp. 15375–15385, 2013.

[30] B. Rossi, V. Venuti, F. D’Amico et al., “Toward an understanding of the thermosensitive behaviour of pH-responsive hydrogels based on cycloexetrins,” Soft Matter, vol. 11, no. 29, pp. 5862–5871, 2015.

[31] S. Ruan, X. Cao, X. Cun et al., “Matrix metalloproteinase-sensitive size-shrinkable nanoparticles for deep tumor penetration and pH triggered doxorubicin release,” Biomaterials, vol. 60, pp. 100–110, 2015.

[32] A. Wang, H. Gao, Y. Sun et al., "Temperature- and pH-responsive nanoparticles of biocompatible polyurethanes for doxorubicin delivery," International Journal of Pharmaceutics, vol. 441, no. 1-2, pp. 30–39, 2013.

[33] A. Kawamura, Y. Hata, T. Miyata, and T. Uragami, “Synthesis of glucose-responsive bioconjugated gel particles using surfactant-free emulsion polymerization,” Colloids and Surfaces B: Biointerfaces, vol. 99, pp. 74–81, 2012.

[34] L. Ren, L. He, T. Sun et al., “Dual-responsive supramolecular hydrogels from water-soluble PEG-grafted copolymers and cycloexetrin,” Macromolecular Bioscience, vol. 9, no. 9, pp. 902–910, 2009.

[35] Y. Qiu and K. Park, "Environment-sensitive hydrogels for drug delivery," Advanced Drug Delivery Reviews, vol. 53, no. 3, pp. 321–339, 2001.

[36] B. Purcell, D. Lobb, M. B. Charati et al., “Injectable and bioreponsive hydrogels for tissue engineering,” Nature Materials, vol. 13, no. 6, pp. 653–661, 2014.

[37] A. Flenning, "Drug delivery: non-invasive drug depot refill," Nature Reviews Drug Discovery, vol. 13, no. 11, pp. 810–811, 2014.

[38] J. M. Anderson, A. Rodriguez, and D. T. Chang, "Foreign body reaction to biomaterials," Seminars in Immunology, vol. 20, no. 2, pp. 86–100, 2008.

[39] R. Londono and S. E. Badyak, "Biologic scaffolds for regenerative medicine: mechanisms of in vivo remodeling," Annals of Biomedical Engineering, vol. 43, no. 3, pp. 577–592, 2015.

[40] Z. Abdullah, T. Saric, H. Kashkar et al., "Serpin-6 expression protects embryonic stem cells from lysis by antigen-specific CTL," The Journal of Immunology, vol. 178, no. 6, pp. 3390–3399, 2007.

[41] S. Bonde, K.-M. Chan, and N. Zavazava, "ES-cell derived hematopoietic cells induce transplantation tolerance," PLoS ONE, vol. 3, no. 9, Article ID e3212, 2008.

[42] S. Bonde and N. Zavazava, "Immunogenicity and engraftment of mouse embryonic stem cells in allogeneic recipients," Stem Cells, vol. 24, no. 10, pp. 2192–2201, 2006.

[43] A. S. Boyd and K. J. Wood, "Variation in MHC expression between undifferentiated mouse ES cells and ES cell-derived insulin-producing cell clusters," Transplantation, vol. 87, no. 9, pp. 1300–1304, 2009.

[44] L. Li, M. L. Baroja, A. Majumdar et al., "Human embryonic stem cells possess immune-privileged properties," Stem Cells, vol. 22, no. 4, pp. 448–456, 2004.

[45] L. Tian, J. W. Catt, C. O’Neill, and N. J. C. King, "Expression of immunoglobulin superfamily cell adhesion molecules on murine embryonic stem cells," Biology of Reproduction, vol. 57, no. 3, pp. 561–568, 1997.

[46] H.-F. Chen, C.-Y. Yu, M.-J. Chen et al., "Characteristic expression of major histocompatibility complex and immune privilege genes in human pluripotent stem cells and their derivatives," Cell Transplantation, vol. 24, no. 5, pp. 845–864, 2015.

[47] S. Kaderreit and A. Trounson, "In vitro immunogenicity of undifferentiated pluripotent stem cells (PSC) and derived lineages," Seminars in Immunopathology, vol. 33, no. 6, pp. 551–562, 2011.

[48] J. I. Pearl, L. S. Keen, M. M. Davis, and J. C. Wu, "Pluripotent stem cells: immune to the immune system?" Science Translational Medicine, vol. 4, no. 164, Article ID 164ps25, 2012.

[49] B. Suárez-Álvarez, R. M. Rodríguez, V. Calvanese et al., "Epigenetic mechanisms regulate MHC and antigen processing molecules in human embryonic and induced pluripotent stem cells," PLoS ONE, vol. 5, no. 4, Article ID e1092, 2010.

[50] F. Fändrich, X. Lin, G. X. Chai et al., "Preimplantation-stage stem cells induce long-term allogeneic graft acceptance without supplementary host conditioning," Nature Medicine, vol. 8, no. 2, pp. 171–178, 2002.

[51] C. A. Koch, P. Geraldes, and J. L. Platt, "Immunosuppression by embryonic stem cells," Stem Cells, vol. 26, no. 1, pp. 89–98, 2008.

[52] L. V. Schnabel, C. M. Abratte, J. C. Schimenti et al., "Induced pluripotent stem cells have similar immunogenic and more potent immunomodulatory properties compared with bone marrow-derived stromal cells in vitro," Regenerative Medicine, vol. 9, no. 5, pp. 621–635, 2014.

[53] M. Drukker, H. Katchman, G. Katz et al., "Human embryonic stem cells and their differentiated derivatives are less susceptible to immune rejection than adult cells," Stem Cells, vol. 24, no. 2, pp. 221–229, 2006.

[54] N. Yachimovich-Cohen, S. Even-Ram, Y. Shufaro, J. Rachmilewitz, and B. Reubinoff, "Human embryonic stem cells suppress T cell responses via arginase I-dependent mechanism," The Journal of Immunology, vol. 184, no. 3, pp. 1300–1308, 2010.

[55] B. Imberti, F. Casiraghi, D. Cugini et al., "Embryonic stem cells, derived either after in vitro fertilization or nuclear transfer, prolong survival of semiallogeneic heart transplants," Journal of Immunology, vol. 186, no. 7, pp. 4164–4174, 2011.

[56] M. Drukker, "Immunogenicity of embryonic stem cells and their progeny," Methods in Enzymology, vol. 420, pp. 391–409, 2006.

[57] C. Ménard, A. A. Hagège, O. Agbulut et al., "Transplantation of cardiac-committed mouse embryonic stem cells to infarcted sheep myocardium: a preclinical study," The Lancet, vol. 366, no. 9490, pp. 1005–1012, 2005.

[58] T. Kofidis, J. L. deBruin, M. Tanaka et al., "They are not stealthy in the heart: embryonic stem cells trigger cell infiltration, humoral and T-lymophocyte-based host immune response," European Journal of Cardio-Thoracic Surgery, vol. 28, no. 3, pp. 461–466, 2005.

[59] J. Nussbaum, E. Minami, M. A. Lafamme et al., "Transplantation of undifferentiated murine embryonic stem cells in the heart: teratoma formation and immune response," The FASEB Journal, vol. 21, no. 7, pp. 1345–1357, 2007.

[60] R.-J. Swijnenburg, M. Tanaka, H. Vogel et al., "Embryonic stem cell immunogenicity increases upon differentiation after transplantation into ischemic myocardium," Circulation, vol. 112, no. 9, supplement, pp. I166–I172, 2005.

[61] B. Imberti, M. Monti, and F. Casiraghi, "Pluripotent stem cells: immune to the immune system?" Annual Review of Biomedical Engineering, vol. 12, pp. 87–117, 2010.
[63] H. Yagi, A. Soto-Gutierrez, B. Parekkadan et al., “Mesenchymal stem cells: mechanisms of immunomodulation and homing,” *Cell Transplantation, vol. 19*, no. 6–7, pp. 667–679, 2010.

[64] L. Fouillard, S. Francois, S. Bouchet, M. Bensidhoum, A. Elmeslmi, and A. Chapel, “Innovative cell therapy in the treatment of serious adverse events related to both chemoradiotherapy protocol and acute myeloid leukemia syndrome: the infusion of mesenchymal stem cells post-treatment reduces hematopoietic toxicity and promotes hematopoietic reconstitution,” *Current Pharmaceutical Biotechnology*, vol. 14, no. 9, pp. 842–848, 2013.

[65] H. M. Lazarus, O. N. Koc, S. M. Devine et al., “Cotransplantation of HLA-identical sibling culture-expanded mesenchymal stem cells and hematopoietic stem cells in hematologic malignancy patients,” *Biology of Blood and Marrow Transplantation, vol. 11*, no. 5, pp. 389–398, 2005.

[66] F. Casiraghi, N. Perico, and G. Remuzzi, “Mesenchymal stromal cells to promote solid organ transplantation tolerance,” *Current Opinion in Organ Transplantation, vol. 18*, no. 1, pp. 51–58, 2013.

[67] N. Perico, F. Casiraghi, M. Introna et al., “Autologous mesenchymal stromal cells and kidney transplantation: a pilot study of safety and clinical feasibility,” *Clinical Journal of the American Society of Nephrology, vol. 6*, no. 2, pp. 412–422, 2011.

[68] J. Y. Weng, X. Du, S. X. Geng et al., “Mesenchymal stem cell as salvage treatment for refractory chronic GVHD,” *Bone Marrow Transplantation, vol. 45*, no. 12, pp. 1732–1740, 2010.

[69] J. A. Plock, J. T. Schneider, W. Zhang et al., “Adipo- and bone marrow-derived mesenchymal stem cells prolong graft survival in vascularized composite allotransplantation,” *Transplantation, vol. 99*, no. 9, pp. 1765–1773, 2015.

[70] C. Ballet, G. Rousssey-Kesler, J.-T. Aubin et al., “Humoral and cellular responses to influenza vaccination in human recipients naturally tolerant to a kidney allograft,” *American Journal of Transplantation, vol. 6*, no. 11, pp. 2796–2801, 2006.

[71] G. W. McCaughan, D. G. Bowen, and P. Bertolino, “Operational tolerance in liver transplantation: shall we predict or promote?” *Liver Transplantation, vol. 19*, no. 9, pp. 933–936, 2013.

[72] T. Kawai, D. H. Sachs, M. Sykes, and A. B. Cosimi, “HLA-mismatched renal transplantation without maintenance immunosuppression,” *The New England Journal of Medicine, vol. 369*, no. 19, pp. 1850–1852, 2013.

[73] J. Leventhal, M. Abecassis, J. Miller et al., “Chimerism and tolerance without GVHD or engraftment syndrome in HLA-mismatched combined kidney and hematopoietic stem cell transplantation,” *Science Translational Medicine, vol. 4*, no. 124, Article ID 42ar28, 2012.

[74] J. D. Scandling, S. Busque, S. Dejbakhsh-Jones et al., “Tolerance and chimerism after renal and hematopoietic-cell transplantation,” *The New England Journal of Medicine, vol. 358*, no. 4, pp. 362–368, 2008.

[75] D. A. Leonard, C. L. Cetrulo, D. A. McGrouther, and D. H. Sachs, “Induction of tolerance of vascularized composite allografts,” *Transplantation, vol. 95*, no. 3, pp. 403–409, 2013.

[76] D. A. Leonard, J. M. Kurtz, C. Mallard et al., “Vascularized composite allograft tolerance across MHC barriers in a large animal model,” *American Journal of Transplantation, vol. 14*, no. 2, pp. 343–355, 2014.

[77] B. N. Brown and S. F. Badyal, “Extracellular matrix as an inductive scaffold for functional tissue reconstruction,” *Translational Research, vol. 163*, no. 4, pp. 268–285, 2014.

[78] M. van der Rest and R. Garrone, “Collagen family of proteins,” *The FASEB Journal, vol. 5*, no. 13, pp. 2814–2823, 1991.

[79] M. Sundaramoorthy, M. Meiyappan, P. Todd, and B. G. Hudson, “Crystal structure of NCI domains: structural basis for type IV collagen assembly in basement membranes,” *Journal of Biological Chemistry, vol. 277*, no. 34, pp. 31142–31153, 2002.

[80] H. L. Birch, C. T. Thorpe, and A. P. Rumian, “Specialisation of extracellular matrix for function in tendons and ligaments,” *Muscles, Ligaments and Tendons Journal, vol. 3*, no. 1, pp. 12–22, 2013.

[81] M. L. Ponce, M. Nomizu, M. C. Delgado et al., “Identification of endothelial cell binding sites on the laminin γ1 chain,” *Circulation Research, vol. 84*, no. 6, pp. 688–694, 1999.

[82] R. V. Iozzo, I. R. Cohen, S. Grassel, and A. D. Murdoch, “The biology of perlecan: the multifaceted heparan sulphate proteoglycan of basement membranes and pericellular matrices,” *Biochemical Journal, vol. 302*, part 3, pp. 625–639, 1994.

[83] R. V. Iozzo and A. D. Murdoch, “Proteoglycans of the extracellular environment: clues from the gene and protein side offer novel perspectives in molecular diversity and function,” *The FASEB Journal, vol. 10*, no. 5, pp. 598–614, 1996.

[84] A. Kanematsu, A. Marui, S. Yamamoto et al., “Type I collagen can function as a reservoir of basic fibroblast growth factor,” *Journal of Controlled Release, vol. 99*, no. 2, pp. 281–292, 2004.

[85] J. Li, Y.-P. Zhang, and R. S. Kirsner, “Angiogenesis in wound repair: angiogenic growth factors and the extracellular matrix,” *Microscopy Research and Technique, vol. 60*, no. 1, pp. 107–114, 2003.

[86] I. Matsuo and C. Kimura-Yoshida, “Extracellular distribution of diffusible growth factors controlled by heparan sulfate proteoglycans during mammalian embryogenesis,” *Philosophical Transactions of the Royal Society B: Biological Sciences, vol. 369*, no. 1657, 2014.

[87] D. J. Tschumperlin, “Matrix, mesenchyme, and mechanotransduction,” *Annals of the American Thoracic Society, vol. 12*, supplement 1, pp. S24–S29, 2015.

[88] M. J. Bissell and J. A. Abdelmohsen, “Dynamic reciprocity: how do extracellular matrix and hormones direct gene expression?” *Progress in Clinical and Biological Research, vol. 249*, pp. 251–262, 1987.

[89] B. N. Brown, J. E. Valentin, A. M. Stewart-Akers, G. P. McCabe, and S. F. Badyal, “Macrophage phenotype and remodeling outcomes in response to biologic scaffolds with and without a cellular component,” *Biomaterials, vol. 30*, no. 8, pp. 1482–1491, 2009.

[90] T. J. Keane, R. Londono, N. J. Turner, and S. F. Badyal, “Consequences of ineffective decellularization of biologic scaffolds on the host response,” *Biomaterials, vol. 33*, no. 6, pp. 1771–1781, 2012.

[91] P. M. Crapo, T. W. Gilbert, and S. F. Badyal, “An overview of tissue and whole organ decellularization processes,” *Biomaterials, vol. 32*, no. 12, pp. 3233–3243, 2011.

[92] J. L. Butterfield, “440 consecutive immediate, implant-based, on-the-host response,” *Biomaterials, vol. 33*, no. 6, pp. 1771–1781, 2012.

[93] N. A. Kissane and K. M. Itani, “A decade of ventral incisional hernia repairs with biologic acellular dermal matrix: what have we learned?” *Plastic and Reconstructive Surgery, vol. 130*, no. 5, supplement 2, pp. 194S–202S, 2012.
[94] D. D. Leventhal and E. A. Pribitkin, “Static facial suspension with surgisis ES (enhanced strength) sling,” Laryngoscope, vol. 118, no. 1, pp. 20–23, 2008.

[95] B. M. Sicari, J. Peter Rubin, C. L. Deartth et al., “An acellular biologic scaffold promotes skeletal muscle formation in mice and humans with volumetric muscle loss,” Science Translational Medicine, vol. 6, no. 234, Article ID 234ra58, 2014.

[96] S. F. Badylak, “Decellularized allogeneic and xenogeneic tissue as a bioscaffold for regenerative medicine: factors that influence the host response,” Annals of Biomedical Engineering, vol. 42, no. 7, pp. 1517–1527, 2014.

[97] J. E. Valentin, A. M. Stewart-Akers, T. W. Gilbert, and S. F. Badylak, “Macrophage participation in the degradation and remodeling of extracellular matrix scaffolds,” Tissue Engineering—Part A, vol. 15, no. 7, pp. 1687–1694, 2009.

[98] T. D. Johnson, R. L. Braden, and K. L. Christians, “Injectable ECM scaffolds for cardiac repair,” Methods in Molecular Biology, vol. 1181, pp. 109–120, 2014.

[99] C. J. Medberry, P. M. Crapo, B. F. Siu et al., “Hydrogels derived from central nervous system extracellular matrix,” Biomaterials, vol. 34, no. 4, pp. 1033–1040, 2013.

[100] M. J. Sawkins, W. Bowen, P. Dhadda et al., “Hydrogels derived from demineralized and decellularized bone extracellular matrix,” Acta Biomaterialia, vol. 9, no. 8, pp. 7865–7873, 2013.

[101] J. L. Ungerleider, T. D. Johnson, N. Rao, and K. L. Christians, “Fabrication and characterization of injectable hydrogels derived from decellularized skeletal and cardiac muscle,” Methods, vol. 84, pp. 53–59, 2015.

[102] D. O. Freytes, R. M. Stoner, and S. F. Badylak, “Uniaxial and biaxial properties of terminally sterilized porcine urinary bladder matrix scaffolds,” Journal of Biomedical Materials Research Part B: Applied Biomaterials, vol. 84, no. 2, pp. 408–414, 2008.

[103] G. A. Gholami, A. Saberi, M. Kadkhodazadeh, R. Amid, and D. Karami, “Comparison of the clinical outcomes of connective tissue and acellular dermal matrix in combination with double papillary flap for root coverage: a 6-month trial,” Dental Research Journal, vol. 10, no. 4, pp. 506–513, 2013.

[104] J. L. Lecheminant and C. Field, “Porcine urinary bladder matrix: a retrospective study and establishment of protocol,” Journal of Wound Care, vol. 21, no. 10, pp. 476–482, 2012.

[105] S. F. Badylak, T. Hoppo, A. Nieponice, T. W. Gilbert, J. M. Davison, and B. A. Jobe, “Esophageal preservation in five male patients after endoscopic inner-layer circumferential resection in the setting of superficial cancer: a regenerative medicine approach with a biologic scaffold,” Tissue Engineering Part A, vol. 17, no. 11-12, pp. 1643–1650, 2011.

[106] S. A. Alpert, E. Y. Cheng, W. E. Kaplan, W. T. Snodgrass, D. T. Wilcox, and B. P. Kropp, “Bladder neck fistula after the complete primary repair of extrophy: a multi-institutional experience,” Journal of Urology, vol. 174, no. 4, part 2, pp. 1687–1689, 2005.

[107] J. M. Ładoś and J. S. Ładoś, “Retrospective analysis of bovine pericardium (Vascu-Guard) for patch closure in carotid endarterectomies,” Annals of Vascular Surgery, vol. 25, no. 5, pp. 646–650, 2011.

[108] J. R. Cintron, H. Abcarian, V. Chaudhry et al., “Treatment of fistula-in-ano using a porcine small intestinal submucosa anal fistula plug,” Techniques in Coloproctology, vol. 17, no. 2, pp. 187–191, 2013.

[109] F. G. Scholl, M. M. Boucek, K. C. Chan, L. Valdes-Cruz, and R. Perryman, “Preliminary experience with cardiac reconstruction using decellularized porcine extracellular matrix scaffold: human applications in congenital heart disease,” World Journal for Pediatric and Congenital Heart Surgery, vol. 1, no. 1, pp. 132–136, 2010.

[110] K. A. Daly, S. Liu, V. Agrawal et al., “The host response to endotoxin-contaminated dermal matrix,” Tissue Engineering Part A, vol. 18, no. 11-12, pp. 1293–1303, 2012.

[111] F. Ambrosio, S. L. Wolf, A. Delitto et al., “The emerging relationship between regenerative medicine and physical therapeutics,” Physical Therapy, vol. 90, no. 12, pp. 1807–1814, 2010.

[112] S. M. Slack, J. L. Bohnert, and T. A. Horbett, “The effects of surface chemistry and coagulation factors on fibrinogen adsorption from plasma,” Annals of the New York Academy of Sciences, vol. 516, pp. 223–234, 1987.

[113] E. C. Carey, C. L. Deartth, S. A. Johnson et al., “In vivo degradation of 14C-labeled porcine dermis biologic scaffold,” Biomaterials, vol. 35, no. 29, pp. 8297–8304, 2014.

[114] T. W. Gilbert, A. M. Stewart-Akers, A. Simmons-Byrd, and S. F. Badylak, “Degradation and remodeling of small intestinal submucosa in canine Achilles tendon repair,” The Journal of Bone & Joint Surgery—American Volume, vol. 89, no. 3, pp. 621–630, 2007.

[115] G. E. Davis, K. J. Bayless, M. J. Davis, and G. A. Meining, “Regulation of tissue injury responses by the exposure of matricryptic sites within extracellular matrix molecules,” The American Journal of Pathology, vol. 156, no. 5, pp. 1489–1498, 2000.

[116] S. Ricard-Blum and L. Ballut, “Matricryptins derived from collagens and proteoglycans,” Frontiers in Bioscience, vol. 16, no. 2, pp. 674–697, 2011.

[117] S. Collaud, T. Warloe, O. Jordan, R. Gurny, and N. Lange, “Clinical evaluation of bioadhesive hydrogels for topical delivery of heylamineoluminale to Barrett’s esophagus,” Journal of Controlled Release, vol. 123, no. 3, pp. 203–210, 2007.

[118] J. K. Kim, H. J. Kim, J.-Y. Chung, J.-H. Lee, S.-B. Young, and Y.-H. Kim, “Natural and synthetic biomaterials for controlled drug delivery,” Archives of Pharmacal Research, vol. 37, no. 1, pp. 60–68, 2014.

[119] J. A. DeQuach, J. E. Lin, C. Cam et al., “Injectable skeletal muscle matrix hydrogel promotes neovascularization and muscle cell infiltration in a hindlimb ischemia model,” European Cells and Materials, vol. 23, pp. 400–412, 2012.

[120] D. M. Faulk, R. Londono, M. T. Wolf et al., “ECM hydrogel coating mitigates the chronic inflammatory response to polypropylene mesh,” Biomaterials, vol. 35, no. 30, pp. 8585–8595, 2014.

[121] T. D. Johnson and K. L. Christians, “Injectable hydrogel therapies and their delivery strategies for treating myocardial infarction,” Expert Opinion on Drug Delivery, vol. 10, no. 1, pp. 59–73, 2013.

[122] M. T. Wolf, K. A. Daly, E. P. Brennan-Pierce et al., “A hydrogel derived from decellularized dermal extracellular matrix,” Biomaterials, vol. 33, no. 29, pp. 7028–7038, 2012.

[123] S. B. Seif-Naraghi, D. Horn, P. J. Schup-Magogin, and K. L. Christians, “Injectable extracellular matrix derived hydrogel provides a platform for enhanced retention and delivery of a heparin-binding growth factor,” Acta Biomaterialia, vol. 8, no. 10, pp. 3695–3703, 2012.

[124] D. O. Freytes, J. Martin, S. S. Velankar, A. S. Lee, and S. F. Badylak, “Preparation and rheological characterization of a gel form of the porcine urinary bladder matrix,” Biomaterials, vol. 29, no. 11, pp. 1630–1637, 2008.
125] Y.-R. Kuo, S. Goto, H.-S. Shih et al., “Mesenchymal stem cells prolong composite tissue allotransplant survival in a swine model,” *Transplantation*, vol. 87, no. 12, pp. 1769–1777, 2009.

126] J. A. Plock, J. T. Schneider, R. Schweizer, and V. S. Gorantla, “Are cultured mesenchymal stromal cells an option for immunomodulation in transplantation?” *Frontiers in Immunology*, vol. 4, article 41, 2013.

127] S. Schlosser, C. Dennler, R. Schweizer et al., “Paracrine effects of mesenchymal stem cells enhance vascular regeneration in ischemic murine skin,” *Microvascular Research*, vol. 83, no. 3, pp. 267–275, 2012.

128] R. H. Lee, A. A. Pulin, M. J. Seo et al., “Intravenous hMSCs improve myocardial infarction in mice because cells emobilized in lung are activated to secrete the anti-inflammatory protein TSG-6,” *Cell Stem Cell*, vol. 5, no. 1, pp. 54–63, 2009.

129] S. Schneeberger, V. S. Gorantla, G. Brandacher et al., “Upper-extremity transplantation using a cell-based protocol to minimize immunosuppression,” *Annals of Surgery*, vol. 257, no. 2, pp. 345–351, 2013.

130] B. G. Ballios, M. J. Cooke, L. Donaldson et al., “A hyaluronan-based injectable hydrogel improves the survival and integration of stem cell progeny following transplantation,” *Stem Cell Reports*, vol. 4, no. 6, pp. 1031–1045, 2015.

131] S. A. Fisher, R. Y. Tam, and M. S. Shoichet, “Tissue mimetics: engineered hydrogel matrices provide biomimetic environments for cell growth,” *Tissue Engineering Part A*, vol. 20, no. 5–6, pp. 895–898, 2014.

132] J. Cortiella, J. Niles, A. Cantu et al., “Influence of acellular natural lung matrix on murine embryonic stem cell differentiation and tissue formation,” *Tissue Engineering Part A*, vol. 16, no. 8, pp. 2565–2580, 2010.

133] S. Khalilian, K. A. Sarhane, M. Tamia et al., “Stem cell-based approaches to improve nerve regeneration: potential implications for reconstructive transplantation?” *Archivum Immunologiae et Therapiae Experimentalis*, vol. 63, no. 1, pp. 15–30, 2015.

134] R. Londoño, B. A. Jobe, T. Hoppo, and S. F. Badylak, “Esophagus and regenerative medicine,” *World Journal of Gastroenterology*, vol. 18, no. 47, pp. 6894–6899, 2012.

135] A. Nieponice, F. F. Ciotola, F. Nachman et al., “Patch esophageoplasty: esophageal reconstruction using biologic scaffolds,” *Annals of Thoracic Surgery*, vol. 97, no. 1, pp. 283–288, 2014.

136] R. S. Sutherland, L. S. Baskin, S. W. Hayward, and G. R. Cunha, “Regeneration of bladder urothelium, smooth muscle, blood vessels and nerves into an acellular tissue matrix,” *Journal of Urology*, vol. 156, no. 2, part 2, pp. 571–577, 1996.

137] J. D. Wood, A. Simmons-Byrd, A. R. Spievack, and S. F. Badylak, “Use of a particulate extracellular matrix bioscaffold for treatment of acquired urinary incontinence in dogs,” *Journal of the American Veterinary Medical Association*, vol. 226, no. 7, pp. 1095–1097, 2005.

138] J. P. Iannotti, M. J. Codsi, Y. W. Kwon, K. Derwin, J. Ciccone, and J. J. Brems, “Porcine small intestine submucosa augmentation of surgical repair of chronic two-tendon rotator cuff tears: a randomized, controlled trial,” *Journal of Bone and Joint Surgery Series: A*, vol. 88, no. 6, pp. 1238–1244, 2006.

139] T. Zantop, T. W. Gilbert, M. C. Yoder, and S. F. Badylak, “Extra-cellular matrix scaffolds are repopulated by bone marrow-derived cells in a mouse model of Achilles tendon reconstruction,” *Journal of Orthopaedic Research*, vol. 24, no. 6, pp. 1299–1309, 2006.

140] V. Agrawal, B. N. Brown, A. J. Beattie, T. W. Gilbert, and S. F. Badylak, “Evidence of innervation following extracellular matrix scaffold-mediated remodelling of muscular tissues,” *Journal of Tissue Engineering and Regenerative Medicine*, vol. 3, no. 8, pp. 590–600, 2009.

141] J. E. Valentin, N. J. Turner, T. W. Gilbert, and S. F. Badylak, “Functional skeletal muscle formation with a biologic scaffold,” *Biomaterials*, vol. 31, no. 29, pp. 7475–7484, 2010.

142] S. Badylak, T. Gilbert, and J. Myers-Irvin, “The extracellular matrix as a biologic scaffold for tissue engineering,” *Tissue Engineering*, pp. 121–143, 2008.

143] E. Bible, F. Dell’Acqua, B. Solanky et al., “Non-invasive imaging of transplanted human neural stem cells and ECM scaffold remodeling in the stroke-damaged rat brain by (19)F- and diffusion-MRI,” *Biomaterials*, vol. 33, no. 10, pp. 2858–2871, 2012.

144] L. Zhang, F. Zhang, Z. Weng et al., “Effect of an inductive hydrogel composed of urinary bladder matrix upon functional recovery following traumatic brain injury,” *Tissue Engineering Part A*, vol. 19, no. 17-18, pp. 1909–1918, 2013.

145] R. A. Allen, L. M. Seltz, H. Jiang et al., “Adrenal extracellular matrix scaffolds support adrenocortical cell proliferation and function in vitro,” *Tissue Engineering Part A*, vol. 16, no. 11, pp. 3363–3374, 2010.

146] T. L. Sellaro, A. Ranade, D. M. Faulk et al., “Maintenance of human hepatocyte function in vitro by liver-derived extracellular matrix gels,” *Tissue Engineering Part A*, vol. 16, no. 3, pp. 1075–1082, 2010.

147] T. L. Sellaro, A. K. Ravindra, D. B. Stolz, and S. F. Badylak, “Maintenance of hepatic sinusoidal endothelial cell phenotype in vitro using organ-specific extracellular matrix scaffolds,” *Tissue Engineering*, vol. 13, no. 9, pp. 2301–2310, 2007.

148] J. M. Singelyn, P. Sundaramurthy, T. D. Johnson et al., “Catheter-deliverable hydrogel derived from decellularized ventricular extracellular matrix increases endogenous cardiomyocytes and preserves cardiac function post-myocardial infarction,” *Journal of the American College of Cardiology*, vol. 59, no. 8, pp. 751–763, 2012.

149] P. M. Crapo, C. J. Medberry, J. E. Reing et al., “Biologic scaffolds composed of central nervous system extracellular matrix,” *Biomaterials*, vol. 33, no. 13, pp. 3539–3547, 2012.

150] M. Szyrikaruk, S. W. P. Kemp, M. D. Wood, T. Gordon, and G. H. Borschel, “Experimental and clinical evidence for use of decellularized nerve allografts in peripheral nerve gap reconstruction,” *Tissue Engineering Part B: Reviews*, vol. 19, no. 1, pp. 83–96, 2013.

151] A. V. Boruch, A. Nieponice, I. R. Qureshi, T. W. Gilbert, and S. F. Badylak, “Constructive remodeling of biologic scaffolds is dependent on early exposure to physiologic bladder filling in a canine partial cystectomy model,” *Journal of Surgical Research*, vol. 161, no. 2, pp. 217–225, 2010.

152] N. J. Turner, A. J. Yates, D. J. Weber et al., “Xenogeneic extracellular matrix as an inductive scaffold for regeneration of a functioning musculotendinous junction,” *Tissue Engineering Part A*, vol. 16, no. 11, pp. 3309–3317, 2010.

153] T. W. Gilbert, D. B. Stolz, F. Biancaniello, A. Simmons-Byrd, and S. F. Badylak, “Production and characterization of ECM powder: implications for tissue engineering applications,” *Biomaterials*, vol. 26, no. 12, pp. 1431–1435, 2005.

154] S. Mazzitelli, G. Luca, F. Mancuso et al., “Production and characterization of engineered alginate-based microparticles
containing ECM powder for cell/tissue engineering applications,” *Acta Biomaterialia*, vol. 7, no. 3, pp. 1050–1062, 2011.

[155] J. De Kock, L. Ceelen, W. De Spiegelaere et al., “Simple and quick method for whole-liver decellularization: a novel in vitro three-dimensional bioengineering tool?” *Archives of Toxicology*, vol. 85, no. 6, pp. 607–612, 2011.

[156] M. He and A. Callanan, “Comparison of methods for whole-organ decellularization in tissue engineering of bioartificial organs,” *Tissue Engineering. Part B. Reviews*, vol. 19, no. 3, pp. 194–208, 2013.

[157] N. T. Remlinger, P. D. Wearden, and T. W. Gilbert, “Procedure for decellularization of porcine heart by retrograde coronary perfusion,” *Journal of Visualized Experiments*, no. 70, Article ID e50059, 2012.

[158] D. C. Sullivan, S. -H. Mirmalek-Sani, D. B. Deegan et al., “Decellularization methods of porcine kidneys for whole organ engineering using a high-throughput system,” *Biomaterials*, vol. 33, no. 31, pp. 7756–7764, 2012.

[159] A. Weymann, N. P. Patil, A. Sabashnikov et al., “Perfusion-decellularization of porcine lung and trachea for respiratory bioengineering,” *Artificial Organs*, 2015.

[160] J. Qu, R. M. Van Hogezand, C. Zhao, B. J. Kuo, and B. T. Carlse, “Decellularization of a fasciocutaneous flap for use as a perfusible scaffold,” *Annals of Plastic Surgery*, vol. 75, no. 1, pp. 112–116, 2015.

[161] S. Farnebo, C. Y. L. Woon, J. A. Bronstein et al., “Decellularized tendon-bone composite grafts for extremity reconstruction: an experimental study,” *Plastic and Reconstructive Surgery*, vol. 133, no. 1, pp. 79–89, 2014.

[162] L. Wang, J. A. Johnson, D. W. Chang, and Q. Zhang, “Decellularized musculofascial extracellular matrix for tissue engineering,” *Biomaterials*, vol. 34, no. 11, pp. 2641–2654, 2013.

[163] B. J. Jank, L. Xiong, P. T. Mose et al., “Engineered composite tissue as a bioartificial limb graft,” *Biomaterials*, vol. 61, pp. 246–256, 2015.

[164] G. Brandacher, V. S. Gorantla, and W. P. A. Lee, “Hand allotransplantation,” *Seminars in Plastic Surgery*, vol. 24, no. 1, pp. 11–17, 2010.

[165] T. D. Johnson, J. A. Dequach, R. Gaetani et al., “Human versus porcine tissue sourcing for an injectable myocardial matrix hydrogel,” *Biomaterials Science*, vol. 2, no. 5, Article ID 60283D, pp. 735–744, 2014.

[166] S. F. Badyalak, “The extracellular matrix as a scaffold for tissue reconstruction,” *Seminars in Cell and Developmental Biology*, vol. 13, no. 5, pp. 377–383, 2002.

[167] S. F. Badyalak and R. M. Nerem, “Progress in tissue engineering and regenerative medicine,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 8, pp. 3285–3286, 2010.

[168] J. A. Plock, J. T. Schnider, M. G. Solari, X. X. Zheng, and V. S. Gorantla, “Perspectives on the use of mesenchymal stem cells in vascularized composite allotransplantation,” *Frontiers in Immunology*, vol. 4, article 175, 2013.