**SPECIAL FOCUS: STRATEGIC DIRECTIONS
IN IMMUNORESPONSIVE BIOMATERIALS IN TISSUE ENGINEERING***

Extracellular Matrix Bioscaffolds as Immunomodulatory Biomaterials

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Suppression of the recipient immune response is a common component of tissue and organ transplantation strategies and has also been used as a method of mitigating the inflammatory and scar tissue response to many biomaterials. It is now recognized, however, that long-term functional tissue replacement not only benefits from an intact host immune response but also depends upon such a response. The present article reviews the limitations associated with the traditionally held view of avoiding the immune response, the ability of acellular biologic scaffold materials to modulate the host immune response and promote a functional tissue replacement outcome, and current strategies within the fields of tissue engineering and biomaterials to develop immune-responsive and immunoregulatory biomaterials.

Keywords: macrophage, ECM bioscaffolds, host response

Introduction

THE HOST RESPONSE to whole organ transplantation has been studied extensively and is well characterized. In contrast, the host response to decellularized tissues and organs (i.e., bioscaffold materials composed of extracellular matrix [ECM]) or to synthetic biomaterials has received much less attention and is relatively poorly understood. Within the last decade, the essential and necessary role of an intact and fully functional immune system in normal development,¹ regeneration,² and tissue homeostasis³⁻⁶ and in the constructive remodeling properties of ECM bioscaffolds⁷⁻⁹ has been recognized. Tissue engineering (TE) strategies that include a biomaterial component but fail to consider the immune response are likely to yield suboptimal outcomes.

The acute host response to any biomaterial involves blood/plasma protein adsorption on the material surface and activation of the innate immune response, including infiltration of neutrophils, macrophages, and the release of a diverse array of inflammatory cell-secreted signaling molecules.^{10,11} Resolution of this pro-inflammatory microenvironment is necessary for a successful clinical outcome following the use of any biomaterial. Normal adult wound healing processes, tissue homeostasis, and normal fetal development easily navigate this transition, partly as a result of the endogenous signals embedded within the ECM which regulate the immune response.¹²⁻¹⁵

For example, when damage denudes the endothelium, von Willebrand Factor embedded in the underlying ECM facilitates rapid tethering and adhesion of platelets.¹⁶ In addition to bioactive factors within the matrix, physical interactions with the ECM can also mediate cell fate. Interactions between subendothelial ECM proteins and platelet surface receptors such as immunoglobulin GPVI and integrins α IIB β 3 and α 2 β 1 drive platelet activation and adhesion, respectively.¹⁷ Mechanotransduction, the mechanisms by which cells translate mechanical stimulus into biochemical responses, has been shown to influence cell fate. Within a tissue, gradations in ECM composition, cross-linking, and three-dimensional arrangement contribute to the biochemical and biophysical environment that regulate cell migration, proliferation, apoptosis, differentiation, and development.^{18,19}

Recently, mechanotransduction has been specifically implicated as a regulator of immune cell phenotype. For example, McWhorter *et al.* demonstrated using a micropatterning approach that cell shape, specifically degree of elongation, modulates macrophage phenotype.²⁰ ECM remodeling in healing and homeostasis thus provides a mechanical feedback loop for the resident and/or infiltrating cells. As the ECM is turned over, particularly during resolution of inflammation, atypically expressed ECM molecules can modulate immune cell activation, differentiation, and persistence. These atypical ECM molecules, termed cryptic peptides, are bioactive

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peptides that are revealed by selective cleavage of ECM proteins by proteases, particularly matrix metalloproteinases.¹⁵ In a murine model of digit amputation, Agrawal *et al.* demonstrated that a C-terminal telopeptide of collagen III α present at the site of amputation recruited progenitor cells, increased calcium deposition, enhanced alkaline phosphatase activity, and amplified osteogenesis.²¹ In a mouse model of lung inflammation, MMP8 or MMP9 cleavage of type I collagen resulted in an acetylated Pro-Gly-Pro peptide that activated CXCR receptors and attracted neutrophils.²²

Altogether, these examples point to an active role of the native ECM in inflammation and healing. In line with these physiological roles, the use of biologic scaffold materials composed of ECM has proven effective, in part, through activation of these native endogenous signals.^{23–26} The present article reviews the limited success associated with the use of synthetic biomaterials, especially in the context of TE applications, and suggests a rationale for the relative effectiveness of ECM-based scaffold materials. In addition, the critical role of the immune system in TE approaches is described and, thus, the increasingly important contribution of immunomodulatory biomaterials.

Immunosuppression: the impact upon organ transplantation

An understanding of the host response to biomaterials benefits from knowledge of the earliest attempts at tissue and organ replacement. The concept of replacing damaged or disease tissues and organs has not been the exclusive pursuit of the TE community, but rather, has been a part of medical practice for millennia. One of the earliest documented cases of tissue and organ transplant, skin grafting, occurred in ~3000 BC as revealed in Sanskrit texts of ancient India.²⁷ Documentation of tissue transplant and grafting that spans the 18th and 19th century includes the use of skin flaps to repair missing nose tissue after sword fights,²⁷ teeth transplants, and the use of cadaveric tissues. It was not until the realization that transplant failure was largely attributed to immunologic events²⁸ that the concept of immunosuppression was extensively investigated. The development of effective immunosuppression techniques allowed for organ transplantation to become commonplace.

Immunosuppression progressed from a cell-centric approach that nonspecifically targeted rapidly dividing cells by irradiation to steroid therapy, to lymphocyte depletion, and eventually to inhibition of selected cytokines.²⁹ These immunosuppression strategies are associated with variable success rates, immune disorders, susceptibility to infections, and significant morbidity. Nonetheless, the discovery of major histocompatibility complexes and the importance of human leukocyte antigen matching led to improved success of immunosuppression compared to prior transplant methods and has allowed for an exponential increase in the number of successfully transplanted organs and tissue grafts during the past 50 years.

The development of “inert biomaterials”

As exhaustive studies were being conducted in the context of tissue and organ transplantation, the biomaterial community was identifying “inert” materials as desirable.^{30–32} Biomaterials such as silicone that were isolated from the surrounding tissue by a defined fibrous capsule were grouped

with materials identified as inert. In hindsight, the very formation of the capsule and associated foreign body response was a manifestation of the host immune reaction toward the biomaterial. Attempts to mitigate fibrous tissue capsule thickness included steroid coatings or coating with cytotoxic agents.^{33,34} Modifications of surface topology, functionalization with various ligands, and design changes that eventually included large (>75 μ M) pores were gradually added to biomaterials in attempts to control the local tissue response.

Arguably, there is no such thing as an “inert biomaterial.” Upon implantation, such biomaterials, usually of synthetic composition, are subjected to a series of well-defined processes characterized as the foreign body reaction (FBR) that ultimately leads to fibrous encapsulation of the implant.³⁵ Implanted medical devices are often isolated from the body by a dense collagenous capsule, which has long been an acceptable form of “biocompatibility” both by regulatory and historical standards. However, the inability to interface with normal host tissue as a result of intervening fibrous tissue eventually leads to diminished function for devices that require close contact with parenchymal cells or neurovascular structures.

Metals and alloys have long been used for orthopedic implants and dental implants, but are typically subject to corrosion, leaching, and adverse immune responses to wear debris.^{36–40} Silicone rubber and natural rubber have been used for breast implant and ocular lens applications, among others, but are associated with oil adhesions and other complications associated with the FBR, including capsular contraction that may eventually necessitate the need for explants.^{41–44} Drug-eluting and other porous materials and sensor-based strategies to direct tissue repair are eventually ineffective if they fail to address the FBR, as the fibrous capsule that will inevitably surround the material will inhibit diffusion or controlled release of drug or sensor-related signals.

It should be recognized that the inevitable inflammatory response that occurs to all biomaterials is virtually the same as the innate immune response, thus setting the stage for the concept of immunomodulatory biomaterials. The biologic processes of vascularization, cell response, fibrous tissue deposition, and the foreign body response have not changed; these events are now simply recognized as part of the host immune response. It is logical therefore that modulation or redirection of the immune response rather than suppression of the immune response is important for long-term biomaterial functionality.³²

Biologic scaffolds as immune modulating biomaterials

Biomaterials composed of ECM and typically derived from xenogeneic tissues have shown notable success promoting constructive and functional tissue remodeling in multiple anatomic sites in both preclinical and clinical studies, including hernia repair applications, esophageal mucosa replacement after cancer resection, volumetric muscle loss treatment, cardiac repair, and mitral valve replacement, among others.^{45–52} It is important to note that ECM scaffolds prepared by methods that remove essentially all cellular remnants (i.e., xenogeneic antigens that would normally elicit a pro-inflammatory response) serve as an inductive niche to influence cell behavior and the downstream tissue remodeling response. Although xenogeneic in tissue origin, there has never been any clinical or histologic evidence of hyperacute or delayed rejection of efficiently decellularized

ECM-derived bioscaffolds. Although no immunosuppressive agents are used with these bioscaffold materials, this does not imply immune privilege. In fact, there is a distinct immune response as described below.

Much is known about the mechanisms by which ECM bioscaffolds promote constructive tissue remodeling, including the fact that upon implantation these bioscaffolds degrade and release chemoattractant,^{53–55} antimicrobial,⁵⁶ and mitogenic peptides,^{53,55,57,58} growth factors,^{57,59} and extracellular vesicles⁶⁰ that contribute to endogenous stem cell recruitment among other bioactive effects. Perhaps most importantly, these scaffolds have been associated with a robust, but favorable host immune response that precedes constructive remodeling outcomes.²³ ECM bioscaffolds modulate the behavior of responding immune cells toward a regulatory anti-inflammatory phenotype.

In 2001, a seminal study conducted by Allman *et al.* showed that xenogeneic-derived ECM bioscaffolds promote a transition in the host innate immune response toward a Th2-restricted response. The study showed that there is indeed a robust host immune response to porcine-derived ECM bioscaffolds when implanted in a murine host, but the cytokine and antibody isotype profile is associated with production of anti-inflammatory cytokines, including interleukin (IL)-4 and IL-10 and noncomplement fixing antibodies, indicators of biomaterial acceptance.⁶¹ Stated differently, ECM bioscaffold materials are not inert, but rather immunomodulatory.

The immunomodulatory effects of ECM bioscaffolds have since been extensively examined. The thoroughness of decellularization of the source tissue is a critical determinant of the ability of these materials to elicit an anti-inflammatory macrophage/T cell (M2-like/Th2-like) host response.^{62,63} In addition, the use of chemical cross-linking,^{8,23} the anatomic origin of the source tissue from which the ECM is derived,⁶⁴ the source animal age,⁶⁵ the terminal sterilization method utilized,⁶⁶ and the supplementary use of NSAIDs⁶⁷ all can markedly affect the host response to ECM bioscaffolds.

For example, drugs that act on COX1/2 have been shown to reduce the constructive remodeling response driven by biologic scaffolds. Dearth *et al.* demonstrated that COX1/2 inhibition by aspirin led to less myogenesis and collagen deposition at the defect site in an animal model of skeletal muscle injury.⁶⁷ *In vitro*, macrophages exposed to aspirin displayed a reduction in ECM-driven secretion of prostaglandins. Aspirin treatment also reduced expression of CD206—a marker of M2-like macrophages. Thus, in clinical practice, the use of NSAIDs alongside ECM scaffolds may dampen the healing process stimulated by the implant. In addition to the biomaterial properties, host-related factors can likewise contribute to the immune response to biomaterials, including age,^{68,69} nutritional status,^{70,71} anatomic site of implantation,^{10,72} and the presence of comorbidities.^{73,74}

In 2009, Valentin *et al.* showed that not only do ECM bioscaffolds elicit a favorable host innate immune response, specifically the macrophage response, but also that this response is required for constructive ECM-mediated tissue remodeling.⁸ Macrophages have recently been recognized as a critical determinant of regeneration in species such as the adult salamander⁷⁵ and in mammals during acute regenerative responses such as following skeletal muscle injury.⁴ Brown *et al.* further showed that the early phenotype profile

of macrophages during the first weeks following ECM implantation can predict downstream remodeling outcomes.²³ It has since been shown that macrophages exposed to degradation products of ECM have a unique phenotype that is associated with suppression of inflammation and high antigen-presenting capabilities,⁷⁶ even in the presence of a harsh pro-inflammatory microenvironment as in ulcerative colitis⁷⁷ or volumetric muscle loss.⁷⁸

Furthermore, the immunomodulatory properties of ECM bioscaffolds act not only by directly influencing macrophage phenotype^{25,26} but also through paracrine effects, mediating macrophage cross-talk with endogenous stem/progenitor cells.²⁵

The immunomodulatory effects of ECM biomaterials include adaptive immune cells, as well as cells of the innate immune system. Sadtler *et al.* have expanded upon the early work of Allman by showing that, in a model of volumetric muscle loss, the remodeling response that follows implantation of an ECM bioscaffold critically relies upon a T helper 2 pathway that guides macrophage polarization. It now appears likely that the cross talk between macrophages and T regulatory cells initiated by ECM degradation products is a critical determinant of downstream remodeling outcomes associated with not only the use of ECM bioscaffolds^{76,79} but also during the native tissue response to disease and injury.^{80,81}

Sicari *et al.* have shown that degradation products of ECM bioscaffolds can directly activate macrophages toward a iNOS⁺/Fizz1⁺ macrophage phenotype.²⁵ Furthermore, Huleihel *et al.*⁸² conducted an exhaustive analysis of macrophage phenotype following exposure to ECM degradation products showing that ECM is consistently associated with downregulation of pro-inflammatory genes and proteins. The ability of ECM bioscaffolds to activate macrophages is well established. Through both direct effects and paracrine-mediated signaling, the ability of ECM bioscaffolds to promote a pro-regenerative microenvironment has been investigated in multiple *in vitro* and preclinical studies. However, the exact mechanisms by which ECM promotes this response have only been partially elucidated. Future work should aim to evaluate the specific components within ECM scaffolds that contribute to this response, including cryptic peptides, topical cues, and miRNA among other components.

Recent work has reinforced the need for caution in drawing broad conclusions regarding the immunomodulatory effects of biomaterials, specifically the effects attributed to macrophage phenotype.⁸³ Although it is clear that macrophage phenotype is a major determinant of the host response, use of a single cell marker or even several markers may be inadequate to accurately characterize the functional and paracrine effects of these cells. Although ideal, it is often impractical to evaluate an exhaustive panel of markers to define macrophage phenotype. Recognizing this limitation, we recommend that researchers choose markers based on their relevancy to the physiologic or pathologic condition being studied. When subsequently reporting the data, researchers should provide a justification for the markers they choose.

Another consideration is that the type of macrophage used for *in vitro* studies can markedly affect results and conclusions. Recent work by Huleihel and colleagues evaluated the use of primary mouse bone marrow-derived macrophages (BMDMs) versus a human monocyte cell line (ThP1s) for *in vitro* activation studies. Drastically different gene

expression profiles between BMDMs and ThP1s after stimulation with LPS+IFN γ , IL-4, or ECM degradation products were reported. Phenotype nomenclature can also be misleading (i.e., M1 vs. M2) and contribute to misinformation regarding cause–effect relationships between biomaterials and the host immune response. Throughout the literature, the terminology “M1” and “M2” is used to describe pro-inflammatory and anti-inflammatory macrophage phenotypes, respectively. However, this dichotomy is an oversimplification. Macrophage phenotype is better defined as a spectrum from pro-inflammatory to anti-inflammatory.

A recent consensus report suggests nomenclature for macrophage phenotype that should minimize such problems.⁷² In their review, Murray *et al.* recommend parameters that should be reported when describing *in vitro* experiments. These parameters include cell source (mouse strain, tissue/organ, pathological condition, etc. as relevant), starting cell number, media and supplements utilized, tissue culture conditions, time in culture, source and concentration of cytokines, macrophage yield, activation conditions, and processing/analysis protocols. Reporting these parameters will permit researchers to directly compare experiments. Moreover, Murray *et al.* propose that macrophage nomenclature be described by the activator utilized, that is, M(IL-4) instead of M2.

Development of immunoresponsive materials

Many of the available immunosuppression strategies that arose before knowledge of the normal immune response to tissue injury and disease had been uncovered. Development of biologic scaffolds and their derivatives and a better understanding of the normal immune response to wound healing, tissue homeostasis, development, and acute regeneration have sparked ongoing investigation into biomaterials and alternative TE approaches that are immunoresponsive, rather than immunosuppressive, to promote a favorable immune response and facilitate functional tissue repair. For example, the use of ECM-coated materials has been investigated as a method to promote the phenotypic switch associated with ECM bioscaffolds instead of the persistent pro-inflammatory response to synthetic materials.

ECM coatings have been shown to mitigate the chronic inflammatory response and associated downstream scar tissue formation after implantation of polypropylene mesh, a synthetic material used for surgical applications for decades.⁸⁴ Faulk *et al.* reported that the addition of an ECM hydrogel coating decreased the number of pro-inflammatory CD86⁺/CD68⁺ macrophages in the vicinity of the polypropylene fibers 2 weeks after implantation. Six months after implantation, the coated polypropylene was associated with less collagen deposition (i.e., fibrosis).

In similar work, using a porcine dermal ECM hydrogel to coat polypropylene meshes reduced cell macrophage accumulation and formation of foreign body giant cells.⁸⁵ At 35 days postimplantation, the ECM coating was fully degraded and replaced with a loose connective tissue. The use of biomimetic ECM components has also been investigated to limit inflammatory responses to synthetic materials. For example, in an *in vitro* model of sterile inflammation, addition of high sulfated hyaluronan reduced macrophage secretion of pro-inflammatory cytokines IL-1 β , IL-6, IL-8, IL-12, and TNF α and induced secretion of the immunoregulatory cyto-

kine IL-10 and the expression of CD163.⁸⁶ Direct coating of surgical meshes with pro-remodeling cytokines such as IL-4 has been associated with improved implant tissue integration.⁸⁷ Implantation of IL-4 coated meshes in mice was associated with an increased M2-like macrophage to M1-like macrophage ratio leading to reduction in the formation of a fibrotic capsule around the implant. The tissue-implant interface has been shown to be an early effector of the responding immune phenotype.⁸⁷ This surface-coating strategy could be pursued to include coatings of biologic scaffold materials, in addition to synthetic materials, to augment the bioscaffold's inherent immunomodulatory effects.

In addition to biomimetic coatings, hybrid hydrogels have also been evaluated for modulating the immune response. Poly(ethylene glycol) (PEG) hydrogels containing a peptide mimic of the TNF α recognition loop on the TNF-receptor 1 were evaluated as a cell encapsulation material.⁸⁸ Because these hydrogels could sequester TNF α , encapsulated cells were protected from this pro-inflammatory cytokine. Similarly, PEG hydrogels containing an inhibitory peptide for the IL-1 receptor were able to protect encapsulated islet cells.⁸⁹ Attempts to combine controlled-release technologies and biomaterials to alter the host immune microenvironment and promote better cell engraftment have also been investigated in preclinical animal studies with success.^{90,91}

Many of the successful cell-centric strategies for tissue engineered constructs have largely been due to the paracrine effects of such stem cells upon the responding immune cell infiltrate, particularly in the case of mesenchymal stem cell (MSC) delivery.^{92,93} MSCs encapsulated in PEG hydrogels secreted PGE2 mediated immunoregulation of macrophages *in vitro* and resolution of the foreign body response *in vivo*. Although the immune response to a synthetic material is a critical design consideration, the incorporation of a natural moiety places design constraints on polymer architecture, dynamics, and stabilization that can affect the long-term functionality of the implant.⁹⁴ It cannot be ruled out that biomimetic strategies merely prolong the inevitable immune response (foreign body response) to a synthetic material. In short summary, immunomodulation is now recognized as an effective method for improving biomaterial performance.

Conclusions

Successful clinical translation of TE and biomaterial-based approaches for functional tissue replacement is critically dependent upon a compatible host response. Immunomodulatory strategies for limiting the FBR and resolving the inflammation following material implantation/cell transplant are at the center of efforts to influence the host–biomaterial interface. The native ECM and ECM-based biomaterials possess signaling molecules that promote such events. Future studies should be aimed at investigation of specific components (both structural and soluble) within clinically used scaffolds that activate the immune response. The reason(s) for the lack of a single documented adverse (i.e., rejection) response to ECM bioscaffolds, even though they are largely xenogeneic in nature, represents an additional area of future investigation. In a broader context, it appears clear that a better understanding of the role of both the innate and adaptive immune systems in the host response to biomaterials, tissue remodeling, and regeneration will help shape the next generation of biomaterials

and will be required to overcome current bottlenecks in the clinical translation pathway for TE and biomaterial-based technologies.

Disclosure Statement

No competing financial interests exist.

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