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REVIEW ARTICLE

The Immune System and Its Contribution to Variability in Regenerative Medicine

Erika M. Moore, PhD,[1] David R. Maestas, Jr., BS,[2] Hannah Y. Comeau, MS,[2] and Jennifer H. Elisseeff, PhD[2]

The immune system plays a critical role in directing tissue repair and regeneration outcomes. Tissue engineering technologies that are designed to promote new tissue growth will therefore be impacted by immune factors that are present in patients both locally at the site of intervention and systemically. The immune state of patients can be influenced by many factors, including infection, nutrition, and other disease comorbidities. As a result, the immune state is highly variable and may be a source of variability in tissue-engineered products in the clinic, which is not found in preclinical models. In this review, we will summarize key immune cells and evidence of their activity in tissue repair and potential in tissue engineering systems. We also discuss how clinical translation of tissue engineering strategies, in particular stem cells, helped elucidate the importance of the immune system. With increased understanding of the immune system's role in repair and tissue engineering systems, it will likely become a therapeutic target and component of future therapies.

Keywords: biomaterials, immune response, immune state, immune variability, clinical translation, tissue engineering

Impact Statement

Clinical translation of tissue-engineered products often yields variability in outcomes of repair. The immune system may be a major contributor to this variability. Each person's immune system is highly plastic and represents a living history of infections, diet, age, sex, and inherited genetic traits, as well as environmental factors. As we seek to design tissue-engineered products, we must consider the influence of the immune system on repair outcomes. Additionally, products can be designed to manipulate the immune system to skew toward a phenotype that promotes a desired repair outcome.

The Immune System Orchestrates Wound Healing and Tissue Repair

The immune system is capable of orchestrating tissue repair and healing. It is broadly categorized into two major components: innate and adaptive immune systems.[1] In response to stimuli, the innate system mobilizes quickly with limited specificity. In parallel, the adaptive system activates more slowly with long-term memory and high specificity. The innate immune system comprises innate sensor cells, including epithelial cells, tissue-resident mast cells, macrophages, plasmacytoid dendritic cells, innate lymphocytes, and general/classical dendritic cells. Additional innate immune cells include basophils, natural killer (NK) cell types, granulocytes (including neutrophils, eosinophils, and basophils), and monocytes. Adaptive immune cells largely include T and B lymphocytes; however, additional cell types such as NKT cells and γδ T cells are of lymphoid origin despite their innate-like functional characteristics. Table 1 highlights the roles of these cells in tissue repair applications in both preclinical and clinical studies. Figure 1 demonstrates a graphical representation of the involvement of these cell types in wound healing.

Stromal Cells Contribute to the Immune Environment in Wound Healing and Tissue Repair

The tissue stroma comprises fibroblasts and the connective tissue matrix. While the stroma has not historically been considered immunologically active, recent studies show that fibroblast subsets can secrete cytokines that interact with the immune system.[30–32] Senescent cells and inflammatory

[1]Department of Materials Science and Engineering, University of Florida, Gainesville, Florida, USA.
[2]Translational Tissue Engineering Center, Johns Hopkins University, Baltimore, Maryland, USA.

Table 1. Summary of Immune Cell Roles in Tissue Healing and Clinical Outcomes

	Role in healing (preclinical)	*Clinical relevance*
Neutrophils	Neutrophil depletion delays/impairs healing[2] Neutrophils enhanced angiogenesis in early wounds[3]	Dominance of neutrophil-derived proteases in recalcitrant venous ulcers, high levels in wound exudate associated with poor wound healing[4]
Innate lymphoid cells (ILCs)	Barrier functions in tissue-dependent contexts dictate the roles of ILCs[5,6] ILC1: proinflammatory through the secretion of IFNγ ILC2: innate immune response to intestinal parasitic worm clearance and ties to pathogenesis of asthma and allergy[7] ILC3: intestinal barrier maintenance, promotion of fibrosis with synthetic biomaterials, or inflammation resolution in the lung[8]	Group 2 ILCs are enriched in atopic dermatitis (AD) skin lesions ILC2s in AD wounds may be a distinct population or in a different state of activation compared with ILC2s present in healthy skin[9] RORγ^{+}-CD127^{+}CD3^{-} ILC infiltration in human skin following wounding with punch biopsy enhances healing[10]
NK cells	Defense against viral infection CD56^{dim} and CD56^{bright} NK cell classifications; CD56^{dim} are cytotoxic, while CD56^{bright} cells secrete mediating cytokines[11] Clearance of senescent cells[12]	In patients undergoing endovascular aneurysm repair (EVAR), lower NK cell numbers correlated with negative long-term outcomes from EVAR (increased mortality)[13] Sleep deprivation reduces NK cell number and NK cell activity[14]
Eosinophils	Produce IL-4 and IL-13 direct stem cell fate in muscle regeneration[15] Secrete IL-4 in liver injury to promote regeneration[16]	In burn wounds, high eosinophil infiltration is linked with increased wound healing. The absence of eosinophil infiltration leads to a poor prognosis and death in patients who did achieve a certain amount of eosinophils within 1 week following the burn[17] However, aged patients with burn wounds present with increased eosinophil infiltration during early stages of healing, resulting in increased healing time compared with younger patients[18]
Monocytes	CCR2^{hi}C$_X$3CR1^{low} monocytes transition to CCR2^{low}C$_X$3CR1^{hi} to enter the injury site[19]	CD33^{+}CD14^{+}CD11b^{+}HLA-DR^{low} monocyte populations increased following hip surgery and an upregulation of *STAT3* signaling correlated with surgical recovery[20]
Macrophages	Required for salamander limb regeneration[21]; M2/alternatively activated macrophage phenotypes M1/proinflammatory macrophage phenotypes[22,23]	M1 macrophages, in chronic venous leg ulcers (CVUs), inhibited tissue repair[24] M2 macrophages are also associated with accelerating bone healing in patients with traumatic brain injury[25]
γδ T cells	Regulate macrophage homing Can induce IL-4 to regulate the macrophage phenotype (specific subsets of γδ T cells within injury)[26,27] Modulate repair mechanisms in the wound environment through expression of IL-17 and IL-22 in CCl_4, which induced liver fibrosis[26,28]	Psoriasis: γδ T cell frequency is increased compared with healthy controls, Psoriasis-associated γδs secrete higher levels of IL-17 than healthy control γδ^{-} T cells[29]
Immunologically activated fibroblasts	Secrete cytokines,[30] orchestrate monocyte differentiation into macrophages[31] Proangiogenic capabilities[32]	IFNγ, MCP-1, GM-CSF, and bFGF were overexpressed by wound fibroblasts in patients with chronic wounds compared with patients with well-healing wounds[33]
CD8^{+} T cells	Depletion of CD8^{+} T cells results in wounds with increased mechanical toughness, linked to regulation fibroblasts and macrophages during wound healing[34]	Elevated CD8^{+} T cells in the patient's blood and at the fracture site were associated with impaired bone repair[35,36]
T_{regs}	Control effector T cell and neutrophil activity Regulate macrophage and monocyte phenotypes to anti-inflammatory states[37–39] In muscle injury, they promote tissue regeneration through the expression of a proregenerative protein, amphiregulin[40]	CD161^{+} T_{regs} are enriched in patients with inflammatory bowel disease (Crohn's disease), suppress inflammation, and accelerate wound closure in the colon[41]
CD4^{+} helper T cells	CD4^{+} T helper cells mediate immune response through cytokine secretion.[1] Three types of CD4^{+} helper cells include the following: T_H1 cells, which dominantly secrete interferon (IFN) and tumor necrosis factor (TNF) T_H2 cells, which secrete IL-4, IL-5, and IL-13 T_H17 cells, which secrete IL-17, IL-21, and IL-22 and follicular helper T cells T_H2 CD4^{+} cells are IL-4-producing T cells that were responsible for enhancing neuronal protection and recovery following CNS injury[42,43]	T_H17 cells are found near breast capsule implants and contribute to IL-17a secretion and fibrosis near the breast capsule implant[44] T_H17 cell frequency and plasma T_H17-associated cytokine concentrations positively correlate with carotid artery plaques[45]
B cells	Secrete IL-10 (intestinal injury)[46]; Enable skin wound closure through the soluble secretion of IL-6 and transforming growth factor beta[47,48]	In renal transplant patients, B cell-mediated immune regulation was characterized by the ratio of IL-10/TNFα expression Patients with poor graft outcomes had a low IL-10/TNFα ratio in transitional B cells[49]

HLA, human leukocyte antigen; IL, interleukin; NK, natural killer; STAT3, signal transducer and activator of transcription 3; T_H2, T helper type 2; T_{reg}, regulatory T cell.

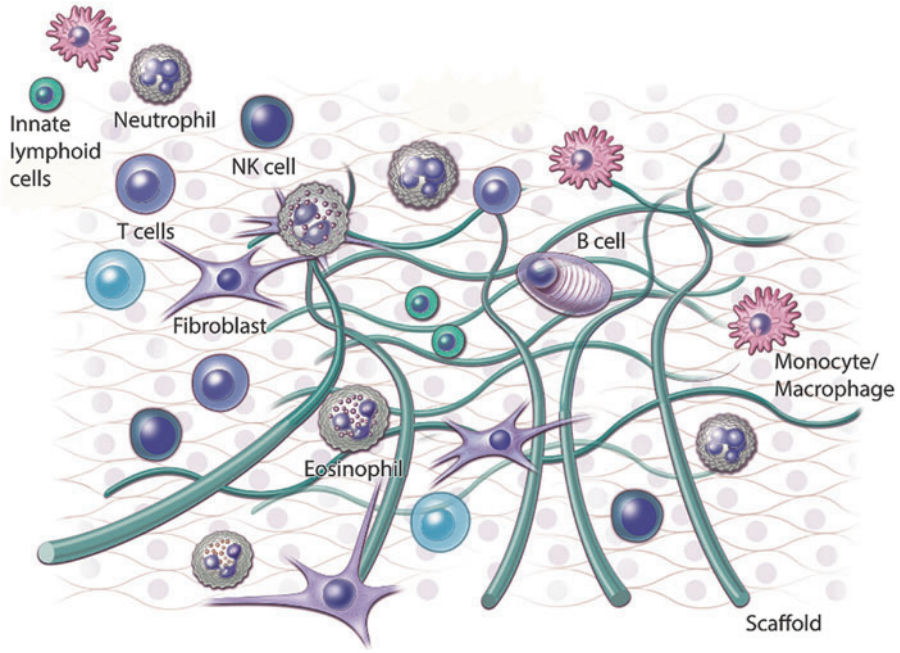

FIG. 1. Several immune cell populations orchestrate wound repair in the presence of a scaffold. This schematic highlights the immune cells known to play a role in clinical outcomes, including NK cells, innate lymphoid cells, T cells, fibroblasts, B cells, eosinophils, and monocytes/ macrophages. NK, natural killer.

fibroblasts are emerging as important regulators in tissue repair and also serve as a connection between traditional immune cells and the stroma. Senescent cells are characterized by cell cycle arrest and secretion of a senescence-associated secretory phenotype (SASP).[50] Senescent cells develop after injury and their SASP is critical to tissue repair.[51,52] Secreted SASP factors include interleukin (IL)-1, IL-6, MIP-1a, and IL-8, as well as extracellular matrix (ECM)-degrading enzymes. These cytokines can influence the local immune response and thus contribute to the immune environment and tissue repair.[53,54]

The phenotype of senescent cells and how they evolve during wound healing remain unknown. It is hypothesized that the SASP may benefit wound healing when present acutely as the inflammatory cytokines stimulate immune cell recruitment and surveillance that enable debris clearance and tissue remodeling. When senescent cells were cleared early after a cutaneous injury (using the p16-3MR mouse model), granulation tissue and blood vessel development decreased in the wound.[51] In both muscle injury and liver fibrosis, the presence of senescent cells limited fibrosis or fibrogenesis and enhanced tissue repair.[12,55] However, senescent cells are also found in atherosclerosis and osteoarthritis lesions and the removal of senescent cells reduces disease pathology.[56,57] These studies suggest that injury-related accumulation of senescent cells can either retard or accelerate wound healing potentially depending on the phenotype and kinetics.[52] Additional work is required to better understand and exploit the stroma and cellular senescence in tissue repair and tissue engineering.

Immunomodulatory Properties of Stem Cells

Mesenchymal stem cells (MSCs) and other adult stem cell types generated excitement in the field of tissue engineering for their ability to proliferate and differentiate into multiple tissue types. Insight from clinical trials using stem cells presented questions regarding their therapeutic mechanisms of action in tissue repair.[58–60] According to Clinicaltrails.gov, there are currently over 5000 clinical trials utilizing stem cells as a therapeutic, with $\sim$1000 of those trials using MSCs specifically.[60] MSCs are being tested for their ability to treat a wide range of clinical challenges, including bone and cartilage repair, neurodegeneration, cardiovascular regeneration, liver disease, diabetes, graft-versus-host disease, and some autoimmune diseases.[60]

While there is strong evidence of stem cell capacity to differentiate into multiple cell types *in vitro*, their activity following implantation into wound models is less clear. Previously, MSCs were thought to facilitate tissue healing by homing to damaged tissue and differentiating into a specific cell phenotype. However, several clinical trials suggested that the immunomodulatory capacity of stem cells was the primary therapeutic mechanism of action.[61,62] Important considerations of cell delivery are viability and localization after injection. When injected *in vivo*, stem cells are primarily found in the lungs and spleen.[63] Both organs, especially the spleen, are immunologically active and may respond to the immunologically active factors in dead cells. Apoptotic and necrotic cells emit damage signals that alter the wound microenvironment and stimulate innate and adaptive immune responses.[64,65]

MSCs reduce inflammation and promote tissue repair through the secretion of multiple factors, including prostaglandin E2, hepatocyte growth factor, and inducible nitric oxide synthase, which diminishes T cell proliferation.[66–69] MSCs also reduced immunoglobulin production in plasma cells through CCL2 (a chemokine).[70] In addition, MSC secretion of leukemia inhibitory factor promotes regulatory T cell (T_{reg}) expansion.[71] In clinical studies of systemic lupus erythematosus (SLE) and Crohn's disease, MSC treatment reduced autoantibody levels in the serum.[72,73] These clinical trials suggest that successful therapeutic response in patients is not primarily due to the differentiation and tissue-

forming capabilities of stem cells but rather a product of cross talk between these cells and the patient's immune system, further supporting the importance of the immune system in tissue engineering technologies.

Biomaterials Can Orchestrate Healing Through Immunomodulation

While stem cell therapies in the clinic suggested immunological therapeutic mechanisms of action in tissue repair, research in biomaterials also points to the immune system in tissue engineering. In clinical studies of the hydrogel system BST-CarGel® for cartilage repair, immunomodulation appeared to be a key mechanism of action.[74–76] Hoemann and Buschmann demonstrated that chitosan hydrogels activated the complement system, an early component of the innate immune system's response, to improve cartilage repair when used in combination with surgical microfracture.[77] It is also likely that poly(ethylene glycol) hydrogels used for cartilage repair also modulated the immune response in addition to concentrating and attracting progenitor cells after microfracture.[78,79]

Biological scaffolds derived from the ECM of tissues create a proregenerative environment. Importantly, research into this class of biomaterials established the importance of the macrophage phenotypes in regeneration outcomes, which have been applied in many clinical indications. Biological scaffolds are used clinically for abdominal repair, wound healing, and rotator cuff repair and are in clinical testing for muscle, cardiac, and soft tissue reconstruction.[80–82] Brown *et al*. found that a biological scaffold derived from the small intestine submucosa–extracellular matrix (SIS-ECM) promoted invading and neighboring macrophages toward an alternatively activated macrophage phenotype.[83,84] In this work, macrophages were described as either classical M1, proinflammatory, or alternative M2, protissue, healing phenotypes. However, cross-linking of the porcine-derived, SIS (CDI-SIS) biological scaffold with carbodiimide inhibited the proregenerative macrophage phenotype and instead induced an M1 phenotype and resulted in decreased repair compared with the SIS-ECM. Additional studies reinforced the link between the macrophage phenotype and tissue remodeling with the biological scaffold in the presence or absence of cells.[83,85] When assessing 14, various, biologically derived surgical meshes, a correlation was found between a positive remodeling outcome and recruitment of higher numbers of M2 macrophages (also characterized by a higher M2/M1 macrophage ratio[84]). An M2 macrophage phenotype is now synonymous with biological scaffold efficacy and tissue regeneration.

Additional studies on biological scaffolds found that an adaptive immune response, specifically a type 2 immune response, is critical for scaffold efficacy and regenerative relevance.[86–88] Following volumetric muscle loss and implantation of biological scaffolds, T helper type 2 (T_H2) cells producing IL-4 infiltrated the wound site. Critically, T_H2 cells aid in orchestrating healing and functional tissue repair; they promoted the M2 macrophage phenotype at the wound site and promoted systemic increases of IL-4 production.

Type 2 immune responses, characterized by IL-4 production, are important for healing in multiple tissues.[43,89] Walsh *et al*. found that IL-4-producing T cells were responsible for enhancing neuronal protection and recovery, following both optic nerve crush injury and spinal cord contusive injury.[42] Importantly, these T cells were stimulated toward a type 2 response independent of the antigen-presenting major histocompatibility complex II (MHC II). Damage-associated molecular patterns from damaged cells following injury were responsible for inducing IL-4-producing T cells. Chawla also found that eosinophils secreting IL-4 stimulate muscle and liver regeneration.[15,16] In a review by Gieseck *et al.*, the authors acknowledge that type 2 signatures are indeed beneficial to wound healing and that future therapeutic targeting against fibrosis should carefully aim to block the pathogenic features of sustained type 2 responses without losing its benefits for wound healing.[90] However, it is critical to recognize that it is not known how the kinetics of a type 2 immune response in healing may or may not be correlated with a type 2 response in fibrosis or even allergic responses. Nevertheless, a type 2 immune response is tied to healing and thus must be considered within the context of regenerative engineering products.

The Human Immune State Is Inherently Variable

The immune state of an individual influences their therapeutic response. Variables that can impact the immune system include, but are not limited to, age; infection; history of infection; allergies; the presence of other injuries, disease, or cancer; and sex.

Variations in immune states are both heritable and nonheritable traits. Heritable traits account for a minority of the variation in a person's immune state[91]; specific examples include sex differences as well as MHC and non-MHC-associated genes. Sex- and hormone-based differences represent some of the most obvious heritable immune differences. Females, with estrogen, have an enhanced and protective humoral immunity response versus males, with testosterone, due to the ability to generate natural antibodies to bacterial infection.[92] Research in MHC and human leukocyte antigen (HLA) complexes links disease presentation to inherited MHC or HLA alleles.[93] Autoimmune diseases such as SLE, ankylosing spondylitis, and type 1 diabetes are all associated with heritable HLA domains and disease presentation.[94] Vaccination and cancer recurrence also critically connect HLA alleles with cancer recurrence risk.[95] It is unknown how HLA may impact the response to tissue trauma and tissue-engineered systems. Nonheritable influences, such as history of infections, antigen/allergen exposure, parasitic infections, and an individual's microbiota, play dominant roles in shaping immune states (Fig. 2). For example, smokers have increased leukocyte counts, reduced serum immunoglobulins, and an increased percentage of autoantibodies, linking the environmental exposure (smoking) to the immune state of the person.[96]

Allergic disease pathways overlap with immunological characteristics of parasitic infections in terms of the influx of T_H2 cells, heavy eosinophil activity, and high immunoglobulin E (IgE) production.[97,98] Moreover, a person's established allergies or their predisposition to development of allergies can significantly influence their immune system response to infections and injuries. Hypersensitivity to otherwise nonharmful environmental agents can influence how a person's immune system may respond to clinical

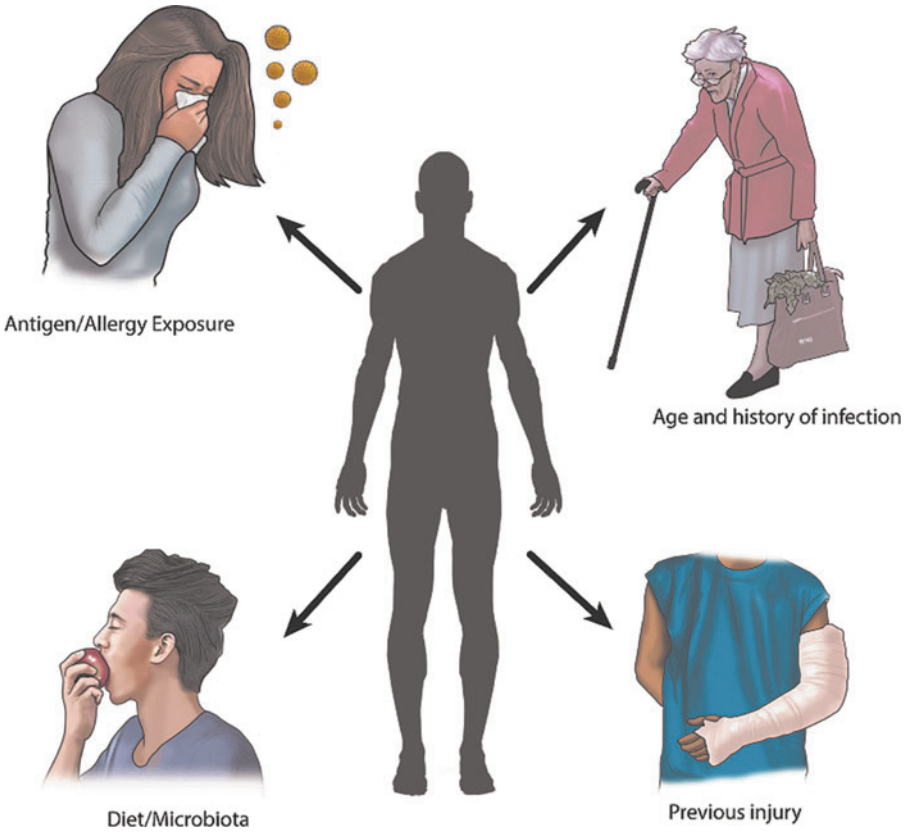

FIG. 2. An individual's nonheritable immune influences include antigen/allergy exposure, age and history of infections, diet/microbiota, and previous injury. Together, nonheritable influences demonstrate significant impact on an individual's immune state.

interventions postinjury and can limit therapeutic options. Biomaterials, especially metals used in orthopedic implants, can have a significantly higher failure rate if a person already possesses an established hypersensitivity or the propensity to develop sensitivity once an implant is in place.[99] While genetic composition may determine the likelihood to develop an allergy, the hygiene hypothesis postulates that a person's early environmental exposure also helps to establish their likelihood of developing hypersensitivity to commonplace environmental agents. Intriguingly, multiple studies demonstrate the inverse association between parasitic infections and chronic or severe allergies.[100] This proposed modification of the hygiene hypothesis proposes that the decline of helminth infections worldwide is associated with the increased prevalence of allergy-related diseases.[101] However, these findings depended on the strain of the parasite as meta-analyses found that some parasitic strains reduced the incidence of asthma, while infection with other strains increased this risk. Regardless, having an established allergic sensitivity or having been previously infected by parasites has lasting effects on a person's subsequent immune responses and should be considered as noninherited aspects of what establishes an individual's immune state and response to injury and tissue engineering systems.

The microbiome and intestinal infections dramatically influence development of lymphoid tissue as well as interactions and phenotyping of the immune system in the gut.[91,102] This is unsurprising as 20% of all lymphocytes reside in the gut. Factors such as obesity, malnutrition, increased caloric intake, and antibiotics influence immunity, specifically the memory T and B cells that develop with age.[102,103] For example, gut microbiota in aged mice have increased clearance of hepatitis B virus (HBV) compared with young mice.[104] Toll-like receptor activation, associated with lipopolysaccharide, allowed aged mice to promote HBV clearance. The history of microbiota in the gut is learned over time, altering each person's immunotype state.[105] The influence of the microbiome on tissue-engineered products has not yet been investigated, but is likely to be a fruitful area for investigation given the role of the microbiome in regulating a patient's immune state.

Age alters the immune state in diverging ways.[91,106–108] The loss of immune cells, reduced diversity of B and T cell clones, and response to the immunological challenge are all altered significantly with age. The variability of the immune state becomes more pronounced with age, indicating a cumulative effect of environmental (nonheritable) exposures. Additionally, the failure to maintain self-tolerance predisposes the aged population to autoimmune disease.[109] Macrophages and dendritic cells display reduced phagocytotic function in aged populations, and immunosenescence occurs in T cells that still possess effector function, but do not proliferate after activation.[110] Importantly, chronic low-grade inflammation occurs with aging, indicating the broad skewing of the immune system. The immunological changes that occur with aging likely impact the clinical responses to tissue-engineered products. Since most preclinical testing of tissue engineering technologies is performed in young animals, the variable performance in clinical studies may be due to the role of aging and variable aging phenotypes of patients.

The notion that each patient's medical history and environment play a large factor in their responses to tissue

healing is widely accepted. Systemic factors in the immune system impact local immune responses and healing. After hip surgery in patients, upregulation of signal transducer and activator of transcription 3 (*STAT3*) in $CD14^+$ monocyte populations correlated with surgical recovery (Table 1).[20] Elevated $CD8^+$ T cells in the patient's blood and at the fracture site correlated with reduced bone repair (Table 1).[35,36] Both examples highlight the influence of the immune system in healing. Personalized medicine has become more widely accepted for drug development and cancer therapies, and future therapies that target the immune system will face high clinical variability due to each patient's personalized immune state and medical history. The immunological variability of each patient helps to explain why many therapeutics fail to achieve similar results in the clinic while achieving success in limited preclinical models.

Conclusion

While the contribution of a patient's medical history and environmental factors is generally viewed as an obvious set of factors, proregenerative and immunomodulatory therapy development by researchers traditionally does not take this into account. Just as a clinician must view each patient's medical history and previous environmental exposure as critical factors in determining treatment regimens, immunotherapy research and engineering product design can also take into consideration these factors. Thus, there is a need to further understand the impact of variability in immune states on tissue-engineered product efficacy. As we seek to advance regenerative engineering products, the immune state and any desired manipulation should inform product design.

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Disclosure Statement

J.H.E. is an inventor on intellectual property related to biological scaffolds and inhibiting fibrosis. J.H.E. is a consultant to ACell and Unity Biotechnology and a founder of Aegeria.

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Address correspondence to:
Erika M. Moore, PhD
Department of Materials Science and Engineering
University of Florida
549 Gale Lemerand Drive
PO BOX 116400
Gainesville, Fl 32611
USA

E-mail: moore.erika@ufl.edu

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