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Biomaterials Strategies for Generating Therapeutic Immune Responses

Sean H. Kelly*, Lucas S. Shores*, Nicole L. Votaw*, and Joel H. Collier

Duke University, Department of Biomedical Engineering

Abstract

Biomaterials employed to raise therapeutic immune responses have become a complex and active field. Historically, vaccines have been developed primarily to fight infectious diseases, but recent years have seen the development of immunologically active biomaterials towards an expanding list of non-infectious diseases and conditions including inflammation, autoimmunity, wounds, cancer, and others. This review structures its discussion of these approaches around a progression from single-target strategies to those that engage increasingly complex and multifactorial immune responses. First the targeting of specific individual cytokines is discussed, both in terms of delivering the cytokines or blocking agents, and in terms of active immunotherapies that raise neutralizing immune responses against such single cytokine targets. Next, non-biological complex drugs such as randomized polyamino acid copolymers are discussed in terms of their ability to raise multiple different therapeutic immune responses, particularly in the context of autoimmunity. Last, biologically derived matrices and materials are discussed in terms of their ability to raise complex immune responses in the context of tissue repair. Collectively, these examples reflect the tremendous diversity of existing approaches and the breadth of opportunities that remain for generating therapeutic immune responses using biomaterials.

Graphical Abstract

Keywords

Immunoengineering; Inflammation; Autoimmunity; Wound Healing; Vaccine

Corresponding Author Joel H. Collier, PhD Associate Professor, Duke University, Biomedical Engineering Department, 101 Science Drive Campus Box 90281, Durham, NC 27708, joel.collier@duke.edu.

*equal contributions

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1. Introduction

Recently there has been an explosion of new research designing biomaterials for intervening in immunological processes to achieve therapeutic results in applications ranging from chronic inflammation to autoimmunity to cancer. This is occurring in part because basic immunology has taken great strides in recent decades, and many underlying mechanisms of immune activation and tolerance have been discovered. This depth of knowledge has begun to approach that which is necessary for a system to be “engineerable,” giving rise to the burgeoning field of immunomodulatory biomaterials[1]. Further, the rapid expansion of available biomaterials systems that have been introduced in the past few decades provides a rich toolbox from which to draw. New immune-active materials span multiple aspects, including size, with nanoparticles on one end of the spectrum and decellularized matrices and other complex bulk materials on the other. They also vary greatly with respect to whether they target a single cytokine, such as those that employ monoclonal antibodies or other single-target biologics, or whether they engage more integrated and complex processes, such as matrices that influence the polarization of macrophages or T cell phenotypes. This review is structured around this spectrum, with single-target interventions on one end and biomaterials affecting broad, multifactorial aspects of immunity on the other end (Figure 1). Paradoxically, strategies that target a single cytokine or factor can have off-target or difficult-to-predict effects, while those that seek to establish a specific therapeutic immune phenotype using multiple factors can produce a more “targeted” overall response. Examples discussed here will cover a range of clinical applications but will generally avoid discussions of infectious disease, the historic purview of vaccines [2,3]; rather, we will emphasize work towards raising therapeutic responses in non-infectious diseases and conditions. Indeed, one of the most exciting aspects of biomaterials’ application with immunology is how they can enable finely tuned immune strategies well beyond traditional vaccination. Cancer immunotherapy is also receiving tremendous interest from a biomaterials perspective, but we will likewise de-emphasize cancer applications in favor of focusing on other therapeutic applications because there exist excellent recent reviews on cancer immunotherapy [4–6]. Additionally, owing to the range of concepts discussed, we will focus on specific, exemplary publications rather than exhaustive lists of all studies in each area.

2. Low complexity: Single cytokine interventions

2.1. Delivering or blocking single cytokines

The first portion of this review will focus on work targeting single cytokines or other factors, with subsequent sections discussing more complex, multi-component, and multi-target strategies. It will present illustrative examples of biomaterials that have been used to perturb specific cytokines, and it will place biomaterials-based approaches in the context of other anti-cytokine technologies.

Immune and inflammatory pathology is characterized by the dysregulation of cellular homeostatic mechanisms [7–9]. Deviance from homeostasis can be triggered by a wide range of conditions, including trauma, infectious disease, inflammation, cancer, congenital disorders, aging, implantation of a biomaterial device, or others. In order to discover

therapeutic targets for intervening in the underlying immune processes of these conditions, work is underway to understand the disease etiology specific to each condition, and to establish which pivotal molecular signals regulate the progression of each disease. For inflammatory conditions, a—relatively discrete set of cytokines—the intercellular chemical signals of the immune system—have emerged as key targets, and biomaterials have been employed to either deliver or block them with appropriate spatial and temporal distributions. Agonistic or antagonistic action against a single cytokine can initiate, alter, or quench signaling cascades that modulate disease. Key mediators of inflammation that have received particular attention within biomaterials strategies include Interleukin 1 beta (IL1 β), IL4, interferon- γ (IFN γ), IL6, IL17, IL23, tumor necrosis factor (TNF), and the classically anti-inflammatory IL10 [10,11]. In the inflammatory milieu, these factors can be upregulated in positive feedback loops when regulatory checkpoints fail. Despite the complexity of inflammatory signaling, the interconnectedness of its networks allows specific targets to be effective therapeutics for treating disease. While the pleiotropic nature of cytokines allows for broad effects when they are singly targeted, such strategies can have negative off-target effects that arise from their systemic administration and their short half-lives in circulation, necessitating technologies that can prolong and localize anti-cytokine signals in a tissue site of interest.

As an archetypical example, IL1 β is a pro-inflammatory cytokine produced throughout the body, central in the inflammatory response to infection [7]. It mediates local inflammation by stimulating the translocation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) to the nucleus where it upregulates pro-inflammatory cytokines, including additional IL1 β , as well as IL6, IL23, and TNF. As one of the master regulators of the inflammatory process, functioning IL1 β is implicated in several chronic and acute inflammatory conditions, such as rheumatoid arthritis (RA) and osteoarthritis (OA) [12–14], as well as in congenital autoinflammatory diseases such as Familial Mediterranean fever (FMF), neonatal onset multisystem inflammatory disease (NOMID), Behçet's disease, and deficiency of the IL1 receptor antagonist (DIRA) [15]. Accordingly, therapeutics targeting IL1 β are the primary or alternative treatment for these diseases. Fortuitously, IL1 β has a natural competitor: the IL1 receptor antagonist (IL1Ra), which regulates IL1 β activity by binding to the IL1 receptor without activating it, providing competitive inhibition for the effector molecule [16,17]. One therapy, anakinra (brand name Kineret), an IL1Ra mutant, consists of daily 100 mg subcutaneous injections, but systemic delivery has disadvantages, including increased susceptibility to serious infection, neutropenia, increased risk of lymphoma, allergic reactions including anaphylaxis, and injection site reactions such as redness and swelling [18–20]. It is the only approved therapy for NOMID, a congenital disease caused by mutations in NLRP3 signaling which leads to excessive inflammasome activation through IL1 β mediated inflammation [21], and it is also approved for patients with moderate to severe RA, but only for those who have failed prior treatments with one or more disease modifying anti-rheumatic drugs [22]. Anakinra has also been evaluated for treating OA via intraarticular injection, but a multi-center study demonstrated no significant difference between it and placebo, attributed to the low residence time of the drug [23].

Owing to challenges with delivering IL1Ra directly, it has become an attractive target for biomaterials-based strategies aiming to improve its biodistribution and pharmacokinetics.

Recent strategies have included biomaterial-IL1Ra conjugates, which work to both prolong the delivery of IL1Ra and to increase or maintain the bioactivity of the molecule. For example, García and colleagues developed a novel nanoparticle system for delivering IL1Ra to the intraarticular space (Figure 2A), which preserved the bioactivity of the protein compared to other hydrophobic synthetic scaffolds (i.e. poly(lactic-co-glycolic acid) (PLGA) [24]. These particles also demonstrated increased retention time in the articular space with a half-life of 3.01 ± 0.09 days for IL1Ra particles compared to 0.96 ± 0.08 days for soluble IL1Ra (Figure 2B). Follow-up work by the same research group demonstrated that modulation of the size of the particle from 500 nm to 900 nm using a poly-hydroxyethylmethacrylate (pHEMA) backbone functionalized with hydrophobic pyridine side chains increased average particle retention time in the knee joint (Figure 2C–D) [25]. The pHEMA particles were then used in an *in vitro* setting with IL1Ra, and it was demonstrated that cells treated with IL1 β and exposed to IL1Ra nanoparticles maintained NF- κ B expression levels similar to those of unstimulated controls, demonstrating protection [26]. These preclinical data are positive indications for the combination of effective localization with high bioactivity, and together they demonstrate different facets of how biomaterials can be employed to deliver single-cytokine targeted biologics.

In another strategy to prolong the residency of IL1Ra within the intraarticular space, elastin-like polypeptides (ELPs) have been investigated both as fusion proteins and as depot-forming materials. ELPs are a class of polypeptides utilizing a repetitive Val-Pro-Gly-Xaa-Gly motif, where Xaa can be any amino acid except proline [27]. ELPs have interesting phase transition properties and can aggregate in response to small changes in temperature near a critical phase transition temperature. Although ELP fusion proteins had lower bioactivity compared to native IL1Ra [28], a depot formed by entrapping anakinra in ELP particles prolonged the drug's residence in the intraarticular space substantially, from 4 hours to more than 5 days in mice [29]. Using this technology Kimmerling et al. demonstrated in a mouse model of post-traumatic arthritis that arthritis progression could be prevented by immediate treatment following trauma.

Not every molecular target has a natural competitive inhibitor like the IL1 β /IL1Ra system, however, and therapeutic materials have been developed using other means for limiting cellular exposure to inflammatory cytokines. For example, the Anseth lab has developed gels of polyethylene glycol (PEG) that antagonize TNF via a short affinity peptide.

The peptide-functionalized PEG hydrogel shielded PC12 cells, mouse islet cells, and human mesenchymal stem cells from the deleterious effects of TNF when the cells were encapsulated in the gel [30]. The Anseth lab has also developed affinity peptide-functionalized PEG hydrogels for sequestering monocyte chemotactic protein 1 (MCP-1), a chemokine that recruits memory T cells, dendritic cells, and monocytes[31]. These gels reduced the release of MCP-1 by encapsulated pancreatic β -cells, suggesting the possibility of using such materials to dampen host immune responses after cell transplantation, where inflammatory reactions initiated by the implantation procedure could damage the cells being delivered.

Some situations require not a diminishment but an enhancement of inflammation, for example cancer immunotherapy. Pradhan et al. recently used PLGA particles functionalized with IL10-targeted siRNA and the toll-like receptor 9 (TLR9) agonist CpG oligodeoxynucleotide (ODN) to boost anti-tumor immunity [32]. Although only one cytokine was targeted, the combination of the IL10-targeted siRNA and the CpG could elicit a Th1 response. In this example, the biomaterial's ability to co-deliver both signals enhanced the effectiveness of each component.

Biomaterials directly intervening in IL1 β and other individual inflammatory cytokines illustrate some of the advantages and disadvantages of single-cytokine targeted therapeutics. Advantages include prolonged delivery, enhanced avidity, relative simplicity of the system (a biologic plus a biomaterial carrier), the ability to co-deliver more than one signal, and increased half-life. Despite the simplicity of such an approach, there are some potential limitations. First, IL1Ra can have off-target effects, such as increasing susceptibility to serious infections and inducing neutropenia[19,20,33]. Second, when delivering a protein-based biologic, it is possible that the biomaterial scaffold itself can augment immune responses against the biologic, against scaffold components, or against neoepitopes created from any linkages between the two. Such biomaterial-augmented immunogenicity can be complex, and a number of physical parameters influencing the immunogenicity of biomaterials scaffolds are being elucidated. Recent work by Andorko et al. demonstrated that the immunogenicity of synthetic scaffolds alters as particles degrade [34], complementing previous work showing that molecular weight [35], size [36], charge [37,38], hydrophobicity [39,40], environmental signals [41], chemical functionality [42–44], biomaterial type [45,46], and shape [47,48] are all factors that can influence the strength and phenotype of anti-biomaterials immune responses. Beyond practical considerations of location and release, the thoughtful design of each of these parameters should be involved in the design of a biomaterial for targeting a specific cytokine.

2.2. Active immunotherapies targeting single cytokines

Biologics, especially monoclonal antibodies, have seen widespread clinical use for anti-cytokine treatments, exemplified by the blockbuster success of adalimumab (Humira), a fully humanized antibody targeting human TNF that has been used clinically to treat a range of diseases that include rheumatoid arthritis[49], Crohn's disease[50], and ankylosing spondylitis[51]. Building on the success of anti-TNF therapies, other antibodies targeting other cytokines such as IL17 have also emerged as new therapeutics. One example, ixekizumab (Taltz) received FDA approval as recently as 2016 for the treatment of plaque psoriasis—with complete control of symptoms in more than 80% of individuals treated [52]. While these treatments demonstrate that specific cytokine targeting has tremendous therapeutic potential, the approach has some disadvantages. Antibody treatments require repeated injections, as frequently as every two weeks, which contributes to patients developing anti-drug antibodies (up to 28% of patients taking Humira [53] and 15.2% of patients taking Taltz [52] developed anti-drug antibodies within one to three years of drug administration). These anti-drug antibodies can be neutralizing, rendering the antibody ineffective.

Current research in biomaterials is poised to build on the success of monoclonal antibody therapy while addressing its limitations. Active immunotherapy, in which antibodies are raised *in situ* against autologous targets, is under development as such an alternative. In active immunotherapy, an immunizing material is used to raise antibody responses against a desired target without generating a damaging or uncontrolled autoimmune response. Among candidates for active immunotherapy, virus-like particles (VLPs) and other self-assembling peptides and proteins are attractive potential platforms. VLPs are assemblies of viral coat proteins lacking the genetic material necessary for viral replication. Although VLPs are not always thought of as “biomaterials”, they share many structural features of biomaterials, being nanoscale particulates composed of self-assembled proteins. These particles can generate robust responses based on the immunogenic structure of the viral capsid, which can display foreign epitopes in a precisely uniform and highly concentrated manner without risk of infection. Prophylactic VLP-based vaccines are already in widespread clinical use, for example Cervarix and Gardasil, but VLPs raising therapeutic responses against harmful self-proteins have yet to be translated significantly to the clinic. We will deemphasize the work that has been done with prophylactic vaccines and instead focus on the use of therapeutic VLPs for the purposes of active immunotherapy.

The advantages of using VLPs and other biomaterials for active immunotherapy are multifaceted. Many VLPs are already well characterized from years of development, they self-assemble by nature, and the design principles that dictate their geometry and size have been well-studied [54,55]. While many icosahedral viruses were utilized in early formulations of VLPs, and icosahedral bacteriophage VLPs are in clinical development, other structures include enveloped viruses, spherical influenza, and HIV [55], which enable the delivery of larger proteins. VLPs contain T cell epitopes, which together with VLPs' capacity for extremely regular and multivalent display can collectively initiate T cell-dependent antibody responses.

The primary obstacle to the development of an active immunotherapy against an autologous target is overcoming immune tolerance to the auto-antigen without triggering autoimmunity; in other words, the capacity to break B cell tolerance and raise therapeutic antibodies without breaking T cell tolerance [56]. Indeed, the maintenance of B cell tolerance in healthy individuals is significantly achieved by the requirement for T cell help for most antibody responses. To overcome this biological barrier to effective auto-antibody response, VLPs, which contain effective viral T cell epitopes, are combined with B cell epitopes from self-antigens. Using VLPs as a biomaterial to co-deliver these two types of epitopes in conjunction with adjuvant has been successful in surmounting B cell tolerance and producing antibodies against self-targets without triggering autoimmunity in the form of cytotoxic T cells [56]. The uniform patterning of antigens on the surface of VLPs can also trigger T cell-independent antibody responses by crosslinking surface immunoglobulin on B cells [57,58]. The combination of these two mechanisms of antibody induction allow for multiple pathways to produce therapeutic neutralizing antibodies.

One VLP that has received interest for designing active immunotherapies is the Q β bacteriophage, a 25 nm diameter virus that naturally infects *E. coli*. Conjugation of whole murine IL17 to the surface of a Q β bacteriophage VLP was shown to produce anti-IL17

antibodies and delay the onset of disease or reduce the severity of symptoms in models of experimental autoimmune myocarditis (Figure 3A–C), autoimmune arthritis (RA) (Figure 3D), and experimental autoimmune encephalitis in mice (Figure 3E) [59,60]. The same bacteriophage VLP technology has been used for vaccination against IL1 β and IL1 α [61]. It has been demonstrated that the IL1 α active immunotherapy had the capacity to limit the development of atherosclerosis in mouse models [62]. Preclinical work demonstrated the efficacy of an anti-IL1 β vaccine in mice [63] using a mutant IL1 β protein with 10,000-fold lower bioactivity than native IL1 β covalently linked to the Q β bacteriophage. The creation of a mutant IL1 β that could still induce an anti-IL1 β response without inducing systemic inflammation had been an obstacle to the prior development of such a vaccine. The IL1 β vaccine has progressed to clinical studies for the treatment of diabetes mellitus type 2 [64]. In an initial study, which combined preclinical and clinical results, 24 rhesus macaques and 36 human patients received the IL1 β vaccine. In both contexts, the vaccine induced neutralizing antibodies without any serious adverse events [64]. Only the highest dose of mutant-IL1 β -Q β -VLP vaccine, 900 μ g administered 6 times over 14 weeks, produced neutralizing antibodies in humans, whereas initial preclinical studies in mice had demonstrated neutralizing antibodies after a single injection and the preclinical study in rhesus macaques had shown induction within 3–4 injections. Encouragingly, participants in this initial clinical trial suffered no adverse side effects over the course of 48 weeks following initial vaccination, and antibody responses had an attractive half-life of about 7 weeks.

While initial results are promising, one challenge for the safe and reliable administration of active immunotherapy is the necessity of an “off” switch if the therapy has unintended side effects, or if treatment needs to be discontinued. Reports to date have shown that auto-antibodies typically have short half-lives between three months and one year without a booster vaccination [65,66]. This means that repeated injection of active immunotherapy agents is required to maintain immunity, and it presents the possibility that simply discontinuing boosting may be sufficient to halt treatment. These results have been recapitulated using VLP technology in rhesus macaques in a study with auto-antibodies targeting CCR5 for protection against a simian immunodeficiency virus [67], and the aforementioned study with mutant IL1-Q β VLPs, but as more studies are conducted in larger human populations the likelihood of contraindications and side effects increases. On the other hand, the development of a long-lived antibody response may be beneficial for individuals living with genetic diseases that require lifelong treatment. In either case, greater understanding of design principles of active immunotherapy platforms will be an important component in the progress of this field, and in this regard biomaterials engineers may be able to contribute.

The development of design principles for active immunotherapy is currently underway, and it is known that VLPs require careful construction and must be altered from their native structure to eliminate naturally immunosuppressive features of the viral capsid. Additionally, the production of VLPs in different organisms has been reported to affect immunogenicity, for example VLPs produced in *E. coli* have been shown to be less immunogenic than those produced in mammalian and yeast cells due to differing glycosylation [55]. It is also important to note that some auto-antibody responses can be elicited from B cells with

limited T cell help, and in fact this may represent a strategy for eliciting antibody response with shorter half-lives [68].

Taking lessons from VLPs and other supramolecular systems, the molecular self-assembly of short synthetic peptides also shows promise as a novel therapeutic vaccine platform by offering a modular, tunable incorporation of mixtures of B and T cell epitopes. Our group has investigated the β -sheet fibrillizing peptide Q11 (QQKFQFQFEQQ), which can be conjugated with a wide range of peptide epitopes. Mixing various Q11 derivatives allows for highly gradated control over epitope concentration in the assembled structure. The platform's capacity to display multiple B and T cell epitopes with stoichiometric control and its amenability to adjustment of the epitope content by simple mixing makes it useful for empirically determining optimal combinations of epitopes in various therapeutic contexts. Recent work by our group has suggested that beyond simple presence or absence of T cell epitopes in the context of B cell epitopes, the relative abundance of T cell epitopes affects the phenotype of the CD4⁺ T cell response by biasing it either toward a higher prevalence of T follicular helper (Tfh) cells or Th1/Th2 cells, while also modulating the general effector function phenotype of antibodies that are produced [69]. By controlling the relative concentration of T cell and B cell epitopes, as well as co-presentation of multiple epitopes in different immune contexts, the duration and magnitude of immune responses and antibody titers could potentially be modulated. Tools such as this could be used to develop a broader understanding of the design principles necessary to maximize the efficacy and safety of active immunotherapies, though much work remains in this regard.

3. Increased complexity: Synthetic biomaterials eliciting multifactorial responses in therapies for autoimmunity

3.1. Autoimmunity challenges

Whereas the previous section discussed strategies focused on targeting single, specific cytokines with biologics or adaptive immunotherapies, this section will focus on biomaterials that function through more complex and multifactorial immunological pathways. These types of biomaterials have been developed towards a range of clinical situations, but particularly for various autoimmune diseases, which we will emphasize here. Currently there are polymer-based immunotherapies in preclinical trials, clinical trials, and in approved treatments that modulate immunity broadly, towards treating a variety of autoimmune diseases including multiple sclerosis (MS), type 1 diabetes (T1D), systemic lupus erythematosus (SLE), and inflammatory bowel diseases such as Crohn's disease.

Autoimmune diseases affect 19% of the world population [70], and their incidence is rising. Caused by a combination of genetic and environmental factors, their etiologies are incompletely understood, and treatment options are limited, so broadly acting immunomodulatory biomaterials could offer significant advancement. Autoimmunity can arise when autoreactive T cells escape deletion by central tolerance mechanisms. Although several peripheral tolerance mechanisms exist to remove or suppress any such autoreactive T cells, in autoimmunity one or more of these also fail. These include anergy, deletion, T cell suppression through the induction of regulatory T cells (Tregs), and anatomical barriers such

as the blood-brain barrier. Initial stages of autoimmunity can engage small numbers of responding cells and small amounts of autoantigen, but many autoimmune diseases are characterized by cyclic episodes with worsening rounds of immune responses. For example, in relapsing-remitting multiple sclerosis, immune cells cross the blood-brain barrier to attack myelin in cyclic episodes that can worsen over time, and inflammatory bowel diseases (IBD) also commonly have a relapsing-remitting course. Although autoimmune diseases are diverse in both their targets and in their clinical progression, they share commonalities, and several aspects have been vigorously pursued for the development of therapeutics. For the randomized amino acid polymers that we focus on in this section, these processes include the presentation of peptide within major histocompatibility complex (MHC) molecules, the inflammatory phenotype of antigen-presenting cells, the polarization of the resultant T helper (Th) cell response, the induction of Treg cells, and the stimulation of therapeutic B cell responses. Notably, autoimmune diseases usually involve innate and adaptive immunity broadly, thus making integrated and broad-acting approaches advantageous.

3.2. Randomized amino acid copolymers

Glatiramer acetate (GA, Copaxone, copolymer 1, cop 1) is a randomized polyamino acid polymer that generates therapeutic immune responses for treating autoimmune disorders of the central nervous system, particularly multiple sclerosis (MS). Like VLPs, GA may not conventionally be identified as a “biomaterial”, but its copolymer construction makes it similar in some respects to other biopolymeric biomaterials, and the complex immune responses it generates raises important considerations for materials design. It is synthesized by the random polymerization of N-carboxyanhydride derivatives of four amino acids: lysine, glutamic acid, tyrosine, and alanine. It is polydisperse, with an average molecular weight between 4.7–11 kDa [71]. The drug, marketed as Copaxone by TEVA Pharmaceuticals, has had an interesting pathway of discovery and application. The copolymers that were originally synthesized were created to mimic myelin basic protein (MBP), with the intention of inducing, not treating, experimental autoimmune encephalomyelitis (EAE) [72]. However, the discoverers of GA were surprised to find that rather than inducing disease, GA protected mice from EAE [72]. Since publication of this finding in 1971, GA has been developed over the ensuing decades, was approved in multiple countries for the treatment of relapsing-remitting MS in 1995, and is now a widely prescribed drug [73]. Recently a generic version marketed as Glatopa was approved by the US-FDA [74], and other generics have been marketed and administered worldwide. Glatimer by Natco Pharma is sold in India and the Ukraine, Probioglat by Probiomed is sold in Mexico, and both Escadra (Raffo Laboratories) and Polimunol GTR (Synthon) are sold in Argentina [75]. Collectively these molecules have been termed glatiramoids [75,76]. Because they are non-biological complex drugs composed of difficult-to-define randomized mixtures of polyamino acids, the specific biological activity of glatiramoids is highly dependent on their manufacturing processes. It has been found that with the exception of Glatopa and Copaxone [77], formulations manufactured with slightly differing processes can induce variable gene expression profiles in responding immune cells [78].

Even though GA itself is a simple molecule, its mechanism of action is complex, in some respects an opposite concept compared with the single-cytokine strategies discussed in the

previous section that seek only to engage one target. GA's multifactorial mechanism of action has been increasingly clarified over the past forty-plus years, and a recent review of these mechanisms counts at least eleven distinct processes that constitute its overall effect in treating multiple sclerosis [79]. These effects can be broken down into three categories, including direct neuroprotection (e.g. the reduction of nerve injury, remyelination, neurogenesis); immunomodulation within the central nervous system; and peripheral immunomodulation outside the central nervous system [79]. We will focus on these latter peripheral immunomodulation mechanisms, as they are illustrative of the complex adaptive immune effects that biomaterials can induce. These immune-specific effects involve most of the essential cells of adaptive immunity, including antigen-presenting cells, T helper cells, and B cells.

With respect to antigen presenting cells, one of GA's proposed mechanisms of action involves GA peptide epitopes competing for MHC class II binding sites with autologous proteins, mainly myelin basic protein. In a patient with MS, peptide epitopes from myelin basic protein and other targets are presented within MHC class II molecules, which stimulate autoreactive T cells that ultimately polarize towards a harmful Th1-type response [71] (Figure 4A). To intervene in this process, GA is believed to promiscuously bind to MHC class II molecules, thus displacing myelin epitopes from the MHC binding groove and blocking myelin-specific T cell responses [71,79,80]. In this process, it is thought that the randomness of each GA peptide strand plays an important role, raising the chances that one or more specific epitopes within GA can out-compete the myelin basic protein epitopes that induce MS. The randomized, complex nature of GA appears to be essential, as specific altered peptide ligands based on myelin basic protein have either been encephalitogenic [81] or ineffective [82] in clinical trials.

Beyond diminishing the presentation of autoreactive peptides in MHC class II, GA also has additional effects on cells of the innate immune system, as it has been shown in dendritic cells from GA-treated MS patients that GA induces them to produce lower levels of the inflammatory cytokines TNF and IL12, along with higher levels of the anti-inflammatory cytokine IL10 [79,83]. Additional diminishment of inflammation arises from GA reducing the secretion of the strongly inflammatory cytokine IL1 β by monocytes [84]. Although GA clearly has strong effects on innate cells, inducing them to decrease their production of inflammatory cytokines and to increase their production of anti-inflammatory cytokines, the fundamental mechanism of how GA achieves this remains to be fully elucidated.

One consequence of the diminished inflammatory phenotype of innate cells elicited by GA is a resultant skewing of T cells away from inflammatory pathways such as Th1 towards the anti-inflammatory Th2 and Th3 pathways [71,74,85]. Th2 cells produce IL4, IL5, IL10, and IL13, resulting in a non-inflammatory phenotype and strong antibody (IgG1 and IgG4) production. Th3 cells, whose differentiation is enhanced by IL4, IL10, and TGF β , are also non-inflammatory, secreting IL10 and TGF β and further inhibiting Th1 and Th2 cells. In contrast, Th1 and Th17 cells are inflammatory, secreting IFN γ , IL17, and other inflammatory cytokines, respectively. This shift from pro-inflammatory Th1 cells towards an anti-inflammatory Th2-profile has been observed not only in animal models and *in vitro*, but also in MS patients receiving GA [86,87]. Additionally, GA has been found to restore the

frequency and function of Treg cells, which are otherwise depleted in MS [88]. In these ways, skewing of T cells participates in the broader anti-inflammatory phenotype that GA elicits, reinforcing and potentiating anti-inflammatory signaling among and between populations of T cells, innate cells, and B cells.

B cell and antibody responses also appear to be involved in the therapeutic effects of GA. In patients, specific antibodies against GA have been detected [89,90]. These antibodies, even though they are directed against GA, appear not to interfere with GA's therapeutic effects. Their isotypes (IgG1 and IgG4) further indicate that a Th1-to-Th2 skewing is induced by GA, and patients with low relapse rates tend to have the highest anti-GA antibody titers, suggesting that the antibodies themselves are therapeutic [91,92]. In sum, GA has been shown to induce therapeutic effects through an extraordinarily broad range of immunological mechanisms.

Glatiramoids are a unique class of materials, yet researchers in the areas of biomaterials and drug delivery might ask a few key questions: What other polyamino acid biomaterials may have similarly broad and therapeutic immunological activities? What design rules could be extracted from GA, other randomized polyamino acids, or other designed polymeric biomaterials? And how can these types of molecules be tailored to maximally achieve broad-acting immunological effects that are optimally suited to treat different diseases? These questions, while fascinating and potentially transformative, largely remain to be definitively answered, as preclinical and clinical data indicate complex design rules. To date GA has been approved only for treating MS, but it has been investigated for treating other diseases, including Huntington's disease (HD) (Fig 4B), Alzheimer's disease (AD) (Fig 4C), macular degeneration (Fig 5D), inflammatory bowel disease (Fig 4E), and even cerebral malaria [93], illustrating how applicable biomaterials raising multifactorial immune responses could be. Towards Huntington's and Alzheimer's diseases, GA may produce neuroprotective and anti-inflammatory responses in the brain through brain-derived neurotrophic factor (BDNF) [94]. One study investigated the effects of GA on BDNF in astrocytes both *in vitro* and in R6/2 and YAC128 transgenic mouse models of HD. GA not only increased BDNF production in astrocytes, but preserved degenerating neurons and motor functions in mice, and increased the overall survival rate compared to controls [94]. In an AD mouse model, GA reduced the plaque formation common in AD, increased neurogenesis, and decreased cognitive decline [95]. Another disease that involves immunological and inflammatory dysregulation is age-related macular degeneration (AMD), which exerts a significant burden on the elderly population. The disease is characterized by the accumulation in the retina of small inflammatory nodules called drusen, leading to progressive blindness. Patients with AMD given GA as a weekly treatment showed a decrease in drusen over 12 weeks compared to placebo, illustrating a potential role for GA in treating AMD [96]. In another example, GA has been investigated for treating IBD, which causes weight loss, intestinal bleeding, diarrhea, and colon damage. In mice with acute experimental colitis, GA treatment reduced and ameliorated these symptoms [97]. TNF and IFN γ levels were depleted, while TGF β and IL10 increased [97], echoing the Th2 biasing that has been observed with GA in the context of treating MS.

Although these therapeutic effects in a range of different inflammatory and autoimmune diseases indicate that GA and similar polyamino acid copolymers may have utility well beyond MS, it remains challenging to tune the complex and multifactorial immune processes to achieve the precise combination of effects on antigen presenting cells, T cells, B cells, and tissue-specific cells that are maximally therapeutic for each disease. Other GA-like polymers have been investigated, with some promising results. In one study, the randomized copolymers VWAK and FYAK [98] were created using solid phase peptide synthesis [99], and FYAK was the most effective in stimulating the production of IL4 and IL10 from T cell lines [98]. Moreover, in experimental autoimmune uveoretinitis (EAU), FYAK was more immunosuppressive than GA, especially at lower concentrations, and it inhibited induction of the disease better than GA [100]. EAU is mediated by IFN γ and IL17 produced by Th1 and Th17 T cells, respectively, and while both GA and FYAK were previously shown to downregulate IFN γ and IL17 secretion, this study revealed that only FYAK inhibited the release of those cytokines in the draining lymph nodes [100], indicating that engineering improvements can be made to randomized polyamino acid copolymers to suit different disease contexts. The FYAK synthetic copolymer was later brought to clinical studies as a 52-mer peptide formulation with N-terminal acetylation. Initial clinical studies had promising results with non-significant reductions in lesion numbers accompanying induction of Th2 associated cytokines [101,102].

Other biomaterials approaches involving the precise mixing and co-delivery of multiple immune factors are also being developed. In one example towards type-I diabetes (T1D), Keselowsky and coworkers developed a PLGA-based microparticle system with an ability to prevent the onset of T1D in non-obese diabetic (NOD) mice [103]. This highly engineered system consisted of phagocytosable microparticles encapsulating vitamin D3 or insulin B(9–23) peptide, plus non-phagocytosable microparticles encapsulating TGF- β 1 and GM-CSF. The four factors together were able to prevent T1D in 40% of the mice receiving the treatment. Another formulation comprising microparticles containing denatured insulin and Puramatrix self-assembling peptide containing GM-CSF and CpG ODN 1826 also were able to protect 40% of NOD mice from T1D, in contrast with controls which uniformly became diabetic [104]. These approaches engage multiple immune pathways yet represent a more tailored approach compared to the random construction of glatiramoids.

4. High complexity: ECM-derived scaffolds inducing therapeutic immune and inflammatory responses

We continue to move towards increasingly complex biomaterials, from synthetic materials in the previous section to biologically sourced matrices and scaffolds in this section. Naturally derived scaffolds such as decellularized extracellular matrices are emerging as another class of materials capable of inducing therapeutic immune responses or productive shifts in immune phenotype. This new application of these materials contrasts with their historical use as matrices for reconstruction, for delivering cells and biological factors, or for controlled release. Although using biologically sourced scaffolds to deliver cells and factors remains an active area of research [105], as does the use of decellularized whole organs in regenerative medicine applications [106], such usage generally lies outside the focus of this

review and will be deemphasized here. Rather, in this section we will emphasize the alternative strategy of using the scaffold to stimulate productive immune and inflammatory responses. Using scaffolds to promote an integrated healing response also diverges from approaches in which scaffolds are loaded with cells prior to implantation, where the scaffold's main function is to promote greater survival, engraftment, and localization of the delivered cells. The immunological activity of the scaffold itself has emerged as an interesting and potentially powerful new dimension to be considered on top of these more established strategies already in development.

4.1. Naturally derived scaffolds show promise for VML in early clinical investigation

Decellularized ECM-based scaffolds have been used in a number of clinical settings, and more than 30 such products are commercially available [107]. In the past, these products have been used primarily to reinforce soft tissue, but naturally derived scaffolds are now emerging as a clinically useful therapeutic in a way that more intentionally engages active biological processes. Recent human studies have used these materials to treat volumetric muscle loss (VML), a condition in which the regenerative capacity of muscle is overwhelmed by the extent of loss. VML typically occurs as a result of traumatic injury, and current treatment options are limited in their ability to restore muscle mass and function. The use of decellularized matrices to promote a constructive healing response in VML patients has shown promise in small-cohort studies [108–110]. In this section, the clinical findings of these studies will be discussed, and a discussion of immunological processes potentially at work follows in sections 4.2 and 4.3.

In 2014, five individuals were treated with urinary bladder matrix (UBM) sheets, which were surgically inserted into the site of muscle loss and sutured to healthy adjacent tissue [108]. This treatment was used in combination with personalized physical therapy both before and after the surgery, with the pre-surgical physical therapy serving as a control to better isolate the effects of the scaffold on muscle repair. New soft tissue formation was observed at the site of the injury at six months post-surgery, and this was accompanied by at least a 25% increase in function in three of the five patients. This study expanded to a total of 13 patients in 2016, with the source of the scaffold being broadened in the eight new patients to additionally include either dermal ECM or small intestinal submucosa (SIS) rather than UBM in some patients [109]. Among the larger 13-patient cohort, there were average improvements in strength of 37.3% and in range-of-motion of 27.1%, while all patients showed an increase in post-operative bulk muscle. Electrodiagnostic analysis was performed on eight of the patients in the study, and among these, five showed postoperative improvement by either nerve conduction study or needle electromyography, suggesting the presence of innervated muscle. One patient in the study showed new tissue growth and functional improvement despite having complete prior atrophy of the hamstring (see Figure 5 for a compilation of data from this patient). The recruitment of perivascular stem cells (PVSCs) is thought to play a role in ECM scaffolds' ability to promote skeletal muscle regeneration, since PVSCs are multipotent cells that have been shown to be involved in the endogenous response to skeletal muscle injury. Here, immunolabeling of postoperative biopsies showed that PVSCs migrated out of their normal anatomic niche, potentially implicating them in new skeletal muscle formation. While these early clinical studies have

small sample sizes and limited controls, they have demonstrated promise in helping patients with traumatic injury who have exhausted current treatment options.

4.2. Scaffold decellularization and immune complexity

While the mechanisms behind these promising early clinical results are not fully understood, preclinical studies involved in the therapeutic development of ECM scaffolds have provided insight. Modulation of the immune system is among the key contributors, and it is this aspect of the response that will be emphasized here (for a recent review of the complete current mechanistic understanding, see [111]). The emerging picture is one in which the elicitation of a particular immune phenotype by acellular scaffolds plays a key role in producing an overall microenvironment that promotes constructive healing. Constructive immune phenotypes are distinguished from inflammatory, rejection-like immune responses directed against the scaffold or its residual cellular debris.

Scaffolds are decellularized before use by a variety of physical and chemical methods [112], which collectively remove initiators of the most adverse immune responses, but they do not eliminate all forms of immunogenicity. Since cells are not a functional component of decellularized scaffolds, there is reduced concern over detrimental cytotoxic T cell responses against the material [113]. However, due to variance in decellularization processes, the degree to which immunogenic cell remnants remain in scaffolds can vary significantly. DNA content has been proposed as a standard measure for evaluating the decellularization of ECM, both because of the practicality of DNA assays, and because of the relationship between remnant DNA and non-constructive responses [114]. Insufficient decellularization of scaffolds may impact the resulting immune phenotypes to these materials negatively. A study showed that macrophage responses to SIS-ECM scaffolds *in vitro* were skewed towards greater inflammatory M1 responses at lower extents of decellularization, while a more thorough decellularization protocol gave a more balanced M1/M2 phenotype [115]. While this effect was not observed when tested in an *in vivo* rat body wall repair model, there were differences in macrophage localization around the implant site, which the authors suggested may be indicative of varied inflammatory responses.

Incompletely decellularized scaffolds retain several types of remnants that increase their complexity and could contribute to the resulting immune phenotype. Damage-associated molecular patterns (DAMPs) are molecules that modulate immune responses by acting via conserved pattern recognition receptors. While DAMPs are typically endogenous molecules that alert the immune system to damage when present in aberrant locations, they could be introduced exogenously via implantation of poorly decellularized scaffolds. Intriguingly, there is limited evidence that DAMPs could potentially contribute to constructive responses. One study found that high mobility group box 1 (HMGB1) was present in both UBM and SIS scaffolds [116]. When UBM was cultured with THP-1 cells, the presence of an HMGB1 inhibitor (glycyrrhizin) caused the cells to upregulate TLR4 mRNA and to secrete more of the pro-inflammatory chemokines CCL2 and CCL4. While this suggests that HMGB1 could promote constructive responses and reduce inflammation, *in vivo* studies examining the effect of HMGB1 are needed to draw such a conclusion and to clarify how this molecule may interact with other parallel pathways in inflammation. The authors of this study noted

variability in HMGB1 content even among samples from the same source tissue; considering the variability in clinical successes, it will be important to further characterize the impact of HMGB1 and other immunogenic cellular remnants on constructive healing responses. As DAMPs represent a diverse class of molecules, it is unclear *a priori* whether each specific DAMP might be harmful, productive, or relatively neutral with regard to healing, or what amounts of each may constitute an appropriate window, and they remain interesting candidates for further investigation.

Another remnant of ECM scaffolds that could impact the resultant immune response are epitopes, such as the galactose-*alpha*-1,3-galactose (alpha gal) epitope. This carbohydrate epitope is present in the mammalian tissues from which natural scaffolds are sourced, including porcine SIS [117], but it is not present in humans, who have large amounts of preexisting, circulating anti-Gal antibodies [113]. These antibodies lead to hyperacute rejection of whole-organ xenografts, and it has been suggested that although host antibody responses are raised against the epitope after SIS implantation, they do not interfere with constructive tissue remodeling [10]. This would form an interesting parallel to the previously discussed glatiramer acetate, for which antibodies generated against the molecule do not interfere with its therapeutic effects.

4.3. Scaffolds promote a specific immune phenotype

Rather than a simple binary consideration of whether a scaffold elicits an adaptive immune response or not, focus has been directed towards more nuanced aspects of immune phenotype. It has become increasingly appreciated that the overall phenotype of any elicited immune response can be a major determinant of success, and that the phenotype of the T-cell response is important. It was observed more than a decade ago, in 2001, that porcine SIS scaffolds elicit Th2-type immune responses after implantation in mice [118]. Cytokine mRNA analysis revealed that IFN γ was decreased significantly in the graft site after implantation of these scaffolds compared with implantation of syngeneic muscle tissue, while IL4 was elevated, both of which are consistent with Th2 polarization. Additionally, the study found that anti-SIS antibodies were primarily of the IgG1 isotype, which also suggests a Th2 polarization.

The macrophage phenotype elicited by naturally derived scaffolds is closely related to the T helper phenotype, and is also likely an important contributor to constructive responses. Temporal macrophage transitions between the pro-inflammatory M1 phenotype and the alternatively-activated M2 phenotype are known to be important events in the regeneration and growth of tissues following injury [119,120]. In muscle, modulation of macrophage phenotype impacts the healing response at least in part through the interaction of macrophage effector molecules with PVSCs. Both M1 and M2 macrophages impact mitogenesis events at different points, and the switch to the M2 phenotype is critical for the differentiation of progenitor cell populations into mature muscle cells. Macrophages also mediate the degradation of implanted ECM scaffolds [121], resulting in the release of bioactive degradation products [111].

The ratio of M1 to M2 macrophages at the implant site has served as a key indicator, with constructive responses associated with an elevated M2:M1 ratio. This facet of innate

immune modulation appears to depend critically on the decellularization of the scaffold. It was shown in a rat abdominal wall repair model that addition of cellular components to the graft led to significantly lower post-implantation M2:M1 ratios, even when the cells used were autologous, and that M2-skewed responses were associated with less scarring and greater constructive remodeling [122]. A comparison of cellular and acellular dermal matrix grafts used for abdominal wall repair in vervet monkeys also found that the use of acellular grafts led to a lower degree of inflammation and more positive outcomes, though M1/M2 phenotypes were not studied [123]. A connection between temporal macrophage transitions, PVSCs, and muscle generation in the response to ECM scaffolds was drawn in a 2016 study from the Badylak group [124]. Using a porcine SIS powder pillow in a murine repair model, they observed high levels of M1-like (iNOS+) macrophages at day 3 following scaffold implantation, followed by a sharp decrease, with significantly lower levels of these cells than in untreated mice at days 7, 14, and 56. Conversely, M2-like (Fizz1+) macrophages became the dominant phenotype in ECM-treated, but not untreated, wounds after this transition. This transition was accompanied temporally by migration of PVSCs out of their perivascular niche and towards ECM-treated defect sites, as well as formation of skeletal muscle myotubes. One limitation of this and other studies of macrophage polarization is the use of limited markers that create a binary distinction between M1 and M2 macrophages, rather than viewing them as a heterogeneous and plastic population.

A recent study by Sadtler et al. [125] provided a key link between the initial observations of adaptive Th2 responses elicited by naturally derived scaffolds [118] and the more recent evidence that macrophage polarization is a critical determinant of constructive remodeling. Using bone and cardiac muscle derived ECM scaffolds in a murine quadriceps VML model, they observed an elevated CD4:CD8 ratio among T cells compared to saline-treated controls at one week post-injury, and T cells upregulated expression of the gene *Il4*, which encodes the IL4 cytokine. This upregulation was lost when ECM scaffolds were used in *Rag1*^{-/-} mice, which lack mature T and B cells. Similarly, macrophage polarization towards the M2 lineage with ECM scaffold treatment was seen in wild type, but not *Rag1*^{-/-} mice. Critically, in *Rag1*^{-/-} mice repopulated with WT CD4 T cells, expression of the key M2 marker CD206 was restored in myeloid cells, but this phenotypic rescue did not occur when the repopulating cells lacked a critical driver of the Th2-phenotype (Rictor) (Figure 6). Furthermore, this result was shown to be IL4-driven, as CD206 expression was greatly diminished in myeloid cells from *Il4ra*^{-/-} mice, which lack the IL4 receptor. These results aligned with a functional test of muscle regeneration in which transfer of WT, but not *Rictor*^{-/-}, CD4 T cells led to an increase in function. Collectively, these results showed that the pro-regenerative M2 macrophage phenotype elicited by ECM scaffolds is dependent on Th2 cells, and that the polarization proceeds through an IL4-dependent pathway. These findings lend more evidence to the concept that Th2 polarization is an important component of a productively immunogenic scaffold in tissue repair applications.

5. Two complementary paths for development: Understanding naturally derived scaffold components and rationally designing synthetic scaffolds

5.1 Introduction

While acellular scaffolds have shown initial clinical promise, as in the aforementioned VML studies, their continued development and expansion into further therapeutic contexts would be aided by a greater degree of engineerability. That is to say, a greater ability to define a desired phenotype for a certain situation and select a scaffold to promote that phenotype. This increase in engineerability would be hastened by the parallel pursuit of two complementary approaches: building a greater understanding of how naturally derived scaffolds work on a component level, and rationally designing and testing synthetic scaffolds.

5.1. Comparative and compositional studies of naturally derived scaffolds

The ECM scaffolds used in preclinical studies are derived from a number of different sources, and available clinical products are also produced from different species and tissues [114]. Matching the anatomical source of ECM with the site in which it will be used is one aspect that might guide ECM selection for particular applications. While studies have largely focused on regenerative applications, some have examined this idea of homologous ECM use in remodeling contexts, but did not show clear and distinct advantages over heterologous ECM scaffolds [126,127]. While not straightforwardly predictable, scaffold origin might have nuanced effects on how scaffolds engage the immune system. A recent *in vitro* analysis of decellularized ECM scaffolds' effects on bone marrow-derived macrophages (BMDMs) found that the tissue source of the scaffolds impacted macrophage phenotype [128]. Scaffolds derived from SIS, brain, esophageal, or colonic ECM caused a shift towards expression of M2 phenotypic markers on treated macrophages, while dermal ECM skewed the macrophages towards the M1 lineage, and no such shift was observed in macrophages treated with UBM or liver- or skeletal muscle-derived ECM.

It is not fully clear to what extent the degree of constructive remodeling can be attributed to compositional differences between variously sourced ECM scaffolds as opposed to the decellularization protocols or any uncharacterized debris from the decellularization process. The decellularization process differs between tissues, and must maintain a careful balance to avoid both poor decellularization and disruption of the ECM's native structure, and even the choice of sterilization method may impact the final product [112,129]. Connecting the composition and structure of decellularized ECM scaffolds to functional *in vivo* results in a precise manner would be a major boon to their clinical use. Unlike synthetic materials that can be designed bottom-up with control over each specific component, decellularized matrices have a high degree of built-in complexity, some of which is stripped out during their isolation from native tissues. Building an understanding of the effects of each component of these products would improve preclinical testing and might someday allow clinicians to select the appropriate scaffold for each therapeutic use.

The Elisseeff lab made an important step in this direction by developing a high-throughput method to link ECM composition and biological activity [130]. In this method, tissues were

In addition to tissue-specific differences in acellular scaffolds, there has been some research into the effects of the species and age of animals from which scaffolds are derived. An assessment of decellularized lung scaffolds from pigs, rats, primates, and humans revealed some interspecies differences after undergoing the same decellularization protocol [132]. For example, glycosaminoglycans made up a higher percentage of total ECM content in decellularized pig scaffolds than the other species, while elastin content in pig scaffolds was the lowest among the four species. While it is important to note that this study used whole decellularized lung, as it was done in the context of regenerative medicine, it suggests that matrix source species might be a variable to consider going forward. Regarding age, the impact of sourcing SIS-ECM from variously aged pigs was the subject of a pair of studies from the Badylak group [133,134]. Of note, the SIS-ECM from younger animals led to higher M2:M1 ratios at 14 days post-surgery in a rat abdominal wall defect model, and the M2-shifted macrophage phenotype was accompanied by more constructive remodeling outcomes in the rats [134].

While a greater understanding of the impacts of the source and composition of naturally derived scaffolds will lend a degree of engineerability to their use, more control may ultimately be afforded by the parallel development of synthetic platforms that combine multiple immune signals. These scaffolds can be more easily tailored due to the control researchers have over the synthesis process, which in turn facilitates the use of design-build-test cycles. The nascent field of immune engineering has designed several materials that allow for control over their immune modulating elements [135–137]. In addition, even long-

studied biomaterials such as alginate, agarose, chitosan, hyaluronic acid, and PLGA can have varying effects on the phenotypic aspects of the immune response, such as T cell polarization [138]. In this way, even seemingly immunologically inert materials may have more subtle and complex interactions with the immune system than is generally recognized. Defined biomaterials also facilitate intervention in specific aspects such as the M1/M2 polarization of macrophages [139–144]; as one recent example, Reeves et al. demonstrated repolarization of M1 to M2 and reversed using controlled release from a coated silk polymer [141]. The silk biomaterials enabled a localized, short-term release of either IFN γ or IL4 to shift macrophage polarization between M1 and M2 [145].

Approaches based on self-assembling peptides, such as the aforementioned Q11 peptide, allow for the incorporation of desired epitopes during synthesis. These materials elicit a relatively unpolarized or moderately Th2-polarized T cell response and strong B-cell/antibody response [69,137,146]. In particular, their ability to raise strong antibody responses without inflammatory adjuvants has received attention [69,146,147]. The ability to co-assemble fibers bearing different epitopes allows for the formation of supramolecular assemblies with precise control over the concentration of each epitope within the assembly; one important immunological consequence of this control that has been demonstrated is that the ratio of B and T cell epitopes can alter the resulting T cell phenotype after immunization [69]. By utilizing an additional fibrillizing peptide domain, protein ligands were also incorporated into Q11-based assemblies while retaining their native activity [148]. This provides the potential to explore several new areas of immune engagement, by attaching conformational protein epitopes or functional proteins such as cytokines or chemokines. The use of solid-phase peptide synthesis allows for precise changes to the material's structure that can alter the resulting immune phenotype. For example, the incorporation of D-amino acids into the self-assembling motif KFE8 (FKFEFKFE) produced nanofibers that, when conjugated to the model epitope ovalbumin (OVA) and used to immunize mice, led to significantly higher levels of IgG, IgM, and IgA than the L-amino acid variant [149]. Such a change might allow for tuning the level of immune response to match the therapeutic context.

The Tirrell group has pioneered the use of another platform for engaging the immune system, peptide amphiphiles (PAs). PAs consist of a lipid tail region joined to a hydrophilic peptide head group, and they self-assemble to form micelles. When an immunogenic epitope is selected as the head group, PAs can elicit self-adjuvanting cytotoxic responses to CD8 epitopes [150] or antibody responses to B cell epitopes [151]. These responses can be further augmented by co-assembling the micelles with TLR agonists [151]. Micelles composed of polymers have also been developed for immune engagement, such as a RAFT-polymerized block copolymer micelle from the Wilson lab [152]. This approach utilized a fatty acid-mimetic core to overcome solubility challenges associated with the small molecule TLR7 agonist imiquimod, and a hydrophilic corona for antigen attachment. Intranasal immunization of mice with OVA and IMQ-loaded micelles led to both cellular and humoral immune responses. In a conceptually related approach, the Irvine group developed interbilayer-crosslinked multilamellar vesicles, which utilize an aqueous core and lipid bilayers to co-deliver antigen and adjuvant, eliciting immune responses after both subcutaneous and pulmonary immunization [153,154]. Hubbell, Swartz, and coworkers have

engineered Pluronic-stabilized polypropylene sulfide nanoparticles, which can be synthesized at ultra-small (25 nm) sizes to target dendritic cells in draining lymph nodes via efficient transport through lymphatic capillaries [155]. When conjugated to antigen, these nanoparticles can act as vaccines, eliciting adaptive immune responses in the absence of adjuvant by activating the complement system. They can also be formulated with additional adjuvant to enhance or alter the resulting immune response [156,157]. The choice of whether and which adjuvants to include in these platforms lends an additional input to their design

In fact, while adjuvants were once referred to as the "immunologist's dirty little secret," they are becoming a truly engineerable component of biomaterial design [158]. Jewell and coworkers have used layer-by-layer assembly to create immune polyelectrolyte multilayers (iPEMs) composed of adjuvant and antigen on gold nanoparticle templates [159,160]. The use of negatively charged nucleic TLR agonists and positively charged peptidic antigens allows for these two immune signals to be codelivered to antigen presenting cells [161], while obviating the need for additional components that could impact the resulting immune response. The synthetic process also provides control over the number of layers in the iPEM, and the process can be modified to use sacrificial templates to form iPEM capsules composed exclusively of antigen and adjuvant [162]. The Esser-Kahn lab has performed studies that explore the synergistic effects of combining multiple adjuvants [163,164]. Interestingly, in immunization against heat-inactivated vaccinia virus, a small-molecule core tri-functionalized with agonists for TLRs 4, 7, and 9 served as a better platform than a simple mixture of the three agonists, generating an antibody response against a larger number of antigens [164]. As with the importance of co-assembling T and B cell epitopes in peptide nanofibers mentioned above, design rules may emerge regarding the use of such adjuvant combinations, though more research is needed.

While the discovery of design principles for material engagement of the immune system have come largely from the use of particulate biomaterials in models of communicable diseases and cancer, these platforms can also be utilized as scaffolds in therapeutic contexts. This flexibility was demonstrated by the recent use of supramolecular Q11 scaffolds in a wound-healing context, in a study which challenged the notion that engagement of the adaptive immune system is harmful to wound healing [165]. In this study, pre-immunization of mice with unadjuvanted OVA-Q11, followed by placement of OVA-Q11 in the wound, did not delay healing of dermal wounds in 3 murine models, including splinted and unsplinted wounds, and C57BL/6 and *ob/ob* mice. Healing occurred despite substantial antibody responses raised by the materials. In contrast, when mice were immunized with OVA-Q11 and CFA, followed by placement of OVA-Q11 and incomplete Freund's adjuvant in the wound, healing was delayed in unsplinted *ob/ob* mice. The difference in healing rates between adjuvanted and non-adjuvanted assemblies highlights the fact that the use of highly immunogenic synthetic materials is compatible with good wound healing, if the correct phenotype of immune response develops. As seen with naturally derived scaffolds, Th2-skewed responses were more conducive to constructive healing, as CD4 T cells from the wound sites of mice given unadjuvanted Q11 scaffolds had significantly higher IL4/IFN γ production ratios.

This study differed from the aforementioned work with naturally derived scaffolds, in that the Q11 scaffolds were not used to improve healing, but rather shown not to interfere. However, while the use of synthetic scaffolds as the primary therapeutic treatment for healing has not yet been demonstrated, they hold promise in two respects. First, they are attractive platforms for continuing to elucidate mechanistic questions about how healing is impacted by elicited immune phenotypes. Second, as this greater understanding develops and researchers are able to more precisely control biomaterials' engagement with the immune system, it can be leveraged to rationally design synthetic scaffolds as therapeutics. In both of these avenues, the engineerability of the synthetic scaffolds is a significant advantage. In contrast to naturally derived scaffolds, the components of synthetic scaffolds can be more precisely defined, and are more readily adjusted. We suggest that the research of synthetic scaffolds in both mechanistic and therapeutic contexts is a promising strategy concurrent to the continued use of naturally derived scaffolds.

6. Conclusions

In this review we discussed immunologically active biomaterials in current development for raising therapeutic responses to treat a range of diseases and disorders, from inflammation to autoimmunity to large wounds. We began with strategies that seek to target one cytokine only, whether by delivering it, blocking it, or inducing an antibody response against it in an active immunotherapy approach. We then progressed to discuss approaches that engage increasingly complex immune responses, focusing on non-biological complex drugs such as glatiramoids and moving on to biologic scaffolds that raise therapeutic responses in applications such as wound healing. In each context, we highlighted the potential for engineered and chemically defined materials to make a contribution both in understanding and in optimizing the multifactorial immune responses that are necessary for maximal therapeutic effect. Clearly, considering the breadth of these examples, immunologically active biomaterials have a broad reach and stand to make significant contributions across biomedicine. In each of these examples, even the seemingly simple ones targeting only one cytokine, the ultimate success depends on first understanding what combined phenotype of the contributing immune cells affords maximal therapeutic effect, and then engineering the delivery of those factors to achieve that effect reproducibly in diverse patient populations. Given the variability in human immunity, this may ultimately be the greatest challenge for widespread development of immunologically active biomaterials, and technologies that reliably function in diverse individuals will achieve the greatest success.

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References

1. Vishwakarma A, Bhise NS, Evangelista MB, Rouwkema J, Dokmeci MR, Ghaemmaghami AM, et al. Engineering Immunomodulatory Biomaterials To Tune the Inflammatory Response. *Trends in Biotechnology*. 2016; 34:470–482. DOI: 10.1016/j.tibtech.2016.03.009 [PubMed: 27138899]

2. van Riet E, Ainai A, Suzuki T, Kersten G, Hasegawa H. Combatting infectious diseases; nanotechnology as a platform for rational vaccine design. *Adv Drug Deliv Rev.* 2014; 74:28–34. DOI: 10.1016/j.addr.2014.05.011 [PubMed: 24862579]
3. Fan Y, Moon JJ. Particulate delivery systems for vaccination against bioterrorism agents and emerging infectious pathogens. *Wiley Interdiscip Rev Nanomed Nanobiotechnol.* 2017; 9:e1403.doi: 10.1002/wnan.1403
4. Koshy ST, Mooney DJ. Biomaterials for enhancing anti-cancer immunity. *Curr Opin Biotechnol.* 2016; 40:1–8. DOI: 10.1016/j.copbio.2016.02.001 [PubMed: 26896596]
5. Melief CJM, van Hall T, Arens R, Ossendorp F, van der Burg SH. Therapeutic cancer vaccines. *J Clin Invest.* 2015; 125:3401–3412. DOI: 10.1172/JCI80009 [PubMed: 26214521]
6. Mehta NK, Moynihan KD, Irvine DJ. Engineering New Approaches to Cancer Vaccines. *Cancer Immunol Res.* 2015; 3:836–843. DOI: 10.1158/2326-6066.CIR-15-0112 [PubMed: 26156157]
7. Mills KHG, Dunne A. Immune modulation: IL-1, master mediator or initiator of inflammation. *Nat Med.* 2009; 15:1363–1364. DOI: 10.1038/nm1209-1363 [PubMed: 19966773]
8. Ku CC, Murakami M, Sakamoto A, Kappler J, Marrack P. Control of Homeostasis of CD8+ Memory T Cells by Opposing Cytokines. *Science.* 2000; 288:675–678. DOI: 10.1126/science.288.5466.675 [PubMed: 10784451]
9. Van Parijs L, Abbas AK. Homeostasis and Self-Tolerance in the Immune System: Turning Lymphocytes off. *Science.* 1998; 280:243–248. DOI: 10.1126/science.280.5361.243 [PubMed: 9535647]
10. Venkatesha S, Dudics S, Acharya B, Moudgil K. Cytokine-Modulating Strategies and Newer Cytokine Targets for Arthritis Therapy. *Int J Mol Sci.* 2014; 16:887–906. DOI: 10.3390/ijms16010887 [PubMed: 25561237]
11. Siebert S, Tsoukas A, Robertson J, McInnes I, Touyz RM. Cytokines as Therapeutic Targets in Rheumatoid Arthritis and Other Inflammatory Diseases. *Pharmacol Rev.* 2015; 67:280–309. DOI: 10.1124/pr.114.009639 [PubMed: 25697599]
12. Goldring MB, Marcu KB. Cartilage homeostasis in health and rheumatic diseases. *Arthritis Res Ther.* 2009; 11:224.doi: 10.1186/ar2592 [PubMed: 19519926]
13. Fukui N, Zhu Y, Maloney WJ, Clohisy J, Sandell LJ. Stimulation of BMP-2 Expression by Pro-Inflammatory Cytokines IL-1 and TNF- α in Normal and Osteoarthritic Chondrocytes. *J Bone Joint Surg.* 2003; 85:59–66. DOI: 10.1002/1529-0131(200106)44:6<1237::AID-ART214>3.0.CO;2-F [PubMed: 12925611]
14. Pelletier JP, Martel Pelletier J, Abramson SB. Osteoarthritis, an inflammatory disease: Potential implication for the selection of new therapeutic targets. *Arthritis Rheumatol.* 2001; 44:1237–1247. DOI: 10.1002/1529-0131(200106)44:6<1237::AID-ART214>3.0.CO;2-F
15. Martinon F, Aksentijevich I. New players driving inflammation in monogenic autoinflammatory diseases. *Nat Rev Rheumatol.* 2015; 11:11–20. DOI: 10.1038/nrrheum.2014.158 [PubMed: 25247411]
16. Palmer G, Guerne PA, Mezin F, Maret M, Guicheux J, Goldring MB, et al. Production of interleukin-1 receptor antagonist by human articular chondrocytes. *Arthritis Res Ther.* 2002; 4:226.doi: 10.1186/ar411
17. Seitz M, Loetscher P, Dewald B, Towbin H, Ceska M, Baggiolini M. Production of interleukin-1 receptor antagonist, inflammatory chemotactic proteins, and prostaglandin E by rheumatoid and osteoarthritic synoviocytes--regulation by IFN-gamma and IL-4. *J Immunol.* 1994; 152:2060–2065. [PubMed: 8120407]
18. Fleischmann R, Stern R, Iqbal I. Anakinra: an inhibitor of IL-1 for the treatment of rheumatoid arthritis. *Expert Opin Biol Ther.* 2005; 4:1333–1344. DOI: 10.1517/14712598.4.8.1333
19. Furst DE. Anakinra: Review of recombinant human interleukin-I receptor antagonist in the treatment of rheumatoid arthritis. *Clin Ther.* 2004; 26:1960–1975. DOI: 10.1016/j.clinthera.2004.12.019 [PubMed: 15823761]
20. Bresnihan B, Alvaro Gracia JM, Cobby M, Doherty M, Domljan Z, Emery P, et al. Treatment of rheumatoid arthritis with recombinant human interleukin-1 receptor antagonist. *Arthritis Rheumatol.* 1998; 41:2196–2204. DOI: 10.1002/1529-0131(199812)41:12<2196::AID-ART15>3.0.CO;2-2

21. Goldbach-Mansky R. Current Status of Understanding the Pathogenesis and Management of Patients With NOMID/CINCA. *Curr Rheumatol Rep*. 2011; 13:123–131. DOI: 10.1007/s11926-011-0165-y [PubMed: 21538043]
22. Zink A. Treatment continuation in patients receiving biological agents or conventional DMARD therapy. *Ann Rheum Dis*. 2005; 64:1274–1279. DOI: 10.1136/ard.2004.031476 [PubMed: 15708884]
23. Chevalier X, Goupille P, Beaulieu AD, Burch FX, Bensen WG, Conrozier T, et al. Intraarticular injection of anakinra in osteoarthritis of the knee: A multicenter, randomized, double-blind, placebo-controlled study. *Arthritis Rheumatol*. 2009; 61:344–352. DOI: 10.1002/art.24096
24. Whitmire RE, Scott Wilson D, Singh A, Levenston ME, Murthy N, García AJ. Self-assembling nanoparticles for intra-articular delivery of anti-inflammatory proteins. *Biomaterials*. 2012; 33:7665–7675. DOI: 10.1016/j.biomaterials.2012.06.101 [PubMed: 22818981]
25. Singh A, Agarwal R, Diaz Ruiz CA, Willett NJ, Wang P, Lee LA, et al. Nanoengineered Particles for Enhanced Intra-Articular Retention and Delivery of Proteins. *Adv Healthc Mater*. 2014; 3:1562–1567. DOI: 10.1002/adhm.201400051 [PubMed: 24687997]
26. Agarwal R, Volkmer TM, Wang P, Lee LA, Wang Q, García AJ. Synthesis of self-assembled IL-1Ra-presenting nanoparticles for the treatment of osteoarthritis. *J Biomed Mater Res A*. 2016; 104:595–599. DOI: 10.1002/jbm.a.35601 [PubMed: 26507256]
27. Meyer DE, Chilkoti A. Purification of recombinant proteins by fusion with thermally-responsive polypeptides. *Nature Biotechnology*. 1999; 17:1112–1115. DOI: 10.1038/15100
28. Shamji MF, Betre H, Kraus VB, Chen J, Chilkoti A, Pichika R, et al. Development and characterization of a fusion protein between thermally responsive elastin-like polypeptide and interleukin-1 receptor antagonist: Sustained release of a local antiinflammatory therapeutic. *Arthritis Rheumatol*. 2007; 56:3650–3661. DOI: 10.1002/art.22952
29. Kimmerling KA, Furman BD, Mangiapani DS, Moverman MA, Sinclair SM, Huebner JL, et al. Sustained intra-articular delivery of IL-1RA from a thermally-responsive elastin-like polypeptide as a therapy for post-traumatic arthritis. *Eur Cell Mater*. 2015; 29:124–140. DOI: 10.22203/eCM.v029a10 [PubMed: 25636786]
30. Lin CC, Metters AT, Anseth KS. Functional PEG peptide hydrogels to modulate local inflammation induced by the pro-inflammatory cytokine TNF α . *Biomaterials*. 2009; 30:4907–4914. DOI: 10.1016/j.biomaterials.2009.05.083 [PubMed: 19560813]
31. Lin CC, Boyer PD, Aimetti AA, Anseth KS. Regulating MCP-1 diffusion in affinity hydrogels for enhancing immuno-isolation. *J Control Release*. 2010; 142:384–391. DOI: 10.1016/j.jconrel.2009.11.022 [PubMed: 19951731]
32. Pradhan P, Qin H, Leleux JA, Gwak D, Sakamaki I, Kwak LW, et al. The effect of combined IL10 siRNA and CpG ODN as pathogen-mimicking microparticles on Th1/Th2 cytokine balance in dendritic cells and protective immunity against B cell lymphoma. *Biomaterials*. 2014; 35:5491–5504. DOI: 10.1016/j.biomaterials.2014.03.039 [PubMed: 24720881]
33. Fleischmann R, Stern R, Iqbal I. Anakinra: an inhibitor of IL-1 for the treatment of rheumatoid arthritis. *Expert Opin Biol Ther*. 2005; 4:1333–1344. DOI: 10.1517/14712598.4.8.1333
34. Andorko JI, Hess KL, Pineault KG, Jewell CM. Intrinsic immunogenicity of rapidly-degradable polymers evolves during degradation. *Acta Biomater*. 2016; 32:24–34. DOI: 10.1016/j.actbio.2015.12.026 [PubMed: 26708710]
35. Scheibner KA, Lutz MA, Boodoo S, Fenton MJ, Powell JD, Horton MR. Hyaluronan Fragments Act as an Endogenous Danger Signal by Engaging TLR2. *J Immunol*. 2006; 177:1272–1281. DOI: 10.4049/jimmunol.177.2.1272 [PubMed: 16818787]
36. Tomi S, Ito J, Vasiliji S, Ogrinc N, Rudolf R, Pelicon P, et al. Size-Dependent Effects of Gold Nanoparticles Uptake on Maturation and Antitumor Functions of Human Dendritic Cells In Vitro. *PLoS ONE*. 2014; 9:e96584.doi: 10.1371/journal.pone.0096584 [PubMed: 24802102]
37. Wen Y, Waltman A, Han H, Collier JH. Switching the Immunogenicity of Peptide Assemblies Using Surface Properties. *ACS Nano*. 2016; 10:9274–9286. DOI: 10.1021/acsnano.6b03409
38. Gallorini S, Berti F, Parente P, Baronio R, Aprea S, D'Oro U, et al. Introduction of Zwitterionic Motifs into Bacterial Polysaccharides Generates TLR2 Agonists Able to Activate APCs. *J Immunol*. 2007; 179:8208–8215. DOI: 10.4049/jimmunol.179.12.8208 [PubMed: 18056364]

39. Moyano DF, Goldsmith M, Solfiell DJ, Landesman-Milo D, Miranda OR, Peer D, et al. Nanoparticle Hydrophobicity Dictates Immune Response. *J Am Chem Soc.* 2012; 134:3965–3967. DOI: 10.1021/ja2108905 [PubMed: 22339432]
40. Seong SY, Matzinger P. Hydrophobicity: an ancient damage-associated molecular pattern that initiates innate immune responses. *Nat Rev Immunol.* 2004; 4:469–478. DOI: 10.1038/nri1372 [PubMed: 15173835]
41. Yoshida M, Babensee JE. Molecular aspects of microparticle phagocytosis by dendritic cells. *J Biomater Sci Polym Ed.* 2012; 17:893–907. DOI: 10.1163/156856206777996844
42. Sunshine JC, Peng DY, Green JJ. Uptake and Transfection with Polymeric Nanoparticles Are Dependent on Polymer End-Group Structure, but Largely Independent of Nanoparticle Physical and Chemical Properties. *Mol Pharm.* 2012; 9:3375–3383. DOI: 10.1021/mp3004176 [PubMed: 22970908]
43. Shankar SP, Petrie TA, García AJ, Babensee JE. Dendritic cell responses to self-assembled monolayers of defined chemistries. *J Biomed Mater Res A.* 2010; 92A:1487–1499. DOI: 10.1002/jbm.a.32487
44. Shankar SP, Chen II, Keselowsky BG, García AJ, Babensee JE. Profiles of carbohydrate ligands associated with adsorbed proteins on self-assembled monolayers of defined chemistries. *J Biomed Mater Res A.* 2010; 92A:1329–1342. DOI: 10.1002/jbm.a.32457
45. Yoshida M, Babensee JE. Differential effects of agarose and poly(lactic-co-glycolic acid) on dendritic cell maturation. *J Biomed Mater Res A.* 2006; 79A:393–408. DOI: 10.1002/jbm.a.30798
46. Norton LW, Park J, Babensee JE. Biomaterial adjuvant effect is attenuated by anti-inflammatory drug delivery or material selection. *J Control Release.* 2010; 146:341–348. DOI: 10.1016/j.jconrel.2010.05.032 [PubMed: 20595029]
47. Sunshine JC, Perica K, Schneck JP, Green JJ. Particle shape dependence of CD8+ T cell activation by artificial antigen presenting cells. *Biomaterials.* 2014; 35:269–277. DOI: 10.1016/j.biomaterials.2013.09.050 [PubMed: 24099710]
48. Bennewitz NL, Babensee JE. The effect of the physical form of poly(lactic-co-glycolic acid) carriers on the humoral immune response to co-delivered antigen. *Biomaterials.* 2005; 26:2991–2999. DOI: 10.1016/j.biomaterials.2004.08.023 [PubMed: 15603794]
49. van de Putte LBA, Atkins C, Malaise M, Sany J, Russell AS, van Riel PLCM, et al. Efficacy and safety of adalimumab as monotherapy in patients with rheumatoid arthritis for whom previous disease modifying antirheumatic drug treatment has failed. *Ann Rheum Dis.* 2004; 63:508–516. DOI: 10.1136/ard.2003.013052 [PubMed: 15082480]
50. Colombel JF, Sandborn WJ, Rutgeerts P, Enns R, Hanauer SB, Panaccione R, et al. Adalimumab for Maintenance of Clinical Response and Remission in Patients With Crohn's Disease: The CHARM Trial. *Gastroenterology.* 2007; 132:52–65. DOI: 10.1053/j.gastro.2006.11.041 [PubMed: 17241859]
51. van der Heijde D, Kivitz A, Schiff MH, Sieper J, Dijkmans BAC, Braun J, et al. Efficacy and safety of adalimumab in patients with ankylosing spondylitis: Results of a multicenter, randomized, double-blind, placebo-controlled trial. *Arthritis Rheumatol.* 2006; 54:2136–2146. DOI: 10.1002/art.21913
52. Gordon KB, Blauvelt A, Papp KA, Langley RG, Luger T, Ohtsuki M, et al. Phase 3 Trials of Ixekizumab in Moderate-to-Severe Plaque Psoriasis. *N Engl J Med.* 2016; 375:345–356. DOI: 10.1056/NEJMoa1512711 [PubMed: 27299809]
53. Bartelds GM, Kriekaert CLM, Nurmohamed MT, van Schouwenburg PA, Lems WF, Twisk JWR, et al. Development of Antidrug Antibodies Against Adalimumab and Association With Disease Activity and Treatment Failure During Long-term Follow-up. *Jama.* 2011; 305:1460–1468. DOI: 10.1001/jama.2011.406 [PubMed: 21486979]
54. Bachmann MF, Jennings GT. Vaccine delivery: a matter of size, geometry, kinetics and molecular patterns. *Nat Rev Immunol.* 2010; 10:787–796. DOI: 10.1038/nri2868 [PubMed: 20948547]
55. Roldão A, Mellado MCM, Castilho LR, Carrondo MJ, Alves PM. Virus-like particles in vaccine development. *Expert Rev Vaccines.* 2014; 9:1149–1176. DOI: 10.1586/erv.10.115

56. Link A, Bachmann MF. Immunodrugs: breaking B- but not T-cell tolerance with therapeutic anticytokine vaccines. *Immunotherapy*. 2010; 2:561–574. DOI: 10.2217/imt.10.30 [PubMed: 20636009]
57. Bachmann M, Rohrer U, Kundig T, Burki K, Hengartner H, Zinkernagel R. The influence of antigen organization on B cell responsiveness. *Science*. 1993; 262:1448–1451. DOI: 10.1126/science.8248784 [PubMed: 8248784]
58. Bachmann MF, Kalinke U, Althage A, Freer G, Burkhart C, Roost HP, et al. The Role of Antibody Concentration and Avidity in Antiviral Protection. *Science*. 1997; 276:2024–2027. DOI: 10.1126/science.276.5321.2024 [PubMed: 9197261]
59. Röhn TA, Jennings GT, Hernandez M, Grest P, Beck M, Zou Y, et al. Vaccination against IL-17 suppresses autoimmune arthritis and encephalomyelitis. *Eur J Immunol*. 2006; 36:2857–2867. DOI: 10.1002/eji.200636658 [PubMed: 17048275]
60. Sonderegger I, Röhn TA, Kurrer MO, Iezzi G, Zou Y, Kastelein RA, et al. Neutralization of IL-17 by active vaccination inhibits IL-23-dependent autoimmune myocarditis. *Eur J Immunol*. 2006; 36:2849–2856. DOI: 10.1002/eji.200636484 [PubMed: 17039570]
61. Spohn G, Keller I, Beck M, Grest P, Jennings GT, Bachmann MF. Active immunization with IL-1 displayed on virus-like particles protects from autoimmune arthritis. *Eur J Immunol*. 2008; 38:877–887. DOI: 10.1002/eji.200737989 [PubMed: 18253928]
62. Tissot AC, Spohn G, Jennings GT, Shamshiev A, Kurrer MO, Windak R, et al. A VLP-based vaccine against interleukin-1 α protects mice from atherosclerosis. *Eur J Immunol*. 2013; 43:716–722. DOI: 10.1002/eji.201242687 [PubMed: 23254454]
63. Spohn G, Schori C, Keller I, Sladko K, Sina C, Guler R, et al. Preclinical efficacy and safety of an anti-IL-1 β vaccine for the treatment of type 2 diabetes. *Mol Ther Methods Clin Dev*. 2014; 1:14048.doi: 10.1038/mtm.2014.48 [PubMed: 26015986]
64. Cavelti-Weder C, Timper K, Seelig E, Keller C, Osranek M, Lässig U, et al. Development of an Interleukin-1 β Vaccine in Patients with Type 2 Diabetes. *Mol Ther*. 2016; 24:1003–1012. DOI: 10.1038/mt.2015.227 [PubMed: 26686385]
65. Semerano L, Assier E, Boissier M-C. Anti-cytokine vaccination: A new biotherapy of autoimmunity? *Autoimmun Rev*. 2012; 11:785–786. DOI: 10.1016/j.autrev.2012.02.003 [PubMed: 22349621]
66. Chackerian B, Fritze KM. Moving towards a new class of vaccines for non-infectious chronic diseases. *Expert Rev Vaccines*. 2016; 15:561–563. DOI: 10.1586/14760584.2016.1159136 [PubMed: 26919571]
67. Chackerian B, Briglio L, Albert PS, Lowy DR, Schiller JT. Induction of Autoantibodies to CCR5 in Macaques and Subsequent Effects upon Challenge with an R5-Tropic Simian/Human Immunodeficiency Virus. *J Virol*. 2004; 78:4037–4047. DOI: 10.1128/JVI.78.8.4037-4047.2004 [PubMed: 15047820]
68. Zabel F, Kundig TM, Bachmann MF. Virus-induced humoral immunity: on how B cell responses are initiated. *Curr Opin Virol*. 2013; 3:357–362. DOI: 10.1016/j.coviro.2013.05.004 [PubMed: 23731601]
69. Pompano RR, Chen J, Verbus EA, Han H, Fridman A, McNeely T, et al. Titration of T-Cell Epitopes within Self-Assembled Vaccines Optimizes CD4+ Helper T Cell and Antibody Outputs. *Adv Healthc Mater*. 2014; 3:1898–1908. DOI: 10.1002/adhm.201400137 [PubMed: 24923735]
70. Lerner A, Jeremias P, Matthias T. The World Incidence and Prevalence of Autoimmune Diseases is Increasing. *International Journal of Celiac Disease*. 2015; 3:151–155. DOI: 10.12691/ijcd-3-4-8
71. Blanchette F, Neuhaus O. Glatiramer Acetate. *J Neurol*. 2008; 255:26–36. DOI: 10.1007/s00415-008-1005-5 [PubMed: 18317674]
72. Teitelbaum D, Meshorer A, Hirshfeld T, Arnon R, Sela M. Suppression of experimental allergic encephalomyelitis by a synthetic polypeptide. *Eur J Immunol*. 1971; 1:242–248. DOI: 10.1002/eji.1830010406 [PubMed: 5157960]
73. Weber MS, Hohlfeld R, Zamvil SS. Mechanism of action of glatiramer acetate in treatment of multiple sclerosis. *Neurotherapeutics*. 2007; 4:647–653. DOI: 10.1016/j.nurt.2007.08.002 [PubMed: 17920545]

74. Anderson J, Bell C, Bishop J, Capila I, Ganguly T, Glajch J, et al. Demonstration of equivalence of a generic glatiramer acetate (Glatopa). J Neurol Sci. 2015; 359:24–34. DOI: 10.1016/j.jns.2015.10.007 [PubMed: 26671082]
75. Weinstein, DV., Schwartz, DR., Grossman, DI., Zeskind, DB., Nicholas, DJM. Non-Biological Complex Drugs. Springer International Publishing; Cham: 2015. Glatiramoids; p. 107-148.
76. Varkony H, Weinstein V, Klinger E, Sterling J, Cooperman H, Komlos T, et al. The glatiramide class of immunomodulator drugs. Expert Opin Pharmacother. 2009; 10:657–668. DOI: 10.1517/14656560902802877 [PubMed: 19245345]
77. D'Alessandro JS, Duffner J, Pradines J, Capila I, Garofalo K, Kaundinya G, et al. Equivalent Gene Expression Profiles between Glatopa and CopaxoneR. PLoS ONE. 2015; 10:e0140299.doi: 10.1371/journal.pone.0140299 [PubMed: 26473741]
78. Towfic F, Funt JM, Fowler KD, Bakshi S, Blaugrund E, Artyomov MN, et al. Comparing the Biological Impact of Glatiramer Acetate with the Biological Impact of a Generic. PLoS ONE. 2014; 9:e83757.doi: 10.1371/journal.pone.0083757 [PubMed: 24421904]
79. Aharoni R. The mechanism of action of glatiramer acetate in multiple sclerosis and beyond. Autoimmun Rev. 2013; 12:543–553. DOI: 10.1016/j.autrev.2012.09.005 [PubMed: 23051633]
80. Weber MS, Prod'homme T, Youssef S, Dunn SE, Rundle CD, Lee L, et al. Type II monocytes modulate T cell mediated central nervous system autoimmune disease. Nat Med. 2007; 13:935–943. DOI: 10.1038/nm1620 [PubMed: 17676050]
81. Bielekova B, Goodwin B, Richert N, Cortese I, Kondo T, Afshar G, et al. Encephalitogenic potential of the myelin basic protein peptide (amino acids 83-99) in multiple sclerosis: Results of a phase II clinical trial with an altered peptide ligand. Nat Med. 2000; 6:1167–1175. DOI: 10.1038/80516 [PubMed: 11017150]
82. Kappos L, Comi G, Panitch H, Oger J, Antel J, Conlon P, et al. Induction of a non-encephalitogenic type 2 T helper-cell autoimmune response in multiple sclerosis after administration of an altered peptide ligand in a placebo-controlled, randomized phase II trial. Nat Med. 2000; 6:1176–1182. DOI: 10.1038/80525 [PubMed: 11017151]
83. Vieira PL, Heystek HC, Wormmeester J, Wierenga EA, Kapsenberg ML. Glatiramer acetate (copolymer-1, copaxone) promotes Th2 cell development and increased IL-10 production through modulation of dendritic cells. J Immunol. 2003; 170:4483–4488. DOI: 10.4049/jimmunol.170.9.4483 [PubMed: 12707324]
84. Burger D, Molnarfi N, Webber MS, Brandt KJ, Benkhoucha M, Gruaz L, et al. Glatiramer acetate increases IL-1 receptor antagonist but decreases T cell-induced IL-1 in human monocytes and multiple sclerosis. Pnas. 2009; 106:4355–4359. [PubMed: 19255448]
85. Racke MK, Lovett-Racke AE, Karandikar NJ. The mechanism of action of glatiramer acetate treatment in multiple sclerosis. Neurology. 2010; 74:S25–S30. DOI: 10.1212/WNL.0b013e3181c97e39 [PubMed: 20038760]
86. Neuhaus O, Farina C, Yassouridis A, Wiendl H, Then Bergh F, Dose T, et al. Multiple sclerosis: Comparison of copolymer-1- reactive T cell lines from treated and untreated subjects reveals cytokine shift from T helper 1 to T helper 2 cells. Pnas. 2000; 97:7452–7457. DOI: 10.1073/pnas.97.13.7452 [PubMed: 10861011]
87. Duda PW, Schmied MC, Cook SL, Krieger JJ, Hafler DA. Glatiramer acetate (CopaxoneR) induces degenerate, Th2-polarized immune responses in patients with multiple sclerosis. J Clin Invest. 2000; 105:967–976. DOI: 10.1172/JCI8970 [PubMed: 10749576]
88. Hong J, Li N, Zhang X, Zheng B, Zhang JZ. Induction of CD4+CD25+ regulatory T cells by copolymer-I through activation of transcription factor Foxp3. Pnas. 2005; 102:6449–6454. DOI: 10.1073/pnas.0502187102 [PubMed: 15851684]
89. Aharoni R, Eilam R, Stock A, Vainshtein A, Shezen E, Gal H, et al. Glatiramer acetate reduces Th-17 inflammation and induces regulatory T-cells in the CNS of mice with relapsing remitting or chronic EAE. J Neuroimmunol. 2010; 225:100–111. DOI: 10.1016/j.jneuroim.2010.04.022 [PubMed: 20554028]
90. Teitelbaum D, Brenner T, Abramsky O, Aharoni R, Sela M, Arnon R. Antibodies to glatiramer acetate do not interfere with its biological functions and therapeutic efficacy. Mult Scler. 2016; 9:592–599. DOI: 10.1191/1352458503ms963oa

91. Brenner T, Arnon R, Sela M, Abramsky O, Meiner Z, Riven-Kreitman R, et al. Humoral and cellular immune responses to Copolymer 1 in multiple sclerosis patients treated with CopaxoneR. *J Neuroimmunol.* 2001; 115:152–160. DOI: 10.1016/S0165-5728(01)00250-8 [PubMed: 11282165]
92. Farina C, Vargas V, Heydari N, Kämpfel T, Meinl E, Hohlfeld R. Treatment with glatiramer acetate induces specific IgG4 antibodies in multiple sclerosis patients. *J Neuroimmunol.* 2002; 123:188–192. DOI: 10.1016/S0165-5728(01)00490-8 [PubMed: 11880163]
93. Lackner P, Part A, Burger C, Dietmann A, Broessner G, Helbok R, et al. Glatiramer acetate reduces the risk for experimental cerebral malaria: a pilot study. *Malar J.* 2009; 8:36.doi: 10.1186/1475-2875-8-36 [PubMed: 19250545]
94. Reick C, Ellrichmann G, Tsai T, Lee DH, Wiese S, Gold R, et al. Expression of brain-derived neurotrophic factor in astrocytes - Beneficial effects of glatiramer acetate in the R6/2 and YAC128 mouse models of Huntington's disease. *Exp Neurol.* 2016; 285:12–23. DOI: 10.1016/j.expneurol.2016.08.012 [PubMed: 27587303]
95. Glatiramer acetate fights against Alzheimer's disease by inducing dendritic-like microglia expressing insulin-like growth factor 1. 2006:1–6.
96. Landa G, Rosen RB, Patel A, Lima VC, Tai KW, Perez VR, et al. Qualitative Spectral OCT/SLO Analysis of Drusen Change in Dry Age-Related Macular Degeneration Patients Treated with Copaxone. *J Ocul Pharmacol Ther.* 2011; 27:77–82. DOI: 10.1089/jop.2010.0109 [PubMed: 21254921]
97. Aharoni B, Kayhan B, Brenner O, Domev H, Labunskay G, Arnon R. Immunomodulatory Therapeutic Effect of Glatiramer Acetate on Several Murine Models of Inflammatory Bowel Disease. *J Pharmacol Exp Ther.* 2006; 318:68–78. DOI: 10.1124/jpet.106.103192 [PubMed: 16624971]
98. Stern JNH, Illés Z, Reddy J, Keskin DB, Sheu E, Fridkis-Hareli M, et al. Amelioration of proteolipid protein 139–151-induced encephalomyelitis in SJL mice by modified amino acid copolymers and their mechanisms. *Pnas.* 2004; 101:11743–11748. DOI: 10.1073/pnas.0403832101 [PubMed: 15292514]
99. Illés Z, Stern JNH, Keskin DB, Reddy J, Brosnan CF, Waldner H, et al. Copolymer effects on microglia and T cells in the central nervous system of humanized mice. *Eur J Immunol.* 2005; 35:3683–3693. DOI: 10.1002/eji.200526121 [PubMed: 16259003]
100. Yin H, Vistica BP, Chan CC, Strominger JL, Gery I. Inhibition of experimental autoimmune uveitis by amino acid copolymers. *J Neuroimmunol.* 2009; 215:43–48. DOI: 10.1016/j.jneuroim.2009.08.002 [PubMed: 19748134]
101. Kovalchin J, Krieger J, Genova M, Collins K, Augustyniak M, Masci A, et al. Results of a phase I study in patients suffering from secondary-progressive multiple sclerosis demonstrating the safety of the amino acid copolymer PI-2301 and a possible induction of an anti-inflammatory cytokine response☆. *J Neuroimmunol.* 2010; 225:153–163. DOI: 10.1016/j.jneuroim.2010.04.008 [PubMed: 20466440]
102. Kovalchin J, Krieger J, Collins K, Genova M, Augustyniak M, Masci A, et al. Safety, Pharmacokinetic, and Pharmacodynamic Evaluations of PI-2301, a Potent Immunomodulator, in a First-in-Human, Single-Ascending-Dose Study in Healthy Volunteers. *J Clin Pharmacol.* 2011; 51:649–660. DOI: 10.1177/0091270010373930 [PubMed: 20940336]
103. Lewis JS, Dolgova NV, Zhang Y, Xia CQ, Wasserfall CH, Atkinson MA, et al. A combination dual-sized microparticle system modulates dendritic cells and prevents type 1 diabetes in prediabetic NOD mice. *Clinical Immunology.* 2015; 160:90–102. DOI: 10.1016/j.clim.2015.03.023 [PubMed: 25842187]
104. Yoon YM, Lewis JS, Carstens MR, Campbell-Thompson M, Wasserfall CH, Atkinson MA, et al. A combination hydrogel microparticle-based vaccine prevents type 1 diabetes in non-obese diabetic mice. *Sci Rep.* 2015; 5:13155.doi: 10.1038/srep13155 [PubMed: 26279095]
105. Wolf MT, Dearth CL, Sonnenberg SB, Lobo EG, Badyak SF. Naturally derived and synthetic scaffolds for skeletal muscle reconstruction. *Adv Drug Deliv Rev.* 2015; 84:208–221. DOI: 10.1016/j.addr.2014.08.011 [PubMed: 25174309]
106. Yu Y, Alkhawaji A, Ding Y, Mei J. Decellularized scaffolds in regenerative medicine. *Oncotarget.* 2016; 7:58671–58683. DOI: 10.18632/oncotarget.10945 [PubMed: 27486772]

107. Brown BN, Badylak SF. Extracellular matrix as an inductive scaffold for functional tissue reconstruction. *Transl Res.* 2014; 163:268–285. DOI: 10.1016/j.trsl.2013.11.003 [PubMed: 24291155]
108. Sicari BM, Rubin JP, Dearth CL, Wolf MT, Ambrosio F, Boninger M, et al. An Acellular Biologic Scaffold Promotes Skeletal Muscle Formation in Mice and Humans with Volumetric Muscle Loss. *Sci Transl Med.* 2014; 6:234ra58–234ra58. DOI: 10.1126/scitranslmed.3008085
109. Dziki J, Badylak S, Yabroudi M, Sicari B, Ambrosio F, Stearns K, et al. An acellular biologic scaffold treatment for volumetric muscle loss: results of a 13-patient cohort study. *NPJ Regen Med.* 2016; 1:16008.doi: 10.1038/npjregenmed.2016.8
110. Han N, Yabroudi MA, Stearns-Reider K, Helkowski W, Sicari BM, Rubin JP, et al. Electrodiagnostic Evaluation of Individuals Implanted With Extracellular Matrix for the Treatment of Volumetric Muscle Injury: Case Series. *Phys Ther.* 2016; 96:540–549. DOI: 10.2522/ptj.20150133 [PubMed: 26564252]
111. Badylak SF, Dziki JL, Sicari BM, Ambrosio F, Boninger ML. Mechanisms by which acellular biologic scaffolds promote functional skeletal muscle restoration. *Biomaterials.* 2016; 103:128–136. DOI: 10.1016/j.biomaterials.2016.06.047 [PubMed: 27376561]
112. Keane TJ, Swinehart IT, Badylak SF. Methods of tissue decellularization used for preparation of biologic scaffolds and in vivo relevance. *Methods.* 2015; 84:25–34. DOI: 10.1016/j.ymeth.2015.03.005 [PubMed: 25791470]
113. Galili, U. Host Response to Biomaterials. Elsevier; 2015. Human Anti-Gal and Anti-Non-Gal Immune Response to Porcine Tissue Implants; p. 239-267.
114. Crapo PM, Gilbert TW, Badylak SF. An overview of tissue and whole organ decellularization processes. *Biomaterials.* 2011; 32:3233–3243. DOI: 10.1016/j.biomaterials.2011.01.057 [PubMed: 21296410]
115. Keane TJ, Londono R, Turner NJ, Badylak SF. Consequences of ineffective decellularization of biologic scaffolds on the host response. *Biomaterials.* 2012; 33:1771–1781. DOI: 10.1016/j.biomaterials.2011.10.054 [PubMed: 22137126]
116. Daly KA, Liu S, Agrawal V, Brown BN, Johnson SA, Medberry CJ, et al. Damage associated molecular patterns within xenogeneic biologic scaffolds and their effects on host remodeling. *Biomaterials.* 2012; 33:91–101. DOI: 10.1016/j.biomaterials.2011.09.040 [PubMed: 21967802]
117. McPherson TB, Liang H, Record RD, Badylak SF. Gal α (1,3)Gal Epitope in Porcine Small Intestinal Submucosa. *Tissue Eng.* 2004; 6:233–239. DOI: 10.1089/10763270050044416
118. Allman AJ, McPherson TB, Badylak SF, Merrill LC, Kallakury B, Sheehan C, et al. Xenogeneic extracellular matrix grafts elicit a Th2-restricted immune response. *Transplantation.* 2001; 71:1631. [PubMed: 11435976]
119. Tidball JG, Vialta SA. Regulatory interactions between muscle and the immune system during muscle regeneration. *Am J Physiol Regul Integr Comp Physiol.* 2010; 298:R1173–R1187. DOI: 10.1152/ajpregu.00735.2009 [PubMed: 20219869]
120. Deng B, Wehling-Henricks M, Vialta SA, Wang Y, Tidball JG. IL-10 Triggers Changes in Macrophage Phenotype That Promote Muscle Growth and Regeneration. *J Immunol.* 2012; 189:3669–3680. DOI: 10.4049/jimmunol.1103180 [PubMed: 22933625]
121. Valentin JE, Stewart-Akers AM, Gilbert TW, Badylak SF. Macrophage Participation in the Degradation and Remodeling of Extracellular Matrix Scaffolds. *Tissue Eng Part A.* 2009; 15:1687–1694. DOI: 10.1089/ten.tea.2008.0419 [PubMed: 19125644]
122. Brown BN, Valentin JE, Stewart-Akers AM, McCabe GP, Badylak SF. Macrophage phenotype and remodeling outcomes in response to biologic scaffolds with and without a cellular component. *Biomaterials.* 2009; 30:1482–1491. DOI: 10.1016/j.biomaterials.2008.11.040 [PubMed: 19121538]
123. Xu H, Wan H, Sandor M, Qi S, Ervin F, Harper JR, et al. Host Response to Human Acellular Dermal Matrix Transplantation in a Primate Model of Abdominal Wall Repair. *Tissue Eng Part A.* 2008; 14:2009–2019. DOI: 10.1089/ten.tea.2007.0316 [PubMed: 18593339]
124. Dziki JL, Sicari BM, Wolf MT, Cramer MC, Badylak SF. Immunomodulation and Mobilization of Progenitor Cells by Extracellular Matrix Bioscaffolds for Volumetric Muscle Loss Treatment. *Tissue Eng Part A.* 2016; 22:1129–1139. DOI: 10.1089/ten.tea.2016.0340 [PubMed: 27562630]

125. Sadtler K, Estrellas K, Allen BW, Wolf MT, Fan H, Tam AJ, et al. Developing a pro-regenerative biomaterial scaffold microenvironment requires T helper 2 cells. *Science*. 2016; 352:366–370. DOI: 10.1126/science.aad9272 [PubMed: 27081073]
126. Crapo PM, Medberry CJ, Reing JE, Tottey S, van der Merwe Y, Jones KE, et al. Biologic scaffolds composed of central nervous system extracellular matrix. *Biomaterials*. 2012; 33:3539–3547. DOI: 10.1016/j.biomaterials.2012.01.044 [PubMed: 22341938]
127. De Ward Aaron KJ, Londono Ricardo ST, Carey Lisa CA, et al. Tissue-Specific Effects of Esophageal Extracellular Matrix. *Tissue Eng Part A*. 2015; 21:2293–2300. DOI: 10.1089/ten.tea.2015.0322 [PubMed: 26192009]
128. Dziki JL, Wang DS, Pineda C, Sicari BM, Rausch T, Badylak SF. Solubilized extracellular matrix bioscaffolds derived from diverse source tissues differentially influence macrophage phenotype. *J Biomed Mater Res A*. 2017; 105:138–147. DOI: 10.1002/jbm.a.35894 [PubMed: 27601305]
129. Dearth CL, Keane TJ, Carruthers CA, Reing JE, Huleihel L, Ranallo CA, et al. The effect of terminal sterilization on the material properties and in vivo remodeling of a porcine dermal biologic scaffold. *Acta Biomater*. 2016; 33:78–87. DOI: 10.1016/j.actbio.2016.01.038 [PubMed: 26826528]
130. Beachley VZ, Wolf MT, Sadtler K, Manda SS, Jacobs H, Blatchley MR, et al. Tissue matrix arrays for high-throughput screening and systems analysis of cell function. *Nat Methods*. 2015; 12:1197–1204. DOI: 10.1038/nmeth.3619 [PubMed: 26480475]
131. Averill MM, Kerkhoff C, Bornfeldt KE. S100A8 and S100A9 in Cardiovascular Biology and Disease. *Arterioscler Thromb Vasc Biol*. 2012; 32:223–229. DOI: 10.1161/ATVBAHA.111.236927 [PubMed: 22095980]
132. Balestrini JL, Gard AL, Gerhold KA, Wilcox EC, Liu A, Schwan J, et al. Comparative biology of decellularized lung matrix: Implications of species mismatch in regenerative medicine. *Biomaterials*. 2016; 102:220–230. DOI: 10.1016/j.biomaterials.2016.06.025 [PubMed: 27344365]
133. Tottey S, Johnson SA, Crapo PM, Reing JE, Zhang L, Jiang H, et al. The effect of source animal age upon extracellular matrix scaffold properties. *Biomaterials*. 2011; 32:128–136. DOI: 10.1016/j.biomaterials.2010.09.006 [PubMed: 20870285]
134. Sicari BM, Johnson SA, Siu BF, Crapo PM, Daly KA, Jiang H, et al. The effect of source animal age upon the in vivo remodeling characteristics of an extracellular matrix scaffold. *Biomaterials*. 2012; 33:5524–5533. DOI: 10.1016/j.biomaterials.2012.04.017 [PubMed: 22575834]
135. Hotaling NA, Tang L, Irvine DJ, Babensee JE. Biomaterial Strategies for Immunomodulation. *Annu Rev Biomed Eng*. 2015; 17:317–349. DOI: 10.1146/annurev-bioeng-071813-104814 [PubMed: 26421896]
136. Wen Y, Collier JH. Supramolecular peptide vaccines: tuning adaptive immunity. *Curr Opin Immunol*. 2015; 35:73–79. DOI: 10.1016/j.coi.2015.06.007 [PubMed: 26163376]
137. Mora Solano C, Collier JH. Engaging adaptive immunity with biomaterials. *J Mater Chem B*. 2014; 2:2409–2421. DOI: 10.1039/C3TB21549K
138. Park J, Gerber MH, Babensee JE. Phenotype and polarization of autologous T cells by biomaterial-treated dendritic cells. *J Biomed Mater Res A*. 2015; 103:170–184. DOI: 10.1002/jbm.a.35150 [PubMed: 24616366]
139. Garg K, Pullen NA, Oskeritzian CA, Ryan JJ, Bowlin GL. Macrophage functional polarization (M1/M2) in response to varying fiber and pore dimensions of electrospun scaffolds. *Biomaterials*. 2013; 34:4439–4451. DOI: 10.1016/j.biomaterials.2013.02.065 [PubMed: 23515178]
140. Wolf MT, Dearth CL, Ranallo CA, LoPresti ST, Carey LE, Daly KA, et al. Macrophage polarization in response to ECM coated polypropylene mesh. *Biomaterials*. 2014; 35:6838–6849. DOI: 10.1016/j.biomaterials.2014.04.115 [PubMed: 24856104]
141. Reeves ARD, Spiller KL, Freytes DO, Vunjak-Novakovic G, Kaplan DL. Controlled release of cytokines using silk-biomaterials for macrophage polarization. *Biomaterials*. 2015; 73:272–283. DOI: 10.1016/j.biomaterials.2015.09.027 [PubMed: 26421484]
142. Vasconcelos DP, Costa M, Amaral IF, Barbosa MA, Águas AP, Barbosa JN. Modulation of the inflammatory response to chitosan through M2 macrophage polarization using pro-resolution

mediators. *Biomaterials*. 2015; 37:116–123. DOI: 10.1016/j.biomaterials.2014.10.035 [PubMed: 25453942]

143. Fuchs AK, Syrovets T, Haas KA, Loos C, Musyanovych A, Mailänder V, et al. Carboxyl- and amino-functionalized polystyrene nanoparticles differentially affect the polarization profile of M1 and M2 macrophage subsets. *Biomaterials*. 2016; 85:78–87. DOI: 10.1016/j.biomaterials.2016.01.064 [PubMed: 26854393]
144. Zanganeh S, Hutter G, Spitler R, Lenkov O, Mahmoudi M, Shaw A, et al. Iron oxide nanoparticles inhibit tumour growth by inducing pro-inflammatory macrophage polarization in tumour tissues. *Nat Nanotechnol*. 2016; 11:986–994. DOI: 10.1038/nnano.2016.168 [PubMed: 27668795]
145. Reeves ARD, Spiller KL, Freytes DO, Vunjak-Novakovic G, Kaplan DL. Controlled release of cytokines using silk-biomaterials for macrophage polarization. *Biomaterials*. 2015; 73:272–283. DOI: 10.1016/j.biomaterials.2015.09.027 [PubMed: 26421484]
146. Chen J, Pompano RR, Santiago FW, Maillat L, Sciammas R, Sun T, et al. The use of self-adjuvanting nanofiber vaccines to elicit high-affinity B cell responses to peptide antigens without inflammation. *Biomaterials*. 2013; 34:8776–8785. DOI: 10.1016/j.biomaterials.2013.07.063 [PubMed: 23953841]
147. Rudra JS, Mishra S, Chong AS, Mitchell RA, Nardin EH, Nussenzweig V, et al. Self-assembled peptide nanofibers raising durable antibody responses against a malaria epitope. *Biomaterials*. 2012; 33:6476–6484. DOI: 10.1016/j.biomaterials.2012.05.041 [PubMed: 22695068]
148. Hudalla GA, Sun T, Gasiorowski JZ, Han H, Tian YF, Chong AS, et al. Gradated assembly of multiple proteins into supramolecular nanomaterials. *Nat Mater*. 2014; 13:829–836. DOI: 10.1038/nmat3998 [PubMed: 24930032]
149. Appavu R, Chesson CB, Koyfman AY, Snook JD, Kohlhapp FJ, Zloza A, et al. Enhancing the Magnitude of Antibody Responses through Biomaterial Stereochemistry. *ACS Biomater Sci Eng*. 2015; 1:601–609. DOI: 10.1021/acsbiomaterials.5b00139
150. Black M, Trent A, Kostenko Y, Lee JS, Olive C, Tirrell M. Self-Assembled Peptide Amphiphile Micelles Containing a Cytotoxic T-Cell Epitope Promote a Protective Immune Response In Vivo. *Adv Mater*. 2012; 24:3845–3849. DOI: 10.1002/adma.201200209 [PubMed: 22550019]
151. Barrett JC, Ulery BD, Trent A, Liang S, David NA, Tirrell MV. Modular Peptide Amphiphile Micelles Improving an Antibody-Mediated Immune Response to Group A Streptococcus. *ACS Biomater Sci Eng*. 2016; acsbiomaterials.6b00422. doi: 10.1021/acsbiomaterials.6b00422
152. Sevimli S, Knight FC, Gilchuk P, Joyce S, Wilson JT. Fatty Acid-Mimetic Micelles for Dual Delivery of Antigens and Imidazoquinoline Adjuvants. *ACS Biomater Sci Eng*. 2017; 3:179–194. DOI: 10.1021/acsbiomaterials.6b00408
153. Moon JJ, Suh H, Bershteyn A, Stephan MT, Liu H, Huang B, et al. Interbilayer-crosslinked multilamellar vesicles as synthetic vaccines for potent humoral and cellular immune responses. *Nat Mater*. 2011; 10:243–251. DOI: 10.1038/nmat2960 [PubMed: 21336265]
154. Li AV, Moon JJ, Abraham W, Suh H, Elkhader J, Seidman MA, et al. Generation of Effector Memory T Cell Based Mucosal and Systemic Immunity with Pulmonary Nanoparticle Vaccination. *Sci Transl Med*. 2013; 5:204ra130–204ra130. DOI: 10.1126/scitranslmed.3006516
155. Reddy ST, van der Vlies AJ, Simeoni E, Angeli V, Randolph GJ, O'Neil CP, et al. Exploiting lymphatic transport and complement activation in nanoparticle vaccines. *Nature Biotechnology*. 2007; 25:1159–1164. DOI: 10.1038/nbt1332
156. Ballester M, Nembrini C, Dhar N, de Titta A, de Piano C, Pasquier M, et al. Nanoparticle conjugation and pulmonary delivery enhance the protective efficacy of Ag85B and CpG against tuberculosis. *Vaccine*. 2011; 29:6959–6966. DOI: 10.1016/j.vaccine.2011.07.039 [PubMed: 21787826]
157. de Titta A, Ballester M, Julier Z, Nembrini C, Jeanbart L, van der Vlies AJ, et al. Nanoparticle conjugation of CpG enhances adjuvancy for cellular immunity and memory recall at low dose. *Pnas*. 2013; 110:19902–19907. DOI: 10.1073/pnas.1313152110 [PubMed: 24248387]
158. Charles J, Janeway A. Pillars Article: Approaching the Asymptote? Evolution and Revolution in Immunology. *Cold Spring Harb Symp Quant Biol*. 1989. 54: 1–13. *J Immunol*. 2013; 191:4475–4487. [PubMed: 24141854]

159. Zhang P, Chiu YC, Tostanoski LH, Jewell CM. Polyelectrolyte Multilayers Assembled Entirely from Immune Signals on Gold Nanoparticle Templates Promote Antigen-Specific T Cell Response. *ACS Nano*. 2015; 9:6465–6477. DOI: 10.1021/acsnano.5b02153 [PubMed: 26035231]
160. Tostanoski LH, Chiu YC, Andorko JI, Guo M, Zeng X, Zhang P, et al. Design of Polyelectrolyte Multilayers to Promote Immunological Tolerance. *ACS Nano*. 2016; 10:9334–9345. DOI: 10.1021/acsnano.6b04001
161. Chiu YC, Gammon JM, Andorko JI, Tostanoski LH, Jewell CM. Assembly and Immunological Processing of Polyelectrolyte Multilayers Composed of Antigens and Adjuvants. *ACS Appl Mater Interfaces*. 2016; 8:18722–18731. DOI: 10.1021/acsami.6b06275 [PubMed: 27380137]
162. Chiu YC, Gammon JM, Andorko JI, Tostanoski LH, Jewell CM. Modular Vaccine Design Using Carrier-Free Capsules Assembled from Polyionic Immune Signals. *ACS Biomater Sci Eng*. 2015; 1:1200–1205. DOI: 10.1021/acsbomaterials.5b00375 [PubMed: 26689147]
163. Ryu KA, Slowinska K, Moore T, Esser-Kahn A. Immune Response Modulation of Conjugated Agonists with Changing Linker Length. *ACS Chem Biol*. 2016; 11:3347–3352. DOI: 10.1021/acscchembio.6b00895 [PubMed: 27749034]
164. Tom JK, Dotsey EY, Wong HY, Stutts L, Moore T, Davies DH, et al. Modulation of Innate Immune Responses via Covalently Linked TLR Agonists. *ACS Cent Sci*. 2015; 1:439–448. DOI: 10.1021/acscentsci.5b00274 [PubMed: 26640818]
165. Vigneswaran, Y., Han, H., De Loera, R., Wen, Y., Zhang, X., Sun, T., et al. Canada: Peptide biomaterials raising adaptive immune responses in wound healing contexts. *J Biomed Mater Res A*; Winner of the Student Award in the Hospital Intern Category, 10th World Biomaterials Congress; May 17–22, 2016; Montreal QC. 2016. p. 1853-1862.
166. Reick C, Ellrichmann G, Tsai T, Lee DH, Wiese S, Gold R, et al. Expression of brain-derived neurotrophic factor in astrocytes - Beneficial effects of glatiramer acetate in the R6/2 and YAC128 mouse models of Huntington's disease. *Exp Neurol*. 2016; 285:12–23. DOI: 10.1016/j.expneurol.2016.08.012 [PubMed: 27587303]
167. Butovsky O, Koronyo-Hamaoui M, Kunis G, Ophir E, Landa G, Cohen H, et al. Glatiramer acetate fights against Alzheimer's disease by inducing dendritic-like microglia expressing insulin-like growth factor 1. *Pnas*. 2006; 103:11784–11789. DOI: 10.1073/pnas.0604681103 [PubMed: 16864778]
168. Landa G, Butovsky O, Shoshani J, Schwartz M, Pollack A. Weekly Vaccination with Copaxone (Glatiramer Acetate) as a Potential Therapy for Dry Age-Related Macular Degeneration. *Curr Eye Res*. 2009; 33:1011–1013. DOI: 10.1080/02713680802484637

Figure 1.

Biomaterials raising therapeutic immune responses have been developed across a spectrum of complexity, from those targeting single cytokines (A) to those with increasingly complex and multifactorial mechanisms of action (B–D). Strategies targeting single cytokines have used monoclonal neutralizing antibodies, nanoparticles that competitively inhibit cytokine activation, and nanoparticles containing siRNA for the targeted cytokine (A). Although directed at a single target, such therapies can have off-target or pleiotropic effects, as a particular cytokine has multiple downstream effects (A, bottom). As a step up in complexity, active immunotherapies can raise polyclonal antibody responses in T-independent or T-dependent processes (B). More complexly, randomized polyamino acid copolymers used to treat autoimmune diseases engage a variety of immune processes including MHC blocking, T-cell biasing, and the induction of therapeutic antibodies (C). Even further, decellularized extracellular matrices can engage in multiple processes including T cell polarization and macrophage polarization (D). Paradoxically, single-target interventions can have off-target or pleiotropic effects (A, bottom), and more complex materials can elicit a reproducible overall phenotype (right). Current research in biomaterials immunology is beginning to elucidate strategies and mechanisms by which biomaterials can reproducibly engage these complex processes.

Figure 2.

The development of a nanoparticle system for delivery IL1Ra to the rat knee joint. Particles of 500 nm diameter were assembled and chemically conjugated with IL1Ra (A) before being tagged with a near-infrared dye and injected into the rat knee joint. Fluorescent intensity was then measured over 14 days and compared to a similarly tagged soluble IL1Ra to determine residence time in the joint (B). In a subsequent study 900 nm particles were developed (C) which maintained bioactivity while increasing total signal retention in the knee as measured by near-infrared fluorescent signal intensity (D). Reproduced with permission from references [24] (A–B), [26] (C), and [25] (D).

Figure 3.

Active Immunotherapy used in mice to treat inflammatory conditions by targeting the cytokine IL17 with a Q β bacteriophage VLP. VLPs conjugated with IL17 elicited high anti-IL17 titers of IgG antibodies (A). In the context of myocarditis, mice receiving IL17-targeted active immunotherapy had (B) reduced clinical scores for inflammation, and (C) decreased immune cell infiltration and fibrosis as demonstrated by Hematoxylin and Eosin staining. Furthermore, in both models of anti-collagen antibody-induced arthritis (D) and Experimental Autoimmune Encephalitis (E) severity and progression of disease was limited in the IL17-targeted treatment group. Reproduced with permission from references [60] (A-C) and [59] (D-E).

Figure 4.

Glatiramer acetate treatment applied to a variety of diseases. (A) Illustration of the mechanism of action of GA, emphasizing MHC blocking, Th2 polarization, and antibody responses. (B) GA in a preclinical R6/2 mouse model of Huntington's Disease (left, naïve mouse, right GA-treated). Arrows indicate brain-derived neurotrophic factor (BDNF)-positive astrocytes, stars indicate brain-derived neurotrophic factor (BDNF)-positive non-astroglial cells. (C) GA in a transgenic (Tg) mouse model of Alzheimer's Disease: CD11b (activated microglia) and A β are more pronounced in the brains of untreated Tg mice (center) versus those treated with GA (right). (D) GA for macular degeneration: fundus photographs before and 12 weeks after weekly GA injections; circles indicate areas of drusen (stained yellow), which were reduced following treatment. (E) GA for inflammatory bowel disease in a dextran sulfate sodium (DSS) colitis mouse model: untreated mice died by day 12, whereas GA-treated mice survived and exhibited repair. Images show histology 10 days after DSS administration in an untreated mouse (top) and 30 days after DSS administration in a GA-treated mouse (bottom), with restored tissue. Reproduced with permission from references [74] (A), [166] (B), [167] (C), [168] (D), and [97] (E).

Figure 5.

Early clinical results of acellular ECM scaffolds for the treatment of volumetric muscle loss. Data from a single patient treated with the porcine dermal matrix XenMatrix (Bard) for a sports-related hamstring injury, is compiled. After pre-operative physical therapy, the patient underwent surgical debridement of scar tissue, followed by implantation of the scaffold in the injury site. Shown is an example of an ECM scaffold sheet similar to that used for this patient (A). The included data show pre- and post-operational images of the patient's hamstring area (B); functional assessments of muscular performance (C); and CT imaging of the treated area (D). Reproduced with permission from references [105] (A) and [109] (B–D).

Figure 6. ECM scaffolds induce Th2-dependent constructive myeloid polarization. Cardiac muscle-derived ECM was implanted into hamstring volumetric muscle loss injuries in wild type (WT), Rag1^{-/-}, and Il4ra^{-/-} mice; and in Rag1^{-/-} mice reconstituted with CD4 T cells from either WT mice (T-WT) or Th2-deficient Rictor^{-/-} mice (T-Rictor^{-/-}). Expression of the M2 macrophage marker CD206 at 3 weeks post-injury indicated that Th2 cells supported M2 polarization of macrophages (A). In particular, CD206 expression was not rescued after repopulation with T cells from a Th2-deficient mouse. Functional treadmill assays at 2 weeks post-injury similarly indicated the importance of Th2 cells (B, distance is normalized to an uninjured control). Function was not restored after repopulation with T cells from a Th2-deficient mouse. Reproduced with permission from reference [125].