Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Engineering self-assembled materials to study and direct immune function

Lisa H. Tostanoski, Christopher M. Jewell

Fischell Department of Bioengineering, University of Maryland, 8228 Paint Branch Drive, College Park, MD 20742, USA
Department of Microbiology and Immunology, University of Maryland School of Medicine, 685 West Baltimore Street, Baltimore, MD 21201, USA
Marlene and Stewart Greenebaum Cancer Center, 22 S. Greene St., Baltimore, MD 21201, USA
United States Department of Veterans Affairs, 10 North Greene Street, Baltimore, MD 21201, USA

Article history:
Received 1 February 2017
Received in revised form 21 March 2017
Accepted 22 March 2017
Available online 6 April 2017

Keywords:
Biomaterial
Nanoparticle, microparticle, micelle, liposome, polyplex, lipoplex, polyelectrolyte multilayer
Immunomodulation
Vaccine and immunotherapy
Autoimmunity and tolerance
Cancer
Non-covalent, hydrophobic, hydrogen bonding, and electrostatic interaction
Nanotechnology
Sensor, diagnostic, and theranostic
Manufacturing, regulatory approval and FDA
Self-assembly

Abstract
The immune system is an awe-inspiring control structure that maintains a delicate and constantly changing balance between pro-immune functions that fight infection and cancer, regulatory or suppressive functions involved in immune tolerance, and homeostatic resting states. These activities are determined by integrating signals in space and time; thus, improving control over the densities, combinations, and durations with which immune signals are delivered is a central goal to better combat infectious disease, cancer, and autoimmunity. Self-assembly presents a unique opportunity to synthesize materials with well-defined compositions and controlled physical arrangement of molecular building blocks. This review highlights strategies exploiting these capabilities to improve the understanding of how precisely-displayed cues interact with immune cells and tissues. We present work centered on fundamental properties that regulate the nature and magnitude of immune response, highlight pre-clinical and clinical applications of self-assembled technologies in vaccines, cancer, and autoimmunity, and describe some of the key manufacturing and regulatory hurdles facing these areas.

© 2017 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
The immune system naturally governs function by integrating the relative concentrations and kinetics of antigens – peptide fragments from pathogens that determine the target of an immune response – along with immune cues that range from nucleic acids, to signaling proteins called cytokines, to small molecule ligands and drugs [11–13]. Multi-disciplinary strategies that bring together immunology, translational perspective, and engineering technologies will be vital in continuing to decode and better direct these interactions. In particular, materials that allow precise control over how signals are encountered – the density or valency, for example – can reveal new knowledge of how immune cells detect and engage pathogens or a vaccine. Similarly, systems with molecular-scale control over the presentation of multiple signals offer the opportunity to exploit and direct function through co-delivery. As these demands for greater spatial and temporal control increase, so does the complexity of candidate vaccines and immunotherapies. Yet across fundamental research, pre-clinical development, and translation to humans, the need for vaccines and immunotherapies that are well defined and can be characterized remains constant; this latter point is an increasing challenge both from manufacturing and regulatory perspectives [2,14–16].

An emerging technology that can enable the rational, tunable, well-defined nature discussed above is self-assembly. In this review, we discuss the unique features of self-assembly as a means to study immune function, to enhance immunosensing and diagnostics, and to improve vaccine and immunotherapy delivery technologies. We begin with brief background on the immune system and the characteristics of self-assembled materials, then describe key examples from recent literature highlighting how the unique advantages of self-assembly are and can be exploited to probe and control immune function. Throughout the review, we emphasize new ways in which self-assembly might be applied to current clinical challenges, as well as some of the hurdles self-assembly faces that limit its feasibility.

1. Introduction

Vaccines are a transformative technology, enabling activation of the immune system to recognize and destroy specific pathogens, and supporting near eradication of diseases such as polio and smallpox [1–3]. Even so, the potential of engineering immune function is far richer than vaccines alone. The immune system is an exquisitely complex control system that is not just a means of activating responses to combat pathogens. Rather, a dynamic balance exists between pro-immune/inflammatory processes, regulatory/suppressive functions, and homeostatic (i.e., resting) activity levels (Fig. 1). In vaccination, a common goal is to induce specific and long-lasting adaptive responses against foreign pathogens for future protection against infection (i.e., immunological memory), while during cancer immunotherapy, one objective is to generate fast-acting killer T cells that destroy existing tumors [2,4,5]. Yet to combat autoimmune disease, where the immune system malfunctions and attacks healthy tissue, a therapy may seek to turn off or suppress particular aspects of inflammatory responses [6,7]. Thus, there is great interest in better understanding the interplay between activated, resting, and regulatory immune functions. Harnessing this knowledge could help overcome the divergent hurdles that continue to persist in infectious disease, cancer, and autoimmunity. For example, HIV undergoes rapid mutation to evade immune recognition and clearance [8], cancer cells secrete suppressive signals to actively subdue anti-tumor immunity [4,9], while during autoimmune diseases, such as multiple sclerosis and diabetes, defects in immune checkpoints lead to inflammation and destruction of self-cells or tissues [6,10]. These nuances highlight the idea that overcoming existing and emerging challenges to public health requires not just generation of immune function, but control over the specific characteristics of immune response. This idea is termed immunomodulation.

![Fig. 1. The immune system operates under a dynamic mix of maintenance processes, pro-immune functions, and tolerogenic functions. The balance between these functions is dictated by how antigen-presenting cells, such as dendritic cells (DCs), encounter antigens and integrate inflammatory or regulatory signals present in the local microenvironment. For example, during infection or inflammatory disease, DCs detect antigen in the presence of inflammatory or danger signals, which drives the expansion of effector T cells (Teff) and triggers antibody responses. In contrast, during tolerance, detection of antigen in the presence of a regulatory environment can lead to the expansion of regulatory T cells (Treg), the induction of anergic T cells (TAn), or the deletion of T cells. Typically, following a perturbation that skews immune function – such as an infection, or the administration of a vaccine – the immune system returns to a resting or homeostatic state.](image-url)

Fig. 1. The immune system operates under a dynamic mix of maintenance processes, pro-immune functions, and tolerogenic functions. The balance between these functions is dictated by how antigen-presenting cells, such as dendritic cells (DCs), encounter antigens and integrate inflammatory or regulatory signals present in the local microenvironment. For example, during infection or inflammatory disease, DCs detect antigen in the presence of inflammatory or danger signals, which drives the expansion of effector T cells (Teff) and triggers antibody responses. In contrast, during tolerance, detection of antigen in the presence of a regulatory environment can lead to the expansion of regulatory T cells (Treg), the induction of anergic T cells (TAn), or the deletion of T cells. Typically, following a perturbation that skews immune function – such as an infection, or the administration of a vaccine – the immune system returns to a resting or homeostatic state.
assembly might help tackle from the viewpoint of manufacturing and the regulatory process.

2. Background

2.1. The immune system initiates, balances, and suppresses immune function

The professional antigen presenting cells (APCs) of our immune system actively survey tissues throughout the body to verify the identity of healthy “self” molecules, cells, and tissues. These processes prevent incorrect attacks by sampling and display of self-antigens in the absence of stimulatory immune cues. A series of regulatory mechanisms also help maintain this “tolerance,” some of which occur during development, while others are ongoing throughout life. Simultaneously, these same APCs sense cues from the surrounding environment, such as inflammatory cytokines [13], and the presence of danger signals common on invading pathogens [17]. APCs, such as dendritic cells (DCs), integrate these signals to control their own cytokine secretion and the expression level of surface proteins that lead to maturation and activation. This information is relayed through: i) recruitment of cells of the innate immune system that secrete chemical signals (i.e., chemokines, cytokines), and ii) interaction with cells of the adaptive immune system in tissues that coordinate immunity, such as lymph nodes (LNs). Innate immune functions, such as engulfment of bacteria and triggering of inflammatory immune cell recruitment can occur in minutes or hours, but is less specific and does not provide immunological memory. In contrast, adaptive responses against pathogens (e.g., viruses, bacteria) develop over days, weeks, or months, drive molecularly-specific destruction and neutralization of pathogens, and can lead to immunological memory that lasts for decades or longer.

Lymphocytes, T cells and B cells, are the major players in exerting the functional effects of adaptive immunity. These cells express surface receptors that bind a target or “cognate” antigen, a peptide moiety for which a particular cell has developed specificity against. Upon recognition of cognate antigen presented in a major histocompatibility protein complex (MHC) by an APC, lymphocytes bind; this antigen display is called “signal 1” (Fig. 1). In the case of intra-cellular antigens, such as those displayed by cells infected by a virus, presentation occurs via the MHC-I pathway, driving the expansion of CD8+ cytotoxic T cells that can directly kill target cells. In contrast, extra-cellular antigens that are engulfed – fragments of bacteria, for example – are presented via the MHC-II pathway to expand CD4+ helper T cells. Importantly, simultaneously, lymphocytes receive cues that guide proliferation and differentiate to enable particular T or B cell functions. For instance, costimulatory markers expressed at different levels on the surface of APCs can engage receptors on lymphocytes during cell-cell interactions, an example of “signal 2”, while the combinations of cytokines present in the local cell environment is now considered “signal 3” [13]. Together, these signals bias lymphocyte development toward specific functions. CD4+ T cells with the same cognate antigen may differentiate toward either pro-inflammatory or tolerogenic phenotypes (Fig. 1). Helper T cells can further interact with B cells, and by working with other APCs, drive B cells to mature and secrete high affinity antibodies that can neutralize extracellular toxins or tag extracellular pathogens for destruction. B and T cell activation share some features, but differences exist that ultimately determine how strongly the antibodies that B cells secrete will bind a pathogen, and the features that these molecules will exhibit, for example, dimerization or transport through mucosal membranes.

Selectively exploiting active, resting, and suppressive immune mechanisms is a critical goal for new vaccines and immunotherapies [18]. The potential to promote cell-mediated (i.e., CD8+ T cell-driven) immunity continues to be particularly advantageous in viral disease and cancer. In these cases, viral antigens or antigens over-expressed on tumor cells are targeted by killer T cells, in some cases, those that arise naturally, and in others, via T cells that are engineered and infused into cancer patients [19,20]. In contrast, the potential to control the phenotype of CD4+ T cells may be a vital capability to promote tolerance during autoimmune disease. In multiple sclerosis, Th1 and Th17 cells that specifically recognize components of myelin – the matrix that insulates and protect neurons – drive inflammation and disease through attack against myelin [21–23]. The capacity to instead expand these myelin-recognizing cells toward regulatory T cells (e.g., TREGS) could enable myelin-specific control of disease, without the broad immunosuppressive effects associated with current clinical therapies. Similarly, regulating metabolic function away from states of extreme activation or suppression, and toward moderated, homeostatic levels, might help address diseases that cause systemic, chronic inflammation or that result in loss of immune tolerance [24,25]. Thus, eliciting better control over the interactions between immune cells and, ultimately, immune function is a core theme in the field [26–28]. Here, we focus on self-assembled materials, which offer a unique opportunity to contribute to this vision.

2.2. Self-assembled materials offer high levels of molecular precision control

Biomaterials have emerged as promising technologies to enhance the spatial and temporal control over immune signal display and delivery [11,12,19,29]. Broadly, biomaterials offer attractive properties, such as delivery of multiple classes of cargo, cell and tissue targeting, protection of payloads from enzymatic degradation, increased circulation time, and defined delivery kinetics [11,12,30,31]. However, there are significant challenges that continue to limit these materials for clinical use, for example, inefficient loading of immunological cargos into carriers, heterogeneous size distribution, and lack of control over the physical arrangement of molecules. Further, the low frequency of success of biomaterials in the clinic over the past decades reveals a need for critical assessment of translational biomaterials research in stringent models, and for ensuring clinically-relevant questions or pathways are targeted. The complexity of many materials approaches also adds hurdles for technologies aimed at human use, as the difficulty in manufacturing, characterizing, and approving these systems can be much greater relative to drug or antibody therapies. This disparity is in part due to the historical experience that manufacturers and regulators have with drugs and antibodies. Lastly, the need for better definition and control is particularly important for applications targeting the immune system, where the signaling pathways control a dynamic equilibrium.

Within the realm of biomaterials, self-assembled materials represent a unique opportunity to generate well-controlled structures from a diverse array of molecular building blocks, including peptides, nucleic acids, lipids, and synthetic polymers (Fig. 2, center) [32,33]. Here, we define self-assembly as spontaneous interactions of these molecules, driven by conversion to more entropically-favored states. These processes can occur over nano-, micro-, and macro-scales via non-covalent forces, such as electrostatic or hydrophobic interactions and, owing to the spontaneous nature, self-assembled materials can often be generated with low energy input and at temperature and pH values in the physiological range. These characteristics are, generally, compatible with inherently less stable biological building blocks.

There are several types of self-assembled materials in the immune engineering field being used to modify the surface of two- or three-dimensional surfaces (e.g., spherical particles, complex micro- or nanoscale topographies), or to directly generate structured particulate materials. Three emerging classes of these materials can be described by the non-covalent interactions that drive self-assembly (Fig. 2, center). First, hydrophobic or amphiphilic molecules often assemble through hydrophobic interactions into micelles, liposomes, or elongated, fibril-like structures. For example, lipids are inherently amphiphilic, making these molecules well-suited for hydrophobic interaction-based assembly, while peptides can be designed to incorporate motifs that fold into secondary structures (e.g., alpha helices, beta sheets) to provide
is deciphering fundamental characteristics of immune response. This
immune interactions
the diverse body of exciting work that comprises this special issue.
ities in the immune engineering
and engineering are also poised more generally to enable new capabil-
on self-assembly, new innovations in materials science, immunology,
clinical and design stages (Fig. 2, bottom). While this review centers
requirements for new vaccines and immunotherapies even in the pre-
cancer, and autoimmunity. We also integrate into the discussion the in-
in applications aimed at clinical translation that span infectious disease,
lines for new vaccines and immunotherapy delivery strategies, and iv)
ii) for immune sensing and diagnostics, iii) to generate design guide-
i) as a tool to interrogate fundamental aspects of immune responses,
structural moieties for rationally-designed synthetic systems that are
understanding provides new basic knowledge to inform the design of
better vaccines, immunotherapies, and carriers for these technologies.
An important example is the use of virus-like particles (VLPs). VLPs
are recombinant proteins designed to self-assemble into particulate
structures after expression in cell culture systems (e.g., yeast, bacteria,
plant cells) that have been engineered to produce the sequences of interest.
These particles mimic native viruses, but cannot replicate and, therefore,
pose lower safety risks compared with live or attenuated vaccines. VLPs are currently used in clinically-approved vaccines to protect humans against human papillomavirus (HPV) and hepatitis B virus (HBV). These clinical uses highlight a key advantage of VLPs, the presentation of sets of antigens in the same physical conformations that is found on native pathogens to maximize immunogenicity. This is in contrast to many other approaches in which the combination of antigens is either poorly controlled, or may result in a consistent arrangement of antigens, but one that differs in spacing, geometry, and shape from that of the native pathogen. This disparity between synthetic platforms and target pathogens can result in poor immunogenicity and efficacy. Despite the advantages of VLPs, a limitation of existing VLPs in the clinic is that the combinations of antigens delivered are not well-defined or well-controlled. Instead, fragments of pathogens are isolated, expressed in recombinant systems (e.g., bacterial cells), then screened for immunogenic potential. While this approach has identified both approved vaccines and promising candidates, the potential to program the combinations of antigens displayed without sacrificing immunogenicity could generate strong responses with greater selectivity.

The efficiency of VLPs is also motivating work to harness specific structural moieties for rationally-designed synthetic systems that are well defined in both formulation and in the specific antigens against which responses are generated. For example, in the context of HIV, synthetic nanoparticles have been used as a tool to interrogate the role of
antigen conformation and valency [47]. This study was motivated by recent reports revealing a portion of an HIV envelope glycoprotein that coats the viral capsid – a trimer composed of three monomers – is critical for recognition of HIV by the immune system. He and colleagues hypothesized that using nanoparticles could enable surface display of the trimer at high density (Fig. 3A) [47]. This report compared the binding affinity of the nanoparticle-displayed trimer to that of a free (i.e., nanoparticle-free) monomer using two well-characterized antibodies. Tests with an antibody that recognizes the monomer, either in monomer or trimer form, revealed low affinity binding to the monomer (Fig. 3B, top left) and higher affinity binding to nanoparticle-displayed trimer (Fig. 3B, top right), as indicated by a dramatic decrease in the dissociation constant ($K_D$) of each binding assay. These results confirmed that nanoparticle display did not interfere with the physical conformation of the trimer, but rather enabled rapid recognition and binding by the antibody through high density presentation of the trimers. This case demonstrates a concrete advantage of the self-assembly-enabled approach: the potential to mimic viral surface presentation of specific antigens to investigate the role of physical arrangement in engaging interactions with biological molecules, like antibodies.

While the research above focused on understanding the display of antigens with higher order structure, self-assembled particles are also being used to understand VLP assembly. For example, the link between amino acid sequence and the integrity and mechanical properties of VLPs have been investigated [48]. In this study, the authors introduced amino acid point mutations into monomers of the minute virus of mice, a virus with a well-characterized structure. Several of the mutations partially or completely inhibited the spontaneous assembly of VLPs. This result underscores the vital role of native, non-covalent interactions between side-chains of amino acids in monomers to drive self-assembly. Further, in formulations that maintained the potential to assemble spontaneously into VLPs, atomic force microscopy studies generally revealed an increase in stiffness when sequences were mutated. Stiffness and other physiochemical properties have been shown to impact T cell activation and proliferation [49,50]. Thus, future studies to elucidate the role of VLP properties in influencing immunomodulatory function, as well as comparisons between VLPs and other synthetic carriers (e.g., polymer emulsions, micelles, polymer-nucleic acid complexes) could inform the design of materials with specific mechanical properties to tune responses for translational applications.

4. New immunosensing and diagnostic applications are enabled by the well-controlled physical arrangement of self-assembled systems

Broadly speaking, immunosensing requires the detection of rare antigens, antibodies, or immune cells among complex, heterogeneous biological samples (e.g., patient blood or serum) to diagnose patients or inform therapeutic interventions. Thus, there is interest in developing strategies that enhance the specificity and sensitivity of detection and screening platforms. This knowledge is important for vaccine and immunotherapy delivery as specific design features may be advantageous depending on the specific cargos to be delivered. One way in which self-assembly is being harnessed along these lines is functionalizing surfaces with reproducible, defined physical arrangements of molecular species. Some of these approaches have involved immobilization of antibodies that specifically recognize key proteins, enzymes, or nucleic acid sequences that are known biomarkers of disease. For example, antibody against an enzyme upregulated in prostate cancer, prostate-specific antigen (PSA), has been used to design an electrochemical sensor [51]. Antibodies consist of two components: a constant region that is conserved across all antibodies (Fc), and a variable “Fab” region that gives antibodies exquisite specificity to bind to a particular molecule. Thus, the sensor construction involved self-assembly of a linker molecule, $\beta$-cyclodextrin, onto a surface, followed by chemical conjugation of the Fc-binding domain to this linker. This approach resulted in well-ordered localization of the antibody on the surface, but left the domain that binds selectively to PSA available for interaction with samples. These sensors provided a high specificity and sensitivity for the detection of the rare PSA antigen in human serum. This was accomplished without fluorescent labeling to amplify the signal that is common in current approaches for detecting this biomarker.

In another study, self-assembly was used to localize antibodies against known antigens of influenza in a particular orientation on surfaces [52]. In this study, Le Brun and colleagues designed a system in which an engineered protein – Protein G, a cell wall-associated protein derived from Streptococcus – is self-assembled onto a gold surface through adsorption mediated by thiol functional groups. Importantly, this engineered Protein G preferentially binds to the Fc region of antibodies with an affinity two orders of magnitude higher than that of binding to Fab regions, facilitating capture of antibody on the surface via the Fc region [52]. This feature allows the variable regions – which...
Engineered self-assembled DNA structures enhance the sensitivity of an immunosensing platform. A) Schematic depiction of physical arrangements of the tetrahedron structure probe (TSP monomer), compared with three controls: i) the single stranded DNA (ssDNA) probe in free form, ii) the unpurified, heterogeneous TSP product, and iii) a polymeric TSP product. In each case, a complementary DNA sequence, linked to a fluorescent reporter, depicted in pink, has been added to show expected degree and orientation of binding. To test the sensitivity of these probe conformations, a uniform quantity of the capture probe, in the four formulations depicted in (A), was deposited on glass substrates. An equivalent mass of the detection probe was added to each well and after an incubation period, excess unbound probe was washed away. The level of fluorescent signal detected could be visualized qualitatively through fluorescent microscopy (B) as well as determined quantitatively (C). Adapted from [54] with permissions.

While these approaches demonstrate some of the advantages of surface immobilization for detection, many platforms – both those driven by self-assembly and those governed by different types of interactions, such as chemical conjugation – have limitations. For example, linking molecules to a surface can alter physical conformation and, as a result, the capacity to bind to an antigen or molecule of interest. In addition, increasing the density of detection molecules (e.g., antibodies) on a surface may offer more binding sites, but these high packing densities can also result in steric hindrance to binding. Thus, some studies have explored self-assembly that integrates linker structures to provide high density arrangements of molecules with predictable orientation and spacing on surfaces [53,54]. In one report, a self-assembling coiled-coil peptide structure was used to display a glycopeptide found on the surface of a tetrahedron-free ssDNA, ii) the unpurified product of self-assembly (unpurified TSP), and iii) a purified structure unrelated to the target structure (TSP polymer) (Fig. 4A). Equivalent doses of the DNA probe were conjugated to glass substrates in the test and control formats just described, then a complementary structure labeled with a fluorophore was incubated with each group, followed by a wash step to remove unbound fluorescent probe. A dramatic signal enhancement using the TSP monomer formulation was observed qualitatively through fluorescence microscopy (Fig. 4B) and quantitatively by fluorescence intensity (Fig. 4C). The TSP monomer exhibited 14-fold increase in signal intensity compared with that of free ssDNA, as well as enhanced signal levels compared with the unpurified or unrelated control structures, described above. These findings support the authors’ hypothesis that oriented conjugation and self-assembly were responsible for the regular spacing of molecules on the substrate. The authors also demonstrated the potential to immobilize multiple classes of molecules, supporting the flexibility of this diagnostic tool. In future studies, the modular nature of such platforms could be exploited to control the distance between ligands by, for example, increasing or decreasing the length of the DNA tetrahedron chains and, by extension, the footprint of the self-assembled structure. In contrast, approaches that use alternative strategies, such as direct conjugation of molecules to a surface, may generate precise control over total ligand bound, but might not offer the same level of control over the spacing or physical arrangement of those ligands. The application of self-assembly to enable the surface-bound display, as well as to control the spacing and valency of antigens could also extend to the design of new strategies to deliver immune cues in vivo, as discussed further in Section 5.2.

5. Self-assembled systems can create design guidelines for new vaccine and immunotherapy strategies

5.1. Physicochemical properties of self-assembled materials help determine to the magnitude and nature of immune response

Self-assembled nano- and micro-scale materials are being used as platforms to explore the relationship between the physical and
chemical characteristics of materials and the magnitude and nature of responses elicited. For example, the role of particle size and shape has been studied, comparing spherical formulations to higher aspect ratio conformations, such as rods or filaments [55–57]. One intriguing theme in this area has been to develop materials that mimic the size and shape of pathogens, such as nanoscale spherical particles that represent viruses, or anisotropic shapes that represent bacteria. The goal is to investigate whether these properties impact immunogenicity and the interactions with immune cells [55–57]. For example, the Scott lab has demonstrated that the size and shape of particles impacts the association of materials with target APC populations, such as DCs, following intra-venous injection in mice [57]. The materials in these studies were self-assembled via hydrophobic interactions using the same co-polymer, poly(ethylene glycol)-bl-poly(propylene sulfide), which enabled the study of different particle geometries with a fixed composition and conserved surface chemistry. Their findings revealed enhanced uptake of spherical particles 113.7 nm in diameter by DCs compared with smaller nanoparticles, 22.5 nm in diameter; the latter were instead found to associate highly with macrophages in the liver. In contrast, fibrous structures formed from the same polymer, termed filomicelles, remained associated with phagocytic cell populations in the blood over time, suggesting an increased circulation time and decreased uptake by phagocytic cells. Together, these results indicate the shape and size of self-assembled particles can alter the biodistribution and retention of nanomaterials. These features and guidelines could be harnessed for translational applications to target particular subsets of cells.

In addition to size and shape, surface properties of self-assembled carriers have been investigated. One recent finding used a platform to generate nanofibrils from peptide monomers that self-assemble through the formation of beta-sheets. These fibrils were used to display defined concentrations and combinations of peptide antigens on the surfaces and to investigate the role of fibril properties in promoting pro-inflammatory responses [58]. Fibrils with a negative zeta potential, a measure of surface charge, were found to exhibit significantly reduced or even undetectable T cell and B cell (i.e., antibody) responses. In contrast, fibril formulations containing equivalent doses of a common model peptide antigen derived from chicken ovalbumin (OVA), SIINFEKL, but with a positive charge, drove potent expansion of antigen-specific T and B cell responses. This finding could be used to inform design criteria – for self-assembled materials, as well as for biomaterials-based approaches more generally. For example, in translational applications where strong pro-inflammatory responses are desired (e.g., infectious disease, cancer immunotherapy), design of nanomaterials with positive surface charge may help further tune the potency and effectiveness of immune response.

An important example of pro-inflammatory signals garnering interest in the clinic is adjuvants, agents designed to amplify the magnitude of immune responses. Current clinical adjuvants include potassium alumnum sulfate (alum), aluminum hydroxide, and mycophenolic acid (MPL) [36,37,39]. However, the mechanism of action of these adjuvants is not fully-understood and they offer limited control over the nature of responses elicited [16,59], motivating exploration of signals that still drive enhanced immunogenicity, but with more definition and molecular specificity. As discussed in Section 2.1, APCs have evolved to detect molecular signatures of pathogens. Pattern recognition receptors, such as toll like receptors (TLRs), detect molecules and structures that are not present in mammalian cells (i.e., “self”), but are common among bacteria and viruses. Agonistic ligands for these receptors – such as lipoprotein components of bacterial cell walls, or distinctive nucleic acid structures frequent in viruses – have emerged as promising stimulatory immune cues to enhance the immunogenicity of candidate vaccines [11,17,60–62]. TLR agonists (TLRas) are well-suited for this function, as they trigger defined molecular pathways to upregulate the expression of activation markers on APCs (signal 2) and drive inflammatory cytokine secretion (signal 3), both of which can promote expansion of pro-inflammatory T cells and trigger potent antibody responses.

Generally, nucleic acids are intriguing molecular building blocks owing to the ability to design predictable structures of DNA or RNA. Fortunately, a number of nucleic acid classes also activate TLRs. Thus, in the self-assemble field, molecular TLR agonists are of great interest. Some of these studies are investigating how the shape of carriers used to deliver TLR ligands [56], or the tunable surface display of TLR ligands [63–65], impacts the degree of DC activation and the cytokine secretion profiles. As discussed in Section 4, DNA sequences can be finely tuned to form defined structures, enabling control over the organization and spacing of tertiary features. For example, dendrimers have been assembled to

---

**Fig. 5.** Programmable density of ligand display enables modulation of the biodistribution and stimulatory capabilities of a molecular adjuvant. A) Schematic of linking a TLR-7/8 agonist to a polymer scaffold to generate assemblies with controlled ligand density. B) Depictions of a polymer coil displaying TLR-7/8 (PC-7/8) and the assembled polymer particle containing TLR-7/8 (PP-7/8) that was observed as ligand density was increased. C) Quantification of the level of inflammatory cytokine IL-12p40 in the draining lymph node following injection of either PC-7/8 or PP-7/8 shown in (B). D) Immunofluorescent staining of draining lymph nodes following injections of small molecule TLR-7/8a (SM 7/8), PC-7/8a, or PP-7/8a. Images show T cells (CD3, blue), macrophages (CD169, red) and signal from the TLR-7/8a (AlexaFluor 488, green) at the indicated timepoints. Adapted from [65] with permissions.
control the loading of a TLR9 ligand, CpG, in nano-assemblies that trigger secretion of an inflammatory cytokine, TNF-α [66]. In another example, CpG was integrated with a helical DNA assembly to form different shapes, including triangle, square, and polygon assemblies. In these structures, increasing the number of sides in the carrier enabled increased loading of CpG per assembly. This control over CpG assembly and, as a result, dose, directly correlated to the level of inflammation measured during incubation with a macrophage cell line [67]. These strategies are just two examples of biomaterials-based approaches to deliver CpG or other TLRs, but they demonstrate the potential of predictable, well-controlled self-assembly of nucleic acids for designer immunogenic materials.

In another example of modulating immunogenic nucleic acid delivery, Lynn et al. controlled the conformation of a TLRa by tuning the display of a small molecule agonist of TLRs 7 and 8 (TLR-7/8a) on a N-(2-hydroxypropyl)methacrylamide (HPMA) polymer scaffold (Fig. 5A) [65]. As the mass of TLR-7/8a per mass of polymer was increased, spontaneous self-assembly of conjugates of TLR-7/8a and polymer was observed. Interestingly, when equivalent doses of TLR-7/8a were administered in either a low density formulation that existed primarily as small, individual polymer coils (PC-7/8a, Fig. 5B) or in a high density formulation that assembled into polymer particles (PP-7/8a, Fig. 5B), the resulting responses differed. Delivery of TLR-7/8a in particulate form in mice drove significantly increased levels of IL-12p40, a key inflammatory cytokine involved in the expansion of pro-inflammatory T cells, compared with an equivalent dose in small polymer coil form (Fig. 5C). This observation was accompanied by an increased level of fluorescently-labeled TLR-7/8a present in draining LNs of mice treated with the particulate form (PP-7/8a), compared with a small molecule formulation (SM 7/8) or the polymer coil form (PC-7/8a) (Fig. 5D). As LNs are key sites of interactions between APCs and lymphocytes, they are a crucial target for candidate vaccines and immunotherapies. Thus, many strategies focus on design of carriers that drain to these sites [68,69] or are carried to LNs after encounter with APCs [11,22,23], or directly access these tissues through targeted introduction of soluble or biomaterials-based formulations [22,70,71]. In the above example, Lynn et al. used a library of candidate materials to interrogate the role of carrier properties in modulating the biodistribution of signals and magnitude of responses.

5.2. Self-assembly facilitates programmable densities of defined combinations of antigens

The previous section demonstrates some of the advantages of self-assembly for adjuvant delivery and parsing out the role of physicochemical features of carriers in the magnitude and nature of responses elicited. This section focuses on antigens, and the ways in which self-assembly is being exploited to link immune outcomes to antigen physical arrangement, combination, and relative dose. Discussion of VLPs (see Section 3) motivates this goal. Although VLPs replicate the high density antigen display and physical conformation of target pathogens, modifications to the amino acid sequences that comprise VLPs – for example, integrate a different antigen of interest into a carrier – can impair nanoparticle formation [48]. These changes may interfere with or inhibit the non-covalent interactions that typically drive uniform self-assembly. Thus, alternative strategies that can condense defined...
were incorporated into fibrils at tunable ratios that could be individually visualized (Fig. 6B). Beyond reporter proteins, new work has also explored defined antigen display properties using peptide antigens: i) a sequence from *Staphylococcus aureus* that can be recognized by B cells, but not T cells, and ii) a peptide sequence that binds to the MHC-II molecules expressed on the surface of T cells of mice termed PADRE [76]. Defined combinations and concentrations of these sequences were incorporated into fibrils (Fig. 7A, top) that could be visualized by transmission electron microscopy (TEM, Fig. 7A, bottom). The authors demonstrate that co-delivery of both sequences in the same nanofiber drove enhanced antibody production when compared with an equivalent dose of each peptide sequence delivered as a mixture of nanofibers incorporating each peptide separately (Fig. 7B). The hypothesized mechanism of action is that co-incorporation of both sequences promotes co-delivery of both peptides to a single B cell. Next, the dose of the B cell sequence was fixed and the dose of PADRE peptide introduced was titrated. After injection in mice, the number of PADRE-specific T cells was found to depend on the amount of PADRE assembled in the nanofibril (Fig. 7C). The nature or phenotype of these specific T cells was further characterized by staining for transcription factors characteristic of three helper T cell types, T follicular helper (Tfh), type 1 helper (Th1), and type 2 helper (Th2) (Fig. 7C). Interestingly, dose-dependent polarization was observed, with higher doses of PADRE promoting Th1 cells, which enhance the magnitude of antibody responses, over Th1/Th2 cells, which promote T cell-mediated immunity. Thus, these findings could be harnessed to program the specific phenotype of immune responses to, for example, promote antibody production to combat extracellular bacteria (i.e., Th1), or promote effector T cell responses (e.g., Th1, Th2) for cancer immunotherapy.

In addition to the nanofiber approach above, other researchers have used alternative self-assembly strategies to control antigen delivery [78–83]. For example, antigens have been linked to hydrophobic peptide sequences that assemble into coiled-coil domains to condense into spherical nanoparticles [84]. Other reports focus on coiled-coiled domains that fold into nanoparticles with tunable ligand display and size by controlling parameters such as pH and salt concentration during folding [85]. A synthetic polymer, poly(2-hydroxyethyl methacrylate) (pHEMA) – which contains a hydrophobic side chain that drives self-assembly – has also been used to complex and condense a protein antigen into nanoparticles with sizes that can be tuned by controlling the concentration of protein incorporated [80]. In parallel, synthetic peptide amphiphiles have been designed to generate fibers or micelle structures [86–89]. For instance, the Tirrell group has demonstrated that peptide amphiphile-based nanofibers, assembled through hydrophobic interactions, drive CD8+ T cell responses against the model epitope SIINFEKL and enhance survival in a subcutaneous melanoma model expressing the same model antigen, B16-OVA [86].

Together, the strategies in this section demonstrate the potential to incorporate defined combinations of peptide antigens into self-assembled nano- or micro-scale materials. This characteristic could prove particularly advantageous in the context of pathogens and diseases which exhibit non-uniform or heterogeneous characteristics within and across patients. From this perspective, eliciting responses against multiple antigens simultaneously could enhance the protective capacity of a prophylactic vaccine, or the efficacy of an immunotherapy. Two current clinical examples include influenza – which undergoes rapid mutation and, as a result, a new formulation of the flu vaccine is required each year – and cancer, in which tumor associated antigens can vary greatly from patient to patient, within a given tumor, and over time. A final consideration is that the immunostimulatory activity of both synthetic VLPs (see Section 3) and self-assembled structures for multivalent delivery of antigens (see Section 5.2) has been demonstrated without the inclusion of an explicit adjuvant in pre-clinical studies. Such intrinsic stimulatory characteristics can be a significant advantage for pro-inflammatory applications. However, the formulations explored that enable presentation of antigen without triggering strong inflammatory responses
could instead be harnessed for alternative applications, such as promoting tolerance to “self” antigens during autoimmune diseases. For example, Shen and colleagues have induced tolerance during a pre-clinical model of rheumatoid arthritis by harnessing a mechanism that the severe acute respiratory syndrome (SARS) virus uses to evade immune surveillance [90]. Thus, rationally-assembled structures could inform the design of therapies for either pro-inflammatory or tolerogenic targets, described in further detail in Section 6.3.

5.3. Self-assembly enables co-delivery of multiple classes of immune cargos to trigger responses through selective molecular pathways

While the previous two sections focused on carrier properties and the delivery of either antigen or adjuvant alone, vaccines and immunotherapies often deliver antigens along with adjuvants or modulatory cues to direct the response to the antigen. This is a central paradigm in vaccines for infectious disease, and also a developing area in cancer immunotherapy, where tumor-associated antigens are delivered with molecular adjuvants (e.g., TLRas) or antibody therapeutics during cancer vaccination regimens. Co-delivery of antigen with one or multiple TLRas [68,91,92] can enhance inflammatory signaling cascades during antigen presentation, promoting the expansion of antigen-specific CD8+ T cells or pro-inflammatory phenotypes of CD4+ T cells (e.g., Th1). In contrast, in the context of autoimmunity, an emerging goal is delivery of “self” antigen with a regulatory immune cue to induce tolerogenic T cell phenotypes, such as regulatory T cells (TREGs). In either case, the principle of co-delivery of antigens and immunomodulatory signals presents a fundamental challenge: coordinated delivery of multiple signals to target cells and tissues in vivo. This hurdle can be compounded by disparate physiochemical properties (e.g., molecular weight, charge) of cargos that results in differences in biodistribution and trafficking after injection. In this section, we will describe strategies that exploit unique characteristics of self-assembled materials to co-deliver immune signals.

Several approaches have emerged to co-assemble and co-deliver antigen and adjuvant using non-covalent interactions, including electrostatic or hydrophobic interactions, and other driving forces. For example, micelles and other particulate strategies have been used to deliver model antigens and either individual TLRas or defined combinations of TLRas [83,93–95]. Interestingly, one example of this approach demonstrates that co-incorporation of antigen and adjuvant enhances the potency of the response with minimal systemic inflammation [96], an off-target side effect often associated with adjuvant delivery. These results highlight an advantage of assemblies that enable co-encapsulation, as simple mixtures do not offer control over how each signal is distributed following injection.

A general advantage of particulate-based systems for co-delivery of immune cues is the potential to target APCs, which have evolved to detect and engulf particulates. This function offers an opportunity to tune uptake and processing of antigens using controlled architectures of self-assembled materials. As discussed in Section 2.1, extra-cellular or “exogenous” antigens are typically processed and presented through an MHC-II pathway, which leads to CD4+ helper T cell responses. Yet, for many applications, expanding CD8+ T cells against an antigen of interest is a critical goal. Thus, strategies that direct the processing and presentation of delivered antigen toward the MHC-I pathway – typically reserved for intra-cellular peptides, such as those formed during degradation of viral particles that have infected host cells – are of key interest. When APCs engulf a pathogen or particle, these materials are generally entrapped in endosomal or lysosomal compartments. This pathway triggers presentation of antigens along the MHC-II pathway to communicate to cells of the adaptive immune system that extracellular, foreign peptides were detected. However, for pathogens requiring CD8+ cytotoxic T cell activity to destroy infected cells, antigens must reach the cytosol of cells to enable presentation through the MHC-I pathway. This process of presentation of endocytosed antigen being presented by the cytosolic MHC I pathway is termed “cross-presentation” [97]. To support this process using synthetic materials, the Swartz and Hubbell groups have reported self-assembling polymersomes that are oxidation sensitive. These assemblies can be loaded with immune signals and, on delivery to cells, promote endosomal escape and cytosolic delivery of antigen or TLR7/8 ligands [98]. In another example, pH sensitive micelles, which self-assemble through hydrophobic interactions among a polymer carrier, were used to study intracellular antigen trafficking to promote cross-presentation of the model antigen OVA [99]. Nanoparticles condensed through hydrophobic interactions were used to entrap OVA, CpG, and a pH sensitive polymer poly(propylacrylic acid). These assemblies exhibited pH-dependent membrane disruption properties, which resulted in enhanced presentation of OVA through the MHC-I pathway compared with simple mixtures of the OVA peptide and the polymer nanoparticles [100,101]. Together, these results highlight opportunities to design self-assembled carriers that target APCs, are responsive to environmental cues, and control how immunological cargo is trafficked in intracellular components.

One driving force of non-covalent self-assembly that has emerged as an approach to organize immune cues into well-controlled assemblies is electrostatic interactions [102–104]. This strategy is particularly well-suited for immunological applications, as many immune signals of interest are inherently charged. For example, nucleic acid ligands of TLRs can serve as an immunostimulatory cargo and facilitate self-assembly through the negative charges of the phosphate backbone. In addition, peptide antigens can exhibit intrinsic charge from amino acid side chains, or peptide antigens can be linked to charged amino acid sequences to alter charge ratio. These properties have been exploited to drive spontaneous (e.g., complexation) or sequential (e.g., layer-by-layer adsorption) of cargos. In one example, layer-by-layer assembly was used to co-assemble and co-deliver a T cell antigen and a B cell antigen for a cancer model [79]. These antigens were modified with lysine residues to confer positive charge and facilitate electrostatic association with a synthetic anionic polymer, γ-polyglutamic acid (γ-PGA). The self-assembled particles drove significant increase in antibody titers, while control formulations without the lysine modifications exhibited significantly diminished responses. These results demonstrate the importance of the cationic modification to drive electrostatic self-assembly, and underscore the synergistic effect observed when multiple antigens were co-delivered [79], consistent with the enhanced effects upon co-delivery of T and B cell antigens on a single nanoparticle, described in Section 5.2 [76].

In other approaches, synthetic polymers have been exploited to co-assemble antigens and TLR agonists via electrostatic assembly. De Geest et al., have reported a polyelectrolyte multilayer strategy to co-deliver antigen and TLR agonists in microcapsules [105]. In this example, OVA is precipitated in a calcium carbonate core, which is then coated in a layer-by-layer fashion with two synthetic polyelectrolytes, poly-L-arginine and dextran sulfate. In some cases, a final layer of CpG was added. These capsules drove significant expansion of transgenic T cells with receptors specific for OVA peptide, as well as secretion of a pro-inflammatory cytokine among these cells. In mice, assembled capsules drove dramatically increased inflammatory cytokine secretion among the CD4+ and CD8+ T cell subsets and enhanced the level of antibody production when compared with soluble OVA, a soluble mixture of OVA and CpG, or capsules that encapsulated OVA only [105]. This result supports a synergistic effect of co-delivery of antigen and adjuvant, enabled by electrostatically-driven co-assembly.

Our lab has recently reported a platform to co-assemble antigens and adjuvants into nanostructured materials constructed entirely from immune signals [106–111]. These immune polyelectrolyte multilayers (“iPEMs”) are built using the electrostatic, layer-by-layer process hallmark of PEMs, yet are unique in that they mimic attractive features of biomaterials, but eliminate all polymer matrices or carriers. This approach simplifies composition, provides modularity and high absolute cargo loadings, and also eliminates intrinsic carrier effects. iPEMs can...
be assembled on gold nanoparticles [109,110], used to form carrier-free hollow microcapsules [106,107,111], or coated on microneedle arrays [108]. To form capsules, a model antigen (SIINFEKL) was linked to cat-ionic arginine residues to confer positive charge, and assembled with an inherently anionic nucleic acid-based TLR3 agonist, polyIC. Thus each cargo, antigen and adjuvant, served both as a functional immune signal and as a structural component that enabled electrostatic assembly and formation of iPEMs upon core removal (Fig. 8A). Layer-by-layer assembly enabled control over the loading as a function of the number of bilayers deposited on a microparticle core. C) Expansion of antigen-specific (i.e., SIIN-specific) T cells following two administration of iPEMs, compared with frequencies in untreated mice or mice given simple mixtures of antigen and adjuvant. D) Survival of mice following challenge with a model of melanoma. E) Microneedle coated with a melanoma antigen (Trp2*) and a TLR9 agonist, CpG and F) delivery of these signals to the skin of mice following microneedle application. G) Expansion of Trp2-specific T cells following two applications of Trp2/CpG coated microneedles (MNs), indicated in red arrows. Panels A-D adapted from [106] and panels E-G adapted from [108] with permissions.

Fig. 8. Tunable, electrostatically-driven assembly and antigens and adjuvants in carrier-free assemblies. A) Schematic of layer-by-layer assembly of antigen and adjuvant to form carrier-free immune polyelectrolyte multilayer “iPEM” capsules. B) Tunable loading of fluorescent antigen and adjuvant into iPEMs as a function of the number of bilayers deposited on a microparticle core. C) Expansion of antigen-specific (i.e., SIIN-specific) T cells following two administration of iPEMs, compared with frequencies in untreated mice or mice given simple mixtures of antigen and adjuvant. D) Survival of mice following challenge with a model of melanoma. E) Microneedle coated with a melanoma antigen (Trp2*) and a TLR9 agonist, CpG and F) delivery of these signals to the skin of mice following microneedle application. G) Expansion of Trp2-specific T cells following two applications of Trp2/CpG coated microneedles (MNs), indicated in red arrows. Panels A-D adapted from [106] and panels E-G adapted from [108] with permissions.

be assembled on gold nanoparticles [109,110], used to form carrier-free hollow microcapsules [106,107,111], or coated on microneedle arrays [108]. To form capsules, a model antigen (SIINFEKL) was linked to cationic arginine residues to confer positive charge, and assembled with an inherently anionic nucleic acid-based TLR3 agonist, polyIC. Thus each cargo, antigen and adjuvant, served both as a functional immune signals and as a structural component that enabled electrostatic assembly and formation of iPEMs upon core removal (Fig. 8A). Layer-by-layer assembly enabled control over the loading as a function of the number of bilayers deposited (Fig. 8B). Compared with an equivalent vaccine composed of a mixture of antigen and adjuvant, components assembled into iPEMs dramatically enhanced the expansion of antigen-specific T cells, indicated by an increased frequency of SIIN-specific CD8+ T cells after both prime and boost injections (Fig. 8C). This increase correlated to prolonged survival when vaccinated mice were challenged with a melanoma expressing SIINFEKL antigen, B16-OVA (Fig. 8D). The flexibility of this platform for cancer vaccination was demonstrated by using microneedle arrays as substrates to assemble iPEMs composed of CpG and a tumor antigen, Trp2 (Fig. 8E). These arrays enabled co-delivery of both signals to the skin of mice (Fig. 8F) and drove significant expansion of tumor-specific CD8+ T cells in following application of the coated arrays (Fig. 8G). Together, examples here demonstrate the potential of the iPEM platform to co-localize immune signals over multiple length-scales and without the inclusion of synthetic polymers or carrier components. This simplicity and modulatory could support the design of well-defined vaccines formulations that facilitate characterization and, ultimately, translation of vaccines and immunotherapies.
6. Pre-clinical studies using self-assembled materials demonstrate exciting translational potential in infection and disease models

6.1. New vaccines and immunotherapies face challenges in both in performance and production

Work described in Section 5 is beginning to reveal design rules for how self-assembled materials interact with immune cells. This section focuses on the translational component of self-assembly, presenting recent examples that involve pre-clinical models and that target current clinical challenges. For example, a fundamental issue in the development of new vaccines and immunotherapies is balancing efficacy and safety. On one extreme, the delivery of live viruses or bacteria can trigger strong protective immune responses, but increases the risk of infecting patients. In contrast, small subunits of pathogens (e.g., short peptide monomers) confer less risk, but are also less immunogenic. This characteristic may result in suboptimal or inadequate responses, necessitating multiple doses and the addition of adjuvants to amplify responses, which complicates the composition and characterization of formulations, and can cause adverse reactions [15, 16, 59].

Traditionally, vaccines have incorporated live, but attenuated or inactivated (e.g., heat-killed) pathogens, often co-delivered with adjuvants, to balance these two factors [1, 3]. However, this approach requires the availability of pathogen in large quantities for manufacturing. Recent developments in the seasonal influenza vaccine also reflect some of these critical challenges associated with prophylactic vaccine manufacturing and distribution. Two general vaccines for influenza have been approved: the first, an injected vaccine formulation, is composed of inactivated virus; the second is a live, but attenuated virus delivered intra-nasally. A recent study to evaluate vaccine effectiveness in children ages 2–17 conducted by the Centers for Disease Control and Prevention (CDC) revealed that the nasal spray formulation exhibited reduced efficacy compared with the injected formulations of the vaccine [112]. This result led the CDC’s Advisory Committee on Immunization Practices, to vote that the nasal spray formulation should not be used in the 2016–2017 season [112].

In addition, despite the reliable level of protective immunity conferred by many vaccines – including flu, there are still significant improvement opportunities for these cases. The current vaccine is primarily generated by growing the virus in chicken eggs, which inevitably takes time to generate in large scale [113], by some estimates a 20–28 week timeline to produce [114]. In contrast, cell culture-based approaches may require roughly half of this duration to produce [114]. The delay with these approaches is particularly relevant to the example of the seasonal influenza vaccine, because the formulation must be changed each year to reflect the strain most likely to spread. Thus, strategies that would allow for rapid and economical production, as well as the flexibility to incorporate antigens to one or more target strains of the virus, could be transformative. Further, incorporation of live, attenuated virus mentioned above can still pose risk of infection, motivating the exploration of synthetic approaches – perhaps that incorporate self-assembly – to recapitulate the structure of pathogens that do not have the potential to replicate.

Another challenge facing vaccines is stability. As an illustration, in one recent study, storage of alum, a clinically-approved adjuvant, for 6 months at 45 °C led to a significant decrease in immunogenicity [77]. This result exemplifies a hurdle for the field: the requirement for a cold chain of refrigeration in order to disseminate vaccines or immunotherapies worldwide. Current clinical options are typically sensitive to both extreme heat and cold; carefully controlled storage is required to maintain the stability of emulsion-based adjuvants and the viability and long-term potency of live, attenuated pathogens. This limitation is particularly relevant because some of the most critical regions to deliver vaccines are in the developing world, where access to healthcare professionals and refrigeration are extremely limited [115, 116]. Self-assembly is already being utilized in this area: one study confirmed that self-assembled nanostructures displaying a peptide epitope of Mycobacterium tuberculosis maintained immunogenicity even after storage for 6 months at 45 °C, compared freshly-prepared doses [77]. This is just one example where self-assembly is being weighed with a specific translational focus. In Sections 6.2 and 6.3 we bring other translationally-geared reports to the forefront, illustrating new self-assembly strategies for either promoting or regulating responses to vaccines and immunotherapies.

6.2. Self-assembled materials generate efficacious responses in pre-clinical models of infection and cancer

One recent approach to minimize risk, but maximize efficacy in a therapeutic context is the use of VLP-based vaccines to incorporate and deliver influenza antigens [36, 113, 114, 117, 118]. The potential for these VLPs to protect against viral challenge with the same strain from which the VLPs are derived (i.e., homologous strain), as well as against challenges with other strains, have been tested [118]. Importantly, the potential to protect against multiple strains could help determine whether candidate vaccine and therapies have the potential to confer broad protection; this question is particularly relevant for influenza, as the virus mutates rapidly to evade immune clearance. While VLPs exhibit demonstrated exciting pre-clinical and clinical success, there are considerations beyond efficacy. The production of recombinant proteins (i.e., VLPs) in cell lines is associated with high cost and low yield, and requires careful purification and characterization to ensure homogeneity, reproducibility, and potency [34, 39, 119]. Thus, ongoing studies aim to enhance the efficiency, yield, and purity of the final vaccine product [82, 84, 119, 120]. In parallel, researchers have also investigated the use of short, synthetic monomers, which are simpler to produce in cell culture compared with full recombinant proteins, or could enable cell-culture-free production. These monomers could then be harnessed to incorporate defined target antigens into nano- or micro-scale materials through self-assembly.

The nanofiber strategies to enable high valency display of model antigen described in Section 5.2 have also been harnessed to elicit responses against disease-relevant antigens. For example, the platform from the Tirrell lab has been extended for immunization against group A streptococcus [89], while Rudra and colleagues demonstrate an approach to trigger antibody responses against a malaria antigen [73]. The driving force of the self-assembly in the latter approach – beta-sheet formation – has also been employed to incorporate a protein from the envelope of West Nile virus (EIII) [121]. In this work, the self-assembling peptide containing a beta-sheet-forming domain spontaneously formed an injectable hydrogel that entrapped EIII to enable sustained, subcutaneous delivery to mice. The hydrogel formulation conferred significant protection in a viral challenge model, with a final survival of 60%, compared with 20% in untreated mice or mice treated with EIII incorporated in a clinical adjuvant, alum [121].

The example just discussed represents an approach using self-assembly to generate a hydrogel that has larger dimensions on the macro scale; along these same lines, others have developed peptide fibers that self-assemble after injection to mimic the antigen “deposits” often formed by conventional emulsion-based adjuvants. The goal of this approach is to generate a persistent source of antigen for prolonged immunostimulation, a partial mechanism of action of alum and other current adjuvants used in the clinic [15]. This fiber-based approach was employed to deliver a hepatitis B antigen with CpG, which triggered enhanced humoral and cellular responses when compared with a formulation containing alum and an equivalent dose of EIII [122]. Together, these results support the potential to use self-assembled materials to generate in vivo depot of antigen and immunostimulatory cues that can enhance immunogenicity. This approach could also simplify depot-like vaccine formulations by incorporating well-defined peptide sequences rather than complex adjuvant systems.
Molecular adjuvants delivered in self-assembled systems are also being explored in disease contexts either with or without antigens. In the latter case, these strategies often exploit the fact that, during disease, the immune system is actively surveying and processing disease-relevant antigens. Yet, the responses to those antigens are not effective in generating responses that combat disease. For example, in cancer, lymph nodes often contain tumor associated antigens that have reached these sites either through passive drainage through the lymphatics or active transport via APCs. However, the tumor microenvironment is often highly immunosuppressive, evading detection and clearance by the immune system [20,22,68,123]. Cells in tumors may alter the expression of key surface markers or secrete regulatory cytokines that suppress tumor-infiltrating immune cells. This reduction in signal 2 and signal 3 effectively reduces the level of “danger” signals, inhibiting the generation of the robust responses needed to clear tumors. Thus, the incorporation of modulatory signals may be able to redirect or skew the types of responses generated against disease-relevant antigens.

One example of an approach to modulate responses in clinically-relevant contexts has been to incorporate molecular adjuvants into self-assembled materials. CpG has been incorporated into multiple self-assembled nanoparticle formulations, through non-covalent interactions with lipids [64], gold nanoparticles [63,124], or synthetic polymers [125]. Broadly, these strategies aim to enhance circulation time as well as target CpG to target cell populations – APCs, like dendritic cells – through nanoparticle-mediated delivery. This approach has been shown to slow tumor growth and enhance survival in a mouse model of melanoma [124,125]. In another example, self-assembly was harnessed to incorporate multiple TLRs, for TLR2 and TLR9, into a nanoparticle with a tumor associated antigen – MUC1, a mucin transmembrane glycoprotein. These nanoparticles were designed to self-assemble through electrostatic interactions to co-deliver these three therapeutic cargos. Treatment of mice with the nanoparticles conferred a synergistic effect on survival in an aggressive melanoma model compared with formulations that contained antigen and a single adjuvant, or antigen alone [126].

Fig. 9. Self-assembly-enabled delivery of DNA ligands to the STING pathway limits autoimmunity and inflammation. A) Mice were induced with a model of multiple sclerosis (EAE) on day 0 and treated at the time of induction with either a vehicle control or DNA nanoparticles (DNPs). Mean clinical score of the level of disease-induced paralysis was determined. B) Mean clinical score of mice induced with EAE as in (A), and either treated with a vehicle or DNPs around the onset of disease-induced paralysis using the regimen depicted. C) The level of inflammatory cytokines in lymph nodes of mice immunized with MOG as in and either injected with a vehicle control or DNPs five times beginning at the time of immunization as in (A).
Adapted from [134] with permissions.

Fig. 10. Electrostatic complexation of immune signals to restrain inflammatory signaling during autoimmunity. A) Schematic depicting the hypothesized mechanism for MOG·GpG complexes. Typically during MS, self-antigen, MOG, is processed and presented by DCs in the presence of excess TLR9 signaling, which drives expansion of self-antigen-specific effector T cells (left). In contrast, co-administration of self MOG peptide – modified with arginine residues to confer positive charge, MOG-RX – with an antagonistic ligand of TLR9, GpG could blunt inflammatory signaling, leading to the development of MOG-specific regulatory T cells (right). B) Nano-scale MOG·GpG complexes exhibited tunable surface charge as a function of the input ratio of MOG peptide to GpG. C) TLR9 signaling was assessed in a reporter cell line following stimulation of cells with GpG and addition of either free MOG-RX, free GpG, or MOG-RX/GpG complexes to investigate the potential to restrain CpG-induced signaling. D) Proliferation of MOG-specific transgenic T cells following co-culture with splenic DCs that were isolated from wild-type mice and treated with CpG and either free MOG-RX, or MOG-RX/GpG complexes.
Adapted from [138] with permissions.
Finally, the electrostatic assembly approaches described in Section 5.3 have also been exploited for pro-immune disease applications by, for example, condensing adjuvant and antigens for either cancer or viral infection [127]. De Geest et al. demonstrate a dramatic enhancement in mouse survival using layer-by-layer assembled capsules to deliver antigen compared with soluble antigen in a model of melanoma. The modular nature of the PEM system was then exploited to instead incorporate an antigen for influenza A. In challenge studies, PEM-mediated delivery of antigen again exhibited an enhanced protective effect over soluble antigen, supporting a role for delivery of antigen in self-assembled particles to enhance protective immune effects [127]. One final example of electrostatic assembly for pro-inflammatory, therapeutic application involves the use of layer-by-layer assembly to co-deliver immune signals for an HIV vaccine administered via transdermal delivery. The Irvine lab has demonstrated an approach to coat microneedle arrays with a degradable cationic polymer, a poly(β-amino-ester), and layers of plasmid DNA encoding for HIV antigens and a TLR3 agonist, polyIC, as the anions [128]. Following microneedle application, the coatings are engineered with a releasable layer to detach from the microneedle substrate and remain in the skin. The co-delivery of these signals was confirmed via immunofluorescent analysis of mouse skin following microneedle application, and the persistence of signals at the site of administration compared with intra-dermal injection at the same site (i.e., mouse ear). Release of films from microneedles drove potent antigen-specific T cell expansion and enhanced antibody titers compared with intramuscular or intradermal injection. Finally, skin penetration and delivery of immune signals was demonstrated in non-human primate skin, supporting the translational potential of this approach in moving toward human disease applications.

6.3 Harnessing self-assembly to regulate immune response and promote tolerance during autoimmunity or transplantation

As discussed earlier, during autoimmune disease, “self” antigens are incorrectly recognized and trigger inflammatory attacks. For example, in multiple sclerosis, peptide fragments from myelin, the matrix that lines neurons, are attacked [129–131]. Delivery of tolerogenic immune signals may be able to redirect immune response against the self-antigens by skewing T cell differentiation away from inflammatory phenotypes and toward regulatory phenotypes. However, the potential to expand therapeutic cell types, such as regulatory T cells, involved in tolerance during active autoimmunity is a significant hurdle. MS and other autoimmune diseases are characterized by excess inflammation, but the development of regulatory cells is dependent on the potential for APCs and, subsequently, lymphocytes to process, present, and recognize self-antigens in the absence of stimulatory immune cues (e.g., signal 2, 3).

Toward the goal of downregulating pro-immune signaling, the Mellor group has described electrostatic condensation of plasmid DNA to promote tolerogenic immune function [132–134]. The nucleic acid

---

Fig. 11. Carrier-free co-localization of self-antigen and a TLR9 regulator promotes tolerance in mouse cells, mouse models of autoimmunity, and samples from human patients. A) Schematic of carrier-free iPEM capsules formulated from an antagonistic ligand of TLR9 (GpG) and myelin self-antigen modified with three arginine residues (MOG-R3). B) iPEMs exhibited tunable relative loading of each cargo as a function of the cargo input to the synthesis process. C) Secretion of an inflammatory cytokine, IL-6, from co-cultures prepared by isolating wild-type splenic DCs from mice and incubating with media alone, soluble CpG alone, or CpG and either soluble GpG, a soluble control oligonucleotide that does not regulate TLR9 signaling (CTRL), iPEMs assembled from MOG-R3 and GpG (MOG-R3/GpG)3, or from MOG-R3 and CTRL, or free MOG-R3. After overnight culture, MOG-specific T cells were isolated from transgenic mice, added to cultures, allowed to incubate for three days, and supernatants were analyzed by ELISA. D) A subset of co-culture samples described in (C) were analyzed for expression of markers of regulatory T cells (CD4+CD25+Foxp3+) by flow cytometry. E) Mice were induced with a model of multiple sclerosis (EAE) and either left untreated or administered two doses of (MOG-R3/GpG)3 iPEMs on days 5 and 10 post induction. The severity of disease-induced paralysis was assessed using a clinical scoring scale. F) Peripheral blood mononuclear cells from a human MS patient were incubated with media alone, (MOG-R3/GpG)3 iPEMs, or (MOG-R3/CTRL)3 iPEMs and metabolic activity was measured using an MTT assay. G) Supernatants from the cultures in (F) were analyzed for the secretion of inflammatory IL-6 using a Luminex assay.

Adapted from [111] with permissions.
cargos are targeted to the stimulator of IFN genes (STING) pathway, which is responsible for producing cytokines that potentiate inflammation. Nucleic acids are condensed through electrostatic assembly with a common polycation, polyethenetimine (PEI) to form polyplexes designed to enhance gene delivery. To test the therapeutic potential of this approach, DNA condensed into NP form (DNPs) were tested in a well-characterized mouse model of multiple sclerosis (MS), experimental autoimmune encephalomyelitis (EAE). In this model, mice are injected with MOG peptide emulsified in a strong pro-inflammatory signal, Complete Freund's Adjuvant (CFA), followed by administration of pertussis toxin to serve as an adjuvant and open the blood-brain-barrier. The pro-inflammatory MOG-specific cells are then able to migrate to the central nervous system (i.e., spinal cord, brain) where they recognize and attack myelin. The result of this attack is progressive paralysis that develops over a few weeks. In this study, a significant decrease in mean clinical score (i.e., reduced disease-induced paralysis) was observed after 5 treatments of DNPs compared with a vehicle control treatment regimen. This effect was observed when treatment was initiated at either the time of disease induction (Fig. 9A) or at the onset of disease symptoms (Fig. 9B). DNPs were shown to restrain the secretion of numerous inflammatory cytokines implicated in disease (Fig. 9C). Importantly, restraint of disease was dependent on delivery of DNA cargo in NP format, as a matched soluble dose caused no effect, supporting the role of self-assembly to enhance delivery of therapeutic immune cargos in vivo.

An intriguing recent idea is to employ biomaterials to co-deliver self-antigen with regulatory immune cues to promote the expansion of cells that are self-antigen-specific, but exert tolerogenic or regulatory functions [111,135–138] instead of inflammatory attacks. This idea is underpinned in part by a fascinating new role of TLR signaling during autoimmune disease. Recent studies have revealed excess signaling through TLRs contributes to the pathogenesis of autoimmunity in both mouse models and human patients [139–145]. Further, work by the Steinman lab has demonstrated the potential for an antagonistic ligand of TLR9, GpG, to partially restrain inflammation and reduce the severity of the symptoms of EAE in mice when administered in soluble form [146,147]. Our lab hypothesized that co-delivery of myelin self-antigen electrostatically assembled with GpG might blunt the TLR9 signaling present during multiple sclerosis and skew T cell responses toward TREGS able to control disease (Fig. 10A).

We have formed polyplex-like structures composed of the GpG signal and myelin antigen (MOG) conjugated to one or two arginine residues to confer positive charge, MOGR1 and MOGR2, respectively, eliminating synthetic components [138]. Varying the input of each

---

**Fig. 12.** A) Schematic representation of encapsulation of a small molecule immunomodulator, tacrolimus, in an enzyme-degradable hydrogel. B) Release kinetics of tacrolimus from the hydrogel represented in (A) when incubated in PBS or in the presence of and enzyme (MMP9) to drive hydrogel degradation. The tacrolimus-containing hydrogel in (A) was injected subcutaneously on the same side as a hind limb transplant in a rat model. Control treatments included no treatment, hydrogel alone (vehicle control), a soluble bolus injection of tacrolimus, and the tacrolimus hydrogel injected on the opposite (i.e., contralateral) side of the hind limb transplant. Graft survival was quantified (C) and could be assessed qualitatively through images comparing transplanted hind limbs from a mouse treated with soluble tacrolimus (a) or the tacrolimus hydrogel formulation (b). Adapted from [151] with permissions.
cargo to the electrostatically-driven self-assembly leads to formation of nano-scale complexes 100-200 nm in diameter with tunable properties, such as loading and zeta potential (i.e., surface charge) (Fig. 10B). MOG/GpG complexes were shown to down-regulate TLR9 signaling – the target ligand of the GpG cargo (Fig. 10C), restrain the proliferation of antigen-specific T cells using a co-culture system with transgenic T cells specific for the MOG peptide (Fig. 10D), and to attenuate EAE.

In parallel, we have adapted the iPEM platform described in Section 5.3 promote tolerance by assembly of MOG and myelin self-antigens (Fig. 11A) [111]. iPEM capsules formed from myelin peptide and GpG enabled tunable absolute and relative cargo loading of each component (Fig. 11B). Interestingly, these MOG/GpG iPEMs promote antigen-specific T cell proliferation in the co-culture system mentioned above. However, the expanding myelin-reactive T cells were found to secrete lower levels of inflammatory cytokines (Fig. 11C) and higher expression levels of markers characteristic of TREGs (Fig. 11D) when compared with a control formulation that incorporated myelin peptide and a nucleotide that does not regulate TLR9 signaling (CTRL) (Fig. 11C-D, orange bars). The finding suggests that iPEMs might promote the expansion of myelin-specific TREGs that control disease in a highly specific manner, rather than acting through broad immunosuppressive pathways. In the EAE model, iPEMs were found to protect 100% of mice from the onset of EAE symptoms (Fig. 11E) [111]. Finally, in samples from human MS patients, iPEMs provided similar benefits to those observed in primary mouse cells (Fig. 11C-D); iPEMs containing MOG and either GpG or CTRL activated cells, as measured by an increase in metabolic activity (Fig. 11F) [111]. However, iPEMs containing GpG restrained inflammatory cytokine secretion relative to CTRL-containing iPEMs (Fig. 11G). These results in human MS patient samples highlight a unique opportunity to regulate TLR signaling to impact human immune cell function.

Another application of interest to promote immune tolerance is transplantation. Following transplant, the host immune system often recognizes the graft – the cells, tissues, or organs transferred – as foreign and mounts an attack. While care is taken to ensure that donors are close matches to recipients, patients are administered life-long regimens of potent immunosuppressive drugs to resist the graft rejection, which can leave these individuals immunocompromised [148]. Approaches to generate durable, specific transplant tolerance could, therefore, dramatically improve patient outcomes and quality of life. Many of the candidate drugs along these lines are highly hydrophobic small molecules. The use of amphiphilic carriers, such as lipids or polymers with hydrophobic residues [149], can allow for incorporation of hydrophobic molecules. The use of amphiphilic carriers, such as lipids or polymers with hydrophobic residues [149], can allow for incorporation of hydrophobic molecules. The use of amphiphilic carriers, such as lipids or polymers with hydrophobic residues [149], can allow for incorporation of hydrophobic molecules. Therefore, dramatically improve patient outcomes and quality of life. Many of the candidate drugs along these lines are highly hydrophobic small molecules. Therefore, dramatically improve patient outcomes and quality of life. Many of the candidate drugs along these lines are highly hydrophobic small molecules. Therefore, dramatically improve patient outcomes and quality of life. Many of the candidate drugs along these lines are highly hydrophobic small molecules.

References
[1] E. De Gregorio, The path forward. Vaccine 33 (2015) 860–863.
[2] T.J. Moyer, A.C. Zmolek, D.J. Irvine, Beyond antigens and adjuvants: formulating future vaccines, J. Clin. Invest. 126 (2016) 799–808.
[3] S. Plotkin, History of vaccination, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 12283–12287.
[4] L. Melero, G. Gaudernack, W. Gerritsen, C. Huber, G. Pariani, S. Scholl, N. Thatcher, J. Wagstaff, C. Zielinski, F. Faulkner, H. Mellstedt. Therapeutic vaccines for cancer: an overview of clinical trials, Nat. Rev. Clin. Oncol. 11 (2014) 509–524.
[5] J.H. Doroshov, S. Kummam, Translational research in oncolgy—10 years of progress and future prospects, Nat. Rev. Clin. Oncol. 11 (2014) 649–662.
[6] C.A. Dendrou, L. Fugger, M.A. Friese, Immunopathology of multiple sclerosis, Nat. Rev. Immunol. 15 (2015) 545–558.
[7] L. Steinman, Inverse vaccination, the opposite of Jenner’s concept, for therapy of autoimmune, J. Intern. Med. 267 (2010) 441–451.
[8] A.J. McMichael, P. Borrow, G.D. Tomaras, N. Goonetilleke, B.F. Haynes, The immune response during acute HIV-1 infection: clues for vaccine development, Nat. Rev. Immunol. 10 (2010) 653–662.
[9] J.A. Feldman, P. Borrow, B. Doherty, N. Goonetilleke, B.F. Haynes, The immune response during acute HIV-1 infection: clues for vaccine development, Nat. Rev. Immunol. 10 (2010) 653–662.
[10] S. Plotkin, History of vaccination, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 12283–12287.
[11] J.A. Feldman, P. Borrow, B. Doherty, N. Goonetilleke, B.F. Haynes, The immune response during acute HIV-1 infection: clues for vaccine development, Nat. Rev. Immunol. 10 (2010) 653–662.
[12] S. Plotkin, History of vaccination, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 12283–12287.
[13] J.A. Feldman, P. Borrow, B. Doherty, N. Goonetilleke, B.F. Haynes, The immune response during acute HIV-1 infection: clues for vaccine development, Nat. Rev. Immunol. 10 (2010) 653–662.
[14] J.A. Feldman, P. Borrow, B. Doherty, N. Goonetilleke, B.F. Haynes, The immune response during acute HIV-1 infection: clues for vaccine development, Nat. Rev. Immunol. 10 (2010) 653–662.
[15] J.A. Feldman, P. Borrow, B. Doherty, N. Goonetilleke, B.F. Haynes, The immune response during acute HIV-1 infection: clues for vaccine development, Nat. Rev. Immunol. 10 (2010) 653–662.
[16] S. Plotkin, History of vaccination, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 12283–12287.
[17] J.A. Feldman, P. Borrow, B. Doherty, N. Goonetilleke, B.F. Haynes, The immune response during acute HIV-1 infection: clues for vaccine development, Nat. Rev. Immunol. 10 (2010) 653–662.
[18] S. Plotkin, History of vaccination, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 12283–12287.
W.J. McAleer, E.B. Buynak, R.Z. Maigetter, D.E. Wampler, W.J. Miller, M.R. Hilleman, D.M. Smith, J.K. Simon, J.R. Baker Jr., Applications of nanotechnology for immunology, Adv. Drug Deliv. Rev. 93 (2015) 168–185.

J. T. Schiller, X. Castellsague, S. M. Garland, A review of clinical trials of human papillomavirus (HPV) vaccine, Adv. Drug Deliv. Rev. 110-111 (2017) 169–187.

J. H. Park, C. M. Jewell, Harnessing biomaterials to engineer the immune microenvironment, Nat. Rev. Cancer 16 (2016) 1557–1570.

J. Chen, R. R. Pompano, F. W. Santiago, L. Maillat, R. Sciammas, T. Sun, H. Han, D. J. Irvine, In situ engineering of the lymph node with adjuvanted nanoparticles reshapes the anti-tumor immune response, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 15745–15750.

J. C. Smith, J. C. M. Jewell, Advanced Drug Delivery Reviews 114 (2017) 60–78.
C.B. Chesson, S. Ekpo-Otu, J.J. Endsley, J.S. Rudra, Biomaterials-based vaccination strategies for the induction of CD+ T cell responses, Adv. Healthc. Mater. 3 (2014) 1200–1208.

T. Sun, H.F. Han, G.A. Hudalla, Y. Wen, R.R. Pompano, J.J. Collier, Thermal stability of self-assembled peptide vaccine materials, Acta Biomater. 30 (2016) 62–71.

P. Li, C.N. Shi, X.Y. Zhang, H.J. Song, C.N. Zhang, W.W. Wang, C.L. B. Song, C. Wang, D.L. Kong, Guanidinylated cationic nanoparticles as robust protein antigen delivery systems and adjuvants for promoting antigen-specific immune responses in vivo, J. Mater. Chem. B 4 (2016) 5068–5072.

Y.F. Liu, Z.Y. Sun, P.G. Gu, Z.H. Huang, Y. Gao, L. Shi, Y.F. Zhao, Y.X. Chen, Y.M. Li, Glycopeptide nan conjugates based on multilayer self-assembly as an antitumor vaccine, Bioconjug. Chem. 26 (2015) 1439–1449.

A. Purwada, F.Y. Tian, W.S. Huang, K.M. Rohrbach, S. Deol, A. August, A. Singh, Self-assembly protein nanoparticles for safer cancer immunotherapy, Adv. Healthc. Mater. 5 (2016) 1413–1419.

M. Tamborini, N. Geib, A. Marrero-Nodarse, M. Jud, J. Hauser, C. Aho, A. Lamelas, A. C. A. Mei-Liu, M.A. Swartz, J.A. Hubbell, Dendritic cell antigenic nanoparticles elicit controlled protective immune response to influenza viruses, Vaccine (2016).

X. Zhang, K.H. Wang, Q.S. Lin, M.H. Zheng, Q. Li, T.T. Li, Q.Y. Hong, Q.R. Zheng, H.Y. Yu, Y. Gu, S.W. Li, N.S. Xia, A shared N-terminal histidine tag for the formation of amphoteric nanoparticles, Biomaterials 11 (2016) 2895–2902.

Z. Luo, P. Li, L.J. Deng, N. Gao, Y. Zhang, H. Pan, L. Liu, C. Wang, L. Cai, Y. Ma. Cationic polypeptide micelle-based antigen delivery system: a simple and robust adjuvant to improve vaccine efficacy, J. Control. Release 170 (2013) 259–267.

Q. Zhao, O. Daugasz, M. Born, D. Burkhard, P. Burkhard, Voltage-induced self-assembly and refolding of a self-assembling protein nanoparticle (SAPNP) malaria vaccine, Methods 60 (2013) 242–247.

R. Ringler, L. Shi, M.A. Swartz, P. Burkhard, Optimizing the refolding conditions of self-assembling polypeptide nanoparticles that serve as retinoic antigen display systems, J. Struct. Biol. 177 (2012) 168–176.

M. Black, A. Trent, Y. Kostenko, J.S. Lee, C. Olive, M. Tirschwell, Self-assembled peptide amphiphile micelles containing a cytotoxic T-cell epitope promote a protective immune response in vivo, Adv. Mater. 24 (2012) 3845–3849.

C.B.Chesson, E.J. Hueselmann, A.T. Lacey, F.K. Kohlhapp, M. Webb, F. Kay, A. Nabatiyan, A. Kamm, J. Miller, E.J. Huelsmann, A.T. Lacek, F.J. Kohlhapp, M.F. Webb, A. Nabatiyan, A. M. Black, A. Trent, Y. Kostenko, J.A. Robinson, A.G. Harmsen, T. Douglas, Biomimetic biomaterials and viral infection, Expert Rev. Vaccines 10 (2011) 1451–1454.

Z.H. Huang, L. Shi, J.W. Ma, Z.Y. Sun, H. Gai, Y.X. Chen, Y.F. Zhao, Y.M. Li, A totally synthetic, self-assembling, adjuvant-free MUC1 glycoprotein vaccine for cancer therapy, J. Am. Chem. Soc. 134 (2012) 8730–8733.

A. Trent, B.D. Uley, M.J. Black, J.C. Barrett, S. Liang, Y. Kostenko, N.A. David, M.V. Tirschwell, Peptide amphiphile micelles self-adjuvant group A streptococcal vaccine, AAPS J. 17 (2015) 380–388.

Z.T. Shen, A.B. Sigalov, SARS coronavirus fusion peptide-derived sequence suppresses collagen-induced arthritis in DBA/1J mice, Sci. Rep. 6 (2016).

C.B. Chesson, C.J. Roy, P.G. Gu, J.S. Rudra, Biomaterials-based vaccination strategies for the induction of CD+ T cell responses, ACS Biomater. Sci. Eng. 1 (2015) 1200–1208.

P.O. Ilyinskii, C.J. Roy, CP. O'Neil, E.A. Browning, L.A. Pittet, D.H. Altreuter, F. Alexis, E.A. Scott, A. Stano, M. Gillard, A.C. Maio-Liu, M.A. Swartz, J.A. Hubbell, Dendritic cell antigenic nanoparticles elicit controlled protective immune response to influenza viruses, Vaccine (2016).

J.T. Wilson, A. Postma, S. Keller, A.J. Convery, P.S. Stayton, pH-Responsive nanoparticle vaccines for dual-delivery of antigens and Immunostimulatory oligonucleotides, ACS Nano 7 (2013) 3912–3925.

J.T. Wilson, A. Postma, S. Keller, A.J. Convery, G. Moad, E. Rizzardo, L. Meagher, J. Clechier, P.S. Stayton, Enhancement of MHC-I antigen presentation via architectural control of pH-responsive, Endosomolytic polymer nanoparticles, AAPS J. (2017) 358–369.

S. de Koker, L.J. De Cock, P. Rivera-Gil, W.J. Parak, R. Auzély Velty, C. Vervaert, J.P. Remon, J. Grooten, B.G. de Geest, Polymeric multilayer capsules delivering body fluids, Adv. Therapeutics, Adv. Drug Deliv. Rev. 63 (2011) 743–753.

Y. Yan, M. Manbcan, F. Caruso, Assembly of layer-by-layer particles and their interactions with biological systems, Chem. Mater. 26 (2014) 452–460.

S.-F. Chong, A. Sexton, R. De Rose, S.J. Kent, A.N. Zeltkin, F. Caruso, A paradigm for peptide vaccine delivery using self-assembling polymeric nanoparticles encapsulated in degradable polyglycolic acid, Biomaterials, Bios 30 (2009) 5178–5186.

B.G. de Geest, M.A. Willart, B.N. Lambrecht, C.Pollard, C. Vervaert, J.P. Remon, J. Grooten, S. de Koker, Surface-engineered polyelectrolyte multilayer capsules: synthetic vaccines mimicking microbial structure and function, Angew. Chem. Int. Ed. 51 (2012) 3862–3866.

Y.C. Chiu, J.M. Gammon, J.I. Andorko, L.H. Tostanoski, C.M. Jewell, Modular vaccine design using carrier-free capsule assembled from Polyionic immune signals, ACS Biomater. Sci. Eng. 1 (2015) 1260–1265.

Y.C. Chiu, J.M. Gammon, J.I. Andorko, L.H. Tostanoski, C.M. Jewell, Assembly and immunological processing of polyelectrolyte multilayers composed of antigens and adjuvants, ACS Appl. Mater. Interfaces 8 (2016) 18722–18731.

Z.Y. Sun, P.G. Gu, J.S. Rudra, Antigenic peptide nanocapsules elicit adjuvant-free CD+ T cell responses, Vaccines 6 (2017) 194.

Y. Yan, M. Bjonmalm, F. Caruso, Assembly of layer-by-layer particles and their in-vitro components as a platform to elicit immune responses to antigens, Expert Rev. Vaccines 15 (2016) 1281–1293.

N. Wibowo, Y.P. Chau, P.G. Gu, J.S. Rudra, Modular engineering of a cationic polymer micelle complex improves CD8+ T cell responses in vaccination against malaria, ACS Nano 10 (2016) 9334–9345.

D.R. Convertine, P.S. Stayton, pH-Responsive nanoparticle vaccines for dual-delivery of antigens and adjuvants, AAPS J. 17 (2015) 380–388.

J. Hammon, D.J. Irvine, Polymer multilayer tattooing for enhanced DNA vaccination strategies, ACS Chem. Biol. 11 (2016) 143–147.

B.M. Friedrich, D.W. Beasley, J.S. Rudra, Supramolecular peptide hydrogel capsules, Biomaterials 30 (2009) 5178–5186.
regulatory T cells accumulate in the CNS but fail to control autoimmune inflammation, Nat. Med. 13 (2007) 423–431.

[130] M.G. Mohammad, V.W. Tsai, M.J. Ruitenberg, M. Hassanpour, H. Li, P.H. Hart, S.N. Breitbart, P.E. Sawchenko, D.A. Brown, Immune cell trafficking from the brain maintains CNS immune tolerance, J. Clin. Invest. 124 (2014) 1228–1241.

[131] M. van Zwaan, R. Huizinga, M.J. Melief, A.F. Wierenga-Wolf, M. van Meurs, J.S. Voerman, K.P. Arbor, H.W. Boddeke, U.E. Hoppeken, C. Meisel, A. Meisel, I. Bechmann, R.Q. Hinzten, B.A. t Hart, S. Amor, J.D. Laman, L.A. Boven, Brain antigens in functionally distinct antigen-presenting cell populations in cervical lymph nodes in MS and EAE, J. Mol. Med. 87 (2009) 273–286.

[132] L. Huang, H.P. Lemos, L. Li, M. Li, P.R. Chandler, B. Baban, T.L. McGaha, B. Ravishankar, J.R. Lee, D.H. Munn, A.L. Mellor, Engineering DNA nanoparticles as Immunomodulatory reagents that activate regulatory T cells, J. Immunol. 188 (2012) 4913–4920.

[133] L. Huang, L. Li, H. Lemos, P.R. Chandler, G. Pacholczyk, B. Baban, G.N. Barber, Y. Hayakawa, T.L. McGaha, B. Ravishankar, D.H. Munn, A.L. Mellor, Cutting edge: DNA sensing via the STING adaptor in myeloid dendritic cells induces potent Tolerogenic responses, J. Immunol. 191 (2013) 3509–3513.

[134] H. Lemos, L. Huang, P.R. Chandler, E. Mohamed, G.N. Barber, Y. Hayakawa, D.H. Munn, A.L. Mellor, Activation of the STING adaptor attenuates experimental autoimmune encephalitis, J. Immunol. 192 (2014) 5571–5578.

[135] R.A. Maldonado, R.A. De, A.S. Ferreira, A.-H. Zhang, R.J. Rossi, P.N. Kotle, A.P. Griset, C. O’Neil, D.H. Altreuter, E. Browning, L. Johnston, O.C. Farokhzad, R. Langer, D.W. Scott, U.H. von Andrian, T.K. Kishimoto, Polymereic synthetic nanoparticles for the induction of antigen-specific immunological tolerance, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) E156–E165.

[136] F.J. Quintana, G. Murugaiyan, M.F. Farez, M. Mitkoffer, A.M. Tukap, E.J. Burns, H.L. Weiner, An endogenous aryl hydrocarbon receptor ligand acts on dendritic cells and T cells to suppress experimental autoimmune encephalomyelitis, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 20768–20773.

[137] L.H. Tostanoski, Y.C. Martinez, Y. Chung, C. Dong, Toll-like receptor 4 signaling in T cells promotes autoimmune inflammation, J. Immunol. 171 (2003) 4920–4925.

[138] K.L. Hess, J.I. Andorko, L.H. Tostanoski, C.M. Jewell, Polyplexes assembled from self-peptides and regulatory nucleic acids blunt toll-like receptor signaling to combat autoimmunity, Biomaterials 118 (2017) 51–62.

[139] R.E. Balashov, L.L. Aung, A. Vaknin-Dembinsky, S. Dhil-Jalbut, H.L. Weiner, Interferon-beta inhibits toll-like receptor 9 processing in multiple sclerosis, Ann. Neurol. 68 (2010) 899–906.

[140] T. Celhrar, R. Magalhaes, A.M. Fairhurst, TL1R7 and TL1R9 in SLE: when sensing self goes wrong, Immunol. Res. 53 (2012) 58–77.

[141] K. Derkow, J.M. Bauer, M. Hecker, B.K. Paap, M. Thamilarasan, D. Kocz, E. Schott, K. Deuschle, J. Bellmann-Strobl, F. Paul, U.K. Zerli, K. Suppe, S. Lehnardt, Multiple sclerosis: modulation of toll-like receptor expression by interferon-beta includes upregulation of TL1R7 in plasmacytoid dendritic cells, PLoS One 8 (2013), e70626.

[142] P.N. Kelly, D.L. Romero, Y. Yang, A.L. Shaffer, D. Chaudhary, S. Robinson, W. Miao, L. Rui, W.F. Westlin, R. Kapeller, L.M. Staudt, Selective interleukin-1 receptor-associated kinase 4 inhibitors for the treatment of autoimmune disorders and lymphoid malignancy, J. Exp. Med. 212 (2015) 2189–2201.

[143] M. Prinz, F. Garbe, H. Schmidt, A. Mildner, I. Gutsch, K. Wolter, M. Piesche, R. Schroers, E. Weiss, C.J. Kirschning, C.D. Rochford, W. Bruck, B. Becher, Innate immunity mediated by TL1R9 modulates pathogenicity in an animal model of multiple sclerosis, J. Clin. Invest. 116 (2006) 456–464.

[144] J.M. Reynolds, G.J. Martinez, Y. Chung, C. Dong, Toll-like receptor 4 signaling in T cells promotes autoimmune inflammation, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 13064–13069.

[145] M. Santoni, K. Andrikou, V. Sotte, A. Bittoni, A. Lanes, E. Piva, A. Conti, M. Nabissi, G. Santoni, S. Cascino, Toll-like receptors and pancreatic diseases: from a pathogenic mechanism to a therapeutical target, Cancer Treat. Rev. 41 (2015) 569–576.

[146] P.P. Ho, P. Fontoura, M. Platten, R.A. Solbiel, J.J. DeVoss, L.Y. Lee, B.A. Kidd, B.H. Tomooka, J. Capers, A. Agarwal, R. Gupta, J. Zernik, M.K. Yee, B.J. Lee, H. Garren, W.H. Robinson, L. Steinman, A suppressive oligodeoxynucleotide enhances the efficacy of myelin cocktail/TL4-tolerizing DNA vaccination and treats autoimmune disease, J. Immunol. 175 (2005) 6226–6234.

[147] P.P. Ho, P. Fontoura, P.J. Ruiz, L. Steinman, H. Garren, An immunomodulatory GpC oligonucleotide for the treatment of autoimmune via the innate and adaptive immune systems, J. Immunol. 171 (2003) 4920–4926.

[148] A.E. Morelli, A.W. Thomson, Tolerogenic dendritic cells and the quest for transplant tolerance, Nat. Rev. Immunol. 7 (2007) 610–621.

[149] H. Yim, W. Park, D. Kim, T.M. Fahmy, K. Nia, A self-assembled polymeric micellar immunomodulator for cancer treatment based on cationic amphiphilic polymers, Biomaterials 35 (2014) 9912–9919.

[150] M.J. Welber, J.B. Matson, V.K. Tamboli, S.I. Stupp, Controlled release of dexamethasone from peptide nanofiber gels to modulate inflammatory response, Biomaterials 33 (2012) 6823–6832.

[151] T. Gajanayake, R. Olariu, F.M. Leclere, A. Dhayani, Z. Yang, A.K. Bongoni, Y. Banz, M.A. Constantinescu, J.M. Karp, P.K. Vermula, R. Rieben, E. Vogelin, A single localized dose of enzyme-responsive hydrogel improves long-term survival of a vascularized composite allograft, Sci. Transl. Med. 6 (2014), 249ra110.