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Therapeutic synthetic and natural materials for immunoengineering

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Immunoengineering is a rapidly evolving field that has been driving innovations in manipulating immune system for new treatment tools and methods. The need for materials for immunoengineering applications has gained significant attention in recent years due to the growing demand for effective therapies that can target and regulate the immune system. Biologics and biomaterials are emerging as promising tools for controlling immune responses, and a wide variety of materials, including proteins, polymers, nanoparticles, and hydrogels, are being developed for this purpose. In this review article, we explore the different types of materials used in immunoengineering applications, their properties and design principles, and highlight the latest therapeutic materials advancements. Recent works in adjuvants, vaccines, immune tolerance, immunotherapy, and tissue models for immunoengineering studies are discussed.

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1 Introduction

Immunoengineering is an interdisciplinary field that aims to design and develop novel immunomodulatory agents, vaccines, and therapeutics by using the fundamentals of bioengineering, materials science, and immunology. Recently, therapeutic materials for a broad range of diseases, including cancer, infectious diseases, and autoimmune disorders have been developed.^{1–3} Immunoengineering efforts have been focused on advancing new approaches to modulate the immune

system's function, either by enhancing or suppressing it, to achieve targeted therapeutic outcomes.⁴

The development of vaccines relies heavily on the ability to design and engineer antigens that can elicit a robust and specific immune response against a particular pathogenic marker. Immunoengineering has enabled the development of novel vaccine platforms, including protein and RNA vaccines, viral vectors, and nanoparticle-based vaccines, which have shown promising results in preclinical and clinical studies.⁵ Immunomodulatory materials including adjuvants alter the immune response to a particular antigen and are added to vaccines. They stimulate the immune system to produce a stronger and longer-lasting response to the vaccine. Commonly used adjuvants include aluminum salts, oil-in-water emulsions such as squalines, biologically-derived materials such as

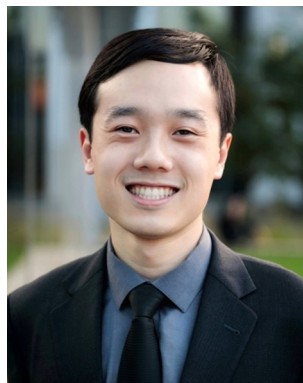
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saponins, and pattern recognition receptor (PRR) agonists like Toll-like receptor (TLR) agonists.⁶ The TLR agonists can be used as immunomodulators to stimulate the immune response to a particular antigen.⁷ Examples of TLR agonists include Toll-like Receptor 7 (TLR7) agonist Imiquimod, TLR9 Agonist CpG, and TLR7 and TLR8 Agonist Resiquimod.^{8,9}

Immunoengineering tools are also used to induce an immune tolerance effect. Immune tolerance induction aims to prevent or treat autoimmune diseases, allergies, and transplant rejection, where the body generates an inappropriate immune response to a non-pathogenic antigen. Strategies to induce immunological tolerance involve the use of engineered cells, nanoparticles, and biomaterials to prevent overactivation of the immune system against self-antigens or non-pathogenic foreign antigens.¹⁰

Another important application of biomaterials in immunoengineering is using antibody- and cytokine-containing

systems to trigger immune response, particularly against cancer. Cancer immunotherapy involves the modulation of the immune system to target and destroy malignant or transformed cells by monoclonal antibodies and cytokine therapies.¹¹ Monoclonal antibodies are particularly useful in blocking certain immune checkpoints that inhibit the cancer-killing activity of T cells. Immune checkpoints are typically cell-surface molecules that dampen the magnitude of an immune response. Examples of monoclonal antibodies used in immuno-oncology include pembrolizumab, nivolumab, and ipilimumab.¹² Cytokines are soluble factors that play a role in regulating the immune response and can be broadly classified into pro- and anti-inflammatory ones. Pro-inflammatory cytokines can be used effectively in immuno-oncology to enhance the activity of T cells or natural killer cells. Examples of cytokines used in immunotherapy include interleukins (e.g., IL-2, IL-12) and



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tolerance and cancer therapies.

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dynamic covalent polymers, self-healing materials, responsive adhesives, sustainable plastics, nanocellulose, polymers for battery applications, biomaterials and developing new synthetic methods for the construction of complex polymeric architectures.

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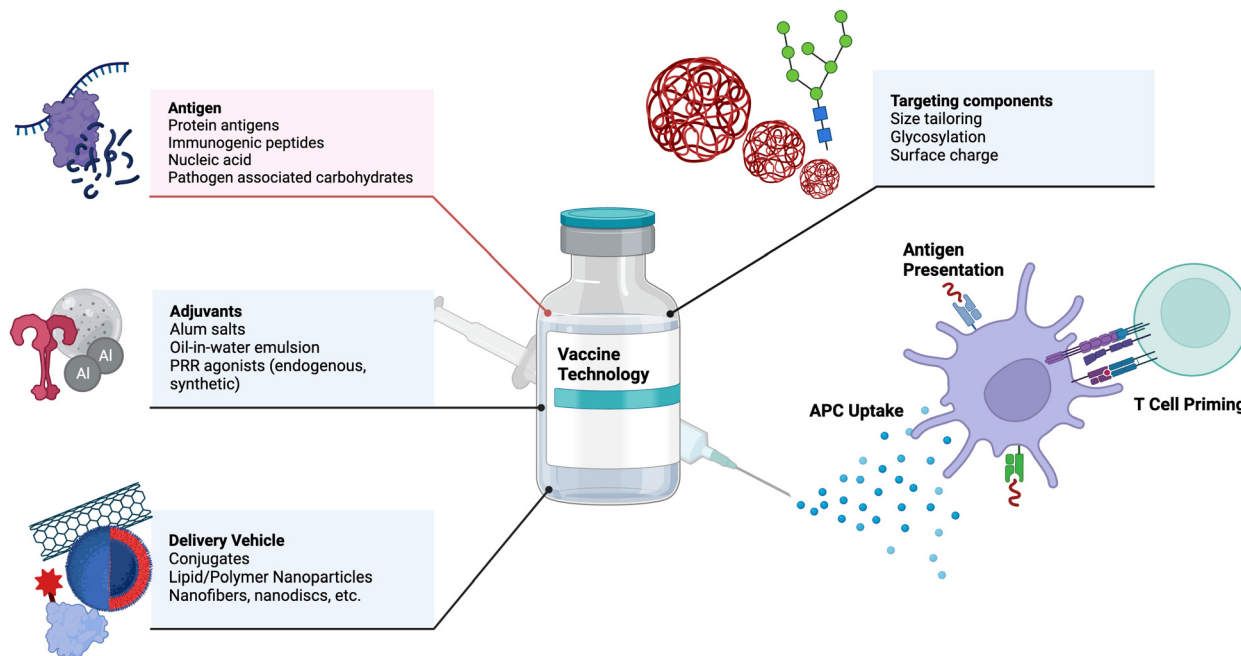


Fig. 1 Key components of vaccines are listed as antigens, adjuvants, delivery vehicles, and targeting components, including examples of each. Upon injection, antigen presenting cells (APCs) uptake vaccine components, present the antigen, and prime adaptive immune cells. This process is depicted above. Adapted from "Common Components of Vaccines", by BioRender.com (2023). Retrieved from <https://app.biorender.com/biorender-templates>.

modulation of pathways of interest. For example, cGAS-STING is a known inflammatory pathway for the development of robust adaptive immunity, but its endogenous ligand, cGAMP (or other cyclic dinucleotides, CDNs), is not suitable for therapeutic applications due to poor drug-like properties. Recent studies have identified non-CDN STING agonists by detecting downstream IFN- γ ²⁶ or interferon regulatory factor (IRF) and nuclear factor kappa B (NF κ B).^{27,28} Other groups use structure-based virtual screens to identify potential modulators.^{29,30} Similar screens have been used to identify agonists of other receptors,^{31,32} or of synergistic receptor agonizing. Once agonists are identified, their structure can be optimized for potency, specificity, and drug-like properties. The continued discovery, optimization, and evaluation of adjuvants can drive the development of more safe and effective vaccines. Current and future research may focus more on identifying molecules or combinations with unique biological properties, like improved secretion of certain cytokines with beneficial effect without increasing others with negative side effects. Examples of several small molecule PRR agonists are depicted in Fig. 2.

Beyond small molecule adjuvants, many researchers are interested in the immunostimulatory properties of macromolecular materials, including polymers and nanoparticles.³³ It is important to note that adjuvants are powerful immunostimulatory molecules and may potentially have consequential off-target effects if not delivered appropriately.³⁴ When appropriately delivered *via* nanoparticle co-encapsulation with or covalent conjugation to antigen, the effects can be localized and specified.³⁵ The optimization of delivery systems remains an active area of research and is detailed in the sections to follow.

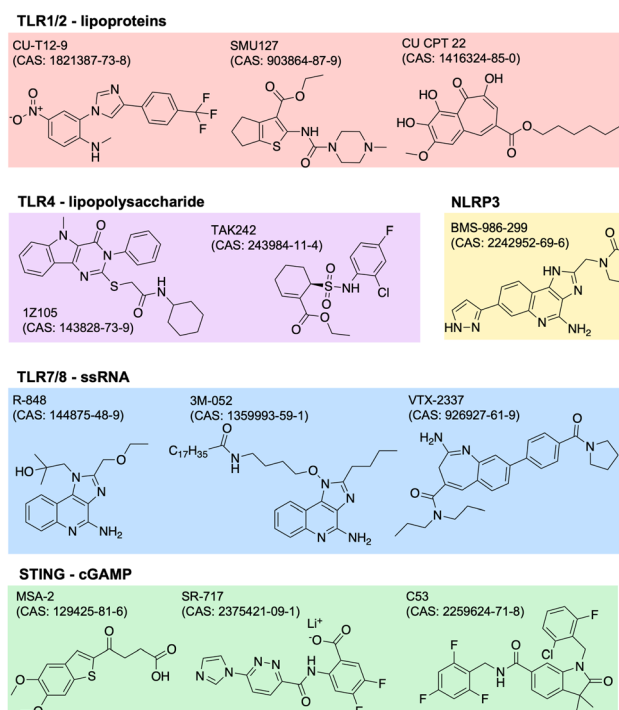


Fig. 2 Small molecule agonists of many PRRs have been identified, including TLR1/2, TLR4, TLR7/8, STING, and NLRP3. Key examples of these are shown, grouped by protein target, with associated CAS numbers. PRR: pattern recognition receptor; TLR: toll-like receptors; PRR: pattern recognition receptor; TLR: toll-like receptor; NLRP3: nucleotide oligomerization domain-like receptor family, pyrin domain containing 3; STING: stimulator of interferon genes.



2.2 Antigen presenting cells

APCs, including dendritic cells (DCs), macrophages, and B cells, are specialized immune cells capable of capturing, processing, and presenting antigens to lymphocytes. Their ability to efficiently process antigens, coupled with their unique capacity to activate T cells, makes them central to initiating adaptive immune responses. Upon encountering antigens derived from pathogens or vaccine formulations, APCs internalize the antigens and present them on their cell surface in association with major histocompatibility complex (MHC) molecules. This process, known as antigen presentation, allows T cells to recognize and respond to specific antigens.³⁶

The DCs, in particular, are regarded as the most potent, or “professional” APCs due to their exceptional antigen-capturing capabilities and ability to prime naive T cells. They capture antigens in peripheral tissues, migrate to lymphoid organs, and present antigens to T cells within specialized regions known as secondary lymphoid organs. By activating T cells, DCs provide critical signals for the differentiation and expansion of antigen-specific T cells, which are instrumental in mounting robust immune responses.³⁷

The success of vaccines relies on the ability of APCs to efficiently capture, process, and present vaccine antigens to immune cells. By mimicking natural infections, vaccines stimulate APCs, leading to the activation of both the innate and adaptive arms of the immune system. APCs recognize PAMPs through PRRs, as detailed above. This recognition triggers a cascade of immune responses, including the production of pro-inflammatory cytokines and the recruitment of immune effector cells. Moreover, APCs also regulate the balance between tolerance and immunity. They possess mechanisms to induce tolerance towards harmless antigens encountered in non-inflammatory, homeostatic settings, preventing unnecessary immune activation and autoimmunity. By modulating the immune response, APCs contribute to the development of long-lasting immune memory, which is essential for effective vaccination strategies.³⁸ Tolerogenic immune modulation is discussed below in more detail. Therefore, the critical role of APCs in vaccine responses cannot be overstated.^{39,40} These specialized immune cells are at the forefront of initiating and shaping adaptive immunity by capturing, processing, and presenting antigens to T cells. A comprehensive understanding of APC biology and their interactions with vaccine antigens is pivotal for designing novel vaccines and improving existing ones. Further research into the precise mechanisms governing APC function and the development of targeted strategies to enhance their efficacy will undoubtedly pave the way for more effective and tailored vaccines against infectious diseases and cancer. Recent advances in this space are detailed below.

2.3 Targeted delivery of antigens

Efficient and selective delivery of antigens to APCs is a key strategy in the development of advanced vaccines and immunotherapies. Targeting antigens specifically to APCs holds great promise for enhancing immune responses, improving vaccine

efficacy, and enabling the development of personalized medicine approaches. This section highlights the importance of targeted antigen delivery to APCs and explores various strategies employed to achieve this objective.

2.3.1 Glycosylation. Glycosylation, the process of attaching sugar molecules to proteins or lipids, has emerged as a valuable tool for enhancing antigen targeting in the development of vaccines. The incorporation of specific glycans onto vaccine antigens can improve their stability, solubility, immunogenicity, and facilitate selective interactions with CLRs on APCs. This section explores the strategies employed to harness their potential in vaccine design.

At the most basic level, targeted glycosylation of single protein antigens can change the way the antigen is recognized and processed by the immune system. This approach dates back to at least the late 1990s, where scientists began thinking about the glycosylation of mucin-1 (MUC1), a glycoprotein heavily overexpressed in some cancers.^{41,42} This approach is still explored today, although with more advanced synthetic techniques. One recent report from Rong *et al.*⁴³ employed *in vitro* glycosylation reactions to attach *N*-acetyl-galactosamine (GalNAc) to SARS-CoV-2 receptor binding domain (RBD) of the spike protein. This specific glycosylation did not lead to an increase in RBD-specific immunoglobulin G (IgG) with respect to native RBD, but, importantly, the serum antibodies exhibited 30–40% higher neutralization capacity. This work highlights the importance in understanding glycosylations to improve vaccinal responses.

More advanced synthetic strategies are also being developed. One recent strategy employed by Wilson *et al.*⁴⁴ used a polymeric glyco-adjuvant to elicit antigen-specific humoral and cellular immune responses. The polymer consists of mannose units and a TLR7 agonist. The mannose units are capable of binding mannose receptor and other C-type lectins on the surface and enable receptor-mediated endocytosis by APCs, while the TLR7 agonist powerfully activates the innate immune system upon endosomal receptor binding. The polymer is reversibly conjugated to protein antigens ovalbumin, circumsporozoite protein, or later SARS-CoV-2 spike protein⁴⁵ *via* a self-immolative linker. Their work demonstrates the importance of antigen and adjuvant targeting in both enhancing the breadth and specificity of the immune response, as well as reducing the side effects of vaccinal immune activation.

The incorporation of glycosylations, synthetic or natural, to enhance antigen targeting offers several advantages. It provides a versatile strategy to precisely direct antigens to APCs, promoting efficient immune recognition and activation. Glycosylation can also confer stability, improve antigen solubility, and prolong antigen circulation time. Moreover, glycosylated antigens can elicit immune responses against specific glycan epitopes, broadening the repertoire of targeted immune responses. However, challenges exist in optimizing glycan structures, ensuring consistency in glycan attachment, and determining the optimal glycosylation sites on antigens.

Further research is needed to elucidate the interactions between glycosylated antigens and APC receptors, allowing for



the rational design of glycan modifications to enhance antigen targeting. Additionally, the development of glycoengineering techniques, such as enzymatic glycan remodeling or chemoenzymatic approaches, will enable precise control over glycan structures and facilitate the production of glycosylated antigens on a larger scale. Integration of glycosylations with other antigen delivery systems, such as nanoparticle-based platforms, could further enhance targeted antigen delivery to APCs and improve vaccine efficacy. We believe better understanding of glycobiology and its subsequent manipulation will drive the next generation of vaccine innovation.

2.3.2 Lymphatic targeting. Vaccines, when targeted to the lymphatic system, can offer enhanced immune responses through lymph nodes, particularly through increased antigen presentation by promoting uptake by lymph node-resident APCs that can go on to prime other immune cells like T cells, leading to a more targeted and specific immune response.⁴⁶ Some vaccine routes of administration, including subcutaneous and intradermal, have been shown to improve lymphatic uptake compared to traditional intramuscular injections.⁴⁷ However, encapsulation of vaccine components into nanoparticles can enhance lymphatic trafficking.^{48,49} While nanoparticles are addressed in detail in Section 4 of this review, it is important to note that the size of nanometer scale carriers can be tailored to increase lymphatic drainage and uptake by certain cell populations, particularly those between 10 and 100 nanometers in diameter.⁵⁰

In a recent report by Chen *et al.*,⁵¹ the group reported a lymph node targeted lipid nanoparticle to deliver mRNA vaccines. They screened a variety of lipids with differing tail lengths, combinations, and linker chemistries, and identified an optimal lipid for accumulation in the injection draining lymph node. They explored the efficacy of their lipid as compared to those in Comirnaty, the U.S. Food and Drug Administration (FDA) approved COVID-19 mRNA vaccine. This work serves as just one example of the beneficial effects of lymph node targeting and the many ways to achieve accumulation. The topic of lymph node targeting nanoparticles has been reviewed further elsewhere.^{52,53} Most modern vaccines are developed for intramuscular administration, and we believe design with lymph node targeting could improve immune responses.

2.4 Viral vectors

Viral vectors are cutting-edge tools for vaccine delivery, leveraging the relatively benign nature of harmless viruses to transport antigenic materials into host cells. By modifying the viral vector to carry specific genes encoding vaccine antigens, they can effectively introduce these antigens into the body, triggering a targeted immune response. The viral vector itself does not cause disease but acts as a delivery vehicle, mimicking a natural infection to stimulate the immune system. This approach has shown promise in generating potent and long-lasting immune responses against various infectious diseases and even certain cancers, paving the way for the development of safer and more effective vaccines with broader applicability.⁵⁴

While viral vectors are promising for some applications, they face limitations in immunogenicity.⁵⁴ There is clear evidence that viral vectors can be dosed repeatedly in immune-privileged tissues like the eye, suggesting that synthetic passivation could expand the use of viral vectors to other tissues.⁵⁵ Some biomaterials approaches show promise in enhancing the efficacy, safety, and specificity of viral vector-based vaccines.⁵⁶ For example, conjugation with stealth polymers like polyethylene glycol (PEG)⁵⁷ or elastin-like polypeptides⁵⁸ have been shown to reduce adeno-associated virus (AAV)-specific antibody-mediated neutralization, specifically protecting against humoral immunity. Other strategies aimed at reducing cellular immunogenicity using conjugation to immunosuppressive materials like zwitterionic phosphoserine.⁵⁹ The future of viral vectors holds immense promise for developing new vaccines and personalized medicine. More efficient, targeted, and safer vaccines against a wide range of infectious diseases and cancer can be developed by using them. The ability to precisely control vaccine delivery, enhance immune responses, and tailor treatments to individual patients' needs opens new avenues for tackling challenging health conditions. As we utilize more biomaterials and their interactions with the immune system, we can expect new novel therapeutic strategies beyond traditional vaccination.

2.5 Synthetic vaccines

Synthetic vaccines offer precise manipulation of antigen structures, improved immunogenicity, and tailored immune responses. Here, we highlight the unique capabilities and design parameters of synthetic vaccines to shed light on this exciting frontier and encourage further research and innovation in this field.

One way to create a synthetic vaccine involves the selection of an immunodominant T cell epitope to drive cellular immunity against pathogens. An interesting strategy was recently published by Cooney *et al.*⁶⁰ using immunogenic peptide epitopes conjugated at high density to a single adjuvant, α -galactosylceramide. Their synthetic route is high-yield and versatile to a variety of peptide antigens. Notably, their work explores the importance of conjugation and antigen:adjuvant ratio, which they demonstrate impacts the NKT cell response and thus tolerability. The synthetic processes detailed here serve as an example of the creative engineering required to develop next-generation synthetic vaccines.

Tsoras *et al.*⁶¹ showed peptide subunit vaccines with increased immunogenicity. However, they are limited in their ability to produce long-lived memory immune responses. Their strategy employs peptide nanoclusters (PNCs), which are suspended clusters of crosslinked peptide antigens. They systematically evaluated how the crosslinking and modification affect the induced cellular immune response. This sort of deliberate study provides a mechanistic understanding, paving the way for the development of next-generation vaccines.

Clearly, peptide antigens and their tailored delivery in the form of synthetic vaccines offer promise in the area of infectious disease protection. There are other examples of



biomaterials strategies to deliver peptides for cancer vaccination, such as macroporous alginate nanoparticles carrying leukemia antigens,⁶² coordinative dendrimers carrying antigenic peptides,⁶³ or peptides conjugated to immune-stimulating agonists.⁶⁴ Further immunological considerations of cancer vaccines are highlighted in the following section.

2.6 Cancer vaccines

Cancer vaccines hold immense promise as a breakthrough in cancer therapy by leveraging the body's immune system to specifically target and eliminate cancer cells. The design of these vaccines includes various components, such as antigen identification, formulation, and adjuvant selection, many of which are familiar from the previous discussion on traditional, antiviral, or antibacterial vaccines. Here, the antigens are tumor-specific or tumor-associated, rather than derived from a particular pathogen. When these antigens can be identified, vaccines can be directed towards them and induce a cancer-antigen-specific immune response.^{65,66} However, antigens are not always uniquely and consistently expressed by cancer cells, particularly in the case of metastatic cancer.⁶⁷ Additionally, because cancer grows otherwise uncontrolled, the immune system is chronically exposed to high levels of these antigens, requiring significant adjuvanting to generate an antigen-specific immune response.⁶⁸ These limitations are important to understand as the scientific community works towards more effective cancer vaccination.

One strategy to overcome the issue of antigen selection is by simply applying adjuvant to the tumor site, hoping to stimulate a local innate immune response that can serve to prime the adaptive immune system in an antigen-specific manner against tumor cells and tumor debris.^{69,70} This strategy can be considered as *in situ* cancer vaccination. This approach worked well for basal cell carcinoma, a type of skin cancer, that can be successfully treated with topical imiquimod cream, a TLR7/8 agonist.^{71,72} Many other groups have been interested in similar strategies employing other adjuvants like CpG (TLR9)^{73,74} or poly I:C (TLR3).^{75,76} However, next generation adjuvant delivery strategy can help direct the immune response and reduce systemic toxicity associated with adjuvant administration. Recent work in this space expands to other adjuvants as well, *e.g.*, Chen *et al.* recently reported the identification and characterization of STING-agonist loaded liposomes that enhance antigen cross-presentation and immune activation.⁷⁷ Other biomaterials innovations help deliver adjuvants intratumorally, including a polypeptide depot that controls release of CpG,⁷⁸ a polymer that binds tumor cells and debris with a polymeric TLR7 agonist,⁷⁹ high-density lipoprotein nanodiscs that co-deliver MPLA and CpG,⁸⁰ and many others that are reviewed extensively elsewhere.^{81–84}

2.7 Tolerogenic vaccination

Tolerogenic vaccination is a type of vaccine designed to induce immune tolerance rather than an effector immune response. Sometimes referred to as inverse vaccination, a term coined by Lawrence Steinman in 2010, these technologies aim to

specifically reduce a pathological adaptive autoimmune response and instead induce immunological tolerance to the antigen.^{85,86} The goal of a tolerogenic vaccine is to re-educate the immune system to recognize self-antigens (components of the body's own tissues) as harmless, thereby reducing or preventing autoimmune reactions. These vaccines often contain specific antigens or proteins associated with the targeted autoimmune disease. By exposing the immune system to these antigens in a controlled manner, tolerogenic vaccines aim to induce immune tolerance and prevent or dampen the immune response against self-antigens. Traditionally, therapies that are used in treating autoimmunity involve broadly immunosuppressive drugs.⁸⁷ However, such broad suppression of the immune system can lead to opportunistic infections and other side effects. Inverse vaccination, on the other hand, aims to induce tolerance in an antigen-specific manner and bypassing the need for broad immune suppression. In recent years, several interesting materials science approaches have shown promise in the ability to induce antigen-specific immunological tolerance.

One such strategy is the use of poly(lactic-co-glycolic acid) (PLGA) particles to carry antigens to APCs. Miller *et al.* published many works on the material, where they used carboxylated, biodegradable PLGA microparticles to bear encephalitogenic peptides. The specific properties of the particles, including 500 nm size, negative surface charge, and disease-specific antigen cocktail, promote APC uptake by mimicking apoptotic debris in size, deliver tolerogenic signals, and enable the presentation of immunologically relevant antigens. In this work, they demonstrated the particles' ability to delay the onset of experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis.⁸⁸ Since then, the group has gone on to expand the microparticle platform for the treatment of other diseases. This includes the encapsulation of gliadin for the treatment of celiac disease,⁸⁹ of insulin, GAD65, and chromogranin A proteins for the prevention of autoimmune diabetes,⁹⁰ and of peanut extract to prevent allergic anaphylaxis.⁹¹ This technology has recently been evaluated in human clinical trials and showed favorable safety and suggestive efficacy results in celiac disease patients.⁹² Many other groups have designed PLGA nanoparticle systems for tolerogenic vaccination based on similar principles and are reviewed in detail elsewhere.⁹³

Other nanoparticle-based strategies have also been employed, the design parameters of which are explained in further detail elsewhere in this review. Recent work by Nguyen *et al.* combines two nanoparticle technologies to elicit protective tolerance in EAE.⁹⁴ Primarily, mesoporous silica nanoparticles (MSN) loaded with a high density of antigen (peptide from myelin oligodendrocyte glycoprotein (MOG)) were administered semi-therapeutically (after disease induction but prior to symptom onset), which prevented disease progression. In order to demonstrate efficacy in a fully therapeutic setting, where symptoms have already developed, MSN-MOG particles were functionalized with cerium oxide nanoparticles (CeNP), which function to scavenge reactive



oxygen species and promote tolerogenic APC phenotype. Together, this work demonstrates unique materials design to fit the constraints of the complex immunological problems at play in EAE disease progression.

Another approach has recently been developed by our lab involving the use of polymerized sugars to target antigens to the immunosuppressive environment of the liver.⁴⁴ The materials, dubbed pGal and pGlu, are based on *N*-acetylgalactosamine and *N*-acetylglucosamine and can be conjugated to proteins to induce antigen-specific tolerance, here for diabetes. In other works, the lab applied the materials to models of skin transplant⁹⁵ and further explored subcutaneous administration for enhanced lymph node trafficking, rather than the liver.⁹⁶ It has remained a question as to whether these approaches could only restrain a developing response, or could contract existing immune aggression, such as in relapsing-remitting multiple sclerosis. Recent work from the lab with pGal-MOG showed that tolerance induction in the face of existing immune aggression was possible.⁹⁷ Similar materials based on polymerized mannose have more recently been published in models of anti-drug antibody responses⁹⁸ and food allergy.⁹⁹ Together, these works provide further evidence for the future of biomaterials in the clinical treatment of autoimmune diseases. With increasing prevalence of autoimmune diseases worldwide, researchers in the coming years will continue to develop a deeper understanding of immunological mechanisms at play and engineer novel therapeutic strategies.

3 Chemical modifications to proteins and nucleic acids

Chemical modification of biologic agents (*e.g.*, recombinant proteins) offers an expanded spectrum of therapeutic applications compared to the use of unmodified biologic agents. Incorporating synthetic elements into biologics is thus an important and quickly evolving field in modern biomedicine. In this section, we will discuss select examples of how synthetic chemistry can be applied to recombinant biologics such as growth factors, cytokines, and antibodies.

3.1 Stealth polymers

Clinical translation of recombinant proteins is hindered by their immunogenicity, short *in vivo* half-life, and stability. In order to enhance the therapeutic properties of proteins, chemical attachment of polyethylene glycol, a process called PEGylation, has been successfully employed over the last 30 years. PEGylation of biologics that do not contain natural half-life extension domains, such as Fc or serum albumin, confers stealth-like properties, limiting their recognition by the immune system, decreasing their clearance rate, and drastically improving pharmacokinetic (PK) and pharmacodynamic (PD) properties. Other major benefits of protein PEGylation are increased protein solubility, higher thermal and chemical stability, and reduced proteolysis.¹⁰⁰ Increased protein solubility, enabled by a high degree of hydrophilicity of PEG

molecules, is particularly important because it allows for biologic therapeutics to be administered at higher doses in smaller volumes with less risk of protein aggregation.¹⁰¹ A summary of the benefits of PEGylation is depicted in Fig. 3.

There are now approximately 30 FDA-approved PEGylated biologics, spanning a diverse range of indications (Table 1). In the majority of these applications (*e.g.*, granulocyte colony-stimulating factor (G-CSF) and growth hormones), PEG chains primarily serve as a synthetic half-life extension domain to improve the PK profile of the biologic.¹⁰² In applications pertaining to enzyme replacement therapies (*e.g.*, alpha-galactosidase A and adenosine deaminase), PEGylation is aimed to reduce the immunogenicity of otherwise 'non-self' enzymes.¹⁰³ The decrease in immunogenicity of the 'non-self' proteins is due to the shielding effect enabled by PEG chains, which minimizes immune complex formation. Despite the fact that the modified protein has reduced immunogenicity upon PEGylation, PEG itself may evoke an immune response, especially after repeated dosing with the PEGylated biologic.¹⁰⁴ Because PEG is a common food additive and widely used in cosmetics, nearly a quarter of healthy individuals have pre-existing anti-PEG antibodies.¹⁰⁵ Such pre-existing anti-PEG immunity is thought to be predominantly mediated by circulating IgM antibodies, but other mechanisms may contribute to the immunogenicity of PEG.¹⁰⁶ Although the immunological mechanisms are not fully understood, the impact of the anti-PEG antibodies on the PK properties is profound. The presence of high levels of antibodies against PEG may result in accelerated blood clearance of the intravenously administered PEGylated biologic by the complement system.¹⁰⁷ Another serious consequence of anti-PEG antibodies is the risk of anaphylactic shock in patients receiving PEGylated biologics.¹⁰⁸

To overcome this limitation, alternative strategies to PEGylation are being studied. One such approach is to expand the hydrodynamic volume of a therapeutic with Pro, Ala, and Ser amino acids (typically hundreds) of residues,¹⁰⁹ a process termed PASylation.¹¹⁰ A recent study demonstrated that PASylation was equally as effective as PEGylation in improving the half-life of liposomes but displayed reduced immunogenicity.¹¹¹ Another study that aimed to reduce the immunogenicity of PEG in a peptide conjugate context utilized poly[oligo(ethylene glycol)methyl ether methacrylate] (POEGMA).¹¹² POEGMA conjugated to exendin, a peptide drug used in the clinic to treat type 2 diabetes, displayed prolonged half-life *in vivo* upon subcutaneous administration and, importantly, did not elicit an immune response against the polymer. In contrast, repeated administration of the PEG-exendin conjugate mounted an antibody response against the PEG moiety, thus significantly reducing its efficacy long-term. Development of alternate synthetic strategies that enhance the PK properties of biologics without causing a neutralizing immune response is warranted.

A majority of the FDA-approved PEGylated biologics were generated using non-site-specific conjugation strategies, resulting in a mixture of polydisperse proteins.¹¹³ The reason for such heterogeneity is that a given protein contains multiple





Fig. 3 Consequences of PEGylation of biologics. When therapeutic proteins are attached to PEG, several key benefits are conferred, including increased hydrophilicity and molecular weight, decreased immunogenicity, and tunable bioactivity. The practical implications of these alterations are graphically depicted.

Table 1 List of FDA-approved PEGylated therapeutics as of May 2023

Entry	Trade name	Active biologic	Manufacturer	Indication	Approval year
1	Elfabrio	α -Galactosidase-A	Chiesi Group & Protalix Bio	Fabry disease	2023
2	Rolvedon	G-CSF	Spectrum Pharmaceuticals	Febrile neutropenia	2022
3	Stimufend	G-CSF	Fresenius Kabi	Febrile neutropenia	2022
4	Fyletra	G-CSF	Amneal Pharmaceuticals	Febrile neutropenia	2022
5	Besremi	IFN- α -2b	PharmaEssentia	Polycythemia vera	2021
6	Skytrofa	Somatrophin	Ascendis Pharma	Growth hormone deficiency	2021
7	Empaveli	Pegcetacoplan	Apellis Pharmaceuticals	PNH	2021
8	Nyvepria	G-CSF	Pfizer	Febrile neutropenia	2020
9	Ziextenxo	G-CSF	Sandoz	Febrile neutropenia	2019
10	Esperoct	Anti-hemophilic factor	Novo Nordisk	Hemophilia A	2019
11	Udenyca	G-CSF	Coherus BioSciences	Febrile neutropenia	2018
12	Palynziq	Phenylalanine ammonia lyase	BioMarin	Phenylketonuria	2018
13	Revcovi	Adenosine deaminase	Leadiant Biosciences	ADA-SCID	2018
14	Revcovi	Adenosine deaminase	Leadiant Biosciences	ADA-SCID	2018
15	Fulphila	G-CSF	Mylan GmbH	Febrile neutropenia	2018
16	Asparlas	L-Asparaginase	Servier Pharmaceuticals	Acute lymphoblastic leukemia	2018
17	Jivi	Anti-hemophilic factor	Bayer	Hemophilia A	2018
18	Rebinyn	Coagulation factor IX	Novo Nordisk	Hemophilia B	2017
19	Adynovate	Anti-hemophilic factor	Baxalta	Hemophilia A	2015
20	Plegridy	IFN- α 1a	Biogen	Relapsing multiple sclerosis	2014
21	Omontys	Erythropoietin receptor agonist	Takeda	Anemia – chronic kidney disease	2012 (withdrawn)
22	Sylatron	IFN- α 2b	Merck	Melanoma	2011
23	Krystexxa	Uricase	Horizon Pharma	Chronic gout	2010
24	Cimzia	Anti-TNF α	UCB	Rheumatoid arthritis & Crohns	2008
25	Mircera	Erythropoietin	Roche	Anemia – chronic kidney disease	2007
26	Somavert	Growth hormone antagonist	Pfizer	Acromegaly	2003
27	Neulasta	G-CSF	Amgen	Febrile neutropenia	2002
28	Pegasys	IFN- α 2a	Roche	Hepatitis C	2002
29	Oncaspar	Asparaginase	Enzon	Acute lymphoblastic leukemia	1994
30	Adagen	Adenosine deaminase	Enzon	ADA-SCID	1990

Abbreviations: ADA, adenosine deaminase; G-CSF, granulocyte colony-stimulating factor; IFN, interferon; SCID, severe combined immunodeficiency disease; TNF, tumor necrosis factor; PNH, Paroxysmal nocturnal hemoglobinuria.

potential reactive lysine residues (the amine of which serves a reactive handle for most PEGylated proteins), thereby posing certain challenges in downstream separation processes.¹¹⁴ Therefore, the development of site-specific PEG conjugation



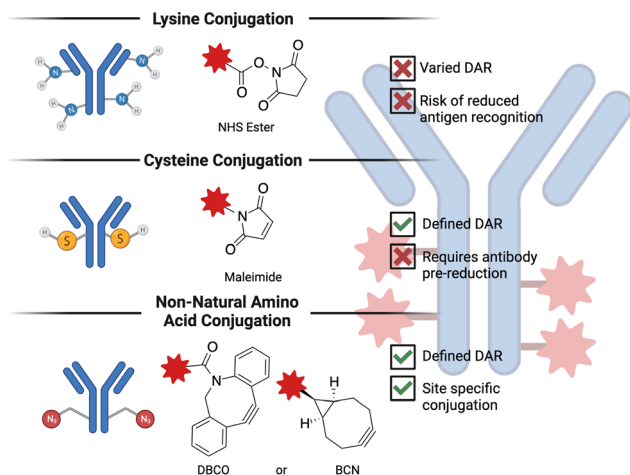


Fig. 4 Antibody drug conjugates are generally synthesized through three main chemistries, including lysine (amine) to NHS ester, cysteine (thiol) to maleimide, or non-natural amino acid (azide) to strained alkyne (DBCO or BCN). Each of the three chemistries are shown here with a high-level description of their key favorable or unfavorable properties. NHS: N-hydroxysuccinimide; DBCO: dibenzocyclooctyne; BCN: bicyclononyne; DAR: drug-antibody ratio.

such as microtubule inhibitors and DNA-damaging agents, were synthesized to be significantly more potent, with IC₅₀ values in the low nanomolar range.¹²⁹ Currently, out of 14 FDA-approved ADCs, 6 carry microtubule inhibitors (auristatins) and 3 carry DNA-damaging agents. It is also worthwhile to note that cytotoxic agents with picomolar range potency have also been tested in the clinic but severe adverse events halted their translation.¹³⁰ This finding illustrates that achieving optimal potency of the payload is important for obtaining favorable results in the clinic.

Instead of synthesizing novel, more potent cytotoxic payloads, researchers have also attempted to combine existing payloads in a single ADC. A dual payload ADC directed against human epidermal growth factor receptor 2 (HER2), a receptor overexpressed in 14–20% of breast cancer patients,¹³¹ has recently been engineered to carry both monomethyl auristatin E (MMAE) and monomethyl auristatin F (MMAF).¹³² Using microbial transglutaminase (MTGase)-mediated transpeptidation, highly homogenous dual-payload ADCs were engineered with well-defined DARs. This method allowed the authors to avoid very high DAR (> 8), which can cause aggregation issues and shorter PK due to increased hydrophobicity of the molecule. Importantly, the dual-drug ADC carrying four copies of MMAE and two copies of MMAF exerted a greater antitumor effect than did the co-administration of two single-drug variants in xenograft mouse models. This strategy highlights the therapeutic potential of the dual-drug ADC format for the treatment of refractory HER2+ breast cancer.

Another promising class of payloads for ADCs are STING agonists. STING agonists are able to induce a broad inflammatory response, particularly type I interferon signature, which leads to activation of T and NK cells.¹³³ Despite very promising preclinical data with STING agonists, systemic administration

has shown limited success in the clinic due to intolerable systemic toxicity. Thus, delivering STING agonists using tumor-specific antibodies can greatly improve their tolerability, eliciting a more localized response. To this end, a STING agonist (IMSA172) has been conjugated to an antibody targeting epidermal growth factor receptor (EGFR), which is overexpressed in a wide range of cancer cells.¹³⁴ The authors demonstrated that STING signaling can be induced in EGFR-expressing cells with a nanomolar-range EC₅₀, but not on EGFR-negative cells. In a syngeneic mouse model of melanoma engineered to express EGFR, the ADC significantly prolonged the survival and was not associated with body weight loss. Importantly, anti-EGFR-IMSA172 also synergized with an immune checkpoint blocking antibody, anti-PD-L1, setting the stage for the clinical development of such STING-carrying ADCs. XMT-2056, a STING agonist conjugated to an anti-HER2 antibody, is already undergoing a Phase I clinical trial in multiple solid tumors.¹³⁵ A more in-depth review of other ADC payloads is available elsewhere.¹³⁰

Perhaps one of the major challenges in ADC development and optimization is the selection of the linker.¹³⁶ The main objective of the linker is to keep the small molecule in the antibody-bound state in circulation (plasma) and release the payload once the ADC is internalized and reaches the endosome/lysosome. Linkers can broadly be classified into cleavable and non-cleavable ones: cleavable linkers release the payload upon specific stimuli such as low intracellular or tumor microenvironment pH (e.g., hydrozone) or proteases (e.g., cathepsin B, which cleaves the valine-citrulline bond).¹²⁹ Non-cleavable linkers, such as succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) rely on complete degradation of the antibody by cytosolic and lysosomal proteases.¹³⁷ Furthermore, optimization of not only linker chemistry but also the linker length and linker steric hindrance can influence the *in vivo* efficacy of ADCs.¹³⁸ Attaching the linker to a more sterically hindered site of the antibody may limit the extracellular cleavage of the cytotoxic drug and enhance the safety profile of the ADC.¹³⁹

Another promising strategy to improve the stability of the ADC in circulation is to employ a tandem-cleavage linker design, in which two sequential enzymatic cleavage events trigger the release of the payload.¹⁴⁰ By using CD79b-targeted MMAE conjugate as the model system, the authors successfully demonstrated that the dipeptide tandem-cleavage linker significantly outperformed the standard vedotin linker in terms of hematopoietic toxicity in rats. While the ADC is in circulation, the cathepsin cleavage site is sterically protected *via* a glucuronide moiety, limiting the premature release of MMAE. However, once the ADC is internalized, glucuronidase removes the protective monosaccharide moiety, exposing the cathepsin cleavage site for subsequent MMAE release. These findings indicate the importance of balancing the stability and sensitivity of the linker to achieve optimal antitumor efficacy with minimal adverse effects.

Another avenue of research in the ADC field is finding complementary combination agents for cancer therapy.¹⁴¹



One class of therapeutics that has shown preclinical potential in synergizing with ADCs is checkpoint inhibitor (CPI) antibodies, (anti-programmed cell death protein 1 (PD-1), anti-programmed cell death ligand 1 (PD-L1), and anti-cytotoxic T-lymphocyte associated protein 4 (CTLA-4)).¹⁴² ADC treatment can increase the quantity of intratumoral T cells whereas CPIs can reinvigorate the T cells that are becoming exhausted or dysfunctional. ADCs can also be rationally combined with other traditional chemotherapies such as tyrosine kinase inhibitors. Indeed, a combination of Flt3-targeting antibody conjugated to MMAF (20D9-ADC) with midostaurin showed enhanced efficacy in preclinical models of acute myeloid leukemia.¹⁴³

3.3 Altering antibody biodistribution via peptide conjugation

In ADCs, the antibody serves as a delivery vehicle for the cytotoxic payload. However, chemical modification of therapeutic antibodies has also been pursued to improve the biodistribution of the antibody itself. In their unmodified form, antibodies do not display an inherent preference to accumulate at the site of disease; rather, their biodistribution is predominantly guided by the antigen expression pattern. Expression of the target antigen in non-diseased tissues often gives rise to immune-related adverse events associated with the neutralization or blockade of the target.¹⁴⁴ To ameliorate this, antibodies can be engineered to achieve higher drug concentration in the tissue of interest, while sparing the healthy organs. For example, CPIs targeting CTLA-4 and PD-L1 were chemically conjugated to an extracellular matrix-binding peptide derived from placenta growth factor-2.¹⁴⁵ In this case, the peptide serves as a retention mechanism for the CPIs that is injected peritumorally. The authors showed that peptide-conjugated CPIs were retained near the tumor and induced minimal off-tumor activity, and significantly decreased the immune-related adverse events. A similar peptide conjugation strategy was applied to a neutralizing antibody against tumor necrosis factor- α (TNF- α), which is used to treat rheumatoid arthritis.¹⁴⁶ In this work, a collagen-binding peptide (CBP) derived from decorin was chemically linked to an anti-TNF- α antibody and administered intravenously. Due to the inflammation in the joints, collagen is exposed to the vascular endothelium, enabling CBP-anti-TNF- α to accumulate in the disease site and greatly enhancing the anti-rheumatic efficacy. In the same work, collagen affinity was conferred to an anti-transforming growth factor- β (TGF- β) antibody, which demonstrated profound anti-fibrotic activity in a model of bleomycin-induced pulmonary fibrosis. These results show that the therapeutic efficacy of monoclonal antibodies can be elevated by chemically conjugating them to the extracellular matrix-binding moieties.

3.4 Glycosylation

While glycosylation was discussed in detail for vaccines, glycans can also be used to improve the targeting of biologic therapeutics to treat infectious diseases. A report from Ye *et al.*¹⁴⁷ details the development of a small interfering RNA (siRNA) conjugate system. They employ both hexavalent mannose to target macrophages and DCs

and *N*-acetylgalactosamine to target hepatocytes for liver-specific infection to treat Marburg virus (MARV) infection. By optimizing glycan structure and combining targeting approaches, the therapeutic strategy provides promising protection in terms of viremia, body weight loss, and ultimately overall survival. This work highlights the importance of rational design of combinatorial approaches in designing the next generation of therapeutics.

4 Nanoparticles

Nanoparticles (NPs) have emerged as a valuable tool in medicine, offering enhanced stability, delivery, and efficacy of therapeutic agents. Due to their unique physical properties, nanometer-scale carriers are capable of encapsulating drugs and biological agents, both within their cores and on their surfaces.¹⁴⁸ These carrier platforms are further functionalized with bioactive coatings, enabling improved circulation, disease diagnosis, and targeted delivery applications. This exciting integration of nanomaterials in medicine is commonly referred to as nanomedicine. Notably, the FDA has already approved a wide range of nanomedicines for cancer treatment, showcasing their potential to revolutionize the field and improve patient outcomes.¹⁴⁹

4.1 Nanoparticle design

4.1.1 Size and charge. The size and charge of nanomaterials affect cellular uptake, transportation through biological barriers, and clearing from the body. Understanding the size- and charge-dependent behavior of nanomaterials is crucial for designing effective biomaterials applications and optimizing their therapeutic or diagnostic potential.¹⁵⁰

NP size greatly influences the properties and behavior of the material in the body. Primarily, it determines the ability of the particles to be uptaken by cells and tissues. Smaller NPs may penetrate tissues more easily, whereas larger ones may be more restricted.¹⁵¹ Similarly, it has been demonstrated that NPs around 25 nm can be transported in interstitial flow to lymph nodes, where they can be delivered to resident cells.⁴⁸ Once there, APC are the primary target cells. DCs uptake NPs through lectin-mediated endocytosis that are under 100 nm. Alternatively, they can uptake particles larger than 200 nm *via* phagocytosis.¹⁵² Many studies have characterized the influence of size on NP distribution and uptake, which should be an important consideration for the development of new NP-based therapies.¹⁵³ As a result, researchers have explored novel approaches to predict and modulate nanoparticle sizes using a variety of technologies. One such approach is to use microfluidic devices, which are often used lipid nanoparticle technologies but can be adapted to polymers as well.¹⁵⁴ Martin *et al.* described the key parameters affecting nanoparticle size and stability and demonstrated reproducible particles ranging from 75 to 200 nm simply by altering polymer concentration in the organic phase. As a result, straightforward modifications can be



made to adjust nanoparticle size based on intended use *e.g.* cellular targeting, lymphatic drainage and circulation time.

Surface charge of NPs also has important implications for the uptake, distribution, and stability of the particles. In general, positively charged particles exhibit superior cell uptake due to electrostatic interactions with negatively charged membranes, but phagocytotic cells can also ingest anionic NPs.¹⁵⁵ Surface charge, as well as chemistry (discussed later), also has an important role in dictating protein adsorption to the surface of the NPs. The changes in surface charge can locally change solution pH, which can have significant impacts on which proteins adsorb and the conformations they adopt.¹⁵⁶ This rapid absorption can alter the stability of NPs in solution and is more significant for positively charged NPs.¹⁵⁷ For individual cases, the surface charge can be intentionally manipulated for specific applications. For example, neutral and negatively charged polymer micelles release their payloads in different gut regions after intragastric administration based on interaction with the gut mucosa.¹⁵⁸ As is true with NP size, it is important to understand and strategically manipulate the surface charge of therapeutic NPs to optimize drug delivery. In the future, scientists will continue to understand how these properties work together to dictate materials properties in living systems.

4.1.2 Surface properties. Nonspecific interactions of nanomaterials with proteins in the physiological conditions result in the formation of a protein corona due to noncovalent interactions such as hydrophobic effect, ionic, hydrogen bonding, and van der Waals interactions. Functionalizing the surface of the nanoparticles with hydrophilic stealth materials such as PEG or zwitterionic molecules prevents nonspecific adhesion of the biomolecules.¹⁵⁹ The immune response can be eliminated by using stealth coatings. Precoating nanoparticle surfaces with proteins such as clusterin and serum albumin has been used to create stealth properties in nanomaterials.¹⁶⁰ These proteins act as a biocompatible shield, effectively camouflaging the nanoparticles from the immune system and reducing their recognition and clearance by the body's defense mechanisms. Clusterin, a multifunctional chaperone protein, can form a protective layer that minimizes opsonization and phagocytosis. On the other hand, serum albumin, a major protein found in blood plasma, offers advantages like prolonged circulation time through interaction with the neonatal Fc receptor (FcRn) and enhanced stability,¹⁶¹ further improving the stealth capabilities of the nanoparticles.¹⁶²

4.1.3 Biodistribution and targeting. The successful transport of the nanomaterials to their intended destinations is greatly influenced by the morphology of the blood and lymphatic vasculature. The intricate network of blood vessels and lymphatic channels determines the pathways through which nanomaterials can navigate and reach their target tissues. In the case of tumor tissues, the situation becomes particularly intriguing. Tumors possess irregular morphologies in their vasculature, primarily due to the excess angiogenic environment present in the tumor microenvironment.¹⁶³ This aberrant vasculature provides an advantageous opportunity for

nanomaterials to selectively accumulate within the tumor, enabling precise and targeted drug delivery or diagnostic imaging. The Enhanced Permeability and Retention (EPR) effect has long been regarded as a pivotal mechanism for the targeted delivery of nanoparticles to tumor tissues.¹⁶⁴ This effect capitalizes on the leaky vasculature and compromised lymphatic drainage commonly observed at tumor sites. The EPR effect enables nanoparticles to pass through the fenestrated blood vessels in tumors, leading to their accumulation in the tumor tissue. However, recent studies have yielded mixed results regarding the actual benefits of the EPR effect. It is now recognized that the rate of tumor formation plays a significant role in shaping the number and morphology of the blood vessels within the tumor microenvironment.

The nanoparticles are designed to specifically target and deliver therapeutic agents to desired cells or tissues. One strategy involves the conjugation of ligands on the surface of nanomaterials, enabling them to anchor to the target cells and promote endocytosis. Ligands such as antibodies, peptides, or aptamers can be attached to the nanoparticles, allowing for precise recognition and binding to specific receptors on the cell surface.¹⁶⁵ This targeted binding enhances the uptake of nanoparticles by the cells, leading to the efficient delivery of bioactive agents encapsulated within the nanoparticles. Through this approach, the release of therapeutic agents can be enhanced, improving their efficacy and minimizing off-target effects, thereby paving the way for more effective and precise treatments in various biomedical applications.

4.2 Lipid nanoparticles & liposomes

Lipid nanoparticle (LNP) technology has witnessed unprecedented growth in recent years, primarily attributed to the success of mRNA LNP-based SARS-CoV-2 vaccines developed by Moderna and Pfizer-BioNTech. LNP technology has rapidly expanded into numerous application areas and, perhaps most importantly, evolved into the premier method for non-viral gene delivery of nucleic acids, especially RNA. In this section, we review the most important characteristics and applications of LNP technology within the context of immunoengineering, highlighting novel and promising developments in the field. Specifically, we delve into the composition of LNPs, the wide array of RNA payloads LNPs can carry, the significance of different chemical properties of RNA payloads, and the application of LNP technology across different disease areas. These advancements underscore the transformative potential of LNPs as immunomodulatory biomaterials, and our exploration aims to provide an understanding of the current state-of-the-art.

4.2.1 Lipid nanoparticle composition. LNPs offer a vast range of possibilities by formulating them with different types of lipids and their combinations. Tuning the composition of an LNP allows researchers to create LNPs with specific properties tailored to meet the desired requirements of each specific use case.^{166,167} Factors such as particle stability, cargo encapsulation, cellular interactions, and immunomodulatory properties are crucial considerations in LNP design. Among the primary constituents of LNPs are cationic lipids, ionizable lipids,



phospholipids, cholesterol, and PEGylated lipids, depicted in Fig. 5. This section will focus on select examples of each of these types of constituents as lipid technology and LNP development have been extensively reviewed elsewhere.¹⁶⁸

Cationic lipids. Nucleic acids, being negatively charged due to their sugar-phosphate backbone, face significant hurdles in crossing the negatively charged cell membrane, owing to electrostatic repulsion. To overcome this obstacle, cationic lipids with permanent positively charged head groups have been developed, enabling effective encapsulation of nucleic acid payloads within LNPs and facilitating their delivery across the cell membrane.

One of the first cationic lipids to be developed for nucleic acid delivery is 1,2-di-*O*-octadecenyl-3-trimethylammonium propane (DOTMA). DOTMA is a quaternary ammonium lipid that was first synthesized in 1987 and has since been extensively employed for both *in vitro* and *in vivo* nucleic acid delivery across various cell types.^{169–171} Lipofectin, a widely used commercialized transfection reagent, constitutes a 1 : 1 (w/w) blend of DOTMA and a fusogenic helper phospholipid called 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE). The incorporation of DOPE enhances stability and delivery efficiency.¹⁷²

The advancement of research on cationic lipids led to the development of 2,3-dioleoyloxy-*N*-[2-(spermincarbamido)ethyl]-*N,N*-dimethyl-1-propanaminium trifluoroacetate (DOSPA), another noteworthy quaternary ammonium lipid

featuring a spermine moiety. DOSPA has been incorporated into Lipofectamine, another widely used commercialized transfection reagent, which comprises a 3 : 1 (w/w) mixture of DOSPA and DOPE.¹⁷³ Lipofectamine has proven highly effective in nucleic acid transfection protocols across a diverse range of cell types and is often considered the default standard for comparison against other methods of exogenous nucleic acid delivery into cells.¹⁷⁴

Ionizable lipids. Ionizable lipids have emerged as a critical element in the formulation of LNPs due to their potential to address toxicity concerns associated with cationic lipids while retaining their beneficial characteristics. Although cationic lipids are generally considered non-toxic at low concentrations, their toxicity becomes significant at higher levels, limiting their effectiveness and clinical applicability.¹⁷⁵ Because the toxicity and immunogenicity of cationic lipids are strongly associated with their positively charged headgroups, lipids with ionizable headgroups have become the predominant component of clinically relevant LNP formulations.¹⁷⁶

Ionizable lipids remain neutral at physiological pH but become positively charged when protonated at low pH, which allows them to maintain the advantageous chemical properties of cationic lipids while exhibiting comparatively reduced toxicity. This feature becomes particularly relevant because nucleic acid LNPs are typically formulated in low pH buffers, enabling efficient complexation of the positively charged, protonated ionizable lipids with the negatively charged nucleic acids.¹⁷⁷ Moreover, after administration, the ionizable lipids generally remain neutral in the extracellular environment until the LNPs are endocytosed into endosomes, thereby minimizing toxicity.¹⁷⁸

As the endosome matures and fuses with the lysosome, the pH of the endolysosomal compartment decreases from 6.8 to 4.5, leading to the protonation of the ionizable lipids within the LNPs.¹⁷⁹ The now positively charged ionizable lipid is then thought to play a crucial role in mediating the endolysosomal escape of the nucleic acid payload by fusing with the endolysosomal membrane, which primarily consists of negatively charged lipids. This fusion results in cationic-anionic lipid pairs that drive the lipid membrane into the inverted hexagonal HII phase, causing the formation of non-lamellar structures, disruption of the bilayer integrity, and release of the nucleic acid payload into the cytoplasm.¹⁸⁰

The quest for an ideal ionizable lipid for efficient nucleic acid delivery yielded a significant breakthrough with the discovery of 1,2-dilinoyleoxy-*N,N*-dimethyl-3-aminopropane (DLinDMA).¹⁸¹ DLinDMA was specifically synthesized for the delivery of siRNA and exhibited promising results when incorporated into LNPs and administered intravenously, enabling effective genetic silencing in the hepatocytes of non-human primates.¹⁸² Further iterative improvements to the linker and headgroup moieties of DLinDMA led to the development of 2,2-dilinoyleyl-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-KC2-DMA), which exhibited significantly enhanced potency and tolerability compared to DLinDMA.¹⁸³ Subsequent efforts



Fig. 5 Chemical structures of lipid nanoparticle components. Lipid nanoparticles are generally made of cationic lipids, ionizable lipids, phospholipids, PEGylated lipids, and cholesterol. Key examples in each of these classes are depicted with structures, names, and CAS numbers.



focused on systematic structural modifications to the amine headgroup in order to modulate the pK_a of ionizable lipids. These studies culminated in the development of (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino) butanoate (DLin-MC3-DMA; MC3), resulting in MC3-based LNPs with more than two orders of magnitude increased potency compared to the original DLinDMA-based LNPs.¹⁸⁴

In an extraordinary milestone, these efforts to develop LNPs for nucleic acid delivery led to the United States Food and Drug Administration (FDA) approval of patisiran (brand name Onpatro) in 2018 for the treatment of polyneuropathy in patients with hereditary transthyretin-mediated amyloidosis. Patisiran is the first-ever FDA-approved siRNA-based drug and utilizes MC3-based LNPs containing siRNA that effectively reduces the production of pathogenic transthyretin protein.^{185–188}

Although MC3-based LNPs were initially optimized for siRNA delivery to the liver, their application for the delivery of other nucleic acids, such as mRNA, faced limitations due to concerns regarding MC3's prolonged tissue half-life and associated toxicity issues.¹⁶⁸ Thus, researchers sought to innovate the next generation of ionizable lipids with enhanced biodegradability, allowing for rapid metabolism and swift elimination from plasma and tissues while retaining potency. One illustrative example involved introducing ester linkages into MC3's hydrocarbon chain region. The introduction of these ester linkages, prone to enzymatic hydrolysis *in vivo* but stable under physiological pH, gave rise to di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate (L319). Remarkably, L319 exhibited substantially improved tolerability and faster clearance compared to MC3, without compromising on potency.¹⁸⁹

In a separate study to engineer LNPs specifically tailored for mRNA delivery, researchers synthesized a novel series of amino lipids, chemically inspired by previous studies on MC3, and systematically identified and optimized structural motifs crucial for efficient mRNA delivery.¹⁹⁰ This pivotal investigation yielded ionizable biodegradable lipids that, when formulated into mRNA LNPs, exhibited higher delivery efficiency through improved endosomal escape. Moreover, these lipids demonstrated a favorable toxicity profile and, for the first time in the field, enabled safe repeat dosing of mRNA LNPs at therapeutically relevant levels without adverse events in non-human primates. Noteworthy examples from this lipid series include heptadecan-9-yl 8-((2-hydroxyethyl)(8-(nonyloxy)-8-oxooctyl)amino) octanoate (lipid 5) and heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino) octanoate (lipid 8; lipid H; SM-102).¹⁹⁰ The same lipid series was then also further screened and optimized for both delivery efficiency and immunogenicity when formulated as mRNA vaccines and administered intramuscularly.¹⁹¹ Of note, SM-102, a lead ionizable lipid characterized in these studies, ended up becoming a key component of Moderna's elasmomeran (brand name Spikevax) COVID-19 vaccine, which contains LNP-encapsulated mRNA coding for an engineered version of the spike protein of SARS-CoV-2.¹⁹² Likewise, a similar biodegradable ionizable lipid called

((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) is a key component of Pfizer-BioNTech's tozinameran (brand name Comirnaty) COVID-19 vaccine.^{193–195} At the time of writing, MC3, SM-102, and ALC-0315 are the only three ionizable lipids to have received FDA approval in a formulation for nucleic acid-based therapies.

Other lipid components (cholesterol, phospholipids, PEGylated lipids). In addition to cationic and ionizable lipids, LNP formulations incorporate other lipid components, including cholesterol, phospholipids, and PEGylated lipids, that are also important for their overall characteristics and functionality.

Cholesterol is often the second-most abundant component on a molar ratio basis and is critical for enhancing particle integrity by filling gaps between lipid tails, thus stabilizing the overall structure.¹⁹⁶ Furthermore, cholesterol has been shown to play a key role in enhancing the fusogenicity of LNPs, which is important for endosomal escape.^{197,198} Although most clinically advanced LNPs use cholesterol in their formulations, there has been increasing interest in using other sterol analogs and derivatives instead of cholesterol to rationally design LNPs with improved nucleic acid delivery.^{199–201}

The phospholipid component of most LNPs is another important structural constituent that promotes LNP fusion with cell membranes. As previously mentioned, DOPE is a phosphoethanolamine often used as a helper lipid in LNP formulations due to its tendency to adopt an inverted hexagonal HII phase, which facilitates endosomal escape.²⁰² Another phospholipid commonly used in LNP formulations is 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC), which is the phosphatidylcholine used as the phospholipid component in both Moderna's mRNA-1273 and Pfizer-BioNTech's BNT162b2 COVID-19 vaccines.²⁰³

Lastly, the PEGylated lipid component plays an important role in determining both the structural and delivery characteristics of the resulting LNPs. First, the molar proportion of PEGylated lipids in the formulation is a major factor in controlling particle size, with higher levels of PEGylated lipids resulting in smaller LNPs.²⁰⁴ Furthermore, PEGylated lipids also contribute greatly to the stability of LNPs both before administration *in vitro*, by decreasing particle aggregation in storage, and after administration *in vivo*, by reducing opsonization by serum proteins and clearance by the kidneys and mononuclear phagocyte system.^{205–207} Notable examples of PEGylated lipids include 1,2-distearoyl-*rac*-glycero-3-methoxypolyethylene glycol-2000 (DSG-PEG 2000), 1,2-dimyristoyl-*rac*-glycero-3-methoxypolyethylene glycol-2000 (DMG-PEG 2000), and 2-[(polyethylene glycol)-2000]-*N,N*-ditetradecylacetamide (ALC-0159). DMG-PEG 2000 and ALC-0159 are the PEGylated lipids used in Moderna's elasmomeran and Pfizer-BioNTech's tozinameran COVID-19 vaccines, respectively.²⁰⁸

4.2.2 Lipid nanoparticle RNA payloads. Although there have been various different types of payloads that have been delivered using LNPs (*e.g.* DNA, peptides/proteins, and small molecules), this review will primarily focus on the most



purification of circRNA remains one of the main challenges limiting the adoption of the technology.²³¹

The saRNA are mRNA transcripts that utilize the self-replication machinery of RNA viruses, usually RNA alpha-viruses, to produce additional copies of the mRNA transcript after being delivered into the cytosol of a cell.²³² Typically, the overall structure of saRNA is very similar to that of mRNA. Both contain a 5' cap, 5' UTR, 3' UTR, and a 3' poly(A) tail. However, within the coding region, rather than only containing the gene of interest, saRNA also encodes for viral machinery used to replicate viral RNA genomes. In wild-type RNA viruses, the genome usually is split into two open reading frames (ORFs). The first ORF contains the genes for the viral replication machinery, while the second ORF contains the genes for the viral structural proteins. In an saRNA molecule, the genes for the viral structural proteins in the second ORF are replaced to instead encode for the desired gene of interest.²³³ After entering a cell, the first ORF on the saRNA is translated to produce the necessary RNA replication machinery, which includes an RNA-dependent RNA polymerase (RdRp). Next, a complementary negative strand copy of the saRNA is created and used as the template for further transcription by the RdRp to create multiple mRNA copies of the second ORF containing the gene of interest.²³⁴ Through this replication process, the intracellular amount of mRNA encoding the gene of interest can be amplified by multiple orders of magnitude. Thus, the dose of saRNA required to achieve a given therapeutic effect is generally lower than that of mRNA. One downside of using saRNA is that the replication process creates double-stranded RNA intermediates that can activate interferon-mediated defense mechanisms, triggering a strong antigen-specific immune response against the gene of interest.²³⁵ While this may be advantageous for some uses such as vaccination, it is extremely limiting for other uses such as protein replacement therapy. Another limitation of saRNA is that the inclusion of the viral replication machinery results in RNA molecules that can be prohibitively long. To overcome this downside, some researchers have experimented with using "trans-amplifying" RNA systems that deliver the viral replication machinery ORF and the gene of interest ORF on separate strands of mRNA.^{236,237}

Considerations for synthesizing mRNA molecules. Unlike siRNA, mRNA is too long and complex to be chemically synthesized. Thus, mRNA is typically synthesized using *in vitro* transcription (IVT), in which the mRNA is transcribed into a linearized DNA template using a cell-free system with an RNAP, usually either the bacteriophage T7 or SP6 RNAP.²²³ Even though IVT was first developed in the 1980s, the development of mRNA-based therapies struggled for many years largely due to the instability and immunogenicity of IVT mRNA.²³⁸ Decades of research later, however, advances in our understanding of RNA chemistry and RNAs interactions with intracellular pathways have led to improved methods for synthesizing more stable and less immunogenic mRNA. Here, we will review the most important considerations to keep in



Fig. 6 Important structural features of mRNA. mRNA synthesized via *in vitro* transcription must recapitulate key structural features of naturally-produced mRNA, such as the 5' cap structure and nucleoside chemical modifications, in order to reduce the immunogenicity of the mRNA and to improve mRNA translation efficacy and stability. These include analogs to uridine in the 5' UTR, coding sequence, and 3' UTR; as well as key analogs for the 5' cap.

mind when synthesizing mRNA molecules, all of which must account for key structural features depicted in Fig. 6.

As previously mentioned, the 5' cap is important for binding to eIF4E and recruiting the rest of the PIC. Naturally-produced cellular mRNA is capped co-transcriptionally in a multi-step process in which a guanidine nucleotide is first covalently attached to the 5' end of the nascent mRNA using a 5' to 5' triphosphate linkage and then methylated on the N7 position by methyltransferases.²³⁹ IVT mRNA can be capped either post-transcriptionally or co-transcriptionally. One of the earliest methods developed for capping IVT mRNA post-transcriptionally utilizes purified Vaccinia virus capping enzyme to attach a 7-methylguanosine to the 5' triphosphate end of the mRNA.^{240–242} Recently, the mRNA capping enzyme from Faustovirus has also been characterized and commercialized for use in post-transcriptional capping, offering advantages over the Vaccinia virus capping enzyme such as improved capping efficiency and stability over a broader range of temperatures.²⁴³ Although post-transcriptional capping is highly efficient, the need to run multiple sequential reactions to generate fully functional IVT mRNA can be a disadvantage



particularly beneficial for immunocompromised individuals who cannot mount their own immune response against pathogens. For example, a previous study has demonstrated that delivering mRNA encoding a broadly neutralizing antibody against the human immunodeficiency virus (HIV) protected mice from subsequent intravenous challenges with HIV.³⁰² Separate work has also explored the delivery of mRNA encoding neutralizing antibodies against various other infectious agents, including respiratory syncytial virus (RSV), Zika virus, and Chikungunya virus.^{303–305} The second approach aims to stimulate active immunity by delivering mRNA-encoding antigens from the pathogen of interest. When a cell internalizes and starts translating this mRNA into protein, the cell becomes a source of the antigen. APCs, namely DCs,³⁰⁶ can then capture and present the antigen, triggering both cellular and humoral immune responses. This approach can also leverage the flexibility of delivery platforms to introduce multiple mRNA sequences, each encoding a distinct antigen, within a single vaccine formulation, allowing for a more robust immune response against a single pathogen or a broader immune response against multiple pathogens or pathogen variants.³⁰⁷ This strategy could be especially useful for vaccinating against pathogens that are known to mutate rapidly, such as influenza. Lastly, of note, saRNA encoding infectious disease antigens are an exceptionally promising technology to use for vaccination because they have been shown to elicit protection equivalent to conventional mRNA vaccines, but require much lower doses.^{235,308} Besides SARS-CoV-2, clinical trials utilizing mRNA LNP-based vaccines have begun for a variety of infectious diseases, including cytomegalovirus, Zika virus, Chikungunya virus, rabies virus, influenza, RSV, human metapneumovirus, and parainfluenza.^{168,273,300}

RNA LNP-based therapies have also shown much promise for applications in cancer immunotherapy. There are three main ways in which RNA LNPs are being developed to treat cancer. First, similar to the passive immunity approach for infectious diseases, mRNA-encoding anticancer antibodies can be delivered using LNPs such that anticancer antibodies are produced by the recipients own cellular machinery. This approach to antibody-based cancer treatments could potentially be more cost-effective, flexible, and logistically simple compared to traditional recombinant protein-based approaches, which often struggle with delivery and manufacturing challenges.^{309,310} Currently, mRNA LNPs encoding for anticancer antibodies are being tested in clinical trials for treating various solid tumors by targeting shared cancer biomarkers such as CLDN6 and CLDN18.2 (NCT04683939, NCT05262530). Second, mRNA can be used in cancer vaccines by encoding either non-mutated or mutated tumor-associated antigens to elicit an antitumor immune response. In one notable example, FixVac is an intravenously administered liposomal RNA cancer vaccine that contains mRNA encoding for four non-mutated antigens prevalent in melanoma. Based on an interim analysis of phase I clinical trial data, FixVac was found to mediate durable antitumor responses in patients with CPI-experienced melanoma, either alone or in combination

with CPI therapy, demonstrating that there are benefits to using cancer vaccines against common non-mutant shared tumor antigens.³¹¹ Using a completely different cancer vaccine approach that is highly specific to each individual patient, mRNA-4157/V940 is a personalized mRNA-based cancer vaccine that encodes for up to 34 patient-specific tumor neoantigens, each of which are computationally predicted based on sequencing analysis of a patient's tumor. Due to promising phase II clinical trial results, in which mRNA-4157/V940 in combination with CPI reduced the risk of recurrence or death by 44% compared to CPI alone in patients with stage III/IV melanoma, mRNA-4157/V940 is moving into phase III clinical trials (NCT05933577). Lastly, LNPs can be used to deliver mRNA encoding cytokines that can reshape the tumor microenvironment from being immunosuppressive to proinflammatory. In one study, the intratumoral administration of an mRNA cocktail encoding for IL-23, IL-36 γ , and OX40L resulted in remarkably durable anticancer immunity in mouse models of cancer.³¹² The therapy has moved into phase I clinical trials as mRNA-2752 for the treatment of various solid tumors (NCT03739931).³¹³ Similarly, intratumoral administration of mRNA encoding IL-12 has also been shown to work well as an anticancer therapy in preclinical models and is now being investigated in clinical trials (NCT03946800, NCT04455620).³¹⁴

4.3 Polymersomes

Polymersomes were first reported by Discher and Eisenberg in the late 1990's, and have since been researched extensively as vehicles for drug delivery and immune modulation.^{315,316} Consisting of amphiphilic polymers, these nanoparticles are treated as the macromolecular analogue to LNPs and liposomes. Morphology of polymersomes are contrasted with other lipid and polymer nanoparticles in Fig. 7. Materials commonly used include polystyrene, polybutadiene or hydrophobic poly(meth)acrylates as the membrane forming block and hydrophilic polymers such as polyethylene glycol or acrylic acid as the corona forming segment. The vesicular morphology is attained by keeping the hydrophilic weight fraction between



Fig. 7 Comparison of morphology and composition of different nanoparticle technologies, which includes both lipid- and polymer-based micelles, vesicles, and nanoparticles, each of which have unique compositions to favor their unique morphology.



known that the disulfide bond is sensitive to the intracellular influx of reducing agents such as cysteine and glutathione.³³³ To this extent, Jia *et al.* developed diblock copolymers consisting of a disulfide bond at the junction between the two polymer segments.³³⁴ In this work, the authors designed polymersomes using standard polymerization techniques and demonstrated the enhanced release of payloads compared to non-reduction sensitive analogs. The materials can be made in high yield with facile purification, representing an improvement over previous formulations often difficult to design due to the instability of the disulfide junction. Importantly, enhanced uptake and release of the auto-quenching fluorophore calcein highlights the utility of this system, which may prove beneficial for delivering more complex macromolecules such as protein and nucleic acid.

Polymer nanoparticles can also be designed to be inherently adjuvanting, as demonstrated by materials designed by Luo *et al.* to stimulate the STING pathway.³³⁵ The authors utilized pH-sensitive tertiary amines over a wide range of pK_a, proposing that an acidification of the endosome leads to transient protonation of the polymer, followed by endosomal rupture *via* the proton-sponge effect. This internal stress in turn activates the STING pathway, leading to a type 1 interferon response and robust CD8⁺ cytotoxic T cell immunity. The upregulation of IDO-1 and CXCL10 was also reported as a validation for STING pathway activation along with phosphorylation of IRF3. To demonstrate its applications, the authors treated B16-OVA, MC38 and TC-1 tumors *via* subcutaneous injection, and reported flattened tumor growth curves and enhanced survival. Enhanced responses were observed when using CPIs for TC-1 treatment.

4.3.4 Clinical translation of polymersomes. Though polymersomes have been proposed to have greater stability than their lipid counterparts, few studies directly compare these two delivery systems.^{320,336} Furthermore, the main issues that prevent the application for polymer nanoparticles in the clinic are primarily in processing, loading efficiency and quality control. As a result, at the time of writing this article, there are no polymersome-based formulations used clinically.

Despite requiring fewer components than LNPs, the macromolecular nature of polymers requires carefully controlled parameters for processing the materials in order to form reproducible morphologies. Due to challenges in materials design along with the uncontrolled nature of self-assembly, extensive purification is required to attain materials of desired size and polydispersity. As a result of this post-processing, there is a substantial loss of synthetic material, making the actual efficiency of production lower than what is theoretically possible.

As mentioned in Section 4.1.1, researchers have designed and utilized microfluidic devices to form reproducible, homogeneous nanoparticles.¹⁵⁴ In addition to carefully modulating the size of polymersomes, microfluidic mixers allow for minimal purification needed prior to injection. As a result, these techniques are critical in improving the scalability of nanoparticle formulations, while retaining narrow polydispersities

needed for clinical approval. Such techniques may prove necessary to implement polymersomes as commercially-relevant formulations.

4.4 Inorganic nanoparticles

Inorganic nanoparticles have emerged as a groundbreaking frontier in medicine, showcasing their remarkable potential to revolutionize diagnostics, therapy, and drug delivery.³³⁷ These nanoscale structures, composed of various materials such as metals, metal oxides, and semiconductors, possess unique physicochemical properties that enable them to interact with biological systems in unprecedented ways. Moreover, their ability to be functionalized with ligands and biomolecules allows for precise targeting and interaction with cellular receptors, paving the way for personalized and highly effective therapeutic interventions.^{338,339} As research in this field continues to advance, the integration of inorganic nanoparticles holds the promise of transforming the landscape of medical treatments, enabling more precise, efficient, and tailored approaches to disease management.

4.4.1 Silica. Silica nanoparticles and porous systems have been used in drug delivery and vaccine development for targeted delivery of therapeutics and enhancing the efficacy of vaccines.³⁴⁰ The porous nature of the silica particles allows for high drug-loading for sustained or controlled drug release. Surface modifications enable targeting specific tissues or cells, increasing drug accumulation at the desired site while minimizing off-target effects. The large surface area of nanoporous silica materials can be functionalized with ligands, antibodies, or peptides to enhance cellular uptake or target specific receptors. These systems are used as carriers for antigens or adjuvants to improve immunogenicity and increase vaccine stability.³⁴¹ A mesoporous silica micro-rod network has been used to encapsulate GM-CSF and CpG through subcutaneous injection. Continuous antigen release caused stimulation of APCs for sustained B cell activation in the lymph node.³⁴² Here, the authors used a model antigen, gonadotropin-releasing hormone (GnRH), and observed formation of anti-GnRH antibodies for a year. They also used a HER2/neu peptide to stimulate immunoreactivity to HER2 on tumor cells. They observed superior results with silica networks compared to bolus and Alum systems and sustained release of antigens enabled eliminating multiple dose requirements for vaccination practices.

4.4.2 Gold. Gold nanoparticles were previously synthesized through reduction of gold ions using a reducing agent and stabilizer by Turkevich³⁴³ and Frens.³⁴⁴ The size of the gold nanoparticles can be controlled by adjusting the concentration of the reactants and the reaction conditions. Gold nanoparticles in various shapes and size range were produced such as nanorods (AuNR),^{345,346} nanocubes (AuNC),³⁴⁷ nanooctahedra (AuNO),³⁴⁸ nanocages (AuNG),³⁴⁹ and nanoplates.³⁵⁰ Their distinct photonic, electronic, and catalytic properties make them suitable for nanomedicine applications such as targeted delivery and diagnostics. Their small size and large surface area allow for efficient uptake by immune cells. The surface of gold



nanoparticles can be functionalized with antigens causing immunogenicity.³⁵¹ The gold nanoparticles can be engineered to have specific shapes, sizes, and surface properties, which can also influence their interaction with immune cells and change antigen presentation efficiency.³⁵² When these antigen-delivering nanoparticles interact with APCs such as DCs, they can be taken up by these cells through endocytosis. The nanoparticles are then processed within the cells, and the antigens are presented to T cells in association with MHC molecules. Activated T cells recognize and eliminate tumor cells expressing the targeted tumor-associated antigens. This immune response can include the activation of cytotoxic T cells, release of cytokines, and recruitment of other immune cells, leading to antitumor effects. In a specific demonstration, tumor mucin associated glycopeptide antigens were immobilized on the surface of the gold nanoparticles to target Dectin-1 on the APCs.³⁵³ The authors showed stimulation of IL-1 β , IL-5, IL-6, interferon- γ , IL-17, IL-21, IL-23, and MIP3 α causing a mixed Th1/Th2/Th17 response. This example demonstrates the therapeutic effects possible *via* precise engineering of gold nanoparticles.

Other inorganic nanoparticles such as calcium phosphate, manganese oxide, iron oxide, and carbon nanomaterials have also been used for immunoengineering applications as discussed elsewhere.³⁵⁴

5 Local immunomodulation

Immunomodulatory materials are used to promote the growth and differentiation of new tissue composed of scaffold materials, biochemical cues, and cellular composite systems.³⁵⁵

One general example is given in a recent work, where local inflammatory response is suppressed by introducing a combination of immunosuppressive enzyme indoleamine 2,3-dioxygenase 1 and galectin-3 protein binding glycans present at the tissue after local injection.³⁵⁶ The authors studied the fused protein system to block gene expression of inflammatory cytokines IL-6 and IL-12p40 on the disease models caused by chronic inflammation such as osteoarthritis and psoriasis. Several other technologies are explored in the following sections.

5.1 Device coatings

Medical device coatings play a pivotal role in enhancing the performance, biocompatibility, and durability of a wide range of healthcare technologies. From implantable devices to diagnostic tools and drug delivery systems, coatings serve as a crucial interface between the medical device and the biological environment. These coatings are meticulously designed to address multifaceted challenges, including improving biocompatibility, preventing infections, promoting tissue integration, and enabling controlled drug release. As the field of medical devices continues to advance, the development and application of innovative coatings have emerged as a dynamic area of research and development, offering new avenues for improving

patient outcomes and revolutionizing the landscape of modern healthcare. Here we discuss recent advances in biomaterial coatings. One primary goal of many device coatings is antimicrobial activity, which can be achieved through a variety of strategies. One recent one, published by Sadrearhami *et al.*, used polydopamine and PEG to encapsulate low amounts of nitric oxide (NO). The coating, which released NO over two days, was able to prevent biofilm attachment in a bacterial culture.³⁵⁷ Another strategy by Piktel *et al.* involved the fabrication of gold nanoparticles of varying morphology, including rods, peanuts, stars, and spheres. Their materials could be coated onto latex urinary catheters and prevent growth of patient-derived bacteria.³⁵⁸ These works stand out as two recent examples of promising antimicrobial device coatings, but tremendous effort is ongoing in the space and has been reviewed in-depth elsewhere.^{359,360} While we have come a long way in understand the foreign body response, further research is needed to understand the immunological interface with 21st century bioelectronics.

5.2 Hydrogels

Hydrogels can face challenges related to immune rejection when implanted into the native tissue environment.^{361,362} The immune system's response to foreign materials, including hydrogels, can lead to inflammation, fibrosis, and ultimately failure of the engineered tissue construct. Understanding and mitigating these immune rejection problems is crucial for successful tissue regeneration applications. This inflammatory reaction can lead to the release of pro-inflammatory cytokines, recruitment of immune cells, and degradation of the hydrogel scaffold and destruction of the cells. The immune response can result in the formation of a fibrous capsule around the hydrogel, limiting its integration with the surrounding tissue.³⁶³

Incorporation of immunomodulatory agents, such as anti-inflammatory drugs or growth factors, into the hydrogel structure can help attenuate the immune response.³⁶⁴ These agents can be released in a controlled manner from the hydrogel, dampening the inflammatory reaction and promoting tissue regeneration. Materials such as these have often been employed to improve outcomes diabetic wound healing, including fibrous mats releasing pioglitazone hydrochloride,³⁶⁵ stimuli-responsive materials releasing metformin,³⁶⁶ or soluble polymeric materials releasing butyrate.³⁶⁷

Co-implantation of immunomodulatory cells with the hydrogel can help regulate the immune response.³⁶⁸ These cells can secrete anti-inflammatory factors and modulate immune cell behavior, promoting a more tolerant environment for the hydrogel. For example, a recent paper by Guo *et al.*³⁶⁹ demonstrated the utility of injectable, conductive hydrogels that degrade over time to delivery myofibroblasts cells to induce muscle regeneration. Their hydrogel material is based on dextran-*graft*-aniline tetramer-*graft*-4-formylbenzoic acid and *N*-carboxyethyl chitosan, which demonstrate rapid self-healing properties attributable to dynamic Schiff base bonds between the formylbenzoic acid and amine group from *N*-carboxyethyl chitosan. The engineering of advanced materials



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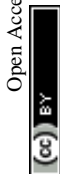
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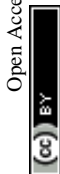
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