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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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interferons (e.g., IFN- α , IFN- γ).¹³ Protein engineering approaches aimed at improving the therapeutic properties of immune checkpoint inhibitors and cytokines remain an active area of research.¹⁴

Nanoparticles can also act as immunomodulators by targeting a particular type of immune cells and delivering antigens, genetic material or other immunomodulators for a variety of disease applications.^{15,16} Types of nanoparticles that are often employed for developing novel immunotherapies include fully synthetic polymers (i.e., polymersomes), lipid nanoparticles, and inorganic nanoparticles.¹⁷ More specific examples of each of these are provided in the following chapters of this review. Broadly speaking, nanomaterials refer to structures (either organized or disorganized) with nanometer scale dimensions ideal for cellular recognition, targeting and uptake. Over the past few decades, advancements in nanotechnology have allowed researchers to design synthetic and biologic nanomaterials for applications in immunoengineering, where precise targeting and modulation is necessary at the cellular and sub-cellular level. Owing to their tunability, nanomaterials present a promising approach to tackling challenges in delivery of payloads such as proteins, nucleic acids and peptides. Nanomaterials can be designed using techniques in synthetic biology (in the context of antibody–drug conjugates and other biologics), organic/inorganic chemistry (in the context of nanoparticles) or a combination of both.

Local immunomodulation refers to the targeted modulation of the immune response at specific tissues or organs, aiming to achieve a balanced and controlled immune reaction.¹⁸ This approach is valuable for addressing various autoimmune disorders, inflammatory conditions, and localized infections, as it allows for tailored interventions without compromising the overall immune system.^{19–21} By fine-tuning immune responses at the site of concern, local immunomodulation holds promise for enhancing therapeutic precision and minimizing systemic side effects in the realm of medical interventions.

In this review, we present recent studies in biomaterials for immunoengineering, including the development of novel

vaccine platforms, adjuvants, immunotherapies, cellular engineering, and tissue models for immunoengineering studies. We also highlight the challenges and opportunities and discuss the potential impact of immunoengineering on human health.

2 Vaccine technologies

Vaccines are one of the most effective medical interventions to prevent the spread of infectious diseases. They work by priming the immune system to recognize and fight off specific pathogens. Their history dates back to 1796 when Dr. Edward Jenner administered the first vaccine against smallpox.²² Since then, our understanding and subsequent design of vaccines has grown tremendously. At their core, vaccines consist of protein antigens to which the immune response is mounted and adjuvants that activate the immune system. Important components of vaccines and examples are depicted in Fig. 1. In this section, we highlight key components of vaccines and important engineering advances in each component, with suggestions for future research.

2.1 Adjuvant design and delivery

Adjuvants are added to vaccines to enhance the immune response. They work by stimulating the innate immune system, which in turn enhances the adaptive immune response. Adjuvants can improve the efficacy of vaccines by increasing the magnitude, duration, and specificity of the immune response. They can also reduce the amount of antigen needed in a vaccine, which can lower the cost of production. There are many types of adjuvants including aluminum salts, oil-in-water emulsions, and others. Aluminum salts, also known as alum, are the most commonly used adjuvants in human vaccines and have been used for over 80 years. They work by forming a depot at the site of injection, which slowly releases the antigen and stimulates the immune system. Oil-in-water emulsions, such as MF59 and AS03, are used in some influenza vaccines and have been shown to improve the immune response in elderly populations.²³ The design of adjuvants is a complex and evolving field. Adjuvants must be safe and effective in stimulating an appropriate immune response for the specific pathogen or disease being targeted. Recent advances in our understanding of the immune system have led to the identification and development of new adjuvants.²⁴ This includes agonists of innate immune PRRs such as TLRs, NOD-like receptors (NLRs), RIG-I-like receptors (RLRs), C-type lectin receptors (CLRs), and AIM2-like receptors (ALRs). PRRs normally function to recognize specific pathogen- or damage-associated molecular patterns (PAMPs, DAMPs).²⁵ The endogenous ligands to many of these receptors have drug-like properties such as stability and solubility, so it is of great scientific and clinical interest to identify and optimize novel adjuvant formulations.

One common way to identify new adjuvants is through the use of high-throughput screening. With a distinct immune activation pathway identified, *in vitro* assays can be optimized to screen thousands of small molecules for their targeted



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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



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



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

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



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	Palmyziq	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.

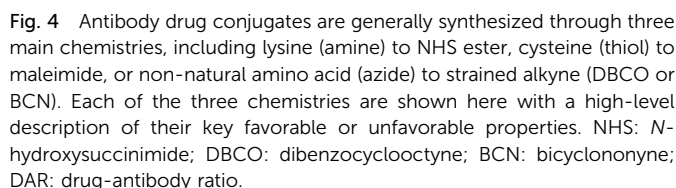
potential reactive lysine residues (the amine of which serves a reactive handle for most PEGylated proteins), thereby posing certain challenges in downstream separation processes.¹¹⁴ Therefore, the development of site-specific PEG conjugation

and the common gamma chain (but not IL-2R- α), which potently activates CD8⁺ T cells and natural killer (NK) cells for antitumor immunity. In THOR-707, Pro 65 a (residue that interacts with IL-2R- α) is replaced by an azide-modified non-natural amino acid, which reacts with a reactive PEG chain in one step. Importantly, in mice, THOR-707 did not cause proliferation of peripheral regulatory T cells but caused a robust expansion and activation of tumor-infiltrating CD8⁺ T cells and NK cells. These two examples demonstrate how site-specific PEGylation can be used to achieve a desired pharmacological profile and elicit certain immunological phenotypes.

3.2 Antibody-drug conjugates

Site-specific PEGylation approaches have recently been applied to interleukin-2 (IL-2), a pleiotropic cytokine that can act both as an immune-activating and immune-suppressing agent, depending on what T cell it activates.¹¹⁸ High-affinity IL-2 binding requires a trimeric receptor complex, consisting of IL-2R- α , IL-2R- β , and the common gamma chain.¹¹⁹ It is hypothesized that increasing the affinity of IL-2 binding towards IL-2R- α and decreasing the binding towards IL-2R- β induces preferential signaling on regulatory T (immune-suppressive) cells over effector cluster of differentiation (CD) 8+ T cells (immune-activating).¹²⁰ To achieve this, copper-free chemistry was utilized to specifically PEGylate Tyr 31 and Thr 51 in IL-2, residues that interact mostly with the β subunit.¹²¹ Such precise conjugation of the PEG chains was enabled by non-natural, azide-bearing amino acids (incorporated *via* genetic code expansion) and the subsequent orthogonal conjugation of PEG moieties *via* a click reaction. Preferential binding of the PEGylated IL-2 to its α receptor subunit over β subunit resulted in sustained activation and proliferation of regulatory T cells, which led to improvement in several autoimmune models such as lupus, rheumatoid arthritis, and *graft-versus-host* disease. A similar site-specific PEGylation approach was applied to IL-2 for the opposite purpose: reduce binding to IL-2R- α and increase the affinity for IL-2R- β .¹²² This engineered IL-2, termed THOR-707, predominantly binds to IL-2R- β

One of the major breakthroughs in ADC research that led to favorable clinical results was the development of highly potent cytotoxic payloads.¹²⁷ One reason that early ADCs failed to demonstrate any clinical improvement compared to the unconjugated chemotherapeutics was the high half-maximal inhibitory concentration (IC₅₀) value of traditional anticancer drugs.¹²⁸ The IC₅₀ values of the initial chemotherapeutics (doxorubicin and methotrexate) used in ADCs was in the range of micromolar potency. The next iteration of cytotoxic payloads,



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

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

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

4.1.1 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

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

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

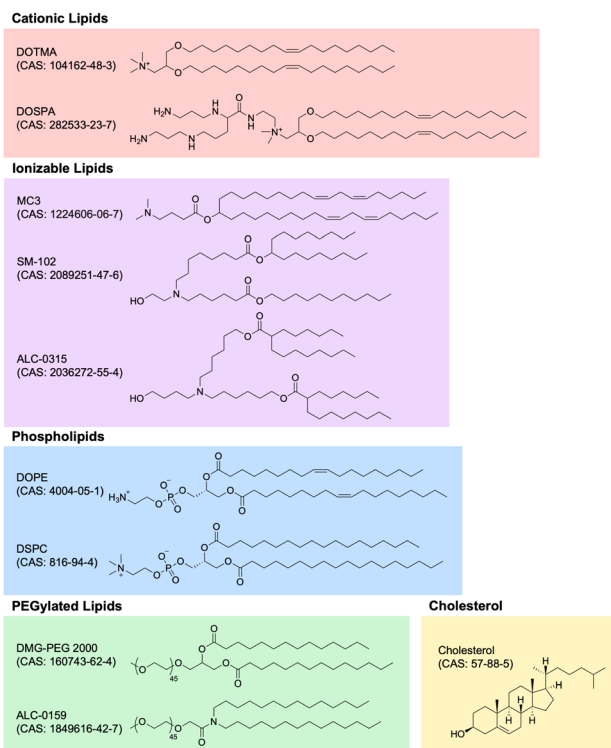


Fig. 5 Chemical structures of lipid nanoparticle components. Lipid nanoparticles are generally made of cationic lipids, ionizable lipids, phospholipids, PEGylated lipids, and cholesterol. Key examples in each of these classes are depicted with structures, names, and CAS numbers.



((4-hydroxybutyl)azanediy)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 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 Modernas elasomeron 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

critical first step for assembling the translation preinitiation complex (PIC).²²⁴ Proteins in the PIC bound to the 5' cap are also responsible for binding to the poly(A)-binding proteins (PABP) that coat the 3' poly(A) tail of the mRNA. This binding interaction circularizes the mRNA, which has been hypothesized to increase translation efficiency *via* ribosome recycling.²²⁵ Once the PIC is fully formed, the small 40S ribosomal subunit then begins scanning through the 5' UTR in a 5' to 3' direction until it encounters the first start codon.²²⁶ Then, the large 60S ribosomal subunit is recruited to the small 40S ribosomal subunit, forming the complete 80S ribosome, which will then translate the coding sequence for the gene of interest.²²⁷ By delivering exogenous mRNA into a cell, it is thus theoretically possible to instruct a cell to produce any protein using a cell's own native translation machinery. Furthermore, delivering exogenous mRNA has numerous advantages over delivering exogenous DNA.²²⁸ First, the mRNA only has to be delivered into the cytoplasm to be translated, whereas DNA requires more challenging delivery into the nucleus in order to be transcribed then translated. Second, mRNA does not integrate into the genome, so there is reduced concern about unintentional mutagenesis resulting from integration, a risk associated with DNA-based gene therapies. Lastly, because mRNA is degraded over time by intracellular nucleases, the mRNA is only transiently active in the cell, which can be beneficial from both a safety and pharmacological perspective.

Although LNP-mediated delivery of mRNA is extremely powerful, there are two additional promising variations on mRNA delivery in development that are worth noting: circular RNA (circRNA) and self-amplifying RNA (saRNA). The circRNA are covalently closed loops of RNA that exhibit much higher stability than linear mRNA due to their circular structure. The two primary pathways for mRNA degradation involve either 3' to 5' exonuclease digestion, after the 3' poly(A) tail has been sufficiently shortened by nonspecific exonuclease digestion, or alternatively, 5' to 3' exonuclease digestion, after the 5' cap has been enzymatically removed by decapping enzymes. Because circRNA lacks both a 5' and 3' end, exonucleases are unable to degrade intact circRNAs. Thus, circRNA degradation must be initiated by other cellular mechanisms, such as those involving endonucleases.²²⁹ The lack of a 5' cap, however, also means that translation initiation cannot proceed *via* the canonical cap-dependent pathway. Instead, circRNAs must rely on cap-independent translation. One of the most commonly employed mechanisms for cap-independent translation is the use of internal ribosome entry sites (IRES), which are RNA elements that fold into secondary and tertiary structures to directly recruit translation initiation factors.²³⁰ There are currently two main methods for synthesizing circRNA. One way is to align the two free ends of a linear piece of RNA, either using a DNA splint or engineered RNA secondary structure and then use a ligase to ligate the two ends together. The second way is to encode a ribozyme, such as a self-splicing group I intron, into the linear RNA sequence and have the ribozyme autocatalyze the RNA circularization. Currently, however, both methods have shortcomings such that the large-scale synthesis and

In recent years, mRNA has become one of the most studied payloads for LNPs, especially after the FDA approval of mRNA-based SARS-CoV-2 vaccines. Indeed, there has been much interest in developing mRNA-based therapies ever since the first proof-of-concept study in 1990 showed that mRNA could be used for direct gene transfer *in vivo*.²²¹ mRNA is a single-stranded RNA molecule that can range anywhere from a few hundred nucleotides to a few thousand nucleotides long. In cells, mRNA is transcribed in the ribosome from a genomic DNA template by RNA polymerases and then exported into the cytoplasm, where the mRNA is then translated by ribosomes.²²² Functioning as a transient “messenger molecule,” mRNA provides ribosomes with the correct sequence in which amino acids should be assembled into proteins. There are a few structural components of mRNA that important for regulating translation: the 5′ cap, the 5′ untranslated region (UTR), the coding sequence for the gene of interest, the 3′ UTR, and the 3′ poly(A) tail.²²³ In cap-dependent translation, recognition of the 5′ cap by eukaryotic translation initiation factor 4E (eIF4E) is a

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



The 3' poly(A) tail is important for translation to occur efficiently and for conferring stability to mRNA. Poly(A) tails can either be added post-transcriptionally by using an *E. coli* poly(A) polymerase or encoded in the DNA template.²⁵⁵ Because enzymatic addition of poly(A) tails results in a distribution of varying poly(A) tail lengths and varies batch-to-batch, using a template-encoded poly(A) tail is generally preferred. Based on

While the 5' and 3' UTRs are non-coding regions of the mRNA, they are nonetheless important for regulating mRNA translation efficiency and stability. mRNA can be regulated by the UTRs in a few different ways. For example, RNA-binding proteins can recognize certain nucleotide primary or secondary structures in the UTRs to regulate subcellular localization and translational control.²⁶⁷ The UTRs also often contain microRNA binding sites that are important for regulating mRNA stability and translation efficiency.²⁶⁸ When producing synthetic IVT mRNA transcripts, there are a few potential UTR engineering approaches if one does not wish to use a genes own native UTR. First, a common strategy that has been demonstrated to work quite well is to swap a genes UTRs with the UTRs of a gene that

As previously mentioned, the use of RNA LNP-based therapies to combat infectious diseases has experienced significant growth and is the most clinically advanced applications of mRNA-based treatments. There are two primary approaches for employing RNA-based therapies in the fight against infectious diseases. The first approach involves providing passive immunity to patients by delivering mRNA that encodes for antibodies that target a particular pathogen. This approach is

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.



4.3.2 Nucleic acid delivery. Charged polymers have been extensively explored in the context of nucleic acid condensation for the delivery of RNA and DNA.³²⁶ As a result, polymersomes incorporating these moieties are highly efficacious for the encapsulation (and subsequent protection) of such payloads.

Redox-responsive polymersomes have also been developed to exploit the reductive environment of the endosome.³³² It is

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

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

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

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



such as these hold tremendous potential for the future of cell therapy.

A crosslinkable norbornene-modified collagen type I hydrogel was used to develop a mechanically tunable environment to regulate T cell phenotype.³⁷⁰ Collagen type 1 was used as an extracellular matrix (ECM) constituent and functionalized with covalently crosslinkable units to control matrix stiffness. The AP-1 pathway was used as a marker for determining T cell phenotype. Slowly relaxing the stiffness of the matrix makes T cell to respond antigens faster at an *in vitro* and *in vivo* xenograft lymphoma model. Tailorable T cell products are expected to be developed by manipulating the ECM mechanics.

5.3 Microneedles

Microneedles have emerged as a tool at the intersection of immunology and engineering as a new tool for vaccination and immunotherapy delivery. These minuscule, minimally invasive structures offer a new way of applying vaccines and immunomodulatory agents and promising improved efficacy, patient compliance, and enhanced immune responses.^{371,372} By harnessing precision fabrication techniques, microneedles enable controlled and targeted delivery of antigens, adjuvants, and other bioactive compounds directly into the skin's immune-rich microenvironment. Microneedles have a multitude of design parameters to be altered for various applications across drug delivery. The composition, morphology, length, and stimuli-responsiveness can all be precisely tuned for different applications.^{373,374} Here, we focus on the recent advances in microneedles for immunomodulatory applications, including vaccination and allergic desensitization.

The concept of microneedle vaccination dates back to the early 1970s, but their widespread adoption has been limited for a variety of factors including manufacturing. The latest advances in manufacturing, combined with cutting edge vaccine technologies, are under investigation. For example, recent work by vander Straeten *et al.*³⁷⁵ reported a 3D printing device capable of fabricating microneedle arrays loaded with LNPs encapsulating mRNA for SARS-CoV-2 vaccination. The resulting arrays demonstrated both robust efficacy and room temperature stability for up to 6 months. Further, the arrays can be fabricated on-site without the need for trained healthcare personnel. Another work by Shin *et al.*³⁷⁶ demonstrates the utility of HPLC in the coating process to produce H3N2 influenza vaccinations that perform comparably to traditional intramuscular vaccines. Together, these works present advancement in multiple areas of vaccine technology and supports the future success of microneedle-based vaccination.

Microneedles are also of interest in allergic desensitization. The same skin-resident APCs are capable of presenting antigen in a pro-tolerogenic manner and can support desensitization to allergens. This therapy is often dubbed subcutaneous or epicutaneous immunotherapy (SCIT or EPIT), depending on the penetration depth of the microneedles. A recent work by Landers *et al.*³⁷⁷ demonstrated the protective effect of peanut antigen-coated steel microneedles in murine models of oral allergic challenge. Interestingly, a similar technology was

employed by Shakya *et al.*³⁷⁸ to deliver the model allergen ovalbumin, which offered protection in a murine model of airway allergy. Finally, more advanced materials science strategies can further improve the therapeutic efficacy and clinical utility of microneedle-based therapies. A recent example by Zhang *et al.*³⁷⁹ reported implantable microneedles with dissolvable components of silk fibroin hydrogel and polyvinyl alcohol, which can offer prolonged antigen exposure to improve clinical outcomes in food allergy. Many other attempts have been made at microneedle-based immunotherapy and are reviewed in-depth elsewhere.³⁸⁰ Clearly, the skin is a powerful immunologically active tissue and can be used to manipulate the balance of immunity in allergy and infectious disease.

6 Conclusions and future perspectives

In this review, we discussed the recent works in the design and synthesis of new biomaterials interacting with the immune response. The vaccine technologies and therapeutic systems for fighting and treating diseases by exploiting the immune system have recently attracted immense interest among the researchers and we have been witnessing the availability of the clinical methods and materials. New immunomodulatory systems including small molecules, biologics and nanoparticle formulations have been finding more applications in clinics. As we research and learn mechanisms of interactions among the synthetic tools and biological events, we are able to engineer new responsive materials that can precisely affect the immune system and their disease mechanisms. While developing new tools for treating the diseases, it is important to understand the quality by design (QbD) principles and regulations during the research for making these therapeutics more efficient and set the translation goals realistic and timely.³⁸¹ Reliability of the results and safety of the new methods are vital for producing more precise and efficient therapies.

Author contributions

All authors contributed to conceptualization, data curation process, analysis, writing, review and editing of the final manuscript.

Conflicts of interest

There are no conflicts to declare.

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