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## Review

# Biomaterial-based immunoengineering to fight COVID-19 and infectious diseases

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## SUMMARY

Infection by SARS-CoV-2 virus often induces the dysregulation of immune responses, tissue damage, and blood clotting. Engineered biomaterials from the nano- to the macroscale can provide targeted drug delivery, controlled drug release, local immunomodulation, enhanced immunity, and other desirable functions to coordinate appropriate immune responses and to repair tissues. Based on the understanding of COVID-19 disease progression and immune responses to SARS-CoV-2, we discuss possible immunotherapeutic strategies and highlight biomaterial approaches from the perspectives of preventive immunization, therapeutic immunomodulation, and tissue healing and regeneration. Successful development of biomaterial platforms for immunization and immunomodulation will not only benefit COVID-19 patients, but also have broad applications for a variety of infectious diseases.

## INTRODUCTION

In 2020, coronavirus disease 2019 (COVID-19) rapidly evolved into a global public health emergency. Coronavirus SARS-CoV-2 was identified as the causative virus of the COVID-19 pandemic, with a much higher infection rate than SARS-CoV of 2003. With over 80 million people infected and 1.7 million deaths globally as of December 2020, there is an urgent need for preventive measures and therapeutic treatments. Currently there is no effective therapy for COVID-19, and for those who recover from the disease, the repair of damaged tissues and organs is challenging. On the preventive side, although significant progress has been made in vaccine development, it is not clear whether a long-term immunity can be achieved.

SARS-CoV-2 gets into cells via the binding of its spike protein to the angiotensin-converting enzyme 2 (ACE2) receptor, which is upregulated in lung epithelial cells with age.<sup>1</sup> The infection of lung tissue may cause overactive inflammatory responses, tissue damage, and the development of acute respiratory disease syndrome (ARDS). Innate immune cells, such as neutrophils and macrophages, are recruited directly to the infection site and release cytokines to stimulate the adaptive immune responses, mediated by T and/or B cells.<sup>2</sup> In healthy people, this process is efficient enough to resolve the infection in a timely manner. However, a dysfunctional and dysregulated immune response to SARS-CoV-2 infection, characterized by monocyte hyperactivation and extensive macrophage/neutrophil infiltration, causes severe lung damage and systemic effects across the body, such as prominent lymphopenia.<sup>3</sup> Compared with patients with mild symptoms, patients in severe and critical conditions exhibit symptoms of hyper-inflammatory cytokine storm. Elevated level of pro-inflammatory cytokines, namely interferon (IFN), tumor necrosis factor (TNF), interleukin-6 (IL-6),

## Progress and potential

The COVID-19 pandemic has significantly affected the global population. The rapid development of preventative vaccines and patient treatment protocols is critical for saving lives. Engineered biomaterials can be used to induce specific immune system responses and improve effectiveness. In this review, we discuss the role of biomaterials in vaccine enhancement, infection treatments, and post-infection tissue regeneration. We identify biomaterial-based examples that help to create more effective and long-term immunization, extend drug release profiles, target specific organs to prevent side effects, control an overactive immune system during infection, and prevent and treat tissue damage. Overall, we recognize an important role for biomaterials in treatments for COVID-19 and the potential to address the challenges of infectious diseases in the future.

and IL-1 $\beta$ , impaired type I IFN responses, and a reduced production of IFN- $\beta$  and IFN- $\alpha$  were identified in more severe patient groups.<sup>3</sup> Increased levels of hyper-inflammatory cytokines may also trigger septic shock-associated multiorgan failure, which has been predominantly observed in elderly populations.<sup>4</sup> Moreover, antibody responses in COVID-19 patients often seem to be weak and short-lived, which is probably caused by dysregulated humoral immune induction and defective formation of germinal centers, where pathogen-specific antibodies are generated and long-lasting memory B cells are derived.<sup>5</sup>

In addition, the SARS-CoV-2 infection and consequent systemic inflammation can cause endothelial dysregulation and coagulation disorder, leading to the disruption of vascular integrity, hypercoagulation, and both arterial and venous thrombosis. Whether the virus can directly attack endothelial cells<sup>6</sup> or whether it infects only pericytes, which are the predominant cell type expressing ACE2 at least in the central nervous system (CNS) and in the heart,<sup>7</sup> is not clear at this point. The most common thrombotic manifestations of COVID-19 are pulmonary embolism, acute limb ischemia, ischemic stroke, and myocardial infarction.<sup>8</sup> COVID-19-related coagulopathies are associated with elevated levels of several coagulation markers, including D-dimer (a fibrin degradation product)<sup>9</sup> and elevated von Willebrand factor antigen (a marker of endothelial cell dysfunction).<sup>10</sup>

Moreover, increased activation of complement, a part of innate immune system, has been suggested to amplify the prothrombotic state.<sup>11</sup> The complement system consists of more than 30 proteins that circulate in the blood as inactive precursors. It uses pattern recognition molecules to directly or indirectly recognize pathogens or injured host cells. It can generate pro-inflammatory mediators, increase the antibody-mediated immunity, or directly attack and destroy the pathogens. The crosstalk between the complement and coagulation system is mediated by mannan-binding lectin serine proteases (MASPs), which are able to convert prothrombin to thrombin (MASP-2)<sup>12</sup> and fibrinogen to fibrin (MASP-1).<sup>13</sup> Recently, it was reported that the N protein of SARS-CoV-2 can bind to MASP-2 and enhance its enzymatic activity.<sup>14</sup> Therefore, it is possible that the thrombotic complications of COVID-19 are mediated, at least in part, by complement hyperactivation.

This ongoing pandemic highlights the critical need and challenges associated with a prompt response to infectious diseases. In this review, we discuss applications of biomaterials on enhancing COVID-19 immunotherapeutics as (1) preventive vaccines, (2) treatments for infection, and (3) healing and regeneration of damaged tissues following the resolution of infection (Figure 1). First, the rapid development of effective, long-term vaccines is critical to control the further spread of emerging and re-emerging infectious diseases. Vaccines as a preventive treatment function by educating the immune system on potential foreign antigens and generating protective neutralizing antibodies as well as memory immune cells. While the development of vaccines has achieved significant progress, the role of biomaterials to elicit adequate immune reaction should not be neglected, especially for the maintenance of long-term immunity and the enhancement of immune responses in aging populations. Biomaterials can support and boost effective immune responses through stabilizing the antigen, organizing antigen presentation patterns, recruiting antigen-presenting cells (APCs), triggering pattern recognition receptors, and/or delivering antigens to the lymph nodes.<sup>15</sup>

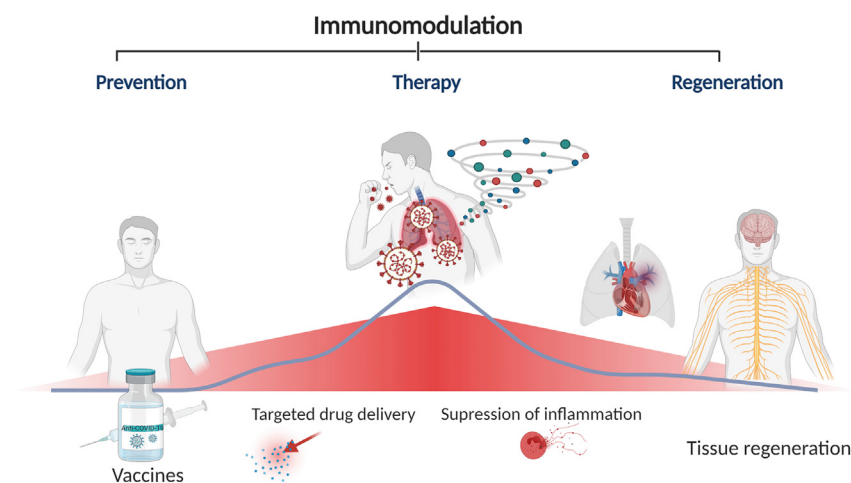
Secondly, if individuals have already been infected, treatments can be categorized into either antiviral or immunomodulatory therapies based on targeting the viruses

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**Figure 1. Immunomodulation to fight COVID-19 at different stages**

Biomaterials can be engineered to enhance vaccines, delivery drugs to treat infections, modulate immune responses, and promote the recovery and/or healing of damaged tissues.

or the host immune system. According to the stage of disease progression and patient's immune response patterns, each strategy has its advantages.<sup>16</sup> Viruses typically induce delayed, ineffective, or dysregulated host immune system reactions. Boosting host antiviral responses with IFN treatments and/or reducing viral load by antivirals represent potential treatments for the early stage of infection.<sup>17</sup> As the infection progresses and host cells actively respond to the virus, the buildup of a highly inflammatory environment can subsequently trigger a cytokine storm. To mitigate an overwhelming immune response, immunomodulators can be used to minimize pathological damage and guide desirable cellular immunity to attack infected cells. Some examples of therapeutic approaches that may support proper immune responses are anti-inflammatory agents (i.e., IL-6 blocker tocilizumab), immunosuppressive drugs (i.e., glucocorticoids), checkpoint inhibitors (i.e., PD-1 inhibitor), and adaptive cell therapies.

Administration of small-molecule antivirals or macromolecule biologics is usually systemic, however, and this global modulation of the immune system may be less specific and have side effects. Therefore, biomaterials can have an impactful role in both directing drug transport and realizing targeted or local immunomodulation.

Finally, concerns related to the long-term side effects of COVID-19 infection on the large population of surviving patients have been raised. Following the clearance of a SARS-CoV-2 infection, the modulation of the immune environment in a tissue-specific manner with biomaterials can be used to support healing and regeneration of damaged tissues to prevent future complications.

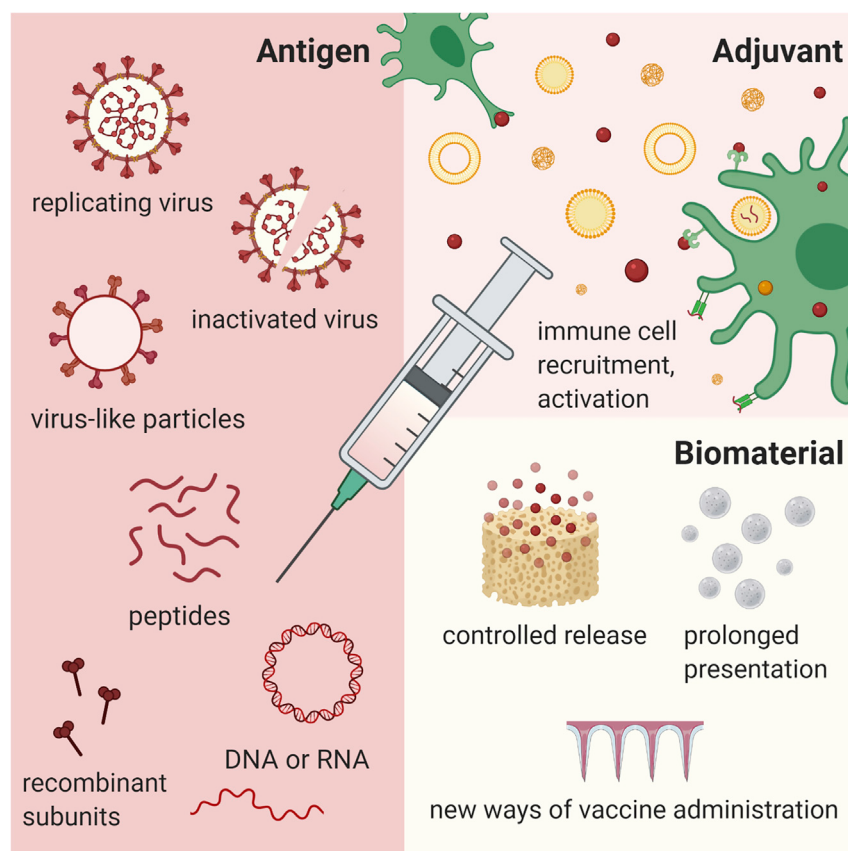
In this review, we focus on biomaterial-based immunoengineering approaches to tackle a variety of pathological problems during different stages of COVID-19 and infectious diseases. Figure 1 depicts the three major stages (vaccine prevention, drug delivery for therapies, and tissue healing and regeneration) where immunomodulation can have significant impact, and outlines the overall strategies at each stage of disease progression.

**Biomaterials-enhanced vaccine delivery to boost immunity***Technological platform of vaccines*

Effective vaccines induce long-term antigen-specific responses by developing long-lived memory T and B cells in addition to plasma cells, which produce antibodies. Variables, such as the type of vaccine and the nature of the targeted pathogen, define the extent of contribution for each of these immune cells. Vaccine development normally takes 10 to 15 years, where the optimal antigen is selected, the vaccine composition is optimized based on the preclinical animal studies, and three phases of clinical studies are performed to evaluate the safety and efficacy of the candidate vaccine. Accelerating COVID-19 vaccine development to condense 15 years into 15 months is very challenging, not only because it is not known which immunogen and which vaccine type will be the most effective in establishing robust long-term immunoprotection, but also due to concerns about the adverse effects that the vaccine might potentially have on vulnerable individuals. One of the safety concerns about vaccines is connected to a phenomenon termed antibody-dependent enhancement (ADE).<sup>18</sup> Instead of inducing neutralizing antibodies that appropriately block a viral entry to the cells, a vaccine might induce the production of non-neutralizing antibodies that facilitate viral interactions with host cells to enhance inflammation and immunopathology. Previous studies on SARS-CoV or MERS-CoV showed that ADE was mainly associated with Th2-biased responses. Therefore, adjuvants promoting Th1 immune responses were preferred when designing COVID-19 vaccines. Other than safety, vaccines in clinical trials must also be effective. The levels of neutralizing antibodies as well as T cell responses, which play an important role in the immune protection against SARS-CoV-2, are evaluated to indicate vaccination efficacy.

Currently, there are more than 60 vaccines against COVID-19 in clinical trials and over 100 vaccines in preclinical phases.<sup>19</sup> Vaccine formulations range from weakened and replicating viruses to inactivated viruses, viral protein subunits, peptides, as well as genetic materials (i.e., DNA and RNA) (Figure 2). Weakened virus-based vaccines are still live viruses, so they can trigger the strong immune responses but require challenging development and optimization to eliminate potential risks. Some COVID-19 vaccine frontrunners are based on inactivated viruses, which are produced through established chemical or heat treatment.<sup>20</sup> Inactivated virus vaccines from Sinopharm and Sinovac Biotech were already approved for emergency use in China.

Protein-based vaccines can be manufactured using a variety of platforms, such as the forced expression of viral proteins in mammalian, bacterial, or yeast cells in bioreactors. These techniques are well developed in pharmaceutical industries but are limited by high costs associated with low yield and essential multistage purification of possible (human) pathogen contamination. Therefore, cost and ease of manufacturing and scale-up should be considered for the global-scale production of potential vaccines. Thus, an alternative method of cell-free production of peptides might be more appropriate; peptide-based vaccines are easier to design, optimize, manufacture, and scale-up to meet the required worldwide mandate. Last but not least, recent clinical data show a great promise on a relatively new class of vaccination by delivering genetic materials, such as DNA and RNA, that induce a host production of immunogenic proteins *in situ*. For example, the two vaccines from Pfizer-BioNTech and Moderna have been approved by the Food and Drug Administration (FDA) for emergency use, and both of them use messenger RNA (mRNA) for viral protein expression.<sup>21</sup> Several other leading COVID-19 vaccines (i.e., Oxford-AstraZeneca, Johnson & Johnson, CanSino) are made from segments of viral DNA, which is packaged inside the adenovirus for delivery into host cells. Recently, a similar adenovirus-based vaccine has been approved for



**Figure 2. Approaches to develop and deliver SARS-CoV-2 vaccines**

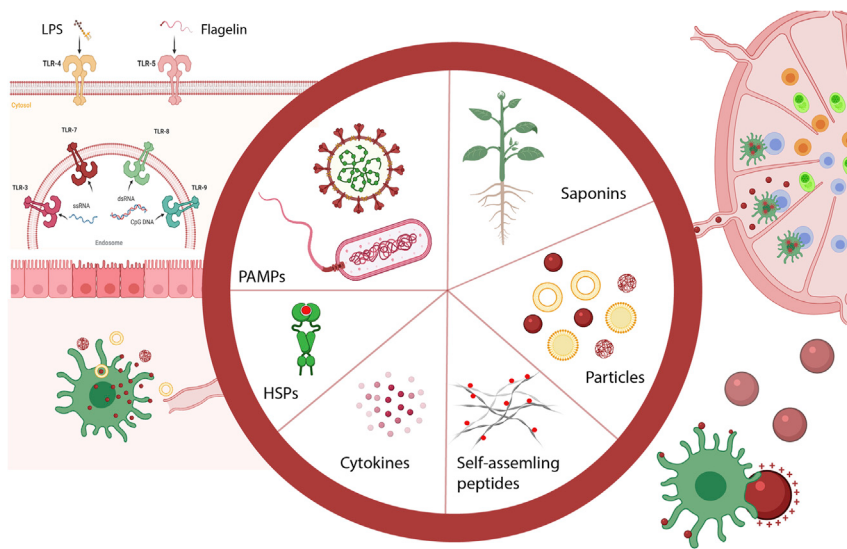
Various virus components can be used as potential vaccines, and biomaterials can be used to facilitate and enhance vaccine delivery.

Ebola. RNA- and DNA-based vaccines can tackle the common issues with scalability, flexibility, and speed of vaccine production against any new pathogens. A major advantage of mRNA vaccines is that mRNA does not need to get into a nucleus as DNA and can be directly translated into proteins within the cytoplasm, which results in higher expression efficiency.<sup>20</sup> Compared with RNA-based vaccines, DNA-based vaccines have the advantage of being more stable. Thus, due to the fragility of RNA, a cold chain ( $-80^{\circ}\text{C}$ ) is usually required for vaccine delivery and storage.

### Vaccine adjuvants and delivery

Subunit vaccines containing purified antigens derived from pathogens are often weakly immunogenic. Without an adjuvant delivery system, subunit proteins or genetic materials are usually subjected to dissociation and elimination before entering host cells.<sup>22</sup> Adjuvant supplementation can enhance the magnitude and durability of immune responses and permit significant dose sparing. Adjuvants are mainly supposed to increase the immune cell recruitment and the production of inflammatory cytokines, enhance antigen uptake and presentation, and also facilitate antigen transport to the draining lymph node to promote the expansion and differentiation of the appropriate T and B cells (Figure 3).

The most commonly used molecular adjuvants are ligands for pattern recognition receptors, such as Toll-like receptors (TLRs), nucleotide binding oligomerization-like receptors, retinoic acid inducible gene 1-like receptors, and C-type lectin-like



**Figure 3. Boosting the vaccination via adjuvant design**

Adjuvant design and their interactions with immune cells. Adjuvants work as immunostimulatory compounds to improve vaccine efficacy by boosting innate and adaptive immunity to support prolonged humoral and cellular response. The central circle is showing classes of molecular adjuvants such as pathogen-associated molecular patterns (PAMPs) and heat shock proteins with bound antigenic peptides (HSPs). Upper left part of the image is depicting Toll-like receptors (TLRs) specific for different PAMPs.

receptors, which are expressed on the cell surface or in endosomal compartments of innate immune cells. These receptors recognize conserved pathogen-associated molecular patterns (PAMPs), such as double-stranded RNA (TLR3), lipopolysaccharide (TLR4), single-stranded RNA (TLR7/8), or unmethylated CpG motifs present in bacterial DNA (TLR9).<sup>23</sup> PAMP recognition by innate immune cells leads to the activation of different immune pathways, including inflammasome, complement, and/or the nuclear factor  $\kappa$ B pathway, which can stimulate the adaptive immune system but may also cause excessive inflammation and toxicity. Therefore, it is critical to carefully select the adjuvant to induce the appropriate type of immune responses to achieve effective vaccination.

Besides pathogen-derived molecules, immunostimulatory adjuvants can be derived from plants. Saponins are steroidal or triterpene glycosides, which can bind to specific carbohydrate receptors on the surface of APCs and promote their activation and production of pro-inflammatory cytokines. Saponins might also interact with cholesterol and facilitate endosomal escape and cross-presentation of antigens and thus activation of CD8 T cells.<sup>24</sup> Saponins containing aldehyde and ketone groups also form imines or Schiff bases with amino groups of receptors on the surface of T cells and serve as co-stimulatory signals for T cell activation.<sup>24</sup> The COVID-19 candidate from Novavax uses Matrix-M, which is nanoparticle adjuvant composed of saponin together with cholesterol and phospholipid, for their protein-based vaccine and achieved high level of antibody production in early clinical trials.<sup>25,26</sup>

Moreover, heat shock proteins with bound antigenic peptides can be utilized as adjuvants to stimulate immune responses.<sup>27</sup> These molecular chaperones enhance both humoral and cellular immune responses by promoting the inflammatory cytokine secretion and facilitating antigen cross-presentation by dendritic cells (DCs) leading to the increased activation of CD8+ T cells.<sup>28</sup>

Immunostimulatory cytokines, such as IL-2, IFN- $\gamma$ , IL-12, and granulocyte-macrophage colony-stimulating factor (GM-CSF) are also studied for their adjuvant function.<sup>29</sup> GM-CSF,<sup>30</sup> for example, promotes the recruitment, maturation, and antigen presentation by DCs and elicits potent immune responses. Soluble, encapsulated, or conjugated administration of these reagents have been tested so far.

Lately, lipophilic statin drugs and small molecules called bisphosphonates have been discovered to have potent adjuvant activities. Bisphosphonates have been extensively used for the treatment of bone resorption diseases, such as osteoporosis. It is shown that they can also inhibit the mevalonate pathway in APCs,<sup>31</sup> which results in reduced lipidation of small GTPases, prolonged antigen presentation, increased T and B cell expansion, and antibody production.<sup>32</sup> In one study, the incorporation of bisphosphonates in nanoparticles was used to increase the targeting of bone metastatic breast tumors in mice<sup>33</sup>; however, its impact on modulating the therapeutic effects upon its interaction with immune cells has not been explored before.

However, molecular adjuvants can diffuse easily to the blood, which absorbs ten times more fluid than the lymph, to cause systemic inflammation or toxicity. Therefore, these adjuvants are often combined with other (bio)materials, namely nanoparticles or liposomes, to improve localization, safety, and efficacy. The size of the particulate adjuvant is an important factor, which can shape the extent of immune response and the protective efficacy of a vaccine. Small molecules drain to the blood, while particles of approximately 9–100 nm preferentially enter the lymphatics and can get directly to the lymph node for antigen presentation. On the other hand, larger particles (0.5–2  $\mu$ m) need to be transported to the lymph node by other cells, such as macrophages.<sup>34</sup> These size limits, however, can be altered by the mechanical properties of the particles. It has been shown that the optimal size for the efficient lymph node uptake is around 50 nm for rigid nanoparticles, while for liposomes it is 150–200 nm.<sup>35</sup> Moreover, the size of immunomodulatory compounds can affect receptor crosslinking on the surface of immune cells or alter the uptake and intracellular processing of the antigen.

Besides the size, the particle charge can affect the immune cell responses. Positively charged particles are more internalized by APCs compared with neutral or negatively charged particles, and are able to induce higher innate and adaptive immune reactions.<sup>36</sup> Moreover, positively charged particles increase the antigen escape from endosomes and consequently cross-presentation of the antigen by APCs to CD8 T cells.<sup>37</sup>

For a long time, alum (aluminum hydroxide and aluminum phosphate) was the only approved adjuvant in the USA. These nanosized crystals can adsorb antigens and aggregate into larger gel clusters upon hydration.<sup>38</sup> The binding of the antigen to alum particles is required for optimal immune responses. This mechanism is based on the electrostatic interaction of negatively charged antigen to the positively charged aluminum hydroxide as well as ligand exchange reactions between antigen phosphate groups and alum hydroxyl groups.<sup>39</sup> Moreover, adsorption of antigen to alum particles slows the antigen intracellular decay and thus prolongs the presentation by APCs.<sup>40</sup> Alum adjuvant ability is often attributed to the establishment of an antigen depot; however, the induction of local production of pro-inflammatory factors, which recruit neutrophils, monocytes, DCs, and macrophages, seems to be the main mechanism of action.<sup>41</sup>

Oil-in-water nano-emulsions, such as MF59, which is composed of small squalene oil droplets (around 160 nm in diameter) stabilized by two non-ionic surfactants Span 85

and Tween 80, can elicit even stronger immune responses than alum. The MF59 immune stimulatory capacity relies on the fully formulated emulsion to recruit and activate APCs, as any of the individual components alone were not able to induce comparable immune responses.<sup>42</sup> Liposomes are another common type of particulate adjuvant, which can enhance immune responses even more robustly than the oil-in-water formulations. For example, the adjuvant system AS01, which can induce strong humoral and cellular immunity, is composed of liposomes of 50–100 nm in diameter from dioleoylphosphatidylcholine, cholesterol with TLR4 agonist monophosphoryl lipid A (MPLA) (a detoxified derivative of lipopolysaccharide), and a saponin QS21. Cholesterol is included in the formulation mainly to reduce the cytotoxic activity of QS21, which would otherwise make holes in cell membranes.<sup>43</sup>

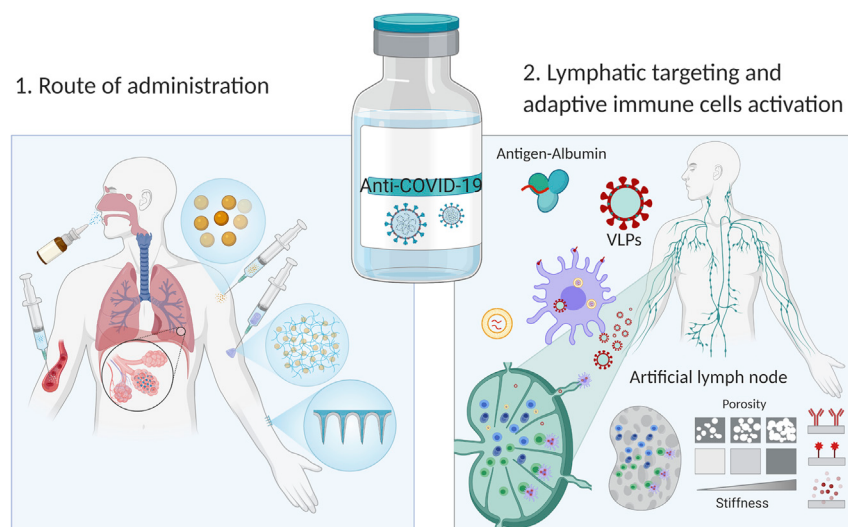
Chitosan produced by partial deacetylation of the natural polysaccharide chitin, can also be utilized as an adjuvant, vaccine stabilizer, or delivery system.<sup>44</sup> This positively charged polymer has strong mucoadhesive properties and exhibits a range of immunological effects from the stimulation of inflammasome to enhanced antibody production and T cell responses. It acts through the binding to cell surface receptors, including macrophage mannose receptor and TLR-2. Internalized chitosan induces mitochondrial stress, the production of reactive oxygen species, and subsequent release of the mitochondrial DNA to the cytosol. This process activates the STING pathway, which responds to the presence of DNA in the cytosol, to induce innate and adaptive immune responses.<sup>45</sup>

Microparticles composed of plant-derived polysaccharide delta inulin with alginate trigger complement activation and, when co-administered with antigen, help mount a robust antigen-specific adaptive immune response consisting of both antibody- and cell-mediated immunity.<sup>46</sup>

Short synthetic peptides, which can self-assemble into long nanofibers, can also be used as adjuvants when coupled to antigenic peptides. The density of T and B cell epitopes displayed on the fibrils can be adjusted to raise optimal adaptive immune responses with minimal local inflammation.<sup>47</sup> This nanofiber vaccine can be administered intranasally to promote expansion of tissue-resident immune cells.<sup>48</sup>

#### *Route of vaccine administration and targeting*

The level of immune responses toward vaccination can also be significantly affected by the route of administration. While most vaccines are injected intramuscularly, subcutaneous delivery with microneedle patches and pulmonary delivery with ventilators are promising alternatives (Figure 4). Biodegradable or dissolvable microneedles provide minimally invasive transdermal delivery and can be as effective as the conventional injection for influenza.<sup>49</sup> Microneedle-based delivery of hepatitis B and influenza vaccines, prepared with silicon crystal or gelatin formulations, respectively, are currently under clinical evaluation.<sup>50</sup> There are recent reports on developing SARS-CoV-2 vaccines that utilize microneedles to deliver spike proteins in mice.<sup>51</sup> Microneedle platforms can be fabricated using low-cost and scalable manufacturing techniques and may be suitable for global self-administration. This would be a convenient way to distribute vaccines for pandemics, such as COVID-19, if the mass production of a formulation can be achieved. Moreover, pulmonary administration empowers respiratory mucosal immunization and can be achieved using a wide range of mucoadhesive nanoparticles. Nasal delivery of inhalable nanoparticulate powders is recently gaining research attention, particularly in vaccine applications, systemic drug delivery for the treatment of pain, and non-invasive brain targeting.<sup>52</sup> Interestingly, recent work has adapted this technology to deliver



**Figure 4. Biomaterials platforms to boost the immunity**

A variety of vaccination techniques can be used to improve patient compliance as well as immunization efficacy. Nasal, pulmonary, intravascular, and subdermal immunizations can be performed through the use of nano- and microparticles, hydrogels, and microneedles. Biomaterials-based engineering strategies can be used to develop more effective vaccines. Particulate vaccines can be designed to drain efficiently to lymph nodes. Alternatively, the hydrogel/scaffold vaccine can act as temporary artificial lymph nodes to provide niches for immune cell programming.

therapeutic peptides, which demonstrates feasibility for the delivery for antigen peptides.<sup>53</sup> The ability to train the immune system at the site of viral entry may be very promising for acute or chronic respiratory infections. In this scenario, T cells can better home to both the lung interstitium and airway lumen, whereas intramuscular immunization-activated T cells are not due to entrapment within the pulmonary vasculature.<sup>54</sup> Some studies have also shown the benefits of having local presence of immune cells at the mucosal layer. For example, pulmonary delivery of tuberculosis vaccine increases antigen transport to draining lymph nodes and elicits antigen-specific mucosal memory.<sup>55</sup>

**Lymphatics targeting.** The lymphatics system is where immune cells are transported and memory cells are generated. Targeting the secondary lymphatic organs, such as spleen and lymph nodes, can improve the efficacy of a vaccine and provoke desirable immune responses. To achieve required spatiotemporal delivery and presentation of antigens and immunomodulatory cues, biomaterials can be designed to enhance lymphatic targeting and generate desirable populations of immune cells with minimal off-target side effects (Figure 4). Biomaterials can be engineered with physical and chemical properties, such as particle size, charge, shape, composition, and even more complicated stimuli-responsive designs.

Simple targeting to draining lymph nodes depends largely on particle size, while more sophisticated complexes are required to integrate into endogenous lymph transport processes.<sup>56</sup> One approach is to utilize carrier proteins, such as the endogenous and most abundant serum protein albumin for longer circulation, improved stability, and better immunogenicity. Modifying vaccine adjuvant CpG oligonucleotides with a lipophilic albumin-binding tail can spontaneously form micelles, which can hitchhike albumin. Combined with the conjugation of an antigen peptide to an albumin-binding domain, this strategy increases lymph node accumulation and

shows 30-fold increases in T cell activation.<sup>57</sup> Instead of albumin hitchhiking, the direct fusion of an antigen peptide to albumin reduces systemic circulation and improved proteolytic stability, as fused proteins traffic to the lymph nodes or are captured by local APCs.<sup>58</sup> With optimized factors, this approach improves vaccine immunogenicity by up to 90-fold and maximizes the responses to viral antigens.<sup>58</sup> SARS-CoV-2 proteins or peptides can be modified with an albumin-binding domain or fused to a carrier protein for lymph node targeting. Elicio Therapeutics repurposed this type of platform and showed an improvement in SARS-CoV-2-specific cellular and humoral immune responses in mice.<sup>59</sup>

Optimal activation of humoral immune responses also relies on appropriate antigen presentation to B cells.<sup>60</sup> To enhance B cell receptor engagement, lipid-based particles can be used as the base platform with the addition of MPLA as an immunostimulatory adjuvant. As simple liposomes are relatively fragile, methods have been developed to extend the lifetime and stability of synthetic vesicles. Polymeric cores of poly(lactide-co-glycolide) (PLGA) enveloped by PEGylated phospholipid bilayers provide support for the lipids and trigger strong antigen-specific immunoglobulin production.<sup>61</sup> Another approach was to engineer interbilayer-crosslinked multilamellar vesicles (ICMVs). These vesicles benefit from stabilized lipids due to the cross-linking of head groups of adjacent lipid bilayers. These ICMVs loaded with antigen cargos are slow to degrade in serum but are susceptible to lipase within intracellular compartments, thus inducing potent T cell and antibody responses.<sup>62</sup> Another category of vesicles to achieve organized antigen presentation is self-assembling nanoparticles. An early study using the glycoprotein of vesicular stomatitis virus (VSV-G) as an antigen has indicated that B cells respond promptly to highly organized VSV-G but are unresponsive to a poorly organized form of the same antigen.<sup>63</sup> Recently, self-assembling melittin-lipid nanoparticles were developed for lymph node targeting, which elicited activation of APCs in lymph nodes and led to a 3.6-fold increase in antigen-specific CD8<sup>+</sup> T cell responses compared with free melittin.<sup>64</sup> Moreover, inspired by viral self-assembling envelope and capsid proteins, virus-like particles (VLPs) arrange immunogens in organized arrays on multivalent nanoparticles. Highly organized display platforms utilize symmetric cage-forming macromolecules, such as enzyme lumazine synthase, ferritin, or self-assembling nanoparticles, to form oligomers.<sup>65</sup> Unlike monomeric antigen, VLPs made of HIV envelope trimer (gp140 trimer) and glycoprotein 120 (gp120) were shown to increase shuttling to follicular DCs (FDCs), concentrate at germinal centers, and enhance B cell activation.<sup>66</sup> The glycosylation helps antigen uptake through an innate immunity-mediated recognition pathway.<sup>66</sup> In addition, more sophisticated nanostructures, such as DNA origami nanoparticles, have been recently designed for nanoscale antigen presentation. Antigen copy number, spacing, affinity, dimensionality, and rigidity of the scaffold were optimized for HIV gp120 to drive functional B cell responses.<sup>67</sup> Furthermore, another research group has used the receptor-binding domain of Middle East respiratory syndrome coronavirus (MERS-CoV) fused with RNA interaction domain and ferritin to construct a self-assembling, protein-folding vehicle.<sup>68</sup> Such VLPs can also be designed with SARS-CoV-2 protein components for the optimal vaccination.

These approaches will provide tools for rapid clinical translation of potent and tissue-specific platforms for modulating pathogen-specific immunization. Recent pioneering work highlights the importance of materials design on nanoparticle-based antigen-delivery platforms to generate specific T cell responses.<sup>69</sup> For instance, the presentation of the same antigen (ovalbumin; [OVA] peptide) using two nanoscale delivery platforms can regulate distinct T cell fates in mice. In this case,

redox-sensitive poly(ethylene glycol)-poly(propylene sulfide) (PEG-PPS) block copolymers as components of a nanocarrier platform. Interestingly, the immobilized presentation of OVA peptide can differentiate naive T cells toward antigen-specific killer (CD8+) T cells, while the intracellular release of the same peptide via PEG-PPS nanoparticles can push T cells toward activated helper (CD4+) T cells. Helper T cells are crucial for cellular immunity as they can provide long-term memory against target antigens.<sup>70</sup> These results demonstrate the importance of material design and highlight the potential of using nanoscale biomaterials to engineer antigen-delivery systems for the induction of desirable T cell responses.

Last but not least, targeting lymph nodes for efficient B cell activation could be through a multistage delivery process. While there is a size preference for lymph node drainage, nanoparticles are still too big to access intra-lymph node canals. Therefore, combinations of nanoparticles and the controlled release of small-molecule cargos via programmed degradable linkers creates a two-step platform to deliver molecules inside the lymph reticular network.<sup>71</sup> Such advanced delivery platforms are able to consider the physiological location of certain immune responses and indicate a path to precision medicine.

**Biomaterials as artificial lymph nodes.** Besides developing particulate platforms to target secondary lymphoid or immunocompetent tissues, biomaterials can be used to create temporary lymph node-like depots to induce immunity against viral infections. Such a platform should be implantable via either injection or surgical placement, while being biocompatible with a controlled biodegradation rate. These macroscale therapeutic devices can be placed at target sites to precisely manage the presentation of antigens and adjuvants, lower required dose to reduce cost, improve therapeutic efficacy, and limit systemic exposure. The main function of such a construct will be the recruitment of adaptive or innate immune cells and education against target antigens. This platform can provide polyclonal activation and the expansion of immune cells to enhance immunogenicity or present specific antigens to induce a more specific immune response. With a grasp of the aspects that affect immune cell function *in vitro*, researchers have tried to focus more on designing 3D niches that offer better mimicry of the immunomodulatory signals. For example, 3D biomaterials have recently been proposed for the *in vivo* modulation of host immune cells.<sup>72</sup> Among various organic- or inorganic-based biomaterials, mesoporous silica-based vaccines represent a promising and versatile platform to present viral antigens, induce immune cell activation, and promote prolonged antibody production, as exemplified in the elegant work that highlights the development of mesoporous silica rod-based pore-forming platforms for *in situ* modulation of host immune cells as a vaccine against infectious diseases.<sup>73</sup> Soft biomaterials, including hydrogel scaffolds, can also offer a versatile platform to deliver a wide variety of therapeutics. High levels of biocompatibility, tunable physicochemical properties, controlled release, and tunable degradation profiles are among the promising characteristics of polymer-based hydrogels. In this context, injectable hydrogels and hydrogel microparticles may be more promising as they can be administered (injected) without surgical implantation. Cryogels and *in situ* pore-forming hydrogels can be used to provide interconnected micropores for the training of immune cells against target antigens. Although these materials are under development for cancer immunotherapies, a simple manipulation of formulations can repurpose these platforms for applications in infectious diseases.

Other than injectability and porous structures, several other criteria, including delivery mechanisms and release rates for each encapsulated immunological

biomolecule, should be considered when developing biomaterial-based vaccines to improve the therapeutic outcomes. Control over the temporal presentation of these molecules can be achieved by tuning the physical (e.g., charge, mesh size, and tortuosity) or chemical (e.g., degradation and bioconjugations) properties of engineered biomaterials. Considering the charge associated with most of antigens and adjuvants, charged polymers can be utilized to enhance the retention of immunological biomolecules. Similar approaches may be used to deliver small-molecule inhibitors but through hydrophobic-hydrophobic interactions. Incorporation of certain chemokines or cytokines can benefit from enhanced affinity through specific interactions with engineered peptide sequences<sup>74</sup> or polysaccharide like heparin.<sup>75</sup> Such an interaction may not only increase the retention, but also increase the biological stability of these proteins.

Mechanical properties of biomaterials are also critical as they can dictate the mobility, activation, and proliferation of recruited immune cells. Under pathological conditions, tissue stiffness increases during the viral infections, which naturally enhances T cell activation and antiviral effects. This process is mediated by changes in the structure, density, and composition of the extracellular matrix (ECM) in the infected tissues as well as in the draining lymph nodes. Experimental observations confirm the stiffening of lymph nodes in rodents from 4 to 40 kPa upon viral infection by lymphocytic choriomeningitis virus (~40 kPa).<sup>76</sup> This distinct stiffness can be replicated using synthetic biomaterials to provide optimal and biologically relevant activation signals for T cells.<sup>77</sup> Designing tissue-inspired biomaterials can help to provide an immune cell-specific microenvironment to ensure prolonged humoral and cellular immunity. The role of specific materials properties, in addition to the release profile, are being investigated when developing biomaterial-based vaccines. The creation of biomaterial-based cell homing sites that can act as a temporary lymph node with similar biological and biophysical signals can moderate the recruitment, residence, training, and fate of immune cells. This may promote immune responses to establish long-term immunity against COVID-19 or other infectious diseases.

## **Delivery of immunotherapy**

### *Current treatments*

The challenge of COVID-19 treatments, among other viral infections, is the balance between antiviral (preventing viral replication, expediting viral clearance, and boosting immune responses) and anti-inflammatory therapies (preventing the exacerbation of a cytokine storm that can lead to systemic complications).<sup>78</sup> Repurposed malaria drugs that interfere with viral entry and propagation, such as hydroxychloroquine, are under investigation for effectiveness against COVID-19. For immunomodulation, antibodies against inflammatory signals (IL-6, IL-1, and IL-2), Janus kinase pathway inhibition, intravenous immunoglobulins, and corticosteroids are under investigation.

Prophylactic anticoagulation treatment with low-molecular-weight heparin (LMWH) is currently recommended to prevent thrombotic complications.<sup>79</sup> However, some COVID-19 patients still developed thrombosis despite prophylaxis with LMWH.<sup>80</sup> Therefore, anti-complement drugs, such as narsoplimab (monoclonal antibody against MASP-2)<sup>81</sup> or eculizumab (monoclonal antibody that blocks complement protein C5)<sup>82</sup> have been tested and showed promising results in reducing endothelial damage and thrombotic risk.

The treatments that are currently under evaluation in clinical trials have been covered in recent reviews.<sup>83</sup> However, these platforms are typically delivered systemically, making the balance between viral clearance and prevention of overactive

immune responses difficult. Combinations of known and clinically approved therapeutics with material- and/or cell-derived components may serve to enhance treatment efficacy through localized delivery and tuned mechanisms. In this way, antiviral or immunotherapeutic regimens can be based on disease trends, such as accumulation in tissues with high ACE2 expression or tissue-specific disease states.

### *Targeted drug delivery to lung and cardiovascular system*

The direct targeting of sites of high virus titers and immune cell accumulation should be able to enhance the antiviral effects and immunomodulation of existing therapies. Even though COVID-19 is primarily a respiratory disease, the sites of infection are not limited to the lungs. The impairment of cardiovascular function and various coagulation disorders in COVID patients are a result of the wide distribution of ACE2 receptors and expansion of viruses within the endothelium and vascular smooth muscle cells.<sup>84</sup> Here, we focus on the strategies to target drug delivery to the lung and cardiovascular systems that are typically inflamed as a result of a SARS-CoV-2 infection.

Firstly, biomimetic particles modified with targeting ligands or antibodies against adhesion molecules expressed on the inflamed tissue can be used for specific drug targeting. The ability to tailor the activity of the immune system, both activation and reduction, may prove efficacious in viral clearance and preventing tissue damage. These techniques are not directly related to the delivery of immunotherapies; however, the modularity of peptide or protein modifications can be easily adapted for novel therapeutic strategies. To resolve inflammatory signaling of blood vessels, one approach involves the delivery of antioxidant enzymes. The delivery of these enzymes alone lacks stability and targeting to achieve functionality at the site of inflammation. To solve this issue, an antioxidant mimetic (EUK-134) may be loaded into PEGylated liposomes conjugated to anti-PECAM-1 antibodies to improve drug stability and specifically target pulmonary ECs; this biomaterial formulation retained enzyme functionality, enhanced desired lung biodistribution, and protected against pulmonary vascular edema in a lipopolysaccharide-induced mouse injury model.<sup>85</sup> Lungs are highly inflamed during ARDS so the local environment is usually acidic and populated with adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1), to attract monocytes and lymphocytes. It has been reported that the use of anti-ICAM-1 antibody-coated poly( $\beta$ -amino ester)/PEG nanoparticles enhances lung targeting and can release anti-inflammatory agents.<sup>86</sup> In this formulation, the incorporation of acid-sensitive poly( $\beta$ -amino ester) domains enables pH-dependent release of drug cargo within acidic, inflamed lungs, which improves the efficacy of TPCA-1 treatment in a murine acute lung injury model, compared with free drug or untargeted drug-loaded nanoparticles. Other strategies to target inflamed vasculature include the conjugation of integrin LFA-1L domain, which binds to ICAM-1, or antibodies against E-selectin to the surface of particles or liposomes (regardless of drug cargo or material).<sup>87</sup> Alternatively, targeting ligands can be replaced with peptides, such as VHPKQHR<sup>88</sup> for VCAM-1-binding or cyclo(1,12)Pen-ITDGEATDSGC (cLABLE) peptide for high-affinity ICAM-1 binding.<sup>89</sup> Similar targeting strategies can be utilized to selectively deliver thrombolytic agents to treat thrombosis and embolism. For example, a modified fibrin-homing peptide (allyl-GGCR(NMe)EKA) has been used to target microenvironment-sensitive nanogels to the blood clots.<sup>90</sup> The acrylamide nanogels contain thrombin-sensitive cross-linkers, which enabled high intra-clot release of encapsulated thrombin inhibitor hirudin. These nanogels showed increased circulation time and bioavailability compared with hirudin alone and were able to efficiently prevent and inhibit clot formation in mouse models of pulmonary embolism and thrombosis. Another strategy

for targeted drug release is based on the increased concentration of hydrogen peroxide by activated platelets at the site of thrombus formation.  $H_2O_2$ -responsive boronate particles show site-specific release of an anti-platelet drug and potent thrombolytic and anti-inflammatory activity without adverse side effects.<sup>91</sup> Further approaches in biomaterial design for thrombosis can be found in a recent review.<sup>92</sup> The lungs possess a unique characteristic of dense capillary networks, which are quite narrow and can only pass one red blood cell (RBC) at a time. Attachment of synthetic nanoparticles onto RBC surface creates a cell hitchhiking platform where nanoparticles are sheared off when squeezing through these narrow capillaries.<sup>93</sup> Non-covalent attachment of nanoparticles to RBCs increases lung accumulation while reducing uptake in other organs, such as liver, spleen, and brain.<sup>94</sup> If combined with anti-ICAM-1 strategies, lung retention of nanoparticles is further improved.<sup>95</sup> Interestingly, by tuning the affinity of nanoparticles to RBCs and making them shear resistant, the target site can be shifted to the spleen where lots of APCs reside. Erythrocyte-driven immune targeting has been developed as a vaccine platform, where erythrocytes deliver antigens selectively to the lung or spleen tissues.<sup>96</sup> This process mimics the natural capture of pathogen by erythrocytes and may not need additional adjuvants to elicit a strong immune response.<sup>96</sup>

Finally, the capacity of SARS-CoV-2 to infect the heart necessitates cardiac tissue targeting for treatments. The use of a cardiac-targeting peptide (CTP)<sup>97</sup> and the CRPPR peptide<sup>98</sup> enhance the delivery and retention of an imaging agent or liposomes, respectively. In addition, a CTP fused to a membrane protein of extracellular vesicles (EVs) improves cardiomyocyte cellular uptake *in vitro* and demonstrates enhanced retention following intramyocardial injection.<sup>99</sup> For protein therapeutic delivery, tannic acid modifications induce aggregation and enhance cardiac tissue accumulation of peptides, viruses, and proteins following intravenous injection; an intravenous treatment of modified basic fibroblast growth factor (bFGF) promotes cardiac recovery, compared with a direct injection of unmodified bFGF, in an *in vivo* model of myocardial ischemia.<sup>100</sup> The presence of markers of tissue damage can also be used as a signal to influence the accumulation of nanoparticles. In one approach, polynorbornene nanoparticles with incorporated MMP-2 and MMP-9 recognition peptides, preferentially aggregate based on matrix metalloprotease (MMP) activity. This interesting approach uses particles <100 nm that can diffuse through leaky vasculature to sites of myocardial damage but do not typically persist within the tissue for long periods of time. The programmed accumulation enables localization and retention up to 28 days at the site of tissue injury.<sup>101</sup> Since MMPs are present during the remodeling phase following acute injury and may be involved in SARS-CoV-2 infections, they may potentially serve as a target to deliver antiviral or immunomodulatory compounds.<sup>102</sup>

#### *Cell-derived drug delivery systems to modulate inflammatory response*

Neutrophils, the most abundant white blood cells in the body, reside in high numbers in the lungs and are the first line of defense against invading pathogens. Upon encountering danger signals, neutrophils quickly migrate to the targeted tissue and release immunomodulatory molecules, which recruit and activate other immune cells. They also internalize pathogens and destroy them in organelles called phagosomes. Moreover, neutrophils can eliminate the invaders by releasing reactive oxygen species and granules containing enzymes and antimicrobial peptides or they can prevent the spreading of pathogens by ensnaring them in web-like structures of DNA and proteins named neutrophil extracellular traps. However, the accumulation and excessive activation of neutrophils, as in case of ARDS in COVID-19 patients, can lead to exacerbated tissue damage and increased risk of thrombosis.<sup>103,104</sup>

To mitigate acute inflammation, activated neutrophils can be selectively targeted with nanoparticles containing agglutinated<sup>105</sup> or denatured proteins,<sup>106</sup> which bind to Fcγ RIII receptors on activated neutrophils.<sup>107</sup> The feasibility of this approach is demonstrated with nanoparticles made from denatured albumin loaded with an anti-inflammatory drug, which are preferentially internalized by activated neutrophils. In contrast, nanoparticles coated by natural albumin are ignored by neutrophils. This highlights the importance of nanoparticle design on generating specific immune responses.<sup>108</sup> Particulate therapeutics can also be used to suppress neutrophil recruitment and accumulation in inflamed tissues. Intravenously injected microparticles can be phagocytosed by circulating neutrophils, which triggers their apoptosis and elimination by the liver. Alternatively, particles captured in the liver and internalized by resident macrophages recruit circulating neutrophils.<sup>109</sup> In both cases, the administration of particles during acute inflammation can decrease the numbers of circulating neutrophils and/or change their homing site, which might be a useful strategy to mitigate inflammation.

In another strategy, cells can be equipped with “cellular backpacks,” polymeric particles loaded with therapeutic agents, that can be attached to the cell membrane with the use of antibodies, cell-adhesive molecules, or by covalent binding to cell surface proteins.<sup>110</sup> The size and shape of polymeric particles can significantly affect cellular backpack stability, because flat disk-like particles are phagocytosed less than spherical particles.<sup>111</sup> In this way, immune cells, such as monocytes,<sup>112</sup> can be utilized to deliver cargo into inflamed tissues. Furthermore, particulate drug delivery systems can be coated with cell membranes derived from different cell types, including platelets<sup>113</sup> or neutrophils,<sup>114</sup> which help reduce their immunogenicity and improve inflammation-targeting capabilities. RBCs and platelets can be fused to create hybrid membrane-cloaked nanoparticles, which show increased circulation half-life and improved homing to inflamed tissues owing to intrinsic RBC and platelet components, respectively.<sup>115</sup> Although adding such a feature seems interesting as it can represent the most natural biointerface, the translational and regulatory pathways appear unclear.

Besides coating nanoparticles or liposomes with cell membranes, EVs secreted by different cell types can be used to deliver drugs and vaccines, introduce targeting, or enhance regeneration. EVs are nanometer-sized phospholipid membrane-enclosed vesicles that contain bioactive components, such as nucleic acids or proteins derived from their parental cells. Based on their surface markers, EVs can be preferentially internalized by distinct cell types and tissues comparably with the membrane-coated biomimetic particles.<sup>116</sup> Among the most studied EVs with pro-regenerative properties are vesicles derived from mesenchymal stem cells (MSCs). These EVs have powerful immunomodulatory effects, which can be beneficial in the treatment of various inflammatory diseases, including ARDS.<sup>117</sup> Moreover, EV content can be further engineered to improve the therapeutic effects. This can be done by decorating EVs with antibodies/targeting ligands or loading them with various therapeutics. Passive loading by co-incubation is suitable mainly for hydrophobic drugs, which can efficiently penetrate the lipid bilayer. On the other hand, active loading techniques, such as electroporation, sonication, co-extrusion, repeated freeze-thawing, or saponin permeabilization, can be advantageous for intermediate or hydrophilic drugs.<sup>118</sup> EV content can also be modified through the genetic engineering of EV-secreting parental cells. For example, EV-targeting efficiency can be increased by fusing targeting peptides or proteins with the C1C2 domain of lactadherin, which binds to phosphatidylserine that is strongly enriched in EV membranes.<sup>119</sup> The EV circulation half-life can be prolonged by overexpressing CD47,

presenting a “don’t eat me” signal, which can protect EVs from being phagocytosed.<sup>120</sup>

In addition to micro- and nanoparticles, artificial cells can be utilized to perform more complex regenerative or immunomodulatory tasks. For instance, artificial neutrophils have been prepared by encapsulating zeolitic imidazolate framework-8 particles with incorporated glucose oxidase and chloroperoxidase within a neutrophil membrane. Thanks to the neutrophil membrane composition, artificial neutrophils are efficiently recruited to the sites of inflammation, where they convert glucose with the use of glucose oxidase to gluconic acid and  $H_2O_2$ . Chloroperoxidase transforms these products into highly reactive HClO which can eradicate infections.<sup>121</sup> Another strategy engineers synthetic MSCs to mimic and apply inherent cell properties. PLGA microparticles are loaded with bioactive factors typically secreted by MSCs and coated with MSC membranes. These synthetic MSCs demonstrate regenerative potential and cryopreservation and lyophilization stability.<sup>122</sup> Alternative biometric designs have implemented super-soft alginate microgels to mimic the shape, mechanical properties, and surface composition of naive and activated T cells.<sup>123</sup> Such a platform has been developed to offer a synthetic tool to investigate the effects of inflammatory cytokines on the fate of MSCs *in vitro*.

Finally, synthetic biology tools can be used to engineer biosensor cells to execute *de novo* antiviral actions. This approach has been explored by utilizing modular chimeric synNotch receptors, which are composed of engineered extracellular receptors specific to the desired antigen, and an intracellular transcription module, which can be tailored to generate user-defined cellular responses. These cellular biosensors can be programmed to secrete IFN- $\beta$  or neutralizing antibodies as an output reaction to the binding of the membrane-bound viral antigen to the extracellular region of the synNotch receptor. Through the genetic engineering process, these cells can mimic, to some extent, the immune cell responses required to suppress infections.<sup>124</sup>

### **Immunomodulation for the healing and regeneration of tissues and organs**

In the previous sections, we discussed the prevention of SARS-CoV-2 viral infection with vaccine supplements and the approaches to enhance viral clearance and mediate inflammatory-related symptoms at a systemic and tissue-specific level with immunotherapies. However, the long-term pathological effects of SARS-CoV-2 infection and the potential to exacerbate or induce future complications, even in mild cases, are just beginning to be explored.<sup>125</sup> A high correlation of symptom severity and mortality in patients with preexisting conditions sheds light on the potential risks of organ trauma. A buildup of fibrotic scar tissues causes many of the leading cardiac and pulmonary diseases, and COVID-19-induced tissue damage may contribute to their exacerbation. The extent, location, and duration of potential tissue damage, as well as the risk of future complications, resulting from COVID-19 infection must be understood and treated accordingly to prevent future trauma in this extensive patient group.

To accomplish this, tissue damage resulting from COVID-19 infection and treatments can be retroactively understood through patient characterization and long-term follow-up; however, this information may not be available in a timely manner. Interestingly, tissue models may be adapted to predict at-risk organ systems, determine the mechanism of pathogenesis, and screen potential therapies in a high-throughput fashion. In this section, we seek to address the long-lasting damage at a tissue-specific level and identify potential post-COVID treatments, specifically

immunomodulatory biomaterial compositions that may support healing and prevent future complications. Several previously developed strategies demonstrate the key role of biomaterials for the targeted and responsive delivery of therapeutic molecules, co-localization of multiple factors, and the induction of specific immune response with relevance for COVID-19 therapeutic development.<sup>126</sup> We focus on the respiratory, cardiovascular, and nervous systems as they represent the primary and more severe long-term tissue damages in COVID-19 survivors. While our discussion focuses on immunomodulation for tissue healing and regeneration, many other widely explored tissue engineering approaches utilizing cell transplantation, scaffold engineering, and drug delivery can also be applied and combined with immunotherapeutic strategies.<sup>126,127</sup>

### Respiratory system

Viral infections induce acute and chronic inflammation that significantly impact lung function, leading to pneumonia and ARDS. Following minor injury, lung epithelium possesses endogenous repair mechanisms, through activated epithelial cell proliferation, that serve to regenerate functional tissue structures. However, severe and extended inflammatory environments (in COVID-19 pneumonia and ARDS) preferentially induce apoptosis in epithelial cells. In these conditions, fibroblasts and inflammatory cells are more likely to dominate the remodeling process and replace damaged tissues with collagen-rich scars that have limited functionality.<sup>128</sup> Pulmonary fibrotic scarring and impacted lung function significantly reduce the quality of life and are present in up to 85% of ARDS survivors.<sup>129,130</sup> Previous clinical data from the SARS outbreak indicate that lung function remains abnormal for up to 3 years following infection. In a different follow-up study, a small group of patients demonstrate that SARS-induced lung scarring persists for up to 15 years after infection.<sup>131</sup>

The extent of prolonged lung dysfunction for COVID-19 survivors has begun to be evaluated, and is expected to pose major issues.<sup>132</sup> The presence of lung abnormalities scales with disease severity and persists even after the treatment and the resolution of symptoms.<sup>133</sup> In addition, a study in April 2020 detected the presence of lung abnormalities via computed tomography scans in asymptomatic COVID-19-positive patients.<sup>134</sup> This troubling discovery indicates the possibility that those unknowingly exposed to COVID-19 and asymptomatic may develop pulmonary fibrosis. An accumulation of scar tissue can lead to significant problems as it contributes to irreversible interstitial lung disease.<sup>135</sup> From the growing clinical insight, it is clear that strategies to address pulmonary scar tissue accumulation and support the restoration of functional tissue are required. Several tissue model systems have been developed to recapitulate key aspects of the respiratory system, such as the air-liquid interfaces and differentiated cell types, and have been characterized for COVID-19 infection.<sup>136,137</sup> Integration with *in vitro* models of fibroblast-mediated scarring<sup>138</sup> may serve to better understand COVID-19-induced fibrosis and evaluate potential therapeutic agents to properly heal scar tissues.

The design criteria of potential tissue-regenerative immunomodulation should reduce chronic fibrotic signaling, reverse scar tissue formation, and restore functional lung tissue. Components of previously reported biomaterial-mediated pulmonary fibrotic treatments may serve as ideal candidates. Anti-fibrotic and anti-inflammatory therapeutics, such as pirfenidone and nintedanib, repurposed from idiopathic pulmonary fibrosis, are already under evaluation for the prevention of COVID-19-induced scarring.<sup>130,139</sup> These treatments target fibroblast proliferation, collagen deposition, and TNF- $\alpha$ /IL-1 $\beta$  pro-inflammatory signaling. In addition, modulation of immune mechanisms, specifically blocking fibrotic fibroblast-immune cell

signaling motifs (CD47 or PD-L1) and cytokines (IL-6 or IL-11), have been implicated for the prevention and reversal of pulmonary scarring.<sup>18,140</sup> Like previously mentioned approaches, the use of biomaterials as delivery vehicles can enhance the efficacy, localization, and retention of delivered therapeutic molecules compared with a systemic delivery. Drug–material formulations can be tailored to achieve novel delivery mechanisms and desirable release rates, with a reduction of systemic side effects. In one approach, the encapsulation of pirfenidone within modular interpenetrating polymeric network microspheres is sufficient to achieve a more therapeutically desired controlled release profile following oral delivery, compared with the clinically available Pirfenex.<sup>141</sup> In another example, the intranasal delivery of IL-10 via hyaluronan and heparin-based hydrogels further decreases inflammatory and fibrotic markers in a bleomycin-induced pulmonary fibrosis mouse model, compared with the delivery of IL-10 alone. This formulation combines the biocompatibility and biodegradability of hyaluronan with the ability of heparin to bind IL-10 to enhance stability and extend the release rate. Interestingly, positive results of the hydrogel–IL-10 treatment as both a preventive and treatment of fibrosis were demonstrated.<sup>142</sup> Biomaterials can also be used to deliver and retain cellular therapeutics within the lungs, with and without active targeting motifs. In a recent example, MSC-laden alginate-based microgels demonstrated enhanced circulation time following intravenous injection and, as seen in most intravenous cellular applications, accumulated within the lungs.<sup>143</sup> MSCs, in particular, have potent immunomodulatory potential and have already been identified for a potential role in COVID-19 therapeutics.<sup>144,145</sup> Alginate networks allow for the diffusion of soluble inflammatory cues into the microgels to induce intrinsic anti-inflammatory stem cell secretion while also protecting against rapid immune clearance.<sup>143</sup> As previously mentioned, cell-based backpacks, based on the homing of specific cell types to fibrotic markers, can also be used to deliver therapeutic payloads to reverse fibrotic scarring.<sup>146</sup> In this study, drug-loaded PLGA/PEG nanoparticles were conjugated to monocytes, which demonstrated MMP-2-induced release and lung injury homing, respectively. This formulation incorporates a multistage targeting and release cascade through specific cell or peptide conjugation to explicitly induce the internalization of astaxanthin/trametinib-loaded nanoparticles within injured alveolar epithelial cells to reverse fibrosis.

### *Cardiovascular system*

As a result of viral infection, cytokine storm, or antiviral treatments, both cardiac tissue and the vascular system may suffer from lasting damage. In cases of a direct viral infection of the heart, a chronic immune response is the main contributor to the degradation of the ECM and induction of fibrosis.<sup>147</sup> Fundamentally, intrinsic heart healing is limited by the lack of cardiomyocyte proliferation and active remodeling processes.<sup>148</sup> Recent reviews have covered the specific mechanisms of COVID-19-induced cardiac damage and risks of long-term damage.<sup>149</sup> The prevalence and unknown duration of cardiovascular complications associated with COVID-19 represents an unmet clinical need; elevated levels of troponin, indicating the presence of acute cardiac injury, were found in 20%–30% of hospitalized COVID-19 patients.<sup>150</sup> Cardiac swelling, scarring, reduced ejection fraction, and high troponin T levels remain even after COVID-19 recovery.<sup>151</sup> Initial studies have identified that markers of cardiac damage and inflammation can last up to at least 10 weeks after initial diagnosis.<sup>152</sup> These symptoms are indicative of more severe complications, namely heart hypoxia, myocarditis, arrhythmias, and eventual heart failure.

Approaches to support the restoration of damaged cardiac tissue following the resolution of a COVID-19 infection are required to reduce the risks of developing chronic conditions. Myocardial infarction treatments that prevent sustained

inflammation-mediated tissue damage and support functional ECM production can potentially address the needs of COVID-19 patients. However, the timing of treatment (presence/absence of viral particles) and mechanism of damage (viral or immune mediated) may affect the immunomodulation required. The localized delivery of anti-inflammatory factors, TIMP-3, IL-10, IL-2, and IL-19, via biomaterial carriers dictates cellular recruitment and survival to improve the extent of healing and regeneration.<sup>153</sup> One example utilizes a poly(ethylene arginylaspartate diglyceride) and heparin coacervate to locally co-deliver FGF-2 and IL-10 with an extended release profile. Intramyocardial injection of this formulation enhances regenerative outcomes following myocardial infarction, compared with free protein or single-protein coacervate delivery.<sup>154</sup> Macrophage polarization toward an M2 regenerative phenotype plays a key role in limiting the extent of tissue damage. In one approach, IL-33 overexpression vectors are delivered intravenously with polyethyleneimine.<sup>155</sup> This delivery mechanism, which utilizes polyethyleneimine complexed with DNA vectors to induce exogenous IL-33 expression, induces specific cell-secreted cytokines as an alternative to cytokine delivery. This treatment enhances the endogenous production of IL-4 and subsequent M2 macrophage polarization to improve myocarditis outcomes in a mouse model. An interesting strategy uses an injectable alginate-based hydrogel to deliver CSF-1 and IL-4 into the heart and demonstrates the potential to promote regenerative macrophages.<sup>156</sup> Recently, the role of B cells in exacerbating inflammation and reducing M2 polarization has been identified;<sup>157</sup> treatments, such as pirfenidone, to address this mechanism may serve as a potential therapeutic.<sup>158</sup> In addition, the role of autoimmunity, in which cardiac proteins can activate and sustain an adaptive immune response, in the context of tissue healing and regeneration has been highlighted.<sup>159</sup> SARS-CoV-2, like other viruses that induce myocarditis, triggers cardiac autoimmunity that prolongs tissue damage, which should be addressed by potential therapies.<sup>160,161</sup> Along these aims, siRNA against CSF-1 is loaded in lipid nanoparticles and delivered intravenously in mouse models of virus- or autoimmune-mediated myocarditis. In both cases, the inhibition of CSF-1 reduces the infiltration of inflammatory monocytes into the heart and is sufficient to lessen the extent of tissue damage.<sup>162</sup> Interestingly, even with a reduced inflammatory response, viral clearance is not hindered.

Immune cells can also promote revascularization of affected tissues. Pro-inflammatory M1 macrophages are important at the beginning of angiogenic process, when they secrete pro-angiogenic factors, such as vascular endothelial growth factor,<sup>163</sup> while M2 macrophages dominate at the later time points when they stabilize blood vessels and promote anastomoses.<sup>164</sup> Thus, immune-instructive biomaterials, which can guide the macrophage phenotype change by sequential release of multiple immunomodulatory factors, might be helpful for vascular regeneration. This strategy was used to promote angiogenesis and regeneration by initial release of IFN- $\gamma$  followed by prolonged release of IL-4 from a decellularized scaffold.

Apart from macrophages, revascularization of damaged tissues can be enhanced by biomaterials concentrating pro-healing T cell subtypes. Animals previously vaccinated with an antigen and Th2 immune response-inducing adjuvant show enhanced vascularization and regeneration of ischemic tissue after receiving the same antigen released from the biomaterial scaffold, which attracts and concentrates antigen-specific CD4<sup>+</sup> Th2 T cells.<sup>165</sup>

### *Nervous system*

Like the aforementioned organ systems, the cells and structure of the CNS is susceptible to viral infection and immunogenic collateral damage.<sup>166</sup> Human brain

organoids have been utilized to characterize the invasion potential of SARS-CoV-2 and identify potential treatments, such as Sofosbuvir.<sup>167</sup> The presence of COVID-19 infection within the CNS induces a wide range of long-term systemic symptoms and risks. Neurological complications resulting from coronavirus infections leads to symptoms, such as fatigue, depression, and musculoskeletal pain that affect quality of life. MERS and SARS follow-up studies identified sustained chronic fatigue symptoms in patients at 12–36 months following the initial infection.<sup>168,169</sup> The risk of direct infection is particularly troubling as viral latency and sustained inflammation is likely. Chronic neural inflammation, namely the overexpression of IL-1, IL-6, TNF- $\alpha$ , IL-1B, and IFN- $\gamma$ , is associated with demyelination and the progression of neurodegenerative diseases, such as Parkinson and Alzheimer disease.<sup>170,171</sup> The persistence of viral load within the CNS, as documented with other coronaviruses, can trigger or contribute to the development of neurological disorders.<sup>172</sup>

Preventative therapeutics must be developed to prevent the chronic degeneration of the CNS and development of neurological diseases. One possible mechanism of treatment is through the modulation of inflammation to support remyelination. Microglia, macrophages, and T regulatory cells intrinsically support the differentiation of oligodendrocytes to regenerate myelin sheaths.<sup>173,174</sup> In an interesting approach that combines targeting moieties with the delivery of immunomodulatory factors, leukemia inhibitory factor is encapsulated in PLGA nanoparticles with NG2 chondroitin sulfate antibodies. These particles target and stimulate the differentiation of oligodendrocyte precursor cells to mature phenotypes *in vitro* and improve the extent of myelination in an *in vivo* mouse model of CNS degeneration.<sup>175</sup>

## CONCLUSIONS AND FUTURE DIRECTIONS

The modulation of immune responses has great potential for the prevention and treatment of infectious diseases, such as COVID-19. However, conventional pharmaceutical interventions lack the spatiotemporal control for robust and precise immune activities. Biomaterials create applicable platforms for enhanced immune responses upon vaccination and targeted and controlled drug delivery, local immunomodulation, and minimally invasive methods for drug administration. Biomaterial-based approaches to guide the immune system to fight viral infections and regenerate damaged tissue can be developed and adopted from other fields, such as cancer immunotherapy and stem cell engineering. Because these platforms are not limited by the type of virus, new combinatorial treatments are valuable in creating more effective treatments for a variety of infectious diseases in any future epidemic or pandemic. Tables 1 and 2 highlight examples of each biomaterial-based approach we discuss in this review for the improvement of preventive immunization, therapeutic treatments, and tissue healing and regeneration. In addition, biomaterials can have broad applications in many other aspects, such as personal protection equipment, diagnosis devices, and organ-on-chip disease modeling.<sup>15</sup>

Regarding COVID-19, there are still more problems to be addressed using immunomodulatory interventions. As the risk for severe illness increases with age, a critical need is the modulation of the immune responses in senior populations. The homeostatic and regenerative capacity of T cells declines with age. Thymic T cell generation, T cell receptor repertoire diversity, and the self-renewing potential of T cell populations reduce throughout the adult lifetime.<sup>176</sup> In addition, coronaviruses often cause reinfections, and it is not clear whether current vaccines are effective in inducing long-term immunity.<sup>177</sup> Therefore, building T memory cell populations via vaccines and promoting effective antigen-specific T cell clonal expansion upon

**Table 1. Biomaterials approaches for various issues of COVID-19**

| Issues                                                                                                    | Biomaterial approaches                         | Biomaterial examples                                                                                                                                                                                                                                                                                                                                                       | Refs           |
|-----------------------------------------------------------------------------------------------------------|------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|
| Insufficient immune responses                                                                             | vaccine adjuvants                              | <ul style="list-style-type: none"> <li>• ligands for PPRs</li> <li>• plant-derived: saponins</li> <li>• heat shock proteins</li> <li>• immunostimulatory cytokines</li> <li>• bisphosphonates</li> <li>• nanoparticles (alum particles, oil-in-water nano-emulsions, liposomes)</li> <li>• self-assembling peptides</li> </ul>                                             | 23–48          |
| Immune cells are densely packed in the secondary lymphatic organs<br>Antigen presentation to immune cells | lymphatics targeting<br>artificial lymph nodes | <ul style="list-style-type: none"> <li>• erythrocyte hitchhiking</li> <li>• albumin hitchhiking or conjugating</li> <li>• lipid-based nanoparticles</li> <li>• self-assembling nanoparticles</li> <li>• virus-like particles</li> <li>• poly(ethylene glycol)-poly(propylene sulfide) (PEG-PPS)</li> <li>• mesoporous silica rods</li> <li>• hydrogel scaffolds</li> </ul> | 56–71<br>71–77 |
| Primary infection site at lung with high virus titer                                                      | lung targeting                                 | <ul style="list-style-type: none"> <li>• anti-ICAM-1 antibody coated</li> <li>• erythrocyte hitchhiking</li> <li>• pulmonary ventilation</li> </ul>                                                                                                                                                                                                                        | 85–96          |
| Excessive inflammation of cardiac tissue/<br>vasculature<br>Thrombosis                                    | cardiovascular targeting                       | <ul style="list-style-type: none"> <li>• PECAM-1 and VCAM-1 antibody conjugation</li> <li>• cardiac-targeting peptides (CTP, CRPPR)</li> <li>• tannic acid-induced aggregation</li> <li>• MMP-2/MMP-9 recognition peptides</li> </ul>                                                                                                                                      | 97–102         |

infection are potential strategies to address weakened and insufficient immune responses. Furthermore, due to the complexity of T cell interaction with major histocompatibility complexes, immunoinformatic studies using data science and machine learning approaches can facilitate the identification of potential B and T cell epitopes for peptide vaccine development.<sup>178,179</sup>

Besides the special consideration for the elderly population, the replication of viruses and increase in viral burden continually stimulate positive-feedback immune responses. This process may cascade into an overactive immune response that results in fatal conditions, such as cytokine storms in some COVID-19 patients. Biomaterial composition or biomaterial-based drug delivery systems can be engineered with bio-responsive components, which can regulate biological processes and the location, timing, and release profile of drug cargo.<sup>15</sup> Integration of artificial intelligence (AI) at the development stage of biomaterial–drug formations can be utilized to determine optimal configurations for ideal release profiles and maximal therapeutic effects.<sup>180</sup> In addition, AI can be used to expedite the development of the next-generation of immunotherapeutics through better design of biomaterials and predictions of cell-specific interactions, efficacy, or risk of adverse events.<sup>181</sup> The appropriate biomaterial strategy will enable the generation of desired immune responses to prevent or treat cytokine storm. In particular, nanomaterials have been implicated by others for their role in COVID-19 therapy due to their modular drug-loading capabilities, delivery mechanisms, tissue targeting, and symptom relief.<sup>108</sup>

Moreover, the transfer of biomaterial designs from bench to bedside is a practical issue, especially for infectious diseases. The scale-up feasibility and manufacturing costs should be taken into consideration while designing biomaterial products. The complexity and simplicity of a biomaterial solutions should be well balanced to enable both reliable clinical outcomes and easy mass production. In addition, the adaptation of biomaterials and/or drug formulations that have already been used in FDA-approved applications can reduce the regulatory burden and time of

**Table 2. Biomaterials approaches toward tissue and organ healing of COVID-19-related issues**

| Issues                                                                  | Biomaterial approaches                               | Biomaterial examples                                                                                                                                                                                                                                                                                                    | Refs                |
|-------------------------------------------------------------------------|------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|
| Damage of lung tissue (reduced lung function)                           | anti-fibrotic immunomodulatory therapies             | <ul style="list-style-type: none"> <li>• microparticles for extended release of anti-inflammatory agents</li> <li>• hydrogel delivery platforms (hyaluronan)</li> <li>• cell-laden alginate microparticles for immunomodulation and lung targeting</li> <li>• PLGA/PEG nanoparticles conjugated to monocytes</li> </ul> | 140–145             |
| Damage of cardiac tissue (reduced heart function)                       | anti-inflammatory agents for macrophage polarization | <ul style="list-style-type: none"> <li>• PEI-mediated gene delivery</li> <li>• injectable alginate hydrogels</li> <li>• lipid nanoparticle siRNA delivery</li> </ul>                                                                                                                                                    | 153,154,156,162,165 |
| Damage to nervous system (increased risk of neurodegenerative diseases) | anti-inflammatory remyelination                      | <ul style="list-style-type: none"> <li>• PLGA nanoparticles conjugated with anti-NG2 chondroitin sulfate antibodies</li> </ul>                                                                                                                                                                                          | 175                 |

approval. Thus, building a history of using biomaterials for various applications will favor fast clinical approval upon an outbreak.

Finally, just as the use of biomaterials for infectious diseases utilizes strategies from other disciplines, biomaterial solutions that can modulate and balance inflammatory and anti-inflammatory responses to COVID-19 will also have translatable applications for cancer vaccines, chronic infections, and tissue repair.

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## AUTHOR CONTRIBUTIONS

All authors made substantial contributions to the content, writing, and editing of the manuscript. Figures were designed by J.Z.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

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