Vaccine delivery: where polymer chemistry meets immunology

“The scene is being set for further advances in polymeric carriers that address many of these challenges and open new opportunities in vaccine delivery.”

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Vaccines are among the most important innovations in human history. The development and widespread implementation of vaccines over the past two centuries have saved billions of lives and have completely eliminated or dramatically reduced the global burden of diseases such as smallpox, polio and diphtheria. However, there remains an urgent and unmet need for vaccines against many diseases that have proven recalcitrant to traditional immunization approaches such as malaria, tuberculosis and HIV, which are collectively responsible for over 4.5 million deaths per year [1]. This challenge persists because vaccines, which have historically been based on killed or weakened forms of a microbe (e.g., virus or bacterium), often fail to elicit the correct type or magnitude of immune response to confer protective immunity. The need to achieve greater control over the magnitude and phenotype of an immune response has catalyzed a shift away from empirical vaccinology toward the rational design of vaccines founded upon a fundamental understanding of how the immune system combats infection. Toward this end, many modern vaccines combine recombinant protein antigens – to define the antigen-specificity of the response – with synthetic immunostimulatory molecules (adjuvants) that engage specific pattern recognition receptors (PRRs) – to generate an appropriate innate immune response to initiate and shape the adaptive response to the antigen. For example, the Cervarix human papillo-

mavirus vaccine contains both recombinant virus like particles and monophosphoryl lipid A, an agonist of TLR-4 [2].

However, this shift toward developing successful synthetic vaccines is associated with several important drug delivery challenges. How can vaccines be delivered to the proper antigen presenting cells? How can vaccines be delivered to the proper pathways within these cell populations? How can vaccines be enriched within lymph nodes – the command centers of an immune response – while minimizing systemic distribution and the risk of potential adverse side effects? How can physicochemically diverse vaccine constituents be packaged and delivered to generate an optimal response? Polymeric delivery systems enable solutions to many of these challenges, and recent advances in polymer chemistry are poised to impact the future of vaccine delivery.

Polymer carriers for ‘bottom-up’ assembly of synthetic vaccines

The efficacy of many successful vaccines based on live or attenuated microbes (e.g., yellow fever vaccine, influenza vaccine) has been attributed to their inherent ability to deliver multiple cues (e.g., antigens, natural agonists of PRRs) to the immune system [3,4]. As such, vaccine formulation processes in which multiple well-defined immunological cues can be packaged into or onto a single particulate delivery vehicle are of particular interest in synthetic vaccine research. These co-delivery approaches allow for simultaneous antigen

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delivery with engagement of multiple PRRs within a single cell, mimicking the natural process of pathogen recognition by antigen presenting cells.

A number of natural and synthetic polymers have been used to encapsulate multiple vaccine components. Most notably, biodegradable poly(lactide-*co*-glycolide; PLGA) micro- and nano-particles have been widely employed in vaccine delivery applications [5], and have recently advanced to clinical trials. While promising, PLGA particles rely on a relatively slow hydrolysis mechanism for drug release and are also associated with an initial phase of 'burst release' in which adsorbed cargo diffuses from the PLGA particles into the surrounding media [6,7]. Collectively, these limitations can make achieving precise spatial and temporal control of vaccine release difficult. Moreover, exposure to organic solvents during particle formation can denature antigens, and achieving efficient cytosolic delivery of cargo often requires blends of PLGA and cationic polymers [8]. Therefore, while PLGA-based particles remain the current gold standard for polymeric vaccine delivery, there is a need for more sophisticated vaccine carriers that confer greater control over the packaging and delivery of immunomodulatory cargo.

Advancements in polymer chemistry are empowering the synthesis of multifunctional polymeric carriers for incorporation of antigens and adjuvants via several mechanisms, widening the field of available tools for achieving tight control over vaccine delivery. In particular, atom transfer radical polymerization and reversible addition-fragmentation chain transfer polymerization have enabled the synthesis of well-defined and monodisperse polymers while allowing for the incorporation of a wide arrange of commercially available and custom monomers for desired functionality and polymer architecture. In recent years, these polymerization chemistries have been used to generate polymers for orthogonal ligation of antigen and adjuvant to reactive pendant groups [9–14], electrostatic complexation of charged immunomodulators (e.g., oligonucleotides) to polyionic segments [9,10,15,16], and/or loading of nonpolar cargo into self-assembled hydrophobic domains of amphiphilic polymers [17]. Importantly, functionalities from multiple monomers can easily be incorporated into a single macromolecule with reversible-deactivation radical polymerization (RDRP). As such, polymeric carriers can be rationally designed with great versatility, allowing for the loading of physiochemically diverse vaccine components into a single vehicle while maintaining control over colloidal characteristics for optimization of vaccine efficacy. For instance, Wilson *et al.* have previously synthesized self-assembled poly[(2-(diethylamino)ethyl methacrylate)-*co*-(pyridyl disulfide ethyl methacrylate)-*b*-(2-propylacrylic acid)-

co-(2-(diethylamino)ethyl methacrylate)-*co*-(butyl methacrylate)] polymer micelles incorporating cationic moieties for immunosimulatory oligonucleotide complexation, pyridyl disulfide moieties for antigen conjugation and membrane destabilizing hydrophobic moieties for mediating cytosolic antigen and adjuvant co-delivery [10]. Similarly, RDRP has also been exploited to synthesize stimulus responsive polymers that self-assemble into vesicular architectures for co-loading of physiochemically diverse antigen and adjuvant [17,18] as well as multifunctional particles with reactive azide or alkyne handles [9,12–14]. Indeed, RDRP techniques are greatly widening the field of accessible vaccine delivery vehicles. Through precise control of polymer molecular weights and functionalities and resulting delivery profiles, great strides are being made toward rational design of vaccine carriers.

Polymeric carriers for targeting cells & tissues of the immune system

Typically, vaccines must be delivered to professional antigen presenting cells (APCs), most notably dendritic cells (DCs) in lymph nodes (LNs). However, compounds below approximately 40–50 kDa, which includes most subunit antigens and molecularly defined adjuvants, are typically rapidly cleared from the injection site into the circulation, often with poor uptake by APCs or accumulation in LNs [19]. Additionally, systemic distribution of adjuvants can result in undesirable systemic inflammation [20]. Polymeric carriers can be designed to improve vaccine targeting and biodistribution properties through several mechanisms. First, large macromolecules and nanoparticles <~100 nm preferentially drain from interstitial space into the lymphatics where they can be efficiently captured by APCs resident in LNs. Reddy *et al.* were the first to design polymeric nanoparticles exploiting this phenomenon, showing that 25 nm poly(propylene sulfide) nanoparticles were internalized by dendritic cells in the lymph node roughly ten-times as efficiently as their 100 nm counterparts [21]. On the other end of the size continuum, large polymeric particles (e.g., PLGA microparticles) or injectable polymer depots allow local retention of cargo at the injection site, which can be endocytosed by resident DCs and 'actively' transported to lymph nodes for priming of an immune response [22,23]. Finally, advances in glycopolymer synthesis have also enabled the design of vaccine carriers that target C-type lectin receptors expressed on macrophages and dendritic cells, including MRC1, DEC-205 and DC-SIGN. This molecular targeting confers an additional level of specificity toward APC populations, while also stimulating innate immunity through engagement of C-type lectin receptors [24].

Stimuli-responsive ‘smart’ polymers for vaccine delivery

Polymers that undergo physiochemical transformations in response to specific internal or external stimuli are especially useful for spatial and temporal control over vaccine release. For delivery of antigens and adjuvants, such ‘smart’ polymers have predominantly exploited changes in endosomal pH or redox potential for triggered or controlled release of cargo to endosomal or cytosolic immunoregulatory machinery. For instance, several groups have developed self-assembling polymers that incorporate pH sensitive amines [9–11,15,16] or reduction sensitive polymer backbones [17,18] as triggers for altering chain solubility. Crucially, these functionalities allow for targeted vaccine delivery to endosomal and/or cytosolic pathways depending on polymer composition and behavior. Additionally, Lynn *et al.* have recently harnessed the lower critical solution temperature behavior of poly(NIPAM) to create injectable polymer solutions that self-assemble *in vivo* into adjuvant and antigen containing microparticles for lymphatic trafficking and accumulation. Specifically, their polymer design incorporated a thermoresponsive poly(NIPAM) block and a hydrophilic block modified with thiazolidine-2-thione moieties and a peptide coil for adjuvant conjugation and antigen attachment respectively, resulting in an easily stored and fully injectable vaccine solution for efficient antigen and adjuvant delivery [25]. Exploiting another type of stimulus, Miyata *et al.* have also previously reported antigen triggered hydrogel release, using competitive antibody binding as a trigger for breaking hydrogel crosslinks [26,27]. Collectively, these approaches enable greater control over both intracellular and extracellular distribution of vaccine components, enhancing immune responses while minimizing toxicity associated with systemic distribution of potentially inflammatory vaccine adjuvants.

Future perspective

Previous investigations in both the fields of immunology and drug delivery have contributed greatly toward the goal of rational design of polymeric vaccine carriers. However, key questions continue to arise regarding the choices of chemistries used for bioconjugation, linker systems, polymer compositions, architectures and resulting physiochemical properties. There is no doubt that such variables can have a critical impact on vaccine efficacy in a number of different applications. With the level of understanding and control being achieved in polymer synthesis, such questions can start to be answered for vaccine delivery systems. Toward this end, it will be important for research

strategies to harness a feedback loop between polymer design and basic knowledge of how polymer properties influence innate and adaptive immune responses. Collaborative efforts among polymer scientists and immunologists will be critical to realizing the full potential of any vaccine delivery system.

This editorial has highlighted many achievements in the application of polymer based vaccine delivery systems. However, many issues with respect to the actual design of the polymeric carrier require much forethought. For example, carriers of a size that would most efficiently be phagocytosed by APCs or exploit lymphatic transport to reach the lymph node would also not be efficiently cleared from the body unless degraded into lower molecular weight components that can be excreted (~40 kDa) [28,29]. Hence, systems that are too slow to degrade or do not biodegrade at all (most radical based polymers) may present associated toxicity, accumulation and undesirable nonspecific immunological responses, rendering them unsuitable for application. At the same time, polymers could also release degradation products (products from biodegradable polymers; pendent groups and resultant polymer backbone for nondegradable polymers) that elicit an inflammatory response that may have unexpected effects on vaccine immunogenicity [30]. Attention must also be given to the synthetic scalability and reproducibility of materials, particularly in infectious disease applications where high safety and low cost are critical. Robust polymer synthetic strategies must be implemented from the point of conception, as materials that cannot be commercially produced at large scales reliably, are not likely to make a significant clinical impact.

The scene is being set for further advances in polymeric carriers that address many of these challenges and open new opportunities in vaccine delivery. New polymeric carriers have been developed with controlled architectures, compositions and hydrodynamic volumes; tunable and stimuli responsive delivery and degradation capacities; cell-targeting properties; and diverse functionalities for delivery of diverse vaccine cargo. These are all aspects that will continue to drive new candidates for development and for clinical consideration in vaccine delivery.

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