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Lipid-Coated Biodegradable Particles as “Synthetic Pathogens” for Vaccine Engineering

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The physicochemical context in which molecules are presented at the surfaces of microbes has tremendous implications for the immune response to vaccination. The spacing and mobility of molecules may control interactions of their receptors, influencing immune cell activation, pathogen uptake, and antigen processing. The chemical environment of antigens also influences the specificity of the humoral immune response, because antibodies recognize antigen in its three-dimensional shape and context. Finally, physical properties of antigen, such as diameter, impact immune response on both a cellular and tissue level. We have constructed “synthetic pathogens” consisting of a biodegradable core polymer coated by a lipid shell to mimic a bilayer-enveloped pathogen. Synthesized in an oil-in-water emulsion, these particles have an average diameter on the order of either 100 nm, mimicking a lipid-enveloped viral pathogen, or 1 micron, mimicking a bacterial pathogen. CryoEM reveals self-assembled lipid layers at the particle surface. With tunable chemical and physical properties, these particles can be used to study the importance of specific properties of biomaterials when used in vaccination. Because all components are biodegradable, the particles may provide a clinically applicable way of implementing structural features of microbes in synthetic vaccines.

I. INTRODUCTION

Antigen-presenting cells (APCs) such as dendritic cells (DCs) recognize pathogens by detecting pathogen-associated molecular patterns (PAMPs) using Toll-like receptors (TLRs) [1]. APCs cross-preset antigen to cytotoxic T lymphocytes 1000-fold more efficiently when the antigen is delivered on a particle rather than in soluble form [2]. Particle size and surface chemistry also play a role in transport of antigen from the periphery to the lymph node, where T and B cells respond to antigen. Particles under 20 nm in size can arrive at the lymph node within hours, draining directly through the lymphatics, whereas particles above 200 nm in size arrive within days, and must be transported by cells [3]. Particles “cloaked” by a hydrophilic shell of poly(ethylene glycol) exhibit greater mobility in mucosal environments [4] and are better able to leave the periphery to enter lymph nodes. Although increasing PEG molecular weight improves the ability of particulate antigen to leave the periphery, this comes at a cost of poorer retention in the draining lymph node [5].

We have developed a biodegradable particulate vaccine carrier of a size that can mimic either viruses (order of 100 nm) or bacteria (order of 1 micron) [6]. The particles have a poly(lactide-co-glycolide) (PLGA) core and a lipid-based shell that includes phospholipids conjugated to 45-monomer PEG chains. The lipid-coated, PEG-cloaked nanoparticles are best suited for conveying lipid-like PAMPs and antigens, though other molecules can be encapsulated into the particle core or chemically coupled to functionalized lipids at the surface. The carriers can serve as models in studies of immune response to pathogens, but are also based on biocompatible materials for potential use as vaccines.

II. PARTICLE SYNTHESIS

Particles are synthesized using an oil-in-water single emulsion or a water-in-oil-in-water double emulsion technique. The hydrophobic phase can be either dichloromethane or chloroform. These solvents are well-suited for particle synthesis because they dissolve lipid and PLGA, and because they are volatile, and can thus be removed from particles by solvent evaporation. An interior aqueous phase may be dispersed into this organic solution of lipid and polymer. The organic phase, in turn, is dispersed into a larger bath of water, with the method of dispersion controlling the particle size distribution. Sonication using a vibrating probe tip outputting 12 Watts yields nanoparticles with an average diameter of 116 nm, whereas homogenization at 12,500 RPM yields microparticles 1-5 μm in diameter, which can be further selected for size by centrifugation. Figure 2 shows the sizes of microparticles and nanoparticles visualized by scanning electron microscopy.

Figure 1. Scanning electron micrographs of microparticles (left) made by homogenization of lipid/polymer solution in water, or nanoparticles (right) made by sonication of the solution.
III. LIPID SEGREGATION TO PARTICLE SURFACES

During dispersion and subsequent evaporation of the organic solvent, the lipid component acts as a surfactant that stabilizes the oil-water interface, finally forming a coating around the solid PLGA microparticles or nanoparticles. Figure 3 shows a cryo-transmission electron micrograph (Cryo-TEM), comparing lipid-coated PLGA with a well-studied lipid-coated silica control [7] to show the similarity in lipid coating. This “lipid surfactant” strategy provides great versatility because of the ease with which the surface chemistry can be modified [8]. The lipid also exhibits two-dimensional fluidity along the particle surface, as detected by fluorescence recovery after photobleaching (FRAP), which shows diffusion of fluorescent lipids into a region within seconds of bleaching with a high-powered laser (Figure 3).

IV. IMPLICATIONS FOR VACCINE DESIGN

As vaccine carriers, these particles mimic the structure and chemistry of pathogen surfaces. PAMPs such as TLR2 agonists and TLR4 agonists are themselves lipid-like molecules, and therefore can be incorporated into the lipid coating by the same self-assembly process as with the other lipid components. This gives the PAMPs a more physiologic lipid context, and a two-dimensional mobility helpful for bringing TLRs in close proximity and induce signaling.

The system also may offer insights into aspects of vaccine design that are not well understood. For example, mechanics of particulate antigen may play a role in antigen transport and processing. Viruses can change their physical properties drastically during the cycle of viral replication—in the case of HIV, by a factor of fourteen [9]. We are investigating this parameter by the juxtaposing our lipid-coated particles with more traditional liposome delivery vehicles.

REFERENCES


Figure 2. Cryo-TEM of lipid-coated silica nanoparticles (left) and biodegradable lipid-coated PLGA nanoparticles (right). Arrows show lipid leaflets directly visualized with this technique.

Figure 3. Fluorescence recovery after photobleaching of a region on a lipid-coated microparticle (red) as compared to the entire particle (blue) and background (green). The lipid coating contains a fluorescent NBD-phospholipid conjugate.

Figure 4. Cryo-TEM showing the dynamic conformations of liposomes lacking a rigid core. Liposomes formed by rehydration of dried lipid, followed by 6 freeze-thaw cycles and 11 passes through a 400 nm track-etch membrane. This treatment yields a size distribution similar to polymer-supported nanoparticles.

In contrast to the rigid spheres shown in Figure 3, liposomes can adopt a variety of conformations, such as rod-like or bowl-like shapes shown in the Cryo-TEM "snapshot" in Figure 5. Our analysis of the effects of particle rigidity in vaccination may be instructive for vaccine design.

These biodegradable lipid-coated particles can be used to implement structural features of pathogens, as a versatile approach to designing vaccine carriers.

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