Design of polyelectrolyte multi-layer vaccines assembled from immune signals on gold nanoparticle templates

Peipei Zhang[1] Yu-Chieh Chiu[1] Joshua M. Gammon[1] James I. Andorko IV[1] and Christopher M. Jewell[1-3]
[1]Fischell Department of Bioengineering, University of Maryland - College Park; [2]Department of Microbiology and Immunology, University of Maryland Medical School; [3]Marlene and Stewart Greenebaum Cancer Center

Statement of Purpose: Modern medicine has benefited tremendously from vaccines, as these treatments have allowed reduction - and in some cases eradication - of disease with just a few doses. New vaccines that promote efficient responses while also helping to direct the specific characteristics of these responses could help address challenges facing candidate vaccine for diseases ranging from malaria to cancer. Nanoparticles and microparticles laden with antigens, adjuvants, or other vaccine components have attracted considerable attention toward this goal as these materials allow spatial and temporal control over the delivery of the incorporated agents. However, the ability to rationally design vaccines that harness the advantages of biomaterials is often hindered by the intrinsic immune function of common biomaterials such as poly(lactide-co-glycolide) or polystyrene. To address this challenge, we reasoned that inert gold nanoparticle (AuNP) templates coated in a layer-by-layer (LbL) fashion might serve as a platform for rationally designing, studying, and exploiting multi-component vaccine formulations without "carrier" effects. The LbL technique has been widely used to create electrostatically-assembled films called polyelectrolyte multilayers (PEMs). Here we describe a platform for assembling well-defined PEMs from immune signals without the need for solvents, mixing, or synthetic polymers employed in many nanoparticle vaccines. This approach could lead to a new platform for rationally-designed vaccines that are potent and play an active role in inducing specific immune responses.

Methods: To prepare PEM-coated AuNPs, AuNPs were coated with combinations of peptide antigen (SIINFEKL, SIIN), and molecular adjuvants such as the inflammatory toll-like receptor (TLR) agonist, polyIC (**Fig. 1A**). These components were used in native states, or modified with charged amino acids to create oppositely-charged cargos to support PEM assembly. For dendritic cell (DC) uptake, activation, and antigen presentation studies, PEM-coated AuNPs were incubated with $CD11c^+$ DCs isolated from mouse spleens. Cells were then stained with antibodies specific for key DC surface makers or for SIIN peptide presented in the context of major histocompatibility complex I (MHC-I), then analyzed by flow cytometry. To assess the impact of DC activation driven by PEM-coated AuNPs, DCs treated with each formulations were co-cultured with CSFE-labeled, antigen-specific $CD8^+$ T cells from OT-I mice. These cells exhibit T cell receptors recognizing SIINFEKL presented in MHC-I. Flow cytometry was then used to assess T cell proliferation.

Results: PEM-coated AuNPs were readily synthesized with tunable properties such as size, surface charge, and cargo loading (**Fig. 1B**). Flow cytometry studies using fluorescently-labeled antigen and adjuvant revealed efficient uptake by DCs in a dose-dependent manner. Treatment with of DCs with these vaccines also increased surface activation makers with 38% of DCs treated with AuNP-$(SIIN/polyIC)_2$ expressing CD40 compared to 13% and 26% of cells treated with buffer, or soluble SIIN, respectively (**Fig. 1C**). PEM-induced activation also correlated to increased presentation of the antigen included in the PEMs (SIIN). These studies indicated that 51% of DCs treated with AuNP-$(SIIN/polyIC)_2$ presented SIINFEKL via MHC-I, compared to 3.6%, 1.0%, and 6.4% of cells treated with buffer, soluble polyIC, or soluble SIIN, respectively. Importantly, only 4% of DCs (baseline values) treated with PEMs assembled from polyIC and an irrelevant peptide (Ctrl) were positive, confirming the antigen-specific delivery capabilities of these PEMs (**Fig. 1C**). In a similar study, PEM-treated DCs were co-cultured with OT-I T cells. Flow cytometry analysis showed that DCs treated with AuNP-$(SIIN/polyIC)_2$ induced potent T cell proliferation (95%) compared with 33% in PEMs containing adjuvant and Ctrl peptides (e.g., AuNP-$(Ctrl/polyIC)_2$). This result further confirms the functional and antigen-specific presentation of antigens in the PEMs.

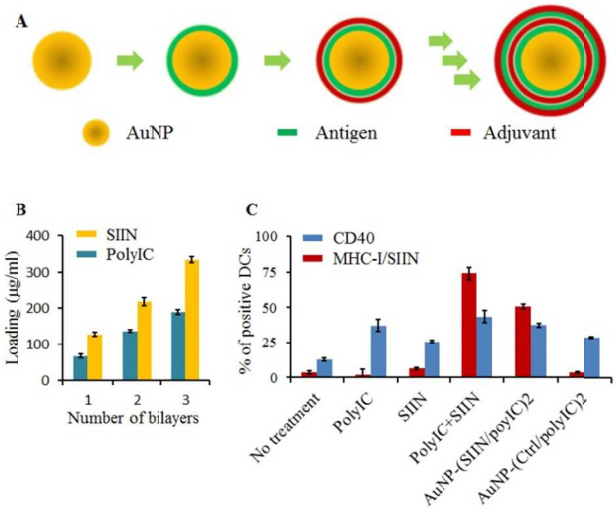

Figure 1. A) Schematic representation of assembling PEMs on AuNPs. B) Loading of SIIN and polyIC versus the number of antigen/adjuvant bilayers. C) Percentage of DCs that present CD40 and MHC-I/SIIN on their surface.

Conclusions: We have developed a vaccine platform for rationally-designing PEMs assembled from immune signals coated on AuNPs. Ongoing studies are investigating the mechanism of adjuvanticity through TLR signaling and the *in vivo* expansion of antigen-specific T cells. This platform could provide more defined control over the types of responses new vaccines elicit.