Design of "Carrier-Free" Polyelectrolyte Multilayer Vaccines Assembled from Immune Signals

Yu-Chieh Chiu[1], Peipei Zhang[1], Joshua M. Gammon[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

Introduction: Despite the tremendous benefits vaccines have provided, vaccines and immunotherapies for challenging diseases such as HIV, cancer, and malaria would benefit from strategies that provide control over the specific type of immune responses that are induced. Biomaterials offer great potential in this area because these agents allow spatial and temporal control over the delivery of drugs and vaccines. However, many biomaterials intrinsically stimulate the immune system without the addition of other signals, leading to a lack of definition in the function and contributions of each vaccine component (e.g., antigen, adjuvant, carrier). Thus, vaccines with highly-defined compositions could provide improved effectiveness and contribute to more rationally-designed vaccination strategies. Toward this goal, we have developed polyelectrolyte multilayer (PEMs) capsules assembled in a layer-by-layer manner entirely from immune signals, and assessed the ability of these materials to activate dendritic cells (DCs) and expand antigen-specific T-cells.

Methods: PEMs were initially assembled on planar substrates using a nucleic acid adjuvant (PolyIC) as a polyanion, and a model antigen from ovalbumin (SIINFEKL) modified with cationic amino acids as a cationic block. Coating conditions were optimized by varying pH and ionic strength of coating solutions. Film thickness and cargo loading were assessed by ellipsometry, UV/VIS spectrophotometry, and confocal microscopy. To prepare capsules, calcium carbonate templates were first synthesized by precipitation of Na_2CO_3 in $CaCl_2$, followed by alternating exposure to SIIN (cationic) and polyIC (anionic) with intermittent centrifugation and wash steps. The coated templates were then exposed to ethylenediaminetetraacetic acid (EDTA) and washed with PBS. For *in vitro* studies, splenic dendritic cells (DCs) were incubated with capsules and stained with antibodies for specific surface markers or that bind SIIN presented in MHC-I. Flow cytometry was then used to analyze capsule uptake, DCs maturation, and antigen presentation. *In vivo* studies were conducted by injecting capsules intra-dermally (*i.d.*) and boosting on D15. T cell response was monitored via MHC-I SIINFEKL tetramer staining.

Results: SIIN and polyIC assembled in to PEMs on templates was a strong function of pH and ionic strength. Assembly as a function of pH revealed that alkaline conditions supported the highest loading densities of SIIN (45.9 ± 1.8 µg/mg) and PolyIC (53.0 ± 4.5 µg/mg). This trend was could be visualized by confocal microscopy (**Fig. 1A**). Capsules were then formed by treating coated templates with EDTA. Following treatment with PEM capsules, up to 80% of DCs internalized capsules, as indicated by fluorescent signal from both polyIC and SIIN

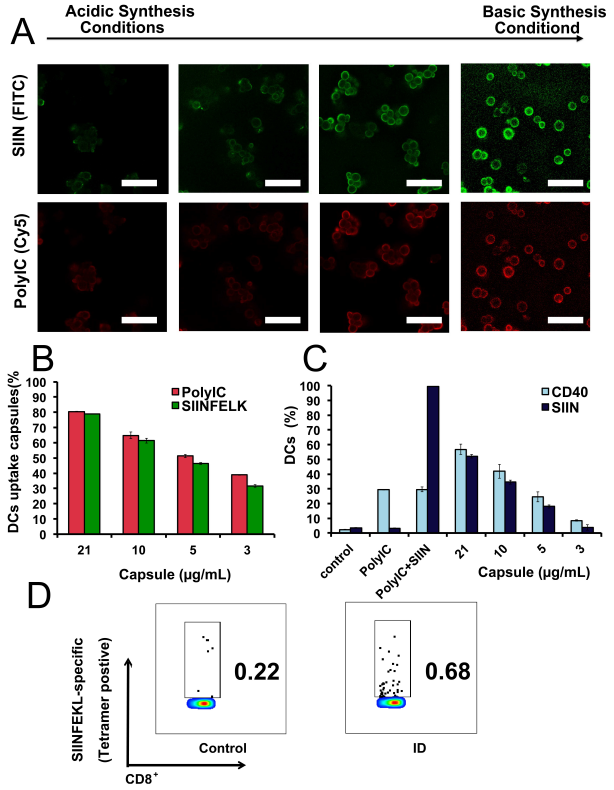

Figure 1. A) PEM templates coated under different pH conditions (scale bar = 20µm). B) DCs internalized each capsule component in a dose-dependent manner, resulting in increased C) DC activation (CD40) and SIIN presentation (via MHC-I). D) Capsules expand SIINFEKL specific $CD8^+$ T cells in mice (Day 29).

during FACS studies (**Fig. 1B**). DCs treated with capsules also exhibited increased maturation markers (e.g. CD40) and SIINFEKL presentation in MHC-I (**Fig. 1C**). In addition, DCs treated with capsules secreted significantly higher levels of inflammatory cytokines (e.g., INF-γ, 43 ± 2 pg; TNF-α, 164 ± 13 pg; IL1-β, 218 ± 6 pg) compared to control groups (43 ± 2 pg, 5 ± 13 pg, and 18 ± 1 pg, respectively). Lastly, mice immunized *i.d.* with capsules exhibited significant increases in the frequency of antigen $CD8^+$ T cells ($p>0.05$ vs. control) (**Fig. 1D**).

Conclusion: PEM microcapsules assembled from molecular adjuvants (PolyIC) and model antigen (SIINFEKL) induce DCs maturation and antigen presentation, and expand antigen specific $CD8^+$ T cells in mice. We are currently investigating PEMs containing one or more classes of adjuvants, and the link between film composition and T cell polarization/function. These well-defined materials could serve as a platform to control the combinations and relative concentrations of immune signals, allowing programming of immune responses with specific characteristics without need for passive carriers.