# Design of "Pathogen-Mimicking" Polyanhydride Adjuvants

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**Statement of Purpose**: Natural infections with pathogens stimulate protective and lasting immune responses. Vaccines have been designed to mimic the immune response associated with an active infection yet avoid the undesirable effects of disease. Polyanhydrides are a class of biomaterials that have demonstrated numerous properties that make them ideal vaccine delivery vehicles. Several studies from our laboratory have shown that polyanhydride particles have the capacity to release fully functional proteins in a controlled fashion while providing adjuvant-like effects by activating immune cells [1-4]. Additionally, polyanhydrides can be fabricated into nanoparticles that can be administered via injection or inhalation. While many chemistry-dependent trends associated with protein stabilization, protein release, and immune activation have been identified [1-4], little work to date has investigated the specific polymer properties responsible for these trends. By identifying these properties, novel and safe adjuvants that more closely mimic the ability to induce a robust the immune responses as do live pathogens can be designed. The goal of this research was to utilize flow cytometry in concert with materials informatics to identify key properties responsible for nanoparticle uptake and downstream activation of murine bone marrow derived dendritic cells (BMDCs). These analyses will facilitate rational design of "pathogen-mimicking" adjuvants for vaccine delivery.

**Materials/Methods:** In this work, polyanhydride copolymers based on sebacic acid (SA), 1,8-bis(*p*-carboxyphenoxy)-3,6-dioxaoctane (CPTEG) and 1,6-bis(*p*-carboxyphenoxy) hexane (CPH) were used. Nanoparticles were fabricated with a nanoprecipitation method. For internalization studies, 630 nm quantum dots (QDs) were encapsulated into the nanoparticles. Bone marrow cells were obtained from C3H/HeOuJ mice and differentiated into DCs as described previously [3]. On day 9 of culture, the DCs were co-cultured with nanoparticles for immune activation and cellular uptake. After 48 h, BMDC surface marker expression and cytokine production were assessed via flow cytometry. Confocal microscopy was used to study particle internalization.

**Results/Discussion:** A library of 12 polymer chemistries were synthesized via a combinatorial melt polycondensation reaction and characterized as described previously [2]. Nanoparticles were fabricated at high throughput as described previously [4]. This investigation revealed that the greatest percentage of BMDCs that were positive for NP-uptake (i.e., QD-positive) were cells cultured with either poly(SA) or 50:50 CPTEG:CPH nanoparticles indicating that less hydrophobic particles were more readily internalized. Consistent with the internalization data, BMDCs stimulated with 50:50 CPTEG:CPH and poly(SA) nanoparticles produced the greatest amount of cytokines and expressed the highest levels of MHC II and co-stimulatory molecules (Figure 1A).

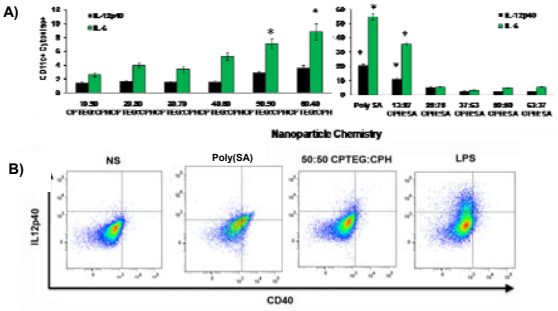


**Figure 1: A) Percentage of cytokine positive BMDCs after treatment with nanoparticles. B) Dot plots of BMDCs; y-axis: IL-12p40 and x-axis: CD40.**

Additional analyses of BMDCs positive for both MHC II or co-stimulatory molecule (CD40, CD86 and MHC II) as well as for cytokine (IL-6 and IL-12p40) revealed that BMDCs stimulated with 50:50 CPTEG:CPH nanoparticles display an activation profile very similar to that of BMDCs stimulated with LPS. In contrast, BMDCs stimulated with poly(SA) appear more similar to non-stimulated BMDCs (Figure 1B). LPS, a major constituent found in the outer membrane of Gram-negative bacteria, is a potent pathogen-associated molecular pattern capable of inducing both robust immune responses and toxic side effects in humans. In contrast, polyanhydride nanoparticles have demonstrated very little toxicity in multiple *in vitro* and *in vivo* studies yet retain the capacity to elicit immune activation [1,3].

To better understand the immune activation patterns observed among the various nanoparticle chemistries, informatics analysis was performed. This analysis demonstrated that 50:50 CPTEG:CPH was "most similar" to LPS. Additionally, the analysis identified specific polymer descriptors that exerted the most influence on particle internalization and BMDC activation.

**Conclusions:** A high throughput approach for investigating the immune activation potential of multiple polymer chemistries demonstrated that both 50:50 CPTEG:CPH and LPS appear to share similar activation patterns. These results are promising first steps in the rational design of "pathogen-mimicking" adjuvants to enhance vaccine efficacy.

**References:** [1] Kipper, M. JBMR 2006;76A:798-810 [2] Torres, MP. Biomaterials 2007;28:108-116 [3] Petersen, LP. Biomaterials 2009;30: 5131-5142 [4] Petersen, LP. J Comb Chem 2010;12: 51-56