In Vitro Activation of Dendritic Cells by Polyanhydride Nanoparticles

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Statement of Purpose: This work is focused on the activation of dendritic cells (DCs) by polyanhydride copolymers based on sebacic acid (SA), 1,8-bis(*p*-carboxyphenoxy)-3,6-dioxaoctane (CPTEG) and 1,6-bis(*p*-carboxyphenoxy)hexane (CPH). These polymers are surface (SA and CPH) and bulk (CPTEG) erodible and have been studied extensively for applications in vaccine delivery and drug therapy [1-4]. These biomaterials are specifically appealing because of their protein friendly environment, tunable release kinetics, and adjuvant properties [1-4]. However, to most effectively design drug or vaccine carriers based on these chemistries, a large parameter space must be investigated to assess the interactions between biomaterials of various chemistries and cells. These time consuming experiments can be greatly expedited through the employment of combinatorial and high throughput techniques, capable of reducing experimental time by 50-fold. The goal of this research is to design high throughput methods for fabrication of combinatorial CPH:SA and CPTEG:CPH nanoparticle libraries and study their effect (cytotoxicity and immune activation) on cellular responses of C3H/HeOuJ DCs.

Materials/Methods: Nanoparticle libraries for studying the cell/biomaterial interactions were fabricated in a multi-vial substrate by utilizing programmable pumps in conjunction with linear actuators as described previously [3,4]. The libraries were characterized by ^{1}H NMR and SEM.

Cells derived from the bone marrow of C3H/HeOuJ mice were utilized in these experiments. The cells were differentiated into DCs and grown as described previously [3]. On day 9 the cells were stimulated with nanoparticles (250, 125, and 62.5µg/mL for cytotoxicity and 125 µg/mL for immune activation) and incubated for 48 h. Following the incubation period, cells were analyzed by the MTT assay to evaluate cytotoxicity and stained for flow cytometry to measure cell surface marker expression of major histocompatibility complex (MHC I and II) and co-stimulatory (CD40, CD86, CD209) molecules. Cell supernatants were collected to assess cytokine (IL-12p40 and IL-6) production as determined by the Luminex assay.

Results: The combinatorially synthesized copolymers were of the desired compositions and molecular weight, in agreement with previous work [2]. A combinatorial library of nanoparticles was fabricated from the polymer library and Figure 1 shows SEM images of two

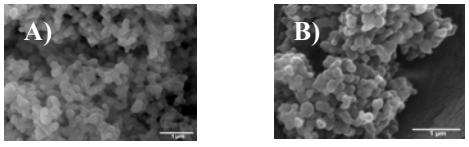

Figure 1: SEM image of A) 50:50 CPH:SA and B) 60:40 CPTEG:CPH nanoparticles.

nanoparticle chemistries. The solvent to non-solvent ratio was optimized at 1:80 for uniform particle size.

Following nanoparticle fabrication, the DCs were stimulated with three different concentrations (250, 125, and 62,5 µg/mL) of twelve CPTEG:CPH and CPH:SA nanoparticle chemistries for 48 h. The nanoparticle-treated cells demonstrated significant viability, only slightly less than the non-treated cells (positive control), with small decreases in viability at the 250 µg/mL concentration. No effect of copolymer chemistry on cytotoxicity was observed. From these studies, the 125 µg/mL concentration was chosen for investigation of DC activation.

The DC activation studies utilized twelve different CPH:SA and CPTEG:CPH nanoparticle chemistries. Interestingly, cell surface marker expression increased with decreasing hydrophobicity of each system (i.e., high SA or high CPTEG content) as shown in Figure 2.

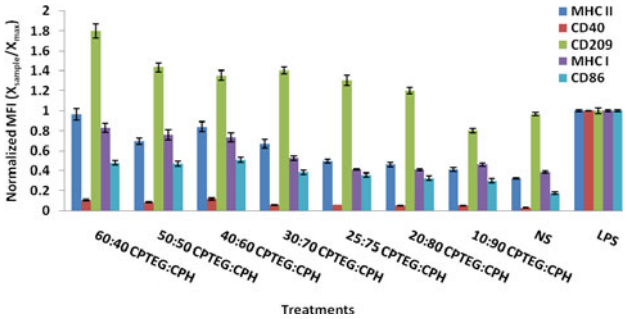

Figure 2: Normalized MFI values of MHC I, MHC II, CD40, CD86, and CD209 for C3H DCs treated with CPTEG:CPH nanoparticles. LPS was used as positive control.

For CD209, MHC I, and MHC II, expression levels met or exceeded that of the positive control, LPS. Cytokine production of IL12p40 and IL6 was assessed and an interesting trend was unveiled, corresponding to the oxygen content in the polymer backbone (↑ O content led to ↑ cytokine production). The numerous findings presented in this work upon testing multiple cell/biomaterial interactions were rapidly identified by utilizing the combinatorial approach. These results demonstrate the adjuvant-like characteristics of this polymer system, which is a valuable attribute for enhancing vaccine efficacy.

Conclusions: The high throughput approach for investigating cytotoxicity and immune activation demonstrated that CPTEG:CPH and CPH:SA nanoparticles are non-toxic at typical concentrations for human use and possess unique adjuvant-like properties. Novel trends were unveiled with the combinatorial approach allowing for rapid optimization of this polymer system. These characteristics along with the tunable release kinetics and protein stabilizing capabilities make this class of biomaterials promising candidates for drug and vaccine delivery.

References: [1] (Kipper, M. JBMR. 2006;76A:798-810) [2] (Torres, MP. Biomat. 2007;28:108-116 [3] Petersen, LP. Biomat. 2009;30: 5131-5142 [4] Petersen, LP. JCC. 2009 accepted.