Amphiphilic Polyanhydride Films for Tissue Engineering

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Statement of Purpose: This work has focused on the use of the amphiphilic polyanhydrides based on 1,8-bis(*p*-carboxyphenoxy)-3,6-dioxaoctane (CPTEG) and 1,6-bis(*p*-carboxyphenoxy) hexane (CPH) as scaffolds. These polymers are capable of sustained protein release, protein stabilization, and are biocompatible/non-mutagenic [1-4]. These properties make them possible candidates for tissue engineering applications. Previous studies with polyanhydride nanoparticles have revealed chemistry dependent trends associated with protein stabilization, protein release, and immune activation [1-4]; however, little work to date has investigated these properties with polyanhydride films. The goal of this research was to study these amphiphilic polyanhydrides for use in tissue engineering applications. Accordingly, protein-film interactions and immune/neural cell-film interactions were investigated.

Materials/Methods: Polyanhydride films were fabricated as previously described [3,4]. Polymer films were loaded with Texas red BSA and monitored for release kinetics (fluorescence imaging) and stabilization of the BSA protein (ELISA) [3].

A 2 day study was carried out with C3He/OuJ bone marrow derived dendritic cells (BMDCs) to investigate immune activation by the films [3]. Flow cytometry and the Luminex bead assay were used to measure cell surface marker expression and cytokine production. Fluorescence microscopy studies were carried out by incubating normal human neural progenitors (NHNPs) on the polymer films loaded with 1% laminin for 7 days. Immunocytochemistry was used to stain the NHNPs for differentiation and cellular adhesion.

Results/Discussion: A library of five polymer chemistries in replicates of three was synthesized via a combinatorial, melt polycondensation reaction [2]. The films (blank, Texas red bovine serum albumin (TRBSA)- or laminin-loaded) were then fabricated in high throughput.

Initial studies examined the chemistry dependence on protein release and stabilization. Figure 1 demonstrates

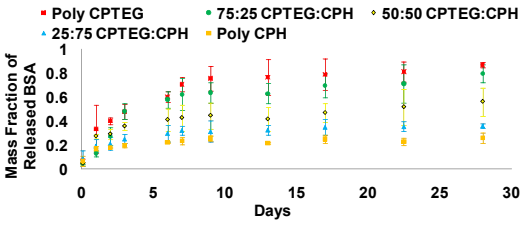

Figure 1: Release of TRBSA from combinatorially fabricated CPTEG:CPH polymer films.

a chemistry dependent trend for release of TRBSA with CPTEG-rich chemistries releasing the fastest. This is consistent with trends associated with protein release from CPTEG:CPH particles [2,3]. Subsequent protein stability studies were performed with a BSA-specific ELISA and indicated that all polymer chemistries preserved the full antigenicity of BSA upon release.

The immune response exhibited by the BMDCs upon stimulation with CPTEG:CPH films was relatively low (compared to the no stimulation (NS) control) for both surface marker expression (MHC I, MHC II, CD40, and CD86) and cytokine production (IL-6, IL-12p40, and TNF-α) as shown in Figure 2A. This suggests that polymer films would remain relatively stealthy to the immune system and not induce inflammation or immune rejection.

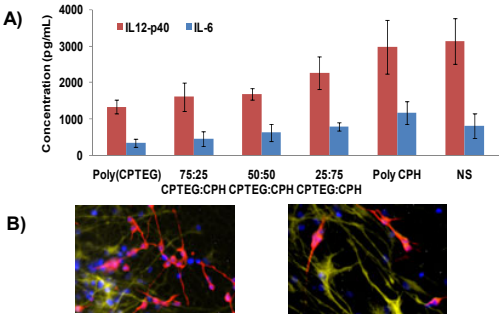

Figure 2: A) Concentration of cytokine produced by BMDCs after incubation with polymer films. B) Microscopy images (left: 50:50CPTEG:CPH film, right: laminin coated coverslip) of NHNPS upon incubation on polymer films (red: TUJ1, blue: DAPI, yellow: GFAP).

Complementary studies were carried out with NHNPs to monitor the effect of the polymer films on differentiation and cellular adhesion. The microscopy results indicated that the CPTEG-rich polymer films prevented cellular adhesion whereas CPH-rich polymer films enabled adhesion. This is likely a result of the ethylene glycol backbone of the CPTEG-rich polymers which has been shown to prevent cellular adhesion in studies with poly(ethylene glycol) [5]. In addition, immunocytochemistry results revealed trends which suggest that the CPTEG-rich polymer films may be influencing cellular differentiation (GFAP and TUJ1 expression), as shown in Figure 2B. These results suggest that polymer chemistry can be used to control neural cell adhesion and differentiation.

Conclusions: The results presented herein have provided a platform to enable the optimization of polyanhydride films for tissue engineering applications. CPH-rich chemistries provided a prolong-sustained drug release, were non-stimulating to BMDCs, and enabled neural cell adhesion; whereas CPTEG-rich chemistries resulted in the most rapid protein release, stabilized the encapsulated protein, were non-stimulating to BMDCs, and prevented neural cell adhesion.

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