High Throughput In vitro Cytotoxicity Screening of Biomaterial Libraries

Latrisha K. Petersen[a], Andrew F. Adler[a], Jon B. Thorstenson[a], Jennifer H. Wilson[c], Michael J. Wannemuehler[c], and Balaji Narasimhan[a]

[a]*Department of Chemical and Biological Engineering, Iowa State University, 2035 Sweeney Hall, Ames, IA 50011-2230, USA*

[c]*Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA 50011, USA*

Statement of Purpose: Polyanhydrides, specifically based on sebacic acid (SA) and 1,6-bis(*p*-carboxyphenoxyhexane) (CPH) copolymers, are a class of biodegradable macromolecules with applications in drug, protein, and vaccine delivery [1]. In order to rationally design carriers based on polyanhydrides for these applications, it is important to consider a large parameter space including polymer chemistry, polymer erosion mechanisms, drug/polymer interactions, and drug release kinetics. To screen this large parameter space, combinatorial methods are invaluable. We have developed a high throughput method for rapid cytotoxicity screening of polyanhydrides using a discrete multi-well substrate created with a novel rapid prototyping method.

Materials/Methods: UV-sensitive Norland Optical Adhesive 81 (NOA) was purchased from Norland Products (Cranbury, NJ). SA and CPH prepolymers were prepared as described before [1].

The discrete multi-well substrates were fabricated by depositing the adhesive in a Petri dish; covering the dish with a prefabricated well design mask and expoing it to UV light for 7 min. The substrate was cleaned of excess NOA and cured again for 15 min.

Libraries of varying concentration or composition of prepolymers of CPH and SA were deposited at high throughput into the multi-well substrates by utilizing robotics. Two programmable syringe pumps (New Era Pump Systems, Farmingdale, NY) in conjunction with three programmable motorized stages arranged orthogonally (Zaber Technologies, Richmond, British Columbia, Canada) served to fully automate depositions. The pumps and syringes were controlled serially by third-party macro software. Following the deposition of the prepolymers, the discrete well substrate was placed in a vacuum oven set at 180 ˚C for 1 h while the polycondensation reaction took place. The libraries were characterized by ^{1}H NMR and IR spectroscopy.

J774, CHO, and Sp2/0 cell lines were incubated in the discrete well substrates for 3 h. Cell viability in the presence of the discrete CPH:SA library was assessed using the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. In this method, viable cells produce solutions that are more absorbent at characteristic wavelengths.

Results/Discussion:

Using a linear varying concentration library of 50:50 CPH:SA copolymers, we established the polymer concentration at which the cells were viable to be 2.8 mg/ml. At this concentration, the polyanhydrides have little to no cytotoxic effect, evidenced by close optical density values for the "no-polymer" control and the polymer-filled wells. Using this concentration, three compositional libraries were prepared and incubated with Sp2/0 myeloma, CHO epithelial, and J774 macrophage cell lines. The results indicate that all cells were viable.

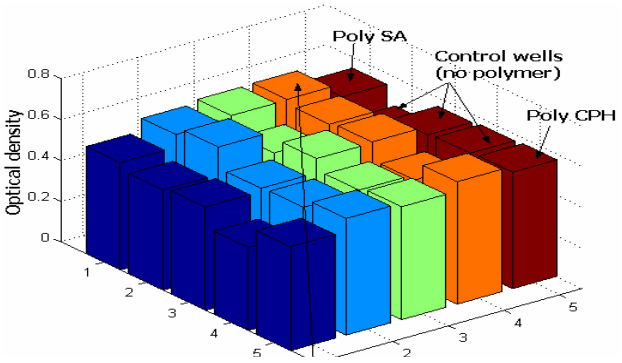

Figure 1. High throughput cytotoxicity screening of polyanhydride libraries

Figure 1 shows the results for the J774A macrophage cell line. Figure 1 also indicates that copolymer composition has a weak effect on modulating cytotoxicity, suggesting that this class of materials is a promising carrier for in vivo applications. This result is consistent with numerous conventional in vitro and in vivo studies that attest to the biocompatibility of polyanhydride systems [3].

Conclusions: We have outlined a high throughput method for prototyping discrete library substrates for rapid screening of polyanhydride cytotoxicity via the MTT assay. The discrete library was rapidly screened with standard myeloma, epithelial, and macrophage cell lines and was found to have no pronounced cytotoxic effect for CPH:SA copolymers at a concentration higher than that expected in human applications[4]. These results add to the large body of evidence supporting the use of polyanhydrides as biocompatible materials for use in drug delivery devices.

References: [1] Shen, EE et al. Biomat. 2001;22:202-210. [2]Harrison, C et al. Micromech Microeng. 2004;14:153-158. [3] Torres, MP et al. Polyanhy Encyc of Chem Proc. 2006;2247-2257. [4] Kipper, MJ et al. J of Biomed Mat Res. 2006;76:798-8