Smooth Muscle Cell Response to a Library of Poly(DTE Carbonate) Co-Polymers

Patrick A. Johnson, Aleksey Demtchouk, Hiral Patel, Larisa Sheihet, Matthew Treiser, Joachim Kohn, Prabhas V. Moghe
New Jersey Center for Biomaterials, Department of Chemical and Biochemical Engineering, Department of Biomedical Engineering, and Department of Chemistry and Chemical Biology, Rutgers University, New Brunswick, NJ 08854, USA

Statement of Purpose: Tyrosine-derived polycarbonates have demonstrated utility as resorbable biomaterials in cardiovascular as well as orthopedic applications. In order to broaden potential applications, a polycarbonate library of co-polymers was developed to systematically vary physical and biological properties through changes in molar ratios of the monomer units. In this study, three chemical moieties were varied: 1) the covalent incorporation of iodine for X-ray visibility, 2) co-polymerization with DT (desaminotyrosyl tyrosine) to increase degradation rates, and 3) co-polymerization of polyethylene glycol (PEG) to inhibit protein adsorption and cell adhesion. While there is some understanding of the physical and biological effects of each component separately, the interplay of these units on physical and biological properties is not well understood. Through a systematic variation of iodine, DT and PEG in the poly(DTE carbonate) library, we sought to elicit the cellular responses of smooth muscle cells on the materials. A rapid screening format was adopted to determine the cell adhesion and motility under uniform cell seeding with minimal material.

Methods: Poly(desaminotyrosyl tyrosine ethyl ester carbonate), abbreviated poly(DTE carbonate), polymers were prepared by solution polymerization in methylene chloride with triphosgene and pyridine of a mixture of iodinated and non-iodinated tyrosine-derived monomers and PEG as previously described.[1,2]. Sixty nanograms of polymer dissolved in methylene chloride were solvent cast into microwells formed from photopolymerization of a thiolene optical adhesive (Norland Products) onto coverglass. The films were UV-sterilized and then wetted for one hour with growth media supplemented with 5% FBS prior to cell seeding. Smooth Muscle Cells were stained with Cell Tracker Green CMFDA (Invitrogen) and seeded at 20,000 cells/cm^2. Cells were allowed to adhere for 1 hour, washed with sterile PBS and imaged by confocal microscopy (Leica TCS SP2). Time-lapse cell migration experiments were carried out in a microscope mounted incubator (PeCon GmbH). For morphological characterization, cells were fixed with 3.7% formaldehyde, permeabilized with 0.1% Triton and stained with Texas Red conjugated phalloidin (Invitrogen).

Table 1: Library of Polycarbonates Evaluated

	% PEG			
poly(DTE Carbonate)	0	4	8	15
poly(DTE Carbonate + 10% DT	0	4	8	
poly(I_2DTE carbonate)	0	4	8	15
poly(I_2DTE carbonate + 10% I_2DT	0	4	8	

Results / Discussion: Smooth muscle cell adhesion to thin films of fourteen members of a poly(DTE carbonate) family of biodegradable polymers was determined using fluorescence imaging. Table 1, groups the polymers in four families, each with varying amounts of PEG. Figure 1 illustrates how cell adhesion varies with the chemistry of the polymer. Cell counts for the poly(DTE carbonate) series showed decreasing number of cells with increased levels of PEG. The I_2DTE carbonate family also showed decreasing cell adhesion as more PEG was introduced but the iodine inhibited the extent of this effect. In contrast the poly(DTE carbonate) + 10% DT series of polymers showed a marked increase in the levels of cell adhesion. This effect was only seen without the presence of iodine. Ongoing studies of water uptake and protein adsorption suggest that water uptake and serum adsorption may play a role in mediating the increased cell adhesion.

Cell migration experiments indicate increased cell motility with the incorporation of PEG for the poly(DTE carbonate) series and showed a maximum at 8% PEG. For the poly(DTE carbonate) + 10% DT series the cell motility was lower and showed a maximum at 4% PEG. This implies that the cells are more strongly adhered to the DT containing polymers. Cells on poly(DTE carbonate) with PEG showed a more compact morphology than cells on poly(DTE carbonate).

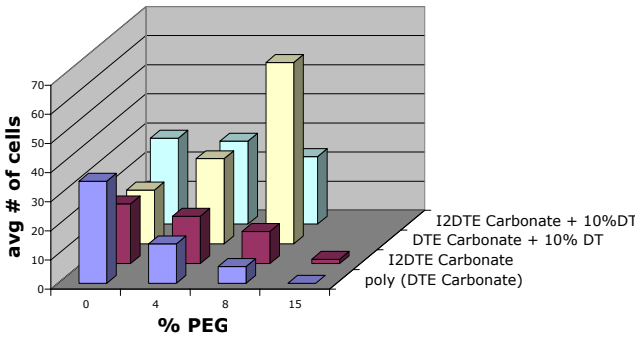

Figure 1: Cell Adhesion to a library of poly(DTE carbonate) polymers.

Conclusions: In conclusion the effects of varying molar ratios of different monomeric units in a polycarbonate library, had complex interactions not necessarily predicted from the known properties of each component. As expected PEG decreased the cell adhesion and iodine limited this effect, which in turn affected cell motility behavior. The incorporation of the DT degradable component significantly enhanced cell adhesion in the presence of 8% PEG.

References:

1. Pulapura S, Kohn J. Biopolymers 1992;32:411-417.
2. Ertel SI, Kohn J. J. Biomed. Mater. Res. 1994;28:919-930.

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