Controlling Polymer Properties and Cellular Interactions through Poly(β-amino ester) Macromer Structure

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Statement of Purpose: Photocrosslinkable and biodegradable materials are being developed for a variety of clinical applications, including scaffolding for tissue engineering and vehicles for drug delivery[1]. Photopolymerization allows for *in vivo* polymerization and control over the reaction both temporally and spatially. Although polymers such as polyanhydrides and poly(propylene fumarate)s are being developed for these applications, polymer synthesis is tedious. This factor, in addition to the difficulty in predicting material behavior from its chemistry and structure, makes developing materials to meet specific design criteria complicated. To allow for more rapid material development, we recently developed a combinatorial library of photocrosslinkable and degradable poly(β-amino ester)s[2] and formed polymers with a wide variety of mechanics and degradation. Here, we describe our work in exploiting modifications in the macromer structure (via branching) to tailor polymer properties and cellular interactions.

Methods: Poly(β-amino ester) macromers were synthesized by mixing 3-methoxypropylamine (TCI America) with 1,6-hexanediol ethoxylate diacrylate (Sigma) and pentaerythritol triacrylate (PETA, Sigma) overnight at 90°C. The ratio of acrylate end groups to amines was kept constant at 1.2:1, but the ratio of diacrylate to triacrylate was varied (100:0, 99:1, 95:5, 90:10). Greater amounts of triacrylate led to crosslinking during synthesis. The photoinitiator 2,2-dimethoxy-2-phenyl acetophenone (Sigma) was added to the liquid macromers at a final concentration of 0.5% (w/w) and samples were polymerized with ultraviolet light exposure (365nm). ATR-FTIR spectra were collected real-time during polymerization to measure conversion of the acrylate double bond (~1630cm^{-1}). Tensile testing was performed on a materials testing machine (Microtester 5848, Instron, Norwood, MA) and unconfined compression testing was completed on a custom made mechanical testing device[3]. Sample degradation was monitored in PBS at 37°C while shaking. Thin films were polymerized on the bottom of 24 well plates in a nitrogen environment and human sarcoma osteoblast-like cells (SaOS-2, ATCC) were added to each well (50,000 cells/well). Cells were fixed in 2.5% glutaraldehyde (Polysciences) and stained with DAPI (Sigma) after 1, 2, and 7 days. Cell areas were measured with NIH ImageJ software and cell numbers were found by counting cell nuclei. Statistical analysis was performed using ANOVA with Tukey's post-hoc among the groups with significance defined as a confidence level of 0.05.

Results/Discussion: The ATR-FTIR showed that the acrylate concentration was constant among the branching variations and that polymerization behavior and final double bond conversion were not influenced by introduction of branching. However, the mechanics in both tension and compression were significantly greater as the concentration of triacrylate increased (Figure 1), from 1.98 ± 0.09 MPa to 3.88 ± 0.20 MPa for 0% to 10% triacrylate in tension. Additionally, after 8 weeks, mass loss of the 90:10 ratio polymer (63.6 ± 2.2%) is slightly greater than the 100:0 ratio (58.5 ± 0.5%). The increased branching may have led to a more densely crosslinked structure and alterations in kinetic chain lengths, which could explain these changes in polymer properties.

The number of cells attached to the various polymers was also controlled by macromer branching at all time points (shown in Figure 1 at 1 week). Cell numbers on the 90:10 and 95:5 polymers were significantly greater than those on the 99:1 and 100:0 polymers. Cell spreading was also affected by the triacrylate concentration (reported as a histogram in Figure 2). The lower ratios are dominated by cells less than 300 μm^2, while the cells spread much better as the ratio increased. These alterations in cell/polymer interactions may be explained by changes in the substrate mechanics as reported by others[4].

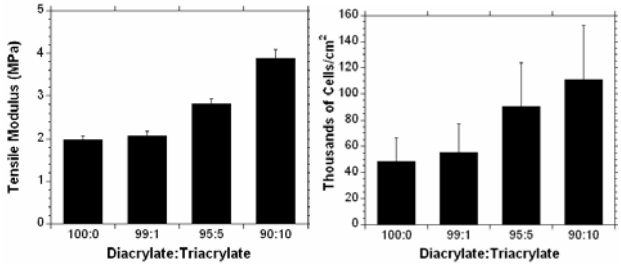

Figure 1. (Left) Tensile moduli of photocured networks with variations in the ratio of diacrylate to triacrylate. (Right) Cell numbers after 1 week of culture.

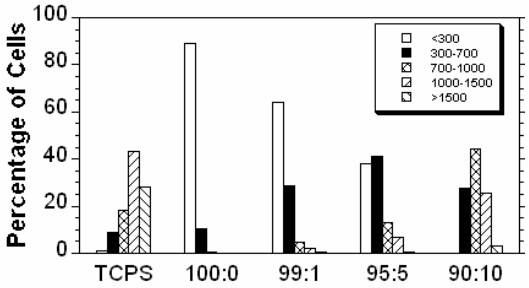

Figure 2. Histogram of cell spreading (μm^2) on polymer films after 1 day of culture.

Conclusions: With the simple addition of a triacrylate component during synthesis, the network structure (via branching) is readily controlled. These changes can alter the polymer bulk properties and cellular interactions, where the moduli and cell adhesion/spreading are enhanced with an increase in network branching.

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References: [1]Anseth KS. MRS Bull. 2002;27:130-136. [2]Anderson DG. Adv Mater. 2006;18:2614-2616. [3]Soltz MA. J Biomech. 1998;31:927-934. [4]Discher DE. Science. 2002;310:130-136.