Synthesis and Characterization of Composite Biodegradable Hydrogel Systems for Controlled Pore Opening

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Statement of Purpose: There are several requirements of an ideal tissue engineering scaffold material. The material must: A) degrade at a rate that allows new tissue formation, B) possess appropriate mechanical properties for the given application, C) be biocompatible, D) promote cell attachment and/or migration, and E) have interconnected pores [1]. In this work, we analyzed the properties of several poly(β-amino ester) (PBAE) biodegradable hydrogels to determine their applicability as tissue engineering scaffolds. We first studied the individual hydrogel systems, and then looked at composite systems that degraded to form a porous scaffold. These composite systems have faster degrading hydrogel particles encapsulated in a slower degrading outer matrix hydrogel. The outer matrix hydrogel provides the mechanical properties for the given application and the porogen particles allow controlled pore opening.

Methods: Macromers were synthesized in an overnight condensation reaction between a diacrylate and amine as outlined in the literature [2]. For this project, diethylene glycol diacrylate (A) and poly(ethylene glycol) 400 diacrylate (H) were used with isobutylamine (#6) to create both A6 and H6 degradable hydrogel systems. Free radical polymerization was carried out through the use of chemical initiators ammonium persulfate and tetramethylethylenediamine. Degradation was characterized by gravimetric analysis of samples immersed in 37°C PBS. Mechanical testing was carried out using a BOSE ELF 3300 Mechanical Testing System. Cytotoxicity data was obtained by exposure of the final degradation products to D1 pluripotent mesenchymal cells followed by MTT assay. To analyze cell attachment to the hydrogel surface, cells were seeded directly onto the hydrogel systems and then analyzed using live/dead staining followed by fluorescent imaging. Porous systems were created by polymerizing the fast degrading material, grounding and sieving the hydrogel to the target size range, dispersing the particles into the macromer used for the outer matrix, and then polymerizing the mixture. 3D characterization of the composite systems was carried out through microCT analysis and SEM imaging.

Results: Degradation plots have been obtained for both systems studied. The A6 system degrades in a period of 3-4 months at a rate of approximately 5%/week, thus making it applicable as an outer matrix material. The H6 system degrades in a time period of 5-7 hours at a rate of approximately 11.5%/hour and, thus, could serve as a porogen material. As expected, the A6 material maintains a significant compressive modulus throughout degradation (>0.2 MPa at 7 weeks) whereas the modulus of the H6 system degrades very rapidly to near 0 MPa in 3 hours. The relative toxicities of all hydrogel degradation byproducts have been studied in comparison to that of PLGA (50:50 carboxylate end group, iv=0.55-0.75 dL/g) using MTT analysis. Based on this analysis, the H6 system has lower toxicity (TC_{50} = 2.20 mg/mL) than that of PLGA (TC_{50} = 1.76 mg/mL), and the A6 is higher (TC_{50} = 0.40 mg/mL). However, this toxicity will be mitigated by the much slower degradation of the A6 system, which will allow byproducts to be cleared away before potentially cytotoxic concentrations are reached. Initial cell attachment studies were carried out on the pure A6 hydrogel systems throughout the first 48 hours of exposure. Figure 1 shows the live/dead assay cell images on the A6 gels at 24 and 48 hours. Cell counting shows substantial viability at both time points 98% and 72%, respectively. Figure 2 shows microCT images of an A6 hydrogel system loaded H6 porogen particles (250-500 μm) at a ratio of 75:25 A6:H6 (by mass). As the H6 porogen material degraded the samples exhibited increased porosity.

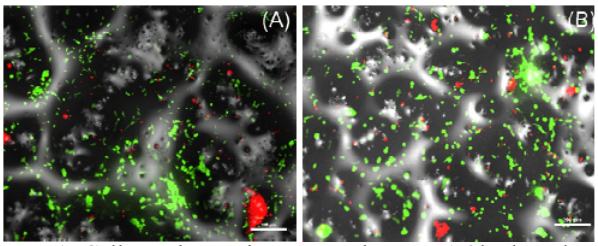

Figure 1: Cell attachment images on the pure A6 hydrogel surface at 24 hours (A), and 48 hours (B).. Scale bar is 200 μm.

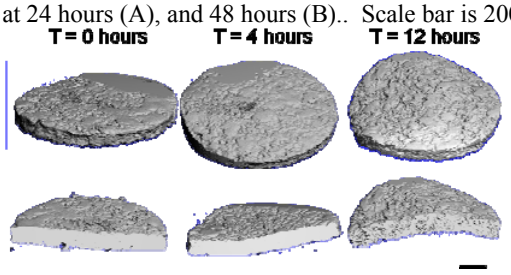

Figure 2: MicroCT images of the composite hydrogel throughout the degradation. Scale bar is 1 mm.

Conclusions: Both the H6 and A6 hydrogel systems were studied; with the knowledge obtained a range of systems with varying degradation and compressive properties can be created. Cell toxicity and attachment studies showed promising results and composite materials displayed controlled pore opening throughout degradation. In conclusion, PBAE biodegradable hydrogels have properties that match or are easily tuned to meet the requirements for a tissue engineering scaffold.

References:

(1) Hutmacher, D.W. Biomaterials (2000:21:2529-2543)
(2) Anderson, DG, et al. Adv Mater. (2006:18:2614-2618)

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