567c Manipulations in Hydrogel Degradation Behavior Enhance Osteoblast Function and Mineralized Tissue Formation

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Toward the end of developing a scaffold to support tissue regeneration, the degradation rate and mass loss profiles of the scaffold are important design parameters. As the scaffold degrades, extracellular matrix fills the void space and, ultimately, the final product is a new living tissue equivalent. In this study, hydrogels were prepared by copolymerizing a degradable macromer, poly(lactic acid)-b-poly(ethylene glycol)-b-poly(lactic acid) endcapped with methacrylate groups (PEG-LA-DM) with a nondegradable macromer, poly(ethylene glycol) dimethacrylate (PEGDM). Copolymer networks consisted of 100:0, 83:17, 67:33, and 50:50 PEGDM:PEG-LA-DM ratios. PEGDM does not degrade on the time scale of these experiments, but the ester linkage will cleave over time in vivo [1]. PEG-LA-DM degrades completely in ~6 weeks in vitro following pseudo-first-order hydrolysis kinetics of the crosslinks [2]. Percent mass-loss was predicted using a previously developed theoretical model in which the hydrolysis kinetics (a model parameter) were based on experimental Young's moduli measured as a function of degradation time for a gel prepared from 10% (w/w) PEG-LA-DA [3]. The model was modified to predict the degradation behavior of copolymerized gels containing degradable and nondegradable crosslinks and shows that when a higher percentage of crosslinks is nondegradable, the rate of mass loss decreases. For example, to reach 10% mass loss, a gel with 100% degradable crosslinks takes only 6 days whereas a gel with 17% degradable crosslinks takes 32 days. Osteoblasts were photoencapsulated in the copolymer hydrogels and cultured for three weeks in vitro. Osteoblast function and mineralized tissue formation was monitored biochemically and histologically throughout the three weeks. In addition, gene expression was analyzed at days 4, 10, and 21. Metabolic activity, a general cell function measure, was enhanced by the presence of the PEG-LA-DM, particularly at day 21 when the 50:50 composition showed 20x more metabolic activity when compared with the 100:0 composition. Proliferation increased with increasing mol% of PEG-LA-DM at days 4 and 10, where the 50:50 composition had 3x and 2.5x higher proliferation rates than the 100:0 composition. In addition, alkaline phosphatase (ALP) production, a specific osteoblast function indicator, was highest for cells encapsulated in the greatest mol% of PEG-LA-DM at all time points, exhibiting nearly a 3-fold greater ALP production when compared to the 100:0 composition. Gene expression of the cultured osteoblasts, normalized to glyceraldehyde-3-phosphate dehydrogenase, was analyzed. Osteopontin and collagen type I gene expression followed similar trends where an increase of gene expression occurred in response to an increase in mol% of PEG-LA-DM. At all timepoints, cells encapsulated in the 50:50 composition exhibited 10x and 4x greater expression for osteopontin and collagen type I, respectively. Finally, as a measure of mineralized tissue formation, calcium and phosphate deposition were analyzed biochemically and with von Kossa staining of histological sections. Mineralized tissue formation increased with increasing mol% of PEG-LA-DM. The mass-loss profile of PEG hydrogels easily can be tailored to control cell function and deposition of mineralized tissue. For example, we have shown through a theoretical model that the incorporation of nondegradable crosslinks into the network slows the mass-loss and eliminates reverse gelation. Osteoblasts encapsulated within a network containing 50% degradable crosslinks exhibited 20x greater metabolic activity at day 21, and about 3x greater proliferation and ALP production at days 4, 10, and 21 when compared to the completely nondegradable system. In addition, gels with degradable crosslinks induced greater osteopontin and collagen type I gene expression and mineralization by encapsulated osteoblasts. By proper design of scaffold degradation behavior, osteoblast function, gene expression, and mineralized tissue formation can be enhanced.

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