Degradation Impacts Chondrocyte Matrix Production in Dynamically Loaded Poly(ethylene glycol)-based Hydrogels

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Statement of Purpose: Photopolymerizable poly(ethylene glycol) (PEG)-based hydrogels are promising for cartilage tissue engineering because they provide a highly hydrated 3D environment that supports maintenance of the chondrocyte phenotype, while their synthetic nature allows the hydrogel chemistry and structure to be tailored. Previous research has demonstrated that non-degradable PEG hydrogels allow for cartilage matrix deposition, however this deposition is often limited to the region surrounding the cell. The incorporation of hydrolytically degradable lactic acid into PEG (PEG-LA) hydrogels was shown in free swelling culture to provide space for cartilage-like tissue evolution as evidenced by the presence of glycosaminoglycans and collagen after 28 days (1). However, articular cartilage experiences dynamic loading *in vivo*, which has been shown to impact the degradation behavior of PEG-LA hydrogels (2). Therefore, this study investigates the impact of degradation in facilitating tissue development comprised of essential cartilage macromolecules and the impact of loading on macroscopic tissue development.

Methods: PEG-LA and PEG were synthesized as reported previously to produce degradable and nondegradable macromers, respectively (3). Chondrocytes isolated from full depth articular cartilage (50 million cells/mL) were mixed with 10% (w/w) macromer and 0.05% (w/w) photoinitiator (Irgacure I2959; Ciba Specialty Chemical, Newport, DE) in PBS and photopolymerized (365 nm, 6 mW/cm^2, 10 minutes) to make 5mm diameter 5mm thick cylindrical constructs. Gels were cultured for 28 days. Free swelling gels were placed on a figure-8 shaker (40 rpm) and loaded gels were placed in a custom bioreactor (2) and subjected to intermittent loading (eight cycles/day of 30 minutes ON/90 minutes OFF) applied by a sinusoidal dynamic unconfined compression at 0.3 Hz with a 15% peak-to-peak strain. Sulfated glycosaminoglycan (GAG) content was measured using dimethylmethylene blue. Collagen content was determined by assaying for hydroxyproline. The compressive moduli were obtained from the linear region of stress-strain curves generated on a mechanical tester (Synergie 100, 10 N; MTS, Eden Prairie, MN). Immunohistochemistry (IHC) was performed with primary antibodies against aggrecan (University of Iowa Developmental Studies Hybridoma Bank, Iowa City, IA), collagen II (US Biologicals, Swampscott, MA), collagen VI (Abcam, Cambridge, MA), and chondroitin-6-sulfate (Chemicon, Billerica, MA).

Results/Discussion: Cell-laden free swelling PEG-LA constructs had increased GAG and collagen contents when compared to non-degradable PEG constructs (Table 1), however the modulus in PEG-LA was significantly decreased with culture time ($p<0.05$) from 23±0.2 to 1.6±0.4 kPa while the PEG modulus was maintained (~70 kPa) with time. Dynamic loading resulted in delayed GAG deposition, but by 28 days was similar to free swelling constructs, and 60% reduced collagen content ($p=0.096$). IHC showed that tissue deposition in non-degradable PEG hydrogels was limited to the pericellular region surrounding the cells, whereas degradable PEG gels had extracellular matrix that more closely resembled that of native articular cartilage for aggrecan (Fig. 1). In addition, cartilage-like tissue was comprised of collagen II throughout the extracellular matrix and collagen VI localized to the pericellullar matrix similar to native cartilage (data not shown).

Table 1. Tissue content within constructs (μg/mg dry construct weight)

Day	PEG: Free Swelling		PEG-LA: Free Swelling		PEG-LA: Loaded	
	GAG	COL	GAG	COL	GAG	COL
0	5±1	26±8	12±4	14±8	12±4	14±8
14	39±13	12±5	80±1	162±43	47±14	15±9
28	51±26	120±170	118±8	348±12	118±23	138±123

GAG=sulfated glycosaminoglycans; COL=collagen

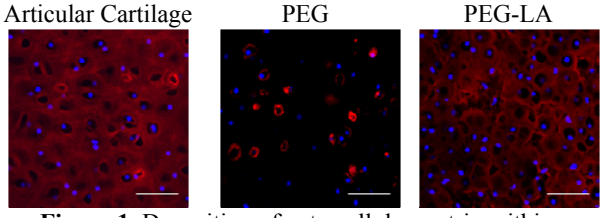

Figure 1. Deposition of extracellular matrix within articular cartilage and free swelling constructs as seen with antibody staining for the hyaluronan binding region of aggrecan. Scale bars represent 50 μm.

Degradation of the hydrogel construct is necessary for macroscopic development of essential cartilage matrix molecules. The mesh size within non-degradable PEG hydrogels is ~150-200 Å, reducing diffusion of large cartilage macromolecules, such as aggrecan aggregates and collagen II, which can reach dimensions of a few microns. Whereas degradable PEG hydrogels supported the macroscopic deposition of these large extracellular matrix molecules. However, when the degradable PEG hydrogels were placed under loading to emulate aspects of the *in vivo* environment, macroscopic tissue defects were observed, suggesting that mechanical loading altered scaffold degradation.

Conclusions: These findings demonstrate that degradation is critical to the development of a macroscopic engineered cartilage, however, the loading environment must be considered during scaffold development. The authors acknowledgment support from the NIH (K22DE016608, R01AR053126, and Leadership Training in Pharmaceutical Biotechnology Program) and Department of Education (GAANN).

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