Tuning Temporal Network Structures to Enhance Neocartilage Formation in Hyaluronic Acid Hydrogels

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Statement of Purpose: Tailoring scaffold degradation mechanisms and rates to coincide with tissue formation is essential for the success of a tissue engineered scaffold. Degradation can affect scaffold properties, cell-scaffold interactions, and the quantity and distribution of extracellular matrix (ECM) proteins. Previously, we showed that neocartilage formation by encapsulated chondrocytes is influenced by network structure and properties[1], and that the chemistry of hyaluronic acid (HA) hydrogels supports and promotes the chondrogenic differentiation of mesenchymal stem cells (MSCs)[2]. By tuning HA network degradation, with the inclusion of hydrolytically degradable components, we show the effects of temporal degradation on scaffold properties and neocartilage formation by encapsulated MSCs.

Methods: The syntheses of methacrylated HA (MeHA) and methacrylated caprolactone HA (MeCLHA) (Figure 1) and acellular characterization (e.g., mechanics, degradation) methods were previously reported[3]. Human MSCs (Lonza) were photoencapsulated (20 million cells/mL) in hydrogels of varying macromer type and wt% (2:0, 1.5:0.5, 1:1, and 1:0 MeHA wt%: MeCLHA wt%) and cultured in chondrogenic media supplemented with 10 ng/ml of TGF-β3 for 2, 5 and 8 weeks *in vitro*. Samples (n=5) were mechanically tested in unconfined compression and digested to determine biochemical content (e.g., DNA, GAG)[4]. Additionally, histological sections were stained for type I and II collagen and chondroitin sulfate[4] to visualize ECM distribution. ANOVA with Tukey's post-hoc test was used to determine significant differences ($p < 0.05$).

Results: Network structures and properties in slowly degrading (enzymatically) HA gels have been shown to influence neocartilage formation by chondrocytes[1]. Specifically, crosslinking density dictates the diffusion of cells, nutrients, waste, and most importantly, newly synthesized ECM proteins that contribute to the construct's mechanical properties. However, ECM distribution is often limited without adequate space for diffusion. In this study, we introduced hydrolytically degradable repeat units of caprolactone between the HA backbone and methacrylate groups to allow for control over hydrogel degradation profiles (e.g., ~19% mass loss for a 2wt% MeHA hydrogel to 100% mass loss for a 2wt% MeCLHA hydrogel after 7 days in PBS).

In copolymer (MeHA and MeCLHA) hydrogels with an initial concentration of 2wt%, increasing MeCLHA content within the hydrogel resulted in increased distribution of chondroitin sulfate after 2 weeks of *in vitro* culture (Figure 1). The increased ECM distribution was also reflected by increases in both the compressive equilibrium modulus (from 24.1±3.7 to 49.1±6.6 kPa) and the GAG/DNA content (from 169±21 to 201±12 μg GAG/μg DNA) after 8 weeks of culture (Figure 1).

Importantly, a gel that starts at 2wt% and decreases to 1wt% is not equivalent to a gel that starts at 1wt%. Specifically, despite an insignificantly higher equilibrium modulus, the 1:0 hydrogels boast a significantly lower GAG/DNA content (Figure 1) at 8 weeks. Furthermore, while total DNA content was similar in all groups, the dimensions of the constructs varied between groups (e.g., 1:1 hydrogels had a diameter of 5.48±0.08 mm and a height of 2.74±0.08 mm compared to 4.44±0.07 mm and 2.4±0.1 mm, respectively, for the 1:0 group). The increased GAG and maintained construct size could be due to a gradual decrease in crosslinking density, compared to constructs that began with a lower crosslinking density alone.

Conclusions: These results indicate that tuning temporal scaffold degradation can be used to control neocartilage production in HA hydrogels. The faster degrading MeCLHA component of the hydrogel creates void spaces, allowing for the deposition and enhanced distribution of newly synthesized ECM proteins, while the MeHA component provides structural support, maintaining size and shape of the scaffold, until it is eventually enzymatically degraded and remodeled by the encapsulated cells.

References: (1) Chung C. JBMR-A. 2006;77:518-25. (2) Chung C. Tissue Eng, in print. (3) Sahoo S. Biomacromolecules. 2008;9:1088-92. (4) Chung C. Tissue Eng. 2008;4:1121-31.

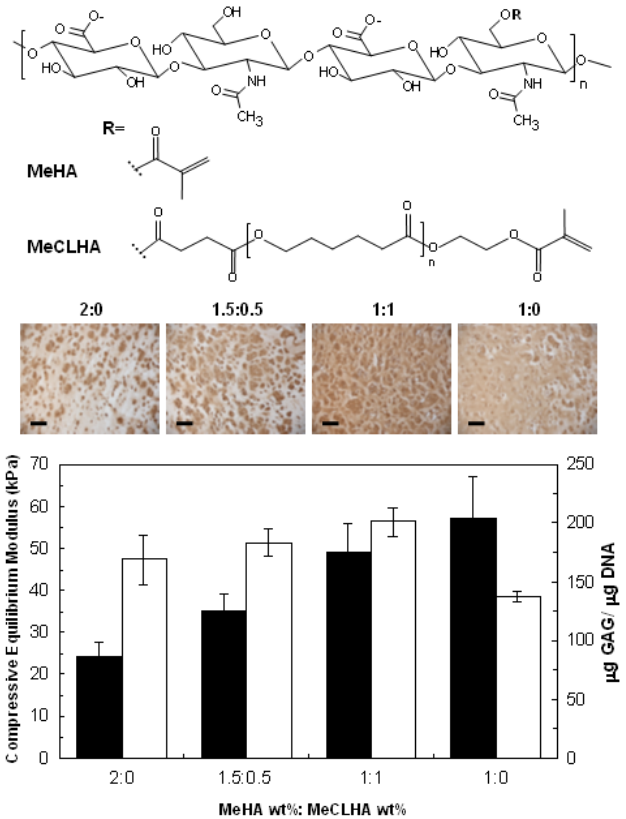

Figure 1. Chemical structures of MeHA and MeCLHA macromers (top). Chondroitin sulfate staining of HA hydrogels after 2 weeks of *in vitro* culture (middle, scale bar = 100μm). Compressive equilibrium modulus (black) and μg GAG/ μg DNA content (white) of HA hydrogels cultured for 8 weeks *in vitro* (bottom).