Neocartilage Formation by Auricular Chondrocytes in Photopolymerizable HA Networks

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Statement of Purpose: The choice of biomaterial and the optimization of scaffold properties are essential in cartilage tissue engineering since they affect chondrocyte proliferation and extracellular matrix (ECM) formation. Hyaluronic acid (HA), a natural polymer found in connective tissue, has many beneficial properties such as recognition by specific cell-surface receptors, roles in cellular processes, degradation by hyaluronidases, and easy modification with photoreactive groups allowing for cell encapsulation. Elisseeff and coworkers[1] first used photopolymerization to suspend and deliver chondrocytes in hydrogels for the treatment of damaged cartilage. In this study, various photocrosslinkable HA hydrogels were characterized and investigated as carriers for auricular chondrocytes for cartilage regeneration.

Methods: Methacrylated HA (MeHA) was synthesized by a previously reported technique[2]. Hydrogels were fabricated by dissolving MeHA (50 to 1100 kDa) at various concentrations (2 to 20 wt%) in PBS containing 0.05 wt% Irgacure 2959 and polymerizing with ultraviolet light for 10 minutes. The compressive moduli of swollen hydrogels were determined on an Instron 5542 mechanical tester. For degradation, HA hydrogels were placed in 100 U hyaluronidase/ml PBS.

Chondrocytes were harvested from the ears of swine and photoencapsulated in various hydrogels by suspension in solutions of 2 to 20 wt% MeHA containing 0.05 wt% I2959 and UV light exposure. Chondrocyte/ hydrogel constructs were implanted subcutaneously in the dorsum of nude mice and harvested after 12 weeks. For biochemistry (n=4), samples were lyophilized and digested in a papain solution overnight at 60ºC. Total GAG and collagen contents were determined using the dimethylmethylene blue dye method and the hydroxyproline assay[3], respectively. Additionally, histological sections were stained for type I and II collagen distributions using the Vectastain ABC kit and the DAB Substrate kit for peroxidase. Anova with Tukey's post-hoc test was used to determine significant differences, ($p < 0.05$).

Results / Discussion: Alterations of HA MW and MeHA concentrations affected precursor solution viscosities and hydrogel properties. With macromer concentrations from 2 to 20 wt%, networks exhibited volumetric swelling ratios ranging from ~42 to 8, compressive moduli ranging from ~2 to over 100 kPa, and degradation times ranging from less than 1 day up to almost 38 days in the presence of 100 U/ml hyaluronidase. A decrease in the volumetric swelling ratio and an increase in both the compressive modulus and time for complete degradation of the networks were found as the macromer concentration was increased (See Figure 1). However, only minor changes in these properties were observed with different MWs.

After 12 weeks, the various constructs consisted of 0 to 0.049 µg CS/ µg wet weight (GAG content) and 0.002 to 0.060 µg collagen/ µg wet weight. The 2 wt%, 50kDa constructs exhibited the greatest GAG and collagen content. Constructs of higher macromer concentration exhibited minimal biochemical content and showed little cell proliferation (see Figure 1). Histological staining showed minimal type I collagen staining for all, and intense, uniform type II collagen staining in the construct with greatest neocartilage production (2 wt%, 50kDa construct). Minimal staining was detected for constructs of higher macromer concentration (see Figure 1).

Conclusions: Macroscopic, biochemical and immuno-histological analysis of the explants indicated both that hydrogel properties influence neocartilage formation and that hydrogels fabricated of the 2 wt% concentration exhibited the greatest neocartilage formation (constructs were shiny and opaque, exhibited the greatest GAG and collagen contents, and showed good distribution of type II collagen) and were most comparable to native cartilage tissue. Constructs fabricated with higher macromer concentrations showed much lower neocartilage formation, which may have been due to restrictions in nutrient transport through the construct, high radical concentration during polymerization, and slower hydrogel degradation, potentially compromising cell viability, growth, and ECM production.

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References: (1) Elisseeff J. Plast. Reconstr. Surg. 1999;104:1014-1022. (2) Smeds KA. J Biomed Mater Res 2001;54:115-21. (3) Burdick JA. Biomacromolecules 2005; 6:386-91.

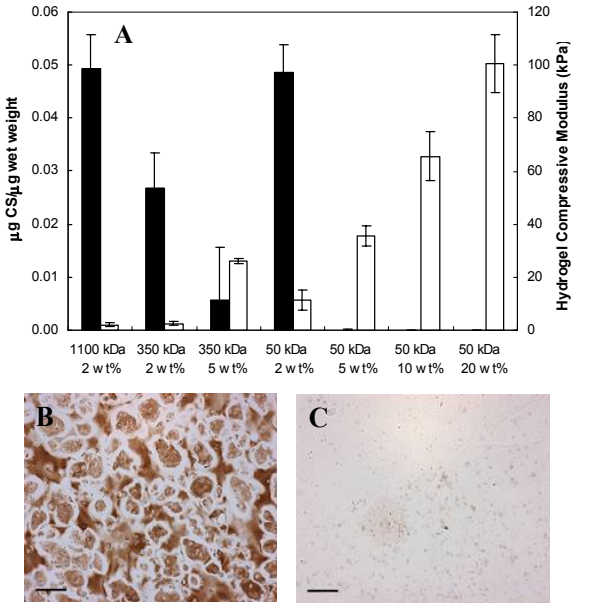

Figure 1: GAG content of explants with respect to wet weight (black) and hydrogel compressive modulus (white) (A). Type II collagen staining for 2 wt% (B) and 10 wt% (C), 50 kDa HA hydrogels after 12 weeks of subcutaneous culture, scale bar = 100µm.