Designing exogenously degradable poly (ethylene glycol) hydrogels for cartilage tissue engineering applications

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Statement of Purpose: Photopolymerizable poly (ethylene glycol) [PEG] hydrogels represent a promising biomaterial for cartilage tissue engineerging applications. Previous work from our group demonstrated that free-radical polymerization, using thiol-norbornenes instead of acrylates, led to a mild encapsulation environment and a depsotition of neo-tissue that closer resembled hyaline cartilage[1]. The degradability of these hydrogels is a crucial feature. Ideally, the rate of degradation would match the rate of new matrix production. Thereby, the mechanical intergrity of the scaffold can be preserved and, at the same time, an adequate space for the uniform deposition of newly synthesized extracellular matrix [ECM] molecules is provided. One promising strategy is to exogenously control degradation as a means to temporally control when and how fast degradation occurs[2]. In the present study, degradable PEG hydrogels were synthesized from a thiol-ene photoclickable hydrogel platform based on caprolactones that can be degraded by exogenous delivery of lipase. Preliminary studies were performed to investiage (a) the degradation profile in a-cellular conditions and (b) the effect of exogenous lipase addition, in varying concentrations, on cartilagenous matrix production and viability of bovine chondrocytes.

Methods: 8-arm PEG (M_W 20 000, JenKemUSA) was functionalized with ε-Caprolactone (Sigma) and, afterwards, conjugated with 5-nobornene-2-carboxylic acid (Sigma). The final product was confirmed by ^{1}H NMR. Full-depth articular cartilage was harvested from skeletally immature calves. Chondrocytes were isolated by digestion in collagenase type II. Cells (>90% viable) were encapsulated in 10% (w/v) gels at $50x10^6$ cells/ml. Gels were photo-polymerized for 7 minutes with 0.05% of photoinitiator I2959 (Ciba Specialty Chemical) and 1000 DA PEG-dithiol as cross-linker (thiol to ene ratio 1:1). The gels were cultured for up to 4 weeks in standard chondrocyte medium, supplemented with 0, 0.05 or 0.1mg/ml amano lipase PS (sigma). Medium was changed and collected thrice weekly. After 0, 14 and 28 days, gels were lyophilized and digested in 0.125mg/ml papain. DNA content was quantified using the Hoechst 33258 dye. The amount of sulfated glycosaminoglycan [GAG] was determined using dimethylmethylene blue. Collagen amount was estimated by determination of the hydroxyl-proline content and type by imunohistochemistry. Cellular viability, after 0, 14 and 28 days, was determined using live/dead staining. Further, a-cellular gels were prepared and cultured in 0, 0.05, 0.1 or 5 mg/ml of lipase in PBS. The wet weight of these gels was determined before the addition of lipase and, afterwards, thrice weekly.

Results: Macromers of PEG-o-caprolactone endcapped with norbornene (PEG-*o*-CAP-NB) were successfully synthesized with the number of caprolactones per arm ranging from 0.93 to 4.75. Solubility in an aqueous solvent, however, was limited to macromers having less than two caprolactones per arm. Initial cellular studies were performed with the PEG-*o*-CAP0.93-NB without or with continuous delivery of lipase. For all conditions, DNA content showed an increasing trend over the course of the study, suggesting cellular proliferation. Cell viability (>70%) was maintained, further confirming the feasibility of this hydrogel platform. The bulk amount of newly synthesized GAG was retained within the hydrogels and a detectable release was only observed during the last week of the study. Newly synthesized total collagen was solely retained within the gels with none detected in the culture medium. Normalization of total GAG and collagen to the DNA content demonstrated extensive neocartilaginous ECM production (figure 1 + 2). Immunohistochemistry confirmed collagen II. No differences were observed in ECM deposition as a function of lipase addition. This observation is attributed to the slow degradation having on average 0.93 CAPS per arm, where no degradation was detected over the time course of this study in a-cellular hydrogels.

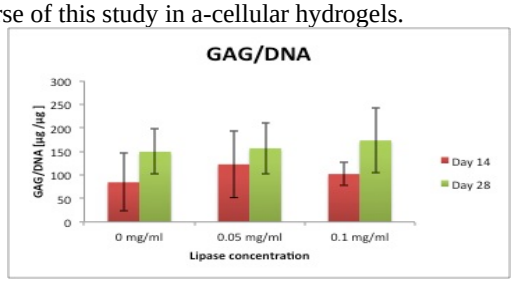

Figure 1: Total GAG (medium + gel) normalized to DNA. Data represent mean (n=4) with standard deviation for error bars.

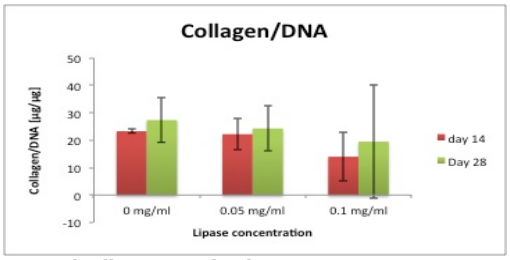

Figure 2: Total collagen normalized to DNA. Data represent mean (n=4) with standard deviation for error bars.

Conclusions: PEG-caprolactone hydrogels formed by photoclickable reactions were established as a viable platform for cartilage tissue engineering. Although significant exogenous degradation was not achieved, the biological data are promising showing viable chondrocytes and matrix production even in the presence of lipase. Extending the length of the CAP to 1.85 per arm demonstrated capability for controlled exogenous lipase-mediated degradation and biological experiments with this modified formulation are currently ongoing.

References:

[1] Roberts JJ. Biomaterials. 2013;34(38):9969-9979.
[2] Rice MA. Tissue Engineering. 2007;13(4):683-691.

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