Cellularly Degradable PEG Hydrogels with Tethered TGF-β1 for Improved Cartilage Engineering

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Statement of Purpose: Healing articular cartilage defects remains a significant clinical challenge due to limited capacity for self-repair. A goal in cartilage tissue engineering is the production of cell-laden constructs possessing sufficient mechanical and biochemical features to restore tissue function. Primary chondrocytes are a versatile cell source for cartilage regeneration due to the ease of isolation and expansion. Differentiated chondrocytes also deposit a matrix more similar to articular cartilage[1], which circumvents some of the problems of mesenchymal stem cell (MSC) derived fibrocartilage.

Previous work has shown that cell-dependent degradation of peptide crosslinked PEG gels accelerates expression of cartilage matrix molecules.[2] Furthermore, covalently tethered TGF-β1 in PEG networks has been shown to enhance the proliferation and ECM deposition of encapsulated chondrocytes in non-degradable systems.[3] Here, we exploit locally dictated cell degradation with the promoting effects of tethered growth factors to create a system that encourages chondrocytes to expedite regeneration processes and generate tissue that more closely captures the biochemical and biomechanical characteristics of native articular cartilage.

Methods: Primary porcine chondrocytes were isolated from the femoral-patellar groove of Yorkshire swine (3-4 months old). Constructs were cultured in hgDMEM with 10% serum. Cells were seeded at 40 million cells/ mL in 40μL syringe tip gels (diameter~ 5mm, thickness ~2mm). Chondrocytes were encapsulated in a 10 wt% 8 arm 10 kDa PEG-norbornene hydrogel that was photopolymerized (30 seconds at 10 mW/cm^2, 365nm). The collagen-derived MMP degradable peptide sequence (KCGPQ*IWGQCK) cross-linker was added at varying stoichiometric ratios (1:1, or 1: 0.5 monomer to linker). We added reactive thiols onto TGFβ and tethered it into the network using a photoinitiated thiol-norbornene polymerization mechanism. PE-25 cells were encapsulated in peptide crosslinked PEG-norbornene gels at 40 million cells/mL with 50 nM tethered TGF-β1. A luciferase assay was performed on PE-25 lysate to find relative bioactivity of the cytokine. Chondrocyte DNA content was determined by Picogreen assay, GAG content was determined by DMMB assay.

Results: In order to ensure covalently tethered TGFβ was not affected by the acidic peptide linker, we neutralized the pH of the linker and confirmed that the cytokine retained its bioactivity in the degradable system using the PE-25 luciferase reporting cell line.

We proceeded by encapsulating chondrocytes in a system with the neutral linker sequence and confirmed it was cleaved by chondrocyte-secreted enzymes using an MMP fluorogenic substrate.[4]

In degradable systems without TGFβ, as shown in figure 1, we observed that by decreasing crosslinking density, encapsulated chondrocytes proliferate more as indicated by increased DNA content and produced more glycosaminoglycans over 14 days.

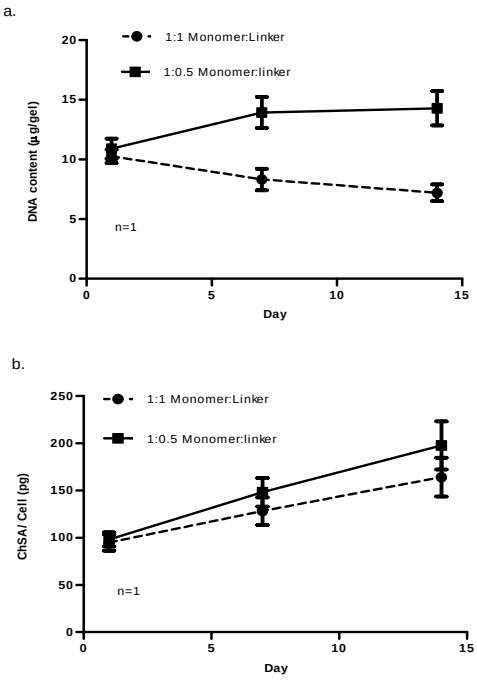

Figure 1. Effect of Crosslinking Density on Chondrocyte Function a) DNA content (μg/gel) with monomer: linker ratio at 1:1 and 1:0.5 b) GAG content/ cell (pg) with monomer: linker ratio at 1:1 and 1:0.5

Conclusions: Covalently tethered TGF-β1 retained bioactivity in our degradable platform as indicated by the PE-25 luciferase reporter system. We confirmed that the chondrocytes were able to degrade the peptide linker using a quenched MMP sensor with the same sequence. Degradable systems with a lower crosslinking density have increased DNA content and allow greater GAG deposition on a per cell basis which is likely due to an increase in volume around the cell to permit ECM expansion. Future studies will investigate crosslinking densities that permit maximal ECM expansion combined with the effects of tethered TGF- β1 on encapsulated chondrocytes in expediting the biochemical and biomechanical maturation process to more closely mimic native articular cartilage.

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