Covalently Tethered Transforming Growth Factor Beta-1 in PEG Hydrogels Expedites Cartilage ECM Production of Encapsulated Primary Chondrocytes.

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Statement of Purpose: Healing articular cartilage defects remains a significant clinical challenge because of its limited capacity for self-repair and a mechanical structure that is difficult to emulate. A goal of 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 source for regeneration due to the ease of cell isolation and expansion. Differentiated chondrocytes also deposit a matrix more similar to articular cartilage, which circumvents some of the problems of mesenchymal stem cell (MSC) derived fibrocartilage.

The cytokine transforming growth factor beta (TGFβ) has been shown to enhance the biochemical maturation of encapsulated chondrocytes when delivered to a 3D construct transiently.[1] Methods to precisely control TGFβ presentation in a local and sustained fashion are important since they can be cross-reactive with multiple cell types and have short serum half-lives.[2] Thus, localized presentation of TGFβ is an important step for developing a device that can be clinically implemented.

In this work, we introduce a reactive thiol onto TGFβ and covalently tether the growth factor into PEG hydrogels using a photoinitiated thiol-norbornene polymerization mechanism. We show that the thiol-ene platform is a suitable 3-D system for the presentation of TGFβ-1 both solubly and covalently tethered using an encapsulated SMAD2 reporter cell line (PE.25 cells express luciferase downstream of a TGFβ responsive promoter).[3] Furthermore, we show that the bioactivity of TGFβ-1 is not affected by thiolation of the protein. We repeated these studies on encapsulated chondrocytes.

Additionally, we took advantage of the fact that TGFβ upregulates the expression of native chondrocyte proteases.[4] We included matrix metalloprotease (MMP) sensitive peptide cross-linkers in our network since it has been shown that cell-dependent degradation of the surrounding PEG network accelerates the turnover of cartilage matrix.[5] We observed the effect of tethered TGFβ on the extracellular matrix production of chondrocytes in both a nondegradable and degradable networks. Our final aim is to create a system that promotes chondrocyte functional properties to expedite regeneration processes and generate tissue that mimics 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). Cells were either immediately encapsulated or allowed to grow to confluency for one passage to garner more cells. Constructs without TGFβ were cultured in serum containing hgDMEM. Constructs with TGFβ were cultured in a serum free hgDMEM with ITS +Premix (BD Biosciences) as the serum substitute. Experiment spanned over 28 days and media was changed every 3 days. Cells were seeded at a minimum density of 20 million cells/ mL in 40μL syringe tip gels (O.D.~ 5mm, thickness ~2mm). Chondrocytes were encapsulated in a 10 wt% 8 arm 10 kDa PEG-norbornene hydrogel that was photopolymerized (60 seconds at 10 mW/cm^2, 365nm) through a radical, step growth scheme. The MMP degradable peptide sequence (KCGPQ*IWGQCK) and 3.5 kDA PEG dithiol (Sigma Aldrich) cross-linkers were added at stoichiometric ratios. PE.25 cells were encapsulated in 10 wt% 8 arm 10 kDA PEG-norbornene gels at 10 million cells/mL in 40 μL gels. Cell-laden gels were exposed to varying concentrations of TGFβ in a soluble (0ng/mL-10ng/mL) or tethered (0nM-25nM) form. A luciferase assay was performed on PE25 lysate to find relative bioactivity of the cytokine. Chondrocyte GAG content was determined by DMMB assay, total collagen content by hydroxyproline assay, and immunohistochemistry was performed with antibodies against mammalian collagen II, collagen I, and aggrecan to characterize articular cartilage ECM expression.

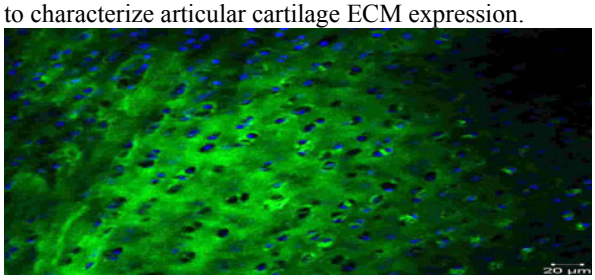

Figure 1. IHC of aggrecan (green) expression of chondrocytes (blue) in a 10 wt% 8 arm 10 kDA thiolene gel with MMP peptide crosslinker after 35 days.

Results and Conclusions: As shown in Figure 1, chondrocytes grown in a degradable thiolene system without TGFβ can generate tissue that resembles native articular cartilage tissue over a long period of time. There is high pericellular aggrecan expression with a high collagen type II:type I ratio which is characteristic of articular cartilage. We also showed that TGFβ-1 can influence luciferase expression of PE.25 cells encapsulated in a thiolene system with a nondegradable dithiol linker when the cytokine was either solubly delivered or tethered to the network. Future studies will look at the specific effects of TGFβ on chondrocytes in expediting the biochemical and biomechanical maturation of articular cartilage.

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