INTRODUCTION

Previous studies have shown that chondrocytes embedded in PEG-based semi-interpenetrating (sIPN) hydrogels can produce hyaline-like cartilage tissue, can produce thick constructs with uniformly distributed cells and extracellular matrix, and can be formulated to have substantial compressive properties [1]. The feasibility of using degradable biocompatible hydrogel scaffolds which are safe for long-term implantation and production of cartilaginous tissue has not been demonstrated. The PEG-dimethacrylate (PEGDM) hydrogel formulation used in the present study contains a degradable PEG-dimethacrylate (PEGDM) and an interpenetrating polyethylene oxide (PEO) polymer component that eventually diffuses from the crosslinked hydrogel and can be excreted from the body. The overall goal of this work is to determine the optimal formulation of the hydrogel that can produce biologically relevant size and shaped cartilage tissue having substantial biochemical composition. To work towards this goal we have the following specific objectives: 1) to determine the effect of the hydrogel formulation and cellular parameters on matrix content and uniformity; 2) to determine whether cartilage tissues of biologically relevant size and shape can be produced using the new hydrogel formulation.

METHODS

Factorial study: A 2^4 full factorial study was conducted to investigate the effects of the PEGDM degradation rate, percent of PEGDM in the formulation, PEO molecular weight (MW), cell seeding density, and chondrocyte source (articular vs. septal) on construct characteristics (Table 1).

Table 1. Factors that were controlled in the factorial study design

<table>
<thead>
<tr>
<th>Factors</th>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEGDM Degradability</td>
<td>rate</td>
<td>fast</td>
</tr>
<tr>
<td>Percentage of PEGDM</td>
<td>40%</td>
<td>80%</td>
</tr>
<tr>
<td>PEO molecular weight</td>
<td>3.4 kDa</td>
<td>100 kDa</td>
</tr>
<tr>
<td>Chondrocyte Seeding Density</td>
<td>100%</td>
<td>50%</td>
</tr>
<tr>
<td>Chondrocyte Isolation Site</td>
<td>N/A</td>
<td>articular septal</td>
</tr>
</tbody>
</table>

Bovine articular cartilage (BAC) tissue was dissected from the patellar groove and condyles and bovine septal cartilage (BSC) tissue was dissected from the septum of 2-3 week old animals. The chondrocytes were isolated by collagenase digestion and mixed with the appropriate hydrogel formulation made at 20% (w/w) total polymer weight in PBS. The photoinitiator 1-cyclohexyl phenyl ketone (HPK; Aldrich) was added to the polymer-cell mixture and photopolymerized under UVA (365 nm) light for 3-5 minutes in the wells of a 48 well plate to produce constructs of 10mm diameter by 2 mm thick. The resulting 32 different cell-embedded hydrogels were cultured in 6-well plates with DMEM media containing 10% FBS and 50 μg/mL L-ascorbic acid for 6 weeks.

Biologically relevant size and shaped constructs: The most promising formulation found in the above factorial study was used to determine whether desired shape constructs can be produced. Cartilage constructs in the shape of a chin implant were produced by polymerizing the hydrogel solution within silicone chin molds and subsequently culturing throughout the entire 6 week culture period producing a rigid implant.

RESULTS

The controlled variables were found to significantly affect the % S-GAG content (per wet weight) in the following order: seeding density > PEO MW > isolation site > % PEGDM; with degradability having an insignificant effect on S-GAG content after 6 weeks of culture. Similarly, the controlled variables were found to significantly affect the collagen content (% per wet weight) in the following order: seeding density > isolation site; with % PEGDM, degradability, and PEO MW having an insignificant effect. An increase in cell seeding density resulted in an approximately 3.3 fold increase in % S-GAG (w/w) content (p<0.0001) and an increase in the MW of PEO caused a 1.3 fold decrease in % S-GAG content (p<0.0001), both of which was also observed by histology (Fig. 1). Constructs produced with septal cells (p<0.0001) or a higher percent of PEGDM (p=0.0008) resulted in a 1.2 fold increase in %S-GAG content. Seeding density also had the largest positive effect on % collagen content (w/w) (p<0.0001), where an increase in seeding density lead to a 2.1 fold increase in collagen content. Septal cells also produced construct with 1.27 x more collagen than articular cells (p=0.0003). PEGDM degradability did not have a significant effect on any of the matrix content parameters.

DISCUSSION

This work has demonstrated that chondrocytes embedded in a degradable PEG-based hydrogel are capable of generating a cartilaginous phenotype similarly to non-degradable PEG-based hydrogel studied previously. These hydrogels are also capable of producing cartilaginous tissue into large and complex shapes for biologically relevant applications.


Acknowledgements: Funded by NIH (1 R43 AR47253-01A1).

Poster #0980

DEGRADABLE PEG-BASED SEMI-INTERPENETRATING HYDROGEL FORMULATIONS FOR CARTILAGE TISSUE ENGINEERING

* Riley, SL; *Dutt, S; **Zhao, X; and Ratcliffe, T

* Advanced Tissue Sciences, La Jolla, CA