GENETICALLY ENGINEERED, ENZYMATICALLY CROSSLINKED ELASTIN-LIKE POLYPEPTIDE GELS FOR CARTILAGE TISSUE REPAIR

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INTRODUCTION

Elastin-like polypeptides (ELP) are artificial polypeptides with unique properties that make them attractive as an injectable scaffold for cartilaginous tissue repair. ELPs consist of oligomeric repeats of the pentapeptide sequence Val-Pro-Gly-Xaa-Gly (Xaa is any amino acid except proline), that occurs naturally in the protein elastin contained in muscle, ligaments, cartilage and numerous other soft tissues (Urry and Prasad 1992). Low-viscosity ELP solutions may be prepared at room temperature for mixing with cells or bioactive factors, and may be easily injected into a cartilage defect (Betre et al. 2002). In the higher temperature of the defect site, the ELP will undergo a phase transition and spontaneously aggregate, forming a gel-like coacervate with mechanical properties dramatically different from that of the ELP in solution. Furthermore, the ELP sequence and chain length can be designed at the molecular level in order to control the mechanical properties of the gel-like coacervate. Nevertheless, crosslinking of the ELP may be essential for attaining mechanical properties that approach that of native cartilaginous tissues.

In prior work, we evaluated the compressive and shear properties of a chemically crosslinked ELP hydrogel and found it possible to attain values similar to that of native cartilaginous tissues (Trabbic-Carlson et al. 2001). The chemical initiators are not conducive to working with cells, however, so that we seek a biocompatible and non-toxic approach to ELP crosslinking for use with tissue repair. In this study, we develop a set of ELP sequences that are capable of enzymatic crosslinking via tissue transglutaminase (tTG). Addition of the enzyme promotes ELP gel formation and cell encapsulation in a biocompatible process, resulting in a biopolymer gel with the potential to promote biological repair as well as contribute to cartilage mechanical function.

METHODS AND MATERIALS

Synthesis of the ELP genes was carried out by recursive directional ligation using standard molecular biology techniques (Meyer and Chilkoti 2002). The resulting genes encoded 112 ELP pentapeptides and were expressed in *E. coli* using a modified pET25b expression vector (Novagen) and purified by inverse transition cycling (Meyer and Chilkoti 2002). The expressed proteins each have a molecular weight of ~47 kDa and amino acid sequences of $[VPGKG(VPGVG)_6]_{16}$ and $[VPGQG(VPGVG)_6]_{16}$ for the lysine and glutamine polymers, respectively. Recombinant tTG was expressed in *E. coli* as a fusion with glutathione S-transferase and purified according to the methods of Lai *et al* (1996).

Fibrochondrocytic cells were isolated from porcine intervertebral disc tissue by digestion with pronase and collagenase. Cells were expanded in culture and resuspended in a 60 mg/ml solution of ELP in a 1:1 ratio. Crosslinking was achieved by adding 1 mg/ml tTG in the presence of 5 mM $CaCl_2$ at room temperature. Gelation was allowed to proceed for 4 h at 37°C with gentle shaking. After the gels had formed, they were placed in individual wells of 12-well untreated polystyrene petri dishes and overlaid with 2 ml of fresh culture medium. Residual cells in the media were counted by a hemocytometer to quantify encapsulation efficiency.

Gel constructs were incubated with gentle shaking at 37°C with 5% CO_2 in 2 ml of Ham's F-12 cell culture medium (10% FBS, penicillin/streptomycin, fungizone) supplemented with ascorbic acid. Fresh media was introduced every 2-3 days and sample gels were harvested for histological evaluation every two weeks over a six week period. Frozen sections were analyzed immunohistochemically for evidence of type I (Sigma, C2456) and type II collagens (DSHB, II-II6B3), to examine important phenotypic characteristics of the cell population over the culture period.

RESULTS AND DISCUSSION

Gels formed as the result of enzymatic crosslinking were completely opaque as compared to the ELP coacervate, which is translucent. The gels were not readily disrupted by mechanical agitation and appeared permanently arrested in the insoluble state. The physical turgidity of these gels appeared greater than that of ELP coacervate but less than that of the chemically crosslinked hydrogels (Trabbic-Carlson et al. 2001); however, the mechanical properties of

the enzymatically-crosslinked hydrogels were not evaluated in a quantitative manner.

Greater than 95% of the cells were found to associate with the ELP gel after the 4 h crosslinking process. Cells were present in all regions of the gels as visualized by nuclear staining with hematoxylin. Consistent with previous observations of fibrochondrocytes in 3D matrices, there was no apparent change in cell number over the course of the study. Additionally, histological sections stained positive for the presence of collagen types I and II as early as 2 weeks of culture. As shown in the figure, virtually all cells in a section stained for type II collagen, whereas a much smaller fraction of the cells were positive for type I collagen. This observation was consistent over the 6 week sampling period. The observed production of collagen indicates that the ELP matrix did not hinder cell viability and acted to preserve the native fibrochondrocyte phenotype.

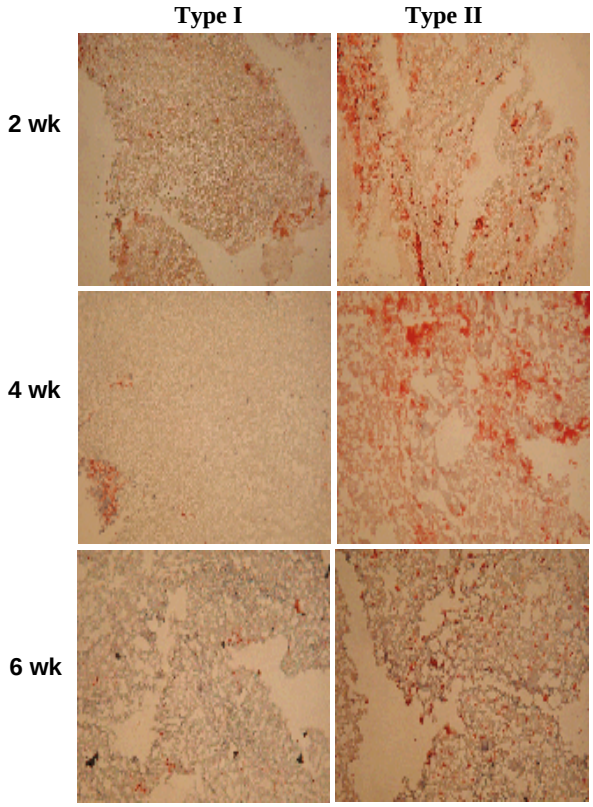

Figure 1. Positive staining for type I and type II collagens using monoclonal antibodies indicates presence of cartilaginous tissue matrix components in the ELP hydrogels

CONCLUSIONS

Synthetically designed ELPs were successfully crosslinked to form hydrated gels by manipulating the enzymatic activity of tissue transglutaminase. These results are consistent with the enzyme-catalyzed gelation events observed by other researchers using Factor XIII (Westhaus and Messersmith 2001). Initial *in vitro* studies indicate that the ELP matrix promotes expression of the fibrochondrocyte phenotype for up to six weeks in culture, as evidenced by the synthesis of two collagens abundant in the cartilage extracellular matrix. An attractive feature of the proposed system is that cells and enzymes may be mixed with the ELP solution prior to gelation, lending itself to use as an injectable, *in situ* gelling scaffold. Importantly, the ELP crosslinks by an enzymatic reaction in which no toxic by-products are formed, resulting in further stiffening of the gel matrix to values comparable to that of native tissue. In addition, the ELP sequence and chain length can be designed at the molecular level to obtain a targeted set of mechanical properties that will be important for promoting functional cartilage repair. Future work is targeted at designed ELP sequences to attain the physical properties of native cartilaginous tissues for repair of degenerated intervertebral disc, and articular cartilage and meniscal defects.

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