Augmentation of Chondrocyte Gene Therapy Using Customized Biomaterials

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Introduction: For almost two decades focal cartilage lesions have been treated using chondrocyte transplantation therapy\textsuperscript{1}. To enhance matrix synthesis by transplanted cells, gene therapy efforts have focused on upregulation of growth factors such as IGF-I\textsuperscript{2,3}. A main limitation of such gene therapy is an initial burst release of growth factor with minimal long term expression. As such, gene therapy would be aided by scaffolds that prevented rapid loss of growth factors, and maximized the duration of exposure to cells to the growth factor. There are many vectors used as vehicle such as adeno-associated virus (AAV), plasmid adeno-associated virus (pAAV) and plasmid DNA (pcDNA). More specifically pAAV/IGF-I has shown to have higher transfection efficiency than pcDNA containing IGF-I\textsuperscript{4}. Our lab has previously shown that grafting peptide sequences from IGF-I Binding Protein-5 (IGFBP-5) to alginate enhances IGF-I binding and GAG metabolism in a ligand-density dependent manner\textsuperscript{5}. The specific goal of this study was to examine ability of this modified alginate to enhance matrix synthesis of constructs receiving IGF-I gene therapy.

Methods: UP LVG alginate was chemically modified\textsuperscript{6} with GGG-KPLL ALL (KPL) peptide sequence identified from IGFBP-5. The final molar concentration of the ligand grafted to alginate was verified with nuclear magnetic resonance (NMR) analysis. Ligand density was varied by mixing modified alginate with unmodified alginate ranging from concentrations from 0 to 70 µM. Articular chondrocytes were harvested from 1-3 day old calves via collagenase digestion. Cells were transfected using two different complexes: FuGENE 6+pAAV/IGF-I (Transfected) and FuGENE 6+pAAV/Empty (Control) for 16 hours. Afterwards, cells were mixed with 2% alginate at different concentrations of KPL and encapsulated in beads formed by extrusion through a 22-gauge needle into a 102 mM CaCl\textsubscript{2} solution. Beads were incubated with DMEM without FBS for 30 days. GAG content was measured using the DMMB dye-binding assay\textsuperscript{7}. The kinetics of GAG accumulation were fit to an establish model of matrix synthesis to calculate steady-state GAG content\textsuperscript{8}, and these steady-state values of GAG synthesis were used to determine the effect of KPL content on chondrocyte matrix synthesis using a generalized variable slope concentration-response model\textsuperscript{9}.

Results: In unmodified alginate, steady state GAG accumulation was 50% greater in transfected chondrocytes than control chondrocytes (Fig.1A). Matrix production increased dramatically in transfected chondrocytes containing the highest concentration of IGF-I binding sites (70 µM) when compared to transfected chondrocytes in unmodified alginate (0 µM) (Fig.1B). The presence of IGF-I binding sites via modification of alginate with KPL also increased GAG accumulation in control chondrocytes, although this effect was far less dramatic than for transfected chondrocytes (Fig. 1C). To compare the effects of KPL on transfected and control chondrocytes, for each cell type, steady-state GAG accumulation was normalized to that in unmodified alginate. For both IGF-I transfected and control chondrocytes, GAG accumulation increased with binding density in a dose-dependent manner (Fig.2 and Table 1). For transfected chondrocytes, the maximal effect of the presence of IGF-I binding sites was a 6.9-fold increase in GAG accumulation, compared to only a 56% increase for control chondrocytes. The density of IGF-I binding sites necessary to achieve half-maximal stimulation (ED\textsubscript{50} was 11.4 µM for transfected chondrocytes and 21.2 µM for control chondrocytes (Table 1).

Discussion: This study demonstrated that a chemically customized alginate with an IGF-I binding sequence from IGFBP-5 enhanced chondrocyte gene therapy, greatly increasing the accumulation of GAG in constructs containing transfected chondrocytes. pAAV/IGF-I transfection increased GAG production by 50% in unmodified alginate (Fig. 1A), while in alginate gels with maximally effective concentrations of IGF-I binding sites, pAAV/IGF-I increased GAG production by more than 600% (Fig. 2). To our knowledge, this is the first report of the use of a customized biomaterial to enhance the effect of gene therapy. Future goals include incorporation of functional mechanical measurements to outcome assessments and in vivo evaluation of constructs.

Significance: This study combines tissue engineering and gene therapy, where customized biomaterials augmented chondrocyte gene therapy. We were able to customize a biomaterial (alginate) to enhance binding of IGF-I, which enhanced GAG production by transfected chondrocytes.

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**Table 1. Parameters of the dose response curve**

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<th>Transfected</th>
<th>Control</th>
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<tr>
<td>EC_{50} (μM)</td>
<td>11.37</td>
<td>21.22</td>
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<tr>
<td>Max/Min</td>
<td>6.86</td>
<td>1.53</td>
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<tr>
<td>R^2</td>
<td>0.99</td>
<td>0.96</td>
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