Effect of Binding Peptide Length and Concentration on Augmentation of IGF-I Gene Therapy for Chondrocytes

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Introduction: For almost two decades focal cartilage lesions have been treated using chondrocyte transplantation therapy [1]. To enhance matrix synthesis by transplanted cells, gene therapy efforts have focused on upregulation of growth factors such as IGF-I [2,3]. Therefore, a number of groups are using gene therapy to enhance IGF-I synthesis locally. However, the main limitation of gene therapy is an initial burst release of growth factor with minimal long term expression, regarding the vector used. 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. Our lab has previously shown that customized alginate, grafted with peptide sequences from the binding pocket from IGF-I Binding Protein-5 (IGFBP-5), combined with transfected chondrocytes with plasmid adeno-associated virus containing IGF-I (pAAV/IGF-I) enhances matrix synthesis in 3D scaffolds in vitro [4]. The goal of this study is to examine the effect of binding peptide length and concentration on IGF-I binding and matrix synthesis, by chondrocytes transfected with pAAV/IGF-I.

Methods: UP LVG alginate was chemically modified [5] with two peptide sequences from IGFBP-5: KPLHALL peptide sequence and shorter sequence ALL. 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 for KPLHALL and 0 to 2000 µM for ALL. 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 KPLHALL or ALL, and encapsulated in beads formed by extrusion through a 22-gauge needle into a 102 mM CaCl2 solution. Constructs (beads ~3 mm diameter) were incubated with DMEM without FBS for 30 days. Immunohistochemistry (IHC) for IGF-I localization was done to constructs on day 30. Syntheses of GAG [6] and hydroxyproline (HYPRO) [7] were used as the primary measure of chondrocyte metabolic activity. The kinetics of GAG and HYPRO accumulation were fit to an establish model of matrix synthesis to calculate steady-state GAG and HYPRO content [8], and these steady-state values of GAG and HYPRO synthesis were used to determine the effect of KPLHALL or ALL content on chondrocyte matrix synthesis using a generalized variable slope concentration-response model [8].

Results: IHC showed the binding and localization of IGF-I in the bead at Day 30. Localization of IGF-I changes with binding density of the residues in both KPLHALL and ALL (Fig.1). IGF-I retention changes with peptide length, where shorter peptide sequence ALL is not as effective at retaining IGF-I when compared to longer sequence KPLHALL (Fig. 1). Transfection with pAAV/IGF-I increases the production of both GAG and HYPRO in the constructs (Fig. 2: A-B; Fig.3: A-B). Ligand density has an effect in both
KPLHALL and ALL peptide sequence, where the highest concentrations of ligand density have higher GAG levels for both ALL and KPLHALL (Fig. 2: A-B). The length of the residues from the IGFBP-5 controls the GAG productions where the longer sequence, KPLHALL, contains 7 times more GAG than the shorter sequence ALL (Fig. 2C). GAG production increased 3.5 fold with 909 µM of ALL, where it increased almost 7 fold only with 11.37 µM of KPLHALL (Fig. 2C).

The presence of IGF-I binding sites also increased HYPRO retention for both KPLHALL and ALL in transfected chondrocytes (Fig. 3: A-B), where the higher concentrations of ligand density have higher HYPRO levels (Fig. 3: A-B). In contrast to GAG, the length of the residues from the IGFBP-I has little effect in HYPRO productions, both the longer sequence KPLHALL and the shorter sequence ALL produced similar levels (Fig. 3C). However, it requires almost 3 times more of ALL to obtain the similar levels of HYPRO than KPLHALL sequence (Fig. 3C).

Discussion: This study demonstrated that a chemically customized alginate with an IGF-I binding sequence from IGFBP-5 enhanced chondrocyte gene therapy. Peptide length influences IGF-I binding and metabolic activity of transfected chondrocytes. Density of binding sites increases GAG and HYPRO production for both peptide sequences. At the same time, peptide length has a greater effect on GAG production than HYPRO. Collectively, peptide length and concentration enables high degree of tuning metabolic activity of chondrocytes.

Significance: This study combines tissue engineering and gene therapy, where customized biomaterials augmented chondrocyte gene therapy. Customized biomaterial (alginate) was able to tune binding of IGF-I to alginate, which affects GAG and HYPRO production by transfected chondrocytes.
**Figure 1**: Immunohistochemistry of constructs for IGF-I at Day 30. Scale bar = 100 μm

**Figure 2**: A) GAG production transfected chondrocytes cultured in KPLHALL peptide sequence B) GAG production transfected chondrocytes cultured in ALL sequence C) GAG Dose Response Curve (R² = 0.99)
Figure 3: A) HYPRO production transfected chondrocytes cultured in KPLHALL peptide sequence B) HYPRO production transfected chondrocytes cultured in ALL sequence C) HYPRO Dose Response Curve (R² = 0.99)