Customized Biomaterials to Enhance Gene Therapy by Augmenting Retention of Target Proteins

IN Aguilar, SB Trippel, S Shi, LJ Bonassar

1 Department of Biomedical Engineering, Cornell University, Ithaca, NY. 2Orthopaedic Surgery, Indiana University, Indianapolis, IN. 3Sibley School of Mechanical and Aerospace Engineering, Cornell University, Ithaca, NY.

ABSTRACT

Gene therapy is used to up-regulate a target protein, but unfortunately the expression of such proteins is highly transient, with rapid decay to sub-therapeutic levels. In this study, biomaterials were customized to enhance gene therapy by binding and enhancing residence time of a target protein, IGF-I. Carbodiimide chemistry was used to modify the material to contain peptides known to bind IGF-I. The biomaterial was able to enhance residence time of IGF-I and increase metabolic activity of target cells.

INTRODUCTION

Gene therapy is used to stimulate reparative activities in damaged tissues. However, gene therapy’s main limitation is the transient expression of the target protein, which rapidly decays to sub-therapeutic levels. As such, gene therapy would be aided by materials that specifically retain the therapeutic protein.

Some of these therapeutic proteins are growth factors, specifically insulin-like growth factor I (IGF-I). IGF-I is known as a potent anabolic regulator of metabolism and has been widely studied as a means to enhance matrix synthesis in different tissues. Our lab has previously shown that grafting peptide sequences from IGF-I binding protein, specifically IGFBP-5, to alginate enhances IGF-I binding and delays protein loss in a ligand-density dependent manner. The goal of this study was to use these materials to enhance IGF-I gene therapy by increasing the duration of exposure of chondrocytes to IGF-I and thereby enhance their matrix synthesis.

EXPERIMENTAL METHODS

Alginate was chosen as delivery material because of its suitability as both drug delivery vehicle and its ability to encapsulate transfected cells. UP LVG alginate was chemically modified through carbodiimide chemistry with GGG-KPLALL (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 of 0 to 70 µM.

Articular chondrocytes were harvested from 1-3 day old calves via collagenase digestion. Plasmid adenoassociated virus (pAAV) was used as a vector due its higher transfection efficiency than plasmid DNA (pcDNA). Also, pAAV does not have the potential of significant inflammatory immune responses for adenoviral vectors and the difficulty to produce high enough titer of adeno-associated virus (rAAV) viral vectors to efficiently transduce chondrocytes. Cells were transfected using two different complexes: FuGENE 6+pAAV/IGF-I (Transfected) and FuGENE 6+pAAV/Empty (Control) for 16 hours. Using this protocol, IGF-I synthesis is enhanced 9-fold over a period of 6 days.

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 CaCl₂ solution. Beads were incubated with DMEM without FBS for 30 days. Synthesis of glycosaminoglycan (GAG) was used as the primary measure of chondrocyte metabolic activity. GAG beads and media using the dye 1,9-dimethylmethylen blue (DMMB) dye-binding assay. The kinetics of GAG accumulation were fit to an established model of matrix synthesis to calculate steady-state GAG content, and these steady-state values of GAG synthesis were used to determine the effect of binding site density on chondrocyte matrix synthesis using a generalized variable slope concentration–response model.

RESULTS AND DISCUSSION

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.1A). 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. 1B). The total GAG release to the media was lower when compare to the total GAG in the constructs for both transfected and controls (Fi.2A, 2B).

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.3A and Table 1A). 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 (EC₅₀) was 11.4 µM for transfected chondrocytes and 21.2 µM for control chondrocytes (Table 1). Similarly,
the amount of GAG in media increase with the concentration of IGF-I binding sites (Fig. 3B and Table 1B). In the absence of IGF-I binding sites, transfection with IGF-I gene increased GAG in media by 20%. At the highest binding site density, transfection increased GAG synthesis by 100% compared to controls (Fig. 3B).

In the absence of IGF-I binding sites, transfection with IGF-I gene increased GAG in media by 20%. At the highest binding site density, transfection increased GAG synthesis by 100% compared to controls (Fig. 3B).

CONCLUSION
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. In alginate gels with maximally effective concentrations of IGF-I binding sites, pAAV/IGF-I increased GAG accumulation by more than 600% (Fig. 3A). To our knowledge, this is the first report of the use of a customized biomaterial to enhance the effect of gene therapy by increasing the retention of the target protein. This technology could be applicable to a wide range of gene targets, particularly growth factors that have known binding sites such as transforming growth factor beta (TGF-β).

REFERENCES
7. Zestawaski W et al. EMBO J. 2001; 14:3638-44

ACKNOWLEDGMENTS
This research is supported by Alfred P. Sloan Foundation, Coleman Foundation, Award NIH/NIAMS F31AR061982, NIH Grant R01 AR047702 and the Department of Veterans Affairs