A Biodegradable Hydrogel Encapsulating IGF-I Transfected Chondrocytes for Cartilage Tissue Engineering

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Statement of Purpose: Hydrogels have been used as 3-D scaffolds to support cell proliferation and new tissue growth for cartilage repair. Recent work has also indicated that gene therapy techniques to transfect chondrocytes with growth factors such as insulin-like growth factor-I (IGF-I) may stimulate the chondrocytes to synthesize more of the extracellular matrix of articular cartilage. We hypothesize that combining a hydrogel scaffold and IGF-I gene therapy is superior to IGF-I gene therapy alone as a technique for cartilage tissue engineering. In this study, we designed and synthesized an injectable, in situ gelable, biocompatible and biodegradable hydrogel based on dextran as a scaffold to encapsulate chondrocytes and support cell proliferation and differentiation. To circumvent the limitations of gene therapy involving viral vectors, we chose to use a non-viral method [1] to deliver IGF-I gene to the target cells.

Methods: Articular cartilage was harvested from the knee joint of adult goats and isolated chondrocytes were cultured in monolayer at 37˚C in humid environment with 5% CO_2. Nearly confluent cells were transfected with pcDNA-humanIGF-I or pcDNA-goatIGF-I three days after cell seeding. Transfection was performed in 10%FBS DMEM with a 3:1 ratio of FuGene6 and DNA for 4 hours. Transfected cells were trypsinized at 1 day post-transfection and stored at 4˚C overnight before encapsulation into hydrogel, or maintained in monolayer culture at 37°C in a humid environment with 5% CO_2.

Dextran (MW 15-20K) was modified by substituting a number of –OH groups with hydrolysable units ended with polymerizable groups. The modified dextran macromer was then dissolved in Dulbecco's Phosphate Buffered Saline containing Irgacure 2959 (0.05 w/w% of dry polymer) at concentration of 13.3 w/v%, and sterilized by filtering through 0.45µm syringe filter. Chondrocytes were suspended in the sterilized macromer solution at 5 million/ml. The cell suspended macromer solution was thereafter added into a 96-well plate, 100µl per well, and illuminated with a 4W long-wave UV lamp for 8 min to form disc-shaped hydrogels. The gels were transferred to 24-well plate, washed twice with cell culture medium, and cultured in fresh medium.

IGF-I and GAG in the conditioned medium was quantified by ELISA and 1,9-dimethylmethylene blue (DMMB) method, respectively. Hydrogel samples were freeze-dried overnight, crushed into small pieces and digested in 250µg/ml proteinase K solution at 62°C for three hours. After digestion, the samples were centrifuged to spin down the polymer pieces. The supernatant was taken for DNA and GAG quantification using PicoGreen® assay and DMMB method, respectively. Monolayer cultured cells were digested with proteinase K solution; and GAG and DNA were quantified using the same methods as above.

Results/Discussion: Chondrocytes encapsulated in the hydrogel maintained 80-90% viability in culture, indicating biocompatibility with these cells.

In monolayer culture, chondrocytes transfected with goat-IGF-I (gIGF-I) or human-IGF-I (hIGF-I) produced significantly higher amounts of IGF-I within the first 2-3 days (Fig. 1) than untransfected cells. However, GAG synthesis between hIGF-I transfected and untransfected was not significantly different for up to 6 days post-transfection (Fig. 2). On the other hand, in a hydrogel culture experiment using a different cell preparation, both hIGF-I and gIGF-I transfected goat chondrocytes cultured in hydrogel produced significantly higher amounts of IGF-I than the control cells; and IGF-I production mainly occurred during the first three days post transfection (Fig.3). However, in contrast to monolayer cells, both gIGF-I and hIGF-I transfected chondrocytes in 3D hydrogel culture produced significantly more GAG than the untransfected cells as of Day 6 (Fig. 4).

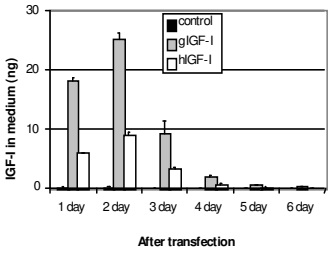

***Figure 1**. IGF-I quantification in the medium of monolayer cultured chondrocytes.*

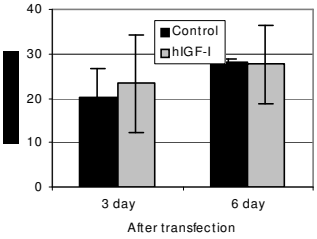

***Figure 2**. Total (cell layer and medium) GAG/DNA quantification of monolayer cultured chondrocytes.*

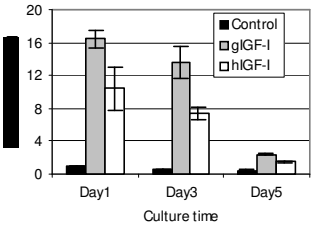

***Figure 3**. IGF-I quantification in the medium culturing chondrocyte-encapsulated chondrocytes.*

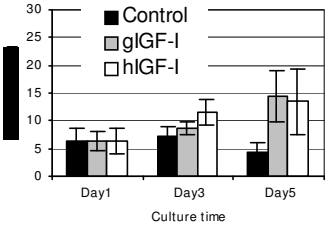

***Figure 4**. Total (medium and hydrogel construct) GAG/DNA values of 3D in vitro culture.*

Conclusions: We tested the feasibility of combining non-viral gene therapy and dextran hydrogel-based tissue engineering of articular cartilage. We found that within the time frame of this study, the 3D hydrogel network provided a suitable environment for the cells to synthesize the extracellular matrix. More importantly, the data suggest that the 3D hydrogel environment may be necessary for the IGF-I transfected chondrocytes to respond to the overexpressed IGF-I protein.

Reference: 1. Madry, H and Trippel, SB, Gene Ther., 2000, 7, 286.
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