In vitro chondrogenic differentiation of rabbit marrow stromal cells encapsulated in oligo(poly(ethylene glycol) fumarate) injectable hydrogel composites

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Statement of Purpose: Hydrogels are promising materials as cell carriers and growth factor delivery systems in cartilage tissue engineering since they have high water content which allows for the transport of nutrients and supplements for cell activity. Hydrogels can also provide temporary supports during a healing process and often used in the form of injectable materials providing a minimally invasive way of implantation [1]. Oligo(poly(ethylene glycol) fumarate) (OPF) macromers, can be crosslinked *in situ* to form hydrogels that are both biocompatible and biodegradable. During crosslinking of OPF, cells and growth factor-loaded microparticles can be encapsulated in the gel network. This study focuses on the evaluation of degradable oligo(poly(ethylene glycol) fumarate) hydrogel composites, which are designed to deliver marrow stromal cells with gelatin microparticles loaded with transforming growth factor-β1 (TGF-β1) and direct the cells to chondrogenic lineage.

Methods: In this study, gelatin microparticles (MPs) were loaded with transforming growth factor β1 (TGF-β1), a cytokine known to promote chondrogenic differentiation of progenitor cells, in order to assess its effects on the function of co-encapsulated marrow stromal cells (MSCs). Rabbit MSCs and loaded MPs were mixed with OPF (Mn 14,500), a poly(ethylene glycol)-diacrylate crosslinker (MW 3400) and the radical initiators ammonium persulfate and N,N,N',N'-tetramethylethylenediamine (0.03 M) and crosslinked at 37°C for 8 minutes to form hydrogel composites. Controls for the study included hydrogels encapsulating only cells and hydrogel composites encapsulating cells and unloaded MPs. After 14 days of *in vitro* culture, constructs were examined for chondrogenic differentiation via quantitative, real-time RT-PCR for expression of genes for type II collagen, type I collagen, and aggrecan. All gene expression was normalized to GAPDH.

Results/Discussion: After 14 days of *in vitro* culture, quantitative RT-PCR results showed that only hydrogel composites containing TGF-β1-loaded MPs had a significant increase in collagen type II and aggrecan gene expression, a characteristics of chondrogenic phenotype, while collagen type I gene expression was consistent over the culture period. Collagen type II expression had 73 fold increase at day 14 and aggrecan showed 93 fold increase at day 7 and increased up to 152 folds as compared with a control group at day 0. The expression level of controls (Day 0) was represented as one. These results suggest that rabbit MSCs remained viable over the culture period and the presence of TGF-β1 in OPF hydrogel composites promoted the chonrogenic differentiation of rabbit MSCs encapsulated in OPF hydrogel composites *in vitro*.

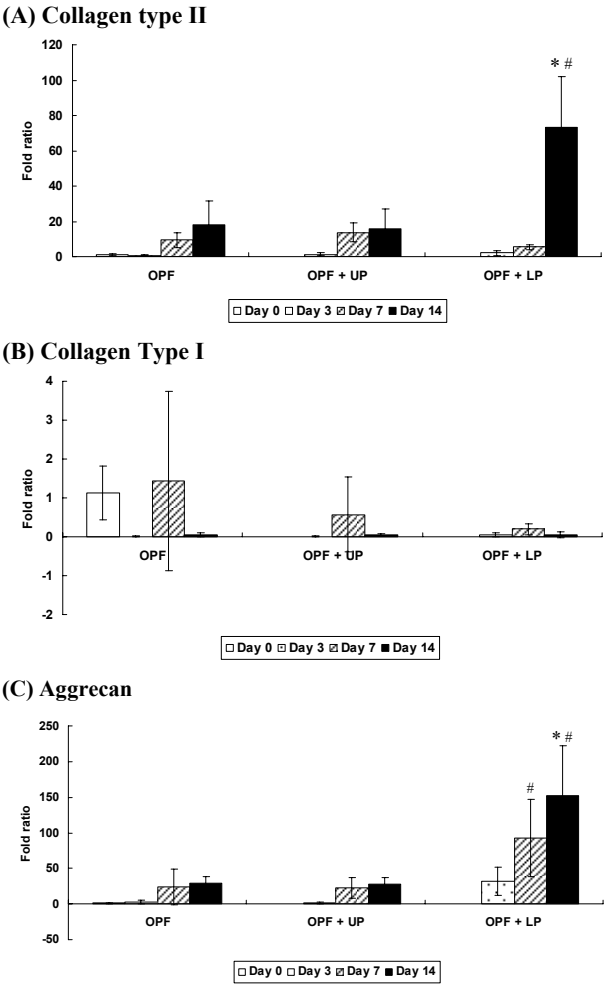

Figure 1. Quantitative analysis of gene expression presented as fold ratio compared to the expression level of controls (Day 0) for OPF hydrogel composites encapsulating MSCs (OPF), MSCs /unloaded microparticles (OPF+UP), or MSCs/TGF-β1-loaded microparticles (OPF+LP). Significantly higher gene expression from day 0 values (controls) are noted with (#). Samples indicated with (*) had significantly higher gene expression than the other two groups ($p<0.05$).

Conclusions: This study investigated OPF hydrogel composites as MSC carriers for cartilage tissue engineering. The gene expression results demonstrate that MSCs retained their ability to respond to growth factors, thus suggesting the potential of OPF hydrogel composites as part of a novel cartilage regeneration strategy involving localized delivery of cells and bioactive molecules.

References: Temenoff JS, Mikos AG. Biomaterials 2000;21:2405-2412.