Co-encapsulation of TGF-β1 into Negatively Charged Oligo(polyethylene glycol) Fumarate (OPF) Hydrogels to Enhance Chondrogenesis

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Introduction
Current methods of repairing damaged cartilage cannot predictably restore the articular surface of the joint. One strategy with great promise to provide functional cartilaginous constructs for repair of damaged articular cartilage is tissue engineering. Previously, our group demonstrated that chondrocytes remained viable when seeded in monolayer on modified OPF hydrogels. These modifications incorporated positive or negative charge into the hydrogels. Chondrocytes seeded on negatively charged hydrogels exhibited significantly greater proliferation and produced more GAG and collagen type II than their neutral and positive counterparts. In this study, we demonstrate that incorporation of negative charged into OPF hydrogels enhances chondrogenesis of bone marrow mesenchymal stem cells (bMSCs) and further that co-encapsulation with TGF-β1 is sufficient to induce equivalent levels of chondrogenesis.

Methods
OPF was synthesized from purified polyethylene glycol with initial MW of 10,000 according to a previously published method. OPF macromer was dissolved to a final concentration of 33% (w/w) in deionized water containing 0.05% (w/w) of a photoinitiator (Irgacure 2959) and 0.33% (w/w) N-vinyl pyrrolidinone (NVP). After filtration, bMSCs at a density of 15 million cells per mL of hydrogel solution. There were 6 experimental groups: neutral hydrogels with (1) high concentration of TGF-β1 [40ng/scaffold], (2) low concentration of TGF-β1 [4ng/scaffold] and (3) with TGF-β1 supplemented in media [10 ng/mL every media change]; and negatively charged hydrogels with (4) high, (5) low and (6) TGF-β1 supplemented in media. To obtain negatively charged hydrogels, 0.916 mM sodium methacrylate (SMA) was added. TGF-β1 was added at appropriate concentration into hydrogel solution. The solution was polymerized using UV light (365 nm) at an intensity of ~8mW/cm² for 10 min. Hydrogels were cut into 4mm diameter, 1mm thick disks, washed in PBS, placed in chondrogenic media consisting of 100 mL of DMEM supplemented with 0.1% BSA plus ITS+ (insulin, transferring, and selenious acid, plus linoleic acid and BSA), proline, Pen/Strp and ascorbic acid and incubated at 37°C for 1, 7, or 21 days. Every 3 days, media was collected and replaced with fresh media. Upon harvest, samples were analyzed for aggregate modulus and GAG content. Single factor analysis of variance (ANOVA) was performed to assess the statistical significance across the groups (p < 0.05).

Results
All neutral hydrogels were significantly stiffer than negatively charged hydrogels (Fig 1A vs. Fig 1B). In the negatively charged hydrogels, the aggregate moduli increased as compared to initial values (Fig. 1A). The increase was significant in the high concentration TGF-β1 group and TGF-β1 in the media group. There were no significant differences between TGF-β1 administration groups at each respective time point.

Conclusions
bMSCs can successfully be incorporated into negatively charged OPF hydrogels and stimulated towards chondrogenesis. Since there were no significant differences between TGF-β1 administration groups, it is reasonable to conclude that initial incorporation of TGF-β1 into these OPF hydrogels is sufficient for stimulating chondrogenesis. Moreover, the mechanical properties of negatively charged OPF hydrogels improve over time.

Significance
The ability to repair damaged cartilage can greatly reduce the number of patients that develop arthritis and can lead to prevention of its degradation. This study addresses some of the shortcomings of current surgical repair techniques of damaged articular cartilage by developing a photopolymerizable hydrogel-cell construct that can be injected and polymerized in debrided areas for cartilage repair via arthroscopic techniques.

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References