Co-encapsulation of TGF-β1 into Negatively Charged Oligo(polyethylene glycol) Fumarate Hydrogels to Enhance Chondrogenesis of Bone Marrow Stromal Cells

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Introduction: Once injured, articular cartilage has a poor intrinsic capacity to heal. Methods currently in use cannot predictably restore the articular surface of the joint. Cartilage tissue engineering is one strategy with great promise that provides functional cartilaginous constructs for use in repair of damaged cartilage. Previously, our group demonstrated that chondrocytes remained viable when seeded in monolayer on charged modified oligo(polyethylene glycol) fumarate (OPF) hydrogels.[1] Chondrocytes seeded on negatively charged hydrogels exhibited significantly greater proliferation and produced more GAG and collagen type II than their neutral and [1] In this study, *we demonstrate that co-encapsulation of TGF-β1 and bone marrow stromal cells (bMSCs) into modified OPF hydrogels is sufficient to induce chondrogenesis and negatively charged hydrogels enhance chondrogenesis of bMSCs.*

Methods: OPF was synthesized from purified polyethylene glycol with initial MW of 10,000 according to a previously published method.[2] 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 were added to 1mL of hydrogel solution. There were 6 experimental groups: neutral hydrogels with (1) high TGF-β1 concentration [20ng/mL], (2) low TGF-β1 concentration [2ng/mL] 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 containing bMSCs. The solution was polymerized using UV light (365 nm) at an intensity of ~8mW/cm^2 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 compressive modulus and GAG content. TGF-β1 concentration in media was recorded throughout culture period using an ELISA kit. Single factor analysis of variance (ANOVA) was performed to assess the statistical significance across the groups ($p < 0.05$).

Results: Figure 1 demonstrates that all neutral hydrogels were significantly more stiff than the negatively charged hydrogels but not statistically different from each other. In the negatively charged hydrogels, there was an increase in compressive moduli when compared to initial values. This increase was significant only in the high concentration TGF-β1 group. There were no significant differences between TGF-β1 concentration groups at their respective time points.

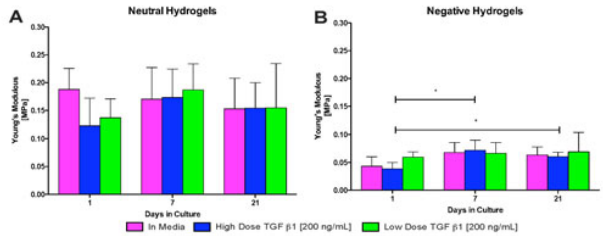

Figure 1: Compressive Moduli (A) Neutral OPF Hydrogels (B) Negatively Charged OPF Hydrogels

Figure 2 demonstrates that MSCs encapsulated in both neutral and negatively charged hydrogels were producing significantly more GAG at day 21 as compared to day 1. Also, the negatively charged hydrogels produced significantly more GAG than the neutral hydrogels by day 21 at all the different TGF-β1 concentrations. No significant differences arose between TGF-β1 concentration groups at any time point with the exception of day 7 in the neutral hydrogels.

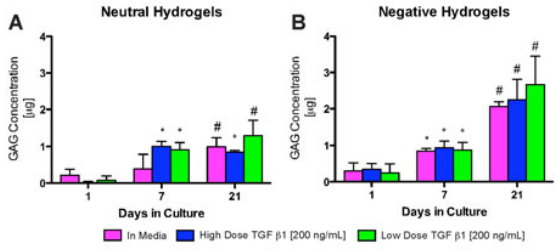

Figure 2: GAG Concentration (A) Neutral OPF Hydrogels (B) Negatively Charged OPF Hydrogels. * significantly different from day 1. # significantly different from day 1 and day 7.

Measurements of TGF-β1 in the media demonstrated that for the first collection the TGF-β1 supplemented media group absorbed TGF-β1 from the media then the concentration collected stabilized, while the high concentration released TGF-β1 initially then stabilized by day 5, and the low concentration group released the same amount throughout.

Conclusions: Since there were no significant differences between TGF-β1 concentration groups, it is reasonable to conclude that an initial incorporation of TGF-β1 into these OPF hydrogels is sufficient for stimulation of chondrogenesis. Moreover, our data demonstrate that negatively charged hydrogels significantly improved chondrocyte matrix production by 21 days.

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References

1. Dadsetan et al. Acta Biomaterials (2010 submitted).
2. Jo S et al. Biomacromolecules 2001;2(1):255-261.