Effect of Hydrogel Charge on Marrow Stromal Cell Chondrogenic Phenotypic Expression

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Introduction: The architecture of scaffolds is a significant factor in cartilage cell function. In this work, the charge and interconnectivity of microporous oligo-(polyethylene glycol) fumarate (OPF) hydrogels were assessed by biochemical analysis for collagen and glycosaminoglycan (GAG) content, and by reverse transcription polymerase chain reaction (RT-PCR) for gene expression. We also investigated the effect of biochemical charge features of the OPF hydrogels had on marrow stromal cell differentiation in chondrogenic media with transforming growth factor beta (TGF-β).
Materials and Methods: OPF was synthesized using polyethylene glycol (PEG) with an initial molecular weight of 10kDa according to a published method.[1]
Hydrogel fabrication: Hydrogels were made by dissolving OPF macromer to a final concentration of 33% (w/w) in deionized water containing 0.05% (w/w) of a photoinitiator (Irgacure 2959, Ciba-Specialty Chemicals) and 0.33% (w/w) N-vinyl pyrrolidinone (NVP). In order to obtain hydrogel with 80% porosity, 1 ml of macromer solution was mixed with 4g of sodium chloride particles (300 μm diameter), and polymerized using 365 nm UV light at an intensity of ~8mW/cm^2 (Blak-Ray) for 10 min. Positive and Negatively charged hydrogels were manufactured by adding the comonomers [2-(Methacryloyloxy)ethyl]-trimethylammonium chloride and methacrylic acid, respectively.
Marrow stromal cell isolation, culture and characterization: MSCs were isolated from the femurs and tibiae of male Sprague Dawely rats according to a previously described method.[2] Prior to cell seeding, samples were disinfected with 70% ethanol for 60 min. The ethanol was then aspirated, and the samples were soaked in sterile PBS for 1 h with three changes, followed by two additional changes of media and incubation over night. Then 25 μl of the cell suspension containing 250,000 cells was seeded onto the hydrogel foams in 24 well plates and incubated for 3 h to allow the cells to attach. About 1 ml of chondrogenic media was added to each well, and the medium was changed every 2-3 days. At days 7 and 28, samples were washed with PBS three times and frozen in one ml dH_2O at -80°C. Samples underwent two freeze/thaw cycles with sonication on ice for 30 min after each cycle prior to analysis. DNA content were determined by the PicoGreen DNA kit (Molecular probes, Eugene, OR) according to the manufacturer's instructions. GAG content was measured at day 28 using a commercially available kit (Blyscan), according to the manufacturer's instructions (Biocolor Ltd, United Kingdom). RT-PCR was carried out for three well characterized markers for chondrogenic differentiation: Aggrecan, Sox-9, and Type II collagen as well as the housekeeping gene GAPDH according to previously published conditions.[3,4]

Results: Figure 1 shows a reverse transcriptase reaction for the hydrogels at 7, 21 days respectively for neutral, positive, and negative hydrogel with 80% pore fraction. This gel reveals the increase in mRNA expression of the three genes studied in a time dependent as well as scaffold dependent manner. From the gels, one can visualize that positively charged hydrogels support a greater increase in early chondrogenic gene expression.

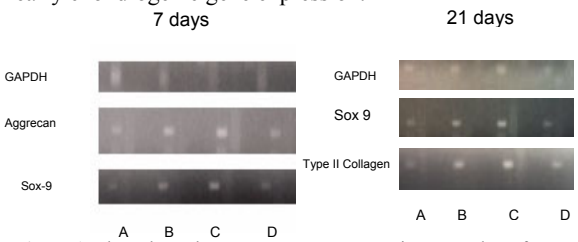

Figure 1: The 7 day gel represents reverse transcriptase products for each of the scaffolds: Tissue culture plate control (A), Neutral Charged hydrogel (B), Positively Charged hydrogel (C), Negatively charged hydrogel (D). The 21 day results are also shown below.

The total number of cells cultured on the porous hydrogels was quantified with a DNA assay. GAG content, an indicator and product of mature chondrocytes were measured and normalized by the total cell number and DNA content for each sample. The difference in the GAG content of the hydrogel scaffolds with different charges was significant ($P<.05$). This indicates that the porous positively charged hydrogel scaffolds supported differentiation of the MSCs to the chondrocyte lineage phenotype.

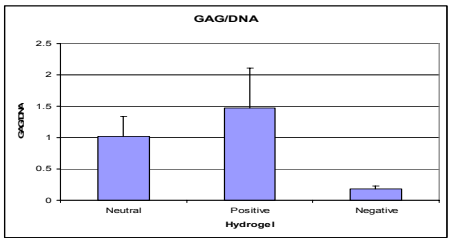

Figure 2: GAG content of MSCs cultured on porous charged OPF hydrogel after 4 weeks.

Conclusions: Our results showed that porous positively charged hydrogels provide a superior microenvironment to support chondrocytic differentiation. Porous hydrogels appeared to support the differentiation of MSCs and may find application in cartilage tissue engineering.

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3-Bryant SJ et al *Biotechnology and bioengineering* 86, 747-755 (2004).
4-Johnstone, B. et al, *Exp Cell Res* 238, 265-72 (1998).

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