Effect of surface electrical charge on chondrogenic differentiation of ADSCs

Fatemeh Babaei[1, 2], Yun-Wah Lam[2], Allan B. Dietz[3], Michael J. Yaszemski[1], and Mahrokh Dadsetan[1].

[1]Department of Orthopedic Surgery, Mayo Clinic, College of Medicine, Rochester, MN, USA
[2] Department of Biology and Chemistry, City University of Hong Kong, Hong Kong SAR, China
[3]Department of Transfusion Medicine, Mayo Clinic, College of Medicine, Rochester, MN, USA

Statement of Purpose: The treatment of cartilage injuries remains one of the most difficult challenges in medicine, because of limited capacity of damaged cartilage to regenerate. In the past decade, new strategies have been developed for the design of biodegradable polymeric compounds which function as a temporary support for the engineering of living constructs in tissue engineering applications. In this study, we use a novel biodegradable, synthetic hydrogel, oligo (polyethylene glycol) fumarate (OPF) conjugated with negatively charged groups as a matrix for adipose-derived mesenchymal stem cells (ADSCs) attachment and differentiation. We have previously shown that OPF is highly biocompatible and permeable to the oxygen and nutrients as well as cell metabolites. In this study, we aim to investigate the effect of hydrogel chemistry and growth factor loading on chondrogenic differentiation of human ADCSs. These scaffolds will be further used for delivery of ADSCs and growth factors to a rabbit knee defect.

Methods: OPF was copolymerized with sodium methacrylate (SMA) as a component for introducing the negative charge into OPF hydrogel. Sodium chloride salt with a particle size of 300µm (diameter) was added to the mixture to provide porosity and cross linked under UV light. ADSCs were seeded on hydrogels by using rotary wall vessel bioreactor for 24hrs. To evaluate the effect of charge on cell proliferation and differentiation, hydrogels with four different charge densities (0%, 10%, 20% and 30% of SMA) were seeded with ADSCs. The resulting hydrogel-cell constructs were transferred to cell culture plates and treated with t TGF-β up to 7 days to check the chondrogenic differentiation of each group. Cell proliferation assay and dynamic mechanical analysis (DMA) were performed on these sets of samples. Statistical analysis was carried out using a Stat View software. All differences were calculated using one-way ANOVA. A *p* value less than 0.05 were deemed significant.

Results: As shown in following Figures, initial cell attachment improved on hydrogels with 30% SMA in the presence of TGF-β, while it was similar on all hydrogel formulations in media without TGF-β. Results from MTS assay demonstrated that ADSCs had higher proliferation rate on hydrogel with 30% SMA. The cell numbers on hydrogel specimens with 30% SMA significantly increased at day7 as compared to hydrogels with 0% SMA in absence of TGF-β. At day 1, cell attachment on hydrogel with 30% SMA was higher than SMA0 in the presence of TGF- β and it significantly increased by day 7.

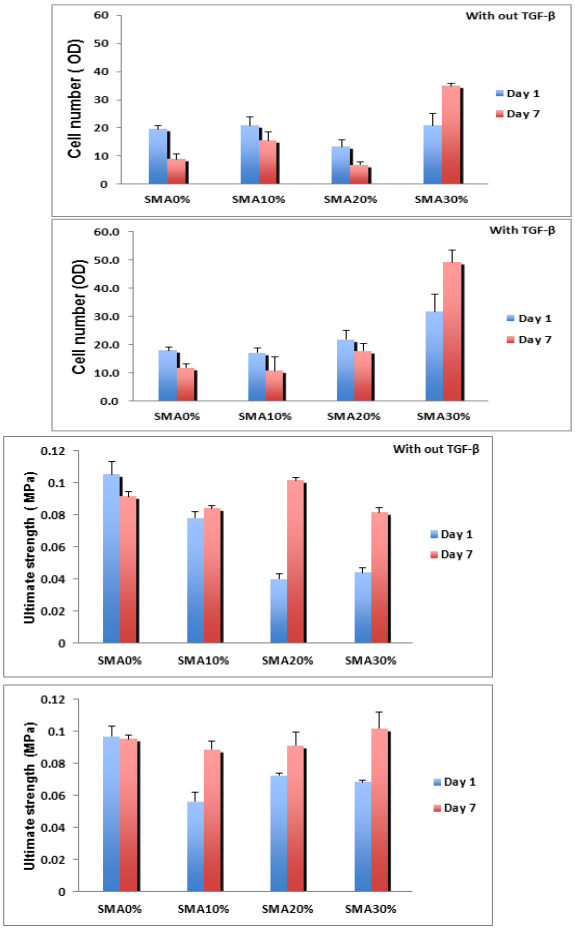

Figure1: Cell numbers and ultimate strength of hydrogel samples seeded with ADSCs. Values represent mean ± standard error (n = 3).

We demonstrated that compressive modulus of the hydrogels decreased with addition of SMA to their formulation. This trend was also seen for ultimate strength. However, ultimate strength of the samples increased over time on hydrogels with higher amounts of SMA, while compressive modulus remained unchanged.

Conclusions: Our data suggest that negatively charged hydrogel support ADSC attachment and proliferation and ultimate strength of the hydrogels seeded with cells improved over time. **Ongoing work**: Chondrogenic differentiation of the cells is currently under investigation in vitro and in vivo.

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