Optimization of Synovium-Derived Stem Cells for Cartilage Tissue Engineering

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INTRODUCTION

The avascular nature of cartilage and the harsh joint loading environment lead to a poor intrinsic healing capacity after injury and the need for cell-based therapies for repair. As a result, cell-based therapies including tissue engineering strategies for growing clinically relevant grafts are being intensively researched. The clinical potential of stem cells has driven forward efforts toward their optimization for tissue engineering applications. Specifically, synovium-derived stem cells (SDSCs) have been shown to have potential for differentiating down a chondrogenic lineage and are thought to aid in articular cartilage repair after damage *in vivo*[1]. In the present study, we analyze the potential of SDSCs as a tissue engineering strategy for growing clinically relevant cartilage grafts. In particular, we investigate the effect of seeding density of SDSCs in 2D culture on development of subsequent tissue properties formed by these cells after seeding in three-dimensional (3D) hydrogel scaffolds as well as the concurrent impact of transient TGF-β3 growth factor introduction in 3D culture.

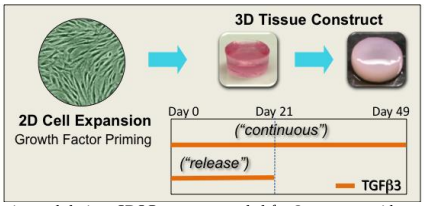

Figure 1. Experimental design. SDSCs were expanded for 2 passages with expansion media containing growth factors. After encapsulation in 3D, constructs were cultured with serum-free chondrogenic media plus TGF-β3. At Day 21, TGF-β3 was removed from half of the groups ('release').

METHODS

Synovium tissue was harvested aseptically from juvenile bovine knee joints (2-4 weeks) (IACUC-exempt), minced and digested for 6 hrs in a 37°C, 5% CO_2 incubator using collagenase type V (494 U/mg). Cells were seeded on tissue-culture treated plastic at low (173 cells/cm^2) and high density (1733 cells/cm^2) and primed for 2 passages with expansion media containing MEM, 10% FBS, pen/strep, that was further supplemented with a growth factor cocktail of 1 ng/ml TGF-β3, 10 ng/ml PDGF-ββ, and 5 ng/ml FGF-2. After passaging, cells were encapsulated in 2% w/v agarose (Type VII, Sigma) at 60 x 10^6 cells/mL and cultured with serum-free chondrogenic media (DMEM, 1% ITS+ Premix, 50 µg/ml L-proline, 0.1 µM dexamethasone, 0.9 mM sodium pyruvate, antibiotics) plus ascorbate (50 µg/ml) and TGF-β3 (10 ng/ml). At Day 21, TGF-β3 was removed from half of the groups ('release'), while the other half continued to receive the growth factor for the remainder of the study ('continuous') (Fig 1). Time points for mechanical, biochemical, and histological analyses occurred at days 0, 21, 35, 49. Mechanical testing was performed using a custom testing device and a stress-relaxation test (for E_Y) followed by dynamic compression (for G*). Glycosaminoglycan (GAG) was measured using a colorimetric assay and collagen content was determined using a standard hydroxyproline (OHP) assay. Samples were prepared for histology, and stained with Picrosirius Red and Alcian Blue to determine the distribution of collagen and GAG, respectively. Statistical analysis was performed using ANOVA and Tukey HSD post-hoc testing with $\alpha<0.05$.

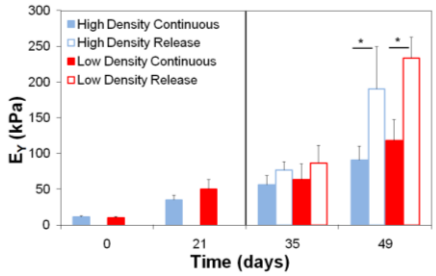

Figure 2. Young's modulus (E_Y) of continuous and release groups over 49 day study period. *p<0.05 as compared to corresponding continuous group (n=5 constructs/group).

RESULTS

Both high and low density release groups at Day 49 (190 ± 58 kPa and 234 ± 29 kPa, respectively) exhibited a significant 2-fold higher Young's modulus (E_Y) as compared to corresponding continuous groups ($p<0.05$) (Fig 2). Therefore, transient application of TGF-β3 to constructs comprised of SDSCs primed with growth factors during expansion led to increased tissue properties over sustained growth factor application, and seeding density during expansion was determined not to be a significant contributor to subsequent tissue development. The increases in material properties were accompanied by corresponding increases in GAG content (Fig. 3). Specifically, a significant 1.5 fold greater % GAG/ww was observed in high density release constructs (5.1 ± 0.42 %/ww) at Day 49 versus the corresponding continuous constructs ($p<0.05$). Collagen content for both groups remained at comparable levels at Day 49 (data not shown). Strong GAG and collagen staining was observed in the central region for the release groups at Day 49, whereas the continuous groups showed more even distribution throughout (Fig 4).

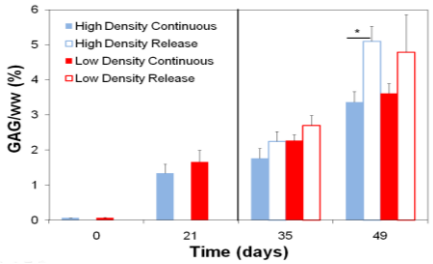

Figure 3. GAG content (%/ww) of continuous and release groups over 49 day study period. *p<0.05 as compared to corresponding continuous group.

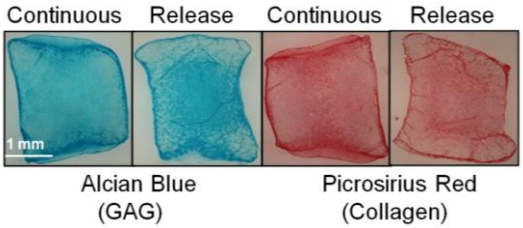

Figure 4. Representative GAG and collagen staining at Day 49.

DISCUSSION

In the current study, the clinical potential of SDSCs as a cartilage replacement source was investigated. Engineered cartilage constructs which received transient application of TGF-β3 exhibited significantly higher mechanical and biochemical properties than those that received constant growth factor stimulation when cultured in agarose hydrogel, a tissue engineered scaffold currently being used for autologous cartilage repair strategies clinically[2]. The 'release' response is similar to that which we have previously reported for juvenile chondrocytes[3]. The tissue properties achieved are closer to native cartilage levels at 7 weeks of culture than those previously reported for cartilage engineering studies utilizing stem cells as a cell source. This study also reports that initial seeding density of SDSCs during expansion does not have a significant impact on tissue development in 3D hydrogel constructs. Our findings demonstrate the potential for SDSCs as a clinically-relevant cell source for regenerative strategies for articular cartilage repair. This work is consistent with approaches used to optimize adipose-derived stem cells for cartilage repair[4]. Future studies will focus on incorporation of physiologic loading bioreactors to expedite functional cartilage growth, as we have previously reported for chondrocytes[5].

ACKNOWLEDGEMENTS

This work was supported by NIH grants AR46568 and AR52871.

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