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# PRIMING OF SYNOVIUM-DERIVED MESENCHYMAL STEM CELLS FOR CARTILAGE TISSUE ENGINEERING

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## INTRODUCTION:

The clinical potential of stem cells has driven forward efforts toward their optimization for tissue engineering applications. The intimal layer of the synovium is composed of two cell types, macrophages and fibroblast-like cells. The fibroblast-like cells, often referred to as synovial-derived mesenchymal stem cells (sMSCs), have the capability to differentiate down a chondrogenic lineage[1]. In addition, *in vivo* tests have shown that synovial cells may be recruited from the synovial membrane to aid in the repair of articular cartilage defects[2].

Cell passaging and priming with chemical or physical factors are often necessary steps in cell-based strategies for regenerative medicine including tissue engineering. To elaborate, culturing of cells on a two dimensional (2D) tissue culture dish provides a platform to increase cell number (expansion) as well as an opportunity to prime cells with chemical and physical stimuli that can induce cell differentiation toward a desired lineage (e.g., chondrogenic potential).[3] In the present study, we investigate the role of chemical priming of sMSCs in 2D culture on development of subsequent tissue properties formed by these cells after seeding in three-dimensional (3D) hydrogel scaffolds. Our findings demonstrate the potential for sMSCs as a clinically-relevant cell source for regenerative strategies for articular cartilage repair.

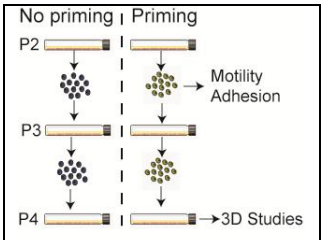


Fig. 1: Experimental design. sMSCs were cultured with and without GFs. Cell morphology and proliferation were examined at each passage. At P2, cell adhesion strength and motility were quantified.

## METHODS:

**Cell isolation:** Synovial tissue was harvested aseptically from juvenile bovine knee joints (2-4 wks), and digested for 3 hrs in a 37°C, 5% $CO_2$ incubator using collagenase type V (696 units/mg). Synoviocytes were isolated and seeded in T-225 flasks (P1) at a density of 0.04 million cells/flask. To obtain a pure population of sMSCs from primary cultures of synoviocytes, a negative isolation procedure was performed using CD14 Dynabeads and Dynal Magnetic Particle Concentrator as described elsewhere[1].

**2-D cell culture:** sMSCs were divided into two groups and plated in T-225 flasks (P2) at 0.04 million cells/flask. Cells in group 1 were cultured with medium containing MEM, 10% FBS, 100 U/ml penicillin, 100 mg/ml streptomycin and amphotericin B. For the cells in group 2, the culture medium was supplemented with a cocktail of growth factors (GFs) to prime the synoviocytes towards a chondrogenic lineage (10 ng/ml TGF-β3, 10ng/ml PDGF-ββ, and 5 ng/ml FGF-2).[4]

The overall experimental design of the study is illustrated in Fig.1. Cell proliferation and shape during culture were monitored via phase-contrast images from passage 2 to 4. To further examine the effects of GF priming in 2D, the speed and adhesion strength of sMSCs were quantified. At P2, sMSCs cultured with and without GF were plated at 37ºC for 1 hr, at a density of 0.1 million cells/ml of culture medium, on sterile glass slides using flexiperm silicone vessels**.** The glass slides were placed in a custom-designed chamber that allowed for temporal visualization of cells. Time-lapse digital microscopy was used to track cell movement at 10 min intervals for 2 hrs. Each group contained 28 cells. The centroid of the cell was measured at the initial and final position (Image J), and the speed was calculated as the displacement of the centroid divided by total elapsed time. For cell adhesion experiments, sMSCs cultured with and without GF were plated on glass slides as described above. The slides were placed in a parallel-plate flow chamber mounted on the same video microscopy system and subjected to step increases of laminar flow

(0.4 $cm^3/s$) of PBS at 1 min intervals. The number of cells attached at each interval was recorded and plotted as a function of wall shear stress (dyne/$cm^2$).

**3-D culture:** At P4, cells were seeded in agarose (2% w/v) discs, 3mm diameter and 2.34mm thickness at a concentration of 60 million cells/ml. Discs were cultured in serum-free medium containing ascorbic acid in the presence or absence of TGF-β3 (10ng/ml). At Day 21, TGF-β3 was removed from 50% of the groups and culture was continued till Day 35. The groups examined are shown in Table 1.

Table 1: Experimental groups examined during Phase II (3D culture).

| | |
|---|---|
| Day 0 | No Priming, Priming |
| Day 21 | No Priming, No Priming + TGF-β3, Priming, Priming + TGF-β3 |
| Day 35 | No Priming, No Priming + TGF-β3 (release and continuous), Priming, Priming + TGF-β3 (release and continuous) |
| Notes | Priming refers to GF application in 2D<br>Release refers to application of GF till day 21<br>Continuous refers to application of GF till day 35 |

**Assays**: The equilibrium Young's modulus ($E_Y$) was determined under unconfined compression at 10% strain. Biochemistry was performed to determine the collagen and glycosaminoglycan (GAG) content using the orthohydroxyproline and dimethylmethylene blue assay, respectively. Samples were prepared for histology, and stained with Picosirius Red and Safranin-O/Fast Green to determine the distribution of collagen and GAG, respectively. Mechanical, biochemical, and histological analyses were performed on Day 0, 21 and 35.

**Statistical analyses:** Statistics were conducted using a 1-way ANOVA (block by time point) followed by Tukey's post-hoc test. For cell adhesion, Fisher's exact tests were performed. A $p < 0.05$ was considered significant. All data is presented as mean ± SD.

## RESULTS:

**2D Culture**: Synovial MSCs cultured without GFs were more spread and elongated, resembling fibroblasts. In contrast, sMSCs cultured with GFs were rounded resembling chondrocytes (Fig. 2). Time to confluence for the sMSCs with GFs was approximately half that of non-GF cultures. The adhesion strength of sMSCs cultured without GFs was significantly higher than cells cultured with GFs and likely contributes to their decreased motility relative to primed cells (Fig. 3).

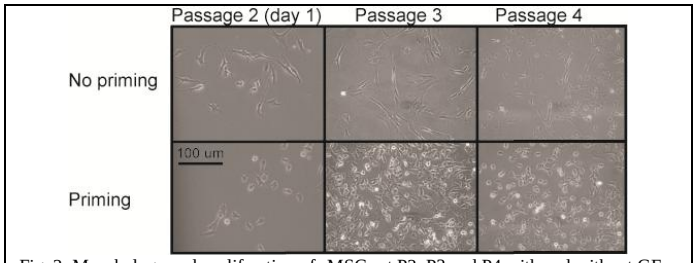

Fig. 2: Morphology and proliferation of sMSCs at P2, P3 and P4 with and without GFs.

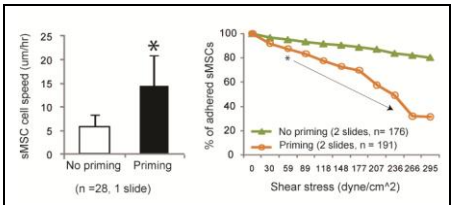

Fig. 3: Effect of GF priming on cell speed and adhesion. Star represents a significant difference between groups.

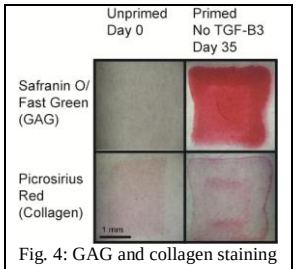

Fig. 4: GAG and collagen staining at Day 0 and Day 35.

**3D Culture:** Strong GAG staining was observed on the periphery of the constructs at Day 35 while collagen was localized towards the inner regions (Fig. 4). Primed, release constructs at day 35 (174 ± 34 kPa) exhibited a 12-fold increase (Fig. 5) in $E_Y$ versus day 35 unprimed constructs ($p < 0.01$). The increases in material properties were accompanied by corresponding increases in GAG content (Fig. 6). Specifically, a 7.6 fold increase in % GAG/WW was observed in primed, release constructs (3.4 ± 0.5% /WW) at Day 35 versus the unprimed Day 35 constructs ($p < 0.0001$). However, no significant differences were observed among groups for % collagen/WW at Day 35 ($p = 0.5$, data not shown).

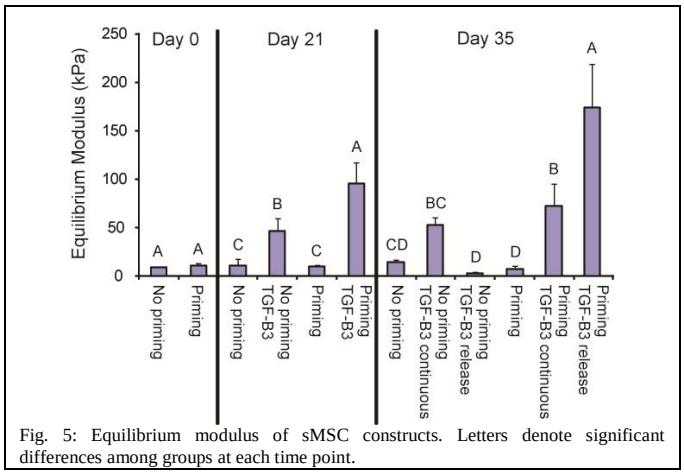

Fig. 5: Equilibrium modulus of sMSC constructs. Letters denote significant differences among groups at each time point.

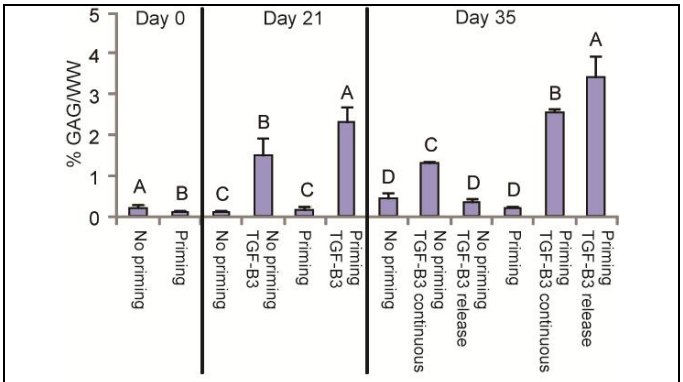

Fig. 6: % GAG/WW of sMSC constructs. Letters denote significant differences among groups at each time point.

## DISCUSSION:

Synovial-derived mesenchymal stem cells are receiving greater attention as a potential cell source for cartilage repair strategies. This study reports that sMSCs cultured in the presence of a GF cocktail of TGF-β3, FGF-2 and PDGF-ββ exhibit higher proliferation in 2D culture while maintaining a rounded morphology, decreased cell adhesion and increased cell motility compared to non-GF expansion. Furthermore, the results demonstrate the significant impact of this GF priming on subsequent tissue production after seeding in 3D culture. A better understanding of the mechanisms that underlie 2D priming-induced enhancement of tissue development will be important for efforts aimed at optimization of sMSCs for cartilage repair strategies.

**ACKNOWLEDGMENTS:** NIH/NIAMS AR46568 and AR52871

**REFERENCES:** 1) Pei+, Differentiation, 2008; 2) Hunziker+, J Bone Joint Surg Am, 1996; 3) Ng+, Tissue Eng Part A, 2009; 4) Barbero+ Arthritis Rheum, 2003