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DIFFERENCES IN ENGINEERED CARTILAGE FROM HUMAN CHONDROCYTES AND MESENCHYMAL STEM CELLS IN PELLET AND CONSTRUCT CULTURE

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INTRODUCTION

Articular cartilage serves as the load-bearing material of joints. One approach to functional tissue engineering is to recapitulate the biochemical and mechanical function of healthy native cartilage *in vitro,* prior to implantation. We have been successful in cultivating engineered cartilage with compressive mechanical properties and glycosaminoglycan (GAG) content near native values by encapsulating chondrocytes or stem cells in a clinically relevant hydrogel [1, 2]. Clinical application of functional engineered cartilage will likely use of chondrocytes (AC) from osteoarthritic tissue or mesenchymal stem cells (MSCs), which have been shown to have chondrogenic potential. That is, it is may be more feasible to differentiate healthy MSCs towards a chondrogenic lineage than to 'reprogram' ACs acquired from an osteoarthritic joint.

There has been growing interest in using human cells to develop engineered tissue for cartilage repair strategies [3-8]; however, many of these studies have been limited to micro-pellet cultures [3-6]. The growth factors used in these studies vary greatly, making it difficult to determine the best method for developing engineered cartilage *in vitro*. BMP2 has been suggested to improve chondrogenesis [3], and bFGF2 may improve collagen type II gene expression [4]. Other studies have suggested that a combination of growth factors is ideal for improving gene expression of aggrecan and collagen [5, 6]. However, gene expression and matrix production in pellet culture does not necessarily indicate that these culture conditions will be ideal for developing functional engineered cartilage that can withstand mechanical loading.

A successful biological repair strategy will need to withstand the mechanical forces experienced *in vivo*. Moreover, human articular cartilage is thick (~7 mm); therefore, an implantable replacement strategy will need to be larger than the tissues developed through pellet culture. Recently, a few studies have shown that engineered cartilage at millimeter-length scale can be cultivated using human cells *in vitro* [7, 8]. The objective of this study was to promote chondrogenesis of human bone-derived MSCs and ACs in pellet and construct culture. We hypothesize that the extracellular matrix production will depend on the growth factors and three-dimensional environment (i.e. pellet or construct).

METHODS AND MATERIALS

AC Culture: Articular cartilage was acquired from a female patient receiving a total knee replacement using an IRB approved protocol (age = 51 years). Chondrocytes (ACs) were acquired by digesting the tissue overnight in DMEM media with 10% serum and collagenase. Cells were expanded in DMEM medium supplemented with 10% serum, 1% 100 U/ml penicillin, 100 mg/ml streptomycin and amphotericin B (Invitrogen Co., Carlsbad, CA), 1 ng/ml TGF-β1, 10 ng/ml PDGF-ββ, and 5 ng/ml bFGF2 [2]. Cells were passaged twice before performing tissue-engineering studies.

Once confluence was reached, cells were trypsinized and micro-pellets were formed for each cell type by centrifugation [9]. Pellets were for cultured for 28 days in chemically-defined media (CM) supplemented with growth factors. The growth factor combinations used in this study were based on preliminary studies that measured GAG production of cells monolayer culture. Therefore, different experimental groups were used for MSC and AC pellets. The experimental groups for AC pellets were: (**AC1**) 10 ng/mL TGFβ3 (control), (**AC2**) 50 ng/mL BMP2, (**AC3**) 50 ng/mL BMP2 and 10 ng/mL bFGF2, and (**AC4**) 50 ng/mL BMP2 and 10 ng/mL PDGFββ.

Passaged cells were encapsulated in 2% w/v low gelling agarose slab (Type VII, Sigma-Aldrich) at a final concentration of 30 x 10^6 cells/mL [1]. Constructs were punched from the slab (2.34 mm thickness) using a 4 mm diameter biopsy punch and cultured in CM

supplemented with growth factors based on the results from the pellet culture. AC-laden constructs were cultured with 10 ng/mL TGFβ3 (control) or 50 ng/mL BMP2 (AC1 and AC2 groups, respectively).

MSC Culture: Bone-derived MSCs were obtained from Cambrex Life Sciences (East Rutherford, NJ). Cells were expanded and prepared for tissue-engineering studies as described above. MSC pellets were cultured in the following groups: (**MSC1**) 10 ng/mL TGFβ3 (control), (**MSC2**) 10 ng/mL TGFβ3 and 50 ng/mL BMP2, and (**MSC3**) 50 ng/mL BMP2 and 10 ng/mL bFGF2. MSC-laden constructs were prepared as described above and cultured with 10 ng/mL TGFβ3 (control) or TGFβ3 and 50 ng/mL BMP2 (MSC1 and MSC2, respectively).

Biochemical analyses were performed on pellets and constructs to measure the DNA, GAG and collagen contents. Cell viability was assessed with the LIVE/DEAD Kit (Invitrogen, Co.). The compressive Young's and dynamic moduli were measured under unconfined compression at 10% strain. Following mechanical testing samples were prepared for biochemical analyses. A one-way ANOVA was performed to evaluate the differences between the biochemical properties across experimental pellet groups. A Student's t-test was performed on mechanical and biochemical properties of constructs.

RESULTS

AC Culture: By day 28, AC pellets cultured in CM supplemented with BMP2 were significantly larger than the control (Fig 1). There were no significant differences in DNA content (Fig 2A). The GAG content of the AC2 and AC3 groups was 65-80% greater than the control (AC1; Fig 2B - *; $p < 0.001$). There was no significant difference in collagen content (normalized by DNA content (g/g): AC1 = 56.6 ± 26.4, AC2 = 64.8 ± 16.9; $p > 0.2$).

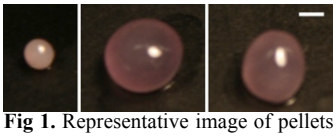

Fig 1. Representative image of pellets from AC1 (left), AC3 (middle) and AC4 (right). Bar = 1 mm.

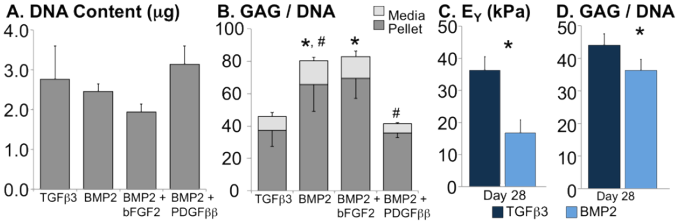

Fig 2. (A) DNA content and (B) GAG content normalized by DNA for AC pellets. * $p < 0.05$ vs. TGFβ3 group pellet GAG; # $p < 0.05$ vs. TGFβ3 group media GAG. (C) Young's modulus and (D) GAG content normalized by DNA for AC-laden constructs. * $p < 0.05$.

Cell viability in agarose was maintained throughout the culture period (Fig 3), and cell proliferation was observed in constructs cultured with TGFβ3 (DNA content at day 28 = 1.2 x 10^{-5} g vs. 8.9 x 10^{-6} g for BMP2; $p < 0.001$). At day 28, the Young's modulus of the TGFβ3 group was 2X greater than the BMP2 group (Fig 2C). The dynamic modulus of the TGFβ3 group (0.22 ± 0.05 MPa) was 2.5X greater than the BMP2 group ($p < 0.01$). The GAG content of the TGFβ3 group 1.62 ± 0.07 %/ww and was 25% greater than the BMP2 group (Fig 2D; $p < 0.01$).

MSC Culture: Pellets in the MSC2 group were visibly larger than the control; similar to the differences observed with AC pellets (Fig 1). The DNA content for MSC2 and MSC3 was approximately 50% greater than the control (Fig 4A; $p < 0.01$). There was a trend for higher GAG production in the MSC2 group (Fig 4B). The collagen content of the control group was below measurable values, which prevented statistical analyses to be performed. The collagen content normalized by DNA was 1.08 ± 0.35 for MSC2 and MSC3 groups (pooled average; $p = 0.9$).

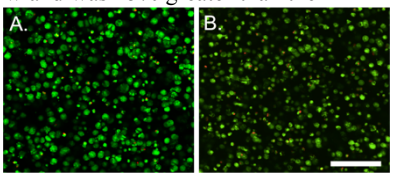

Fig 3. Cell viability for (A) AC1 and (B) AC2 constructs at day 28. Living cells are green; dead cells are red. Bar = 150 μm.

Matrix production by MSCs encapsulated in agarose was slower than ACs, as noted by lower compressive moduli and biochemical properties. By day 35, the Young's modulus of the TGFβ3 group was 21.7 ± 5.3 kPa and was not significantly different from the TGFβ3 + BMP2 group (21.0 ± 2.4 kPa; $p = 0.8$). The GAG and collagen contents normalized by wet weight were 0.7 ± 0.1% and 1.5 ± 0.4%, respectively (pooled average; $p > 0.6$).

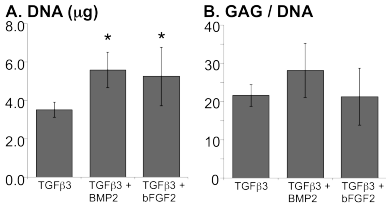

Fig 4. (A) DNA content and (B) GAG content normalized by DNA. * $p < 0.05$ vs. TGFβ3 group.

DISCUSSION

This study evaluated the effect of commonly used growth factors in three-dimensional culture of engineered cartilage. The results shown here are comparable to previous studies that have suggested improved GAG production by culturing pellets in the presence of BMP2 [3, 5]. Interestingly, the difference in growth factor culture did not translate to improved mechanical and biochemical properties in hydrogel scaffold culture. That is, for AC-laden constructs, the TGFβ3 group deposited more extracellular matrix and had better mechanical properties than the BMP2 group (Fig 2B vs. 2D). The TGFβ3 group continued to deposit extracellular matrix as noted by a Young's modulus of 153.6 ± 20.0 kPa by day 63 (data not shown). Differences between pellet and construct culture were observed for both AC and MSC cultures. It is possible that the differences between the matrix deposition in pellets and constructs are due to a lack of cell-cell interactions or due to the cells maintaining a rounder morphology in the construct.

Perrier et al. suggested that a combination of TGFβ3 and BMP2 may be better for inducing MSCs towards a chondrogenic lineage. Our results conditionally support their findings. That is, we observed a slight, but not significant, increase in GAG production in pellet culture; however, this difference did not translate to improved mechanical properties MSC-laden constructs. Therefore, it is likely that more cost effective measures (i.e. less growth factors) can be utilized for chondrogenesis of bone-derived MSC in hydrogel cultures.

Cartilage tissue-engineering has been successful in demonstrating native values using animal cell sources [1, 2]. Recent work has focused on applying these findings to cultivating a biological replacement strategy with human cells. This study demonstrated significant differences in extracellular matrix production between pellet and construct culture. These results are important for culturing clinically-relevant functional engineered cartilage with mechanical and biochemical properties towards healthy native tissue.

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