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Chondrocytes and Bone Marrow Staromal Cells Exhibit Differential Responses to Mechanical Stimulation and Cytokine Challenge

J. M. Taboas [1] and R. S. Tuan [1]

1 Introduction

Bone marrow mesenchymal stem cells (BMSC) are considered a promising cell type to produce engineered cartilage. Several studies have focused on developing conditions that promote BMSC differentiation along specific lineages and on optimizing matrix synthesis and/or accumulation. Though easier to expand *ex vivo* than tissue specific differentiated cells, BMSCs have demonstrated lower regenerative capacity and unstable phenotype. Recent work has elucidated a crucial role for BMSCs in regulating the regenerative potential of differentiated host cells, such as in myocardial regeneration. This indirect role of BMSCs can be leveraged for skeletal tissue engineering using BMSC + differentiated cell approaches. To do so, the differential responses of BMSCs vs. differentiated cells to culture conditions and simulated host environments, such as cytokine challenge and mechanical forces, must be understood. The objective of this study was to elucidate the differential response of chondrocytes vs. bone marrow stromal cells cultured in a chondrogenic three-dimensional (3D) environment to cytokine, growth factor, and hydrostatic loading challenge similar to that experienced by engineered cartilage constructs in vivo.

2 Materials and Methods

Bovine chondrocytes and human BMSCs were cast in 1.25% alginate beads (≈40 μl each) at $2x10^6$ and $10x10^6$ cells/ml respectively and cultured in α-MEM supplemented with 10% FBS and antibiotics. Gels were hydrostatically loaded daily 2 h/day using a sinusoidal waveform of 1 Hz at 13 MPa peak pressure. During the first 7 days of loading, cultures were supplemented with biofactors (5 ng/ml): inflammatory cytokines TNFα and IL1β, and growth factors FGF2 and TGFβ. Proliferation (**Fig. 1**) and real time PCR assays (**Fig. 2**, subset of transcript results shown) were performed 24 hours after the 15th day of loading on this 2x6 factorial design. Transcripts representative of three functional groups were analyzed: a) chondrogenic differentiated activity (chondrogenic transcription factors and cartilage matrix specific transcripts), b) matrix remodeling (degradation enzymes and their inhibitors), and c) biofactor production (Cox2, Vegf, FGF2, TGFβ).

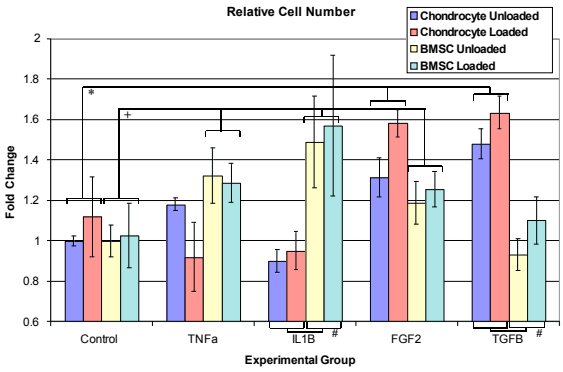

Figure 1 : Effects of hydrostatic loading and medium supplements on chondrocyte and BMSC proliferation. Results are normalized to unloaded control conditions for chondrocytes and BMSCs respectively. Symbols highlight significant differences at $p<0.5$ from control chondrocytes (*), from control BMSCs (+), and between chondrocytes and BMSCs (#).

3 Results

The proliferative response of BMSCs to biofactors was opposite that of chondrocytes. BMSC proliferation significantly increased in response to

[1]Cartilage Biology and Orthopaedics Branch, NIAMS, NIH Bethesda, Maryland 48109, USA

cytokines whereas chondrocyte numbers exhibited a decreasing trend ($p<0.05$). Conversely, chondrocyte proliferation significantly increased under TFG while no significant change occurred in BMSCs ($p<0.05$). Only FGF increased proliferation in both cell types ($p<0.05$). Hydrostatic load displayed a trend towards increasing proliferation under all biofactor treatments except TNFα, where loading decreased cell number in both cell types.

Cytokine effects on gene expression varied between cell types. As expected, IL1β and TNFα increased remodeling gene expression in chondrocytes (MMPs and TIMPs), but unexpectedly did not increase Cox2. In BMSCs, these cytokines also increased MMP1 (+220-360%). But in contrast to chondrocytes, they did not have a strong effect on TIMPs, while they did increase Cox2 (+40-110%). Interestingly, BMSCs had a differential response to IL1β vs. TNFα in that IL1β also decreased matrix transcripts (Col1, Col2). Increased MMP expression was not correlated with a hypertrophic phenotype in chondrocytes, as cytokines consistently decreased Col10 expression (Col10 not detected in BMSCs).

Across all medium treatments, hydrostatic loading displayed a number of consistent effects on gene expression in each cell type. In chondrocytes, loading was chondro-protective in that it decreased MMPs and further increased TIMPs even under cytokine treatment (TIMP1 +60%), without a strong effect on Cox2 and differentiated activity or differentiation state (Sox9, FN). In BMSCs, loading consistently decreased Cox2 and the master differentiation marker Sox9, but had no consistent effect on differentiated activity (Col1 and Col2).

Growth factors also displayed opposing regulation patterns in chondrocytes vs. BMSCs. In serum containing medium, TGFβ decreased differentiated activity in chondrocytes relative to controls (Col1 -60%, Col2 -30%, Link -30%, AG -50%), while inducing the greatest increase in Cox2 (+800%) and matrix remodeling genes (MMPs, ADAMTS and inhibitors TIMPs). In BMSCs, TGFβ increased differentiated activity markers (Col1 +75%, Col2 +180%) and decreased Cox2 (-200%) and MMP1 (-140%). Sox9 was also differentially regulated, up 40% in chondrocytes vs. down 30% in BMSCs. Interestingly, loading inverted TGF effects on BMSC differentiated activity. TGFβ may have undesirable effects when coupled with hydrostatic pressure stimulation in BMSC chondrogenic differentiation. FGF2 served as a greater stimulator than TGFβ of cartilage differentiated activity in chondrocytes (Col2 +62% and AG +40%) while also inhibiting Cox2 (-30%), MMP, and TIMP expression. However in BMSCs, FGF2 was detrimental to matrix transcript expression.

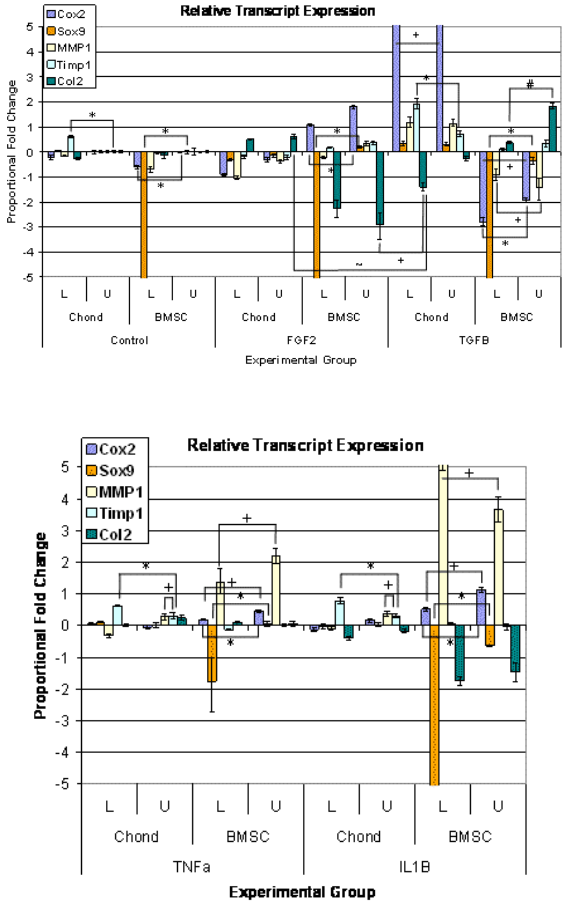

Figure 2 : Effects of hydrostatic loading and medium supplements on chondrocyte and BMSC gene expression. Results normalized to unloaded control conditions for Chondrocytes and BMSCs respectively. L = loaded for 2 hrs daily with 1 Hz sinusoidal waveform at 13 MPa peak, U = Unloaded controls. Chond = chondrocyte gels, BMSC = stem cell gels. Supplements at 5 ng/ml. Symbols highlight significant differences at $p<0.5$ for loading

effects across all supplement treatments (*), loading effects unique to a supplement treatment (#), supplement effects relative to controls irrespective of loading treatment (+), and between two different supplements (~).

4 Conclusion

This work uncovers antithetic responses of chondrocytes vs. BMSCs to physiologic levels of cytokines, growth factors, and mechanical forces present in injured skeletal tissue that are of significance for tissue regeneration therapies. In general, BMSCs were found to be more sensitive to cytokines than chondrocytes in terms of proliferation, biofactor production, and remodeling factor secretion. Paracrine signaling molecule production in BMSCs appeared more sensitive to hydrostatic loading than matrix synthesis. Thus, BMSCs may be suitable for tissue regeneration in an inflammatory environment, primed to grow in but not contribute to inflammation. This work serves as a basis for improving current cell based regeneration therapies and developing combined stem cell + differentiated cell engineered constructs.

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