Conditioned Media Enhance the Osteogenic and Chondrogenic Differentiation of Mesenchymal Stem Cells

Scott Maxson, Karen J.L. Burg

Department of Bioengineering and Institute for Biological Interfaces of Engineering, Clemson University, Clemson, SC

Statement of Purpose: Osteoarthritis is the most common form of arthritis and affects an estimated 26.9 million US adults (Lawrence et al., 2008). Osteochondral tissue engineering provides a potential treatment option for osteoarthritis; however the optimal culture conditions that allow the development of a clinically viable tissue-engineered device have not been determined. Chondrocytes and osteoblasts share a complex relationship during the development of the human skeletal system which, in part, can be attributed to the complicated cytokine and growth factor signaling pathways that exist between the two cell populations. Paracrine signaling may be modulated *in vitro* to find optimal culturing conditions and improve our ability to tune mesenchymal stem cell (MSC) differentiation for tissue engineering applications, more specifically for osteochondral tissue engineering. The objectives of this study were to determine the effect of chondrocyte conditioned media on MSC differentiation to an osteoblast phenotype and to determine the effect of osteoblast conditioned media (CM) on MSC differentiation to a chondrocyte phenotype.

Methods: Murine MSCs were cultured in two different groups: a chondrogenic group and an osteogenic group. The cells in the chondrogenic group were encapsulated in alginate beads (a common material used for cartilage tissue engineering) and cultured in chondrogenic differentiation medium. The cells in the osteogenic group were seeded on porous scaffolds made of 75/25 poly-L-lactide/polycaprolactone containing 10% hydroxyapatite and cultured in osteogenic differentiation medium. The experimental samples from the chondrogenic group were given CM from the osteogenic group, at concentrations of 25% and 50%. Similarly, the samples from the osteogenic group were given CM from the chondrogenic group, at concentrations of 25% and 50%. Several controls were implemented: MSCs were grown on 2D plates without differentiation medium, on scaffolds or in alginate without differentiation medium, on scaffolds or in alginate with only the appropriate differentiation medium, and on scaffolds or in alginate with the appropriate unconditioned differentiation medium mixed with 25% or 50% of the "opposite" unconditioned differentiation medium. Osteogenic differentiation was analyzed by measuring ALP activity and using RT-PCR to assess osteocalcin and bone sialoprotein expression. Chondrogenic differentiation was analyzed by measuring the sGAG production and using RT-PCR to analyze the sox9 and aggrecan expression.

Results: By Day 21, the osteogenic group samples that were administered 25% and 50% chondrocyte conditioned medium showed higher alkaline phosphatase (ALP) activity than the controls that were not administered CM, with the 50% CM samples showing the highest activity. Additionally, on both Days 14 and 21, the cells that were given chondrocyte CM had higher osteocalcin expression than the controls. By Day 21, the bone sialoprotein expression was higher in the samples given chondrocyte CM. On Days 14 and 21, samples in the chondrogenic group that were administered osteoblast CM at a concentration of 50% produced higher sGAG than the controls. The aggrecan expression was significantly higher in the samples given 50% CM as compared to the controls (Figure 1). Finally, the cells given 50% CM had a higher expression of sox9 on Days 14 and 21. Another finding of the study was that the cells that were encapsulated in alginate, but not given differentiation media showed similar levels of differentiation as the samples that were administered differentiation media, suggesting the material played a large role in the chondrogenic differentiation.

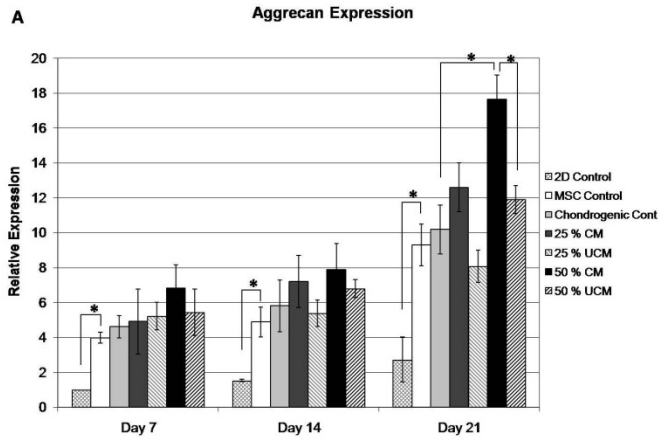

Figure 1: Results of the RT-PCR for the chondrogenic marker aggrecan. The results are reported as a relative expression to GAPDH using the ΔΔCt method. The other differentiation markers showed similar patterns. Error bars represent standard deviation. Asterisks (*) signify a statistically significant difference ($p < 0.05$).

Conclusions: This study showed that CM from differentiating chondrocytes can enhance the differentiation of MSCs toward osteoblasts and that CM from differentiating osteoblasts can enhance the differentiation of MSCs toward chondrocytes. The enhanced osteogenic differentiation is likely due to soluble factors that are released by the chondrogenic cells such as BMP-2, BMP-7, and transglutaminase enzymes. Similarly, the enhanced chondrogenic differentiation is likely due to soluble factors that are released by the osteogenic cells such as TGF-β1 and BMP-2. Results from this work may assist in optimizing culturing conditions for MSCs and may contribute to the development of clinically viable tissue engineered osteochondral devices.

References: Lawrence, R.C., et al. Arthritis Rheum. 2008; 58:26-35.

Acknowledgements: Hunter Endowment