Encapsulated Mesenchymal Stromal Cells for Osteoarthritis Treatment

Ileana Marrero – Berríos, ME; Sarah E Salter, BS; Rene S Schloss, PhD; Martin L Yarmush, MD PhD

Department of Biomedical Engineering, Rutgers University, Piscataway, NJ

Background

Osteoarthritis:

•Osteoarthritis (OA) is the most common form of arthritis characterized by a slow progressive degeneration of articular cartilage.

•Considered the most prevalent joint disease in adults, affecting more than 12.4 million over the age of 65 in the U.S.

•Increased rate of incidence in astronauts returning from space due to cartilage degeneration kick started by micro-gravity environments

•Although OA is considered a non-inflammatory joint disease, pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) have been implicated as key mediators in the disease.

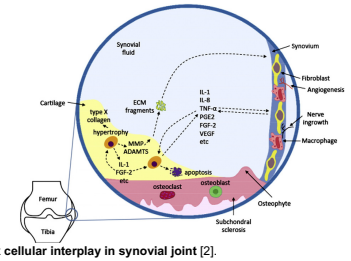


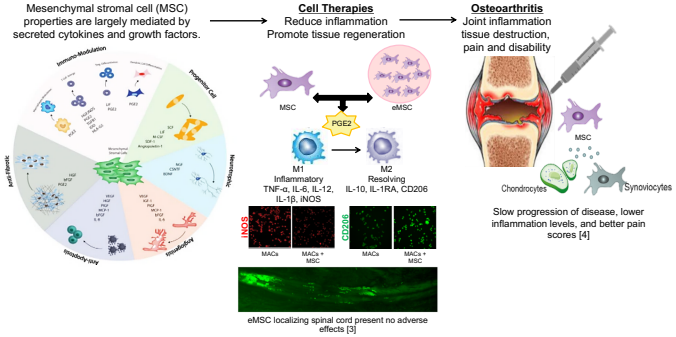
Figure 1: Complex cellular interplay in synovial joint [2].

Current treatments:

•Existing treatments alleviate symptoms initially; however, they are not able to alter disease progression and disease development eventually proceeds.

New approaches with cell-based therapies:

•One approach to alter OA progression has been the administration of mesenchymal stromal cells (MSCs) into the arthritic joint. MSC secrete numerous anti-inflammatory and regenerative factors that could modulate the inflammatory state in OA.



•Nevertheless, this treatment requires injection of millions of cells and lacks long-term efficacy.

•Therefore, a strategy to deliver and localize MSCs while promoting their chondrogeneic AND anti-inflammatory function is needed.

Objectives

- To establish an *in vitro* model of osteoarthritic chondrocytes.
- To ascertain whether encapsulated MSC (eMSCs) can provide sustained reduction of OA mediated joint inflammation and destruction, and promote re-growth and healing by optimizing the encapsulation conditions to promote MSC anti-inflammatory and chondrogeneic function.
- To identify key mediators of MSC or eMSC anti-inflammatory action in an *in vitro* model of OA.

Methods

Alginate encapsulation of MSCs:

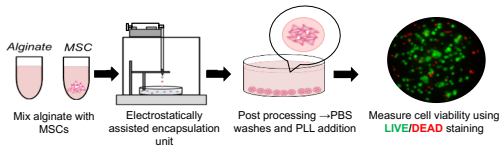


Figure 3: Schematic of encapsulation and analysis procedure.

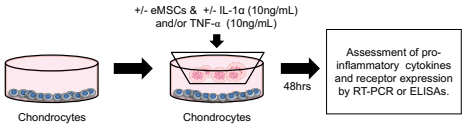


Figure 4: Experimental setup for *in vitro* co-culture studies of MSCs chondrocytes. 2.2 % alginate eMSCs' anti-inflammatory effect on IL-1α and/or TNF-α stimulated chondrocytes as a model of OA. Using IL-1α and/or TNF-α will produce an OA-like environment and test the immunomodulatory properties of eMSCs.

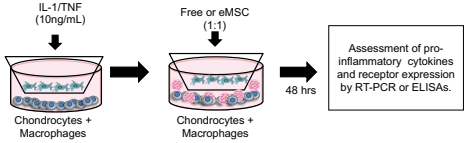


Figure 5: Experimental setup for *in vitro* co-culture studies of MSCs chondrocytes. 2.2 % alginate eMSCs' anti-inflammatory effect on IL-1α and/or TNF-α stimulated chondrocytes and macrophages as a comprehensive model of OA. Using IL-1α and/or TNF-α will produce an OA-like environment and test the immunomodulatory properties of eMSCs.

Results

Free and eMSC effect on osteoarthritic chondrocytes:

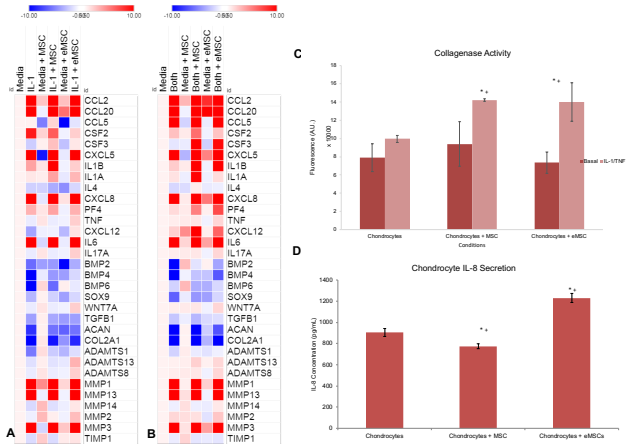


Figure 6: Inflammatory cytokine, protease, and chondrogenic gene expression panel of chondrocytes in the presence of free MSCs and eMSC. Inflammatory stimuli such as IL-1α (**A**) and/or TNF-α (**B**) induced the upregulation of pro-inflammatory cytokine and proteases while downregulating pro-chondrogenic gene expression. **C-D** examples of collagenase activity and IL-8 secretion changes with IL-1α or IL-1α /TNF-α treated chondrocytes in the presence of free or eMSCs. The presence of IL-1α/TNF-α enhanced pro-inflammatory cytokine and protease gene expression, predominantly in eMSC treated groups. Data represents the fold regulation changes compared to basal chondrocytes of N=3, n=9 pooled samples.

Results (cont.)

Free and eMSC PGE2 secretion in co-culture with chondrocytes and its effect on OA chondrocytes:

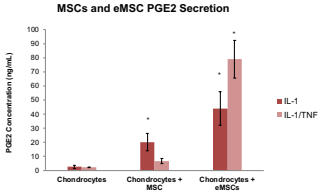


Figure 7: MSC PGE2 secretion in co-culture with chondrocytes. Free and eMSC secrete high levels of PGE2 in the presence of IL-1α or IL-1α /TNF-α stimuli. PGE2 works as an immunomodulatory molecule on macrophages and might have similar effects on chondrocytes. Data represents N=2, n=2-3 replicates. *significantly different from all conditions (p<0.5)

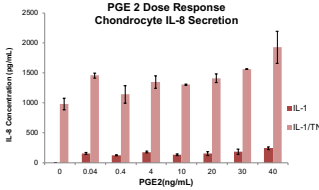


Figure 8: PGE2 dose response. Chondrocyte IL-8 secretion in response to OA stimuli and PGE2 dose response. Increasing levels of PGE2 did not attenuate chondrocyte IL-8 secretion. Data represents n=3 replicates.

Addition of macrophages to the OA *in vitro* model:

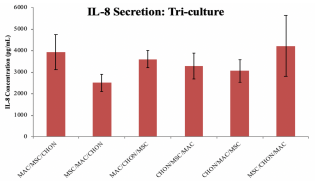


Figure 9: Multiculture system with chondrocytes, macrophages and MSCs. IL-8 Secretion was compared among various combinations of inflamed co-cultures that included chondrocytes, macrophages, and MSCs. Co-cultures varied by stacking order in order to test the hypothesis that order would vary overall IL-8 secretion. No significant difference was found between the various combinations. Data represents n=3 replicates.

Conclusions and Future Work

- An *in vitro* model of osteoarthritic chondrocytes was established using key mediators of OA inflammation such as IL-1α and TNF-α. When used in combination the inflammatory response and downregulation of pro-chondrogenic genes is exacerbated creating an OA-like environment.
- Free and eMSC treatment of stimulated chondrocytes promoted the downregulation of pro-inflammatory genes and the upregulation of pro-chondrogenic genes, marginally.
- In response to OA inflammatory mediators, free and eMSC secrete multiple factors and immunomodulatory molecules, such as PGE2.
- PGE2 is considered to have anti-inflammatory effects on several cell types, including M1 macrophages. However, it promotes an inflammatory response in chondrocytes. Since free and eMSC secrete high levels of PGE2, this results might suggest why the MSC treatment was not effective.
- In a multiculture system with MSCs, OA macrophages and chondrocytes, the system is the same so long as all three cell types are present on separate monolayers.
- Further studies to characterize the potential of MSC as a treatment for OA are needed. In the future, we will study overall biochemical changes after the introduction of macrophages and the effect of the timing and dose of MSC using the *in vitro* OA model.

References

1. Lawrence, R. C., et al. (2008). "Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part II." Arthritis Rheum.**58**(1):26-35
2. Ramachandran, V., Wang, R., Ramachandran, S. S., Ahmed, A. S., Phan, K., & Antonsen, E. L. (2018). Effects of spaceflight on cartilage: implications on spinal physiology. *Journal of Spine Surgery*, *4*(2), 433–445. doi: 10.21037/jss.2018.04.07
3. Hedbom, E. and H. J. Hauselmann (2002). "Molecular aspects of pathogenesis in osteoarthritis: the role of inflammation." Cell Mol Life Sci **59**(1): 45-53.
4. Barminko, J., et al (2011). " Encapsulated Mesenchymal Stromal Cells for In-Vivo Transplantation." Biotechnol Bioeng **108** (11): 2747-2758
5. Jo, C. H., et al. (2014). "Intra-Articular Injection of Mesenchymal Stem Cells for the Treatment of Osteoarthritis of the Knee: A Proof-of-Concept Clinical Trial." Stem Cells **32**(5): 1254-1266.

Acknowledgements

New Jersey Space Grant Consortium Fellowship

Aresty Spring Research Fellowship