Material-Based Cues that Influence Mesenchymal Stem Cell Differentiation for Use in Cartilage Scaffolds

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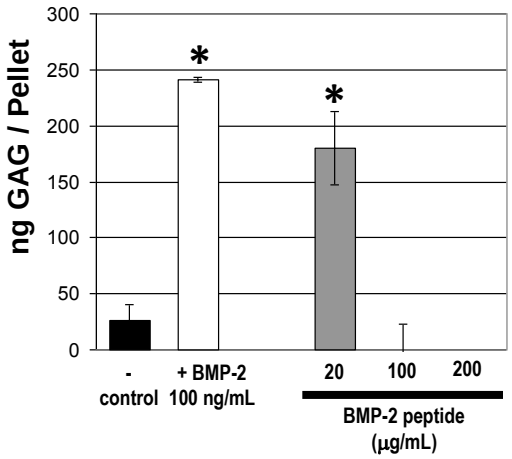
Introduction: Healthy articular cartilage covers and cushions the moving surfaces inside joints. Because articular cartilage is avascular, it has limited capacity to self-heal, allowing damage to persist, or even spread. Tissue engineers have developed a variety of biomaterials for cartilage replacement. These materials consist of a structural scaffold, cells, and bioactive molecules to direct tissue regeneration. Mesenchymal stem cells (MSCs) have emerged as a promising cell source because they can be harvested without injury and have a high proliferation capacity. Expanded MSCs are typically differentiated to the cartilage phenotype through the use of soluble factors such as transforming growth factor-beta 3 (TGF-β3) and bone morphogenetic protein 2 (BMP-2).[1]

We are developing an artificial protein scaffold that directs cell differentiation and maintains the desired phenotype based on embedded biochemical cues. Benefits to material-based cues include: 1) decreased culture times; 2) prevention of undesired differentiation states in vivo; 3) increase in local surface concentration of factors, resulting in a decrease in total amount of factor needed; and 4) possible application for 3D patterning of complex tissues. In addition to the differentiation cues, our protein design consists of resilin mechanical domains interspersed with crosslinking sites. This design provides structural integrity and allows the control of material properties.

Methods: *E. coli* is the expression host for the artificial proteins. Human mesenchymal stem cells (hMSCs) were purchased from Lonza. Growth factors were purchased from Peprotech. Chondrogenic differentiation was assessed using a modified pellet culture in a 96-well plate.[2] After seven days in culture, the pellets were digested with papain, and the amount of glycosaminoglycans (GAGs) were quantified using a dimethylmethylene blue colorometric assay. A student's t-test was used, and an * represents $p < 0.05$ compared to the negative control.

Results: We investigated three peptide sequences for their potential to differentiate hMSCs into cartilage cells. The first is a peptide sequence derived from the knuckle epitope of BMP-2. This peptide was previously shown to increase alkaline phosphatase activity of osteoprogenitor cells after three days of culture.[3] In our study, the BMP-2 peptide was added to the pellet culture media at 20, 100 and 200 μg/mL. For a negative control, hMSCs were cultured in a basal medium without peptide or growth factor. hMSCs cultured with 100 ng/mL BMP-2 growth factor added to the basal medium served as a positive control. As shown in the figure, cells cultured with 20 μg/mL of the BMP-2 peptide produced seven times more GAG/pellet than the negative control and were not significantly different compared to the positive control.



The second set of peptides we investigated is derived from the TGF-β1 growth factor and was shown to promote wound healing in human foreskin fibroblasts after one day of incubation.[4] Based on these studies, we examined two TGF-derived peptides (TP1 and TP2). The positive control cells were cultured with 10 ng/mL of TGF-β3 added to the basal medium and exhibited a 4.5-fold increase over the negative control. hMSCs cultured with TP1 (0.01-1.0 μM) had 7-9 times higher GAG response compared to the negative control. Adding TP2 at 0.01 μM resulted in a 9-fold increase over the negative control.

The BMP-2 and TGF-β1 peptides are currently being incorporated into our artificial proteins as material-based cues and are being evaluated for their ability to induce chondrogenesis within the context of our protein-based scaffold.

Conclusions: These studies illustrate that the BMP-2 and TGF-β1 peptides induce hMSCs to produce cartilage matrix. As such, these peptides are strong candidates for material-based cues in a variety of cartilage-inducing scaffolds (e.g. poly(ethylene glycol), alginate, etc). Further understanding of the ways in which these cues affect phenotype will be valuable in production of osteochondral grafts and stratified cartilage tissues.

References:

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2) Penick. Biotechniques. 2005; 39: 687-691.
3) Saito. BBA-Proteins & Proteomics. 2003; 1651: 60-67.
4) Lam. Proceedings of the 26th Annual International Conference of the IEEE EMBS. San Francisco, CA, USA September 1-5, 2004