Material-Based Cues that Influence Mesenchymal Stem Cell Differentiation to Cartilage

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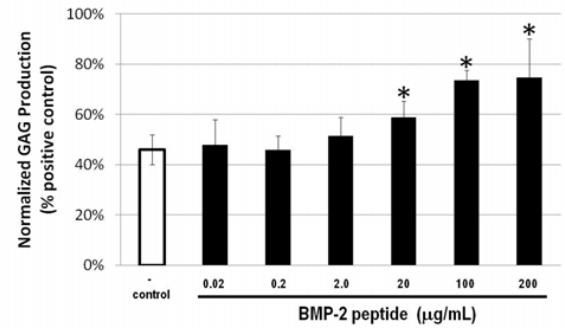
Introduction: Healthy articular cartilage covers and cushions the moving surfaces inside joints. Because articular cartilage is avascular, it has limited capacity to self-heal, allowing damage to persist, or even spread. Tissue engineers have developed a variety of biomaterials for cartilage replacement. 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 matrix that directs cell differentiation and maintains the desired phenotype based on embedded biochemical cues. Benefits to material-based cues include: 1) prevention of undesired differentiation states *in vivo*; 2) increase in local surface concentration of factors, resulting in a decrease in total amount of factor needed; and 3) 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.

This study has two aims: 1) identify short peptide sequences for use as bioactive domains in the protein material, and 2) characterize effect of the bioactive domains on chondrogenesis of human mesenchymal stem cells (hMSCs).

Methods: Growth factors were purchased from Peprotech and hMSCs were purchased from Lonza. Cells were cultured utilizing a modified pellet culture in a 96-well plate.[2] After culture, the pellets were digested with papain, and glycosaminoglycans (GAGs) were quantified using a dimethylmethylene blue assay. The hydroxyproline content was measured with the chloramine T reagent/p-dimethylaminobenzaldehyde assay, and DNA was quantified utilizing the Hoechst dye. Alkaline phosphatase (AP) activity of the sample medium was measured using the p-nitrophenyl phosphate substrate. A student's t-test was used, and an * represents $p < 0.05$ compared to the negative control.

Results: We investigated peptide sequences for their potential to differentiate hMSCs into cartilage. The most promising of these peptides is 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 medium at concentrations ranging from 0.02 to 200 μg/mL. The normalized GAG production of cells cultured with >100 μg/mL of the peptide was 74% of the positive control (cells cultured with 200 ng/mL of BMP-2 growth factor). The effect of the peptide (100 μg/mL) on normalized GAG and collagen production and AP activity were studied over time. GAG production was measured in the presence and absence of TGF-β signaling.



Conclusions: The BMP peptide promotes normalized GAG production in pellet culture over time, and its effect on GAG production is comparable to the full-length BMP-2 positive control. The BMP peptide has little to no effect on the production of collagen. The BMP peptide does not increase the AP activity compared to the negative control (medium with no growth factors or peptides), suggesting it does not promote hypertrophy. Finally, the BMP peptide does not enhance GAG production in TGF-β3 mediated chondrogenesis, whereas BMP-2 does, suggesting different biological mechanisms.

These studies illustrate that the BMP-2 peptide induced hMSCs to produce cartilage matrix without additional growth factors present in the media. As such, this peptide is a strong candidate for material-based cues in a variety of cartilage-inducing scaffolds (e.g. poly(ethylene glycol), alginate, etc). The BMP-2 peptide has been incorporated into our artificial proteins as a material-based cue and is currently being evaluated for its ability to induce chondrogenesis within a matrix.

References:

1) Heng BC. Stem Cells. 2004; 22: 1152-1167.
2) Penick. Biotechniques. 2005; 39: 687-691.
3) Saito. BBA-Proteins & Proteomics. 2003; 1651: 60-67.