Introduction: Multipotential adult mesenchymal stem cells (MSCs) can differentiate into various mesenchymal cell lineages, including bone, cartilage and adipose, and are believed to play an important role in tissue repair and regeneration. In vitro chondrogenesis of MSCs requires a 3D cell culture system and growth factor stimulation in serum-free medium. We previously reported a synergistic enhancement on the chondrogenic differentiation of MSC pellet cultures in response to co-treatment with two members of the transforming growth factor beta (TGF-β) superfamily (TGF-β3 and bone morphogenetic protein-4 (BMP-4)). Biochemical analysis and immunohistochemistry demonstrated that treatment of MSC pellets with BMP-4+TGF-β3 significantly enhanced the deposition of the cartilage-specific extracellular matrix molecules collagen type II (Col II) and aggrecan when compared with BMP-4 or TGF-β3 alone. Here, we report on the optimization of a cell based tissue engineering approach for creating cartilaginous tissues rich in extracellular matrix molecules from MSCs in a 3D hydrogel scaffold in response to TGF-β3, BMP-4, or a combination of BMP-4+TGF-β3.

Materials and Methods: Low passage, bone marrow-derived human MSCs were seeded in ionically crosslinked alginate hydrogels as previously reported [1]. Pellets and hydrogels were cultured in (1) chondrogenic medium (CM), (2) CM supplemented with 100ng/mL BMP-4, (3) CM supplemented with 10ng/mL TGF-β3, or (4) CM supplemented with BMP-4+TGF-β3 (BMP-4+TGF-β3; 100ng/mL and 10ng/mL, respectively). Medium was changed every three days. Samples were harvested at specific time points, and MTT assay, sulfated glycosaminoglycan (sGAG) and hydroxyproline (HyPro) assays, histology and immunohistochemistry, and mechanical testing were performed to assess cell metabolic activity, matrix production, and mechanical properties.

Results: Cell proliferation was determined via the MTT assay at D28 and normalized to data obtained from hydrogels immediately after formation. Culturing hydrogels in CM medium alone resulted in a significant amount of cell death (0.48±0.13-fold increase relative to D0) while no significant change in cell proliferation was observed for hydrogels cultured in medium supplemented with BMP-4 (0.88±0.27-fold increase relative to D0) or TGF-β3 (1.09±0.22-fold increase relative to D0). Supplementation of CM medium with BMP-4+TGF-β3 resulted in a significant increase in cell number (2.44±0.80-fold increase relative to the D0 control).

At D28, the total sGAG content for each hydrogel was determined and normalized to the total DNA content per hydrogel and reported as sGAG/DNA (μg/μg) (Fig.1). Relative to the hydrogel cultures treated with CM alone (0.02±0.03 sGAG/DNA (μg/μg)), all other conditions induced a significant increase in sGAG production. The TGF-β3 treated hydrogels produced slightly more sGAG (0.16±0.05 sGAG/DNA (μg/μg)) than the BMP-4 treated hydrogels (0.12±0.07 sGAG/DNA (μg/μg)), though this difference was not significant. The largest sGAG accumulation was observed in the BMP-4+TGF-β3 treated cultures (0.59±0.38 sGAG/DNA (μg/μg)) and this was a significant increase relative to the BMP-4 or TGF-β3 treated hydrogels. The total collagen content of the TGF-β3 and BMP-4+TGF-β3 hydrogels was determined for the D28 and D60 cultures. The CM and BMP-4 treated samples (D28) did not produce enough HyPro to be tested. Total HyPro content/DNA of the hydrogels was normalized to TGF-β3, and reported as fold increase relative to TGF-β3. At D28, a significant increase in HyPro content was observed for the BMP-4+TGF-β3 treated gels (3.40±1.44-fold increase relative to TGF-β3) (Fig.1). This trend continued at D60 where BMP-4+TGF-β3 supplementation of the CM differentiation medium resulted in a 4.40±2.28-fold increase in HyPro content, although this difference between D28 and D60 was not significant.

Hematoxylin and eosin-stained constructs showed morphologically distinct chondrocyte-like round cells evenly distributed throughout all growth factor treated hydrogels (Fig.2). Alcian blue staining intensities correlated with total sGAG levels. Staining intensity was weakest for BMP-4 treated constructs, and strongest for BMP-4+TGF-β3 treated constructs (Fig.2). Col II was uniformly deposited at high levels throughout the BMP-4+TGF-β3 treated hydrogels. Col II accumulation was more uniformly distributed throughout the TGF-β3 treated gels, although the staining was less intense than in the BMP-4+TGF-β3 treated hydrogels (Fig.2).

The equilibrium mechanical properties of the MSC hydrogel constructs depended upon growth factor treatment and length of in vitro culture. No appreciable values could be determined for all of the D28 hydrogels or the CM or BMP-4 treated D60 hydrogels, so these conditions were excluded from the mechanical testing study. The equilibrium Young’s modulus of the TGF-β3 treated constructs was found to be 4.44±2.15kPa, whereas the BMP-4+TGF-β3 samples were significantly stiffer with a Young's modulus of 17.11±7.28kPa.

Discussion: Based on cell proliferation, sGAG and collagen production, and mechanical properties, BMP-4 and TGF-β3 co-treatment was shown to synergistically enhance chondrogenesis of MSCs in comparison to BMP-4 or TGF-β3 individually.


Acknowledgements: CK Kuo and MT Morgan contributed to this work equally. CK Kuo current address: Department of Biomedical Engineering, Tufts University, Medford, MA. This research was supported by the Intramural Research Program of the NIH, NIAMS.