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Hyaluronic acid facilitates chondrogenesis and matrix deposition of human adipose derived mesenchymal stem cells and human chondrocytes co-cultures

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Abstract:
Clinical success on cartilage regeneration could be achieved by using available biomaterials and cell-based approaches. In this study, we have developed a composite gel based on collagen/hyaluronic acid (ColI-HA) as ideal, physiologically representative 3D support for *in vitro* chondrogenesis of human adipose-derived mesenchymal stem cells (hAMSCs) co-cultured with human articular chondrocytes (hAC). The incorporation of hyaluronic acid (HA) attempted to provide an additional stimulus to the hAMSCs for chondrogenesis and extracellular matrix deposition. ColI-HA gels were fabricated by directly mixing different amounts of HA (0 – 5 %) into collagen solution before gelation. hACs and hAMSCs were co-cultured at different ratios from 100 % to 0 % in steps of 25 %. Thus, five different co-culture groups were tested in the various ColI-HA 3D matrices. HA greatly impacted the cell viability and proliferation as well as the mechanical properties of the ColI-HA gel. The effective Young's modulus changed from 5.8 to 9.0 kPa with increasing concentrations of HA in the gel. In addition, significantly higher amounts of glycosaminoglycan (GAG) were detected that seemed to be dependent on HA content. The highest HA concentration used (5 %) resulted in the lowest Collagen type X (Col10) expression for most of the cell culture groups. Unexpectedly, culturing in these gels was also associated with decreased SOX9 and Collagen type II (Col2) expression, while Collagen type III (Col3) and metalloproteinase 13 notably increased. By using 1 % HA, a positive effect on SOX9 expression was observed in the co-culture groups. In addition, a significant increase in GAGs production was also detected. Regarding co-culturing, the group with 25 % hAMSCs + 75 % hACs was the most chondrogenic one considering SOX9 and Col2 expression as well as GAGs production. This group showed negligible Col10 expression after 35 days of culture independently of the gel used. It also featured the highest effective Young's modulus (9.9 kPa) when cultivated in the 1 % HA matrix. Concerning the level of dissolved oxygen *in situ*, the groups with a higher amount of hAMSCs showed lower oxygen levels (40 – 58 % O_2) compared to hACs (63 – 74 % O_2). This might be attributed to the higher cellular metabolism and proliferation rate of the hAMSCs. Interestingly, lower oxygen was detected in the HA-containing gels when compared to plain collagen. This may contribute to the better chondrogenesis observed in the groups. Altogether, our results indicated that HA may favour chondrogenesis, but its effect highly depends on the concentration used. Additionally, co-culture of hACs with hAMSCs also favours chondrogenesis and especially increases extracellular matrix production and decreases hypertrophy.

Keywords: co-culture, human adipose-derived mesenchymal stem cells, human chondrocytes, hyaluronic acid, chondrogenesis