hMSC and Fibroblast Dispersion Cultures in Chitosan-based Injectable Hydrogels for Cartilage Regeneration

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Statement of Purpose: Cartilage could be ravaged by various diseases and regular physical activities; osteoarthritis is the most common joint disorder requiring significant medical aid. Tissue regeneration techniques offer alternative strategies, but require seeding immuno-compatible cells to colonize biodegradable scaffolds to circumvent immune rejection. Hydrogels are desired for cartilage scaffolding because of the possibility of providing both liquid and solid phases similar to in vivo conditions. Further, injectable hydrogels offer a minimally invasive alternative to arthroscopic surgeries and ease of incorporation of cells and biologically active agents. Adult human mesenchymal stem cells (hMSC) are differentiated in pellet cultures which is difficult for use in the injectable form. The objectives of this study were to investigate the differentiation of hMSCs and an autologous human foreskin fibroblast (hFF-1) cell source in dispersion cultures on thermosensitive chitosan-based hydrogel to regenerate cartilage. We also investigated the possibility of forming chitosan hydrogels in combination with gelatin and hyaluronic acid and effects on mechanical properties with different concentrations.

Methods: Compressive and cyclical tests were performed on the hydrogels of different concentrations by using an in-house fitting that allowed testing at the physiological environment. hFF-1s were purchased from ATCC and hMSCs were purchased from Lonza Walkersville, Inc. Cells were cultured in suspension form by adapting an environment utilized for adult human mesenchymal stem cells (hMSC) differentiation into chondrocytes in different concentrations of chitosan-gelatin hydrogels for twenty eight days. Multiple cultures were grown up to four weeks and cells, supernatants, and the matrix were analyzed by different methods. The cultures were harvested for RT-PCR, histology, IHC, and FACS analyses. Supernatants were examined for collagen, and matrix metalloproteinase (MMP -MMP 2/9, MMP 3, MMP 13) activity using fluorogenic substrates. H&E, Masson's trichrome and alcian blue (pH 1.0) stains were used for histology. Aggrecan, collagen type II, and Sox-9 were assessed by IHC. Cells were stained with anti-CD44-PE and anti-CD151-FITC for FACS. ELISA was performed on cell culture supernatants from hMSC and fibroblast cultures to assess protein secretion.

Results: Increasing chitosan concentration and the gelatin concentration, significantly improved the compressive modulus while increasing the rate of gelation and ability to hold together when tested for large quantities. It was also possible to form anisotropic gels with different gradations of chitosan-gelatin and chitosan-gelatin-hyaluronic acid gels. Also, cyclical tests showed the possibility of these materials holding together after ten cycles. Premixing cells and solution before gelation allowed uniform distribution, with affecting viability. In the supernatants, hFF-1 hydrogel cultures showed a significant increase in collagen type II, comparable to that hMSCs at day 21. Analysis of cultures without cells suggested the observed increase in the hydrogel cultures is not attributed to leaching of gelatin. Fresh hFF-1s had much higher CD44 expression than hMSCs. Yet, no significant effect was observed in either cell type when cultured on different conditions, suggesting that at seven days hydrogels do not influence CD44 expression. CD151 was expressed in hFF-1s but not in hMSCs. Similar to CD44 were observed when cells cultured on different conditions were tested. The histological appearances were similar in the differentiation cultures of hFF-1 and hMSC, showing increased nuclear size relative to the cytoplasm, an indication of chondrocytes. MMP-2/9 activity was similar in both hMSC and hFF-1 cultures.

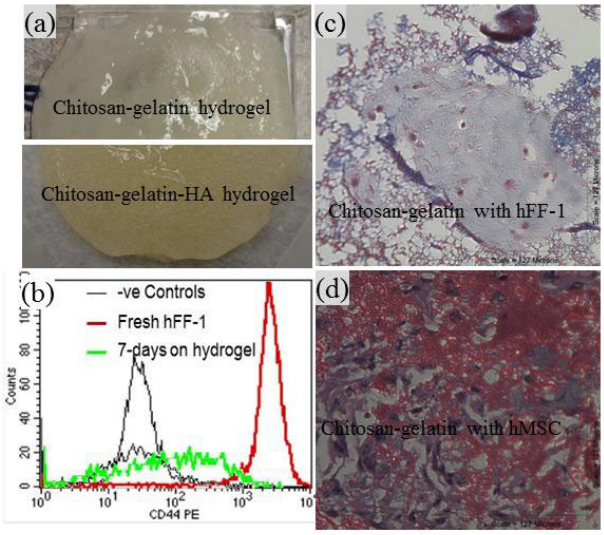

Figure. Effect of chitosan-gelatin and HA on hMSC and hFF-1 differentiation. (a) hydrogel preparations showing their ability to hold shape. (b) flow cytometry histogram showing the changes in CD44 expression in hFF-1. (c) micrograph of trichrome stained 4-week hFF-1 culture and (d) micrograph of H/E stained 4-week hMSC culture.

Conclusions: Increasing compressive strength of the hydrogels is suggested to be resultant of increasing chitosan concentration. The fibroblast line displayed similar characteristics to the hMSCs after 28 days of culture in chondrogenic induction medium. The cell morphology and collagen distribution studies displayed the cells and collagen in the ECM produced by each cell line could be observed as embedding in the chitosan-gelatin tissue. Further, the cells were observed to be similar in shape with chondrocytes. Both the control and experimental forms of the fibroblasts followed the same progression of Collagen Type II as the hMSC pellet cultures. In summary, hFF-1 cells showed similar trends to the hMSCs after twenty eight days of culture.

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