Collagen-alginate-hyaluronan composite hydrogels for effective culture and maintenance of chondrocytes

Chinmaya Mahapatra[1,2], Guang Zhen Jin[1,2], Hae-Won Kim[1,2,3*]

[1]Institute of Tissue Regeneration Engineering (ITREN), Dankook University, Cheonan 330-714, South Korea.
[2]Department of Nanobiomedical Science & BK21 PLUS NBM Global Research Center for Regenerative Medicine, Dankook University, Cheonan 330-714, South Korea.
[3]Department of Biomaterials Science, School of Dentistry, Dankook University, Cheonan, 330–714, South Korea
*E-mail: kimhw@dku.edu

Statement of Purpose:
Clinical treatment for cartilage damage due to injury, old age, disease and arthritis is primarily limited by slow rate of proliferation and the possible dedifferentiation of cells[1]. The three dimensional (3D) culture system mimicking chondrocyte-specific migration domains provides the proper conditions for cell proliferation and synthesis of cartilage ECM such as proteoglycans. In this study we provide an engineered composite hydrogel matrix made of Alginate- Hylaronic Acid -Collagen type I (Alg-HA-Col) and show the matrix has appropriate physicochemical and mechanical properties to enable the proliferation of chondrocytes and the long-term maintenance of their phenotypes, which ultimately useful for cartilage engineering.

Methods: Hydrogels were fabricated by mixing Alg, Col and HA at ratios of 2.1:0.4:0.2 (wt %). For comparison group, Col-free gel was also prepared. The gel was allowed to mature and crosslink with the addition of 50mM $CaCl_2$ for 15 min at 37ºC at 5% CO_2. Dynamic mechanical analyzer and FT-IR were used to analyze mechanical and chemical properties. Crosslinking behavior was observed by differential scanning calorimetry. The rat costo-chondral chondrocytes were primarily isolated and cultured in the hydrogels for 21 days. Cellular viability and proliferation was observed by calcien AM/EtBr staining and MTS assay. The GAG synthesis and collagen type-II protein expression were observed by alcian, toluidine blue staining and anti-collagen type-II conjugated FITC immunostaining. Chondrogenic gene expression for SOX-9, AGG (aggrecan), and COLII (collagen type-II) were analyzed by real time PCR.

Results: Mechanical analysis of the hydrogels showed increased storage (E') and loss modulus (E'') in case of Col-added composite hydrogel. Further observation at 3 weeks of cell-cultured groups showed significant difference in tan delta value potentially due to the increased extracellular GAG production and re-modulation of microenvironment. FT-IR spectrum and TG analysis of the composite hydrogel showed higher chemical and thermal stability. The composite hydrogel showed increased cell viability and proliferation with extensive spreading of chondrocytes. Safranin 'O' staining of the cellular constructs in the composite hydrogel demonstrated the maintenance of chondrocyte phenotype even up to 21 days (Fig. 1). The composite hydrogel stimulated the production of cartilage specific extracellular matrix, as revealed by the immunuostaining of alcian blue, toluidine blue and collagen type-II. The mRNA expression of AGG and COLII was also enhanced at 7 and 21 days whilst that of SOX-9 was down-regulated at 21 days for the composite hydrogel.

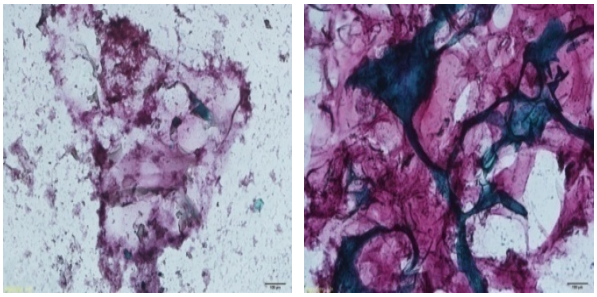

Fig. 1. (a)MTS assay for proliferation; (b) Safranin 'O' staining of composite hydrogel at 3weeks.

Conclusions: The Col-Alg-HA composite hydrogels were prepared to have proper physicochemical and mechanical properties of the ECM for chondrocyte culture. The chondrocytes cultured within the gel matrix were shown to proliferate actively, and to preserve the phenotypes for a long-term period over 3 weeks. The matrix is thus considered as useful 3D matrix for cartilage tissue engineering.

References:
1.Jin GZ, Kim JJ, Park JH, Seo SJ, Kim JH, Lee EJ, Kim HW. Tissue Eng Part A. 2014; 20:895-904