Study of Biomimetic Aggrecan Materials for Chondrocytes Culture

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Statement of Purpose: Due to the limited self-renewal ability of cartilage tissue, the tissue engineering approach, culturing of chondrocytes on a scaffold and then subsequently implanting into the defect is considered a promising alternative strategy for cartilage regeneration. However, chondrocyte cultivation *in vitro* is difficult in maintaining their shape and function because of the ease of dedifferentiation. Both Type II collagen (COLL II) and aggrecan, which consists of hyaluronic acid (HA) and chondroitin sulfate (CS), compose the native extracellular matrix (ECM) and serve as a structural scaffold for cell adhesion and growth. Many recent studies have shown that these major components have great influences on maintaining the function and shape of chondrocytes while cultured *in vitro*. Thus, it's reasonable to expect the potentially benefits of chondrocyte cultivation *in vitro* on the scaffolds composed of collagen and aggrecan materials. In this study, chondrocytic cells from bovine articular cartilage were selected and cultured *in vitro* by a floating stirred bioreactor. The characterization and its influences of the biomimetic aggrecan materials with or without COLL II on the performances of chondrocytes were discussed.

Methods: COLL II was extracted from bovine articular cartilage by pepsin digestion and subsequently purified by salt precipitation. Both HA and CS were first reacted with sodium periodate ($NaIO_4$) in aqueous solution respectively at room temperature in the dark. The excess $NaIO_4$ was then deactivated by adding ethylene glycol and subsequently removed under dialysis. A biomimetic aggrecan material was produced through the grafting of the aldehyde-containing hyaluronic acid and chondroitin sulfate by using 1, 6-diaminohexane as cross-linking agent. After removal of the ungrafted CS, the sodium cyanoborohydrate ($NaCNBH_3$) was added for better CS immobilization to HA.The chondrocytes were isolated from adult bovine articular cartilage using pronase E (2 mg/mL) and collagenase Type II (2 mg/mL) for enzymatic digestion. Chondrocytes resuspended in F-12 medium with seeding density 5×10^4 cell/mL were cultured either without matrix material, with biomimetic aggrecan material added, or biomimic aggrecan material mixed with COLL II in a floating stirred bioreactor. The cell proliferation and phenotypic expression were further examined by immunofluorescence staining.

Results: A periodate activation method was used to create reactive aldehyde groups of HA and CS. Additionally, the mechanism of the grafting reactions has been studied using ^{1}H-NMR spectroscopy. The disappearing of the peak around 7~8 ppm, which corresponds to the reactive aldehyde group in the glucuronic acid moiety of HA and CS molecule, after cross-linked with diamine suggested the coupling between these two materials.

The observations of CS stained with cupromeronic blue under TEM showed that CS from covalent bonding group was seemingly integrated to the HA domain as native aggrecans, whereas, CS in blended group was loosely arranged.

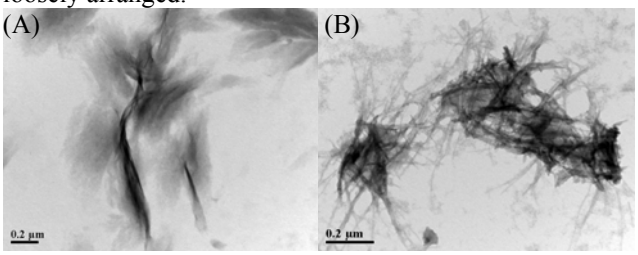

Fig. 1. TEM micrographs of (A) HA covalent bonded with CS; (B) HA blended with CS.

From the immunofluorescence results, the less compact of chondocytes cells, which cultured in a floating stirred bioreactor as biomimetic aggrecan added with or without COLL II, suggested the well adhesion cells on matrix materials. On the other hand, the chondocytes cultured without any matrix materials addition were self-assembled and the newly formed COLL II were also observed. These result suggested that the functionality of chondrocytes cultured *in vitro* could still well maintained.

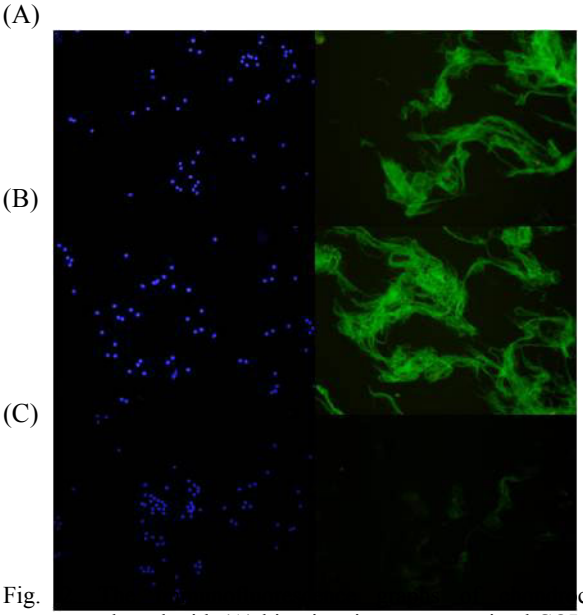

Fig. cytes cultured with (A) biomimetic aggrecan mixed COLL II, (B) COLL II, or (C) control group.

Conclusions: Our preliminary results indicated that the CS well integrated to the HA domain through diamime cross-linked and resembled the configurations of native aggrecans. With the addition of biomimetic aggrecan and COLL II while culturing the chondrocytes *in vitro*, the immunofluorescence result showed potential in cell proliferation and maintaining of cell functionality.

References:

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