CHITOSAN-BASED HYALURONIC ACID HYBRID POLYMER FIBER FOR A NOVEL THREE DIMENSIONAL SCAFFOLD IN CARTILAGE TISSUE ENGINEERING

Yamane, S; lwasaki, N; Majima, T; Funakoshi, T; Masuko, T; Minami, A; Nishimura, S
Department of Orthopaedic Surgery, Hokkaido University School of Medicine, Sapporo, Japan. +81-11-706-5937. FAX:+81-11-706-6054, s-yamane@med.hokudai.ac.jp

[Introduction] In cartilage tissue engineering, a number of studies have shown the importance of selecting the appropriate biomaterials as scaffolds for chondrocyte attachment, for supporting its proliferation and for its synthesis of extra-cellular matrix (ECM). Although a variety of biomaterials, including naturally occurring and synthetic, have been introduced as potential scaffolds for cartilage repair, we believe that the ideal cell-carrier substance should be one which closely mimics the natural environment in the cartilage-specific ECM. To mimic the ECM, we focused on the application of hyaluronic acid to a scaffold material. In this study, we hypothesized that chitosan-based hyaluronic acid hybrid polymer fibers and to evaluate the synthesis of ECM in the new material.

[Materials and Methods] Polymer fiber preparation: Chitosan polymer fibers (Chitosan-based group) and chitosan-based hyaluronic acid hybrid polymer fibers (HA 0.04% group, Chitosan coated with hyaluronic acid 0.04%; HA 0.07% group, Chitosan coated hyaluronic acid 0.07%) were initially prepared in our laboratory by the wetspinning method. The diameter of each fiber was 0.03 mm. Cell adhesion study: Chondrocytes were isolated from the articular surfaces of a Japanese white rabbit (2.0 kg) using a method described by Ochi, et al. The fibrous samples were cut into 10 mm pieces and packed in Teflon tubes (25mm length, 4.8mm inner diameter) and then 0.1ml of chondrocyte suspension containing 0.5 x 10^6 cells was loaded on the column at room temperature. The cells were allowed to adhere in a humidified incubator (37°C 4% CO2 95% air) for 1 hour. Each column was gently rinsed with 1ml of 1M phosphate-buffered saline, and the number of unattached cells in the rinsed solution was quantified by microscopic observation using a hemocytometer. In vitro chondrocyte culture: The polymer fiber sheets were fixed in a polyvinylpyrrolidone with six layers (8 x 8 mm, 1mm thickness). These three dimensional (3-D) scaffolds were used for chondrocyte culture and further investigations. Chondrocyte suspension containing 0.6 x 10^6 cells was embedded on the scaffolds. DNA isolation and semiquantitative RT-PCR analysis: RNA was extracted from samples incubated for 14 days with the TRIspin method described by Reno et al. Semiquantitative RT-PCR analysis was performed as previously reported by Hart et al. with rabbit articular cartilage specific primer sets for type I, II collagen, aggregan, and a housekeeping gene, glyceraldehydes-3-phosphate dehydrogenase (GAPDH). Data were expressed as a normalized ratio by comparing the integrated density (experimental integrated density / GAPDH integrated density) of mRNA for ECM molecules to the GAPDH; lane 2, type II collagen; lane 3, aggrecan; lane 4, type I collagen.

[Discussion and Conclusions] Although several studies have demonstrated the chondrogenic potential of glycosaminoglycans (GAGs)-augmented chitosan hydrogels and porous scaffolds, these materials do not have enough mechanical strength. To overcome this limitation, we have developed an original polymer fiber as a fundamental material for 3-D fabric. This new fabric based on chitosan-hyaluronic acid hybrid polymers will make an ideal scaffold with adequate strength for cartilage tissue engineering. The current study demonstrated that the adhesivity of chondrocytes and mRNA level of aggregan were significantly higher on a chitosan-based hyaluronic acid hybrid polymer than on a chitosan polymer. The ideal cell carrier substance should be one, which closely mimics the natural environment in the articular cartilage matrix. Glycosaminoglycans, which are part of the cartilage ECM components, play a critical role in regulating expression of the chondrocyte phenotype and in supporting chondrogenesis both in vitro and in vivo. Therefore, use of hyaluronic acid, which is one of the major GAGs in articular cartilage, as a component of a cartilage scaffold appears to be a reasonable approach for enhancing chondrogenesis. Consequently, chitosan-based hyaluronic acid hybrid polymer fibers show great potential as a desirable biomaterial for regenerating tissue scaffolds.

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**Laboratory for Bio-Macromolecular Chemistry, Hokkaido University, Sapporo, Japan.

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