Evaluation of Bio-mimic Collagen II/HA copolymer as scaffold for Chondrocytes

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Introduction: Articular cartilage has a limited self-repairing ability after injury and the healing of articular cartilage is still a clinical problem. Many approaches are being developed for the healing of articular cartilage, like excission, or the use of perichondreal autografts. However, these remedies could cause the formation of the fibro-cartilagenous tissue. Tissue engineering of cartilage, in which biocompatible scaffolds are cultured with chondrocytes to prepare hyaline-like tissue, may provide a more adequate choice. The physico-chemical features of the matrices appreciably influence the differentiation and activity of its cellular constituents. In this study, we have attempted to prepare the matrices which imitate articular cartilage, yet may overcome fibro-cartilaginous tissue problem (Frenkel S et al., J Bone Jt Surg 1997;79:831-6). Collagen II is the majority of extracellular matrix in articular cartilage (K. von der Mark, Academic Press, 1999, pp. 3-29). To obtain a bio-mimic ECM matrix, the collagen-based matrix could be prepared by addition of other biomolecules, such as hyaluronan or proteoglycan. In fact, hyaluronan, which is an important component of cartilage, combines with collagen II and other components to form the extracellular matrix of cartilage. Besides, hyaluronic acid could stimulate the synthesis of proteoglycan and chondroitin sulfate by chondrocyte. In this research we have fabricated a series of bio-mimic copolymers that composed of collagen type II and hyaluronic acid. The feasibility of this bio-mimic collagen/HA scaffold in vitro is under evaluation for application on chondrocytes culture.

Methods: To obtain the copolymer, we used $NaBH_4$ and $NaIO_4$ to modify HA to yield the reducing ends of HA for the coupling of collagen II. Hyaluronan with 220KD was used in this study. The modified HA was used to react with collagen II-NH_2 in synthesizing the collagen II-*co*-HA copolymer. The final modified hyaluronan was dissolved in sodium tetraborate decahydrate solution (0.05M, pH 8.8) and mixed with collagen II solutions (0.1 mg/ml). After the mixture was stirred for 30min at room temperature, $NaCNBH_3$ was charged into the solution to reduce the Schiff's base. The reaction was terminated after 48 h of stirring, by adding acetic acid to attain pH 4. The collagen II –co-HA copolymer was obtained by centrifuging with centrifugal filter.

Results/Discussion: The existence of aldehyde group in final modified hyaluronan could be confirmed by H^1-NMR. The localization of HA on the collagen fibrils was examined with electron microscopy. Lectin conjugated on the surface of nanogold could bind with the *N*-acetylglucosamine which was the unit of HA. According to TEM observations, the results could reveal that hyaluronan had bonded onto collagen II fiber. The AFM observation showed that the diameter of collagen II –co-HA copolymer was bigger than pure collagen II fiber. From SEM observation and MTT assay, it could revealed that using the copolymer to manufacture a high-density micromass cultures could maintain the chondrocyte phenotype after three weeks in vitro culture.

Fig. NMR spectrum of HA-CHO

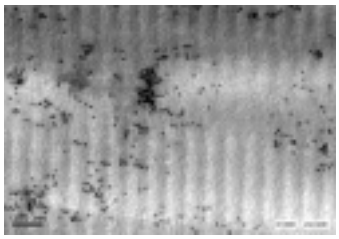

Fig. 2 TEM of nanogold labeled HA in collagen II fibrils

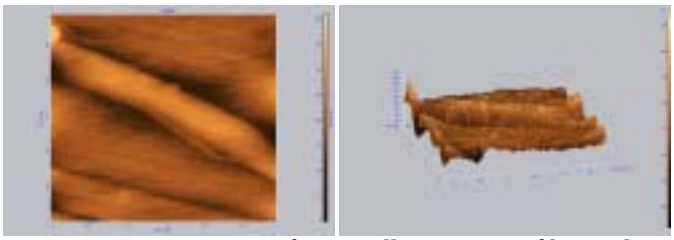

Fig. 3 Fig.3 AFM image of pure collagen type II fiber and collagen II-HA copolymer

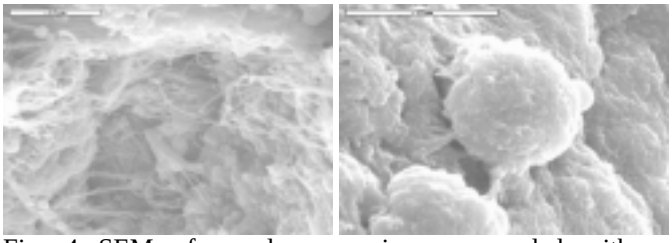

Fig. 4 SEM of copolymer mciromass, seeded with chondrocytes, after 21 days in culture.

Conclusions

The preliminary results demonstrated that collagen-g-HA copolymer was synthesized by the current process. The existence of the reducing ends in the modified HA was confirmed by H1-NMR. From AFM and TEM observations, the modified HA has bonded onto the collagen type II fiber by reacting between with collagen II-NH2. Chondrocytes cultured in the copolymer in vitro could maintain their phenotype in vitro culture. In this study, we fabrication a bio-mimic collagen II/HA copolymer and confirm it can sustain the phenotype for three weeks at least. The feasibility of this copolymer is still under evaluation for application on articular repair.