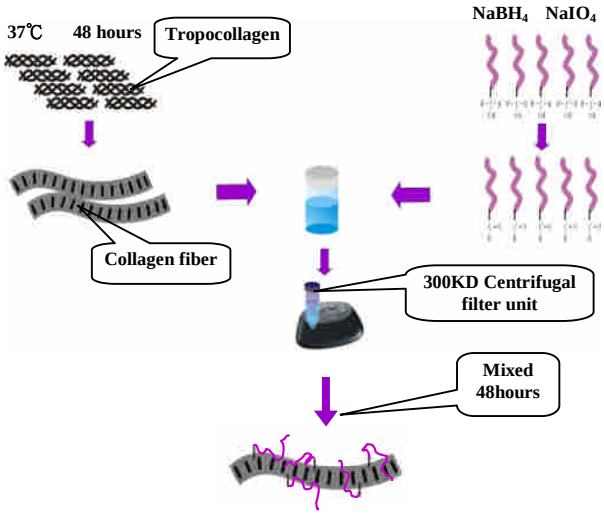
Bio-mimic Collagen II-co-HA Copolymer for Chondrocytes Culture

Shwu Jen Chang, Shyh Ming Kuo, Chen Zen Yang, Cheng-Wen Lan
Department of Biomedical Enginnering, I-SHOU University, Kaohsiung County, Taiwan

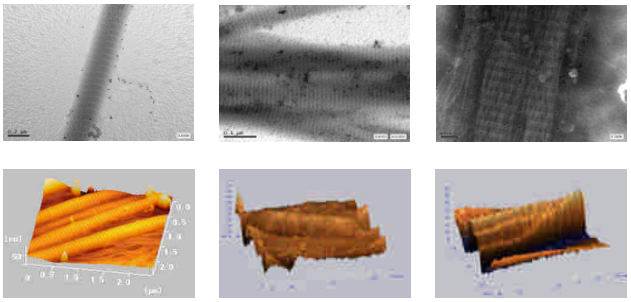
Introduction: The development of new biomaterial scaffolds for regenerative medicine and tissue engineering requires a better understanding of how a cell interacts with its surrounding extracellular matrix (ECM). Since bindings of ECM proteins and their respective membrane receptors occur in nanometer scale, ideal bio-matrix should be designed and fabricated by a bottom-up process. Type II collagen, as the major structural component of the extracellular matrix of articular cartilage, has provided critical domains as sites for the molecular interactions involving in fibril formation, entanglement and binding with other components such as hyaluronan and some proteoglycans to form articular cartilage. Hyaluronic acid, which is a negatively charged polysaccharide and also an important element in extracellular matrix, has marvelous viscoelastic ability, and high water retention capacity. The aim of this project is to develop 3-D matrices composed of nano-collagen fibrils and to study their effects on the regulation of chondroccyte differentiation.

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. The modified HA was used to react with collagen II-NH_2 in synthesizing the collagen II-*co*-HA copolymer. The collagen-*co*-HA copolymer was prepared according to the following scheme.



Results/Discussion: The existence of the reducing ends in the modified HA was confirmed by H^1-NMR (Fig. 1). From AFM and TEM observations, the modified HA has bonded onto the collagen type II fiber. We have prepared a novel copolymer, collagen II-*co*-HA, which was synthesized by reacting collagen-NH_2 with the aldehyde end group of HA. The preliminary result indicates that we have synthesized the bio-copolymer through the current process.

Figure 1. NMR spectrum of (a) 220KD HA (b) 220KD HA-CHO (c) 10KD HA (d) 10KD HA-CHO.



(a) Collagen fiber (b)Collagen-co-HA (HA 220kDa) (c) Collagen-co-HA (HA 10kDa)

Figure 2. TEM and AFM images of (a) pure collagen II, (b) 220kD HA/collagen II and (c) 10kD HA/collagen II.

Pure collagen fiber	402.3 ± 42.7 nm
220 kD HA/Collagen fiber	550.8 ± 43.7 nm
10 kD HA/Collagen fiber	414.9 ± 34.0 nm

Table I. The diameter of collagen II and collagen II-co-HA fibers.

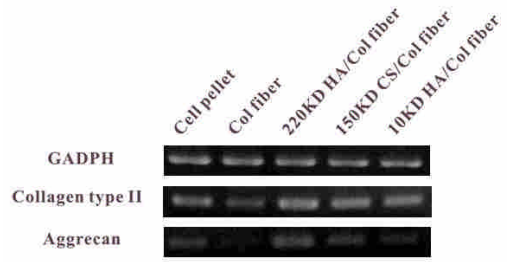


Figure 3. A reverse transcriptase PCR analysis of type II collagen and aggrecan gene expression in articular chondrocytes.

Conclusions: The results demonstrated that collagen-g-HA copolymers were 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. Bio-mimic copolymers were evaluated for their capacity to maintain chondrocyte proliferation and differentiation in vitro. After 21-day culture, the chondrocytes in 220kDa HA-co-collagen II expressed more mRNA transcripts for type II collagen and aggrecan than in control collagen II fibers.

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