Bovine achilles tendon collagen sponge modified with TGF-β1 maintained phenotype of chondrocytes *in vitro*

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**INTRODUCTION:** Articular cartilage offers loading carriage and lubrication. The self repair of cartilage is very limited.¹ Chondrocytes in cartilage are responsible for the synthesis and maintenance of extracellular matrix, which is composed of collagen network, proteoglycan, other proteins and glycoproteins. For hyaline-like cartilage tissue regeneration, constructs based on chondrocytes and biomaterial scaffolds were developed. One of the keys for success is to select suitable materials for fabrication of the scaffolds.² For this purpose, a new source of collagen from bovine achilles tendon was utilized as scaffold material. The main composition of bovine achilles tendon is collagen type I. For maintaining phenotypes of chondrocytes in scaffolds better, a recombinant human transforming growth factor-β1(TGF-β1) was bonded onto collagen type I by chemical processes (Figure 1). We purpose the modification of collagen scaffolds could keep phenotypes of chondrocytes during a long culture time.

**MATERIALS AND METHODS:** The bovine achilles tendon collagen (Sigma) dissolved in 0.05M acetic acid and 15% alcohol into the reserve of 1% colloidal solution. Collagen solution was replaced into a diameter 12 mm mold, freeze-dried at -80°C for 24 hours and bovine achilles tendon collagen sponge (BATCS) were harvested. BATCS was put into 250 ng/ml of TGF-β1(Biotechnology) solution containing 20mM 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride(EDC, Sigma) and 50 mM Nhydroxy - succinimide(NHS, Sigma) at 4°C for 2 hours, wash with water twice, freeze-dried sponges at -80°C for 24 hours, and BATCS modified by TGF-β1 (BATCS-TGF) were obtained. According to the results of DNA content (Figure 2), the similar proliferation of BATCS and BATCS-TGF with time was observed. The total collagen in BATCS-TGF was obtained (Figure 3) at 7 day. The more proteoglycan deposited in BATCS-TGF than those in BATCS were determined (P<0.05, Figure 2). The chondrocytes are round shape, secret collagen and proteoglycan and up-regular gene expression of collagen type II, aggrecan and Sox9. The round cell shape in BATCS-TGF was determined at 7 day (Figure 4) at 7 day. The more proteoglycan deposited in BATCS-TGF than those in BATCS were determined at 14 day was observed (Figure 5). The more proteoglycan deposited in BATCS-TGF than one in BATCS at 14 day was observed (Figure 6). According above results, bovine achilles tendon collagen sponge after modified with TGF-β1 maintain phenotype of chondrocytes *in vitro* was demonstrated. It can feasibly offer new biomaterials for cartilage regeneration.

**REFERENCES:**


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