Differentiation of Human Mesenchymal Stem Cells into Ligament Tissue on Collagen-Elastin Nanofibers with Appliance of Cyclic Strain

Jae Hyung Kim[1,2], Il-Hoon Kim[1], Hwal Suh[1,2], Si-Nae Park[1,2],
[1]Department of Medical Engineering, College of Medicine, [2]National BK21 Project Team of Nanobiomaterials for Cell-Based Implants, Yonsei University, Seoul 120-752, Korea.

Statement of Purpose: Ligament reconstruction surgery is still facing major obstacles to overcome, such as tendonitis, stress shielding effect and muscle atrophy. In order to accomplish successful regeneration of ligament tissues, therefore, tissue engineering is considered as an alternative approach (Altman GH. Biomaterials 2002; 23:4131). We have previously fabricated aligned collagen-elastin nanofibers by using electrospinning method and EDC-crosslinkage for the development of biomimicking ligament tissue engineered constructs. Mechanical forces play a central role in physiology of various tissues. Several investigator have reported that cyclic mechanical stretch increases extracellular matrix (ECM) production in cultured fibroblasts on flexible membrane (Breen EC. J Appl. Physiol. 2000; 88: 203-209). In this study, human mesenchymal stem cells (hMSCs) were cultured on aligned collagen-elastin nanofiibers and cyclically strained for promoting stem cell differentiation toward ligament lineage.

Methods:

1. Materials

Type I atelocollagen was extracted from calf skin using pepsin treatment and salt precipitation as previously described (Gentleman E. Biomaterials 2003; 24:3805-13). Collagen precipitate was then lyophilized at -40 ºC and kept at -20 ºC for application. Elastin (from bovine neck ligament) and 1-ethyl-(3-3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2. Fabrication of Nanofiber

Type I atelocollagen and elastin (8:2, w/w) were dissolved together in 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) with the concentration of 7% (w/v). This was applied in order to develop biomimicking ligament tissue engineered constructs. The collagen-elastin solution was then loaded into a syringe with an 18-gauge needle and placed in a syringe pump for metered dispensing at a rate of 2.5 ml/hr. The positive output lead of a high voltage supply set to 20 kV was attached to a needle. The target mandrel was rotated at approximately 3500 rpm for deposition of fibrils along the axis of rotation.

3. Cell Culture on Collagen-Elastin Nanofiber

We cultured hMSCs on collagen-elastin nanofibers using a spinner flask at 60 rpm for 8 hours.

4. Application of Cyclic Mechanical Strain and Measurement of Alignment Nanofibers with hMSCs seeded were strained at 0.75 Hz in a CO_2 incubator. Cyclic strain was applied for 6, 12, 18 or 24 hours. Cyclic strain was also applied for 12 hours at 1.5 Hz to observe an effect of change in frequency.

5. RT-PCR

The attached cells were analyzed by reverse transcription-polymerase chain reaction (RT-PCR) for the mRNA expression of type I collagen, type II collagen, type III collagen, tenascin-C and bone sialoprotein (BSP).

Results/Discussion:

We have previously observed and characterized collagen-elastin nanofibers produced by an electrospinning method. Using advantages of this method, we were able to produce well-aligned nanofibers for applications of mechanical strain.

Cells were seeded in a spinner flask, and then different stretching times of 0, 6, 12, 18 and 24 hours were applied at a frequency of 0.75 Hz. All experiments were operated for a 24-hour period. Here we tried to find optimal stretching time for a period. Based on RT-PCR analysis, it was found out that mRNA expression of type I collagen and type III collagen increased as stretching time increased up to 12 hours.

Following outcomes obtained, an optimal stretching time was settled at 12 hours and 1.5 Hz of frequency was applied for a 24-hour period. As the frequency increased, mRNA expression of type I collagen increased. Interestingly, the expression of BSP at 1.5 Hz showed a remarkable decrease comparing to that at 0.75 Hz.

Conclusions: Results demonstrate that aligned collagen-elatin nanofibers can provide suitable biomaterial scaffoldings for supporting stem cell differentiation by mechanical strain. We have attempted to set up an optimal stretching time and frequency for effective induction of hMSCs to ligament tissues. These results will be applied for long-term generation of cyclic strain on further study, in order to observe whether mechanical properties affect to differentiation into ligament tissues.