Electrospun Collagen-Elastin Nanofibers: a Biomimetic Extracellular Matrix for Smooth Muscle Cell Proliferation

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Introduction

The media of the blood vessels is composed of multiple layers of smooth muscle cells (SMCs) within a surrounding extracellular matrix (ECM) composed of collagen types and , and elastin fibers. The SMCs and collagen fibrils have a marked circumferential orientation (Boland ED. Front Biosci. 2004;9:1422-1432).

In this study, a biomimetic ECM was developed using aligned collagen-elastin nanofibers by electrospinning. Produced scaffolds were stabilized by crosslinking with the N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC). Considering the applications for vascular tissue engineering, proliferation of SMCs on the fabricated nanofibers was evaluated.

Methods and Methods

Unless noted, all reagents were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). Solutions of type atelocollagen from bovine skin (Dalim Tissen Inc., Seoul, Korea) and elastin from bovine neck ligament were prepared by dissolving them in 1,1,1,3,3,3-hexafluoro-2-propanol (HFP). The concentration of a mixture of collagen and elastin (8:2) in the solutions was 7 or 9% (w/v). Fibers have been spun at 20 kV, with a flow rate of 2.5 mL/h and a collecting distance of 8~10 cm. The cylindrical mandrel rotated at 4500~4830 rpm. Fabricated fibers were crosslinked by 50 mM EDC solution (EtOH:H_2O = 95:5) for 12 h at room temperature.

The morphological characterization of nanofibers and cell/scaffold hybrids was carried out by scanning electron microscopy (SEM, S-800, Hitachi, Tokyo, Japan) (Cha JM. Artif Organs. 2006;30:250-258).

The human aortic vascular smooth muscle cell line T/G HA-VSMC was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in ATCC complete growth medium. The prepared scaffolds were sterilized and prewet with 70% ethanol for up to 24 h. Then, the scaffolds were soaked in growth medium overnight. The cells were then seeded to the scaffolds in a spinner flask (60 rpm) and incubated for 20 h in a CO_2 incubator.

After 1, 3, 5, and 7 d of culture, conventional hexosaminidase assays were performed to measure cell proliferation (Cha JM. Artif Organs. 2006;30:250-258).

Results and Discussion

The electrospun collagen-elastin fibers are non-woven, highly porous mesh. Beaded fibers, thin filaments of fibers with a ribbon-like appearance were produced at concentration of 7%. Only at concentration of 9%, long and uniform fibers were fabricated. By increasing the concentration of the solution, the average size of the fibers increased. The 7 and 9% collagen-elastin nanofibers contained an average fiber diameter of 0.448±0.024 μm and 0.744±0.103 μm, respectively (n = 50).

It was observed that the collected fibers were partially aligned. It is expected that varying the rotating speed of the mandrel can influence the degree of orientation of the fibers obtained. The electrospun collagen-elastin lacked the morphological characteristics like the D-period of collagen or the filament-like folds of elastin. As a consequence, it was not possible to distinguish the proteins in the fiber (Buttafoco L. Biomaterials. 2006;27:724-734).

After crosslinking, the porous and aligned structures of the collagen-elastin nanofibers were retained.

We examined the biological properties of electrospun collagen-elastin in tissue culture experiments. The increasing number of SMCs on the scaffolds during the culture period demonstrates that proliferation occurred on the scaffolds. SEM observation revealed that the scaffolds were densely populated with the SMCs. SMCs grew as a confluent layer on top of the nanofibers after 7 d of culture. Within 7 d of seeding, the cells infiltrate the electrospun matrix and are well integrated into the network.

Conclusions

We developed aligned collagen-elastin (8:2) nanofibers by electrospinning. By EDC crosslinking, more stabilized scaffolds were obtained. The scaffold provided favorable condition to proliferate human aortic vascular SMCs.

References

[1] Boland ED, et al. Front Biosci. 2004;9:1422-1432.
[2] Buttafoco L, et al. Biomaterials. 2006;27:724-734.
[3] Cha JM, et al. Artif Organs. 2006;30:250-258.