A collagen/vascular smooth muscle cells (SMCs) incorporating elastic scaffold for tissue-engineered vascular graft

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Statement of Purpose: This study was focused on a collagen/cell mixture seeding method to improve the adhesion and proliferation of vascular smooth muscle cells (SMCs) in tubular porous scaffolds for vascular grafts application. Recently, poly(L-lactide-co-ε-caprolactone) (PLCL) copolymers have been applied as a biomaterial for vascular graft due to the high elastic property. We previously reported that tubular PLCL scaffolds were fabricated as a mechanically active artificial blood vessel by an extrusion-particulate leaching technique. However, these extruded PLCL scaffolds caused a few problems to be improved in respect of not only the cell seeding efficiency and cell in-growth. In this study, we sought to introduce a hydrogel seeding method for the enhancement of SMCs seeding and proliferation into tubular PLCL scaffolds.

Methods: Tubular porous scaffolds (ID: 4 mm, OD: 6 mm) were fabricated from a biodegradable elastic polymer, poly(L-lactide-co-ε-caprolactone) (PLCL) (50:50, Mn 1.58 × 10^5), by an extrusion/particulate leaching method (pore size : 50~100 μm, 100~200 μm, and 300~500 μm). SMCs incorporating collagen solution was infiltrated in PLCL scaffolds under vacuum and incubated for 1 h at 37 ^oC to form collagenous gel. The optimal condition of the seeding process was determined by investigating cell viability and cell adhesion rate depending on pressure and gelation time. Cell adhesion and cell viability were determined by Water-soluble Tetrazolium salts (WST) assay and Lactate dehydrogenase (LDH) assay, respectively.

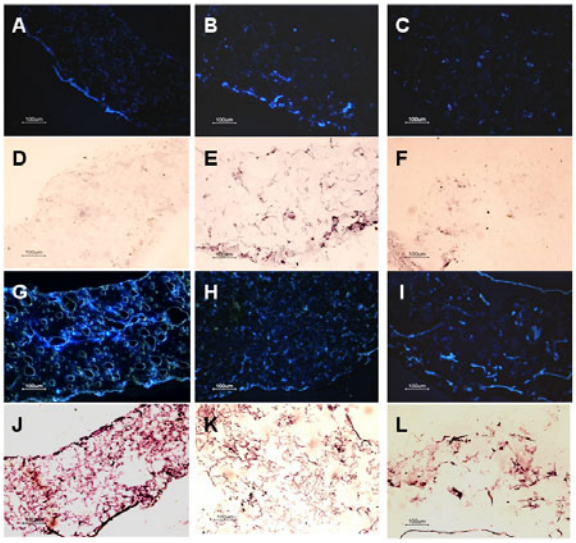

Figure 1. DAPI staining (A~C,G~I) and histological analysis (Van Gieson solution) (D~F,J~L) of SMCs grown on PLCL scaffolds for 4 weeks; pore size of (A,D,G,J) 50~100 μm, (B,E,H,K) 100~200 μm, and (C,F,I,L) 300~500 μm. (A~F) collagen unmodified scaffolds, (G~L) collagen modified scaffolds. The scale bars indicate 100μm (magnification X 60).

Results: Collagen/SMCs incorporating PLCL scaffolds proved to maintain elasticity and flexibility. Results from SEM image analysis showed that collagen was infiltrated into the inside of the scaffolds. Cell adhesion and proliferation efficiency increased in collagen/SMCs incorporating PLCL scaffolds as compared with PLCL scaffolds in which only SMCs were seeded. From SEM image and histological analysis, we further found that SMCs grew in the inside as well as on the surface of collagen/SMC incorporating scaffolds and the cells continued to grow as a monolayer on collagen fibers. In particular, cell proliferation and elastin contents were the highest in a PLCL scaffold with 50~100 μm pore size than any other scaffolds used in this experiment.

Conclusions: Extensive efforts have been made to develop scaffolds able to support and promote tissue growth for clinical appplicatons. In this study, we have evaluated the adhesion, growth and ECM expression of SMCs on collagen modified scaffolds of different diameters. It was found that cell morphology pictured by scanning electron microscopy substantiates the structural properties, collagen fiber prosses very high surface-to-area ratios. Higher cell viability suggests that under our vacuum seeding condition, the type I-A collagen nanofibers provide favorable growth conditions and survival for SMCs. This appears to be advantageous that scaffold may aid in the viability and expansion of cells and enhance the rate of tissue formation.

Such an environment is similar to that provided by the web like collagen fibers in which able surfaces tend to have higer amounts of extracellular matrix proteins. The highest cell adhesion and proliferation was observed in the collagen modified scaffolds with the pore diameter of 50~100 μm.

References:

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