Enhanced ECM regeneration in Mechano-active vascular tissue engineering

Kim, YH[1], Lee, J[1], Jung, H[1], Lim, JI[2], Kim, S-H[2], Kim, SH[2]
[1]Dept. of Materials Science & Engineering, Gwangju Institute of Science and Technology, Gwangju 500-712, Korea
[2]Biomaterials Research Center, Korea Institute of Science and Technology P.O. Box 131,Cheongryang, Seoul 130-650,Korea

Introduction

Many recent studies have reported that mechanical stimuli enhanced the development and function of engineered vessel tissues[1,2]. For such stimuli-enhanced (mechano-active) tissue engineering, it is necessary to develop very elastic scaffolds that can withstand cyclic mechanical strain without cracking or significant permanent deformation. We applied very elastic poly(lactide-co-caprolactone) (PLCL) scaffolds for mechano-active vascular tissue engineering[3,4] to culture VSMCs (vascular smooth muscle cells) and ECs (enthothelial cells) on a pulsatile-flow bioreactor[5] to regenerate native-like vascular tissues..

Materials and Methods

PLCL (50:50, Mn~280,000) polymer was processed into cylindrical PLCL scaffolds (ID 4mm, porosity 98%) by a extrusion/particulate leaching technique. Aortic SMCs were seeded onto PLCL scaffolds and cultured in vitro for up to 7 weeks on a bioreactor under a pulsatile flow with 120 beats/minute (bpm) and 8 % radial distention. Then, ECs were seeded into the lumen and cultured further. The scaffold/cells conducts were analyzed by SEM, haematoxylin and eosin (H&E) staining, Messon's trichrome (M&T) staining, Western blotting. The characteristics of regenerated vessels were evaluated in terms of collagen and elastin contents and burst strengths.

Results and Discussion

Tubular PLCL scaffolds demonstrated a complete resilience under cyclic strain in culture medium for the initial 2 weeks. The cell number in the engineered tissues continuously increased for up to 5 weeks in both static and pulsatile flow culture groups. The regenerated grafts showed well developed SM tissues within the scaffolds left. Collagen and elastin production was enhanced for pulsatile flow group compared with static culture group (Fig. 1). The collagen or elastin contents reached about the half values of native vessels. From the Western blotting analysis, the phenotypes of SMC were better maintained for pulsatile flow group while those for static culture group were slowly decayed. Burst strength of engineered vessels was increased on culture to reach the values of native vessels **of rabbit arota** (Table 1). An implantation test using animal models of regenerated vessels is in plan.

Conclusions .

SMCs were seeded onto very elastic PLCL scaffolds and cultured on a pulsatile perfusion reactor (mechano-active tissue engineering). A mechanical stimulation encouraged SMCs to maintain their phenotypes and enhanced elastin production. The rubber-like elasticity of PLCL applied in this study might have contributed to transfer the mechanical signal to cultured cells completely to reveal the effect of mechanical stimuli on tissue engineering. The regenerated vessels exhibited the burst strength similar to native vessels.

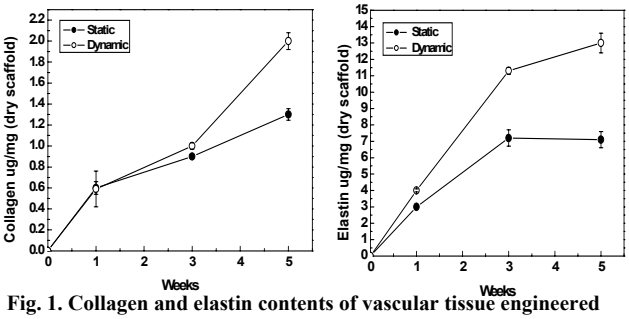

Fig. 1. Collagen and elastin contents of vascular tissue engineered under dynamic stimulation or static condition.

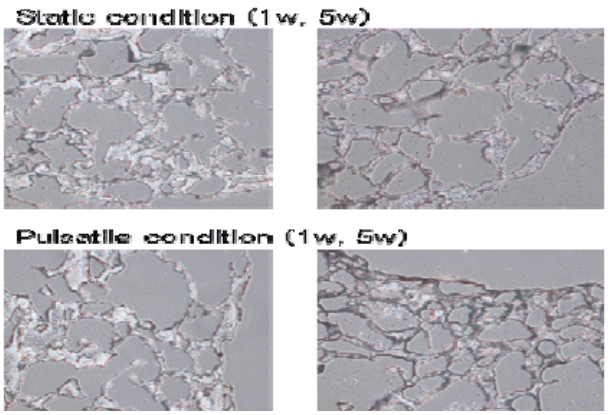

Fig. 2. Elastin staining of regenerated scaffolds

Table 1. Characteristics of blood vessels and regenerated tissue

	Un-seeded scaffolds	Regenerated vessels	Native vessels	Acellular native vessels
Burst strength	0.12 MPa 890 mmHg	0.16 1,220	0.20 1,480	0.15 1,160
Collagen (ug/mg)	0	2-3	5-6	
Elastin (ug/mg)	0	12-20	36	

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