An Elastic Fibrous Scaffold for Cardiovascular Tissue Engineering

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Statement of Purpose: The purpose of this study is to use biodegradable scaffolds and stem cells for abdominal blood vessel surgery. We used a novel fibrous scaffold fabricated from poly(L-lactide-*co*-ε-caprolactone) (PLCL) by a gel spinning technique. Bone marrow mononuclear cells were employed as a stem cell source for efficient vascular tissue engineering.

Methods: Fabrication of elastic fibrous scaffolds. A biodegradable polymer, poly (L-lactide-*co*-ε-caprolactone) (PLCL) was precipitated in non-solvent (methanol) using solvent phase separation method and, at the same time, a tubular scaffold was fabricated by a rotating shaft in the methanol. Scaffolds were fabricated by changing conditions such as gel flow rate, gel concentration, and salts-PLCL weight ratio. **Analysis of morphology and mechanical strength.** To investigate microfiber structure and interconnectivity, scaffolds were analyzed by scanning electronic microscope (SEM). The measurement of mechanical properties was tested by Instron. ***In vivo* canine study.** Scaffolds were fabricated as a double-layered form in order to avoid blood leakage. Briefly, inner lumen of scaffolds were coated by PLCL solution with 20μm salts and salts were removed after gel spinning. Bone marrow (30cc) was aspirated in canine femur and mononuclear cells were isolated by gradient-density method using Histopaque 1077 (Sigma co.) 1ml bone marrow mononuclear cell suspension in serum-free Endothelial basic media-2 was seeded in the fibrous scaffold (ca. 5 cm).

Results/Discussion: Morphology of fibrous scaffolds. Fibrous scaffolds fabricated by gel spinning had micro-fibrous structure as shown in Fig.1. The diameters of microfiber increased gradually depending on PLCL gel concentration.(data was not shown) Interconnectivity between fibers could help cells and biomolecules to interact each other.

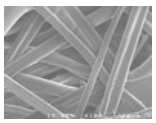

Figure 1. SEM image of fibrous scaffold. Outer surface of fibrous scaffold.

Mechanical property of fibrous scaffolds. Mechanical properties in circumferential direction of fibrous scaffolds were stronger than those of longitudinal direction. The trend is shown in scaffolds of every gel concentration group. **Degradation rate and tissue in-growth of fibrous scaffolds in mouse.** In both groups (gel-spun and extruded scaffolds), molecular weights decreased relatively fast and there is no significant difference. After 153 days later, molecular weights degraded. Though hematotoxylin and eosin stain, tissue in-growth was observed 41days after implantation. ***In vivo* canine study.** Implanted scaffolds were harvested after 4weeks. From immunohistochemisrty of von Willebrand Factor (vWF), angiogenesis was detected in the scaffolds. (Fig. 2. (c))

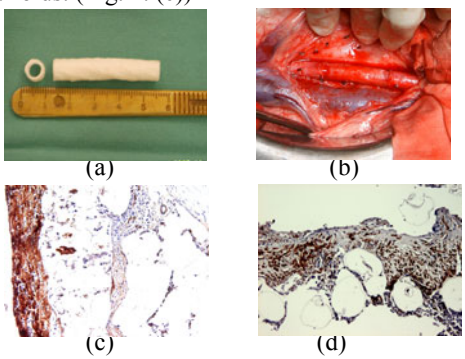

Figure 2. *In vivo* canine study. (a) fibrous scaffold; (b) Implantation; (c) immunohistochemisty of von willebrand factor; (d) immunohistochemisty of smooth muscle α-actin

Von Willebrand Factor (vWF) is known as a marker of endothelial cells. There were vWF-positive cells lined in the lumen of the implanted scaffold. The result is a very important indicator for tissue regeneration. In addition, α-SM actin-positive smooth muscle cells were also observed in the inside of the scaffolds (Fig. 2(d)).

Conclusions: In this study, the characterization and *in vivo* experiment of fibrous scaffolds were investigated. As a result, fibrous scaffolds have proper morphology and mechanical strength. These characteristics were depended on the conditions such as gel concentration, flow rate and salt-PLCL weight ratio. From *in vivo* study, PLCL fibrous scaffolds showed the possibility of regeneration in the blood vessel environment. Thus, this elastic fibrous scaffold could be a good graft for clinical application of vascular tissue engineering in the future.

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References: 1. S. I. Jeong, B. S. Kim, Y. M. Lee, K. J. Ihn, S. H. Kim, Y. H. Kim Biomacromolecules; 2004; 5: 1303-1309 2. M.R. Williamson, E.F. Adams, A.G.A. Coombes Biomaterials; 2006; 27: 1019-1026