Abstract of Invited Speech 5

Development and Characterization of Biodegradable Polymeric Scaffolds for Cardiovascular Tissue Engineering

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1. Introduction

The Congenital heart disease (CHD) has remained to be the major cause of infant death accounted for 48.1% of all infant mortality in the United States. Some patients with severe CHD may need the reconstruction of heart with use of polymer prosthetic graft such as Dacron or PTF. Although prosthetic stents have widely been used for cardiovascular reconstruction, it is susceptible to complication such as infection which potentially interferes the healing process. Recently, researches have actively been conducted in the field of vascular tissue engineering, mainly to develop engineered artificial vascular tissues [1], however, the technology is still developing and fundamental researches still need to be performed to construct artificial tissues that have biological and physical properties comparable with natural tissues.

In the present work, cylindrical porous scaffolds were developed using biodegradable polymer PLCL by the freeze-drying method. A hybrid construct of the tubular scaffold and MSC or MSC/ES cell sheets was also fabricated successfully. Then, the mechanical and biological properties were characterized. The melt-spinning method was also utilized to construct a different type of cylindrical scaffold having a fibrous structure with randomly aligned PLCL fibers. The mechanical properties were characterized and compared with those of the porous scaffold.

2. Experimental

Porous polymeric tubular scaffolds were fabricated from poly(L-lactic acid-ε-caprolactone) (PLCL) by using the combination method of the solid liquid phase separation and freeze-drying techniques. Briefly, PLCL (75/25) (BMG Co.) granules were dissolved in 1.4-dioxane solvent (6% w/v) and then a frozen Teflon tube was dipped into the polymer solution and pulled at a constant rate. Then the graft was freeze-dried at -50oC for 1 night. Finally, the tubular graft could be pulled out from the Teflon tubes. The above procedure was repeated to obtain double and triple

layers of tubular scaffolds.

For a biological examination, co-culture cell sheets were prepared from human mesenchymal stem cells (HMSCs) (UE6E7TE, Riken RBC) and human pulmonary artery endothelial cells (HPAECs) (KA-4109, Kurabo) at cell ratio of 1:1 into 24 multi-wells of the temperature-responsive dishes (CellSeed Inc.). For comparsion, monoculture cell sheets of HMSCs were also prepared in the same culture condition. Two pieces of the cell sheets were layered on the surface of each of the tubular scaffolds. These scaffolds with cell sheets were then cultured for 11 days in a medium. Cell proliferation was monitored by the colorimetric assay using a cell counting kit (Dojindo). After incubating for 2 hours, the absorbance was measured at 450 nm with a microplate reader (Perkin Elmer). The expression of angiogenic gene was also characterized using the real-time polymerase chain reaction (RT-PCR).

Ring tensile test was performed at a displacement rate of 1 mm/min by a conventional testing machine (EZ Test, Shimadzu Co.). Force and displacement were recorded in a personal computer. The circumferential strain, ε, was then calculated from the recorded data. Microstructures of the specimens were also observed by a field-emission electron microscope (FE-SEM, Hitachi).

Microfibrous cylindrical scaffolds were also fabricated from the same PLCL with the melting temperature of 163 °C using a commercial cotton candy machine (EA-WA2805, Azuma Engineering Co.). Two gram of PLCL granules were put into the spinning head of the machine. The spinning head has a fixed rotating speed of 1800 rpm and heating temperature. PLCL fibers were formed at temperature of 180°C, recorded by a digital thermometer. They were immediately collected on the surface of a Teflon rod (ϕ 5 mm) and then dried for one night. Ring tensile tests and FE-SEM observation were also conducted to characterize the tensile mechanical properties and microstructural morphology.

3. Results and Discussion

The overview, surface morphology, and layered structure of the porous tubular scaffolds fabricated by the solid-liquid phase separation and freeze-drying method are shown in Fig.1. It was found that pores were evenly distributed on the surface and continuous porous structures were generated in the thickness direction. This kind of porous structure was thought to be ideal for cell proliferation and formation of extra-cellular matrices (ECM). It was also seen that a double-layer structure was successfully fabricated by the current technique. However, a thin wall was generated between the inner and outer layers, which may prevent cells from growing from outer to inner (or from inner to outer) through the interlayer.

The overview, surface morphology, and layered structure of the fibrous tubular scaffolds fabricated by the melt-spinning method are shown in Fig.2. It was found that the micro-fibers were randomly distributed and gaps were generated between the distributed fibers. These gaps created a porous structure with continuous pores which is considered to be an ideal structure for cell proliferation and ECM formation.

The tensile strengths of the single, double and triple-layer porous scaffolds are shown in Fig.3.

It is clearly seen that the increasing layer number corresponded to the increase of tensile strength. It is worth noting that the strength of the triple-layer scaffold is very close to that of native vessels (about1700 kPa). It is thus concluded that the layered structure can be the candidate for a scaffold for cardiovascular regenerative medicine in which the mechanical properties of the scaffold must be equivalent to those of native vessels. The tensile strength of the fibrous scaffold is also compared with the porous scaffold in Fig.3. It was found that the fibrous scaffold has higher strength than the porous scaffold, although it is still lower than that of native vessels.

Cell proliferation behavior is shown in Fig.4. It was found that the co-cultur system promoted higher proliferation rate. Moreover, the angiogenic potential of the co-culture was superior to that of monoculture.

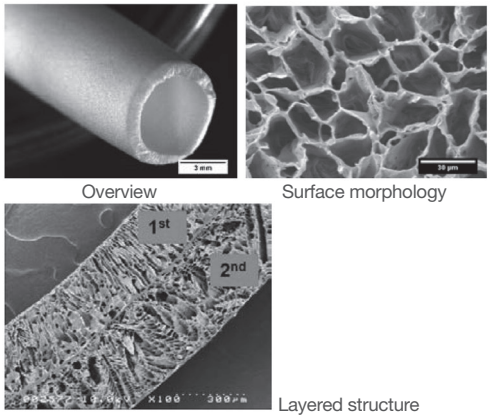

Fig.1 Porous tubular scaffold

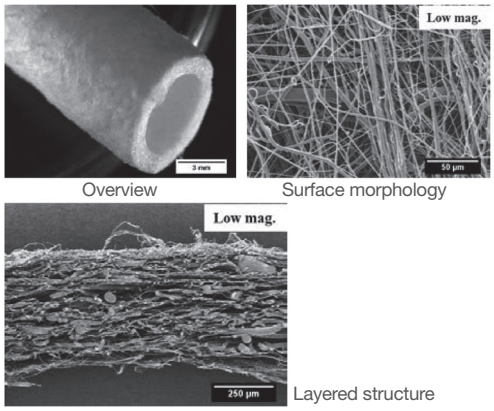

Fig.2 Fibrous tubular scaffold

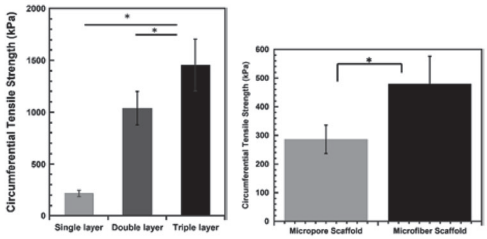

Fig.3 Comparison of tensile strength

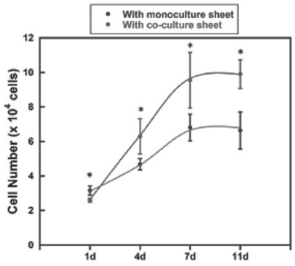

Fig.4 Cell proliferation behavior

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