P16

The Behavior of Co-culture Cell Sheet on Porous Tubular Polymer Scaffold for Blood Vessel Tissue Engineering

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Abstract
The aim of this study is to develop hybrid tubular structure of poly-co-lactide-caprolactone (PLCL) and mesenchymal stem cells (MSCs). Such graft was constructed by layering MSC sheets on porous PLCL tubular graft. Cell proliferation assay confirmed that the number of cells increased steadily during 11 days of *in vitro* study. Moreover, it was also found that co-culture cell sheet of MSCs and endothelial cells (ECs) exhibited higher proliferation rate than that of MSCs' monoculture cell sheet, indicating that the co-culture cell sheet can modulate higher proliferation of cells. Tensile mechanical testing of the hybrid tubes was also conducted and it was found that the mechanical properties of the hybrid tube with co-culture cell sheets tended to be larger than those of the tube with monoculture sheets.

1. Introduction

Recently, the cell sheet technology has been proven to repair partial tissue defects including myocardium[1], cartilage[2], and vascular[3]. However, it is difficult to construct organ-like 3D structures using the cell sheets because high cell density of a thick layered cell sheet may trigger necrosis due to poor supply of oxygen and nutrient without blood vessels[4]. Therefore, the aim of this study is to develop a hybrid graft by layering cell sheet on polymer tubular scaffold for cardiovascular tissue engineering. The hybrid graft was fabricated from polymer tube composed of PLCL and two layers of cell sheet which wrapping around the polymer tube.

2. Materials and Methods

Polymer tube was fabricated from PLCL by using the solid-liquid phase separation method, followed by the freeze drying method. Co-culture cell sheets were prepared by seeding $5x10^4$ cells composed of HMSCs (UE6E7TE, Riken RBC, Japan) and HPAECs (KA-4109, Kurabo,Japan) at cell ratio of 1:1 into 24 multi wells temperature-responsive dishes (*Upcell* ®, CellSeed Inc., Tokyo, Japan). After 4 days culturing in 37°C/5%CO_2 condition, cell sheets were detached by utilizing a membrane (*cell shifter* ®, CellSeed Inc., Tokyo, Japan) and 2 layers of cell sheets were attached on the PLCL tubular scaffold (Fig. 1). Those hybrid grafts were cultured for 11 days in a mixed medium of the cell growth medium and endothelial growth medium (50:50).

The tensile tests of ring specimens prepared from the hybrid grafts were performed using a compact tabletop testing machine with 10 N load cell and a crosshead speed of 1 mm/min to investigate the variation behavior of the mechanical properties during *in vitro* study. The microstructures of the specimens were also observed using FE-SEM (Hitachi Ltd., S-4100). Cell proliferation assay was performed using the Cell Counting Kit (Dojindo Laboratories, Kumamoto, Japan). Angiogenic expression was identified using real-time polymerase chain reaction (RT-PCR) (Takara Bio, Shiga, Japan).

3. Result and Discussion

To assess the effect of cell sheet layered on a tubular scaffold to the mechanical properties, ring tensile test was performed. The results were shown in Figure 1. In general, the typical stress-strain curve, elastic modulus and circumferential tensile strength of the three construct were insignificantly different, indicating that both of monoculture cell sheet and co-culture cell sheet did not alter the original mechanical strength of the scaffold. The estimated burst pressure during in vitro course was summarized in Figure 1d. All constructs have estimated burst pressure more 150 mmHg. This value is more than the normal blood pressure of human which is in the range of 120 mmHg.

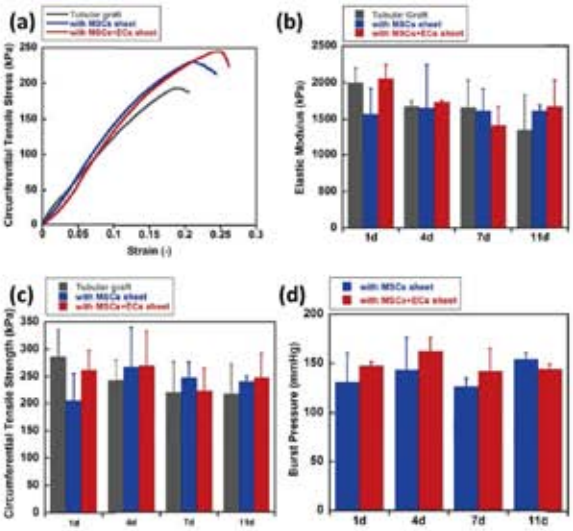

Figure 1. Mechanical Characterization

Figure 2 showed microstructural observation of the three constructs. Tubular scaffold has porous structure with average diameter of 21 μm. The cross sectional image of tubular scaffold showed elongated pores connected to each other. Both of monoculture and co-culture cell sheet exhibited similar cell spreading behavior. Cell sheet covered the outer wall of tubular scaffold, making the pores unobservable. The interconnected pores were able to facilitate cell infiltration (as observed in right panel images), thus enabling cell spreading on the inner wall of the tubular scaffold.

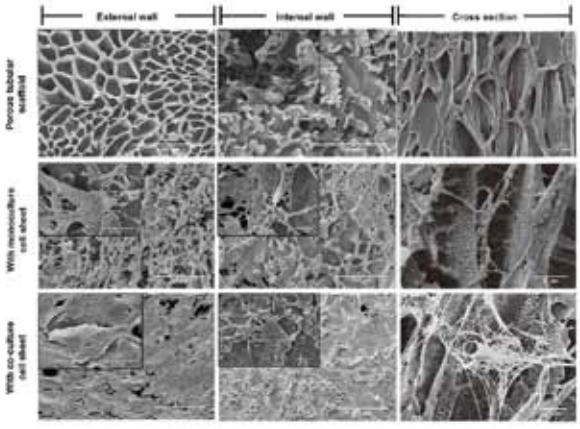

Figure 2. SEM observation

The exact number of cells within tubular scaffold was summarized in Figure 3a. During in vitro course, it was obviously seen that cells in co-culture system proliferate higher than that in monoculture system. This result suggested that co-culturing cells of endothelial cells and mesenchymal stem cells may induce one of the cell type to proliferate faster.

The effect of co-culture system over vasculogenic gene were further studied. Figure 3b

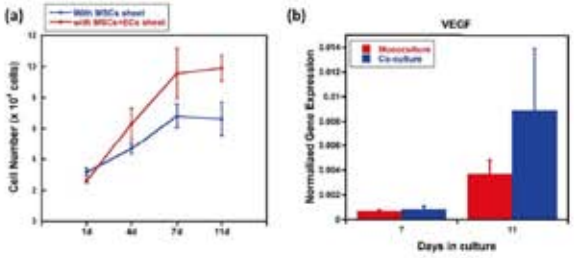

Figure 3. (a) Cell growth and (b) Gene expression of angiogenesis

showed gene expression contained in both of monoculture system and co-culture system. The expression of vascular endothelial growth factor (VEGF) in co-culture system was higher than that in monoculture system, particularly at days 11. The presence of VEGF is responsible in stimulating the formation of new blood vessels[5]. These results indicate that co-culture system could promote angiogenesis which is crucial in maturation of tissue engneered blood vessel.

4. Conclusion

In the present study, a tubular graft was successfully fabricated as a hybrid structure of PLCL porous tubular scaffold and co-culture cell sheets of MSCs and ECs for vascular tissue engineering. The co-culture system exhibited higher mechanical properties and proliferation rate than the mono-culture system. It is thus concluded that the co-culturing with ECs can be one of the important factors to maintain the active proliferation of MSCs.

References

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