Tissue Engineered Blood Vessels by Combining Cell Sheet Engineering and Electrospinning Technology

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Statement of Purpose: Tissue engineering offers an attractive approach to building vascular grafts, particularly for small diameter (<5 mm) vessels. The basic approach is to create vessels by combining autologous cells with a natural and/or synthetic scaffold under suitable culture conditions, resulting in a tubular construct that can be implanted in vivo. Coating the vascular lumen of synthetic grafts with endothelial cells (ECs) has been shown to prevent acute thrombosis. Smooth muscle cells (SMCs) contribute to contractility/tone and accelerate tissue maturation, which provides mechanical stability of the engineered blood vessels. We have previously developed a bilayered vascular scaffold that allows for EC adhesion onto the luminal surface and homogenous infiltration of SMCs into the outer layer. Moreover, this cellularized scaffold provides sufficient mechanical properties that withstand physiologically relevant vascular conditions [1]. However, uniform and effective cell seeding is challenging as individual cells can be detached and removed from the vascular scaffold. In this study, we investigated whether engineered cell sheet could be combined with our engineered vascular grafts to fabricate a more mature smooth muscle layer as compared to the conventional cell seeding method. We hypothesize that smooth muscle cell sheet will provide completely preserved cell-to-cell junction and extracellular matrix (ECM) [2].

Methods: Electrospun vascular scaffold was prepared using collagen type I mixed with poly(ε-caprolactone) (PCL) in a 1:1 ratio and dissolved in HFP at 5% (w/v) [1]. Vascular SMCs were isolated from sheep femoral artery tissue biopsy and cultured by passage 5. SMCs were seeded at 4×10^6 cells/100 mm of Upcell™ culture dish to fabricate a cell sheet. After 5 days in culture, cell-seeded dishes were transferred to incubator set in 20°C for 30 min to obtain the cell sheets. The detached cell sheets were wrapped around electrospun tubular scaffolds up to three layers, and the scaffolds were incubated for 1 day prior to preconditioning. The cellularized scaffolds were preconditioned in a pulsatile perfusion bioreactor system to enhance the tissue maturation by flow through the scaffold and progressive increase of the pulsatile amplitude every 24 h over the course of 5 days. After 5-day preconditioning, the scaffolds were evaluated by determining gene expression, specific protein expression, and cell apoptosis.

Results: Primary SMCs were confirmed by the expression of α-SMA and SM-MHC. The electrospun vascular scaffold (5-μm fiber diameter) was able to combine with the fabricated smooth muscle cell sheet. Combination of cell sheet and electrospun vascular scaffolds enhanced expression of the mature SMC markers, including α-smooth muscle actin (α-SMA), myosin light chain kinase (MLCK), and connexin 43, while statically cell-seeded scaffolds minimally expressed these markers. Moreover, preconditioning by the perfusion bioreactor maintained cell viability of SMC layer, while cell viability under the static culture condition decreased over time.

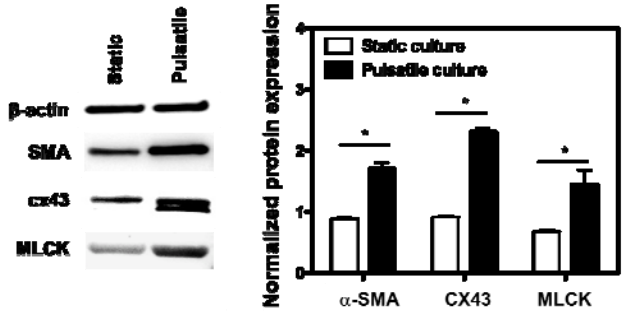

Figure 1. SMC specific marker expression at 5 days after static culture or preconditioning by pulsatile bioreactor: western blot and quantitative data of the cell sheet-vascular scaffolds (n = 3, $*P<0.05$).

Conclusions: We developed a method to construct a vascular scaffold with a more mature smooth muscle layer by employing the cell sheet technology. The engineered cell sheet, wrapped around the vascular scaffold, was able to provide a mature SMC layer that expressed strong cell-to-cell junction marker and contractile protein. In addition, preconditioning of the cell sheet covered vascular scaffold maintained cell viability and infiltration into the scaffold.

References:

[1] Ju, Y.M., et al., Bilayered scaffold for engineering cellularized blood vessels. Biomaterials, 2010;31(15):4313-21.
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