Tissue Engineered Blood Vessels Lined with Late-Outgrowth EPCs from Coronary Artery Disease Patients

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Statement of Purpose: An intact endothelial lining along the lumen of small diameter (< 4 mm) tissue engineered blood vessels (TEBVs) is required to prevent thrombosis *in vivo*. Flow-mediated vasodilation also requires the presence of a confluent and functional endothelial layer. Previous work from our group indicates that late-outgrowth, peripheral blood-derived endothelial progenitor cells from coronary artery disease patients (CAD EPCs) behave similarly to endothelium from healthy patients when seeded over synthetic substrates *in vitro*[1] and *in vivo*[2], and in co-culture with human aortic smooth muscle cells *in vitro*[3]. CAD EPCs were isolated in a minimally invasive procedure and may be expanded *ex vivo* to produce a viable source of endothelium for TEBVs from a patient population targeted to receive cardiovascular procedures. In this study, CAD EPCs were used in a TEBV comprised of a dense collagen gel scaffold and human neonatal dermal fibroblasts (hNDFs) to successfully create an endothelialized, vasoreactive TEBV after one week of culture.

Methods: Dense collagen gel scaffolds yield TEBVs with high mechanical strength that are perfused immediately after fabrication[4,5]. Primary human neonatal dermal fibroblasts (hNDFs) are added to a 2.05 mg/mL rat-tail collagen I solution at $5x10^5$ cells/mL. The solution is added to a 3-cc syringe mold with a 0.81-mm stainless steel mandrel through the center and allowed to gel for 30 minutes at room temperature. Next, the gel is placed onto sterilized tissue paper covering a 0.8 μm nylon mesh and suspended for 10 minutes to remove 95% of the water content, yielding a mechanically strong, dense collagen gel TEBV. A solution of $5x10^5$ CAD EPCs is added to the lumen and rotated at 10 rph for 30 minutes at 37 °C. Endothelialized TEBVs are immediately perfused in a continuous, steady, laminar flow loop at a physiological vascular wall shear stress of 6.8 dynes/cm^2 at 37 °C for 7 days.

Results: Endothelialized TEBVs with a 0.81-mm inner diameter exhibit an average wall thickness of 0.4 mm and an average burst pressure of 1770 ± 42 mmHg after 7 days of culture (Fig. 1A) with >85% adhesion of CAD EPCs (Fig. 1B).

Table 1: Endothelialized TEBV after 7 Days	
Constriction after 1 μM Phe	-5.0 ± 1.1% Baseline
Dilation after 1 μM Ach	+14.3 ± 0.5% Baseline
Burst pressure	1770 ± 42 mm Hg

Preliminary vasoconstriction and vasodilation studies were performed on TEBVs lined with CAD EPCs after 7 days (Table 1). Exposure to 1 μM phenylephrine (Phe) caused vasoconstriction response of 5.0 ± 1.1% initial TEBV diameter. Subsequent exposure to 1 μM acetylcholine (Ach) elicited a vasodilation response of 20.2 ± 0.9% of the phenylephrine-contracted TEBV diameter (14.3 ± 0.5% baseline diameter).

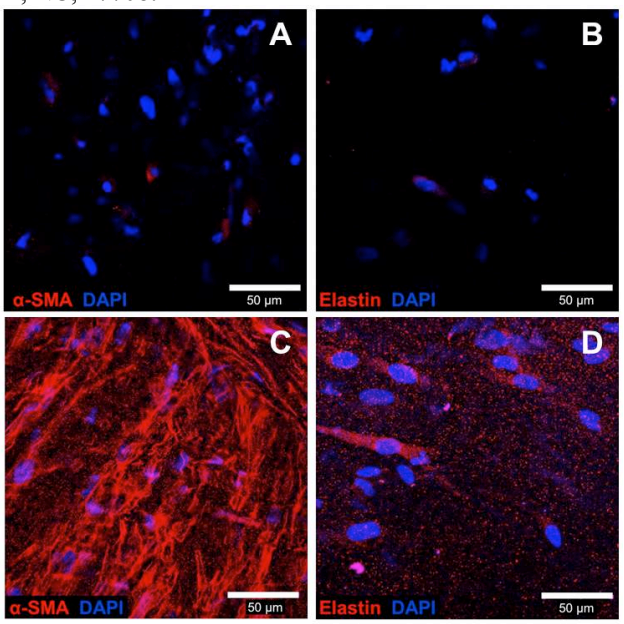

Figure 1: Vascular wall hNDFs increase expression of α-SMA and elastin after 7 days of culture in a continuous, steady, laminar flow loop (C, D) compared to TEBVs in static culture for 24 hours (A, B).

After 7 days, hNDFs within the TEBV wall express α-smooth muscle actin (α-SMA), indicating differentiation toward the myofibroblast phenotype (Figure 1). TEBVs lined with CAD EPCs also indicate elastin expression after 7 days of steady, laminar perfusion.

Conclusions: TEBVs created from hNDFs embedded in dense collagen gel scaffolds were lined with a >85% confluent layer of CAD EPCs. Endothelialized TEBVs yielded high burst pressures after one week of steady, laminar perfusion at physiological shear stresses. Vascular wall hNDFs expressed α -SMA and elastin, indicating differentiation toward a contractile phenotype after 7 days. Finally, endothelialized TEBVs demonstrate endothelium-dependent vasodilation and endothelium-independent vasoconstriction after only one week of perfusion. Future work will measure TEBV vasoreactivity after 14 days of culture and evaluate endothelial nitric oxide production after exposure to varying dosages of phenylephrine and acetylcholine. This study demonstrates that endothelium-dependent vasoreactivity is a critical step toward confirmation of a healthy and functional endothelial layer in a TEBV. Creation of a vasoreactive TEBV *in vitro* is an essential first step in the development of vascular models for the study of drug responses.

References: 1)Stroncek JD. Tissue Eng Part A. 2009; 15:3473-3486. 2)Stroncek JD. Acta Biomater 2012; 8:201-208. 3)Fernandez CE. Acta Biomater 2013; http://dx.doi.org/10.1016/j.actbio.2013.10.004. 4)Ghezzi CE. Biomaterials 2013; 34:1954-1956. 5)Micol LA. Biomaterials 2011; 32:1543-1548.