**Vascular Substitutes with Physiologic Compliance and Co-expression of Elastin and Collagen**

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**Introduction:** Compliance mismatch is a significant challenge to vascular graft patency. We hypothesized that vascular cells cultured on elastomeric scaffolds subjected to pulsatile flow would give rise to vascular substitutes with physiologic compliance and ECM synthesis. Primary baboon vascular cells synthesized elastin and collagen simultaneously when cultured in porous PGS scaffolds. *In vitro* co-expression of elastin and collagen by adult mammalian smooth muscle cells (SMCs) is relatively rare in engineered vasculature. Elastin expression in this study is enhanced by coculture of SMCs with circulating endothelial progenitor cells (EPCs, also from adult baboons), which coordinate mural cell functions during angiogenesis and vasculogenesis. The response of the elastomeric scaffold, composed of poly(glycerol sebacate) (PGS), to cyclic strain may also contribute significantly to physiologic compliance and ECM synthesis.

**Methods:** Tubular PGS scaffolds with macro- and micropores were fabricated by optimizing a previously published method (Gao, 2006). In short, polyolefin was substituted for paraffin as the mandrel material during scaffold fabrication to reduce flaws in scaffold geometry and potential paraffin and solvent contamination.

Vascular EPCs and SMCs were obtained from juvenile male baboons. Scaffolds were cultured with EPCs, SMCs, or coculture of both under pulsatile flow. EPCs were introduced on day 7 for coculture constructs. All scaffolds were seeded with the same cell density. Medium was MCDB 131-based, with 10% fetal bovine serum, 1% L-glutamine, and 2% gentamycin. Permutations of ascorbic acid, growth factors (EGF, bFGF, IGF, VEGF), and hydrocortisone were included when EPCs were present in culture. Constructs were cut into 0.2-in (0.005-m) segments on day 21 for evaluation by histology, electron microscopy, and mechanical testing.

Prepared tissue sections were stained with Masson's trichrome stain to detect collagen and Verhoff's stain to detect elastin. The presence of elastin was confirmed by dissolving construct segments in 0.1 N sodium hydroxide at 98°C (371 K) and centrifuging to pellet any insoluble protein. As an additional verification, the pellet's amino acid content was analyzed and compared to that of elastin.

Luminal and cross-sectional samples of constructs were prepared, mounted, and imaged by electron microscopy. Luminal confluence and cell distribution across the thickness of the construct were compared qualitatively.

Uniaxial mechanical testing was completed immediately after construct removal from the bioreactor and sectioning. Segments were tested to failure using an Instron 5842 uniaxial mechanical tester with a 1.1-lbf (5.0-N) load cell (Instron, Norwood, MA). Video capture of mechanical testing was collated with force-extension data and segment dimensions to calculate ultimate tensile stress, strain, elastic modulus, and compliance. Compliance was defined as the change in lumen cross-sectional area from zero to maximum stress as a percentage of the original luminal area.

**Results/Discussion:** After 3 weeks of culture in the bioreactor, the coculture constructs each had a completely cellular lumen with cells well-dispersed throughout the scaffold. Luminal confluence and cell distribution were superior in the coculture constructs. A circumferential collagen band completely surrounded the lumen in all constructs, and elastin was evident in coculture and SMC-only constructs from Verhoff's stain and amino acid analysis of construct remnants after sodium hydroxide digestion. The presence of SMCs, with or without EPCs, improved mechanical properties toward physiologic values. The compliance of coculture constructs was in the physiologic range (Fig. 1), as compared to human artery compliance at physiologic pressures (L'Heureux, 2006).

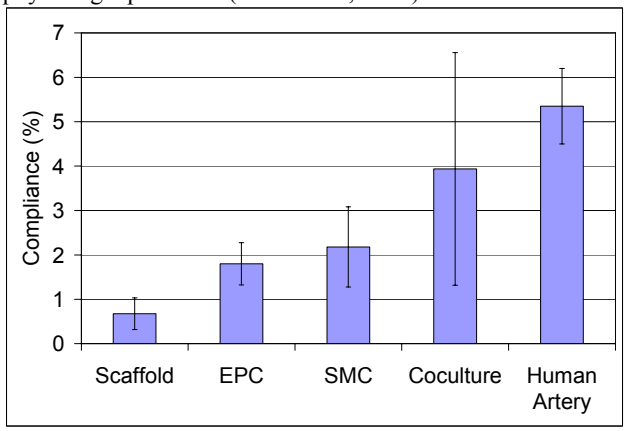


**Figure 1.** Compliance of coculture scaffolds was comparable to that of the human arteries.

**Conclusions:** Our research demonstrates that compliant scaffolds cultured with adult vascular cells can yield engineered vascular substitutes with physiologic compliance. This study also characterizes the advantages of engineering vasculature from EPCs, which increased elastin synthesis, apparently without compromising SMC proliferation of collagen synthesis. Optimization of the culture medium, mechanical conditioning, and longer culture time may lead to robust and non-thrombogenic vascular substitutes.

**References:**

Gao J, *et al*. Tissue Eng. 2006; 12:917-25.
L'Heureux N, *et al*. Nat Med. 2006; 12:361-5.