Small-caliber Vessel Grafts Made from Human Umbilical Veins
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Introduction
Human umbilical cords contain valveless, unbranched veins (HUV) which have recently been suggested as starting materials for tissue-engineered small-caliber vessel grafts [1]. Endothelium-denuded HUV (denHUV) could be completed by the recipient's own endothelial cells to create an immunologically inert graft with contractile function and with antithrombogenic properties. This study explored techniques to efficiently denude human umbilical veins (HUV) and to use them as scaffolds for endothelial cell seeding in a perfusion bioreactor.

Materials and Methods
HUV were dissected and flushed with carbogen at 60 ml/min to destroy the endothelium by dehydration. Vessel function was assessed in an organ bath before and after denudation. Reductive metabolism was measured by tetrazolium dye reduction (TDR). denHUV were mounted in a perfusion bioreactor and perfused with 20 ml/min using M199 + 20% fetal calf serum. HUV endothelial cells were cultured to confluence, fluorescence-labelled, and seeded at a density of 5E6 cells/ml. denHUV were rotated 90° every 4 min for 1h. The constructs were then perfused for another 3h at 20 ml/min.

Results
Denudation by dehydration successfully removed the endothelium without affecting the integrity of the smooth muscle layer as judged by histology. Denudation did not significantly affect TDR (OD HUV 0.79±0.21 vs. denHUV 0.87±0.17, p=0.361, t-test). Contractile responses to KCl (control 45.1±26.3 vs. denHUV 37.6±18.8 mN, p=0.829, ANOVA) and to serotonin (control 62.2±28.5 vs. denHUV 58.4±27.2 mN, p=0.894, ANOVA) were also unaffected. Seeding resulted in a smooth, flow-resistant neoendothelium (Fig. 1).

Discussion and Conclusions
Denudation by dehydration is a simple and effective method to denude HUV without reducing contractile responses or reductive metabolism. denHUV can be seeded with endothelial cells, resulting in small-caliber vessel grafts with a flow-resistant custom neoendothelium which we intend to develop as grafts for revascularization.


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Disclosures
none