Developing Human Umbilical Vein as Living Scaffolds for Vascular Tissue Engineering

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Objective

- Approx. 7% of eligible patients lack suitable autologous grafts for coronary artery bypass surgery. Tissue-engineered grafts could avoid the drawbacks of xenografts and of synthetic vessel replacements
- Vascular tissue engineering procedures usually combine biological or biocompatible scaffolds and autologous cells
- Human umbilical veins (HUV) are valveless, unbranched small caliber vessels which can be obtained in large numbers and without ethical concerns
- We have recently suggested to use endothelium-denuded HUV (denHUV) as “living scaffolds“ for autologous endothelial cells*
- This study explored techniques to efficiently denude HUV and to seed them in a perfusion bioreactor

* Hoenicka et al., *Tissue Eng.* 13:219 (2007); Hoenicka et al., *Biomaterials* 29:1075 (2008)

Methods

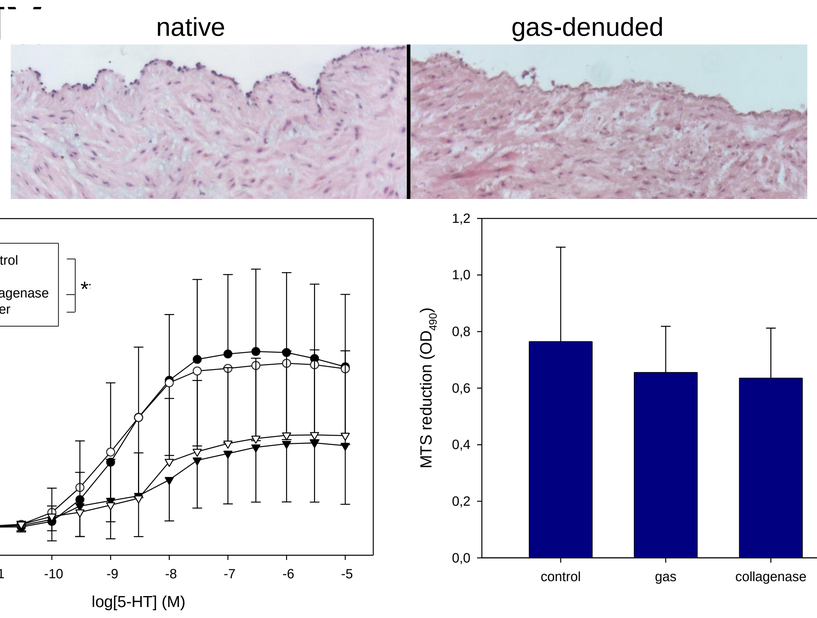
Endothelium removal

- HUV were denuded by collagenase treatment (0.1%, 20 min, 37 C), by hypotonic lysis (distilled water, 5 min, RT), or by dehydration (carbogen at 60 ml/min, 10 min, RT)
- Contractile function was determined in an organ bath
- Viability was assessed by tetrazolium dye reduction
- Mechanical properties were determined in an uniaxial tensile testing rig

Seeding

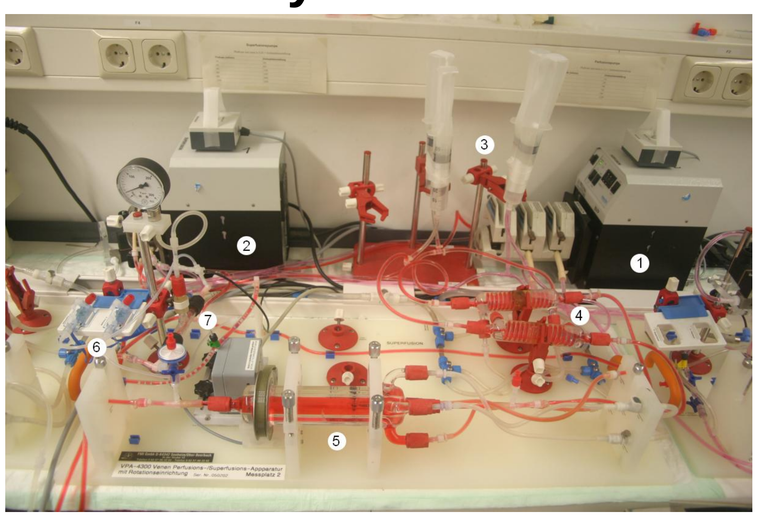
- denHUV were mounted in a bioreactor and perfused with M199+20% FCS at 20 ml/min
- Cells were seeded at a density of 5E6 cells/ml (~5E5 cells/cm^2)
- Vessels were rotated 90 every 3 min for one hour
- After 3 hours of perfusion, vessels were fixed and analysed

Contractile Function and Viability of denHUV



Denudation conditions were optimized until essentially all endothelial cells were removed safely from the vessels, as shown for gas denudation in the photomicrographs (top). Contractile responses to serotonin (5-HT) were determined before and after denudation (left panel). Responses of gas-denuded vessels did not differ from native vessels, whereas both collagenase-treated and distilled water-treated vessels showed significantly lower maximum responses ($p<0.001$, ANOVA). Reductive capacities were not altered by any of the treatments (right panel)

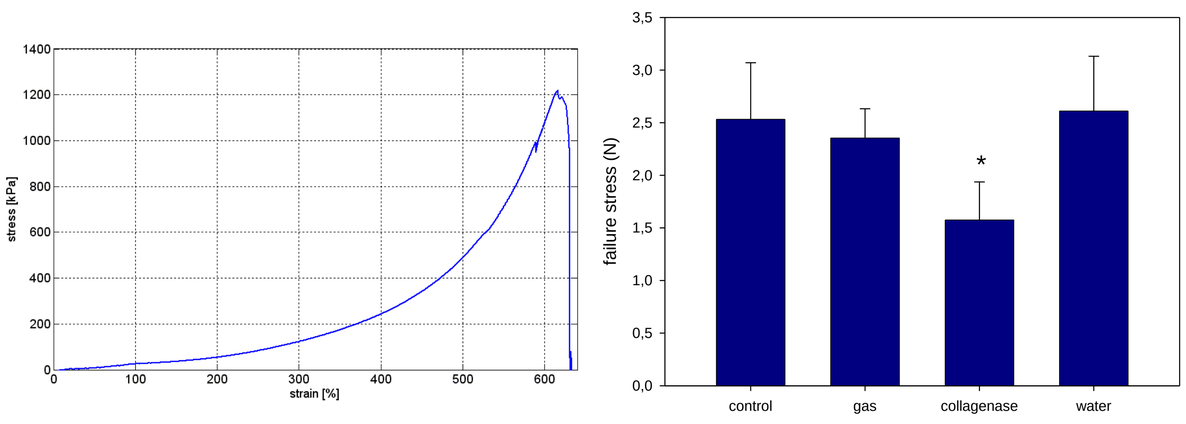
Perfusion system



1. perfusion pump
2. superfusion pump
3. compliance chambers
4. heat exchangers
5. vessel chamber
6. pressure probes
7. starling resistors

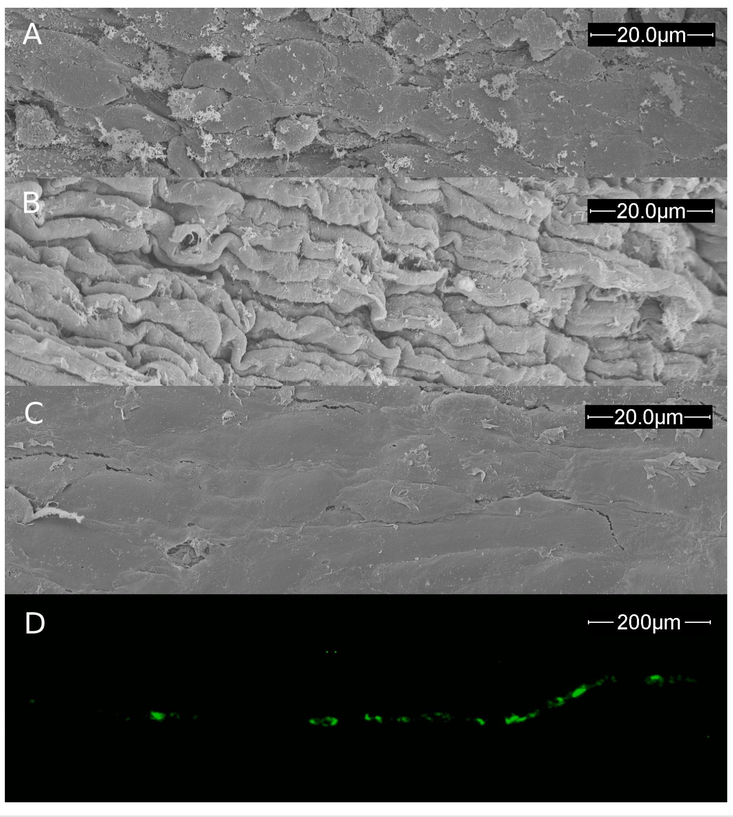
denHUV were mounted in vessel chambers. Peristaltic pumps perfused (20 ml/min) and superfused (40 ml/min) the vessels with M199+20% FCS. Medium was gassed with 20% oxygen and 5% carbon dioxide via membrane oxygenators. Total medium volume was 300 ml. Compliance chambers were adjusted to provide venous pulsatile flow. Steppers allowed to rotate the vessel chambers via driving belts.

Mechanical Properties of denHUV



Stress-strain relationships were recorded in a tensile testing rig (left panel). Vessel rings were distended at 10 mm/min until they failed. denHUV, just like HUV and other vessels, showed a biphasic stress-strain relationship. Young's modulus was determined at the steepest slope as 900 kPa. Computed burst pressure was approx. 1000 mm Hg (133 kPa). Failure stresses were not altered by gas denudation and by osmotic lysis, whereas collagenase treatment significantly weakened the matrix (right panel, $p=0.007$, ANOVA).

Seeding Results



(A-C) scanning electron microscope images. (A) intact endothelium of native HUV. (B) surface of denHUV, showing the structure of the basal membrane. (C) neoendothelium after seeding denHUV with isolated HUVEC. Note the size and shape difference compared to native endothelium. (D) Cells were labeled before seeding with fluorescent dyes to visualize their appearance under a UV microscope. Image shows cross section of seeded denHUV. Freshly seeded HUVEC are shown in green and form a confluent monolayer on top of the basement membrane.

Conclusions

- Denudation by a stream of gas is a gentle and reproducible method to denude HUV
- Contractile function, reductive capacity, and mechanical properties are not affected by this procedure
- Allogeneic endothelial cells form a confluent and flow-resistant monolayer on denHUV
- denHUV may thus serve as living scaffolds to generate a non-immunogenic small caliber vessel graft using the graft recipient's own endothelial cells

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