LCL-GRAFT™ - Innovative Large and Small Caliber Vascular Grafts for Coronary Bypass and Peripheral Vascular Surgery.

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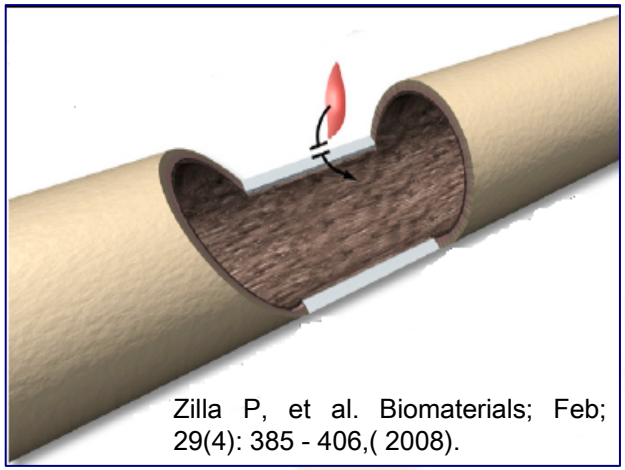
Introduction

The Clinical Problem: Cardiovascular disease are the leading cause of death in the United States and is estimated to cost the US economy over $300 billion per year. The major problem of cardiovascular disease is due to atherosclerosis, the formation of lipid plague, inside the vascular vessels. As this lipid plague increases in size, it can eventually block the passage of blood flow. Atherosclerosis of the coronary vessels in the heart leads to ischemic heart disease and myocardial infarction, while that of peripheral vasculature leads to ischemia and loss of extremities.

The solution: In order to bypass the blood circulation, there is a need for a small artificial conduits.

Our Objective



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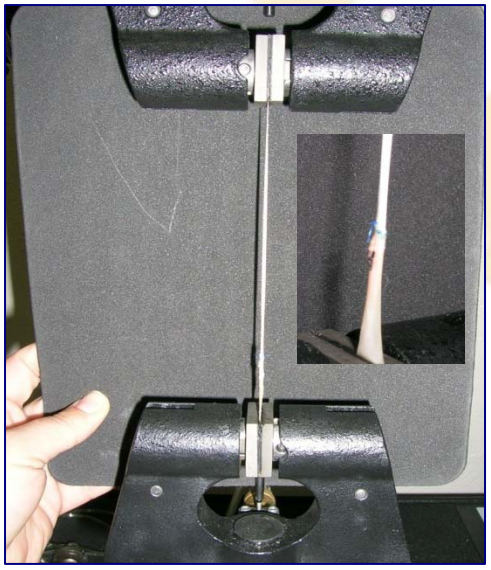


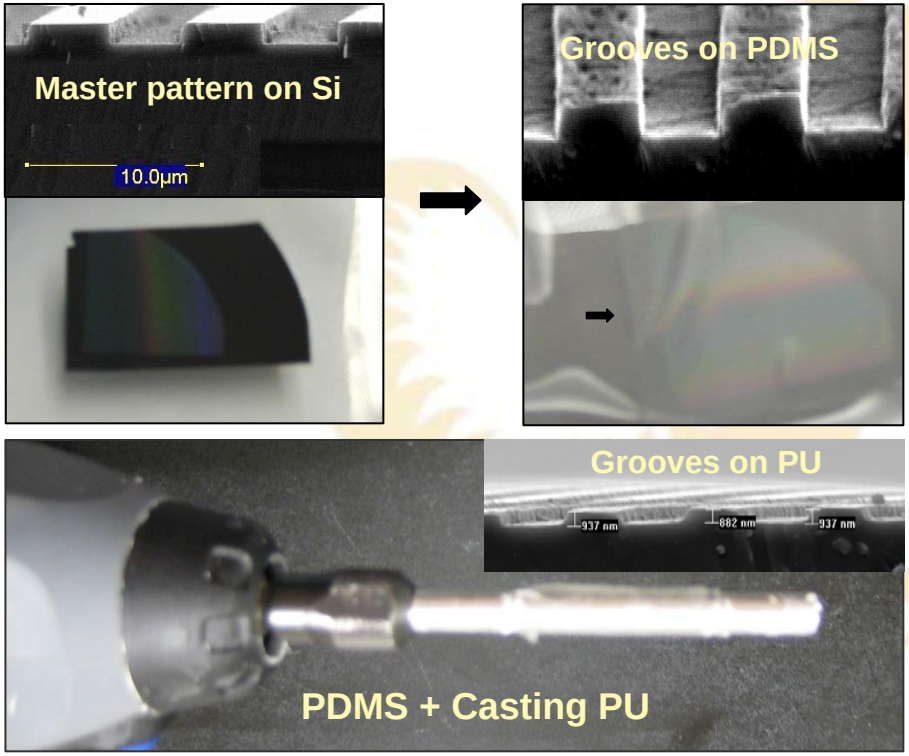
Instron testing of rabbit aorta sutured to PU graft

Fabrication of a vascular graft that:

- Functions for more than 5 years
- Is a synthetic "off the shelf " prototype with compliance that matches the one of native artery.
- Is thromboresitant / has a non-thrombogenic surface.
- Facilitates "endothelialization" (surgeon's choice) in the OR setting (sodding within 1 hour) and by "fallout seeding" through circulating EPC.
- Exhibits enhanced shear resistance to prevent loss of endothelial cell coverage.

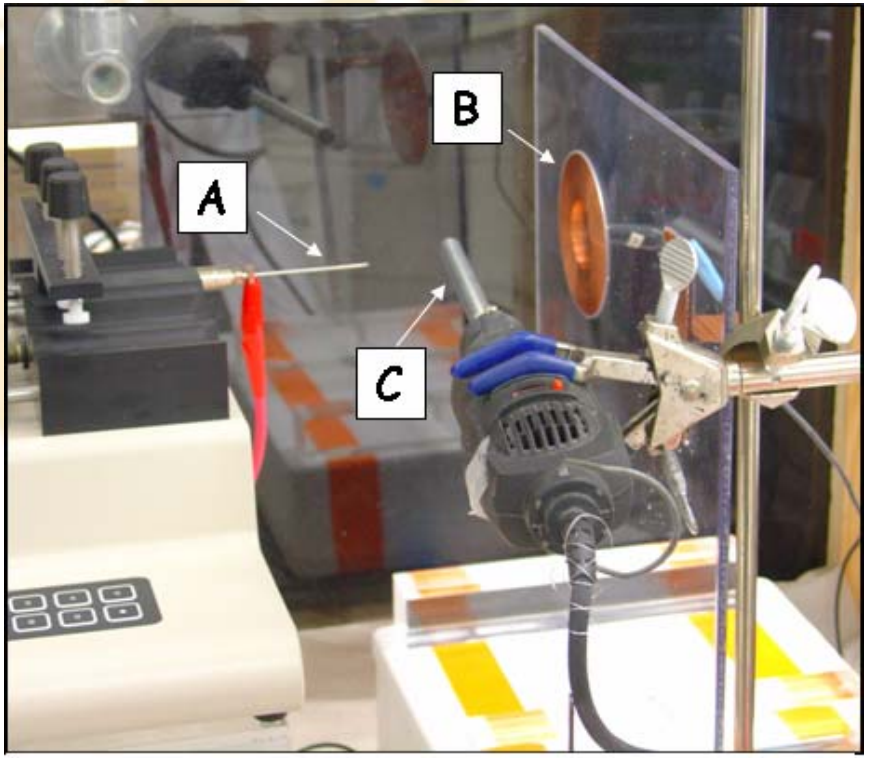
Method: Fabrication of PU Hybrid Graft

Fabrication of the graft lumen by Spin Casting.



On a microfabrication silicon wafer with a grooved pattern we cast a PDMS layer, which is attached to a drill. On top of the PDMS we cast a PU solution while the drill is rotating. As a result we get a PU tube with a grooved pattern in the lumen. We then electrospin a PU solution on the exterior side resulting in aligned microfibers.

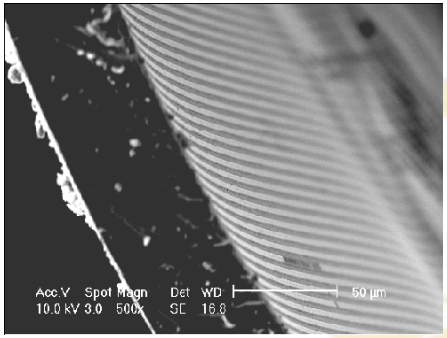
Fabrication of the graft exterior by Electrospinning.

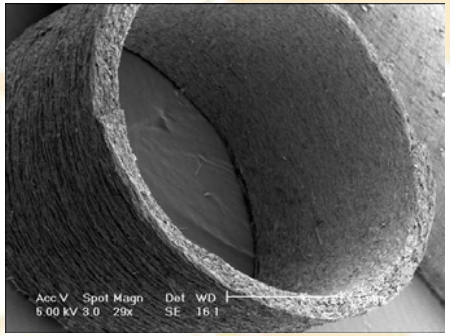


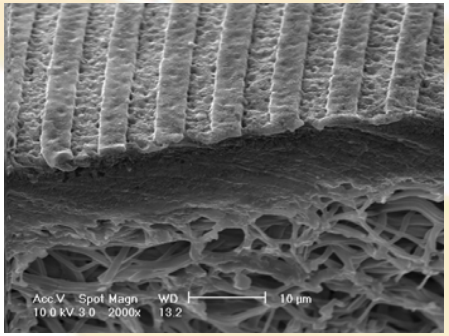
Electrospinning Instrument generating an electrical field between A (syringe with metal needle, positive pole) and B (a copper negative Pole). The Matrigel is injected by the automatic syringe pump and the fibers are collected: 1) on the rotator (C), generating aligned fibers. 2) On the slide placed on B, generating random fibers.

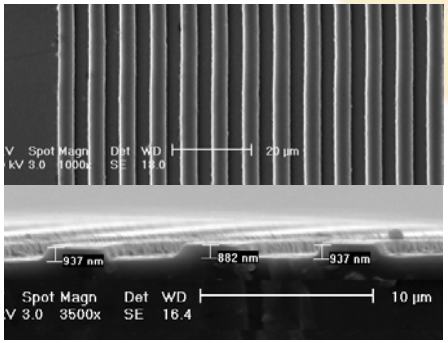
Micro-Textured Lumen of the Vascular Graft

Small diameter PU graft:,
d~ 4mm, L~4.5cm

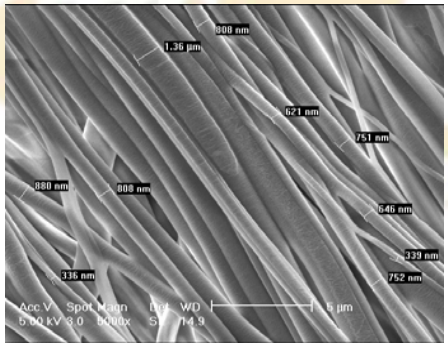




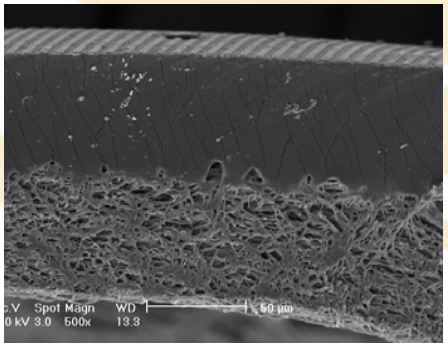




Aligned micro-grooved pattern on the graft lumen. (5mmX5mmX1mm)



Aligned fibers on the graft exterior. (~1mm)

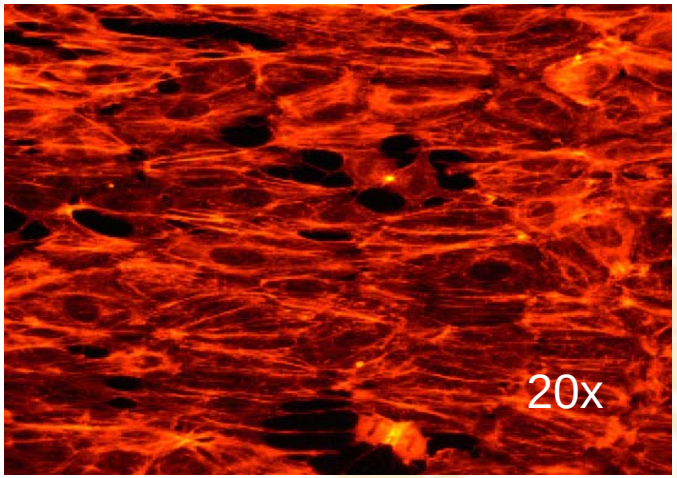


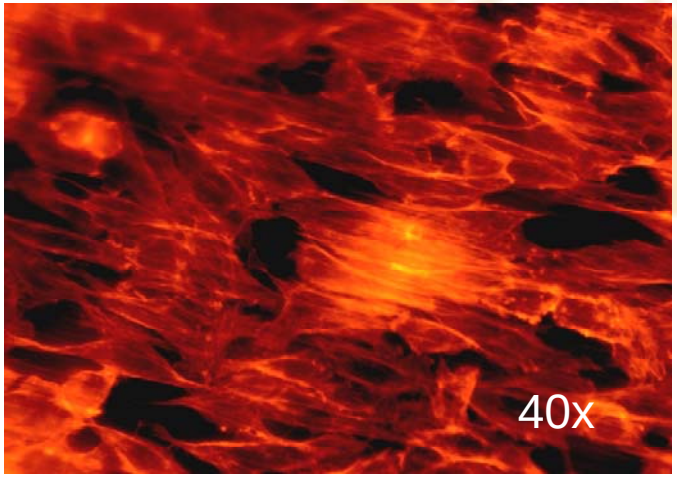
Hybrid Graft

The hybrid LCL graft realizes both micro-grooves to guide cell alignment and micro-fibrous structure to maintain the mechanical properties, which are similar to those of native vessels.

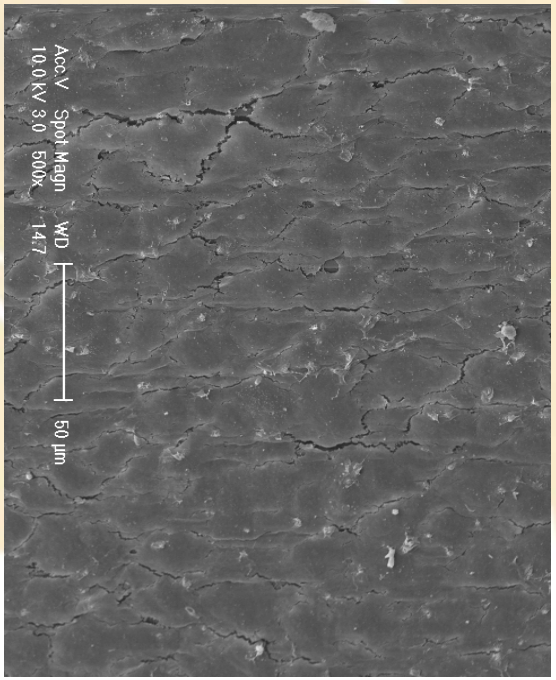
Endothelialization of the Vascular Graft

Mimicking Naturally Aligned Endothelium on the Luminal Surface of the PU Tube !

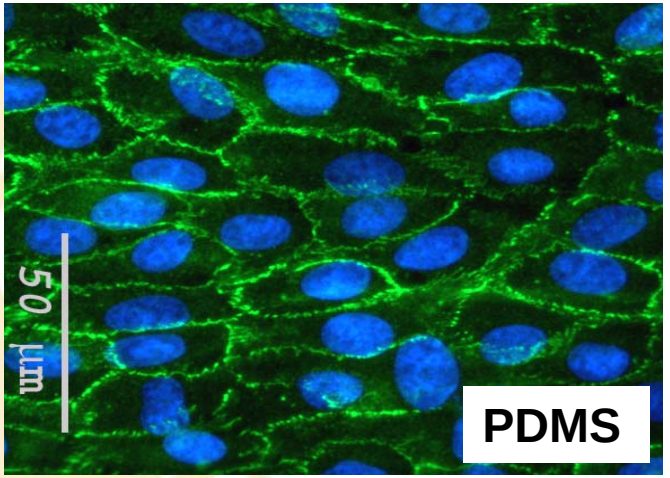


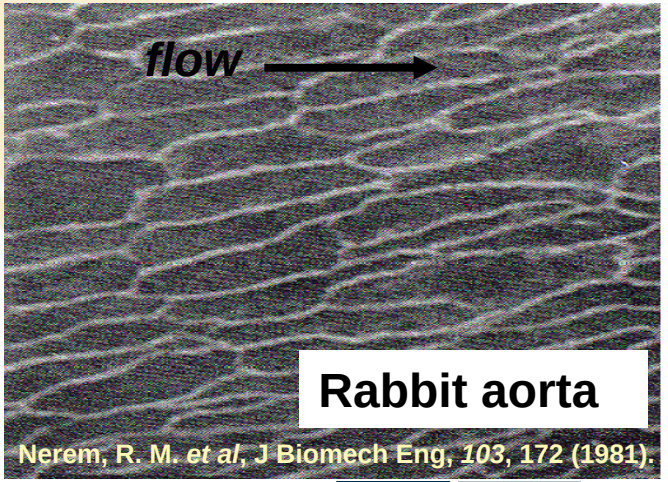


groove direction

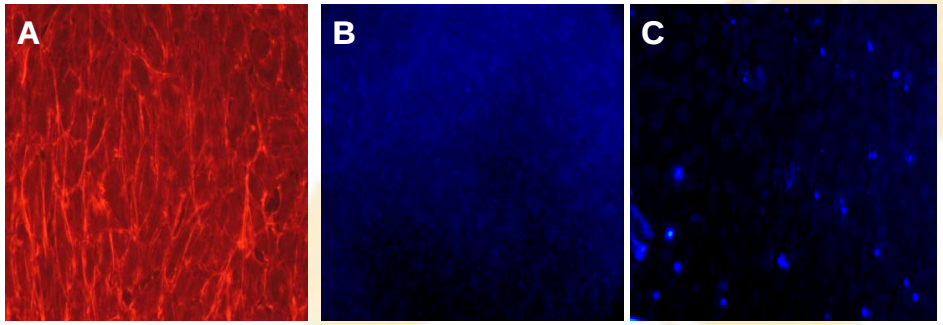


PU groove tube

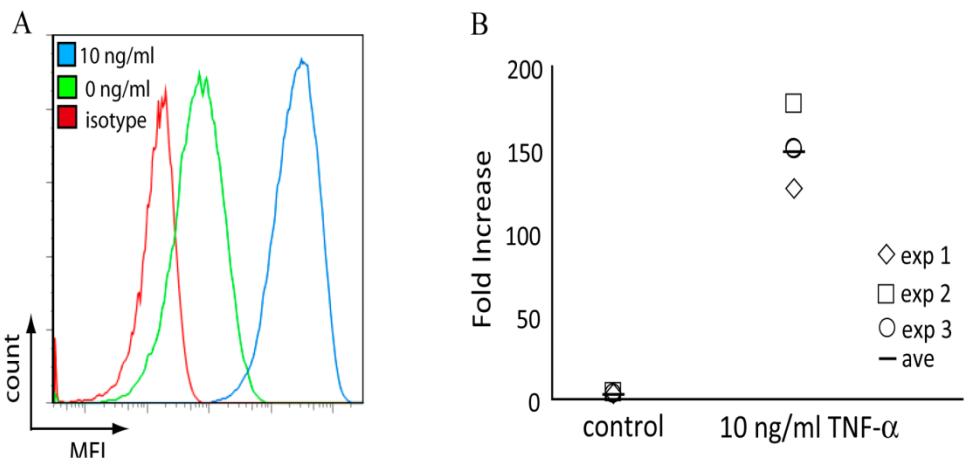




Anti - thrombogenicity of Vascular Graft



Adhesion of monocytes to endothelialized PU graft. (A) Endothelial monolayer (red:phalloidin), (B) naive monolayer, stained for BBZ-loaded monocytes, (negative control), (C) TNF-α stimulated endothelial monolayer, stained for BBZ-loaded monocytes (bright blue spots) . The endothelial monolayer (A, red: Phalloidin) was intrinsically non-thrombogenic (B: no monocytes adhered) and needed to be activated by incubation with the inflammatory cytokine, 10 ng/ml of tumor necrosis factor-α (TNF-α), to observe monocyte adhesion (C).

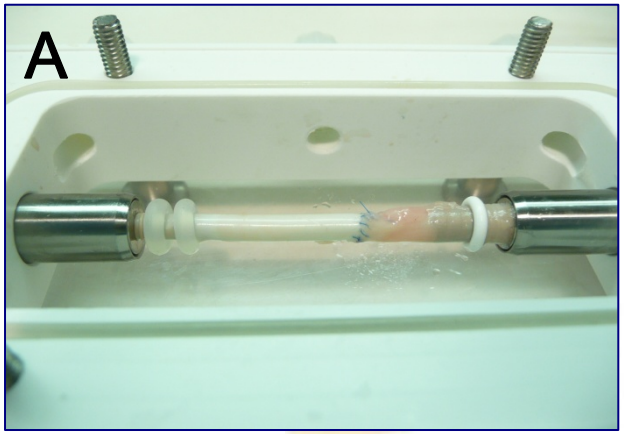


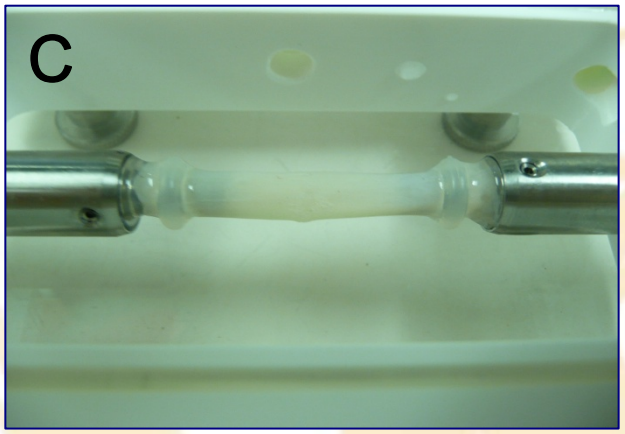
TNF-α induced ICAM-I expression of EA.hy926 endothelial cells grown on PU graft surface. Elevated ICAM-1 levels were found only when the cells were stimulated with TNF-α. Thus, without TNF-α, the cells remained their phenotype and function on our prosthetic PU grafts.

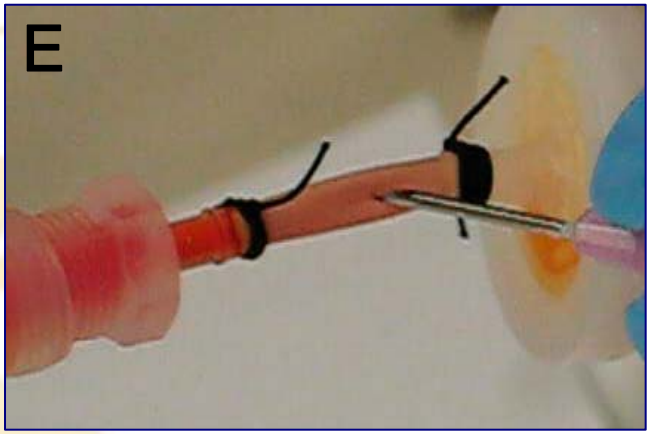
Endothelialized PU Grafts under Pulsatile Flow Condition - Bioreactor System

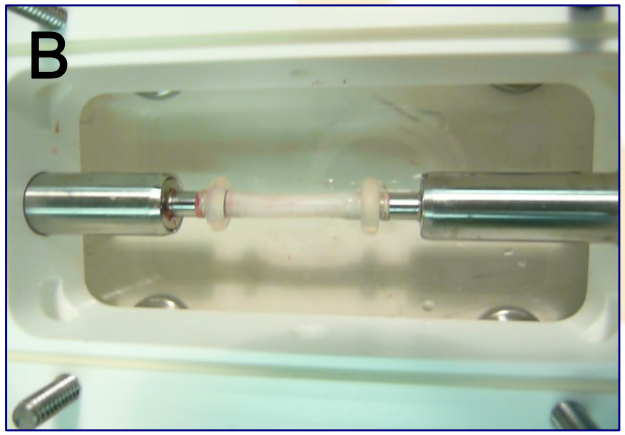
The adhesion strength of endothelialized polyurethane graft was tested in vitro in a TGT-flow system.

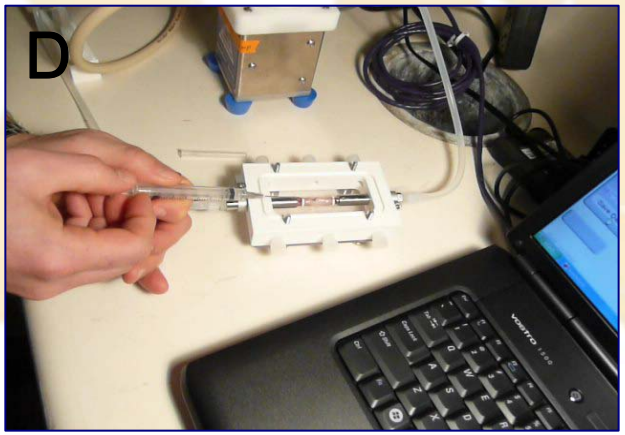
PU Graft Suture to Rabbit Aorta under Flow

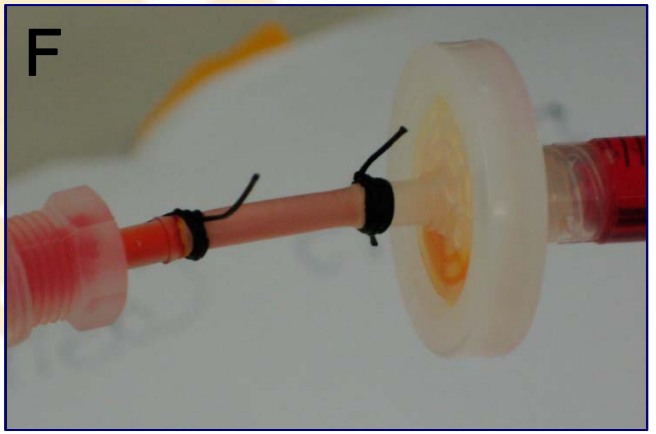








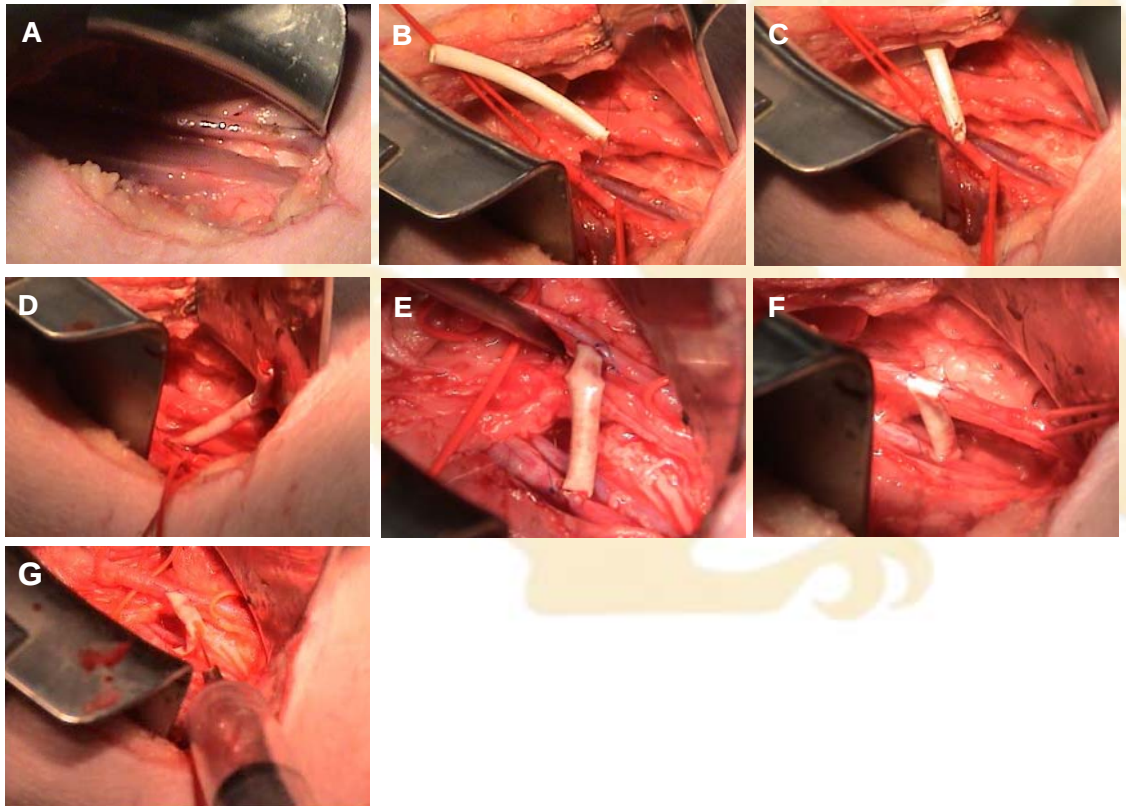




(A) Rabbit aorta sutured to PU graft under pulsatile flow (120/60) after 2 h. (B) PU graft under pulse flow (C) Rabbit aorta under pulsatile flow (D) Puncture Test of PU – Aorta under flow (E-F) Puncture Test to the PU graft results in no leakage.

Preliminary In Vivo Studies in Pigs

Experienced surgeons at Hahnemann Hospital perform the *in vivo* evaluation of the PU LCL- Grafts.



Implantation of a PU graft in pigs. **A)** Implantation site, top: jugular vein, bottom: carotid artery; **B)** implanted PU tube being sutured to the carotid artery, (side I); **C)** PU graft sutured to the carotid artery; **D)** blood coming through the unsutured site (side II) of the artery-PU tube; **E & F)** Anastomosis sutured PU implant, at the top is the jugular vein, at the bottom is the carotid artery; **G)** Withdrawal of blood from the PU implant.

Conclusions

- The “LCL Graft™” is a novel highly compliant small caliber vascular graft made of improved, oxidation-resistant polyurethane with mechanical properties similar to those of native blood vessels.
- Physical and chemical modifications to the lumenal surface were designed to concomitantly limit thrombogenicity and enhance endothelial cell attachment.
- Following successful testing in vitro we have started animal experimentations in vivo.

Acknowledgement

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Endothelialization of Vascular Graft

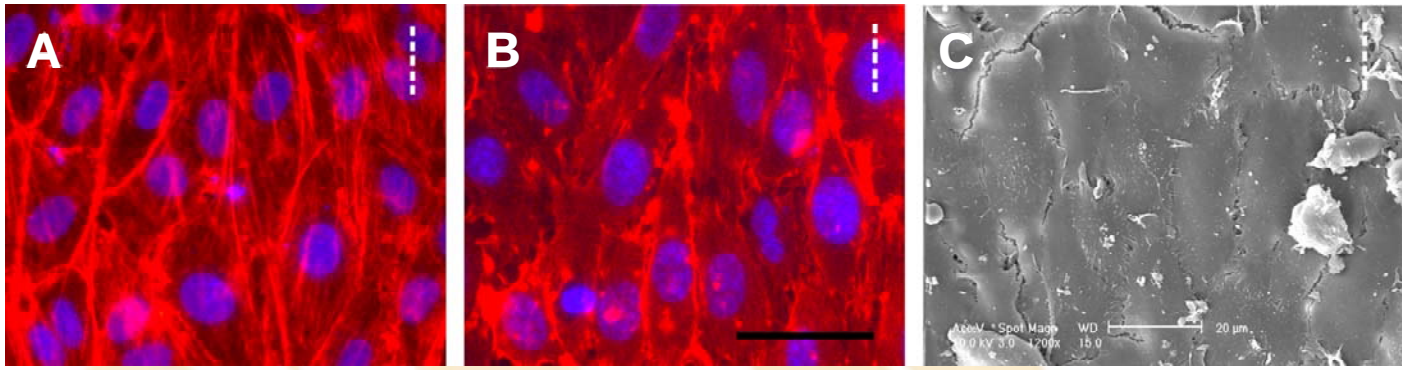

Figure 3. Fluorescence images of endothelial monolayer formed on the lumen of electrospun grafts after 7 days in culture. F-actin microfilaments and nuclei are in red and blue, respectively. Dashed lines guide the direction of microfibers. Scale bar is 50 μm for all images. (A) Bovine aortic and (B) human umbilical vein-derived EA.hy926 endothelial cells maintained their alignment parallel to the fiber direction at confluence. (C) SEM image confirmed the uniform coverage of cells on the graft lumen as demonstrated in the EA.hy926 monolayer. The alignment of cell shape followed the microfiber direction shown by dashed line.