UNIVERSITÉ DE GENÈVE

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ERICE, Italy
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Vascular Tissue Engineering using Biodegradable Synthetic Nano Scaffolds

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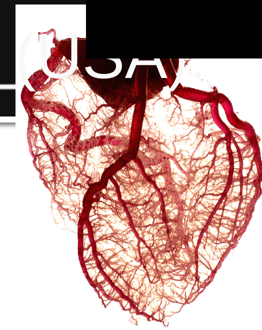
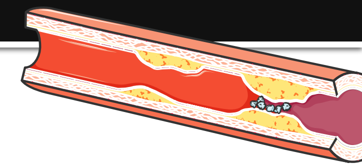
HUG Hôpitaux Universitaires de Genève

CARDIOVASCULAR RESEARCH GENEVA

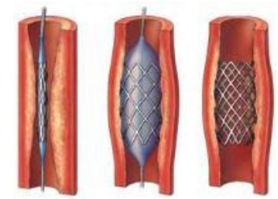
BACKGROUND: Clinical Needs [redacted]



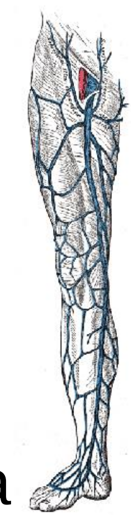


- Coronary surgery: 500,000 / year
- Vascular surgery: 1,500,000 / year
- Access surgery: 350,000 / year

Existing vascular prosthesis show poor results in the small calibre

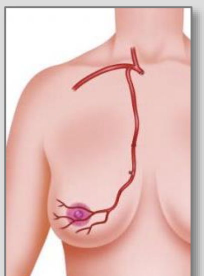
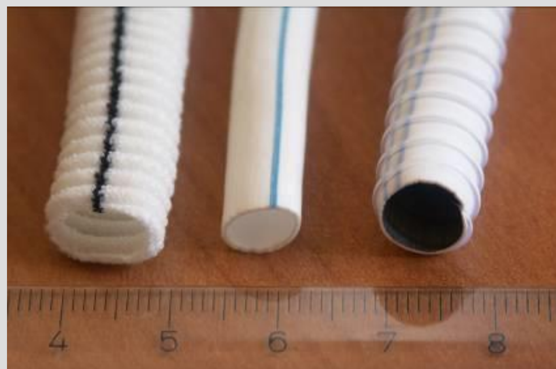
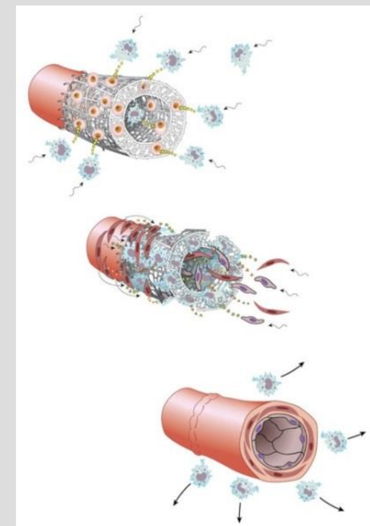


Mostly done with autologous grafts requiring:
- Availability of autologous arteries and veins:
  these are diseased or previously used in 30% of patients
- Additional harvesting surgery
- Increased OR time and surgeons
- Adding morbidity to the patient
- Risk of infection (200 Mio $ / year)



Therefore small calibre, shelf-ready, vascular prostheses a needed as alternatives to autologous grafts for better clinical outcomes

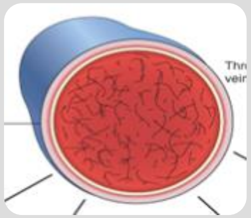
Next Generation Vascular Grafts

Currently Used Grafts		The Future
Autologous Vascular Material	Non-degradable Synthetic Grafts	Vascular Regeneration
 		
Availability	Availability	Availability
Clinical Outcome	Clinical Outcome	Clinical Outcome

Next Generation Vascular Grafts

Design Strategies

Biostable



Failure (<6mm)

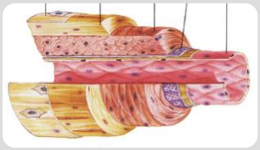
Synthetic Materials

Availability

Pre-seeded with cells

Cost & Time

Biodegradable



Long Term Efficacy of Natural Tissues

Natural Materials

Source

No cells

Shelf Ready

Our Strategy

Synthetic Biodegradable Vascular Grafts

Part I

Can Synthetic Biodegradable Vascular Grafts Work?

de Valence S, Tille JC, Mugnai D, Mrowczynski W, Gurny R, Möller M, Walpoth BH.
Long term performance of polycaprolactone vascular grafts in a rat abdominal aorta replacement model.
Biomaterials. 2012 Jan; 33(1):38-47.

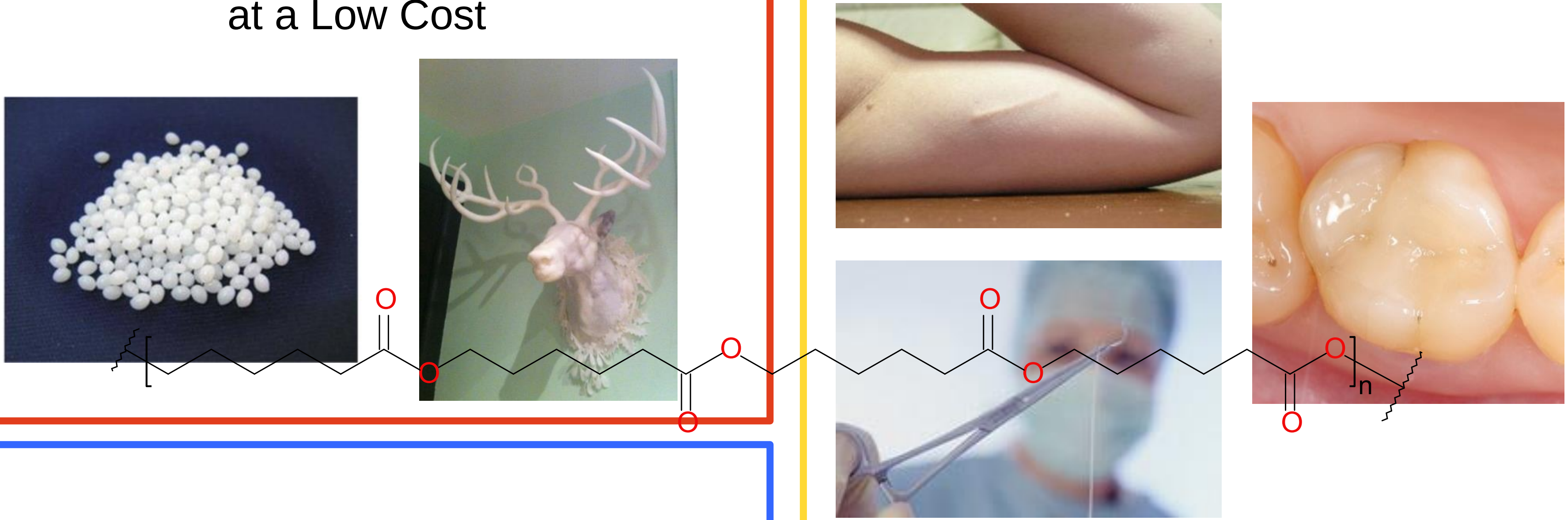
Polycaprolactone (PCL)

Availability

Available in Large Quantities
at a Low Cost

Biocompatible

FDA approved



Biodegradable

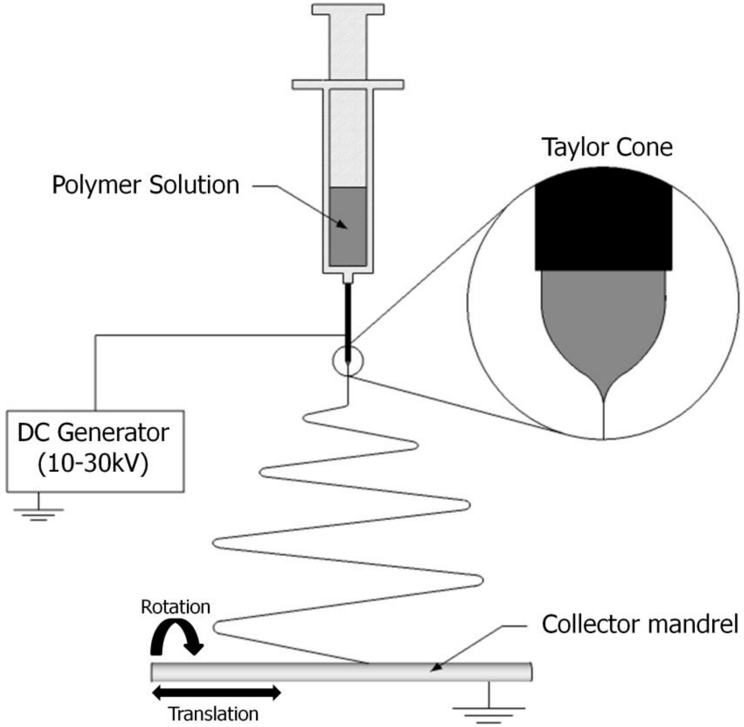
Slow degradation rate (>2years)
Non-toxic byproducts

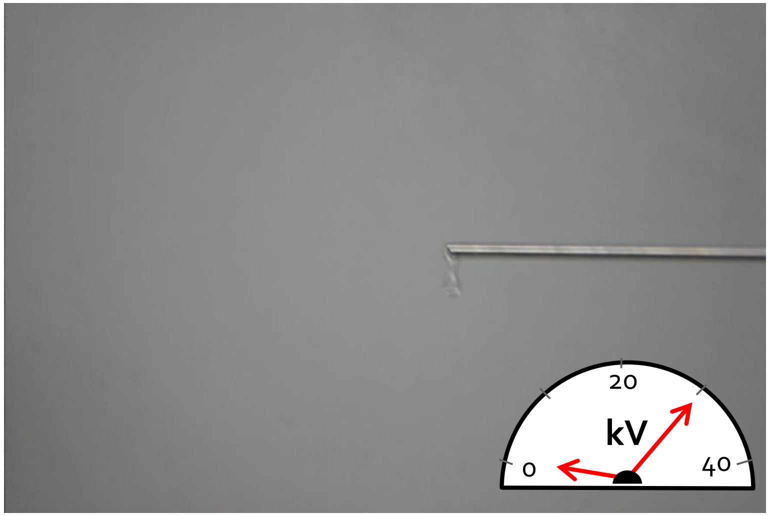
Mechanical Strength

Excellent Intrinsic
Mechanical Properties

Electrospinning

Generate polymeric micro- and nano-fibers with an electrical field

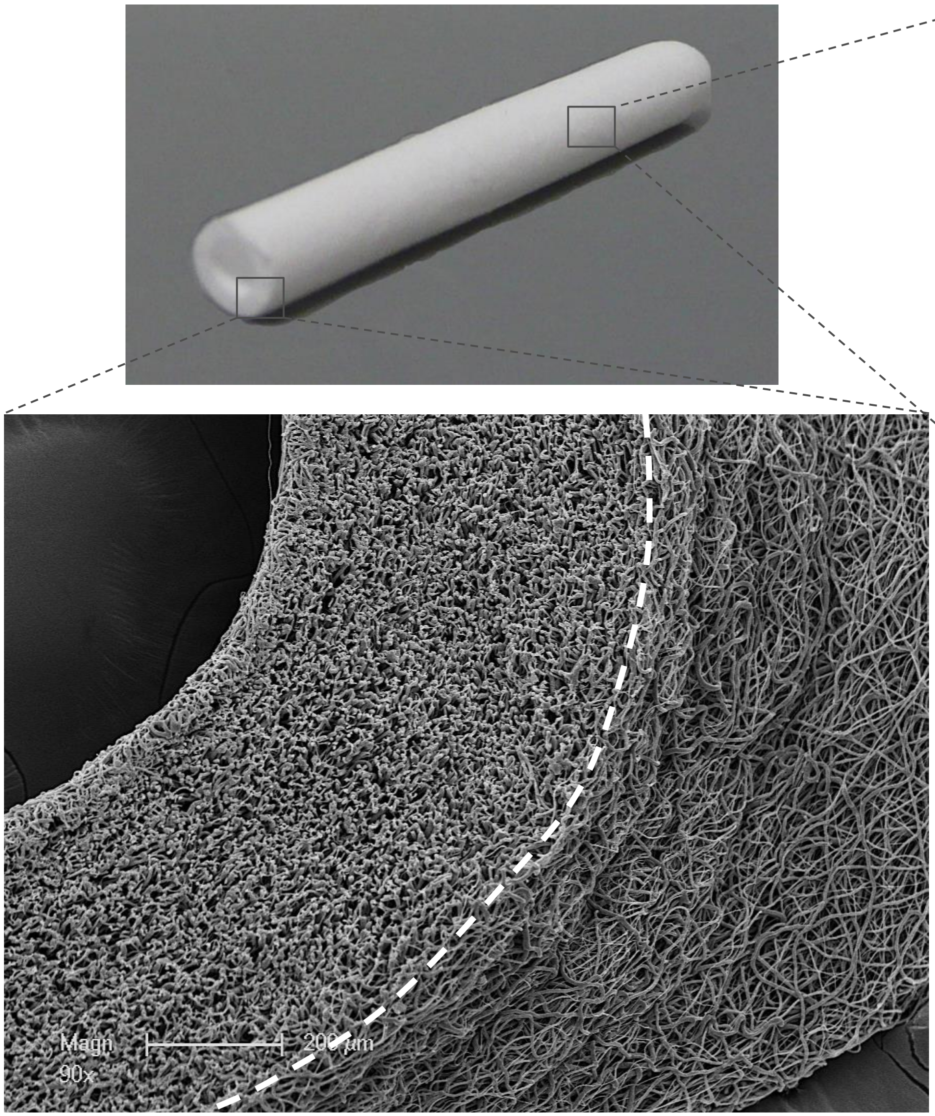


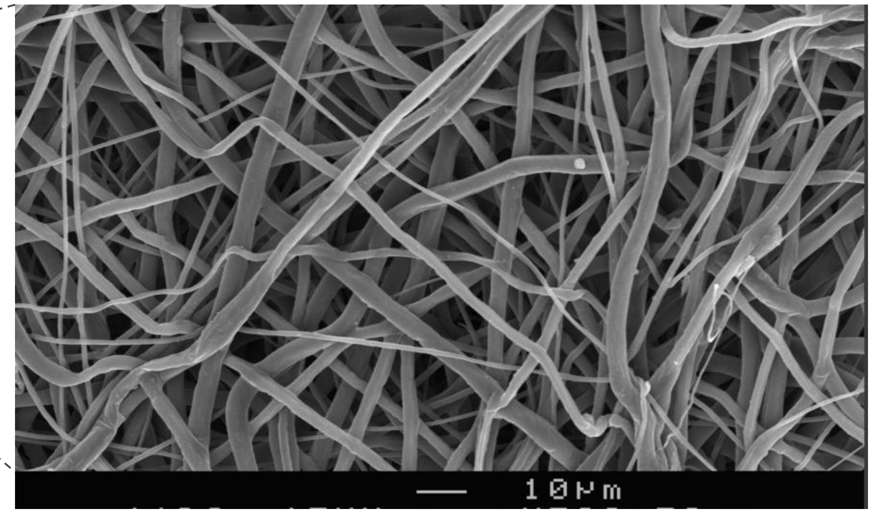


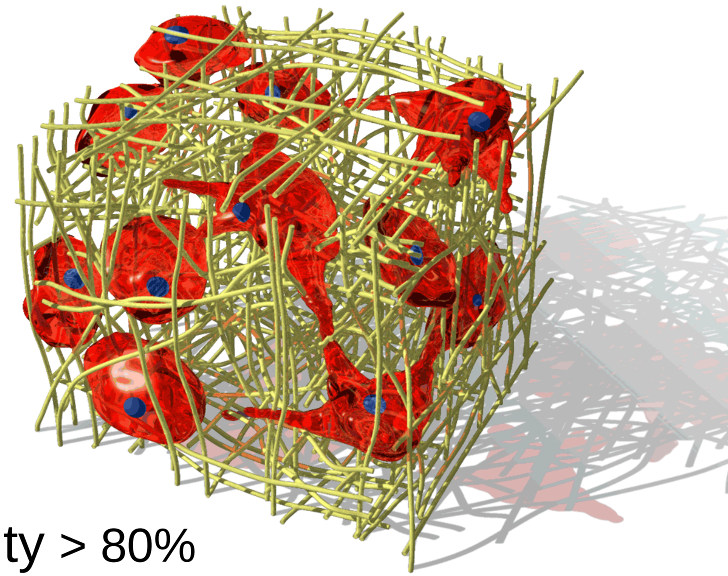
Polymer Solution
15% Poly(ε-caprolactone)(PCL)
in Chloroform / Ethanol (7:3)

de Valence et al. Biomaterials 2012

The graft







Porosity > 80%

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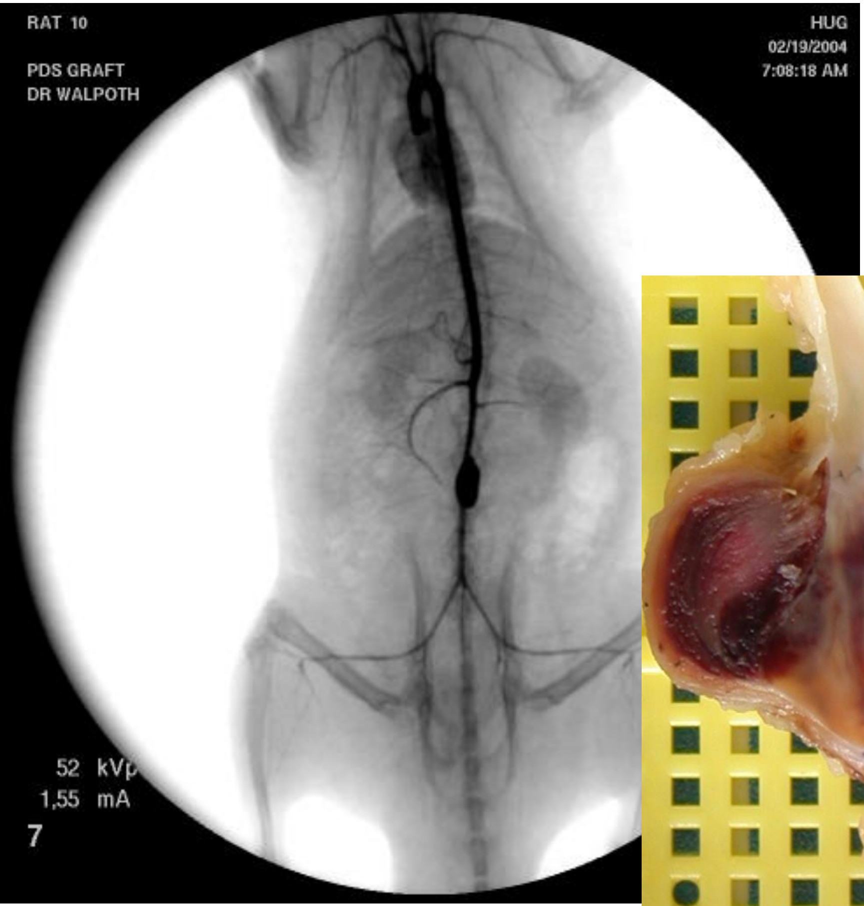
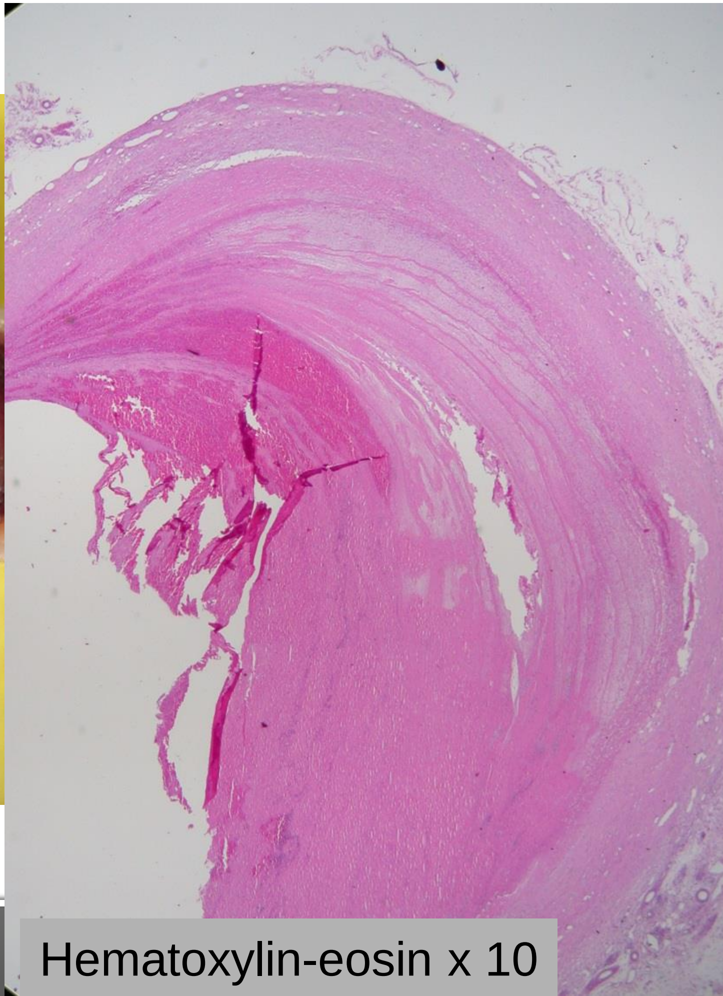
PDS GRAFT INTERPOSITION (n=3)

(after 3 weeks implantation)





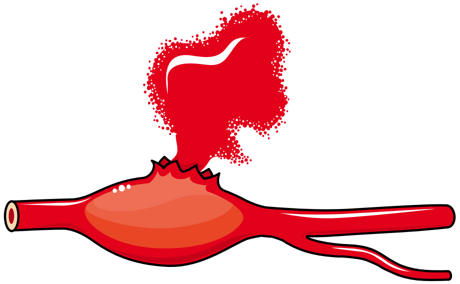
Hematoxylin-eosin x 10

Mechanical Properties

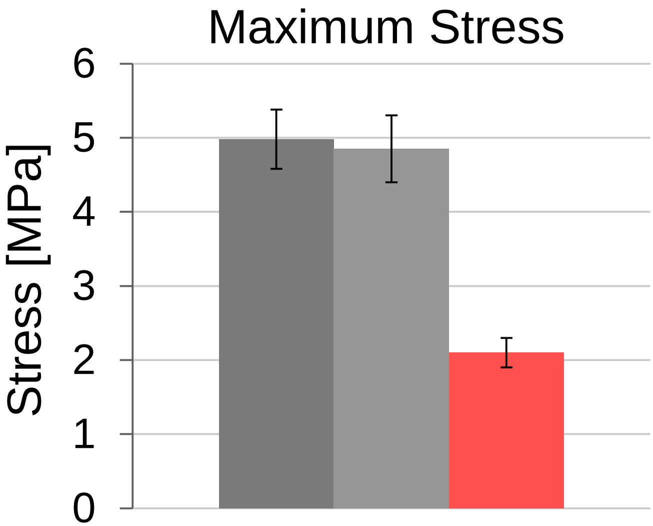
Mechanical Stress

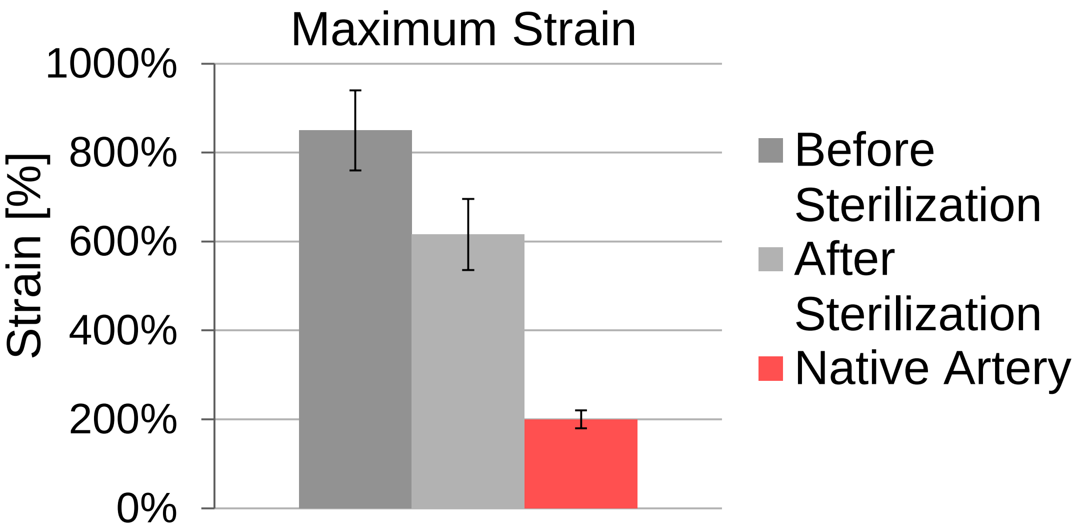
Blood pressure 50–200mmHg
Pulsatile flow
Suturing

Mechanical Failure



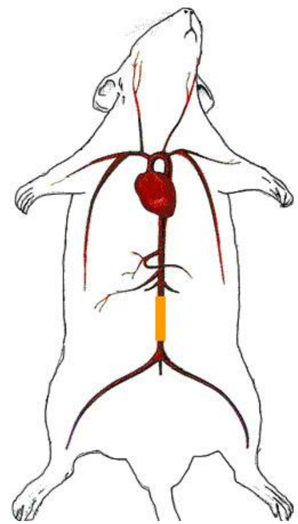
Sterilization method: gamma irradiation (25 kGy)

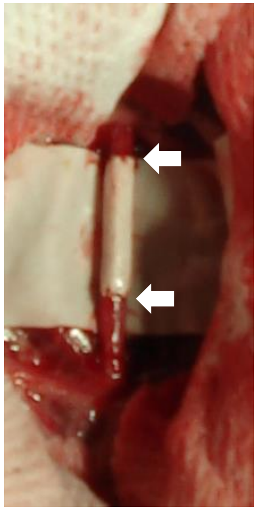




The In Vivo Model

Rat Infrarenal Abdominal Aorta Replacement Model





(n=3)

1.5 3 6 months 12 18 months

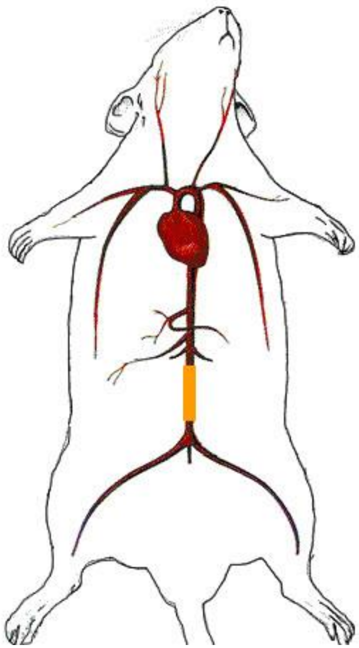
Previous Study: Pektok E, Nottelet B, Tille JC, Gurny R, Kalangos A, Möller M, et al. *Circulation*. 2008;118:2563-70

New Study: de Valence S, Tille JC, Mugnai D, Mrowczynski W, Gurny R, Möller M, Walpoth BH. *Biomaterials*. 2012; 33:38-47

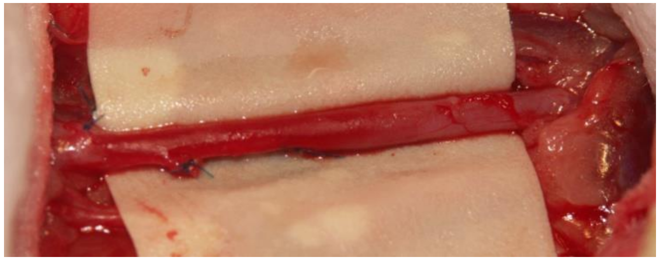
Investigation Tools

- MicoCT
- Digital Subtraction Angiography
- Scanning Electron Microscopy
- Histology
- Gel Permeation Chromatography
- High Resolution Ultrasound

Implantation

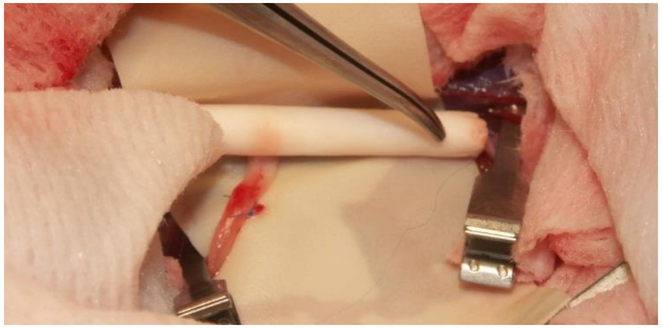


Under an operative microscope
(10x magnification)



Isolated aorta

10/0 nylon interrupted sutures
6-8 sutures per anastomosis



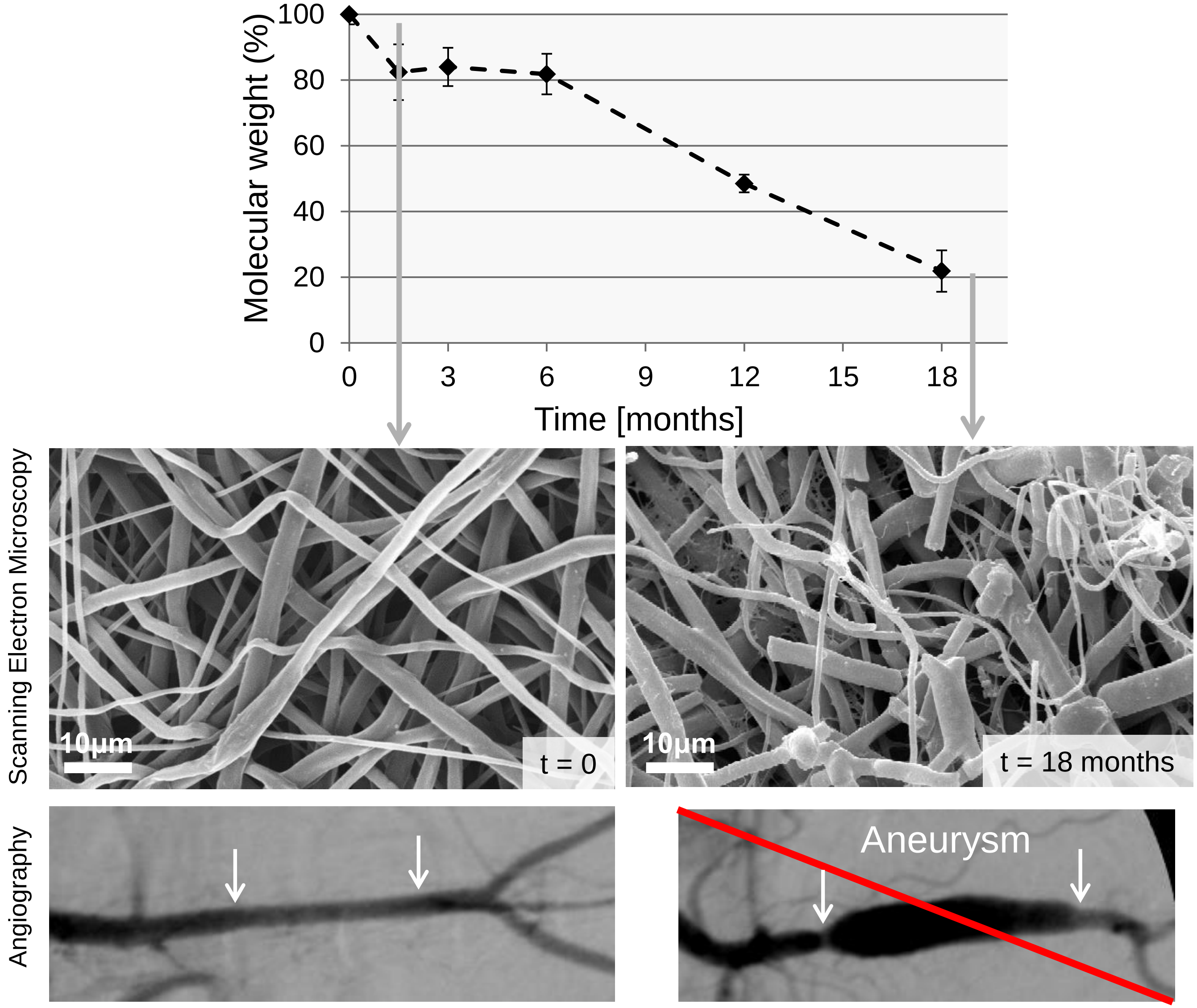
The graft is connected to the aorta by two end-to-end anastomoses

Bulk Properties

Polymer Degradation

Bulk Properties

- 80% polymer degradation
- Good structural integrity



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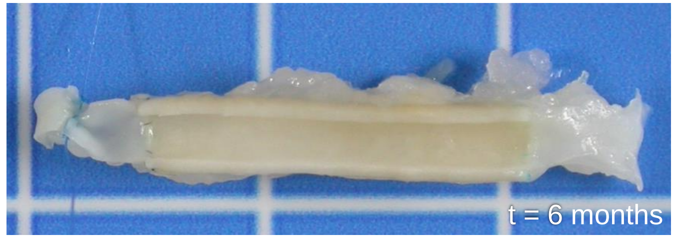
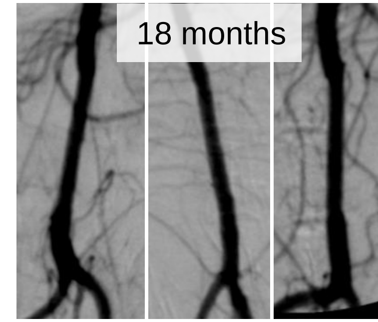
Endothelialization

Bulk Properties
- 80% polymer degradation
- Good structural integrity

Luminal Surface
- 100% Patency
- No Thrombosis
- Rapid and Stable Endothelialization

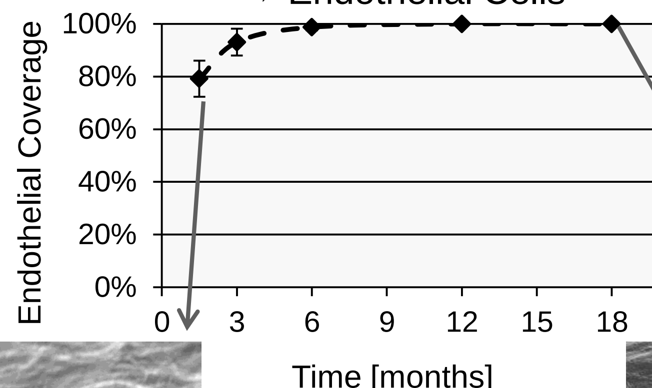


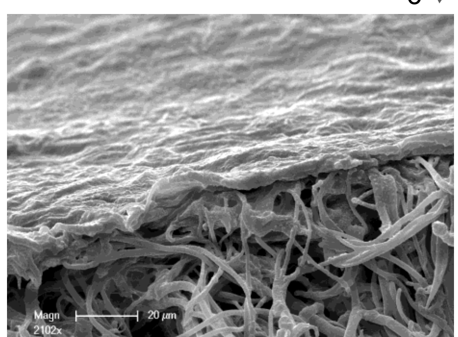
Smooth Luminal Surface
⇨ No Thrombosis

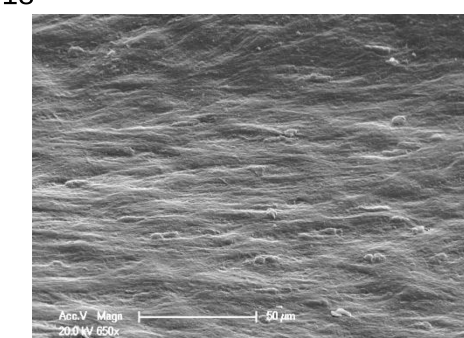


No Occlusions
⇨ 100% Patency

Best anti-thrombotic surface
⇨ Endothelial Cells







Rapid and Stable Endothelialization

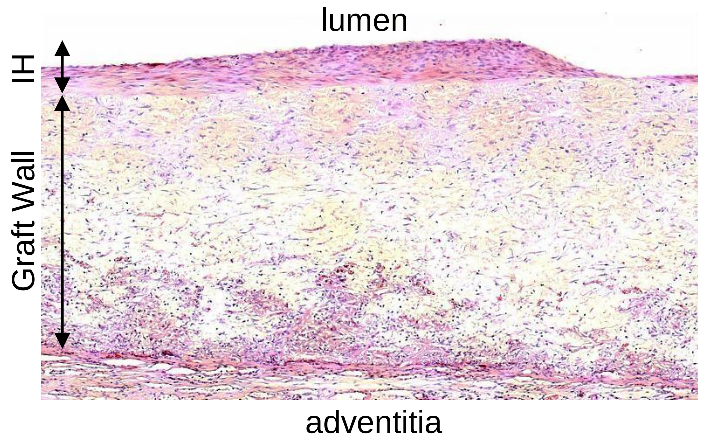
de Valence et al. Biomaterials 2012

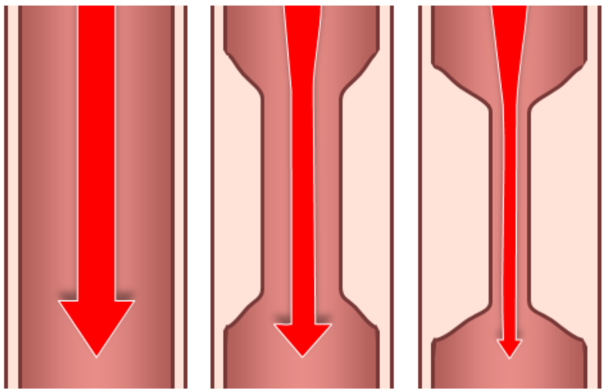
Intimal Hyperplasia (IH)

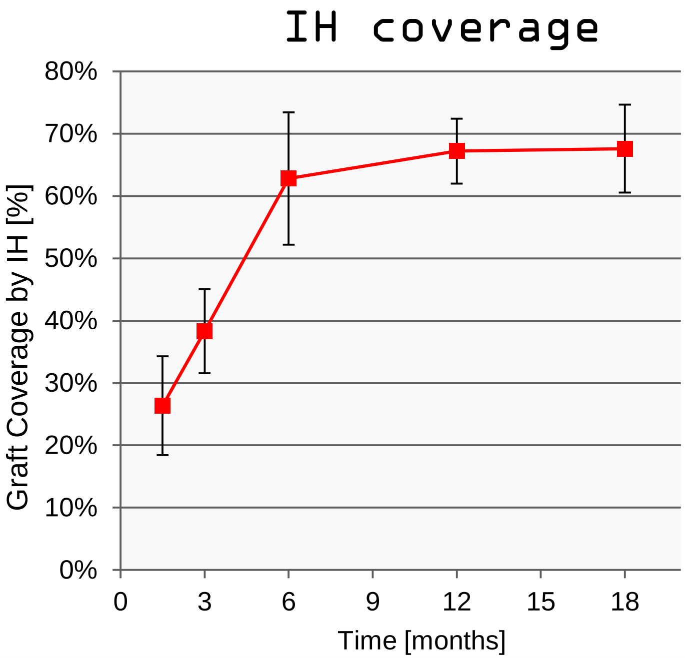
Bulk Properties
- 80% polymer degradation
- Good structural integrity

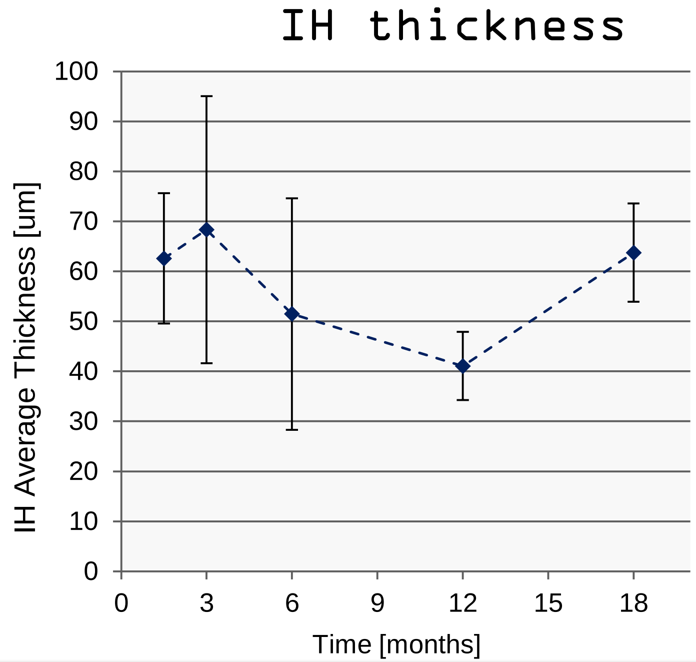
Luminal Surface
- 100% Patency
- No Thrombosis
- Rapid and Stable Endothelialization

Limited Intimal Hyperplasia



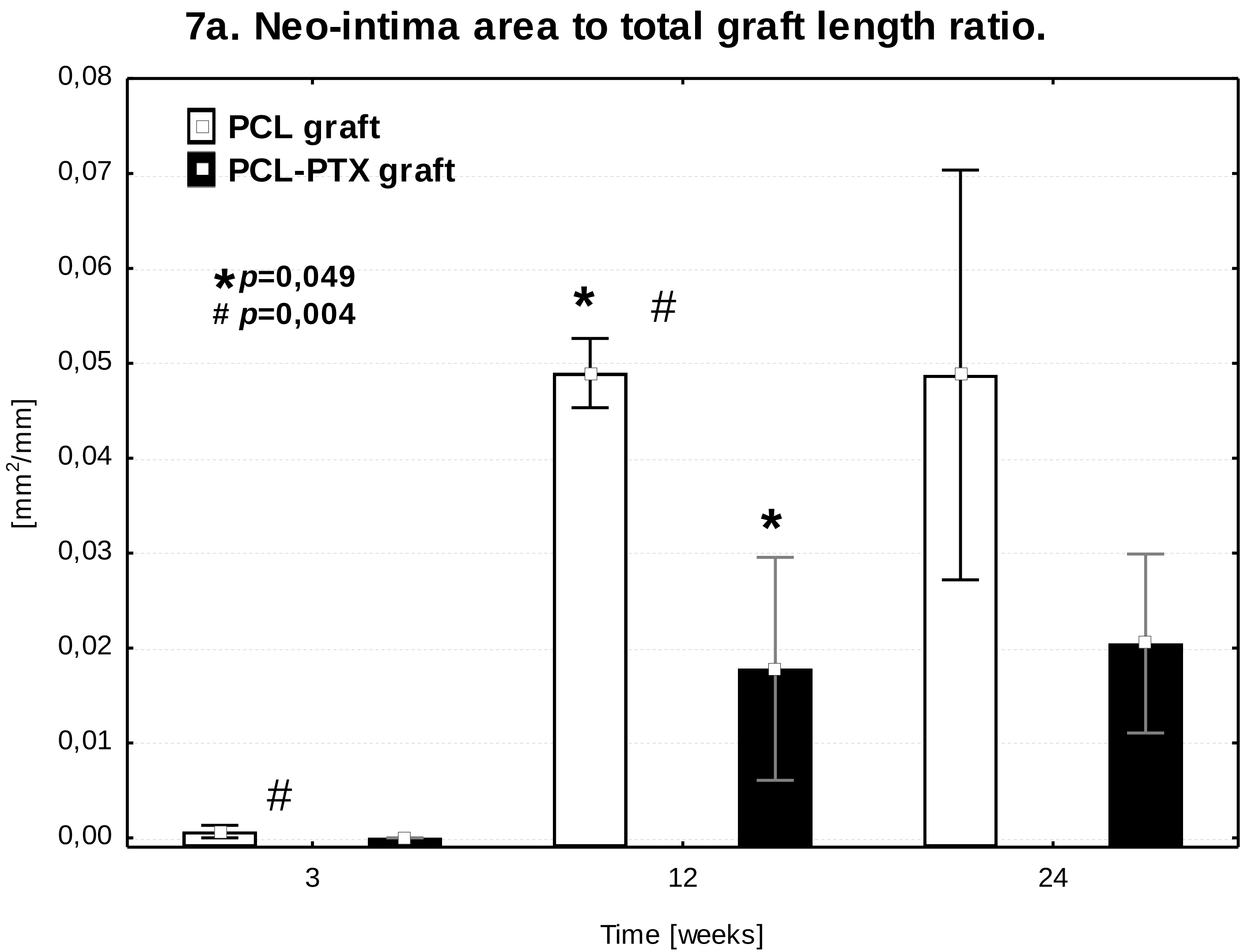






de Valence et al. Biomaterials 2012

Intimal Hyperplasia Reduction



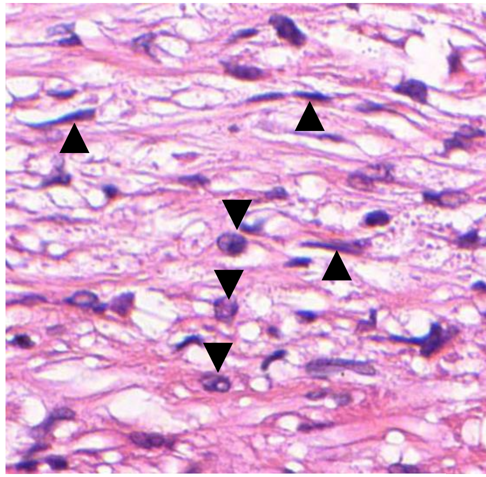
Innocente F. et al. *Circulation.* 2009;120[suppl 1]:S37–S45

Cell Types

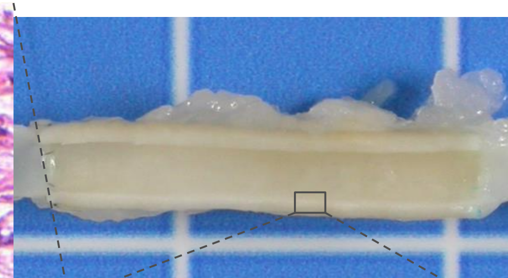
Bulk Properties
- 80% polymer degradation
- Good structural integrity

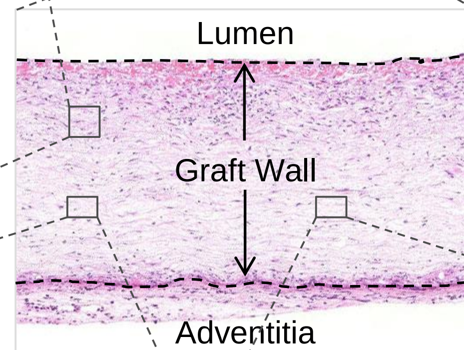
Luminal Surface
- 100% Patency
- No Thrombosis
- Rapid and Stable Endothelialization
- Limited Intimal Hyperplasia

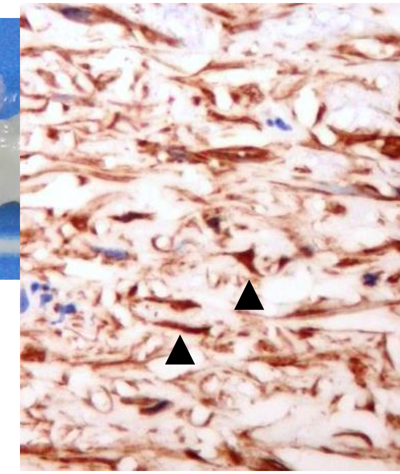
Graft Wall
- Macrophages
- Myofibroblasts
- Capillaries
- Collagen



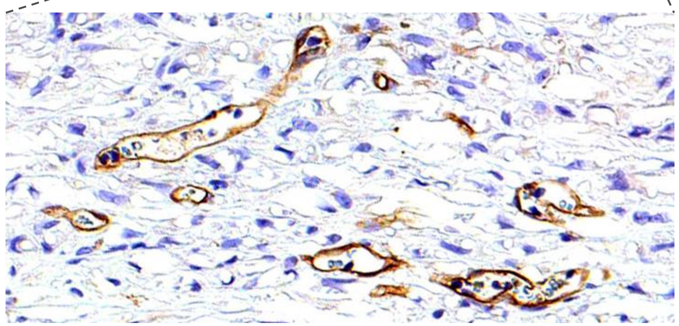
Macrophages ▼
Fibroblasts ▲
H&E stain (purple: cell nuclei)



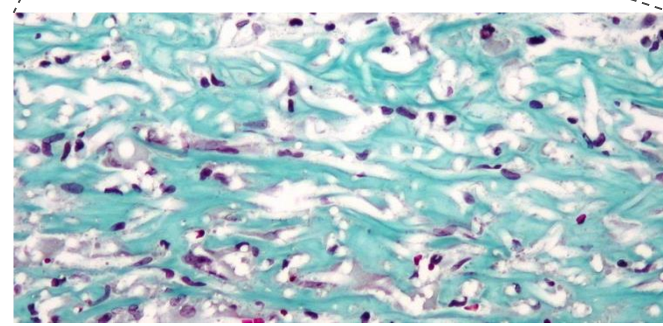




MyoFibroblasts ▲
α-smooth muscle actin stain
(brown: myofibroblasts)



Capillaries
CD34 stain
(brown: endothelial cells)



Collagen
Miller-Masson stain
(green: collagen)

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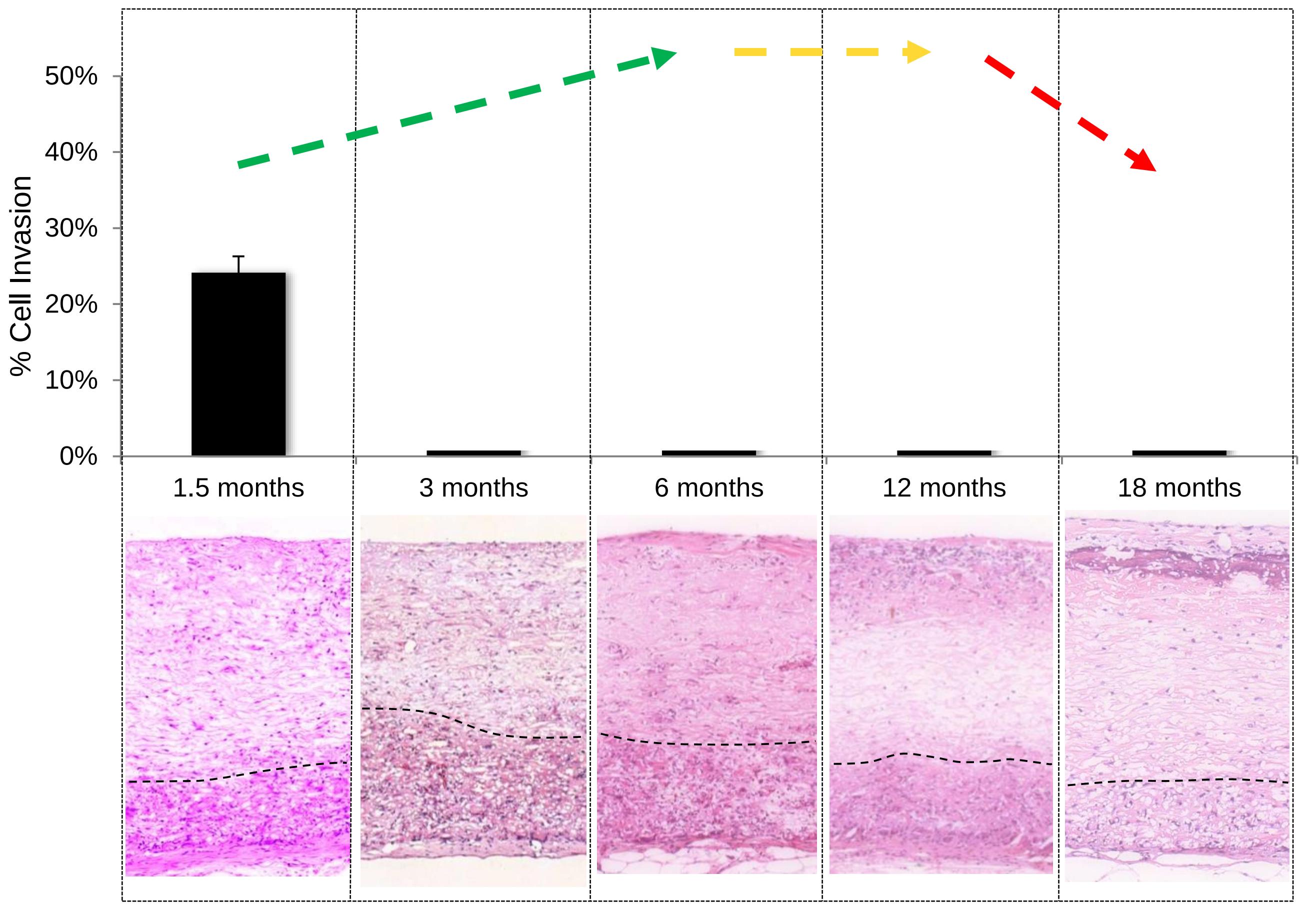
Cell Invasion

Bulk Properties
- 80% polymer degradation
- Good structural integrity

Luminal Surface
- 100% Patency
- No Thrombosis
- Rapid and Stable Endothelialization
- Limited Intimal Hyperplasia

Graft Wall
- Macrophages
- Myofibroblasts
- Capillaries
- Collagen



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Calcifications

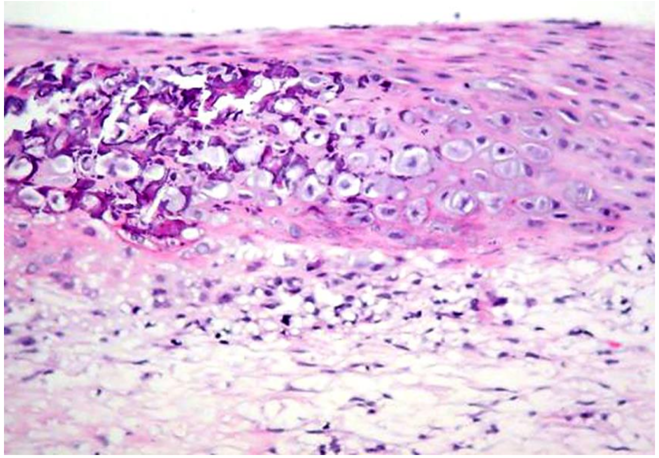
Bulk Properties
- 80% polymer degradation
- Good structural integrity

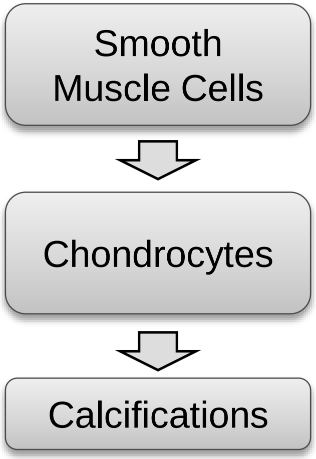
Luminal Surface
- 100% Patency
- No Thrombosis
- Rapid and Stable Endothelialization
- Limited Intimal Hyperplasia

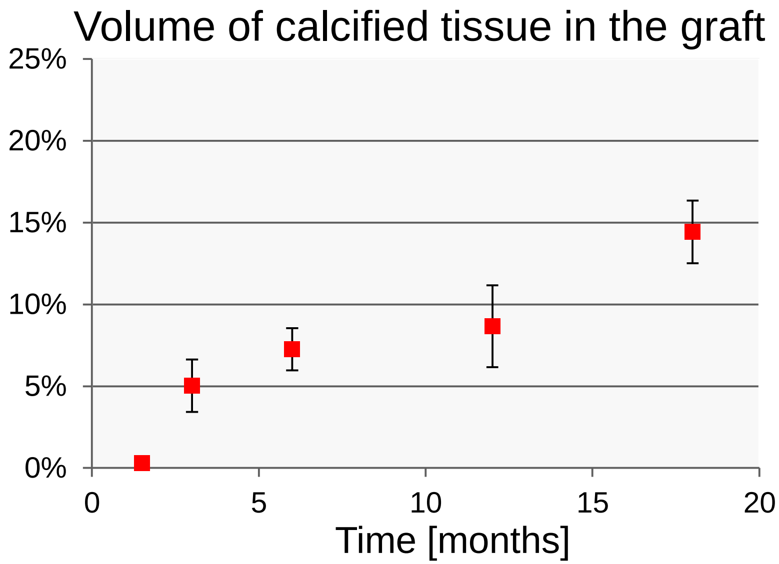
Graft Wall
- Macrophages
- Myofibroblasts
- Capillaries
- Collagen
- Calcifications

MicroCT

Chondroid Metaplasia







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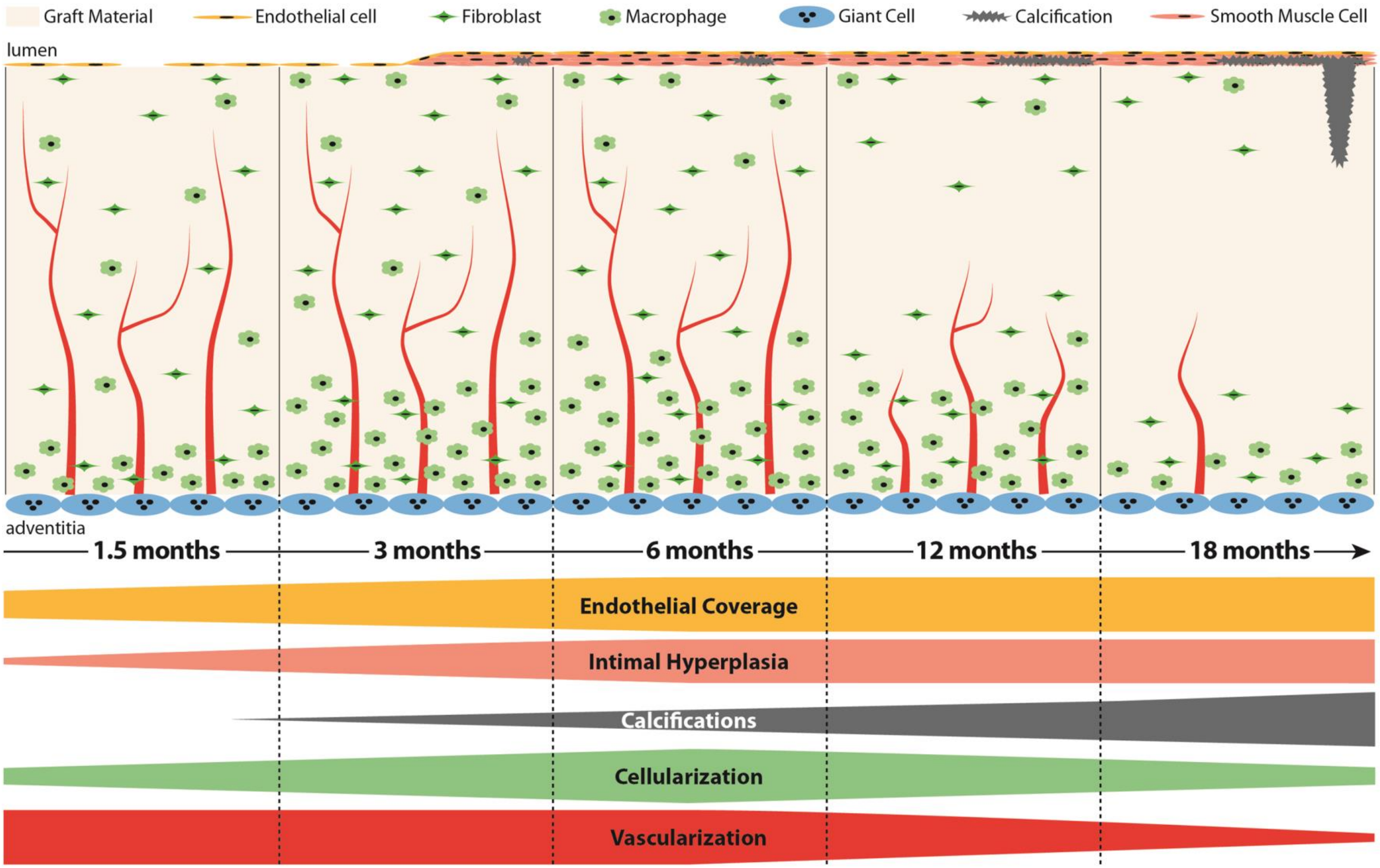
Overview



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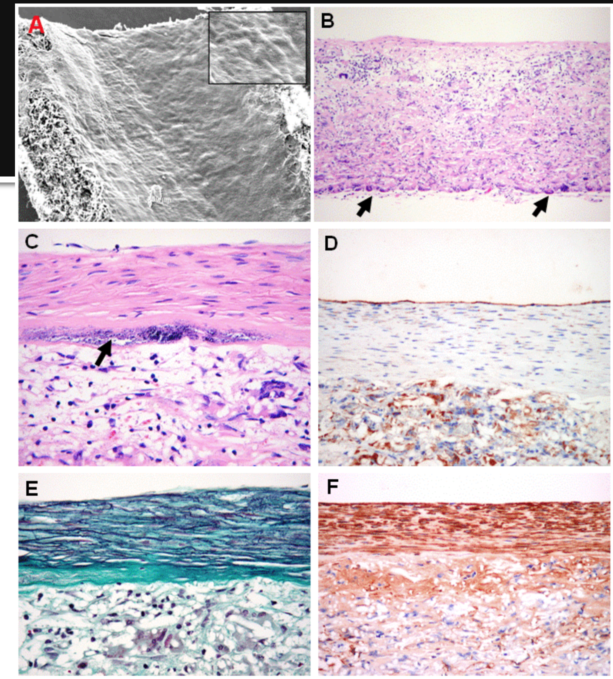
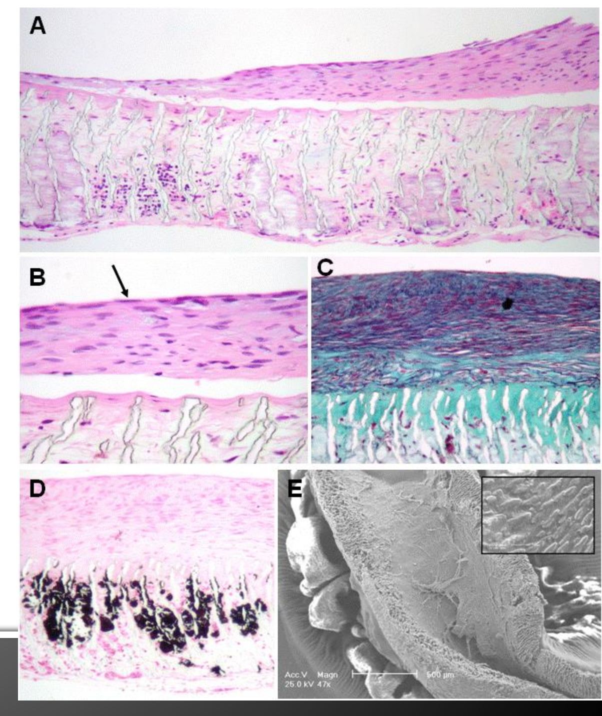
Comparison with ePTFE

PCL



	PCL (n=8)	ePTFE (n=6)	Significance
Survival (months)	14.8±2.9	16.0±3.1	p=0.17
Patency (%)	100	67	p = 0.46
Endothelialization (%)	100 ± 0.0	99.6 ± 1.0	p = 0.92
Compliance (%/mmHg)	8.2 ± 1.0	5.7 ± 0.7	p = 0.01
Calcification (% volume)	7.0 ± 5.0	15.8 ± 3.2	p = 0.04
Intimal Hyperplasia (μm)	51.6 ± 20.7	76.9 ± 36.9	p = 0.09
Cellular Ingrowth	32.1 ± 9.2	10.8 ± 4.0	p < 0.001

ePTFE



Mugnai et al. J Thorac Cardiovasc Surg 2012

Can Synthetic Biodegradable Vascular Grafts Work?

YES

- Perfect patency and functionality over the lifetime of a rat
- No aneurysms despite polymer degradation
- No stenosis linked to intimal hyperplasia or thrombosis
- Better long term performance than ePTFE in the rat model

BUT Two Main Issues

- Unstable tissue regeneration in the graft wall on the long term
- Chondroid metaplasia and spreading of calcifications

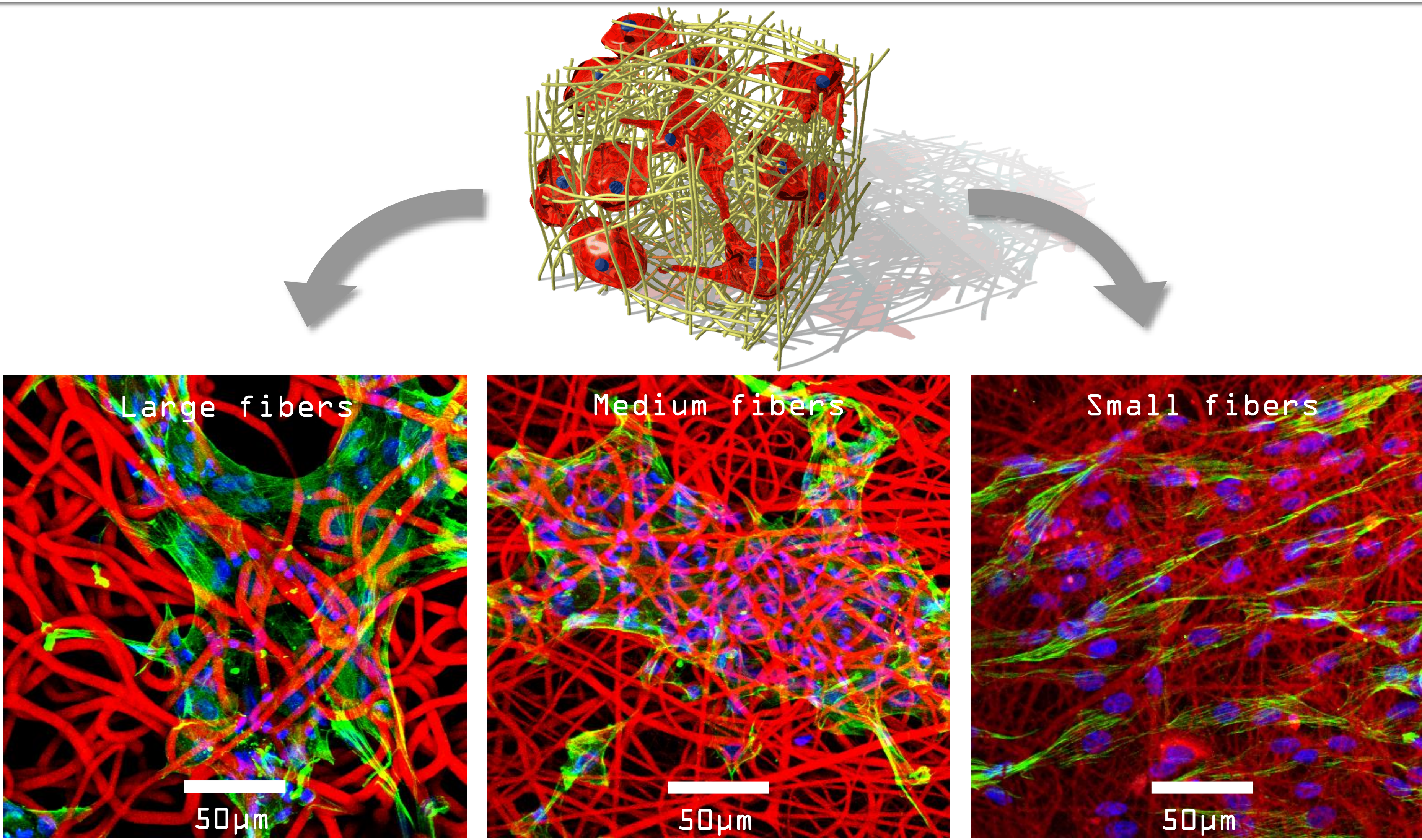
Importance of Long Term Studies
to Identify Unforeseen Issues

Part II

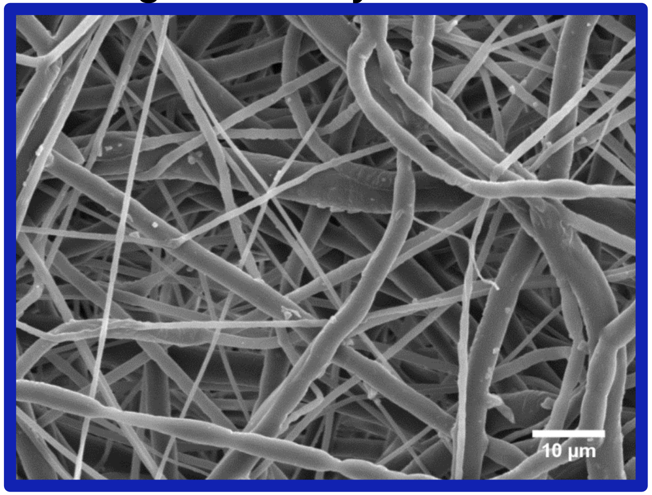
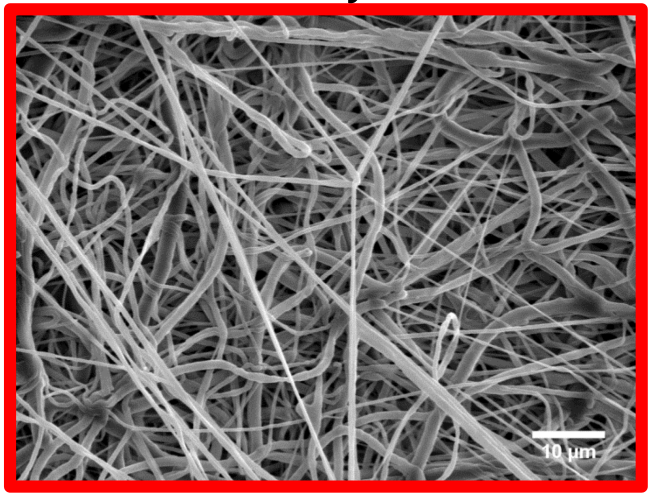
Can the Outcome be Improved by Tuning the Scaffold Micro-Architecture?

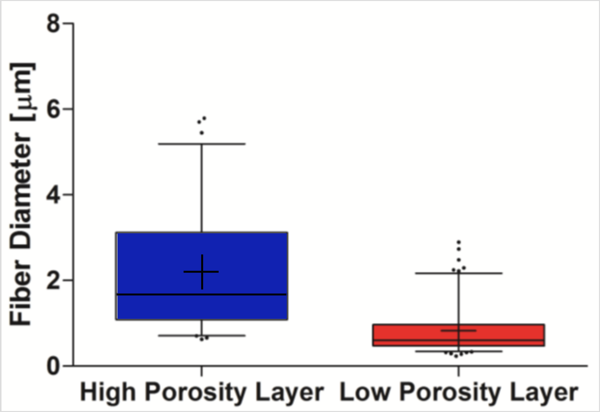
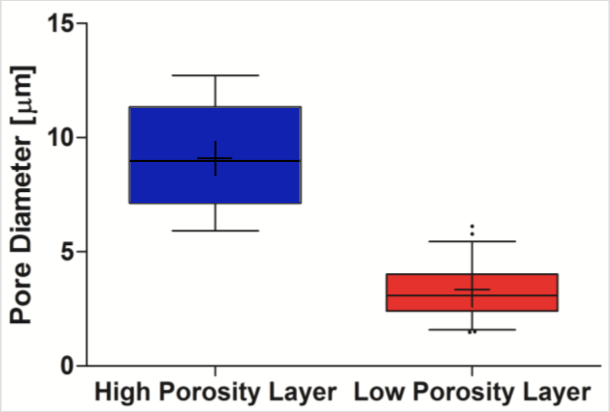
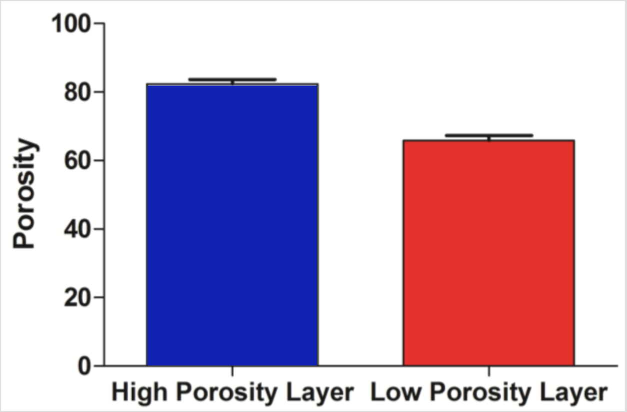
de Valence S, Tille JC, Giliberto JP, Mrowczynski W, Gurny R, Walpoth BH, Möller M. Advantages of bilayered vascular grafts for surgical applicability and tissue regeneration. *Acta Biomaterialia*. 2012 Nov; 8(11):3914-20.

Micro-Architecture



2 Types of Scaffolds

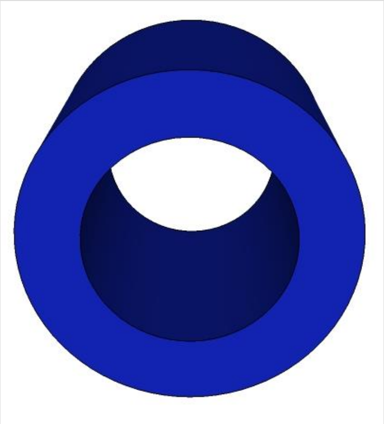
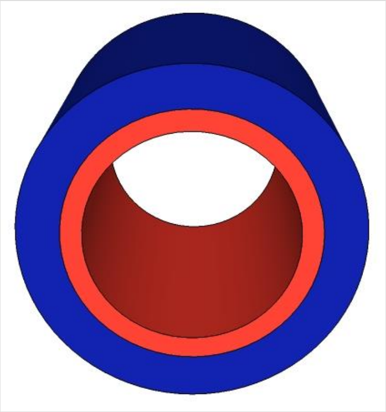
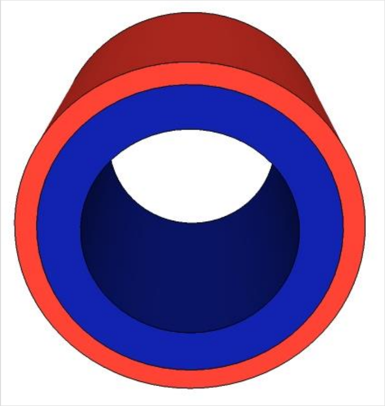
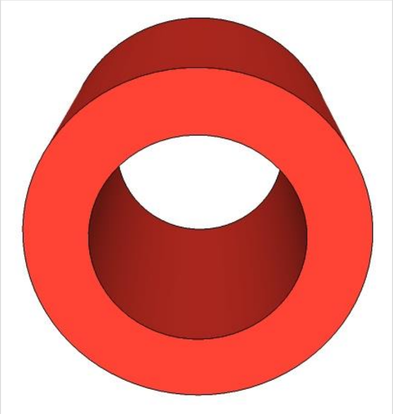
High Porosity Scaffold	Low Porosity Scaffold
	

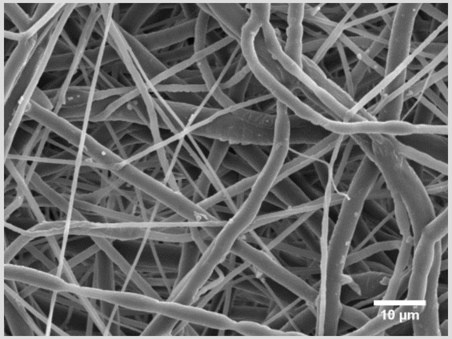
Fiber Diameter	Pore Diameter	Total Porosity
		

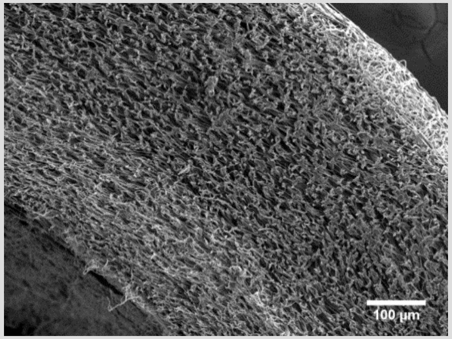
de Valence et al. Acta Biomaterialia 2012

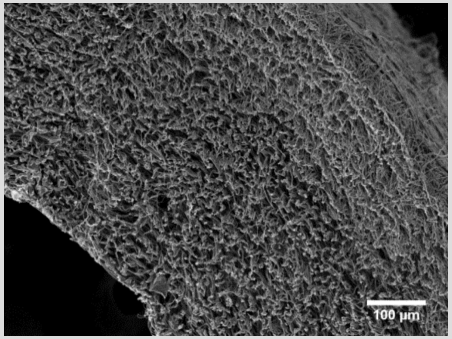
4 types of grafts

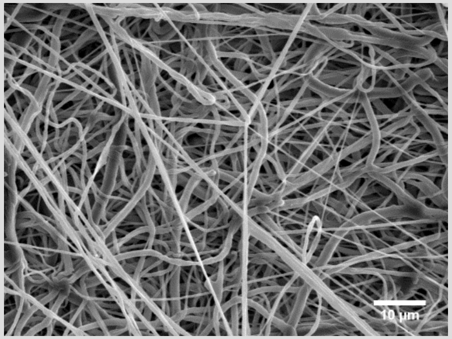
No Barrier	Inside Barrier	Outside Barrier	Only Barrier









No Barrier	Inside Barrier	Outside Barrier	Only Barrier
Layer thickness	Layer thicknesses	Layer thicknesses	Layer thickness
600 µm	400/200 µm	200/400 µm	600 µm

de Valence et al. Acta Biomaterialia 2012

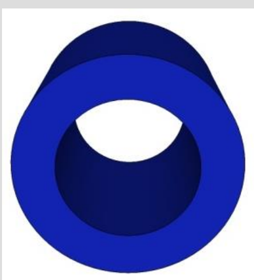
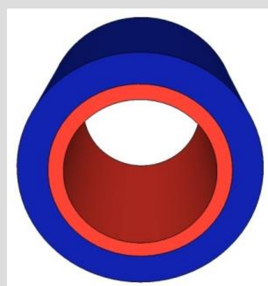
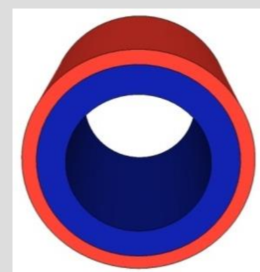
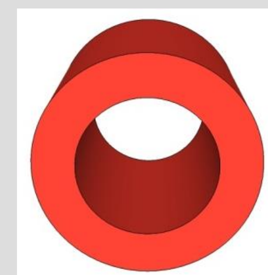
In Vitro Blood Leakage

Pore size

Cell infiltration ⬄ Blood leakage

Experiment

Amount of leakage of heparinized blood through the graft wall, under flow, at 120 mmHg

No Barrier	Inside Barrier	Outside Barrier	Only Barrier
			
$0.87\ ml \cdot min^{-1} \cdot cm^{-2}$	$0.17\ ml \cdot min^{-1} \cdot cm^{-2}$	$0.25\ ml \cdot min^{-1} \cdot cm^{-2}$	$0.09\ ml \cdot min^{-1} \cdot cm^{-2}$
			
	÷5	÷3.5	÷10

Scaffold Micro-architecture Can Effectively Reduce Blood Leakage

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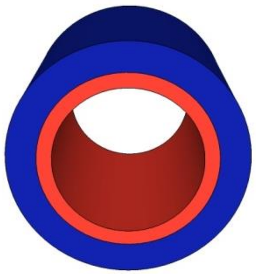
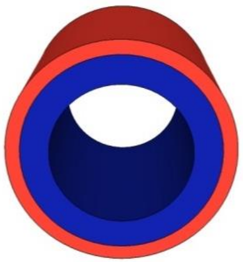
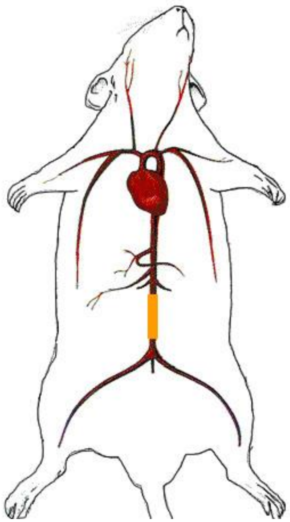
Implantation in the Rat Model

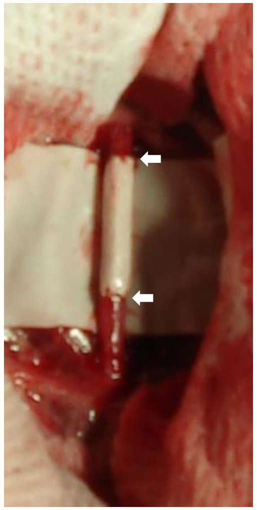
Inside Barrier



Outside Barrier







3 12 weeks

(n=5)

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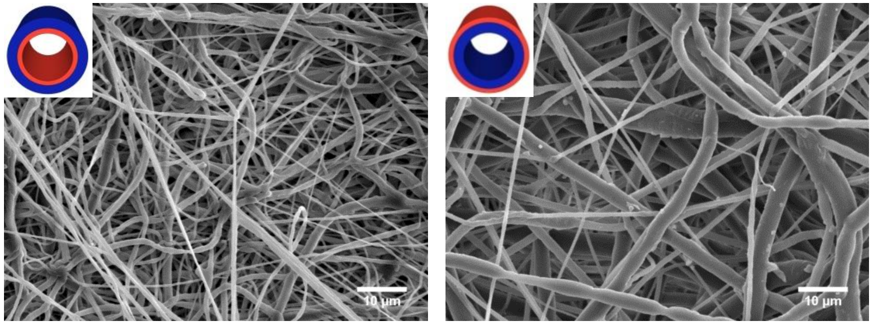
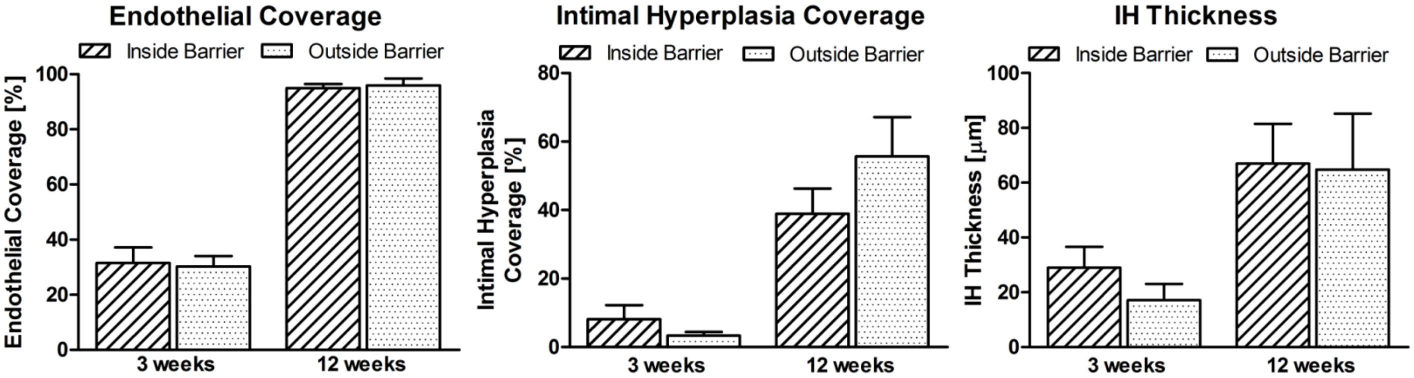
Endothelialization & IH

Luminal Surface

- No difference in endothelialization
- Little IH



Inside Barrier Outside Barrier



Endothelial Coverage – No difference
Intimal Hyperplasia – No difference

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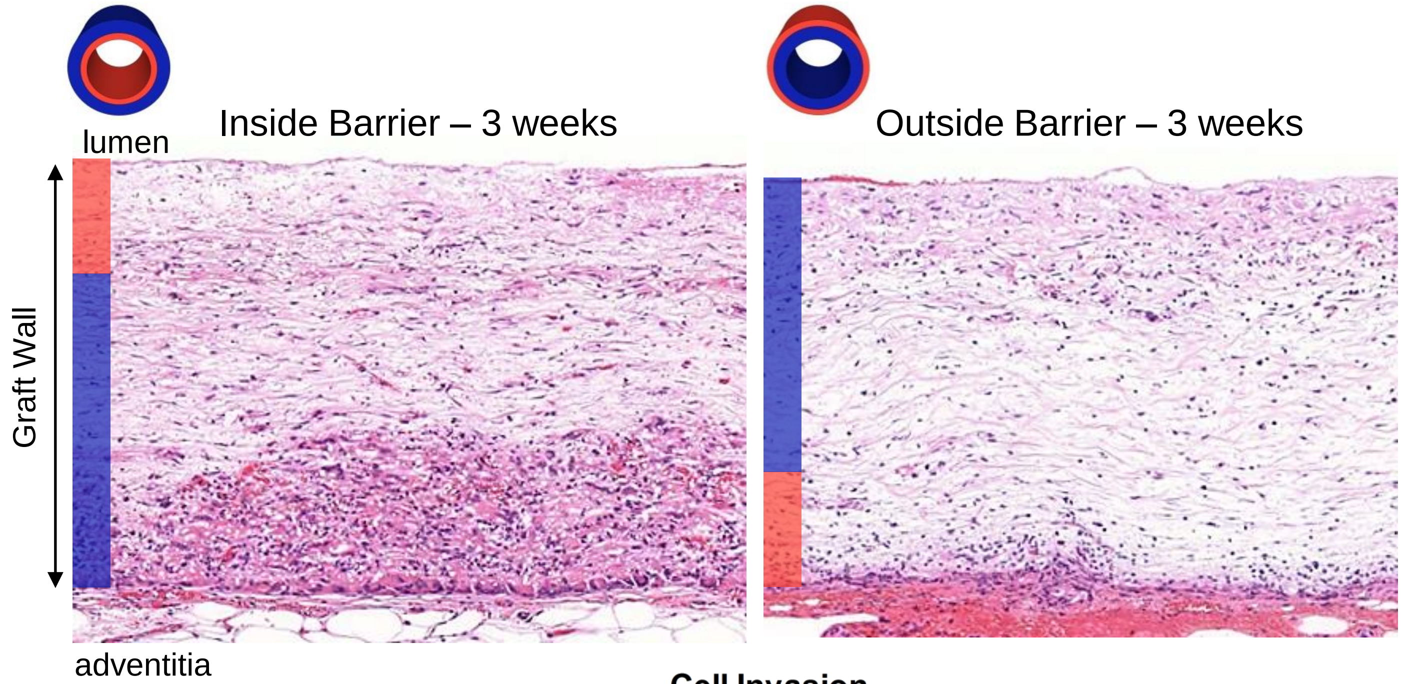
Cell Invasion

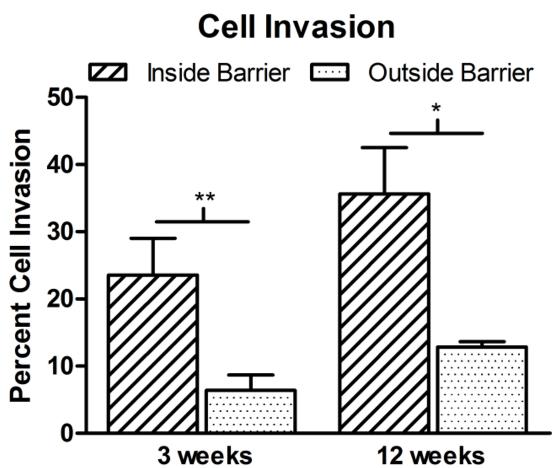
Luminal Surface

- No difference in endothelialization
- Little IH

Graft Wall

- Cell invasion is inhibited by outside barrier
- Cells come mainly from the adventitia





de Valence et al. Acta Biomaterialia 2012

Does the Outcome Depend on the Micro-Architecture?

YES

- Micro-architecture is an effective means to control blood leakage
- Too small pores will impede cell infiltration and tissue regeneration
- Cell infiltration comes mainly from the adventitia so micro-architecture should be designed with this in consideration

AND

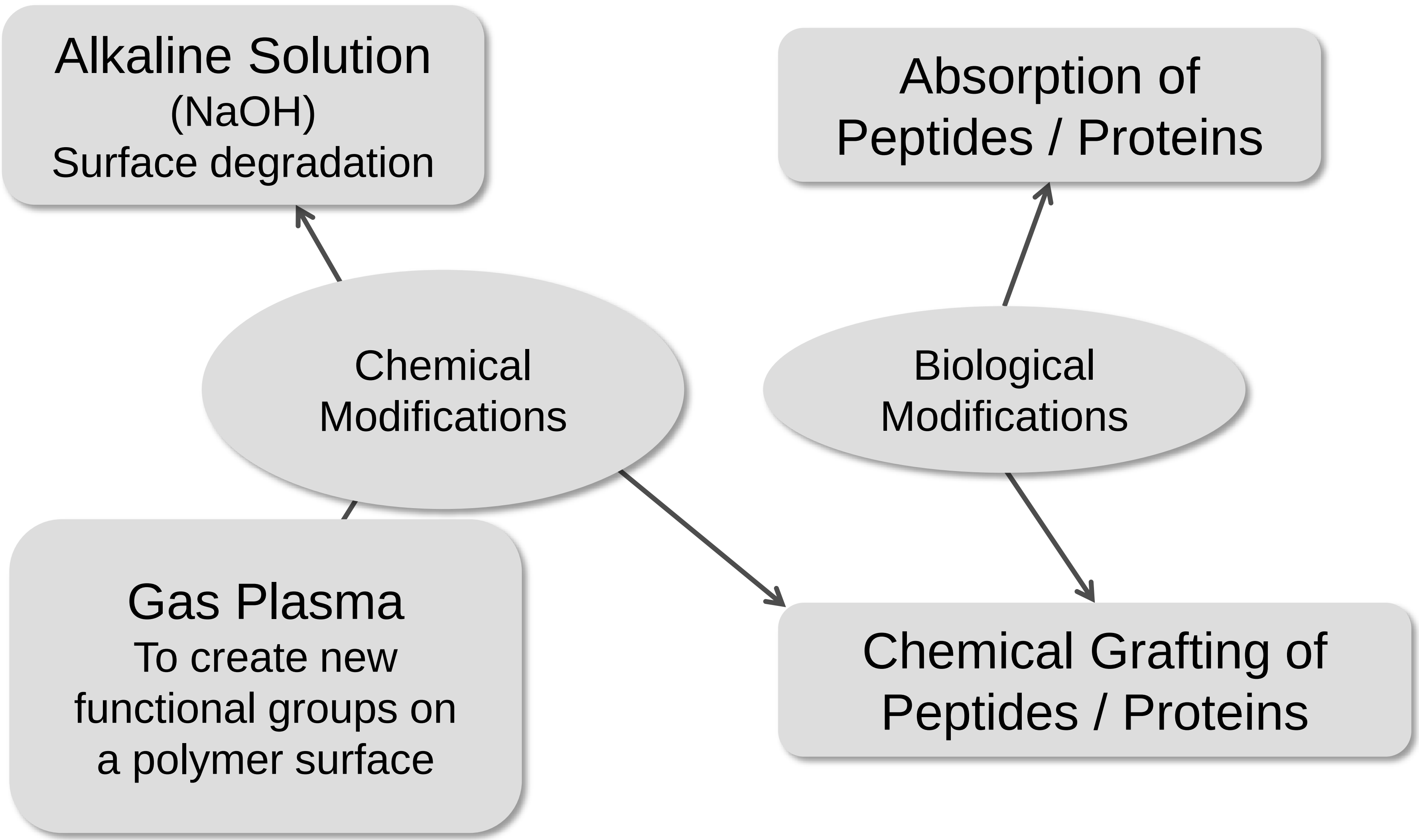
- Multiple layer grafts can easily be produced by electrospinning

Part III

Can Tissue Regeneration be Improved by Tuning the Surface Properties of the Scaffold?

de Valence S, Tille JC, Chaabane C, Gurny R, Bochaton-Piallat ML, Walpoth BH, Möller M.
Plasma treatment for improving cell biocompatibility of a biodegradable polymer scaffold for vascular graft applications.
To be submitted

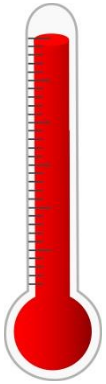
Polymer Surface Modification

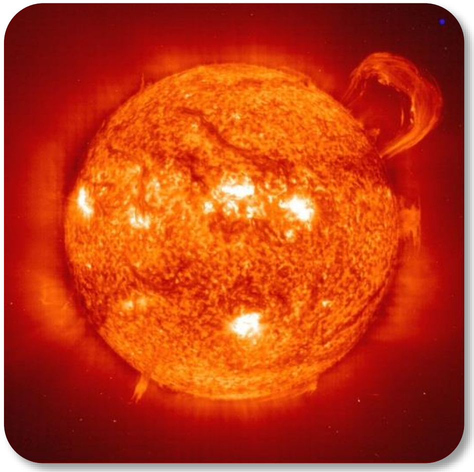


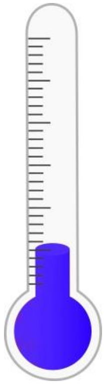
Jiao YP and Cui FZ. Biomedical Materials 2007

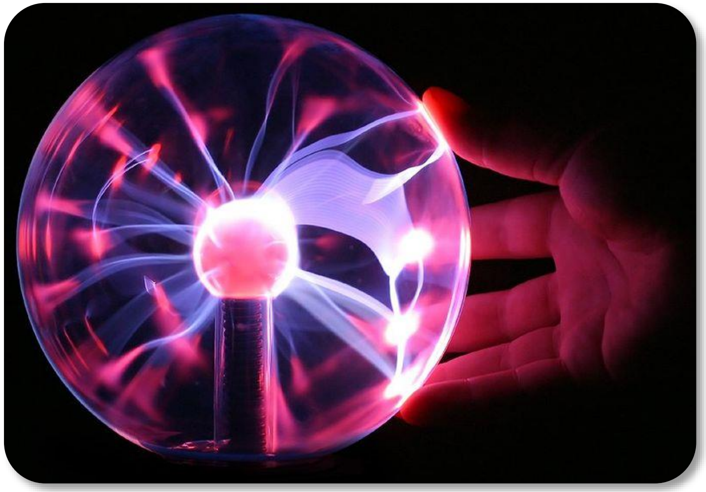
Gas Plasma – 4^{th} state of matter

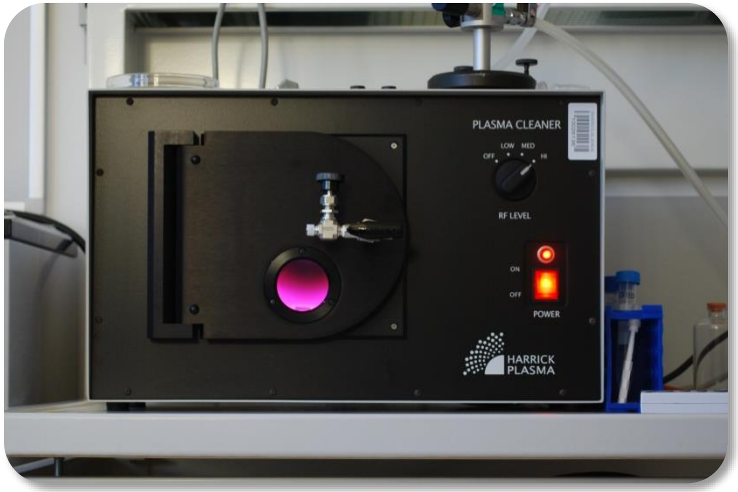
Plasma: partially ionized gas



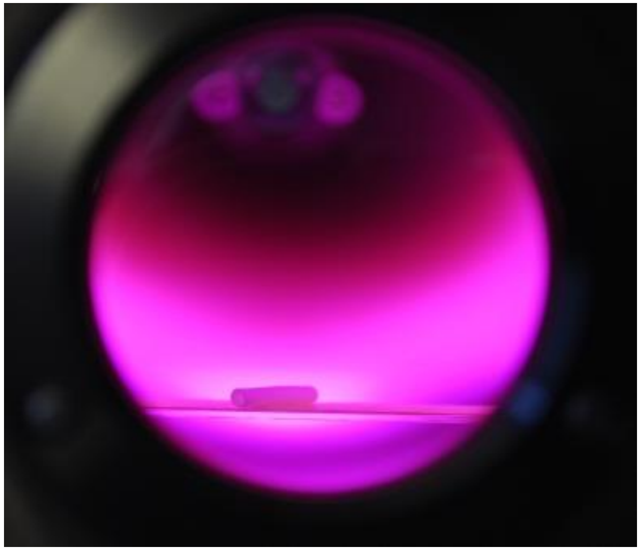


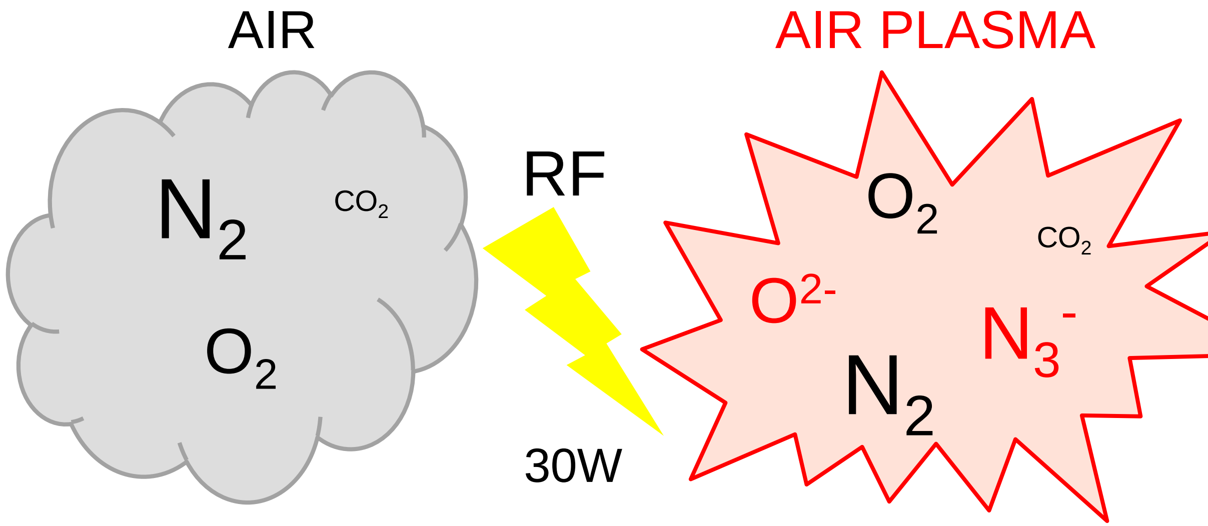




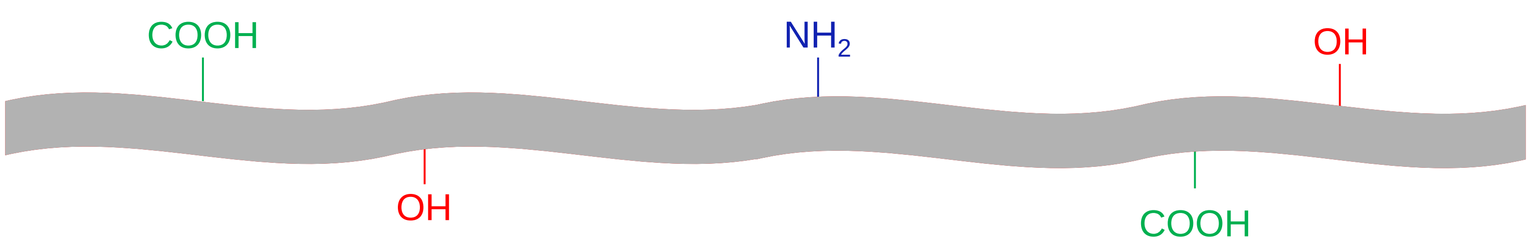


Plasma Modification





Plasma Treated PCL

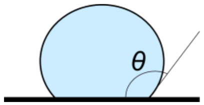


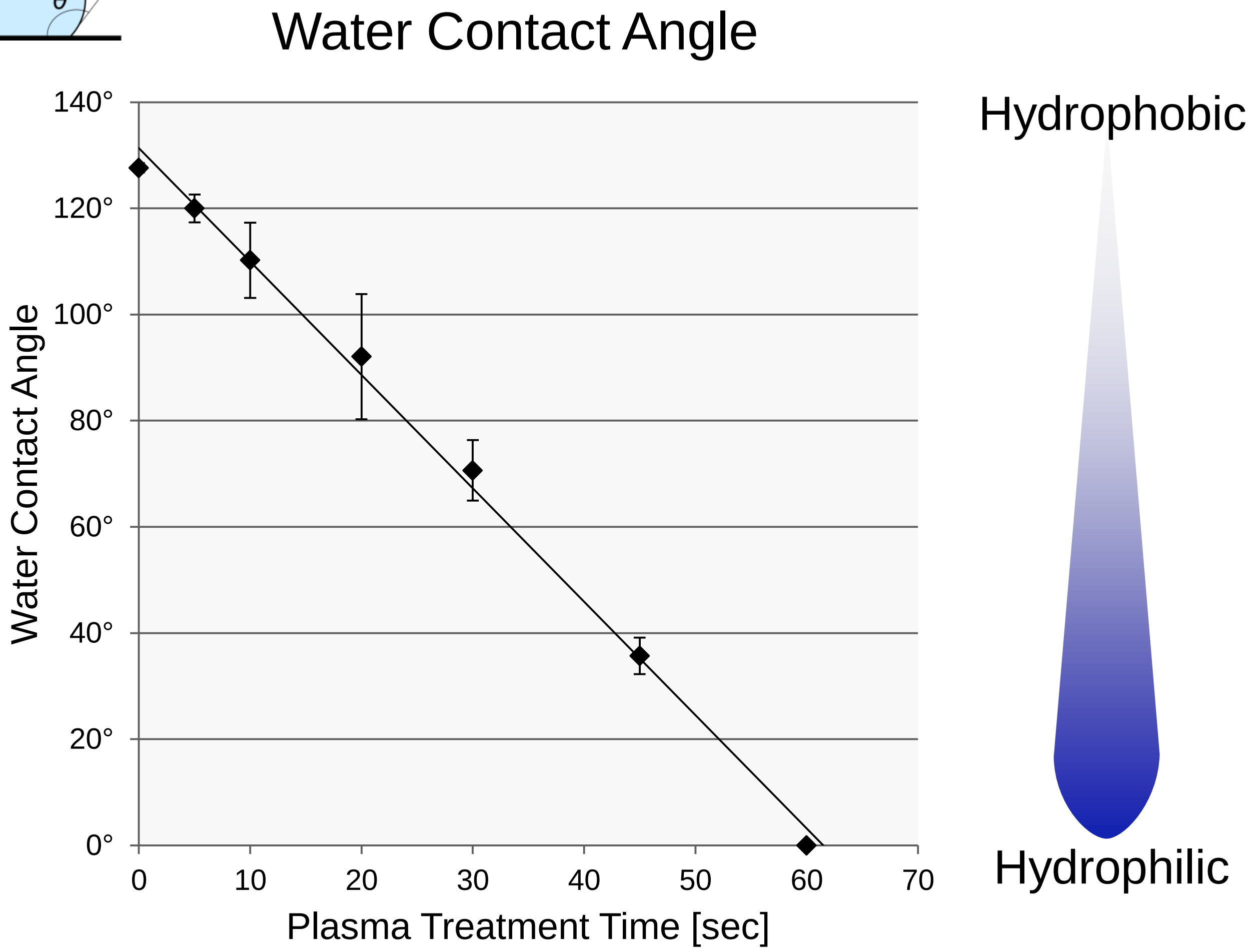
Hydrophobic → Hydrophilic

Changes in Hydrophilicity

Characterization

Increased Hydrophilicity





Cell Morphology

Characterization

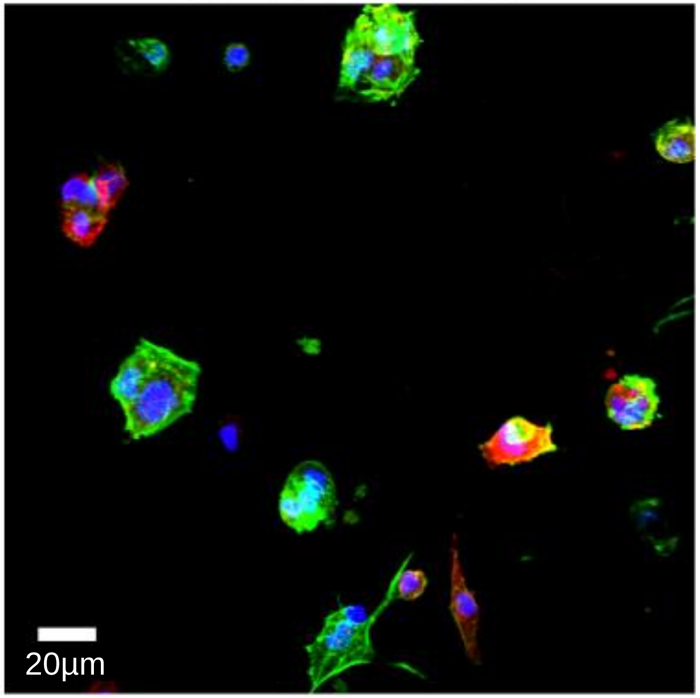
- Increased hydrophilicity
- No change in fiber morphology

In Vitro

- Outspread cell morphology

After 3 days of culture

No Treatment



Blue – nuclei
Green – smooth muscle actin
Red – S100A4 – migratory and proliferation phenotype marker

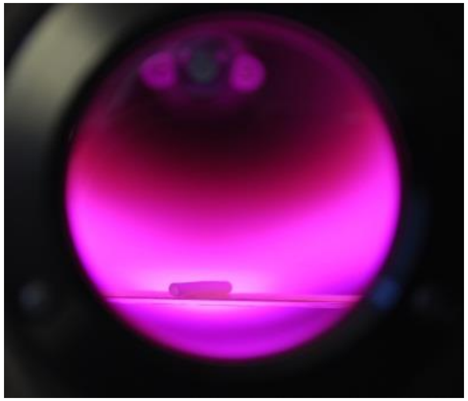
Vascular Implantations

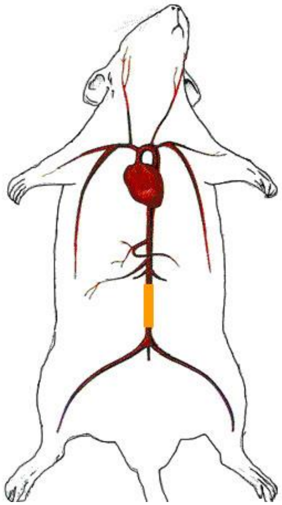
Characterization
- Increased hydrophilicity
- No change in fiber morphology

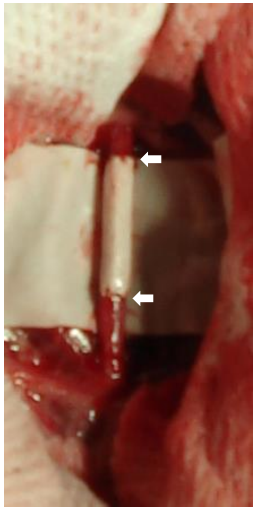
In Vitro
- Outspread cell morphology

Vascular Implants

Plasma Treated Grafts
vs.
Untreated Grafts







3 weeks
n=5

In Vivo

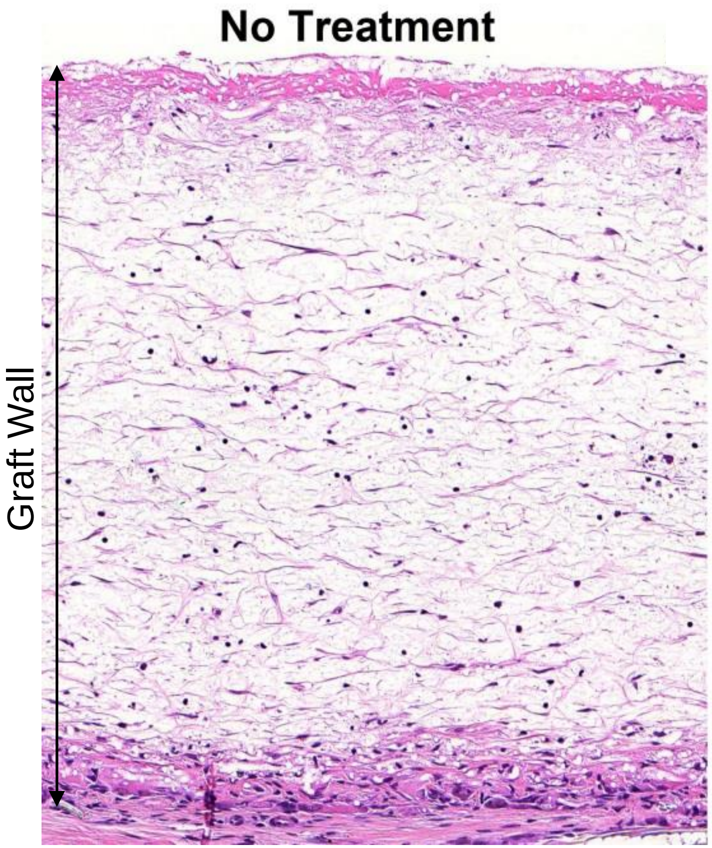
Cell Invasion

Characterization
- Increased hydrophilicity
- No change in fiber morphology

In Vitro
- Outspread cell morphology

Vascular Implants
- Increased cellularization

3 weeks



Increased Cell Invasion in Plasma Treated Grafts

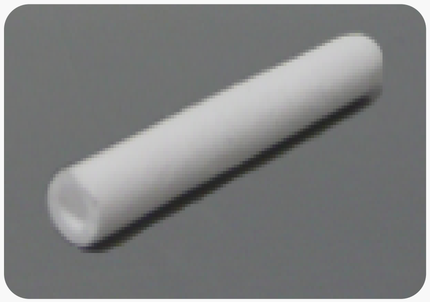
Can Tissue Regeneration be Improved by Tuning the Surface Properties?

YES

- Increased hydrophilicity improves cell-scaffold affinity
  - Cell morphology in vitro
  - Cellular infiltration and neo-vascularization in vivo

Step by Step Towards a Successful Graft

Evaluation Tools and Methods



Polycaprolactone Electrospun Vascular Grafts

- ✓ Long term outcome in the rat
- ✓ Mechanical integrity
- ✗ Unstable neo-tissue long term
- ✗ Calcifications

Excellent Potential but Improvements Needed

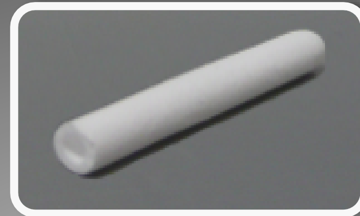
Micro-Architecture

- ✓ Barrier layer can reduce blood leakage
- ✓ Inside barrier layer does not impede tissue regeneration

Surface Modifications

- ✓ eased hydrophilicity
- ✓ Improved cell affinity
- ✓ Improved tissue regeneration

Further Developments



Tissue Regeneration

Micro-Architecture

Surface Modifications

Biomaterial or Combination of Biomaterials

Drug or Growth Factor Release

Smooth Muscle Cell Pre-Seeding

Thrombosis

Drug Release
- Heparin
- Thrombin Inhibitor
- Nitric Oxide Donor

Endothelial Cell Pre-Seeding

Calcifications

Better Tissue Regeneration

Anti-Calcic Drug Release

PEER REVIEWED PUBLICATIONS

FNS Project 3200-119822

Drug eluting cardiovascular prosthesis using nano-fibre structured biodegradable polymers

Pektok E, Nottelet B, Tille J-C, Gurny R, Kalangos A, Moeller M, Walpoth BH. Degradation and healing characteristics of small-diameter poly(ε-caprolactone) vascular grafts in the rat systemic arterial circulation ***Circulation***. 2008;118:2563-2570

Innocente F, Mandracchia D, Pektok E, Nottelet B, Tille J-C, de Valence S, Faggian G, Mazzucco A, Kalangos A, Gurny R, Moeller M, Walpoth BH. Paclitaxel-eluting biodegradable synthetic vascular prostheses: a step towards reduction of neointima formation? ***Circulation***. 2009;120[suppl 1]:S37–S45

B. Nottelet, E. Pektok, D. Mandracchia, E. Pektok, J-C. Tille, B. Walpoth, R. Gurny, M. Möller Factorial design optimization and *in vivo* feasibility of poly(caprolactone)-micro-and nanofiber-based small diameter vascular grafts. ***J Biomed Mater Res*** *A*. 2009 Jun 15;89(4):865-75.

Sarra de Valence; Jean-Christophe Tille; Damiano Mugnai; Wojciech Mrowczynski; Robert Gurny; Michael Möller; Beat H Walpoth. Long term performance of polycaprolactone vascular grafts in a rat abdominal aorta replacement model. ***Biomaterials*** 33 (2012) 38-47.

de Valence S, Tille JC, Giliberto JP, Mrowczynski W, Gurny R, Walpoth BH, Möller M. Advantages of bilayered vascular grafts for surgical applicability and tissue regeneration. **Acta Biomater**. 2012 Jul 6. Epub ahead of print

Mugnai D, Tille JC, Mrówczyński W, de Valence S, Montet X, Möller M, Walpoth BH. Experimental non-inferiority trial of synthetic small-caliber biodegradable versus stable vascular grafts. ***J Thorac Cardiovasc Surg***. 2012 Oct 22. doi:pii: S0022-5223(12)01208-1. 10.1016/j.jtcvs.2012.09.054. [Epub ahead of print]

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THANK YOU