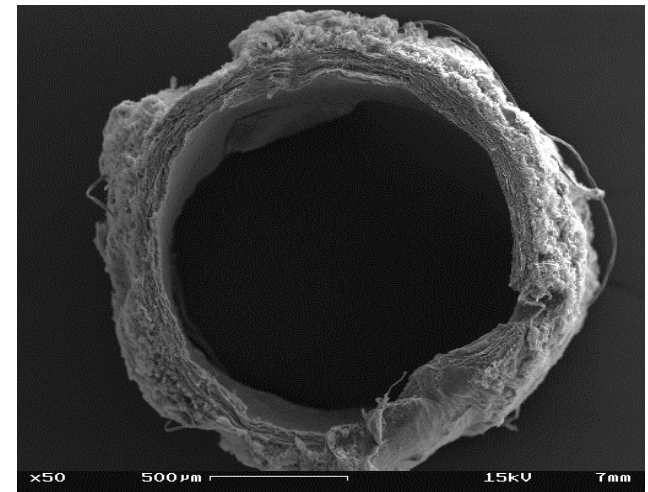

Sterilization of biological tissues using low energy electron irradiation

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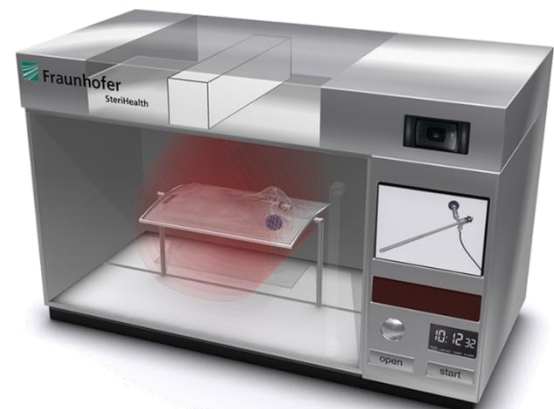


Sterilization procedures for biological tissues

Procedure	Limitations for biological tissues
Autoclaving	Denaturation of proteins / loss of tissue architecture
Chemical Sterilization	Impaired biocompatibility
Ionizing Irradiation	
Gamma irradiation	Impairment of mechanical properties
high-energy electron irradiation	High shielding necessary

Low Energy Electron Irradiation - LEEI

- › On-site sterilization possible
- › Irradiation inside the final packaging
- › Short irradiation time
- › Irradiation under atmospheric pressure
- › No heat development inside the sample (< 40 °C)
- › Sterilization of medical products according to DIN EN ISO 11137



<https://www.sterihealth.de/>

Part I: Sterilization of Vascular Grafts

Introduction

- › Replacement of dysfunctional vessels or infected grafts
- › Large diameter vessels are often replaced by polymeric vascular grafts
- › Small diameter vessels (< 8 mm) are preferentially replaced by autologous veins
- › Allografts and xenografts are infrequently used: Challenge - Sterility

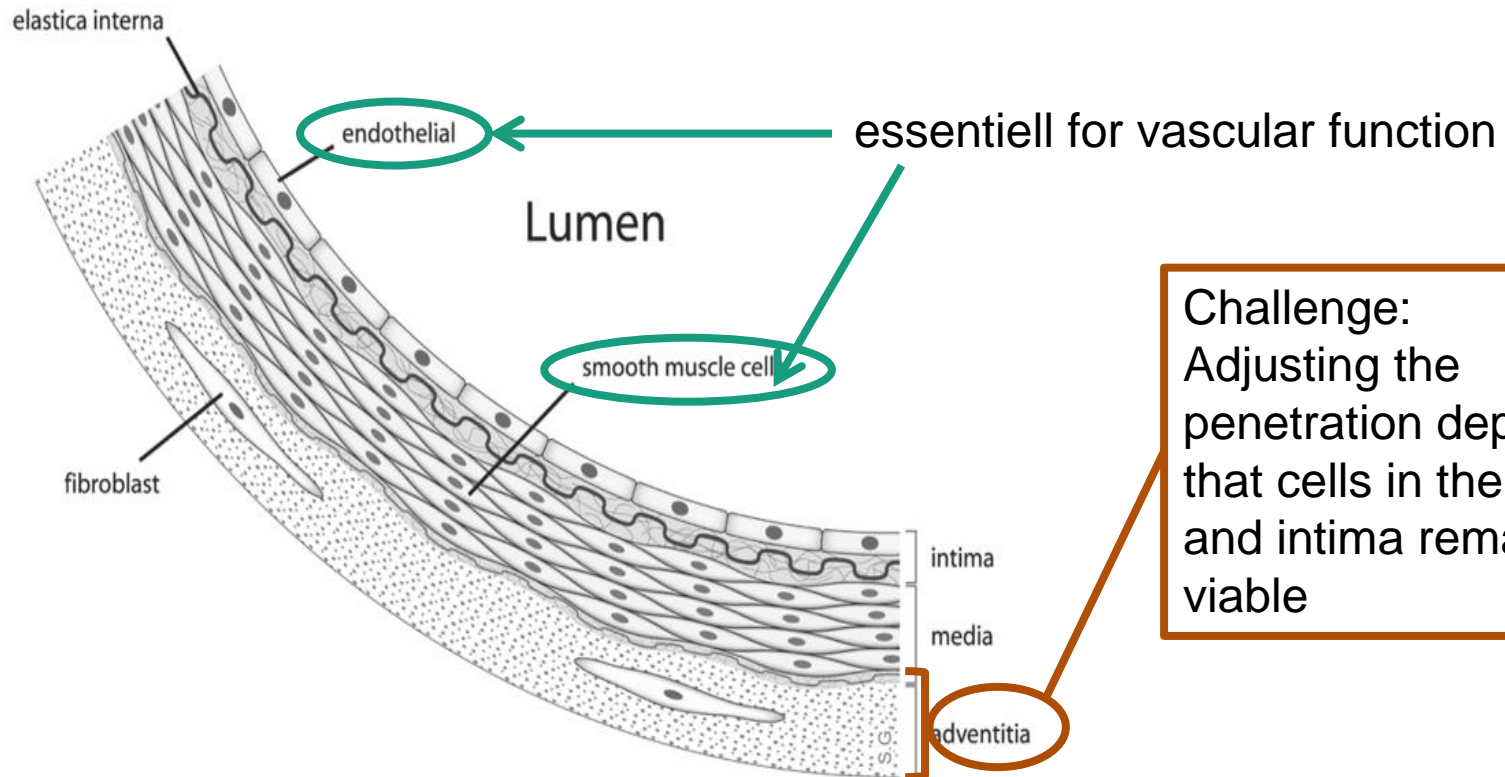


Various vascular prosthesis

(Source: Rotes Kreuz Krankenhaus
Gefäßzentrum Bremen;
<http://www.gefaesszentrum-bremen.de/lexikon/gefaessprothese>)

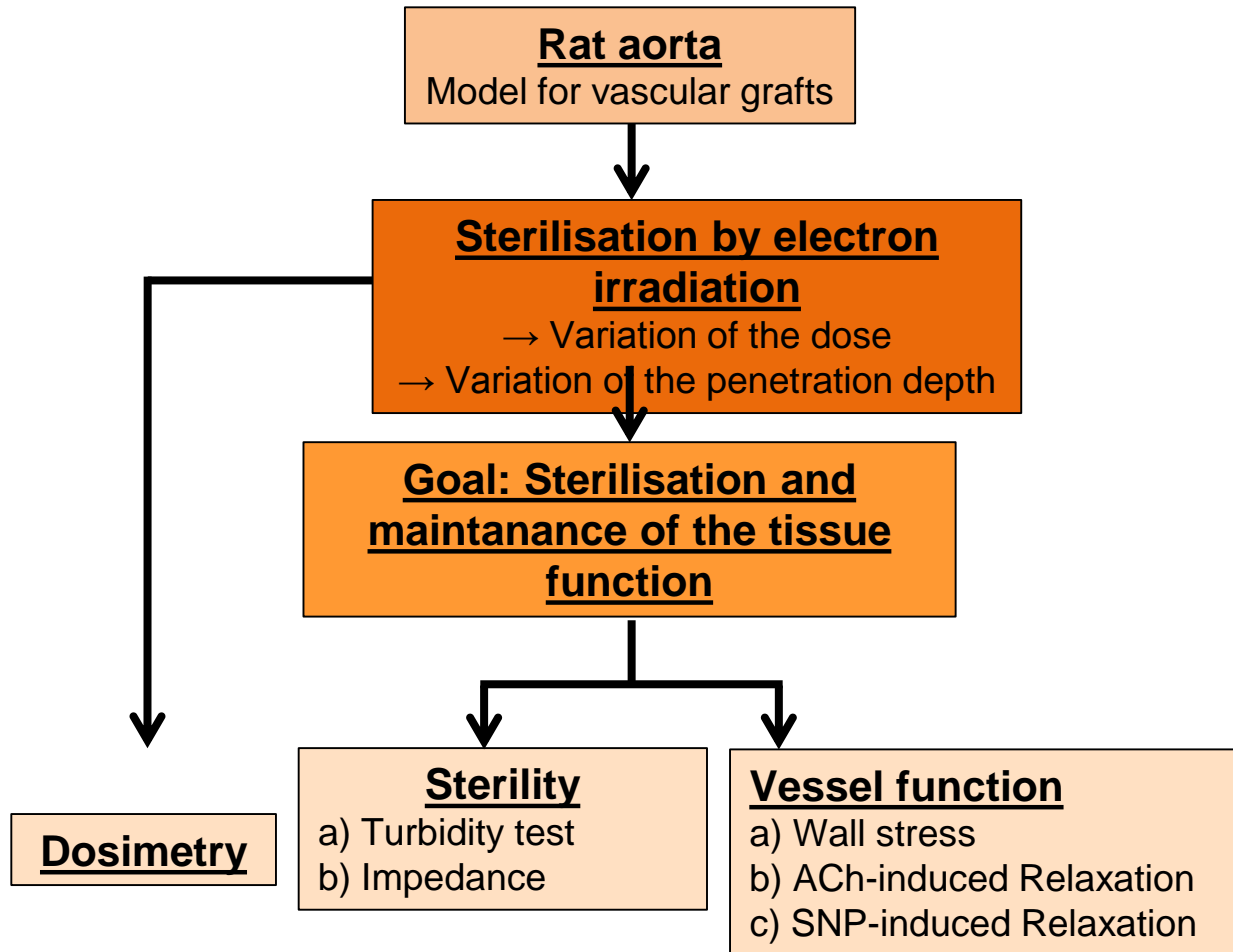
Introduction

Wall structure of blood vessels



Source: S.A.I. Ghesquiere (2006) The Role of Phospholipases in Atherosclerosis, UPM Universitaire Pers Maastricht, ISBN 9789052785301

Experimental approach



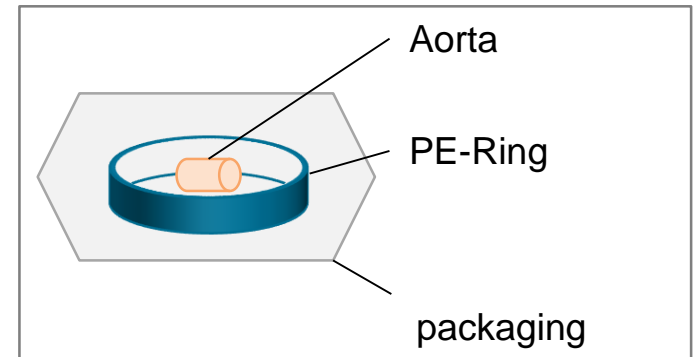
Experimental approach

Low-energy electron irradiation:

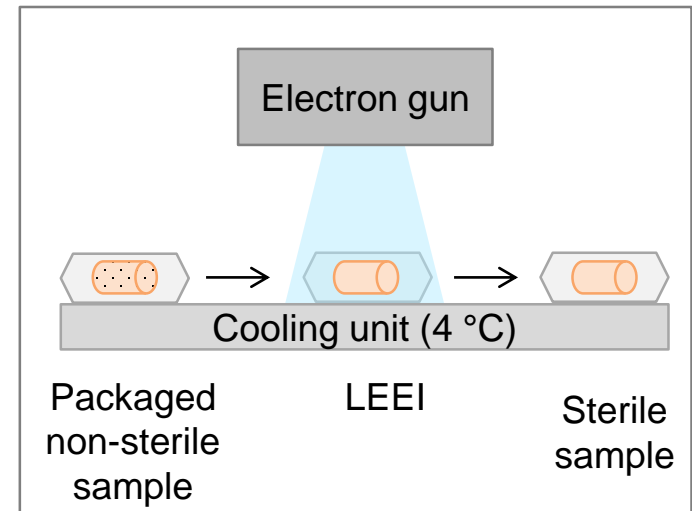
- Sample 1: penetration depth of max. 18 μm only irradiation of the Adventitia (outer layer)
- Sample 2: penetration depth ca. 110 μm irradiation of the complete vessel wall
- 2 doses:
 - ca. 21 kGy
 - ca. 42 kGy
 - untreated control (FEP)
 - physiological control (4 °C)
- Treatment from both sides

subsequently:

Assessment of the sterility and vessel function



Schematic diagram of the packaged aorta

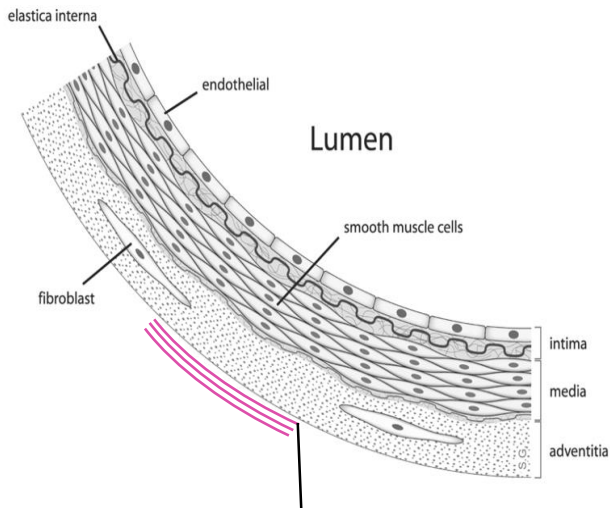


Schematic diagram of the LEEI

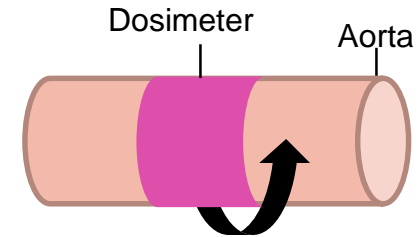
Sterilization concept of aortic vascular grafts

Parameter optimization, so that

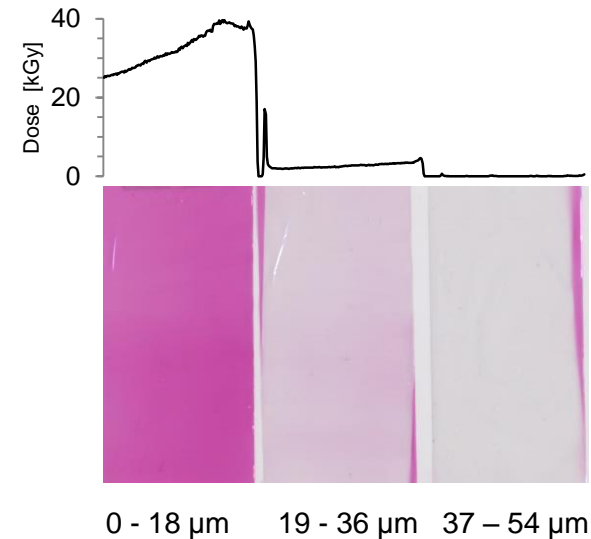
- › Homogenous irradiation
- › Surface dose 21 kGy
- › Penetration depth <math>< 18 \mu\text{m}</math>



3 layers dosimeter foil



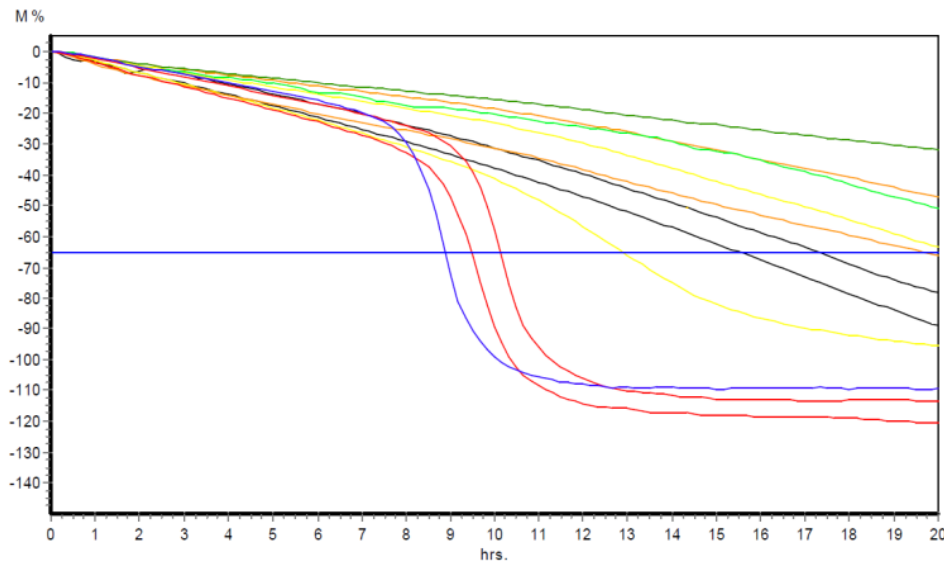
Calibration



S.A.I. Ghesquiere (2006), UPM Universitaire Pers Maastricht, ISBN 9789052785301

Sterility assessment - Methods

- Turbidity testing and impedance measurements (Bac Trac 4100, SY-LAB Geräte):
 - Metabolic activity and growth of bacteria → CO₂
 - lowering of the impedance



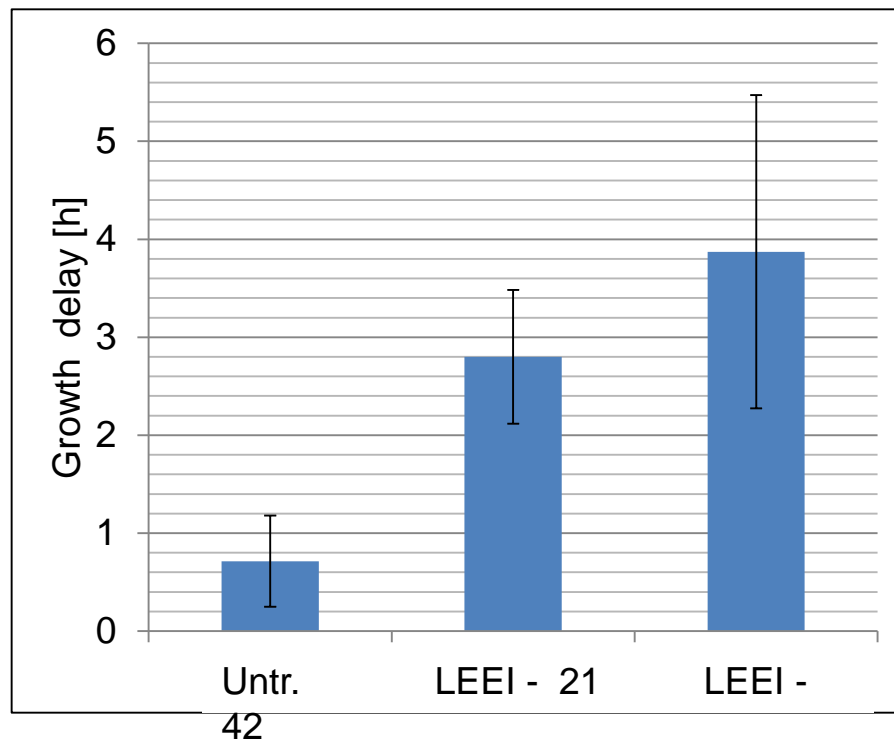
Measured curves of sterile and non-sterile samples



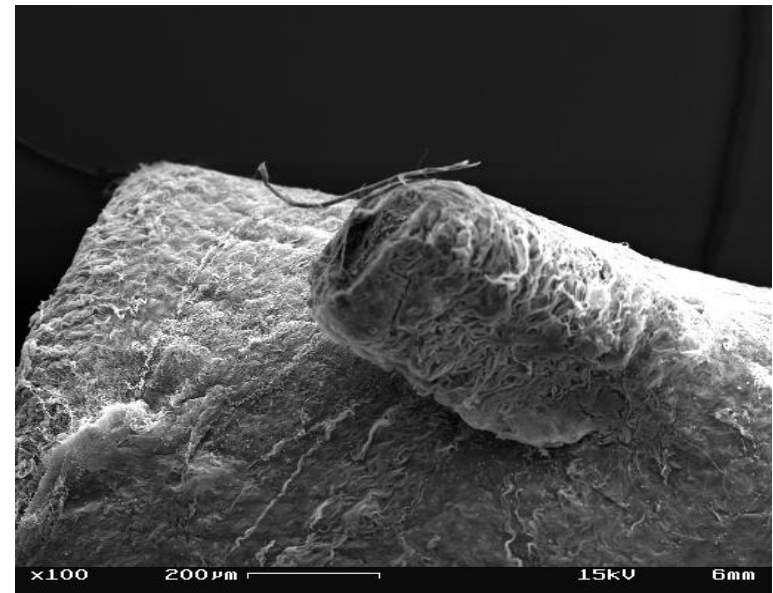
Sterile (left) and non-sterile (right) samples

Sterility assessment - Results

Sterility assessment: growth delay of non-sterile samples

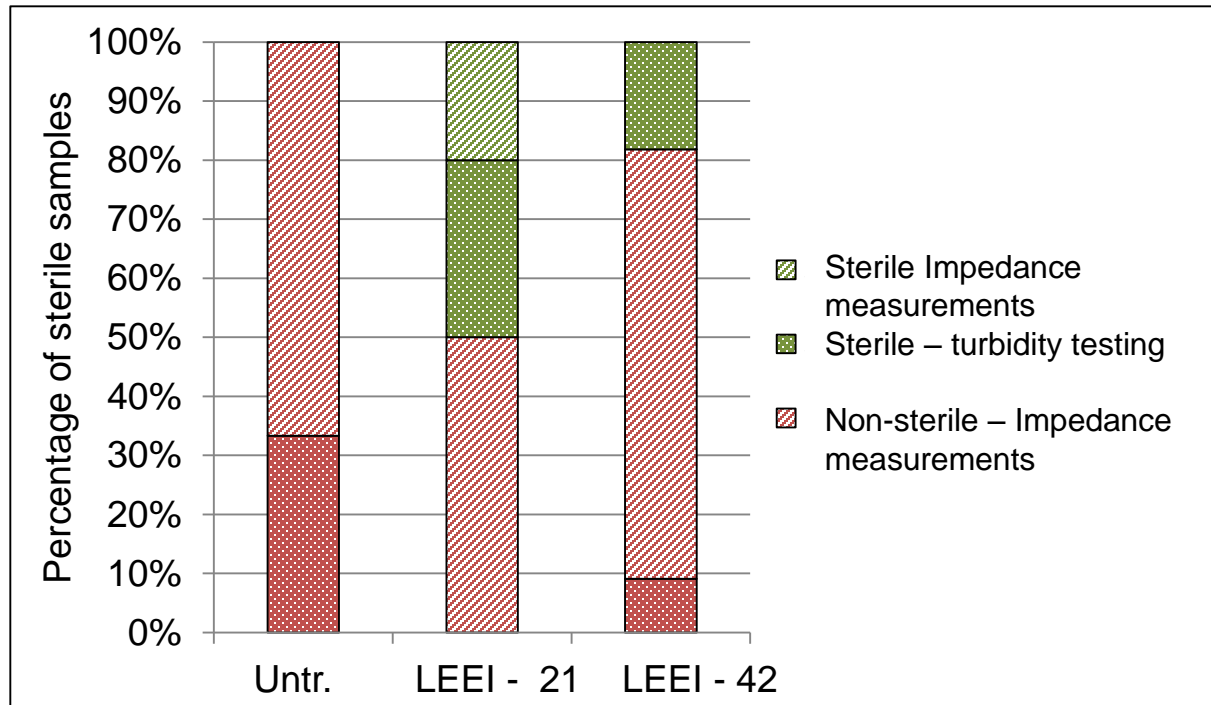


Challenge:



Sterility assessment - Results

Sterility assessment: Increase of sterile samples after LEEI



- Up to 50% of the samples sterile
- Sterilization only on the surface of the sample possible

Vascular function – Experimental approach

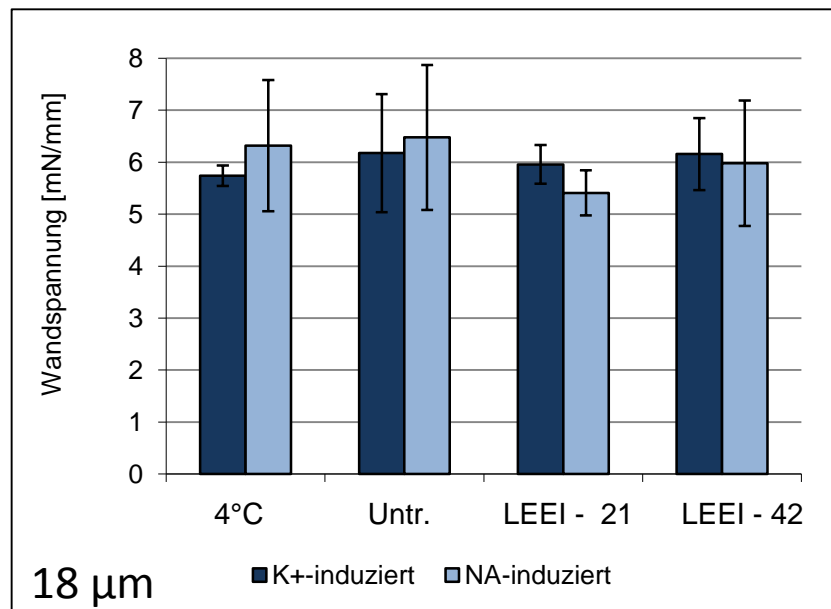
- Myograph 610 M (DMT) at 37 °C
- Fixation of the aorta on the grip which is coupled to a force measurement unit
- Measurement of the vessel function by stimulation of the cells with the following reagents:
 - contraction:
 - high potassium-ion concentration (K^+)
 - Noradrenaline (NA)
 - Relaxation:
 - Acetylcholine (ACh)
 - Natriumnitroprussid (SNP)



Myograph

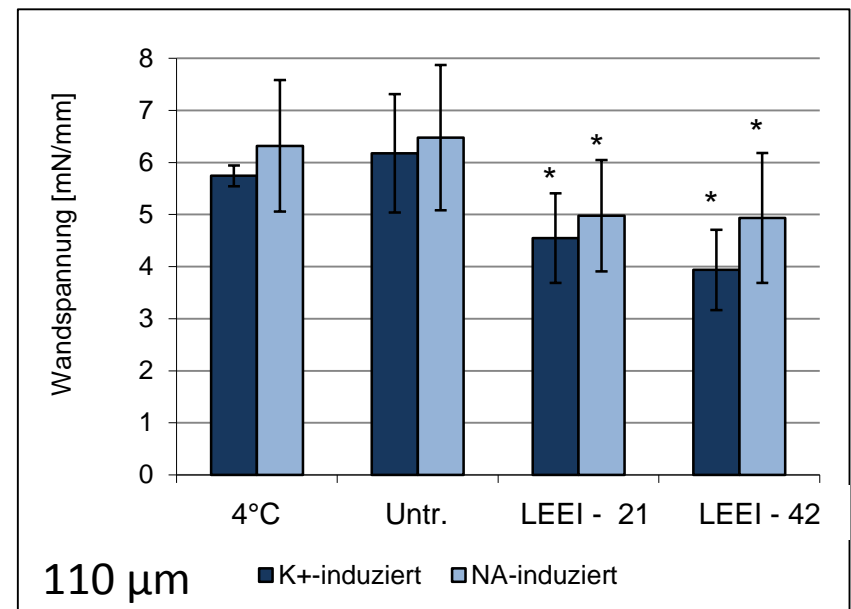
Vascular function - Results

Vascular function: Wall stress as a measure for vascular contraction



→ no changes

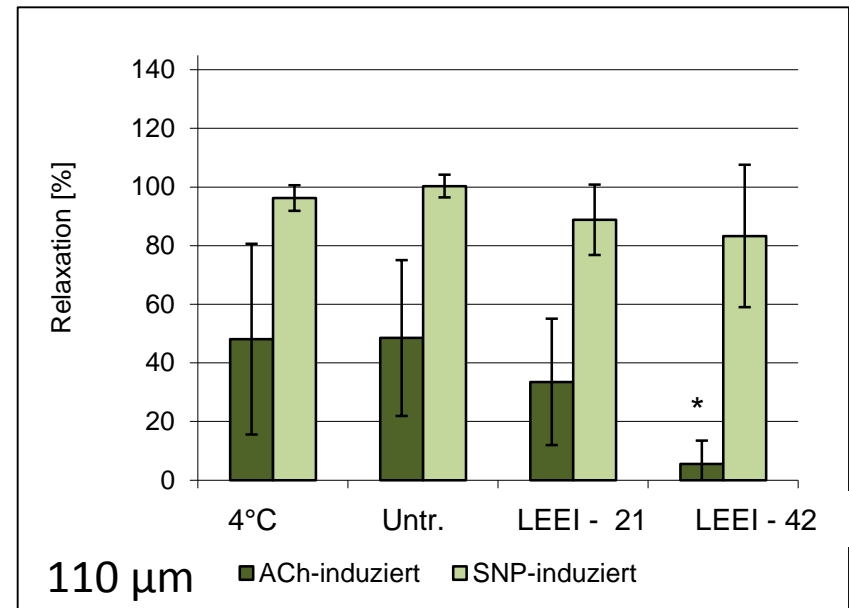
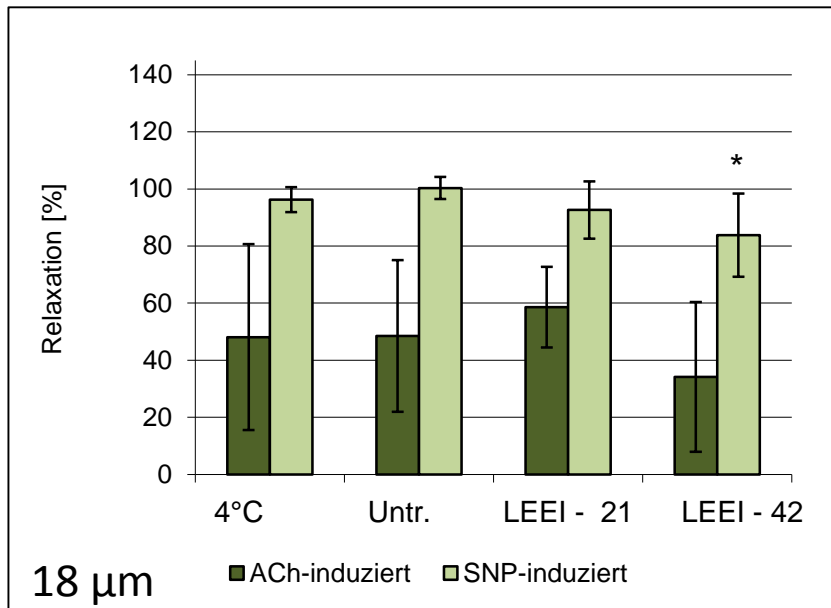
* P < 0,05 compared to untreated



→ decrease with increasing doses

Vascular function - Results

Vessel function: Relaxation



- SNP-induced: no changes
- ACh-induced: decrease after 42 kGy

- SNP-induced: no changes
- ACh-induced: decrease after 42 kGy

Vascular functionality after LEEI

Rat-Aorta

Penetration depth	18 μm		110 μm	
	21 kGy	42 kGy	21 kGy	42 kGy
Sterility	+	+	O	+
Wall stress	O	O	-	-
Endothelial independent relaxation	O	-	O	(-)
Endothelial dependent relaxation	O	(-)	(-)	-

+ positive impact
 O no impact
 - negative impact
 (-) not significant

➤ Sterilization of rat aortae by LEEI possible. However, the penetration depth needs to be adjusted to keep the endothelial layer unaffected

Part II: Sterilization of Pericardial Tissue

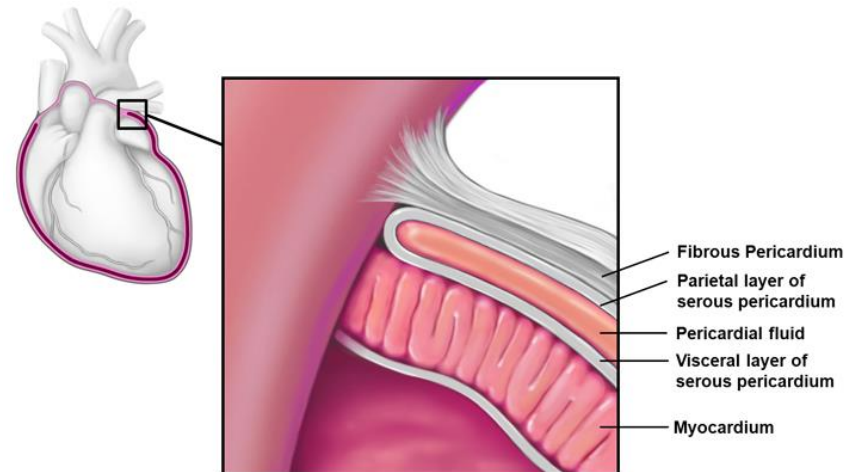
Pericardium-based tissue transplants

Clinical Applications

- › Cardiac surgery (Heart valve prosthesis, Cardiac reconstruction, closure of pericardial sac)
- › Vascular reconstruction
- › Abdominal wall and diaphragm defects
- › Orbital repair
- › Dural repair
- › Tendon and ligament augmentation



Herzzentrum Dresden



Phelan et al., *Pericardial Disease*, 2015:
<http://www.clevelandclinicmeded.com/medicalpubs/disease-management/cardiology/pericardial-disease/>

Heart Valve Replacements

~250 000 heart valve replacements annually worldwide

Mechanical heart valves

Patients < 65 years



Biological heart valves

Bioprosthetic heart valves

Patients > 65 years

Homografts

Patients 35 – 65 years

Crosslinking of collagen-rich tissues

- › Accelerated biodegradation or insufficient mechanical strength
- › Gold standard: pre-treatment with glutaraldehyde
 - › Major limitations
 - › Cytotoxicity
 - › Calcium deposition
- › Alternative treatments
 - › Crosslinkers - carbodiimides, genipin
 - › Radical scavengers - glucose, ascorbate, riboflavin
 - › **Low energy electron irradiation**

Tissue preparation



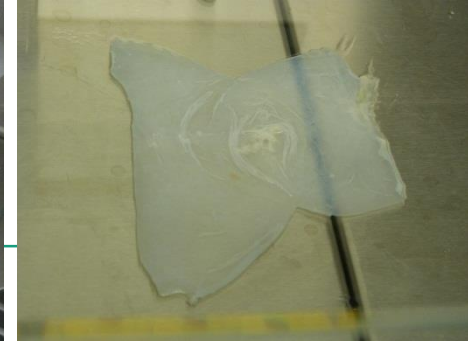
Native pericardium



removal of PI. pericardia



removal of cells



dissected pericardium

Samples:

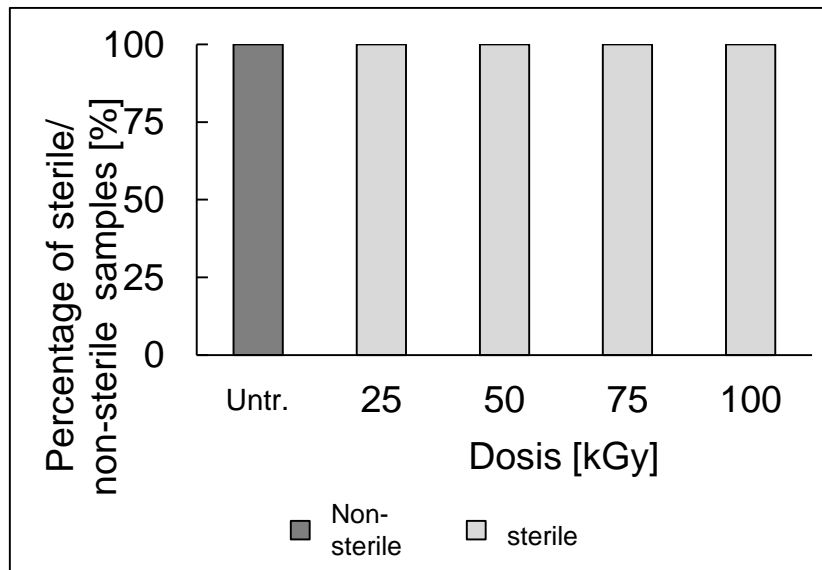
untr.	Untreated control
GA	Glutaraldehyde-crosslinking (0,2 % 30 min)
LEEI 10	Low-energy electron irradiation with a dose of 10 kGy
	⋮
LEEI 500	Low-energy electron irradiation with a dose of 500 kGy



Handling of the samples during irradiation

Sterility assessment - Results

- untreated samples non-sterile
- Reproducible sterilization above 25 kGy

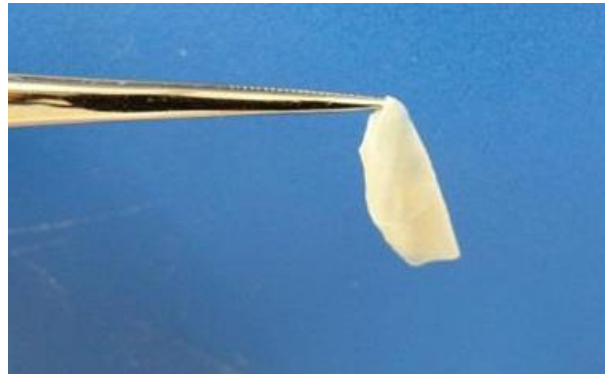


n = 5

Change of Appearance- Results



Untreated



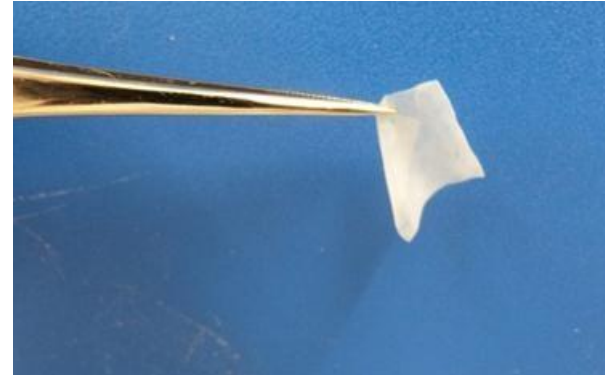
GA



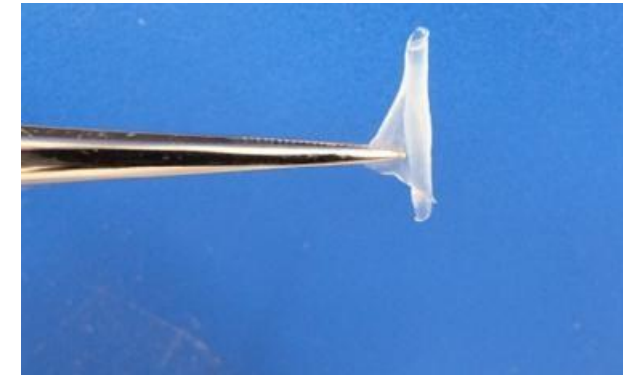
EB 100 kGy



EB 200 kGy



EB 250 kGy



EB 500 kGy

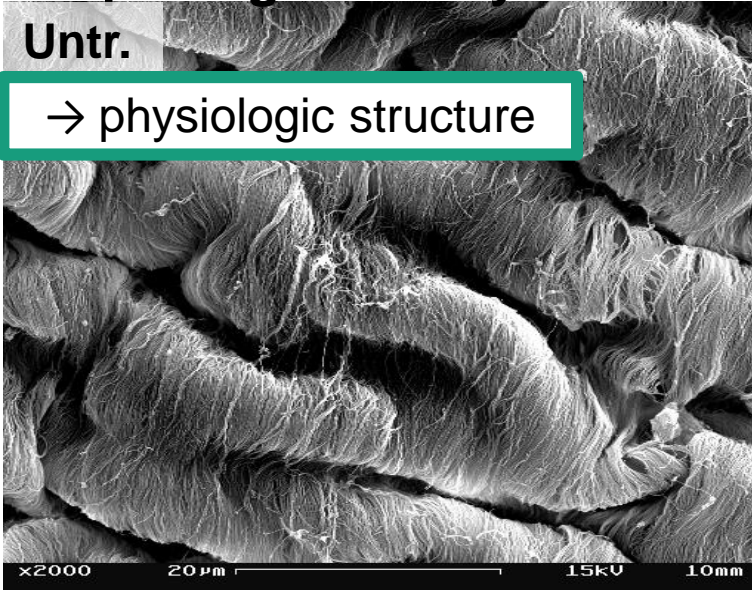
- Pericardium becomes stiffer with increasing dose (indication of cross-linking)
- Color change: more yellowish and more transparent with increasing dose

Morphological analysis - Results

2000x

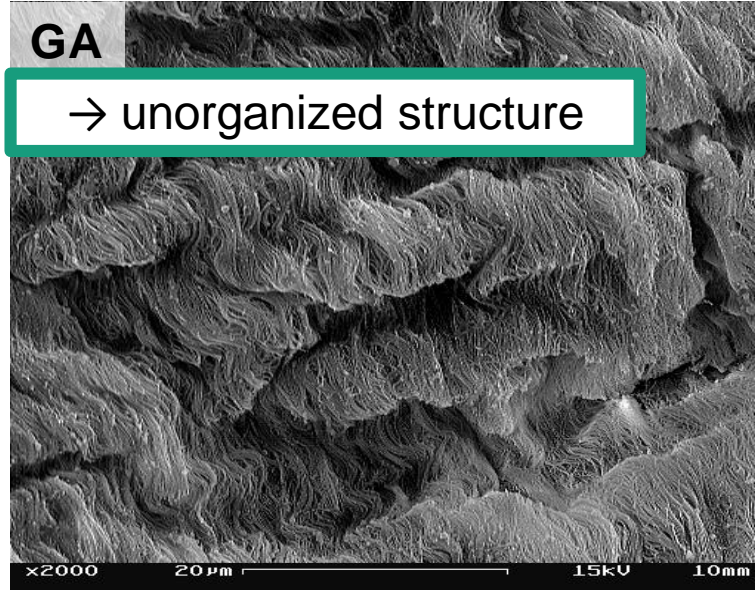
Untr.

→ physiologic structure



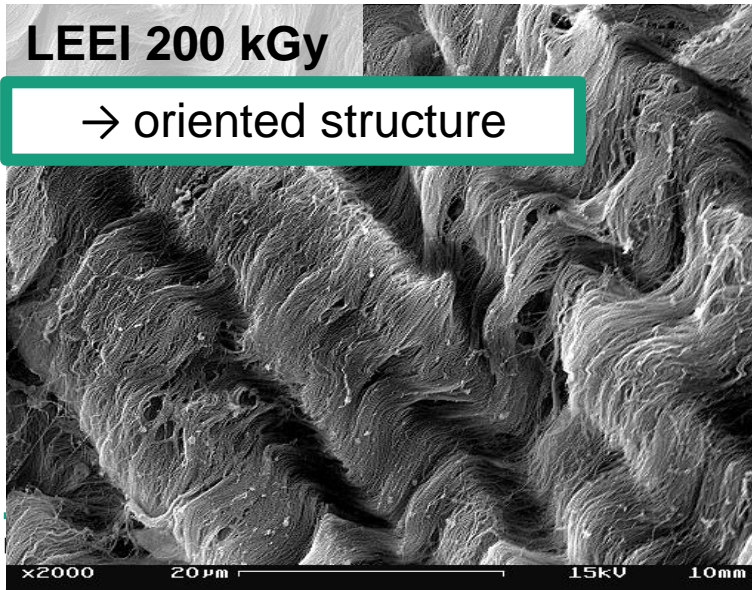
GA

→ unorganized structure



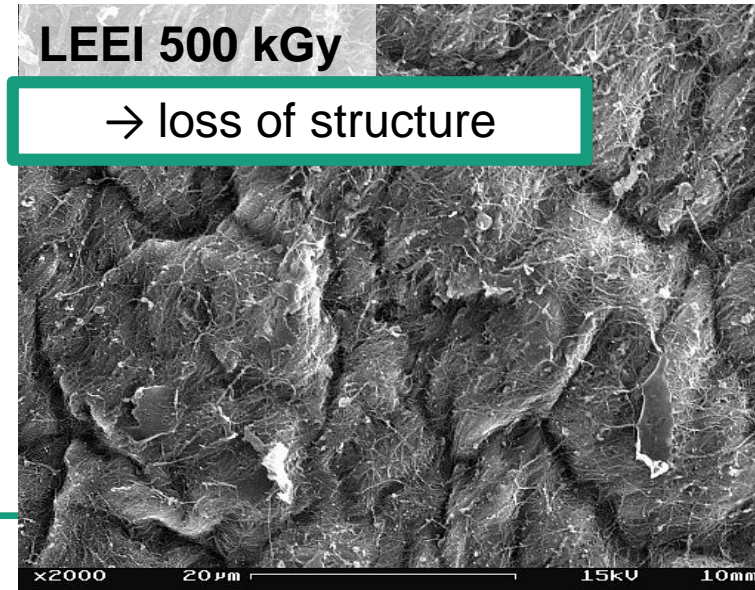
LEEI 200 kGy

→ oriented structure



LEEI 500 kGy

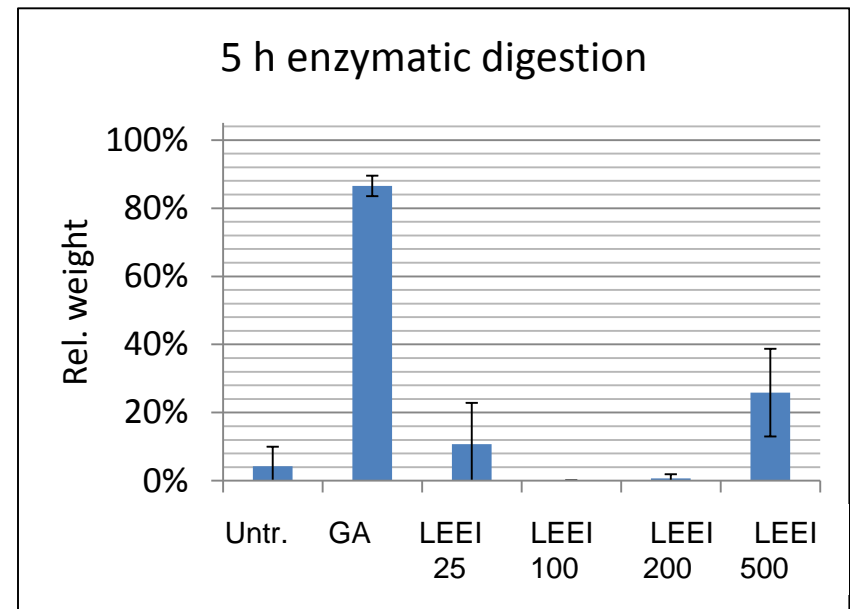
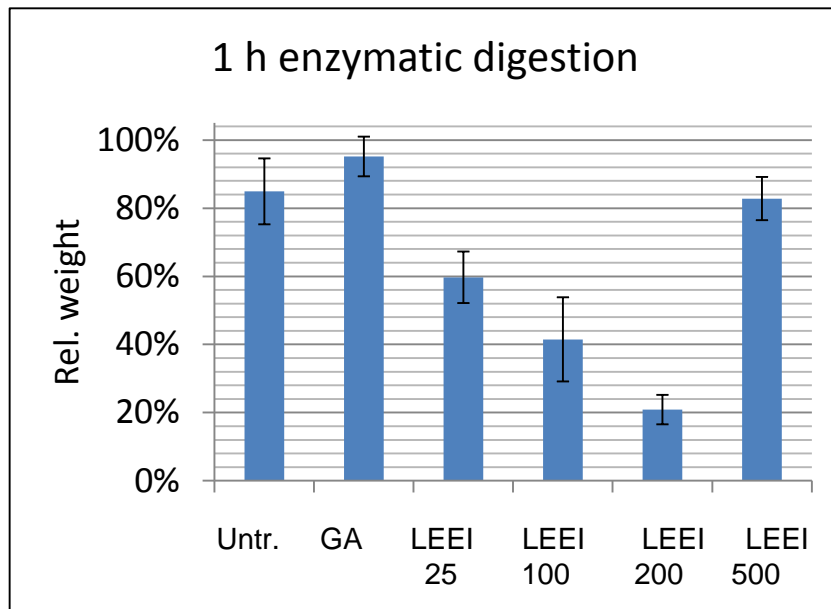
→ loss of structure



Experimental approach – *in vitro* biodegradability

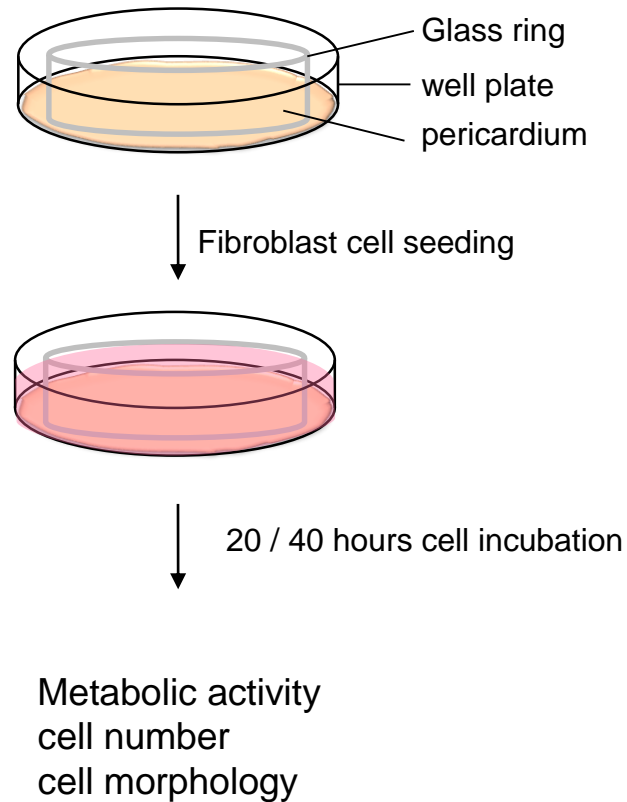
- One hour incubation in collagenase solution:
 - 0.6 U/ml collagenase in TRIS-Buffer, 37 °C, pH 7.4
- Analysis of the wet weight before and after incubation
- Calculation of the mass relative to the initial mass before digestion

In vitro biodegradability - Results



- EB 100 und EB 200 are rapidly digested, EB 500 is stable
- GA very stable, even after 5 h digestion
- An extra treatment should be added

Experimental Approach – Cytocompatibility Test

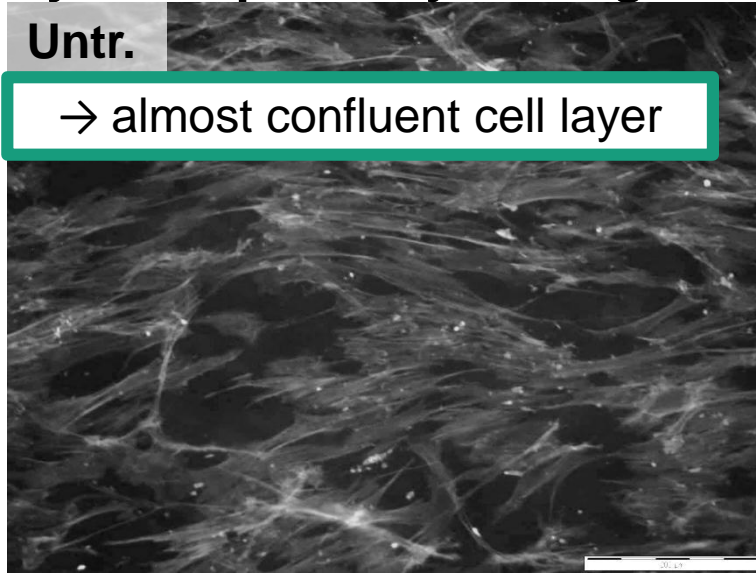


Cytocompatibility testing - results

100x

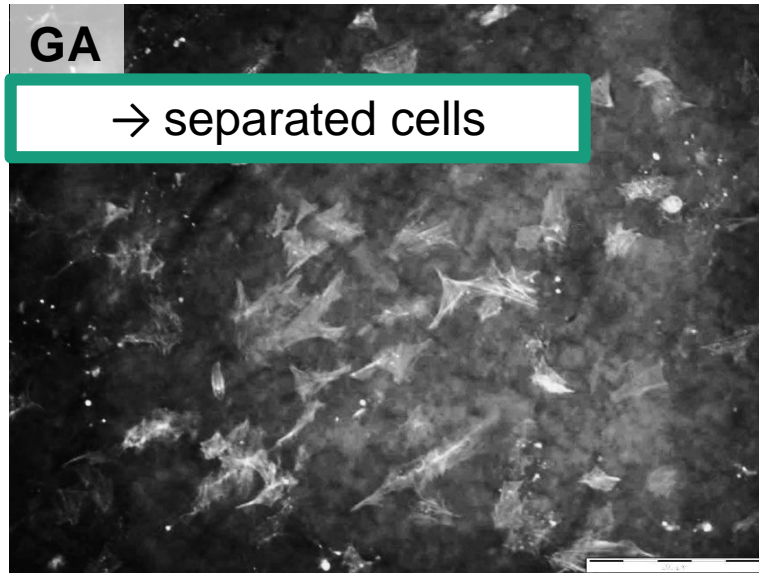
Untr.

→ almost confluent cell layer



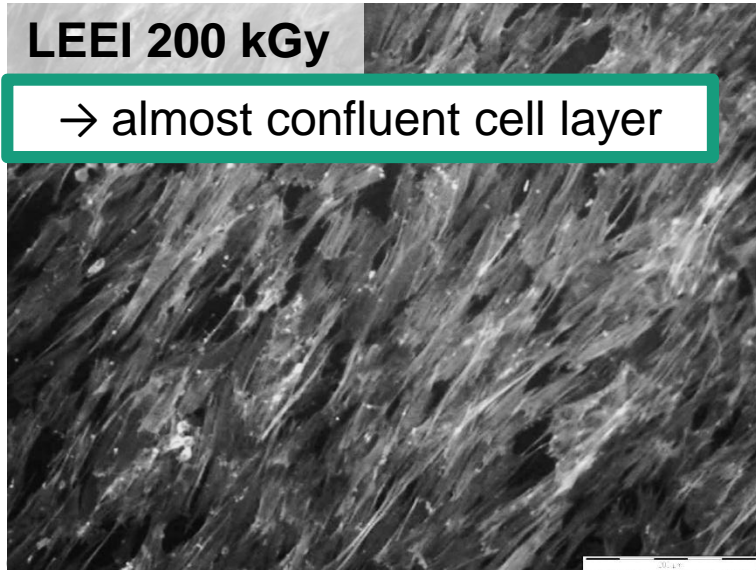
GA

→ separated cells



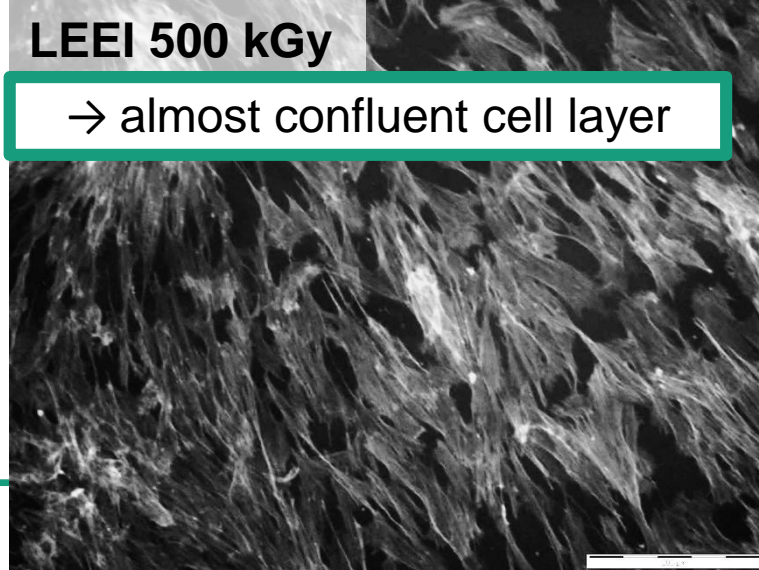
LEEI 200 kGy

→ almost confluent cell layer

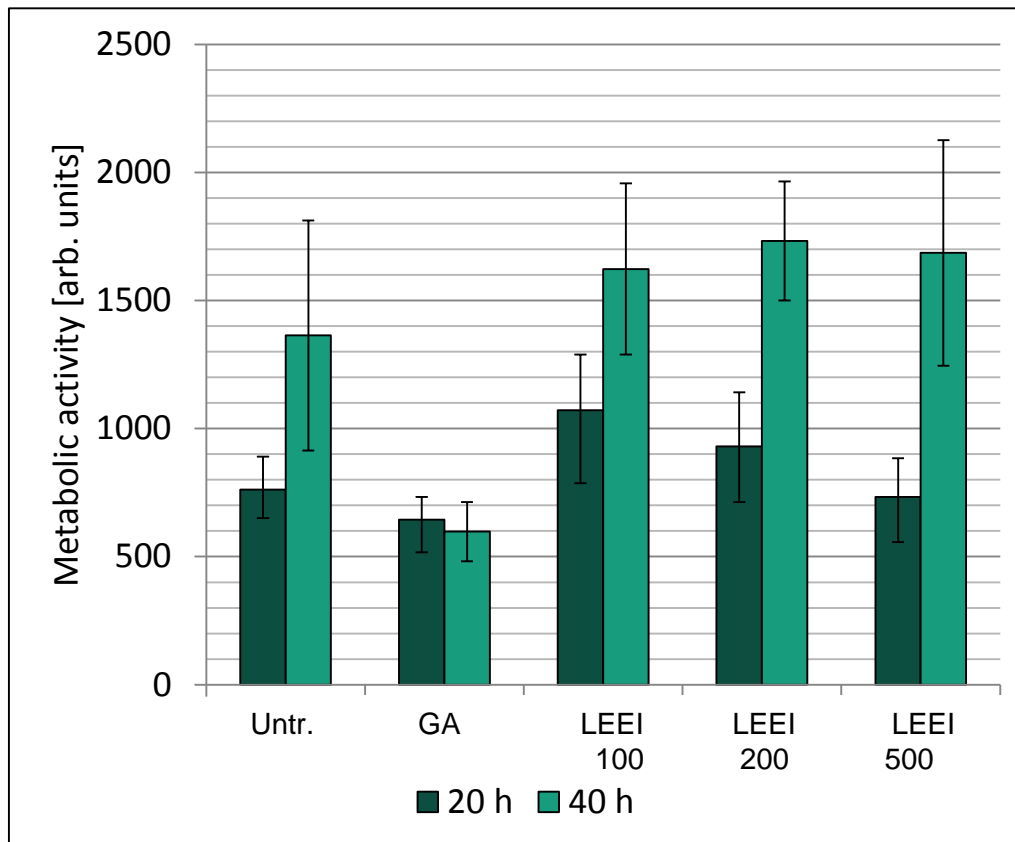


LEEI 500 kGy

→ almost confluent cell layer



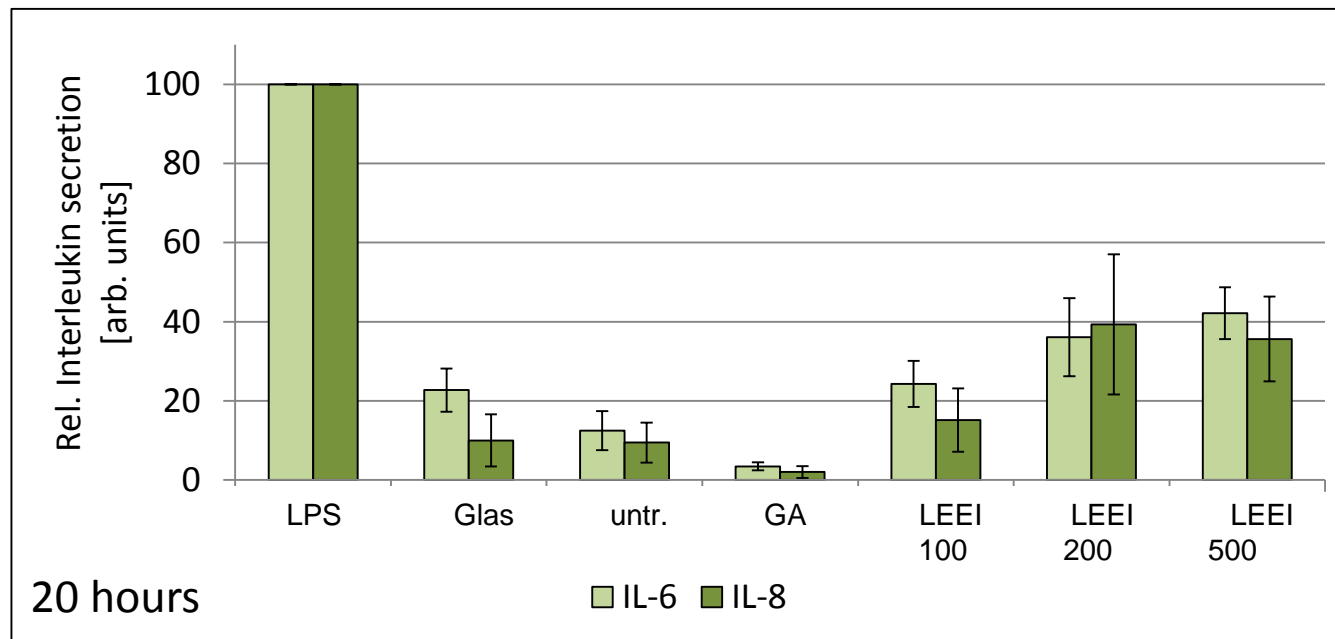
Cytocompatibility - Results



GA: no proliferation
untr + EB: proliferation

Immunogenicity- Results

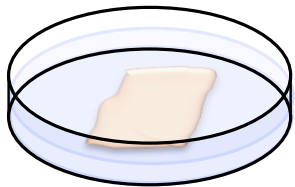
- Determination of the secreted pro-inflammatory Interleukins IL-6 und IL-8 in the cell culture supernatant



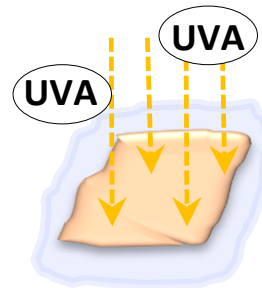
- Untr. > GA
- Untr. < EB

Novel SULEI procedure for sterilization and crosslinking of transplantable tissues

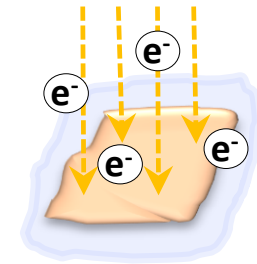
Example: pericardial tissue



Decellularization



UVA treatment,
pericardium soaked in
riboflavin



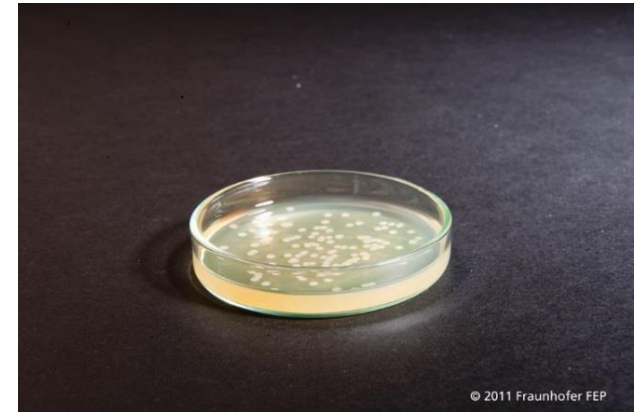
Modification and
sterilization by low energy
electron irradiation

IP Reference number: 10 2016 121 982.7

Bioburden determination sterility assessment

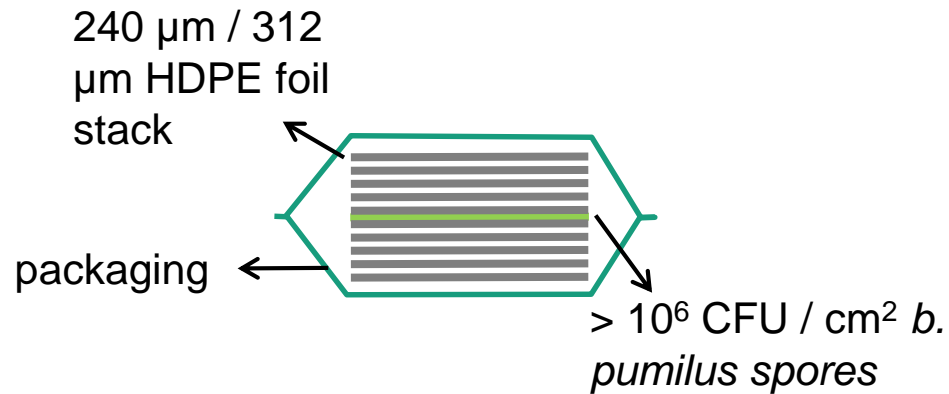
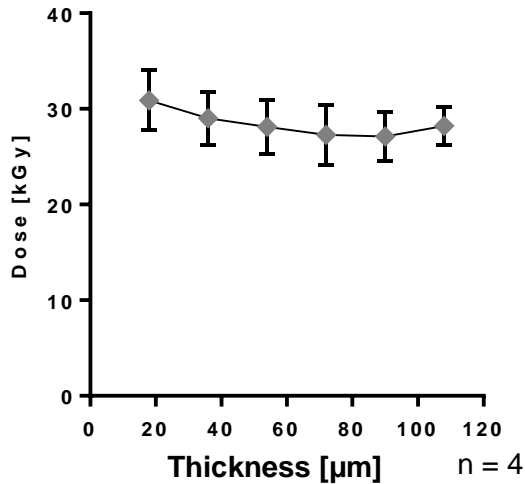
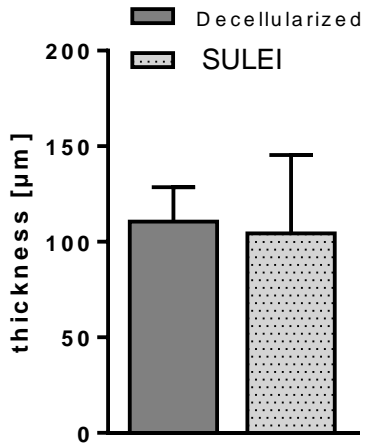
Bioburden: $5.1 \cdot 10^5 \pm 4.6 \cdot 10^5$ CFU/cm² (n=20)

Treatment	sterile	Non-sterile	n
Glutaraldehyde	28 %	72 %	29
Riboflavin / UV	0 %	100 %	30
SULEI	100 %	0 %	30



Walker et al., in preparation

Sterilization depth

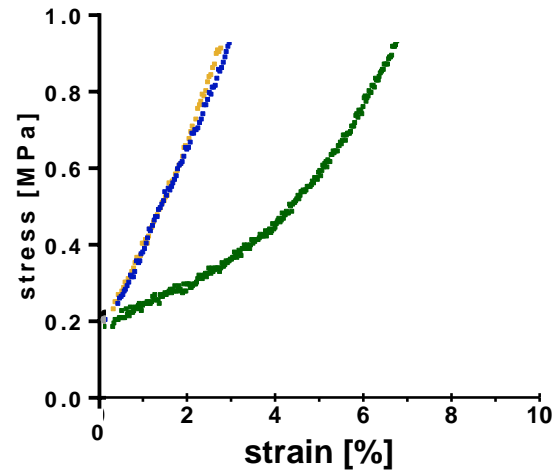
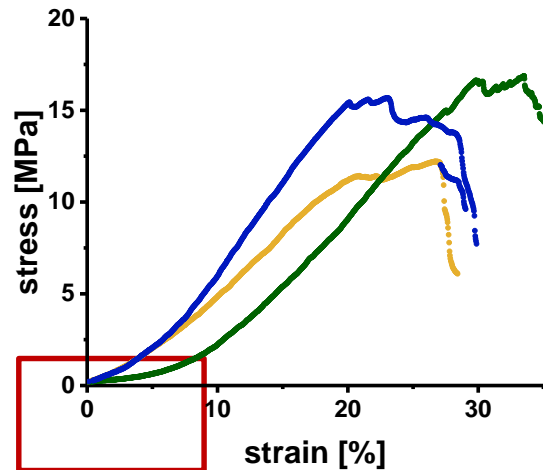


Thickness HDPE stack [μm]	Inoculum [CFU/cm ²]	CFU/cm ² after LEEI	Log-Reduction	Turbidity
240	$2.2 \cdot 10^6$	< 1	n.a.	-
312	$2.2 \cdot 10^6$	$5.7 \cdot 10^5 \pm 8.1 \cdot 10^5$	0.5	+

n = 3, n.a. – not applicable

Walker et al., in preparation

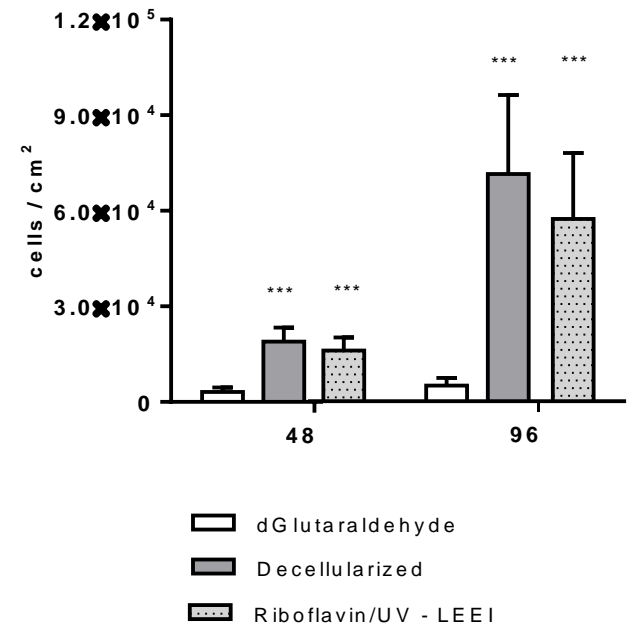
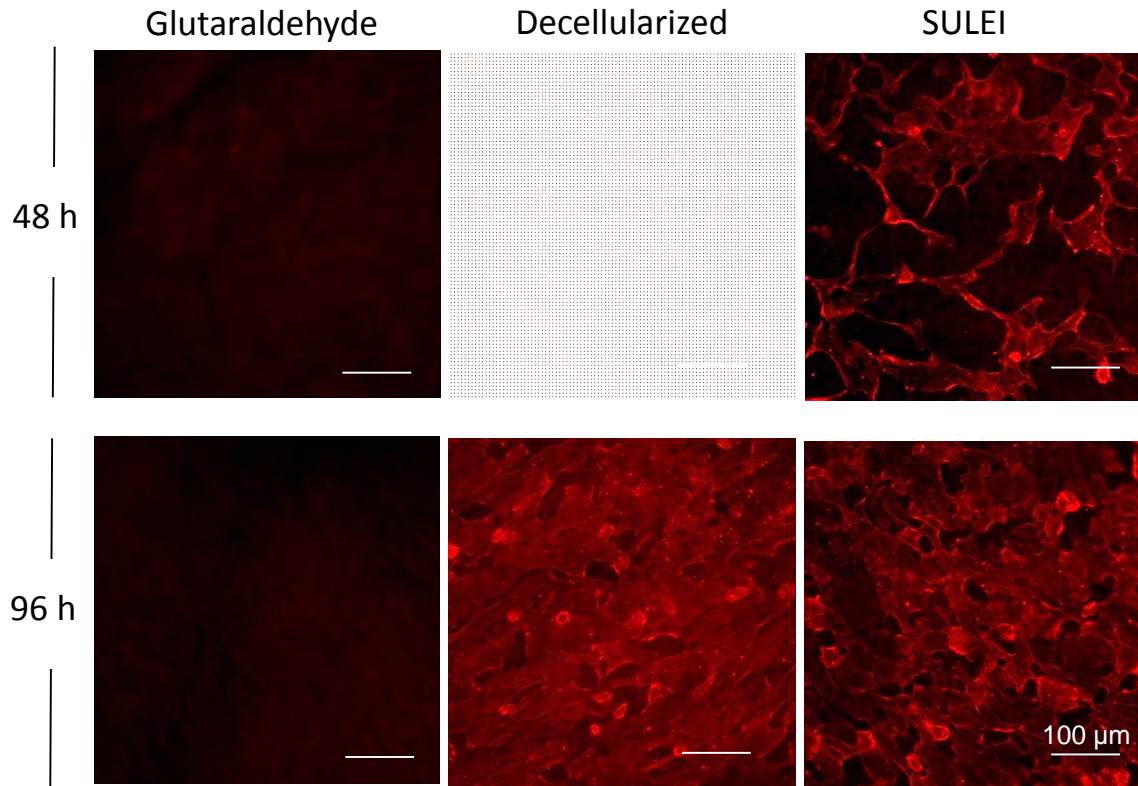
Biomechanical properties



- Glutaraldehyde
- Decellularized
- SULEI

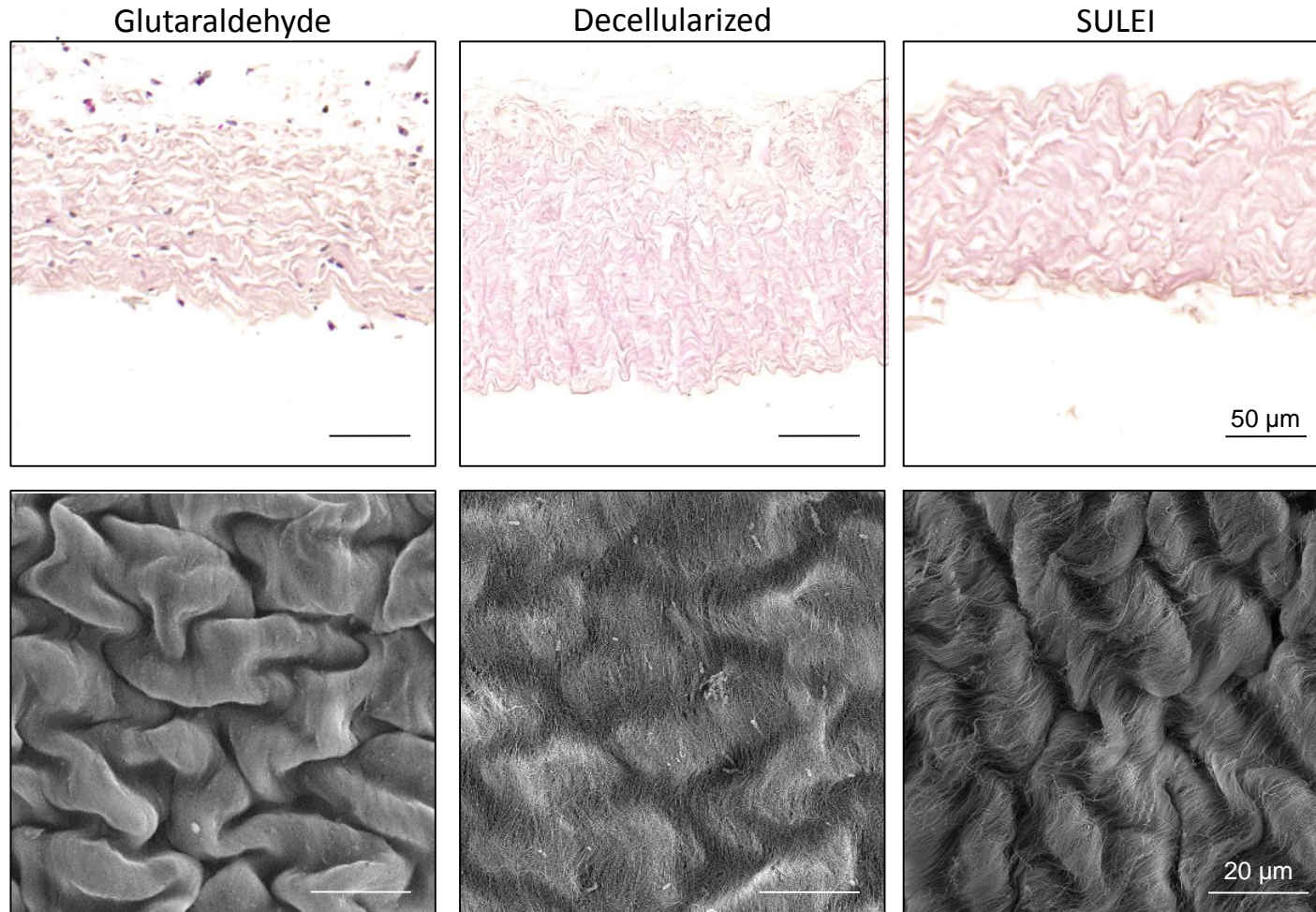
n = 8
Walker et al., in preparation

Cytocompatibility



Walker et al., in preparation

Morphology



Conclusions and outlook

Low-energy electron beam tissue treatment

→ targeted modification:

- Crosslinking predominates until 100 kGy:
 - Pericardium: increase bending stiffness, shrinkage and elasticity
 - Aorta: reduction of wall tension by crosslinking in the vessel wall
- Degradation predominates from 200 kGy:
 - Pericardium: tears at low tension in tensile test
- Sensitive control of the penetration depth

Conclusions and outlook

- Feasibility of sterilization of biological tissues using LEEI was shown using two models
 - Rat aorta as a model for vascular grafts
 - Porcine pericardium as a model for pericardium-based tissue transplants
- Major functional parameters of the tissue could be maintained
- Validation of the process in progress

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Pharmaceutical Technology, Universität Leipzig

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Dr. Michael Hacker

Institute of Anatomy, TU Dresden

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Department of Cardiac Surgery, TU Dresden

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Aline Jakob

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