

Department of Medical Technologies & Regenerative Medicine

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Cardiovascular Tissue Engineering

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Tübingen

Cardiovascular Tissue Engineering

- 1) Introduction
- 2) Polymers
- 3) Processing
- 4) Tissue-engineering approaches
- 5) In vivo performance

Introduction

Introduction

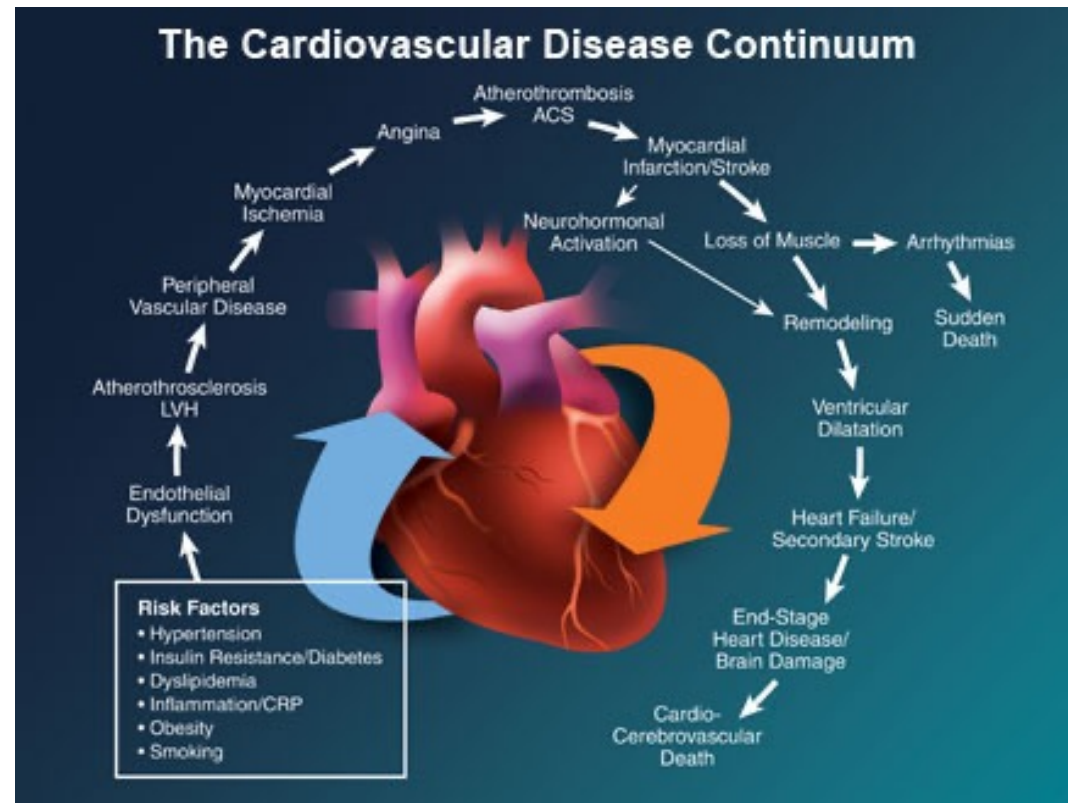
Cardiovascular diseases

Leading cause of death globally

Prevalence of cardiac and metabolic events ↑

Heart valve replacement and bypass surgeries ↑

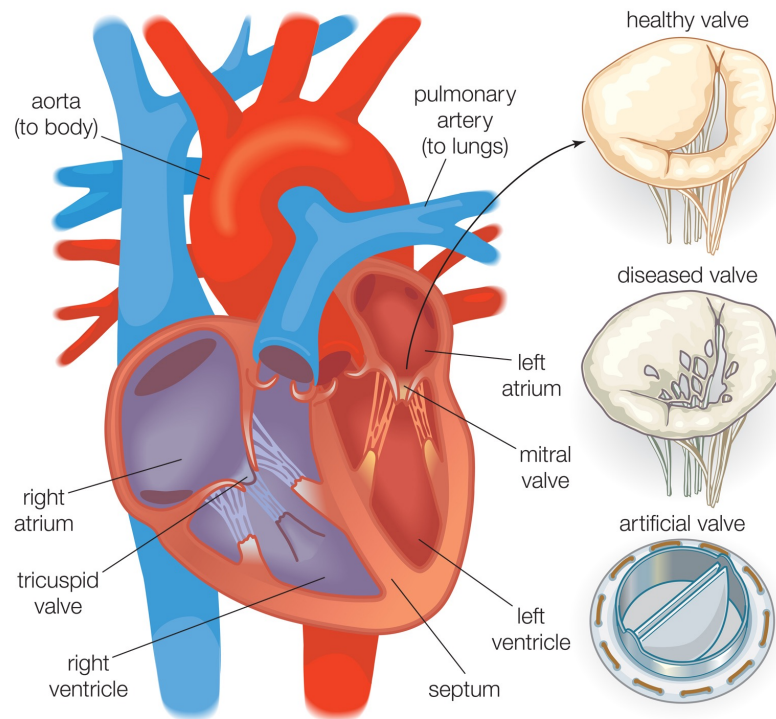
Patients suffer from repeated interventions & complications



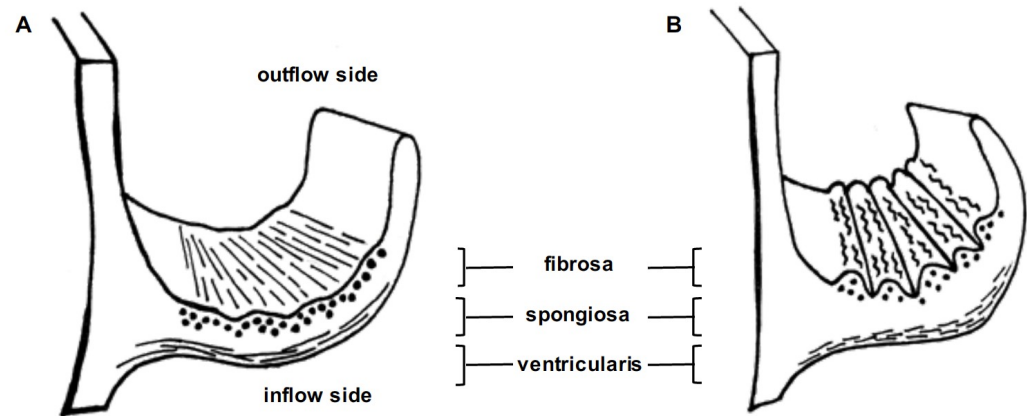
<https://healthlifemedia.com/healthy/what-is-cardiovascular-disease/>

Cardiovascular Implants

Heart valve replacement – anatomy



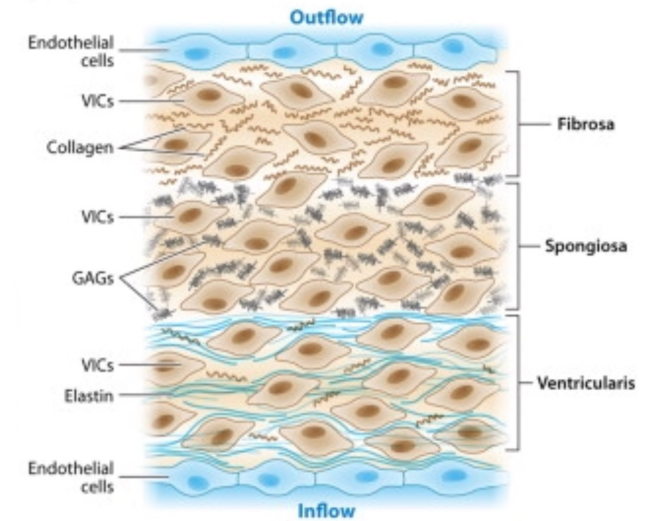
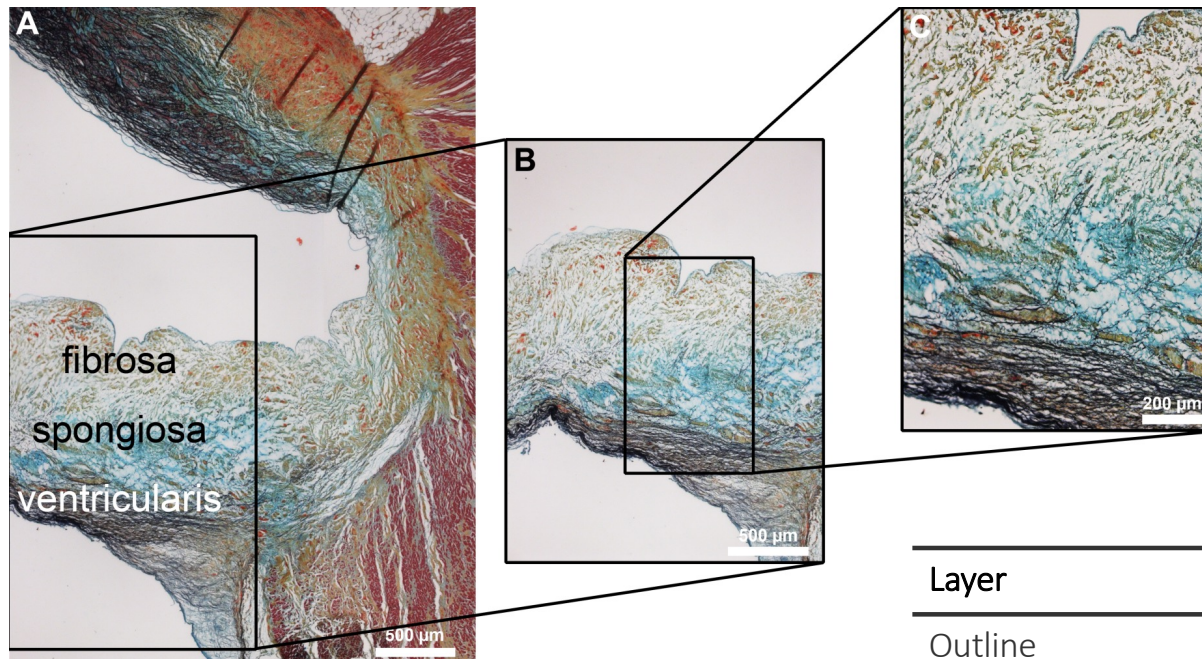
© 2010 Encyclopædia Britannica, Inc.



Layer	ECM components
Fibrosa (F)	circumferentially aligned collagen
Spongiosa (S)	collagen, proteoglycans
Ventricularis (V)	radially aligned elastin

Cardiovascular Implants

Heart valve replacement – anatomy



Schoen et al, 2001, Cardiovascular Pathology

Layer	Cell types
Outline	Valvular endothelial cells (VECs)
Inside	Valvular interstitial cells (VICs)

Cardiovascular Implants

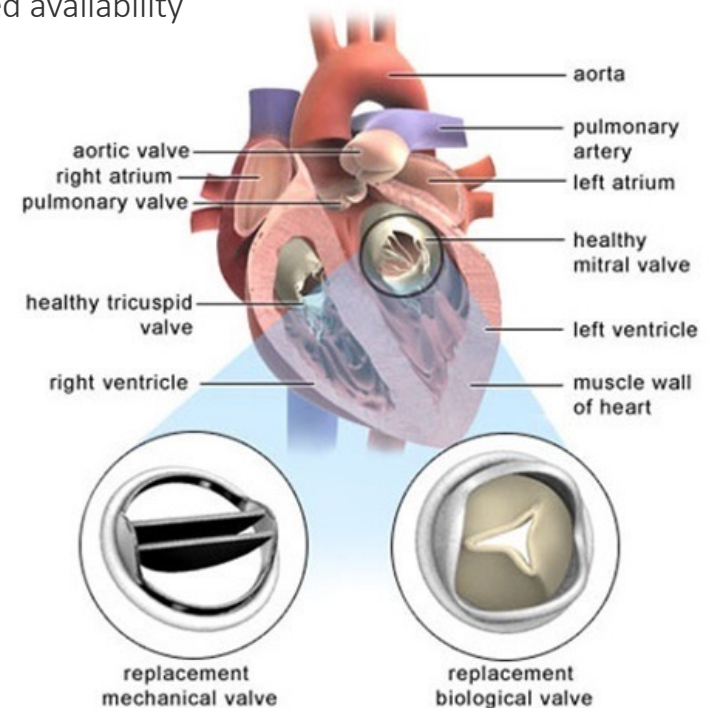
Heart valve replacement

Autologous donor valve (homograft) represents the ideal replacement - limited availability

>> mechanical or bioprosthetic valve replacements

First heart valve transplantation >50 years ago

	Mechanical valves	Bioprosthetic valves
Material	synthetic	Xenogenic or allogenic donor valves
Indication	< 70 years	> 65 years
Life-span	long	10-15 years
Risks	Thrombosis	Immune-reaction
Co-medication	Life-long anticoagulants	none

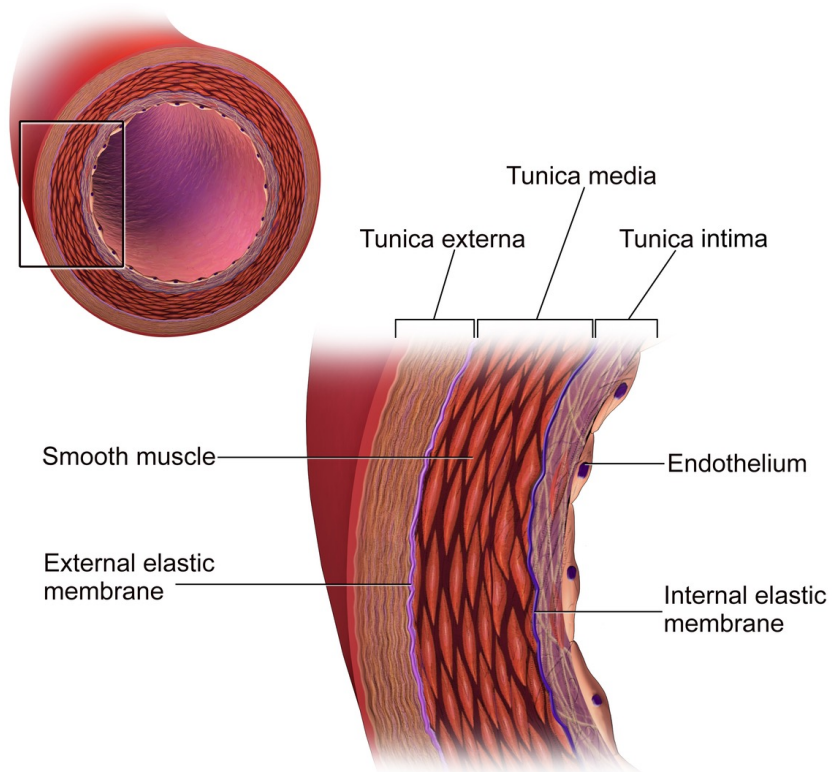


apexeyecareclinic.com

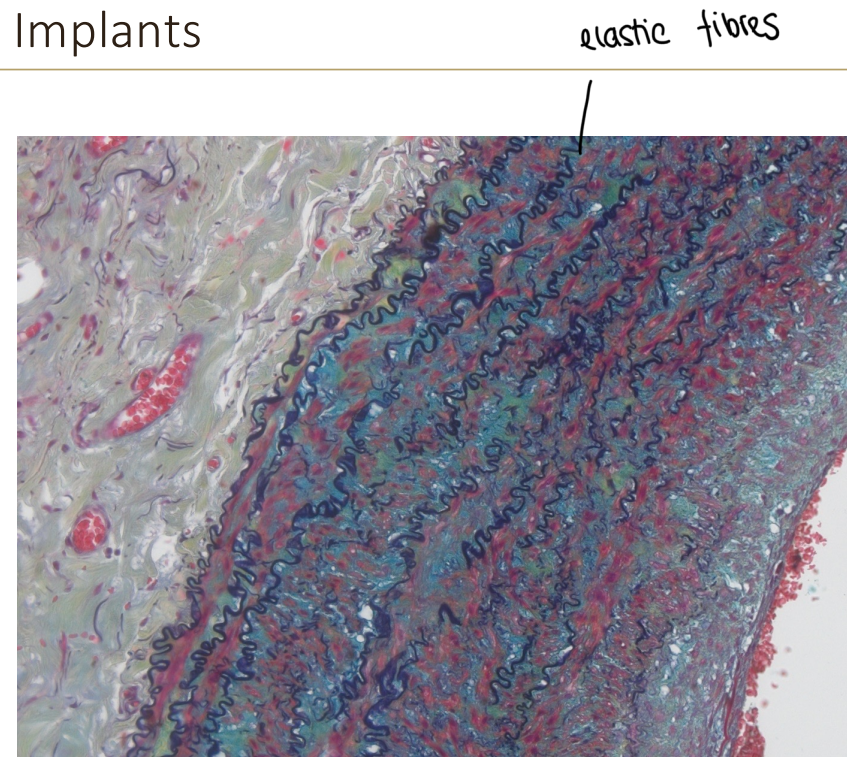
↳ usually decellularized

Cardiovascular Implants

Vascular grafts – anatomy



https://commons.wikimedia.org/wiki/File:Blausen_0055_ArteryWallStructure.png



Layer	Cell types
Intima	Endothelial cells
Tunica media	Smooth muscle cells

Cardiovascular Implants

Vascular grafts (VGs)

Indicated for vessel occlusion/narrowing, where ever pharmaceutical treatment or stent insertion are insufficient or for reconstitution of arteries or veins

>> autologous arteries or veins or synthetic conduits

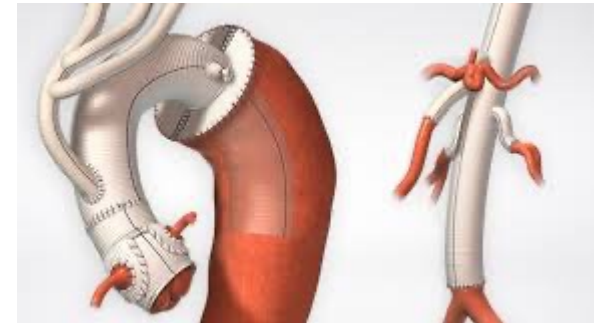
Coronary artery bypass graft (CABG)
saphenous vein
radial, mammary arteries

Dacron®, Teflon®

Synthetic materials show good mid-term results in large and medium diameter replacements

Long-term failure (infection, thrombosis), especially in small diameter VGs < 6mm

4 hold to keep it open

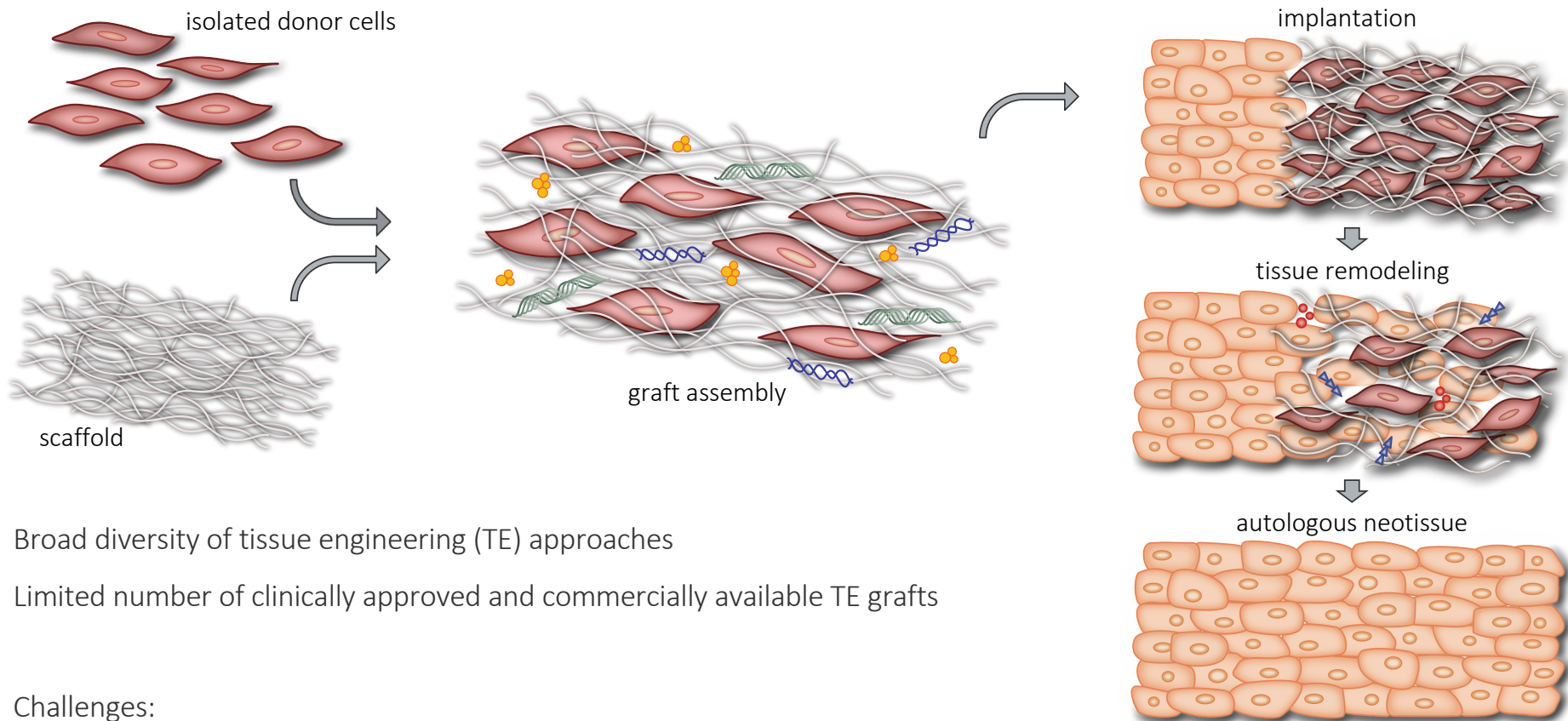


terumo-cvs.com



goremedical.com

Tissue engineering



Broad diversity of tissue engineering (TE) approaches

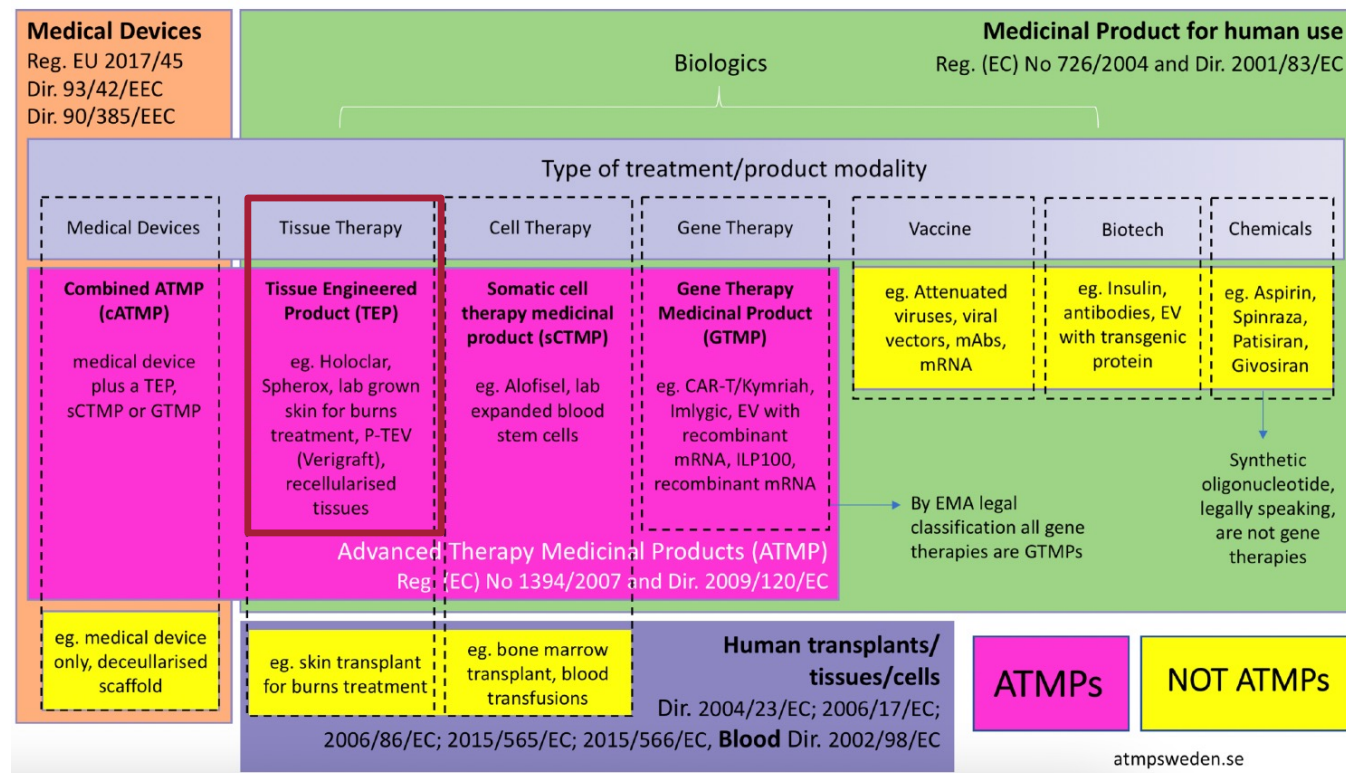
Limited number of clinically approved and commercially available TE grafts

Challenges:

>> better understanding of the underlying developmental & regulating mechanisms

Tissue engineering

Advanced therapeutic medicinal products (ATMPs) → classification



Tissue engineering

In vitro tissue engineering

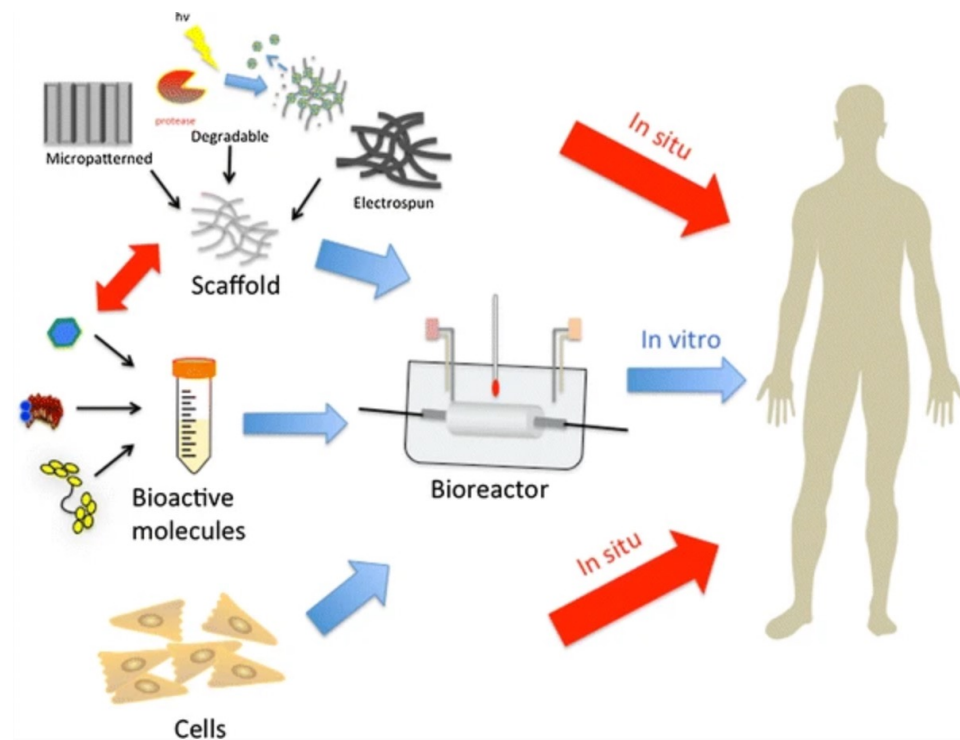
Classic approach: cells are seeded on a scaffold and cultured in vitro before implantation

Self-Assembly (TESA)

Scaffold-free assembly of cells as sheet or in molds

In situ tissue engineering

Cell-free constructs as implants, recellularization and remodeling occurs in vivo



Sengupta et al, Annals Biomed Engineering, 2014

Tissue engineering

In vitro vs in situ tissue engineering

	<i>In vitro</i> tissue engineering	<i>In situ</i> tissue engineering
Availability off-the-shelf	Possible	More likely
Scalability	Difficult	Easier
Ease of clinical translation	Complex	May be easier
Biomaterials	Extensively used	Extensively used
Bioreactors	Used	Not commonly used
Chemical factors	Used	Used
Cells	Used	Not commonly used
Cost-effectiveness	Less	More
Disease modeling	Yes	N/A
Drug screening modeling	Yes	N/A

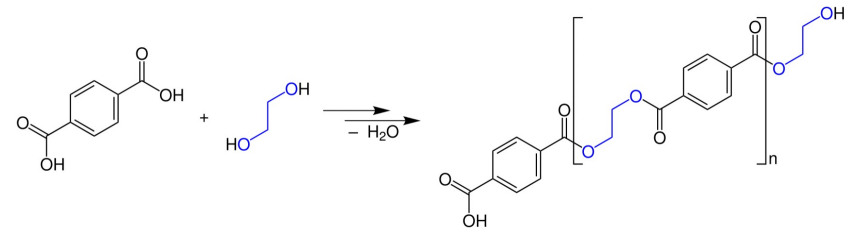
Sengupta et al, Annals Biomed Engineering, 2014

Polymers

Polymers

‘Poly’ = many + ‘meros’ = parts

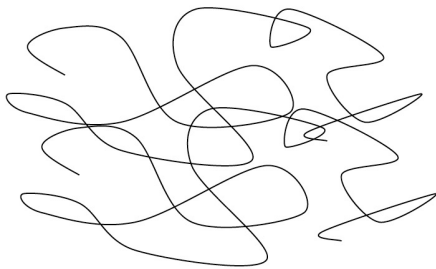
>> Macromolecule consisting of many small molecules (monomers)



Polymerization

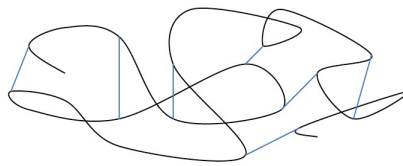
- 1) Chain growth: Radical polymerization, Cationic/Anionic polymerization
- 2) Step growth: Polycondensation, Polyaddition

Polymer structure



Thermoplasts

heatable & coolable
reshaping possible
linear or branched chains
though



Thermoset plastics

degrade before melting
no reshaping
crosslinked
high stiffness



Semi-crystalline

melting point
glass-transition (T_g)
opaque
well-ordered



Amorphous

glass-transition (T_g)
transparent
randomly oriented

Polymers

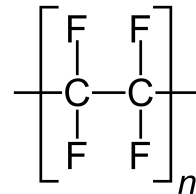
Non-degradable polymers for synthetic approaches

Expanded Polytetrafluoroethylene (ePTFE)

First synthetic polymer for vascular grafts (Gore, 1969)

Teflon®

chemically inert

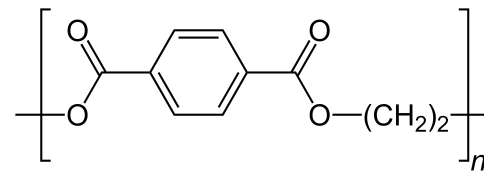


Bbraun.com

Polyethylene terephthalate (PET)

Dacron®

Superior biomechanical properties/flexibility



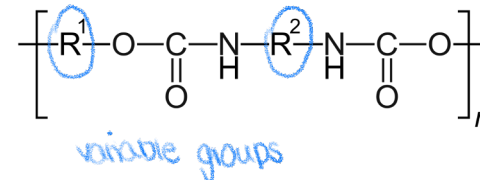
Lemaitre.com

Polyurethane

Hard (diisocyanate) and soft (diol) domain

Tunable porosity and biomechanical properties

Toxic degradation products



>> non-adhesive, generalized immune response (macrophages & T-cells), platelet aggregation

Polymers

Degradable polymers

Main mechanism: enzymatic or oxidative hydrolysis of ester groups (crosslink, side chain or backbone degradation)

Polyglycolic acid (PGA)

rapid degradation (60-90 days), influences biomechanical properties, poor tissue interaction

Poly-lactic acid (PLA)

degrades over several years, hydrophobic, stiffer than PGA, good endothelialization

Polycaprolactone (PCL)

long-term, hydrophobic, sustainable biomechanical properties

Polyglycerol sebacate (PGS)

fast degradation (within 30 days) *f.e. for self-removable sutures*

Poly (lactide-co-glycolide) (PLGA), Poly-L-lactic acid (PLLA)...

>> some are poorly adhesive, ineffective seeding

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7314574/>

Polymers

Biopolymers

>> naturally provide **binding sites** for cell adhesion, high biocompatibility, biodegradability

Collagen

most abundant, e.g. isolation from rat tail

Various types (fibrillar, intercellular)

Low mechanical properties & thrombogenicity for TEVG (crosslinking or co-polymerization w/ fibronectin, elastin..)

Fibrin *more stable*

Glycoprotein, cleavage from fibrinogen (blood plasma)

Source of growth factors, cytokines & chemokines

Silk

Silk fibroin (e.g. from silkworm)

Degradable, anti-thrombogenic

Chitosan

Linear polysaccharide (derived from shellfish)

Polymers

Biopolymers - Hydrogels

>> allow for substrates with simplified integration of cells, growth factors, nutrients etc. & application for bioprinting

Gelatin

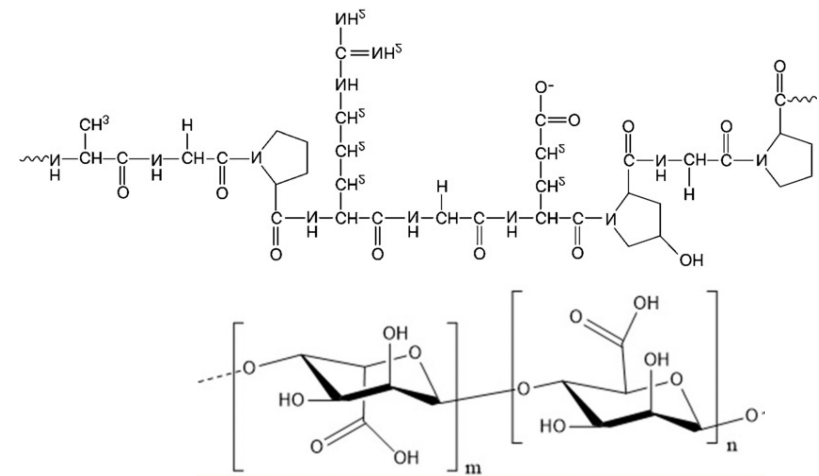
Biocompatible & biodegradable, fully absorbable
Lack of 3D structural integrity

Alginate

Biocompatible & biodegradable, non-thrombogenic
Low protein absorption

Nanocellulose

Linear polymer of glucose, derived from bacteria or woods
Biocompatible & good mechano-biological features
Water absorption & retention
Enhances cell adhesion and growth



<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7314574/>

Processing

Processing

Extrusion

→ heat & compress

Feed zone

Preheating of plastic
Constant screw depth

Compression zone

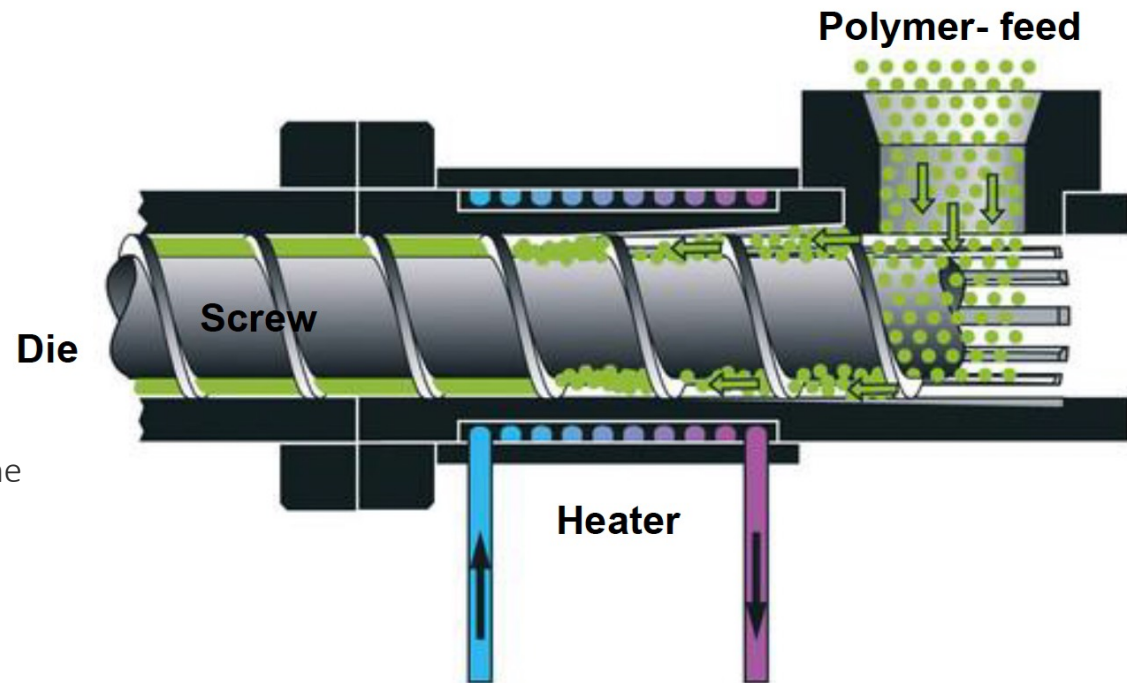
Screw depth constantly decreases
Compaction of the plastic
Air pockets are squeezed back to the feed zone

Metering zone

Constant but smaller screw depth
Melt homogenization
Constant material flow rate with uniform temperature & pressure
Shape of die defines the shape of the extrusion product

Injection moulding

Melt is injected into a mould (hydraulic system for opening and closing)



Processing

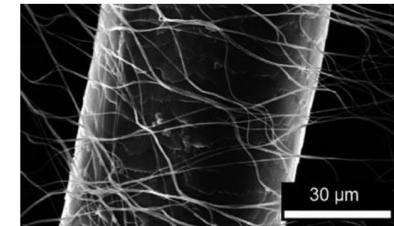
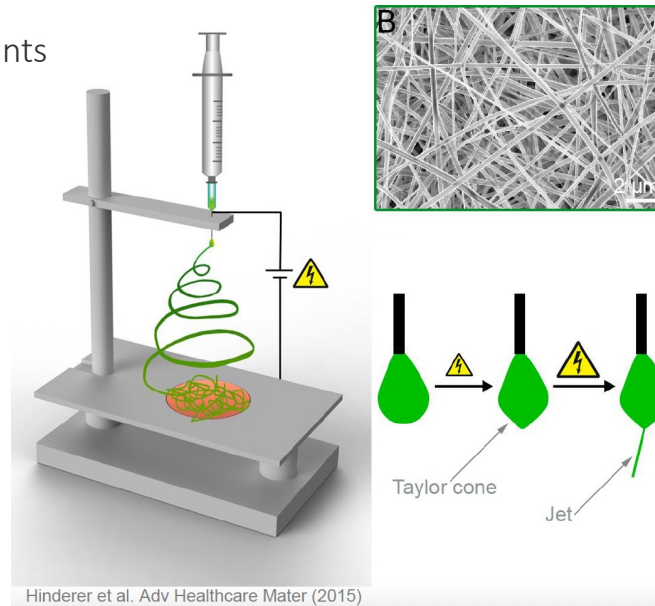
Electrospinning *for meshes*

Production of nanofibers of a polymer solution or melts via electric forces/ an electric field

- >> under high voltage a liquid droplet can get charged and stretched
- >> point of eruption: Taylor cone
- >> upon high molecular cohesion: charged liquid jet ($\neq$ electrospraying)

No need for high temperature / chemical coagulants

- >> suited for large molecules



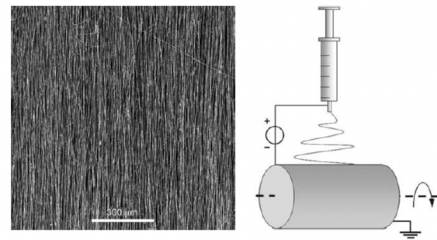
Processing

Electrospinning – Fiber morphology

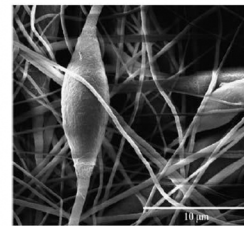
should usually be avoided

/

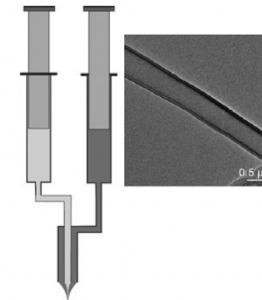
random vs. aligned



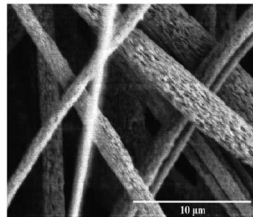
beads



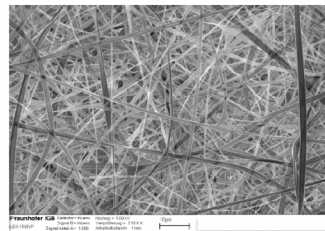
core-shell



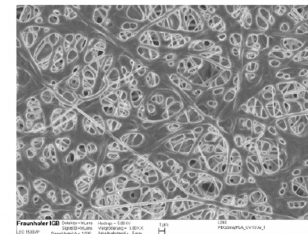
porous fibers



tape-like



crosslinked



>> influenced by: voltage, collector distance, solvent, humidity, polymer concentration, nozzle diameter, flow rate, device...

Processing

Additive manufacturing

Rapid and easy fabrication of complex structures & materials

'on-demand' & personalization

Principles

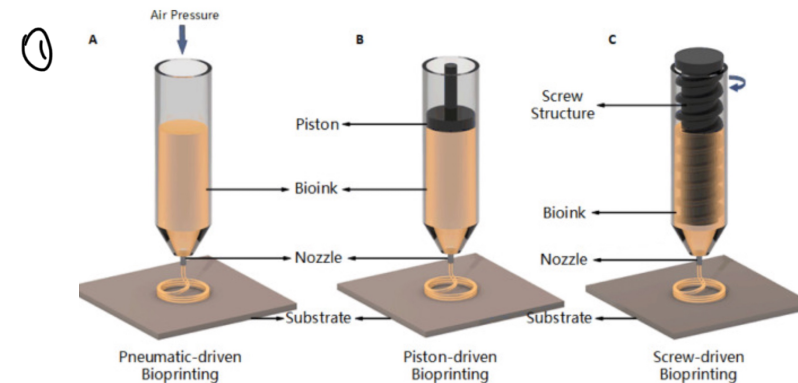
- ① Extrusion-based
- ② Droplet-based
- ③ Photocuring-based

Bioprinting

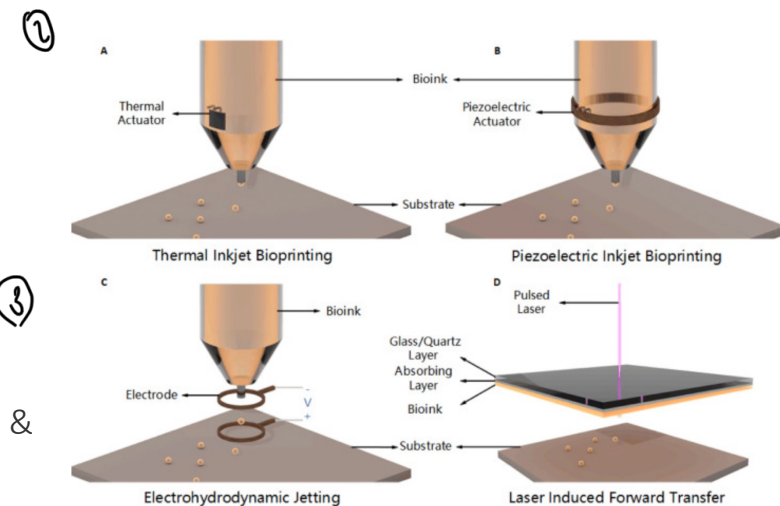
>> 3D printing with bioinks

Bioink: Cells (+ biopolymer substrate)

Requires combination of computational modelling, material science & biology



single droplets:



Photocuring

Processing

Others

>> Knitted/Woven structures

>> Foaming

>> Gelation

>> Phase inversion

Processing

Functionalization

Adhesion enhancers

growth factors e.g. VEGF, EGF, FGF

fibronectin, collagen, fibrin, gelatin

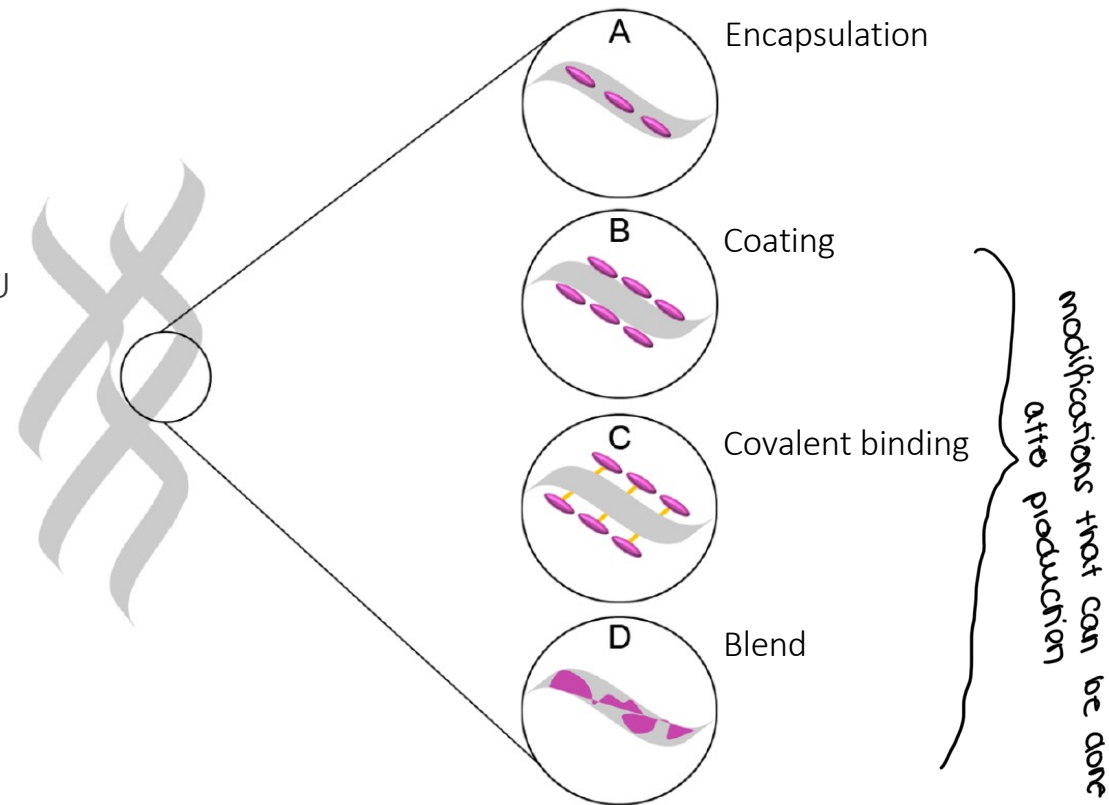
peptides

increasing porosity e.g. by second polymer coating by PU

Anti-thrombogenic coating

Heparin

Polarization (hydrophilic, hydrophobic)



Hinderer et al. AdvHealthcareMater, 2015

Tissue engineering approaches

TE Approaches

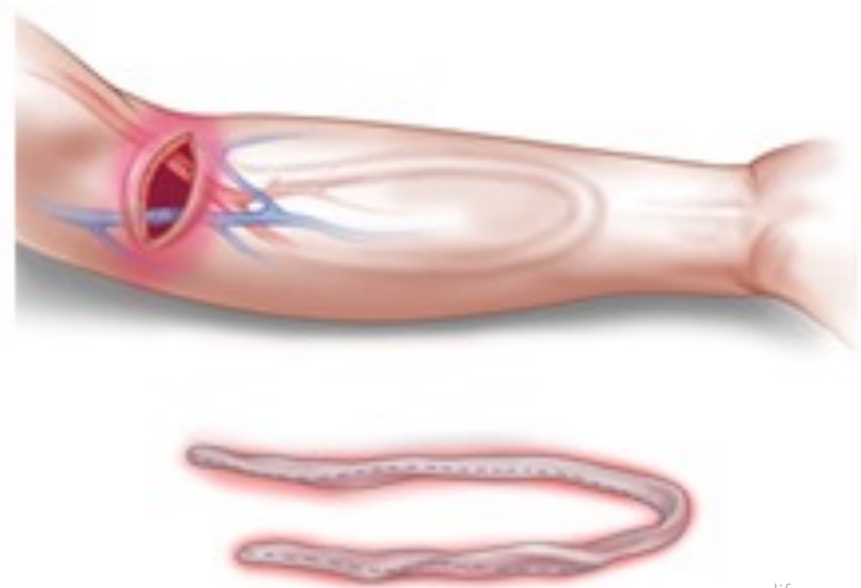
Vascular grafts

Decellularized xeno or allografts

CryoVein/Artery[®], blood vessel allografts

Artegraft[®], bovine carotid

ProCol[®], bovine mesenteric vein



cryolife.com

Downsides



TE Approaches

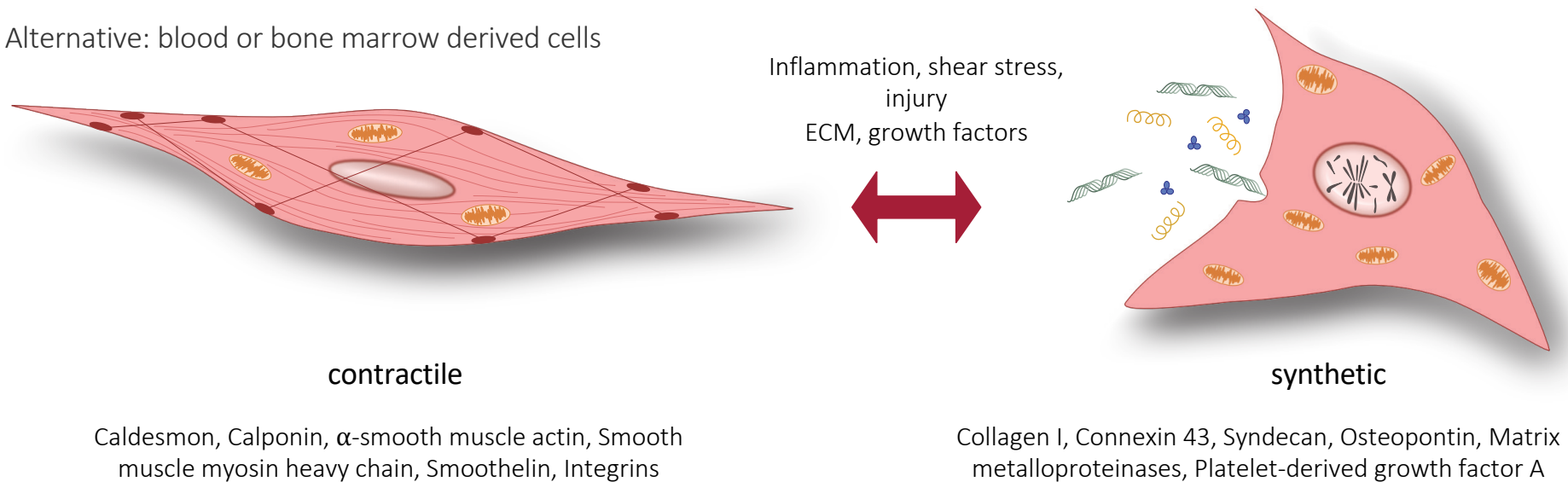
Vascular grafts

Smooth muscle cells *main cell type of vessels*

Originate from distinct vascular and non-vascular anatomical regions

Contractile or synthetic phenotype

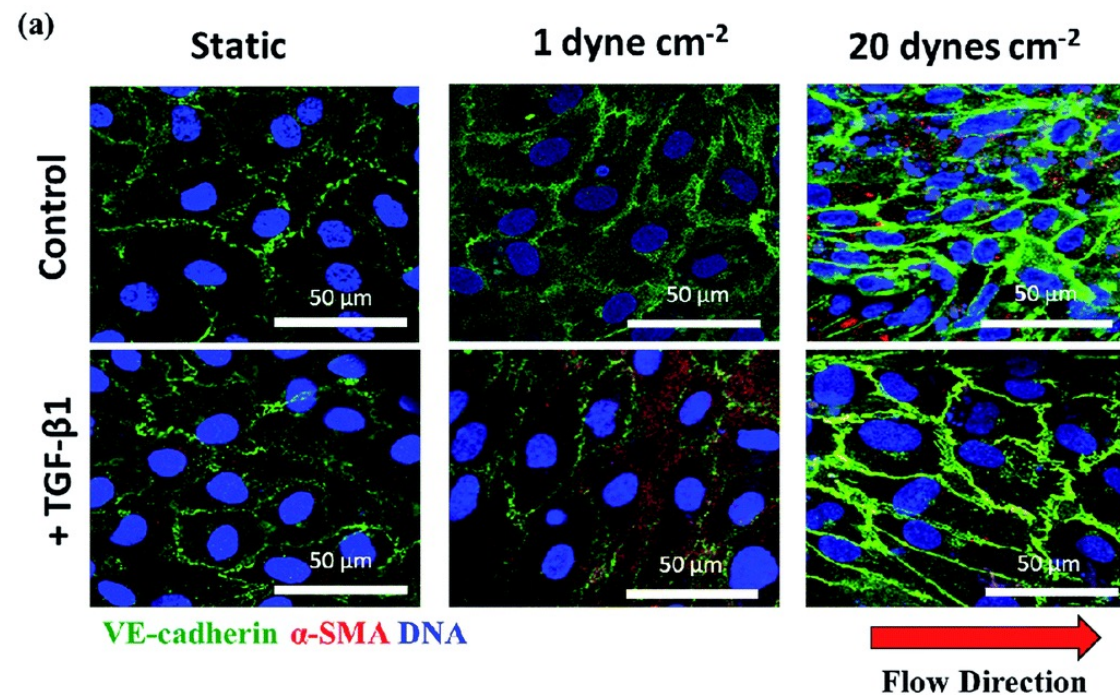
Alternative: blood or bone marrow derived cells



TE Approaches

Vascular grafts

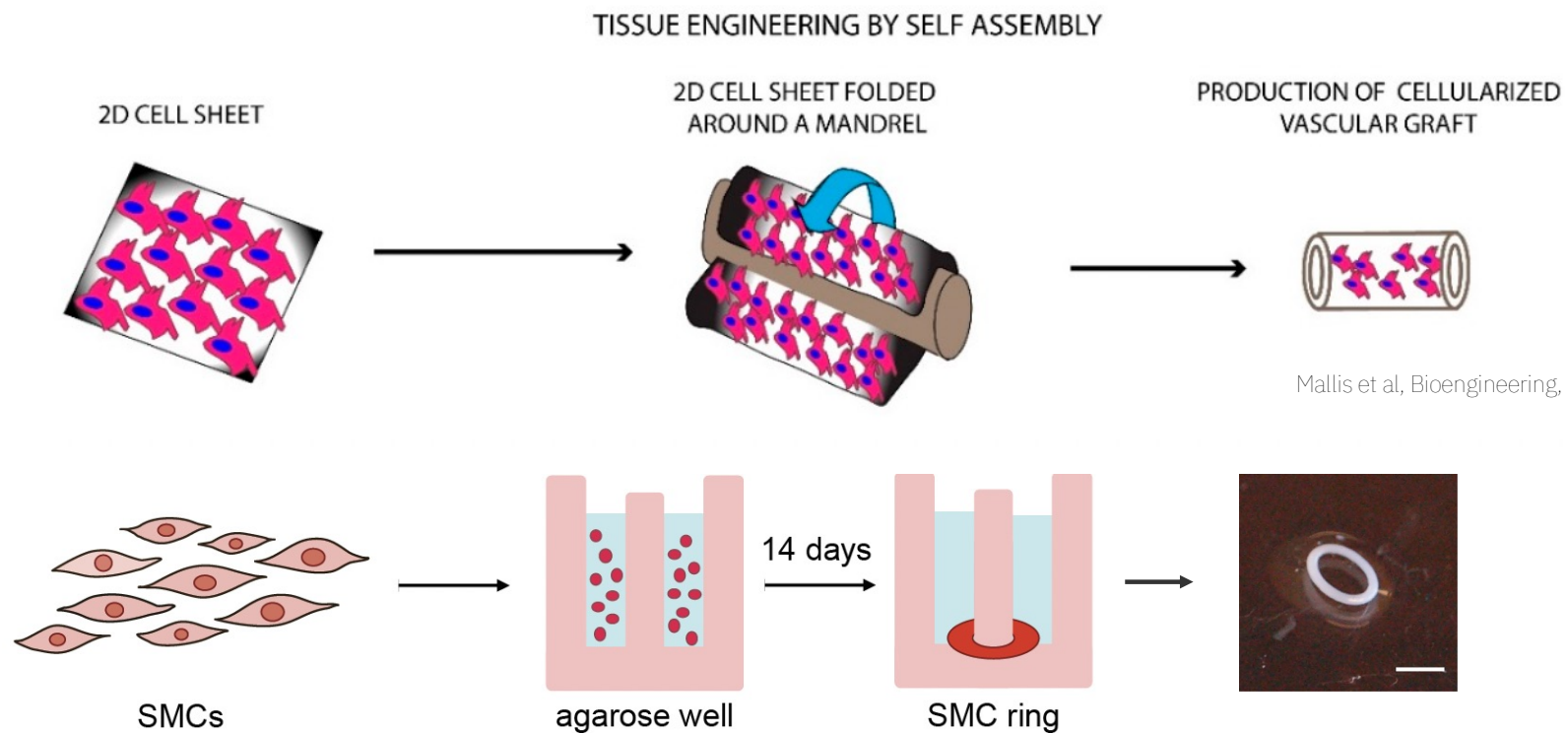
Static vs dynamic conditions → flow is needed for proper cell characteristics / function



TE Approaches

Vascular grafts

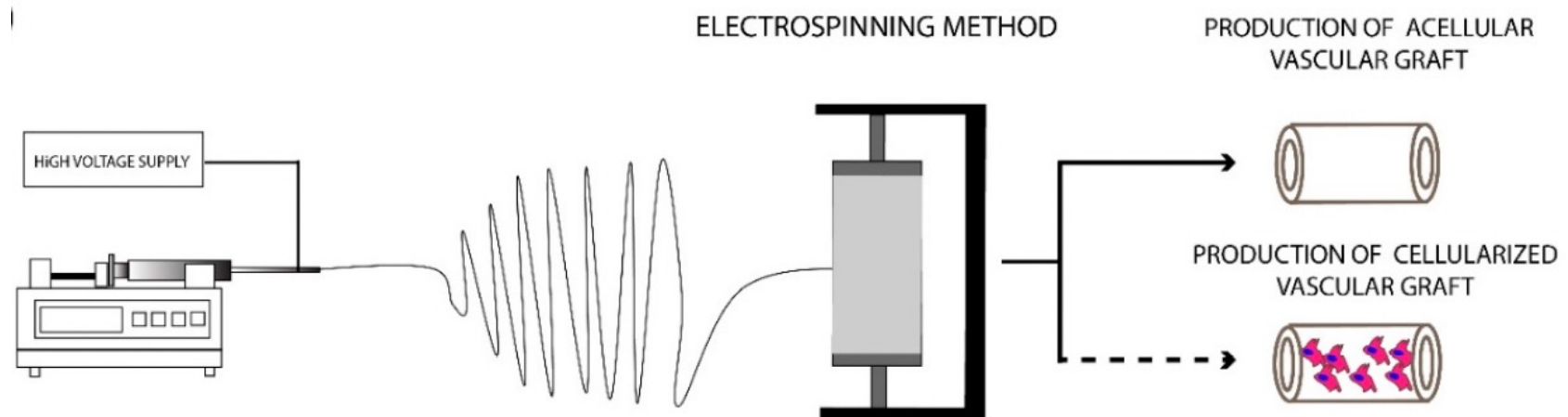
Self assembly of SMCs or IPS-derived cells



TE Approaches

Vascular grafts

Electrospun (hybrid) composites

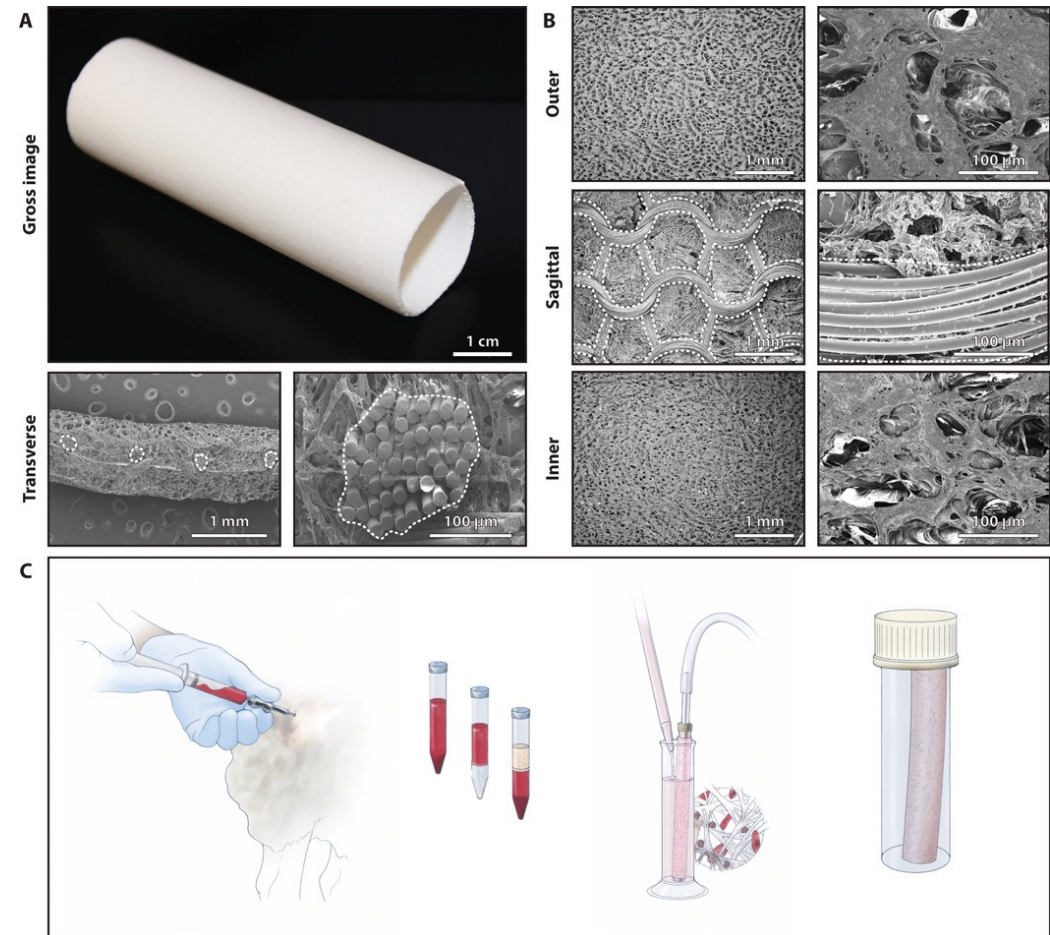


TE Approaches

Vascular grafts

PGA fibers and a copolymer of caprolactone and lactide (PCLA)

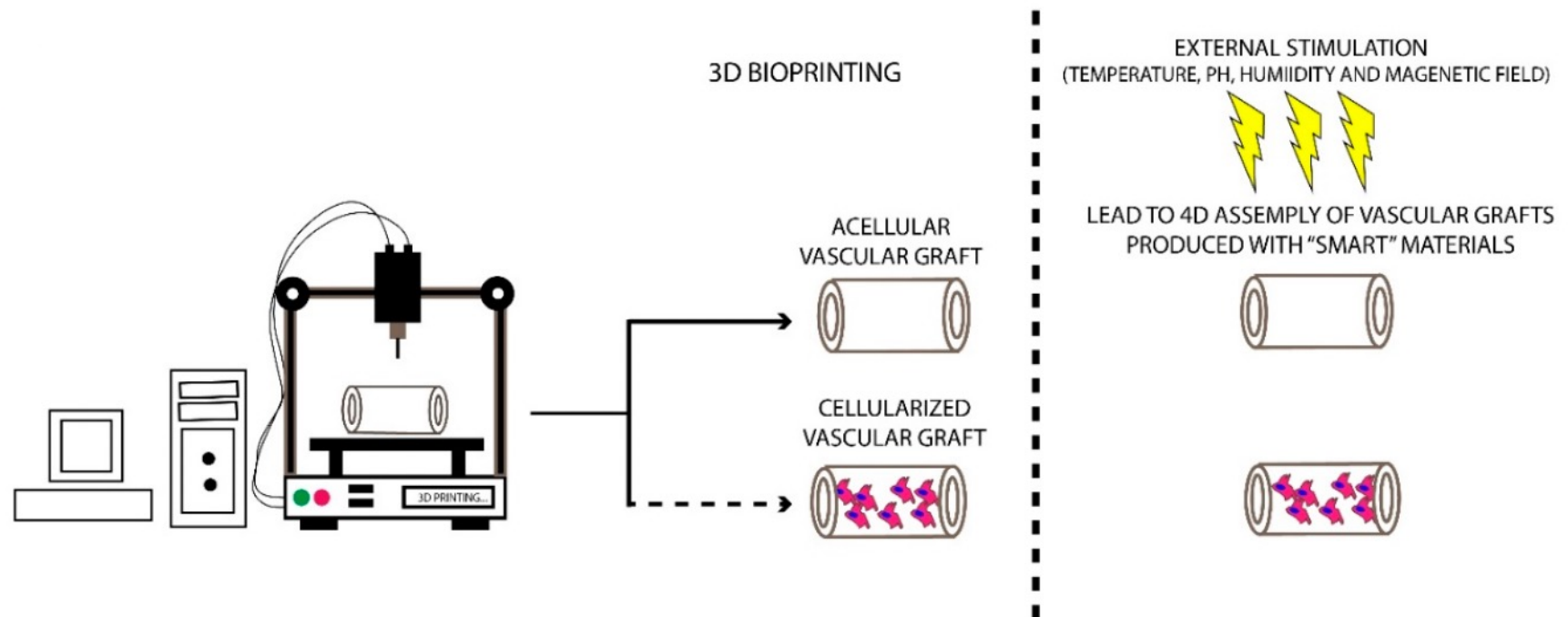
Seeded with bone marrow derived mononuclear cells



TE Approaches

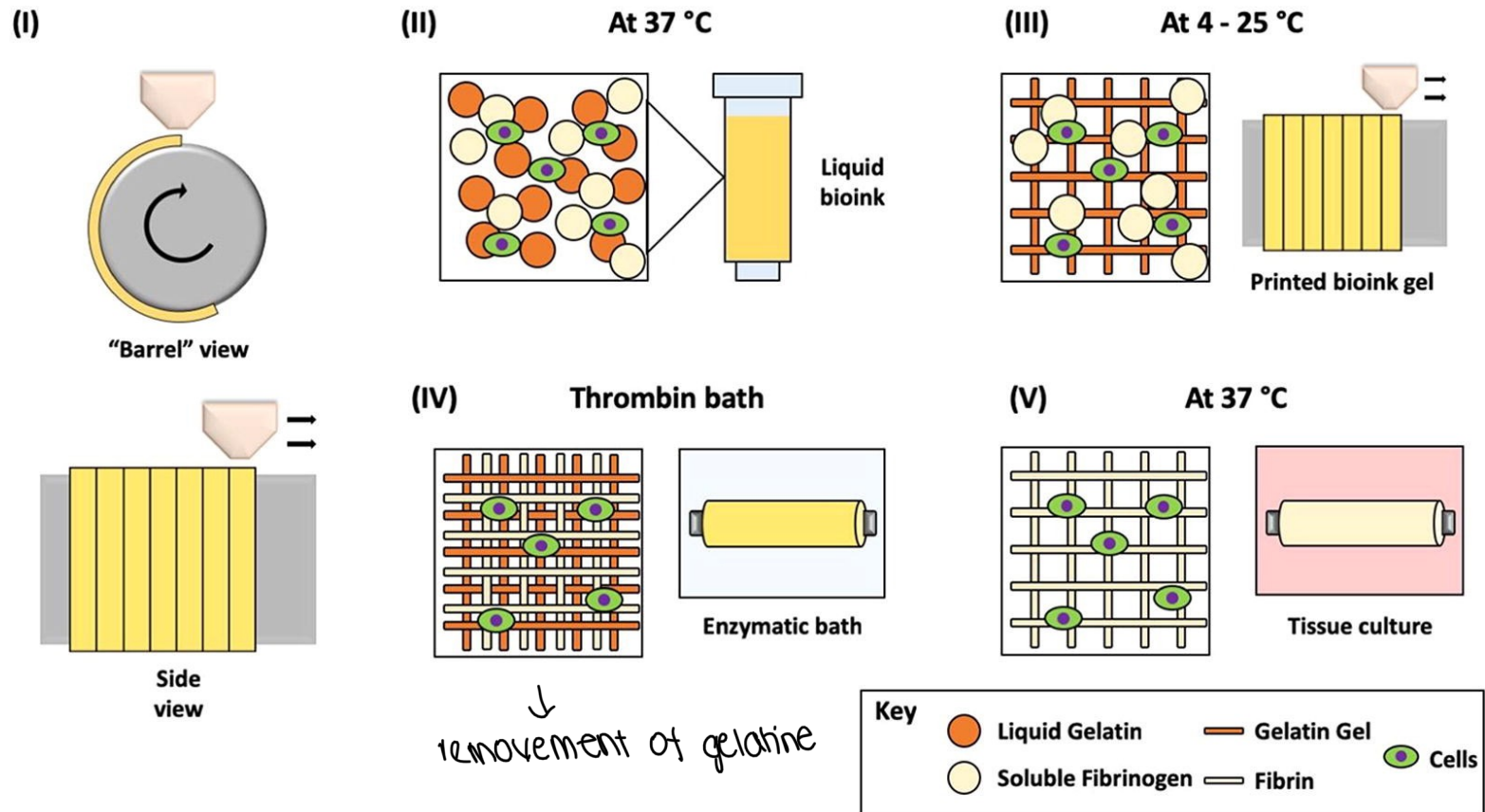
Vascular grafts

Bioprinting



TE Approaches

3D printed vascular grafts



→ direct spinning of fibrinogen is not possible, as not stable enough

TE Approaches

Heart valve

Decellularized leaflets

Xenografts

Allografts

In vitro or in situ tissue engineering

TE Approaches

Decellularized heart valves

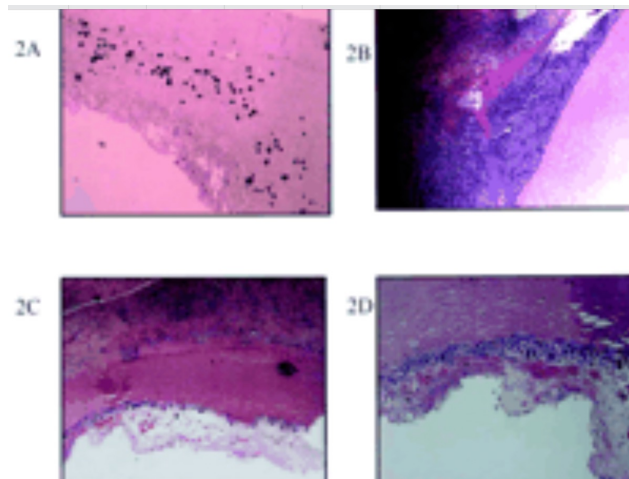
Synergraft®

Decellularized porcine heart valve (=Xenograft)

Animal model (sheep): good acceptance

Clinical trial: 3 of for pediatric patients died due to severe inflammation (within the first year after implantation)

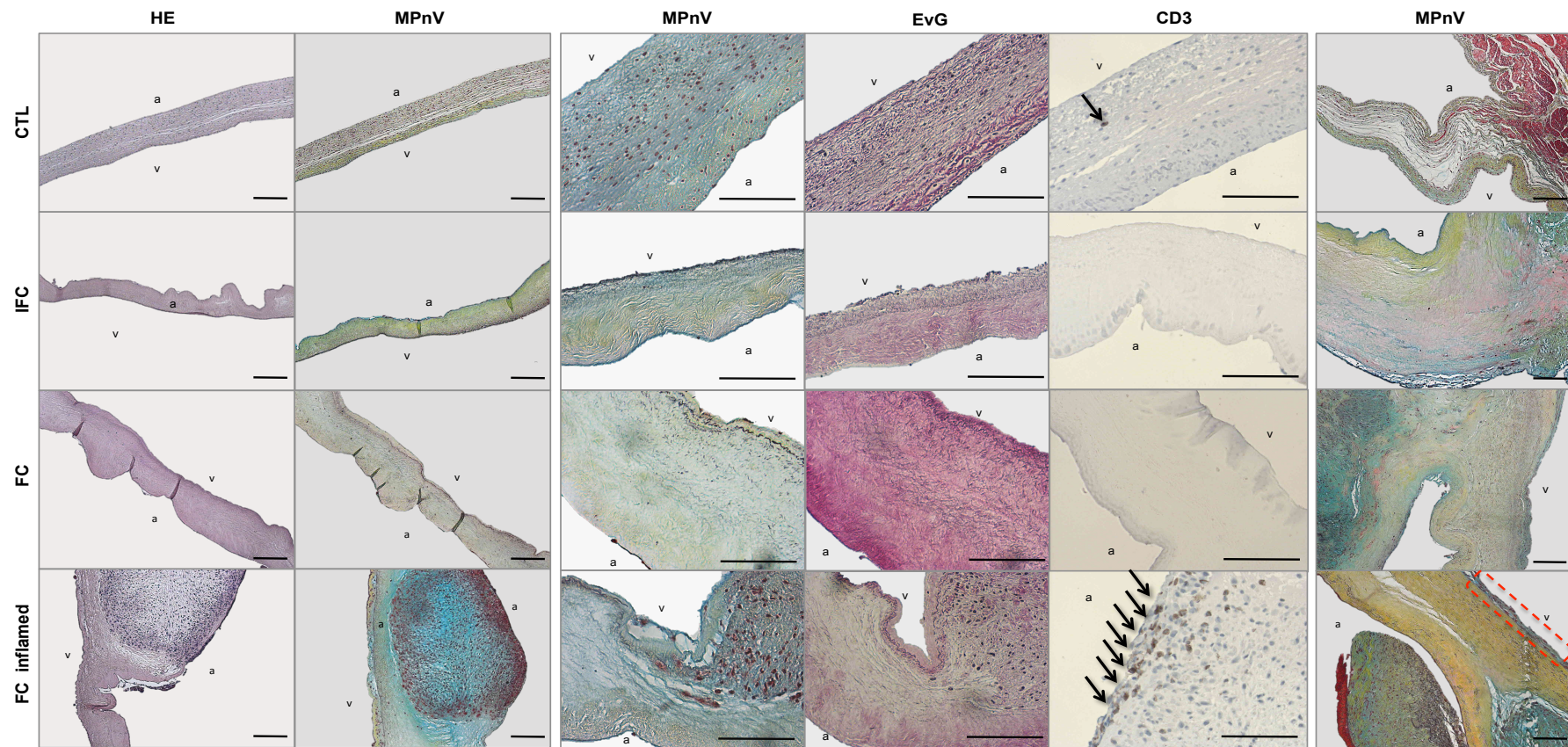
>> no in vivo recellularization and immediate immune reaction to the xenogeneic collagen matrix



4 glycosylation of collagen
is problematic

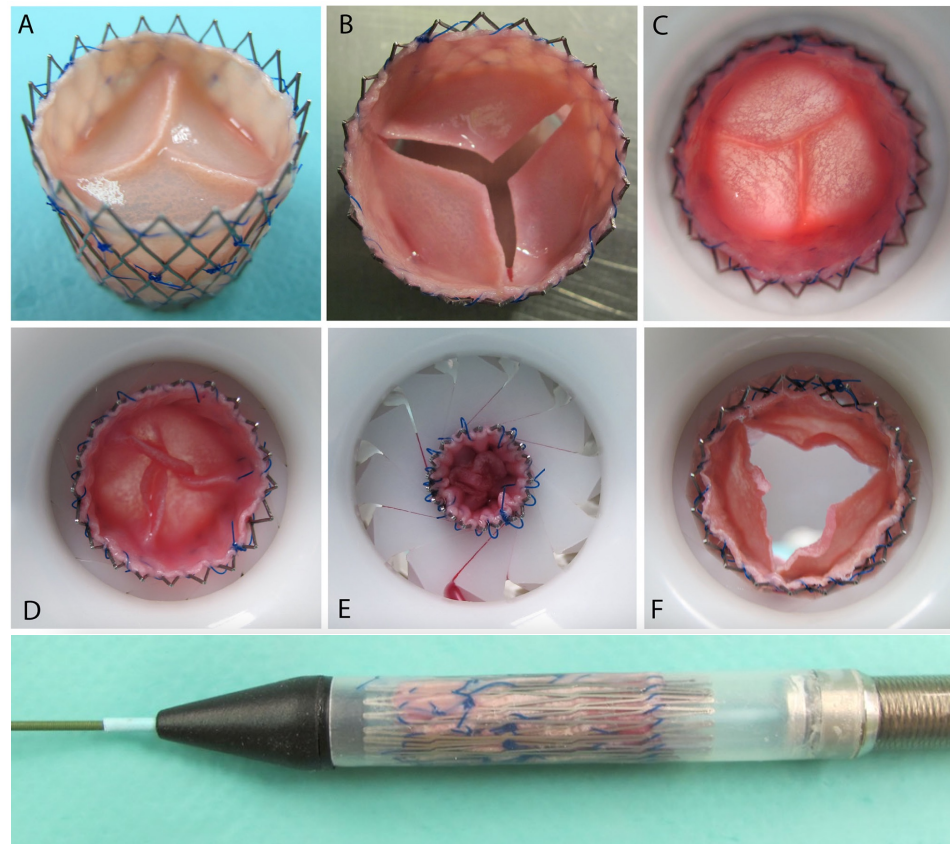
TE Approaches

Decellularized/Cryopreserved heart valve



TE Approaches

In vitro TE



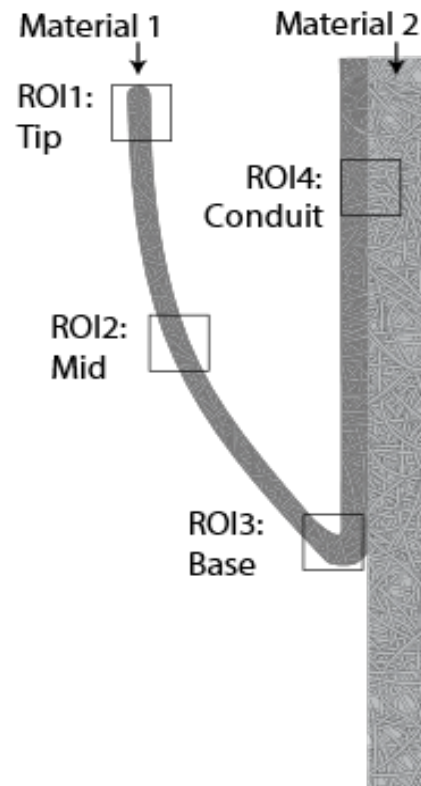
- decellularize
- culture in vitro already let some cells grow on

→ minimally-invasive procedure

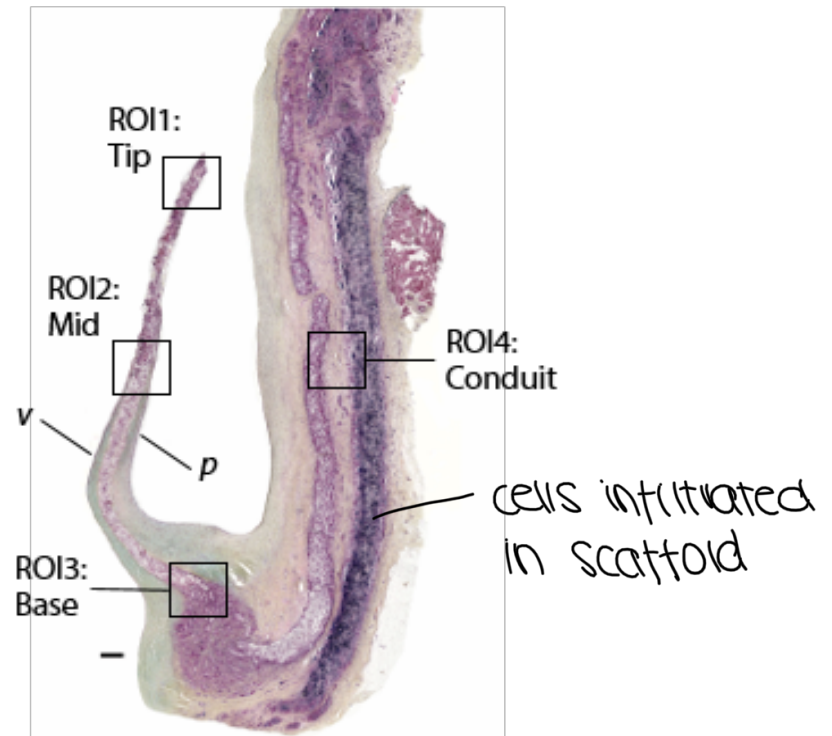
Spriesterbach et al, Cardiovasc Interv and Ther, 2017

TE Approaches

In situ TE

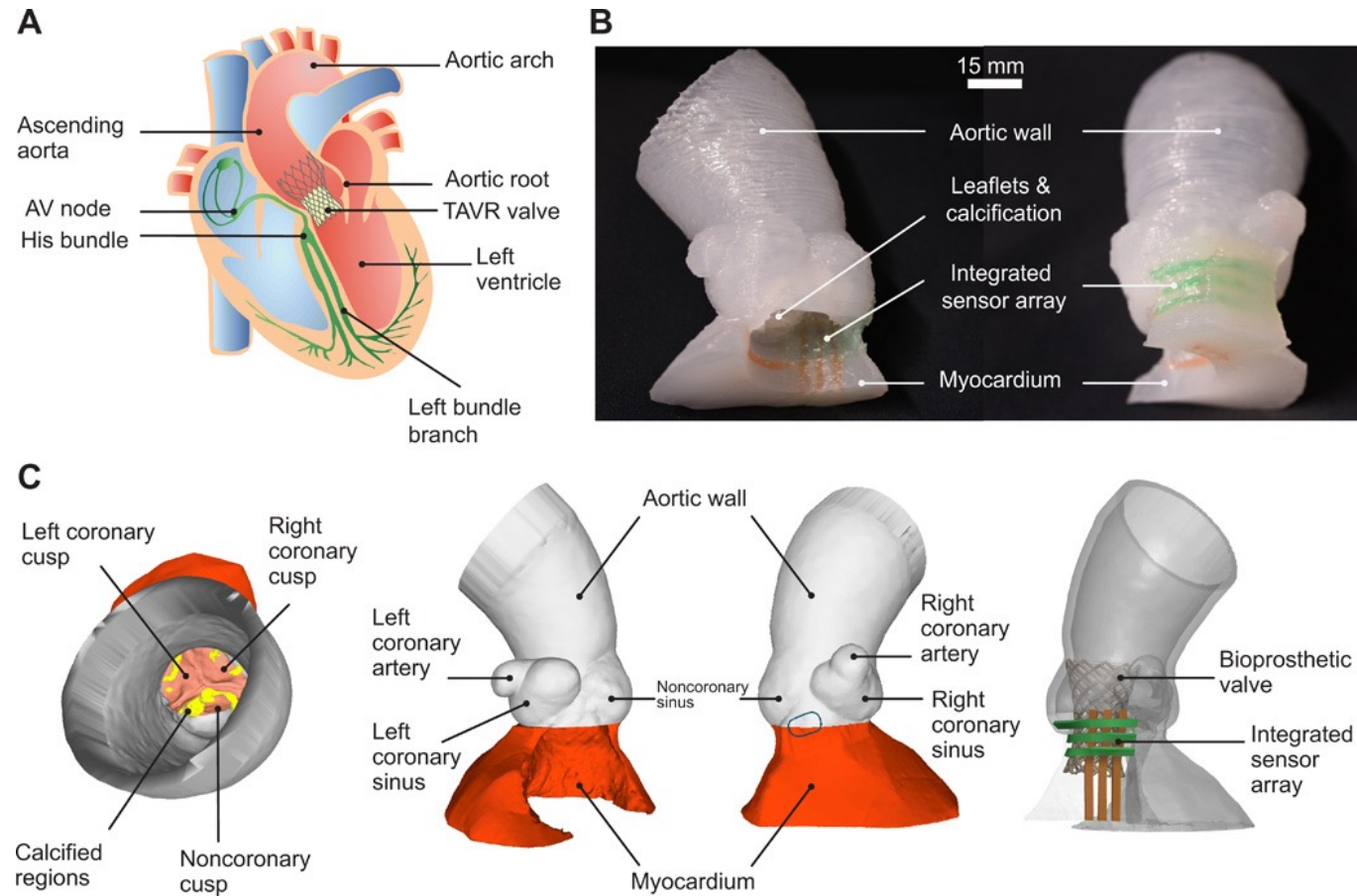


after 2 years



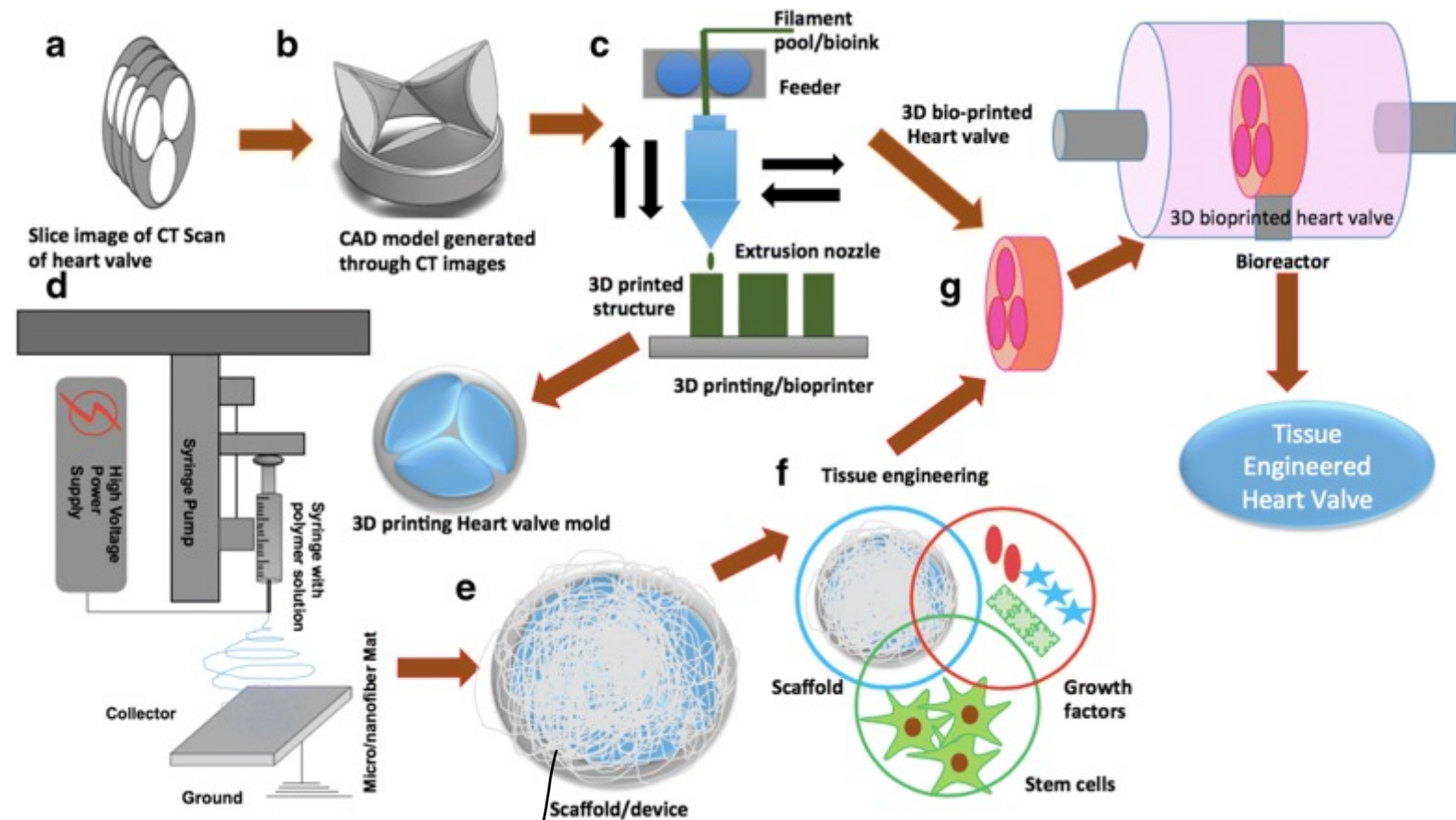
TE Approaches

Bioprinting



TE Approaches

Personalized in vitro TE



for different mechanical properties
e.g. mesh sites

In vivo performance

In vivo performance

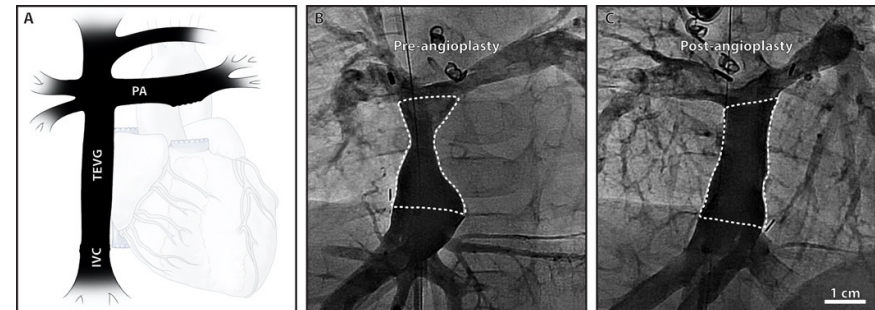
Function

Biocompatibility

Cytotoxicity, immune response

Vital parameters

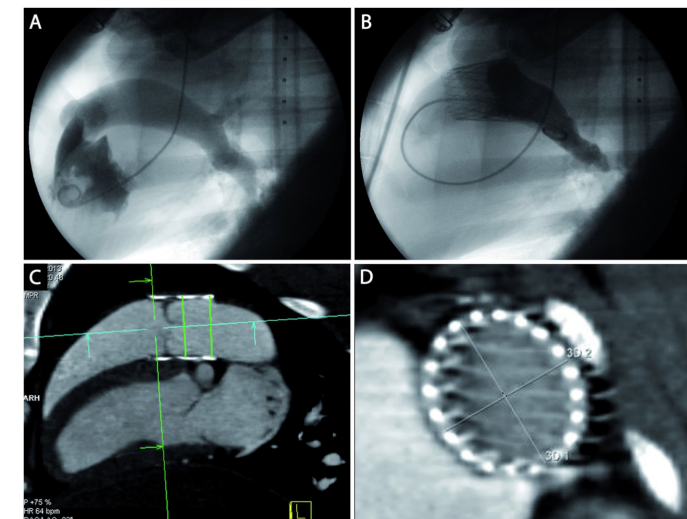
(Micro-)CT, Angiogram, Doppler ultrasound, MRI, Ejection fraction



Drewe et al, Science Transl Med, 2020

Haemodynamics / Echocardiography

		IFC	FC	P value
RVOT v_{max} [m/sec]		1.28 ± 0.13	1.46 ± 0.39	0.3567
Gradient [mmHg]		13.74 ± 5.58	19.18 ± 13.33	0.4155
RV ED diameter [mm]		18.3 ± 3.6	2.75 ± 6.6	0.0493
PI		1.60 ± 0.89	1.67 ± 1.37	
B				
		IFC S-3 (36)	FC S-4 (40)	
RVOT v_{max}	Echo	1.2	1.0	[m/sec]
	MRI	1.2	0.8	[m/sec]
PI	Echo	1	1	[grading]
	MRI	1.02%	0.42%	[%]



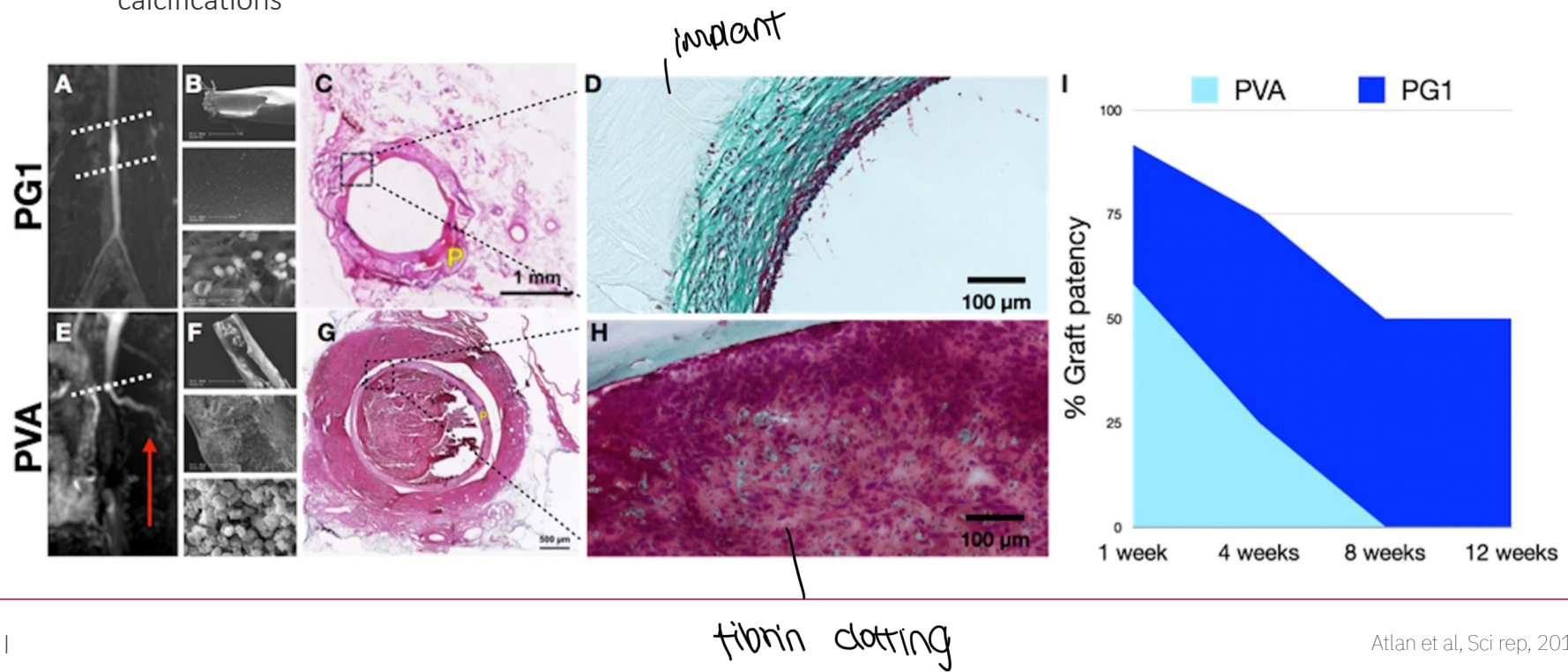
Spriesterbach et al, Cardiovasc Interv and Ther, 2017

In vivo performance

Function

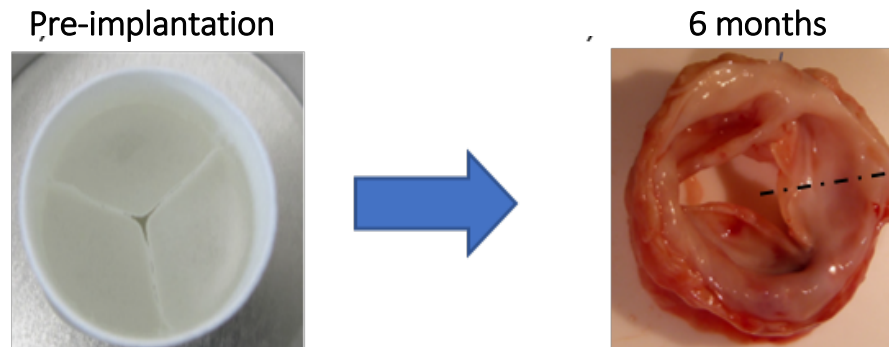
Long term performance & durability

thrombogenesis
occlusion
calcifications



In vivo performance

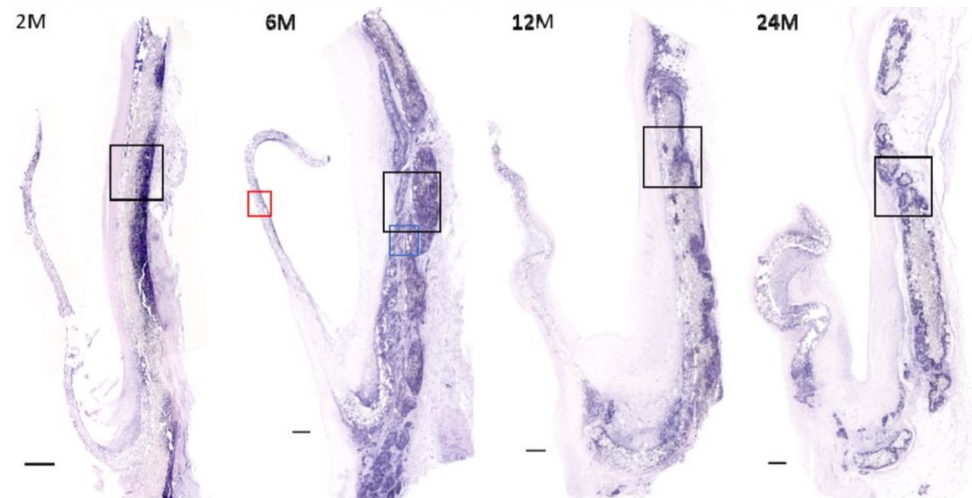
Tissue remodeling



Newly formed tissue structures & cell types

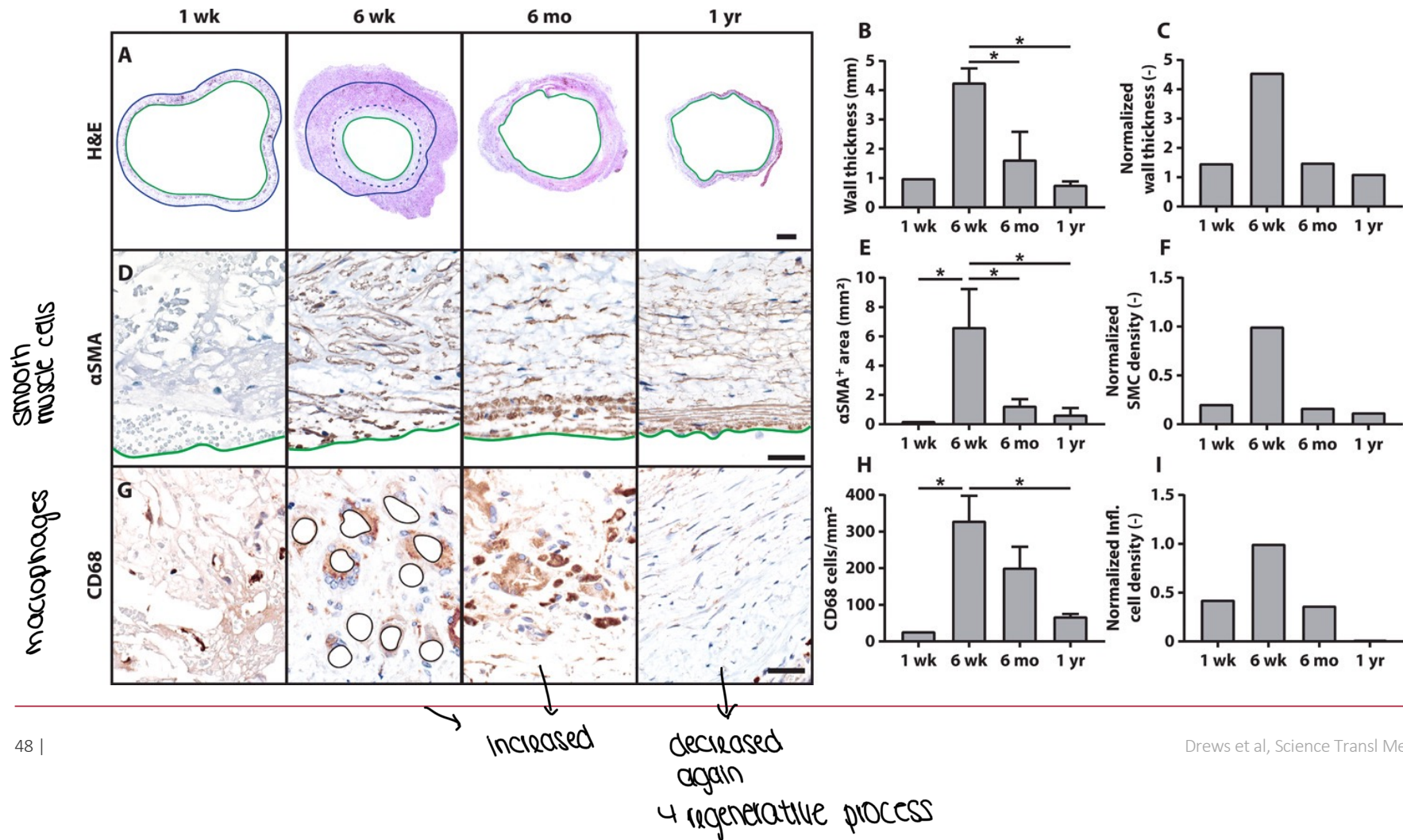
>> histological staining, IF staining

extracellular matrix structures
tissue infiltrating cells
immune cells
enzyme activity



In vivo performance

Tissue remodeling



↳ innate immune reaction, then remodeling

In vivo performance

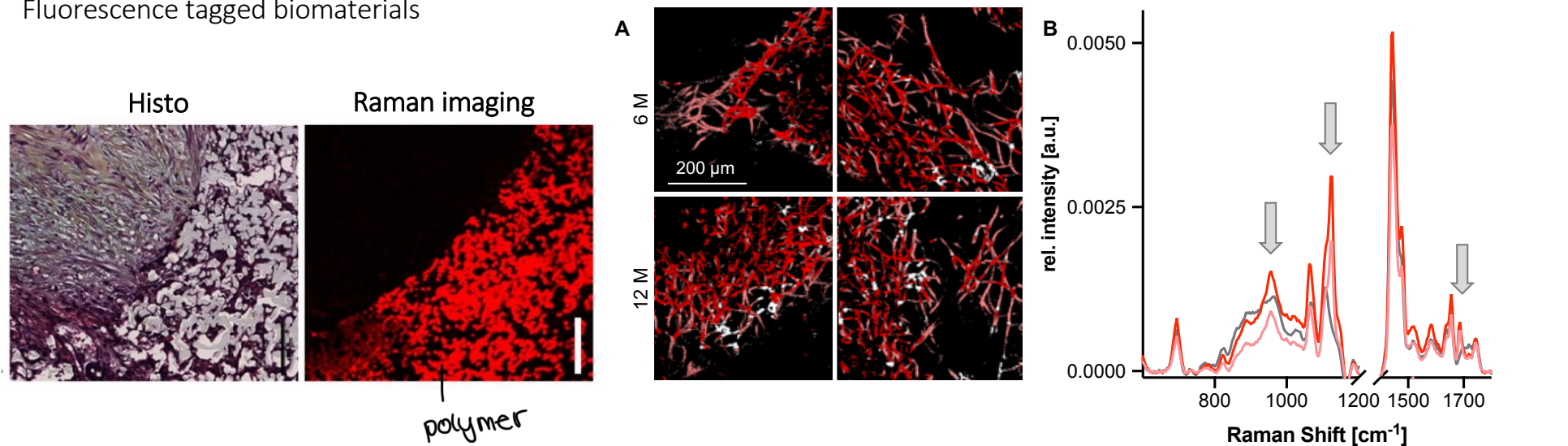
Degradation

>> How would you determine implant degradation?

Contrast in histological staining

Mass loss (in vitro/in vivo) → weight, gel electrophoresis

Fluorescence tagged biomaterials



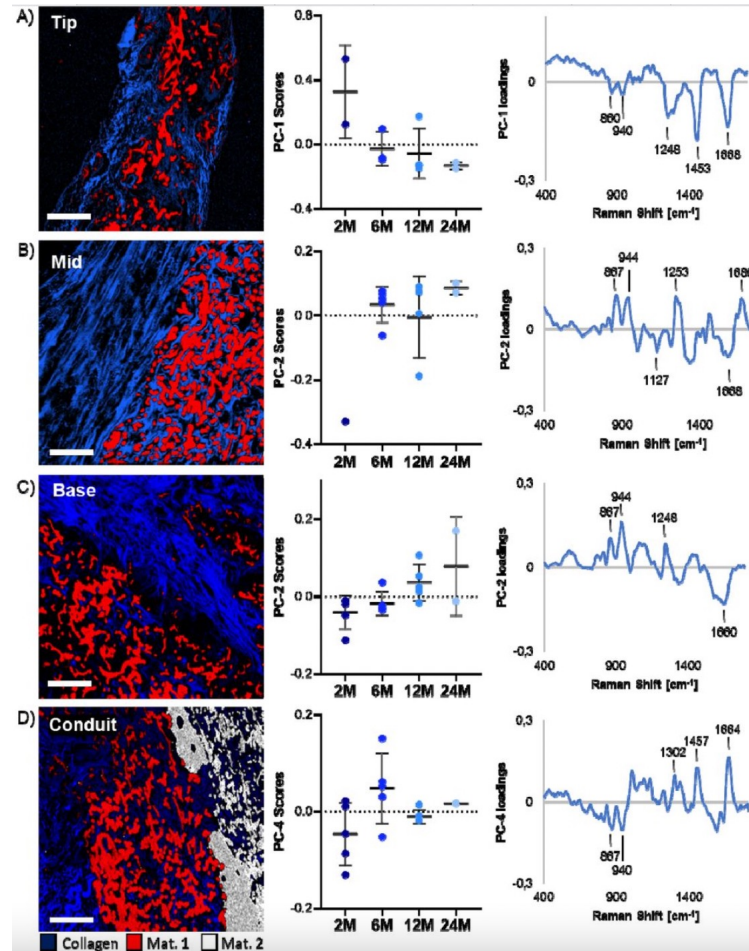
4 structure information
4 molecular composition via
spectral information

In vivo performance

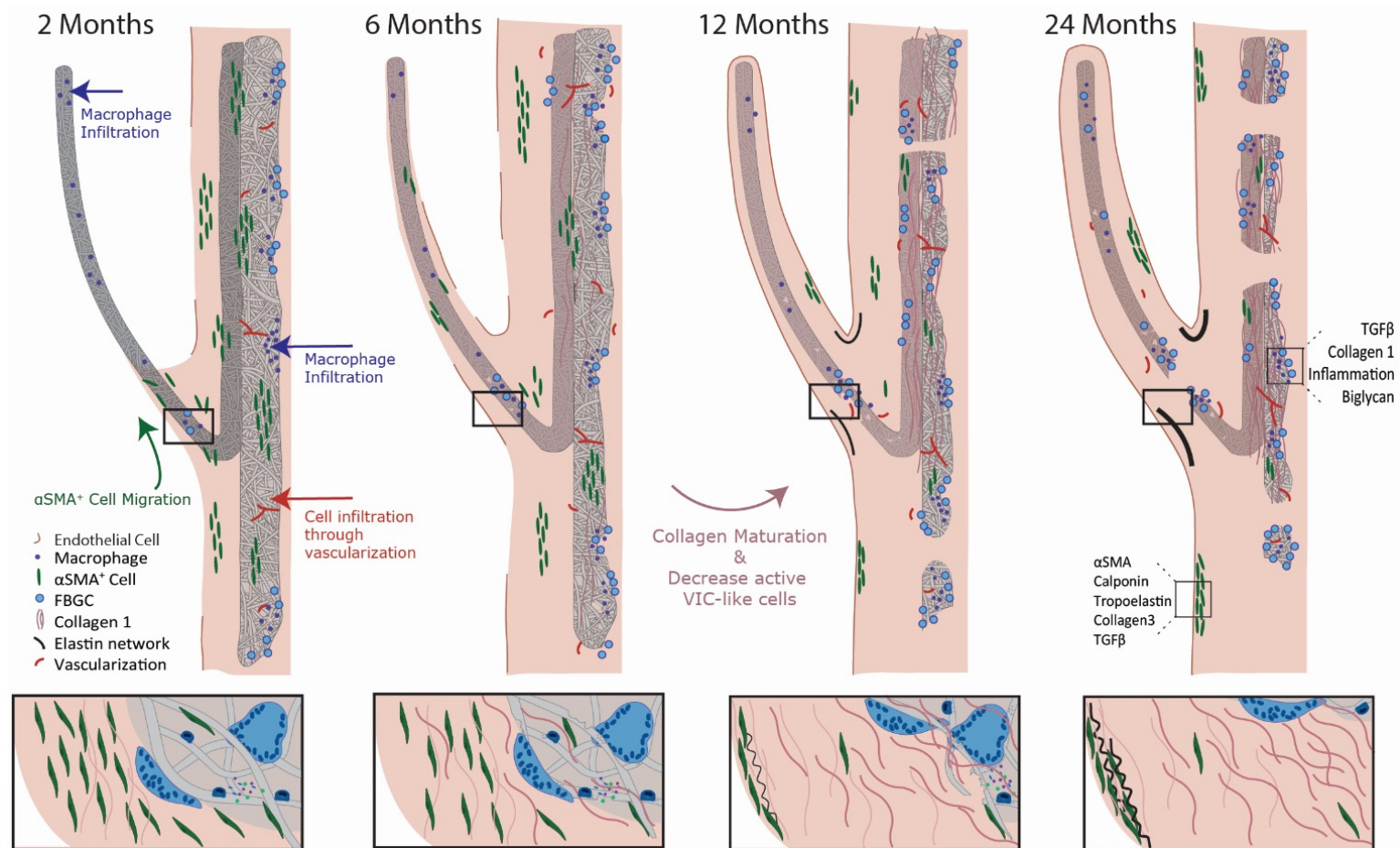
Collagen maturation

collagen = blue

synthetic structures = red



In vivo performance



Thank you for your attention!

julia.marzi@uni-tuebingen.de
www.schenke-layland-lab.com