Sviluppo di nuovi dispositivi biotecnologici per la validazione di nuovi approcci terapeutici

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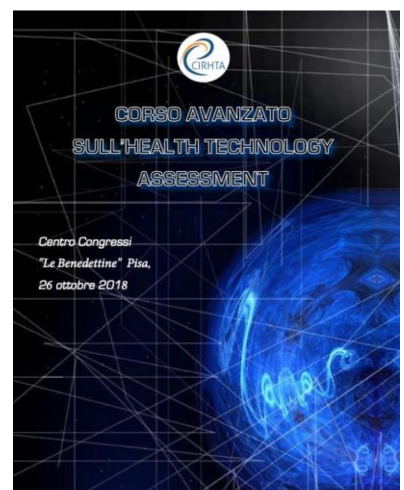
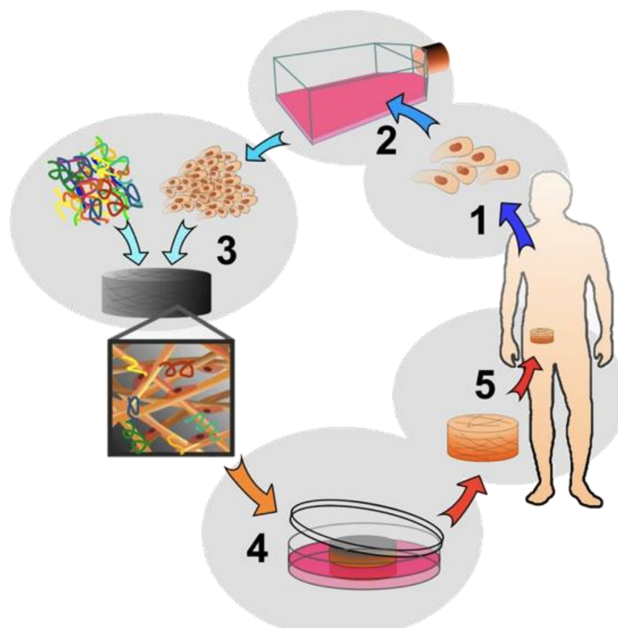


Regenerative medicine

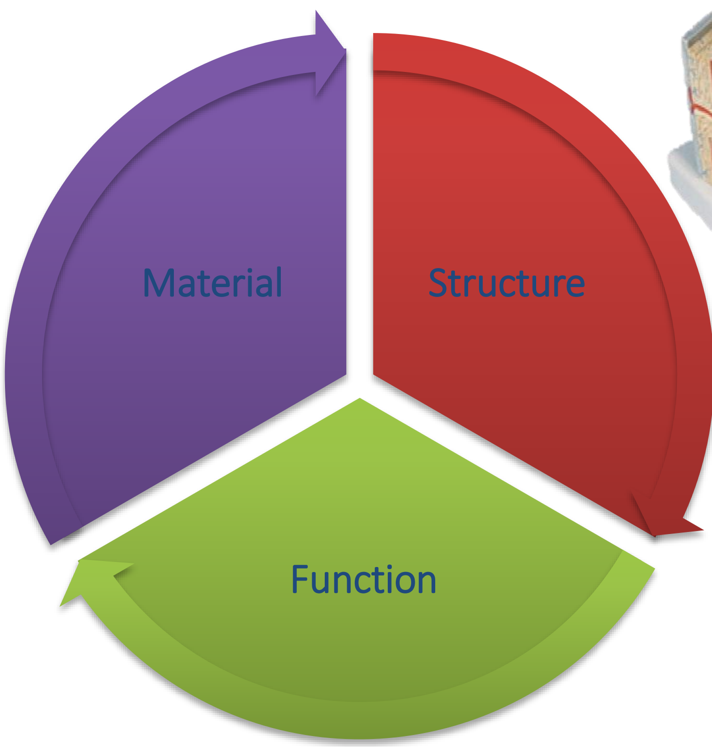
the application of tissue science, tissue engineering, and related biological and engineering principles that restore the structure and function of damaged tissues and organs

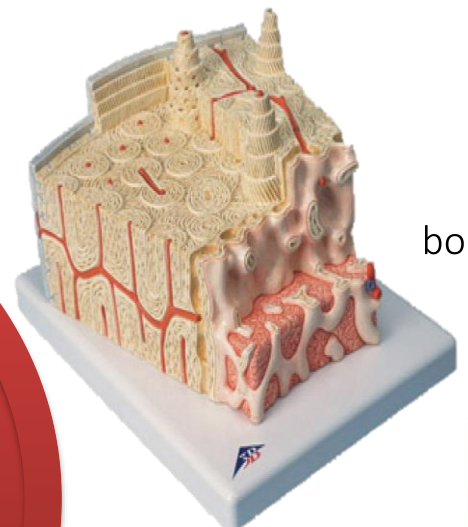


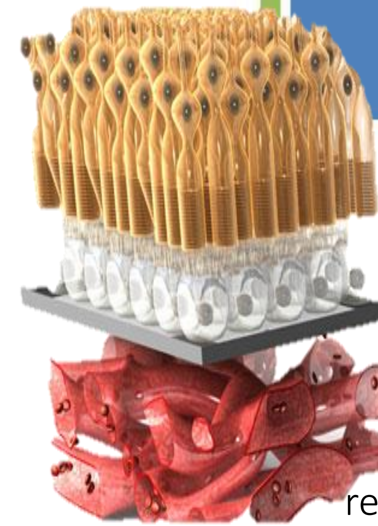
U.S. department of health and human services, 2006:
A New Vision - A Future for Regenerative Medicine,

Living tissues

- Multiscale and multimaterial structure

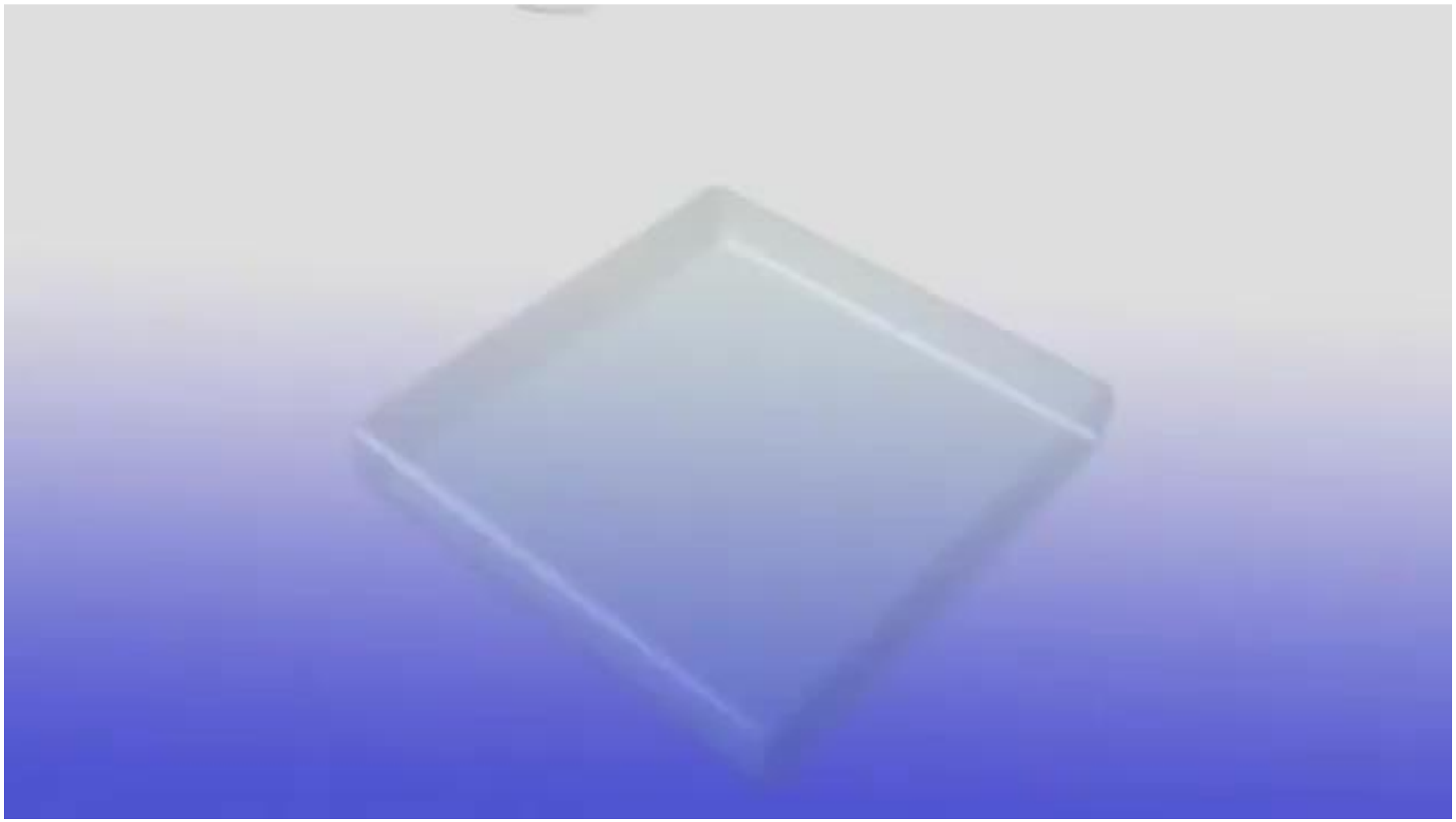



bone


retina

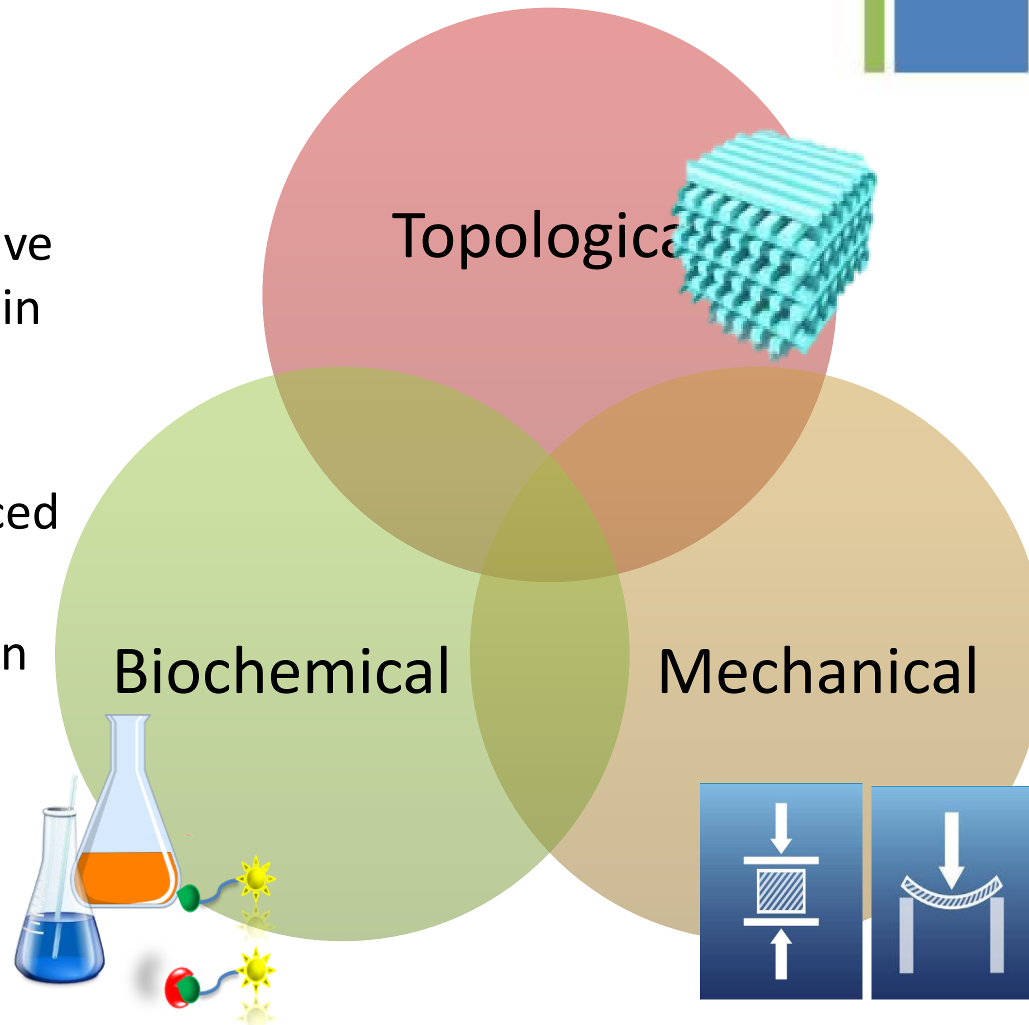

blood
vesses

Vozzi G and Ahluwalia A. *Microfabrication for tissue engineering: rethinking the cells-on-a scaffold approach*, J Mat Chem 17 (13), 1248-1254



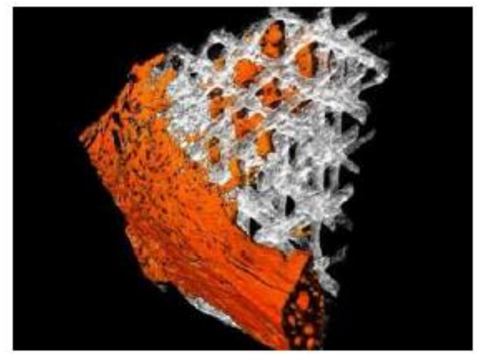
Biofabrication of human organs

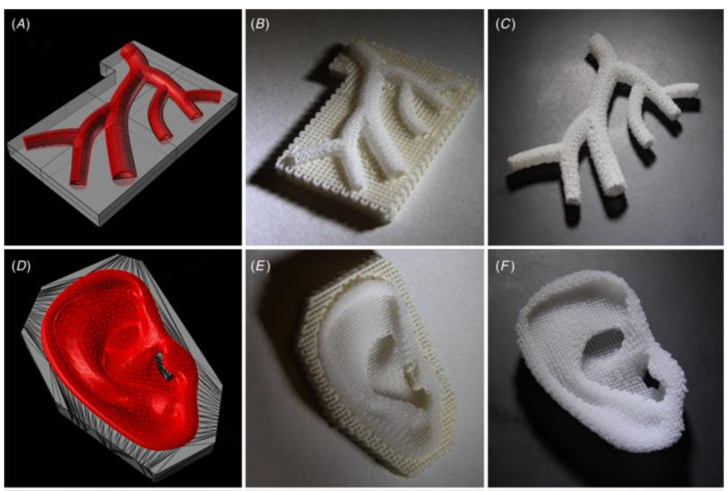
- Mimicking the stimuli that drive growth and functions of cells in living tissues
- Tailor the scaffold properties using combinations of advanced fabrication technologies
- Allowing the tissue maturation into appropriate bioreactors



What is a scaffold?

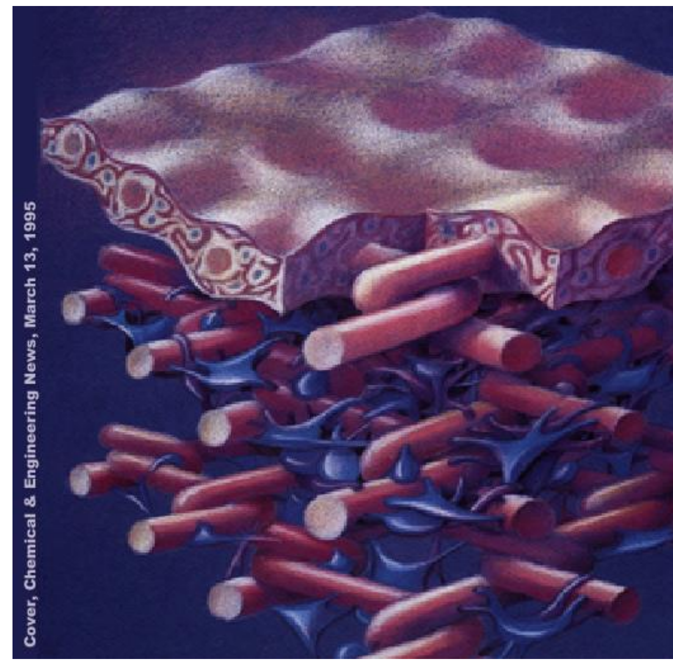
Scaffold is a **temporary** 3D polymeric structure that **mimics** the m**echanical**, **structural**, and **biochemical** properties of the extracellular matrix (ECM) of natural tissue supporting 3D tissue growth

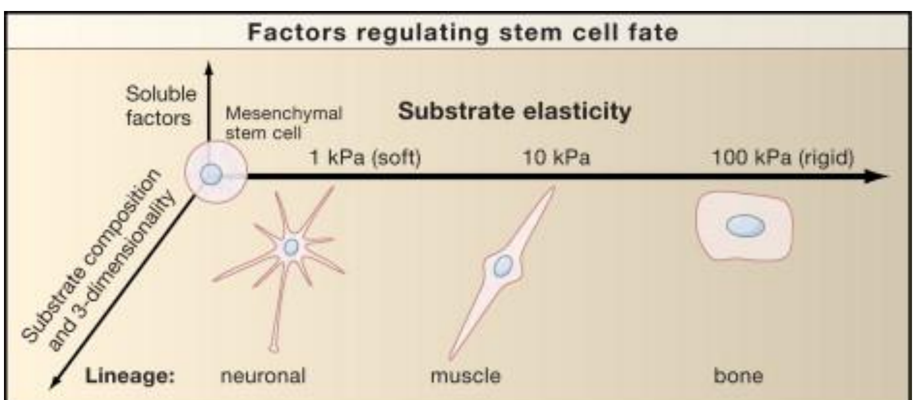




Biochemical stimulus

- Synthetic biomaterials with ligands
- Natural biomaterials
- Decellularized Tissue





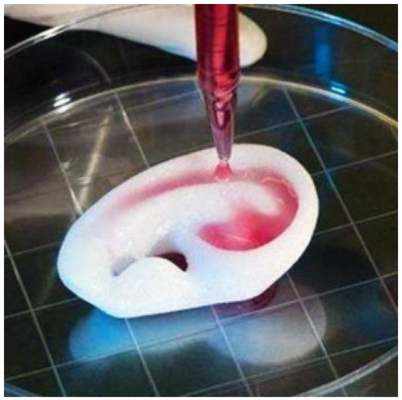
Even-Ram s et al, Matrix Control of Stem Cell Fate, Cell. Volume 126, Issue 4, 25 August 2006, Pages 645–647

Biochemical stimulus

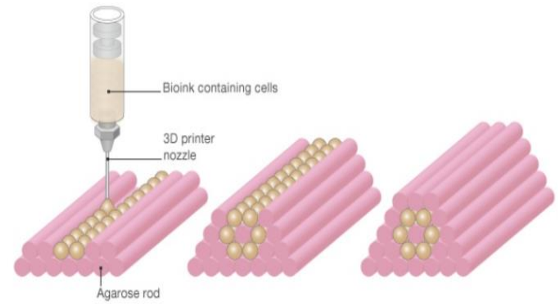
Biomaterial	Reproducibility	Processability	Biochemical features	Mechanical feature
Synthetic	⋎	⋎	X	X
Natural	X	X	⋎	⋎
Decellularized	X	X	⋎	⋎

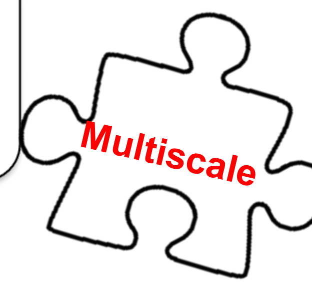
There is no one ideal material for tissue model….

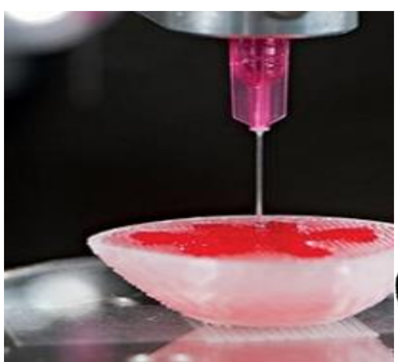
Why Rapid Prototyping in organ/tissue regeneration ?

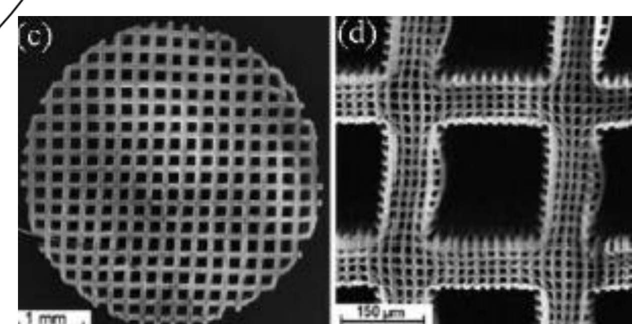


- Bottom up approach
- Control from micro to nano architecture
- Processing of several living and non living materials
- Customized 3D tissue applications

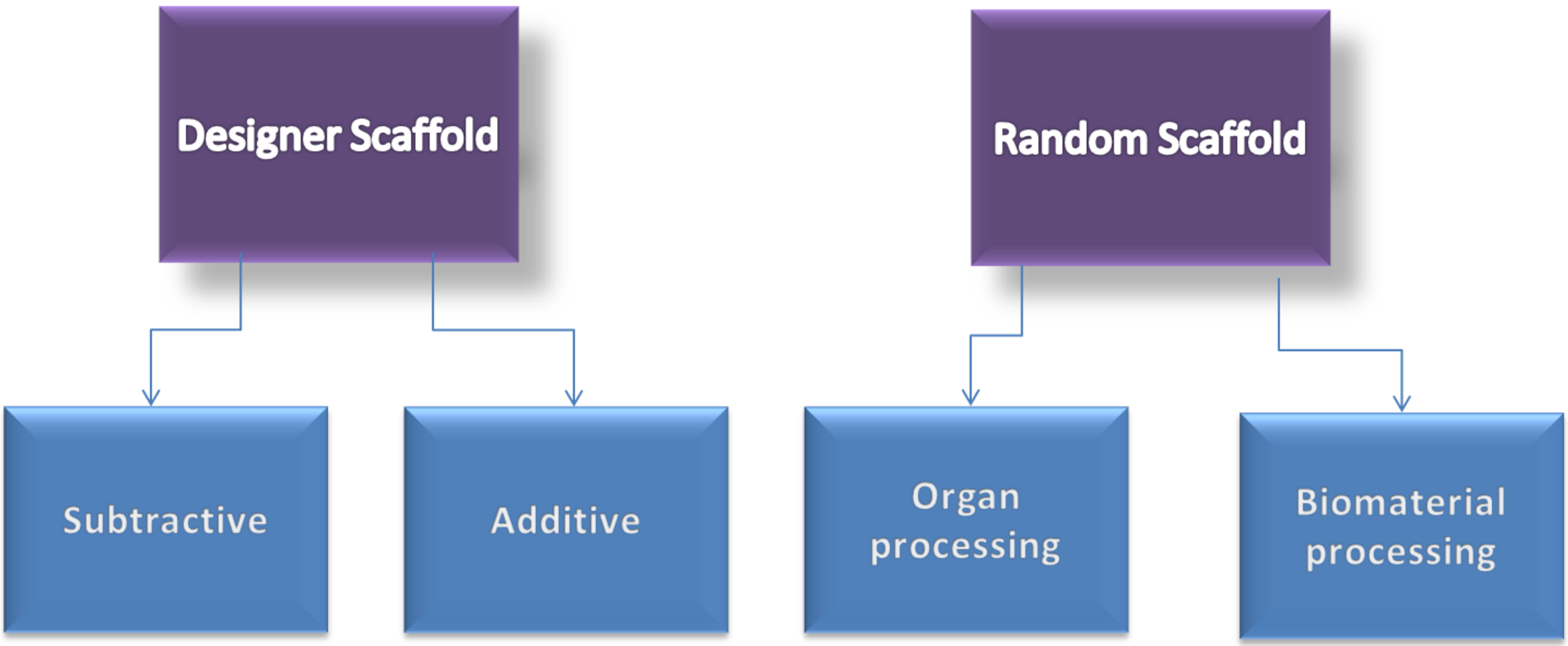








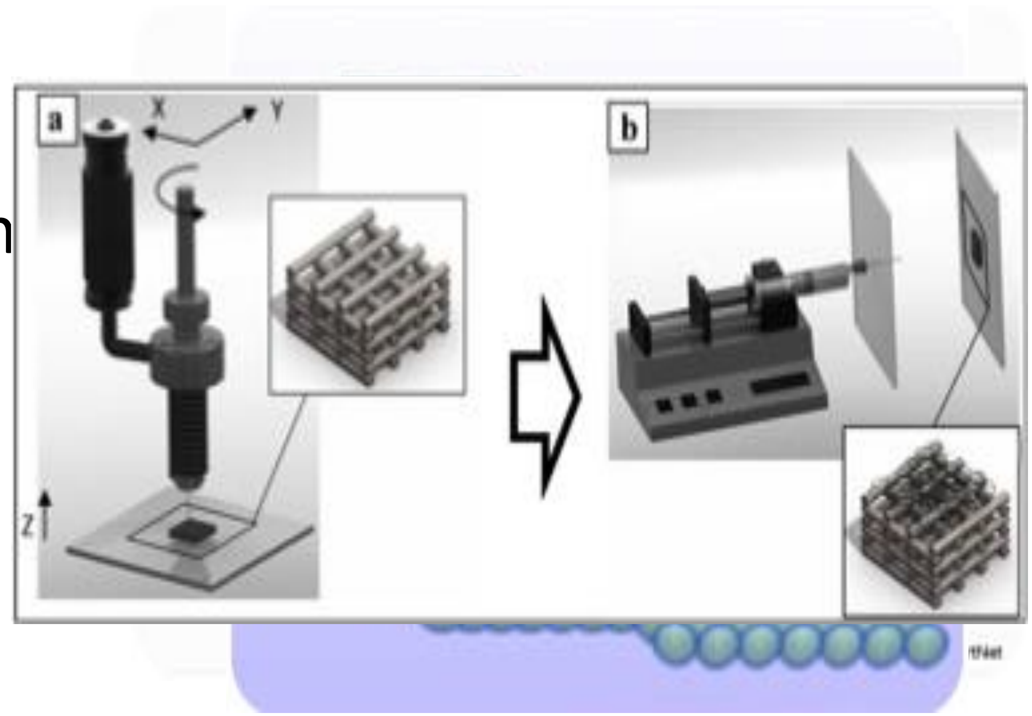
Scaffold fabrication techniques



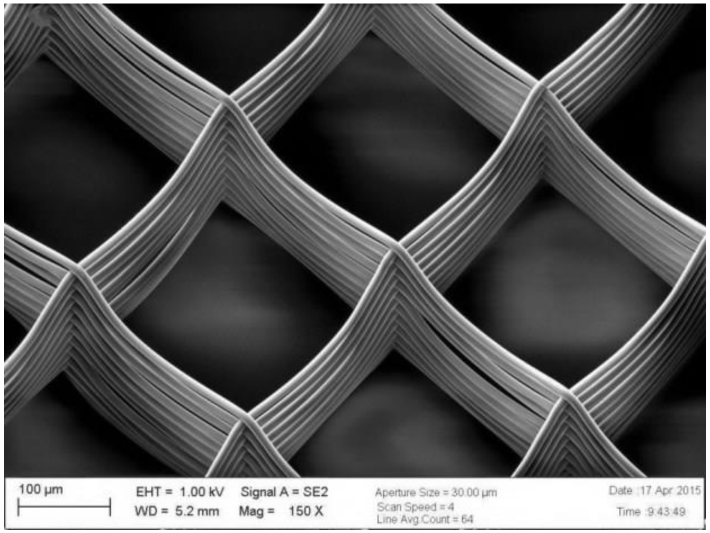
Scaffold fabrication techniques

Three main groups:

- o laser systems
- o nozzle based systen
- o extrusion systems
- o hybrid systems



Scaffolds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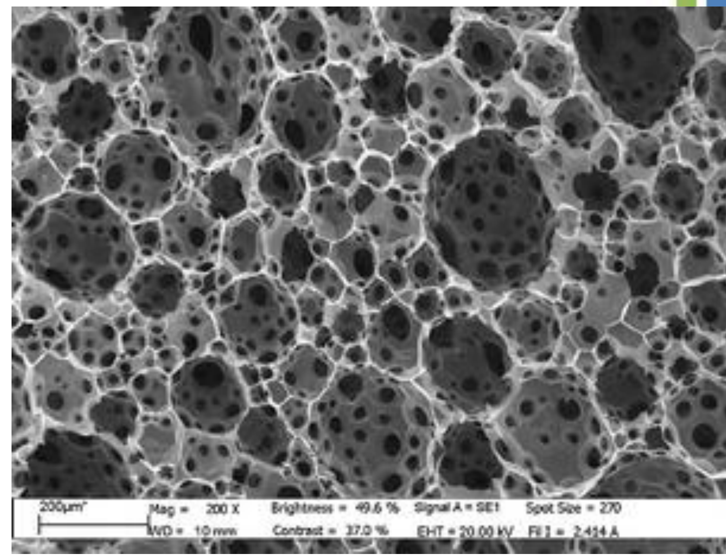
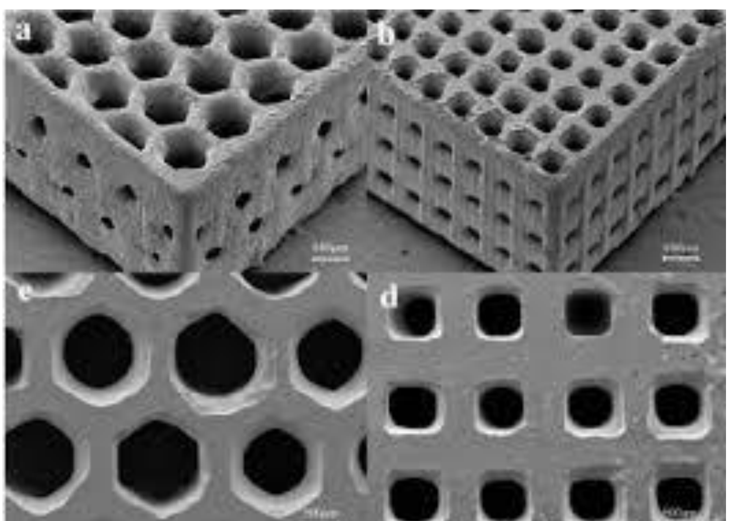
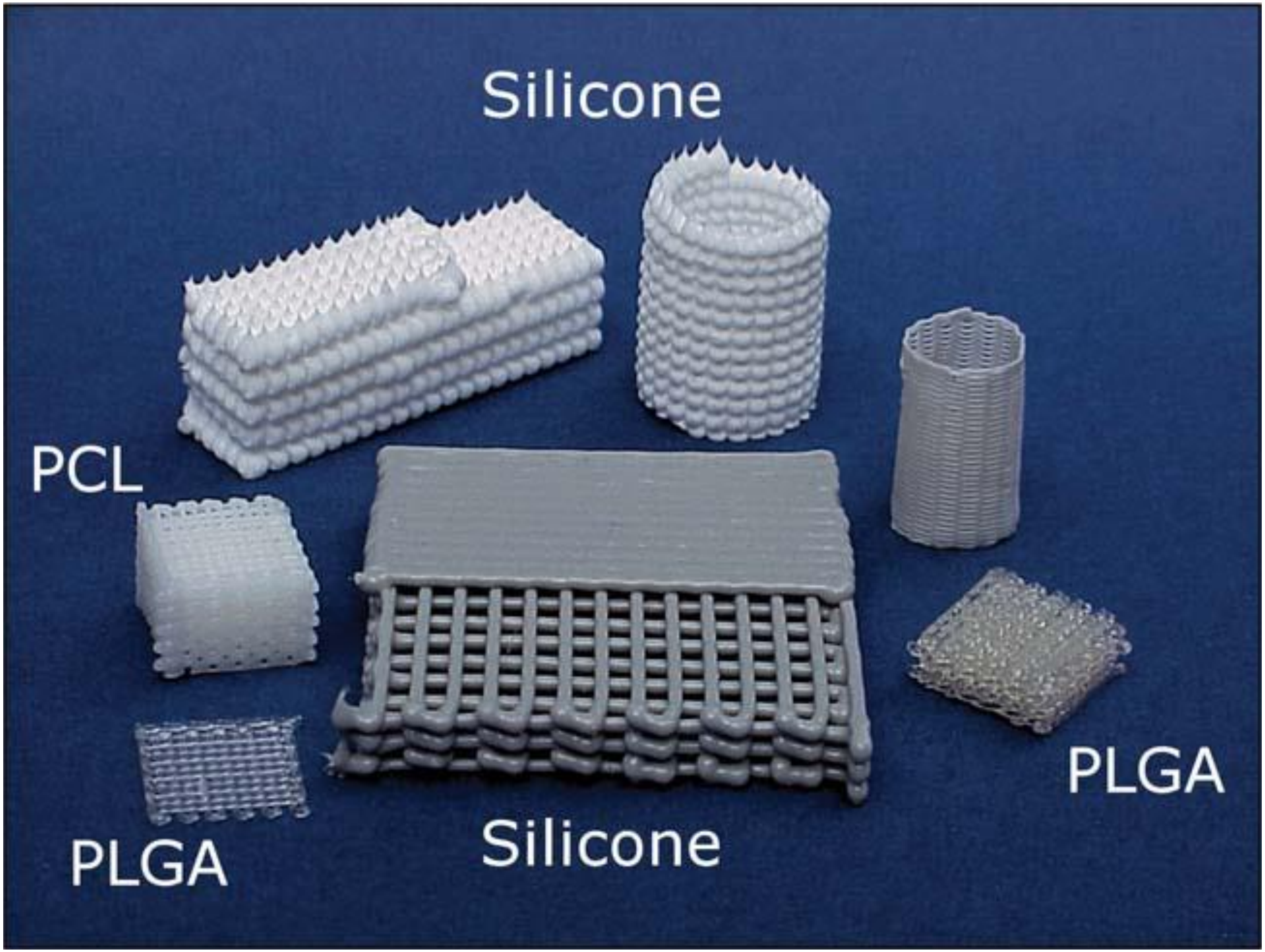
EHT = 1.00 kV
Signal A = SE2
WD = 5.2 mm
Mag = 150 X
Aperture Size = 30.00 µm
Scan Speed = 4
Line Avg.Count = 64
Date :17 Apr 2015
Time :9:43:49


200µm
Mag = 200 X
WD = 10 mm
Brightness = 49.6 %
Contrast = 37.0 %
Signal A = SE1
EHT = 20.00 kV
Spot Size = 270

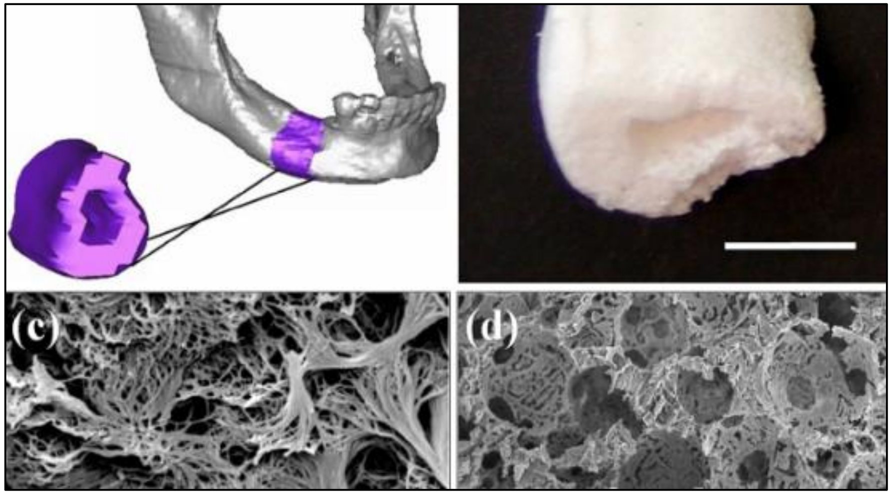

a
b
c
d

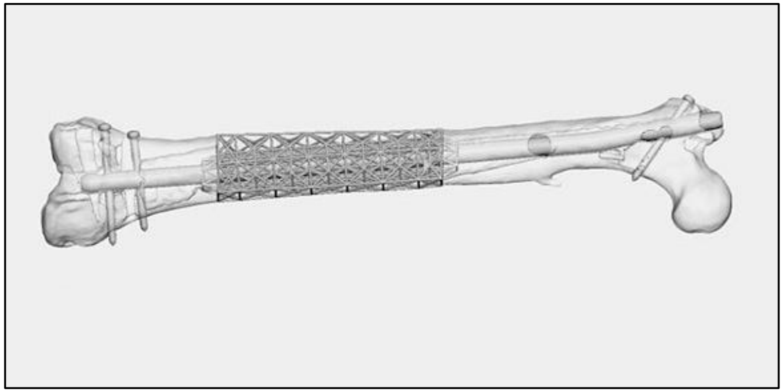
Scaffolds



RP: towards patient specific scaffold

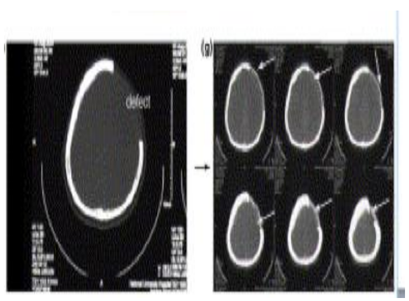
RP creates **patient-specfic** solutions that perfectly mimic the physical shape to potentially restore the original functionality of a tissue

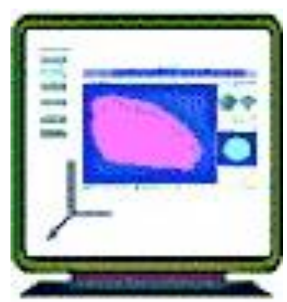


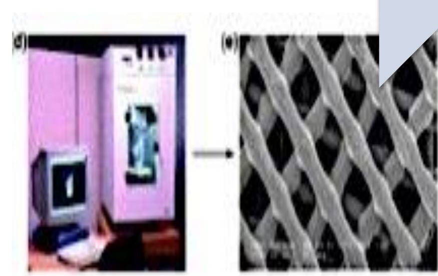


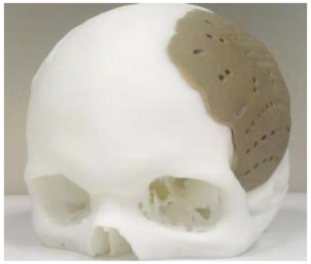
RP workflow :
from patient images to the 3D model

- Images acquisition (CT, MRI or 3D ultrasound)
- Segmentation and model creation
- Design customized structure
- Print preparation and optimization
- 3D printed model





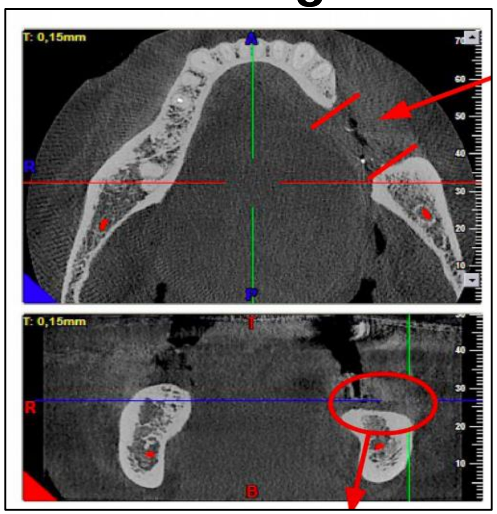




Computer aided design (CAD) software are the key of this workflow !

Segmentation procedure

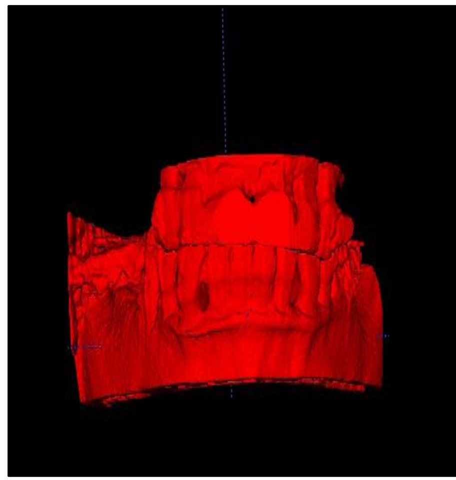
CT images



Defect

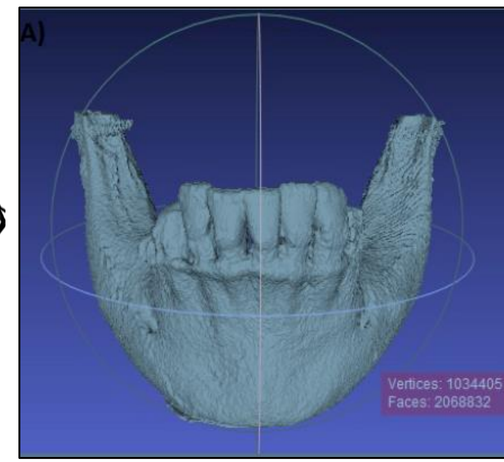
Defect

Segmentation



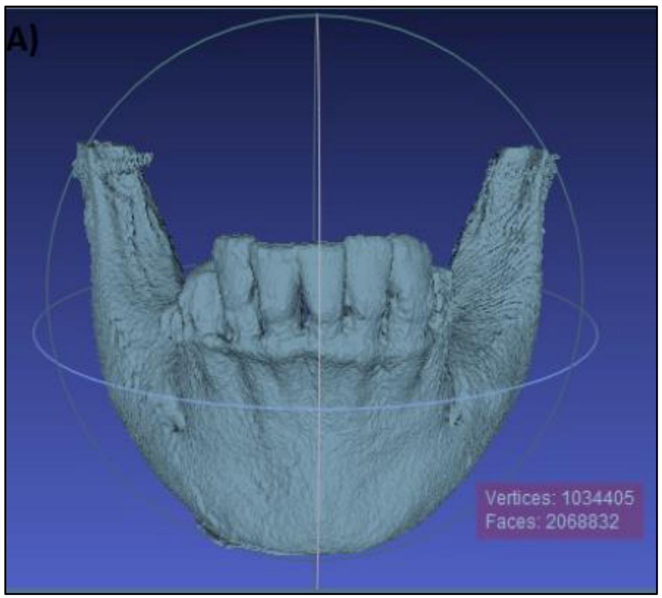
itk-SNAP

Elimination of artifacts

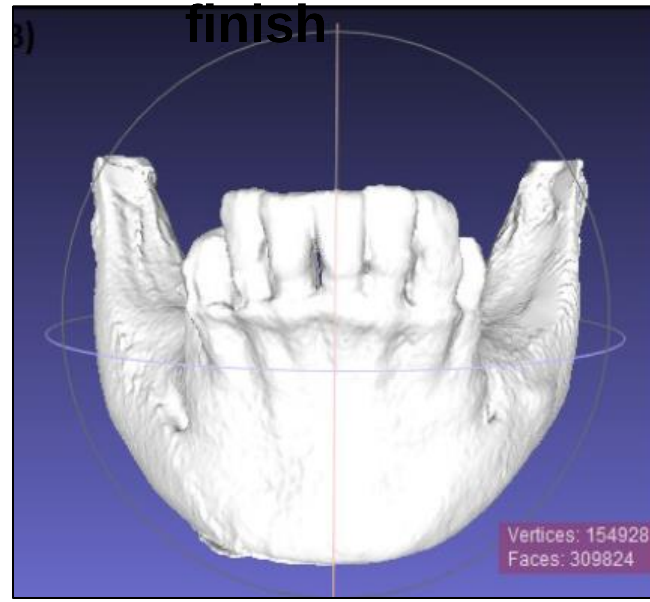


AUTODESK® MESHMIXER®

3D model correction



surface finish



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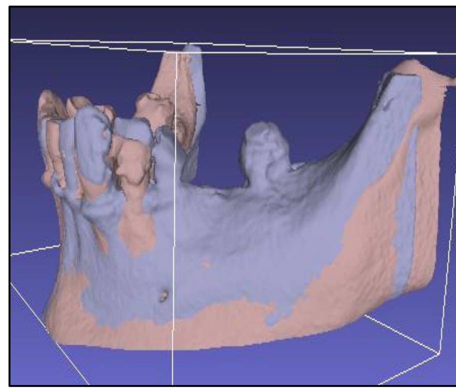
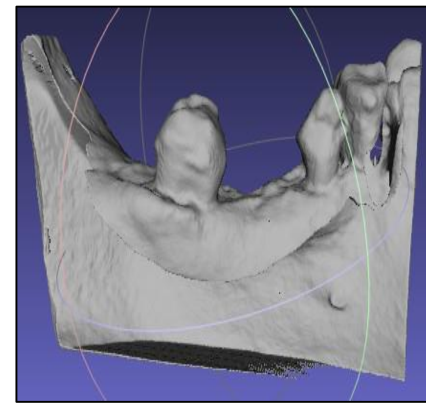
Identification of defect

Mirroring

Alignment



Subtraction



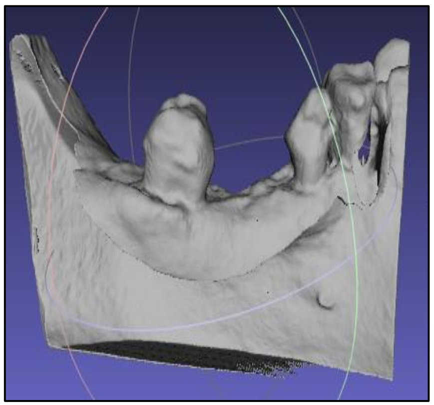
Segmentation and Registration Toolkit

Align Tool

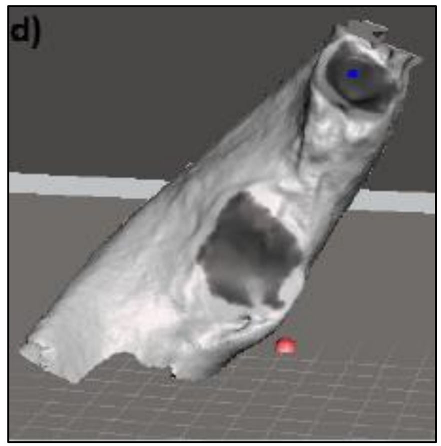
Visualization Toolkit

Preparing to print

Segmented model



Model optimization



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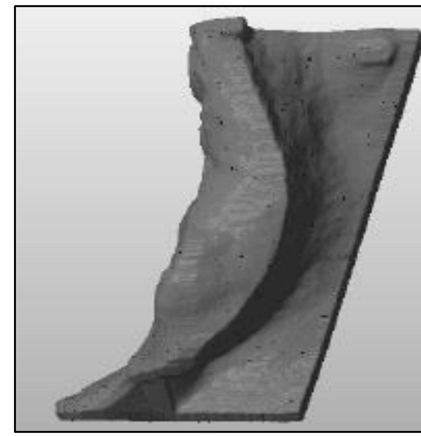
Slicing



netFabb®

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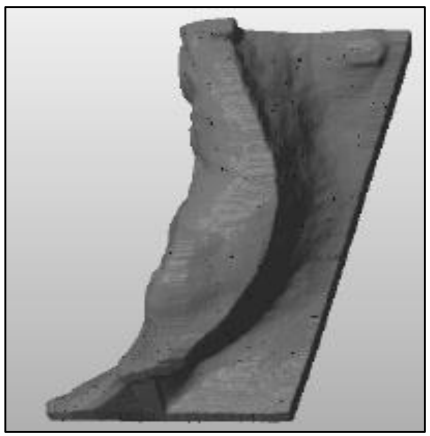
Printing the model

3D CAD model



FDM printer

Printed defect

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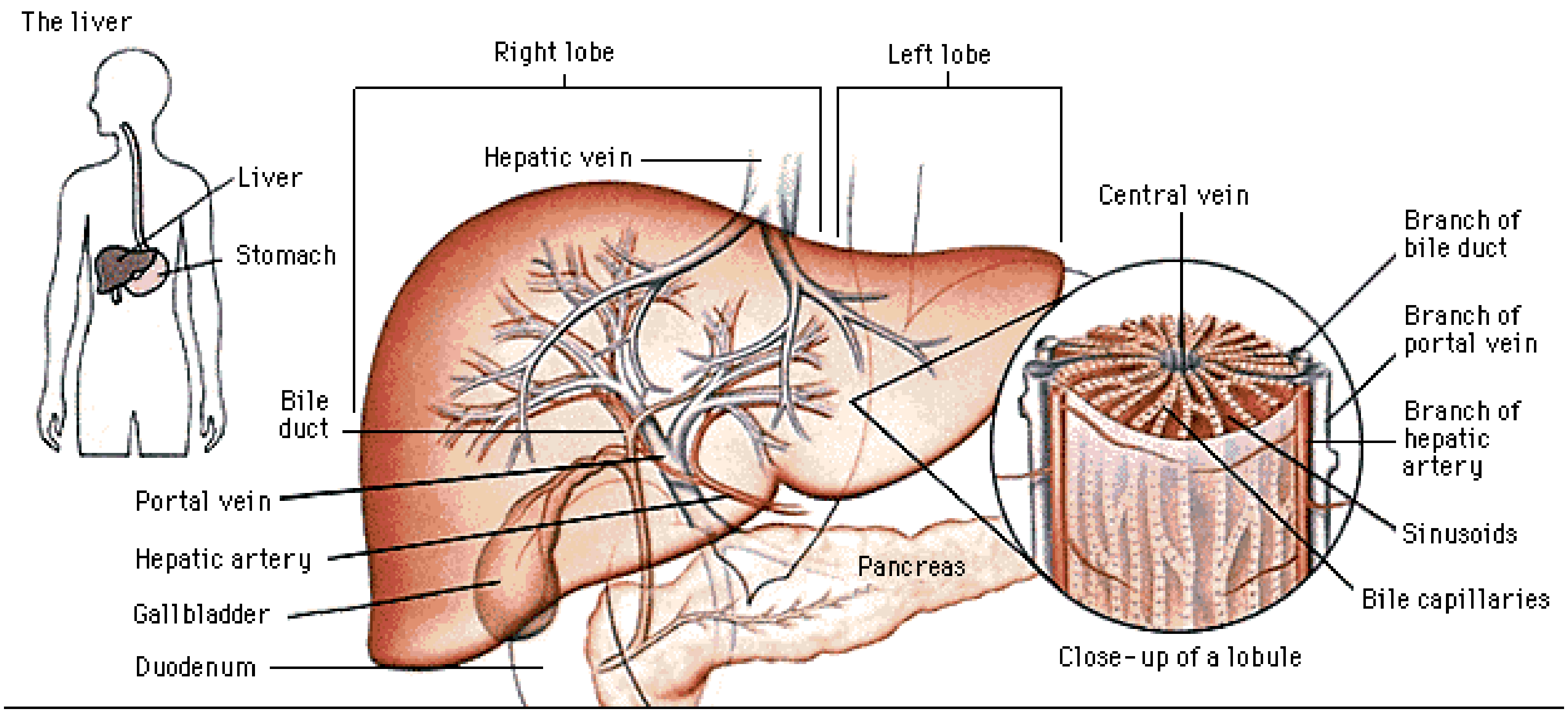
Living tissues: multiscale e multimaterial



Multimaterial processing

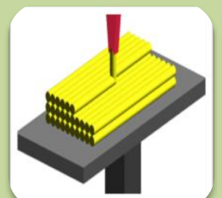
2-Dimensional

Lithography and Soft-Lithography

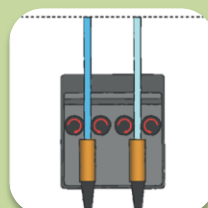
Soft-Molecular Imprinting

Electrospinning

3-Dimensional



PAMsquare



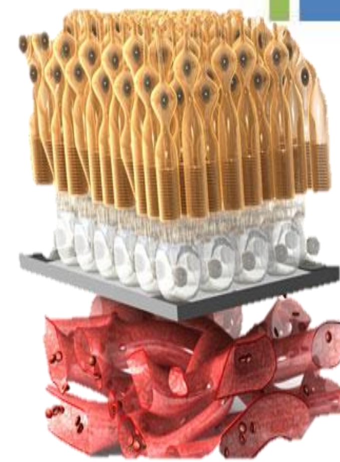
Thermoplastic extrusion

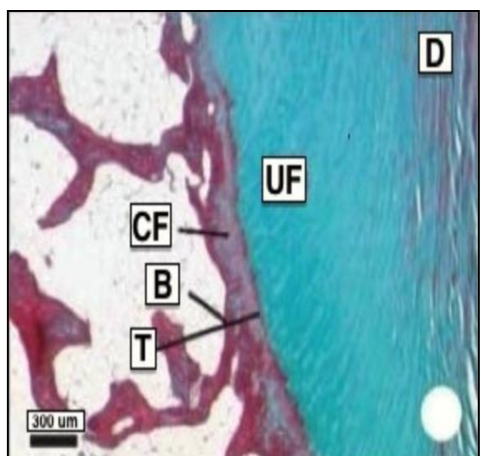
Inkjet Printing

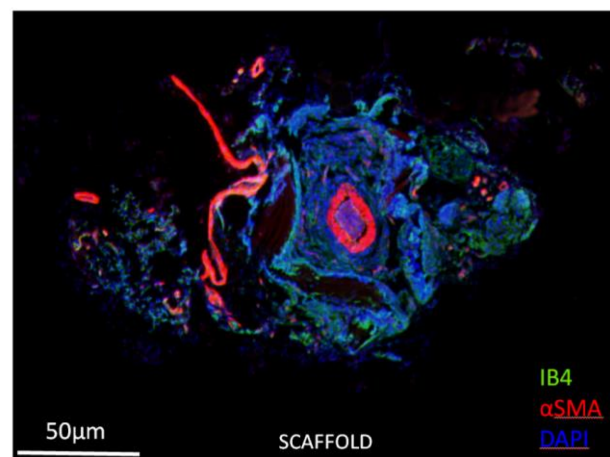
Combination of 2D and 3D Technologies

Multimaterial biofabrication for tiisue model

- Human skin
- Bone ligament interface
- Angiogenesis
- Retinal Pigment Epithelium of human eye







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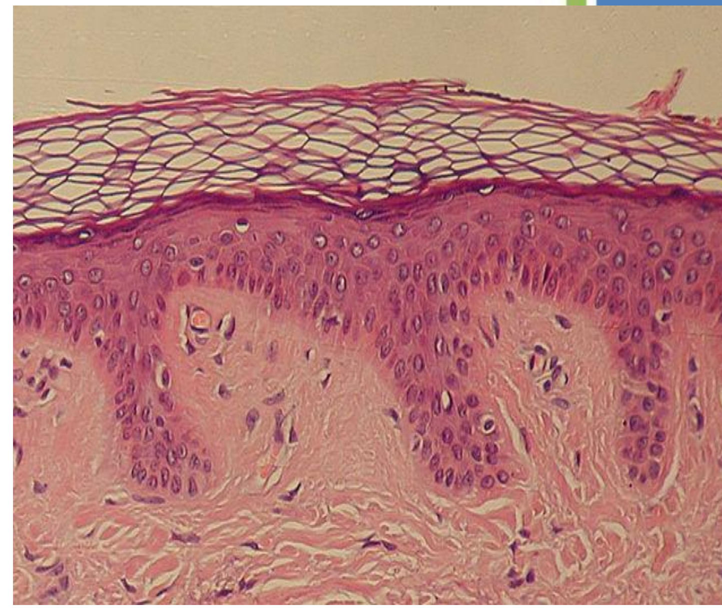
Aim of the work

- Validate keratin from chicken feathers as biomaterial
- Propose an added-value products from animal waste
- In vitro model of human skin

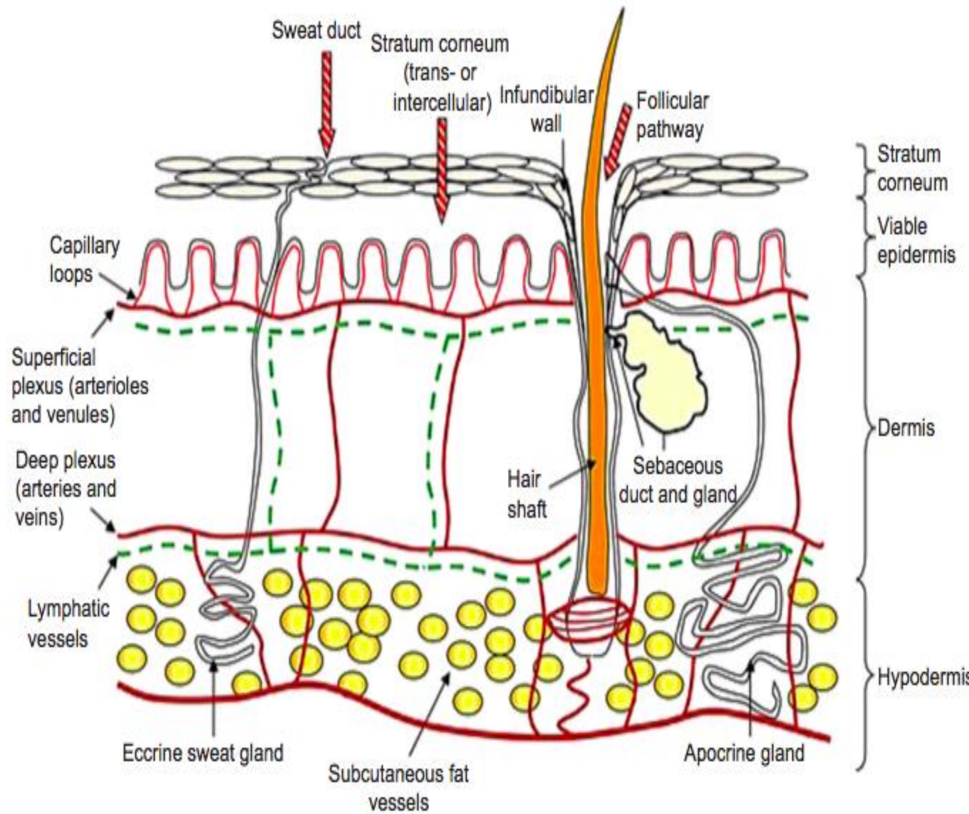


IN VITRO MODEL OF HUMAN SKIN

De Maria C. et al. *Hydroxylated Keratin from chicken feather as novel substrate for in vitro tissue model fabrication.*
Biofabrication 2017 – Beijing, China, Oct 15-18 2017

Skin in vitro model

- Applications
  - Topical formulation development
  - Skin irritancy and toxicity
  - Skin disease model
- Requirements
  - Availability (storage)
  - easiness of use
  - cost
  - limitations (functions, including mechanical properties and ethics)



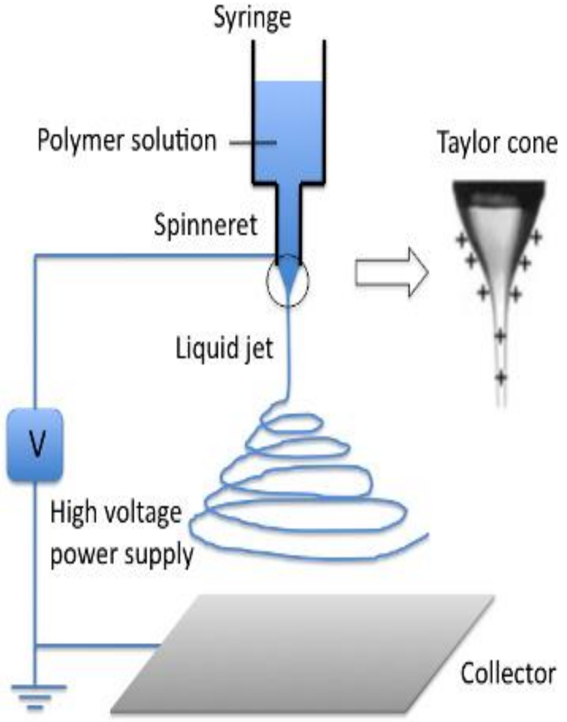
Skin model: state of the art

Table 1 Skin models

Model	Advantages	Disadvantages
Human skin		
In vivo	Gold standard	Often precluded for ethical and practical reasons
Ex vivo skin	Best surrogate for in vivo humans	Not readily available, variability
Animal skin		
In vivo	Reasonably easy to obtain animals, can be scaled up to humans, hairless species available	Pigs: similar barrier to humans, but difficult to handle Rodents: different barrier properties from humans
In vivo chimeric model	Human skin xenografts on mice allows testing on living human skin	Technically difficult
Ex vivo skin	Easy to obtain	Different barrier properties, variability
Artificial membranes		
Simple polymeric models	Useful for studying basic diffusion mechanisms, consistent and homogenous	Not representative of human skin
Lipid-based models	Useful for screening	Not representative of human skin
Reconstructed skin models		
Reconstructed human epidermis	Built-in barrier properties	Usually more permeable than human skin
Living skin equivalents	Can be engineered to include a range of normal or disease features	Usually more permeable than human skin

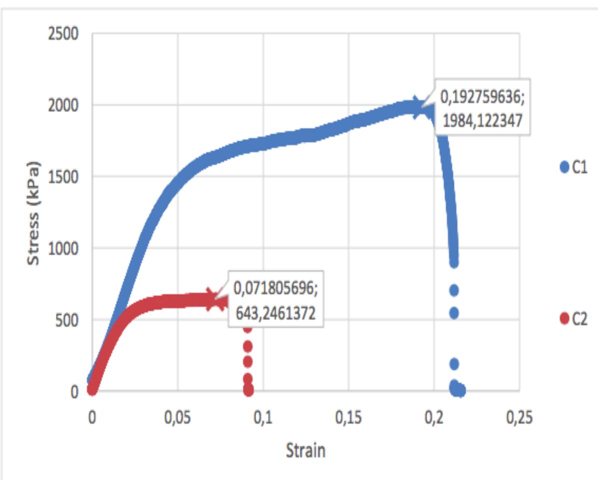
Abd E. et al. *Skin models for the testing of transdermal drugs.* Clin Pharmacol. 2016 Oct 19;8:163-176.

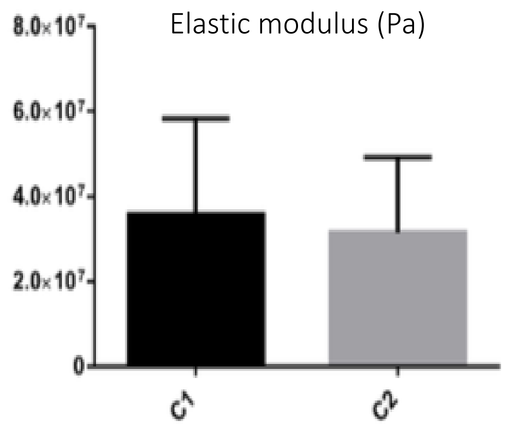
Keratin-based electrospun films

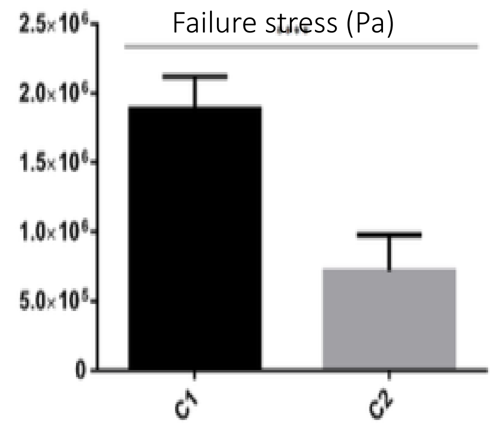


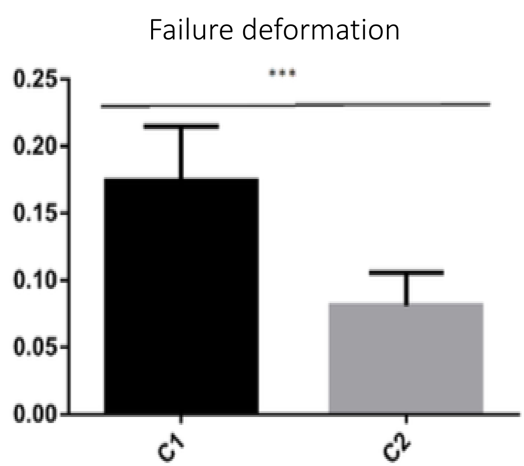
Mechanical characterization

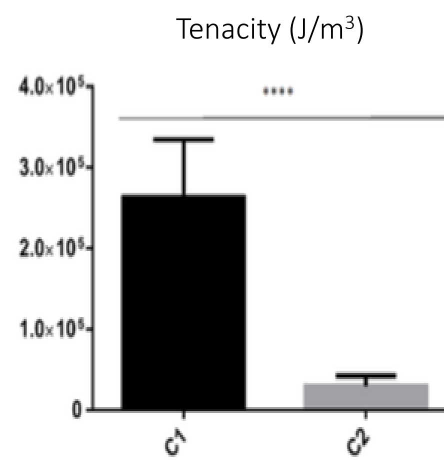
Composition	Distance (cm)	Flow (ml/h)	Voltage(kV)	TIme (h)
2:1	20	1	30	2
1:1	20	1	30	2







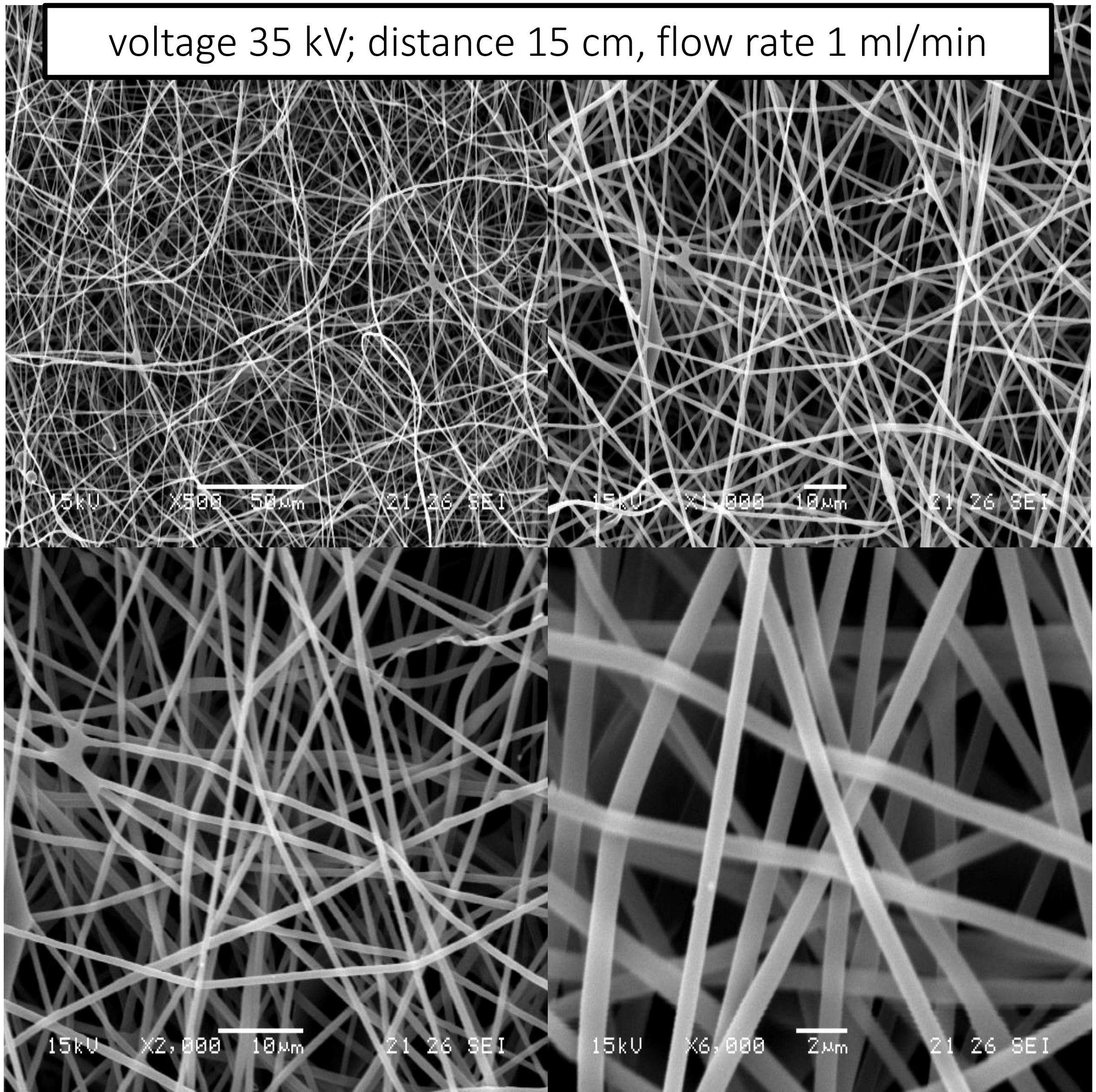


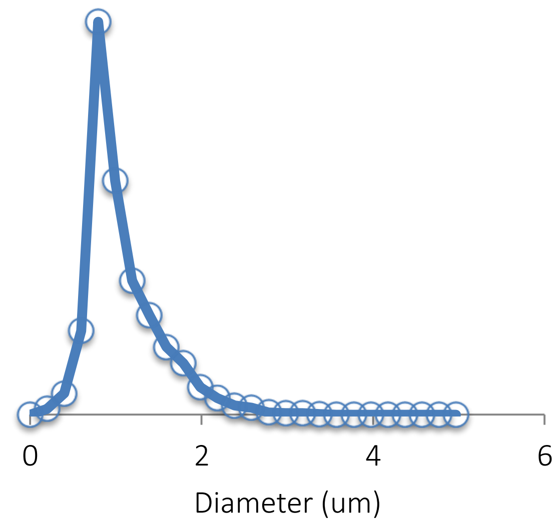


The elastic modulus of electrospun structures is in the range [30-70] MPa, similar to skin

Fibers analysis

voltage 35 kV; distance 15 cm, flow rate 1 ml/min





Uniform orientation
Porosity: 48%

Analysed with ImageJ

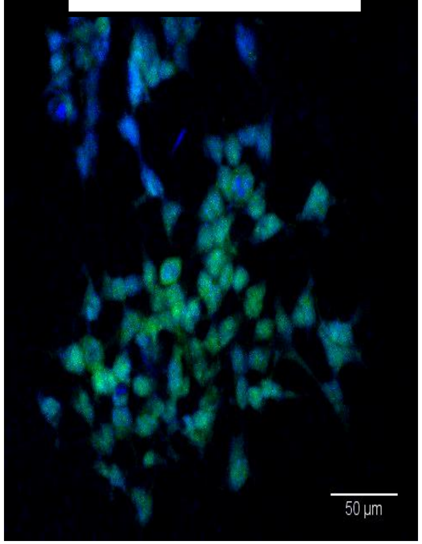
Biological validation

- Tests were conducted on spuns obtained with the following parameters:
  voltage 35 kV; distance 15 cm, flow rate 1 ml/min
- <u>Human epithelial</u> HEK-293T cells,
- <u>Rat neuronal</u> RT4-P6D2T cells
- Density 20000 cells/well in 12well plate on keratin/gelatin electrospun
- Cultured in DMEM-10%FBS (Invitrogen) for 1, 2 and 4 days.

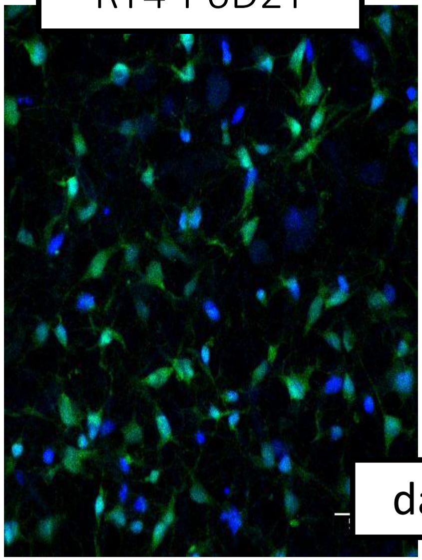
Cell vitality

- CellTracker ™Green CMFDA (5µM in PBS, Invitrogen) was added for 1,5 hours before fixation

HEK-293T

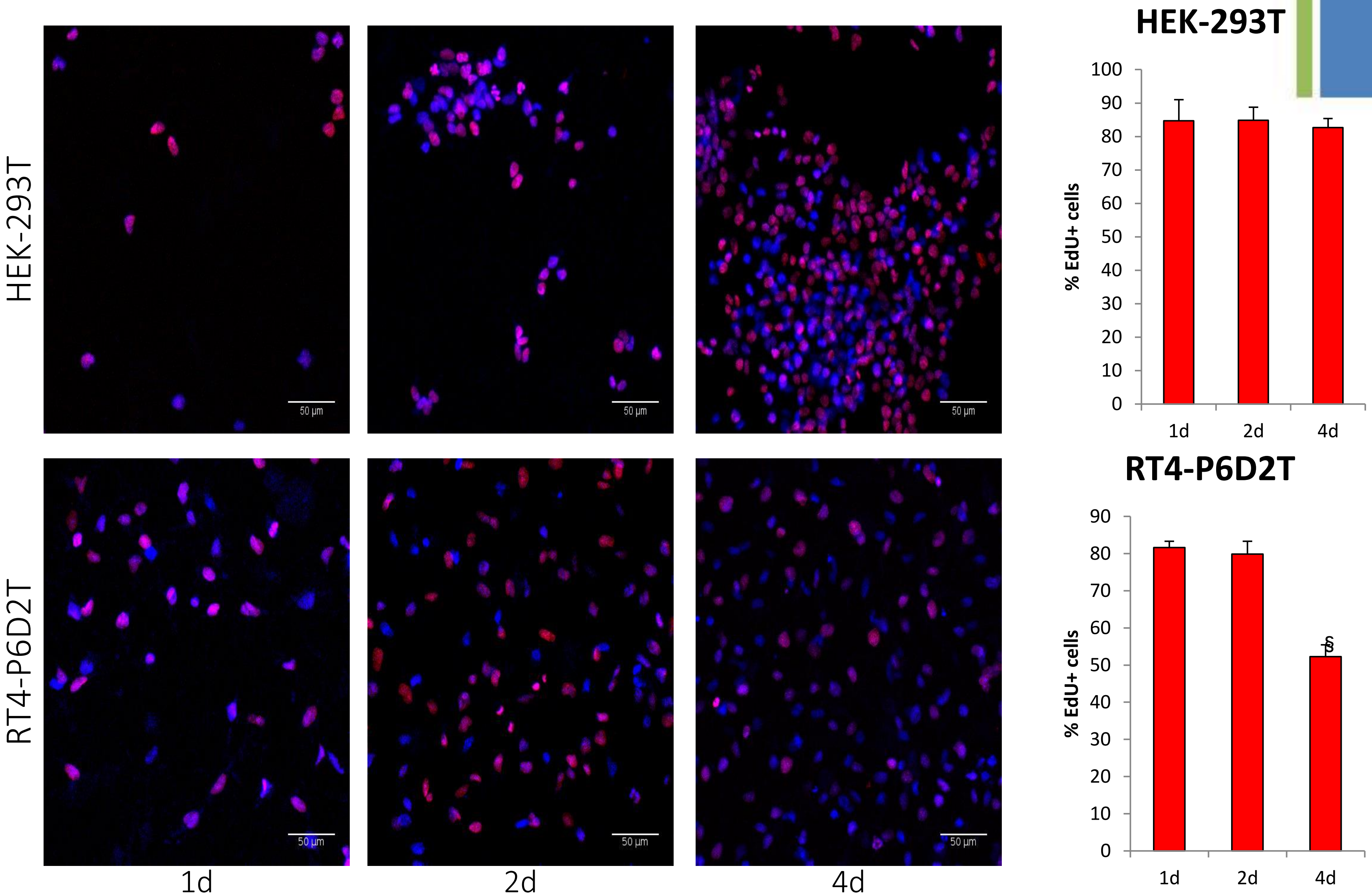


RT4-P6D2T



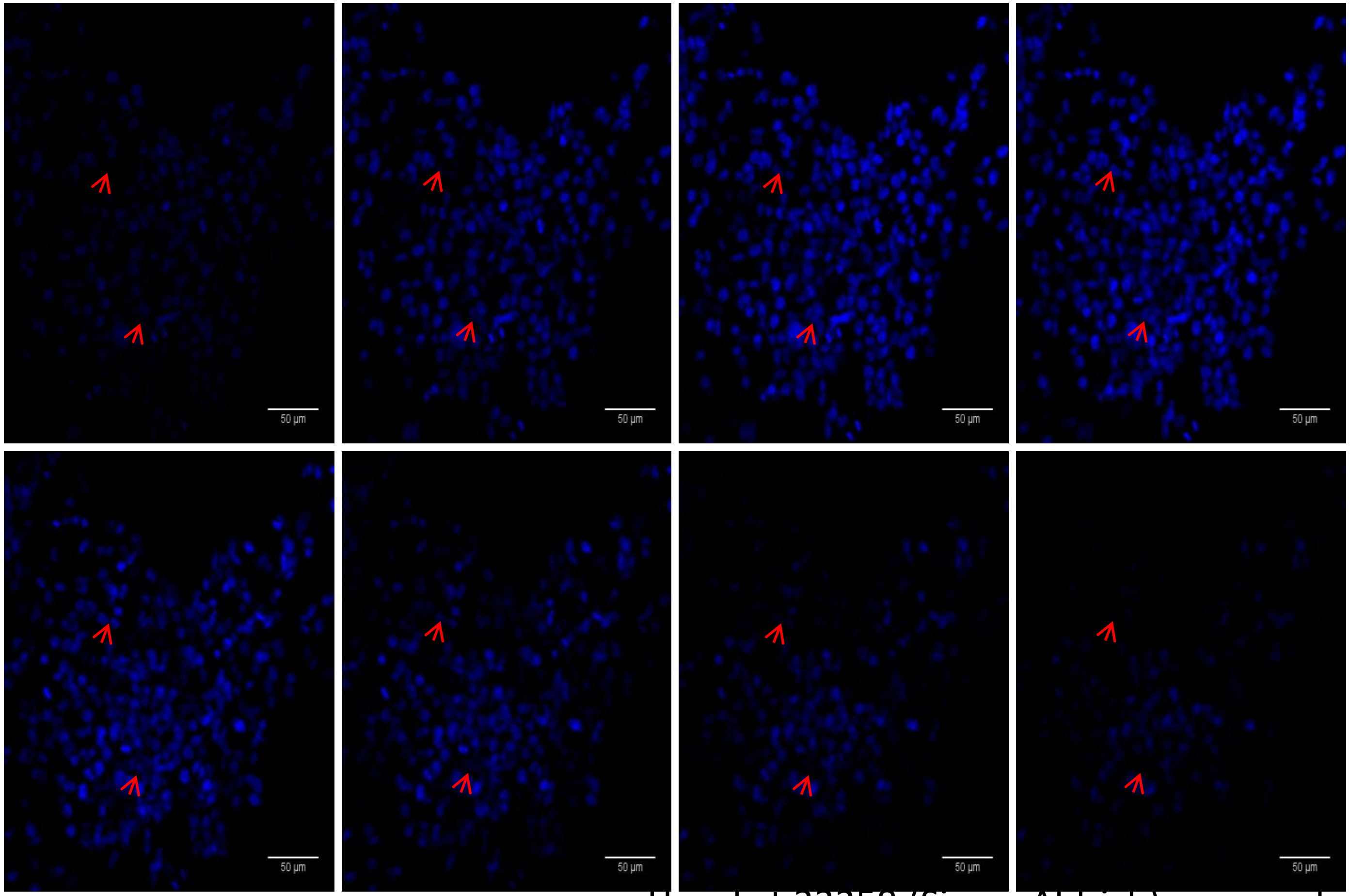
day4

EdU Proliferation assay



Cell penetration into the scaffold

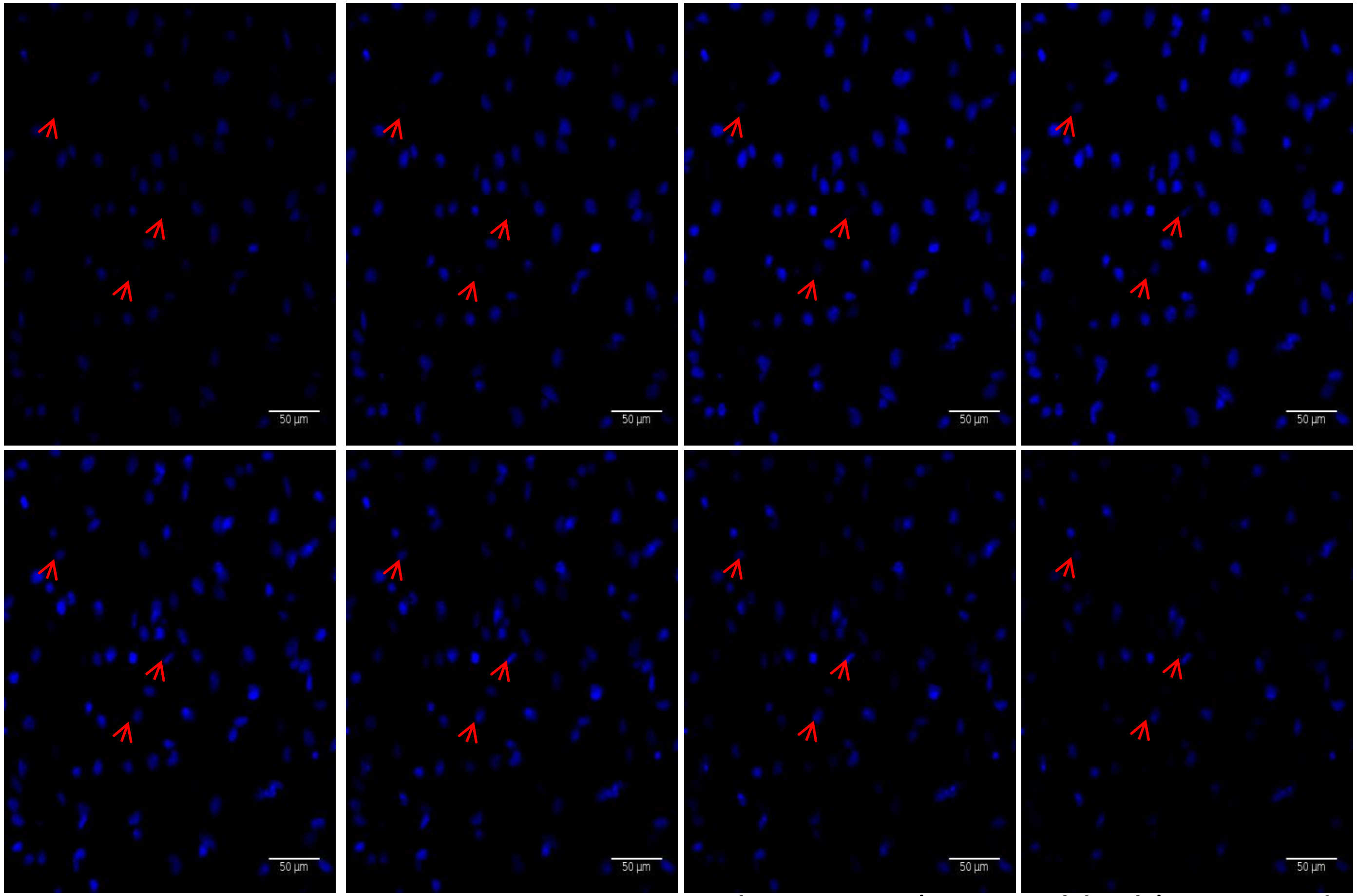
- HEK-293T at day4



Hoechst 33258 (Sigma Aldrich) was used as nuclear counterstain

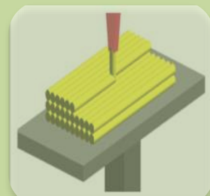
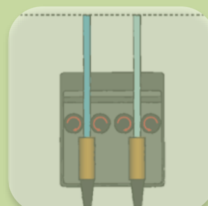
Cell penetration into the scaffold

- RT4P6D2T at day5



Hoechst 33258 (Sigma Aldrich) was used as nuclear counterstain

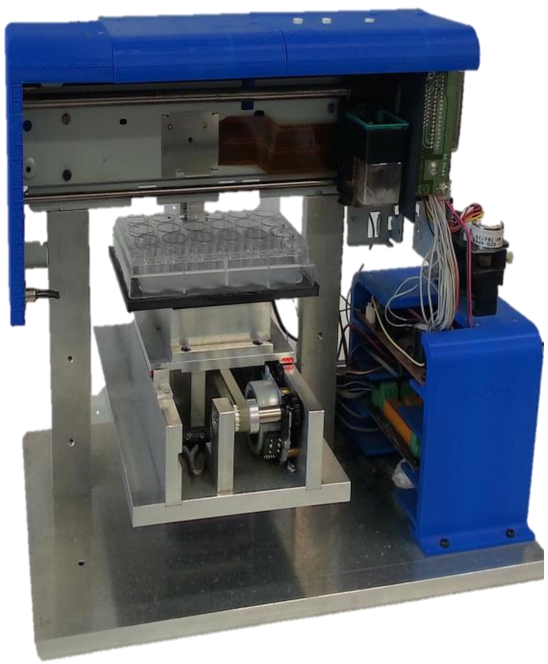
Multimaterial processing

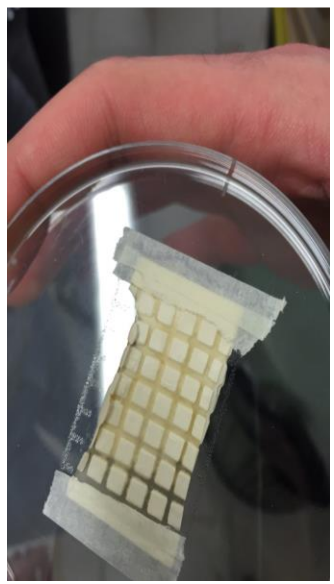
2-Dimensional		3-Dimensional	
	Lithography and Soft-Lithography		PAMsquare
	Soft-Molecular Imprinting		Thermoplastic extrusion
	Electrospinning		Inkjet Printing

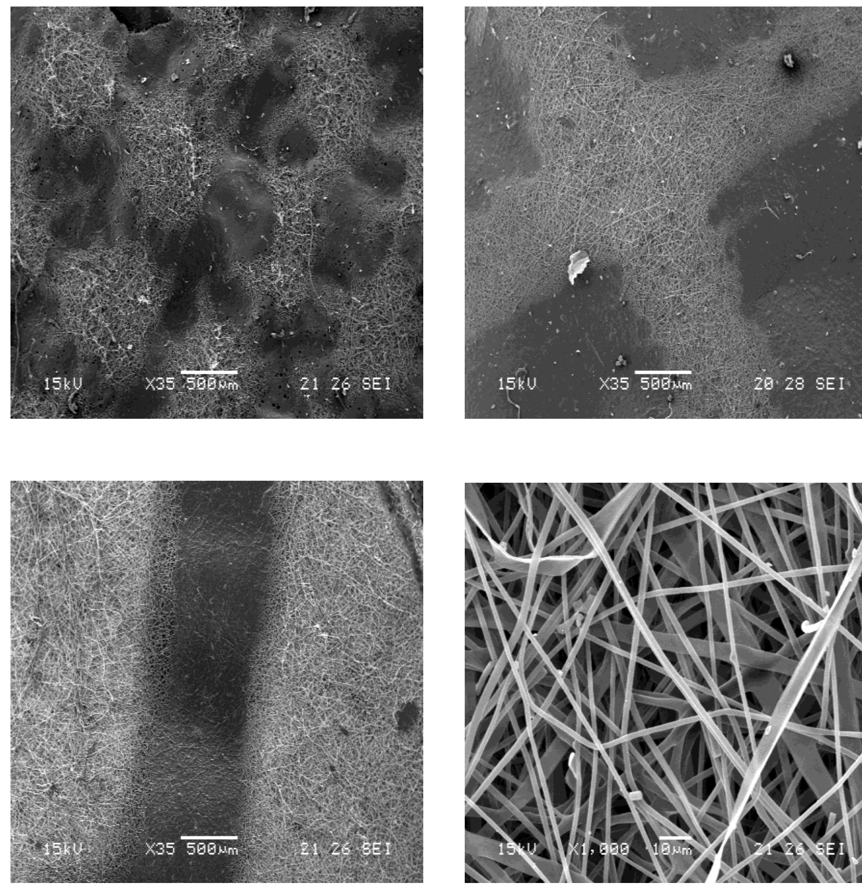
Combination of 2D and 3D Technologies

Multiscale and multimaterial scaffold

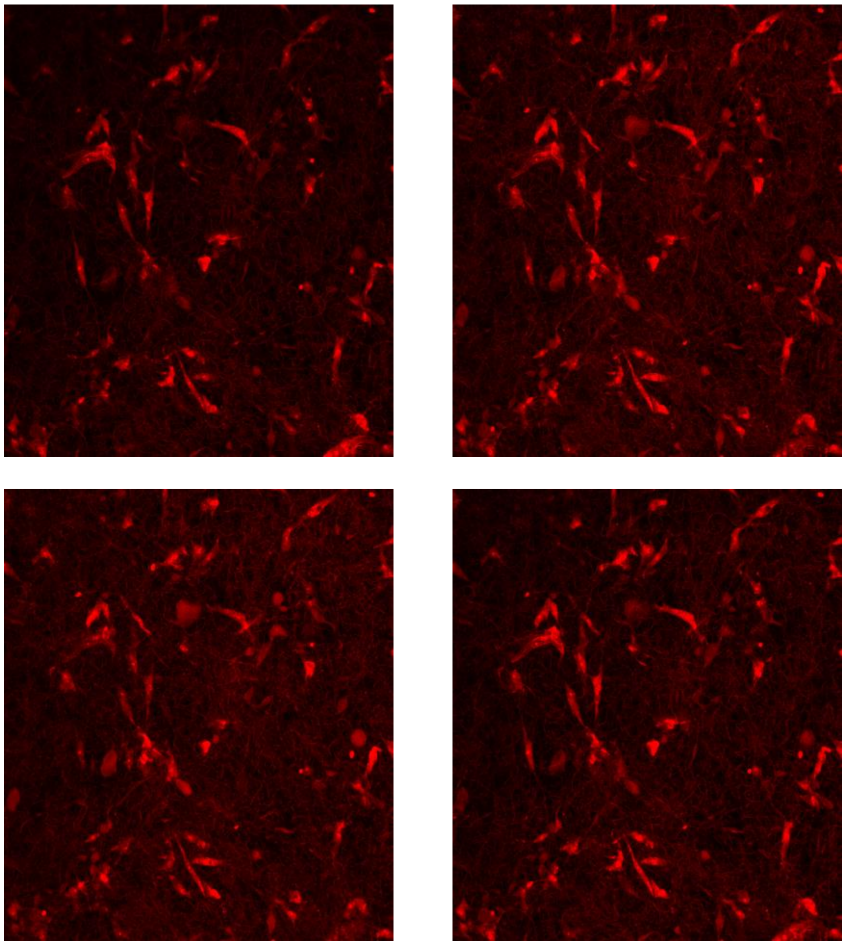
- Electrospinning and inkjet printing
- Introducing micro features on to nanofibers

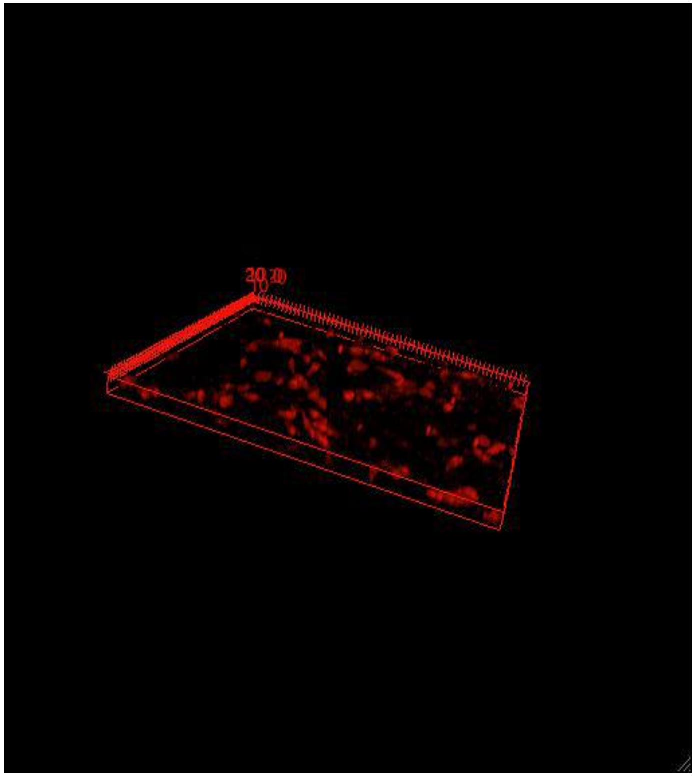


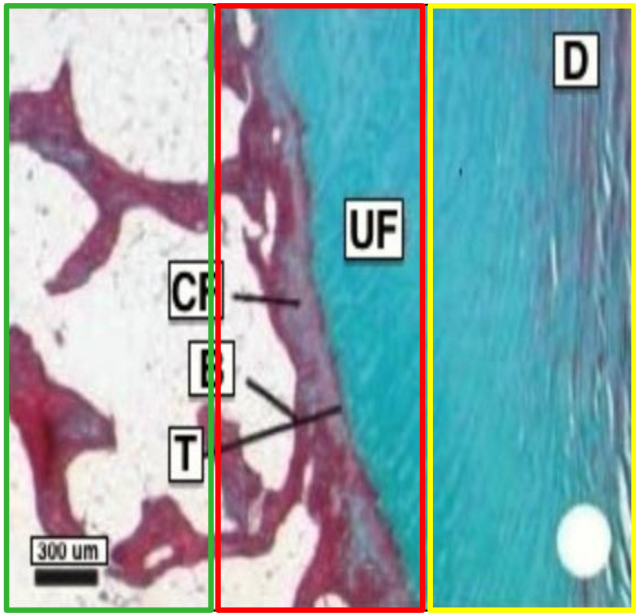




Multiscale and multimaterial scaffold





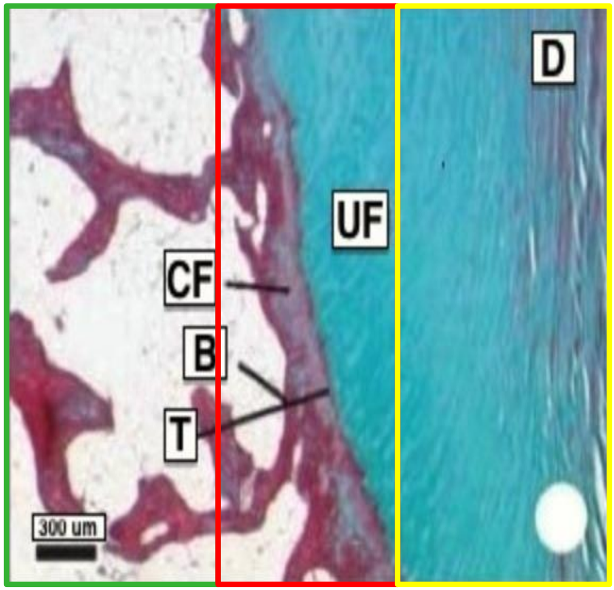


BONE LIGAMENT INTERFACE

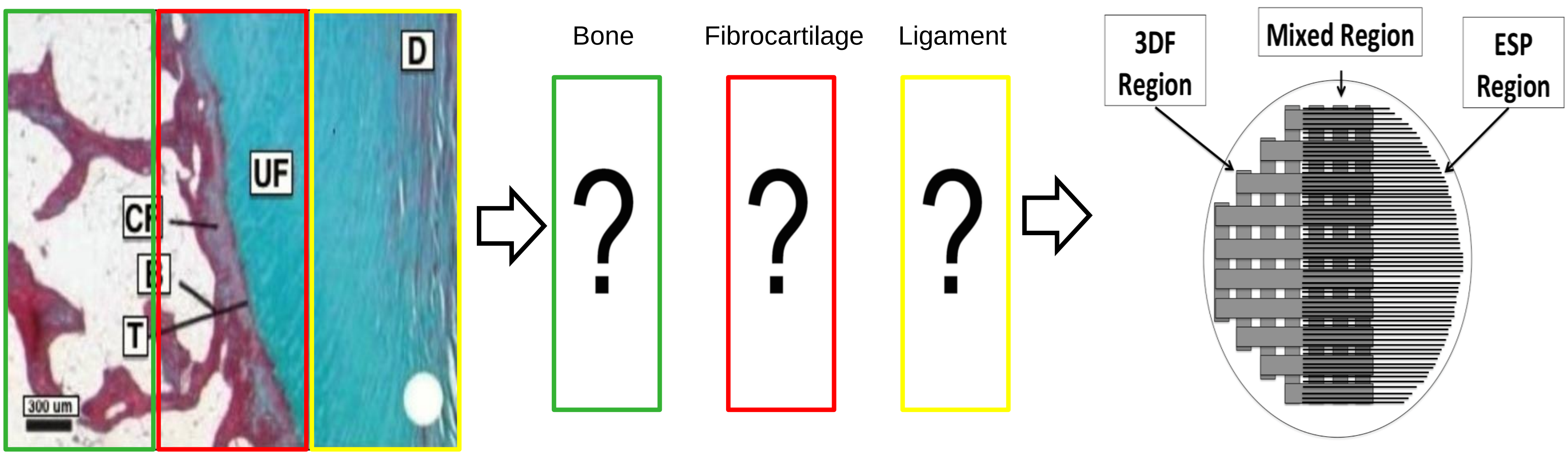
Criscenti G. et al. *Triphasic scaffolds for the regeneration of the bone-ligament interface*. Biofabrication. 2016 Jan 29;8(1):015009.

Bone-ligament interface

- Enthesis:
  - also know as “insertion” or “attachment” site, or “osteotendinous” or “osteoligamentous” junction is the region where a tendon, ligament or joint capsule attaches to bone
- Fibrocartilagious enthesis
  - three different regions at the bone-tendon interface:
    1. Dense fibrous connective tissue (D)
    2. Uncalcified / calcified fibrocartilage(UF/CF)
    3. Bone (B)
  - **Gradient of topological, chemical and mechanical properties**



Triphasic scaffold for bone-ligament interface



Multimaterial processing

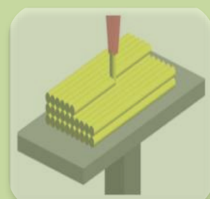
2-Dimensional

Lithography and Soft-Lithography

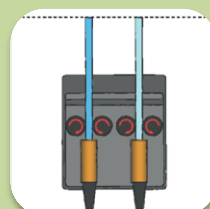
Soft-Molecular Imprinting

Electrospinning

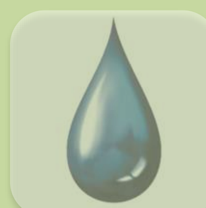
3-Dimensional



PAMsquare



Thermoplastic extrusion

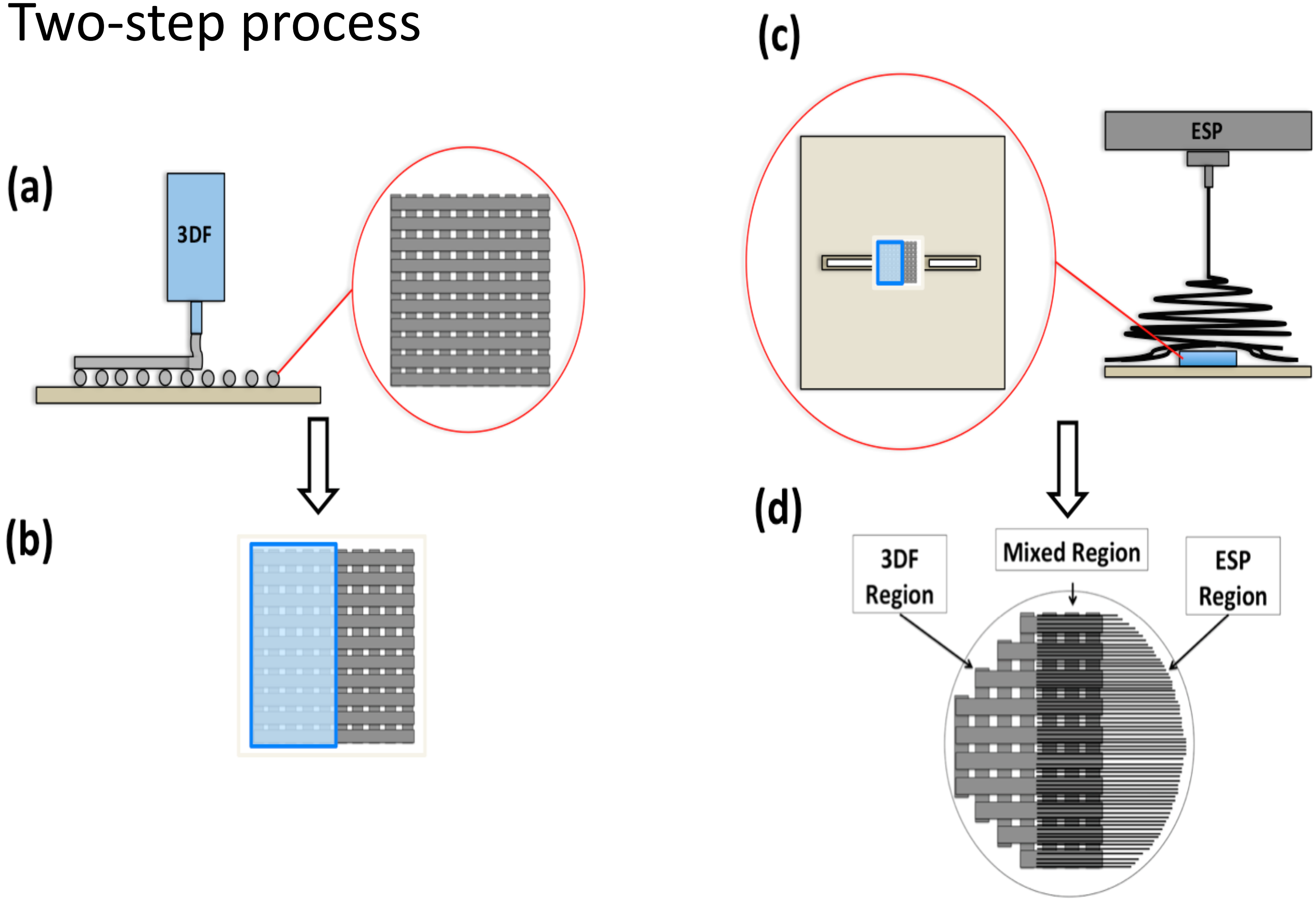


Inkjet Printing

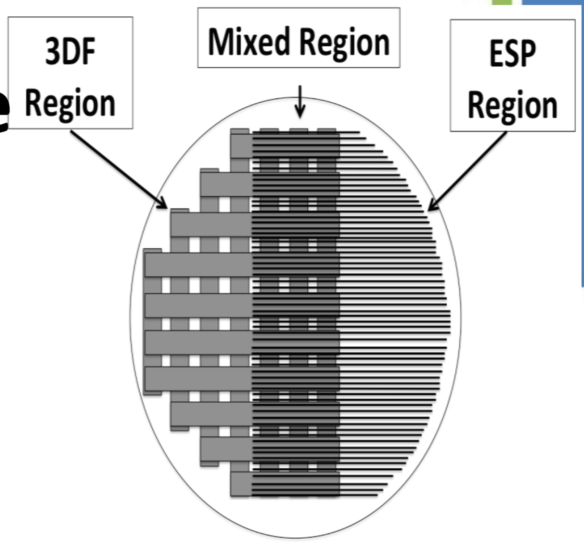
Combination of 2D and 3D Technologies

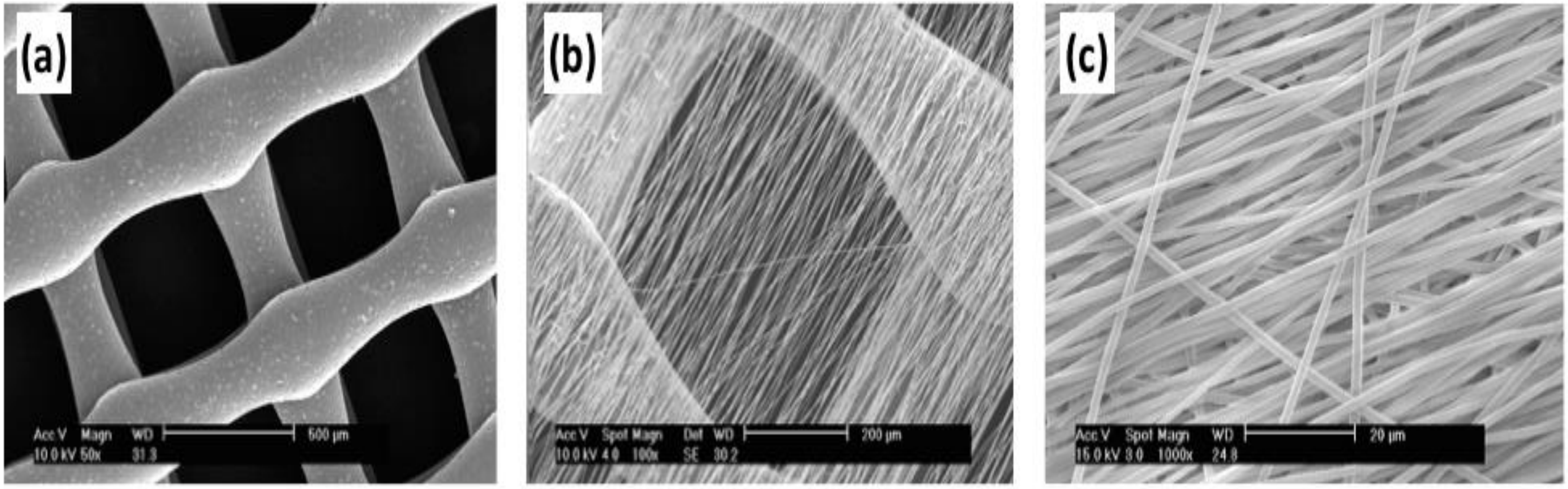
Triphasic scaffold for bone-ligament interface

- Two-step process



Triphasic scaffold for bone-ligament interface



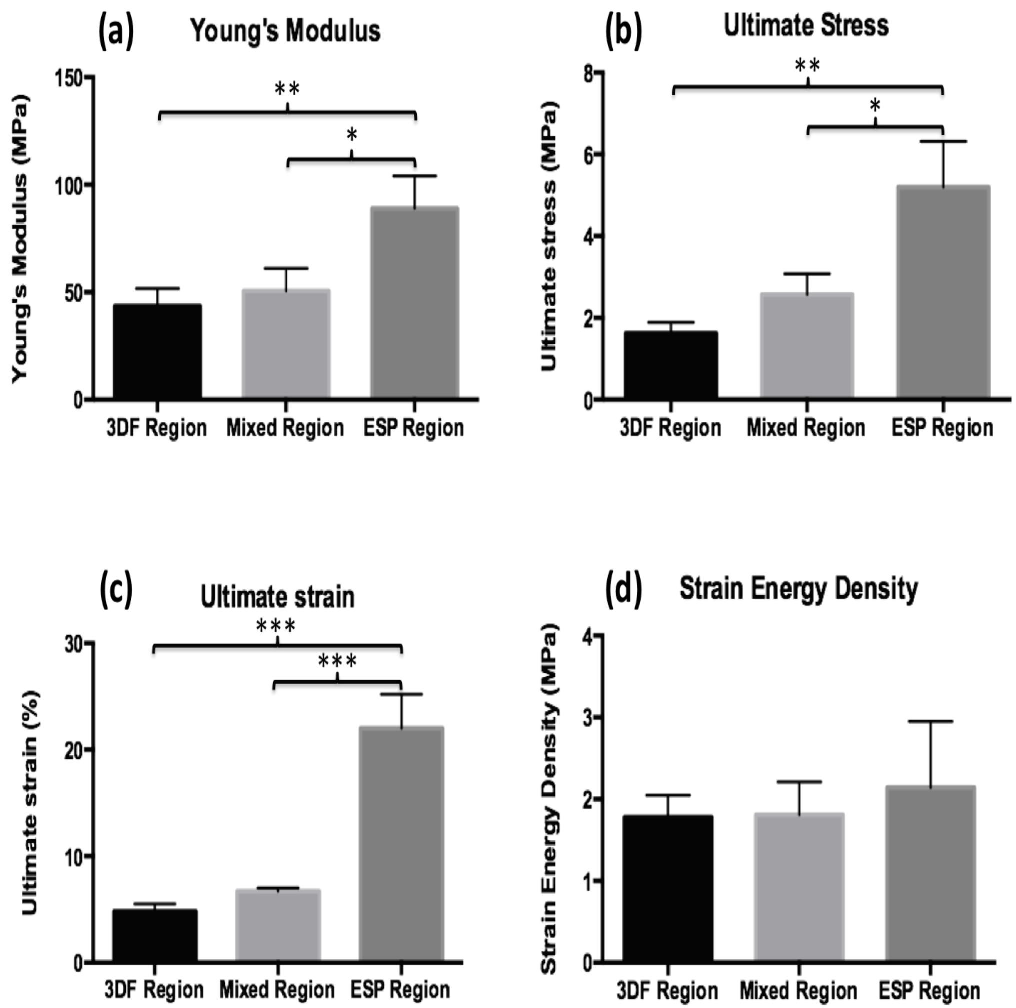


P = 77 ± 1.7%

P = 68.9 ± 3.1%

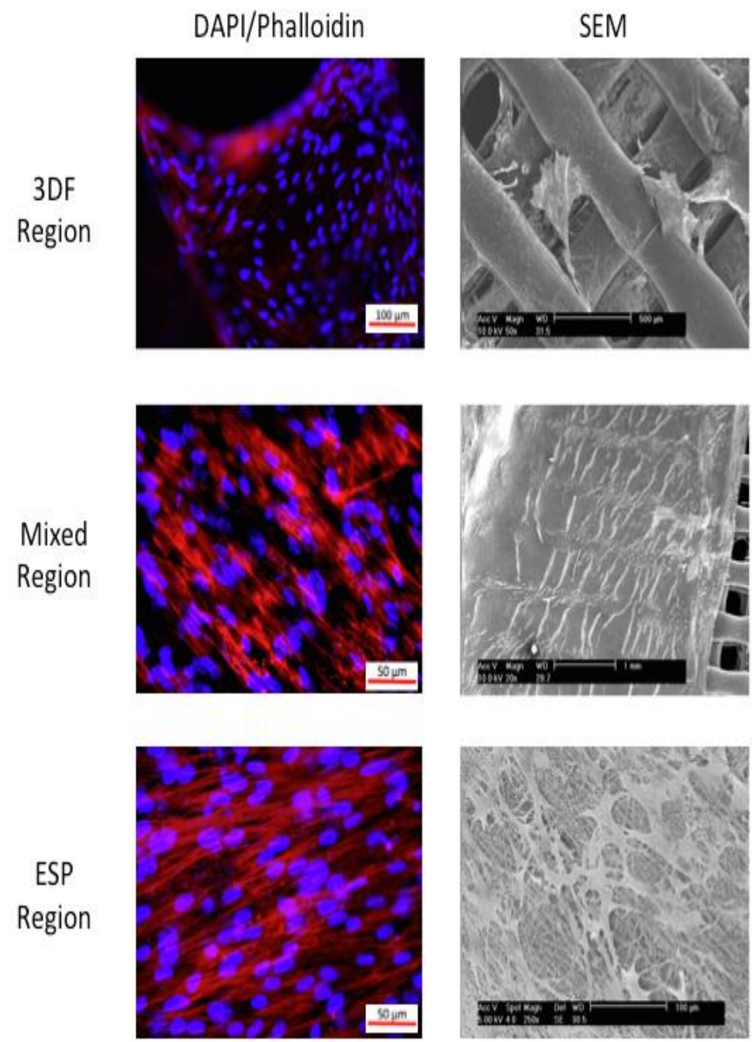
P = 95.9 ± 1.6%

Triphasic scaffold for bone-ligament interface



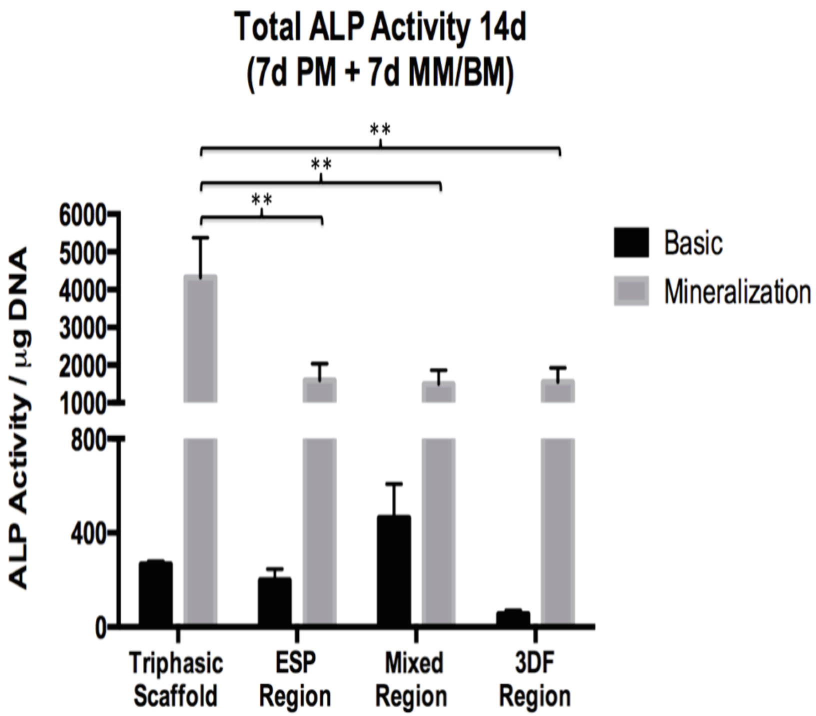
Triphasic scaffold for bone-ligament interface

- hMSCs are an easily available cell source at the implantation site in orthopaedic applications
- hMSCs were homogeneously distributed on the entire scaffold and at the interface areas
- In the ESP mesh cells are well spread, whereas on the 3DF region they connect the fibers by bridging the pores.

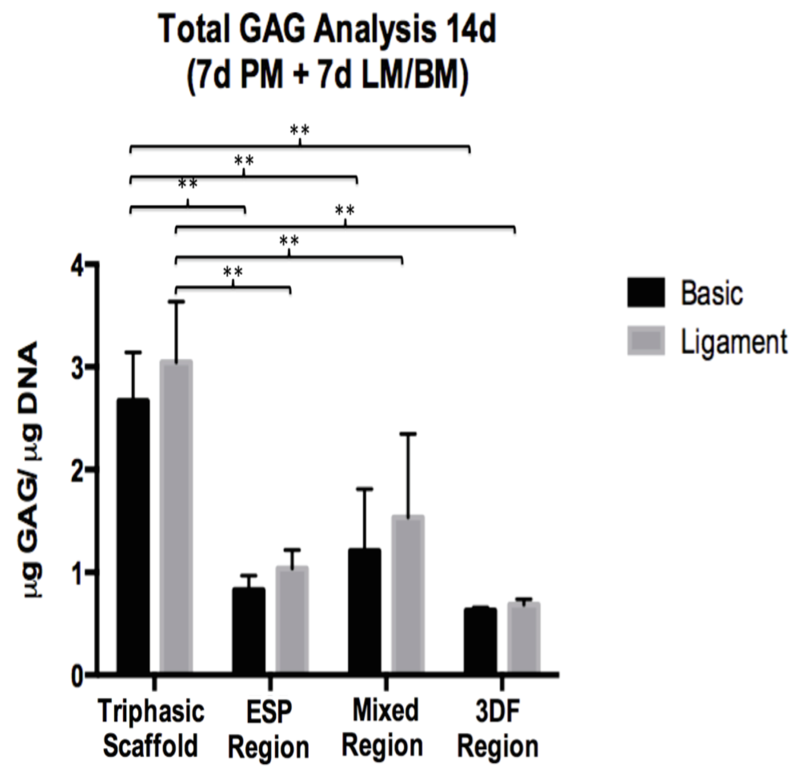


+

Triphasic scaffold for bone-ligament interface

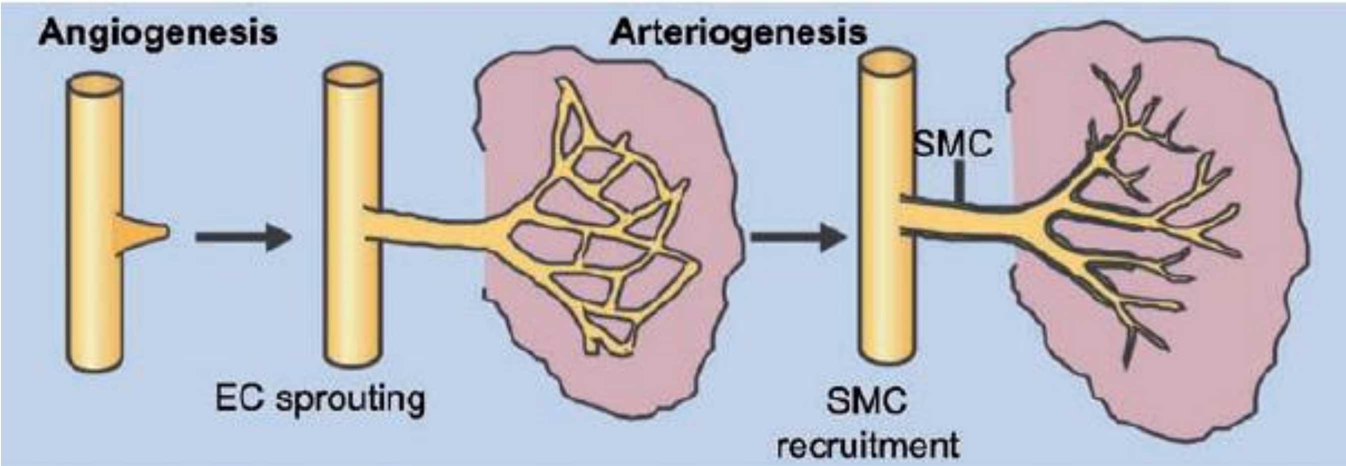


Total ALP activity after 7 day is PM and other 7 days in BM/MM. Controls were represented by scaffolds composed by only one region, thus being only eletrospun, 3DF and mixed structures.



Total GAGs after 7 day is PM and other 7 days in BM/LM. Controls were represented by scaffolds composed by only one region, thus being only electrospun, 3DF and mixed structures.

+



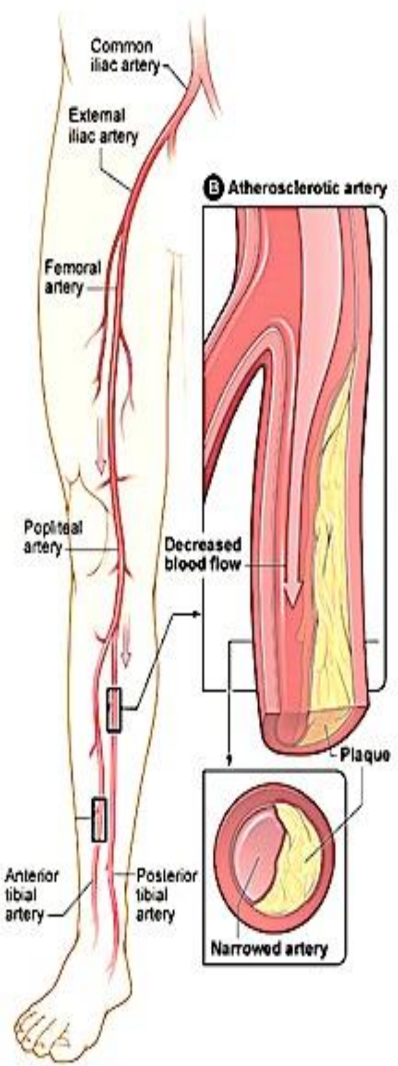
STIMULATING REPARATIVE ANGIOGENESIS FOR PERIPEHRAL ARTERY DISEASES

Carrabba et al.
Design, fabrication and perivascular implantation of bioactive scaffolds engineered with human adventitial progenitor cells for stimulation of arteriogenesis in peripheral ischemia.
Biofabrication. 2016 Mar 24;8(1):015020.

+

Peripheral artery disease

- Incidence over 50 years of:
  - 1 in 5 man
  - 1 in 8 woman
- Critical Limb Ischemia
- Traditional medical approach:
  - Drugs
  - Surgical angioplasty
- Tissue Engineering:
  Design and development of a scaffold able to stimulate reparative angiogenesis in a mouse model of peripheral ischemia



Multimaterial processing

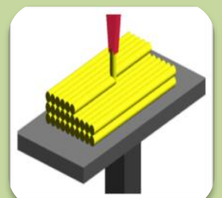
2-Dimensional

Lithography and Soft-Lithography

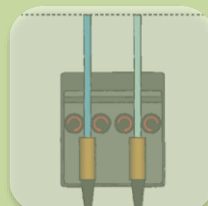
Soft-Molecular Imprinting

Electrospinning

3-Dimensional



PAMsquare



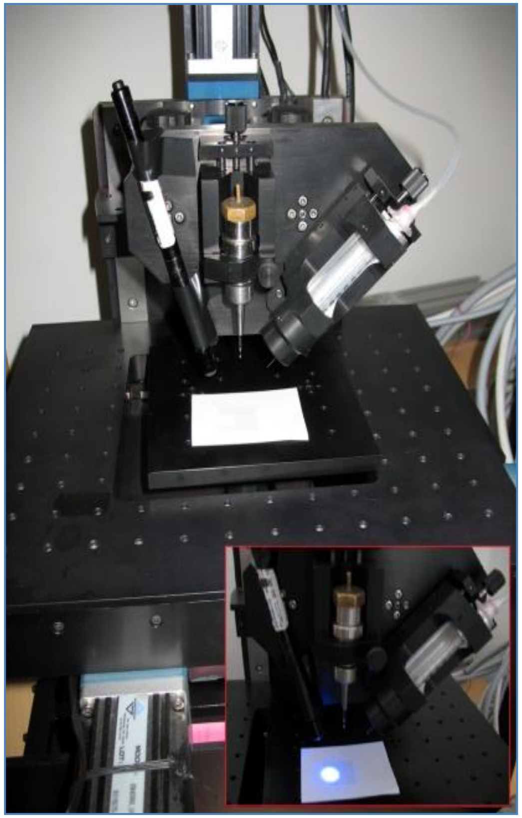
Thermoplastic extrusion

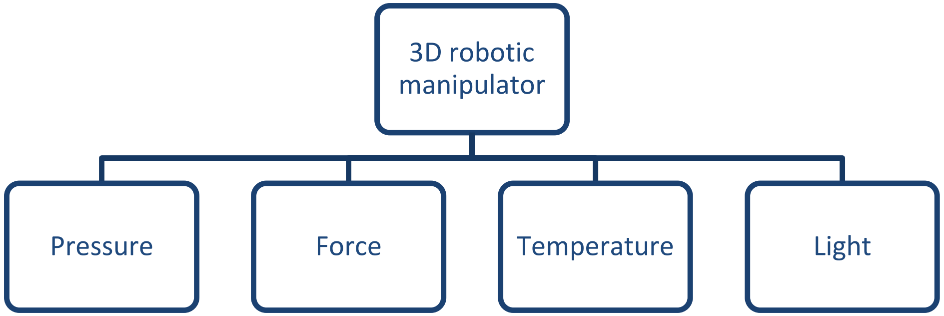
Inkjet Printing

Combination of 2D and 3D Technologies

PAM^2

- Modular CAD/CAM system
- A 3-axes robotic manipulator:
  - position ±50 mm;
  - velocity 0-15 mm/s;
  - resolution 1 µm;
  - different extrusion modules;
  - layer-by-layer processing.

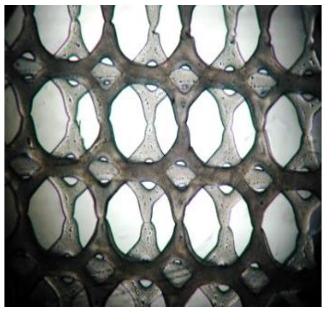


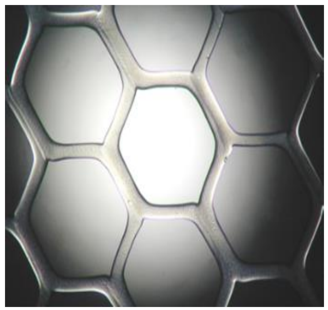


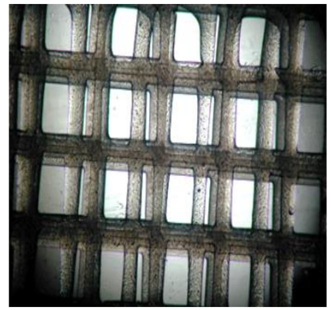
Tirella A, De Maria C, Criscenti G, Vozzi G, Ahluwalia A. The PAM^2 system: a multilevel approach for fabrication of complex three-dimensional microstructures. Rapid Prototyping J 2012;18(4):5-5

PAM²

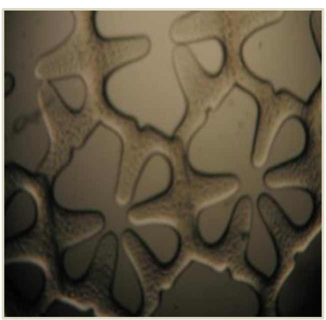
Polyester structures

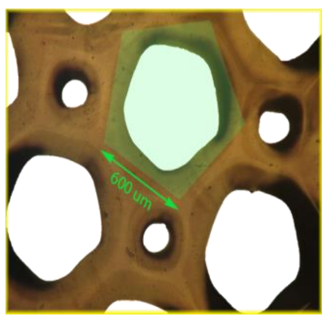


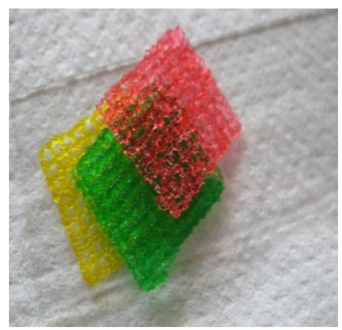




Natural polymer hydrogel structures

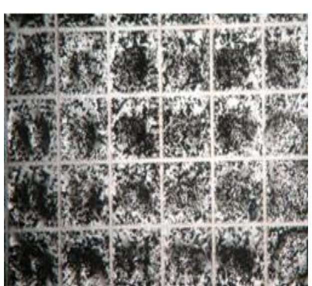


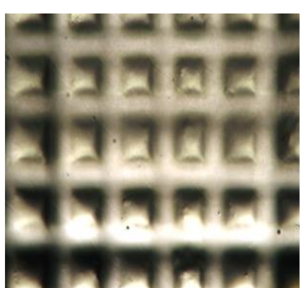


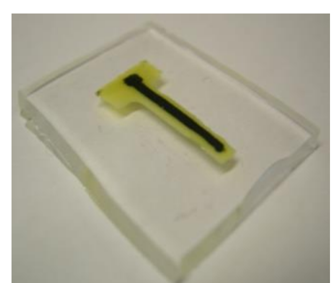


Gel-in-gel printing
(self assembling polypeptide gels)

Laser ablation dry and wet structures

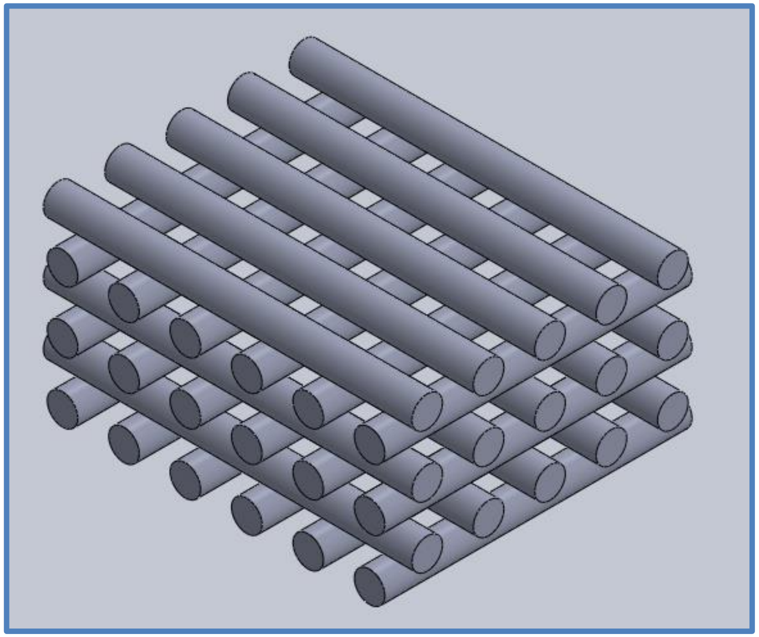




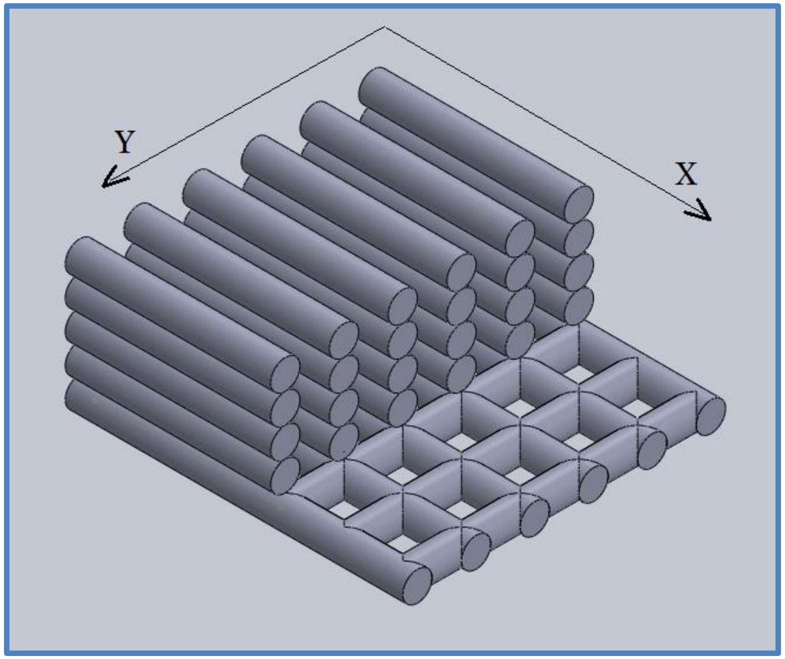


Polymeric actuators
(condutive and dielectric ink)

Polymer structures



Woodpile structure

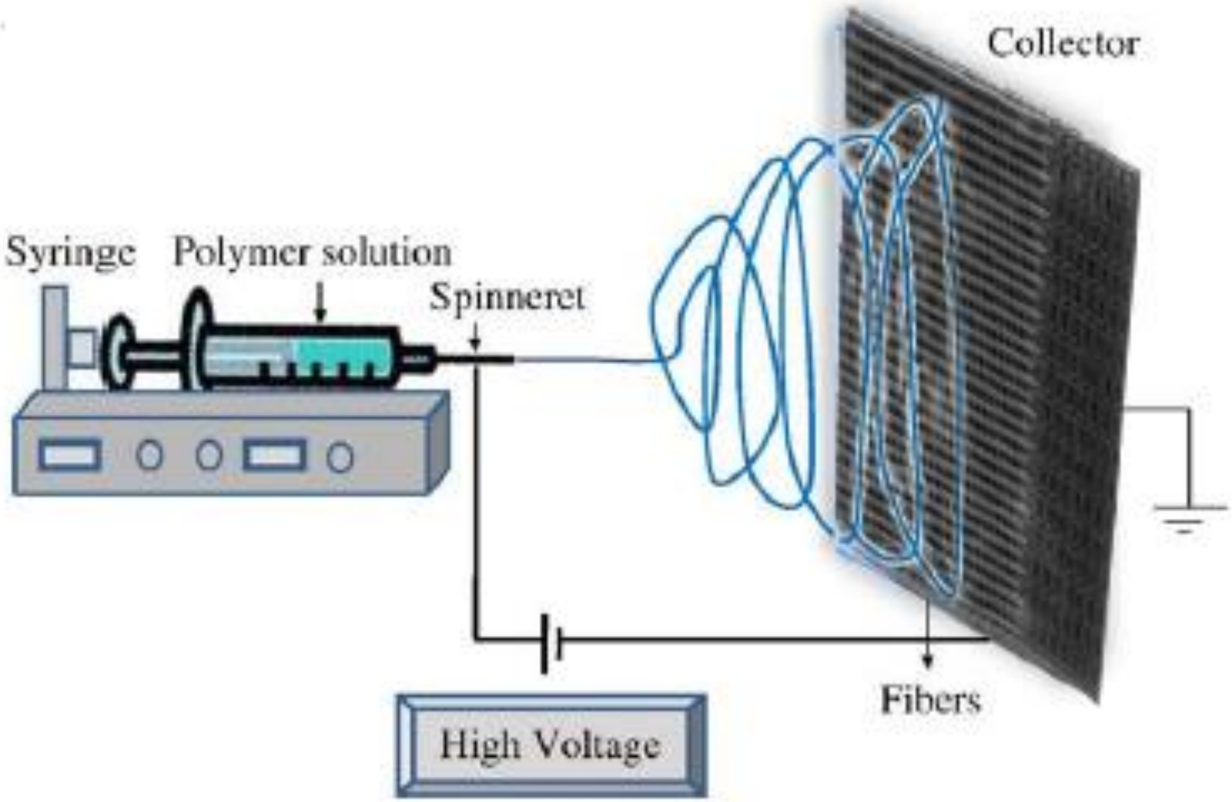


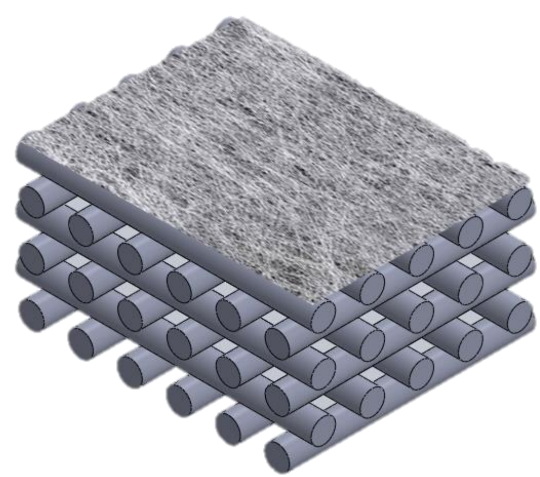
Channel structure

- PCL 10% w/v in chloroform
- Glass needle (⃠90 μm)
- Extrusion pressure 8kPa
- Deposition plate velocity 9 mm/s

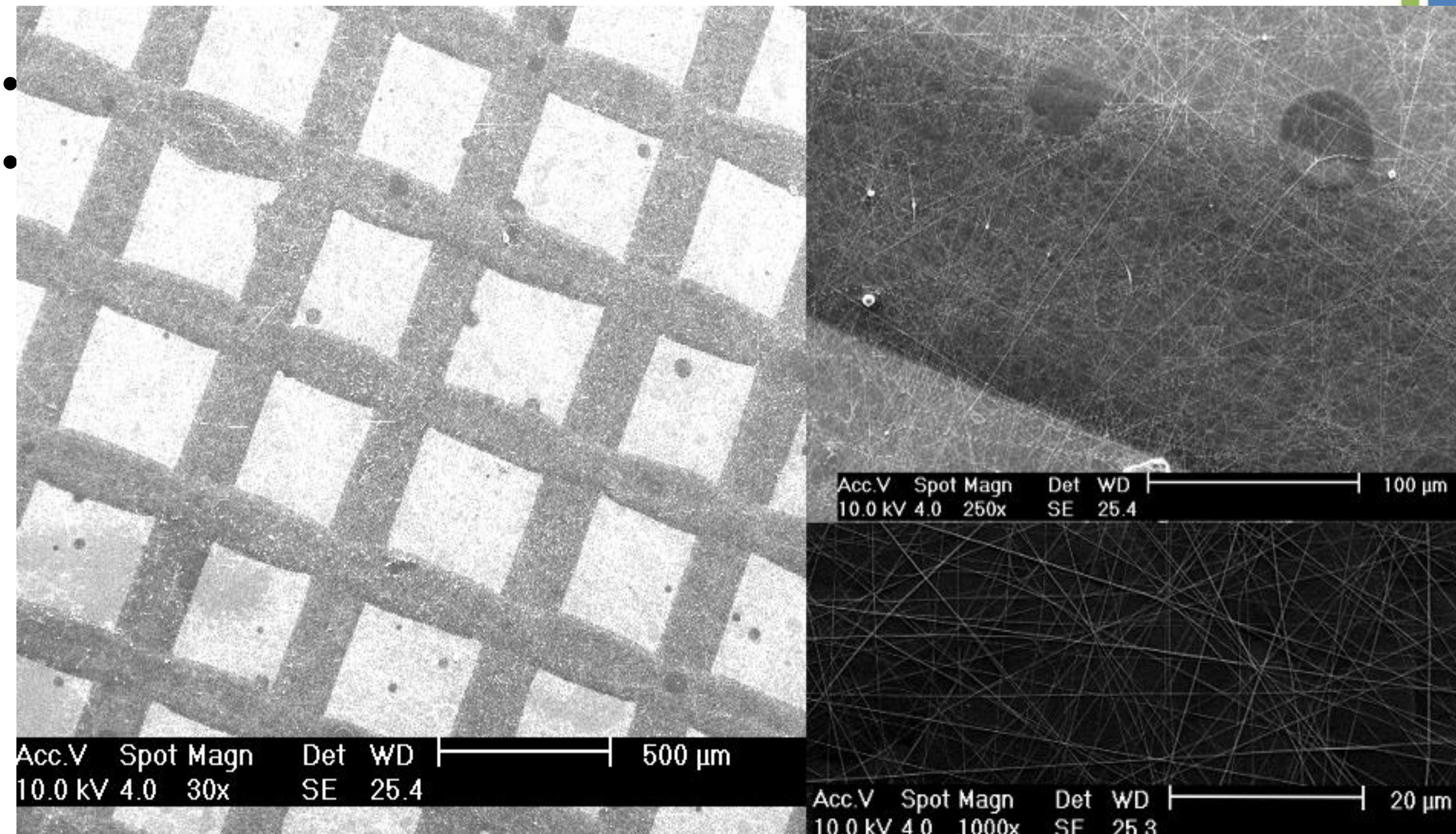
- 6 layers
- Total height 70±5μm

Electrospinning enhanced PAM[2]





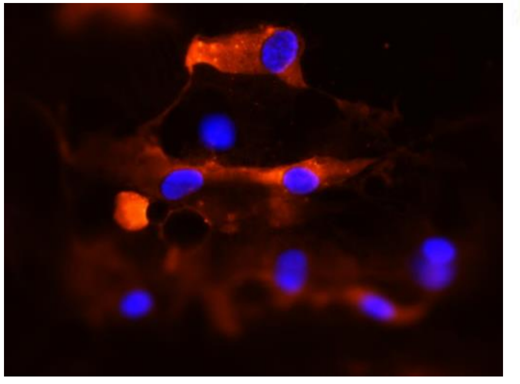
Electrospinning enhanced PAM^2

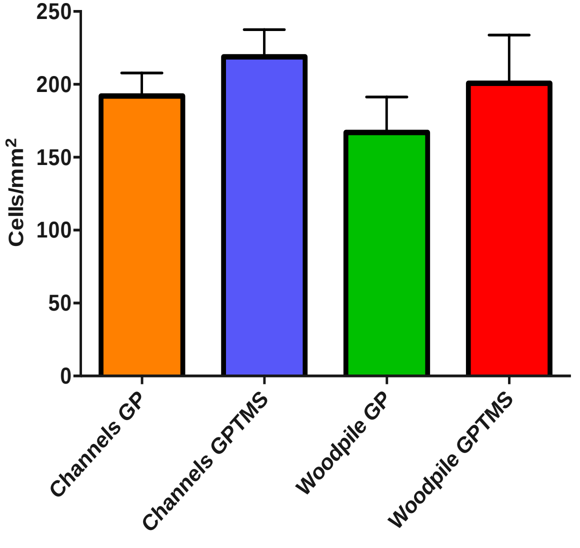


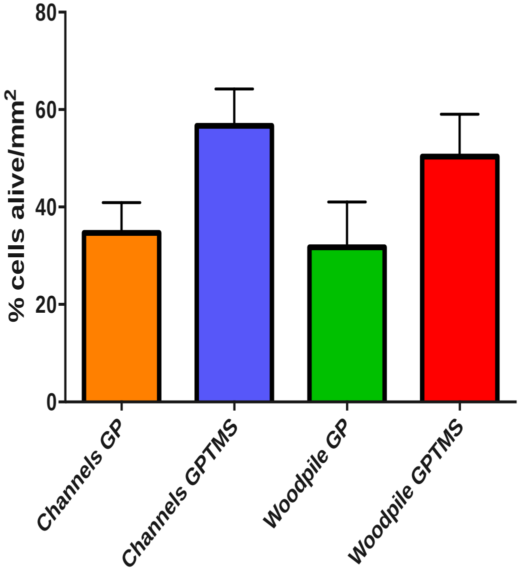
- Flow 1ml/h
- Time of spinning 12 min

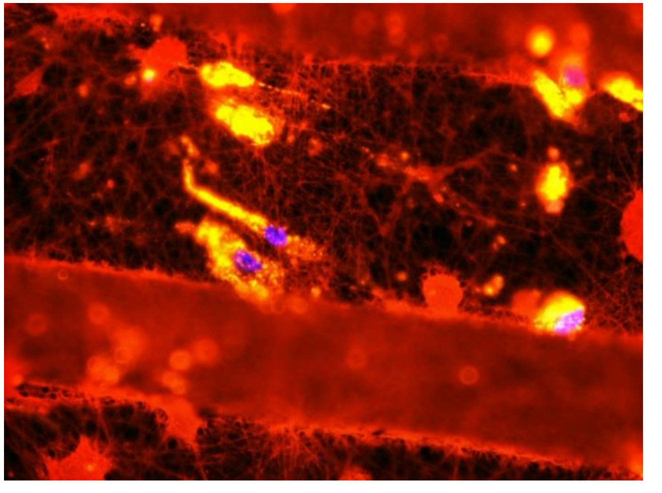
In vitro assays

- **SVP** (saphenous vein pericytes) from **human** donors
- 10000 cells/well in 250 µl of complete media





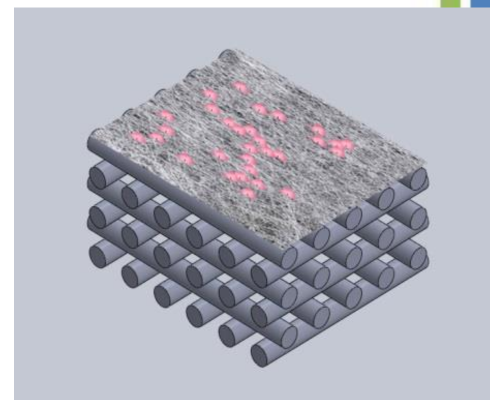


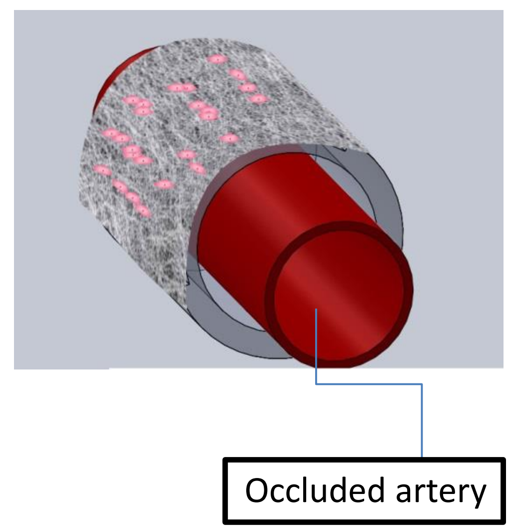


In-vivo assays

- **Channel** structures crosslinked with **GPTMS**

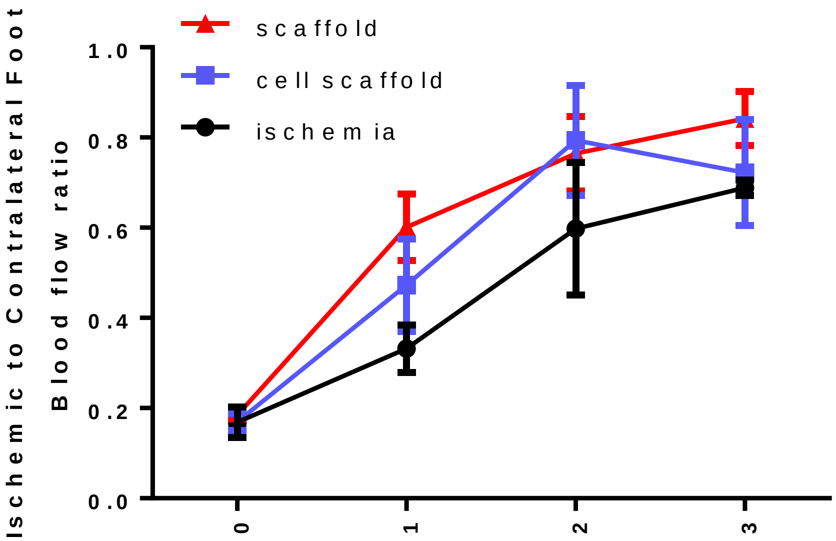
- 3 groups of CD1 mice subject to Limb Ischemia at femoral artery:
  - scaffold implatation
  - scaffold, seeded with SVP, implantation
  - control
- End point 3 weeks



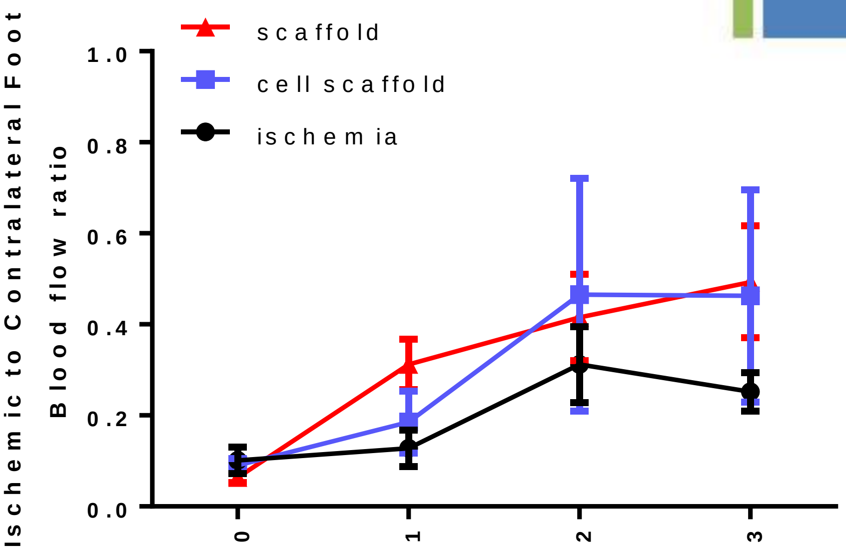


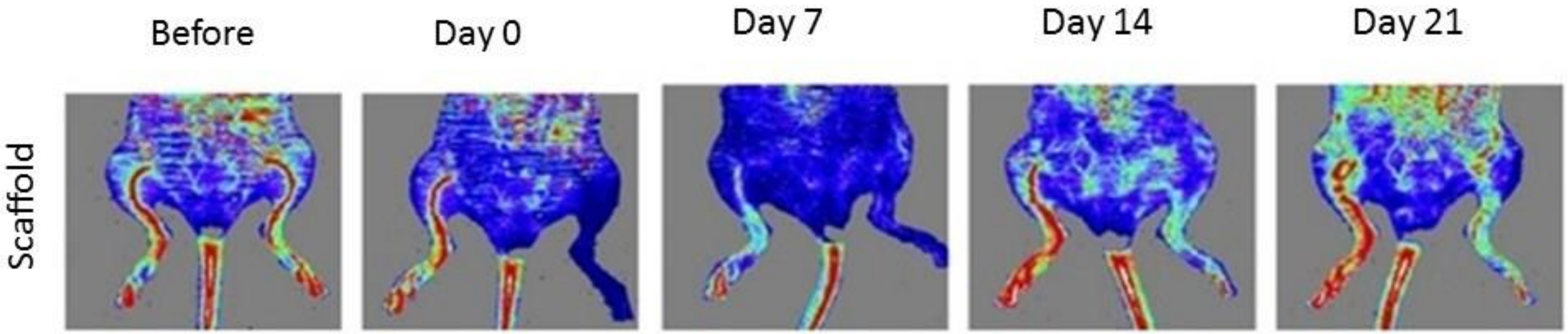
Laser doppler

Area of scaffold implantation

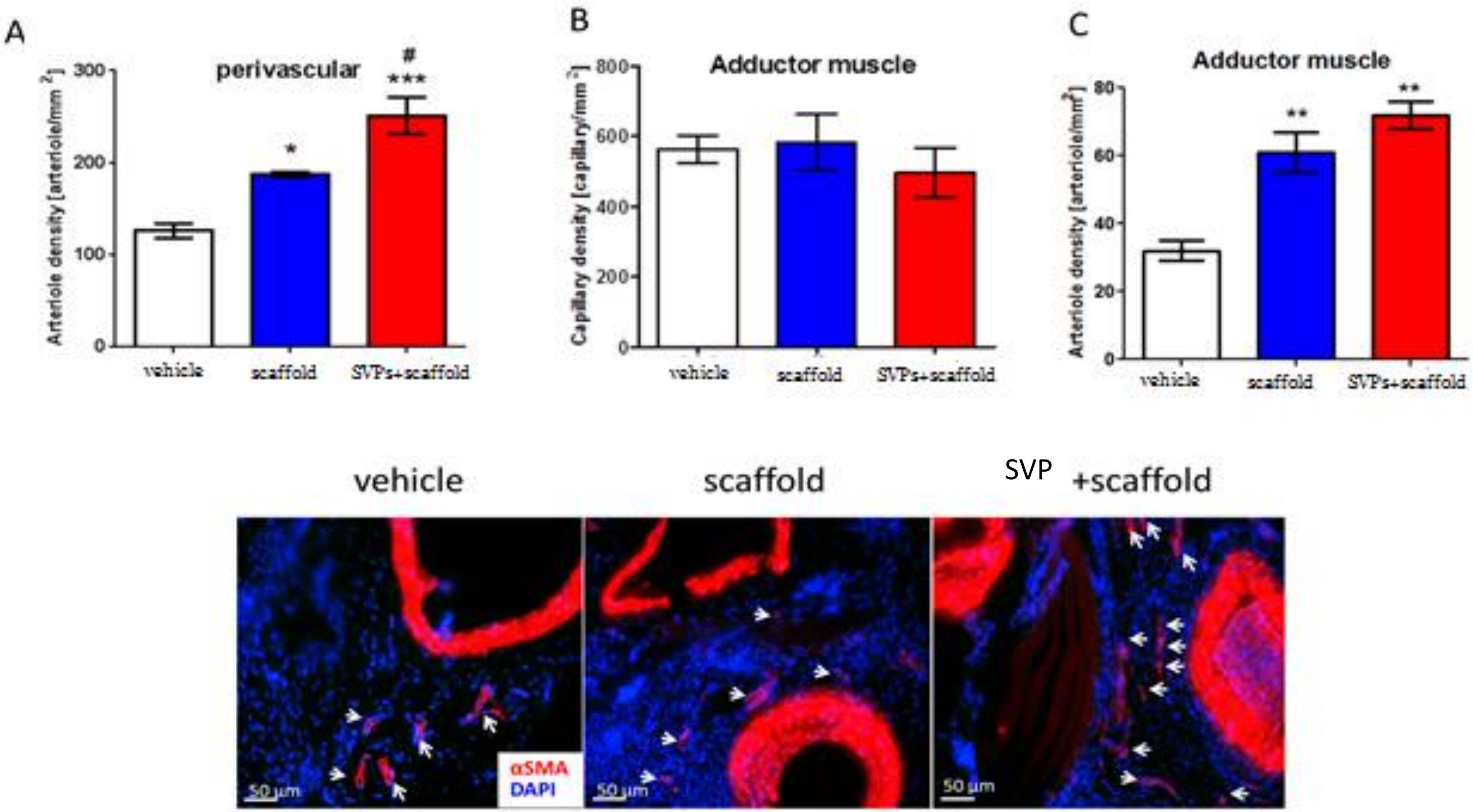


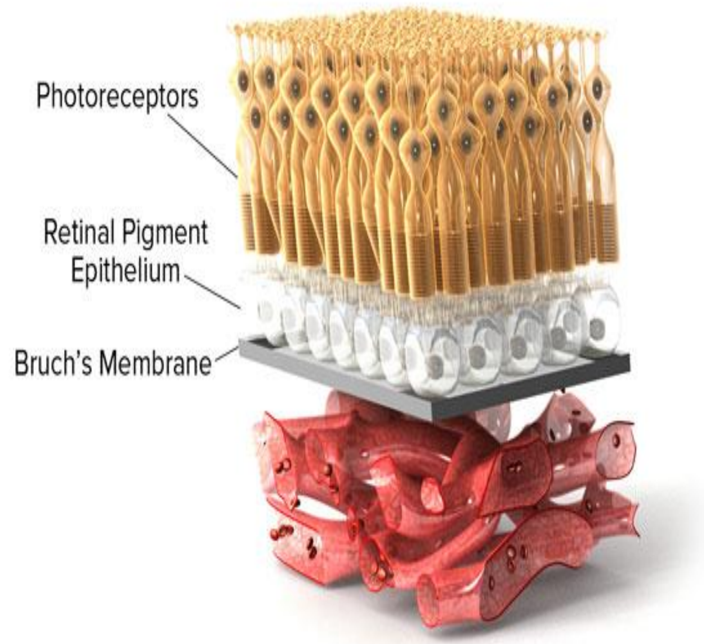
Foot





Immunohistochemistry



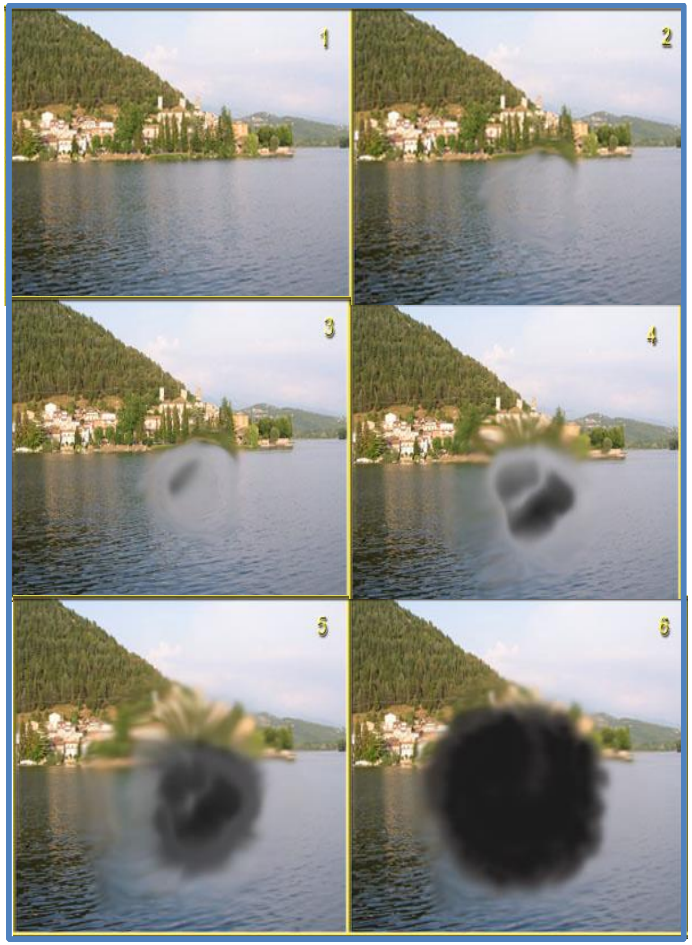


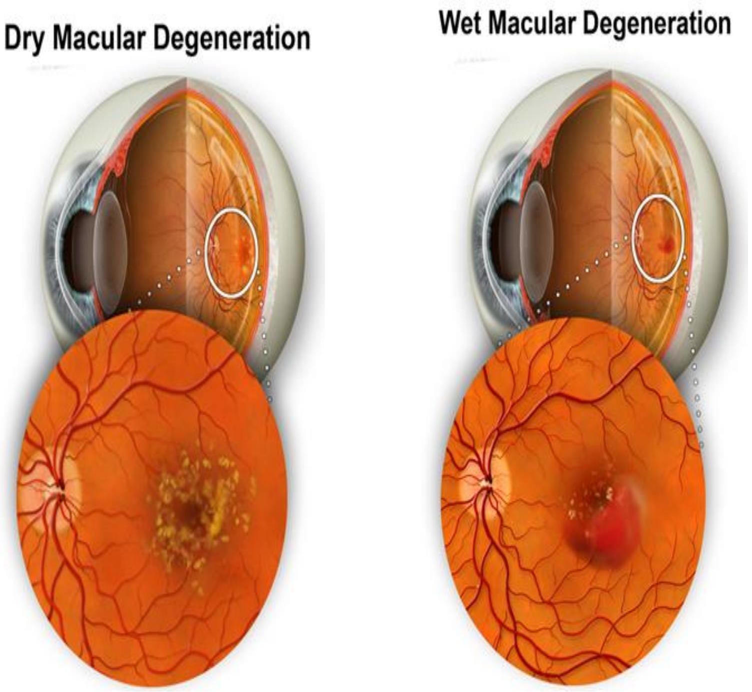
BIOMEMBRANE

BIOENGINEERED IN VITRO MODEL OF RETINAL PIGMENT EPITHELIUM OF HUMAN EYE

Background

- Age-related macular degeneration





Multimaterial processing

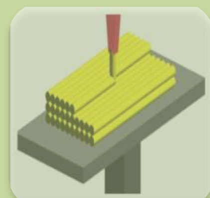
2-Dimensional

Lithography and Soft-Lithography

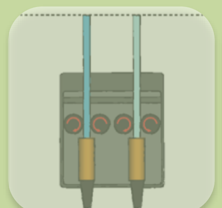
Soft-Molecular Imprinting

Electrospinning

3-Dimensional



PAMsquare



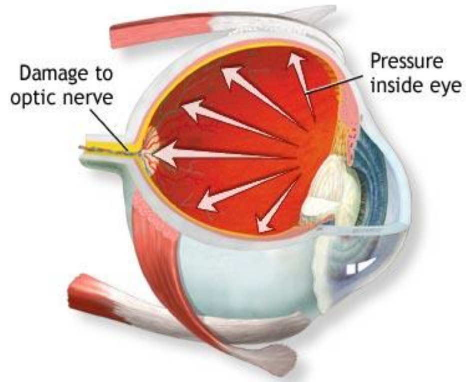
Thermoplastic extrusion

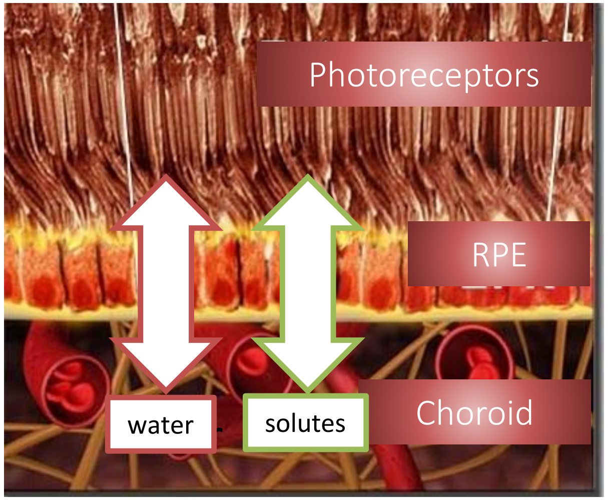
Inkjet Printing

Combination of 2D and 3D Technologies

Bruch’s membrane characteristics

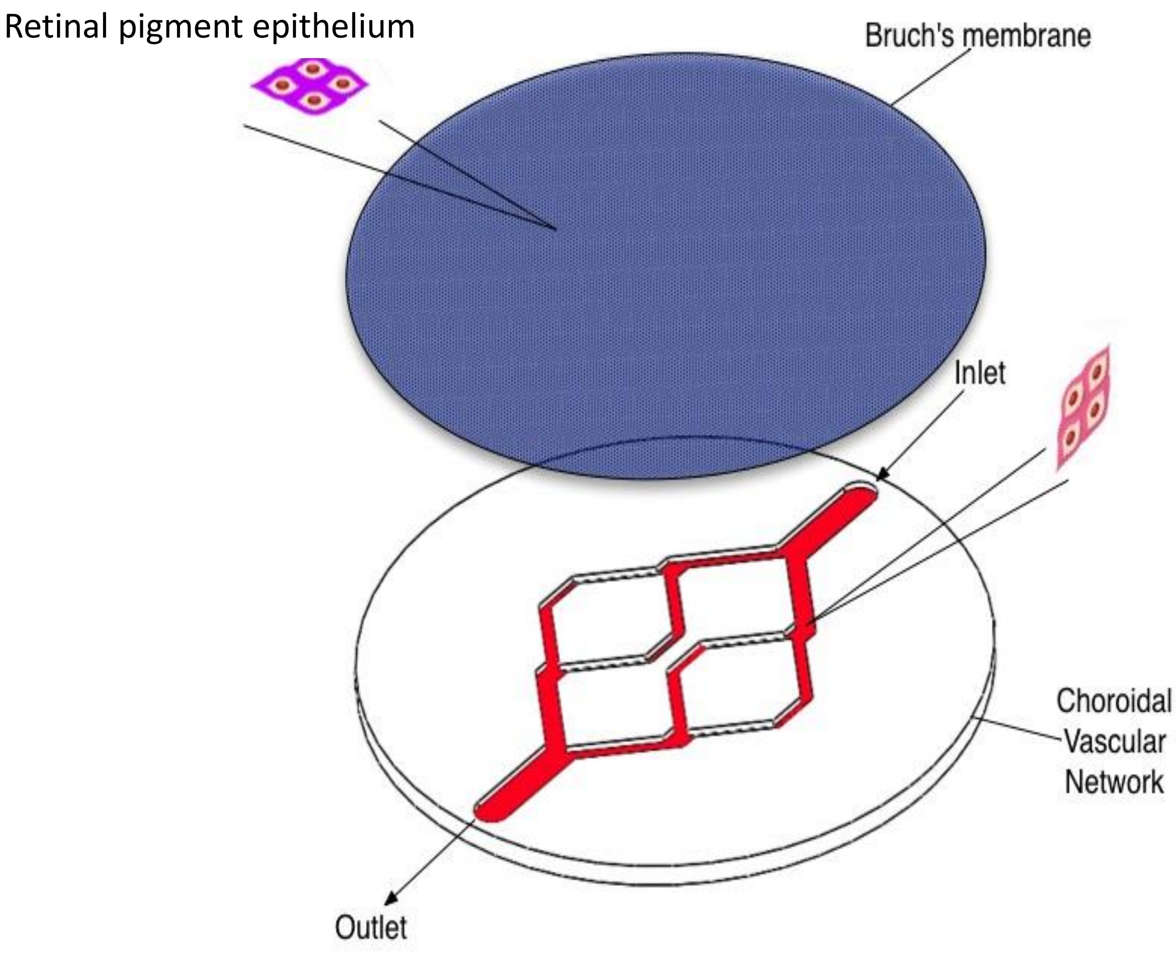
- Structural role
  - Mechanical resistance, agaist intraocular pressure
  - Elastic modulus 7-19 MPa
  - No changes during AMD
- Transport role
  - Convective transport (hydraulic resistance)
  - Diffusion driven process
  - Changes during AMD
    (in vitro model of the pathology)
- Electrospun meshes are a good model of Bruch’s membrane





Hotaling NA et al. *Nanofiber Scaffold-Based Tissue-Engineered Retinal Pigment Epithelium to Treat Degenerative Eye Diseases.* J Ocul Pharmacol Ther. 2016 Jun;32(5):272-85.

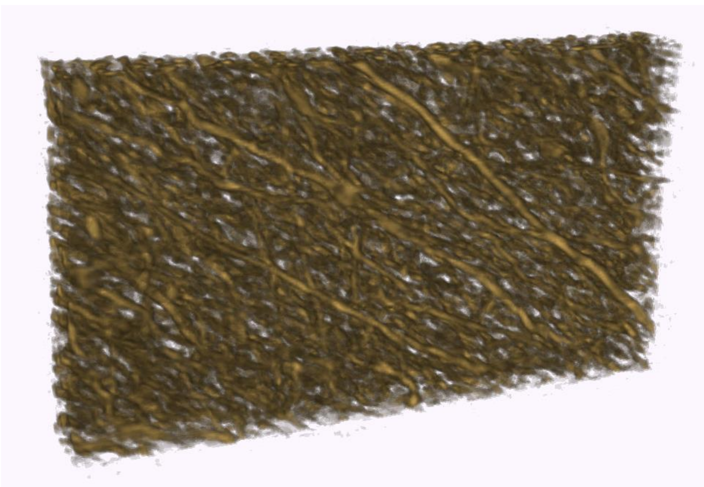
Objective

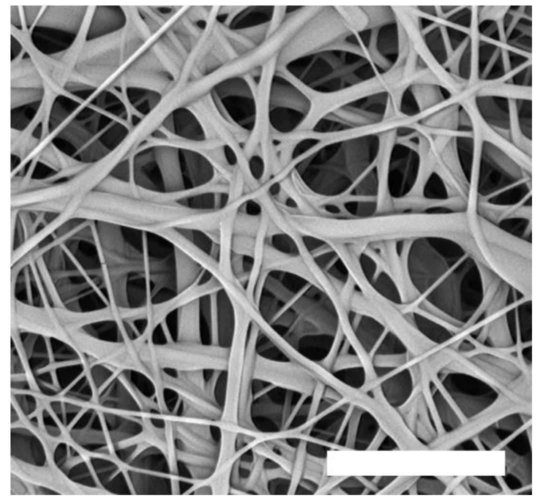


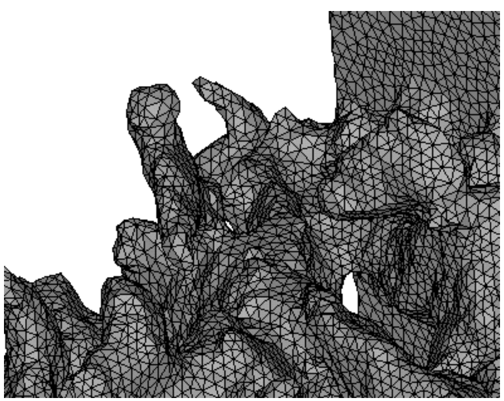
Retinal pigment epithelium
Bruch's membrane
Inlet
Choroidal
Vascular
Network
Outlet

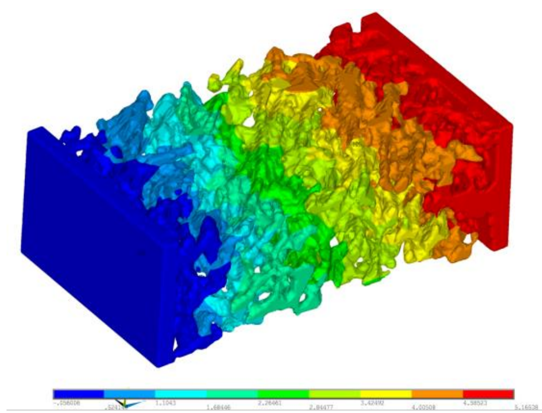
Electrospun mat analysis

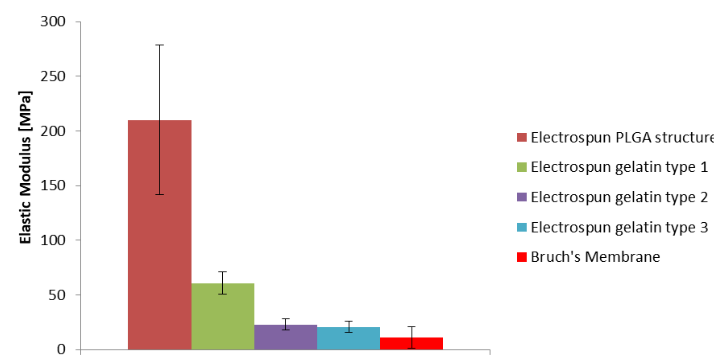
Realistic representation of the membrane structure was obtained through micro-computed tomography (μCT) (scan resolution 0.57 μm) and SEM



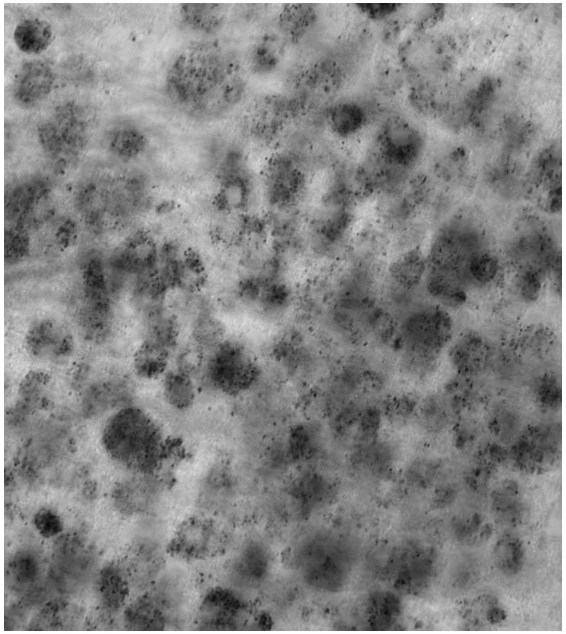




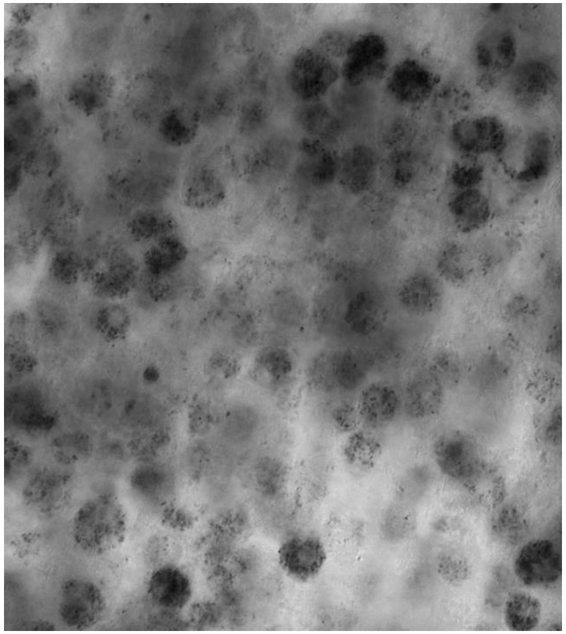




Culture hESc-RPE



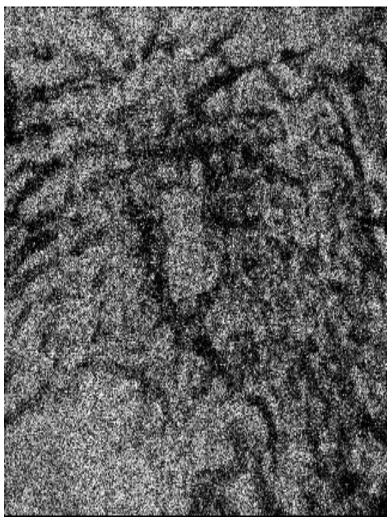
In vivo



In vitro

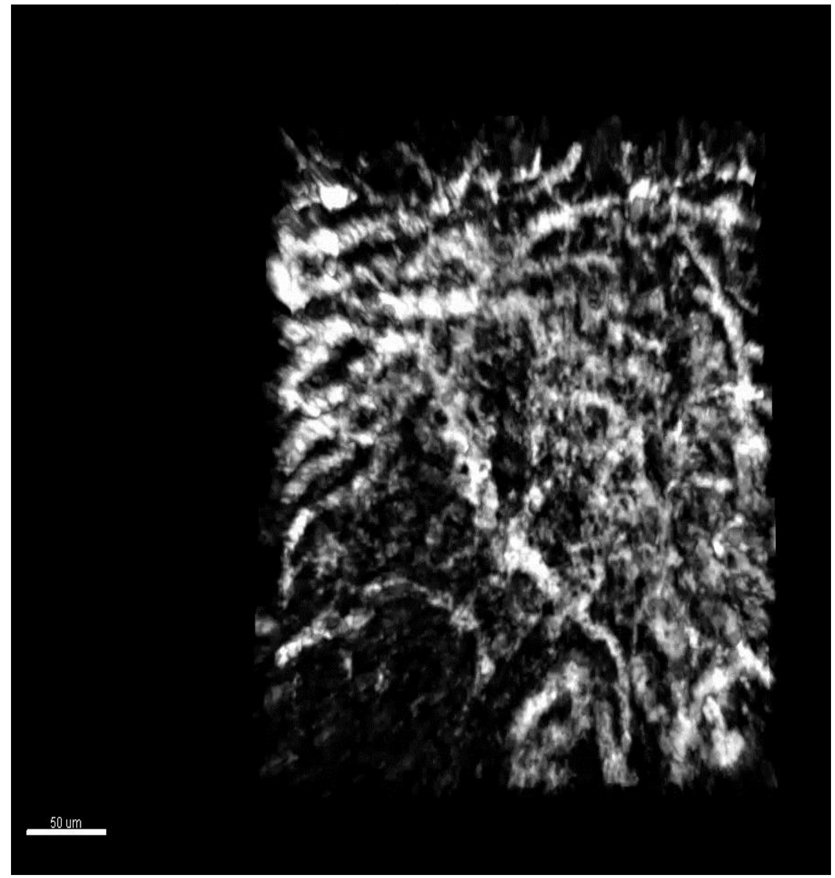
3D PROCESSING

Raw data



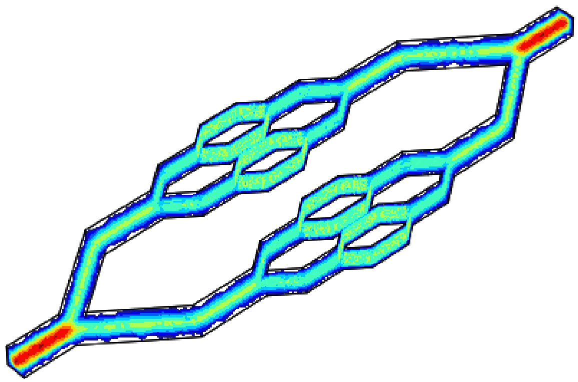
Processed

3D reconstruction +

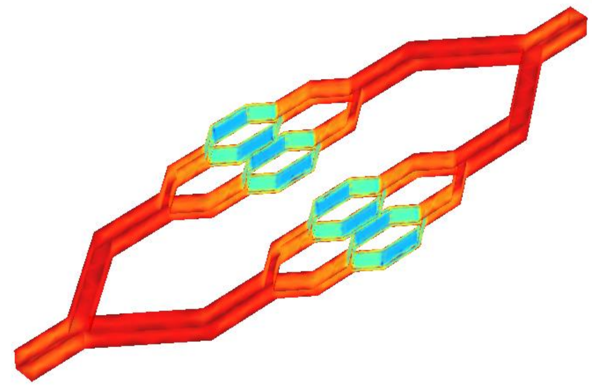


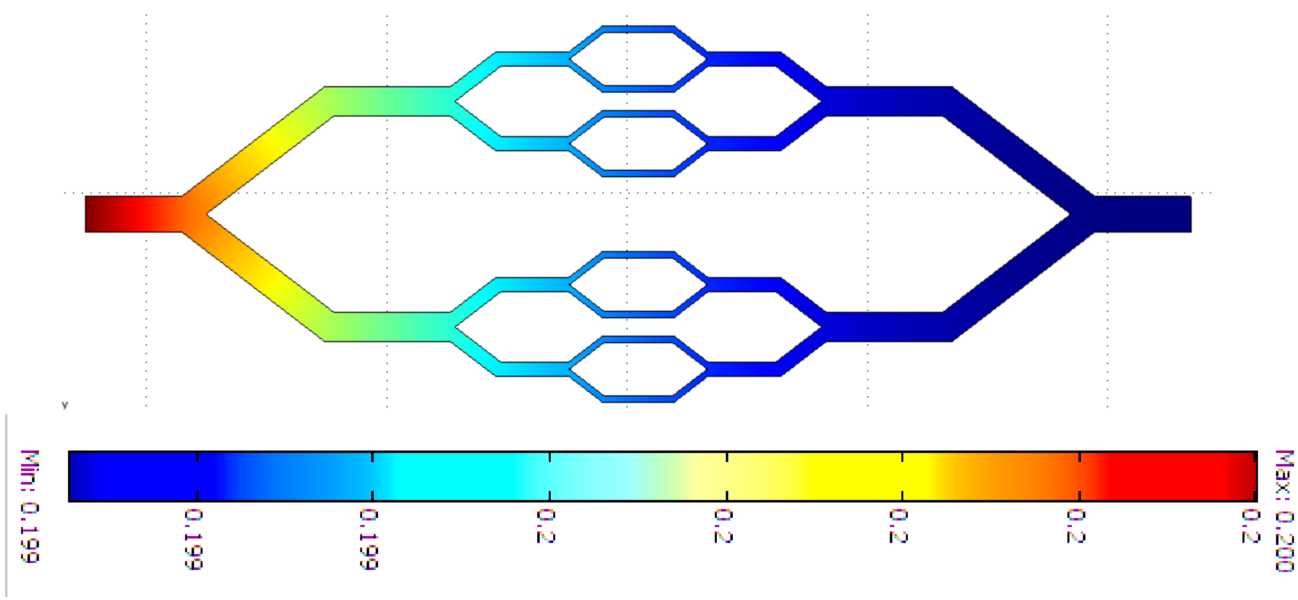
Vascular network model

Velocity field



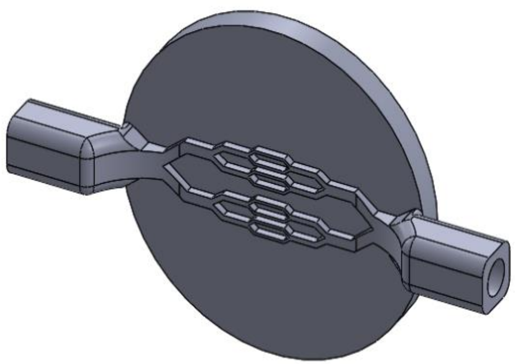
Viscous force



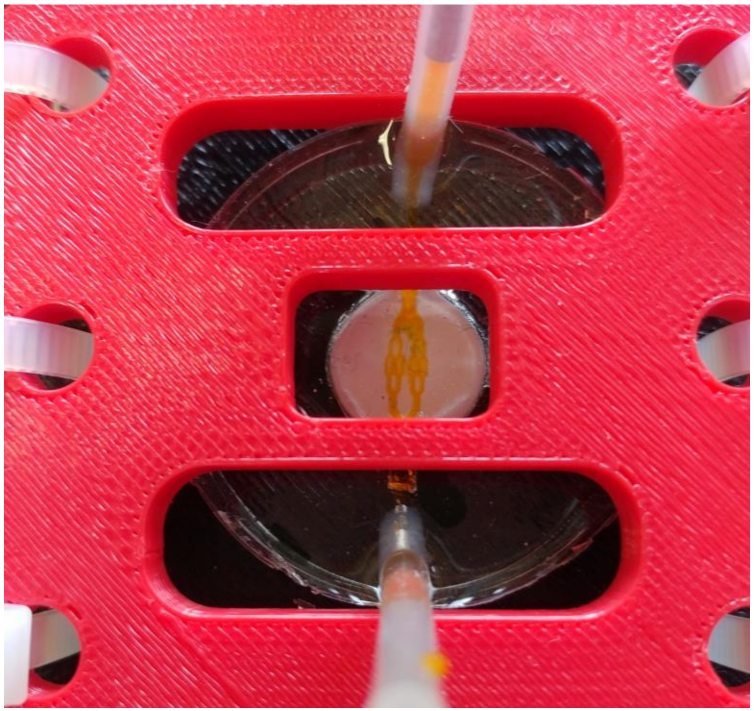


Mimicking blood vessels

3D model realised with Stereolithographic Printer Formlabs Form2



Mimicking blood vessels



+

IMAGO (MAECI-AMEXIC)

Thanks for your attention!

Biofabrication Group

www.centropiaggio.unipi.it/research/biofabrication.html

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