Centro E. Piaggio
bioengineering and robotics research center

IN SUPREMÆ DIGNITATIS 1343

Biofabrication

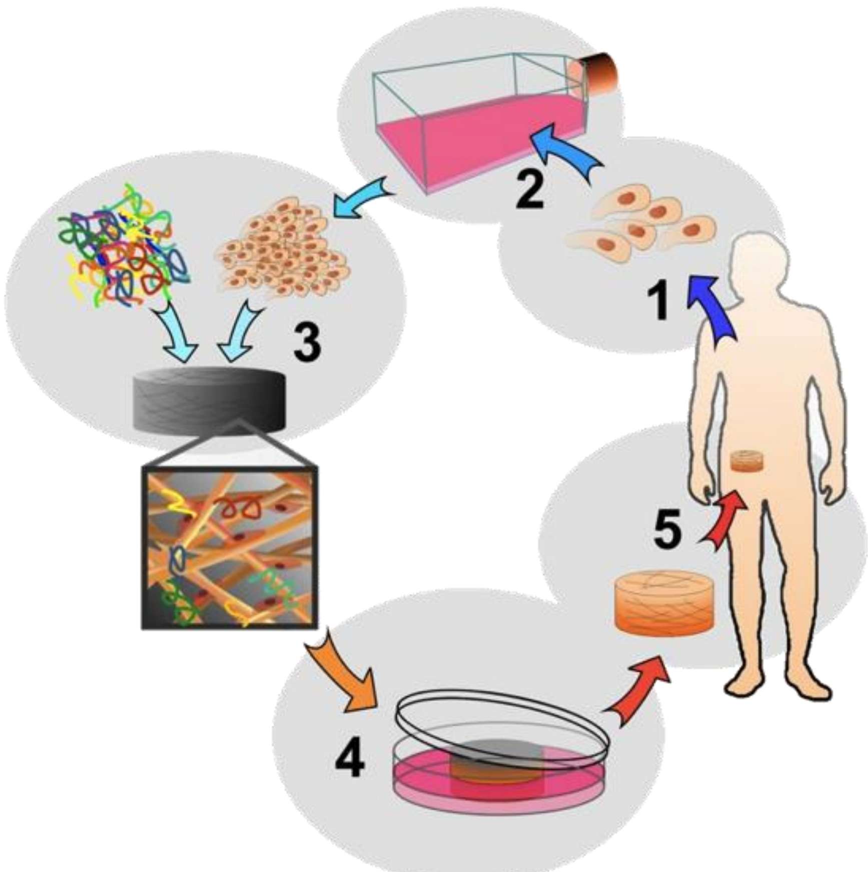
Tissue engineering

- *an interdisciplinary field that applies the principles of engineering and life sciences towards the development of biological substitutes that restore, maintain, or improve biological tissue function or a whole organ*

Langer R, Vacanti JP. *Tissue engineering*. Science 1993, **260**(5110), 920–926

Tissue engineering

- Classic paradigm



Regenerative medicine

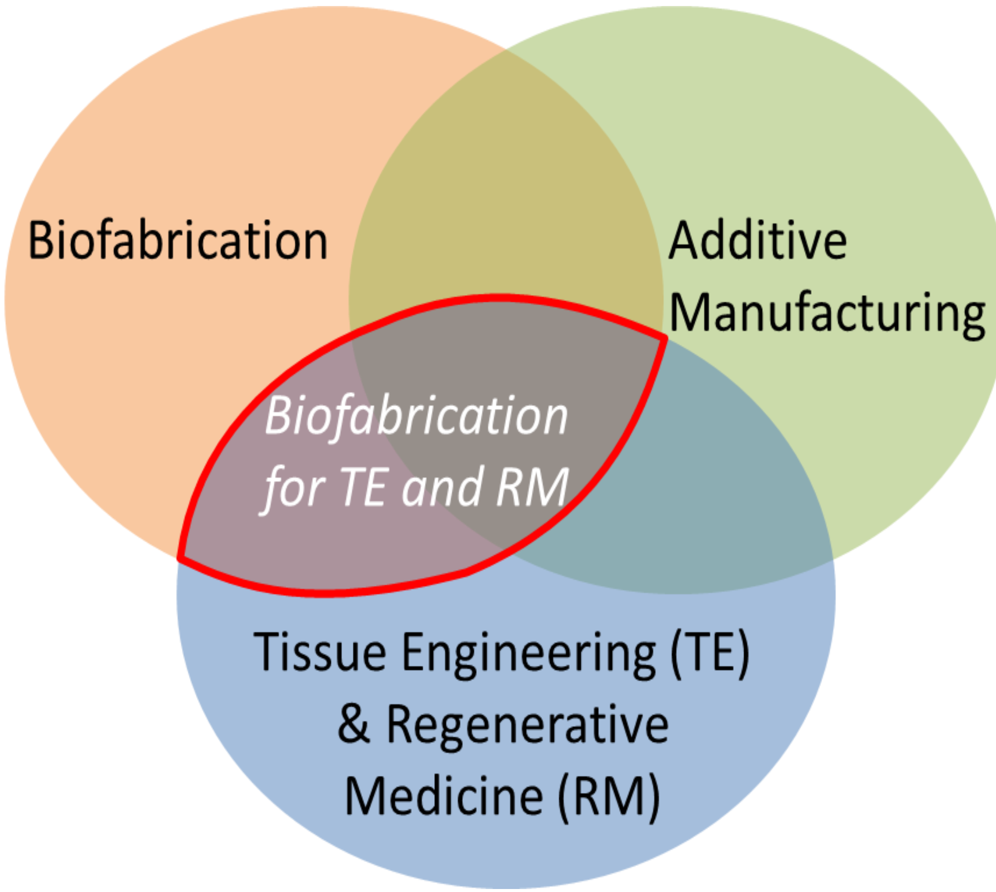
- the application of tissue science, tissue engineering, and related biological and engineering principles that restore the structure and function of damaged tissues and organs

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

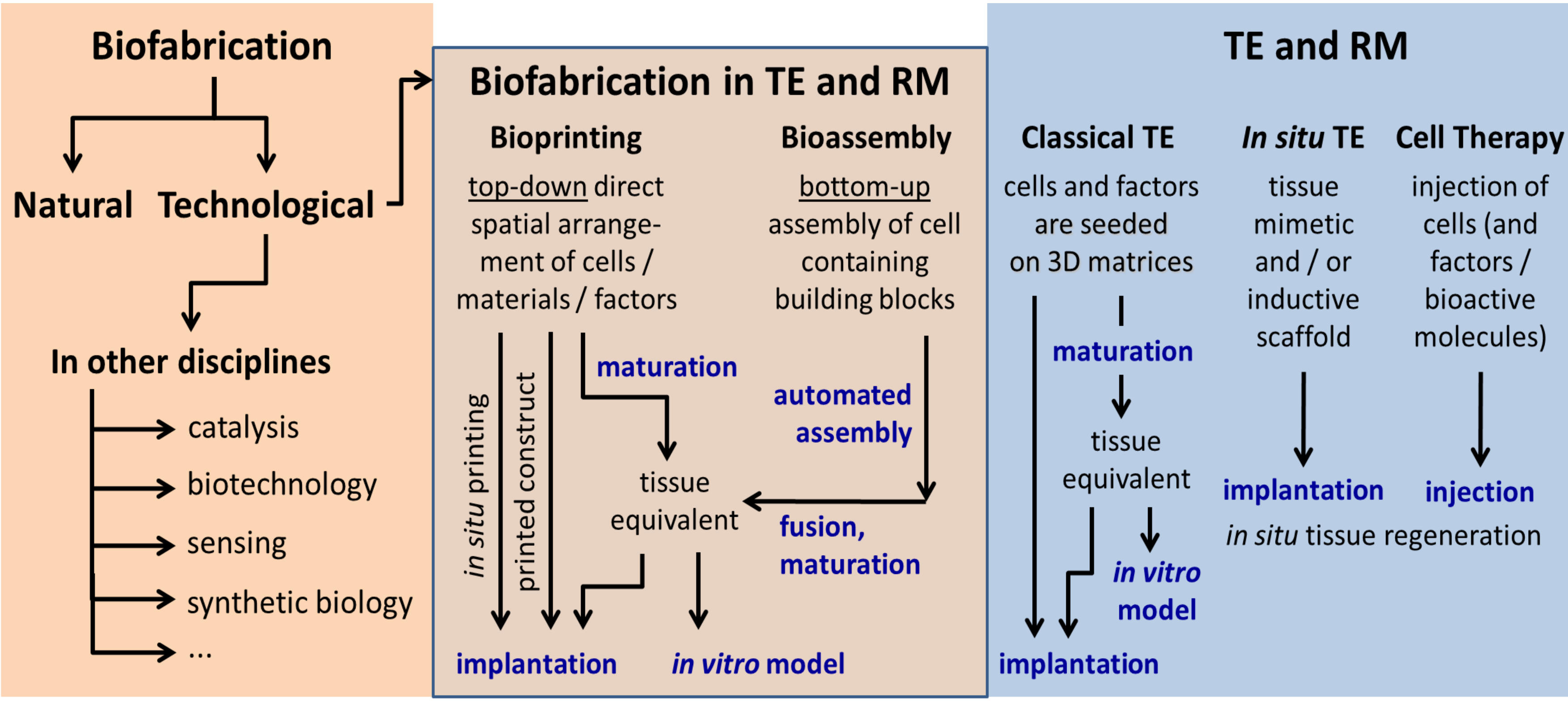
Biofabrication

- *the generation of biologically functional products with structural organization from living cells, micro-tissues or hybrid tissue constructs, bioactive molecules or biomaterials either through top-down (Bioprinting) or bottom-up (Bioassembly) strategies and subsequent tissue maturation processes.*

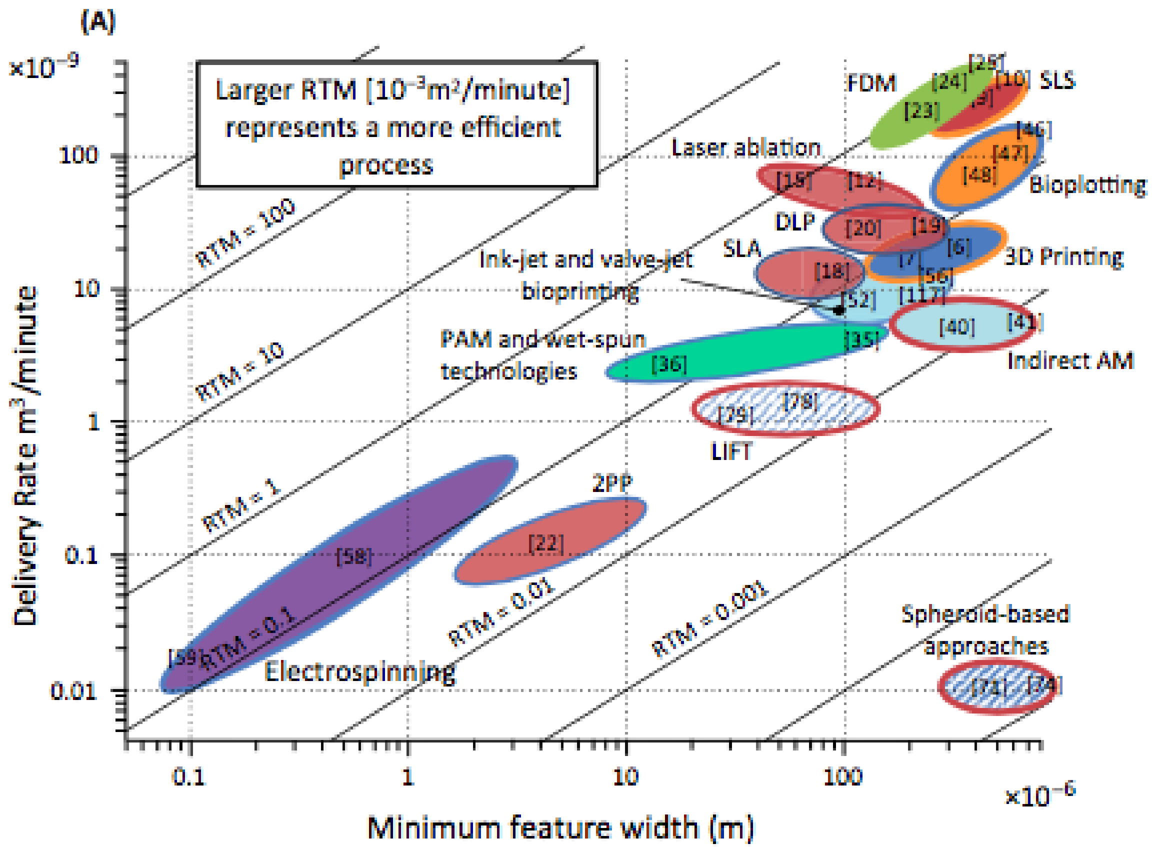
Biofabrication



Biofabrication

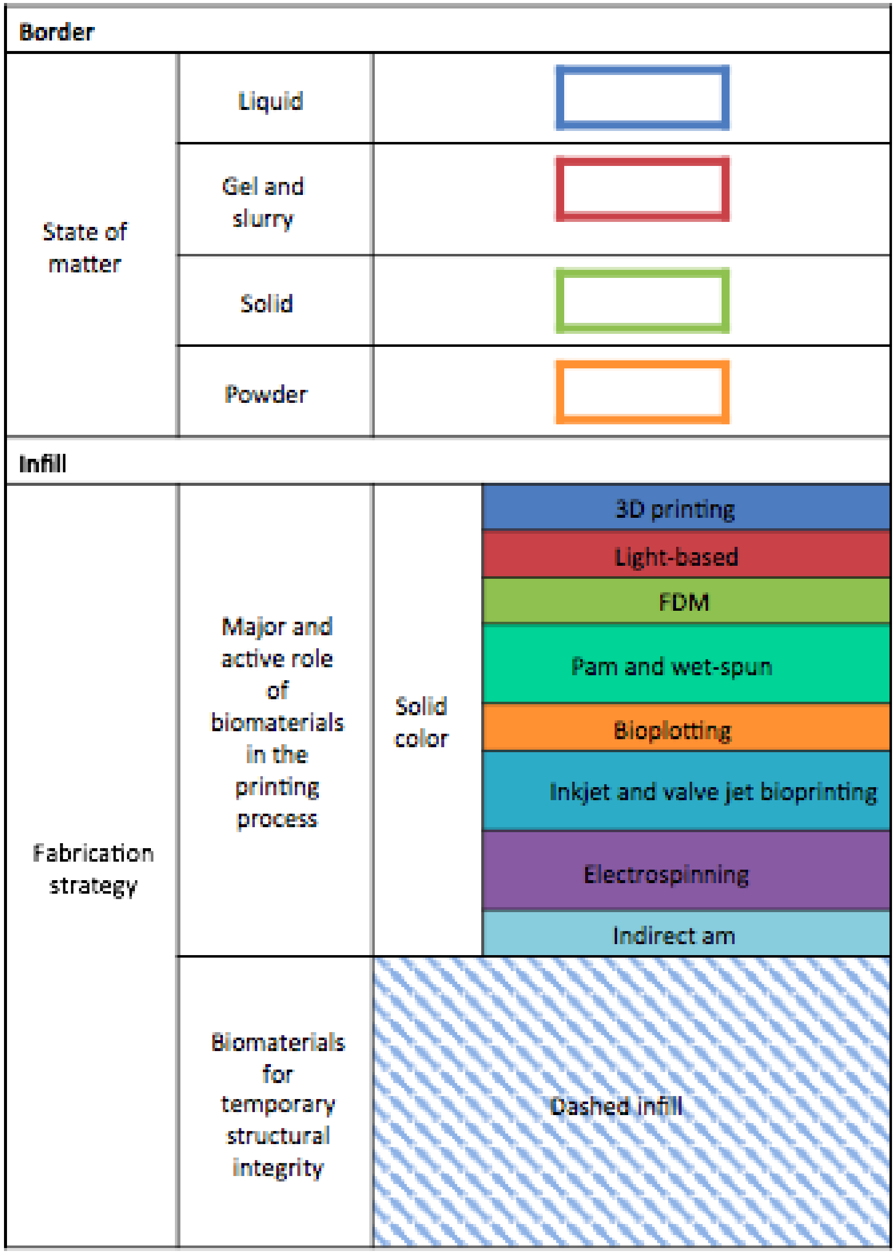


Biofabrication chart



$$RTM = \frac{Spatial\ resolution}{Time\ for\ manufacturing} \cong R \cdot P = \frac{1}{d} \cdot \frac{V}{t}$$

(B)



Trends in Biotechnology

CellPress REVIEWS

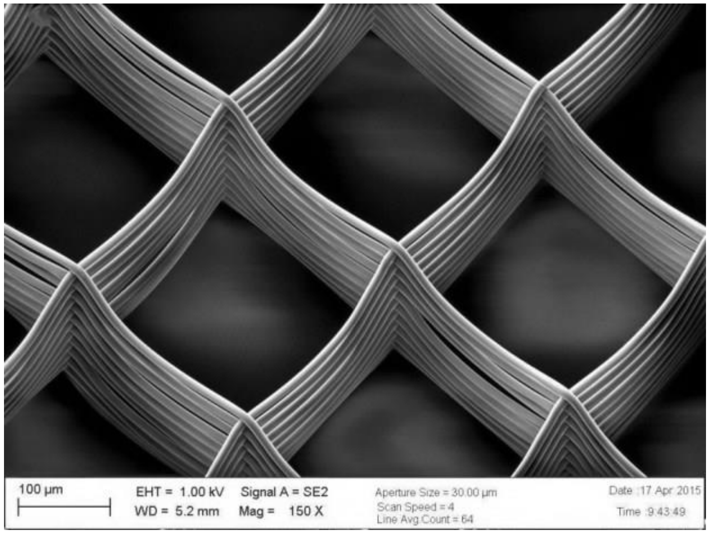
Feature Review

Biofabrication: A Guide to Technology and Terminology

Lorenzo Moroni,[1,*,@,@] Thomas Boland,[2] Jason A. Burdick,[3] Carmelo De Maria,[4] Brian Derby,[5] Gabor Forgacs,[6,7] Jürgen Groll,[8] Qing Li,[9] Jos Malda,[10,11] Vladimir A. Mironov,[12,13] Carlos Mota,[1] Makoto Nakamura,[14] Wenmiao Shu,[15] Shoji Takeuchi,[16] Tim B.F. Woodfield,[17] Tao Xu,[18] James J. Yoo,[19] and Giovanni Vozzi[4]

BIOFABRICATION AT RESEARCH CENTER E. PIAGGIO

Scaffolds


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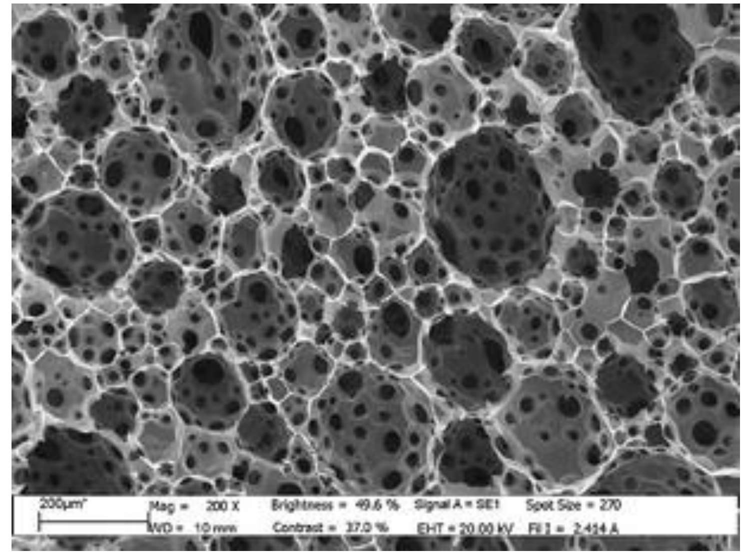
Line Avg.Count = 64
Date :17 Apr 2015
Time :9:43:49


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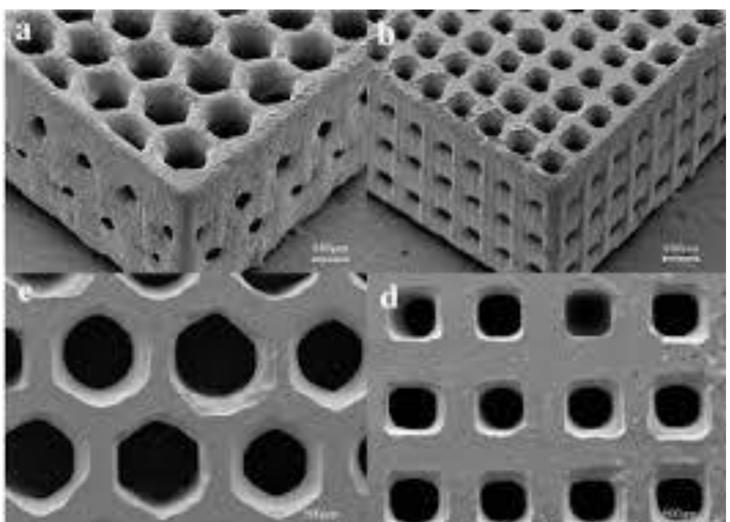
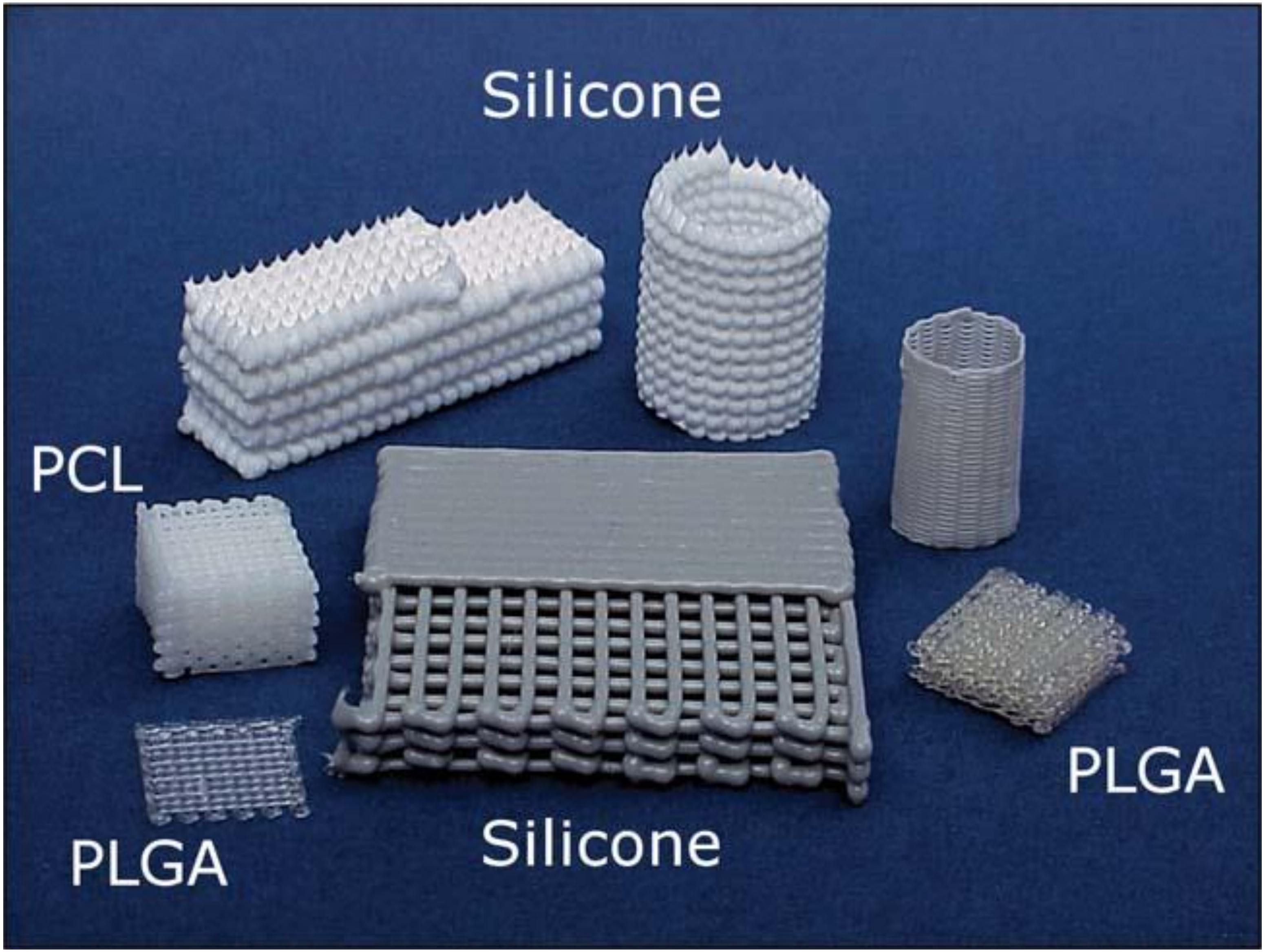
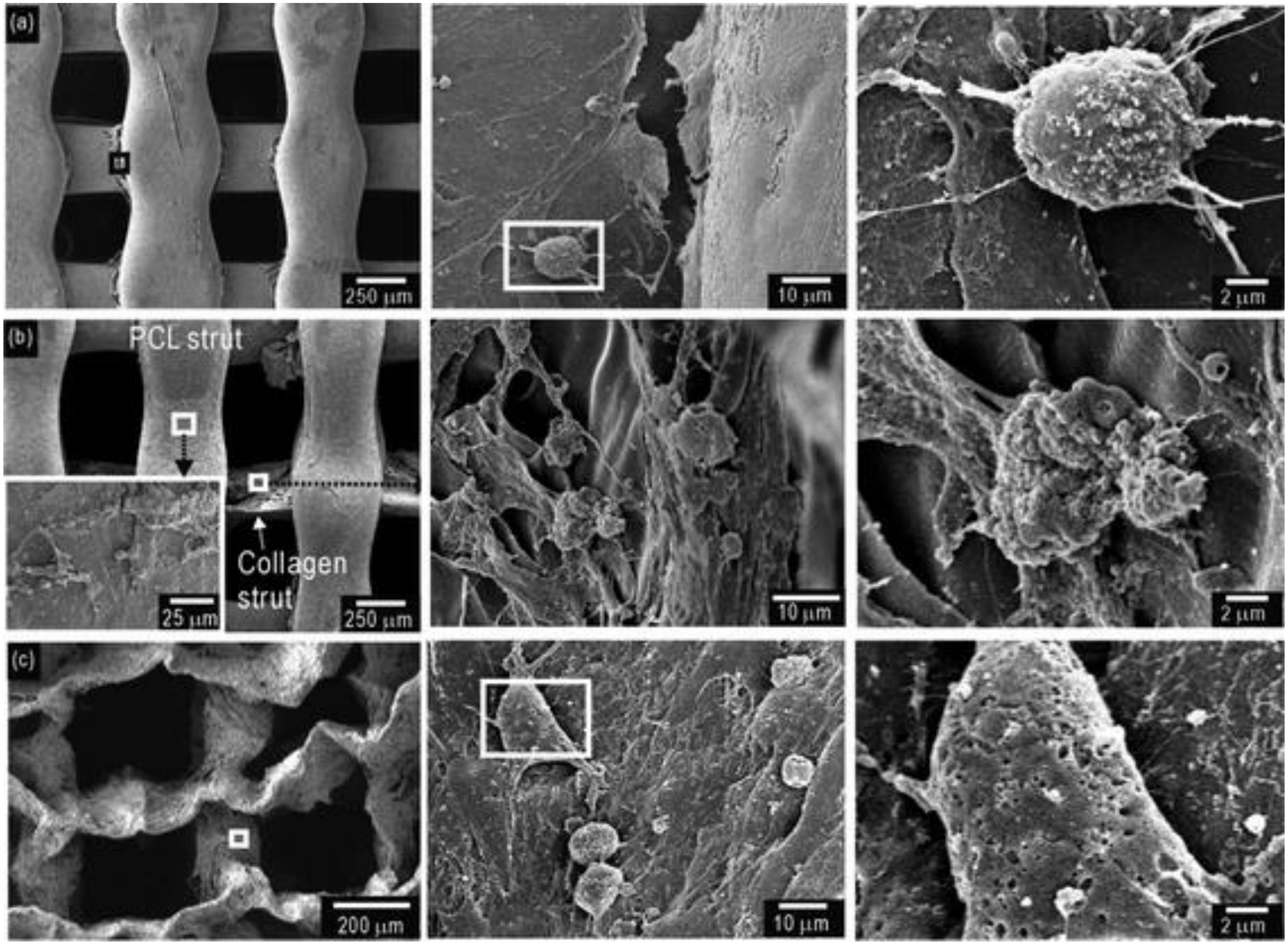

a
b
c
d

Scaffolds

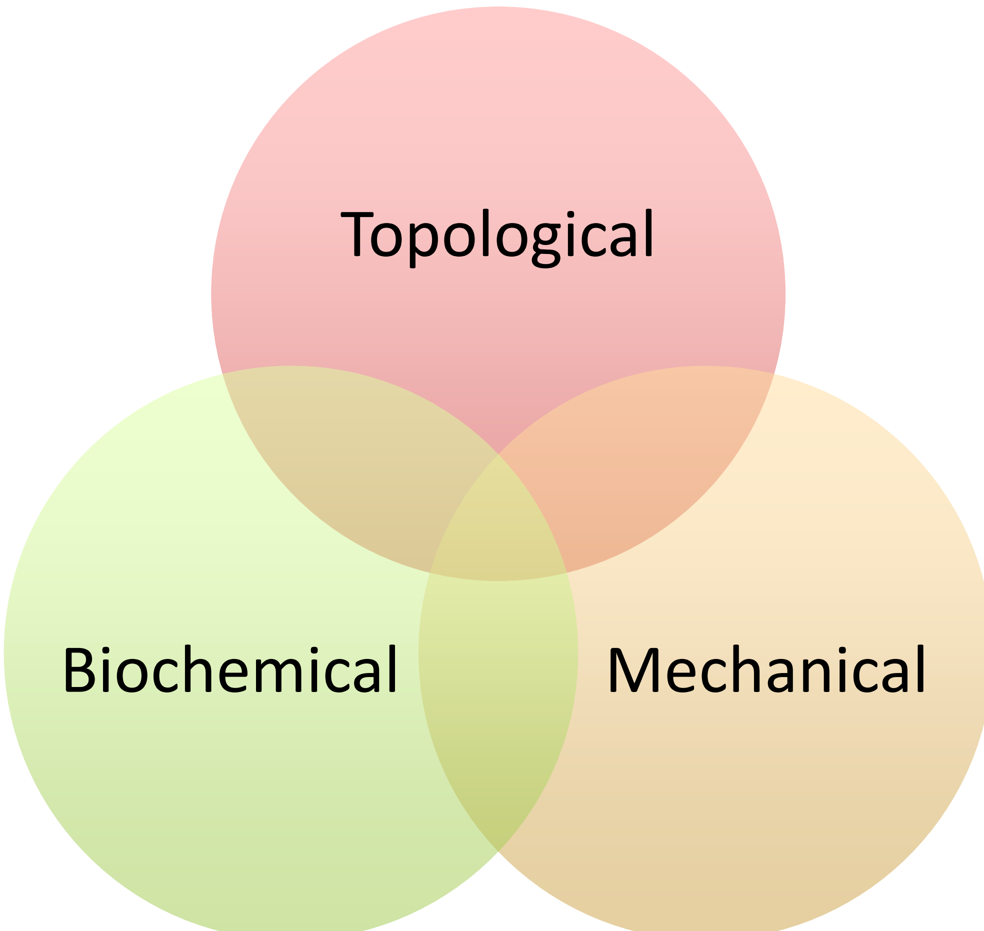


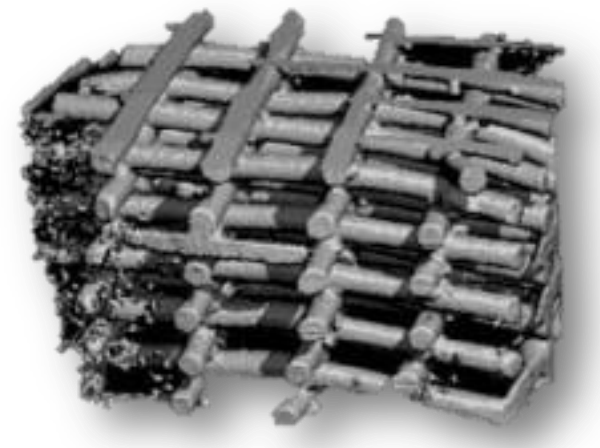
Scaffold



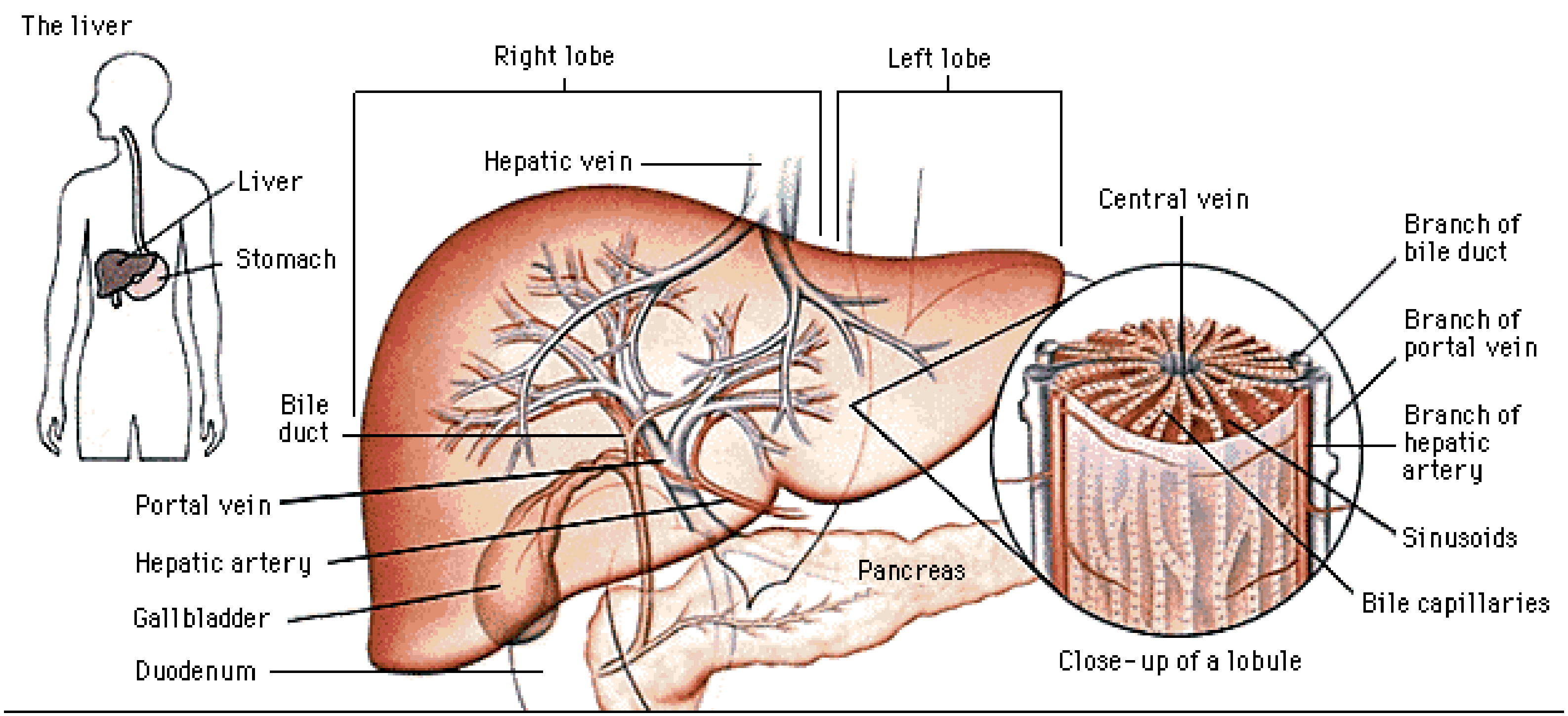
(a)
250 μm
10 μm
2 μm
(b)
PCL strut
Collagen strut
25 μm
250 μm
10 μm
2 μm
(c)
200 μm
10 μm
2 μm

Scaffold cues





Living tissues: multiscale e multimaterial



Multimaterial Processing

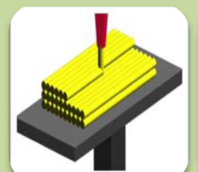
2-Dimensional

Lithography and Soft-Lithography

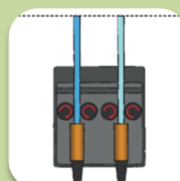
Soft-Molecular Imprinting

Electrospinning

3-Dimensional



PAMsquare

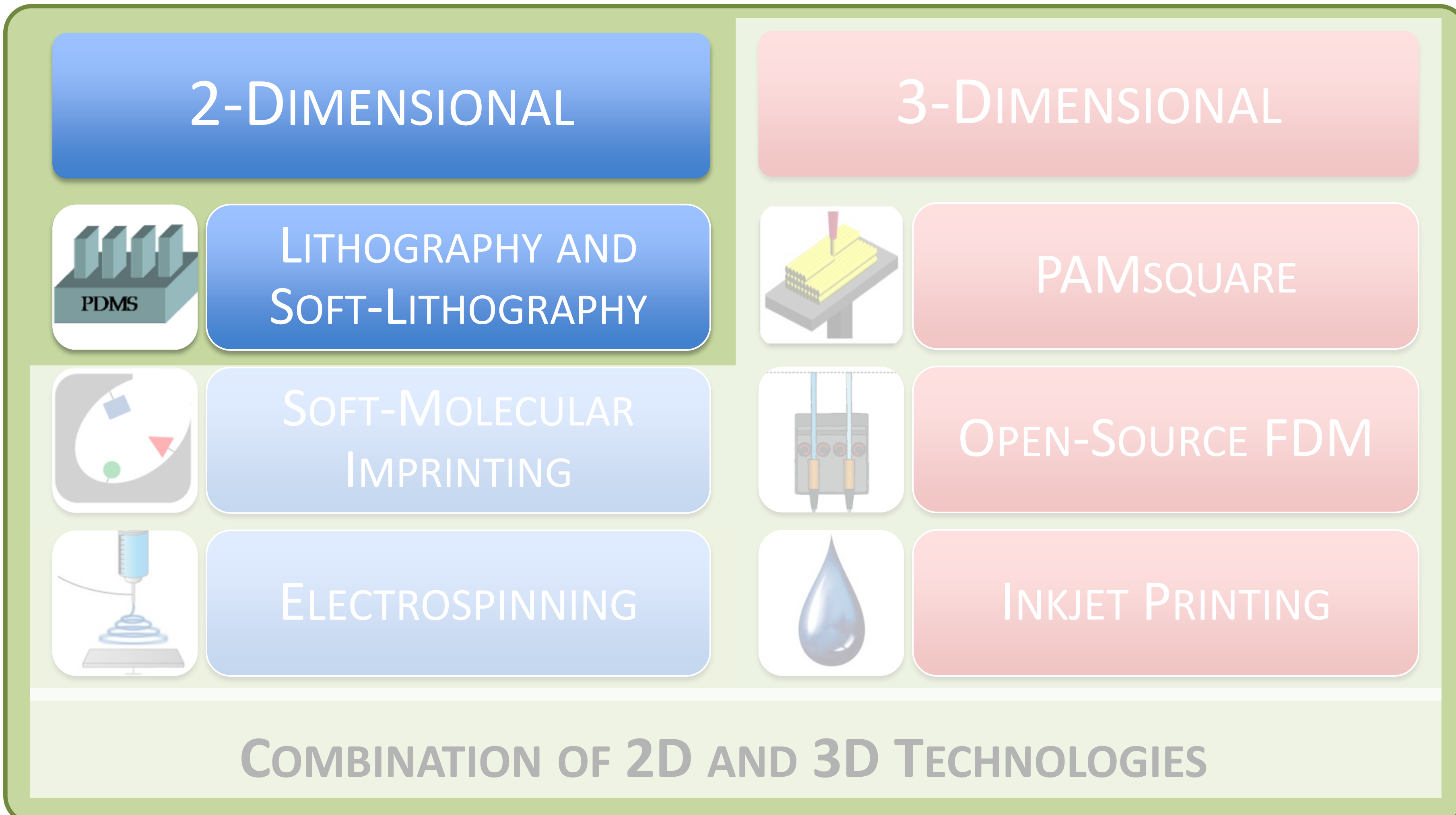


Open-Source FDM

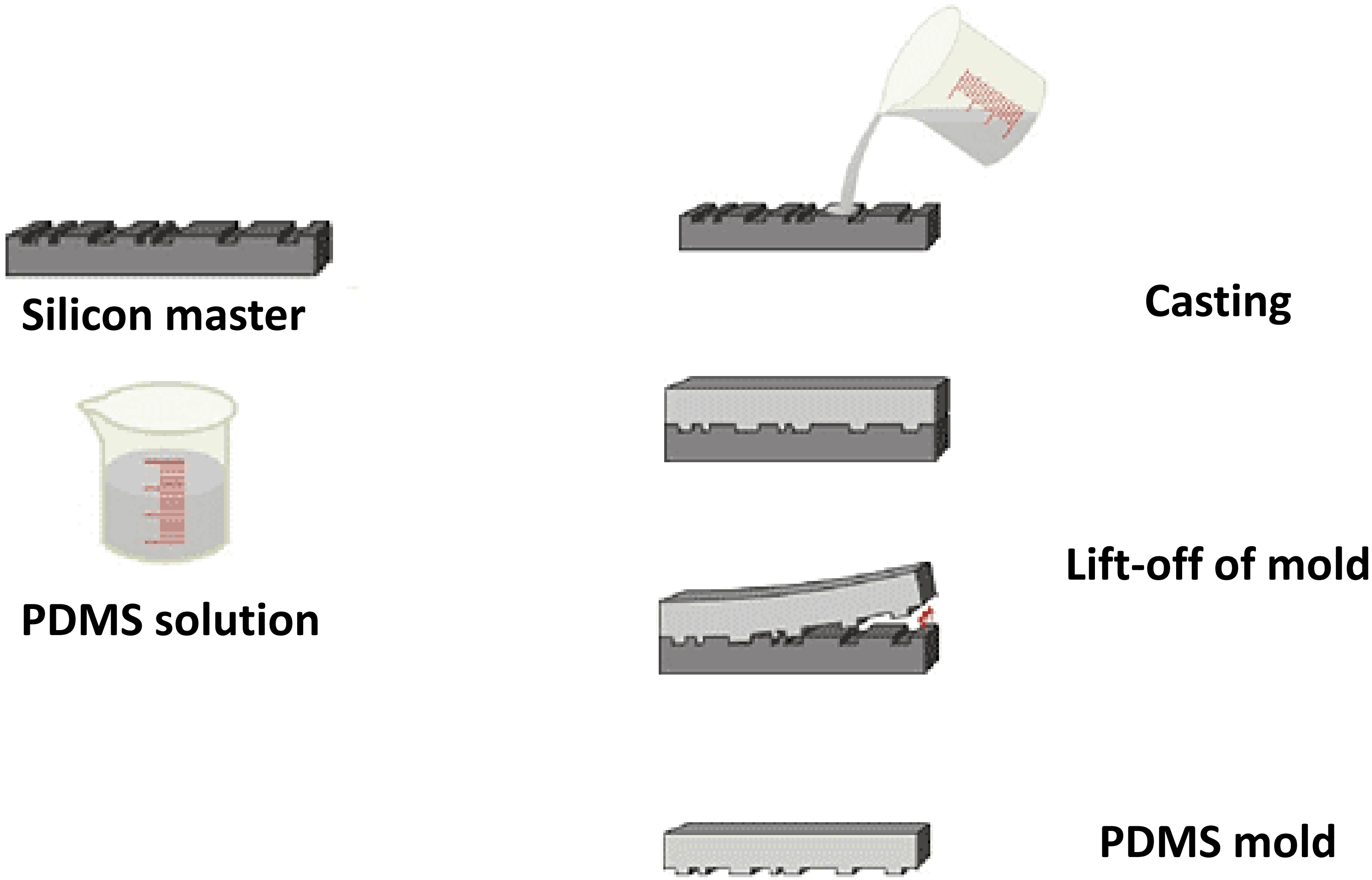
Inkjet Printing

Combination of 2D and 3D Technologies

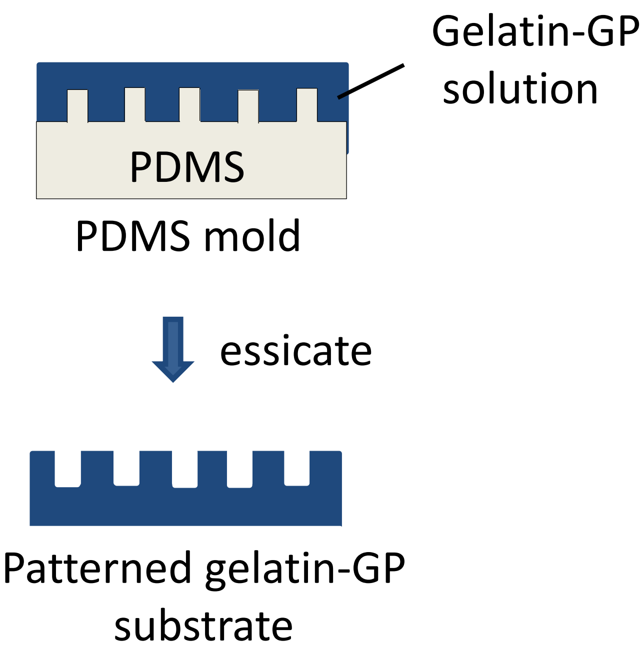
Lithography and Soft-Lithography

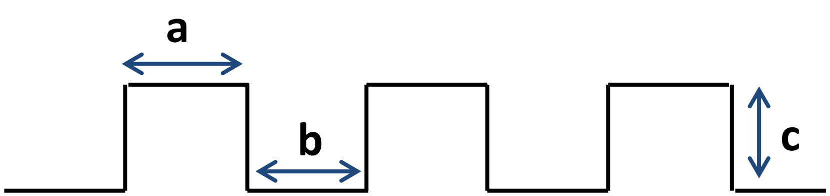


Soft-lithography process



+ Micro-pattering of gelatin-GP scaffolds





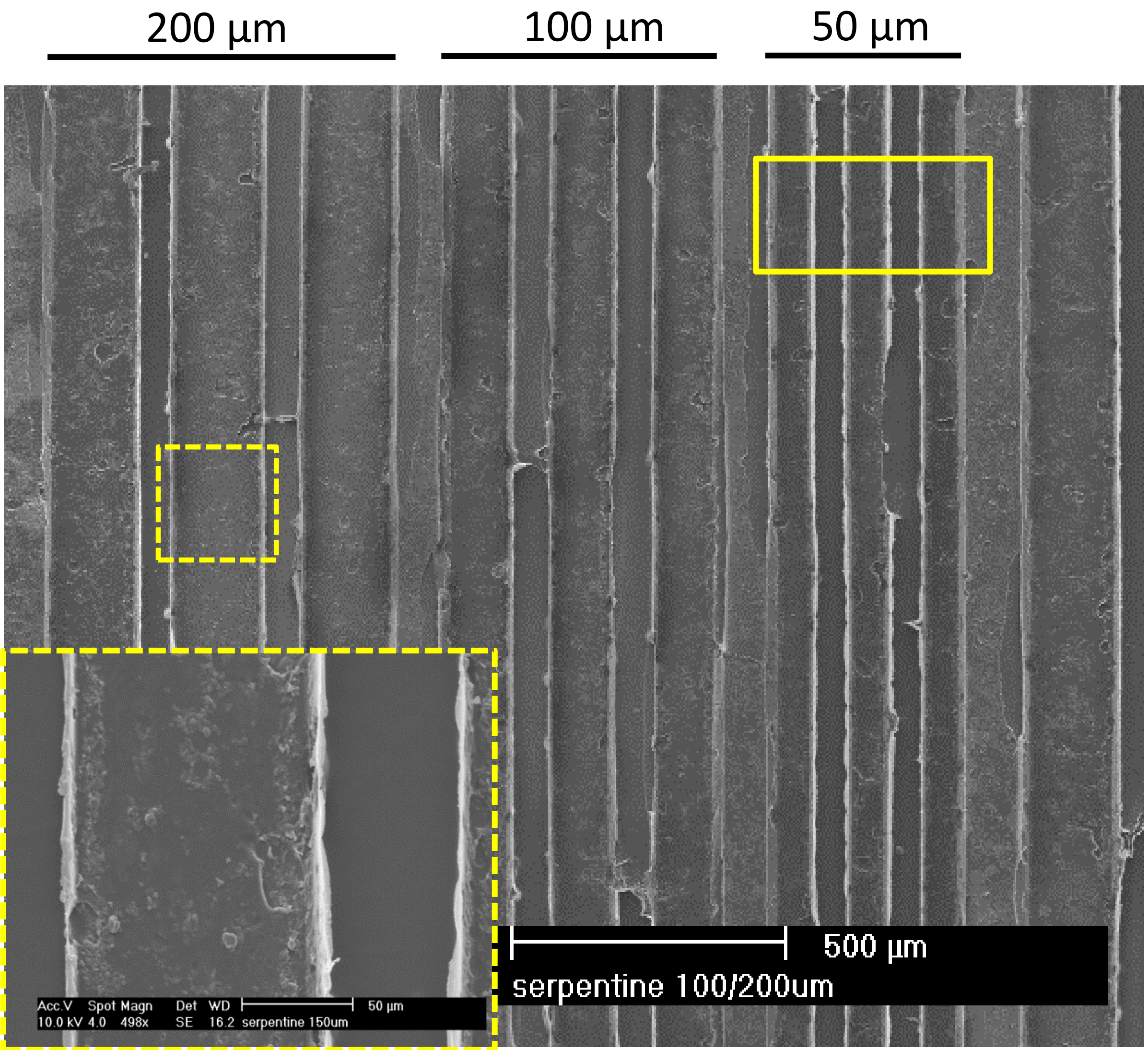
a= 50-100-200 µm wide strips

b= 100 µm apart

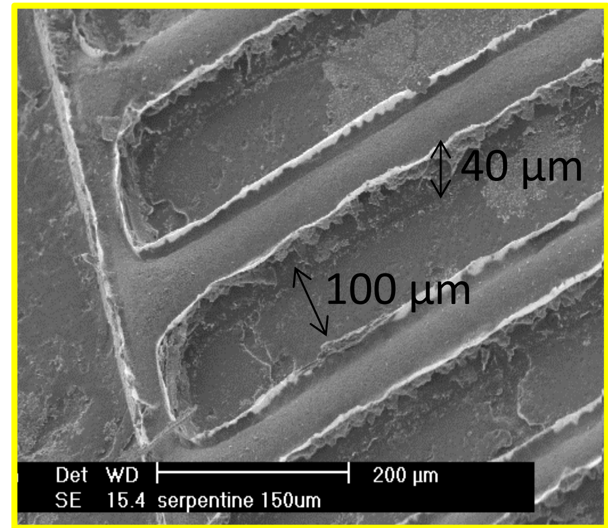
c= 40 µm

Micro-pattering of gelatin-GP scaffolds

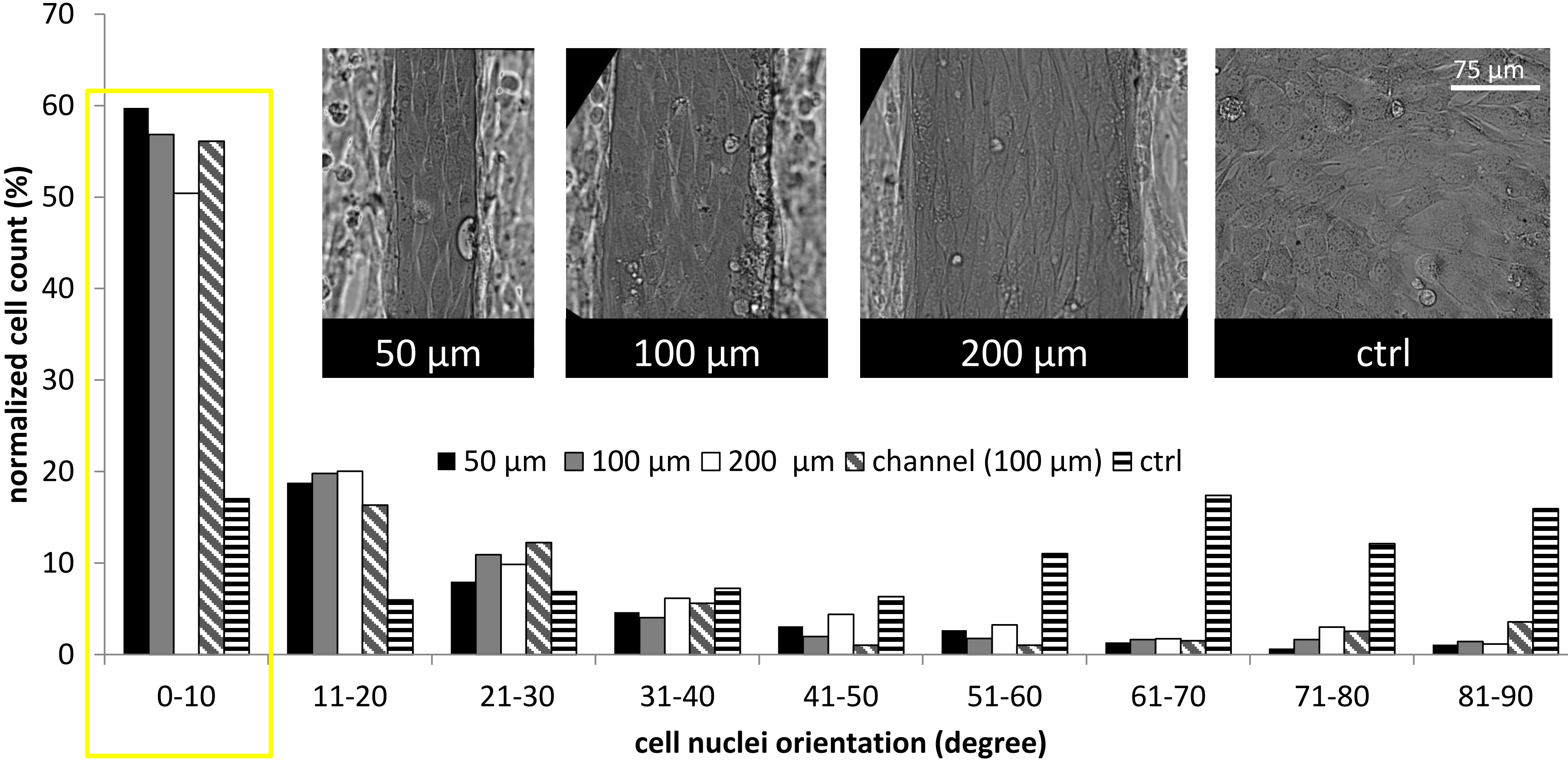
Graded patterned substrates were used to follow myoblasts and myotubes orientation



LATERAL VIEW

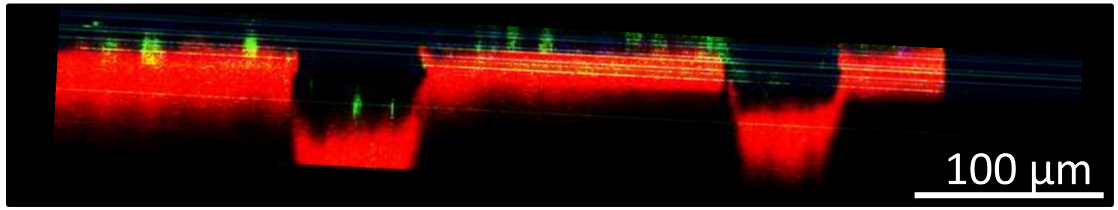


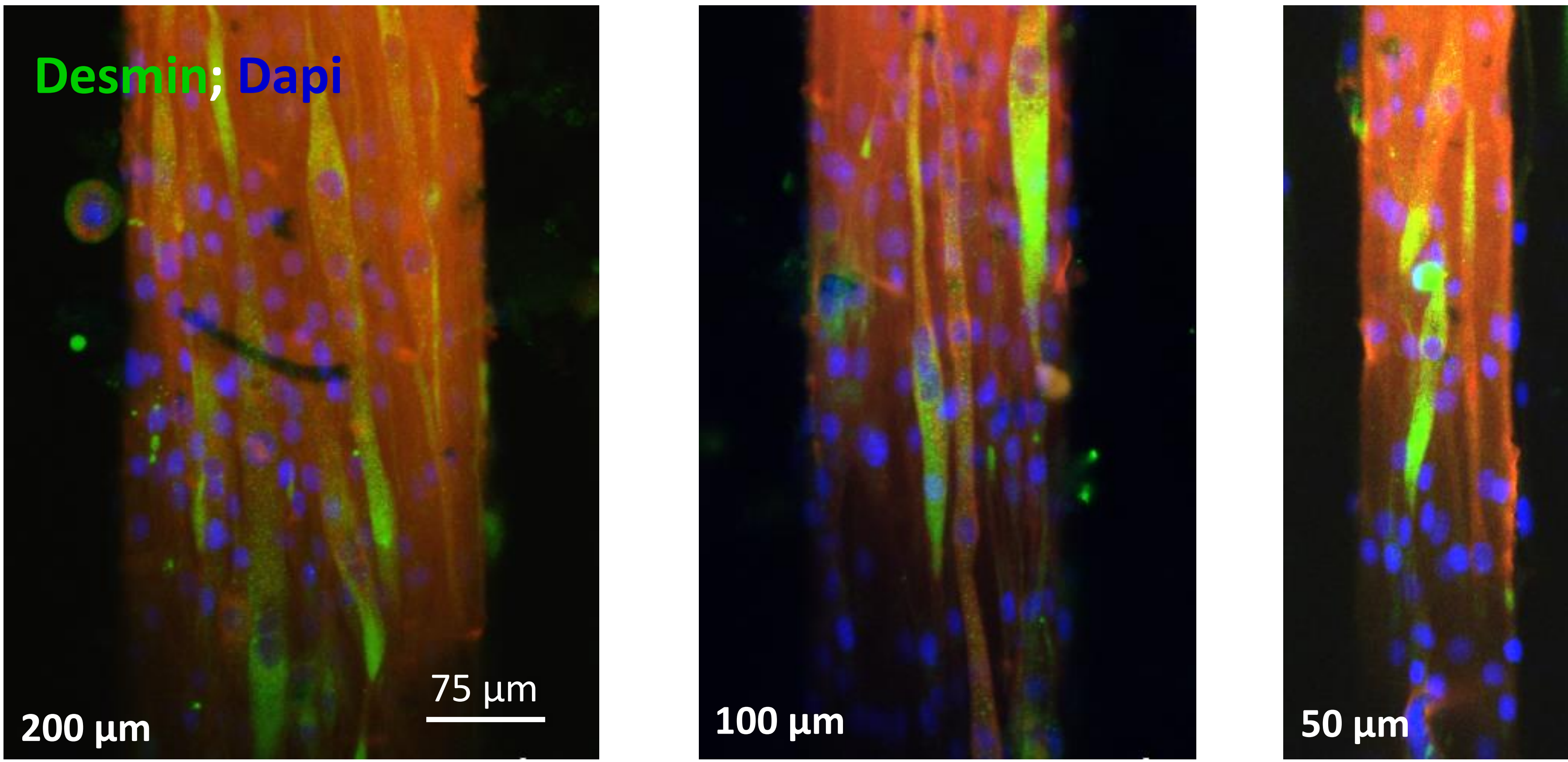
+ C212 myoblasts orientation on patterned structures



C2C12 myoblasts orientation is preferentially restricted within 10° relative to the direction of the structure

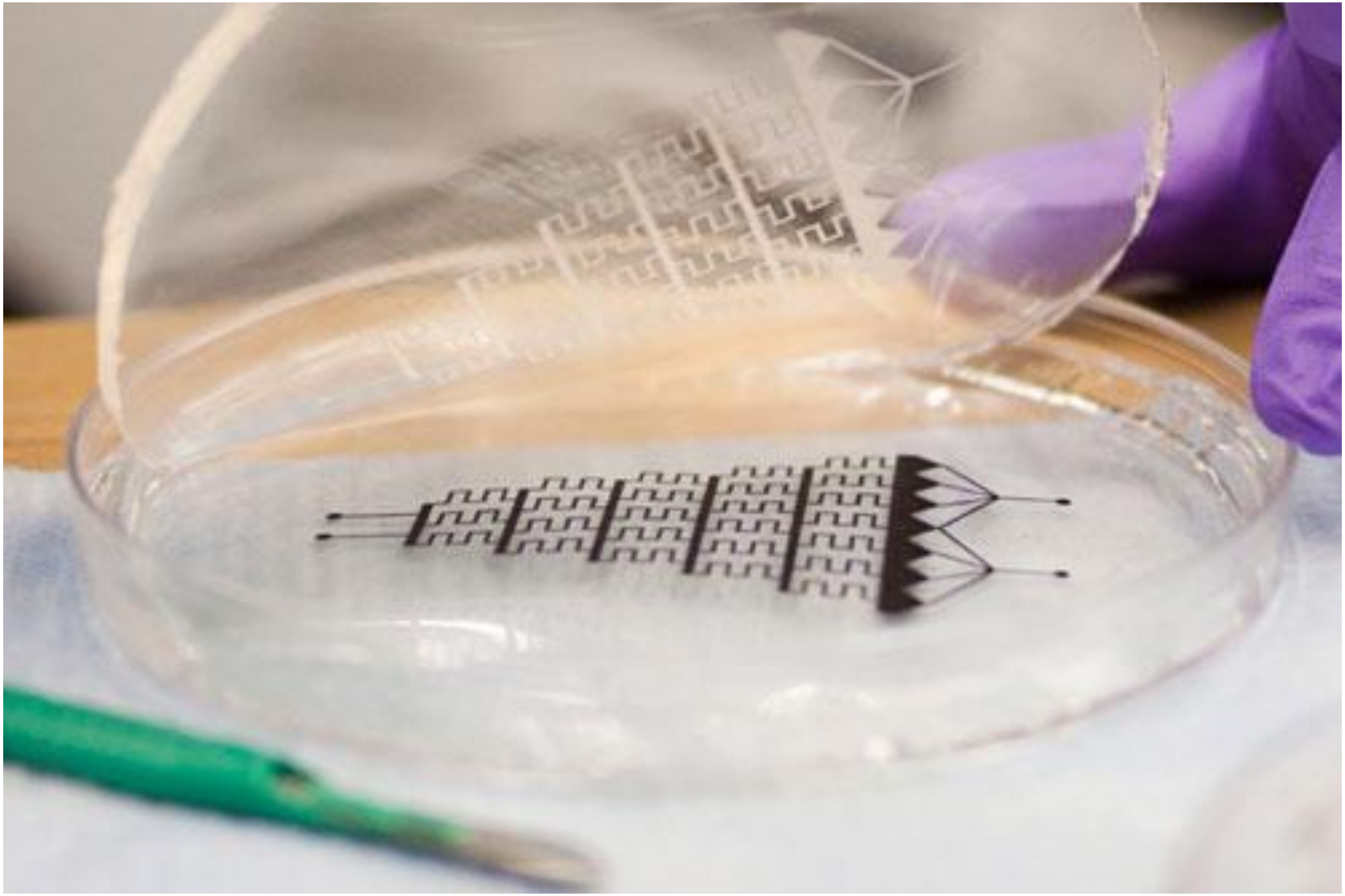
C212 myoblasts orientation on patterned structures



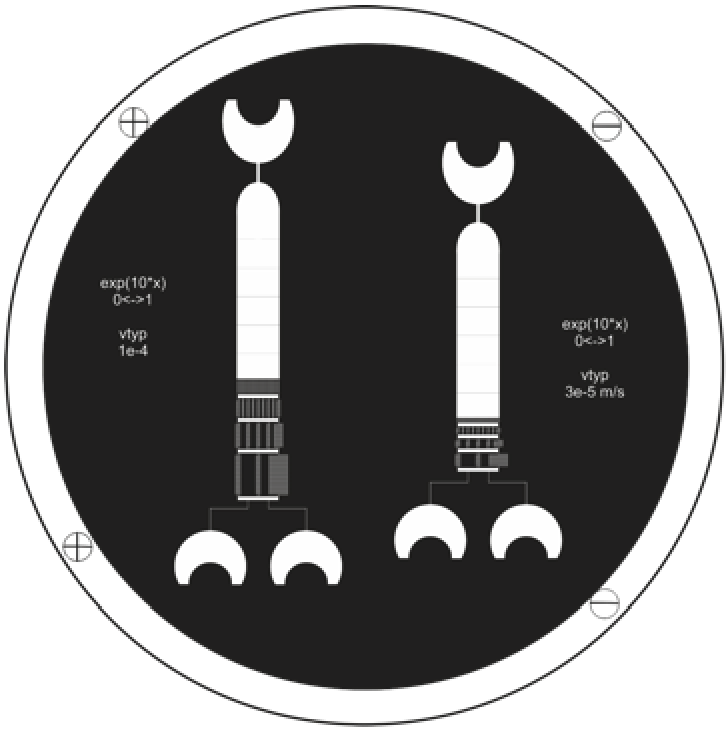


C2C12 myotubes are orientated on micropatterned substrates

Microfluidic device fabrication

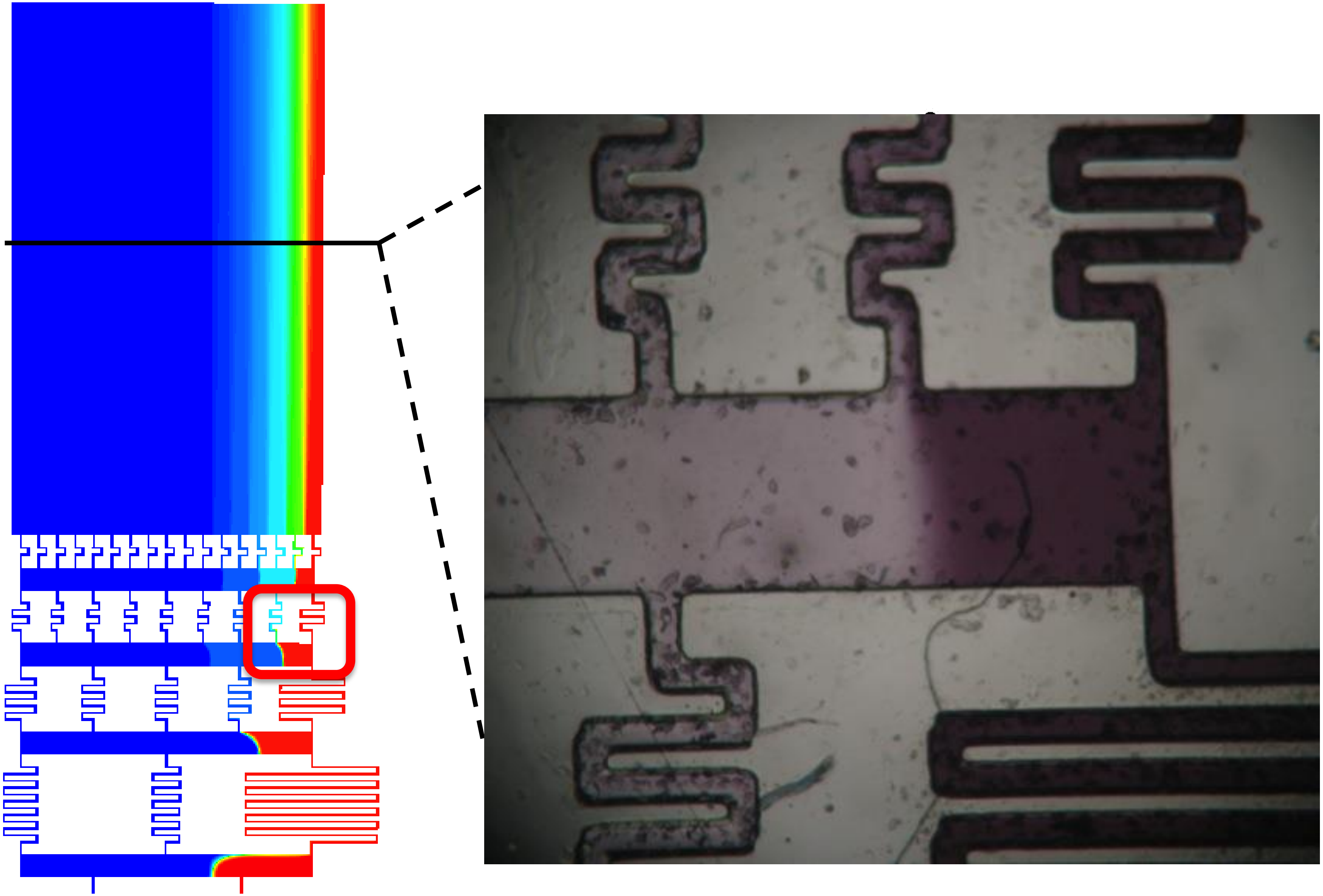


Microfluidic device fabrication

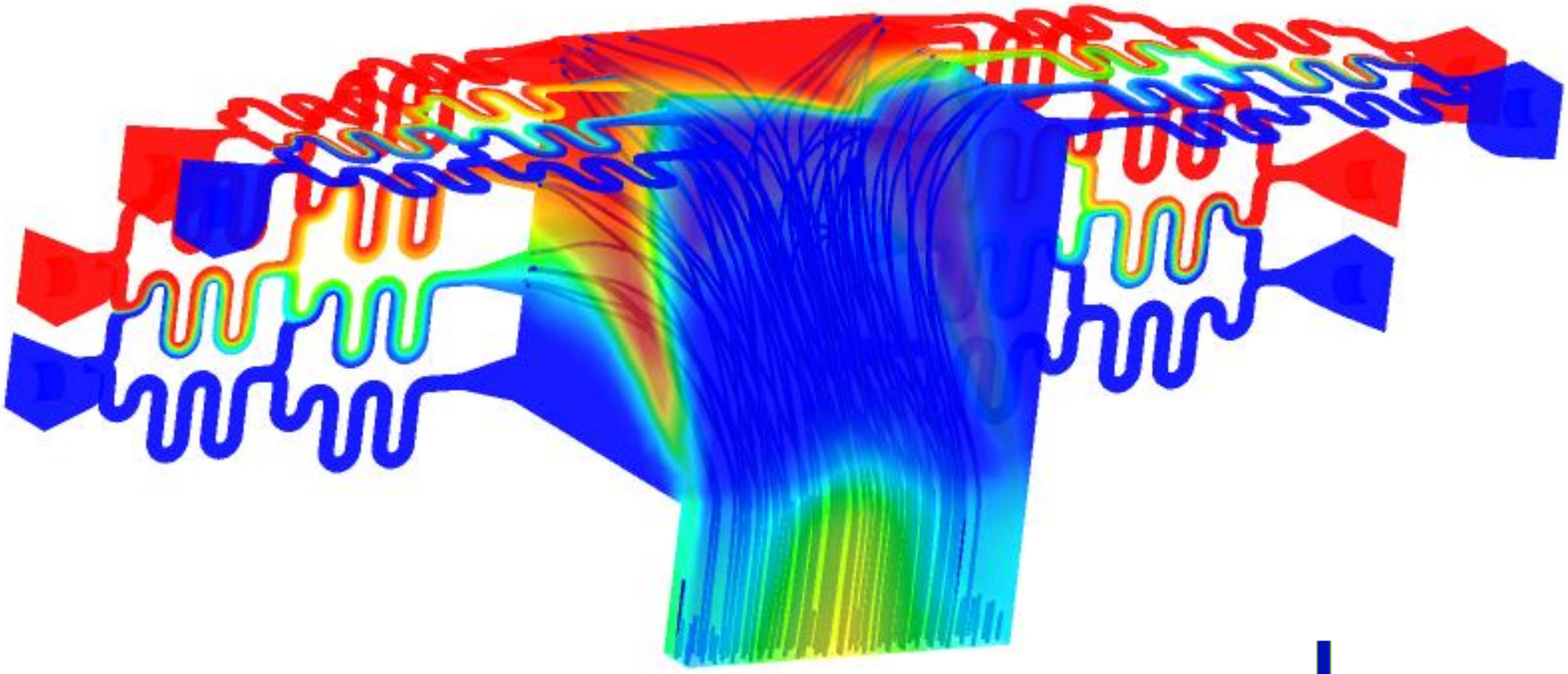


Silicon Wafer with SU-8 structure

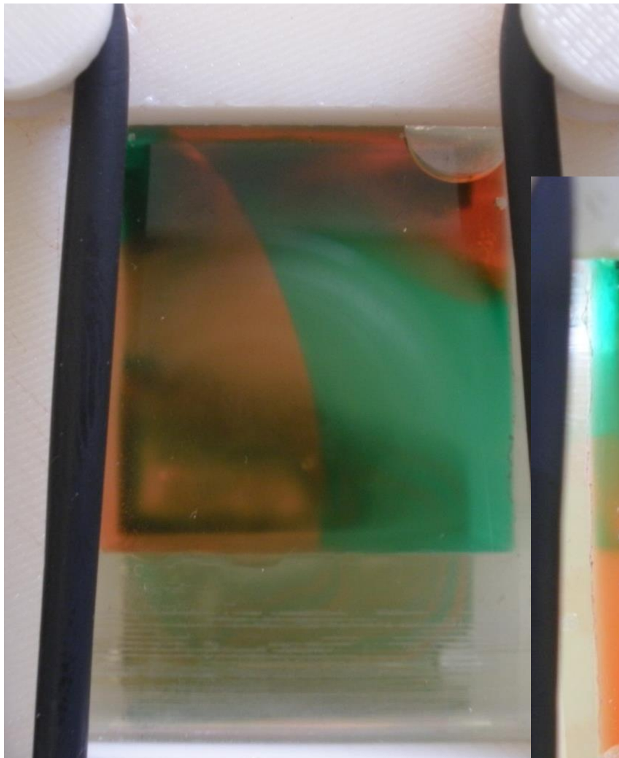
Experimental vs simulated

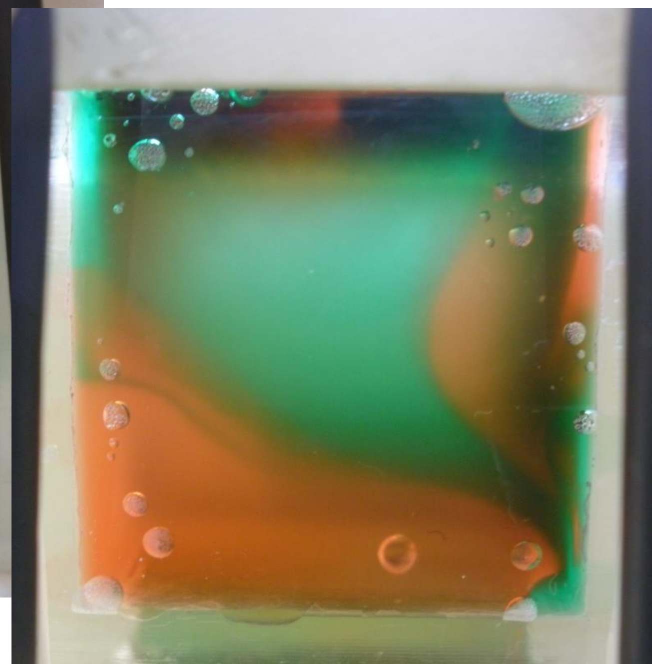


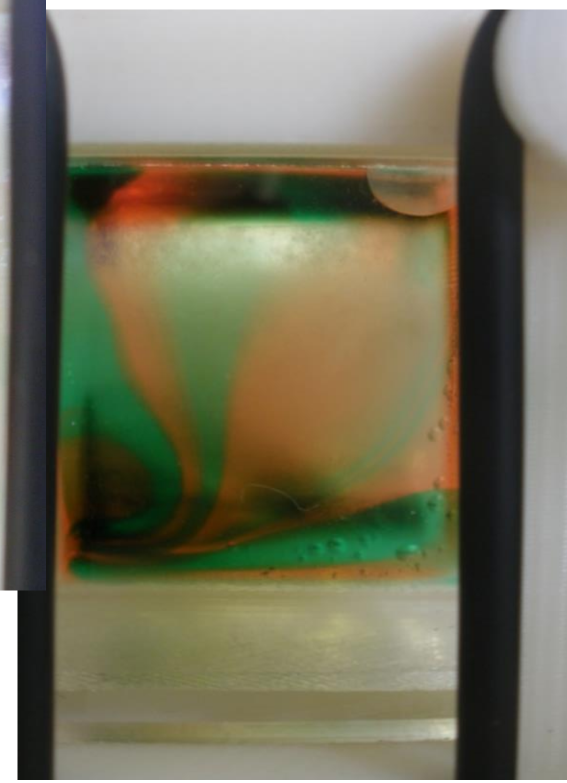
3D Concentration gradient maker



Graded stiffness substrates

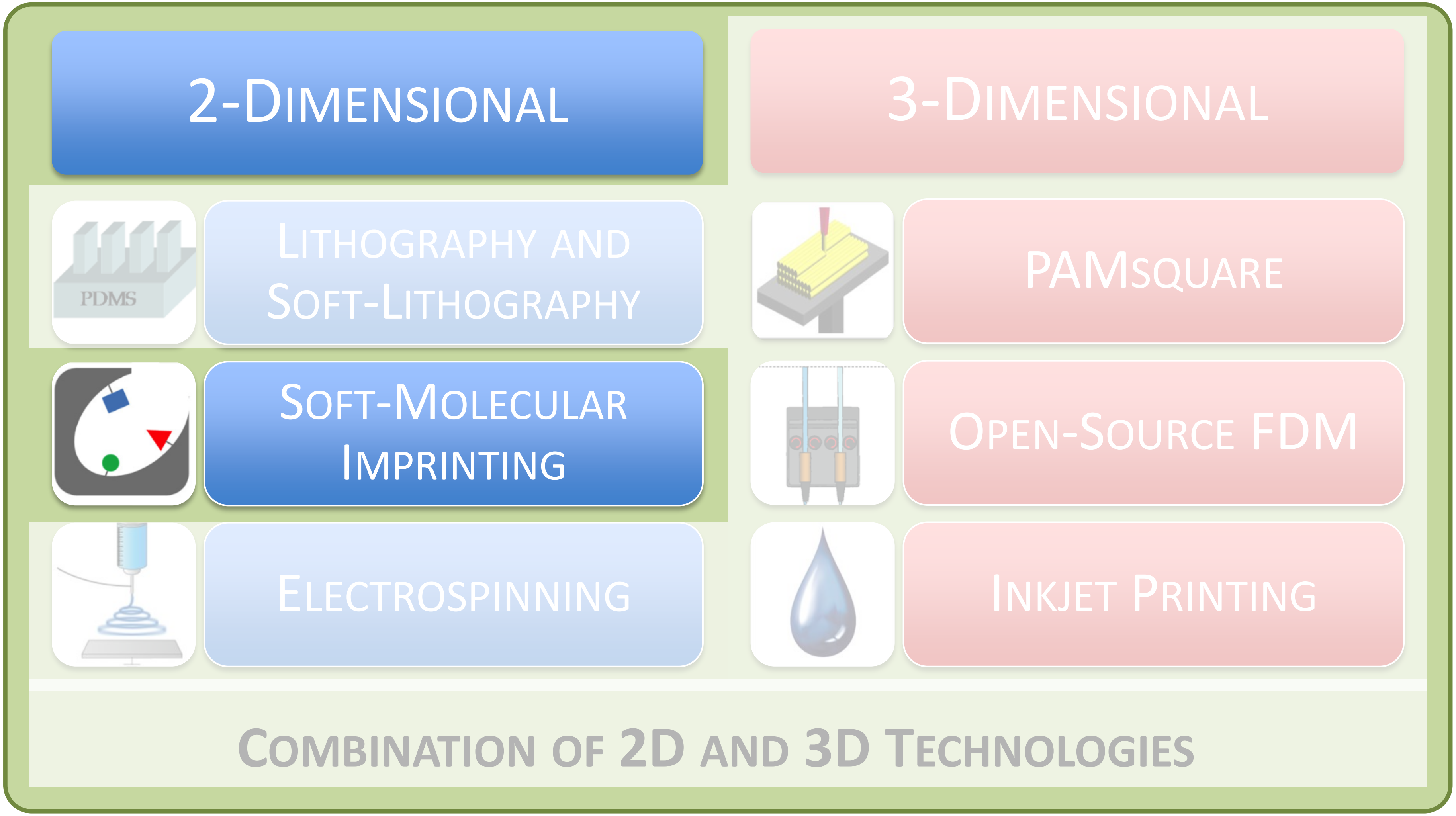






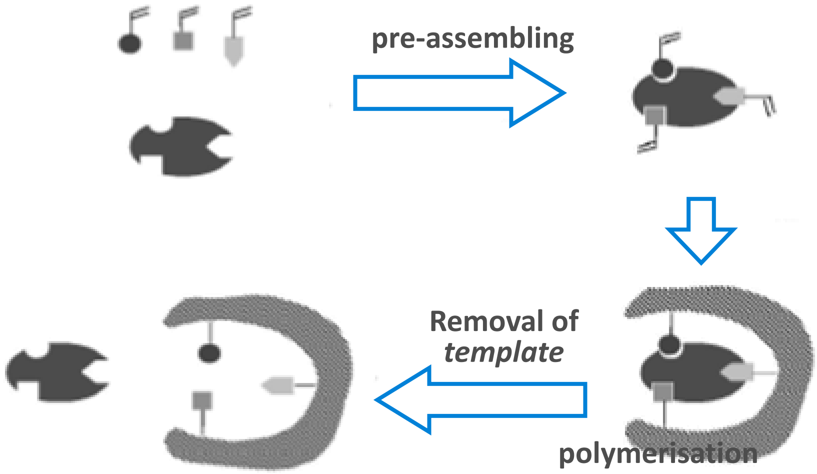
High number of different patterns generable in a single device

Soft-MI

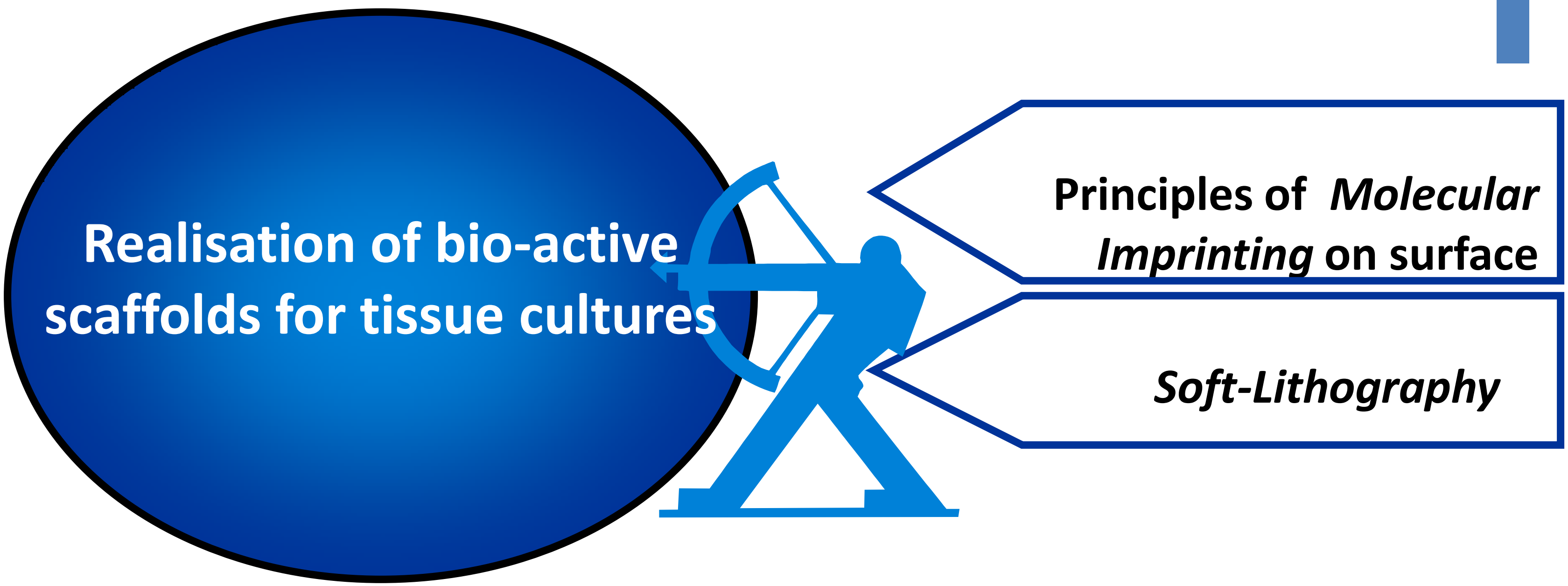

2-Dimensional
3-Dimensional
PDMS
Lithography and Soft-Lithography
PAMsquare
Soft-Molecular Imprinting
Open-Source FDM
Electrospinning
Inkjet Printing
Combination of 2D and 3D Technologies

Molecular Imprinting

- Molecular Imprinting is a technology that allows to realise matrix or surface, usually made of organic polymers, with specificic and selective sites of recognition of a selected molecule (template) thanks to the steric and chemical complementarity
  - covalent interactions
  - reversible not covalent interactions



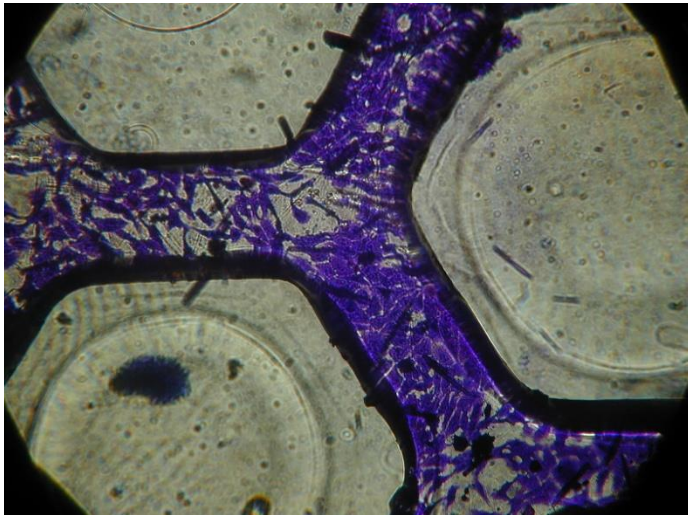
SOFT-MI

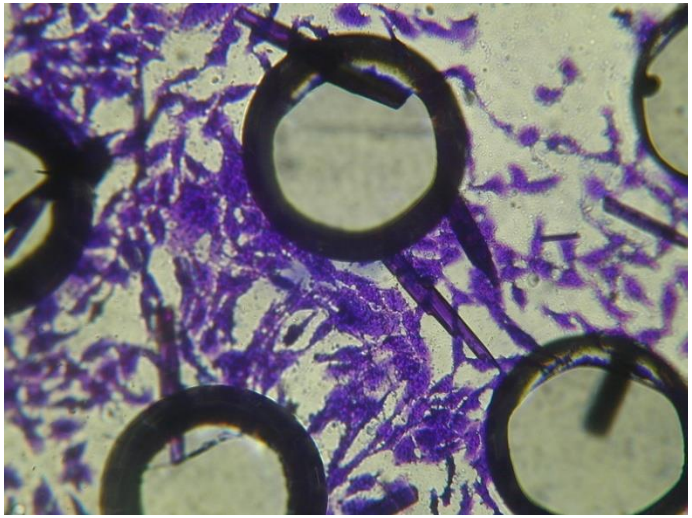


1. **Fabrication of PDMS mold**
2. **modification of its superficial chemical properties**
3. **functionalisation of its surface**
4. **cell culture test**

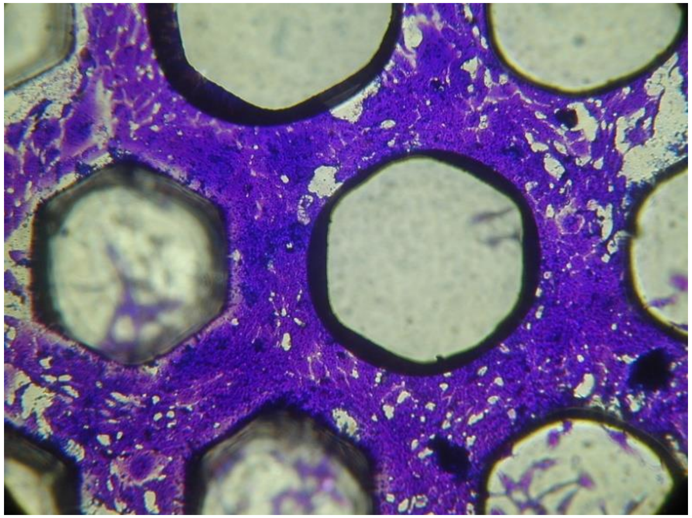
Vozzi G et al Biotechnol Bioeng. 2010;106(5):804-17.

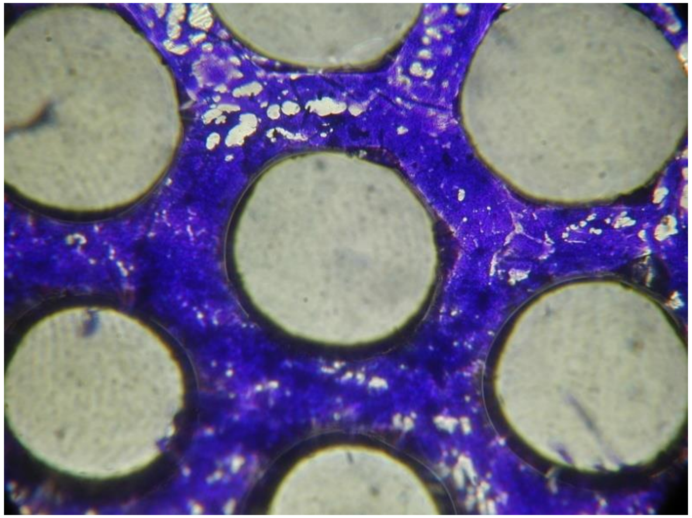
Imprinting cells





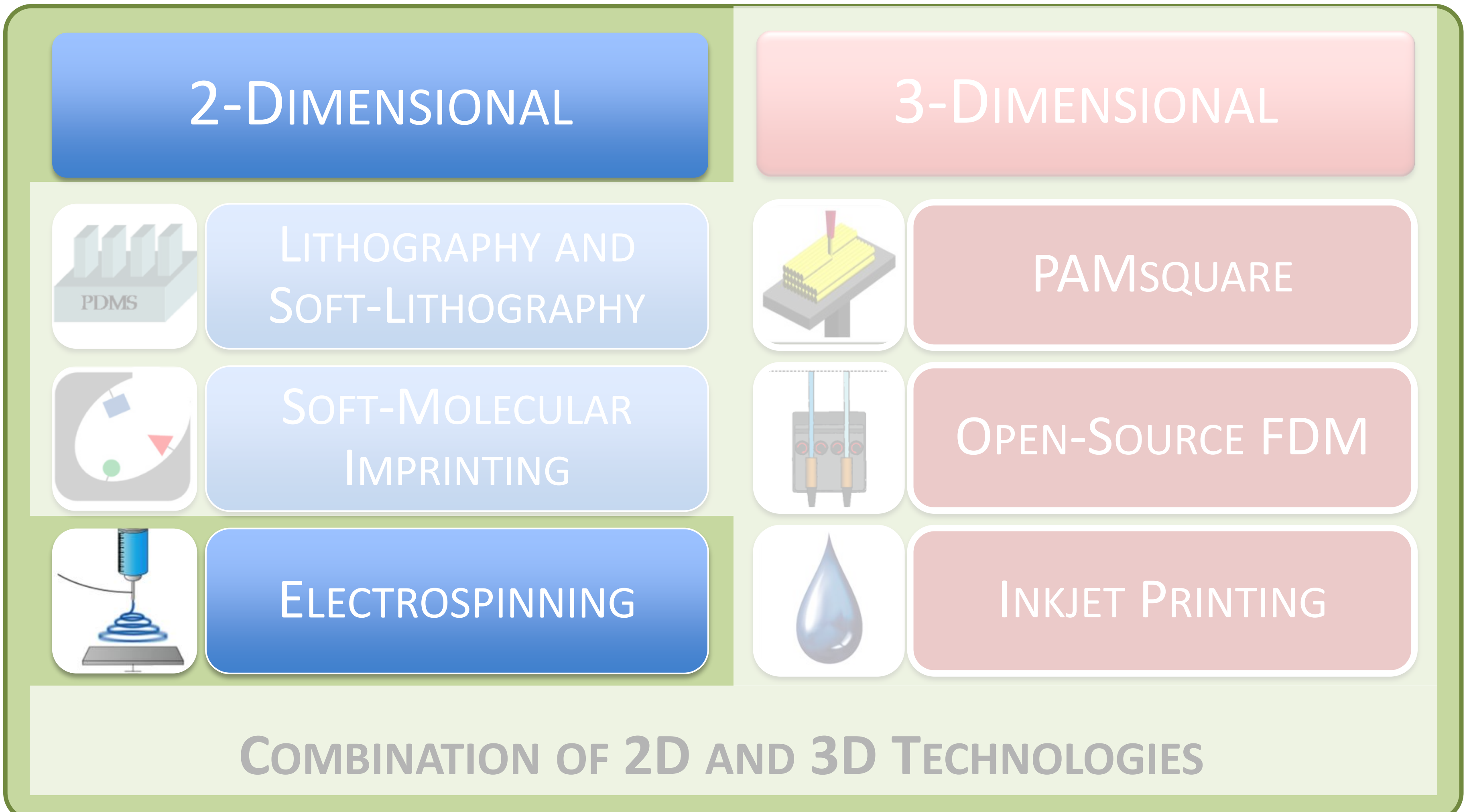
48h



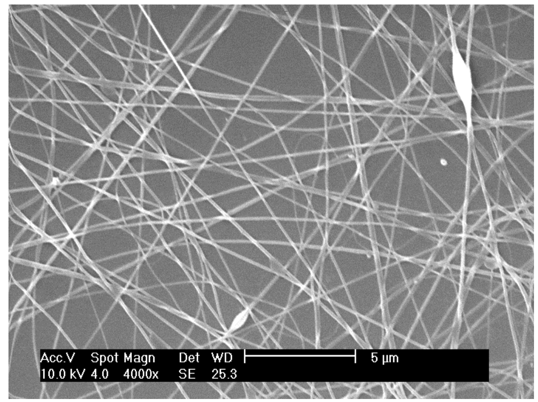


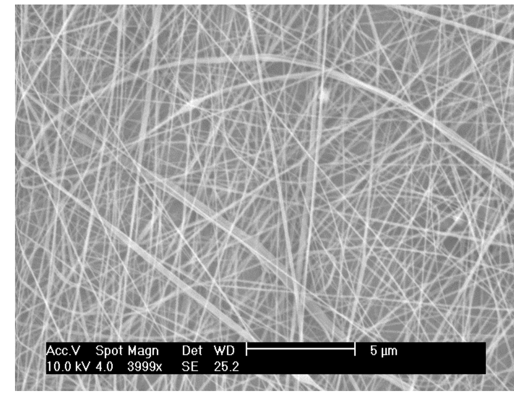
72h

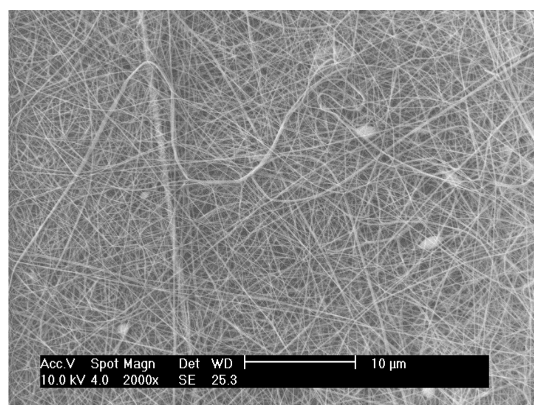
Electrospinning

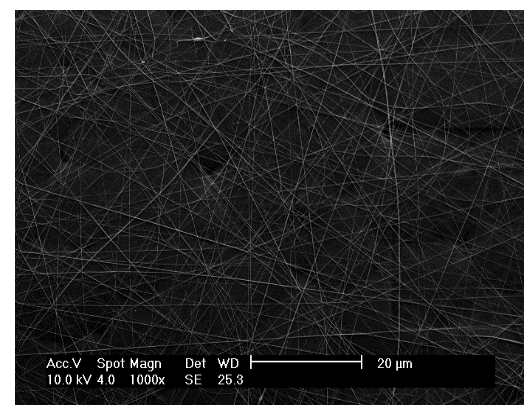


Electrospinning

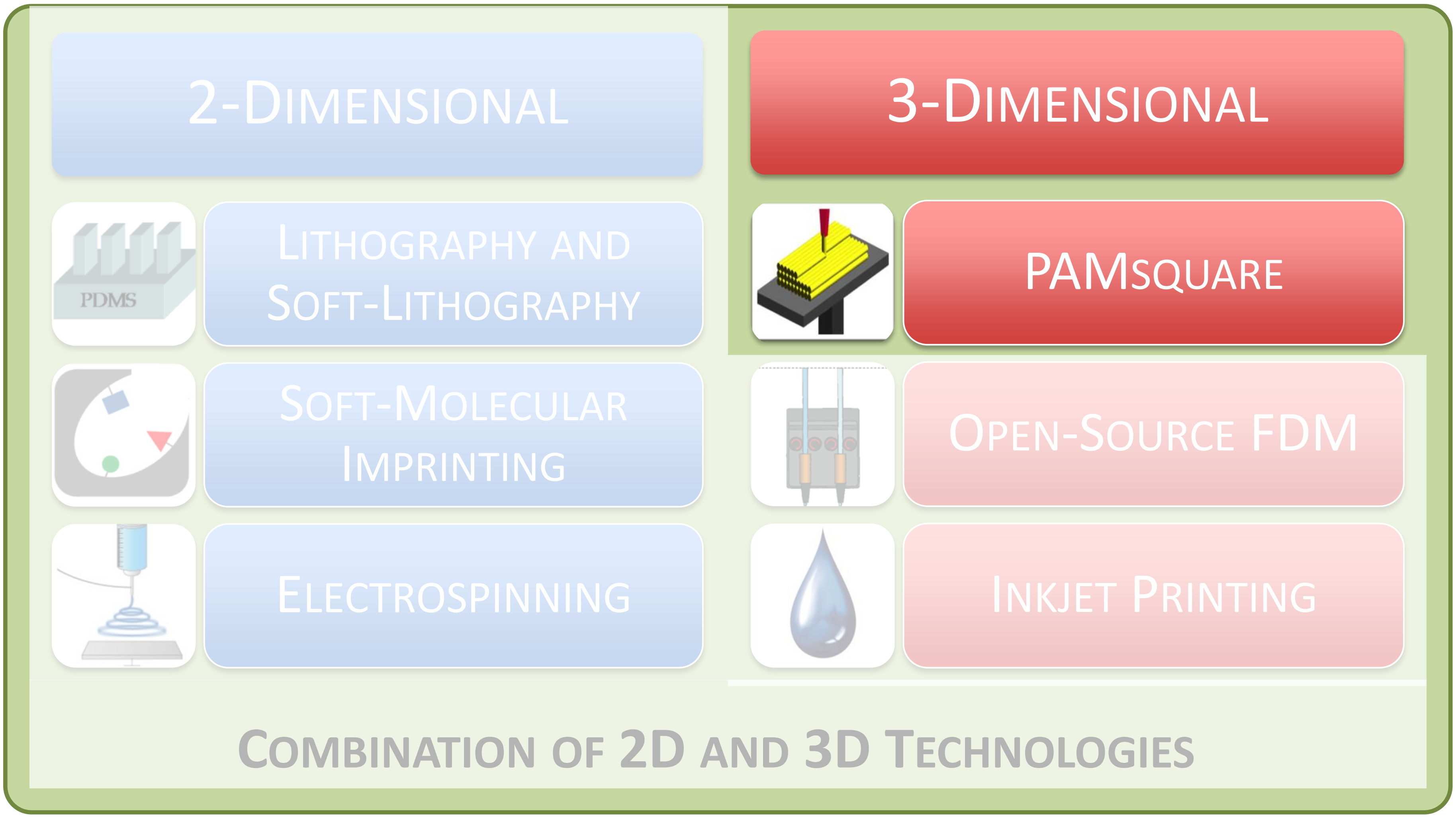






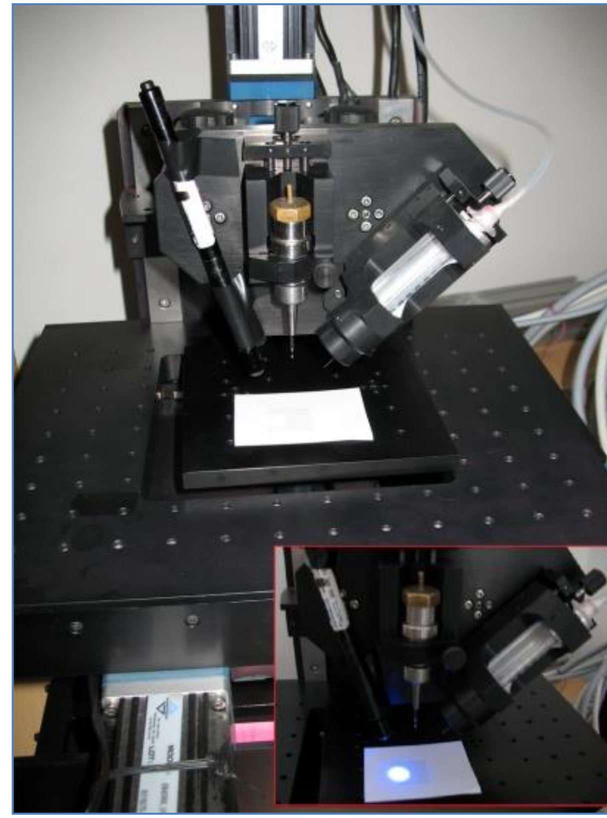


PAMsquare


2-Dimensional
3-Dimensional
PDMS
Lithography and Soft-Lithography
PAMsquare
Soft-Molecular Imprinting
Open-Source FDM
Electrospinning
Inkjet Printing
Combination of 2D and 3D Technologies

PAM^2

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



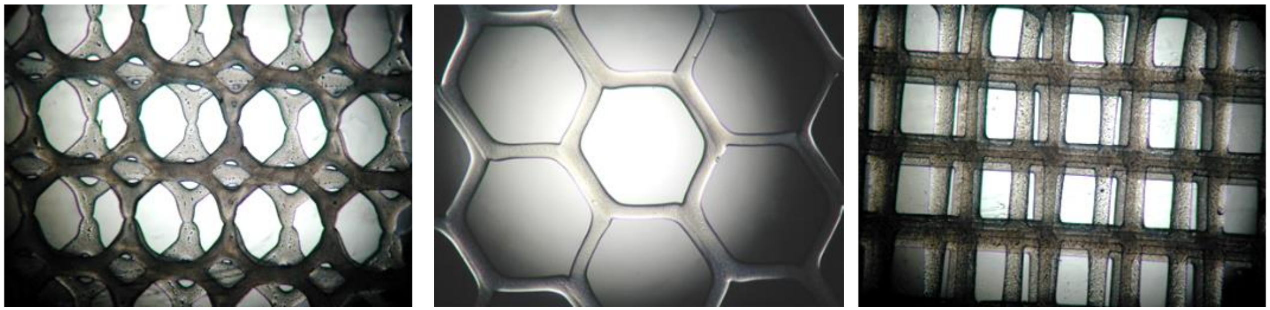
3D robotic stage

- Pressure
- Force
- Temperature
- Light

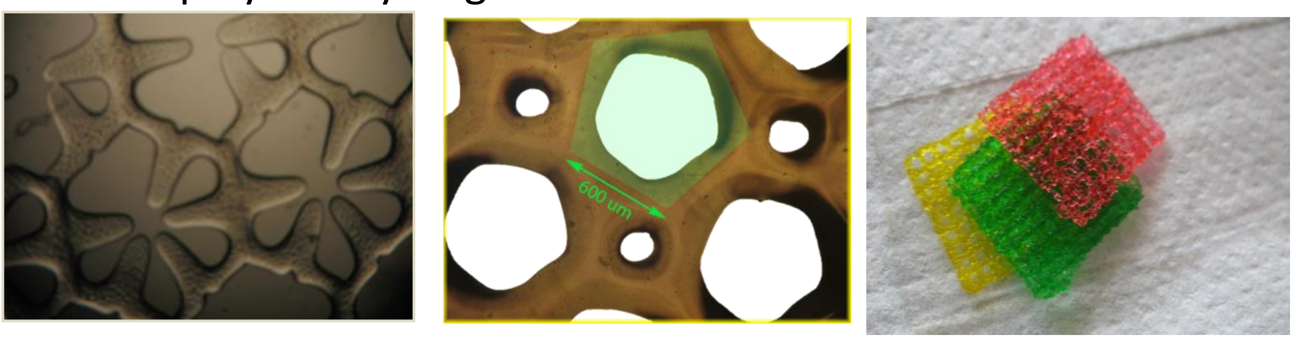
Tirella A, De Maria C, Criscenti G, Vozzi G, Ahluwalia A. The PAM^2 system: a multilevel approach for fabrication of complex three-dimensional microstructures. Rapid Prototyping J 2012;18(4):5-5

PAM^2

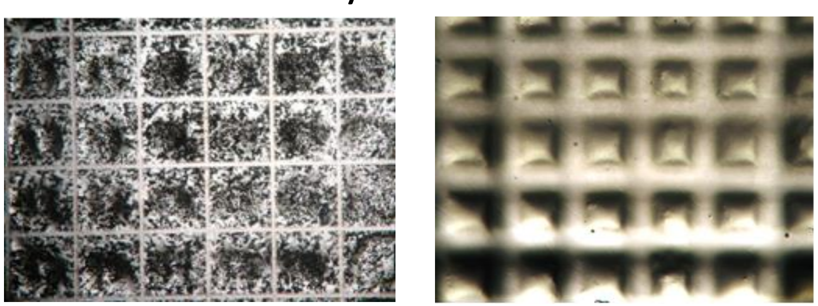
Polyester structures



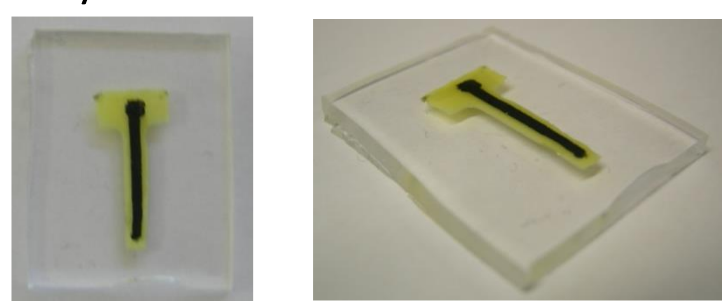
Natural polymer hydrogel structures



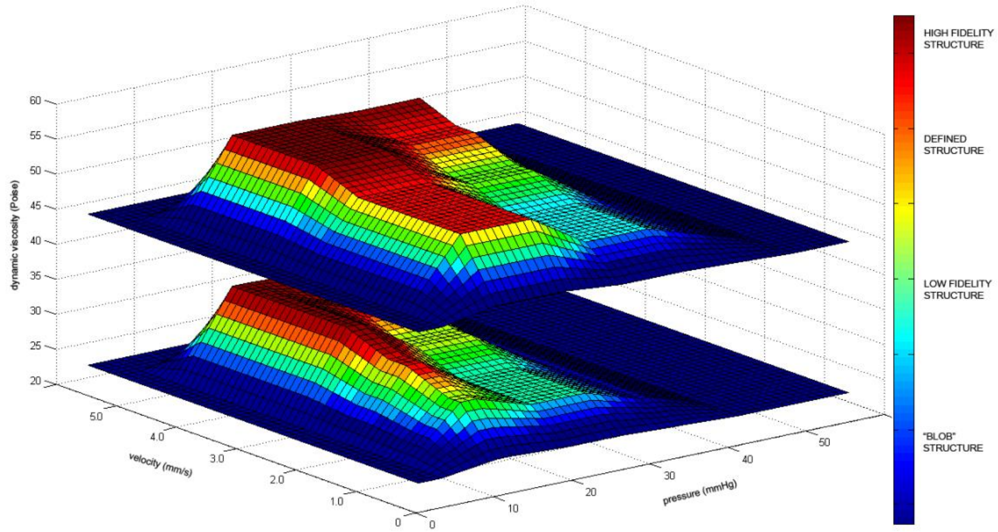
Laser ablation dry and wet structures

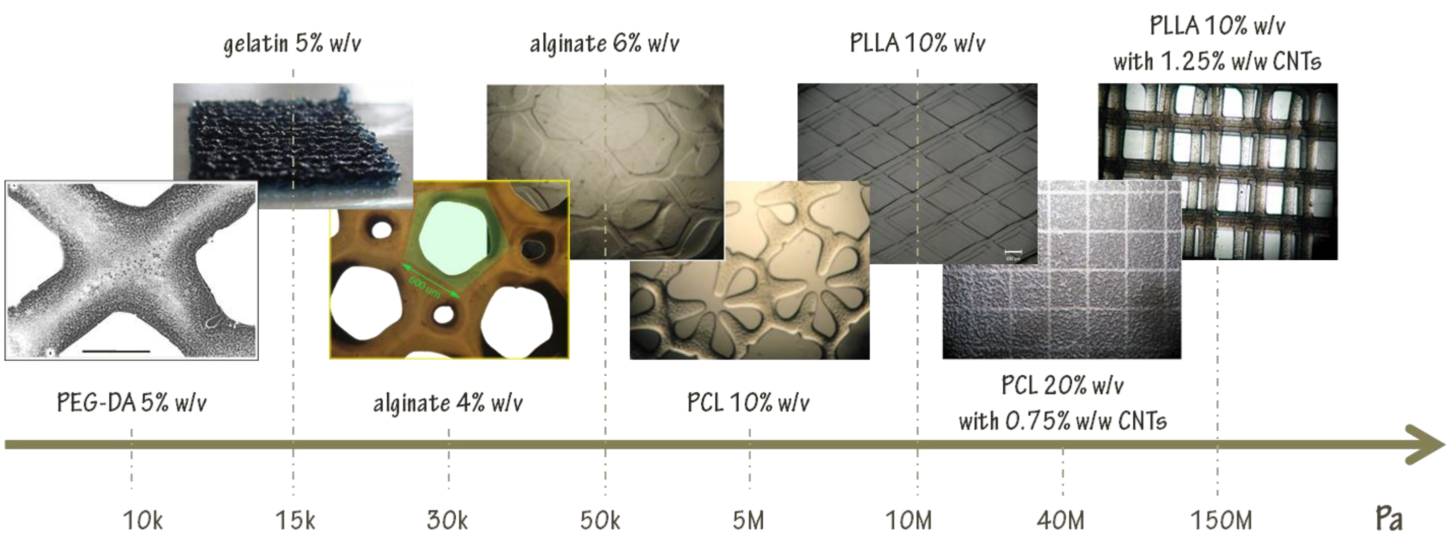


Polymeric actuators



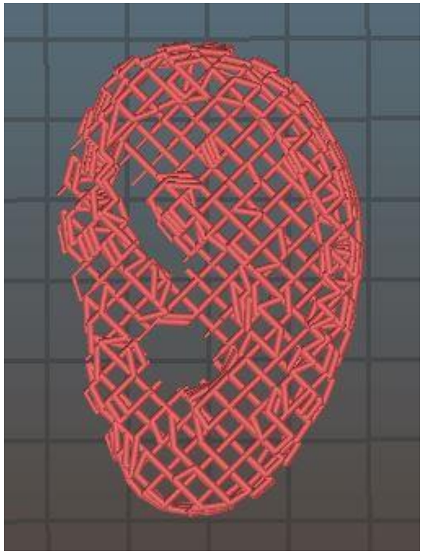
Multi-tuning Bioactive scaffold



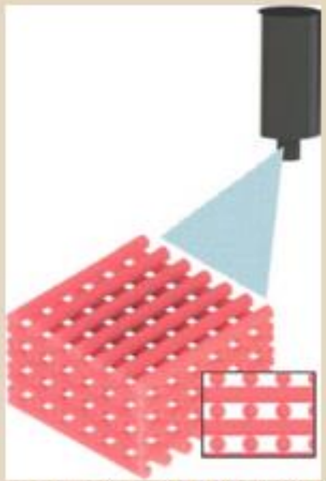
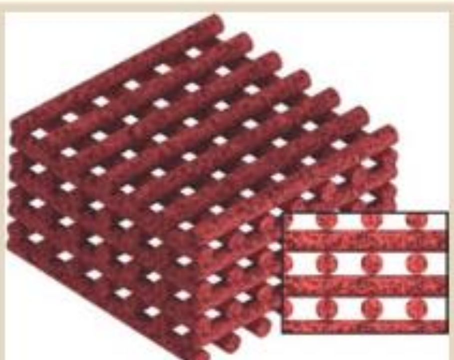


Hydrogel plotting

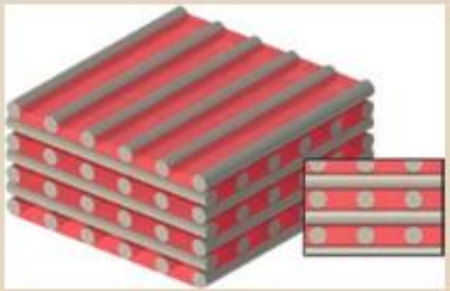
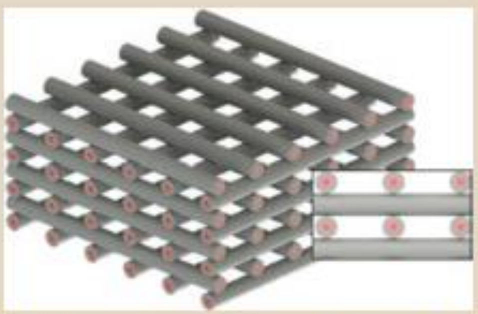
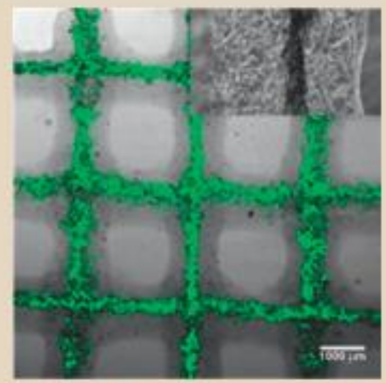
- Self-assembling ph-sensitive polypetide gel
- Printing gel-in-gel



Strategies for hydrogel plotting

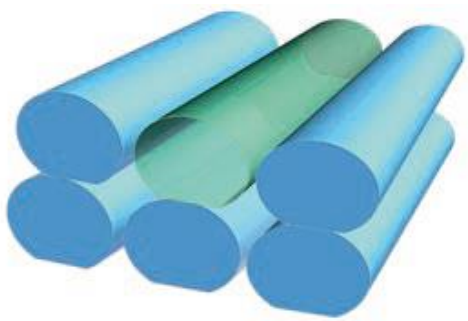
Table I. Principles of fabricating volumetric tissue constructs by extrusion bioprinting approaches and respective examples. The cell-laden bioink strands are shown in red in the schematics.		
Category	**Principle**	**Example**
1		
Technical solutions	 Technical procedure for scaffold stabilization (e.g., $CaCl_2$ aerosol spray enabling cross-linking *in situ*)	 Three-dimensional printed alginate structure, fabricated via continuous platform-lowering into stabilizing cross-linking solution, resembling a vascular tube (tube diameter 10 mm, height ca. 35 mm).[4]
2		
Internal stabilization	 Internal stabilization of hydrogel (red) strands by blending with additional polymer material(s) (black)	 Nanofibrillated cellulose-alginate bioink (80:20) printed in the shape of a human ear with high shape fidelity (dimensions ca. 20 × 25 × 10 mm^3). This blend offered excellent properties for printing of chondrocytes.[5]

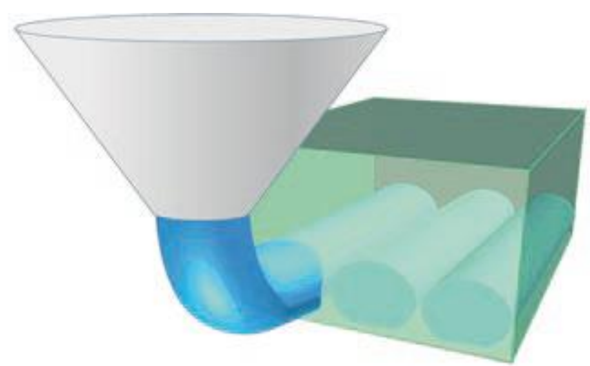
Strategies for hydrogel plotting

3		
External stabilization	 External structure stabilization of cell-laden hydrogel (red) by a second, stiffer biomaterial (gray)	 Three-dimensional printing of an ear with a PCL frame. The auricular cartilage region is colored red, and the lobe fat tissue is blue (dimensions ca. $20 \times 25 \times 8$ mm^3).[6]
4		
Core–shell morphology	 Modification of strand morphology by core–shell (core: red; shell: gray) setup based on two different (cell-laden) materials	 Three-dimensional printed core–shell scaffold with fluorescently labeled cells (green) in the core surrounded by the shell (gray). The inset illustrates the core–shell morphology of such scaffolds in bright-field microscopy. Stability to the construct was provided by cross-linking the shell components by ionic cross-linking and photocuring. Printing of a cube with $20 \times 20 \times 20$ mm^3 without cells was demonstrated.[7]

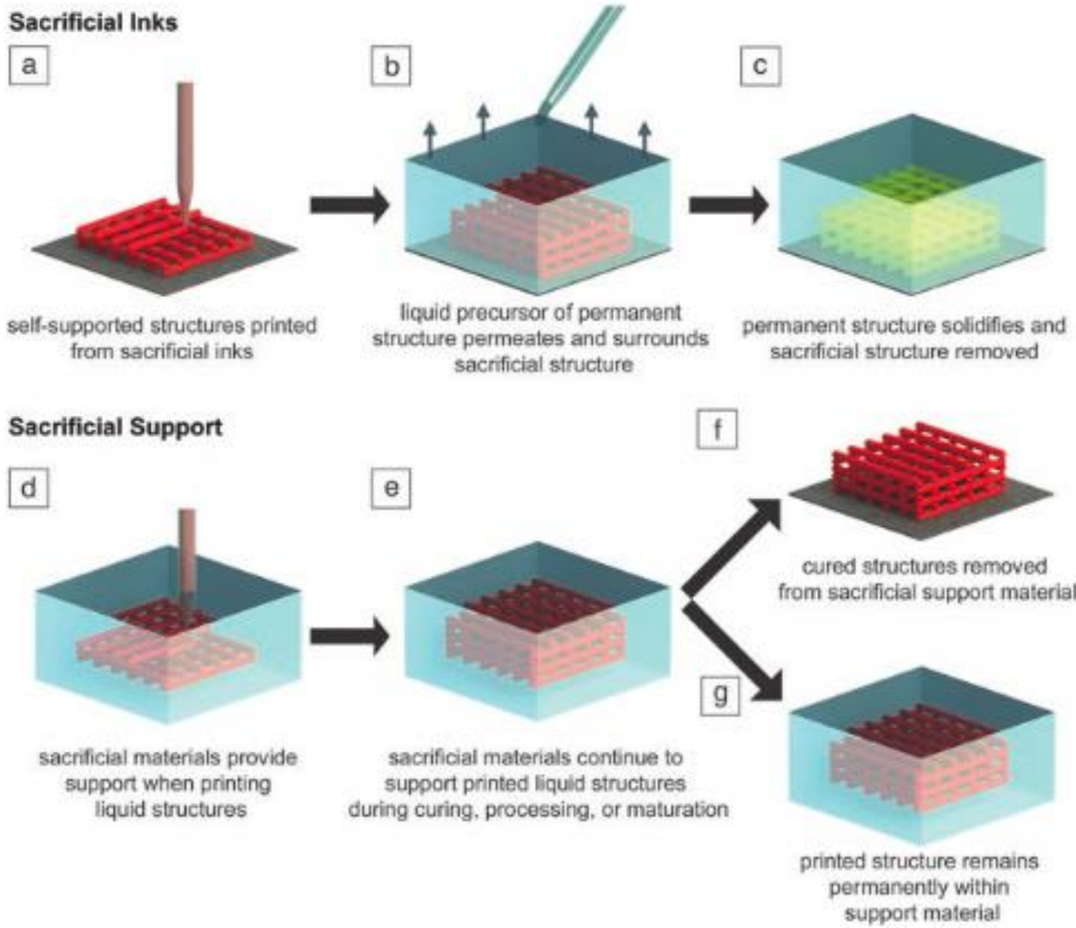
Strategies for hydrogel plotting

- External stabilization
  - Sacrificial inks co-printed with bioinks
  - Bioinks printed into sacrificial medium

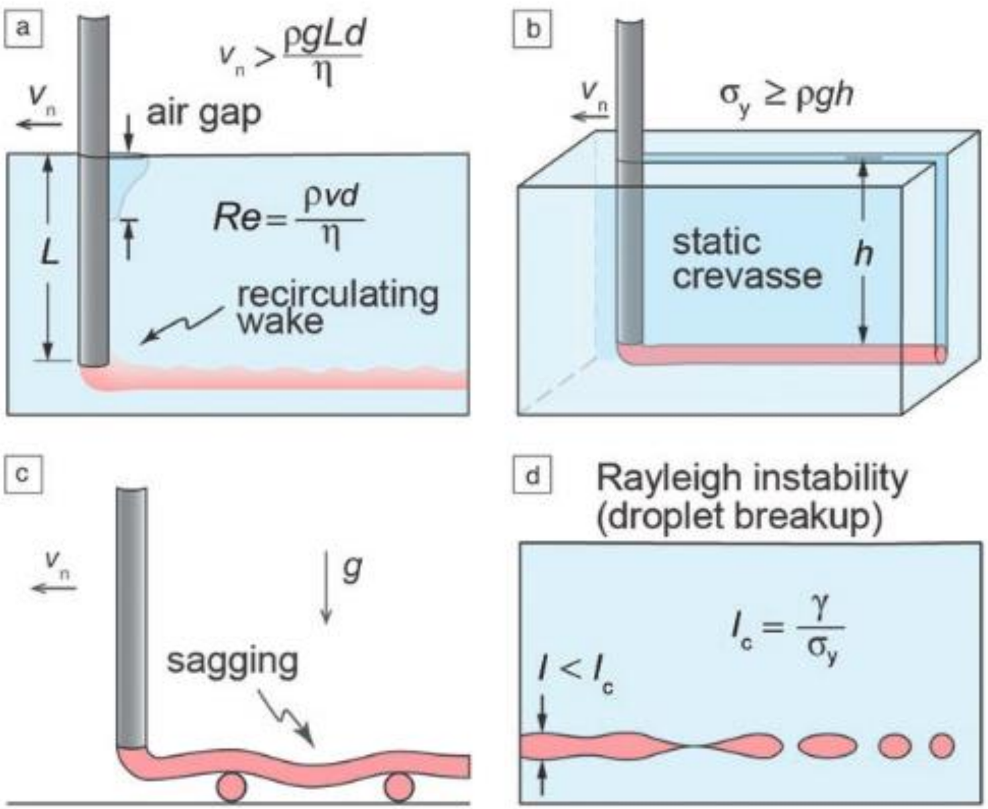


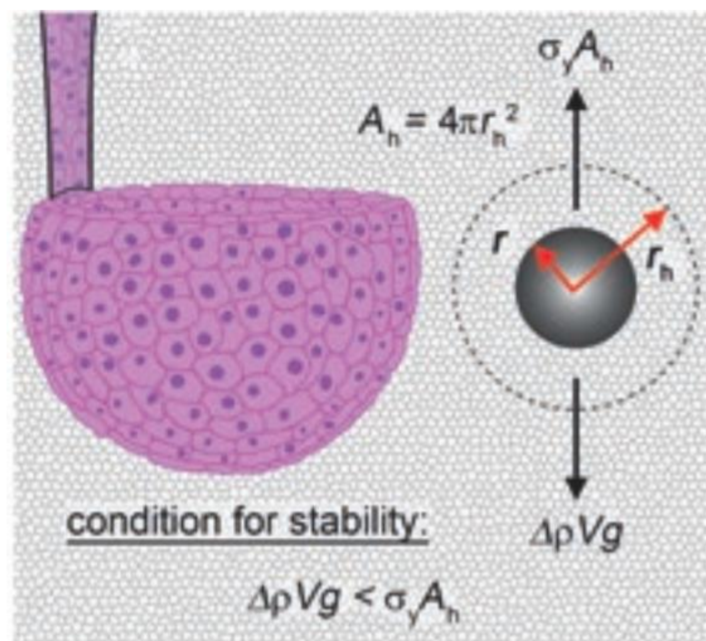


Strategies for hydrogel plotting



Plotting into a sacrificial support





Printing cell laden hydrogel

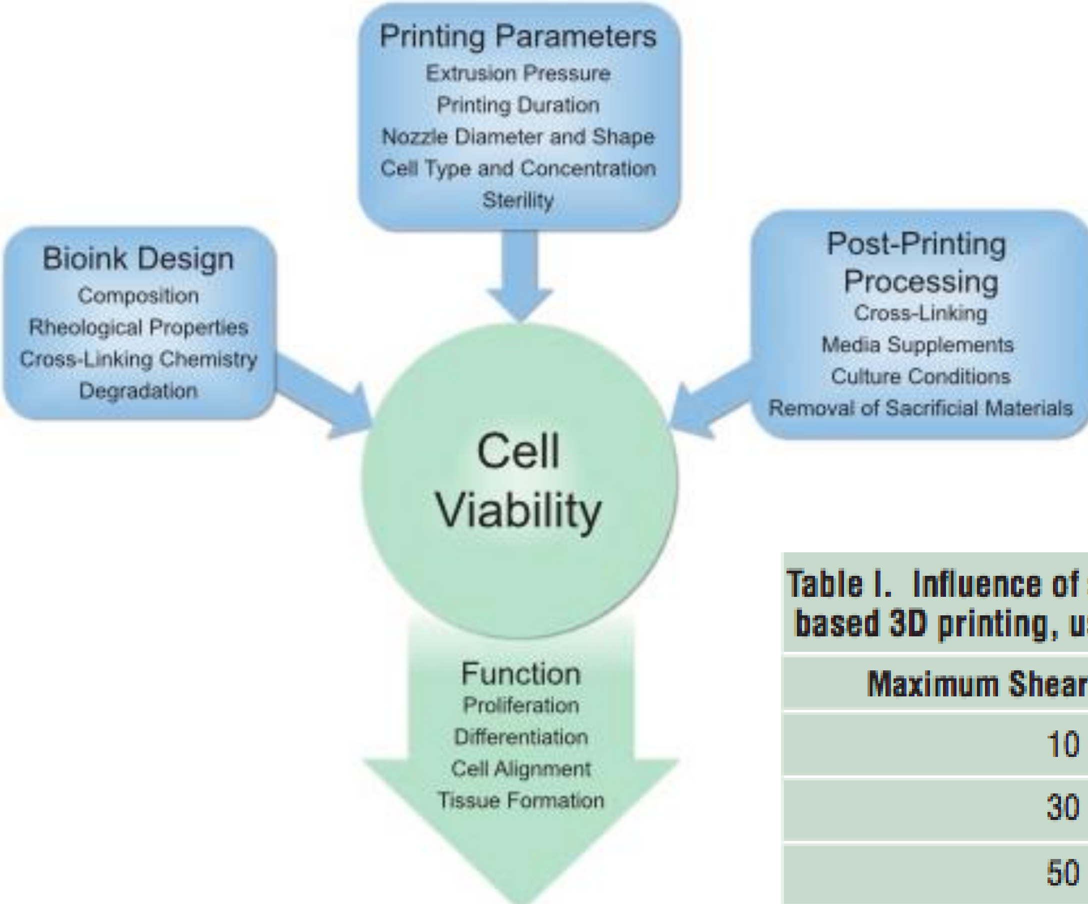
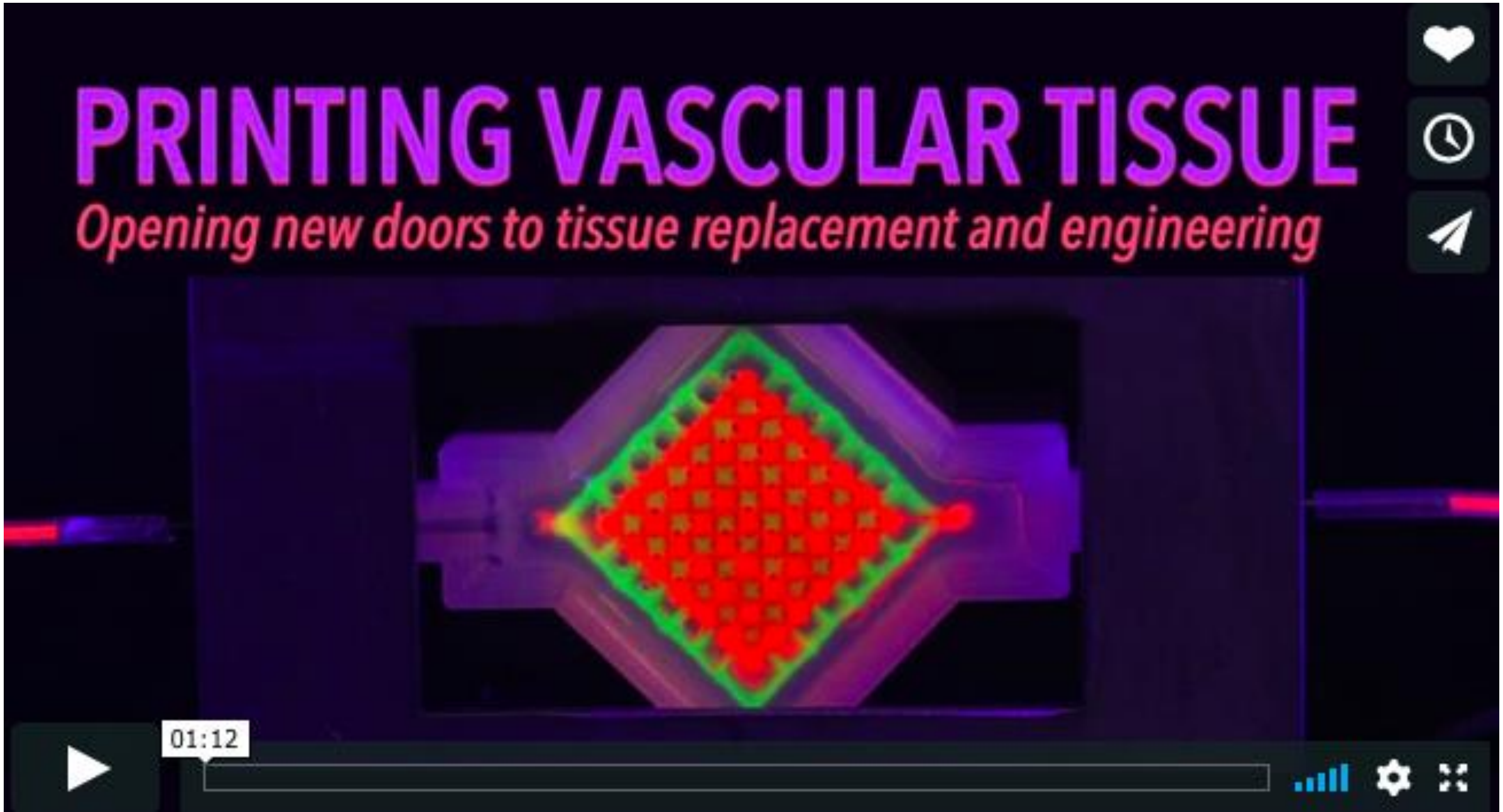


Table I. Influence of shear stress on cell viability during extrusion-based 3D printing, using a pneumatic system with a microvalve.[46]

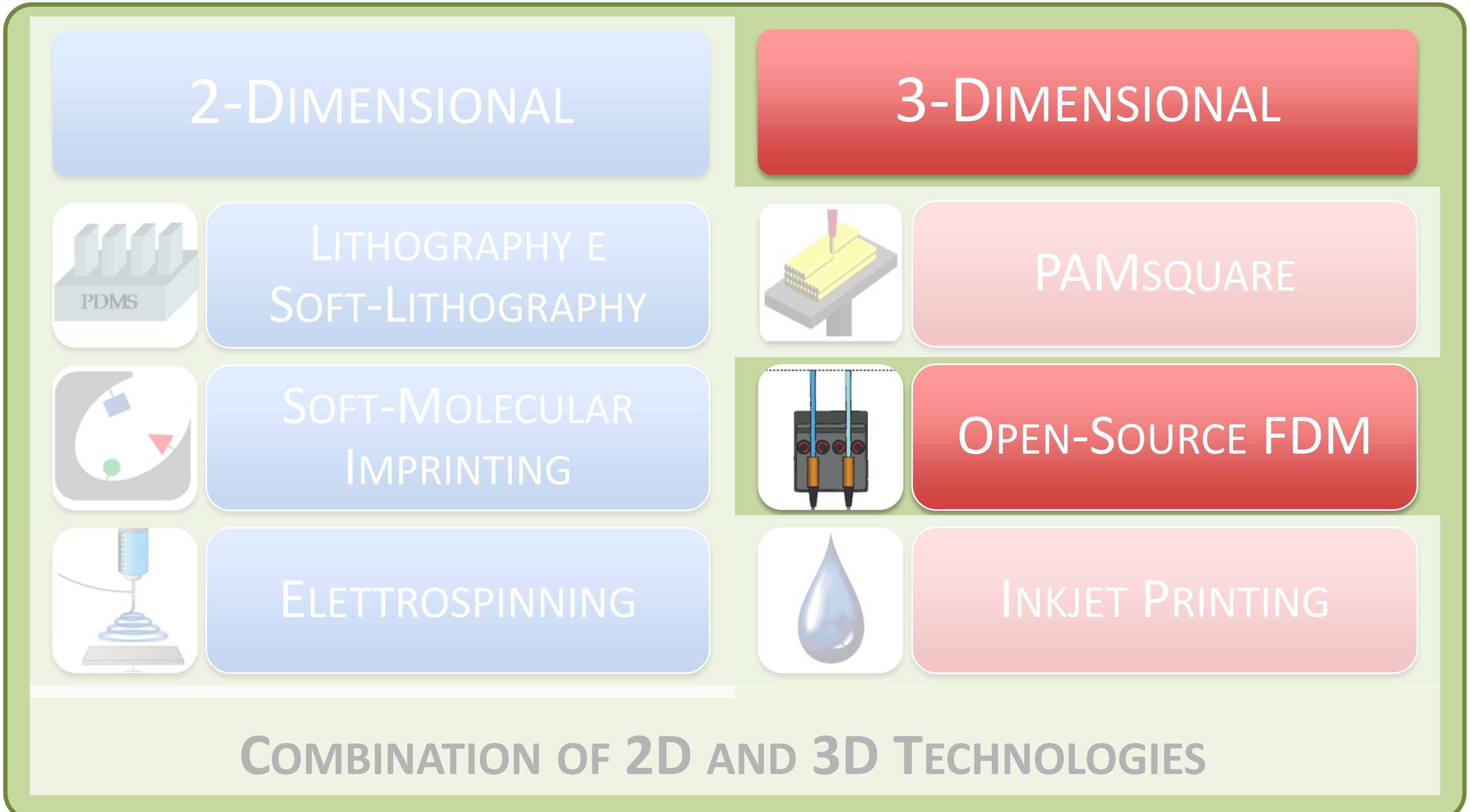
Maximum Shear Stress (kPa)	Viability (%)
10	90
30	80
50	70
130	60

Challenges in cell printing

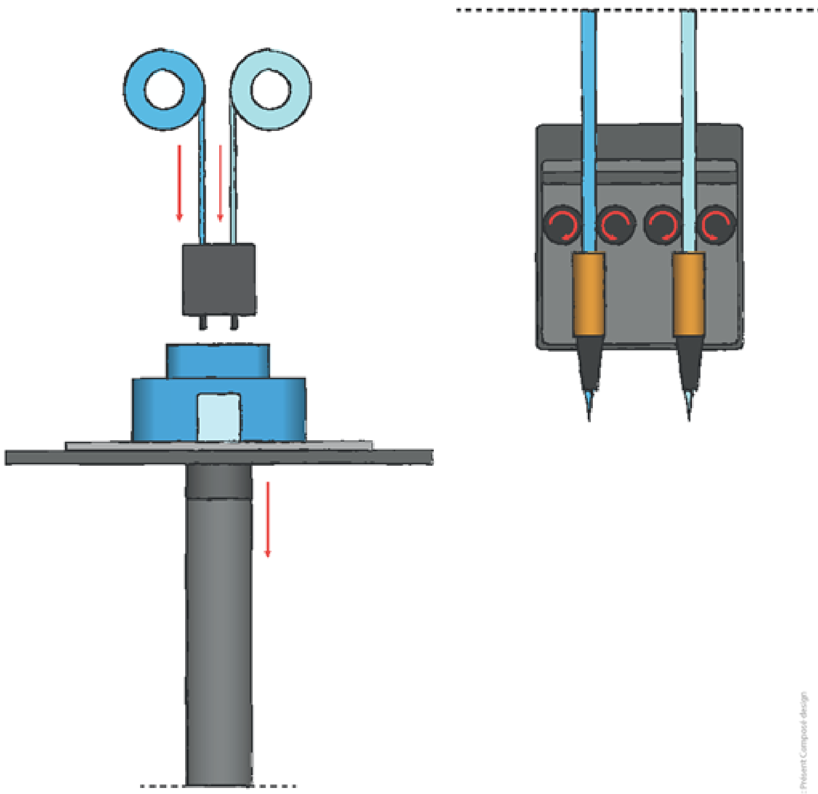
- https://wyss.harvard.edu/media-post/printing-vascular-tissue/



Open-Source FDM



Fused Deposition Modeling



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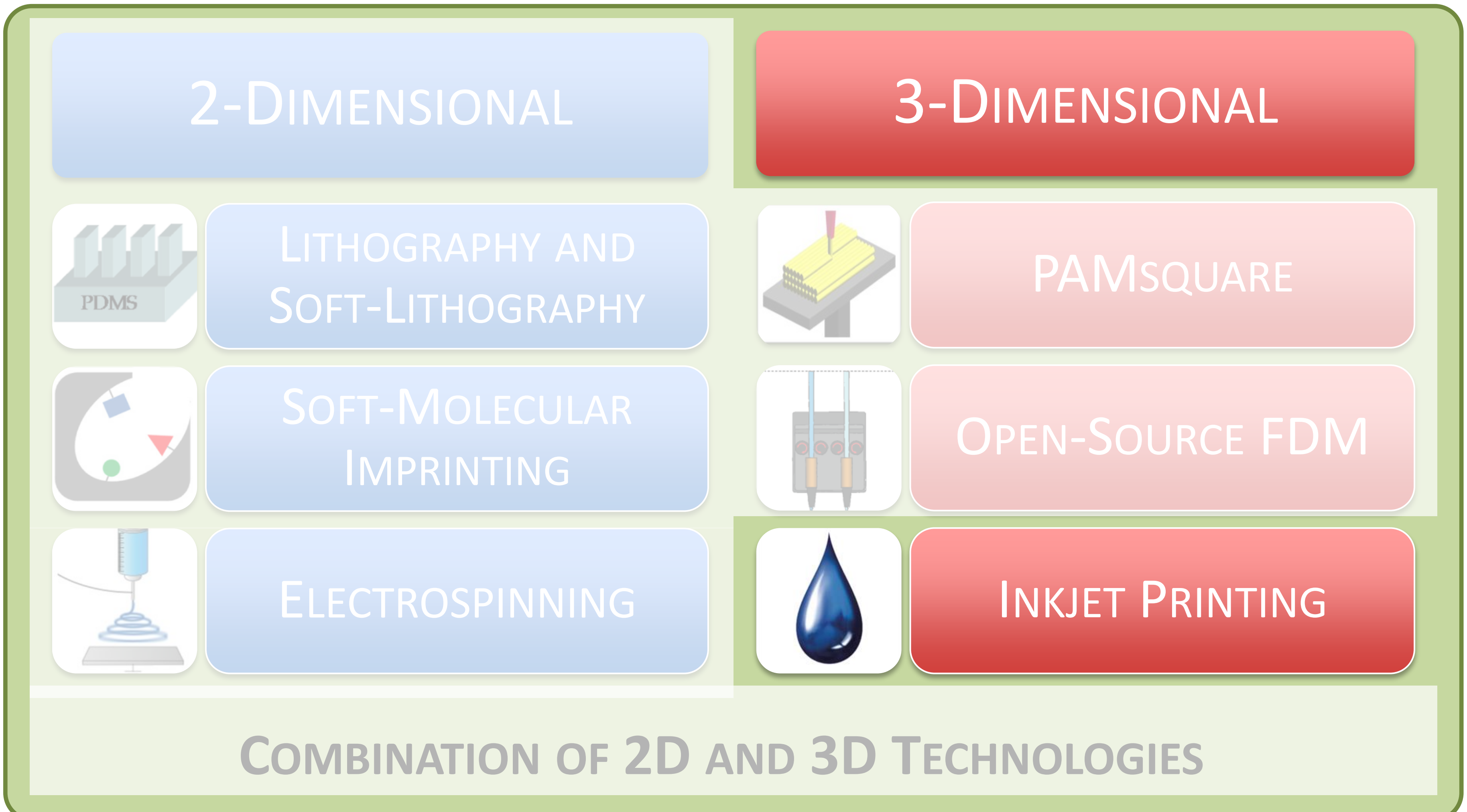
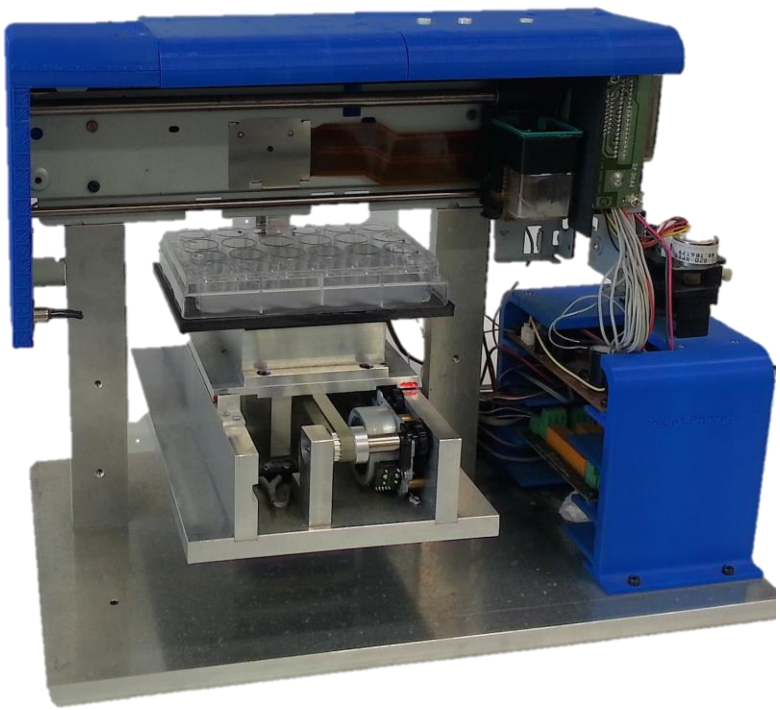
THE IDEAS FACTORY

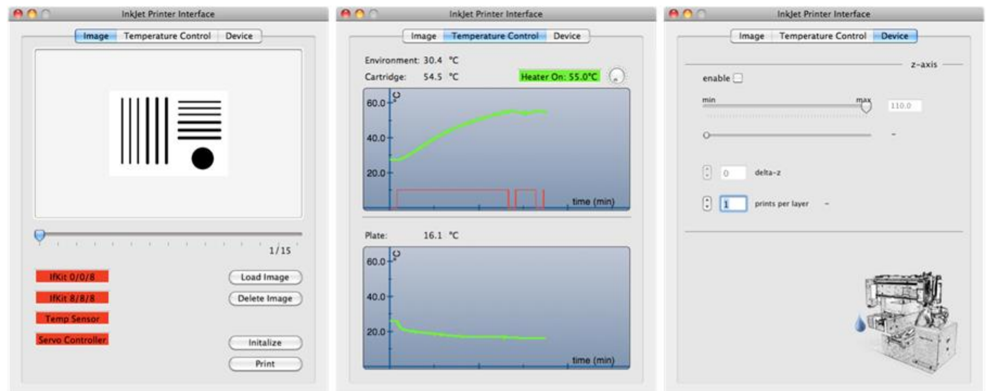
Polymeric structures for bacterial cell growth for cellulose production

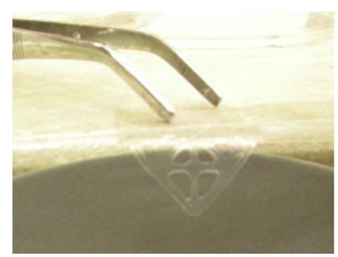
Inket Printing

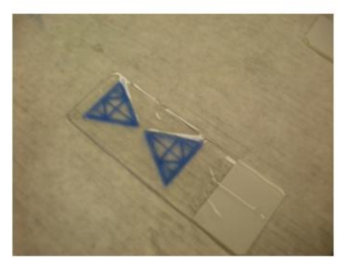


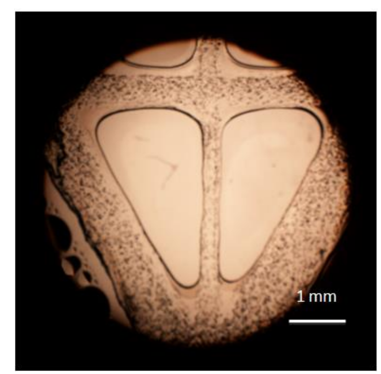
Penelope Ink-Jet printer



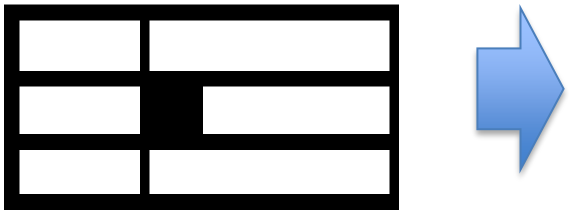








Printable Smart Scaffolds

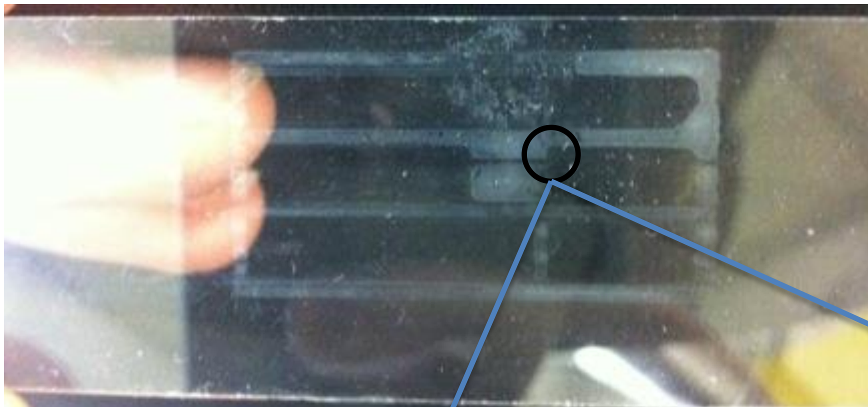


Structure not altered by 24 h at 60°C in water.

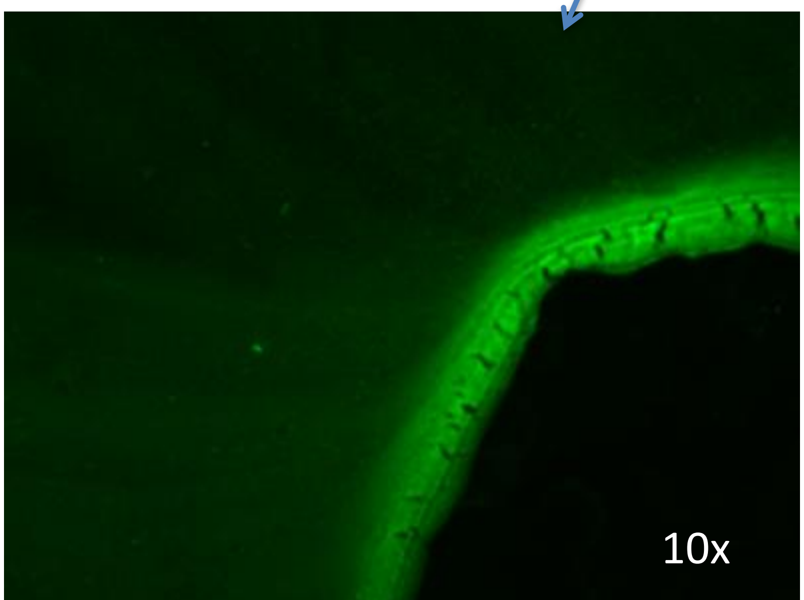
Also GPTMS silanol groups are able to bond to glass, so delamination is unlikely.

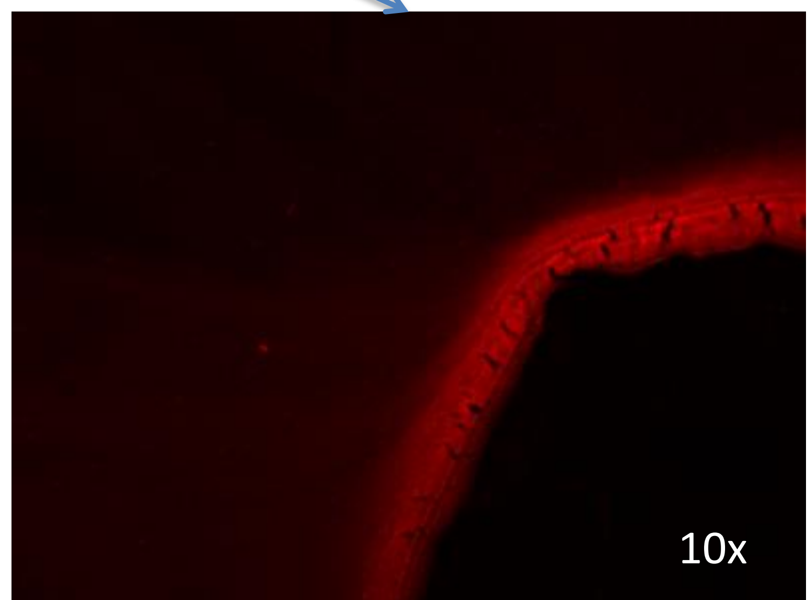
Swelling effects are minimal.

Printable Smart Scaffolds



Both Red and Green fluorescence detected in the structure

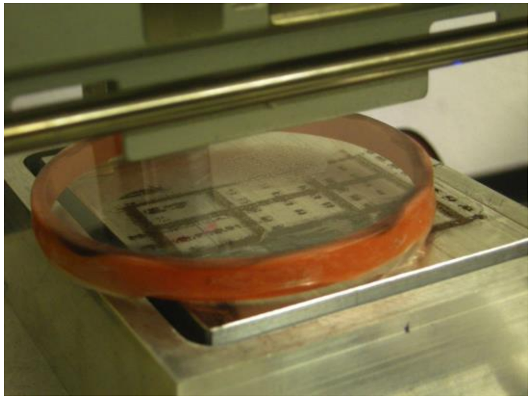




Nanoparticles are within the gel, even after 24 h at 60 degrees.

Inkjet printer - application

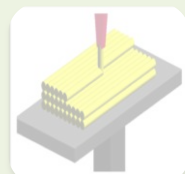
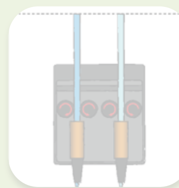
- CNTs for compliant and transparent electrodes for polymeric actuators
  - 0.01 SWNTs in 1% SDS in water
  - Problems with surfactants



In collaboration with Eng. Carpi’s group

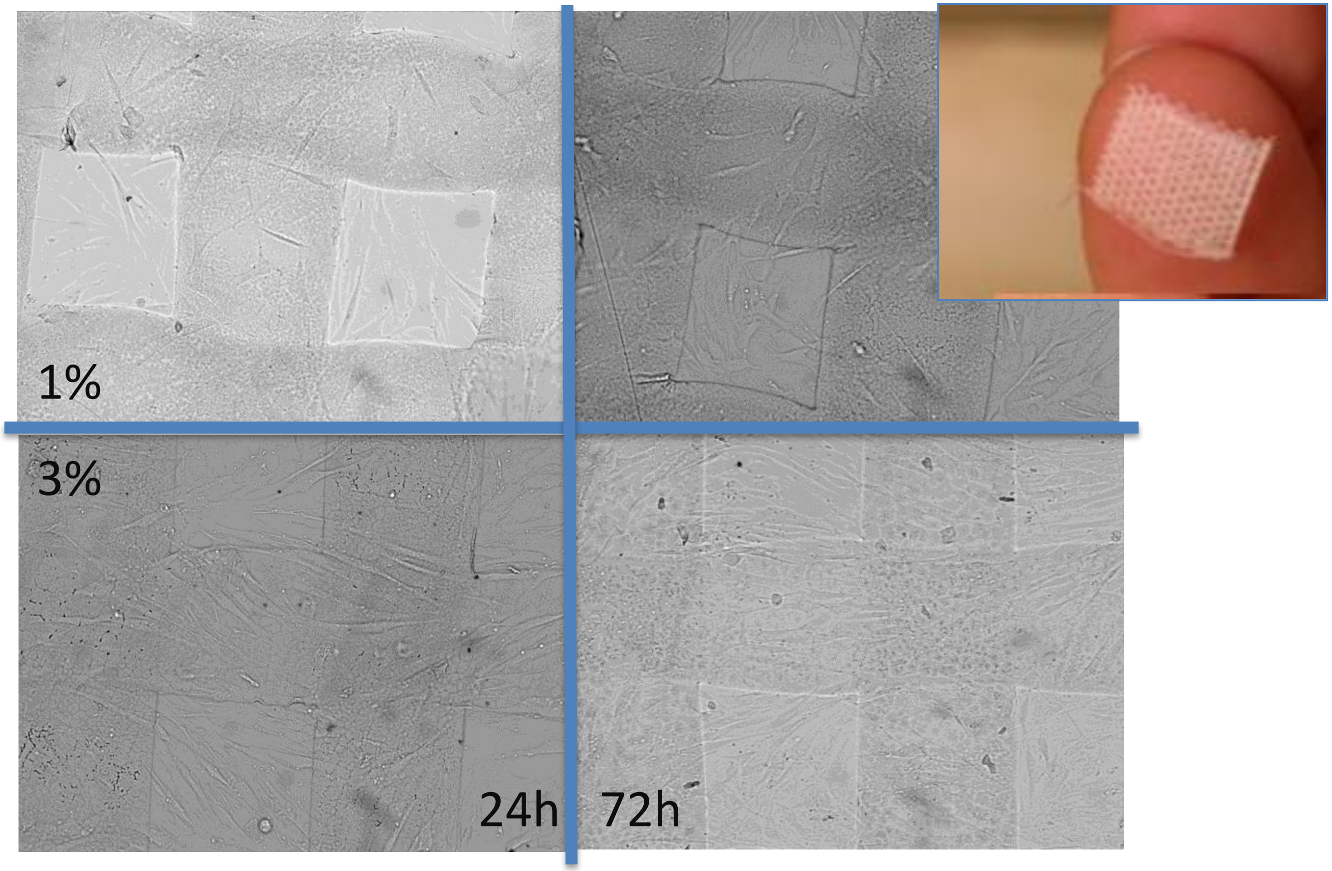
Combination of 2D and 3D Technologies

2-Dimensional	3-Dimensional
Lithography e Soft-Lithography	PAMsquare
Soft-Molecular Imprinting	Open-Source FDM
Electrospinning	Inkjet Printing

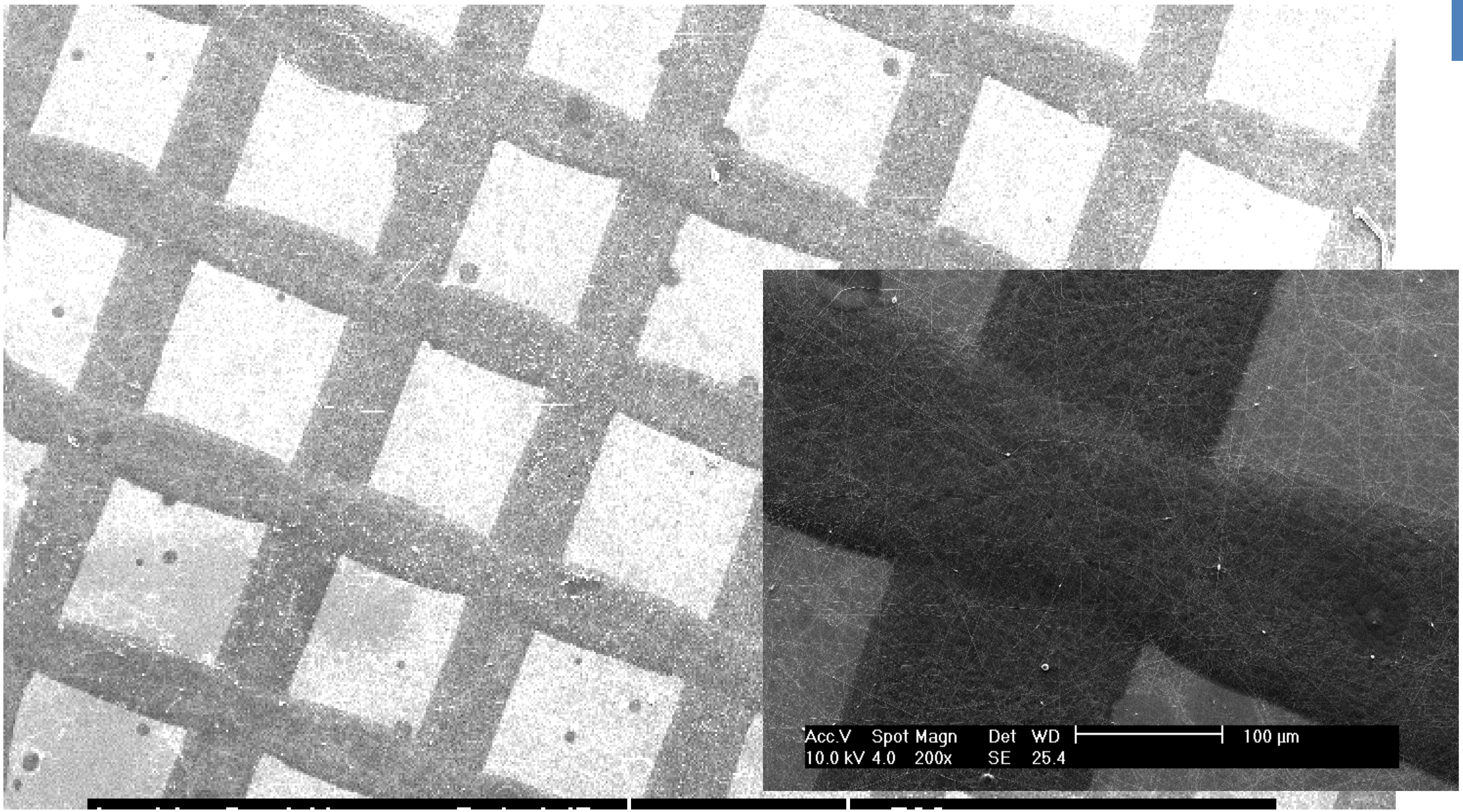





Combination of 2D and 3D Technologies

PAM & Inkjet

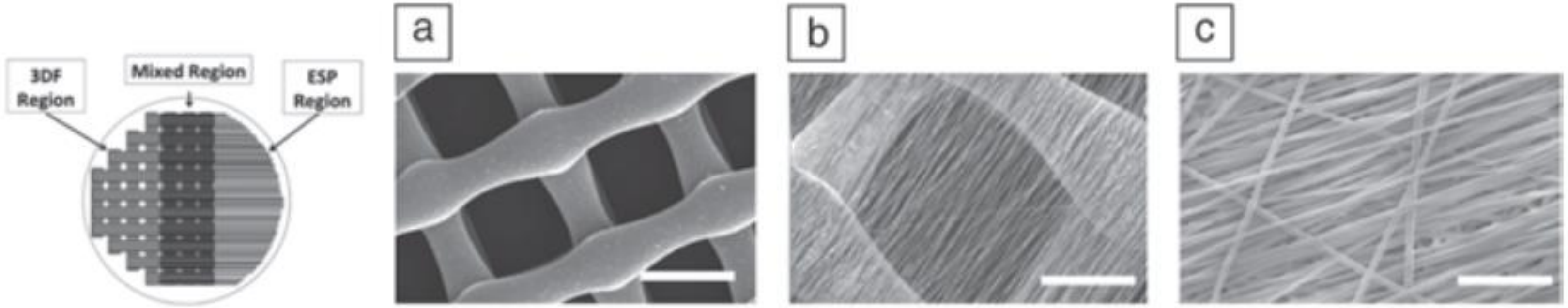


PAM^2 & Electrospinning

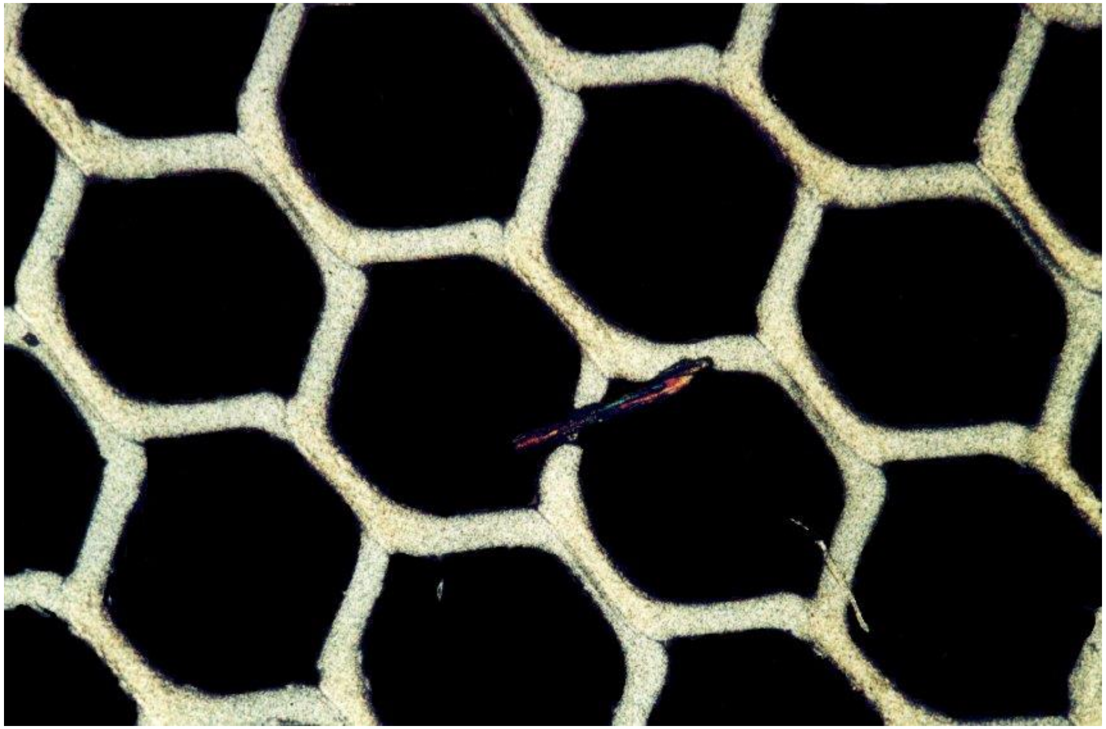


In combination with inkjet printing

Bioextruder & electrospinning

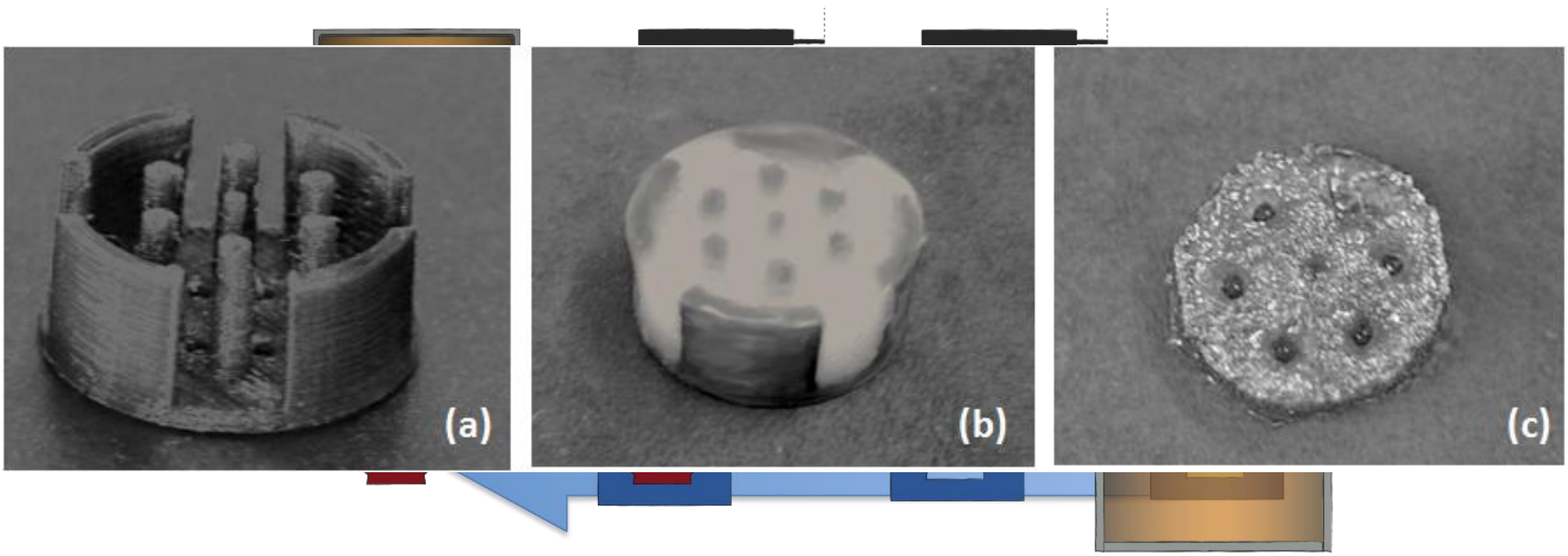


SOFT MI & PAM

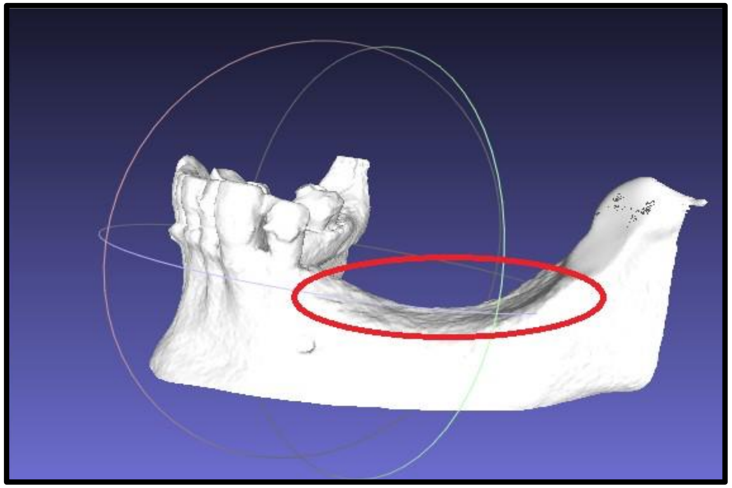


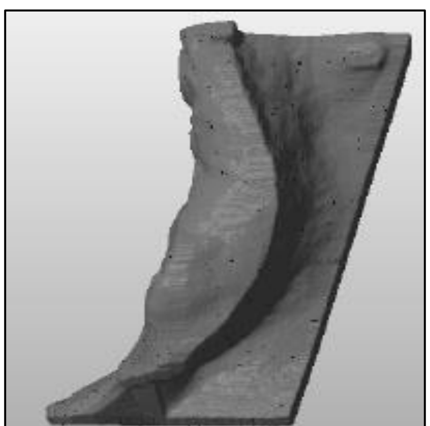
Indirect Rapid Prototyping (iRP)

- Molds realised with RP devices (CAD/CAM)
- Casting of the desired (bio-)material
- Extraction of the final object



Patient specific iRP





Multimaterial and Multiscale Rapid Prototyping of Patient-Specific Scaffold
A De Acutis, C De Maria, G Vozzi Advances in Science and Technology 100, 151-158

SCAFFOLD CHARACTERISATION

Scaffold Characterisation

- Mechanical Characterization
  - Zwick Roell Uniaxial Testing Machine
  - Trasduttori isometrico e isotonico Ugo Basile
- Surface Characterization
  - Kelvin Probe
  - Contact Angle
- Rheological Characterization
  - Rheometer Rheostress
- Optical Microscopy
- Finite Element Modelling

Living Reference Work Entry
3D Printing and Biofabrication
Part of the series Reference Series in Biomedical Engineering pp 1-25

Date: 28 August 2017 Latest Version

Characterization of Additive Manufactured Scaffolds

Giuseppe Criscenti , Carmelo De Maria , Giovanni Vozzi, Lorenzo Moroni