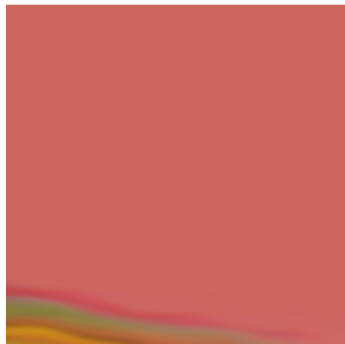
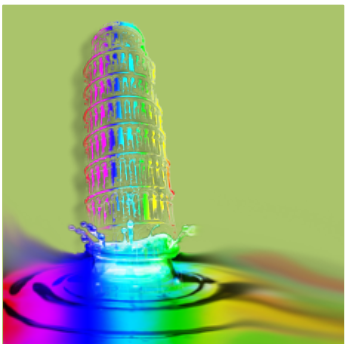
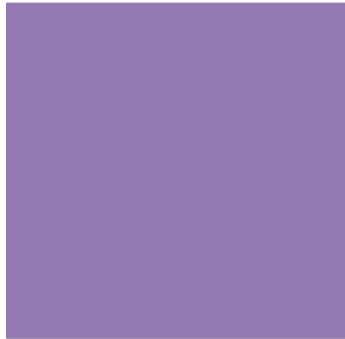




Centro E. Piaggio  
bioengineering and robotics research center

# Neural Tissue Engineering

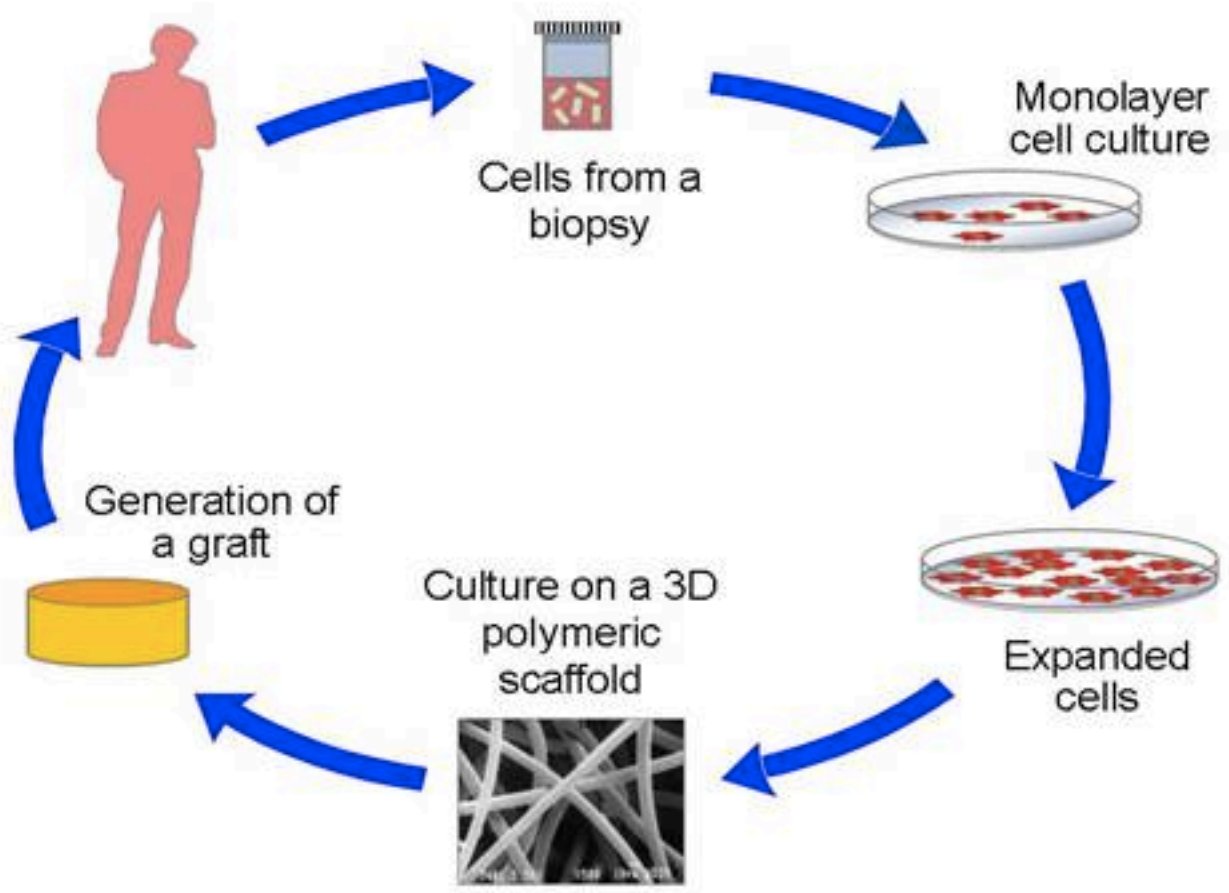


---

**Giovanni Vozi**  
Research Center “E. Piaggio”,  
Dipartimento di Ingegneria dell’Informazione  
University of Pisa



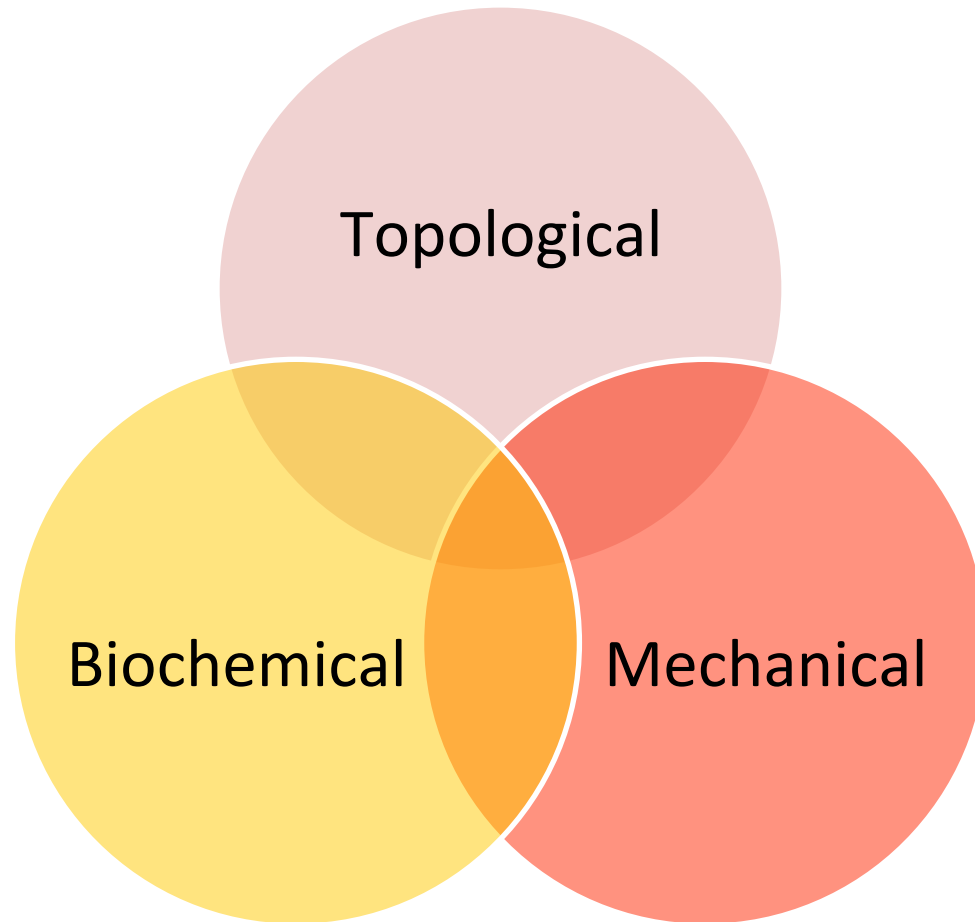
# Basic principles of Tissue Engineering



# + How we may mimic natural tissue?



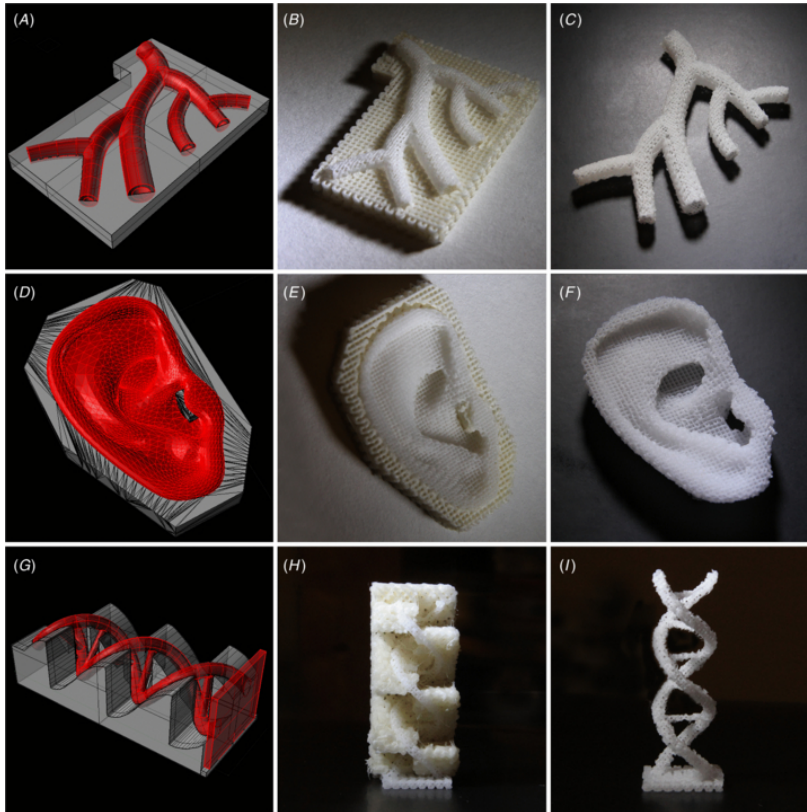
Three main simuli



+

# What is a scaffold?

Polymeric structure topologically well-defined and modulating biochemical and mechanical signals typical of natural tissue, i.e. ***a 3D structure which supports 3D tissue growth***





# What are the features of an ideal scaffold?



- Biocompatible, cell adhesive, bioerodable and *bioactive*
- Mechanical properties *similar* to those of natural tissue
- Optimal meso, micro and nano pores
- Well-defined, or *quantifiable* topology at meso- micro- and nanoscales



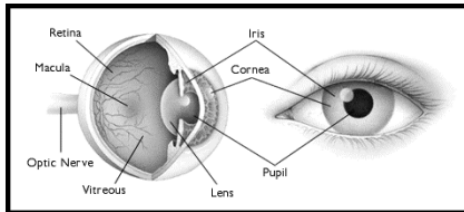
# Designer or Random?



**Structure**

**Function**

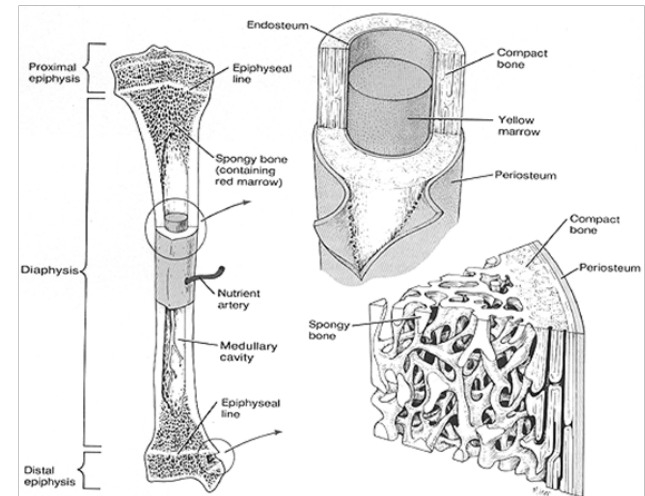
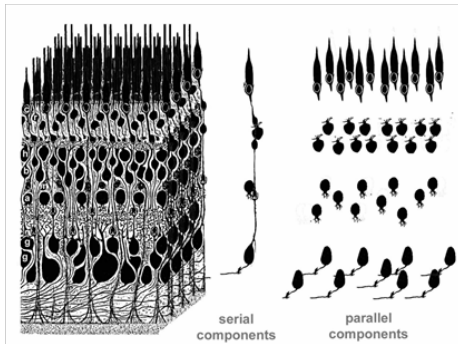
**Retina**



**Liver**

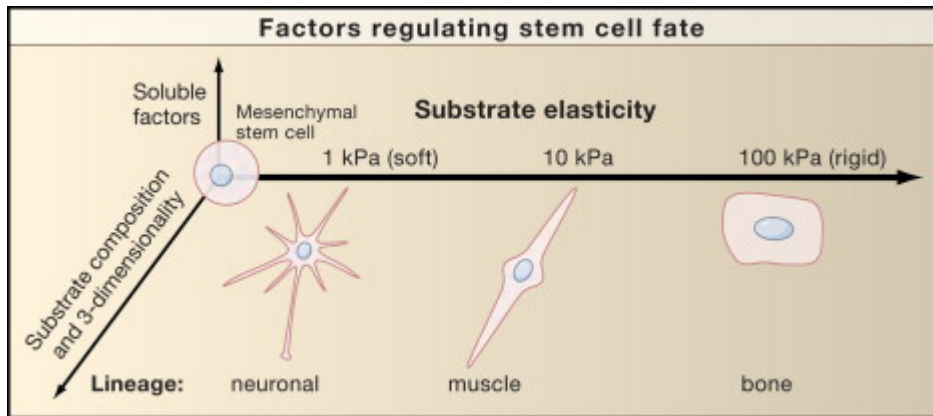
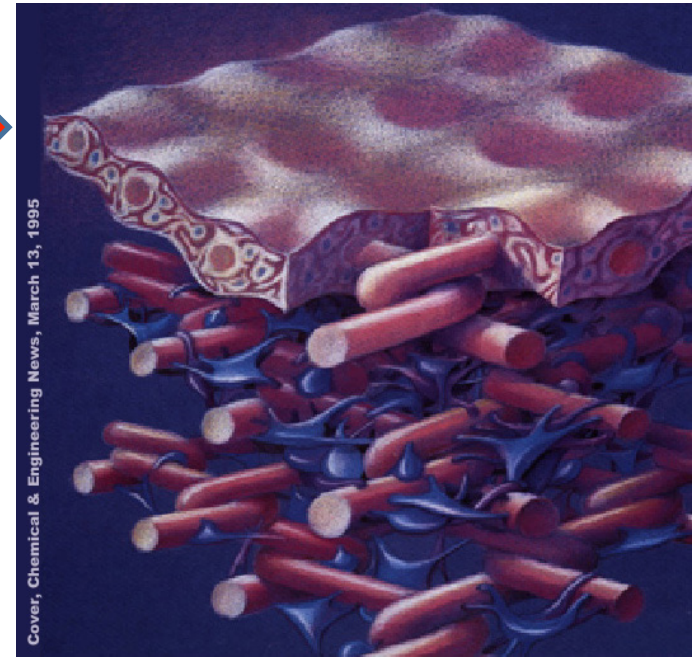
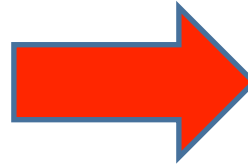


**Bone**



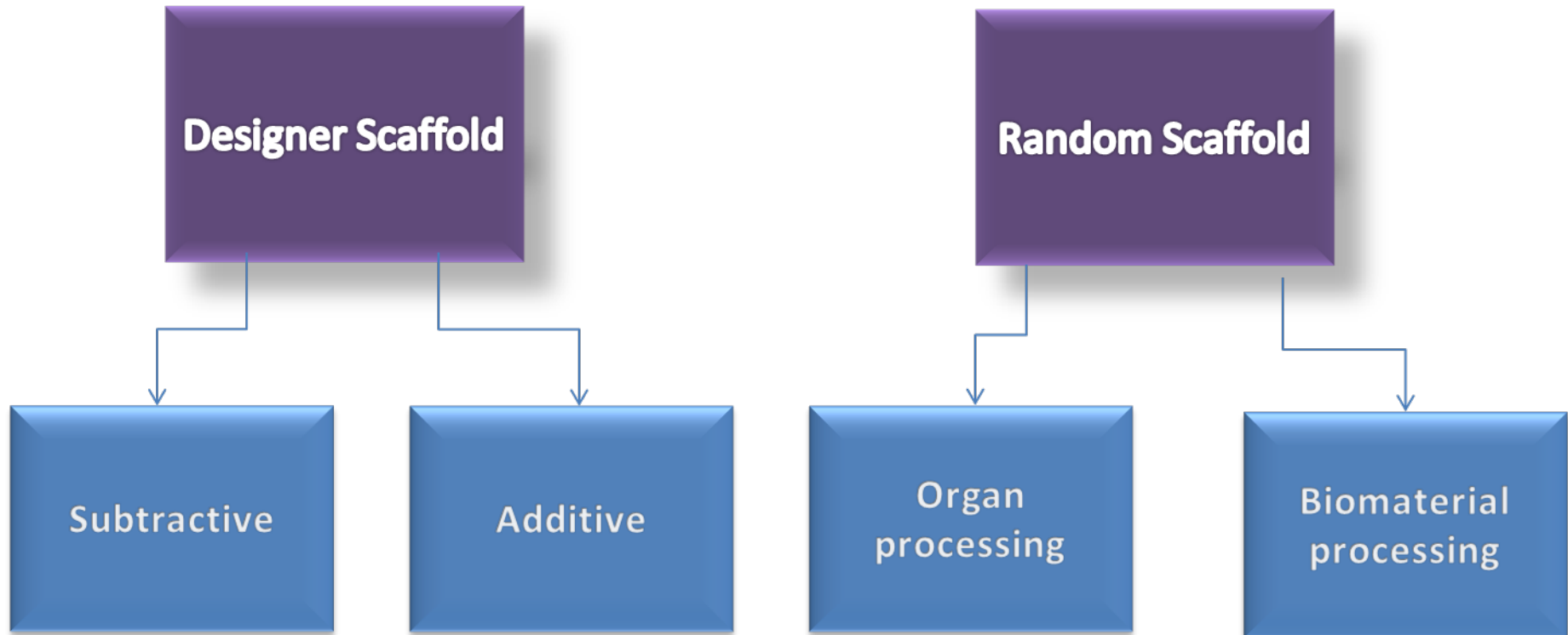
# + Biochemical stimuli in scaffolds

- Synthetic biomaterials with ligands
- Natural biomaterials
- Decellularized Tissue



+

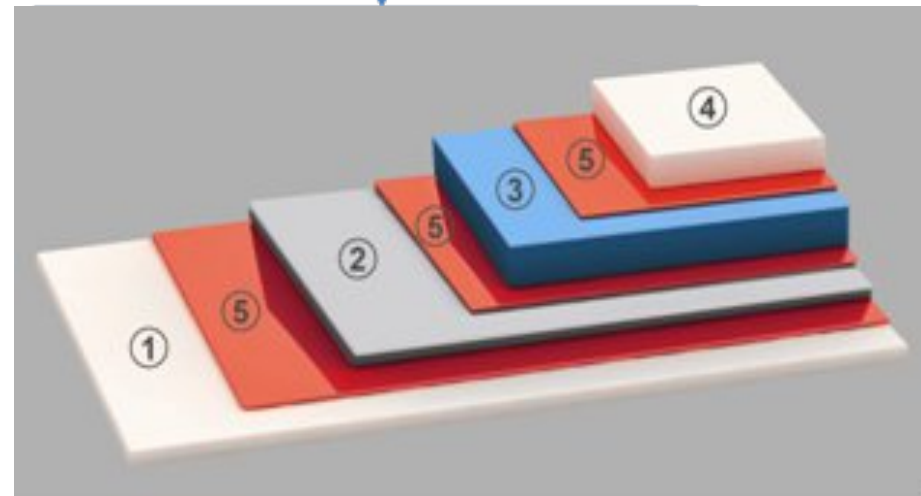
# Methods for generating MS stimuli in scaffolds





+

# Designer Scaffold

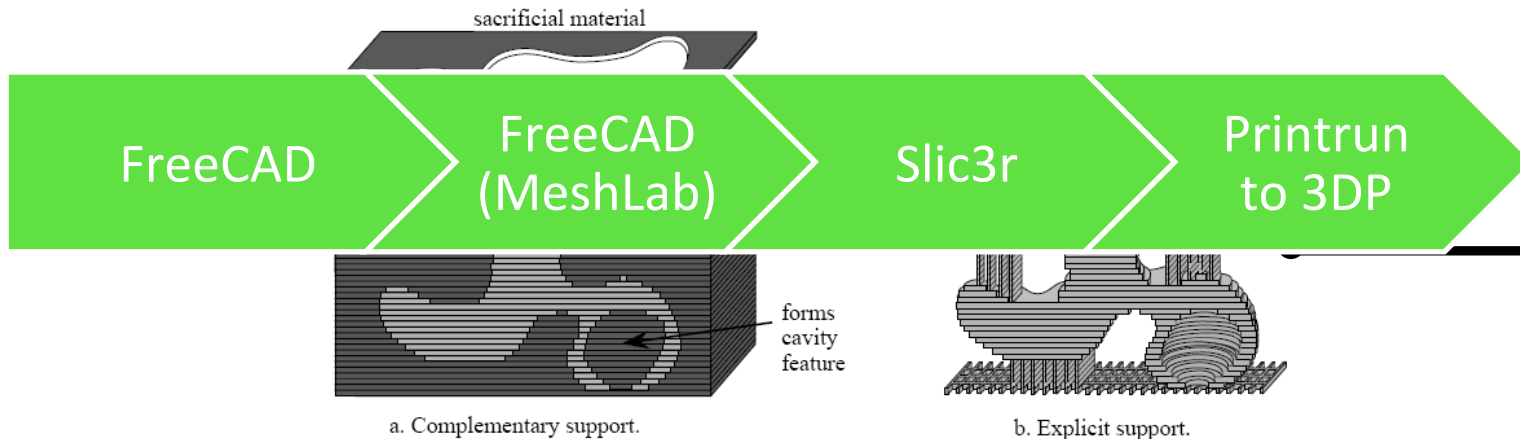
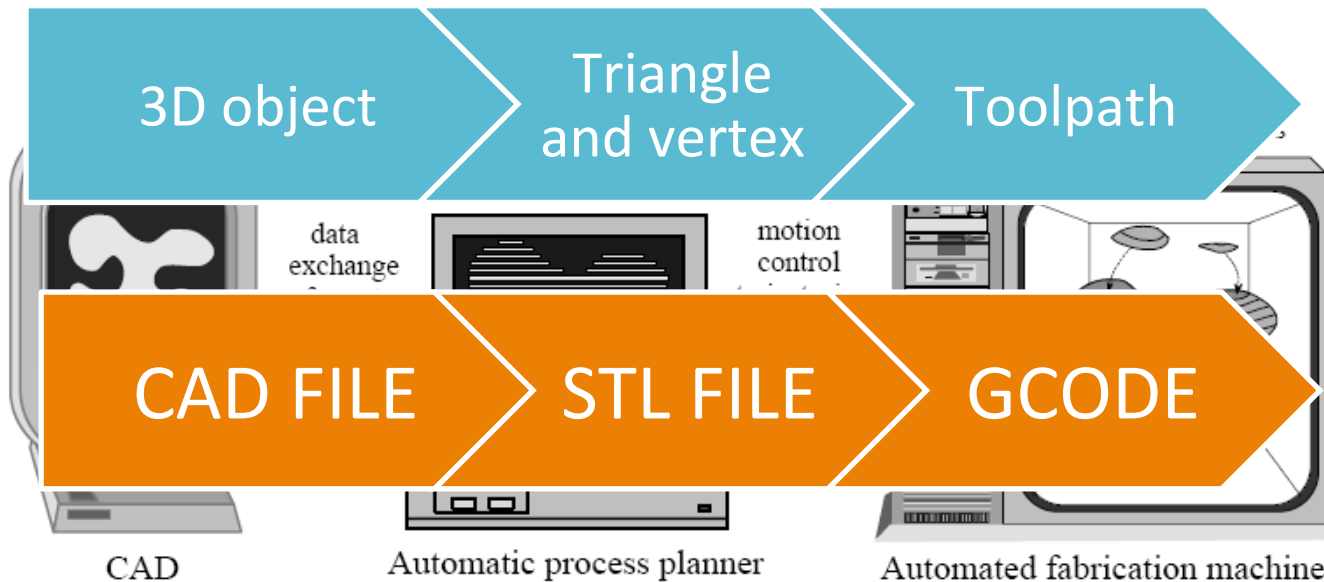


+ Designer Scaffold

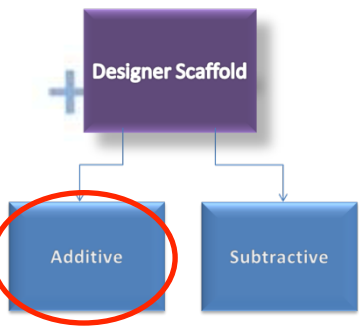
# Additive = rapid prototyping

Additive

Subtractive



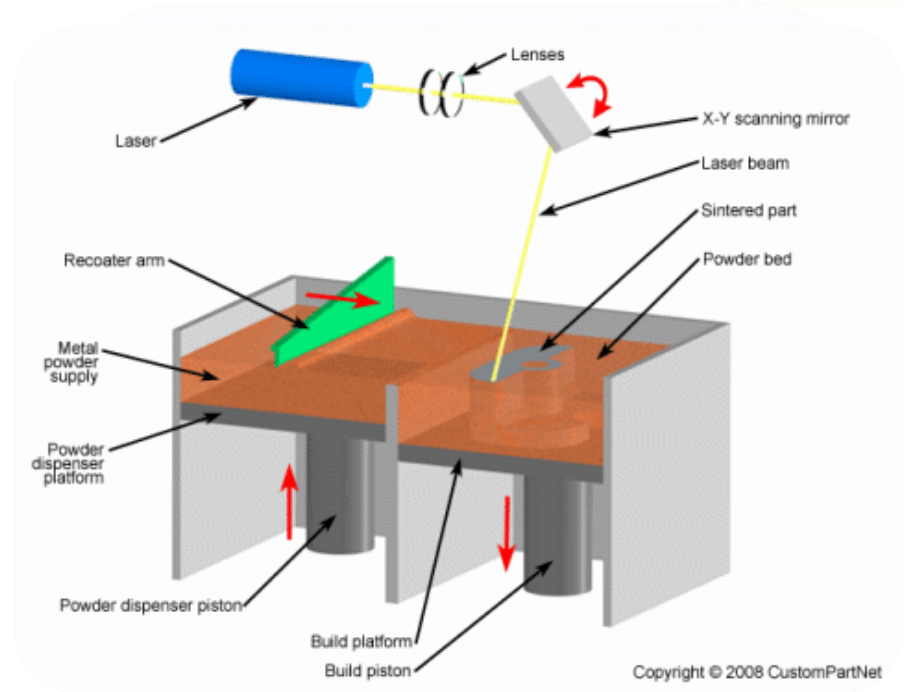
# Designer Scaffold



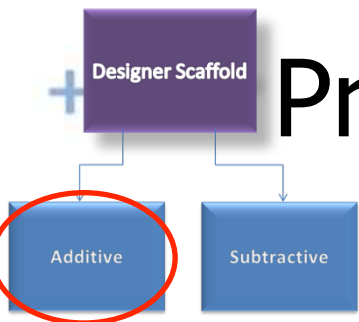
Three main groups:

- laser systems
- nozzle based systems
- direct writing systems

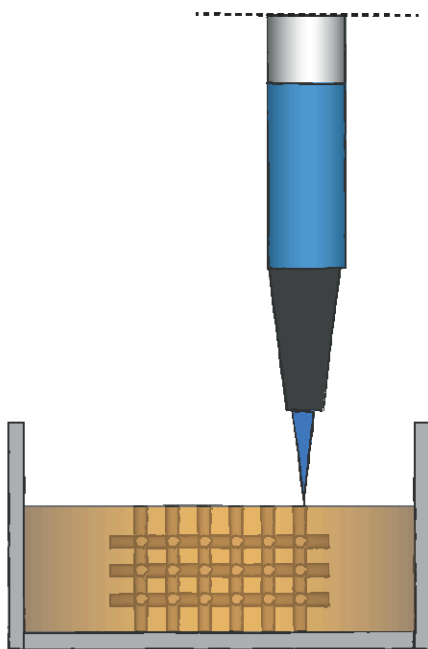
*Materials?*  
*Speed?*  
*Price?*  
*Fidelity?*



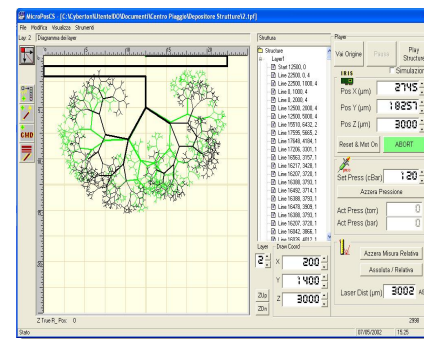
# Pressure Assisted Microsyringe (PAM)



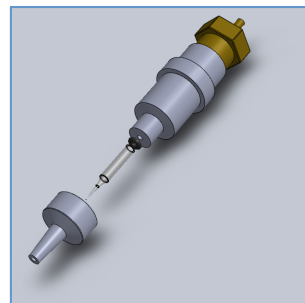
Regulated air flow



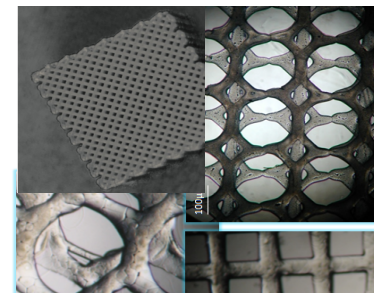
PAM system



Software



Syringe design

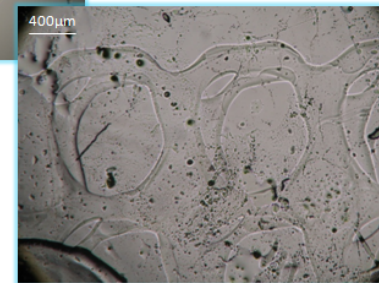
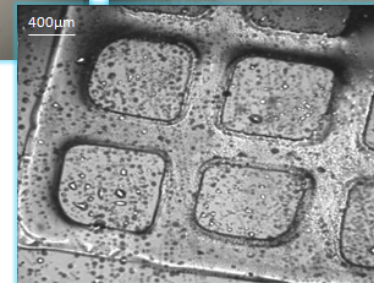
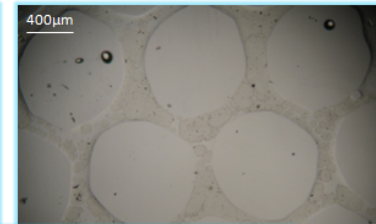
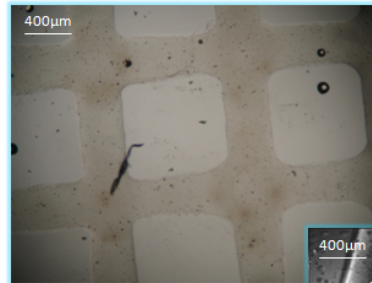
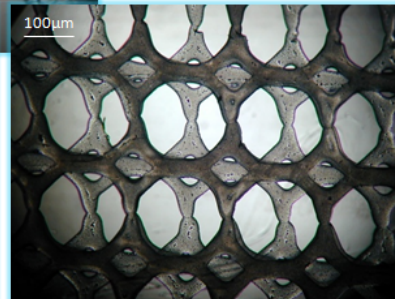
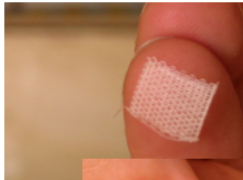
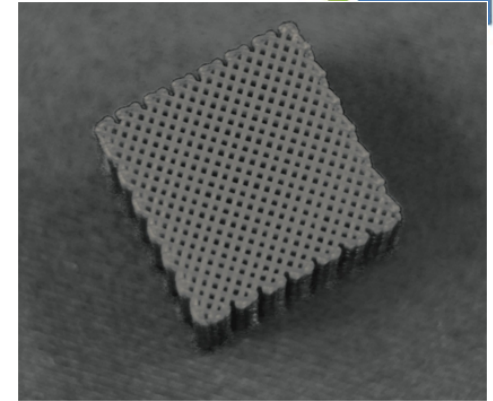
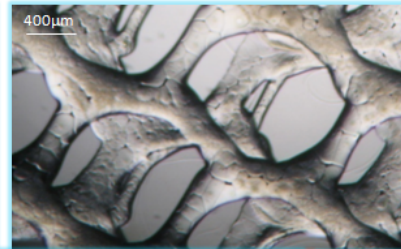
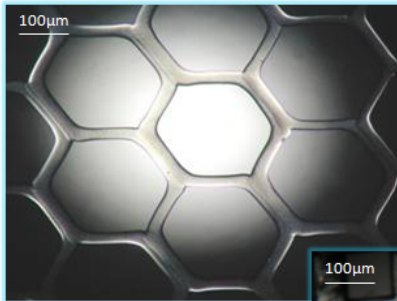
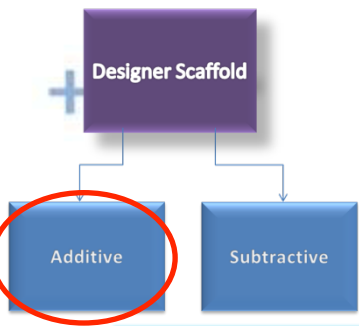


Software

Materials?  
Speed?  
Price?  
Fidelity?

Vozzi, Previti, De Rossi, *Tissue Engineering*, 8, 34, 2002. Vozzi, Flaim, Ahluwalia, Bhatia, *Biomaterials*, 24, 2533, 2003

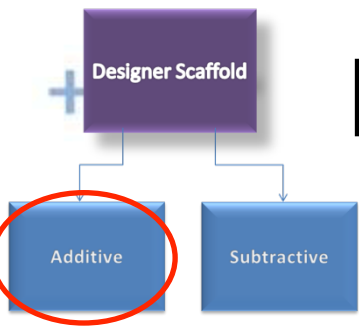
# Scaffolds with PAM



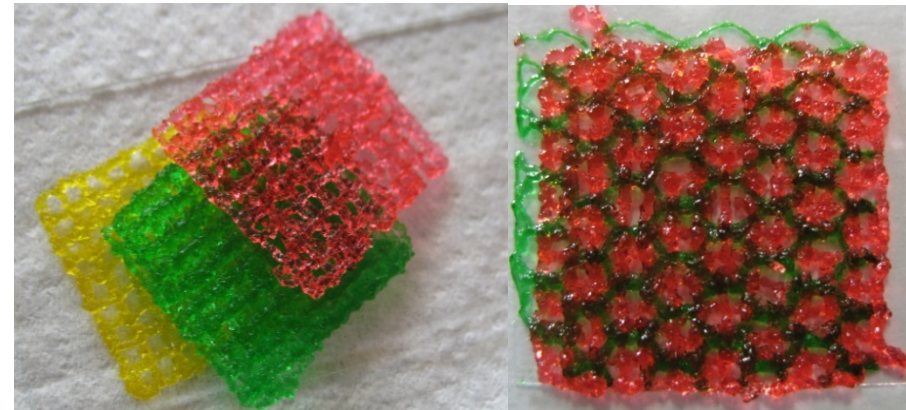
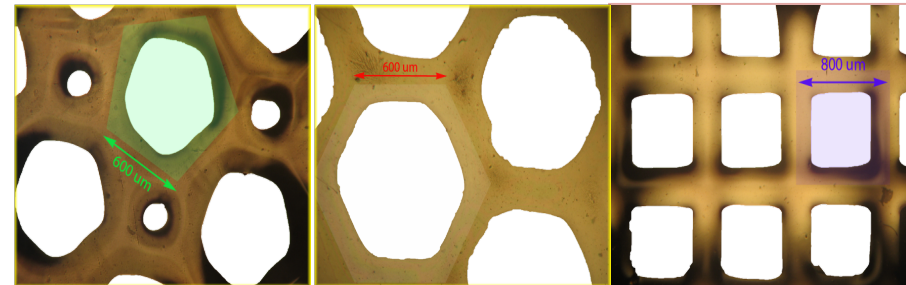
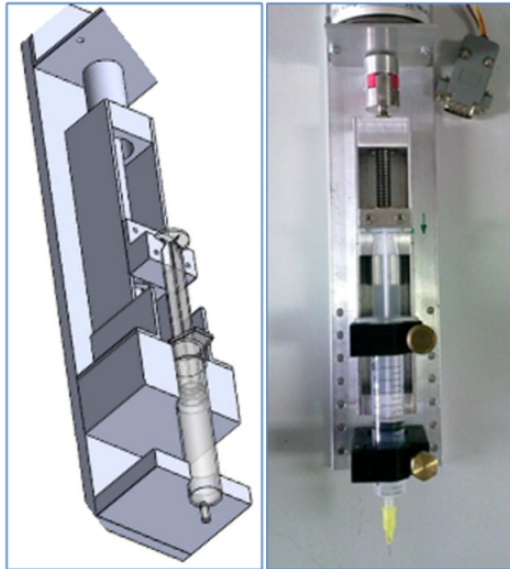
Materials?  
Speed?  
Price?  
Fidelity?

Vozzi et al, *JBMRA*, 71A, 326, 2004.  
Mariani et al., *Tissue Eng.* 12, 547, 2006.  
Bianchi et. Al. *JBMR* 81, 462, 2007.

# Piston Assisted Microsyringe (PAM2)

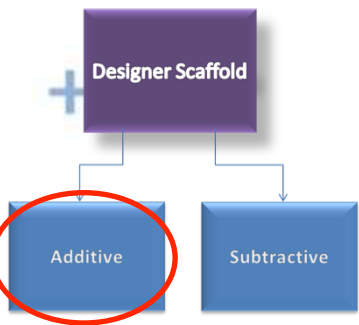


Plunger driven



Materials?  
Speed?  
Price?  
Fidelity?

Vozzi, G., Tirella, A., Ahluwalia, A., *Computer-Aided Tissue Engineering*, Springer (2010); Tirella, De Maria, Vozzi, Ahluwalia *Rapid Prot. J* (2012); Tirella, Orsini, Vozzi, Ahluwalia *Biofabrication* (2009),



# The PAM2 system

## Robotic 3 axis micropositioner.

- ✓ PAM
- ✓ PAM2
- ✓ Diode laser
- ✓ Temperature control
- ✓ PAM<sup>2</sup> software

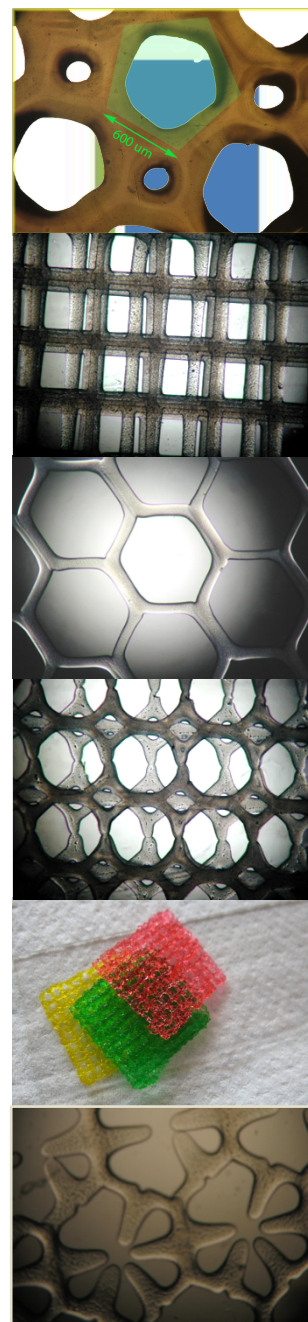
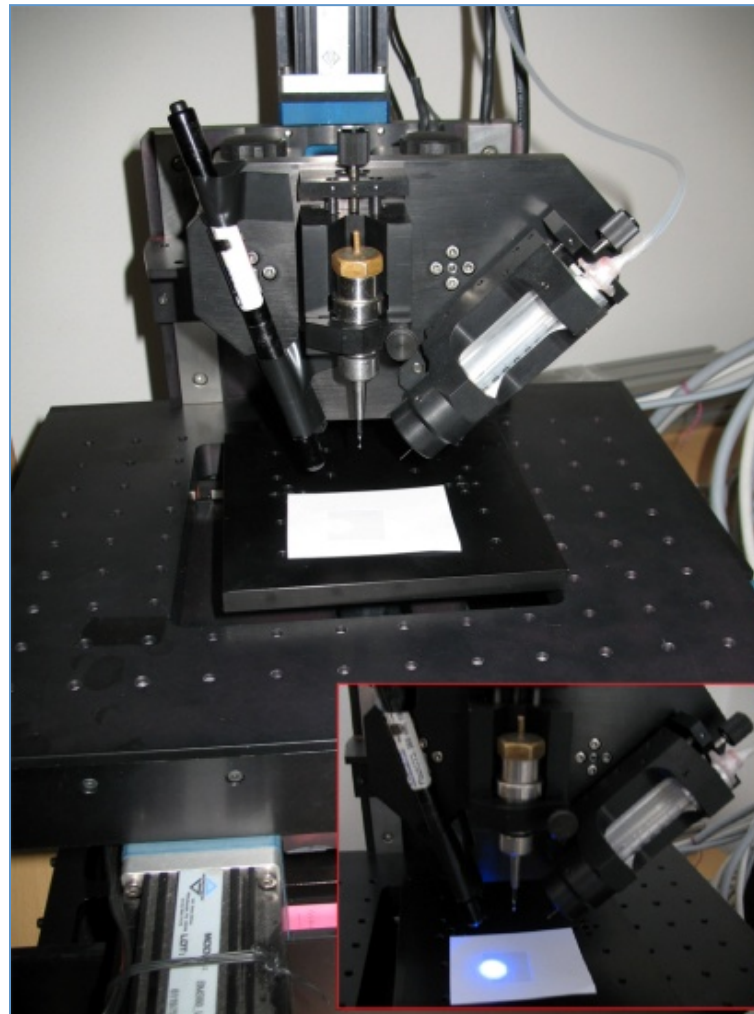
- 4 Position controlled brushless motors (resolution of 10 μm ± 1 μm)
- Working space 100×100×80 mm
- Working velocity 1-15 mm ·s<sup>-1</sup>
- Design of z-stage to locate several modules

Materials?

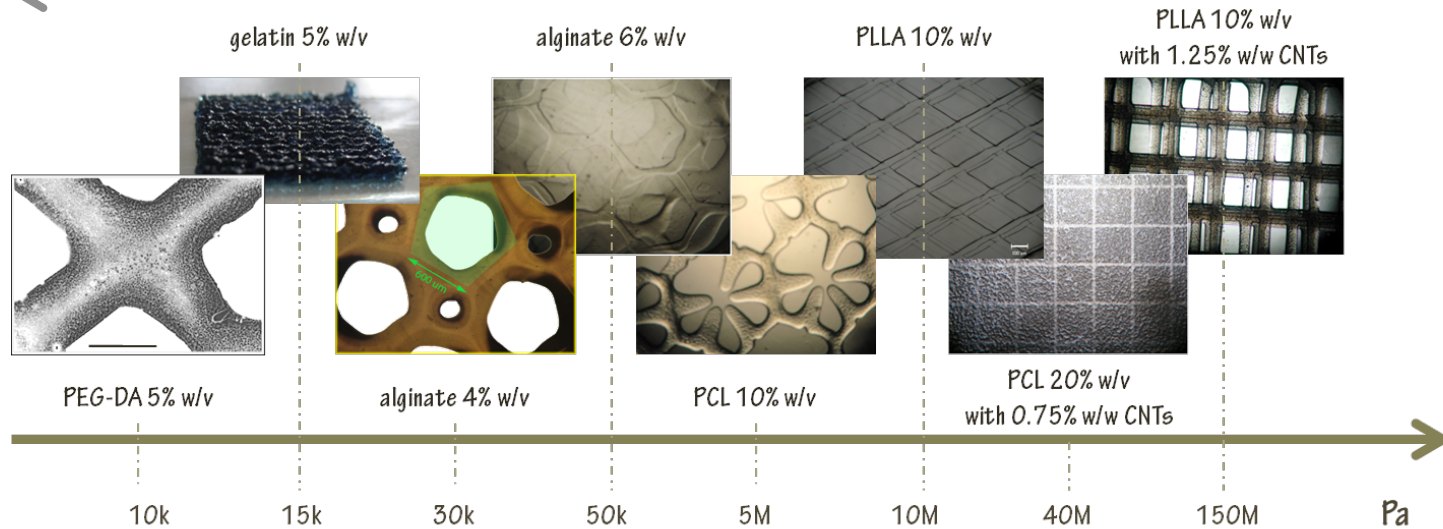
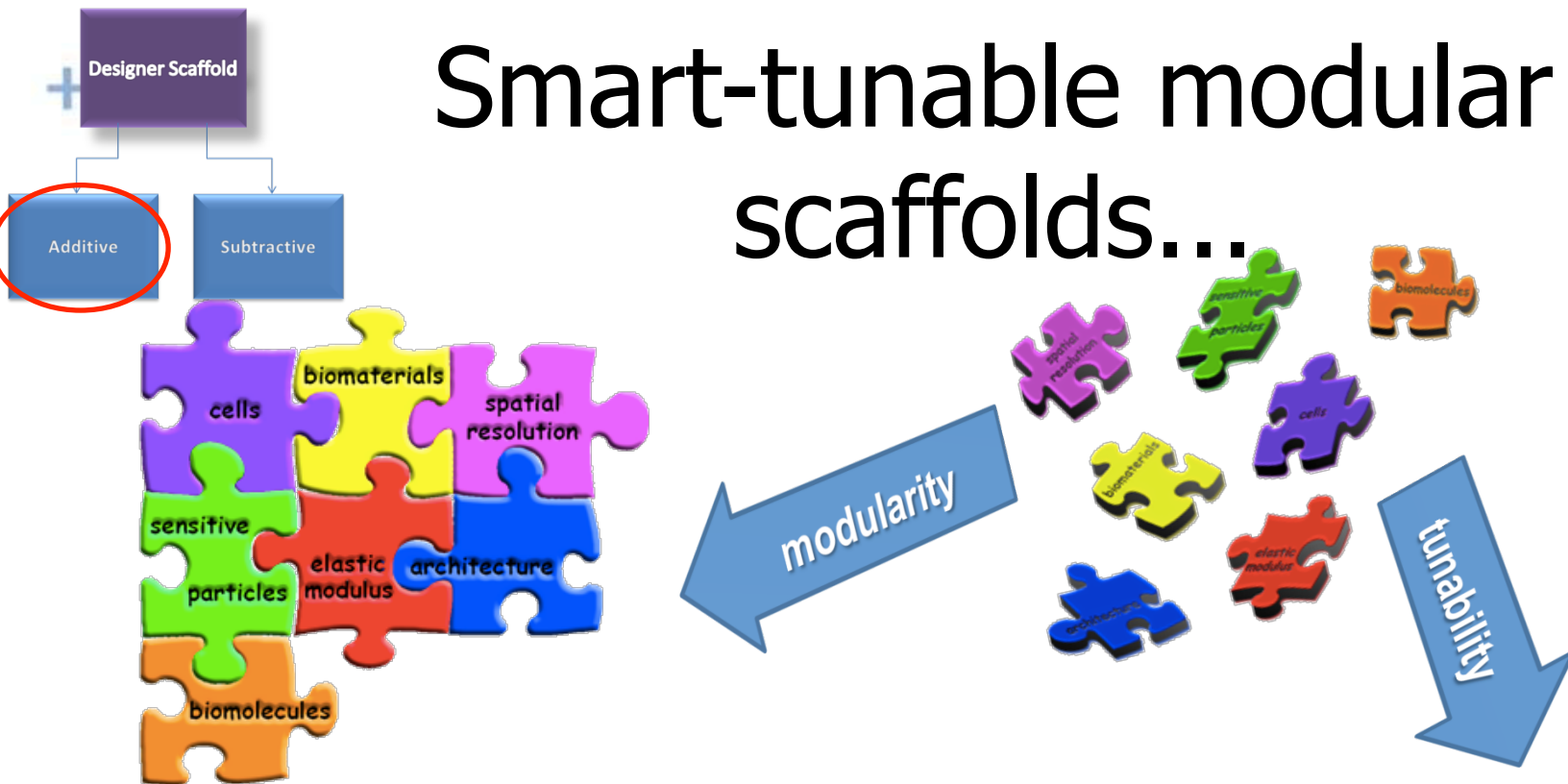
Speed?

Price?

Fidelity?



# Smart-tunable modular scaffolds...



Development of a modular microfabrication system to engineer complex tissues

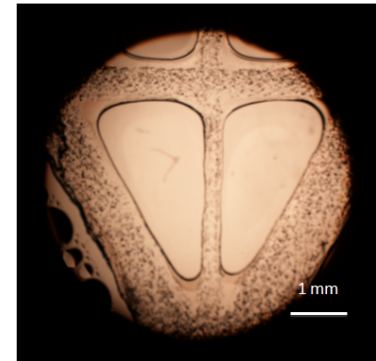
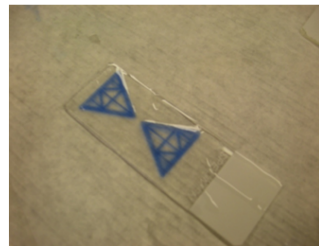
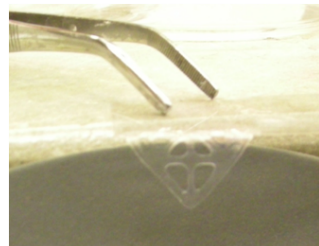
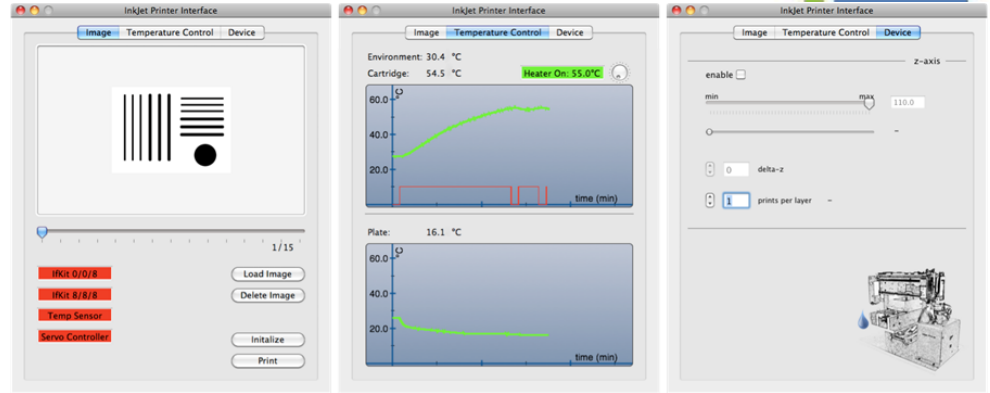
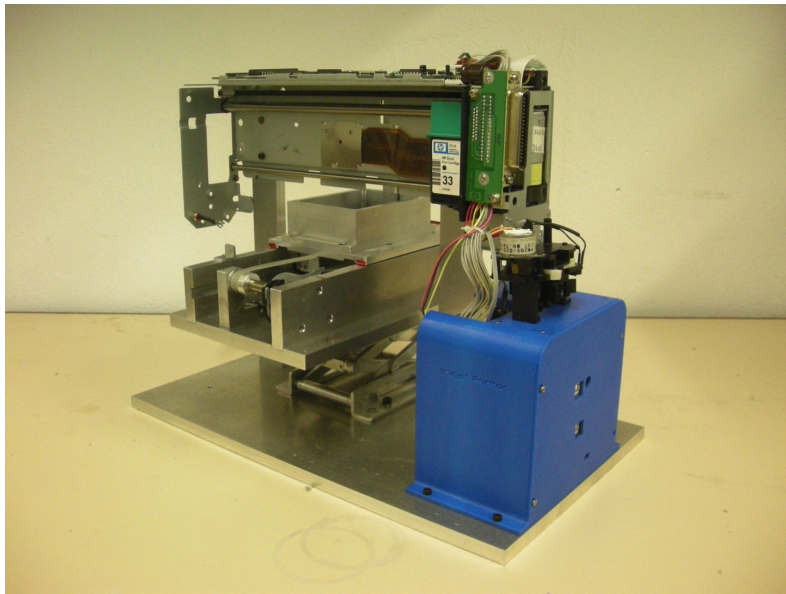


+ Designer Scaffold

# Penelope Ink-Jet printer

Additive

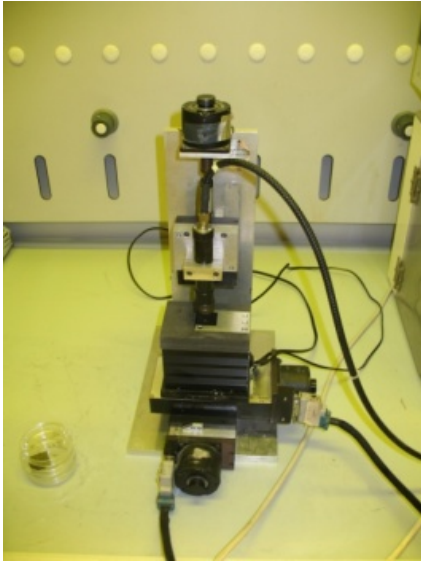
Subtractive



Materials?  
Speed?  
Price?  
Fidelity?

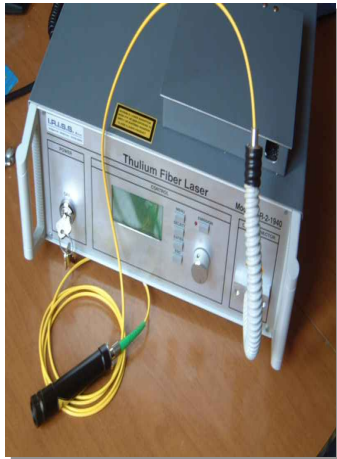


# $\mu$ Laser System



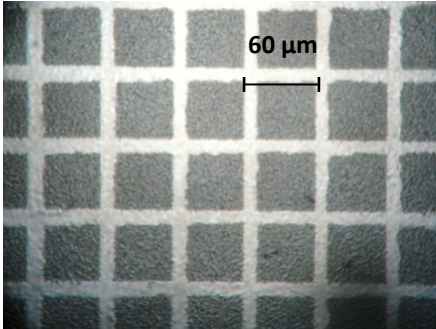
CAD/CAM system, 3-axes control of:

- position,  $\pm 25$  mm;
- velocity, 0-4.5 mm/s;
- resolution, 1  $\mu$ m;
- accuracy and repeatability.
- Thulium laser (1920 nm wavelength, 2W emission power):
  - Control of power emission
  - Layer-by-layer processing.

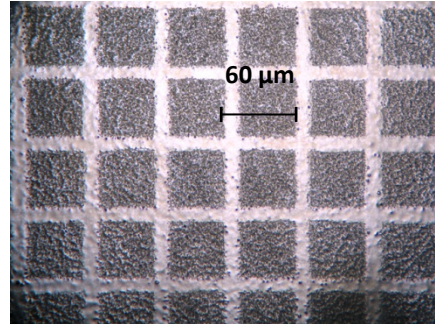




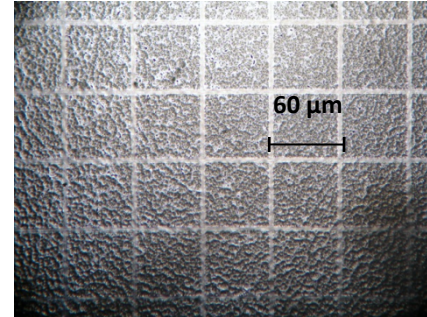
# $\mu$ Laser Structures



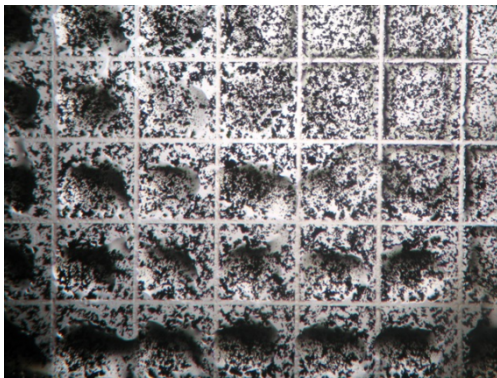
20 % PCL x-y velocity 1,25 mm/s



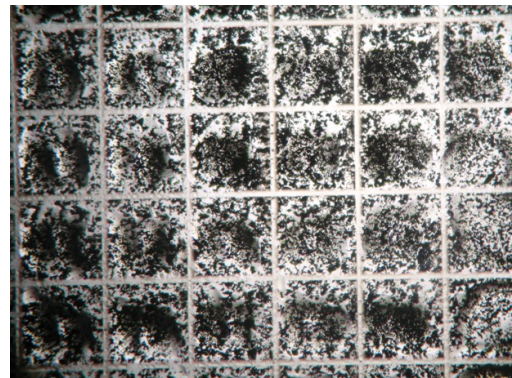
20 % PCL x-y velocity 2,15 mm/s



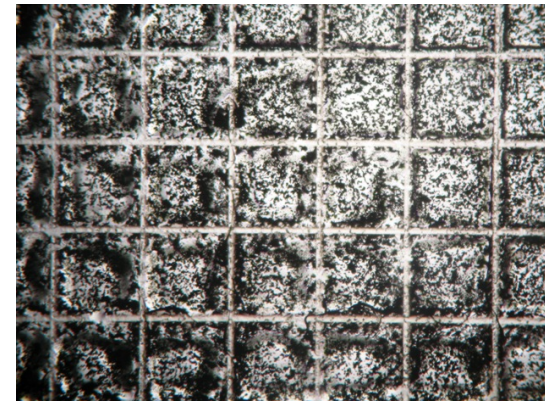
20 % PCL x-y velocity 3,34 mm/s



20 % PLGA+ 1.25% carbon black  
x-y velocity 1,25 mm/s



20 % PLGA+ 1.25% carbon black  
x-y velocity 2.15 mm/s

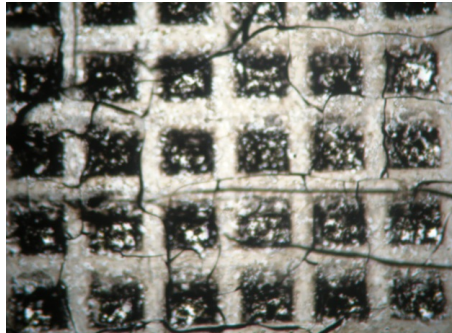


20 % PLGA+ 1.25% carbon black  
x-y velocity 3.34 mm/s

60  $\mu$ m

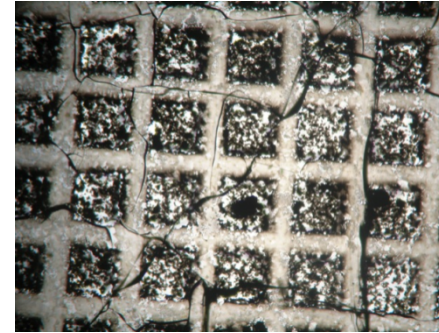


# $\mu$ Laser Structures



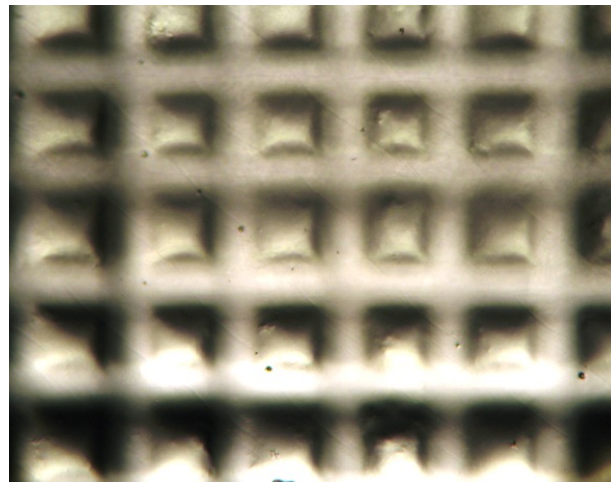
60  $\mu$ m

**20 % PLGA+ 1.25% carbon nanotubes**  
x-y velocity 1,25 mm/s



60  $\mu$ m

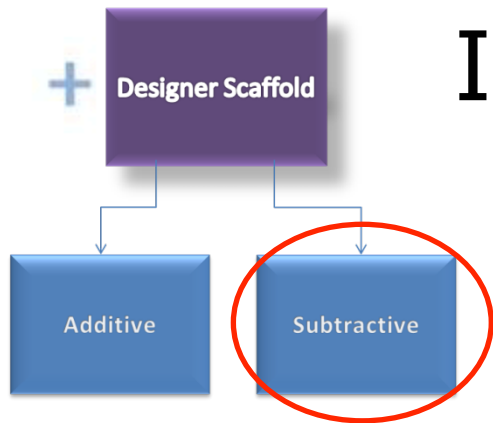
**20 % PLGA+ 1.25%Carbon nanotubes**  
x-y velocity 2.15 mm/s



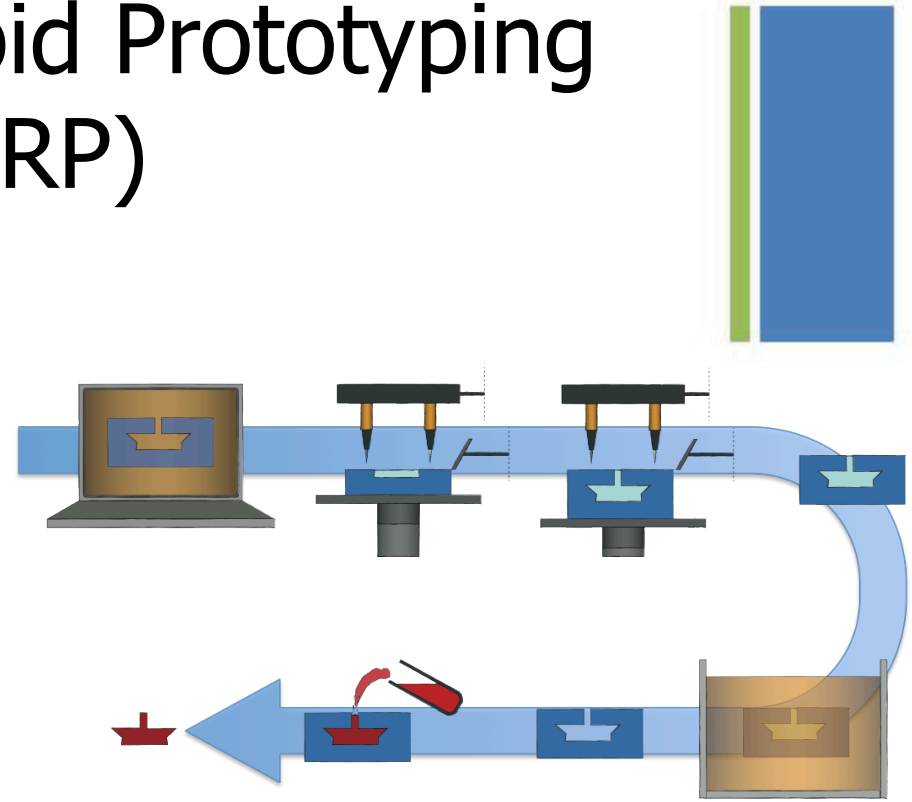
60  $\mu$ m

**1 % Agarose**  
x-y velocity 3.34 mm/s

# Indirect Rapid Prototyping (iRP)



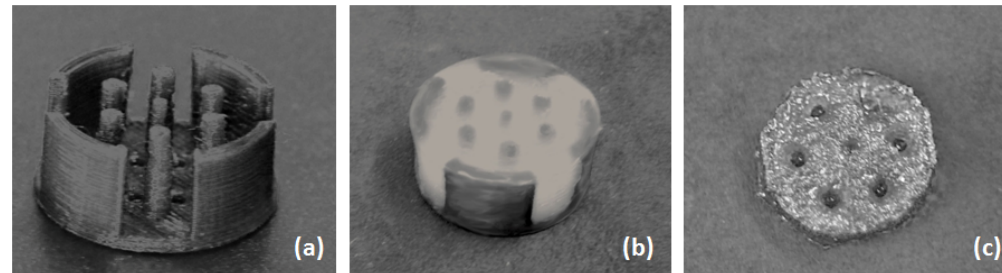
- Molds realised with RP devices (CAD/CAM)
- Casting of the desired (bio-) material
- Extraction of the final object



Advantages?  
Limitations?

Materials?  
Speed?  
Price?  
Fidelity?

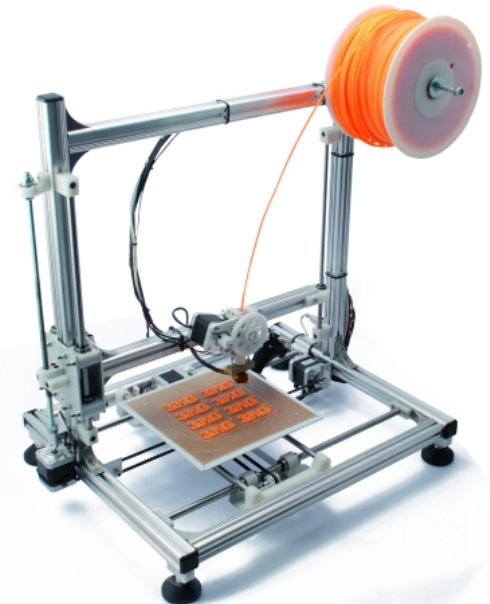
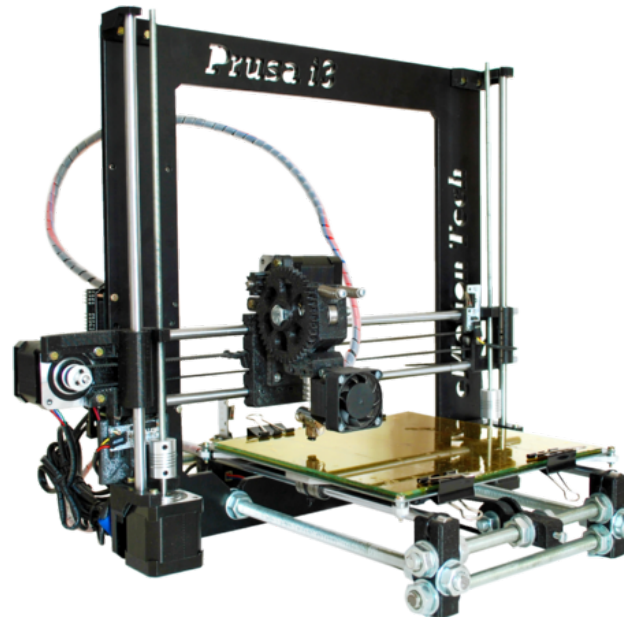
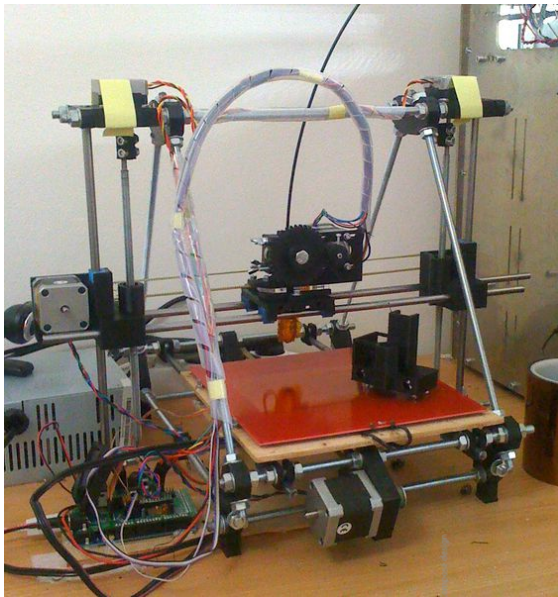
DW Hutmacher et al., Trends in Biotechnology, 22(7): 354 – 362, 2004



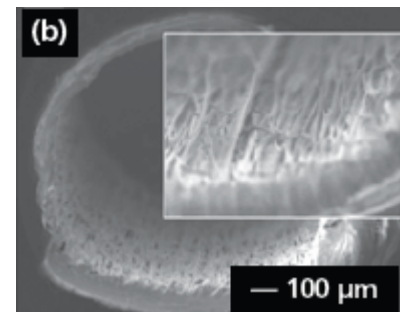
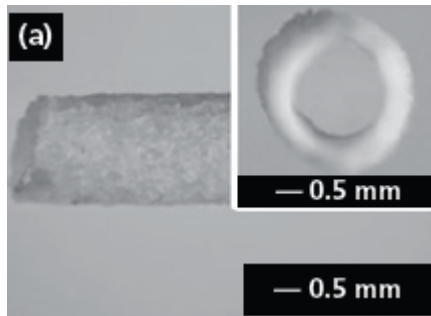
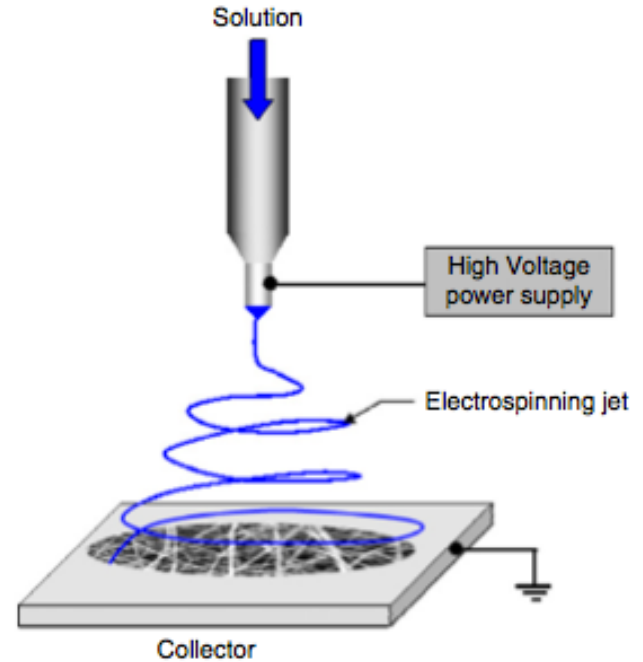
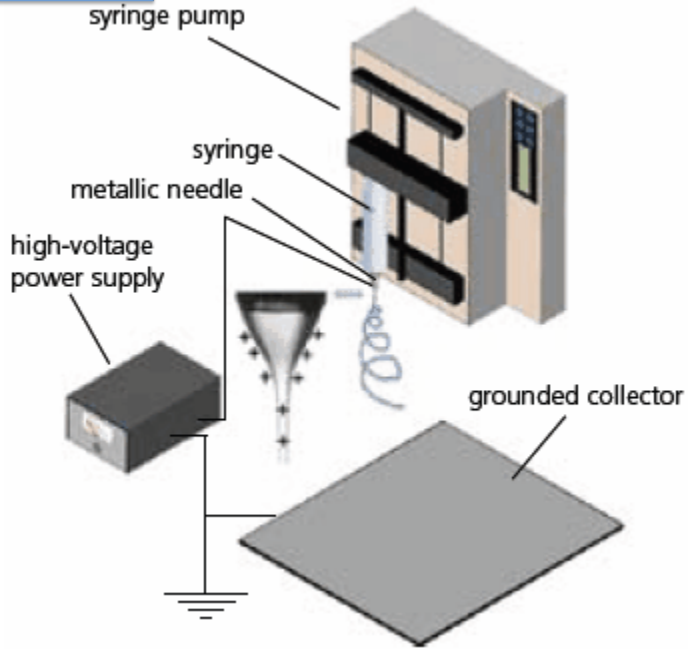
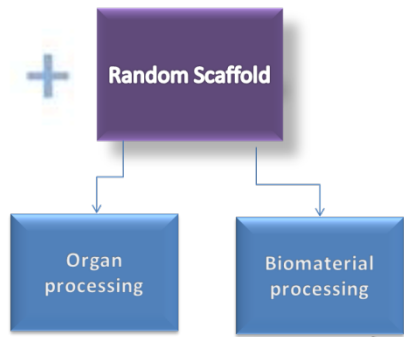
# + Open source FDM machine: RepRap Project



- RepRap is first general-purpose self-replicating manufacturing machine.
- An open source project with several forks



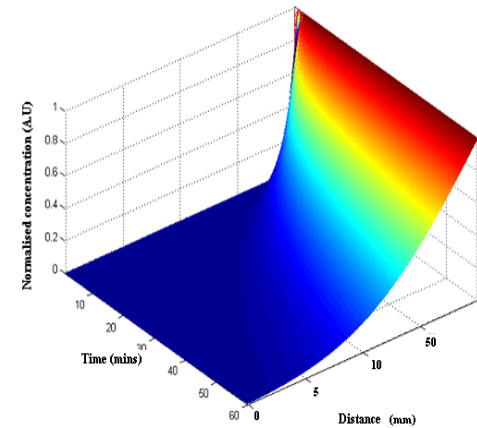
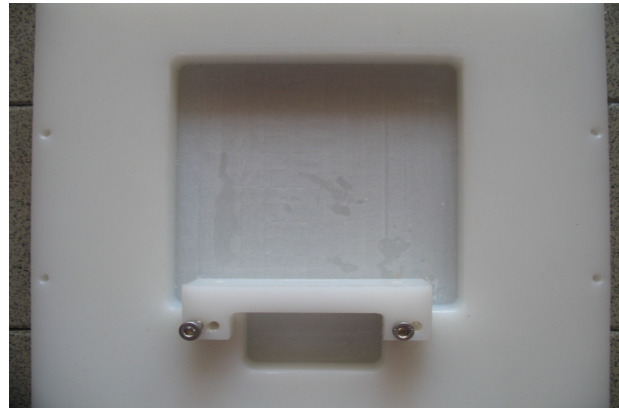
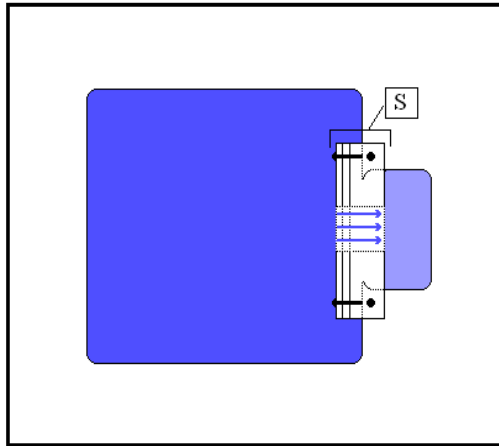
# Electrospinning



Price?  
Materials?  
Speed?  
Repeatability?



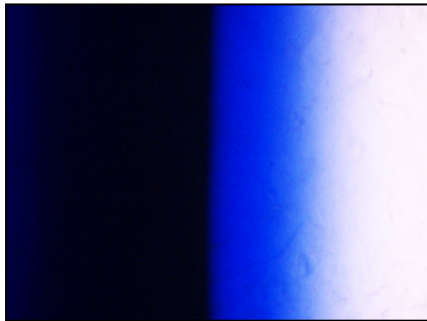
# Chemical Gradient Concentration



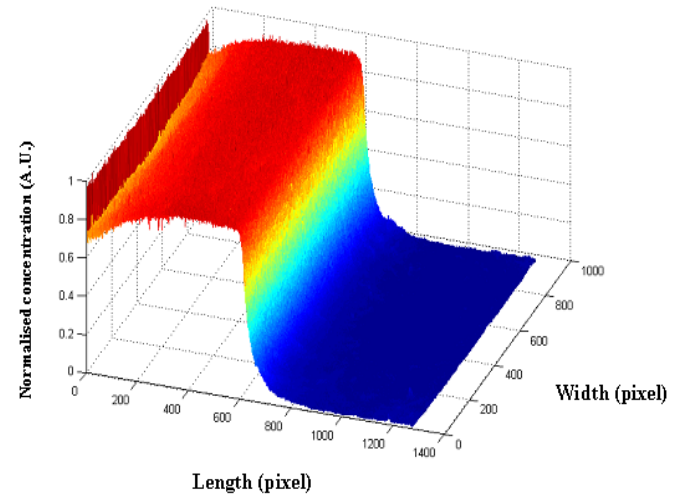




# Chemical Gradient Concentration

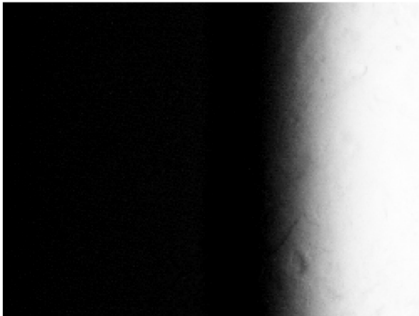


100  $\mu\text{m}$

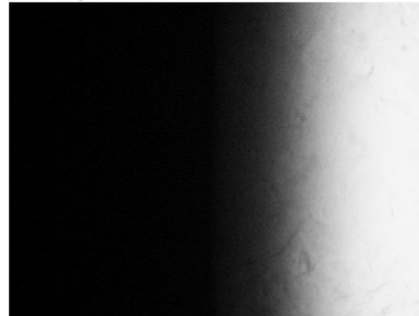


(b)

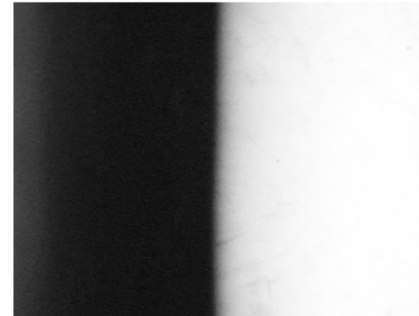
red channel



green channel



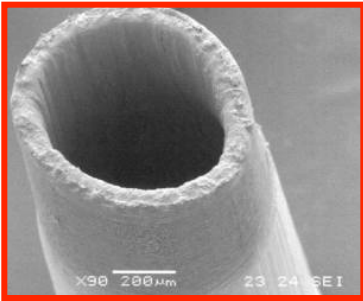
blue channel



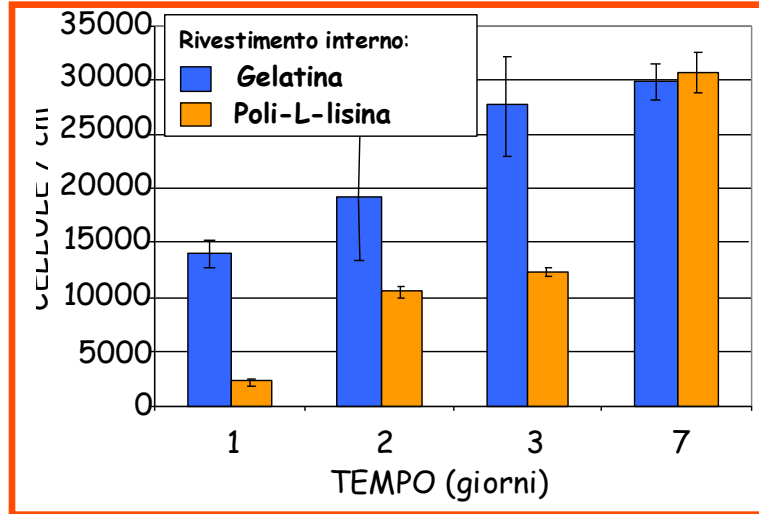
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# Production of Hollow Fibers

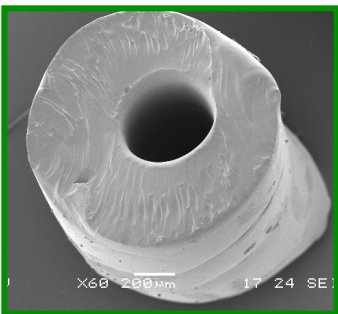
## Topology and cell adhesion with Neuroblastoma cell line (S5Y5)



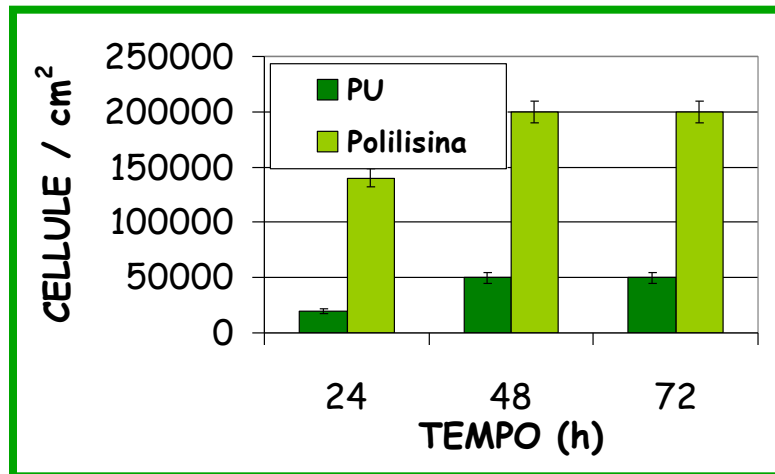
**PCL**  
( $M_n = 80,000$ ;  
 $T_m = 57.5^\circ\text{C}$ ;  
 $\Delta H_m = 84 \text{ J/g}$ )



- Dint = 500-2000  $\mu\text{m}$
- Wall Thickness = 50-200  $\mu\text{m}$



**PU**  
( $M_n = 77,300$ ;  
 $T_m = 59.8^\circ\text{C}$ ;  
 $\Delta H_m = 1.6 \text{ J/g}$ )



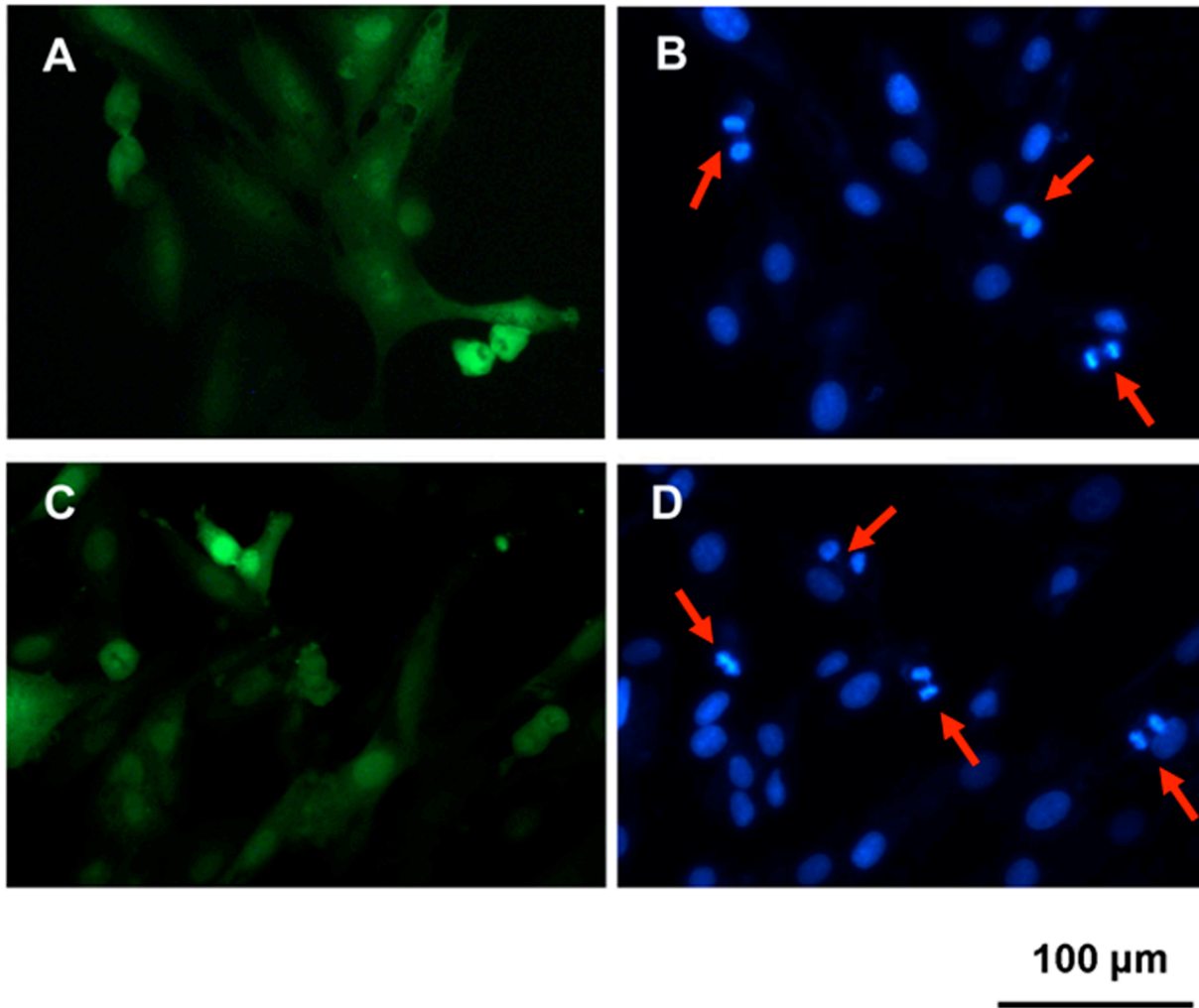
Saturation of cell density at 48 h

- Dint = 300-450  $\mu\text{m}$
- Wall Thickness = 300-400  $\mu\text{m}$



+

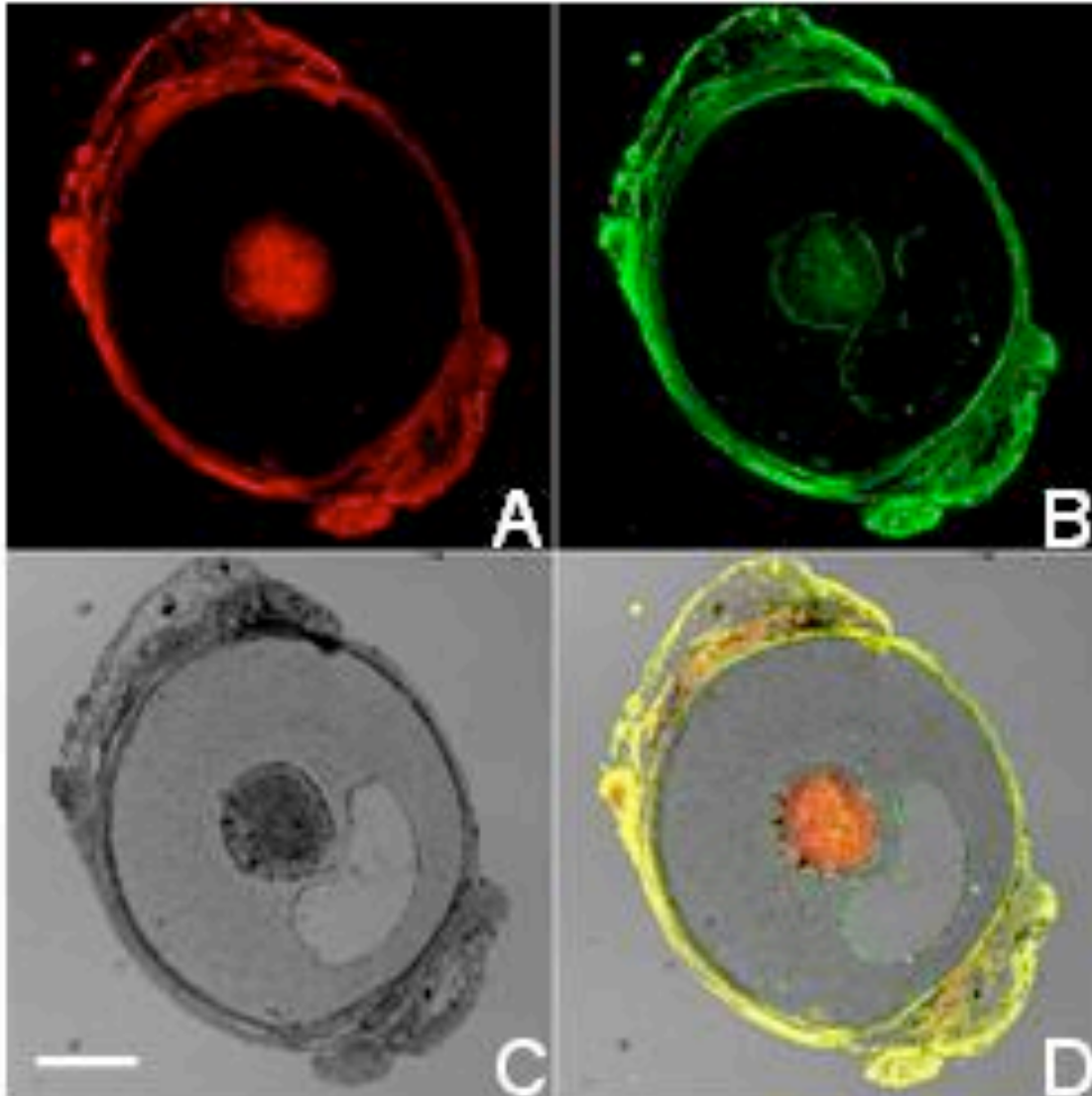
# Production of Hollow Fibers



Fluorescence microscopy images of NOBEC-GFP cells (3 days in vitro) on PCL films (A, B) and after nuclear DAPI staining (C, D). DAPI staining binds strongly to DNA and it is useful to label cell nuclei. DAPI staining can be used to visualize cells during mitosis phases (indicated by red arrows).

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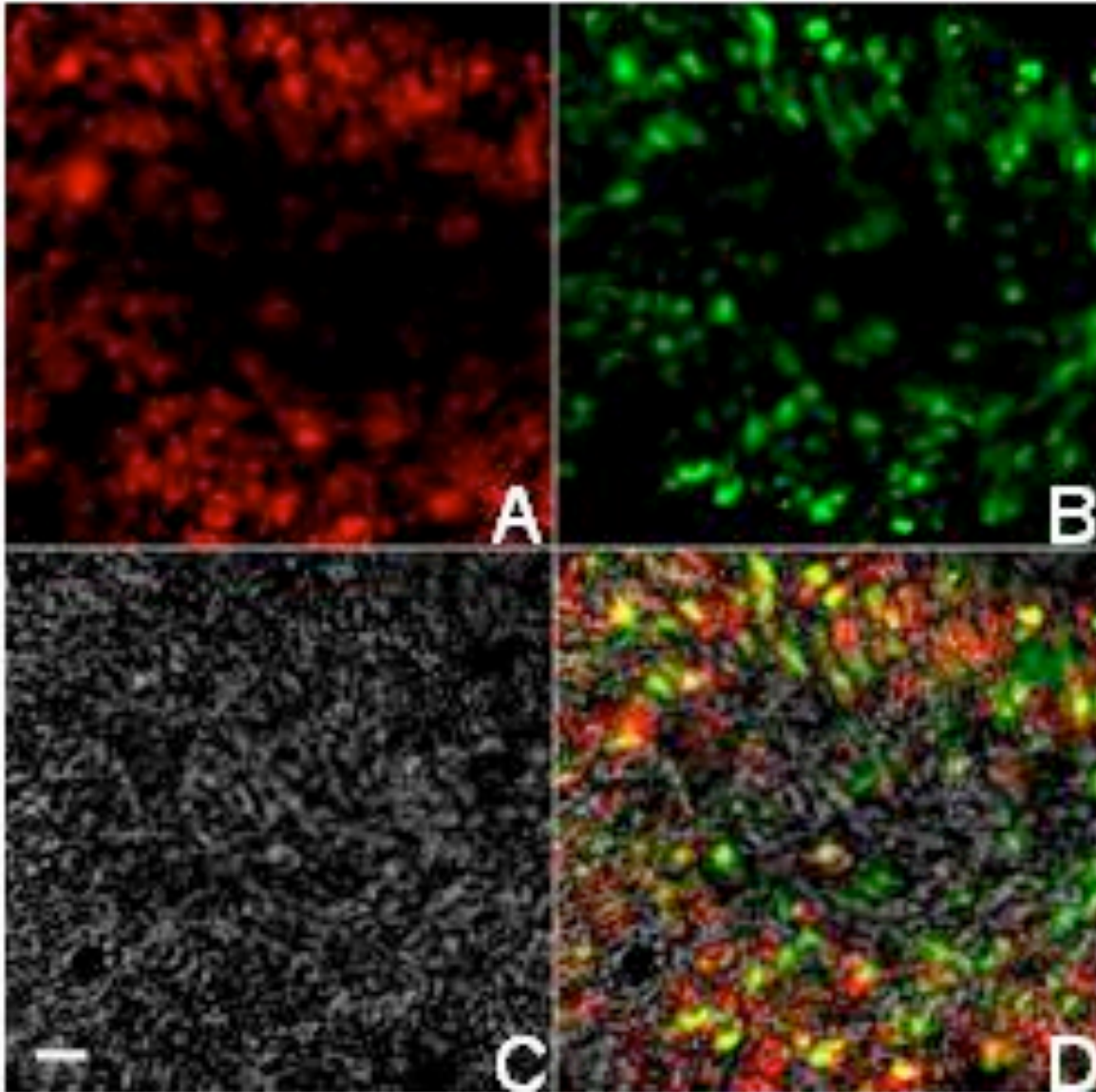
# Production of Hollow Fibers



Confocal imaging of regenerated nerve fibers inside the PCL guides immunolabelled with the axonal marker anti-NF-200kD antibody (A) and the Schwann cell marker anti-S100 antibody (B); bright field (C) and merge (D). Nerves were withdrawn six months after reconstruction of a 1.5 cm gap in the rat median nerve. The presence of a fascicle of regenerated fibers in the inner part of the conduit can be clearly detected. Bar indicates 200  $\mu\text{m}$ .

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# Production of Hollow Fibers



Higher magnification confocal imaging of regenerated nerve fibers inside the PCL guides (6 months postoperative after 1.5 cm gap repair in the rat median nerve): (A) Anti-NF-200kD (axonal marker) immunolabelling; (B) Anti-S100 (Schwann cell marker) immunolabelling; (C) Bright field; (D) Merge. The advanced maturation stage of nerve fibers formed by axons (green) surrounded by glial sheaths can be detected. Bar indicates 10  $\mu$ m.



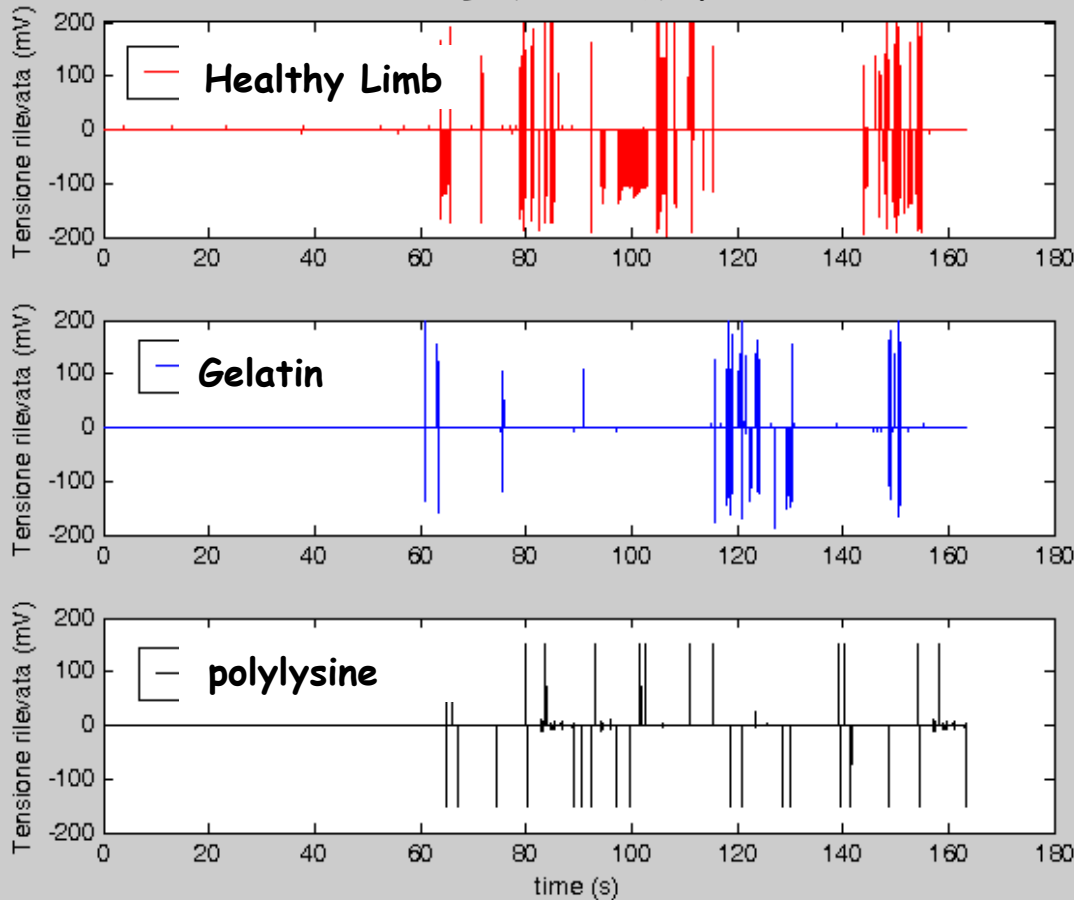
# Results of in-vivo implantation of PCL hollow fibers

## Electromyographic analysis

*Regeneration of peroneal nerve of Wistar rats at 6 months*



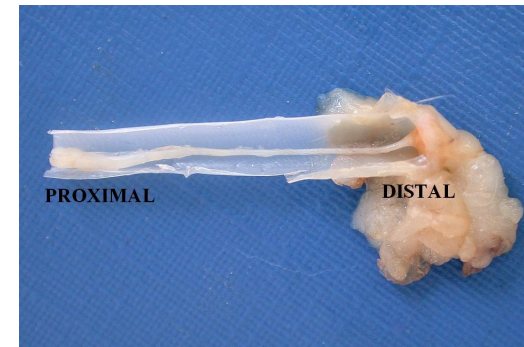
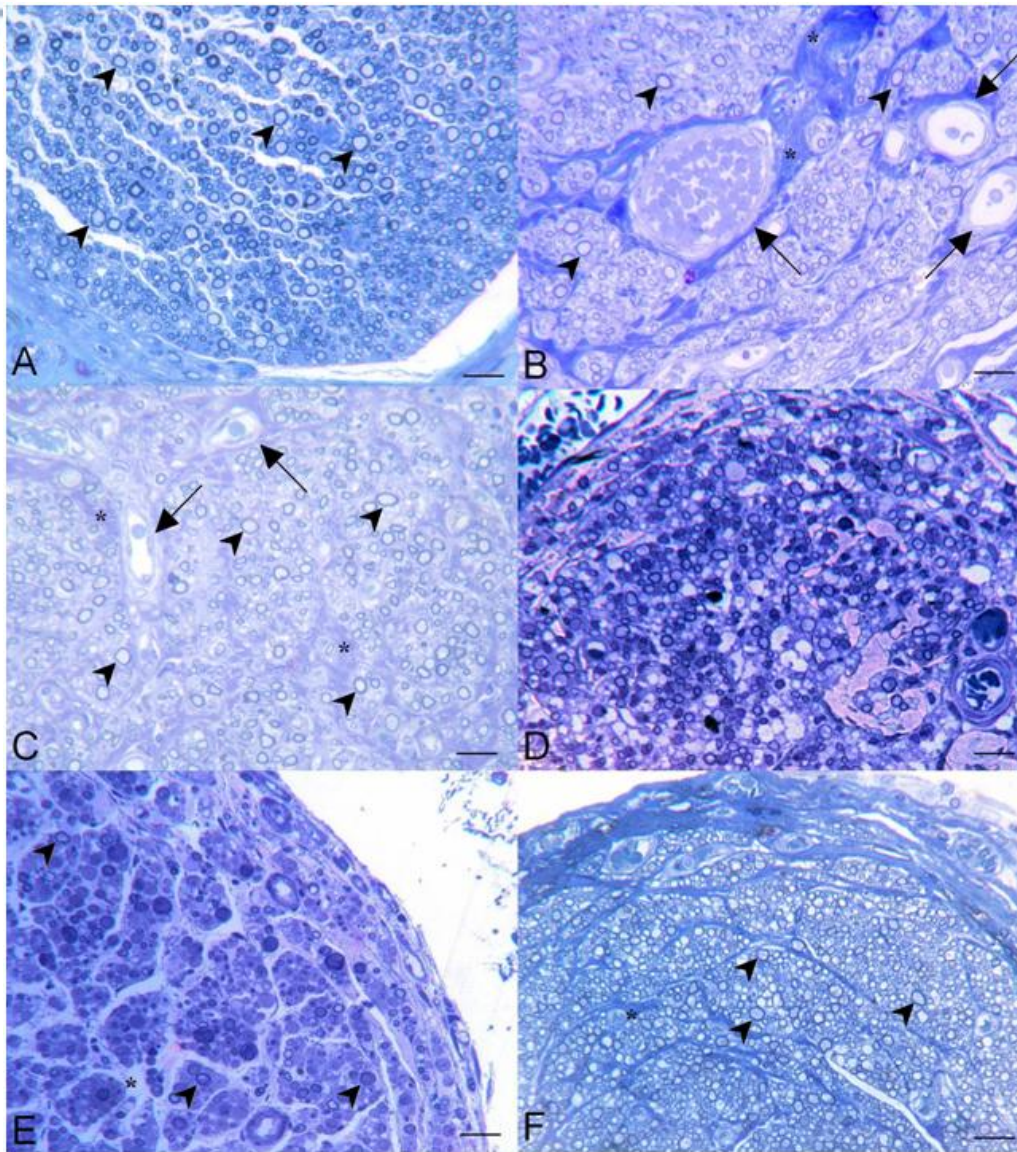
### PCL Hollow Fiber



### Methods:

*3 surface electrodes connected to BIOPAC System to measure the difference of potential induced in muscle fibers after physical stimulation.*

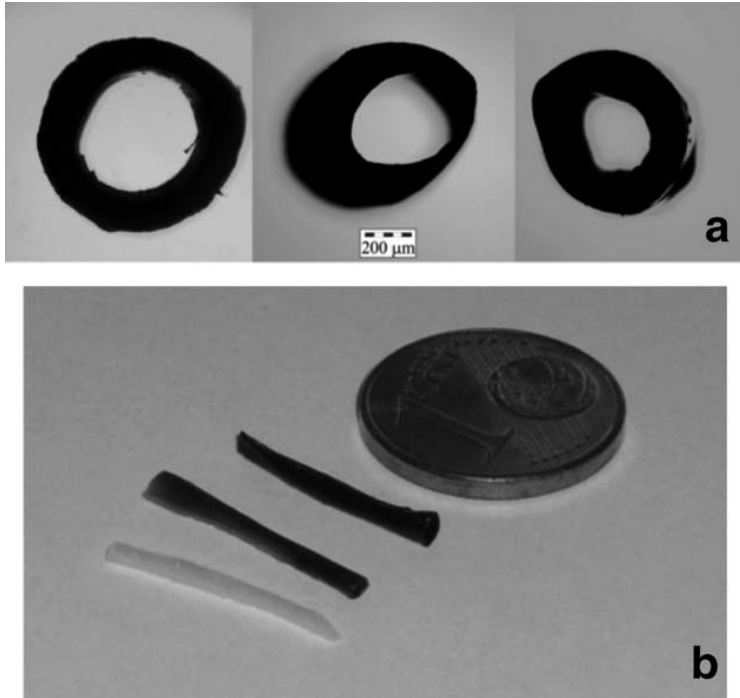




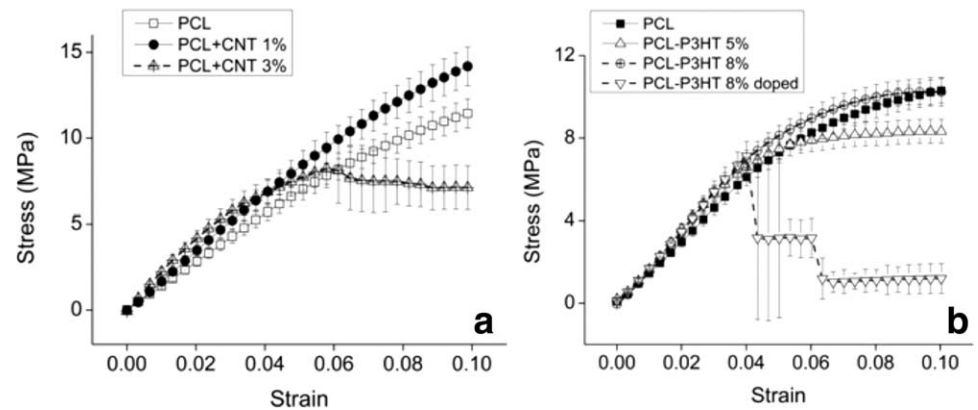
Regenerated nerves within the polymeric scaffold (a,c,e) and distal stumps (b,d,f) at 30 (a,b), 60 (b,c) and 160 days (d,e) after surgery. Regenerated nerve are composed of numerous small, tightly packed fibers with a thin myelin sheath. Medium fibers are evident at 60 days (c) and scattered large fibers are evident at 160 days (f). Numerous small caliber blood vessels are also evident in a. Multifocal regenerating fibers are evident in distal stumps in each groups (arrows). Moderate endoneurial fibrosis is evident, associated with multifocal axonal degenerations (f). Bar= 30  $\mu$ m (a); 15  $\mu$ m (c,d,e); 8  $\mu$ m (b,f).



# Conductive hollow fibers



**FIGURE 2.** (a) Cross section of CNT/PCL hollow fibers with different dimension and (b) hollow fibers made of PCL, P3HT/PCL, and CNT/PCL.



**FIGURE 3.** Stress-strain graphs of (a) CNT/PCL (b) P3HT/PCL hollow fibers.





# Conductive hollow fibers

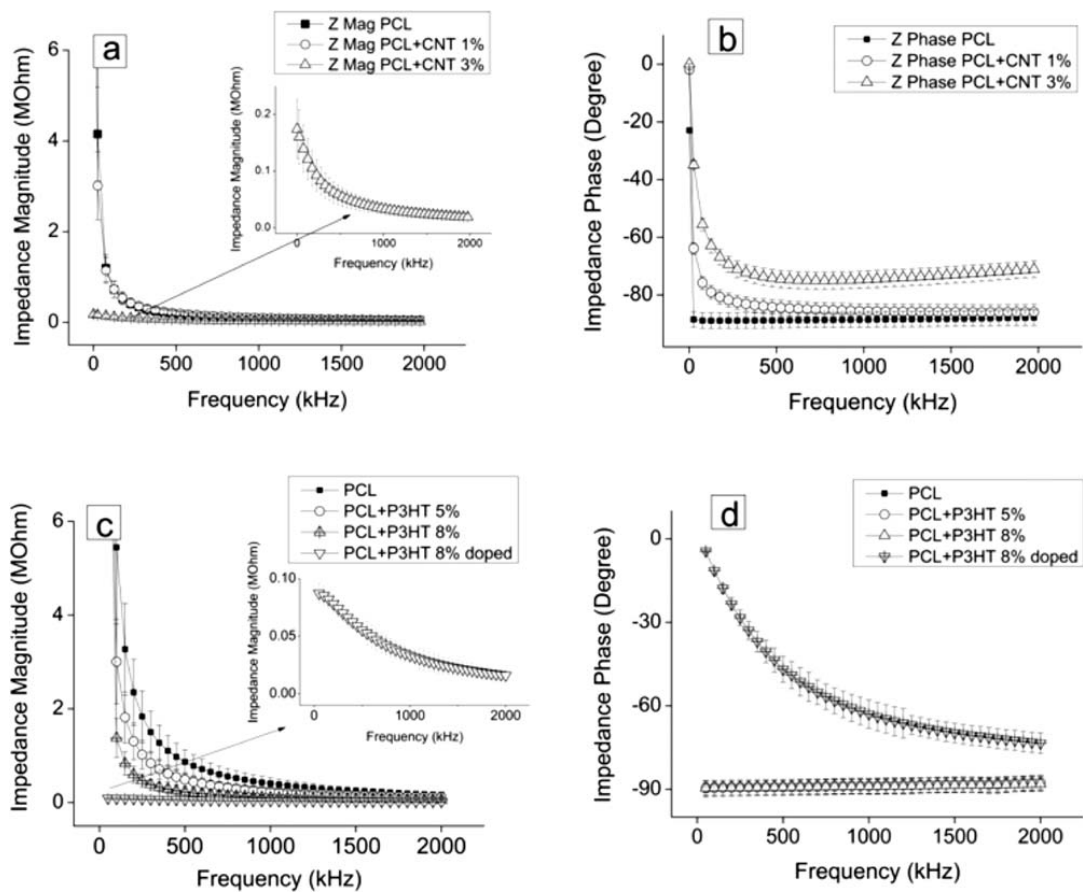
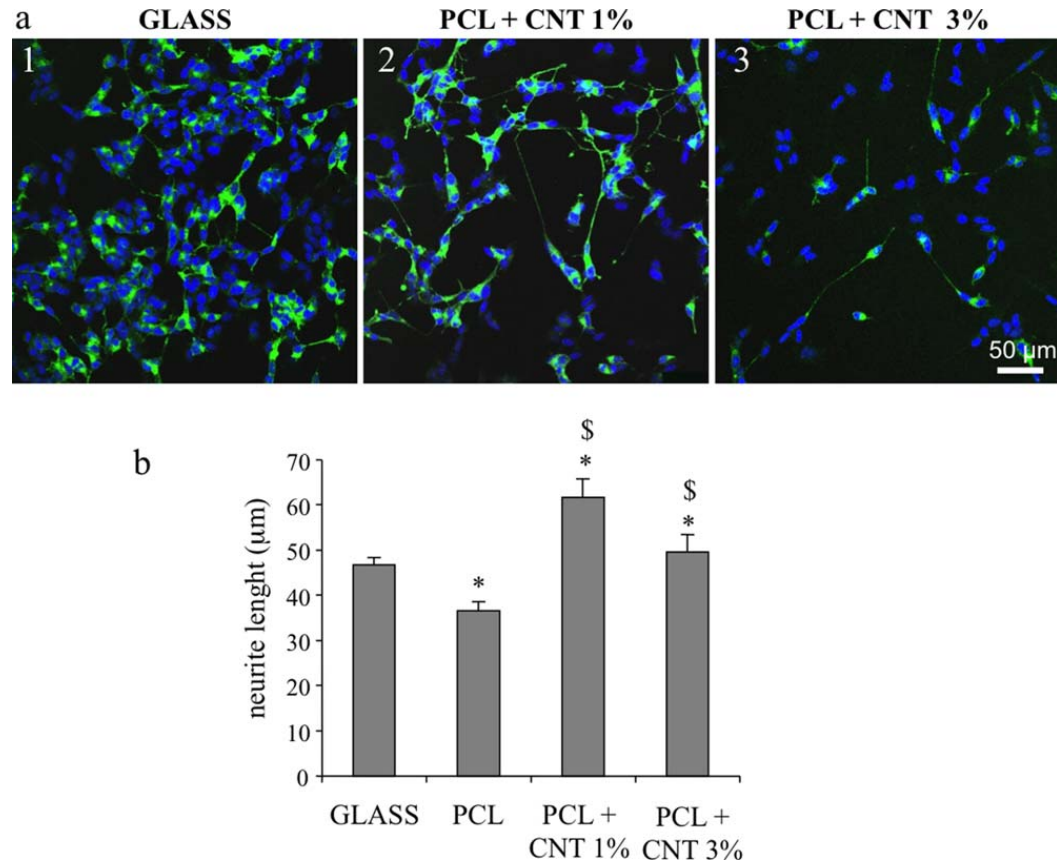


FIGURE 4. Impedance (a) magnitude and (b) phase profiles of CNT/PCL hollow fiber and (c) magnitude (d) phase profiles of P3HT/PCL hollow fiber.



# Conductive hollow fibers



**FIGURE 6.** (a) Representative images of SH-SY5Y cells stained with synaptophysin (green) after 1 week in culture with retinoic acid (RA) on (1) cover glass, (2) PCL + 1 wt % CNT, and (3) PCL + 3 wt % CNT. Nuclei were stained with DAPI. Scale bar = 50  $\mu$ m. (b) Quantification of neurite length by tracing method. Data are shown as mean  $\pm$  s.e.m. of three independent replicates. \*Significance against glass \* at  $p < 0.05$ ; \$ Significance against PCL at  $p < 0.05$  (ANOVA test). [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

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# Future of Live Scaffold Fabrication

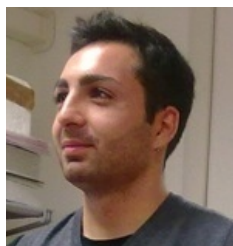




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