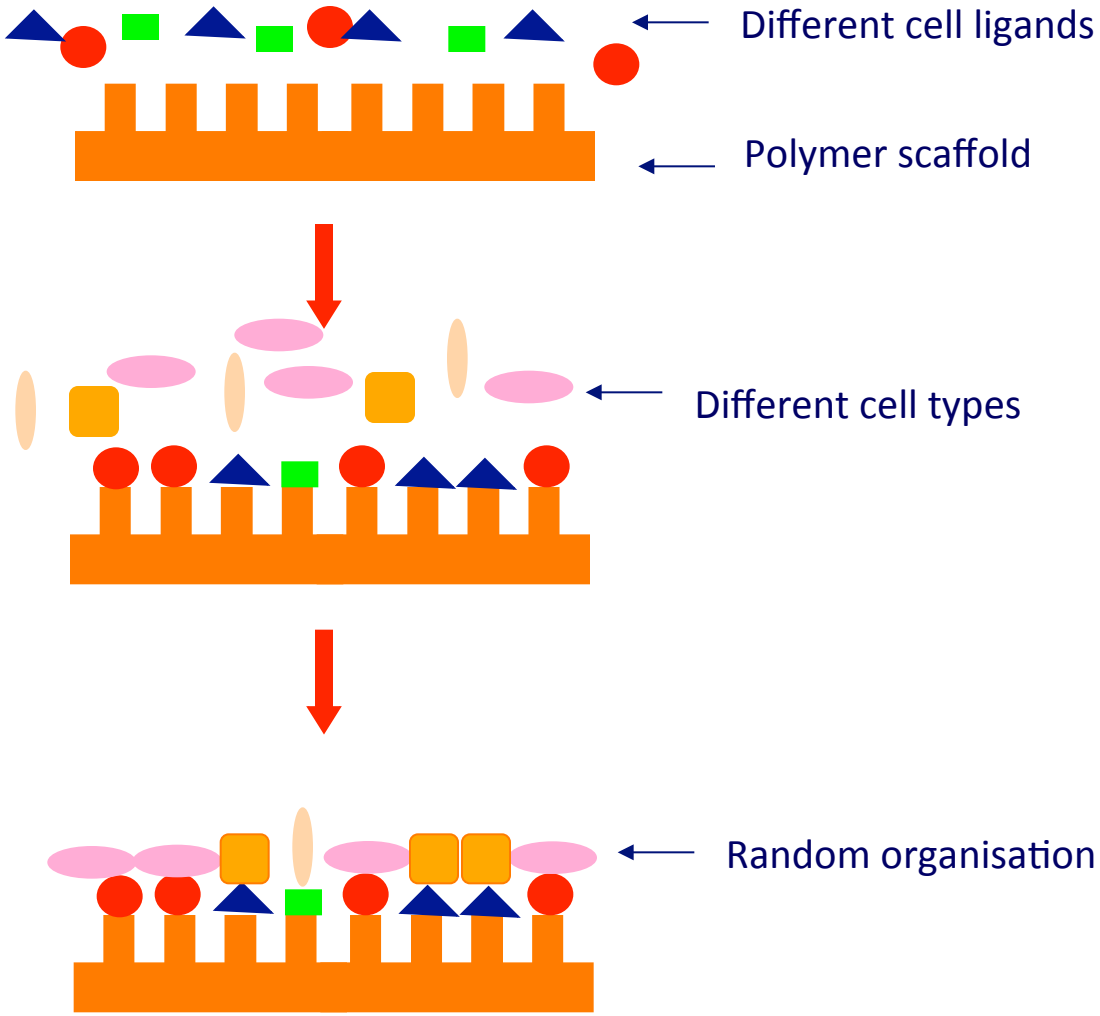
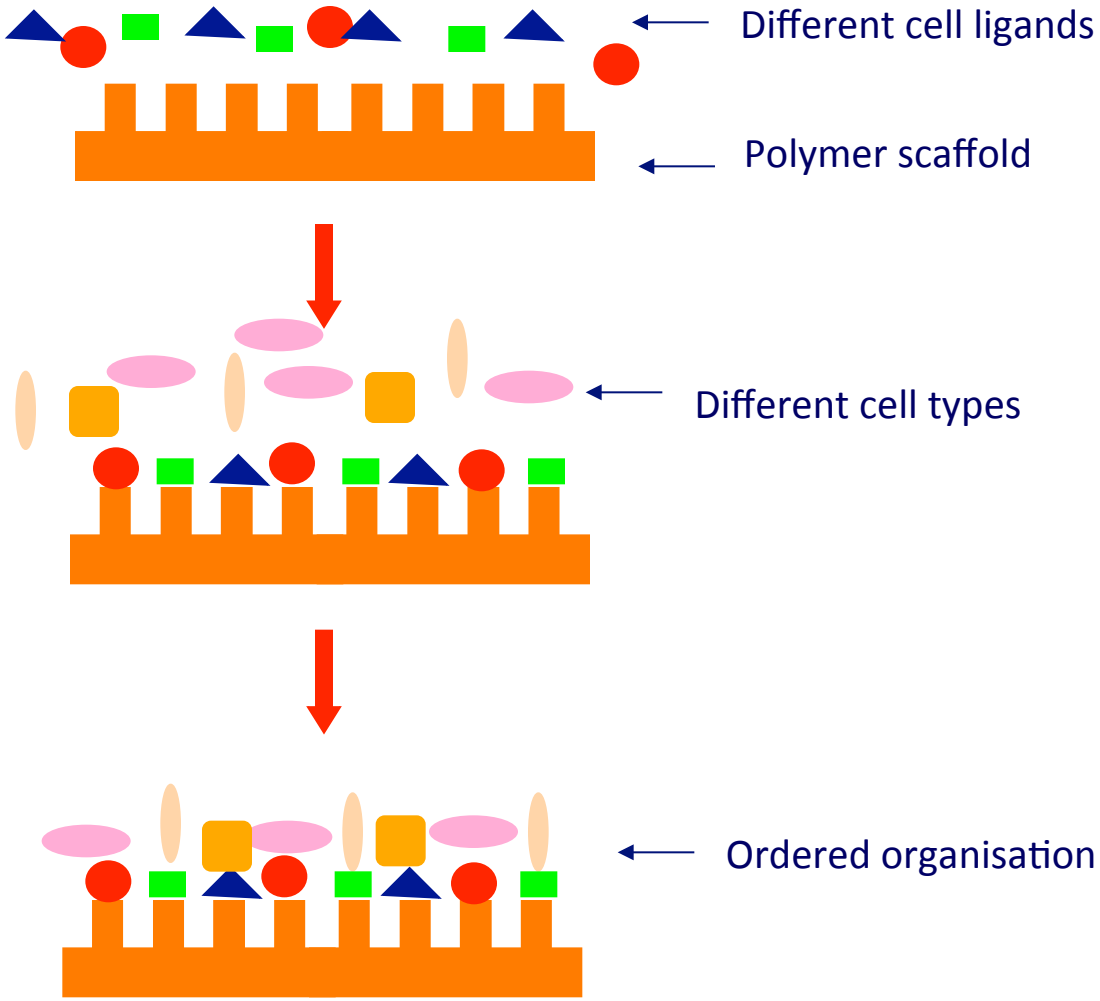
Introduzione alla fabbricazione 3D

g.vozzi@centropiaggio.unipi.it

Nano-structured Microstructures

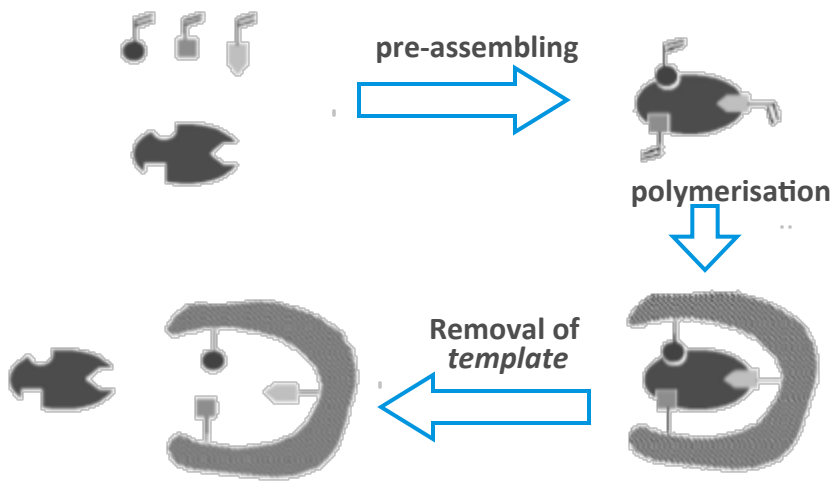


Nano-structured Microstructures



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**



approachs:

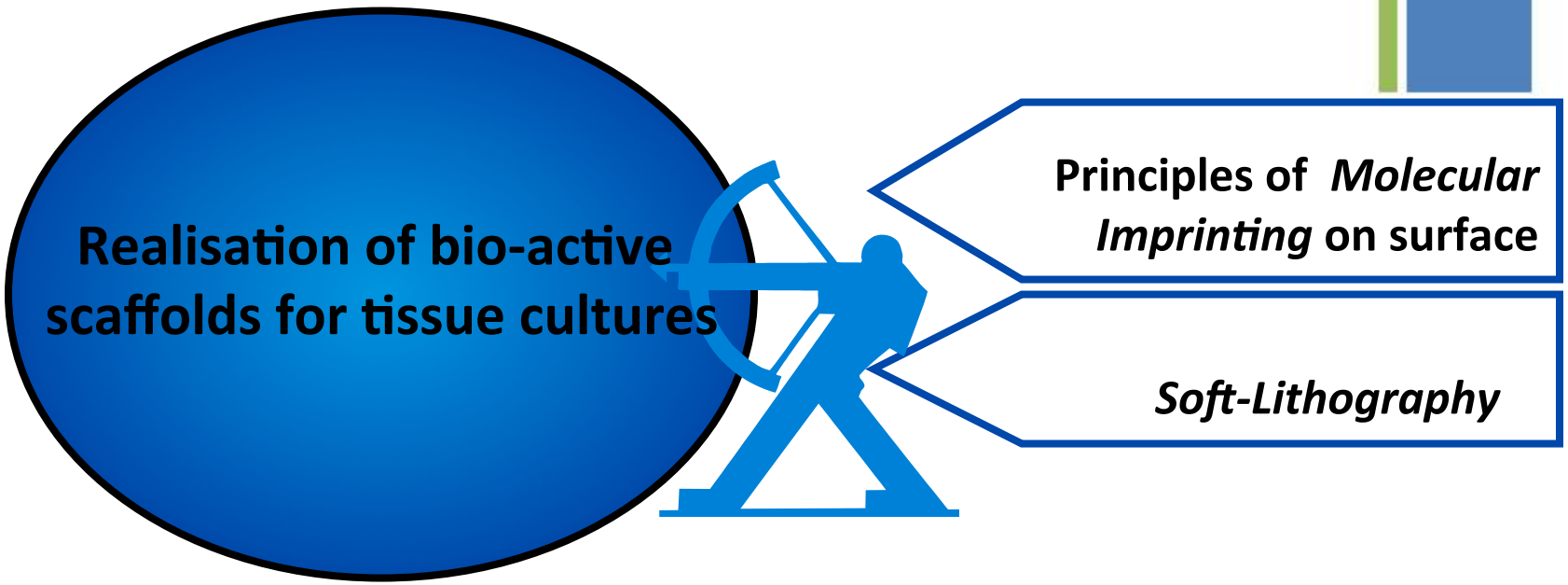
- **covalent interactions**

  (ex. For esters and ketonesi; G. Wulff e K. Shea)

- **reversible not covalent interactions**

  (ex. Ionic or hydrogen bond; K. Mosbach)

SOFT-MI

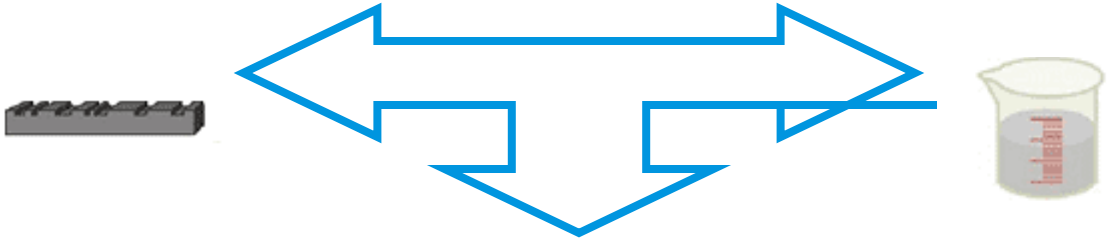


Method:

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

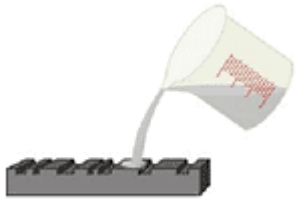
Vozzi G et al Biotechnol Bioeng. 2010;106(5):804-17.

Realisation of mold



master

PDMS solution

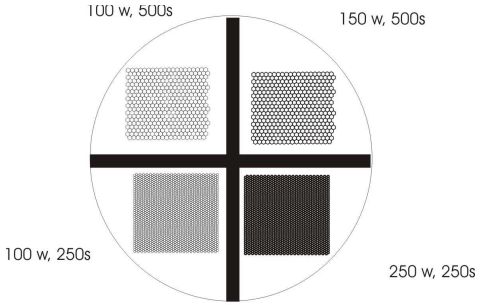


casting

Lift-off of mold

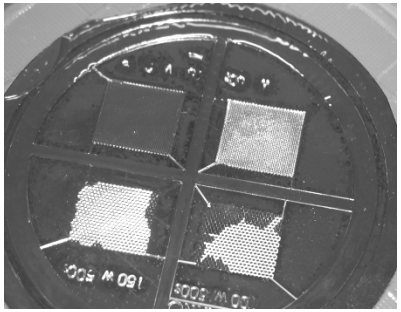
PDMS mold

Realisation of mold



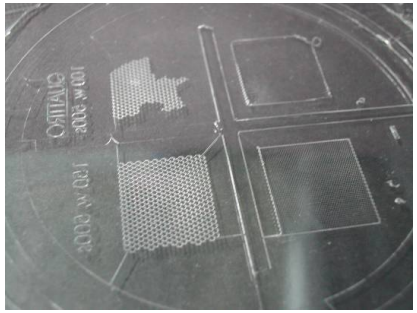
1

Mask layout



2

master



3

PDMS mold

Techniques of surface modification

Surface of PDMS is Hydrophobic

Physical and/or chemical treatment in order to increase wettability of PDMS

On the basis of state of art…

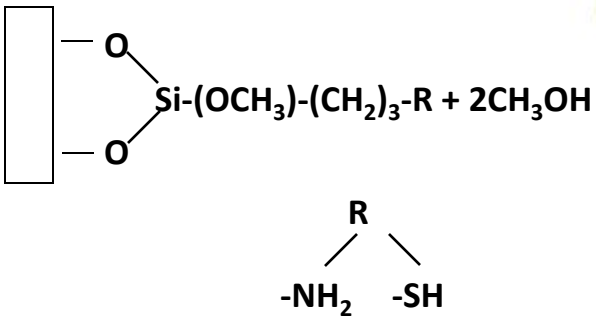
1. UV Exposure (λ = 350 nm)
2. Treatment with Argon plasma

On the basis of experimental experience…

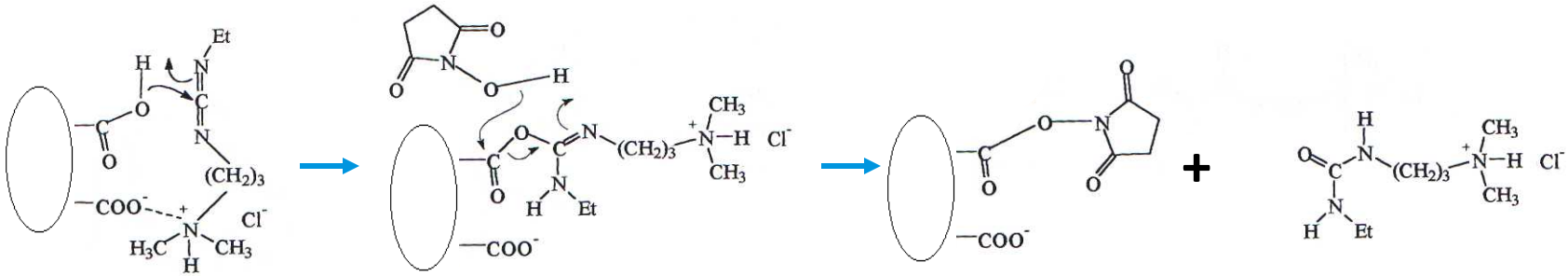
3. Dipping in Pirhana solution (H_2SO_4:H_2O_2 30% m/m in water = 3:1 v/v)
4. Dipping in H_2O_2 30% m/m in water

Functionalisation of mold

- **Derivatisation with poly-functional silanes in toluene solution**
  - 3-aminopropyl-trimethoxysilane
    H_2N-$(CH_2)_3$-Si-$(OCH_3)_3$
  - 3-mercaptopropyl-trimethosilane
    HS-$(CH_2)_3$-Si-$(OCH_3)_3$



- **Activation of reaction between Carboxylic and nucleophilic groups**
  solution of N-ethyl-N'-(3-dimethylamminopropyl)-carbodiimmide (EDC) and N-hydroxysuccinimmide (NHS) in water

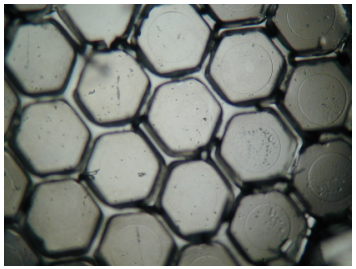


Cell adhesion test

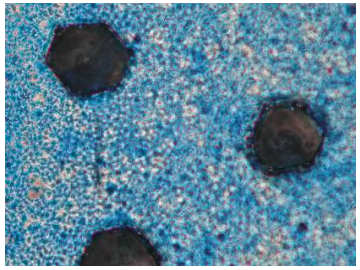
protocol:

- **Fabrication of PDMS mold functionalised with a protein (Gelatin)**
- **Realisation of polymer *scaffold with micromolding method using* 20% polymethylmethacrylate (PMMA) solution in $CHCl_3$**
- **Rebonding of protein with imprinted scaffold**
- **Cell seeding with Murine Fibroblasts (NIH-3T3)**
- **Cell Fixing and staining at different time intervals (6/24/48 h)**

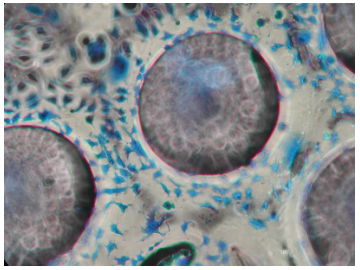
Example of PMMA scaffold



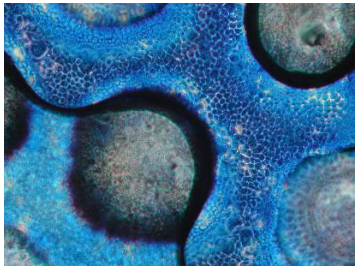
Cell adhesion and Proliferation



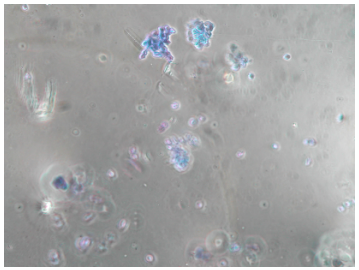
UV exposure
amino-silane
48 h



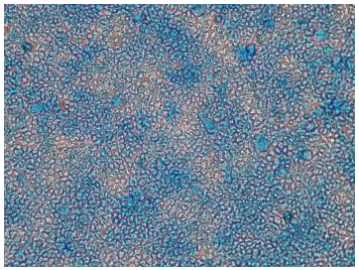
Pirhana solution
mercapto-silane
48 h



Argon Plasma
mercapto-silano
48 h

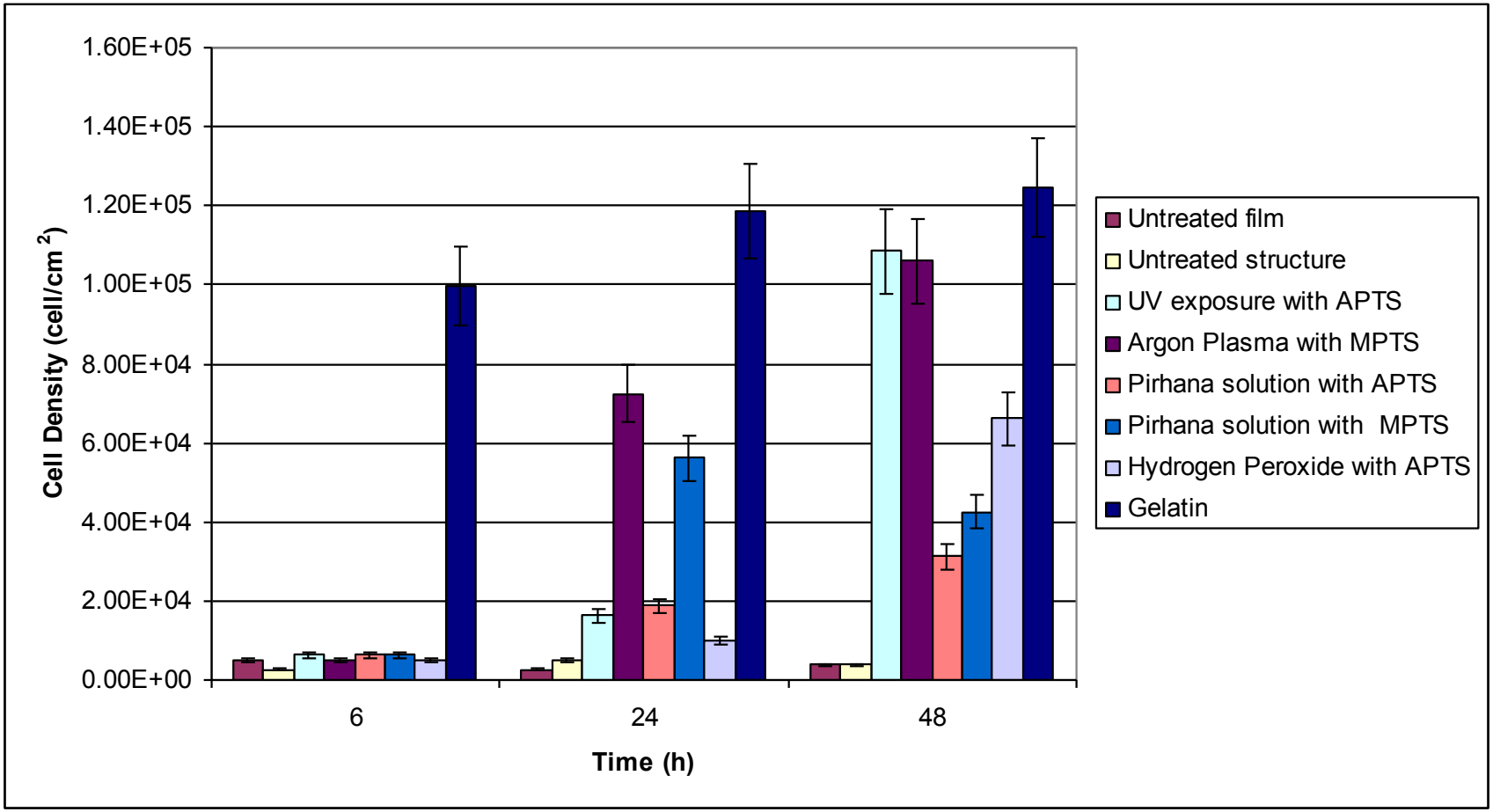


Control (Film of PMMA)
48 h



Control (Gelatin)
48 h

Synthesis of results



Imprinting cells

PDMS mold

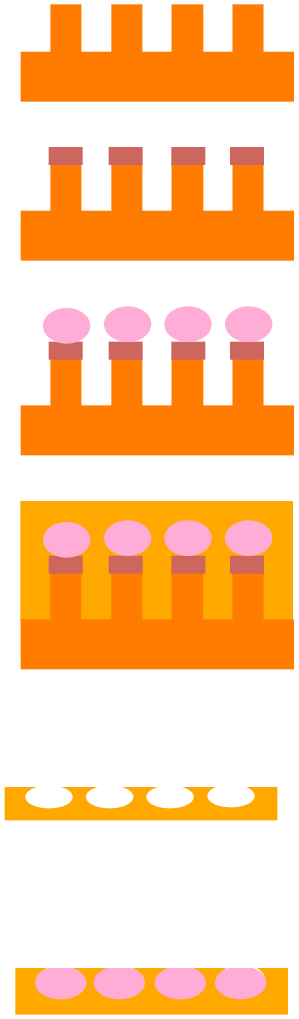
Functionalisation of PDMS mold

Cell Seeding and fixing

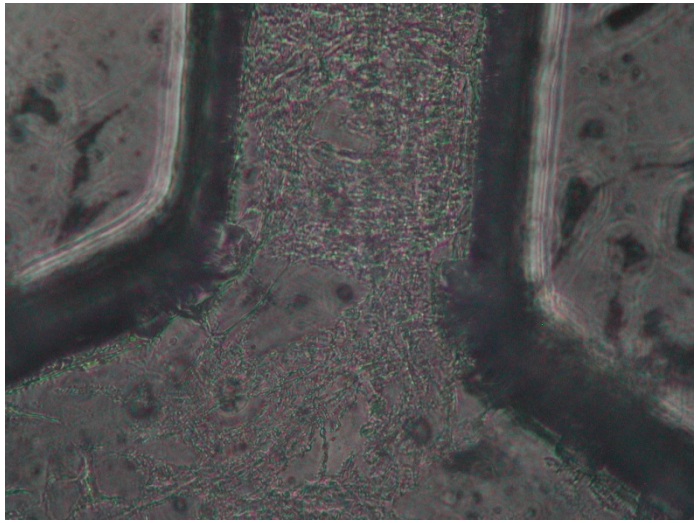
Polymer Casting

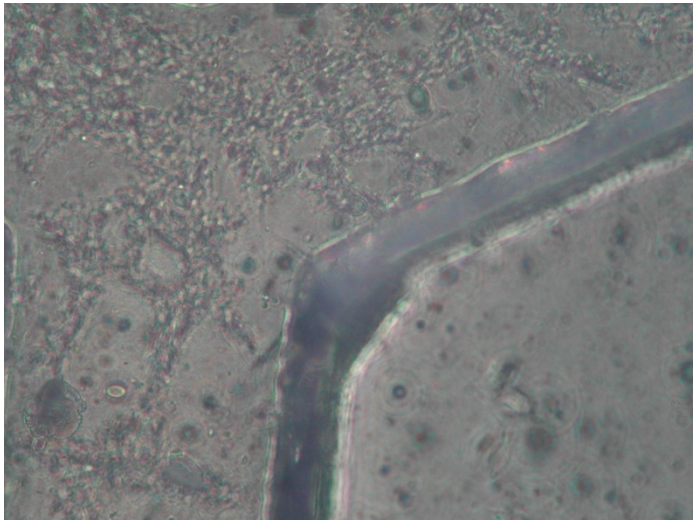
Realisation of Polymer Scaffold

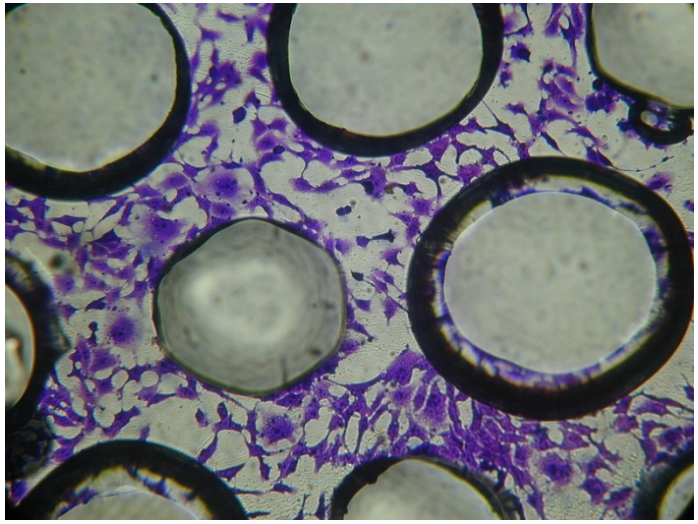
Cell Seeding on functionalised polymer scaffold



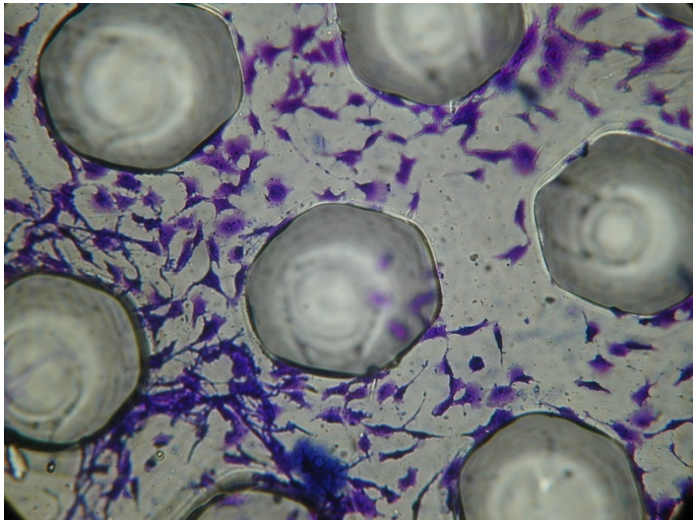
Imprinting cells



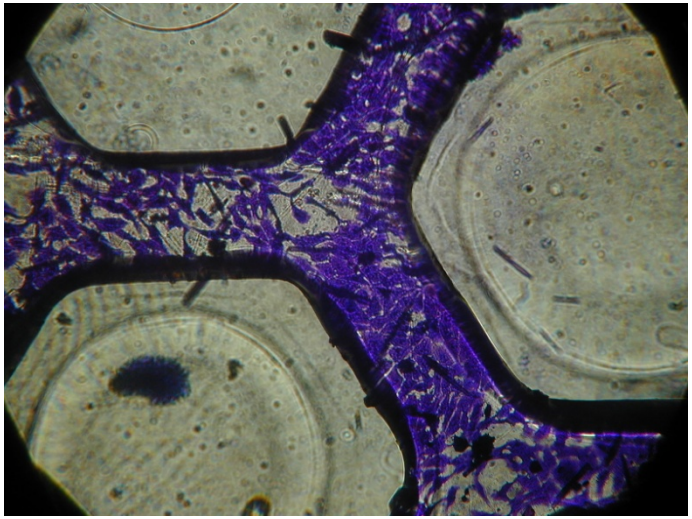




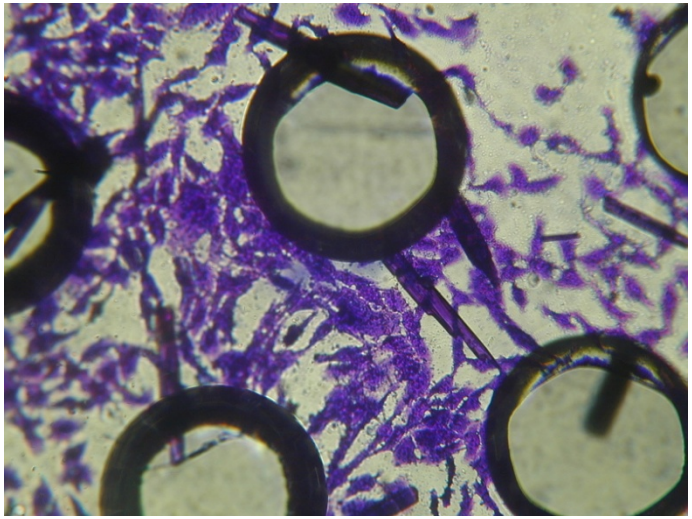
24h

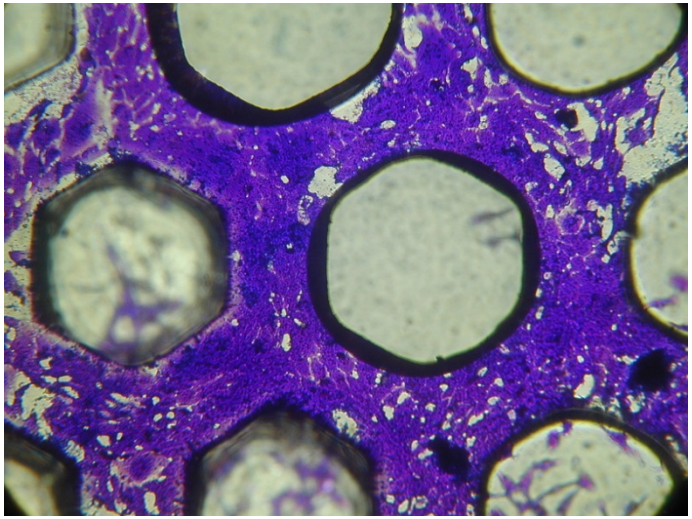


Imprinting cells

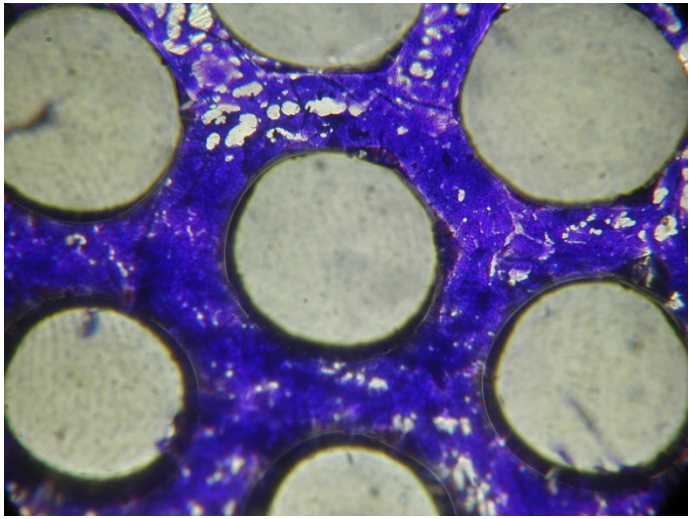


48h

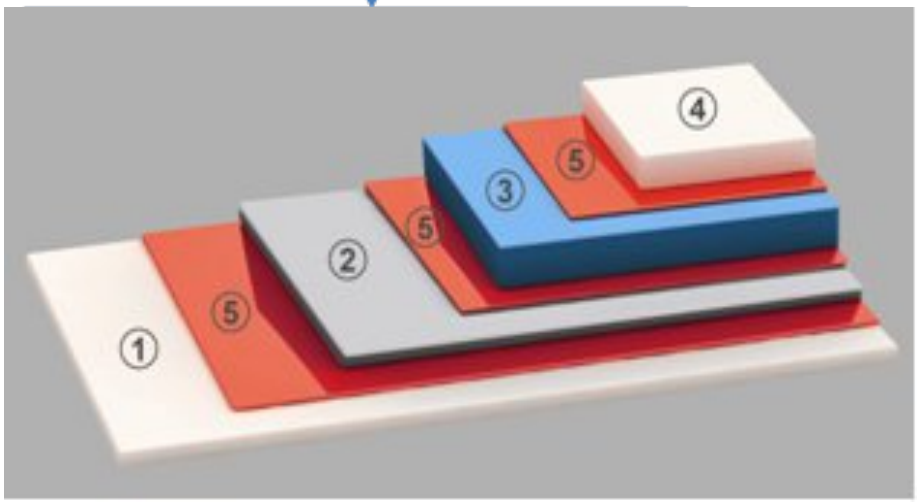


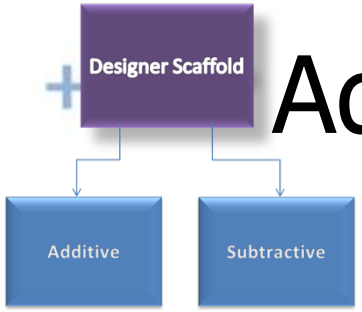


72h



Designer Scaffold

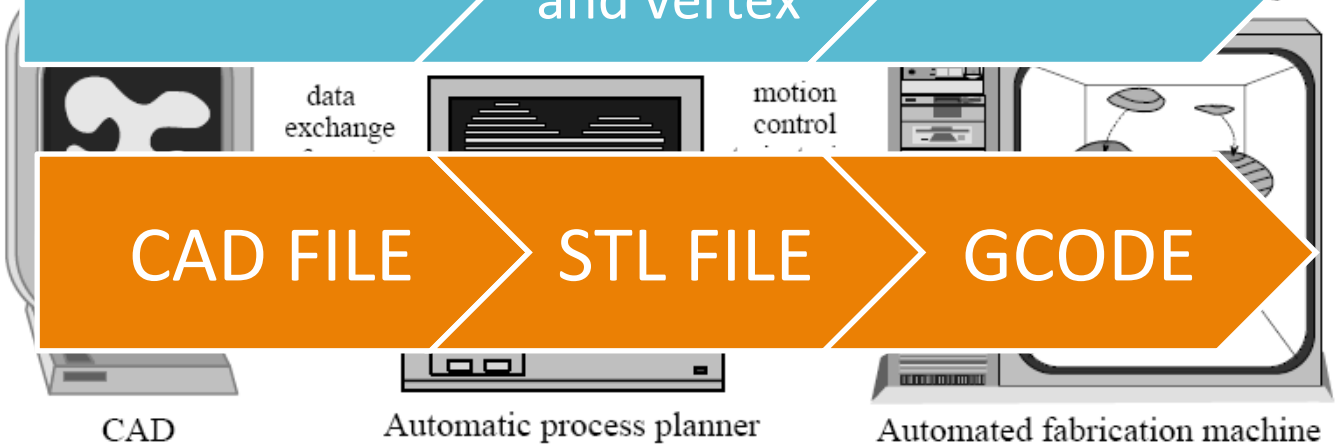




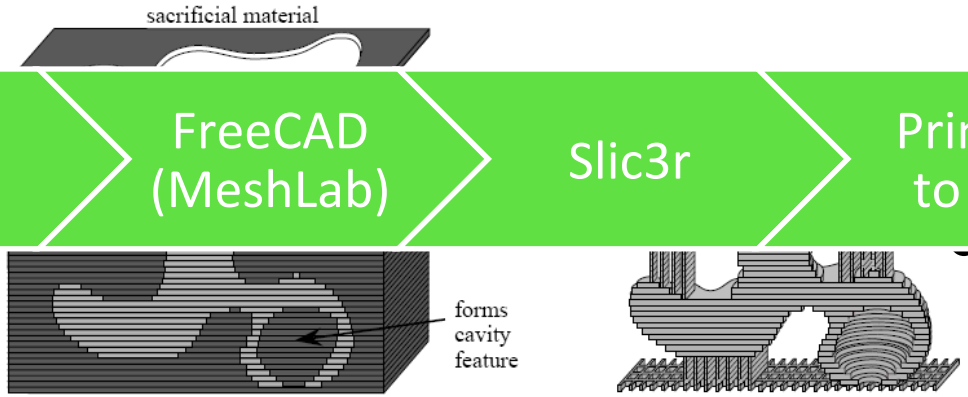
Additive = rapid prototyping

3D object → Triangle and vertex → Toolpath

CAD FILE → STL FILE → GCODE



FreeCAD → FreeCAD (MeshLab) → Slic3r → Printrun to 3DP



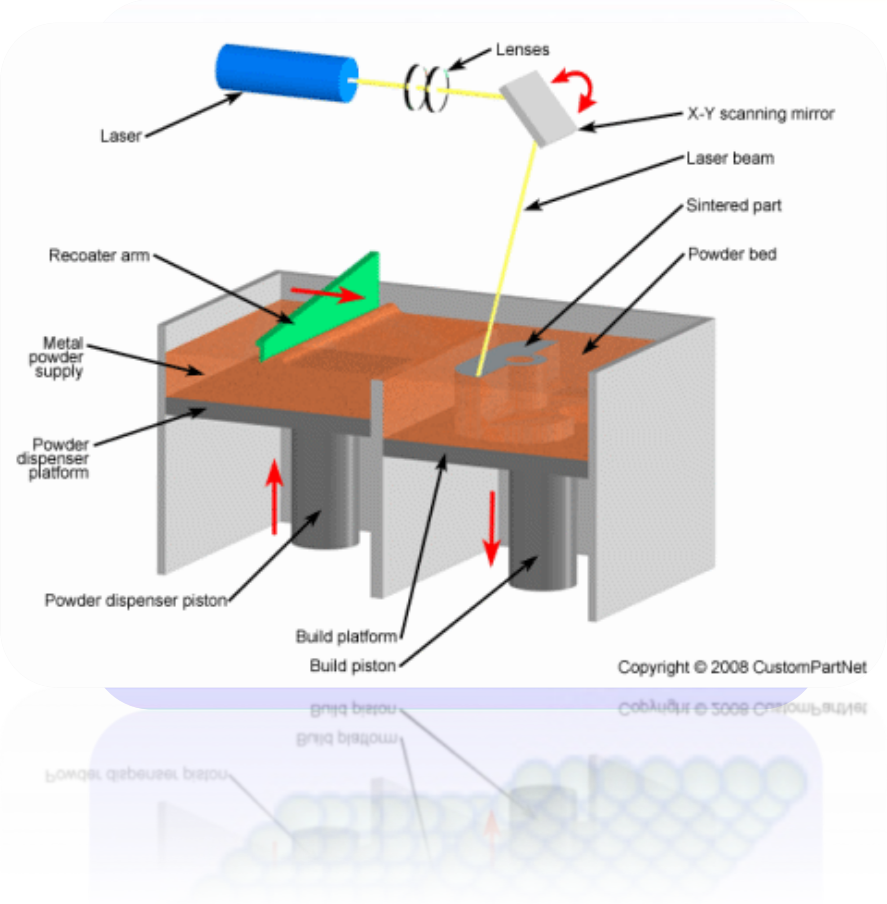
a. Complementary support.

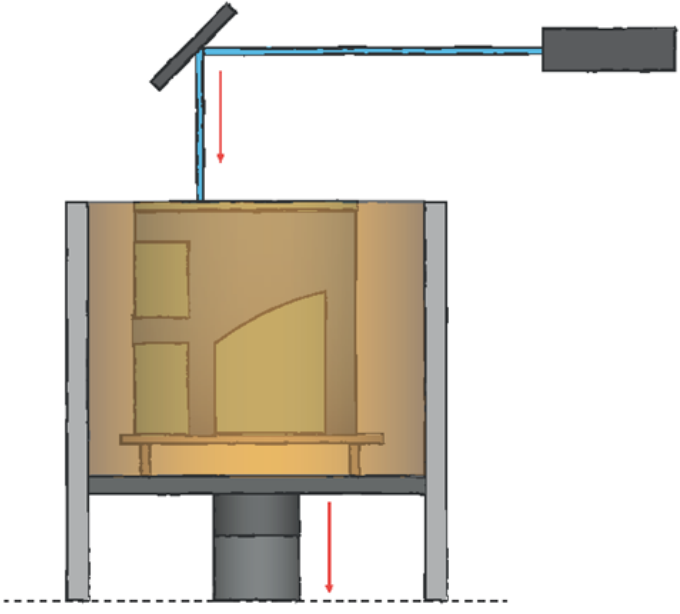
b. Explicit support.

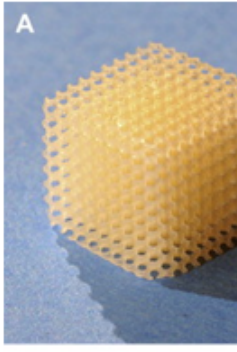
Rapid Prototyping (RP) Techniques

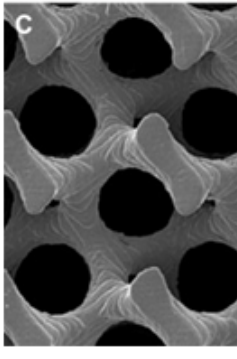
Three main groups:

- laser systems
- nozzle based systems
- direct writing systems

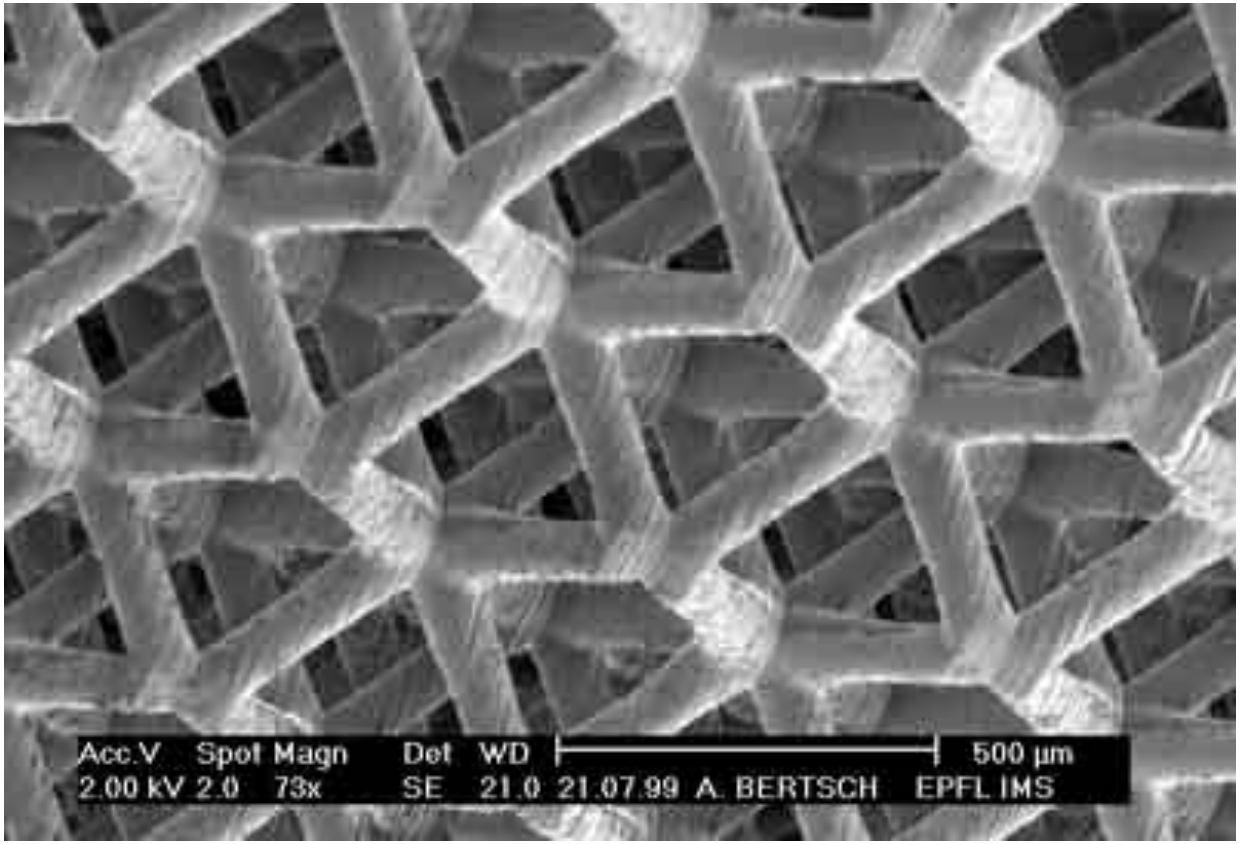


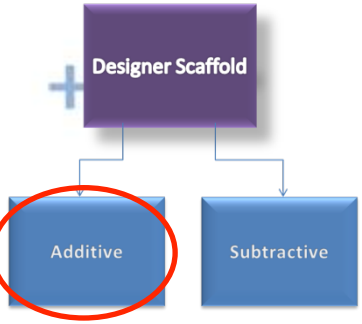



A

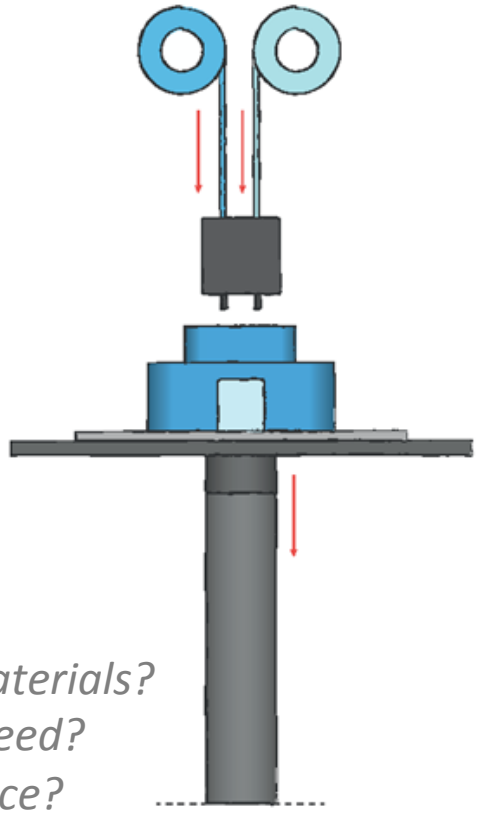

C

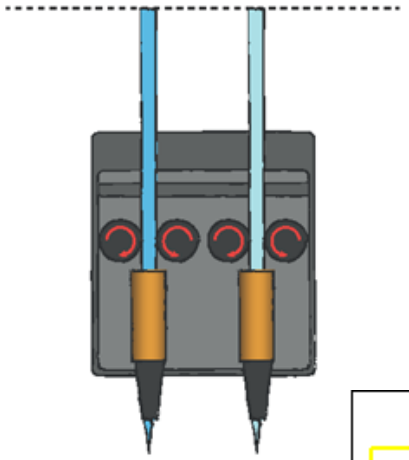
Risultati

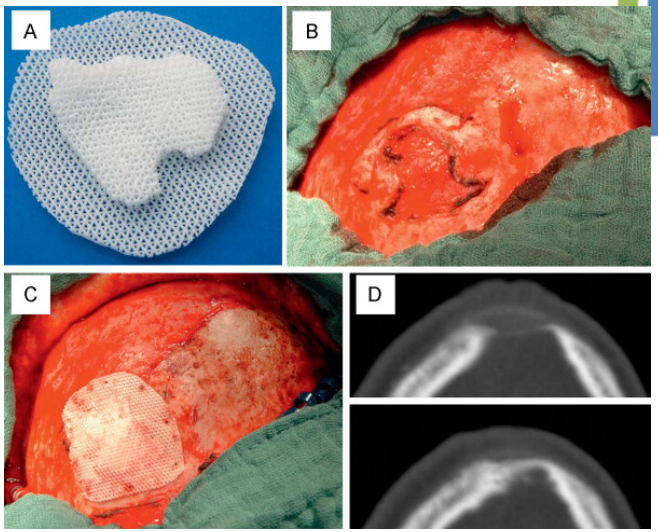

Acc.V Spot Magn Det WD
500 µm
2.00 kV 2.0 73x SE 21.0 21.07.99 A. BERTSCH EPFL IMS



Fused Deposition Modeling







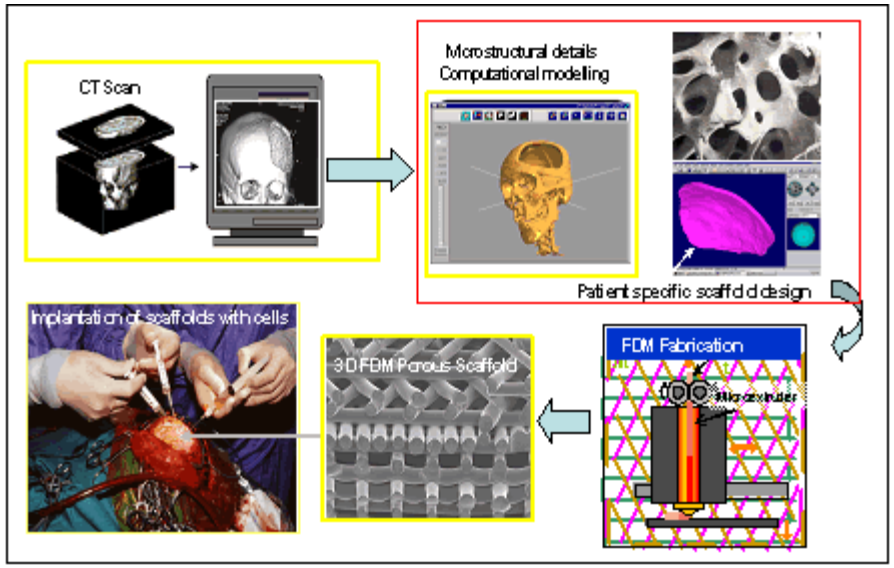
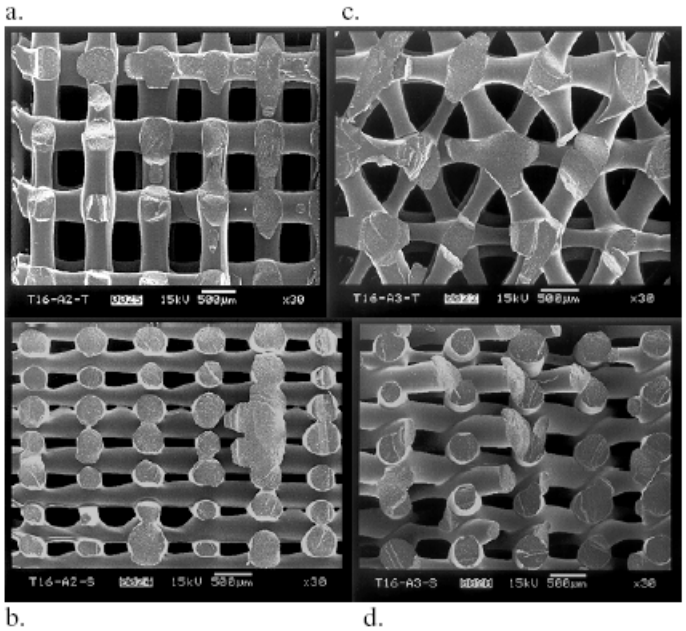


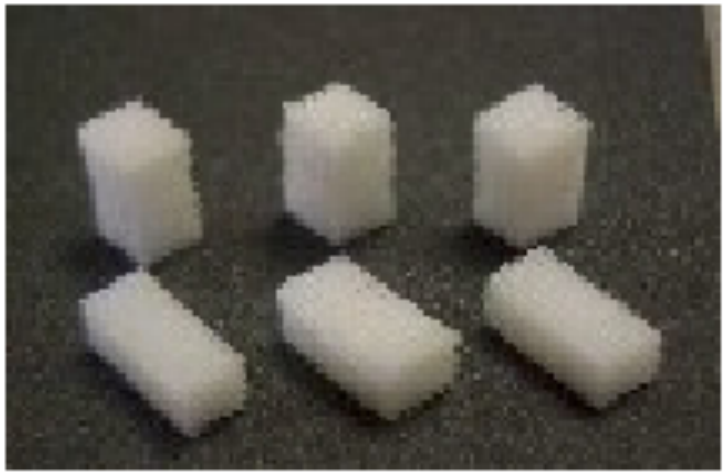
Figure 1: Platform technology for patient specific scaffolds TE.

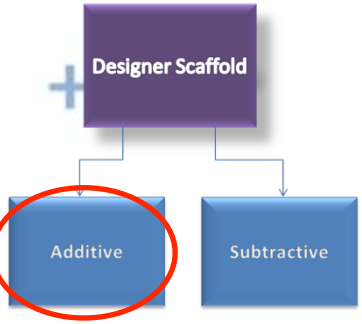
Materials?
Speed?
Price?
Fidelity?

Hutmacher & coworkers

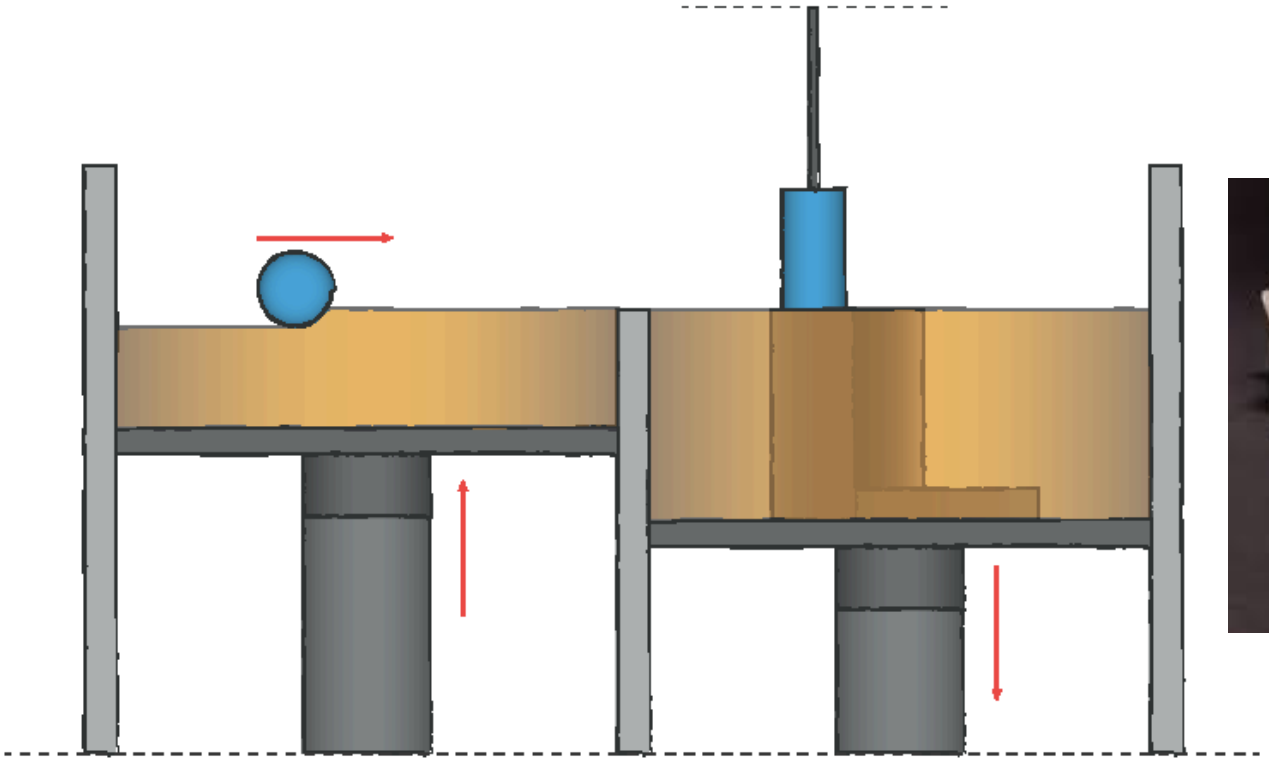
Risultati





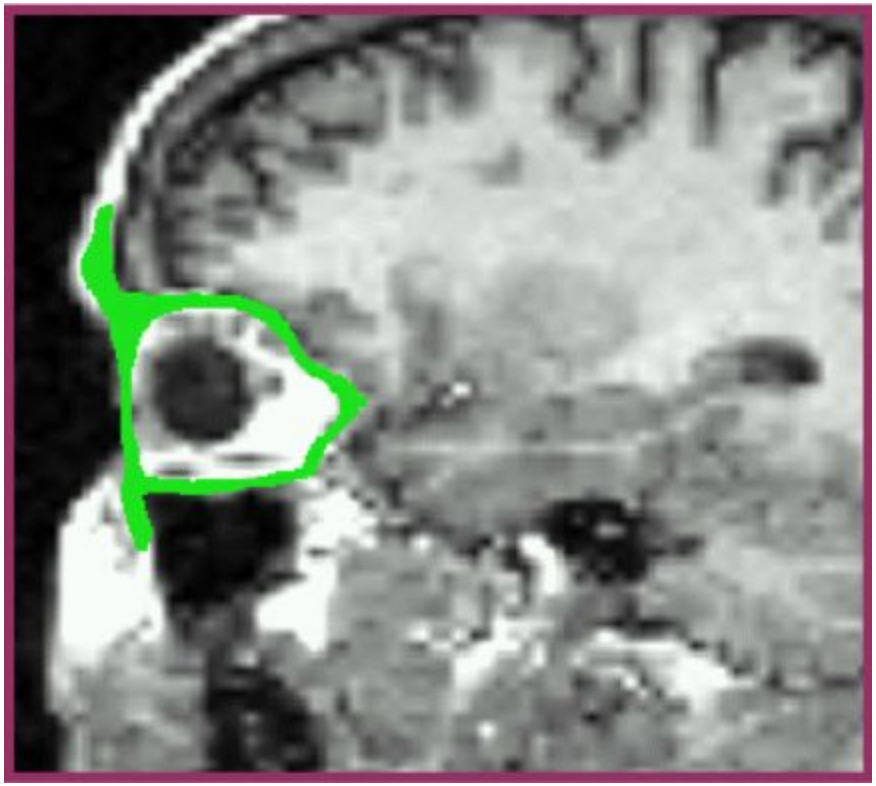


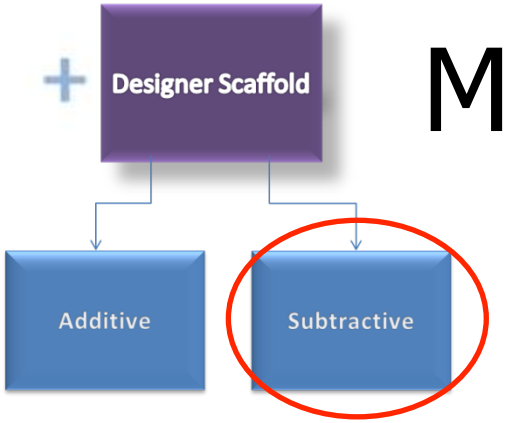
3D Printing™



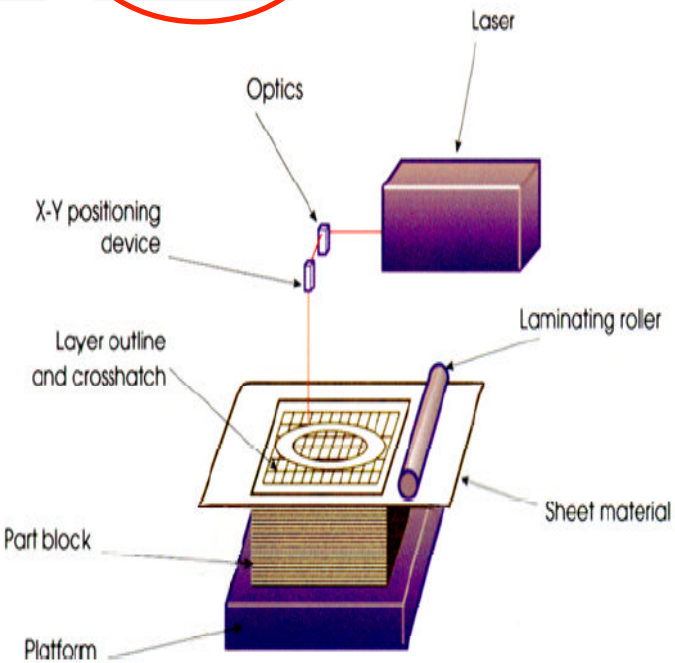
Materials?
Speed?
Price?
Fidelity?

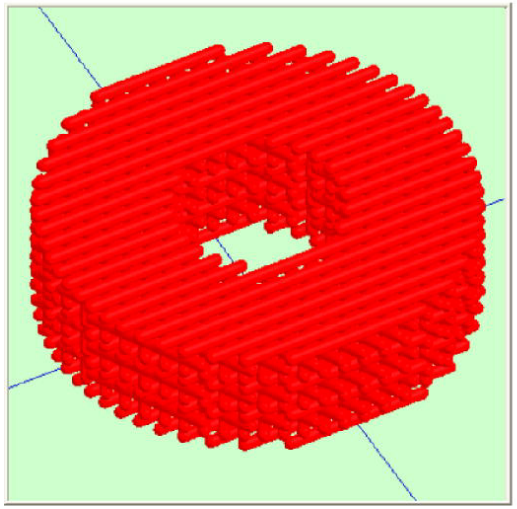
Risultati

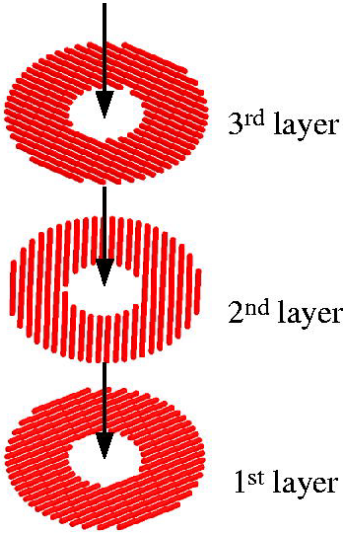




Membrane Lamination



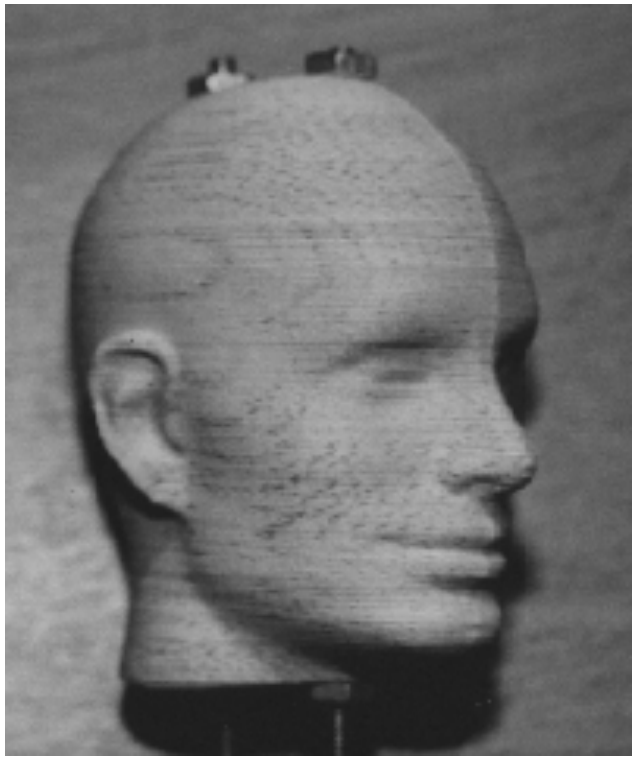




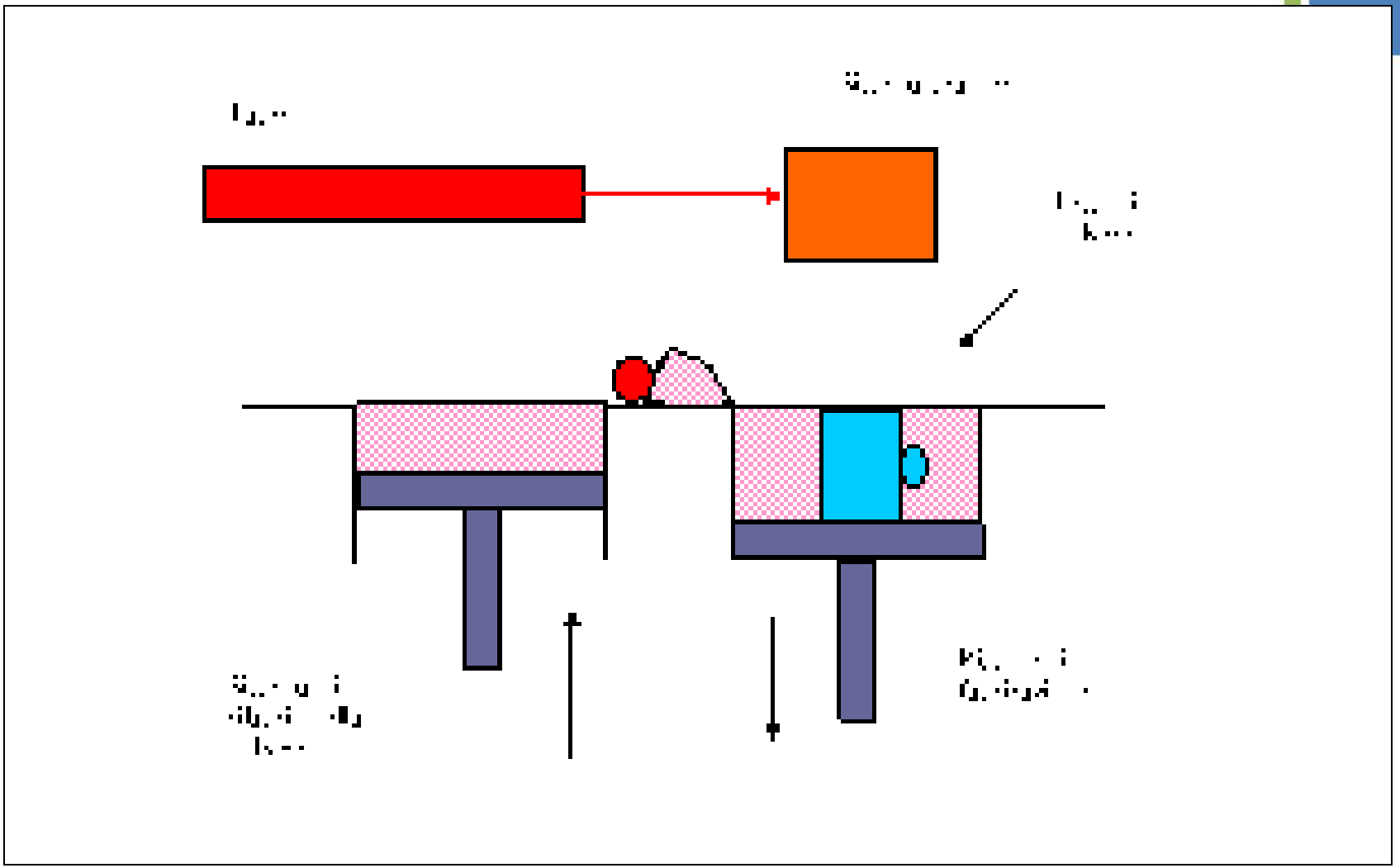
Laser as a cutter

Materials?
Speed?
Price?
Fidelity?

Risultati

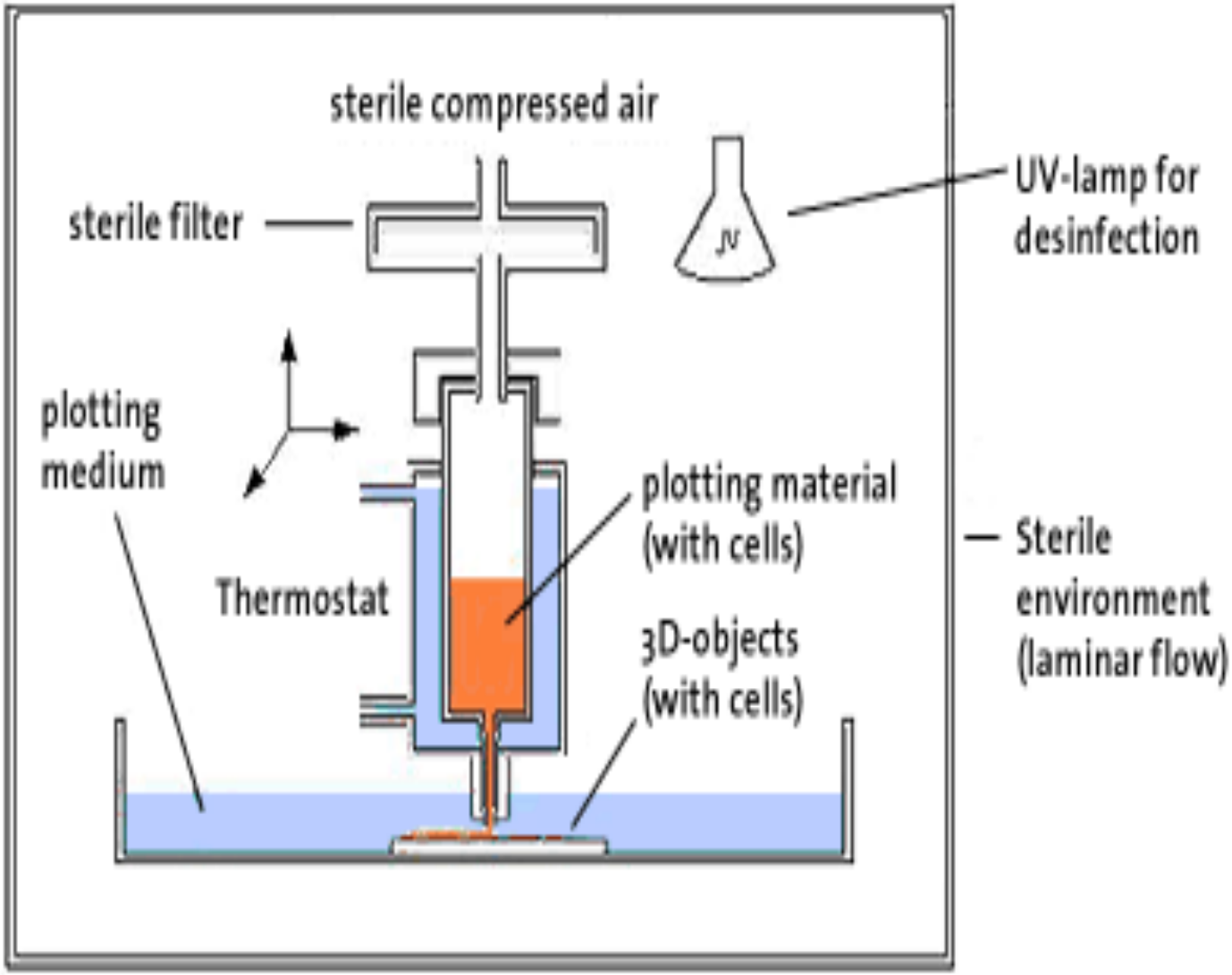


Laser Sinterising

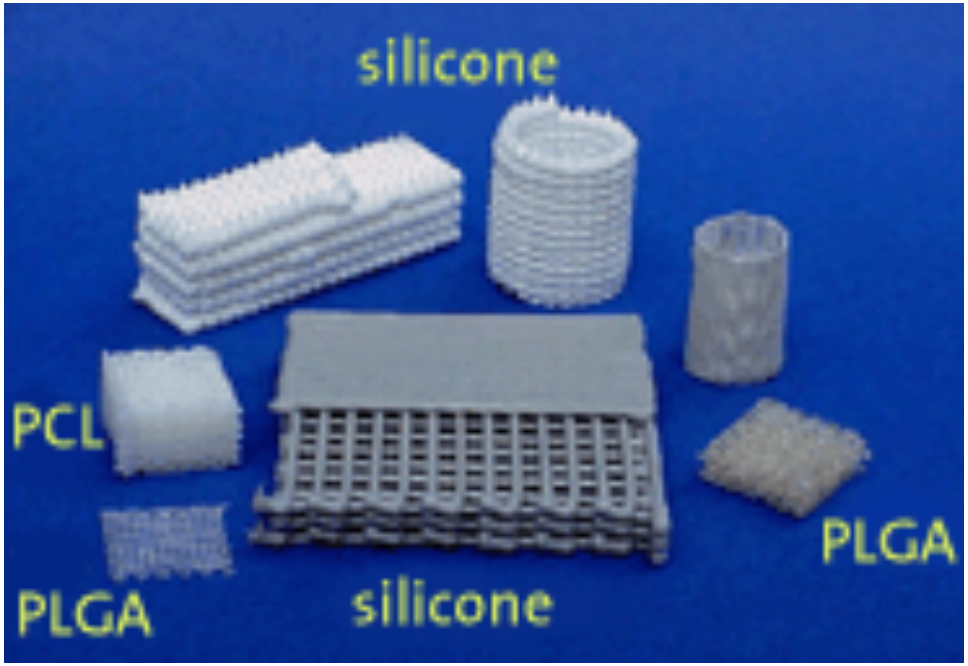


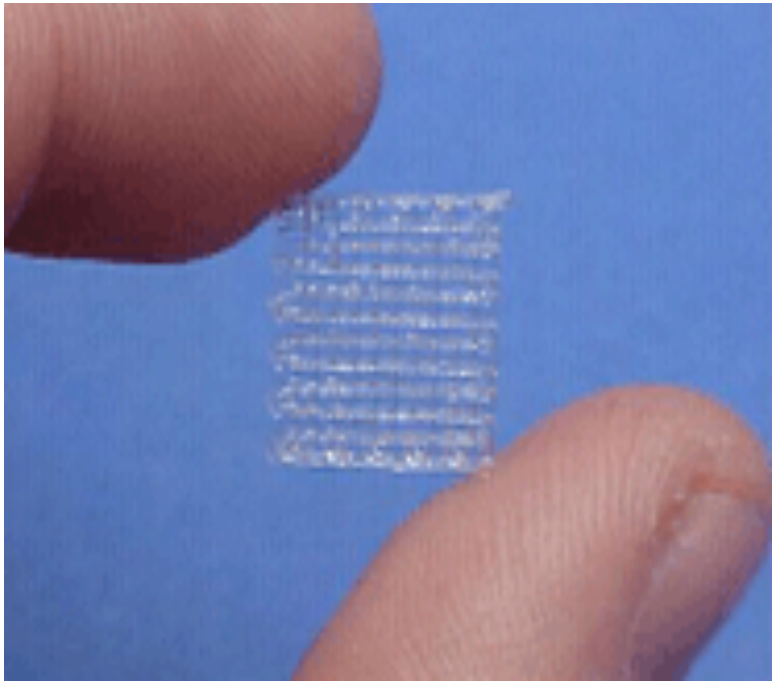
Risultati

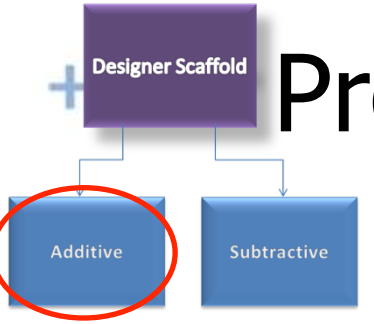
Bioplotter


sterile compressed air
sterile filter
UV-lamp for desinfection
plotting medium
plotting material (with cells)
Thermostat
3D-objects (with cells)
Sterile environment (laminar flow)

Risultati

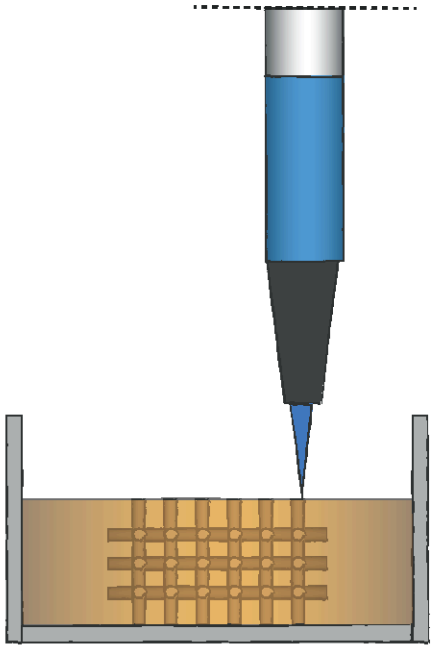




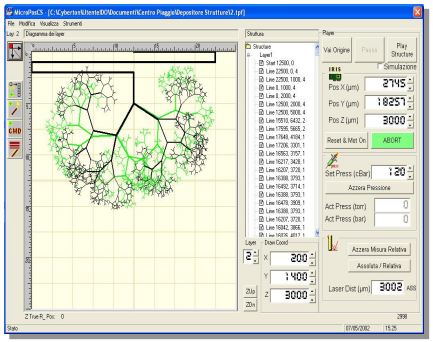


Pressure Assisted Microsyringe (PAM)

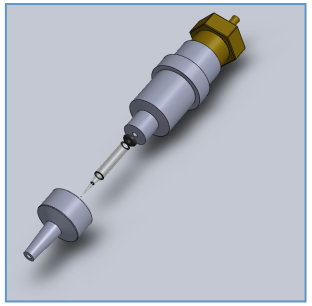
Regulated air flow



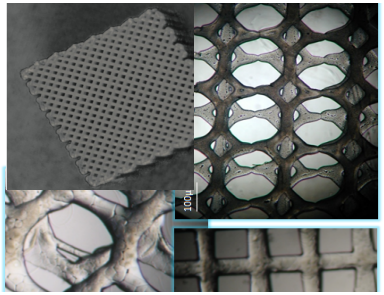
PAM system



Software



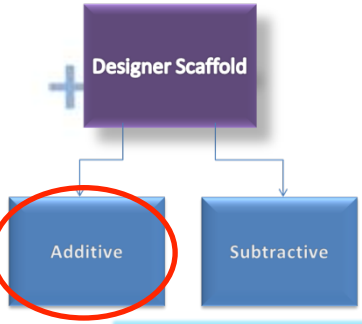
Syringe design



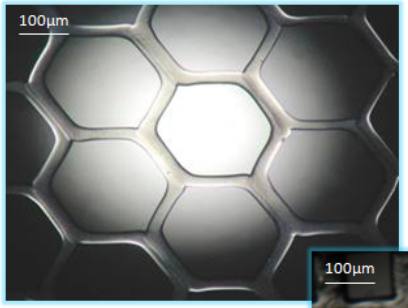
Software

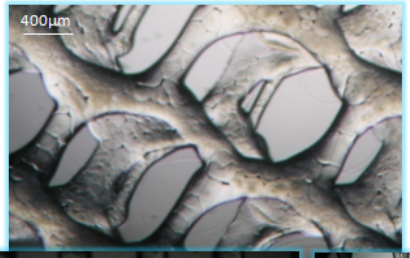
Materials?
Speed?
Price?
Fidelity?

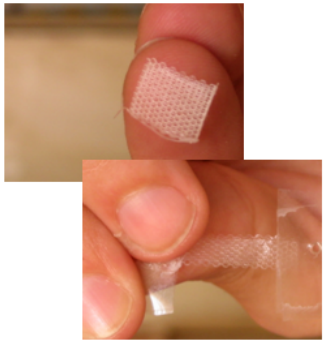
Vozzi, Previti, De Rossi,. Tissue Engineering, 8, 34, 2002. Vozzi , Flaim, Ahluwalia, Bhatia , Biomaterials, 24, 2533, 2003

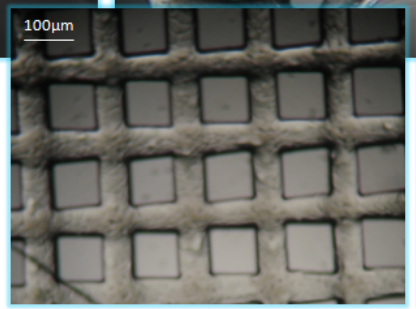


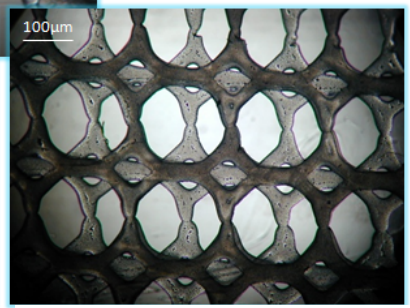
Scaffolds with PAM

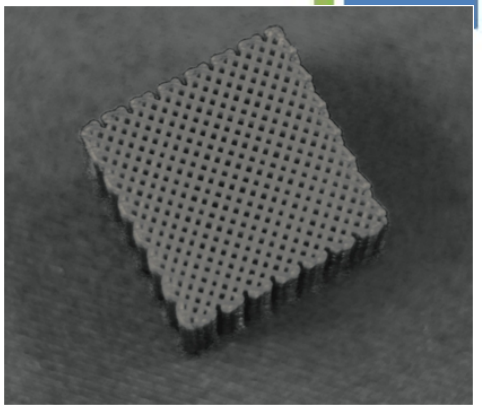


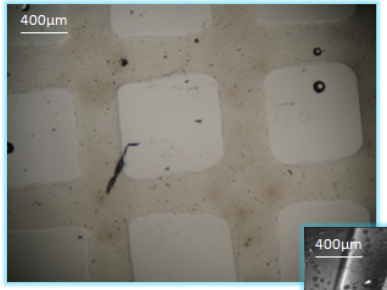


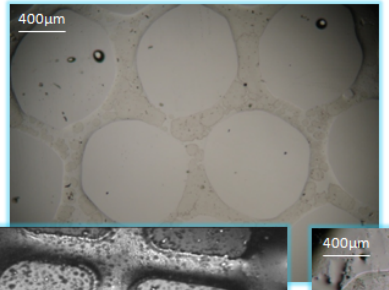


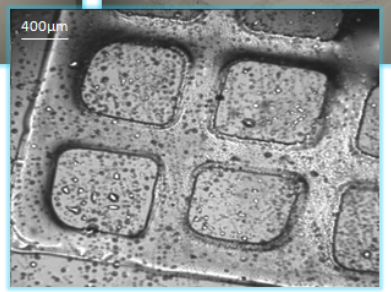


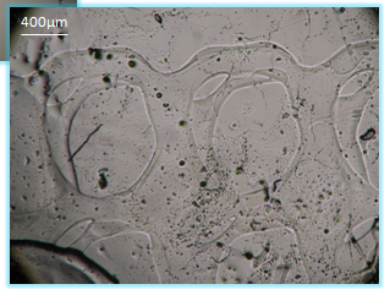






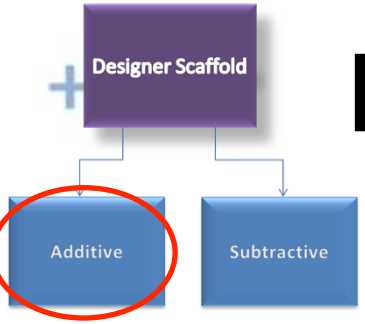






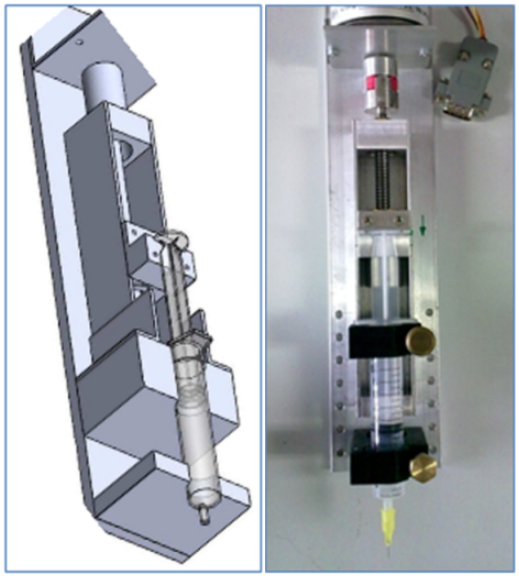
Materials?
Speed?
Price?
Fidelity?

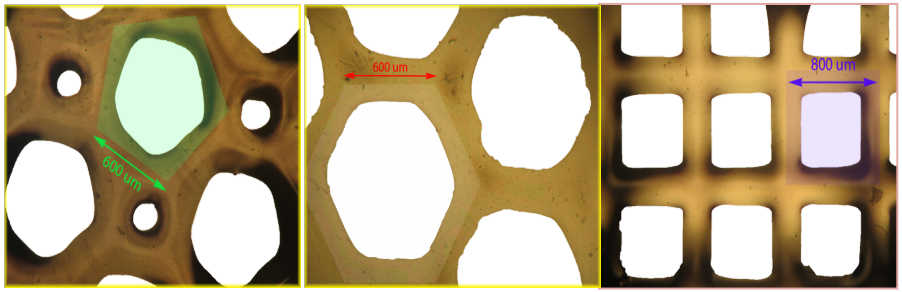
Vozzi et al, JBMRA, 71A, 326, 2004.
Mariani et al., Tissue Eng. 12, 547, 2006.
Bianchi et. Al. JBMR 81, 462, 2007.

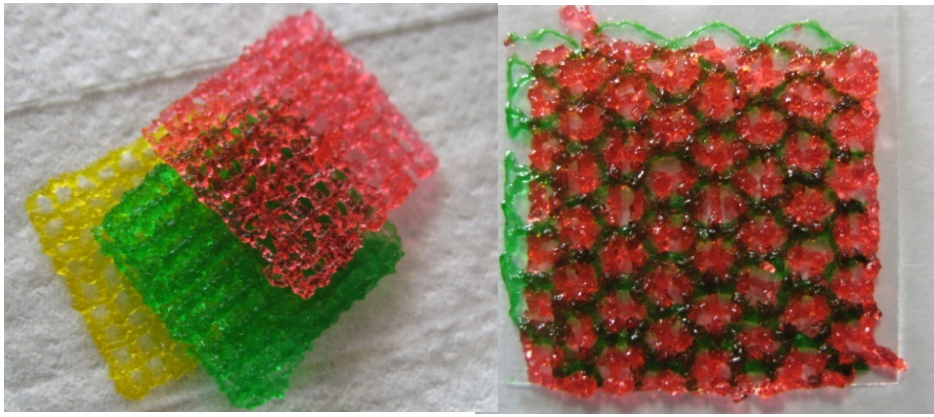


Piston Assisted Microsyringe (PAM2)

Plunger driven

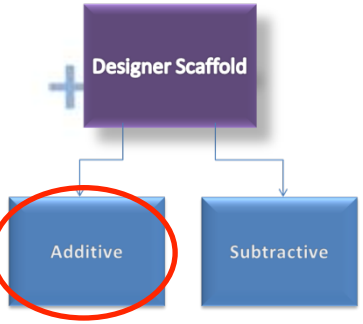






Materials?
Speed?
Price?
Fidelity?

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),



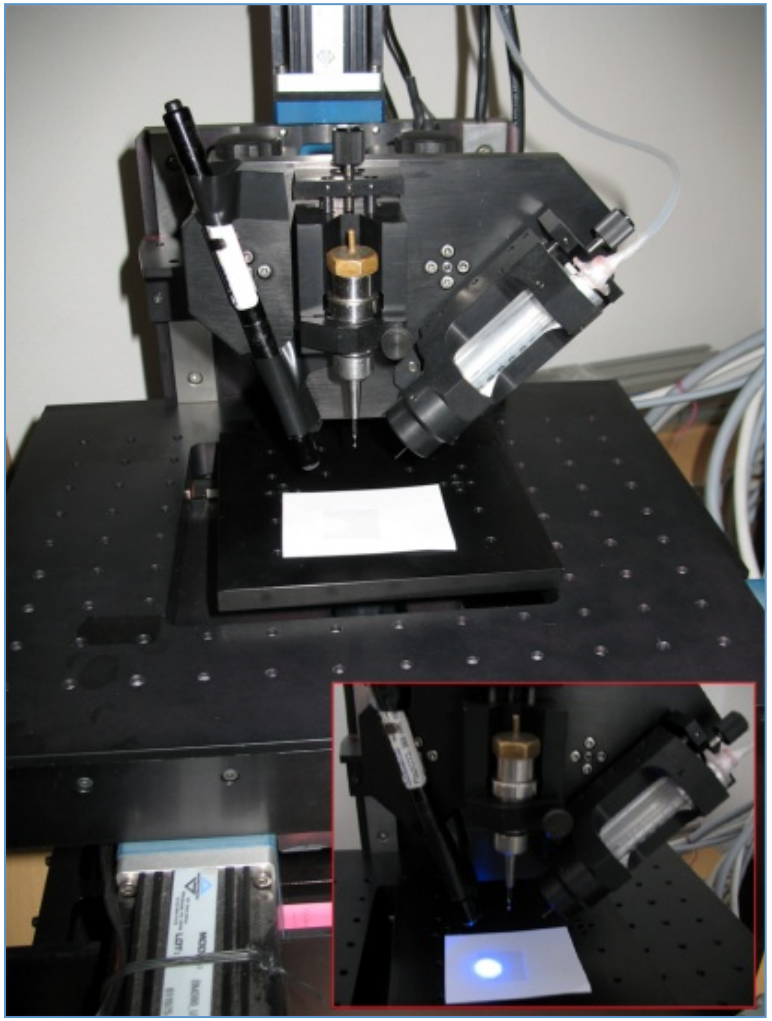
The PAM2 system

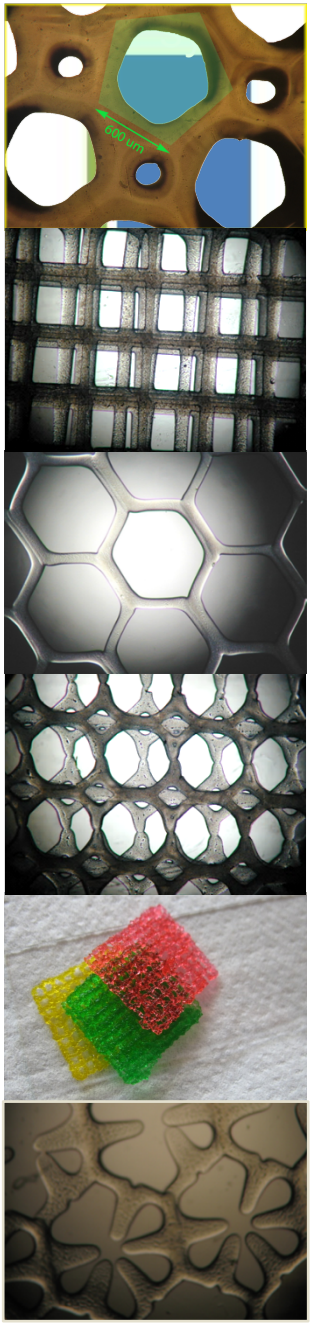
Robotic 3 axis micropositioner.

- ✓ PAM
- ✓ PAM2
- ✓ Diode laser
- ✓ Temperature control
- ✓ PAM^2 software

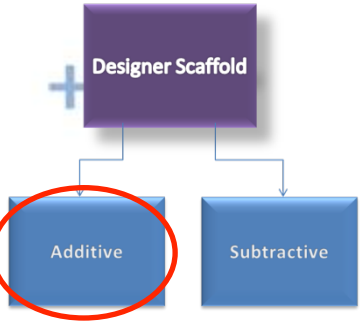
- 4 Position controlled brushless motors (resolution of 10 µm ± 1 µm)
- Working space 100×100×80 mm
- Working velocity 1-15 mm $\cdot s^{-1}$
- Design of *z*-stage to locate several modules

Materials?
Speed?
Price?
Fidelity?



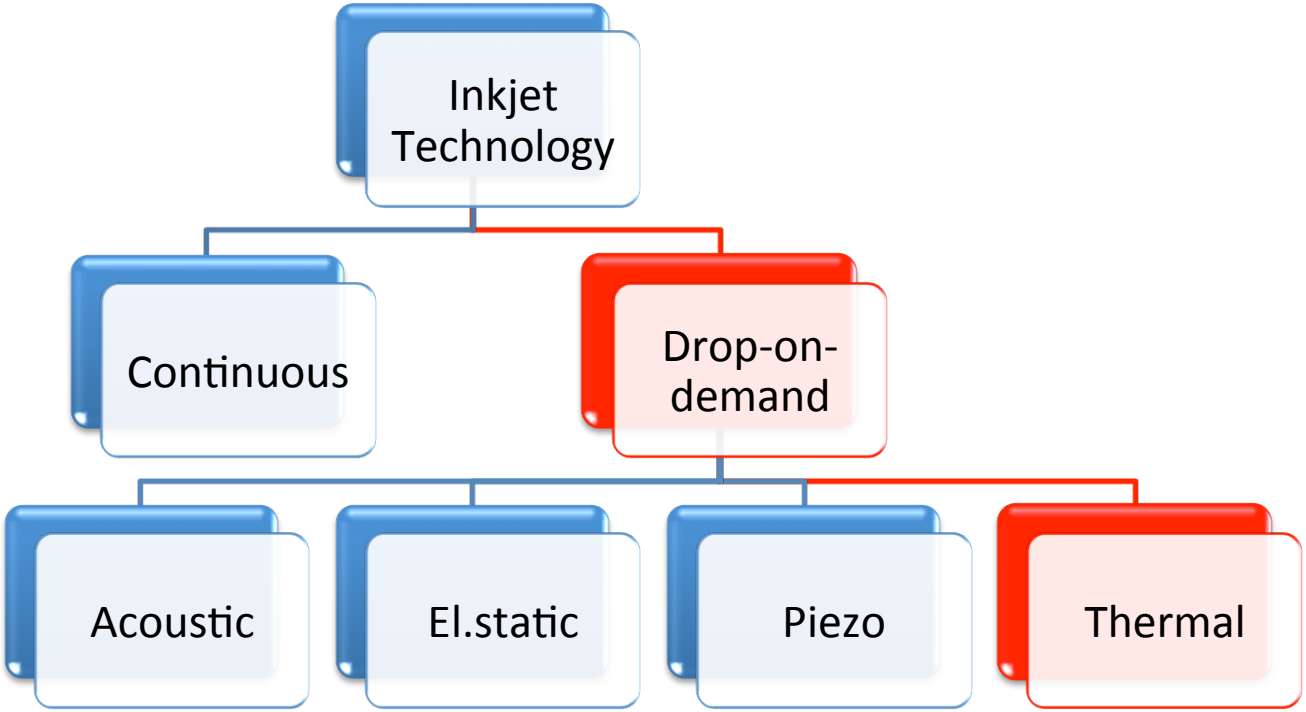


Tirella, De Maria, Vozzi, Ahluwalia Rapid Prot. J (2012);

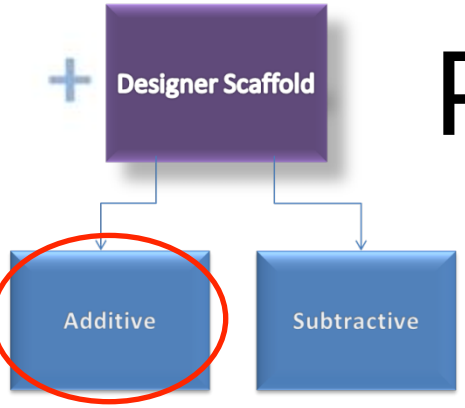


Inkjet Printing

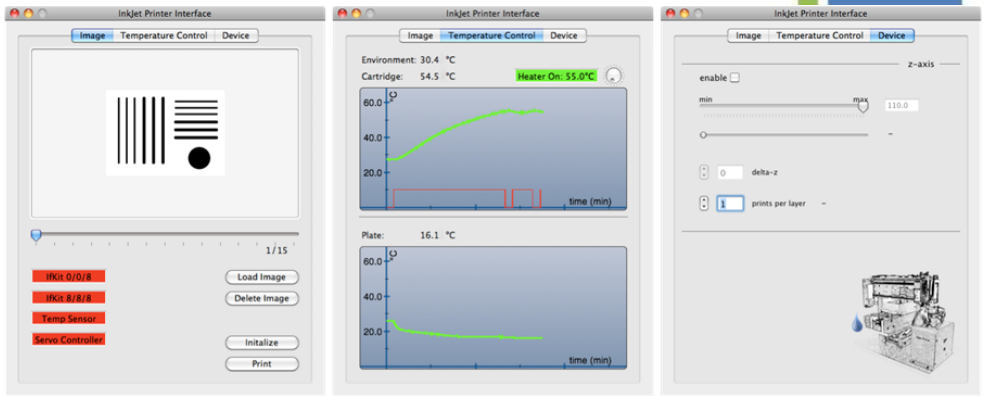
Inkjet technology is a ***contact free dot matrix printing*** procedure. Ink is issued from a small aperture directly onto a specific position on a substrate

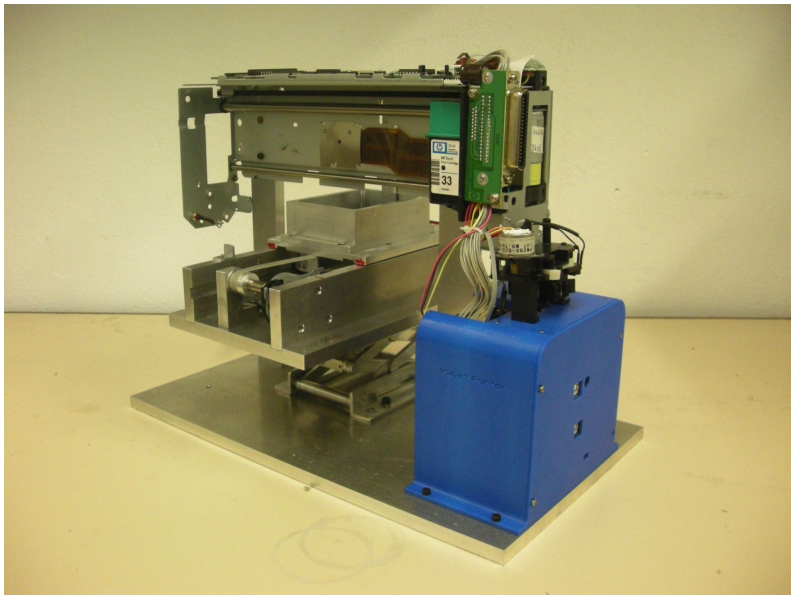


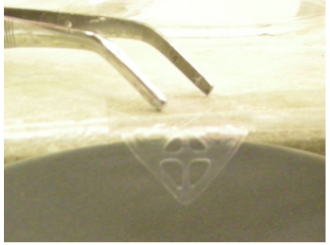
Materials?
Speed?
Price?
Fidelity?

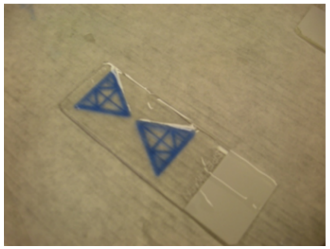


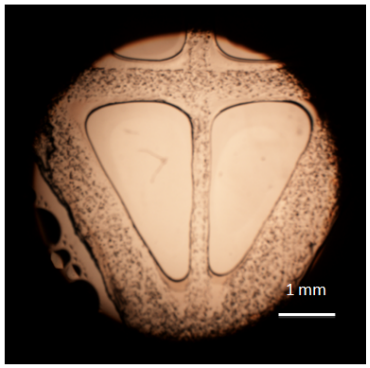
Penelope Ink-Jet printer







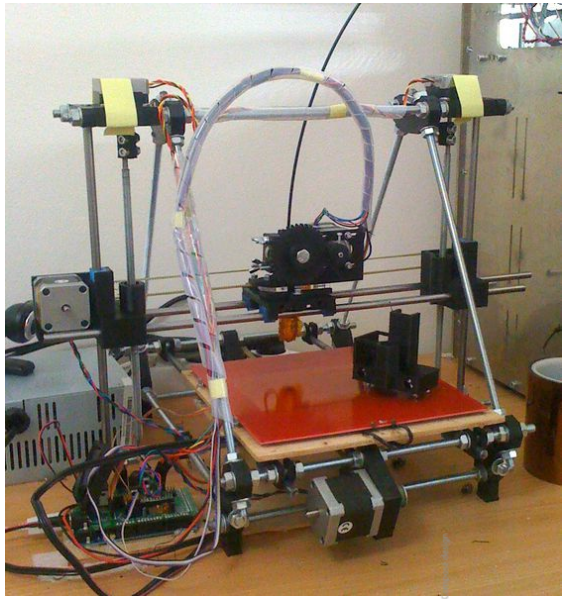


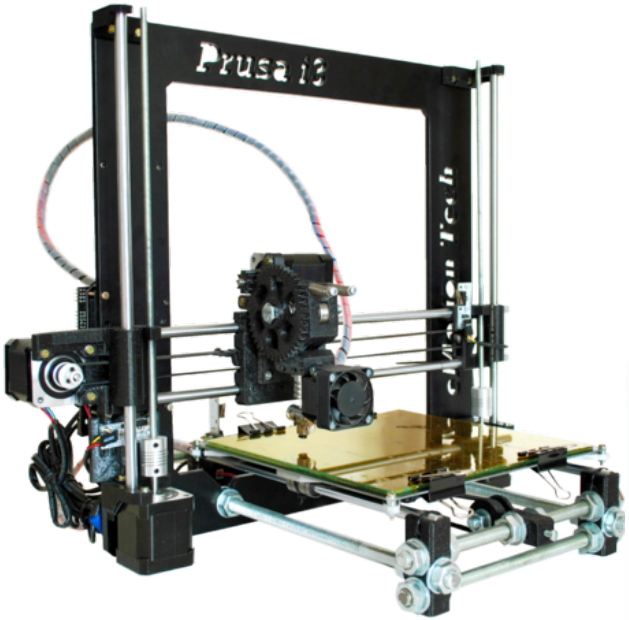


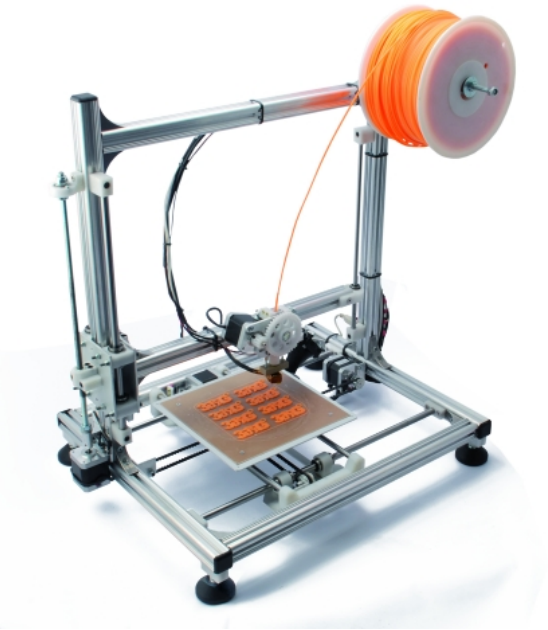
Materials?
Speed?
Price?
Fidelity?

Open source FDM machine: RepRap Project

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

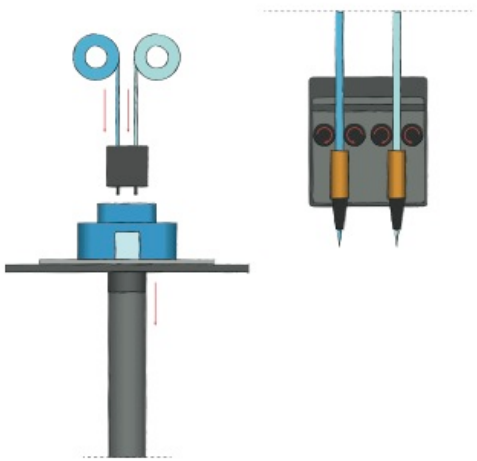


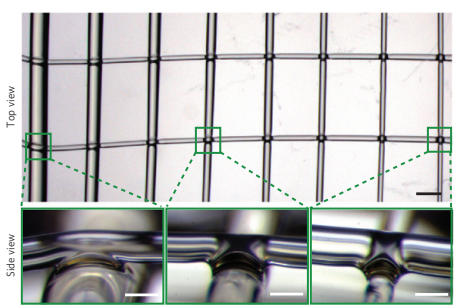


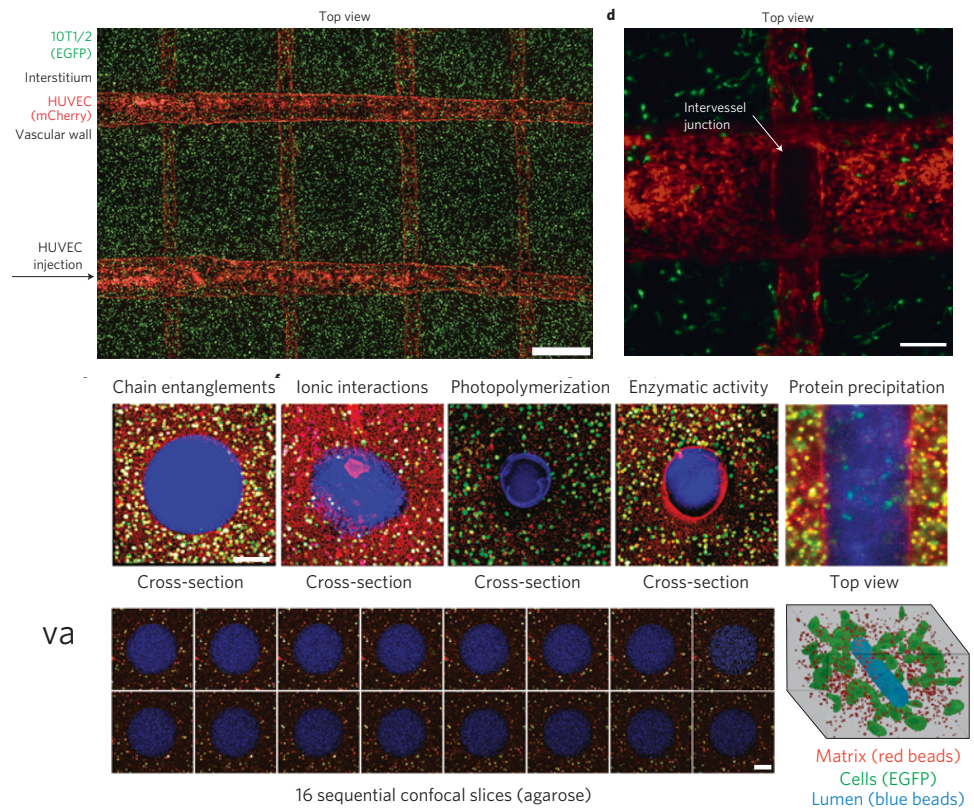


iRP using Open Source FDM

Extruded material	Casted Materials	Extraction method	Resolution (µm)
Carbohydrate-glass (with a subsequent coating with PDLGA)	Agarose, Alginate, PEG, Fibrin, Matrigel	Water dissolution	500

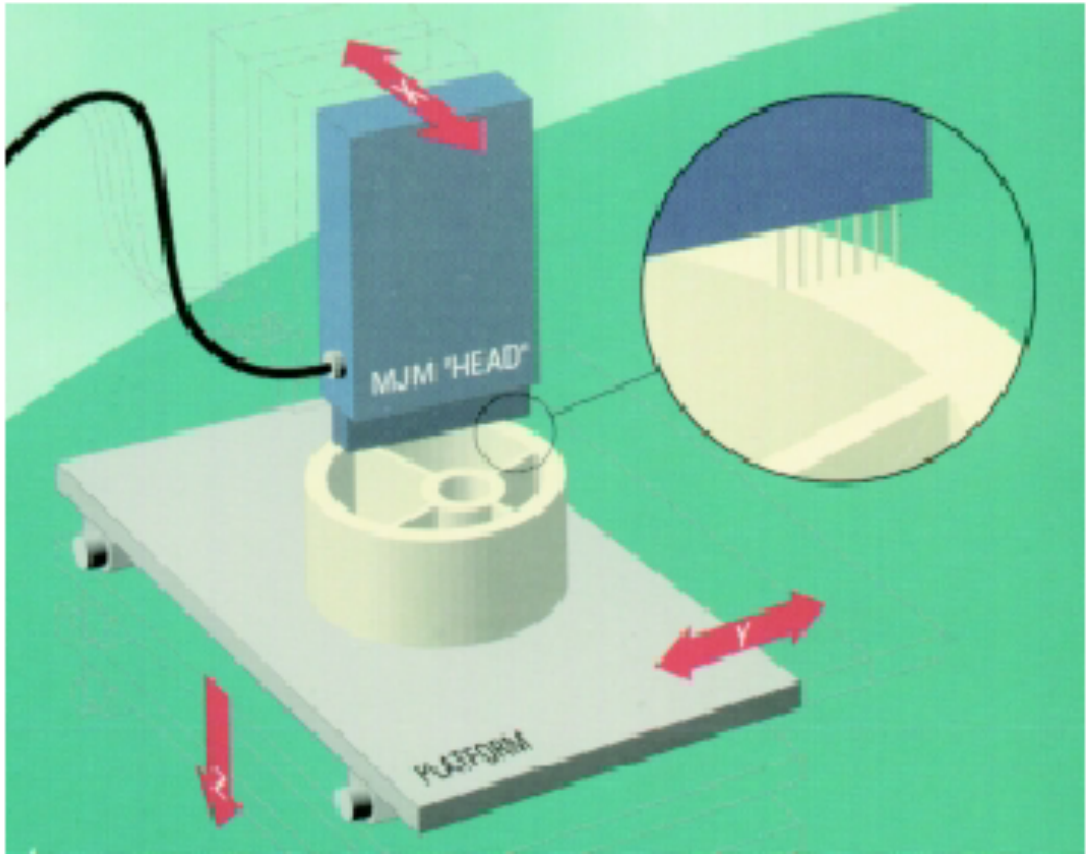




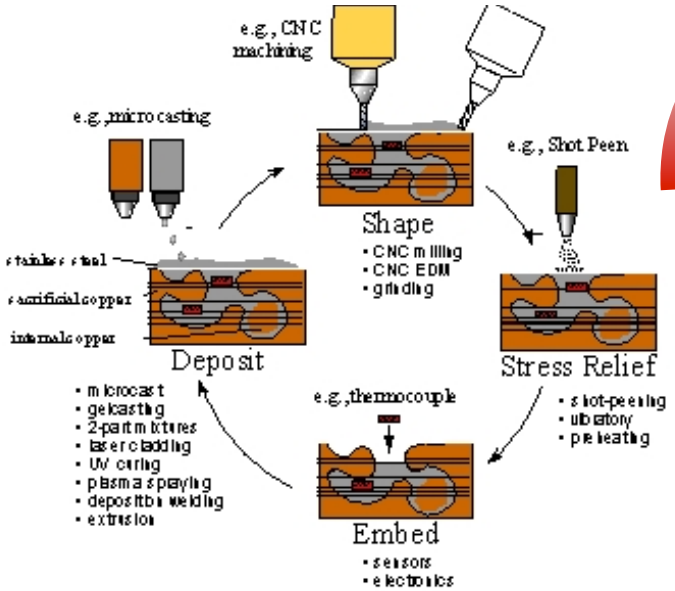


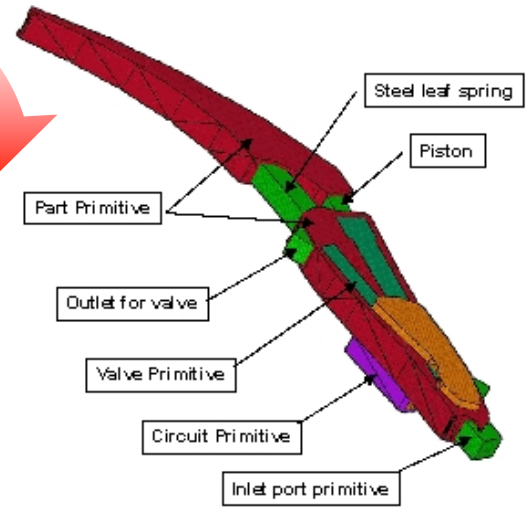
JS Miller et al, Nature Materials Letters: Published online 1 July 2012

3D System Multi-jet Modeling

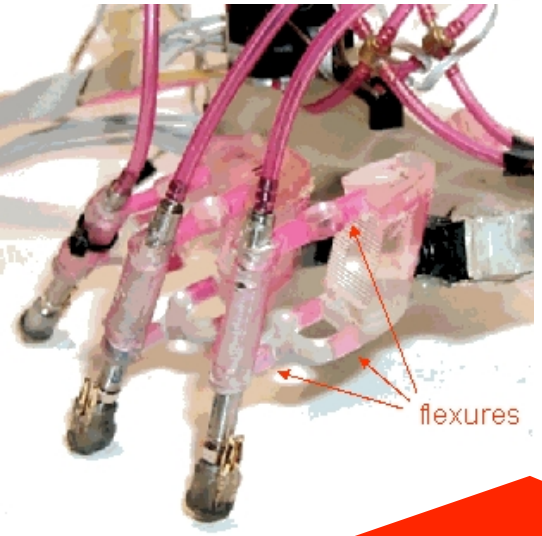


Shape Deposition Manufacturing

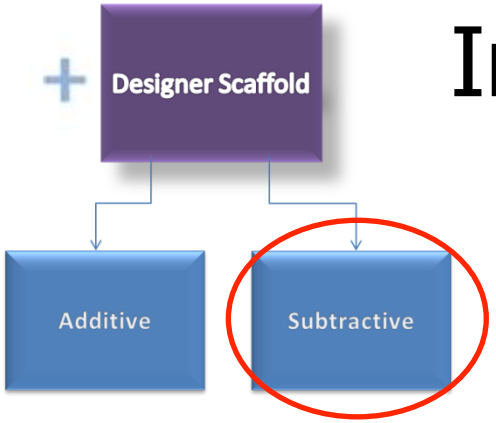




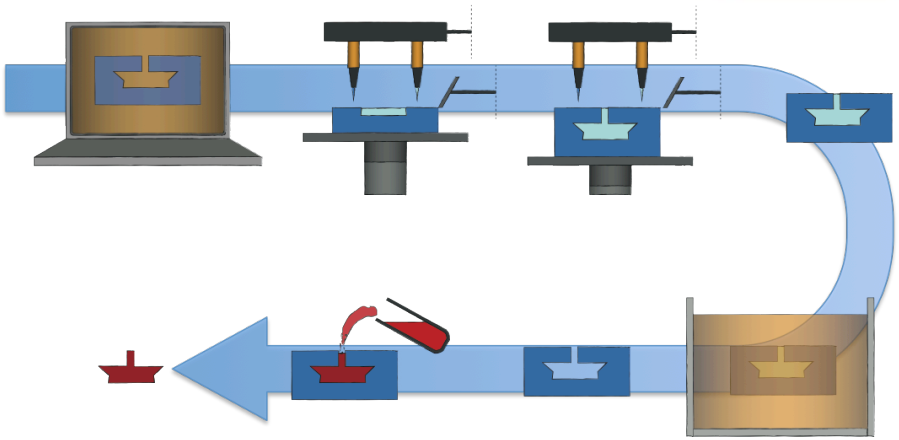
Designer composes the design from library of primitives, including embedded components



embedding sensors and actuators



Indirect Rapid Prototyping (iRP)



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

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

Advantages?
Limitations?

Materials?
Speed?
Price?
Fidelity?