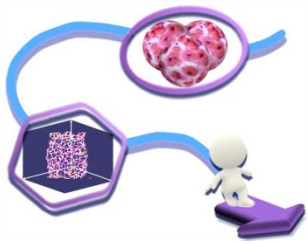
LO SCAFFOLD

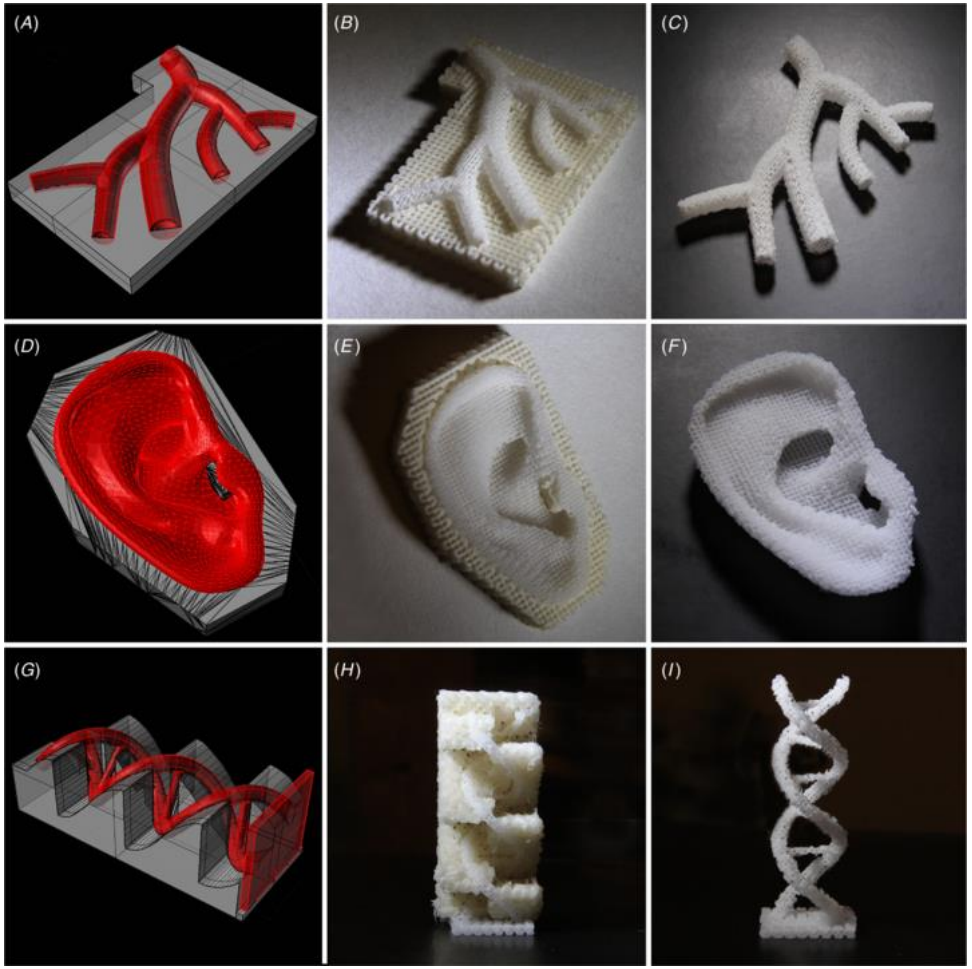
Outline

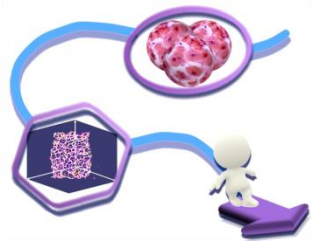
- Scaffold *definition*
- Scaffold *requirements*
- History of scaffold fabrication
- New approaches in scaffold design: Bioprinting, Nano-in-Micro
- Scaffold *characterisation*



What is a scaffold?

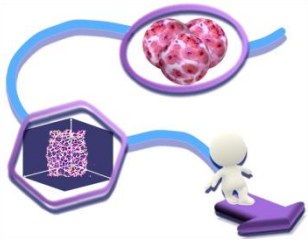
A 3D structure which supports 3D tissue growth



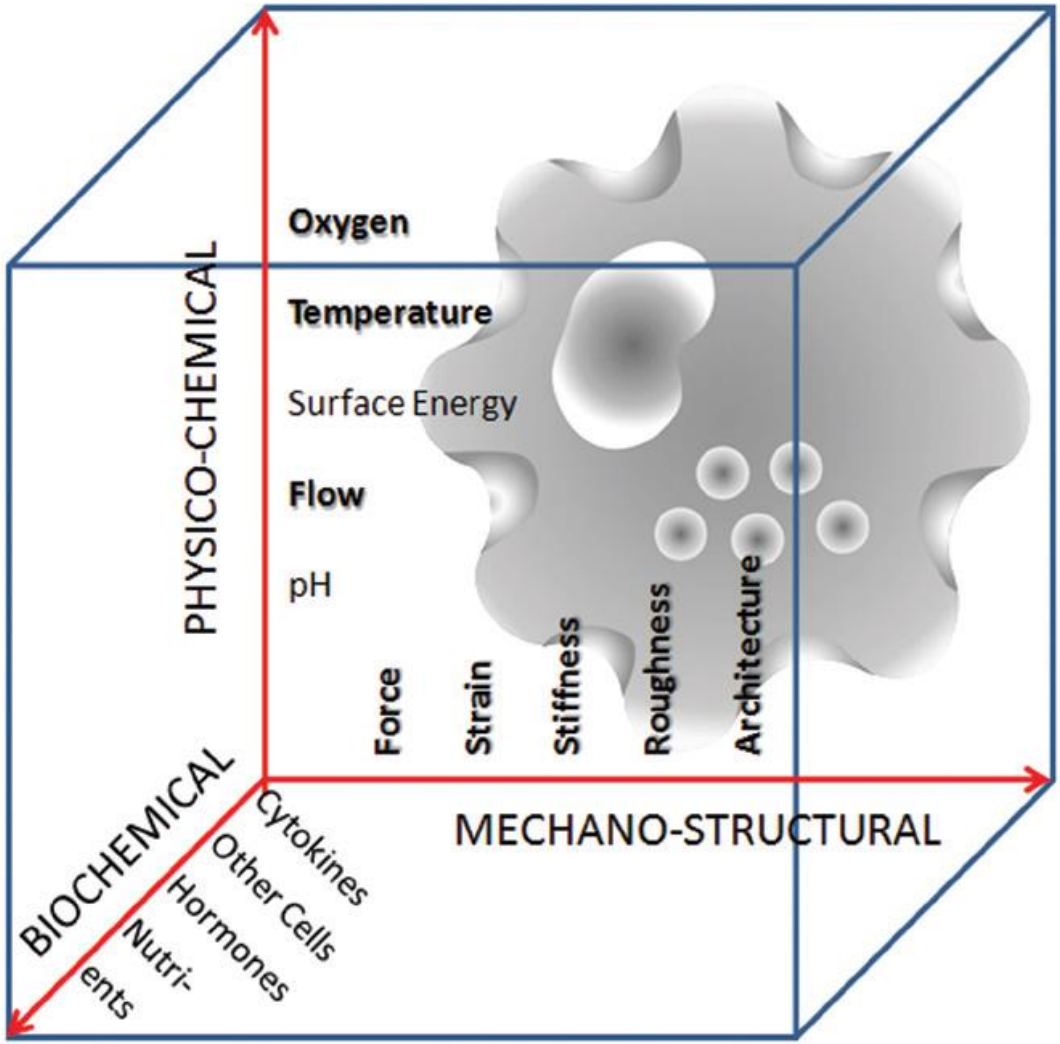


What are the features of an ideal scaffold?

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



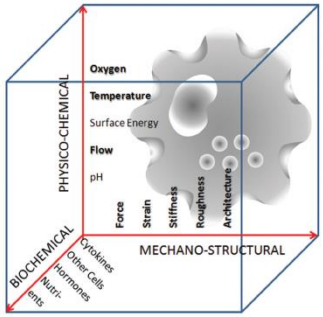
Stimuli- the tripartite axis



Engineering Quasi-Vivo In Vitro Organ Models. Sbrana & Ahluwalia. Methods Adv Exp Med Biol. 2012;745:138-53.

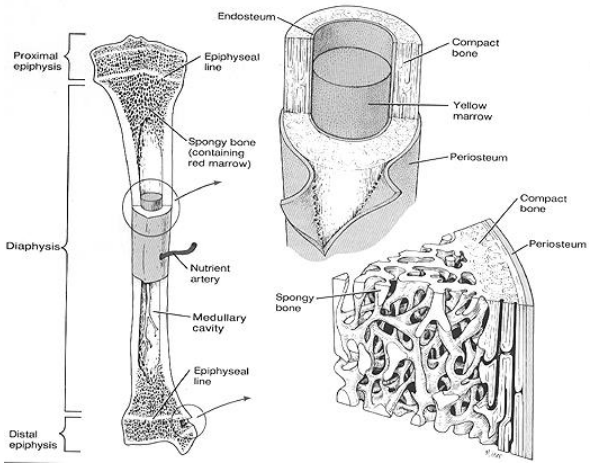
Extracellular matrix features

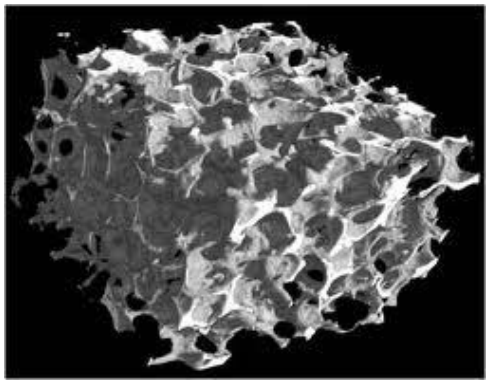
- High degree of porosity
- Appropriate pore size
- High surface to volume ratio
- High degree of pore interconnectivity
- Biochemical factors & ECM features able to guide cell function

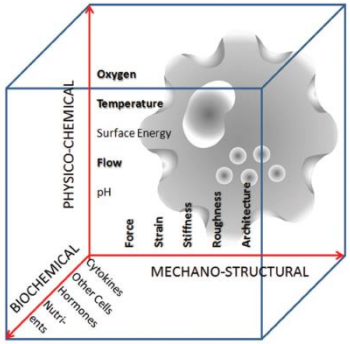


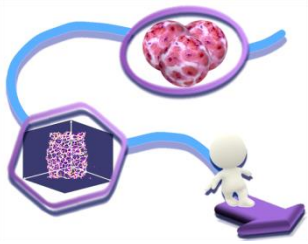
We need a bottom–up approach

Mechano-structural stimulii

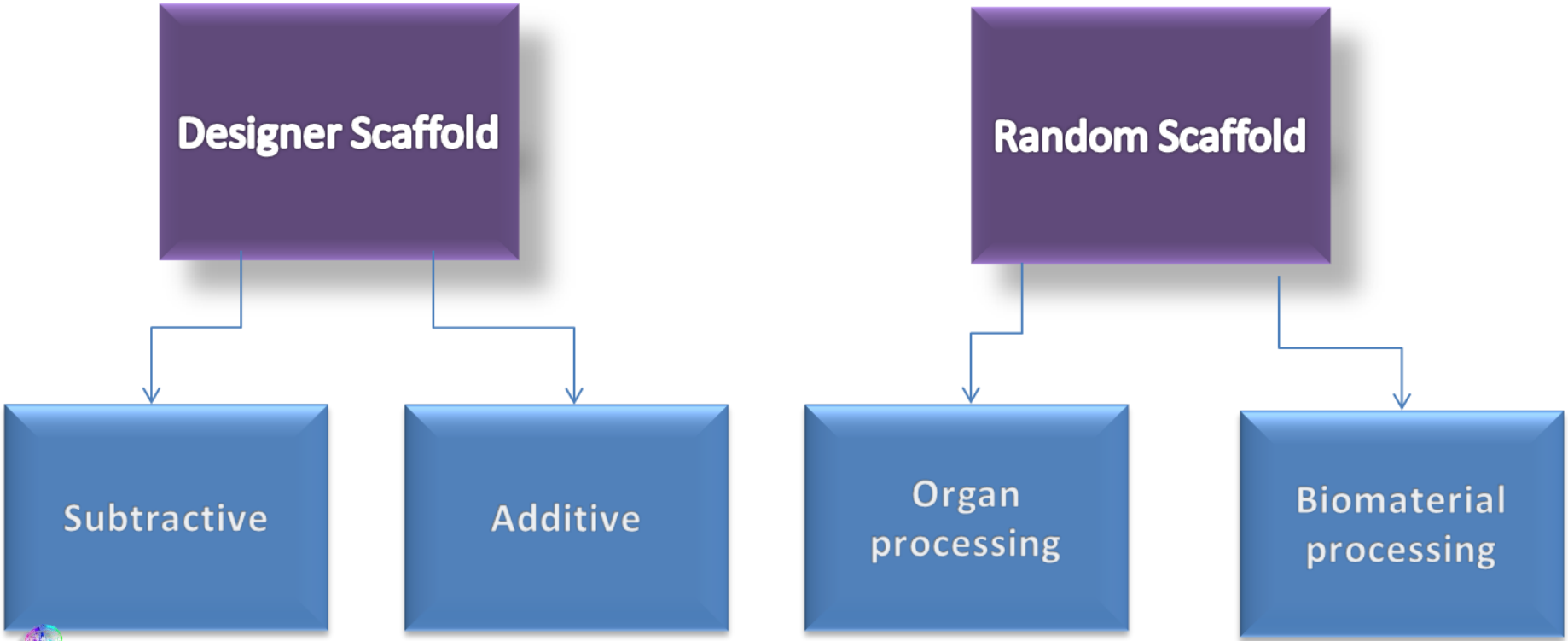


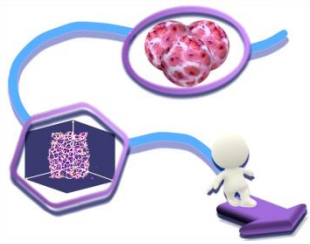






Methods for generating MS stimuli in scaffolds

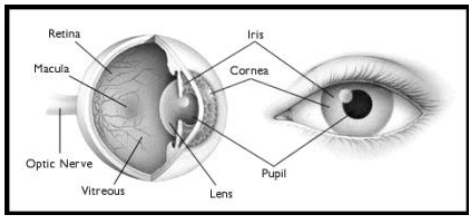


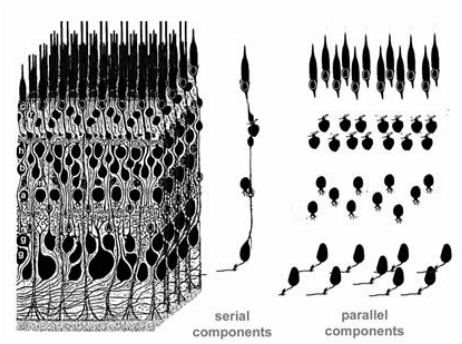


Designer or Random?

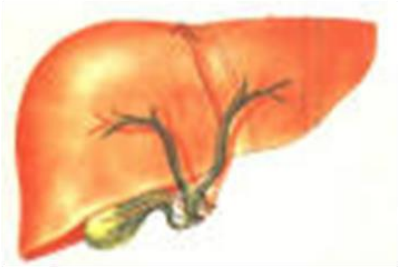
Structure ⇄ **Function**

Retina

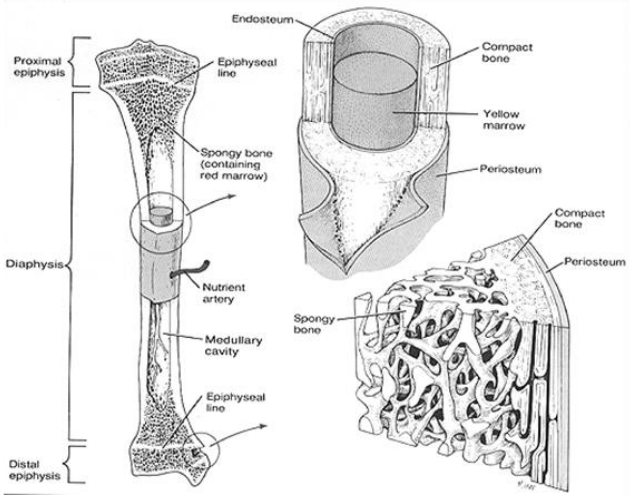




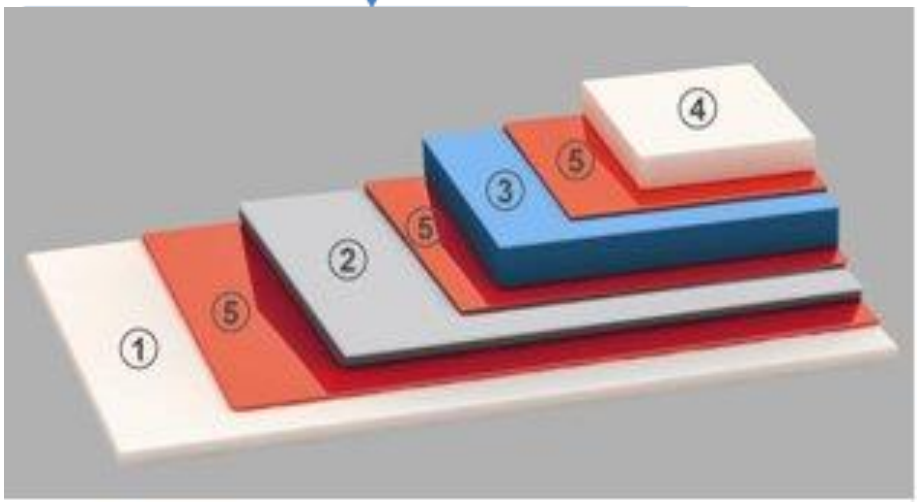
Liver

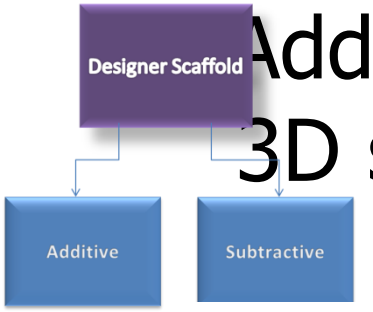


Bone

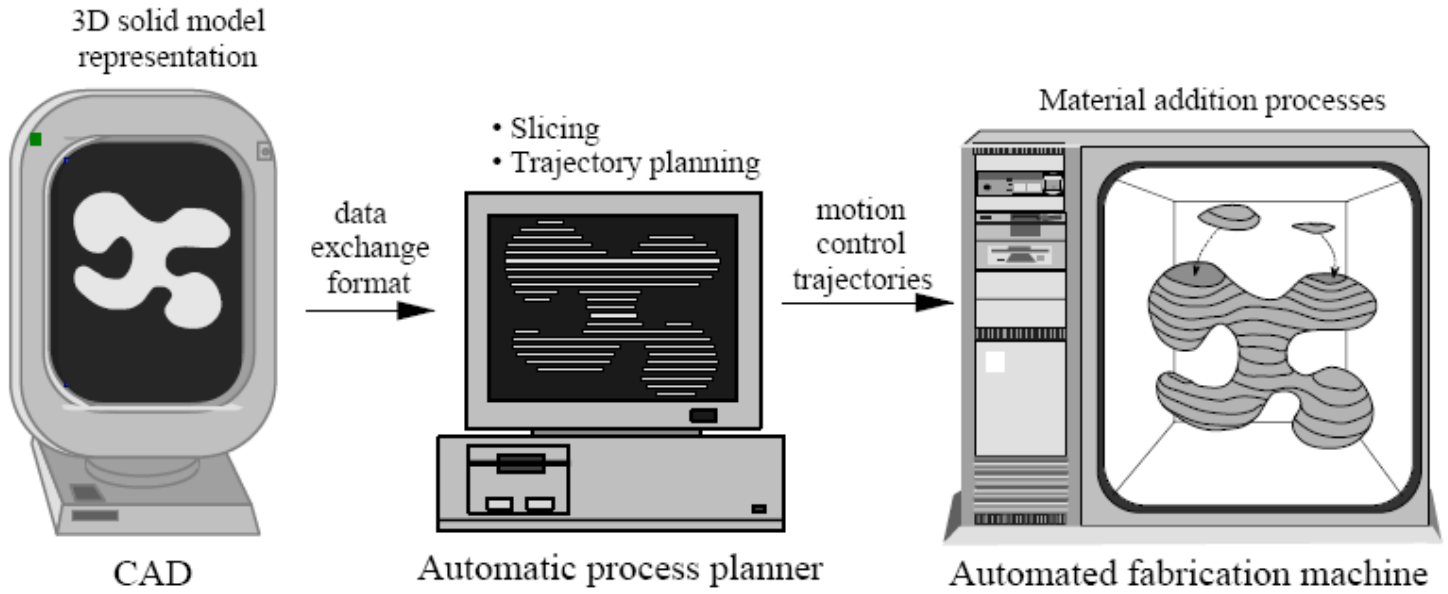


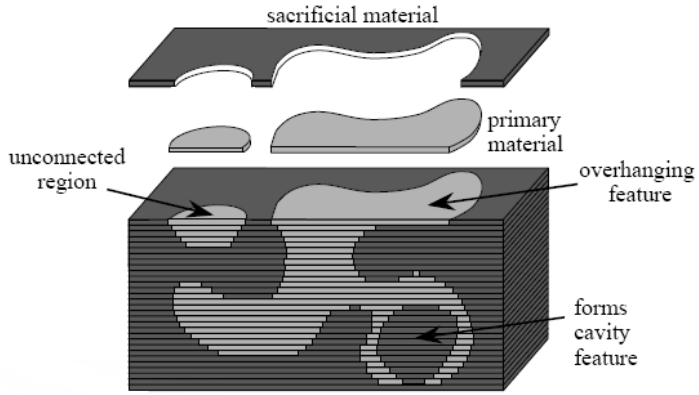
Designer Scaffold



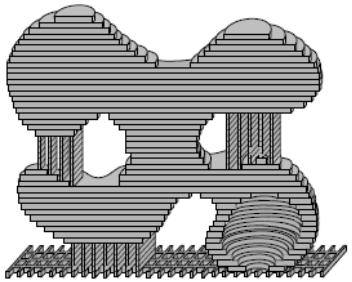


Additive = rapid prototyping (from object to 3D scan to slicing to layer by layer printing)

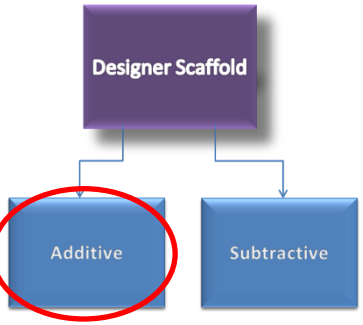




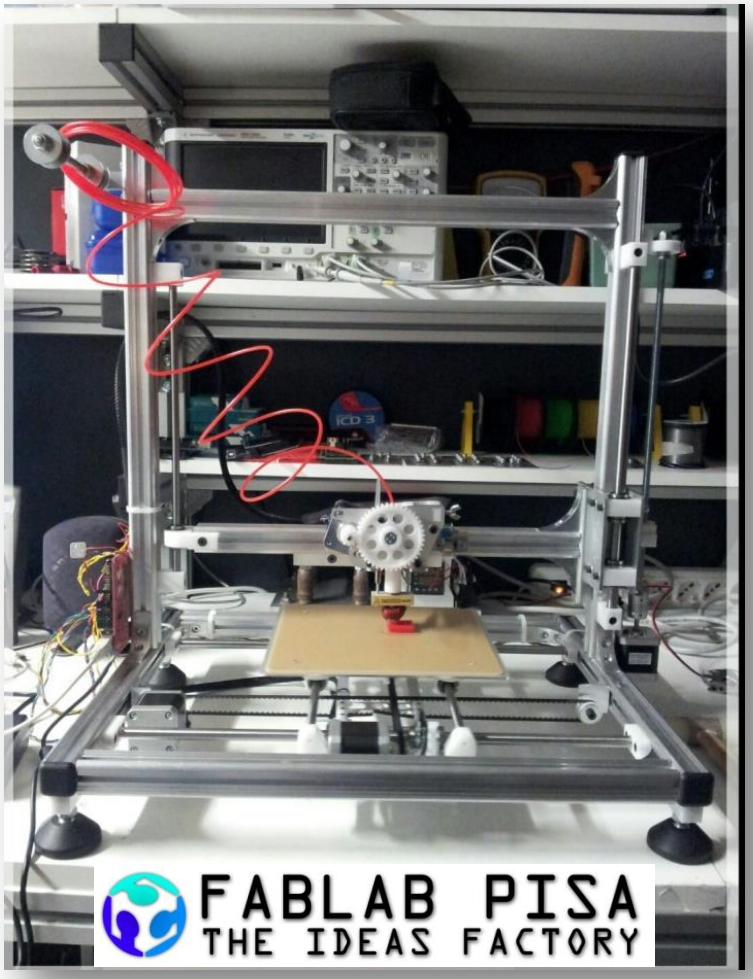
a. Complementary support.

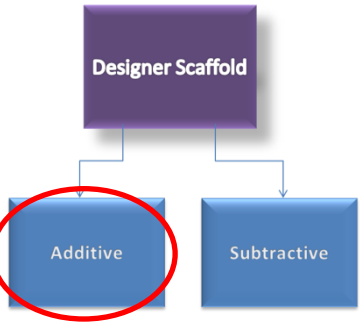


b. Explicit support.



3D Printing/Digital Fabrication & RP

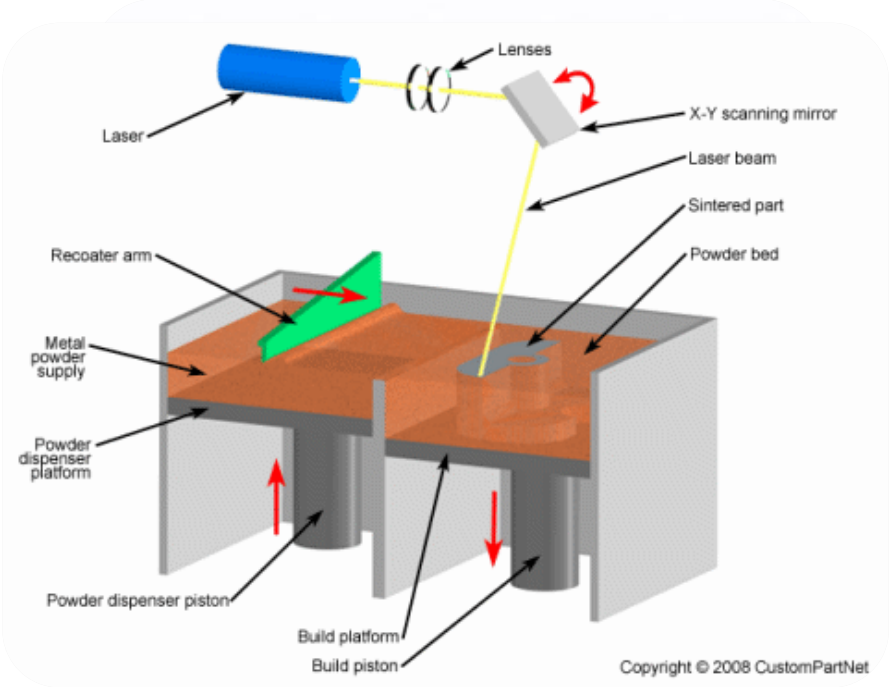




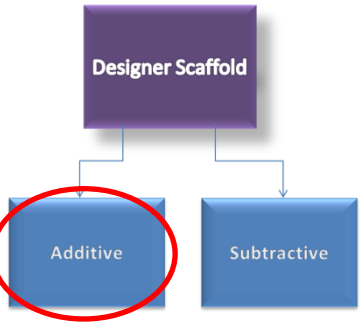
Designer Scaffold

Three main groups:

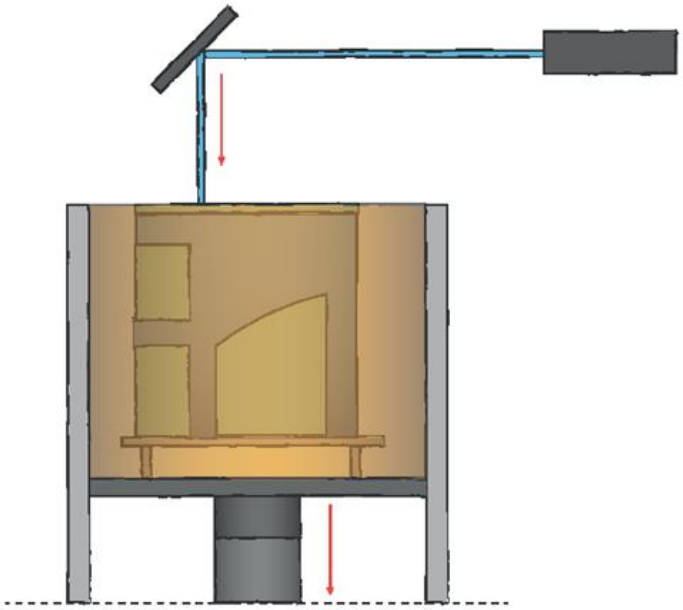
- laser systems
- nozzle based systems
- direct writing systems

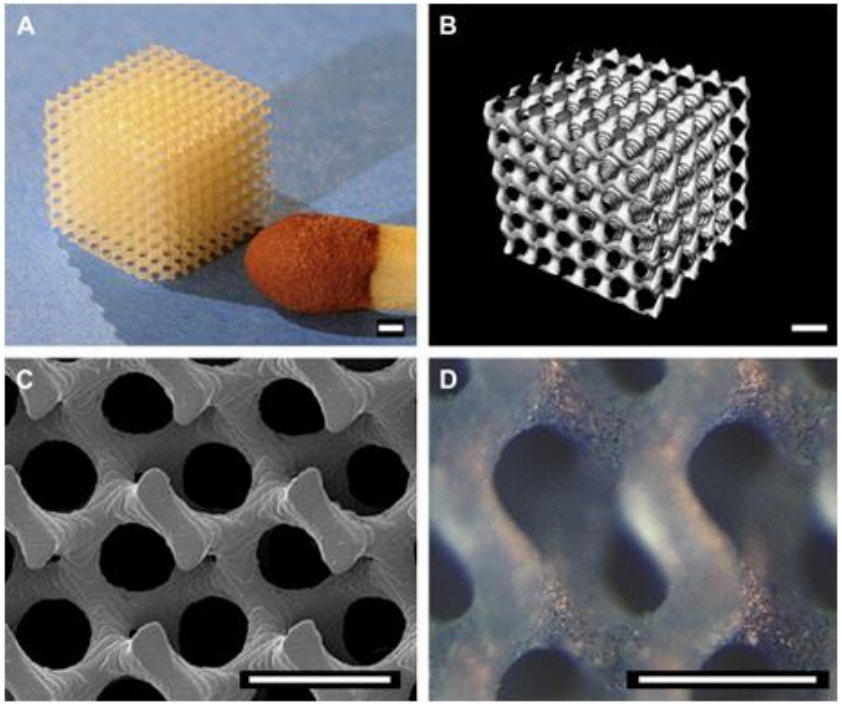


Materials?
Speed?
Price?
Fidelity?



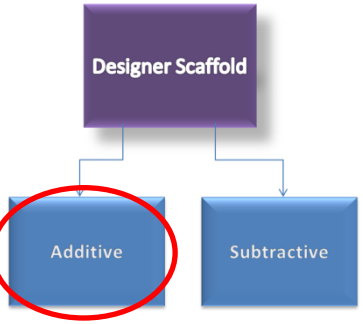
Stereolithography



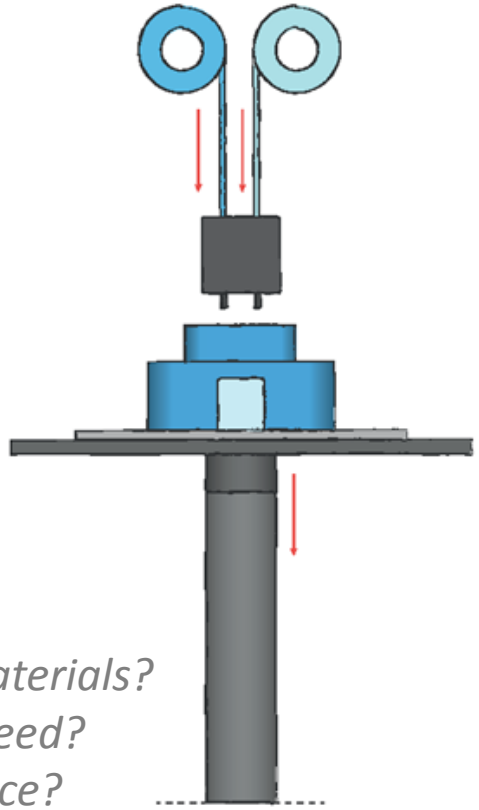


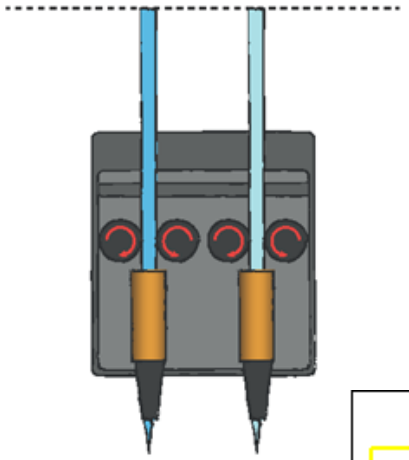
Materials?
Speed?
Price?
Fidelity?

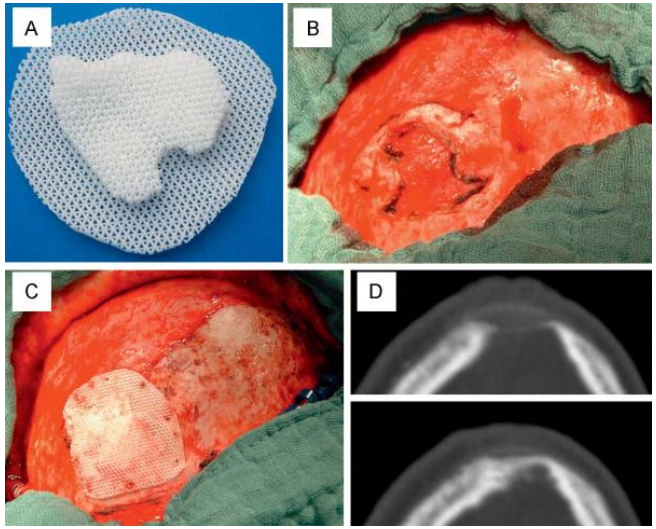
Laser for polymerisation of liquid monomer or resin



Fused Deposition Modeling







Materials?
Speed?
Price?
Fidelity?

Hutmacher & coworkers

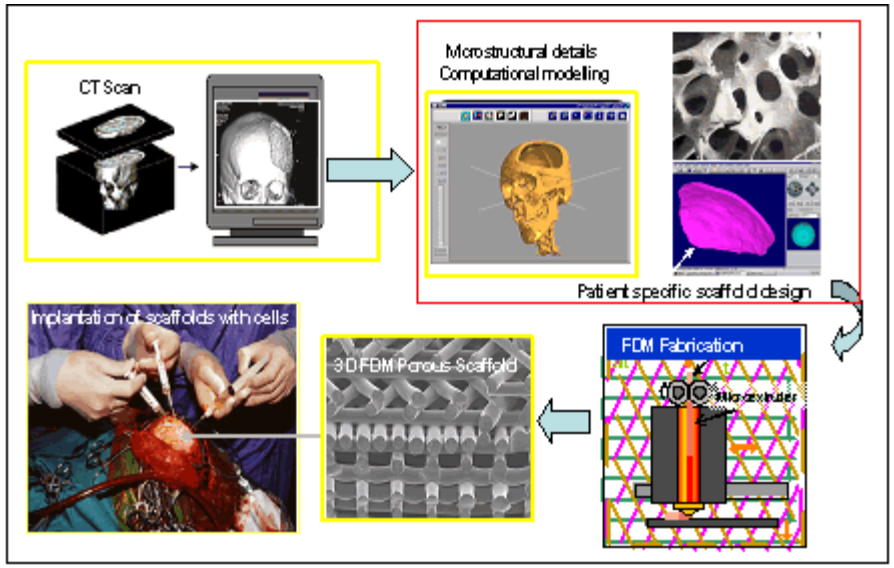
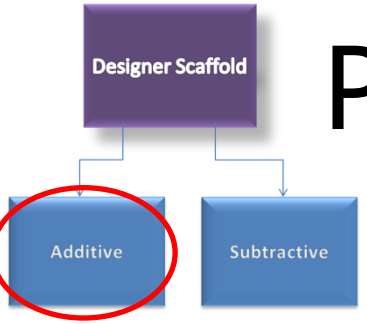
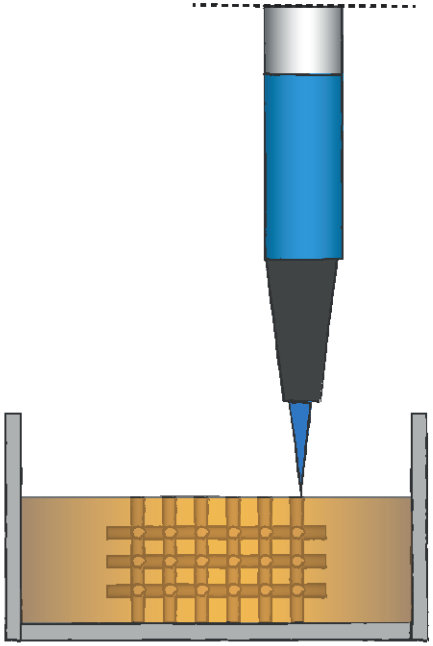


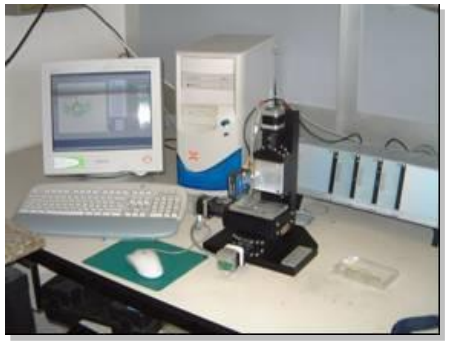
Figure 1: Platform technology for patient specific scaffolds TE.



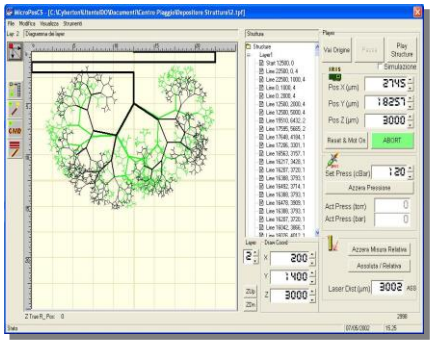
Pressure Assisted Microsyringe (PAM)

Regulated air flow

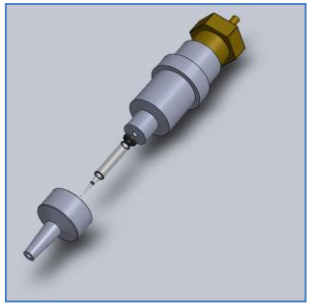




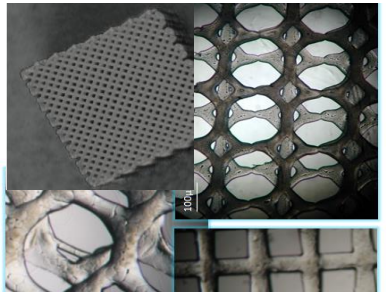
PAM system



Software



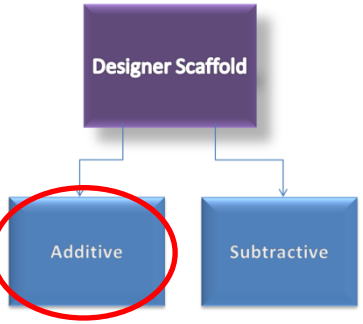
Syringe design



Software

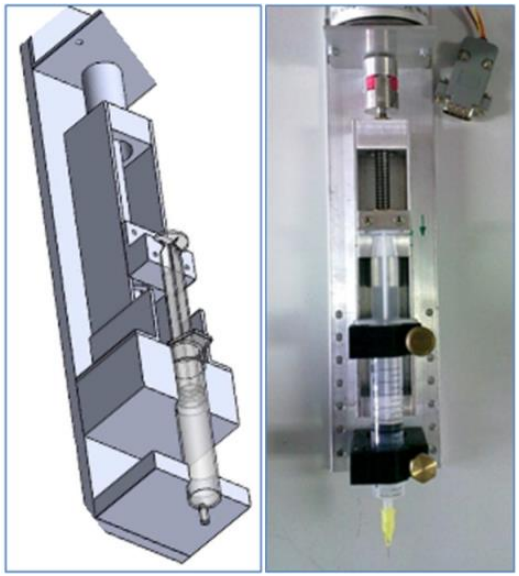
Materials?
Speed?
Price?
Fidelity?

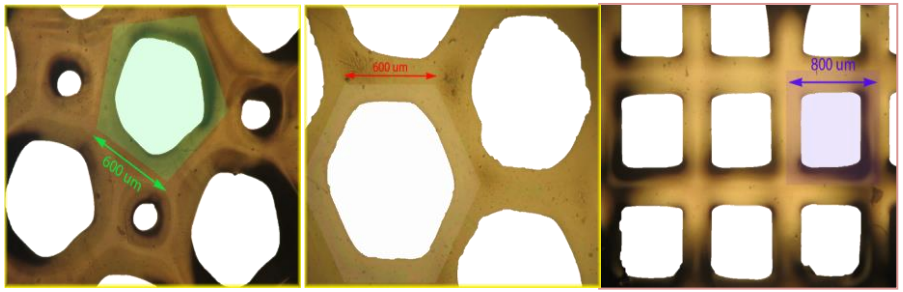
Vozzi et al,. Tissue Engineering, 8, 34, 2002. Vozzi et al , Biomaterials, 24, 2533, 2003, Vozzi et al, JBMRA, 71A, 326, 2004. Mariani et al., Tissue Eng. 12, 547, 2006. Bianchi et. Al. JBMR 81, 462, 2007.

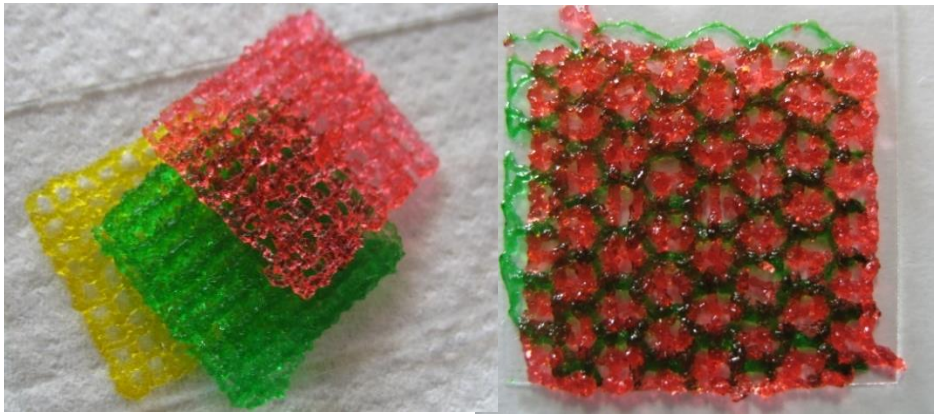


Piston Assisted Microsyringe (PAM2)

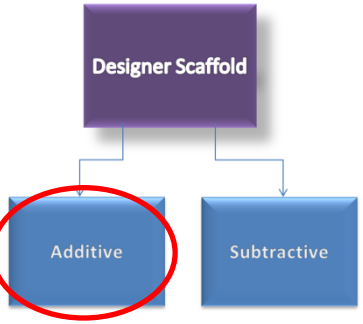
Plunger driven







Materials?
Speed?
Price?
Fidelity?



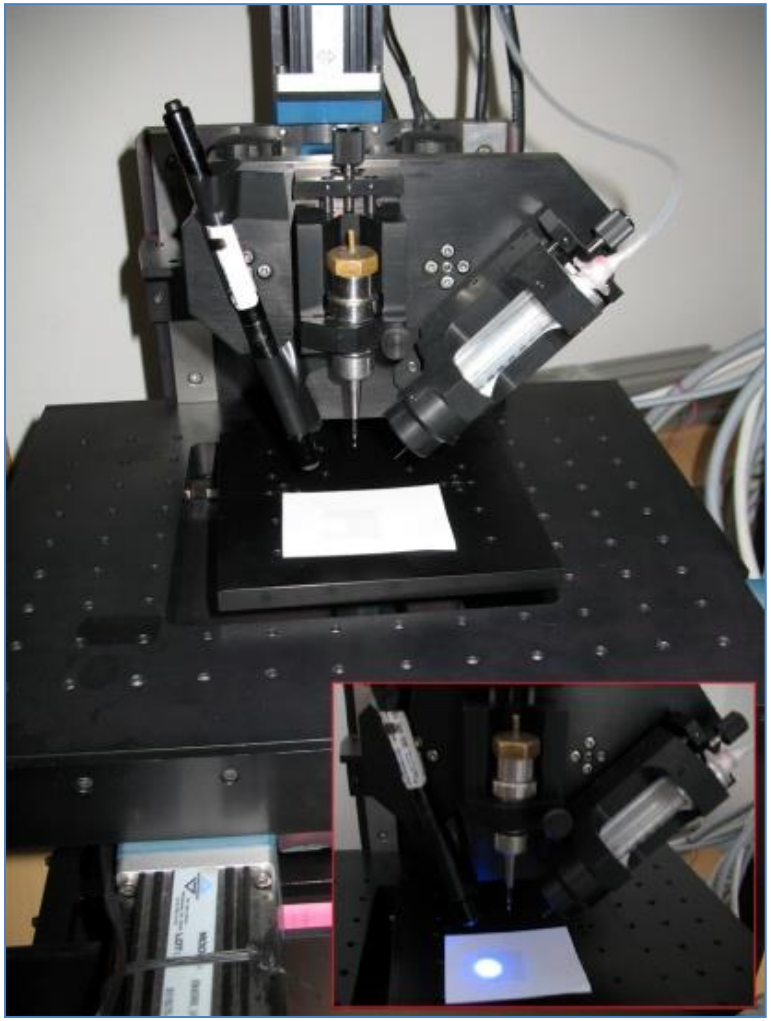
The PAM2 system

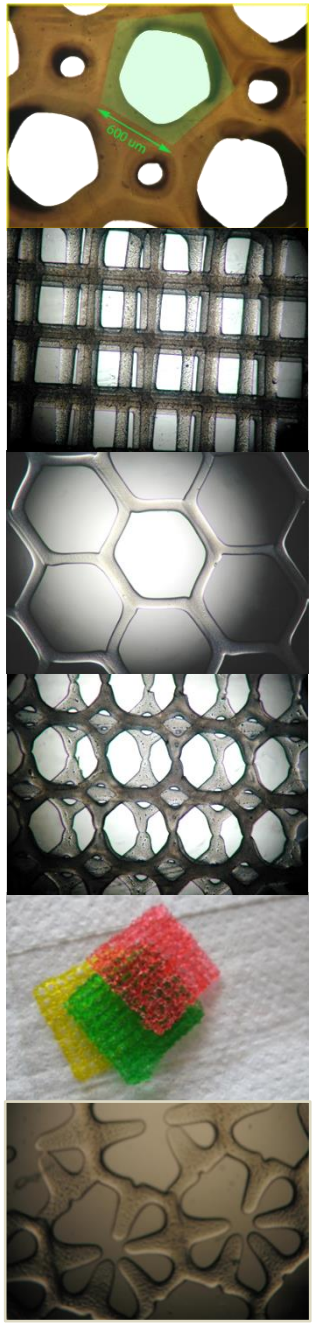
Robotic 3 axis micropositioner.

- ✓ 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

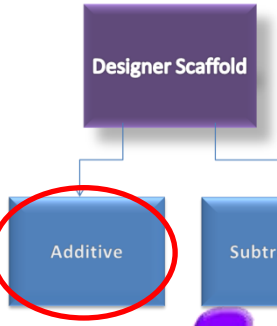
Materials?
Speed?
Price?
Fidelity?

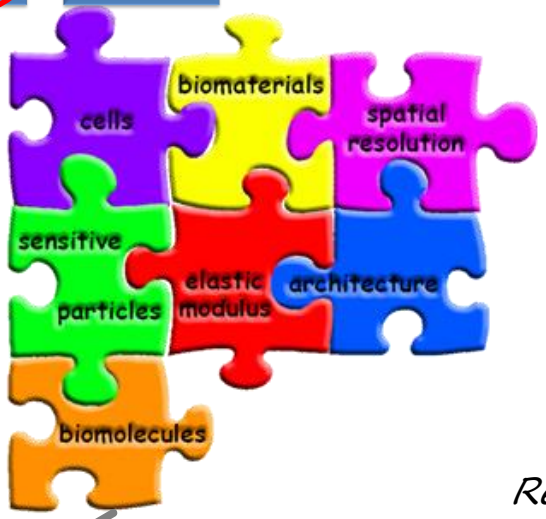


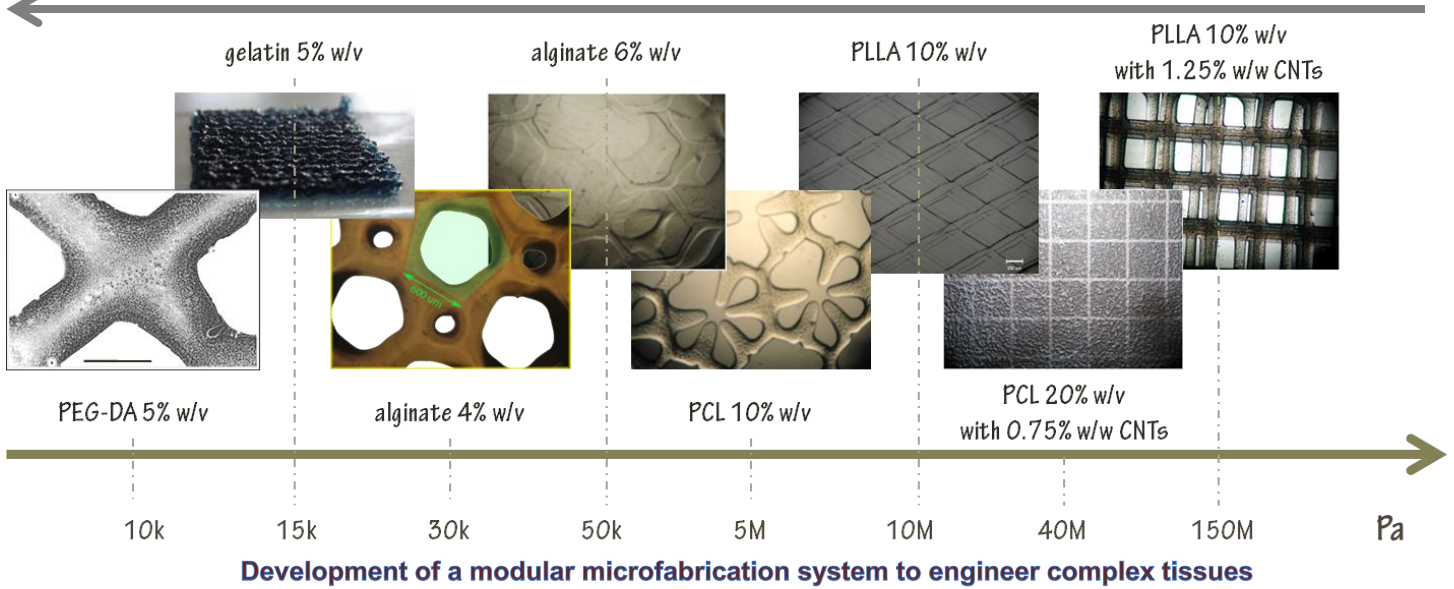


Tirella, De Maria, Vozzi, Ahluwalia Rapid Prot. J (2012);

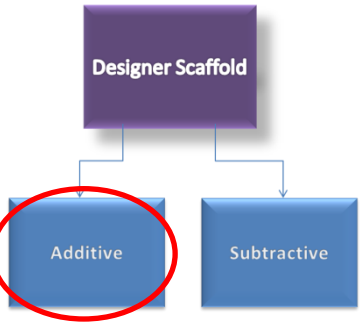
Smart-tunable modular scaffolds...





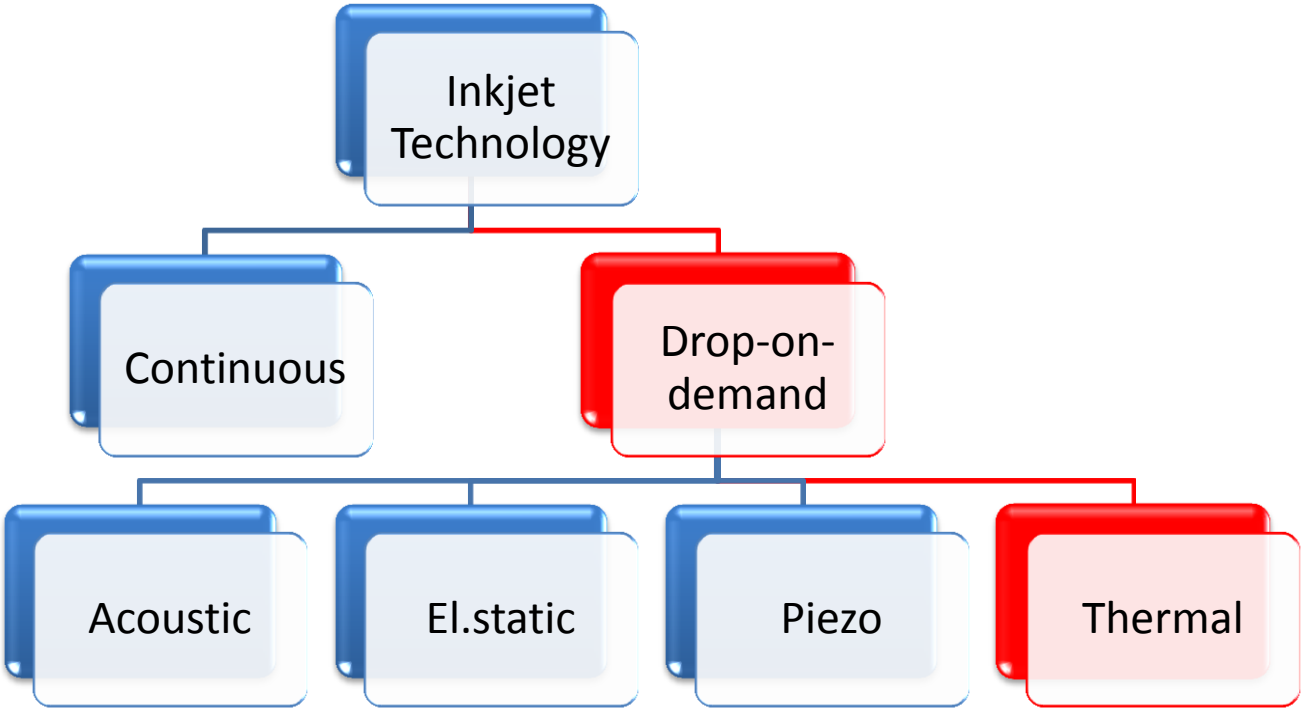


Development of a modular microfabrication system to engineer complex tissues

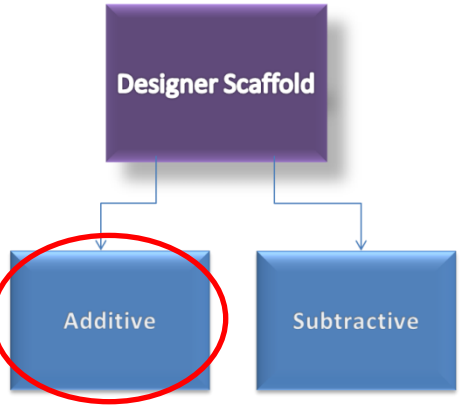


Inkjet Printing

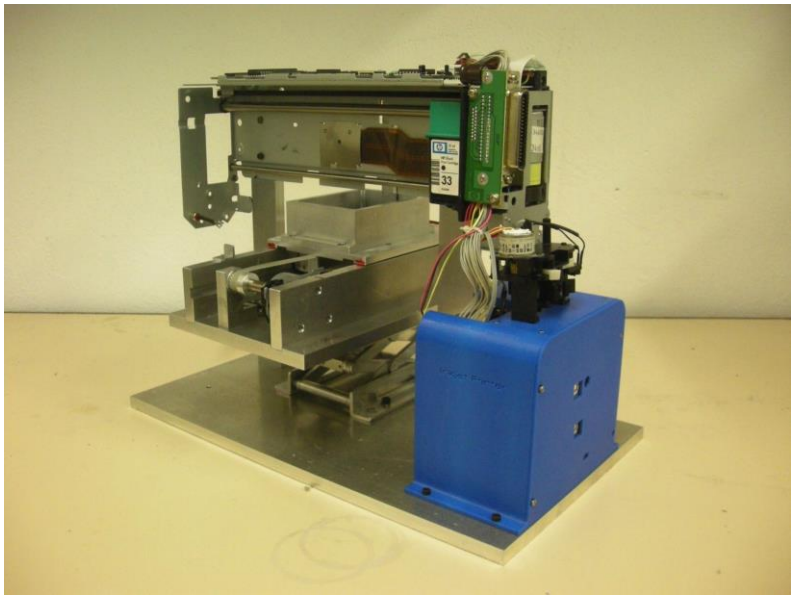
Inkjet technology is a ***contact free dot matrix printing*** procedure. Ink is issued from a small aperture directly onto a specific position on a substrate

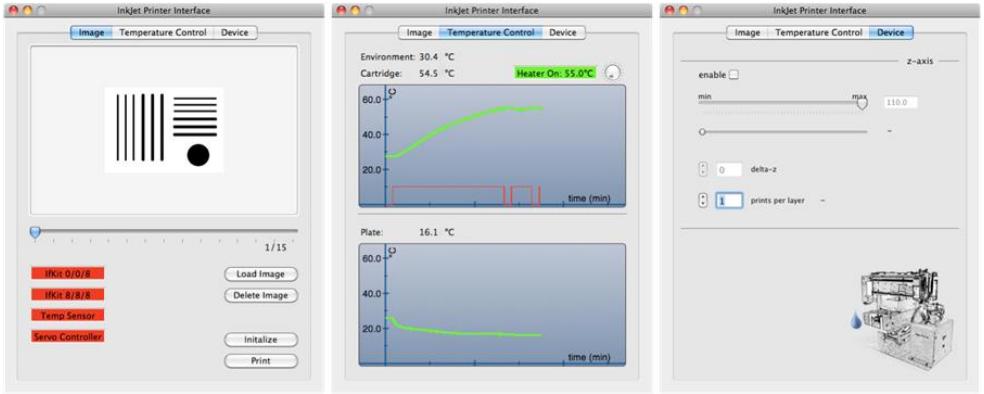


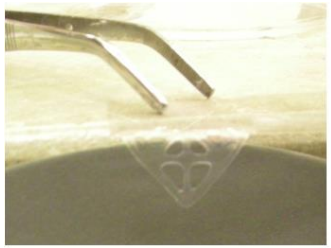
Materials?
Speed?
Price?
Fidelity?

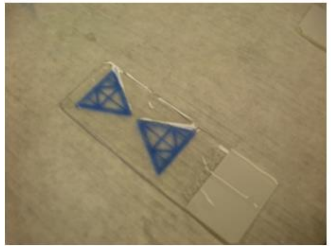


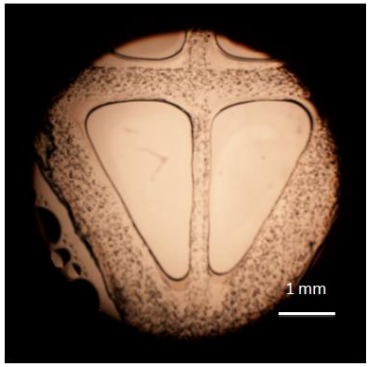
Penelope Ink-Jet printer





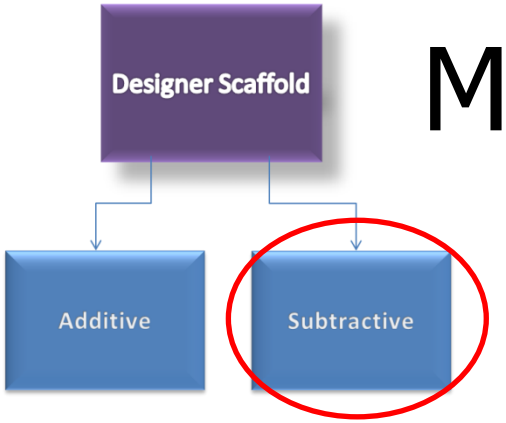


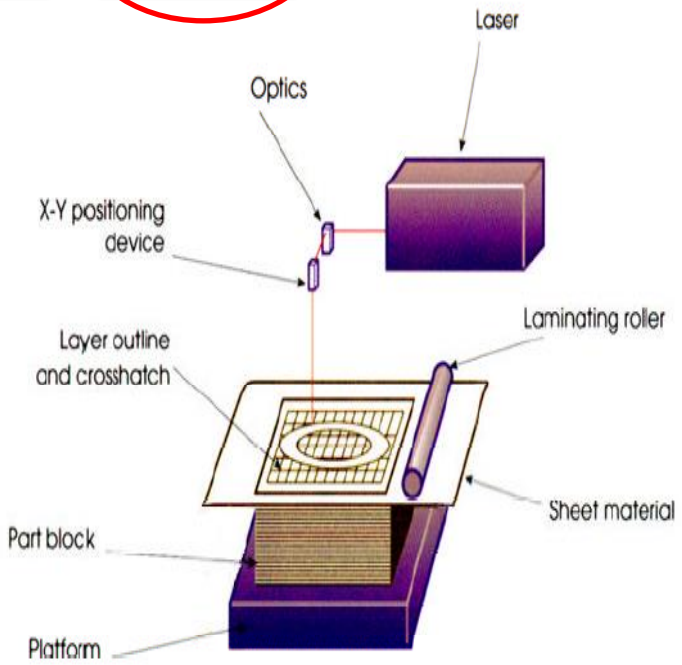


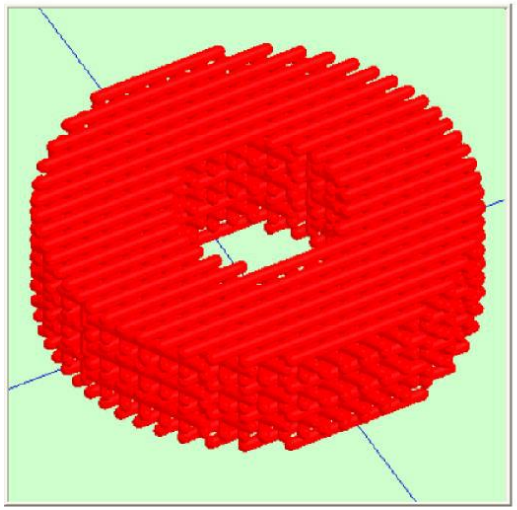


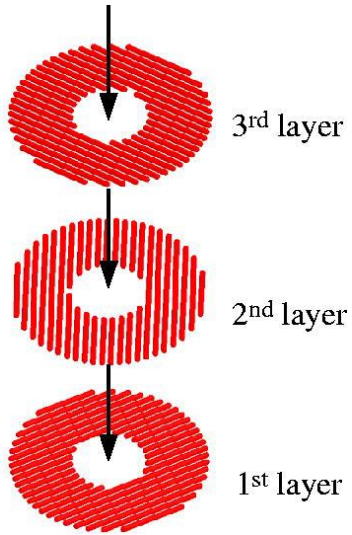
Materials?
Speed?
Price?
Fidelity?

Membrane Lamination



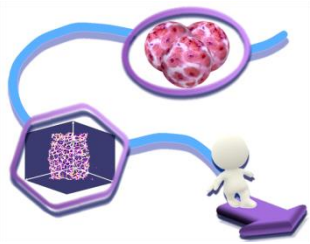






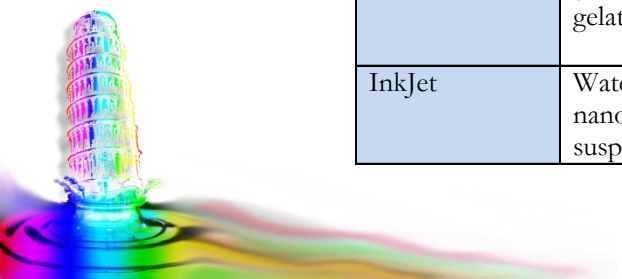
Laser as a cutter

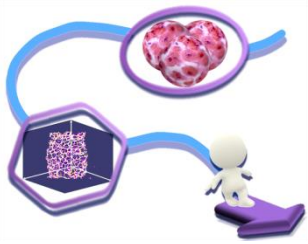
Materials?
Speed?
Price?
Fidelity?



Technique	Material used	RTM ratio (cm^3/min)	Resolution (μm)	Cells used	Limits
Membrane Lamination	Bioerodable polymers (PLA, PLGA, etc), bio-ceramics	Low (<1)	1000	Osteoblasts	Structures not really porous, low resolution
Laser Sintering	Calcium Phosphates, polymers (PLA, PLGA, etc)	Medium to high	< 400	Osteoblasts	Presence of polymeric grains and of excess solvent
Photo-polymerisation	Photo-polymeric resins	0.5 (medium)	250	Osteoblasts	Use of photo sensitive polymers and initiators which may be toxic
Fused Deposition Modelling	Bioerodable polymers (PLA, PLGA,etc)	7 (very high)	200	Various types	Limited to non thermo labile materials. Layered structure very evident
3D™ Printing	Bioerodable polymers, (PLA, PLGA, etc) and hydroxyapatite	Medium (about 1)	300	Various types, mainly skeletal	Presence of polymeric grains and of excess solvent
iRP	Bioerodable polymers (PLA, PLGA, etc), collagen	0.1 (low)	300	Various types	Complex to realise, build materials limited, low fidelity.
PAM[2]	Bioerodable polymers (PLA, PLGA, etc) and gels (alginate, gelatin)	1 (medium)	5-100	Neurons, endothelial cells, fibroblasts, hepatocytes, muscle	Highly water soluble materials cannot be used. Extrusion head very small.
InkJet	Water, solvents, nanoparticle suspensions	Very low (<0.01)	10	Various	Only low viscosity liquids.

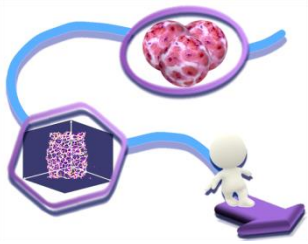
confronto





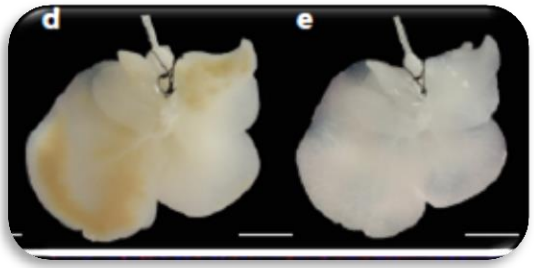
Summary

- Resolution vs manufacturing time trade off
- Softness (and wetness) vs resolution and fidelity trade off



Random Scaffold

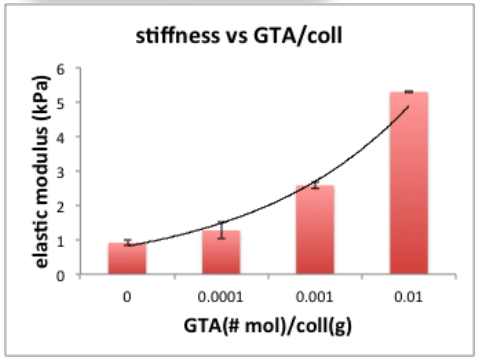
Organ processing

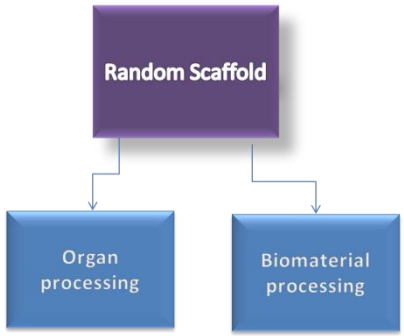


Uygun et al, Nature Med, 2010.

Mattei. et al, Biomat. Acta, 2013

Biomaterial processing

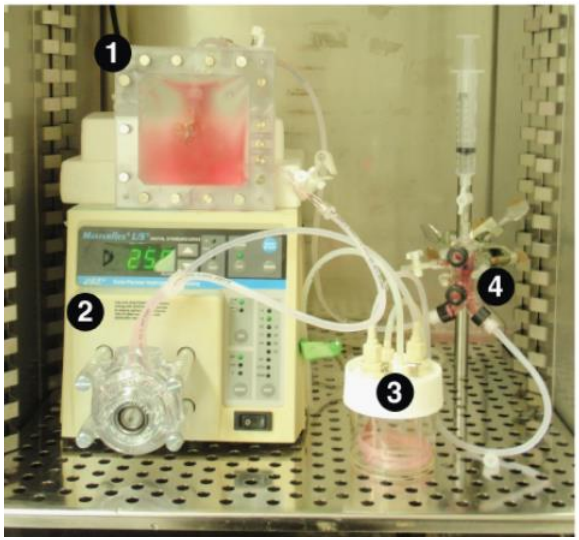




Organ Processing

Whole Organ Perfusion

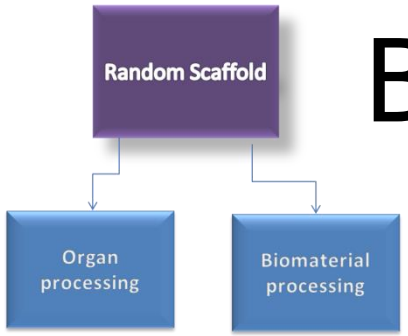
- Detergents
- Intact microvasculature
- Slow and costly



Tissue Decellularization

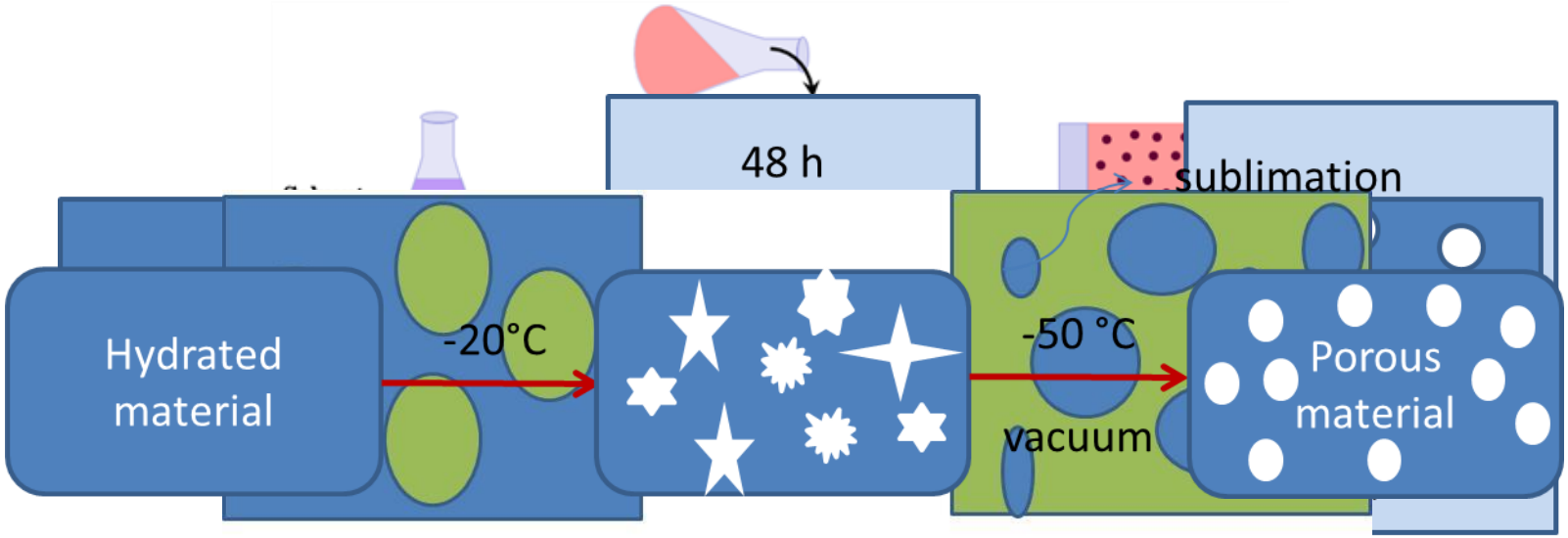
- Detergents
- Rapid, less wasteful

Price?
Materials?
Speed?
Repeatibility ?



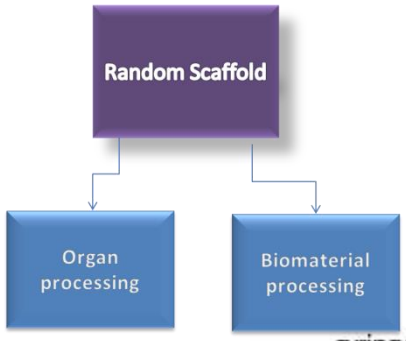
Biomaterial Processing

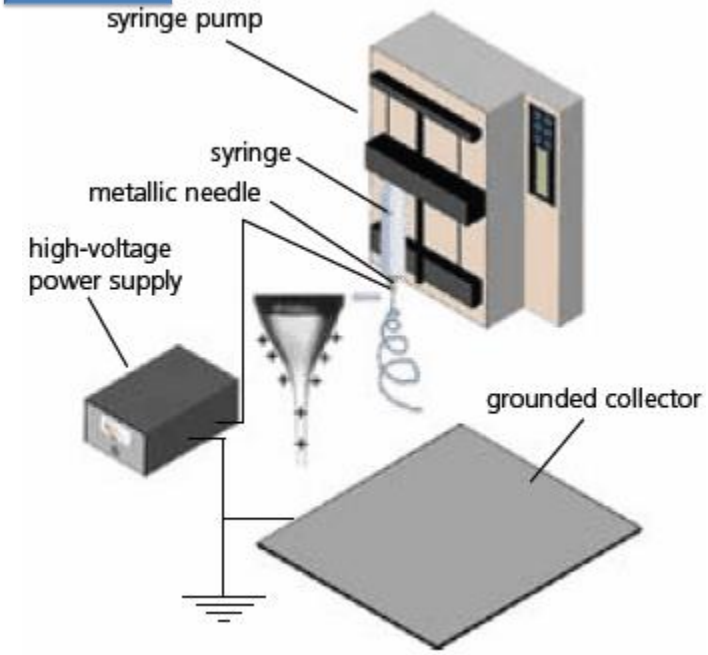
- ***Freeze drying***
- ***Phase separation***
- ***Gas foaming***
- ***Salt leaching***

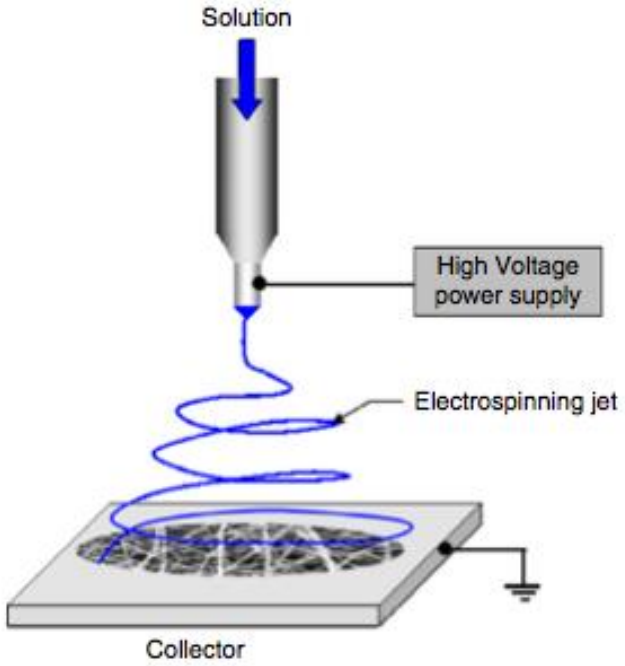


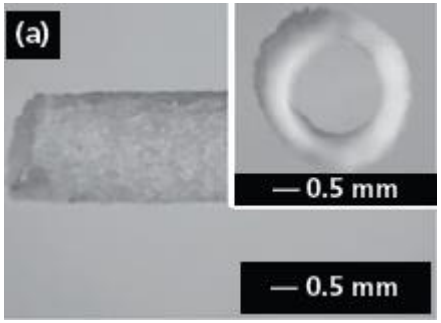
Price?
Materials?
Speed?
Repeatibility ?

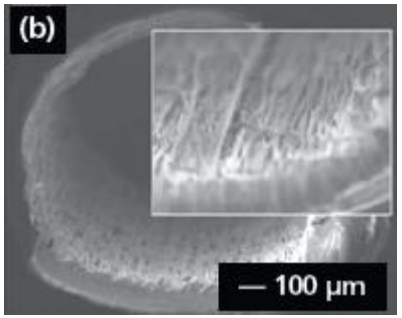
Electrospinning





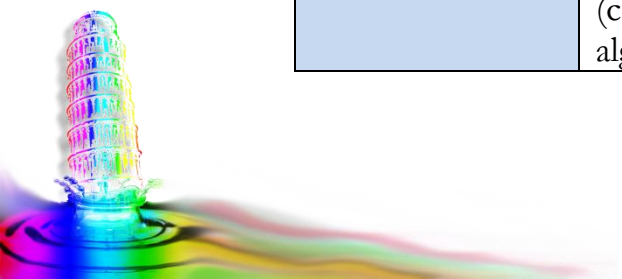


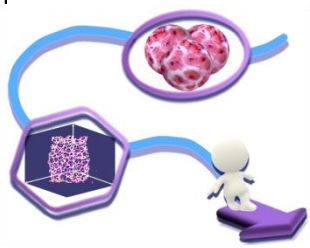




Price?
Materials?
Speed?
Repeatibility ?

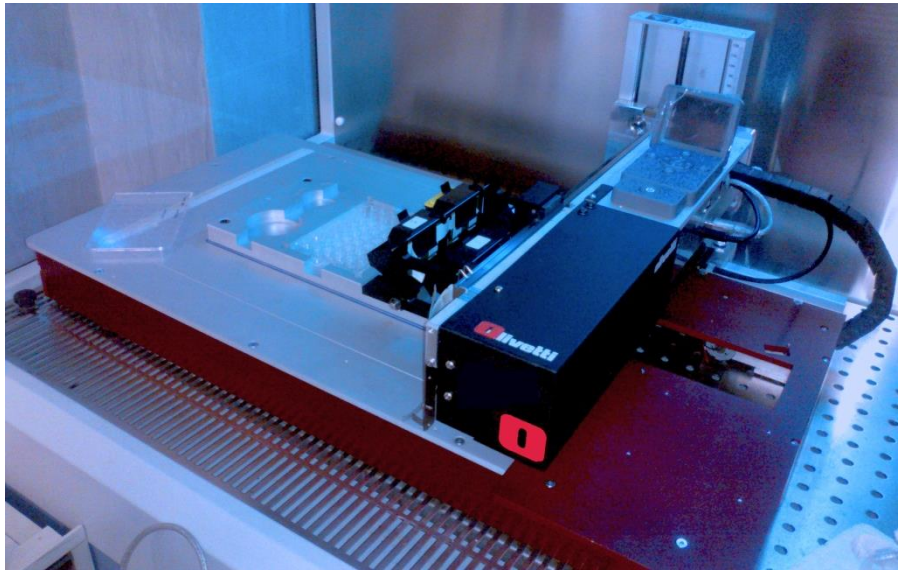
Technique	Material used	RTM ratio (cm^2/min)	Cells used	Limits
Freeze drying	Proteins, carbohydrates, polyesters, hydroxyapatite	High	Variety	Wide distribution of pore size
Phase Inversion	Polyesters, PVA, polyurethanes, biogels (gelatin)	High	Variety	Low interconnectivity, difficult to control pore size
Salt leaching	Polyesters, polyurethanes, hydroxyapatite	High	Variety	Salt residues, limited connectivity
Gas foaming	Polyesters, PVA, polyurethanes, biogels (gelatin)	High	Variety	Quite expensive
Whole organ decell	Organs	High	Heart, liver, lung, etc	Whose organ? Detergents are aggressive
Tissue decell	Pieces of tissue	High	Many	
Electrospinning	Bioerodable polymers (PLA, PLGA, etc), proteins and gels (collagen, alginate, gelatin)	Very low (<1)	Variety	Gives rise to pseudo 3D "squashed" scaffolds





Cell Printing

- Cell Printing (Boland-inkjet)
- Organ Printing (Mironov-Forgacs)
- Living Inks, bioinks, bioprinter, bioplotter



Olivetti NanoBioJet

Cell dispensers and Bioprinters

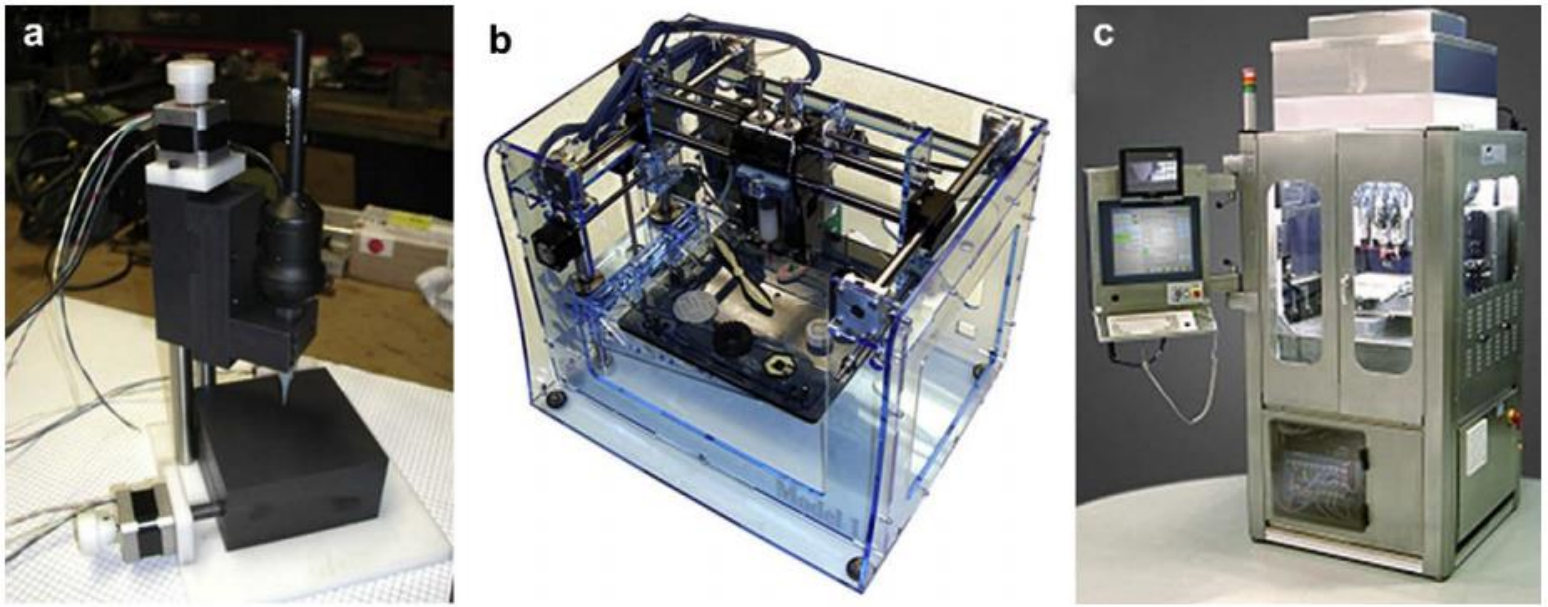
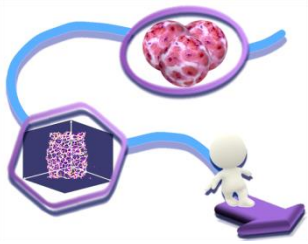


Fig. 3. Bioprinters: a) 3D dispensing Laboratory Bioprinter – 'LBP' (designed by Neatco, Toronto, Canada in cooperation with MUSC Bioprinting Research Center, Charleston, SC); b) 3D robotic printer – 'Fabber' (designed by Cornell University, USA); c) 3D robotic industrial bioprinter — 'BioAssembly Tool' (designed by Sciperio/nScript, Orlando, USA).



Organ Printing using *cell suspensions* as a *material*

V. Mironov et al. Biomaterials 30 (2009) 2164–2174

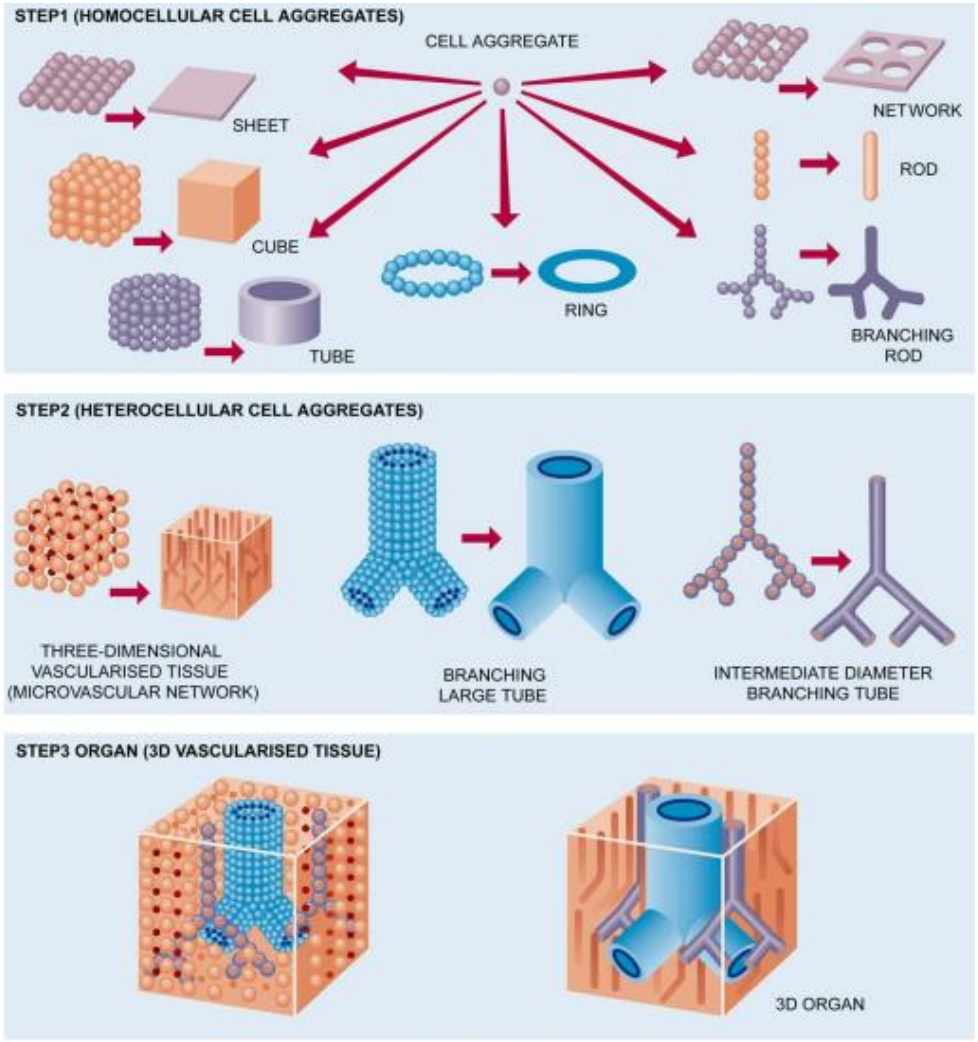


Fig. 4. Roadmap for organ printing.

fusion is a ubiquitous process during embryonic development and can be recapitulated in vitro [45]. It has been shown that the kinetics of tissue fusion of two rounded embryonic heart cushion tissue explants placed in an hanging drop fits perfectly to fusion kinetics described for two droplets of fluids [46]. Moreover, based

physical laws and Malcolm Steinberg's "differential adhesion hypothesis" [28–30]. From another point, motile living cells, cytoskeleton and number, and redistribution and activation of cell adhesion receptors are also essential for the tissue fusion process [46,47]. The accumulation of ECM and associated restriction of cell

Live scaffold fabrication

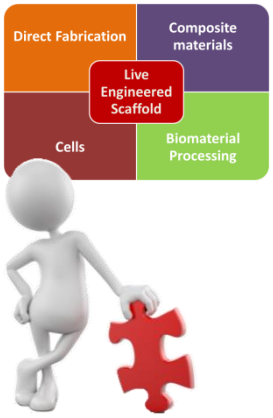
Direct Fabrication

Composite materials

Live Engineered Scaffold

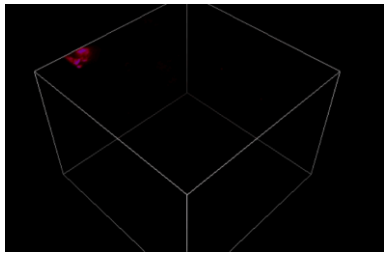
Cells

Biomaterial Processing



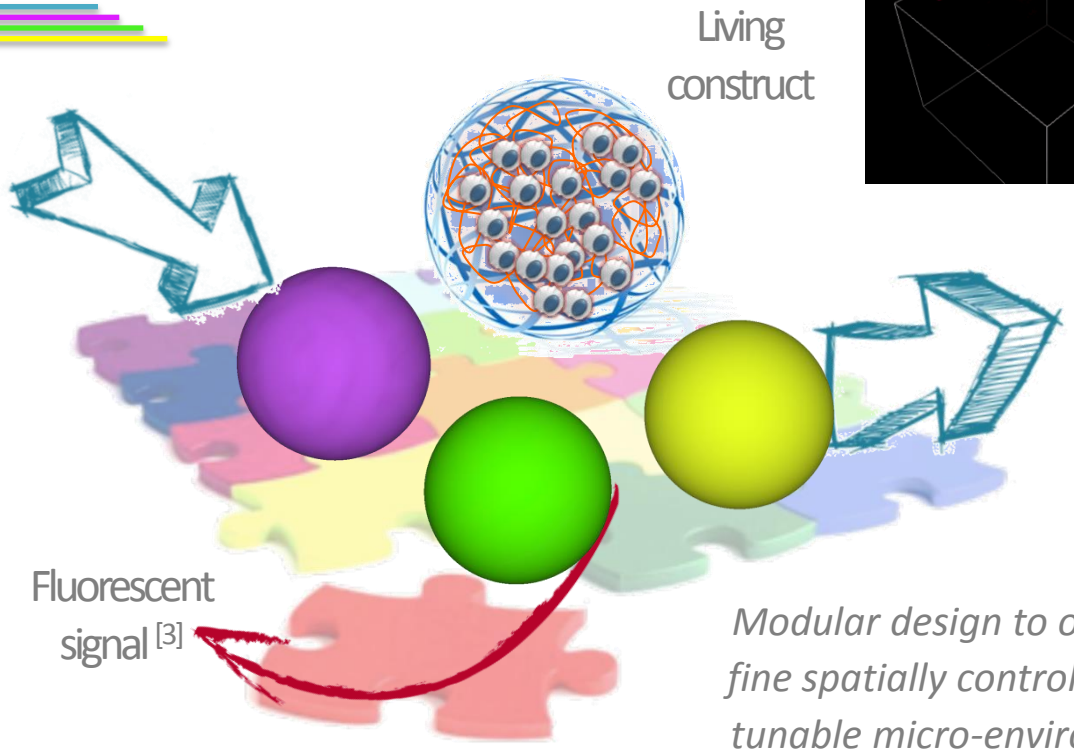
Nano-in-micro (NIM) Live Scaffold Fabrication

Recreate an *in vitro* microsystem able to interact and monitor living constructs in a non-invasive manner



Assembling:

- Living micro-spheres with controlled mechanical and properties and biomimetic composition;
- Having:
  - Cells
  - Tissue matrix
  - Release of known moieties (e.g. ROS, exogenous molecules)
  - Scavenger properties
  - Sensitive detectors[3]

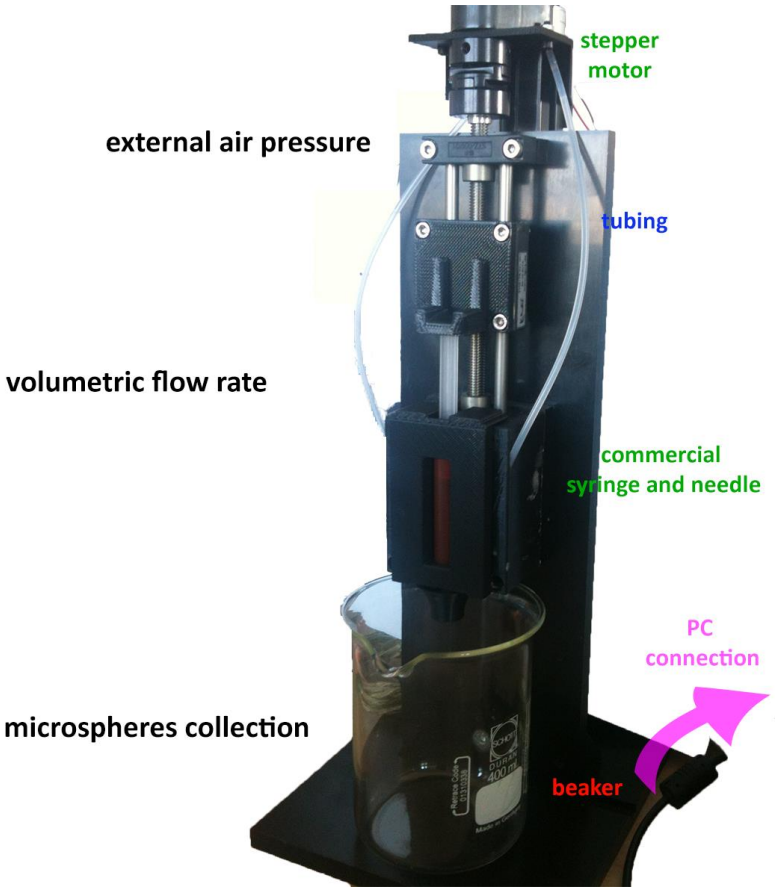


Modular design to obtain a fine spatially controlled and tunable micro-environment

[1] Tirella et al. JBMA 2013 ; [2] Mattei el all Biomat. Acta,; [3] Tirella , La Marca, Ahluwalia , Soft Matter submitted; [4] La Marca et al., ESB 2013.

Spherical Hydrogel Generator

Sensitive/Functional domains can be easily fabricated controlling sphere dimension, shape and composition



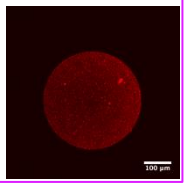
Size controlled hydrogel micro-spheres as function of system working parameters and solution properties:

- ✓ Solution viscosity (e.g. alginate w/v ratio, NPs concentration, cell concentration)
- ✓ Nozzle diameter
- ✓ Volumetric flow rate
- ✓ External air flow

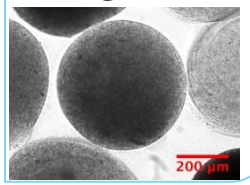
Shape is fixed via rapid physical gelation, e.g. for alginate microspheres form a gel in a beaker containing a 0.1 M $CaCl_2$ solution in water.

Alginate

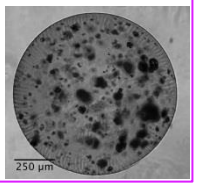
Alginate+NPs



Alginate+matrix

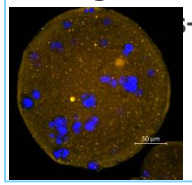


Alginate+matrix+cells



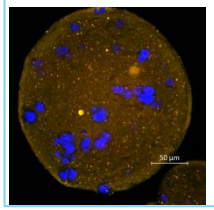
Alginate+matrix+

+NPs



NIM Live Scaffold

Alginate+cell+NPs



200 µm spheres immersed in buffering solutions

A. pH reversibility detection

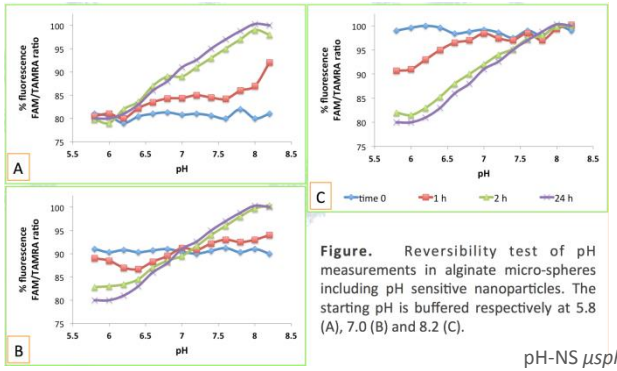
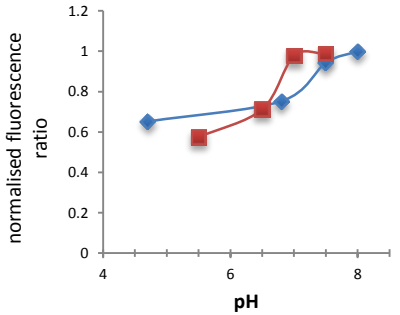
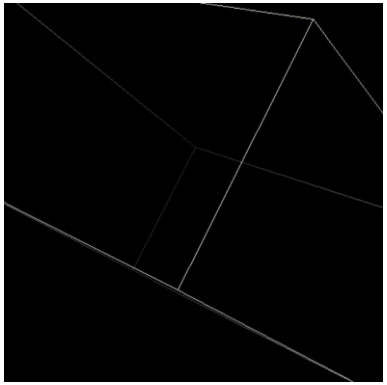


Figure. Reversibility test of pH measurements in alginate micro-spheres including pH sensitive nanoparticles. The starting pH is buffered respectively at 5.8 (A), 7.0 (B) and 8.2 (C).

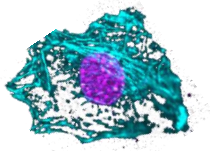
B. Calibration curve (spectrofluorimeter vs confocal acquisition)



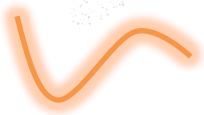


pH-NS *µsphere*
Confocal acquisition

Alginate hydrogel µsphere

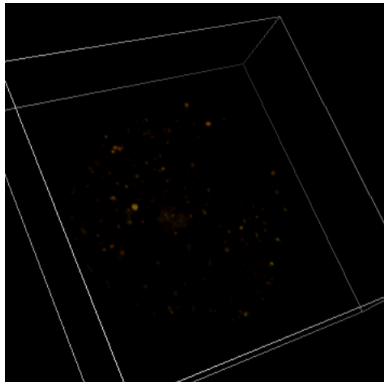


hepatocytes

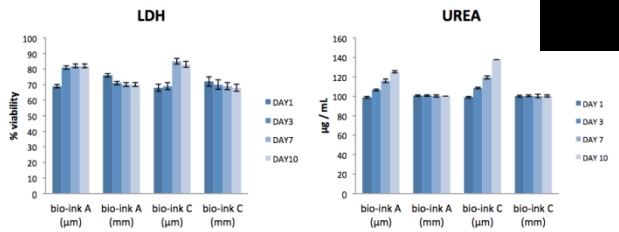


Digested liver matrix

Ratiometric pH-NSs



ddECM/pH-NS / HepG2
µsphere
Confocal acquisition
DAPI / **pH-sensitive** / **pH-reference**



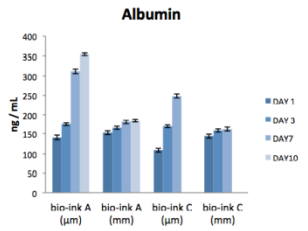
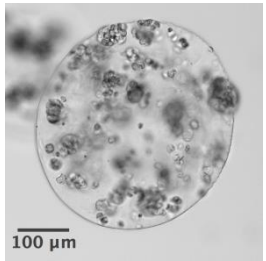


Figure 4. Metabolic analysis: (A) Lactate dehydrogenase Triton as positive control.; (B) Urea; (C) Albumin. Mean ± SE from three different experiments.

pH-NS/HepG2 *µsphere*
BF image



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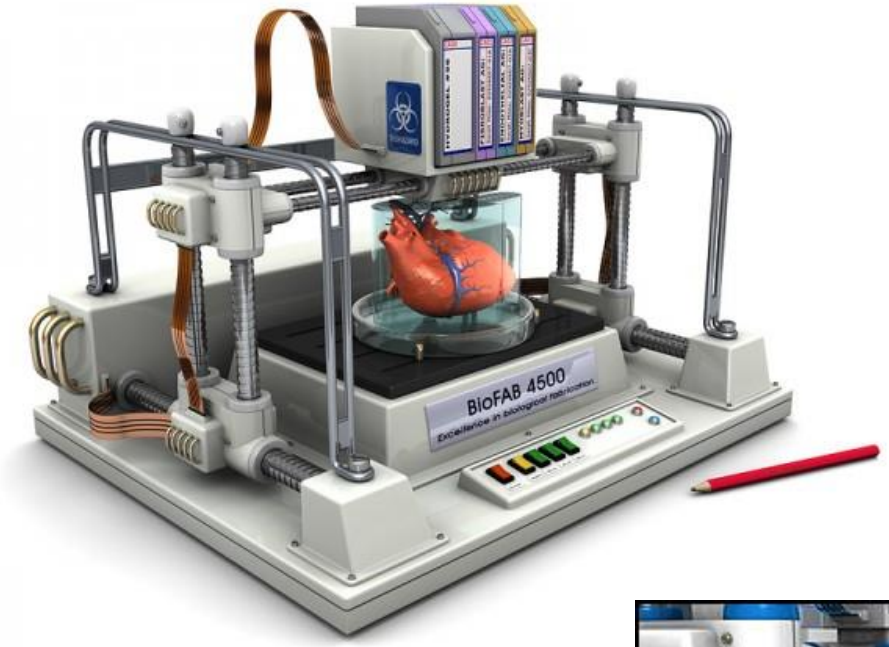
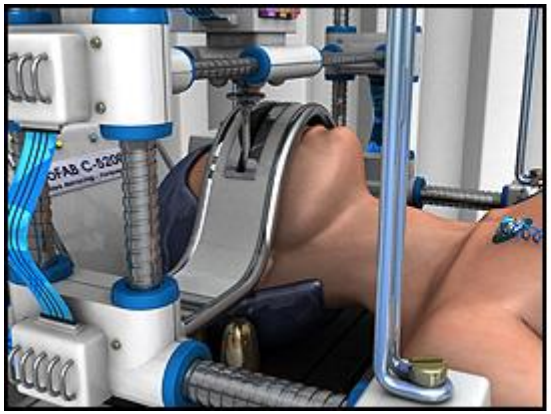
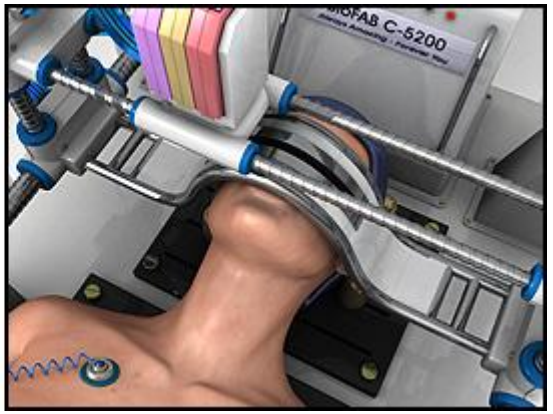
Future of Live Scaffold Fabrication







Concept: European Bioprinting Network

Scaffold Characterisation

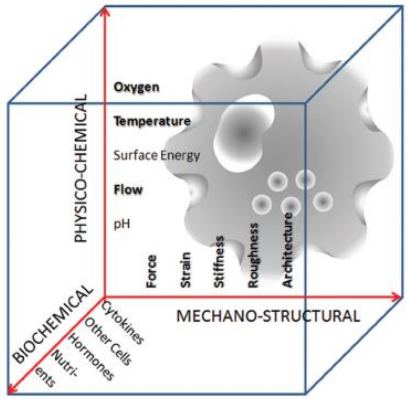
Without cells

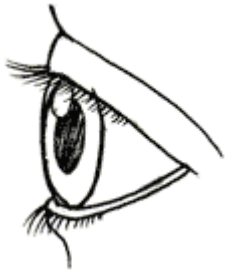
- ***Topological*** (porosity, interconnectivity, & related scaffold features)
- ***Physico-chemical*** (swelling, degradation, ligand release, presentation, ligand localisation)
- ***Mechanical***: compressive, tensile, viscoelastic

With cells

- In-vitro
- Quasi-vivo
- In-vivo

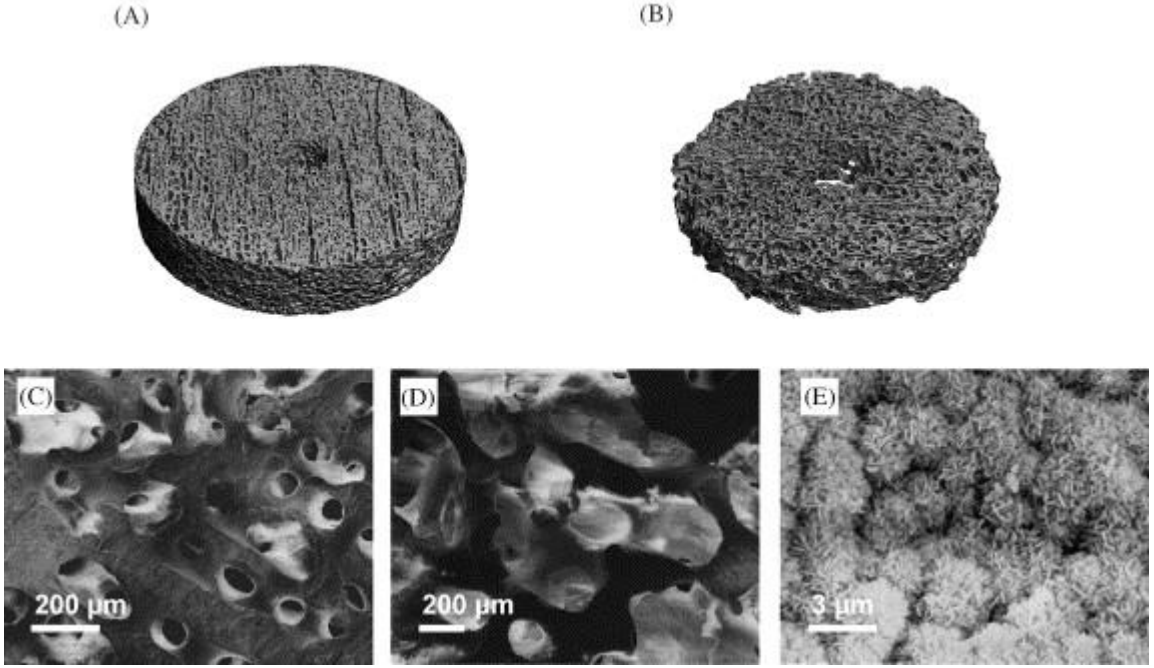
Wet vs. Dry





Scaffold Characterisation Topological

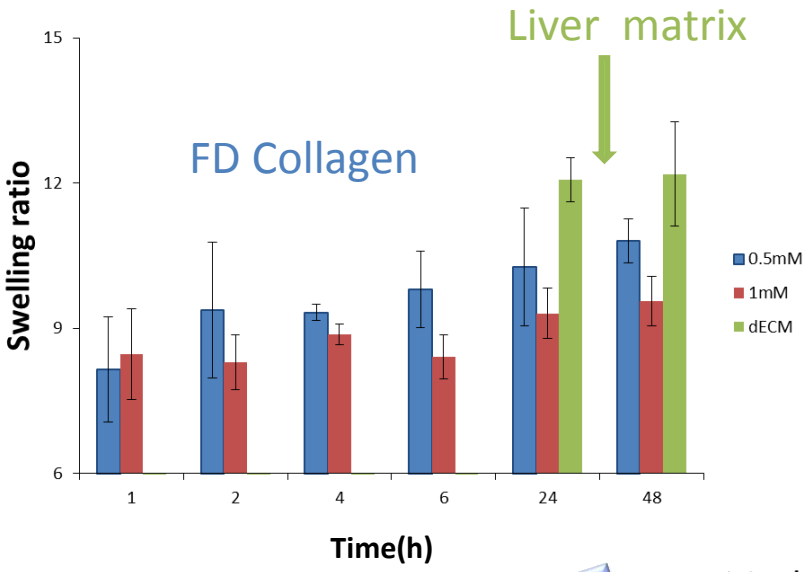
Dry methods



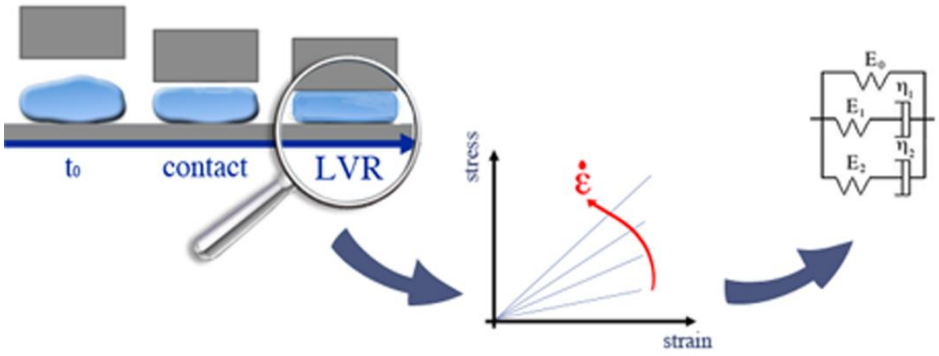
µCT scan of a 200-µm (A) and 500-µm (B) pore scaffolds. SEM micrographs depicting the scaffold architecture of the 200-µm (C) and 500-µm (D) pore scaffolds. In (E) is shown a representative higher magnification image of the scaffold walls as they appear on both types of scaffolds.

Scaffold Characterisation (wet)

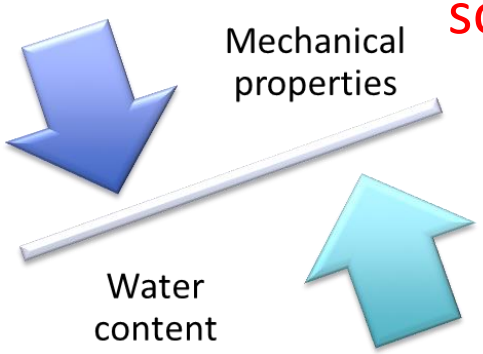
Swelling



Mechanical



Mechanical characterisation of soft wet materials



Tirella, Mattei, Ahluwalia, Strain rate viscoelastic analysis of soft and highly hydrated biomaterials, JBMRA, 2013

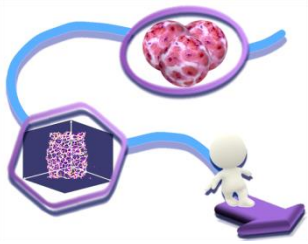
The problem of characterising living scaffolds

They are alive

They are 3D

Small features

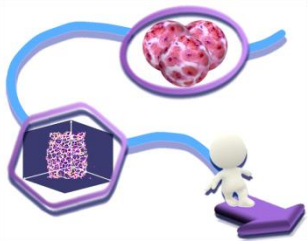
High resolution, non destructive, fast



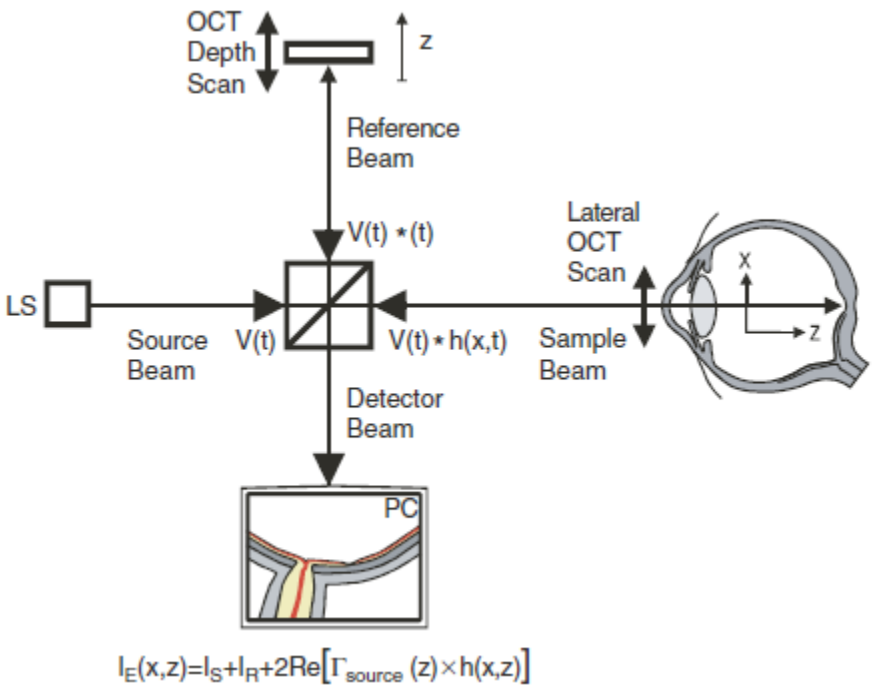
3D characterization

Technique	Principle	Depth	Lateral (micron)	Label
Ultrasound (20 MHz)	Acoustic impedance	20 cm	250	no
Microscope	Phase/Transmittance	100 μm	5-10	no
Fluorescent microscope	Fluorescent lablel	50 μm	5	yes
Confocal	Laser scanning, confocal planes	100-200 μm	1	yes
OCT	Interferometry (optical impedance)	Several mm	100	no

Resolution vs. depth of penetration



OCT



Fercher et al.
Rep. Prog. Phys. 666, 239, 2003

Measures difference in path length between reference and sample beam. Highly focused white light source . The back-scattered light travels to the detector where the unique phase delay for each wavelength is detected. Depth information is acquired using a Fast Fourier Transformation .