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

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 Scaffold
- Subtractive
- Additive

Random Scaffold
- Organ processing
- Biomaterial processing
Designer or Random?

Structure

Retina

Liver

Bone

Function
Designer Scaffold
Additive = rapid prototyping (from object to 3D scan to slicing to layer by layer printing)
3D Printing/Digital Fabrication & RP

Designer Scaffold

Additive
Subtractive

FABLAB PISA THE IDEAS FACTORY
Three main groups:
- laser systems
- nozzle based systems
- direct writing systems

Materials?  
Speed?  
Price?  
Fidelity?
Stereolithography

Laser for polymerisation of liquid monomer or resin

Materials?
Speed?
Price?
Fidelity?
Fused Deposition Modeling

Hutmacher & coworkers

Materials?
Speed?
Price?
Fidelity?

Figure 1: Platform technology for patient specific scaffolds TE.
Pressure Assisted Microsyringe (PAM)

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 ·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...

Resolution, fidelity, viscosity

Deveopment of a modular microfabrication system to engineer complex tissues
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.
Penelope Ink-Jet printer

Materials?
Speed?
Price?
Fidelity?
Membrane Lamination

Laser as a cutter

Materials?
Speed?
Price?
Fidelity?
<table>
<thead>
<tr>
<th>Technique</th>
<th>Material used</th>
<th>RTM ratio (cm$^3$/min)</th>
<th>Resolution (μm)</th>
<th>Cells used</th>
<th>Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane Lamination</td>
<td>Bioerodable polymers (PLA, PLGA, etc), bioceramics</td>
<td>Low (&lt;1)</td>
<td>1000</td>
<td>Osteoblasts</td>
<td>Structures not really porous, low resolution</td>
</tr>
<tr>
<td>Laser Sintering</td>
<td>Calcium Phosphates, polymers (PLA, PLGA, etc)</td>
<td>Medium to high</td>
<td>&lt; 400</td>
<td>Osteoblasts</td>
<td>Presence of polymeric grains and of excess solvent</td>
</tr>
<tr>
<td>Photo-polymerisation</td>
<td>Photo-polymeric resins</td>
<td>0.5 (medium)</td>
<td>250</td>
<td>Osteoblasts</td>
<td>Use of photosensitive polymers and initiators which may be toxic</td>
</tr>
<tr>
<td>Fused Deposition Modelling</td>
<td>Bioerodable polymers (PLA, PLGA, etc)</td>
<td>7 (very high)</td>
<td>200</td>
<td>Various</td>
<td>Limited to non thermo labile materials. Layered structure very evident</td>
</tr>
<tr>
<td>3D™ Printing</td>
<td>Bioerodable polymers, (PLA, PLGA, etc) and hydroxyapatite</td>
<td>Medium (about 1)</td>
<td>300</td>
<td>Various, mainly skeletal</td>
<td>Presence of polymeric grains and of excess solvent</td>
</tr>
<tr>
<td>iRP</td>
<td>Bioerodable polymers (PLA, PLGA, etc), collagen</td>
<td>0.1 (low)</td>
<td>300</td>
<td>Various</td>
<td>Complex to realise, build materials limited, low fidelity,</td>
</tr>
<tr>
<td>PAM²</td>
<td>Bioerodable polymers (PLA, PLGA, etc) and gels (alginate, gelatin)</td>
<td>1 (medium)</td>
<td>5-100</td>
<td>Neurons, endothelial cells, fibroblasts, hepatocytes, muscle</td>
<td>Highly water soluble materials cannot be used. Extrusion head very small.</td>
</tr>
<tr>
<td>InkJet</td>
<td>Water, solvents, nanoparticle suspensions</td>
<td>Very low (&lt;0.01)</td>
<td>10</td>
<td>Various</td>
<td>Only low viscosity liquids.</td>
</tr>
</tbody>
</table>
Summary

• Resolution vs manufacturing time trade off
• Softness (and wetness) vs resolution and fidelity trade off
Organ Processing

Whole Organ Perfusion
- Detergents
- Intact microvasculature
- Slow and costly

Tissue Decellularization
- Detergents
- Rapid, less wasteful
Biomaterial Processing

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

Price?
Materials?
Speed?
Repeatability?
Electrospinning

Price?
Materials?
Speed?
Repeatability?
<table>
<thead>
<tr>
<th>Technique</th>
<th>Material used</th>
<th>RTM ratio (cm²/min)</th>
<th>Cells used</th>
<th>Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freeze drying</td>
<td>Proteins, carbohydrates, polyesters, hydroxyapatite</td>
<td>High</td>
<td>Variety</td>
<td>Wide distribution of pore size</td>
</tr>
<tr>
<td>Phase Inversion</td>
<td>Polyesters, PVA, polyurethanes, biogels (gelatin)</td>
<td>High</td>
<td>Variety</td>
<td>Low interconnectivity, difficult to control pore size</td>
</tr>
<tr>
<td>Salt leaching</td>
<td>Polyesters, polyurethanes, hydroxyapatite</td>
<td>High</td>
<td>Variety</td>
<td>Salt residues, limited connectivity</td>
</tr>
<tr>
<td>Gas foaming</td>
<td>Polyesters, PVA, polyurethanes, biogels (gelatin)</td>
<td>High</td>
<td>Variety</td>
<td>Quite expensive</td>
</tr>
<tr>
<td>Whole organ decell</td>
<td>Organs</td>
<td>High</td>
<td>Heart, liver, lung, etc</td>
<td>Whose organ? Detergents are aggressive</td>
</tr>
<tr>
<td>Tissue decell</td>
<td>Pieces of tissue</td>
<td>High</td>
<td>Many</td>
<td></td>
</tr>
<tr>
<td>Electrospinning</td>
<td>Bioerodable polymers (PLA, PLGA, etc), proteins and gels (collagen, alginate, gelatin)</td>
<td>Very low (&lt;1)</td>
<td>Variety</td>
<td>Gives rise to pseudo 3D “squashed” scaffolds</td>
</tr>
</tbody>
</table>
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

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**

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 CaCl₂ solution in water.
NIM Live Scaffold

200 μm spheres immersed in buffering solutions
A. pH reversibility detection

B. Calibration curve (spectrofluorimeter vs 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. Reversibility test of pH measurements in alginate micro-spheres including pH sensitive nanoparticles. The starting pH is buffered respectively at 5.6 (A), 7.0 (B) and 8.2 (C).

pH-NS μsphere
Confocal acquisition

LDH
UREA
Albumin
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
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

FD Collagen

Liver matrix

Swelling ratio

Time (h)

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

<table>
<thead>
<tr>
<th>Technique</th>
<th>Principle</th>
<th>Depth</th>
<th>Lateral (micron)</th>
<th>Label</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrasound (20 MHz)</td>
<td>Acoustic impedance</td>
<td>20 cm</td>
<td>250</td>
<td>no</td>
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<tr>
<td>Microscope</td>
<td>Phase/Transmitance</td>
<td>100 μm</td>
<td>5-10</td>
<td>no</td>
</tr>
<tr>
<td>Fluorescent microscope</td>
<td>Fluorescent label</td>
<td>50 μm</td>
<td>5</td>
<td>yes</td>
</tr>
<tr>
<td>Confocal</td>
<td>Laser scanning, confocal planes</td>
<td>100-200 μm</td>
<td>1</td>
<td>yes</td>
</tr>
<tr>
<td>OCT</td>
<td>Interferometry (optical impedance)</td>
<td>Several mm</td>
<td>100</td>
<td>no</td>
</tr>
</tbody>
</table>
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.

Fercher et al.