Hybrid scaffold manufacturing for tissue engineering applications

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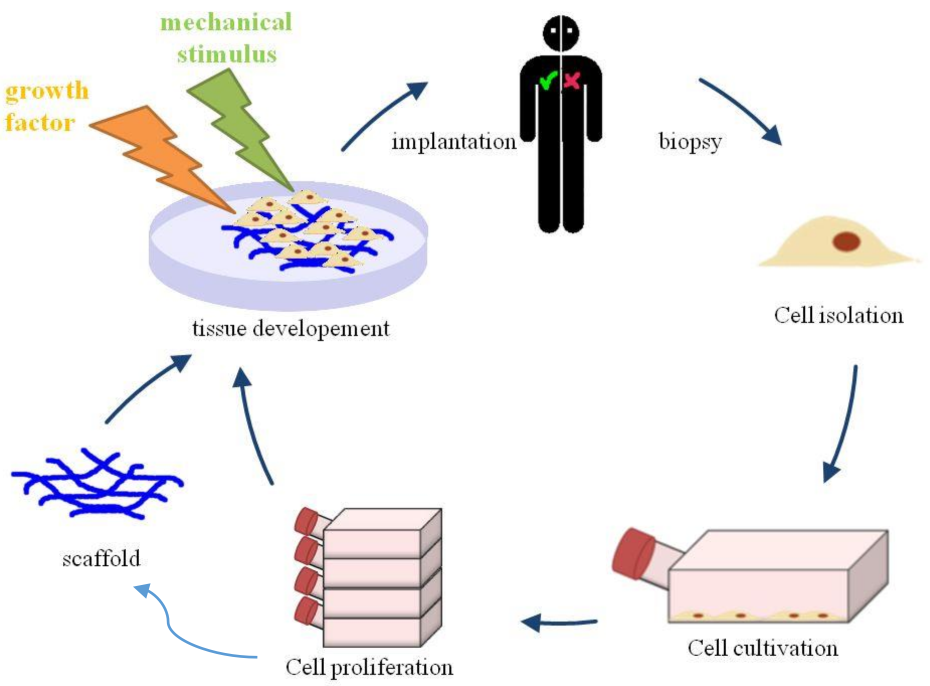
Introduction: Tissue Engineering

- Tissue Engineering: “A field of study in which knowledge about cells, cellular environment, and materials join together in order to improve or regenerate living tissue and its function”.

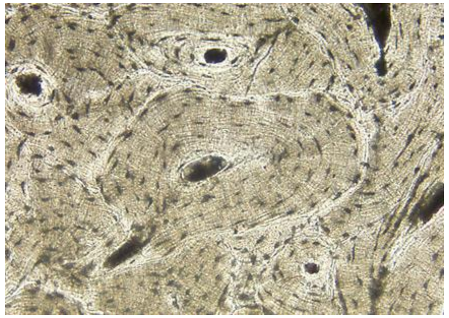


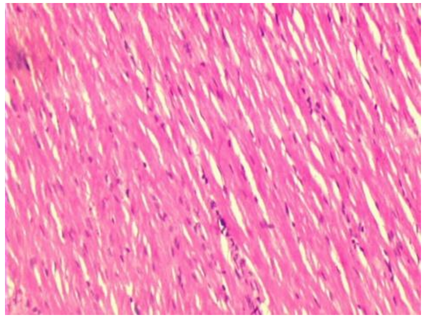
Introduction: Cell cultures

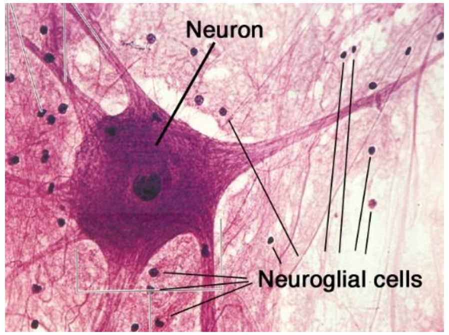
- Cell cultures of different cell types, such as osteoblasts and neurons are grown in laboratories for tissue engineering and cellular therapies applications.

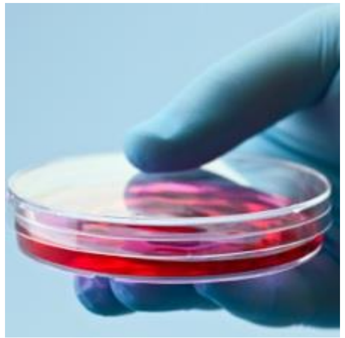
- These tissues have the objective of replacing damaged tissue (epithelial, connective, muscular, neural).

- Petri dishes (2D) are commonly employed for cell cultures. However, tissues *in vivo* are not limited by a 2D structure, they are rather found in an intricate 3D structure, which in turn forms the extra cellular matrix (ECM).





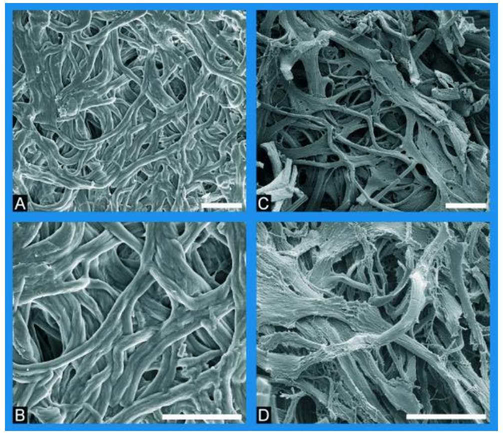


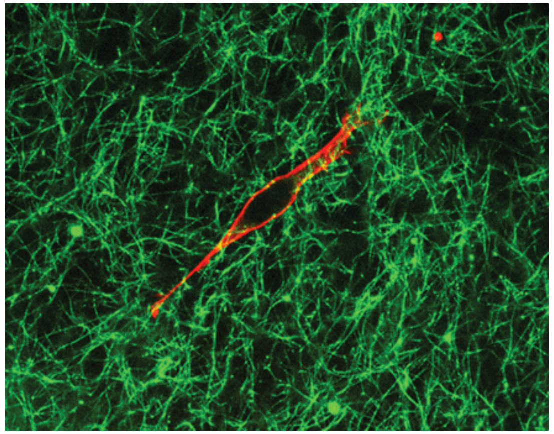


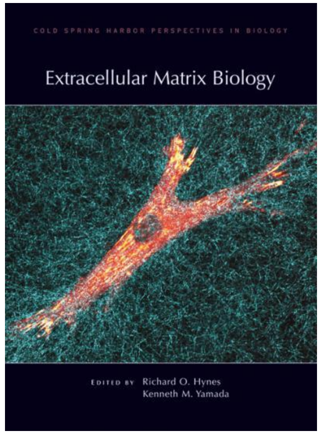
Extra Cellular Matrix

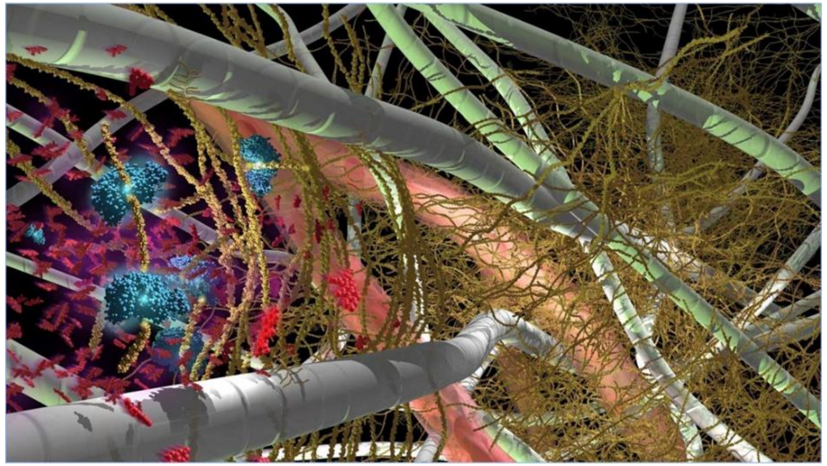
- Some functions of the ECM are to provide support for the cellular adhesion, allow for communication, and differentiation towards a specific cell type.

- Cells grown in traditional 2D Petri dishes may not develop and behave in the same manner as their *in vivo* counterparts, due to a lack of appropriate ECM signaling, such as biochemical or physical cues.





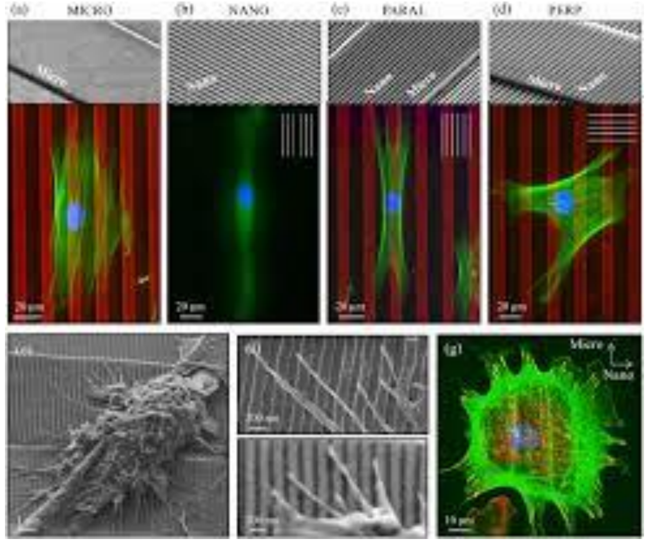


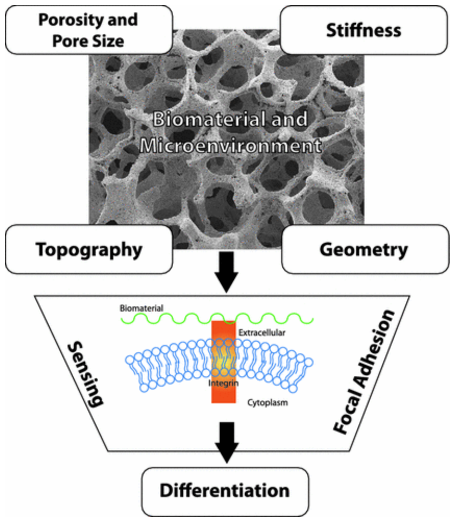


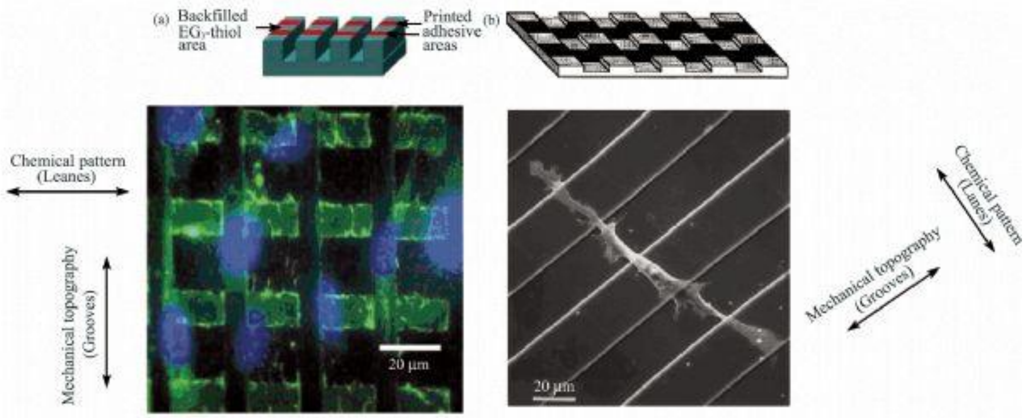
Extra Cellular Matrix

- ECM topography, stiffness, geometry and chemistry play an important role in cellular fate.

- For example, the Young Modulus for the ECM of osteoblast differs greatly from the Young Modulus of the neuronal ECM.



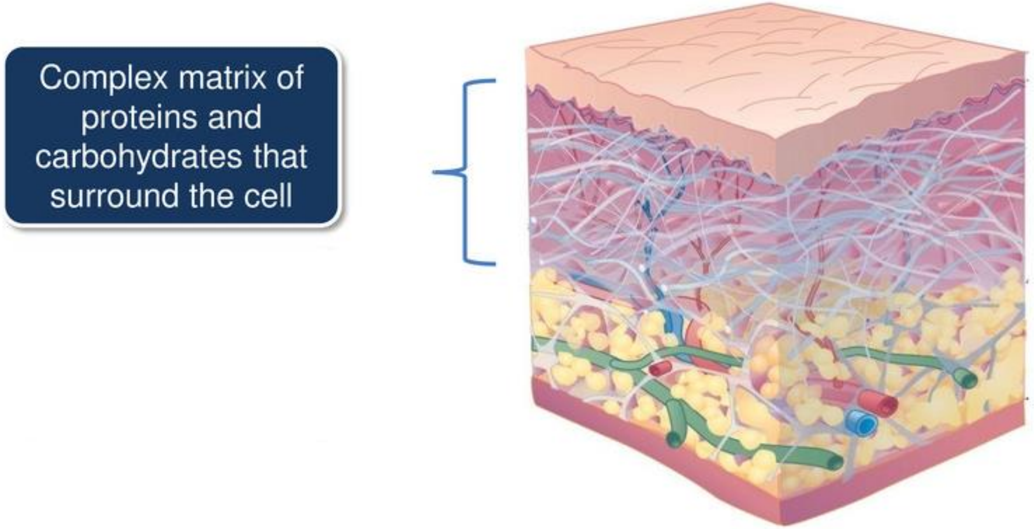




3D scaffolds for Tissue Engineering

- Therefore, it is of great interest to develop materials and techniques that allow us to fabricate scaffolds in an intrinsically 3D structure so they could better approximate *in vivo* ECM.

- Important aspects to consider when building scaffolds for tissue engineering applications are:
    - Mechanical stability
    - Porosity
    - Surface chemistry
    - Topography
    - Biocompatibility
    - Pore interconnectivity



Research areas involved (Engineering + Life Sciences)

Two general approaches in 3D bioprinting

Scaffold based

- Scaffolds are first manufactured, cells are then seeded and cultivated on the scaffold.
- The scaffold provides a framework for cells to attach, proliferate, and even orient their growth.

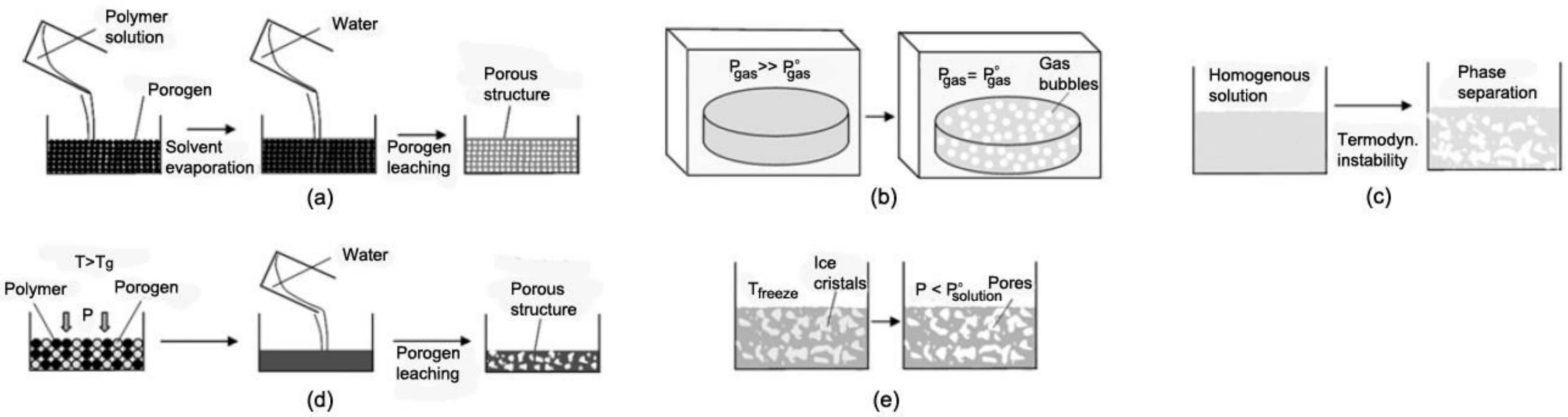
Scaffold free

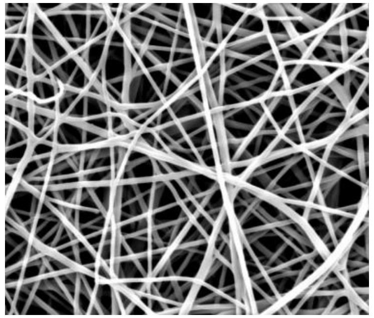
- Cells are placed in a three-dimensional way, using hydrogels as supporting structure.
- This approach relays on the ability of cells to form their own ECM.

Conventional Scaffold Fabrication Techniques

Conventional scaffold fabrication techniques

- Solvent casting
- Particulate leaching
- Gas foaming
- Phase separation
- Melt molding
- Freeze drying
- Electrospinning





Solvent toxicity and pore size control

Not great control in porous size and geometry

Cells seeded afterwards scaffold fabrication

Ning Zhu and Xiongbiao Chen (2013). Biofabrication of Tissue Scaffolds, Advances in Biomaterials Science and Biomedical Applications, InTech, DOI: 10.5772/54125.

2D biofabrication techniques

- Laser-based (1999)
  - Laser-Guided Direct Writing (LGDW)
  - Matrix Assisted Pulsed Laser Evaporation Direct Write (MAPLE DW)
  - Biological Laser Printing (BioLP)
  - Laser Assited Bioprinting (LAB)

- Inkjet printing (2003)
  - Thermal inkjet
  - Electrostatic inkjet
  - Piezoelectric inkjet

Materials:

- Matrigel
- Alginate
- Fibrin
- Collagen
- PVA
- Agarose

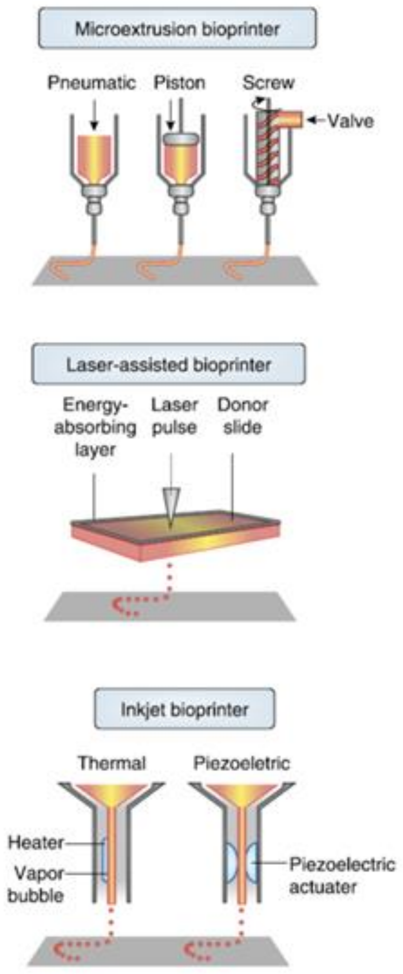
Cells:

- Osteoblast
- Endothelial
- Proteins
- HeLa
- Fibroblasts

Towards 3D bioprinting

Additive Manufacturing (Rapid Prototyping)

- A) Nozzle-based (extrusion/dispension)
  - Fused deposition modeling (FDM)
  - Precision extrusion manufacturing (PEM)
- B) Laser-based (photopolymerization)
  - Selective Laser Syntering (SLS)
  - Stereolithography (SLA)
  - µ-SLA
  - Multi-photon polymerization
- C) Printer-based
  - 3DP
  - Inkjet printing



Nozzle-based

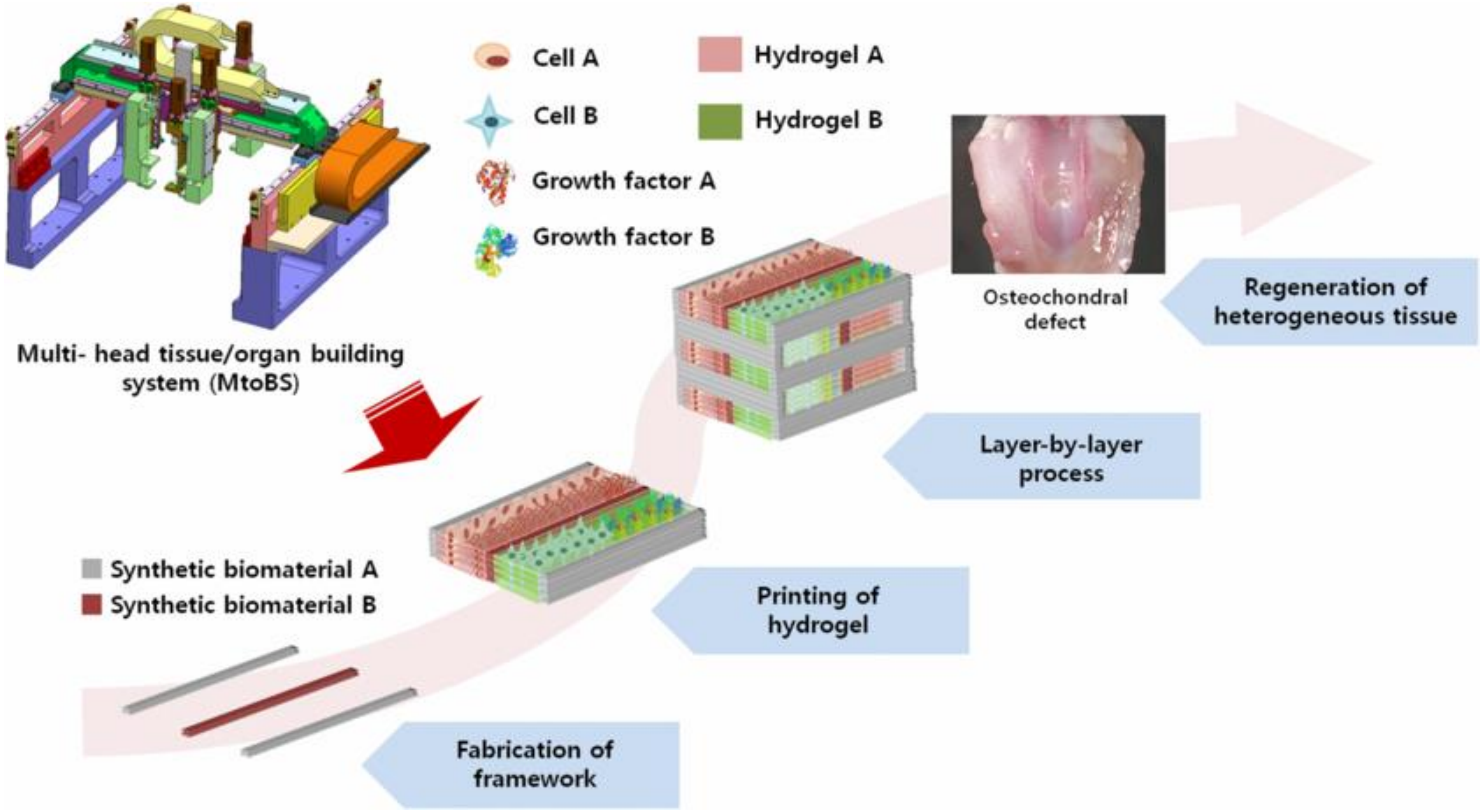
Nozzle-based rapid prototyping techniques for TE

- Melting process
  - Fused deposition modeling (FDM)
  - 3D fibre deposition
  - Precision extrusion deposition (PED)
  - Precision extrusion manufacturing (PEM)
  - Multiple jet solidification (MJS)
- Non melting process
  - Pressure-assisted microsyringe (PAM)
  - Low-temperature deposition modeling (LDM)
  - 3D-Bioplotter

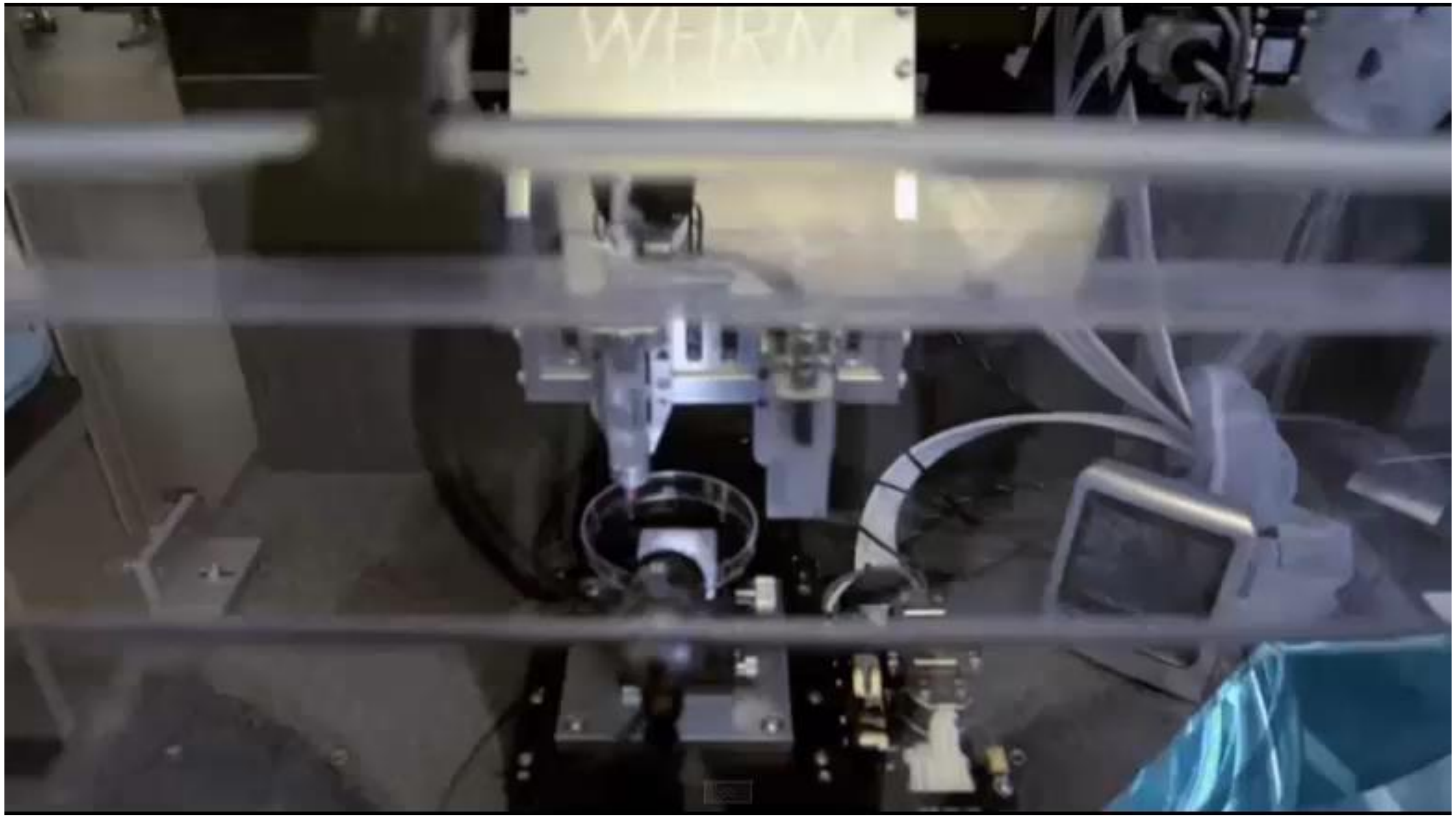
- Materials:
  - PCL
  - PLA
  - PEG
  - Gelatin
  - Hyaluronic acid
  - Alginate
  - Fibrinogen
  - Chitosan
  - Collagen
  - Hydroxiapatite
  - PLGA
  - Matrigel
  - PEO
  - Methylcellulose
  - Agarose

- **Cells:**
- Adipose-derived stromal cells (ADSC)
- Hepatocytes
- HepG2
- Human intestinal epithelial cells
- NIH 3T3
- Chondrocytes
- Multipotent stromal cells (MSCs)
- Bone marrow stromal cells (BMSCs)
- Human fibroblasts
- Bovine aortic endothelial cells (BAECs)
- Endothelial cells
- Schwann cells
- Neurons

Nozzle-based techniques for TE



Nozzle-based RP for TE



Nozzle-based rapid prototyping limitations

- Limited resolution (45 - 1600 µm)
- Limited mechanical strength
- Smooth surface
- Low accuracy
- Slow processing
- High equipment purchase costs

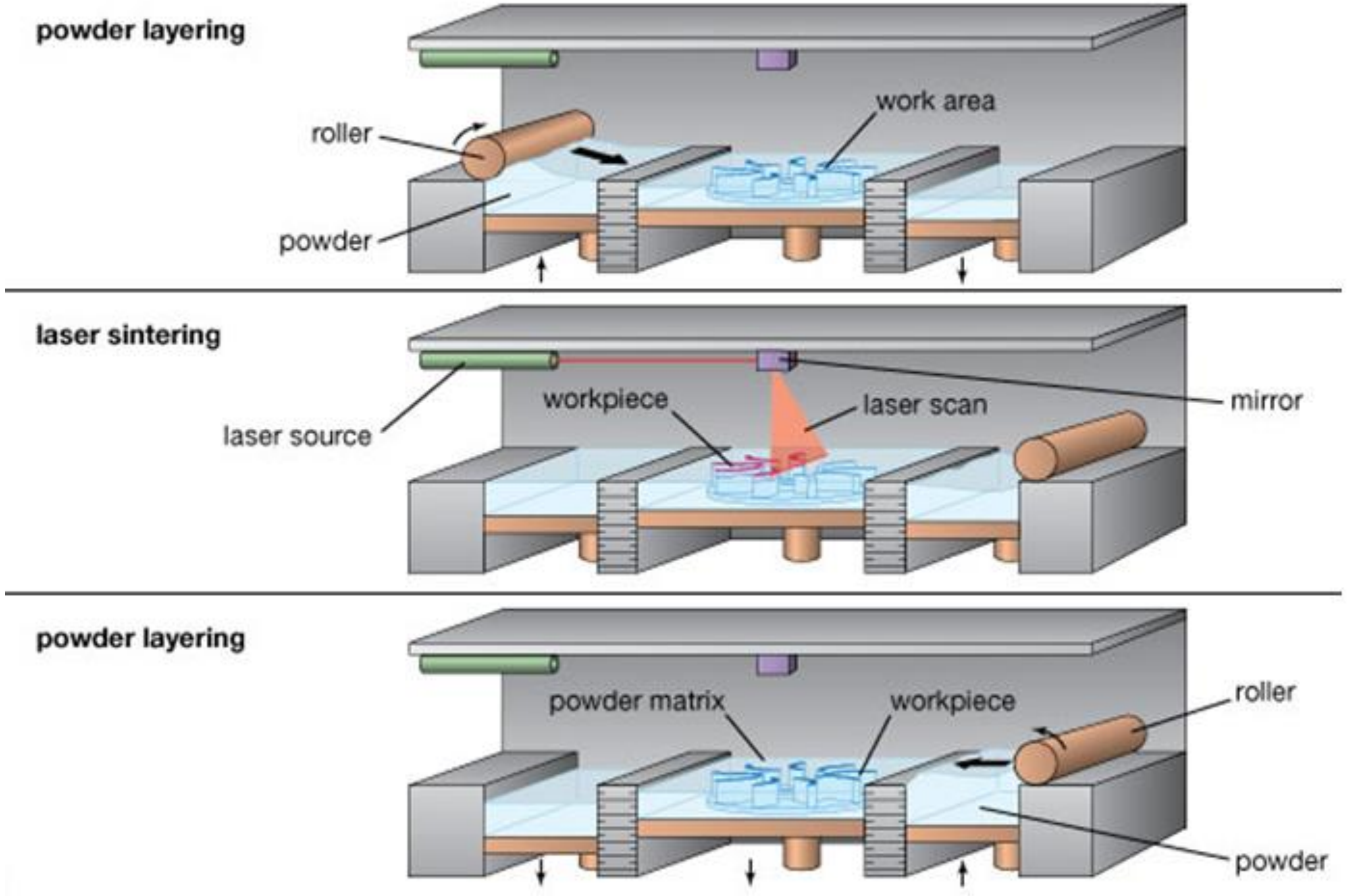
Laser-based

Laser-based rapid prototyping techniques for TE

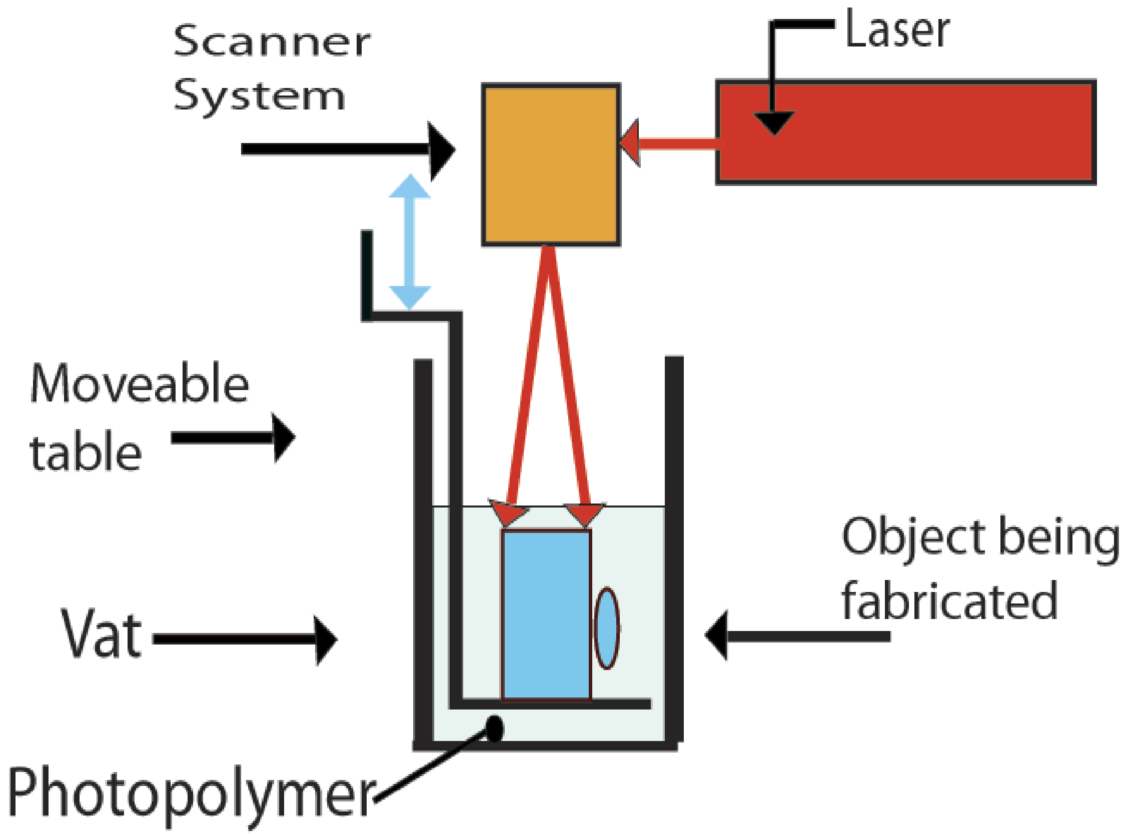
- Laser-based RP
  - Selective Laser Sintering (SLS)
    - Only non-cell laden scaffolds
  - Stereolithography (SLA)
    - Liquid photosensitive polymer
    - Compatible with hydrogels
  - Micro Stereolithography (μ-SLA)
  - Two-photon polymerization (2PP)
    - Combination with Laser-Induced Forward Transfer
  - Multiphoton polymerization (MPP)

Laser-based rapid prototyping techniques for TE

SLS



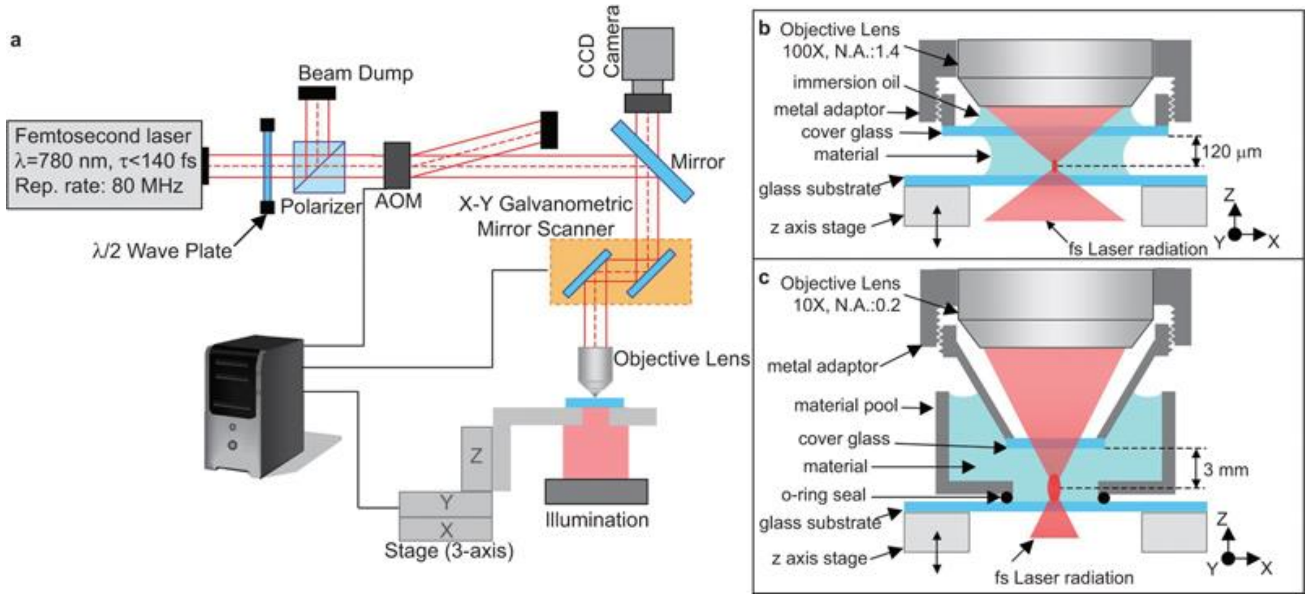
Laser-based rapid prototyping techniques for TE

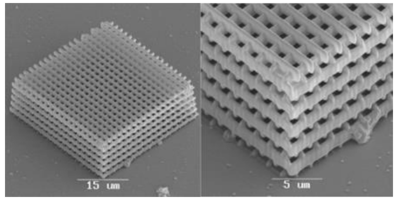


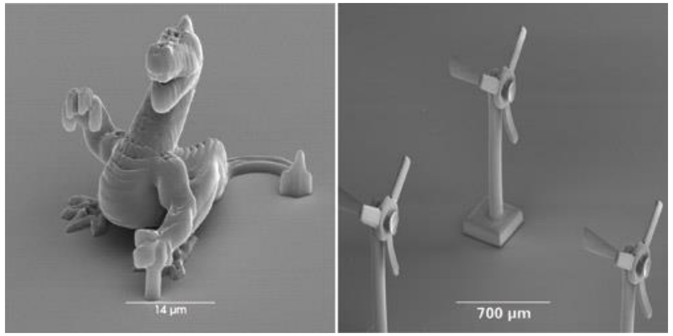
Stereolithography (SLA)

Laser-based rapid prototyping techniques for TE

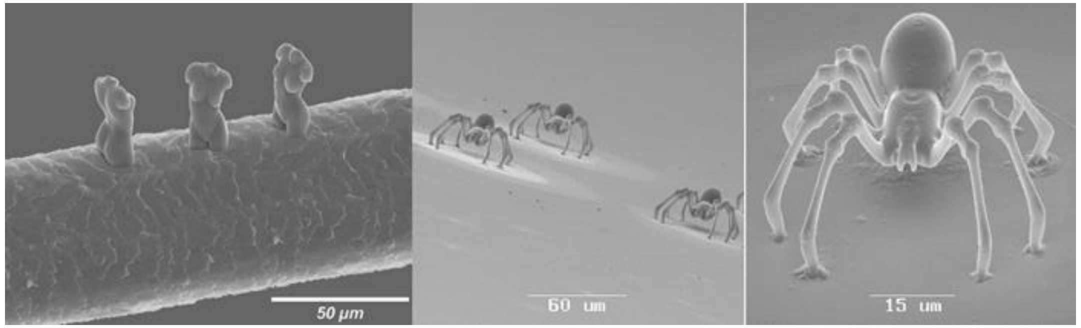
- Two-photon polymerization







Resolution: 100 – 200 nm



Laser-based rapid prototyping techniques for TE

- Selective Laser Syntering (SLS)
- Stereolithography (SLA)
- Micro-stereolithography (μ-SLA)
- Two-photon polymerization (2PP)
- Multi-photon polymerization (MPP)

- Hydrogel Materials
  - Gelatin-methacrylate
  - Hyaluronic acid-methacrylate
  - Agarose
  - HEMA
  - PEG-D(M)A
  - Alginate
  - PEG-DA
  - Fibronectin
  - Bovine serum
  - BSA

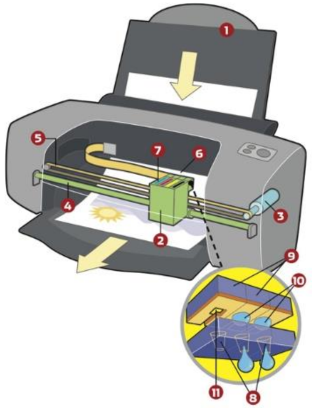
- Encapsulated cells
  - Fibroblasts (NIH-3T3)
  - Human hepatoma cells (HepG2)
  - Hepatocytes
  - Human dermal fibroblasts
  - Marrow stromal cells
  - Chondrocytes

Printer-based

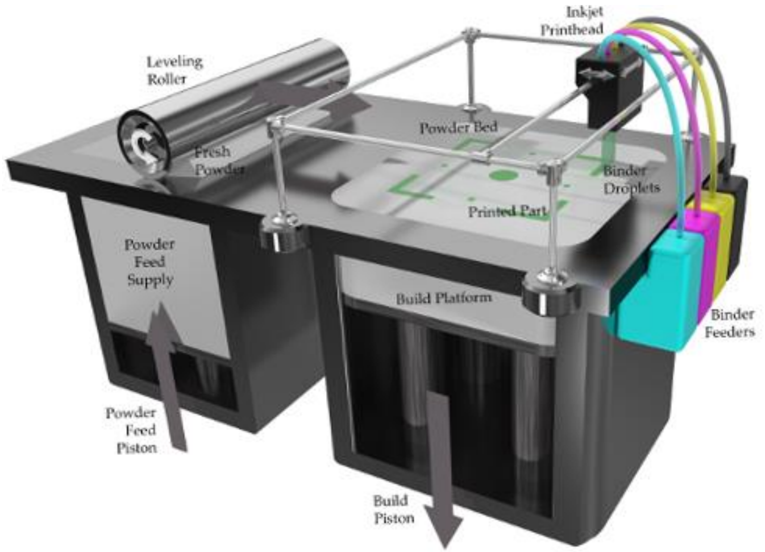
Printer-based rapid prototyping for TE

- Inkjet
  - Hydrogels
- 3DP™
  - Ceramic
  - Metal
  - Metal-ceramic composites
  - Polymers (PCL, PLA, PEO)

Inkjet



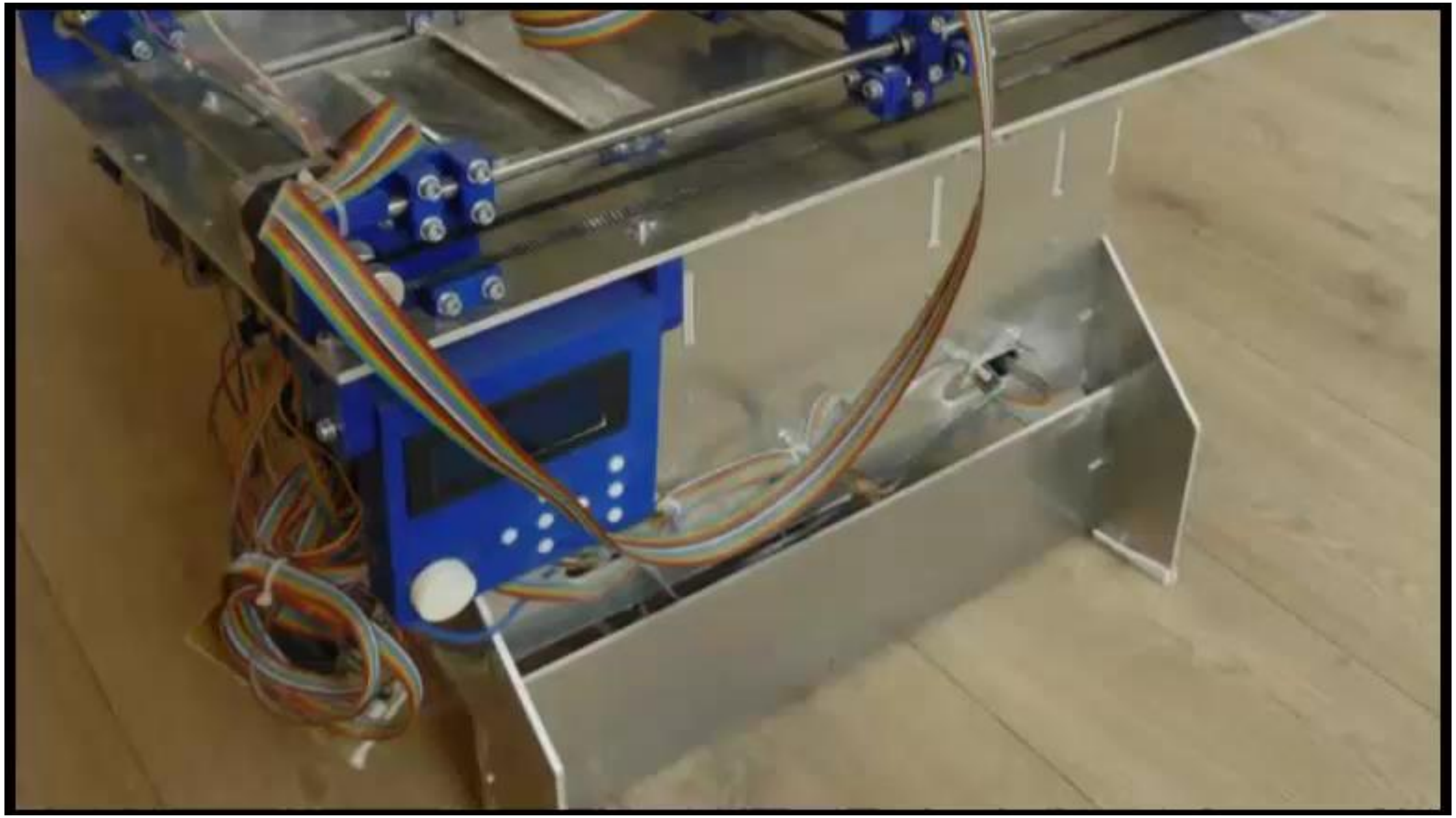
3DP



Requires biocompatible powder-binder systems

Inkjet rapid prototyping for TE

3DP

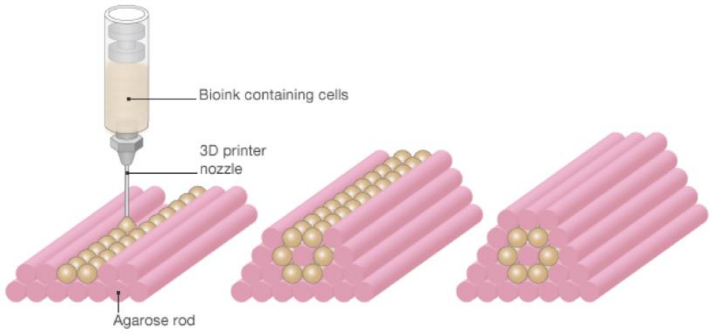


Printer-based rapid prototyping limitations

- Mechanical strength (post-processing often required)
- Powder entrapment
- Resolution 100 µm
- Binder droplet size and accuracy of drop placement
- Clogging of small binders jets
- Pore sizes of the fabricated scaffolds limited by powder grain size.
  - Available pore sizes 50 µm -250 µm

Current trends on scaffold manufacturing for tissue enginering applications

- Bioprinting (extrusion)
  - Cell-seeded alginate hidrogel
  - Spheroids with several cell types
  - Extrusion based + cell-seeded hidrogel
    - Decellularized ECM (dECM)

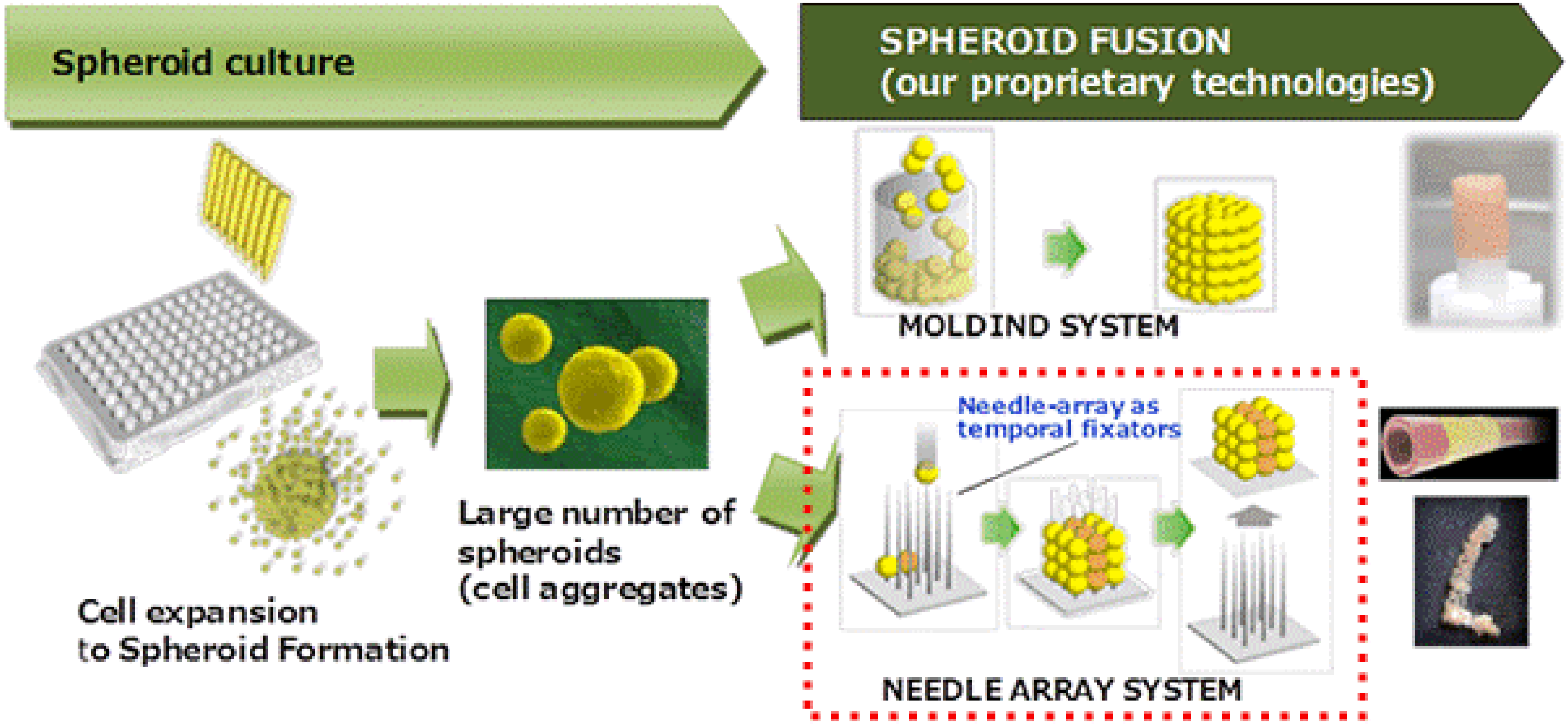


- Ink-jet printing

- Powder printing

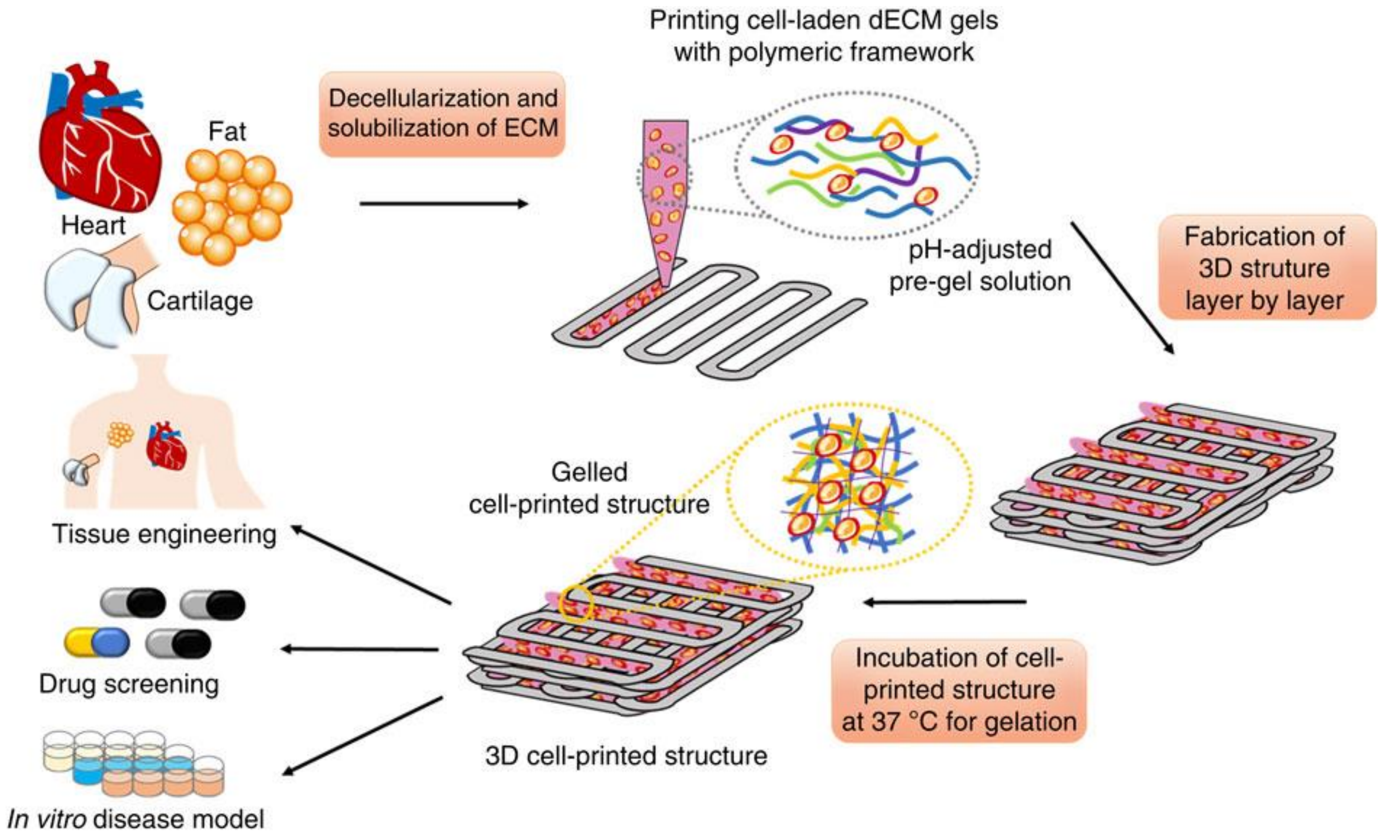
- Multi-photon polymerization
  - Combination with laser-induced forward transfer (LIFT)

Bioprinting trend example: Spheroids for vasculature generation



Bioprinting: Extrusion-based + Ceel seeded hydrogel

- Decellularized ECM (dECM)

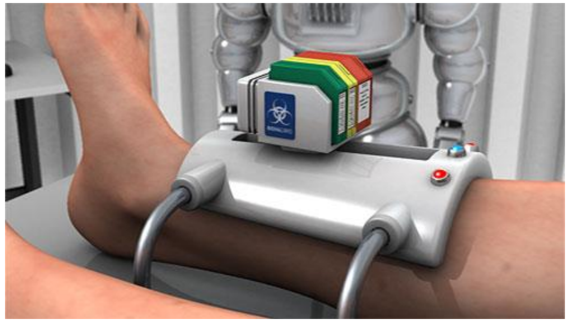


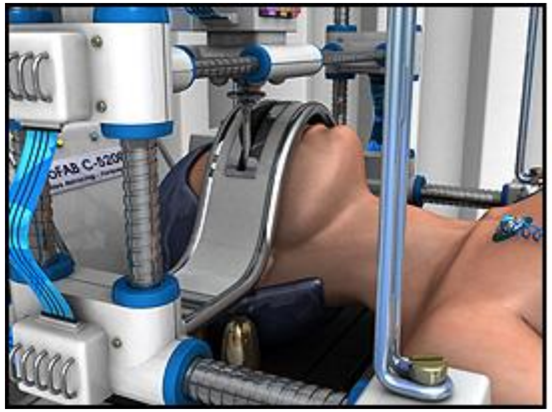
Commercially available machines for 3D printing

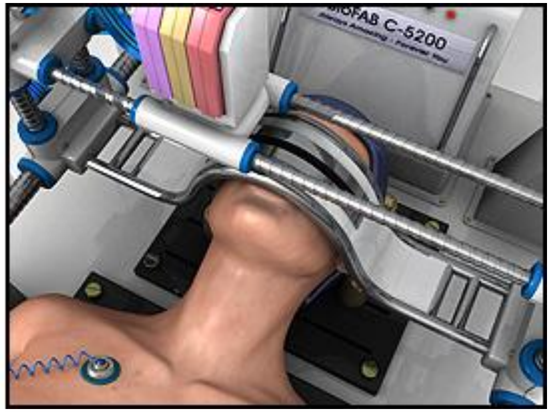
- 3D-Bioplotter from EnvisionTEC
- Fab@Home (Hydrogel compatible)
- Formiga P100 from EOS (CO2 laser syntering machine)
- ZPrinter® 310 Plus
  - Composite materials
  - Direct casting
  - Elastomers

Future work

- In situ Bioprinting

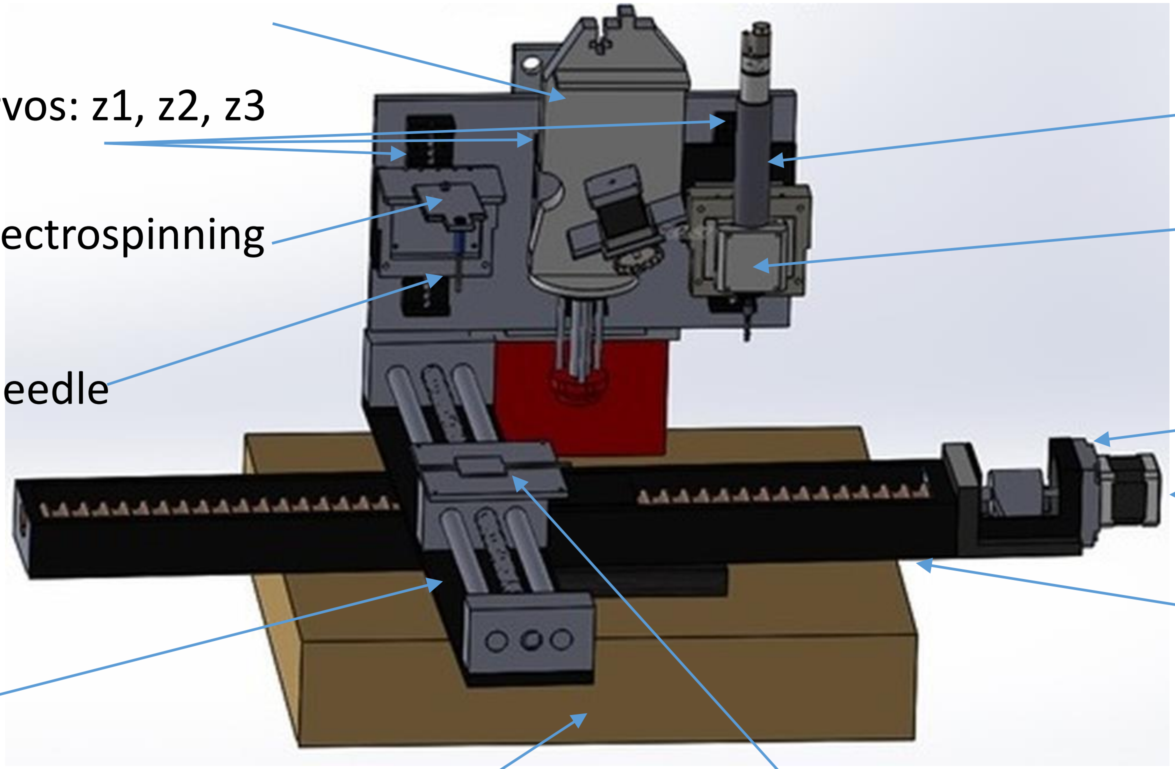




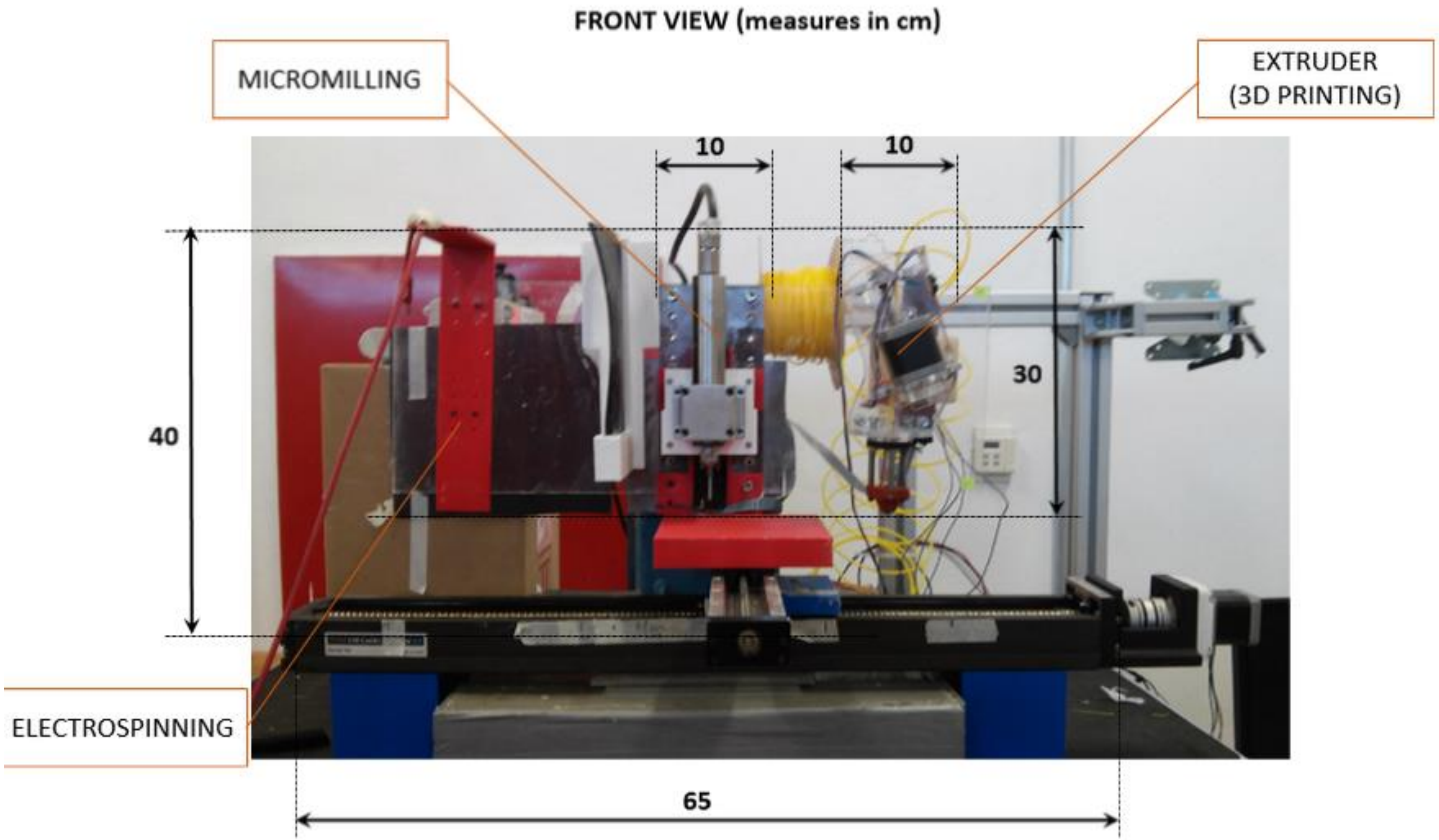


Current work at ITESM

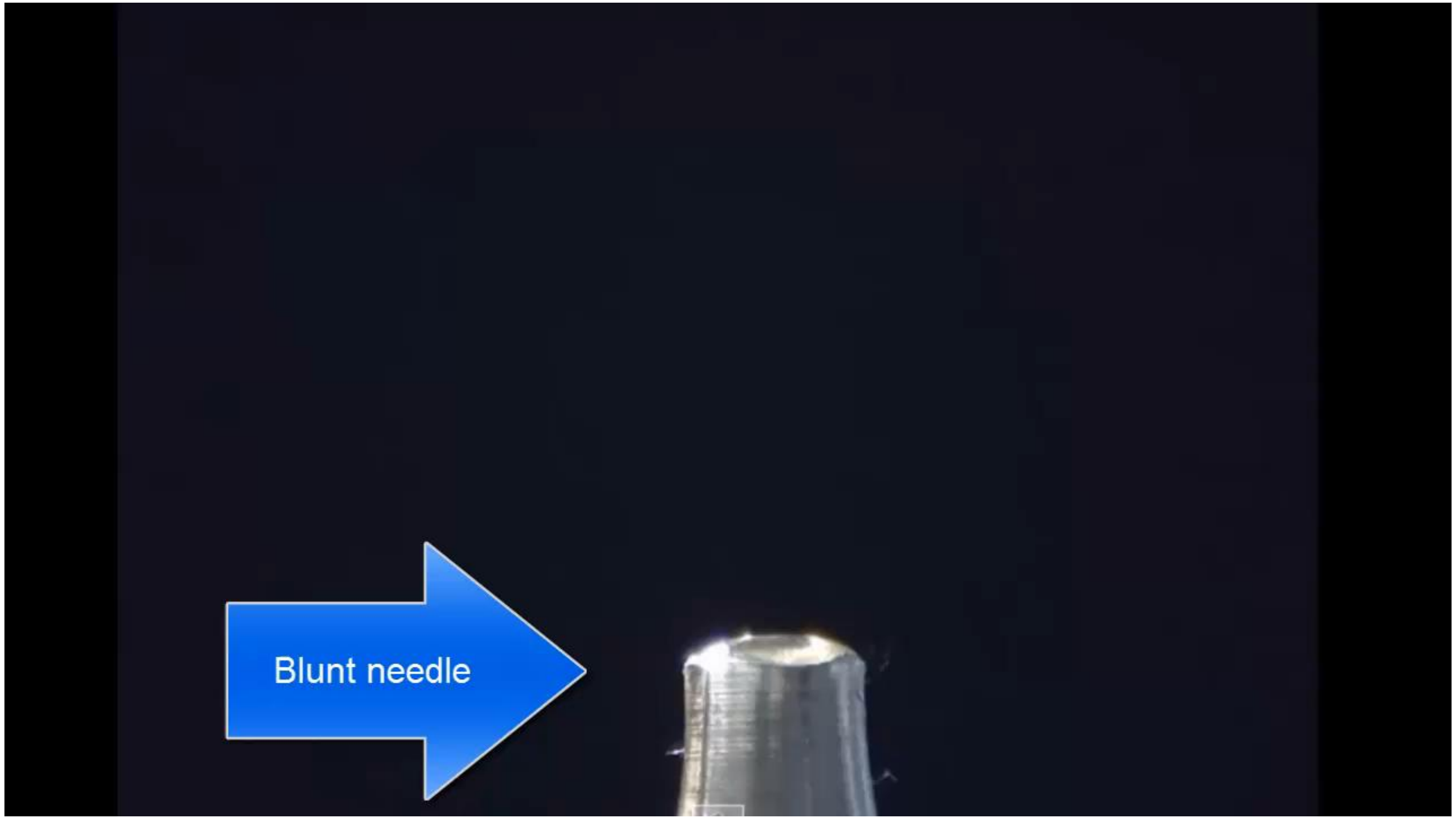
Current work on hybrid scaffold manufacturing at ITESM Monterrey



Current work on hybrid scaffold manufacturing at ITESM Monterrey

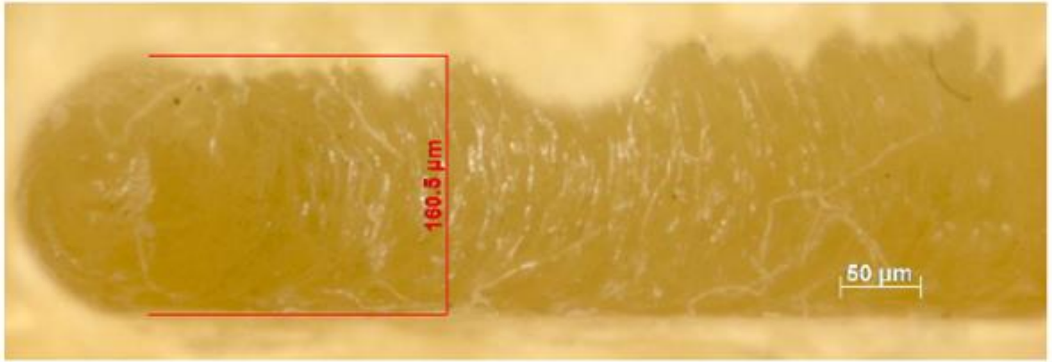


Electrospinning



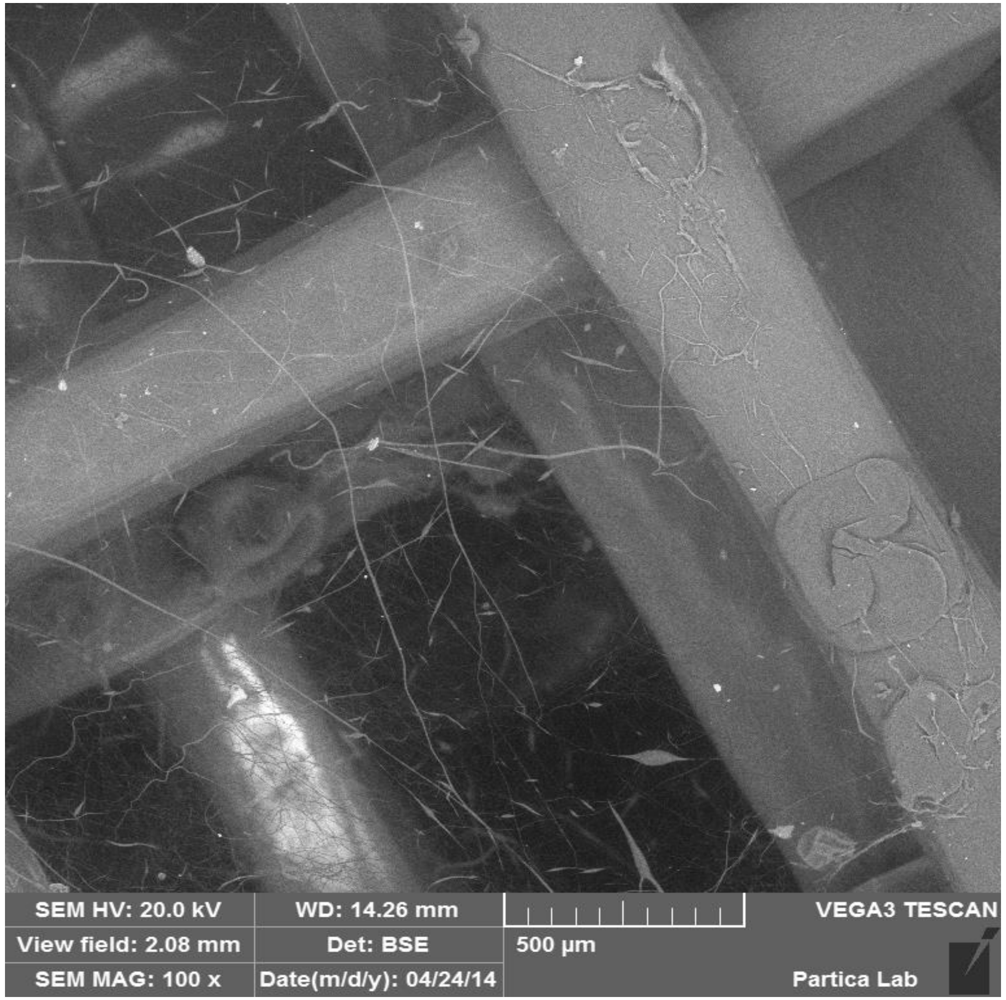
Results

- Micromilling
- 3D printing + Electrospinning

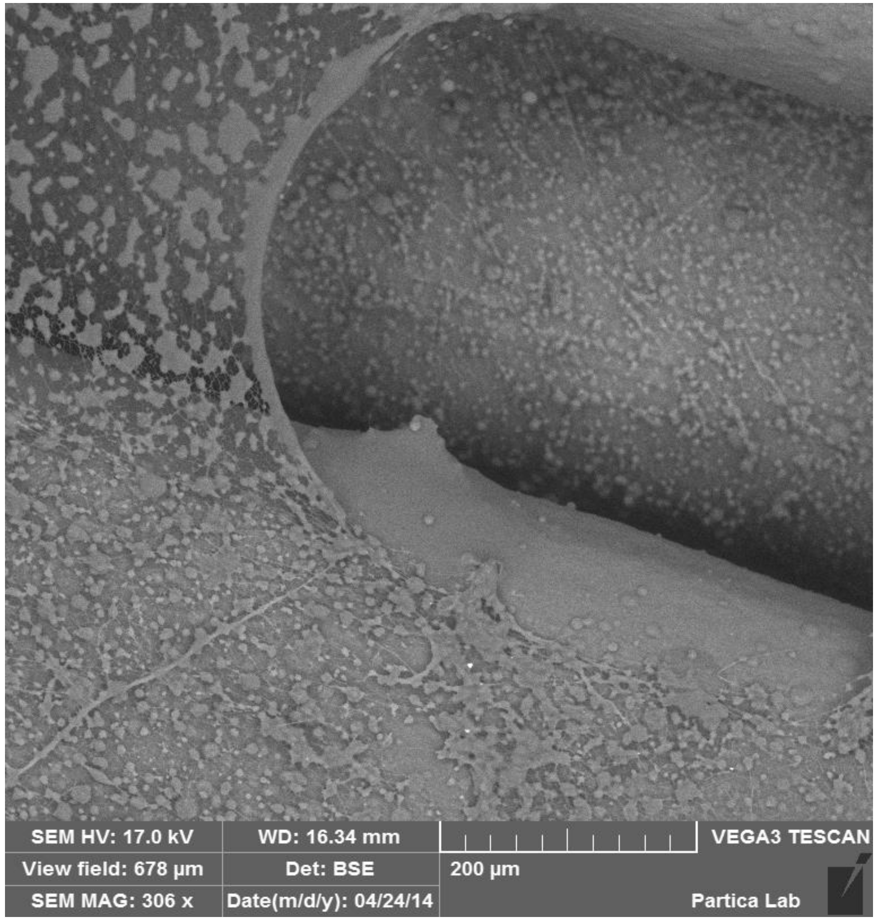


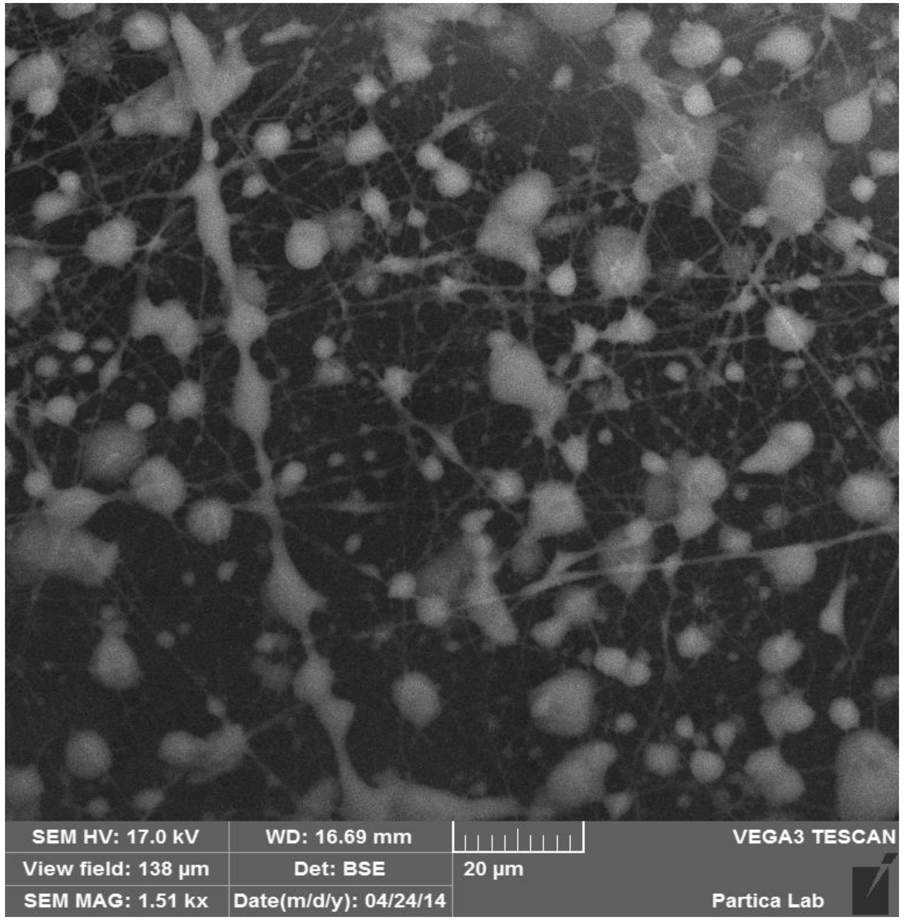
Combining 3D printing and electrospinning

- Thick fibers: PLA
- Thin fibers: Electrospun PCL



Combining 3D printing and electrospinning





Thank you

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