Nano and Microscale 3D Bioprinting: An Enabling Technology for Precision Regenerative Medicine

Shaochen Chen, PhD
Professor of NanoEngineering and Bioengineering Departments
Co-Director, Biomaterials and Tissue Engineering Center
Institute of Engineering in Medicine (IEM)
University of California, San Diego
Outline

I. Introduction & Motivation

II. Micro and Nanoscale 3D Bioprinting
   • Processes
   • Biomaterials

III. What Can 3D Bioprinting Achieve?
   • Creating Designer Pores and Shapes
   • Printing Heterogeneous Scaffolds
   • Digital Patterning Mechanical Properties
   • Bioprinting with Cell Encapsulation

IV. Concluding Remarks
**THE NEW ERA OF REGENERATIVE MEDICINE**

Dozens of biotech companies and university labs are developing ways to replace or regenerate failed body parts. Here are a few of the projects:

**BONE**

Bone-growth factors or stem cells are inserted into a porous material cut to a specific shape, creating new jaws or limbs. A product that creates shinbones is in clinical trials.

**COMPANIES:** Creative Biomolecules, Orquest, Sulzer Orthopedics Biologics, Genetics Institute, Osiris Therapeutics, Regeneron.

**SKIN**

Organogenesis’ Apligraf, a human-skin equivalent, is the first engineered body part to win FDA approval, initially for leg ulcers. Other skins are in the works for foot ulcers and burns.

**COMPANIES:** Organogenesis, Advanced Tissue Sciences, Integra LifeSciences, LifeCell, Ortec International.

**PANCREAS**

Insulin-manufacturing cells are harvested from pigs, encapsulated in membranes, and injected into the abdomen. The method has been tested in animals and could be in human trials in two years.

**COMPANIES:** BioHybrid Technologies, Neocrin, Circe Biomedical

**HEART VALVES, ARTERIES, AND VEINS**

A 10-year initiative to build a heart has just started. Genetically engineered proteins have been successfully used to regrow blood vessels.

**COMPANIES:** Organogenesis, Advanced Tissue Sciences, Genetech, LifeCell, Reprogenesis.

**TEETH**

Enamel matrix proteins are used to fill cavities. It works in dogs; human trials are a few years away.

**COMPANIES:** Biora, Atrix Laboratories, Creative BioMolecules.

**BREAST**

In preclinical studies, several companies have been able to create a cosmetic nipple by inserting a ball of cartilage. Researchers are now trying to grow a whole cosmetic breast.

**COMPANIES:** Reprogenesis, Integra LifeSciences.

**LIVER**

A spongy membrane is built up and then seeded with liver cells. Organs the size of a dime have been grown, but a full-size liver could take 10 years due to its complexity.

**COMPANIES:** Advanced Tissue Sciences, Human Organ Sciences, Organogenesis.

**CARTILAGE**

A product is already on the market that regrows knee cartilage. A chest has been grown for a boy and a human ear on a mouse.

**COMPANIES:** Genzyme Tissue, Biomatrix, Integra LifeSciences, Advanced Tissue Sciences, ReGen Biologics, Osiris Therapeutics

**SPINAL CORD NERVES**

Scientists are investigating nerve-growth factors, injecting them at the site of damage to encourage regeneration or seeding them along biodegradable filaments and implanting them. Rats have been made to walk again.

**COMPANIES:** Acorda, Regeneron, CytoTherapeutics, Guilford Pharmaceuticals.
Tissue Engineering Approaches

- ECM Biomolecules & Growth Factors
- Mechanical Stimulation
- Gene Delivery
- Physical Guidance

Engineered Tissue

- Repair of Injury
- Drug Screening Microenvironment
- Wound Healing
- Organ Replacement
Conventional 3D Printing

A typical nozzle-based bioprinter
- ~300 μm resolution
- slow printing speed

http://mariakonovalenko.wordpress.com

Laser Stereolithography
- μm and nm resolution
- slow printing speed

Two-Photon 3D NanoPrinting

**Mechanism:**
1. Absorb two photons simultaneously
2. Only the central portion of the focal point is polymerized

Nano-Printing of Scaffolds

- Multi-layer logpile scaffold
- Nano/sub-micron dot array
- Gradient dot array

Scanless and Continuous 3D Printing

Complex 3D Array with Smooth Sidewalls

Continuous printing is key to eliminate artificial interfaces between drops and layers in nozzle-based 3D printing.

1,000 times faster than a conventional bioprinter!

Materials Used for 3D Printing

• **Poly(ethylene glycol) Diacrylate (PEGDA)**
  – Photo-crosslinkable
  – Tunable mechanical properties
  – Nontoxic, non-immunogenic
  – Can be chemically modified (i.e., RGD, heparan sulfates)

• **Gelatin Methacrylate (GelMA)**
  – Biodegradable
  – Cell adhesive

• **Hyaluronic Acid (HA)**
  – Biodegradable
  – Cell adhesive if covalently functionalized with a cell-adhesive protein (e.g. laminin using EDC-NHS)
Physical Control in Direct Printing of 3D Scaffolds

Digital image may come from
- Computer design (CAD)
- CT scan
- MRI scan

Scaffolds with various pore shapes

- A large surface area favors cell attachment and growth;
- A large pore volume accommodates and delivers a sufficient number of cells;
- High porosity for easy diffusion of nutrients, transport & vascularization.

Physical Control: Functions-on-a-Chip

3D printed neuron conduits in HA

Competing Cues on-a-Chip: Grooves and NGF gradient for hippocampal cell


Chemical Control: Scaffolds with a Chemical Gradient

Red and green fluorescent particles encapsulated in PEGDA

HA Scaffold with two gradients running in opposite directions. Red and green fluorescent microparticles were mixed in a prepolymer solution before printing (scale bar = 1 mm)

Polydiacetylene (PDA) nanoparticles (green) are installed in PEGDA hydrogel matrix (grey) with liver-mimetic 3D structure fabricated by 3D printing. The PDA nanoparticle surface is made of a π-conjugated polymer with alternating double- and triple-bond groups in the main polymer chain.

Gou, Qu, Zhu, Xiang, Yang, Zhang, Wei, and Chen, *Nature Communications, 5: 3774 (2014)*
Mechanical Control: Patterned Stiffness

Cell Alignment on Stiffness-Patterned Hydrogel

In vitro cell migration. A7R5 smooth muscle cells show migration to the stiffer regions patterned by the digital plasmonic patterning method.

Fluorescent staining shows their actin (red) and nuclei (cyan).

Mechanical: Scaffolds With a Negative Poisson’s Ratio

News Report by: Science Daily, R & D Magazine, Health Science, Plus Magazine...
Biological Control: Cell-Laden 3D Scaffolds

Summary

- Light-based 3D printing approaches offer micro and/or nano-scale resolution,
- BioPrinting allows direct-write of 3D, novel, biomimetic biological scaffolds with control of chemical, biological, and mechanical properties:
  - porosity, shape, stiffness, Poisson ratio
  - chemical composition
  - cell encapsulation
- A variety of biopolymers (e.g. PEG, GelMA, HA) can be used for precision tissue models \textit{(in vivo and in vitro)}
Acknowledgement

**Former Group Trainees:**
Shifeng Li (Life Technologies)   Carlos Aguilar (MIT Lincoln Lab)
Dongbing Shao (IBM)             Senthil Theppakuttai (SV Probe)
Yi Lu (3M)                      Arvind Battula (Schlumberger)
Jamil Wakil (IBM)               Shaomin Wu (Spansion)
David Fozdar (SandRidge Energy) JinWoo Lee (Assist Prof, Gachon Univ)
Andrew Wang (China Energy)      Pranav Soman (Assist Prof, Syracuse Univ)
Wande Zhang (Bank of America)   Leo Han (Assist Prof, Drexel Univ)
Paul Qu (Investigator, Novartis Inst)

**Current PhD Students/Postdoc Researchers:**
John Warner         Kolin Hribar    Justin Liu       Peter Chung
Wei Zhu             Michelle Ma     Ray Wang         Kyle Meggs

**Collaborators:**
Shu Chien (UCSD)    Inder Verma (Salk)
Adam Engler (UCSD)  Ali Khademhosseini (Harvard)
Andrew McCulloch (UCSD) Farah Sheikh (UCSD)
Yang Xu (UCSD)      Kang Zhang (UCSD)
Sponsors

**NSF** (CAREER award and Scalable Nanomanufacturing program)

**NIH** (Edward Nagy Award for 3D Bioprinting, R21 Bioprinting of Stem Cells)

**ONR** (Young Investigator award), **AFOSR** (nano-optics), **CDMRP**

**CIRM** (3D Bioprinting of hESCs)
You are welcome to visit us at UC San Diego!