Bioprint Design & Use of Imaging Part 1

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Prof. Angela Panoskaltsis-Mortari’s BMEn 5361, 3D Bioprinting
Part 1 – Design & Imaging

- Tissue engineering
- Bioprinting
- Design considerations
- Imaging modalities
- Segmentation software
- *In Part 2 we will look at some examples from the literature.*
Tissue Engineering

- **Tissue Engineering Components:**
  - The type or types of living cells being implanted (e.g. somatic, embryonic stem cells, adult stem cells, or induced-pluripotent stem cells).
  - Type of scaffolds supporting the cells (i.e. the mechanical cues provided to the cells).
  - Type of drugs, extra-cellular matrix (ECM), and growth factors conditioning the cells, (the additives that provide chemical cues to the cells).

- **3D Printing:**
  - Computer assisted process for depositing biomaterials and living cells in a determinate configuration in order to produce a defined 3D biological structure.
  - Bioinks consist of various polymer materials, cells and additives.

Three Approaches to Tissue Building…

Our focus today

Bioprinting Methods

(A) Inkjet Bioprinting

(B) Microextrusion Bioprinting

(C) Laser-assisted Bioprinting

(D) Stereolithography Bioprinting

Advantages of Bioprinting...

- Anatomically correct constructs from medical imaging data.
- Porous structures.
- Co-culturing of multiple cell types.
- Precise patterning of cells and ECM.
- Controlled deliver of growth factor and genes.
- Potential for high-throughput fabrication.
- Challenge – suitable vascularization.
  - Diffusion length of oxygen/nutrients is 100–200 µm.
Pathway for Bioprinting 3D Tissue

Workflow

1) Design – What are you trying to achieve? What bioinks and printing method?
2) Images – Useful from the subcellular to organ level. Apply CAD tools for segmentation, freeform and other space-filling methods.
3) Slicer – G-code generation for controlling toolpath, speed, valves, droplet patterns, laser pulse, photoinitiator lights (*e.g. Ingracure and other gels*), temperature etc.
4) Special setups – Extruder, tips, light source (specific nm), pressure & calibration, cooling (*Pluronic Gel*) etc.
5) Bioprinting
6) Bioreactor – Incubation, nutrients, growth factors, oxygen supply, environment, etc. (*Your 4th dimension –time!*)
7) Observation – Fluorescent and transmitted light (*confocal microscopy, bright-field, dark-field, confocal laser microscopy* etc.).
   - Automatic imaging with control of ambient air, humidity and temperature.
8) Characterization – Histology, growth, mechanical properties etc.
Design Considerations

Design Considerations for Bioprinting

Data Processing
- Data from Medical Imaging
- CAD Drawing
- 2 layer Tracing
- Computer Simulated Data

Bio-ink Formulation
- Coordinate control
  - G Code
  - Processing Parameters
- Cell Surface Modification
  - DNA template conjugated
- Cell Modification
- Conjugating Stimulus Responsive Unit
  - Temperature
  - pH
  - MMP
- Adding Property Enhancing Units
  - Electrical (nanowires, carbon nanotubes, etc)
- Biomaterial Modification

Process Compatibility Consideration
- Orifice-Based Process
  - Extrusion/Microvalve
  - Inkjet Printing
- Orifice & Cell size
- Jetting-Based Process
  - Inkjet Printing
  - Laser-Induced Forward Transfer
- Viscosity, Surface tension & Jetting

Design Factors...

What are involved?
- Biomaterial
- Cells
- Growth factors

What happened?
- Effect of print process on materials
  - Radical
  - Thermal
  - Shear
  - Process-induced Damage
  - Setup’s restriction
  - Orifice size

How to print?
- Technology: Extrusion, Inkjet, LIFT, Stereolithography
- Print toolpath: Coordinate Control, Interface between different technology

What can be printed?
- Any virtual shape
- Droplets VS Strands
- 2D patterning
- Anatomical Shape
- Heterogeneous material
  - Heterogeneous Cell
  - Heterogeneous Biomaterial
  - Patterned growth factor

Figure 1. A design map showing varies design factors in bioprinting which is essential for evaluation during the pre-printing stage.

Table 1. Highlighting various design considerations that attributes to the printing of engineered biological constructs.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Considerations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shape and Resolution</td>
<td>Anatomical resemblances Material formed as droplet, strand, spots etc. Layer thickness</td>
</tr>
<tr>
<td>Material Heterogeneity</td>
<td>Cell Source</td>
</tr>
<tr>
<td>Cells</td>
<td>• Autologous, Allogeneic or Xenogeneic</td>
</tr>
<tr>
<td>• Single Cells</td>
<td></td>
</tr>
<tr>
<td>• Cell aggregates (alias Tissue Spheroids)</td>
<td></td>
</tr>
<tr>
<td>• Multiple cell types</td>
<td></td>
</tr>
<tr>
<td>Biomaterials</td>
<td>Cell Type</td>
</tr>
<tr>
<td>• Hydrogel Collagen, Gelatin (and derivatives), Agarose, Alginate, Fibrin e.t.c.</td>
<td>• Sterm Cells or Specialized Cells</td>
</tr>
<tr>
<td>• Thermoplastic</td>
<td></td>
</tr>
<tr>
<td>PLGA, PCL, PLA, e.t.c.</td>
<td></td>
</tr>
<tr>
<td>Growth factor [43]</td>
<td></td>
</tr>
<tr>
<td>• Patterning and precise location</td>
<td></td>
</tr>
<tr>
<td>Cellular-material remodeling dynamism</td>
<td></td>
</tr>
<tr>
<td>Cell Activities [44, 45]</td>
<td></td>
</tr>
<tr>
<td>• Cell fusion, sorting</td>
<td></td>
</tr>
<tr>
<td>Material Changes [47]</td>
<td></td>
</tr>
<tr>
<td>• Degradation</td>
<td>Structural Changes</td>
</tr>
<tr>
<td>• Shape change (shrinkage or expansion)</td>
<td>Time evolution</td>
</tr>
<tr>
<td>Cell-Material Interaction [16, 46]</td>
<td></td>
</tr>
<tr>
<td>• Cell remodeling</td>
<td></td>
</tr>
</tbody>
</table>

Shape Fidelity...

Figure 3. A bioprinting process that uses combination of different printing strategies to improve print fidelity and resolution.
Table 2. Resolution of different bioprinting technologies from droplet forming technologies to techniques that produces continuous strands.

<table>
<thead>
<tr>
<th>Types of Technique</th>
<th>Resolution</th>
<th>Form of material deposition</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piezoelectric / Thermal Inkjet Printing</td>
<td>100 μm</td>
<td>- Droplets jetted onto substrate</td>
<td>[26,102,103]</td>
</tr>
<tr>
<td>Electro Hydrodynamic Jetting</td>
<td>10–20 μm</td>
<td></td>
<td>[104–108]</td>
</tr>
<tr>
<td>Acoustic Droplet Ejection</td>
<td>37–150 μm</td>
<td>- Continuous droplets</td>
<td>[109,110]</td>
</tr>
<tr>
<td>BioLP / AFA-LIFT / MAPLE-DW</td>
<td>10–100 μm</td>
<td>- Deposited to form line</td>
<td>[27,65,72,111]</td>
</tr>
<tr>
<td>Mechanical/Pneumatic Extrusion</td>
<td>15–400 μm</td>
<td>- Extrude continuous hydrogel line</td>
<td>[17,19,51,54,68,70,83,86,112–116]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Continuous droplets</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Deposited to form line</td>
<td></td>
</tr>
<tr>
<td>Laser Guided Direct Writing</td>
<td>100 nm – 10 μm</td>
<td>Single cell manipulation</td>
<td>[117,118]</td>
</tr>
<tr>
<td>Stereolithography (SLA)</td>
<td>~1 mm</td>
<td>- Shapes (line/dot) form through selective curing of photopolymer</td>
<td>[119]</td>
</tr>
<tr>
<td>Digital Light Processing (DLP)</td>
<td>20–200 μm</td>
<td></td>
<td>[120,121]</td>
</tr>
<tr>
<td>Properties</td>
<td>Design Consideration</td>
<td>Selections Affecting Tissue Construct Properties</td>
<td></td>
</tr>
<tr>
<td>---------------</td>
<td>--------------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Mechanical</td>
<td>Structural integrity, internal architecture stability, strength, and stiffness</td>
<td>Bioink selection, internal architecture, porosity and pore distribution, bioprinting modality</td>
<td></td>
</tr>
<tr>
<td>Biological</td>
<td>Cell loading, cell distribution, nutrition, cell attachment and growth, cell viability and differentiation, cell—cell and cell—matrix interactions, cell aggregation, and tissue formation</td>
<td>Layout, pore size, interconnectivity, vasculature, cell density, bioink selection, bioprinted cell types</td>
<td></td>
</tr>
<tr>
<td>Geometric</td>
<td>Anatomical fit, tissue topology</td>
<td>External geometry, tissue density</td>
<td></td>
</tr>
<tr>
<td>Transport</td>
<td>Nutrient and oxygen delivery, waste removal, growth factor, and drug delivery</td>
<td>Interconnectivity and permeability, bioink selection</td>
<td></td>
</tr>
<tr>
<td>Bioprinting</td>
<td>Environmental conditions during bioprinting, bioprinting parameters, control, and resolution</td>
<td>Bioink and bioprinting modality selection, bioprinter</td>
<td></td>
</tr>
</tbody>
</table>

(Modified from Gomez, 2007.)
Design Techniques...

- Underlying methods in CAD systems:
  - Constructive solid geometry (solid primitives and boolean operators)
  - Boundary representation (vertices, edges and faces)
  - Spacial enumeration (cubic elements)

- Image–based design
- Implicit surfaces
- Space–filling curves
- Irregular porous structures

Library of CAD-based Primitives

Lay-down Patterns

Honeycomb pores

Hilbert recursive curves

## Different Fiber Arrangements for Extrusion

<table>
<thead>
<tr>
<th>Typology</th>
<th>Arrangement</th>
<th>Schematic diagram</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single material scaffolds</td>
<td>Homogeneous fiber spacing</td>
<td><img src="image" alt="Schematic" /></td>
<td>[48,51,52,65]</td>
</tr>
<tr>
<td>Double layer configuration</td>
<td></td>
<td><img src="image" alt="Schematic" /></td>
<td>[52]</td>
</tr>
<tr>
<td>Staggered fiber spacing</td>
<td></td>
<td><img src="image" alt="Schematic" /></td>
<td>[51,55,64]</td>
</tr>
<tr>
<td>Pore size gradients</td>
<td></td>
<td><img src="image" alt="Schematic" /></td>
<td>[65]</td>
</tr>
<tr>
<td>Hybrid scaffolds</td>
<td>Bi/multimaterial</td>
<td><img src="image" alt="Schematic" /></td>
<td>[36,117]</td>
</tr>
</tbody>
</table>

Our focus today

Some Common Imaging Methods

- **Magnetic Resonance Imaging (MRI)**
  - Human max. is 3T (Tesla) – resolution of 250µm x 250µm 0.5mm.
  - High spatial resolution µMRI, 7–10T, 5–200µm.
  - Magnetic nanoparticles.

- **Computed tomography (CT)** – *Computer Axial Tomography*
  - Typical resolution of 0.24 – 0.3mm.
  - µCT, resolution of 1–200µm.

- **Ultrasound**
  - Resolution of 1mm x 1.mm x 0.2mm.

- **PET** – Positron emission tomography
- **SPECT** – Single photon emission computed tomography
- **Optical Coherence Tomography (OCT)**
- Traditional optical techniques.
Magnetic Resonance Imaging (MRI) & Computed tomography (CT)
Ultrasound

Mayo Foundation for Medical Education and Research
Positron Emission Tomography (PET)

CT scan/PET Scan/ Combined
Mayo Foundation for Medical Education and Research
Segmentation

Purpose
- To delineate and isolate anatomical features within an imaging database—e.g. bone, cartilage, soft tissue, edema; muscle, lung, brain & other organs, and tumors.

Method
- Extract images from DICOM files (ITK-Snap, Onis) and possible deidentifying them for HIPPA regulations (DICOMCleaner).
- Segmentation Software (ITK-Snap, Materialise Mimics, Materialise 3-matic).
  - Pre-segmentation Phase – identify parts of image as foreground and background.
  - Active Contour Phase – manual and semiautomatic methods.
- Editing and fixing mesh files (.STL) – Autodesk Meshmixer.
- Slicer software – Simplify3D and Repetier.
  - G-coding for the specific bioprinter – e.g. Slic3R (printer customized interface to control what happens in a sequence of control steps.)
Main Anatomical Planes

- Sagittal or Median
- Parasagittal (Yellow)
- Frontal or Coronal
- Transverse or Axial
Segmentation with ITK-Snap (freeware)

Manual Segmentation…
Semiautomatic – Contrast Adjustment...
“Balloon” Placement & Inflation...
3D Rendering...
Import the STL Mesh file generated by ITK-Snap.

Edit feature – here slicing in a plane, bottom view.
Summary

- Tissue engineering
- Bioprinting
- Design considerations
- Imaging modalities
- Segmentation software