Bioprint Design & Use of Imaging

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Prof. Angela Panoskaltsis-Mortari’s BMEn 5361,
3D Bioprinting

Design & Imaging

- Approaches to Tissue Engineering.
- Bioprinting Workflow.
- Design Considerations:
  - Design Factors.
  - Shape Fidelity.
  - Design Techniques.
- Image J1 & J2 Software.
- Role of Imaging & Methods.
- Segmentation
- Tool Paths.
- Bioprinter Software.
- Example – Segmenting Chronic Wounds.

Three Approaches to Tissue Building...

**Workflow**

1. **Extruder** - 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. **Biorutting**
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.

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**Design Considerations**

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**Design Factors…**

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Table 2. Resolution of different bioprinting technologies from droplet forming techniques to techniques that produces continuous strands.

<table>
<thead>
<tr>
<th>Types of Technique</th>
<th>Resolution (μm)</th>
<th>Form of material deposition</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piezoelectric / Thermal Inkjet Printing</td>
<td>100–1,000</td>
<td>Droplets printed onto substrate</td>
<td>[61,102,150]</td>
</tr>
<tr>
<td>Electro Hydrodynamic Jetting</td>
<td>10–100</td>
<td>Continuous jets</td>
<td>[70,106,109]</td>
</tr>
<tr>
<td>(ELGP) AM: FDM / FFF</td>
<td>10–100</td>
<td>Degraded to form lines</td>
<td>[71,66,72,77]</td>
</tr>
<tr>
<td>Dielectric Rheostatic Extrusion</td>
<td>1–500</td>
<td>Hydrogel lines</td>
<td>[17,151,154,158]</td>
</tr>
<tr>
<td>Laser Coded Direct Writing</td>
<td>100 nm – 10 μm</td>
<td>Single cell manipulation</td>
<td>[17,73]</td>
</tr>
<tr>
<td>Sintering Photography (SIP)</td>
<td>1 μm</td>
<td>Spheres / micro/nano</td>
<td>[119]</td>
</tr>
<tr>
<td>Digital Light Processing (DLP)</td>
<td>20–200</td>
<td>Through selective curing of photopolymer</td>
<td>[102]</td>
</tr>
</tbody>
</table>
Table 2.1 Tissue Construct Design Consideration and Parameter Selections Affecting Tissue Construct Properties

<table>
<thead>
<tr>
<th>Properties</th>
<th>Design Consideration</th>
<th>Selections Affecting Tissue Construct Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanical</td>
<td>Structural integrity, internal architecture stability, strength, and stiffness</td>
<td>Bone strength, internal architecture, porosity and pore distribution, bioprinting modality</td>
</tr>
<tr>
<td>Biological</td>
<td>Cell loading, cell distribution, nutrition, cell attachment and growth, cell viability and attachment to matrix interactions, cell-cell interaction</td>
<td>Local, pore size, interconnectivity, calcification, cell viability, bone selection, bioprinted cell types</td>
</tr>
<tr>
<td>Geometric</td>
<td>Avascular tissue topology, tissue topography</td>
<td>Tissue geometry, tissue density, interconnectivity and permeability, biodegradation</td>
</tr>
<tr>
<td>Transport</td>
<td>Nutrient and oxygen delivery, waste removal, growth factor, and drug delivery</td>
<td>Biodegradation parameters, control, and resolution</td>
</tr>
<tr>
<td>Bioprinting</td>
<td>Environmental conditions during bioprinting</td>
<td>Bone and bioprinting modularity parameters, bioprinting</td>
</tr>
</tbody>
</table>


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

Lay-down Patterns...

Honeycomb pores

Hilbert recursive curves

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Different Fiber Arrangements for Extrusion

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Image J1 & J2 Software

- Image processing software developed by the NIH.
- Display, edit, analyze, process, save and print color and grayscale images.
- Able to read TIFF, PNG, GIF, JPEG, BMP, DICOM and FITS files.
- Calculate area and pixel value statistics of user-defined selections and intensity-threshold of objects.
- Measure distances and angles.

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- Create density histograms and line profile plots.
- Supports standard image processing functions such as logical and arithmetical operations between images, contrast manipulation, convolution, Fourier analysis, sharpening, smoothing, edge detection, and median filtering.
- Geometric transformations such as scaling, rotation, and flips.
- Useful in evaluating 3D printability of gels through image analysis of lattice structures.

Role of Imaging

- 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 (less useful in bioprinting)
  - Resolution of 1mm x 1mm x 0.2mm.
- PET – Positron emission tomography
- SPECT – Single photon emission computed tomography
- Optical Coherence Tomography (OCT)
- Traditional optical techniques.
**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...

Editing with Meshmixer (freeware)...

Import the STL Mesh file generated by ITK-Snap.

Edit feature - here slicing in a plane, bottom view.
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Toolpaths

- Bioprinting rasters may be in Cartesian vs parametric* form.
  - Consider extrusion-based (EBB) rather than droplet-base (DBB) or laser-based bioprinting (LBB) which are less common and based on manufacturer specific tool paths.

- Why use one method vs the other?
  - Issues arise with resulting printed gradients as excess accumulation of bioink can occur at directional changes.
  - Parametric* modeling/toolpath may be helpful for lumen and other hollow shape object printing.
  - Control of porosity (e.g. bones)

*Parametric implies a variable is dependent on other variables – commonly used to express the coordinates of the points that make up a geometric object such as a curve or surface.

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A. Design of a continuous toolpath and bilayer bioprinted vertebra.
B. Toolpath for graded wound device and bilayer printed device.
C. Comparison of toolpath using cartesian vs parametric coordinates.

Toolpath in Cartesian Coordinates...

A. CAD model of aorta with controlled material composition along the parametric distance u.
B. Femur model with toolpath for controlled porosity along the distance u; sample double layer structure bioprinted using sodium alginate hydrogels.

Toolpath in Parametric Form...

Bioprinter Software

*Repetier Slicer & G-Code Generation…*

Open Repetier Host and load your CAD File.

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Set Layer Height and Nozzle Diameter…

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Set Speed, Pressure and Temperature

Print pressure and temperature are set in BioBots software after the sliced G code file is saved and uploaded.

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Segmenting Chronic Wounds

Literature Learning...

Fig. 1. Measuring wounds using (a) a contact and (b) a non-contact method.


Region-Based
- Region growing - Wound segmentation can be obtained based on the intensity similarity of wound pixels.
- Active Contours without Edges (Chan–Vese Model) – this algorithm detects objects based on region similarities, not relying on gradients.

Edge-Based Methods
- Most chronic wounds have clear and distinguished boundaries, which make it possible to perform the segmentation based on the edges in the images.
- Livewire (Intelligent Scissors) – a seed point is chosen, and the mouse controlled pointer follows the edges, allowing exclusion of detached skin (outlier edges).
- Parametric Active Contours – Snakes – A deformable energy minimizing curve.

Texture-Based Methods
- Houhou–Thiran–Bresson model (HTB)

Workflow & Wound Type Examples...


2D Plane Segmentation Methods...

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