Overview and Challenges of Bioprinting

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S. Presnell has the following potential conflict of interest in relation to this program/presentation:

- Former employee of company that developed and used 3D bioprinting in the production of human tissue products (Organovo, Inc.)
- Shareholder of Organovo, Inc.
Bioprinters, Bioink, and Bioprinting

- **Bioprinter.** An automated instrument for the spatially-controlled deposition of biological materials
- **Bioink.** Biological materials formulated and configured to be dispensed from Bioprinters
- **Bioprinting.** The practice of generating biological outputs through the deposition of one or more Bioink(s) with a Bioprinter

“Biological”–biomaterial, biomaterial + cells, cells?
Why Bioprinting?

• Unit-to-Unit reproducibility
  • Intralot
  • Lot-to-Lot

• Incorporation of living cells (and void spaces) during fabrication
  • More uniform distribution throughout construct (vs. seeding pre-formed scaffolds)

• Spatial Patterning in 3D
  • Hybrid / composite structures
  • Multiple unique components
  • Feature size / resolution
History of the Art

- 2003: Modification of ink-jet printers to controllably dispense cells
- 2003: Automated placement of cell aggregates to create 3D structures
- 2004: Automated extrusion of cells in hydrogel to create 3D structures
A: Live/dead assay on printed BAECs. The image was taken after 3 days incubation (5% CO₂, 37°C), and shows that the endothelial cells attached to the Matrigel™. B: A strip of smooth muscle cells printed onto a collagen gel.

Wilson and Boland 2003 The Anatomical Record A. 272A:491


Smith et al 2004 Tissue Engineering 10, 1566
<table>
<thead>
<tr>
<th>Technology</th>
<th>Mode of Operation</th>
<th>Cell Compatibility</th>
<th>Limitation(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fused Deposition Modeling (FDM)</td>
<td>Extrusion of synthetic thermopolymer, ceramic, or metal at high heat (140-250°C)</td>
<td>Not Compatible</td>
<td>High Temperature</td>
</tr>
<tr>
<td>Selective Laser Sintering (SLS)</td>
<td>Laser-mediated layer-by-layer fusion of polymer, ceramic, or metal in powder form</td>
<td>Not Compatible</td>
<td>High Temperature, Laser Exposure</td>
</tr>
<tr>
<td>Stereolithography (SLA)</td>
<td>Light-directed (UV or Laser) layer-by-layer crosslinking of photocurable polymer</td>
<td>Selectively</td>
<td>Laser/UV Light Exposure</td>
</tr>
<tr>
<td></td>
<td>or pre-polymer in viscous solution</td>
<td>Compatible</td>
<td>Exposure to cross-linking agents</td>
</tr>
<tr>
<td>Laser Initiated Forward Transfer (LIFT)</td>
<td>Indirect laser-mediated transfer of ink solution coated onto a metal or</td>
<td>Selectively</td>
<td>Cell-Material Compatibility</td>
</tr>
<tr>
<td></td>
<td>metal oxide coated surface</td>
<td>Compatible</td>
<td>Mechanical Forces</td>
</tr>
<tr>
<td>Extrusion or Direct Ink Writing (DIW)</td>
<td>Direct extrusion of high-viscosity solutions, hydrogels, and colloidal suspensions</td>
<td>Selectively</td>
<td>Cell-Material Compatibility</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Compatible</td>
<td>Mechanical Forces</td>
</tr>
<tr>
<td>Droplet-based Printing (Ink-jet, etc.)</td>
<td>Deposition of low-viscosity colloidal or cell suspensions in droplet form at</td>
<td>Selectively</td>
<td>Shear Forces, Cell-Material</td>
</tr>
<tr>
<td></td>
<td>high shear rates</td>
<td>Compatible</td>
<td>Compatibility</td>
</tr>
</tbody>
</table>
Bioprinters: Adaptable 3D Printing Technologies

- Cell-compatible 3D printing technologies
  - Extrusion-based bioprinting
  - Droplet-based bioprinting
  - Laser-based bioprinting

### Bioprinters: Comparing the Most Common Modes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Extrusion, or DIW</th>
<th>Droplet-based</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolution</td>
<td>&gt; ~150 µm</td>
<td>~50 µm</td>
</tr>
<tr>
<td>Compatible Ink Viscosity</td>
<td>30 – 6 x 10⁷ mP•s</td>
<td>&lt;10mP•s</td>
</tr>
<tr>
<td>Shear</td>
<td>Highly variable; &lt;10⁵ – 10⁶ s⁻¹</td>
<td>10⁵ – 10⁶ s⁻¹</td>
</tr>
</tbody>
</table>

## Ink/Bioink Formulations

<table>
<thead>
<tr>
<th>Composition</th>
<th>Examples</th>
<th>Printer Compatibility</th>
<th>Considerations for Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymer-based with no live cells</td>
<td>Polycaprolactone (PCL)</td>
<td>FDM; SLS; SLA</td>
<td>High temperature requirements; Cytotoxicity of organic solvents</td>
</tr>
<tr>
<td></td>
<td>Polylactic Acid (PLA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell-laden Hydrogels</td>
<td>Natural -- Alginate, Collagen, Gelatin, Fibrin, or Hyaluronic Acid + Cells;</td>
<td>Extrusion / DIW; Droplet-based / Ink-jet; SLA; LIFT</td>
<td>Mechanical properties of finished product; Biological impact of cell-material interface; Potential cytotoxicity of cross-linking agents; Requirements for tunability of material; Stability of material over time at 37°C; Immunogenicity of material (if intended for implant)</td>
</tr>
<tr>
<td></td>
<td>dECM + natural or synthetic polymer</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Synthetic -- Pluronic or Polyethylene Glycol (PEG) + Cells;</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Synthetic-Natural hybrid materials (GelMA; Alginate-Gelatin blends)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell Suspensions or Aggregates</td>
<td>Cells in liquid suspension (media, etc.); Multi-cellular aggregates or spheroids; Cell pastes or slurries</td>
<td>Droplet-based / Ink-jet; Extrusion / DIW</td>
<td>Cell number requirements; Dependence on cell-cell interactions and self-assembly</td>
</tr>
</tbody>
</table>
Application-Specific Bioink Considerations

End product requirements?

• Cellularity at time of use
  • Cell concentration in bioink
  • Maturation time

• Physical properties at time of use
  • Mechanical strength
  • Barrier function
  • Size

• Functional considerations
  • Physiologic relevance

Ink-Jet Printing of Mammalian Cells (2017)

Extrusion Printing (2016-2018)

Multi-material, multi-mode 3D construction


Extrusion-based Bioprinter Examples (Commercially Available)
Key Questions for Establishing Requirements for Tissue Design

- What are the primary target tissue functions or attributes you are trying to create?
- Which cell type(s) will be required to achieve this?
- Is there an existing Bioprinter/Bioink strategy that is compatible?
  - Cell and biomaterial tolerances
  - Requirements for resolution and overall size
- What is the print substrate and how might it impact all of the above?
- How “finished” must the product be at the time of fabrication?
  - Immediate function vs. gain-of-function over time
- What format is required by the end-user of the product?
## Print-on-Demand vs. Print-and-Mature

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<th>Print-on-Demand</th>
<th>Print-and-Mature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ready for use shortly after production (&lt;24 hrs)</td>
<td>Significant maturation time required before use (days-weeks)</td>
</tr>
<tr>
<td>May require higher cell number and density to attain desired functionality</td>
<td>May rely in part on cell expansion and/or the development of cell-cell interactions over time for maturation</td>
</tr>
<tr>
<td>Target physical strength must be achieved immediately after production</td>
<td>Target physical strength developed over time through ECM deposition and/or changing properties of biomaterial components</td>
</tr>
<tr>
<td>Biomaterial components may play a major role in achieving / maintaining structure and physical properties</td>
<td>Cellular components may play a major role in meeting functional requirements</td>
</tr>
</tbody>
</table>
Application Highlight: Compound-induced Liver Injury

Bioprinted human liver tissue was constructed from (3) major liver cell types: hepatocytes, hepatic stellate cells, and endothelial cells.

14-day exposure to methotrexate (MTX) or thioacetamide (TAA)

Measurements:
- Liver tissue function (ALT, Albumin)
- General toxicity (LDH)
- Histology (injury and fibrosis)

Use of Multicellular Bioprinted Tissue Enabled Detection of Complex Injury (Fibrosis)

Adverse effects of compound exposure were detectable biochemically and histologically, with both nodular (NF) and pericellular (PF) fibrosis.

Current and Potential Uses of Bioprinted Liver Tissue

Current uses of bioprinted human liver tissue “surrogates”

• Assessment of compound effects in normal human liver tissue
• Assessment of compound effects in compromised liver tissue
  • Bioprinted tissues with Fatty Liver Disease (NAFLD)-like features
  • Bioprinted tissues with advanced fibrosis

Future directions

• Integration of bioprinted liver into multi-tissue “chip” system for systems approach (for example: liver–gut–kidney)
• Transplantable liver “patches” for augmentation of damaged or diseased organs
Application Highlight: Bioprinted Human Intestine

Human small intestine was constructed from primary epithelial cells and intestinal myofibroblasts.

Madden et al. iScience 2:156-167 (2018)
Bioprinted Intestine Develops Barrier Function and Directional Transporter Activity

Madden et al. iScience 2:156-167 (2018)
Bioprinted Intestine Detects Compound-induced Injury Biochemically and Histologically

Madden et al. iScience 2:156-167 (2018)
Potential Uses for Bioprinted Intestine

• Assessment of potential adverse compound effects
  • Epithelial toxicity
  • Effects on barrier function
• Screening for compounds that prevent or repair intestinal injury
• Inflammation and infectious disease
• As components of multi-tissue systems for absorption/excretion
• Microbiome studies
Current Challenges in Bioprinting

- Raw Materials
  - Availability
  - Quantity
  - Quality
- Compatibility of hardware, chemistry, and cells
  - Impact of reagents, physical forces, and printing environment on biology
- Scale
  - Feature size / resolution
  - Overall product size
  - Fabrication time requirements
- Other considerations (analytical challenges, qualification/validation, costs)
The Importance of Starting Materials: Balancing Biological Relevance and Reproducibility

Madden et al. iScience 2:156-167 (2018)
Future Perspective

- Bioprinters
  - Continued development of multi-modal, hybrid systems
  - Integrated automation to replace some manual steps
  - In-process monitoring of key parameters that impact outcome

- Bioinks
  - More (relevant) cells
  - Ready-to-use Cell/Biomaterial mixtures
  - Integrated sensors for in-process and post-processing measurements
  - Cross-platform compatibility

The New Frontier: Tissue Maintenance and Analysis

- Systems for the care and feeding of bioprinted tissues
- Integration of physiologically-relevant tissues and fluidics
- Analytical tools that can resolve outcomes
  - From multiple cell types
  - In a 3D configuration
  - Under dynamic conditions
References

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