BIOINK DEVELOPMENT & PRINTABILITY FOR COMPLEX 3D PRINTING

Sang Jin Lee, Ph.D.

Definition of 3D Bioprinting

- Medical Imaging (CT, MRI, PET, etc.)
- Reverse Engineering (3D CAD/CAM)
- Biomaterial Science
- Cell Sources

3D Bioprinting

Clinically Applicable Living Tissue & Organ Constructs
3D Bioprinting Workflow

- Generation of 3D freeform shaped constructs with precision
  - Multiple cell types, biomaterials, drugs
- High strength constructs:
  - Hydrogels and polymers (~12)
- Printing resolution:
  - Cell printing: ≥ 50 µm
  - Structural polymer printing: ≥ 2 µm

Integrated Tissue-Organ Printing (ITOP) System

ITOP can concurrently print synthetic biodegradable polymers and cell-laden hydrogels in a single tissue construct with clinically applicable size and shape with structural integrity for tissue engineering applications.

- Generation of 3D freeform shaped constructs with precision
- High strength constructs:
  - Hydrogels and polymers (~12)
- Printing resolution:
  - Cell printing: ≥ 50 µm
  - Structural polymer printing: ≥ 2 µm
3D Bioprinting - Bioinks

- A major challenge for tissue and organ engineering is the production of 3D biomimetic, cellular tissue constructs of clinically relevant size and shape with structural integrity.
- 3D bioprinting can print cell-laden hydrogels to manufacture complex, multicellular living tissue constructs that mimic the structure of native tissues.
- Bioinks provide the biological microenvironment needed for the successful delivery of cells and biomaterials to discrete locations within 3D structures.
- To improve and enhance the significance and innovation of this approach, it is critical to develop standardized bioink systems.
### Hydrogel-based Extrusion Bioprinting

**Required properties of bioinks**

I. Formulation & cell encapsulation
   - Cell-laden bioink

II. In the syringe:
   - Homogeneous cell suspension
     - Culture medium
     - Cell-laden bioink
     - Dispensing module (pneumatic or syringe)
   - Nozzle (10-400 µm)

III. Through the nozzle:
   - Extrudability, uniformity, shear thinning (thixotropy)

IV. Crosslinking: structural integrity & construct elasticity

V. In culture: Dimensional stability, cell phenotype & differentiation

**Working Definitions of Printability**

1. **Extrudability**
   - How difficult is it to extrude the bioink?
   - Pressure required to extrude the bioink at a given flowrate

2. **Extrusion uniformity/accuracy**
   - Are the extrusion lines straight and uniform?
   - Length of an extrusion line’s edge relative to a perfectly uniform filament

3. **Structural integrity**
   - Does the bioink hold its shape after extrusion?
   - Height of a printed structure

**Biological properties**

- Non-toxic
- Supporting cell growth
- Maintaining cell phenotype
- Accelerating tissue formation

**Determination of Printing Parameters**

- Gillispie et al., Biofabrication. 2020
Hydrogels Available for Bioprinting

- Hydrogels
  - Collagen
  - Fibrinogen
  - Hyaluronic acid (HA)
  - Sodium alginate
  - Gelatin
  - Methyl cellulose
  - Gellan gum
  - Chitosan
  - Agarose
  - Xanthan gum
  - Poly(ethylene glycol) (PEG)
  - Pluronic F127

Quantitative printability measurement is needed
- Most often, printability is described qualitatively
- Rheological measurement for the extrudability and shear-thinning property of materials
- Yet, predicting the final shape of a printed construct have been inconclusive
- The relationships between rheology and other aspects of printability are not fully understood - rheology cannot yet be used as a proxy for printability
- Direct measures of printability are currently needed in order to confirm the suitability of bioinks for specific bioprinting applications.

Strategy of Bioink Development

<table>
<thead>
<tr>
<th>Bioink preparation</th>
<th>Measurement tools</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rheological test</td>
<td>Viscoelastic (G, G'), yielding recovery, &amp; shear thinning</td>
</tr>
<tr>
<td></td>
<td>Printing of artifact structures</td>
<td>Tube height, wall thickness, Pr, pore area, filament width, turn accuracy, deflection angle, etc.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Determination of printability</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Quantification by image analysis</td>
</tr>
</tbody>
</table>
Bioink Artifact for Printability Measurement

The artifact possesses excellent ease of use, printing in less than 10 min, using less than 0.4 mL of bioink, and an automated image analysis process.

Selected Testing Bioink Formulations

<table>
<thead>
<tr>
<th>Abbr.</th>
<th>Formulation</th>
<th>Selection criteria</th>
<th>Printing conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pressure (kPa)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Flowrate (mm³/min)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Feedrate (mm/s/min)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Layer height (µm)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nozzle size (µm)</td>
</tr>
<tr>
<td>PF</td>
<td>40% Pluronic F127</td>
<td>Standard bioink</td>
<td>258</td>
</tr>
<tr>
<td>GG/GM</td>
<td>1.2% Gellan Gum + 4% GelMA</td>
<td>Testing formulation</td>
<td>164</td>
</tr>
<tr>
<td>Alg-Lap-RD</td>
<td>1% Alginate + 6% Laponite RD</td>
<td>2nd high printability comparator</td>
<td>140</td>
</tr>
<tr>
<td>Alg-Lap-EP</td>
<td>1% Alginate + 6% Laponite EP</td>
<td>Testing formulation</td>
<td>75</td>
</tr>
<tr>
<td>ALG</td>
<td>7% Alginate</td>
<td>Viscose hydrogel</td>
<td>742</td>
</tr>
<tr>
<td>MC</td>
<td>8% Methylcellulose</td>
<td>Poor shape fidelity</td>
<td>602</td>
</tr>
<tr>
<td>HA</td>
<td>3% Hyaluronic Acid</td>
<td>Poor shape fidelity</td>
<td>174</td>
</tr>
</tbody>
</table>
5-layer Tube (Side View)

5-layer Tube (Top View)

Gillispie et al., under review
**Crosshatch**

![Crosshatch Diagram]

**4-Angled Pattern**

![4-Angled Pattern Diagram]
**Overhang Collapse**

![Image of overhang collapse]

**Printability Outcomes**

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<tr>
<td></td>
<td>Tube height</td>
</tr>
<tr>
<td>PF (standard)</td>
<td>+++</td>
</tr>
<tr>
<td>GG/GM</td>
<td>++</td>
</tr>
<tr>
<td>Alg-Lap-RD</td>
<td>+++</td>
</tr>
<tr>
<td>Alg-Lap-EP</td>
<td>+++</td>
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<tr>
<td>ALG</td>
<td>+</td>
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<tr>
<td>MC</td>
<td>+</td>
</tr>
<tr>
<td>HA</td>
<td>+</td>
</tr>
</tbody>
</table>

+++ Good; ++ Intermediate; + Poor; n/d: not detectable
Rheological Properties

Rheology vs. Printability (Linear Regression Analysis)
Rheology vs. Printability (Linear Regression Analysis)

Rheological Properties vs. Printability

- Rheological measurement is valuable insight into the bioink’s shear-thinning, viscoelastic, yielding, and recovery properties
- Loss modulus ($G''$) is not predictive of printing outcomes
- Rheological measures are not predictive of uniformity, except, low $G''$ may be an indicator of poor uniformity
- No rheological parameter alone was able to predict relative printability
- Printing outcomes must be measured directly rather than inferred from rheology

Thus, standardization of printability measurement is essential for bioink development
Effect of Cell Density on Printability

Effect of Cell Density on Rheological Properties
Effect of Cell Density on Printability

- The effect of cell density on printing outcomes have been investigated in the GelMA/GG composite bioink.
- No effect on printability was seen for cell densities up to $40 \times 10^6$ cells/mL.
- Rheological measures showed some variation between the bioinks with different cell densities.
- Both storage modulus ($G'$) and loss modulus ($G''$) increased moderately as cell density increased.
- Yield stress showed slight changes, initially increasing as cells were introduced at $5 \times 10^6$/mL and then decreasing from there as cell density increased.
- All bioinks showed similar shear-thinning abilities with analogous $K$ and $n$ constants.
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Cell biologists
- Lauren West-Livingston, PhD
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- Gregory J. Gillispie, PhD
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- Juntae Huh, BS

Mechanical engineers
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- Eric Renteria, BS

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