A 3D bioprinting system and plasma-surface modification to fabricate tissue engineering scaffolds

Abstract: The achievement of tissue engineering can be highly depending on the capability to generate complicated, cell seeded three dimensional (3D) micro/nano-structures. So, various fabrication techniques that can be used to precisely design the architecture and topography of scaffolding materials will signify a key aspect of multi-functional tissue engineering. Previous methods for obtaining scaffolds based on top-down are often not satisfactory to produce complex micro/nano-structures due to the lack of control on scaffold architecture, porosity, and cellular interactions. However, a bioprinting method can be used to design sophisticated 3D tissue scaffolds that can be engineered to mimic the tissue architecture using computer aided approach. Also, in recent, the method has been modified and optimized to fabricate scaffolds using various natural biopolymers (collagen, alginate, and chitosan etc.). Variation of the topological structure and polymer concentration allowed tailoring the physical and biological properties of the scaffolds. In this presentation, the 3D bioprinting supplemented with a newly designed plasma treatment for attaining highly bioactive and functional scaffolds for tissue engineering applications will be introduced. Moreover, various in vivo and in vitro results will show that the fabricated scaffolds can carry out their structural and biological functionality.
A 3D bioprinting system and plasma-surface modification to fabricate tissue engineering scaffolds

GeunHyung Kim
Sungkyunkwan University (SKKU)
May 25, 2017

• February 12, 2013
  President Barack Obama
  • State of the Union Address

“Last year we created our first manufacturing innovation institute in Youngstown, Ohio... where workers are mastering 3-D printing that has the potential to revolutionize the way we make almost everything.”
Microscale 3-D Printing

Inks made from different types of materials, precisely applied, are greatly expanding the kinds of things that can be printed.

<table>
<thead>
<tr>
<th>Breakthrough</th>
<th>Why It Matters</th>
<th>Key Players</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-D printing that uses multiple materials to create objects such as biological tissue with blood vessels.</td>
<td>Making biological materials with desired functions could lead to artificial organs and novel cyborg parts.</td>
<td>Jennifer Lewis, Harvard University</td>
</tr>
</tbody>
</table>

Table 1

<table>
<thead>
<tr>
<th>Mean Hardness (kPa)</th>
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</thead>
<tbody>
<tr>
<td>44.85 ± 2.68</td>
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<tr>
<td>38.93 ± 3.00</td>
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<tr>
<td>42.40 ± 2.87</td>
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<tr>
<td>45.47 ± 1.95</td>
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<tr>
<td>41.33 ± 4.36</td>
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<tr>
<td>46.80 ± 4.72</td>
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<tr>
<td>40.67 ± 1.13</td>
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<tr>
<td>38.50 ± 1.73</td>
</tr>
<tr>
<td>40.10 ± 2.42</td>
</tr>
<tr>
<td>39.27 ± 3.26</td>
</tr>
</tbody>
</table>
3D printing?

- **3D CAD model & STL file**
- **Slicing software**
- **3D printing**
- **3D Object**

**Advantages**
- Idea develops in reality, faster than any other process.
- It can save time and money by making 3D model compare to any other process.
- It is tool-less operation, layer by layer manufacturing process.
- Almost any types of complex model can make.
- It makes perfect replica of an object.
- It is helpful of R&D before large scale operation for detection any type of flaws.
- It can be used as a test model or some time final product.

**Disadvantages**
- Required additional material layer manufacturing process.
- Much more time required for making 3D model.
- It has some limitation of raw materials.
- It has some size limitation, basically big scale models.
- It's a machine, so it can make errors or low accuracy.
- Manufacturing limitation.

“David after two years staying in USA”
Applications of 3D printing

Fashion

Food
Architecture!!!!

http://architect.co.kr/archives/35125

Surgery!!!!
Advantages of bioprinting

1. Simple to use
2. Enabling researchers to generate geometrically well-defined scaffolds in a rapid and inexpensive manner
3. Allowing high-throughput generation of replicas of spatially and temporally well-controlled complex constructs
4. Providing 3D complexity by multilayer printing

By S. Taneqhe et al., Trends in Biotechnology 31, 2013
1. Medical devices
   (non-biocompatible materials)

2. Medical implants
   (biocompatible but not biodegradable)
Roles of scaffolds

1. A framework to support cell migration into the defect from surrounding tissues.

2. A delivery vehicle for exogenous cells, growth factors, and genes.

3. A matrix for cell adhesion, proliferation, and differentiation.

4. Structurally stable.

5. A barrier to prevent the infiltration of surrounding tissue that may impede the process of regeneration.
3. Tissue scaffolds (bio-compatible, biodegradable, and bio-absorbable)
For the reconstruction of the orbital walls and craniosynostosis

To heal fractured orbital floors

Overcoming strategies for the conventional bioprinting
Ex. 1. Collagen for dermis regeneration

To confirm the suitability of newly developed 3D collagen scaffold, human keratinocytes and fibroblasts were (1) submerged and co-cultured for 7 days and then (2) air-liquid cultured for another 7 days.

Ex. 2. Collagen/PCL hybrid scaffold for improving mechanical properties without loss osteogenesis

Ref: G.B. Kim et al., J Mater Chem, 2012
- Exposure time (120 min)
- Oxygen flow rate (10 sccm)
- Plasma power levels of 10 and 30 W

4. Cell-embedded scaffolds
(Basic idea for cell-laden scaffolds; organ printing)

The successful development of a 3D tissue requires optimization of each key parameter

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### 4. Cell-embedded scaffolds

**4.1. Requirement for cell-printing: bio-inks**

<table>
<thead>
<tr>
<th>Methods/Printing</th>
<th>Methods</th>
<th>Bioink materials</th>
<th>Collating conditions</th>
<th>Cell type</th>
<th>Cell viability</th>
<th>Geometry of printed structure</th>
<th>Pros and Cons</th>
<th>Notes</th>
</tr>
</thead>
</table>
| 3D cell printing | Animal | 5-10% gelatine + 25-35% alginate | 1. animal; 2-10 MCT15 (mesenchymal precursor cells) | Human MSC (hMSC) | In vitro | 90% viability | Enhanced 3D printability | 36-38
|                   | Human   | 5-10% gelatine + 25-35% alginate | 1. animal; 2-10 MCT15 (mesenchymal precursor cells) | Human MSC (hMSC) | In vitro | 90% viability | Enhanced 3D printability | 36-38
|                   | Human   | 5-10% gelatine + 25-35% alginate | 1. animal; 2-10 MCT15 (mesenchymal precursor cells) | Human MSC (hMSC) | In vitro | 90% viability | Enhanced 3D printability | 36-38

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<table>
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<tr>
<th>Method</th>
<th>Printing</th>
<th>Biopolymers materials</th>
<th>Cell viability</th>
<th>Geometry of printed structures</th>
<th>Pros and Cons</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-temperature stage</td>
<td>3.5 wt% alginate + 0.5 wt% CaCl₂ solution (1:1)</td>
<td>Human primary skin, Compatible cells (mesenchymal and +90%) (fibroblasts)</td>
<td></td>
<td></td>
<td>Outstanding biomechanical properties and high cell viability.</td>
<td>[9]</td>
</tr>
<tr>
<td>Electro-fluid Electrokinetically assisted 3D microjet (EK3D) cell printing</td>
<td>3.5 wt% alginate + 0.5 wt% CaCl₂ solution (1:1)</td>
<td>Human primary skin, Compatible cells (mesenchymal and +90%) (fibroblasts)</td>
<td></td>
<td></td>
<td>Highly porous cell-laden alginate scaffolds with a Balance ratio of 0.5</td>
<td>[30]</td>
</tr>
<tr>
<td>Extrusion-based</td>
<td>3.5 wt% alginate + 0.5 wt% CaCl₂ solution (1:1)</td>
<td>Human primary skin, Compatible cells (mesenchymal and +90%) (fibroblasts)</td>
<td></td>
<td></td>
<td>Homogeneous cell distribution and 100% pore biocompatibility.</td>
<td>[31]</td>
</tr>
<tr>
<td>Hybrid 3D Conventional Transfection printing (multi-printing)</td>
<td>PCL (Mw 70,000-90,000) for scaffold printing</td>
<td>Human hepatocytes, High potential cell expansion (4.5 fold)</td>
<td></td>
<td></td>
<td>Enhanced printing stability and orientation; increased coherence between layers.</td>
<td>[32]</td>
</tr>
<tr>
<td></td>
<td>4 wt% alginate, alginate solution (1:1)</td>
<td>Human primary skin, Compatible cells (mesenchymal and +90%) (fibroblasts)</td>
<td></td>
<td></td>
<td>Highly improved 3D printability and mechanical properties with high cell viability.</td>
<td>[33]</td>
</tr>
<tr>
<td></td>
<td>0.5 wt% CaCl₂ solution (1:1)</td>
<td>Human primary skin, Compatible cells (mesenchymal and +90%) (fibroblasts)</td>
<td></td>
<td></td>
<td>Enhanced formation of mesenchymal cells derived from MSCs without BrdU incorporation.</td>
<td>[34]</td>
</tr>
</tbody>
</table>

References:

**Printability vs. Biocompatibility**

- **Ideal inks for 3D printing**
  - Advanced Bioinks
  - Traditional Bioinks

- **Biofabrication Window**
  - Ideal cell culture hydrogels
  - Cytotoxicity
  - Cell adhesion
  - Proliferation

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*Images and diagrams not fully visible in the text.*
4. Cell-laden scaffolds
4.2. Requirements for cell-printing: microporous structure

- 1. Micro-pore structure
- 2. Vasculatures
- 3. 3D structures

1. Micro-pore structure
2. Vasculatures
3. 3D structures: large scale structures

4. Cell-embedded scaffolds
(4.3. Requirements for cell-printing: Processes)

**Inkjet-printing**

Inkjet cell printers: thermal, piezoelectric, and acoustic inkjet printers using heat, piezoelectric, and acoustic wave actuators, respectively, to dispense cell-embedded microdroplets

**Bio-plotting**

Cell-embedded 3D printing with microextrusion: pneumatic or mechanical dispensing system to extrude bioink in a line

**Laser-assisting**

Laser-assisted bioprinting: Nozzle-free printing of bioinks containing cells using lasers with a high resolution, accuracy, and precision
Table. Advantages and disadvantages of basic 3D cell printing techniques

<table>
<thead>
<tr>
<th>Techniques</th>
<th>Laser-assisted</th>
<th>Inkjet</th>
<th>Microinjection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advantages</td>
<td>Single cell manipulation</td>
<td>High cell viability</td>
<td>High mechanical properties</td>
</tr>
<tr>
<td></td>
<td>Nozzle free</td>
<td>Noncontact nozzle</td>
<td>Short fabrication time</td>
</tr>
<tr>
<td></td>
<td>Usage of high viscosity biosink</td>
<td>Printed cell patterns using different cell types</td>
<td>Printing of various types and viscosities of biosink</td>
</tr>
<tr>
<td></td>
<td>High resolution</td>
<td>Multicell heterogeneous constructs</td>
<td>Wide range of biocompatible materials</td>
</tr>
<tr>
<td></td>
<td>High accuracy</td>
<td>High thoroughput</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High gelation speed</td>
<td>High gelation speed</td>
<td></td>
</tr>
<tr>
<td>Disadvantages</td>
<td>Low mechanical properties</td>
<td>Low mechanical and structural integrity</td>
<td>Low cell viability due to nozzle wall shear stress and mechanical stress</td>
</tr>
<tr>
<td></td>
<td>Long fabrication time</td>
<td>Long fabrication time</td>
<td>Low accuracy</td>
</tr>
<tr>
<td></td>
<td>Damage cells due to heat generated from laser energy</td>
<td>Low upper limit for viscosity of biosink</td>
<td>Cell death due to changes in dispensing pressure and biosink concentration</td>
</tr>
<tr>
<td></td>
<td>Aggregate in the final tissue construct</td>
<td>Cell aggregation</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Clogging of the nozzle orifice</td>
<td></td>
</tr>
</tbody>
</table>


Bio-printing in SKKU (Bioink: M13 Bacteriophage)

G. H. Kim et al., Acta Biomater., 2015
Bio-printing in SKKU (Structure: Mechanical stability)

Conclusion

1. Cell-printing system can enable to generate various 3D tissue architects precisely.

2. The system can be used as one of highly efficient cell delivering methods for constructing multifarious tissue/organ systems.

3. However, it has various overcoming issues such as limited bioinks, low mechanical stability, and poor controllable complex 3D shape-ability etc.
Thank you!!