Lecture #10:
Scaffold Fabrication and Tailoring
<table>
<thead>
<tr>
<th></th>
<th>Scaffold Formation</th>
<th>Cell Seeding</th>
<th>Cell Adhesion</th>
<th>Scaffold Degradation</th>
<th>Stiffness</th>
<th>Natural or Synthetic</th>
<th>Signaling Cues</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Collagen</strong></td>
<td>forms hydrogel at 37°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>natural, often animal source</td>
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<tr>
<td><strong>Fibrin</strong></td>
<td></td>
<td>cells can be encapsulated in situ</td>
<td></td>
<td></td>
<td>soft gel; tunable by controlling conc.</td>
<td></td>
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<tr>
<td><strong>Alginate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>hydrolysis</td>
<td>natural, plant source</td>
<td></td>
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<tr>
<td><strong>PLGA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>blank slate; signaling peptides can be added</td>
</tr>
<tr>
<td><strong>PEG</strong></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td><strong>Small Intestine Submucosa</strong></td>
<td>Remove cells from animal tissue</td>
<td></td>
<td></td>
<td></td>
<td>protease mediated; release of growth factors and antibiotics</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Altering Hydrogel Chemistry: Adding Function to PEG Hydrogels
Collagen-Mimetic Peptide
MSC Differentiation

Heparin-functionalized PEG hydrogels
MSC Differentiation

Retention of BMP-2 and Fibronectin within gels

Benoit et al., Biomaterials, 2006
Screening Small Functional Groups

Benoit et al., Nat Materials, 2008
Screening Small Functional Groups

Benoit et al., *Nat Materials*, 2008

- **Oil red**, **PPARG** = markers of adipocytes (fat cells)
- **Osteopontin**, **CBFA1** = markers of osteocytes (bone cells)

- White circles = phosphate group
- Black diamonds = t-butyl group

- Oil red, **PPARG** = markers of adipocytes (fat cells)
  Osteopontin, **CBFA1** = markers of osteocytes (bone cells)
Topography and 3D architecture
Cellular Length scale: topography

• Topography
  – Pore size
    • Controls size of cells that can enter material
    • Degradation rate
    • Mechanical properties
    • Material isotropy
    • Cell fate
  – Pores formed by:
    • Particulate leaching with salt or polymer particles in polymer solution, which are dissolved to leave pores
    • Freeze drying to form ice crystals that may be sublimated to leave pores

Porous polymer scaffold with global pores ~250 um, local pores 10-100 um
Cellular-Supracellular Length scale: topography and architecture

- **Shape of material**
  - biomaterial shapes that mimic the shape of the tissue.
  - Biomaterial shapes that follow function

Agarose gel shaped as patella
Hung CT et. al. *J. Biomech* 2003

PGA shaped as trileaflet heart valve
http://www.mate.tue.nl/mate/pdfs/3803.pdf
Cellular-Supracellular Length scale: topography and architecture

- Methods for dictating architecture
The Science Fiction Version…
Cellular-Supracellular Length scale: topography and architecture

• Methods for dictating architecture
  – Solid free form (SFF) fabrication
• Binder printing and sintering
• Fused deposition modeling
• Sterolithography
  - Micromachining and micromolding
Scaffold Architecture

• 3-D binder printing

Figure 16.14
3-D printed biomaterial scaffolds. A layer of a scaffold is formed by printing the "glue" in a desired pattern onto a thin layer of powder polymer. Additional powder polymer is deposited onto the printed layer, and the process is repeated to form a three-dimensional scaffold. From [199].

3-D printing manufactured scaffold

3D Printing

http://www.youtube.com/watch?v=u7h09dTVkdw
Selective Laser Sintering
Selective Laser Sintering of Anatomical Bone Scaffold

Hollister, Nat Materials, 2005
Selective Laser Sintering of Anatomical Bone Scaffold

Modeled (left) and fabricated (right) PCL scaffold

Imaged growth of bone (white) into scaffold (blue)

Hollister, Nat Materials, 2005
Scaffold Architecture

- Fused Deposition Modeling
Fused Deposition Modeling

http://www.pbs.org/video/2192351751/

https://www.thingiverse.com/

MakerBot
Stereolithography - also not science fiction

http://www.youtube.com/watch?v=tiYNfB9Oi9M
Scaffold Architecture

• SFF fabrication
  – Stereolithography

Figure 16.12 Building parts with light. Stereolithography is an example of a solid free-form fabrication technique. A layer of liquid polymer is turned into a solid by a pattern of light. The stage is then lowered so that the next layer of the structure can be solidified. Through repetition of this process, complex three-dimensional structures can be formed.

Hydroxyapatite structure formed using stereolithography

Chu/Orton, Ann N Y Acad Sci 2002
Photopatterned hydrogels containing cells

PEGDA + cells  + Photoinitiator + light  → Hydrogel + entrapped cells

Add flow

Liu & Bhatia, Biomedical Microdevices 2002
Liu+ FASEB J. 2007; Chen/Underhill+ Biomaterials. 2006
Photopatterned Layers

Liu & Bhatia, Biomedical Microdevices 2002
Liu+ FASEB J. 2007; Chen/Underhill+ Biomaterials. 2006
3D Plotting of Hydrogels

<table>
<thead>
<tr>
<th>Plotting material</th>
<th>Plotting medium</th>
<th>Temperature plotting material (°C)</th>
<th>Temperature medium (°C)</th>
<th>plotting</th>
<th>Nozzle type</th>
<th>Inner (mm)</th>
<th>nozzle diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gelatin 10%</td>
<td>Silicone oil</td>
<td>40</td>
<td>3</td>
<td></td>
<td>Standard medical injection needle</td>
<td>1.50</td>
<td></td>
</tr>
<tr>
<td>Agar 5%</td>
<td>Gelatin 4.00%</td>
<td>70</td>
<td>20</td>
<td></td>
<td>Cyanoacrylate tip with teflon inlay</td>
<td>0.15</td>
<td></td>
</tr>
</tbody>
</table>

Landers+, Biomaterials, 2002
Omnidirectional Printing of 3D Microvascular Networks

Willie Wu, Adam DeConinck, and Jennifer A. Lewis*

Nature is replete with examples of microvascular systems that enable efficient fluid flow and distribution for autonomic healing, cooling, and energy harvesting. Emulating these systems in functional materials is of considerable interest for emerging applications in self-healing,[1-3] tissue engineering,[4-7] organ printing,[8,9] microfluidics,[10,11] and biomedical devices.[12] In one example, skin-like mimics containing synthetic microvascular networks filled with healing agents demonstrated repeated repair of damage in a single location.[2,3,13] In another example, tissue engineering constructs containing both embedded cells and a planar array of microchannels were developed to facilitate the delivery of nutrient-laden fluids that the reservoir, void space is generated locally and filled by the migration of liquid from the fluid capping layer (Figure 1b). The liquid filler is designed to have identical chemical functionality, yet a significantly lower viscosity than the photopolymerizable reservoir. Thus, any voids produced during printing are immediately filled. After printing is completed, the gel reservoir and fluid filler are solidified via photo-polymerization to form a mechanically robust, chemically crosslinked matrix (Figure 1c). Because the fugitive ink has not been chemically modified, it can be subsequently removed by liquefaction at 4 °C under a modest vacuum to yield the desired microchannel network within the matrix (Figure 1d,e).
Pluronic Micelles/Gel

Unimer

Micelle

Gel

CMC, CMT

Increasing $c$, $T$

PO block

EO block
Figure 3, 4

(a) Plot showing the ratio of channel diameter to nozzle diameter for 200 μm and 30 μm nozzles as a function of pressure (psi).

(b) (a) t* = 0, (b) t* = 1, (c) t* = 5, (d) t* = 10 images showing the progression of polymerization at different times.

(c) Graph showing normalized intensity versus distance from the channel wall (μm).

(d) Graph showing the variation of normalized intensity over time (s).

(e) Plot depicting the fillers, matrix, and cured states with their respective concentrations (wt %) and storage modulus (G', E' (Pa)).