Bio-scaffolding for Regenerative Medicine

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Investigating Scaffolds as a Physicist

- History of two regenerative organs:
  - Esophagus
  - Bladder
- Material requirements for a bio-scaffold.
- Historical comparison of Synthetic v. Extra Cellular Matrix.
- Material experiments to identify structural requirements of bio-scaffolds.
The Rise and Promise of Regenerative Scaffolding

Exponential Growth

Through the Use of Two Primary Bio Scaffolds

- Extracellular Matrix
- Silicone Rubber Compound
Fundamental Scaffolds for Organs

- Extra Cellular Matrix (ECM) –
  - Connective tissue that is absent of living cells.
  - Protein fibers embedded in an amorphous mixture of huge protein-polysaccharide molecules. Mostly collagen. MW of 470 kDa.

Felt-like microstructure

Can be conditioned in a bio-reactor, and shaped

*For example to form an Esophagus*
Fibronectin

- A Glycoprotein that among other things is the primary protein which binds to ECM.
- The major contributor to cell adhesion on ECM.
- Has approx. a Molecular Weight of 230–250 kDa.

R. Langer et. al 2007
Both dense and loose connective tissue are derived from cells called fibroblasts which secrete the extracellular matrix. Fibronectin from stem cells are seeded. Fibronectin forms covalent bonds with the ECM seeping through the felt-like structure. ECM Scaffold biodegrades. Covalent bonds weaken as ECM Scaffold biodegrades. New native Epithelial layer of the esophagus is formed.
Mechanism for ECM Cell Binding

Reilly et al. 2009

Fibronectin on ECM Surface
Mechanical Properties of ECM

- Performed on a Perkin Elmer 7 DMA.
- Mesh Size calculated by the Canal and Peppas Equation.

\[
\xi = u_{2,s}^{-1/3} l C_m^{1/2} n^{1/2}
\]

<table>
<thead>
<tr>
<th>(%) PEGDM\textsuperscript{b}</th>
<th>(q^c)</th>
<th>Compressive Modulus (kPa)</th>
<th>Mesh Size (Å)</th>
<th>(q) (w/cells)\textsuperscript{c}</th>
<th>Compressive Modulus (kPa) (w/cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>9.3 ± 1.0</td>
<td>34 ± 3</td>
<td>140</td>
<td>12.6 ± 0.2</td>
<td>30 ± 1</td>
</tr>
<tr>
<td>20</td>
<td>5.2 ± 0.1</td>
<td>360 ± 14</td>
<td>60</td>
<td>6.4 ± 0.05</td>
<td>260 ± 30</td>
</tr>
<tr>
<td>30</td>
<td>4.5 ± 0.1</td>
<td>940 ± 60</td>
<td>50</td>
<td>5.2 ± 0.2</td>
<td>400 ± 100</td>
</tr>
<tr>
<td>40</td>
<td>4.2 ± 0.1</td>
<td>1370 ± 20</td>
<td>40</td>
<td>—</td>
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</tr>
</tbody>
</table>

S.J. Bryant et. al 2001
Fundamental Scaffolds for Organ

- Pig stomach used and prepared.
- Cylinders of ECM formed and placed in a bioreactor.
- The Epithelial layer of a patient’s esophagus removed like “pulling a sock inside out”. (Dr. Niall Turner, McGowan Institute of Regenerative Medicine)
- The newly formed bioscaffolds is replaced.
- The process of creating a new esophagus begins.
Uses for Epithelial Scaffold Replacement

- Already performed successful at the University of Pittsburg hospital on 5 patients. (see visual results below)
- Can cure “Barrett’s Esophagus”.
- Can prevent early cancer tumors from spreading.

Badylak et. al 2011
Synthetic Solution for Bioscaffolding

- Polystyrene for directing of stem cells and simulation of vascular system.
- Cured Silicone for efficient mechanical properties and biocompatibility.
- Porosity for fibronectin receptors.
Polydimethylsiloxane (PDMS)

Why PDMS?

1. Polymer chains have loose entanglement when MW is high.
2. Viscosities can be low enough during processing to allow for discreet channels.
3. Elasticity increases with shear force.
4. Can be patterned like a plastic for vascular mapping.
Comparison of ECM and PDMS

- ECM is 50 Microns.
- PDMS sheets are up to 2mm.
- A scaling facture is needed for comparison:

\[
a_{\text{pore}} = n \pi d l t
\]

- \( a_{\text{pore}} \) = dimensionless ratio of internal pore surface area per sample membrane surface area of a given thickness
- \( n \) = number density (# of pores / membrane surface area)
- \( \pi \) = \( \pi \) (pi)
- \( d \) = average pore diameter
- \( l \) = membrane thickness
- \( t \) = pore tortuosity factor

Thus:

\[
l_2 = \frac{(n\pi d l t)_1}{(n\pi d t)_2}
\]
Polydimethylsiloxane (PDMS)

- $E' = 435$ kPA
- $\tan \delta = 0.0002$
- Chemical structure: $\text{CH}_3[\text{Si(CH}_3)_2\text{O}]_n\text{Si(CH}_3)_3$
- Hydrophobic, requiring pore structure

All Tests run on at TA instrument Q 800 in rapid compression mode
Frequency 2Hz
Temperature 100°C
Strain 1%
ECM Surface Density Map at 20X

Annotated by area
PDMS Surface Density Map at 20X

Annotated by area
Results

- The total area covered by porosity in the PDMS is 1865 µm²
- The total area covered by porosity in the ECM is 1689 µm²
- Average pore size for PDMS is 1.973227 µm²
- Average pore size for ECM is 1.140332 µm²

What does this tell us?
The total area of porosity can be reduced in PDMS if the porosity size is appropriately decreased.
Importance of Porosity

- Porosity provides expansion space.
- Porosity provides binding sites.
- Porosity aids in bio destruction for degradability.
- Small pore size for optimization to avoid shear collapse.

Mike Adams et al.
Fibronectic Behavior

- Non-entangled branching.
- Appears when in contact with the polymer or ECM.
- Exhibits first strain hardening, then strain softening.
Fibronectic Behavior

Daniel Fletcher UC Santa Barbara.
Summary

- Fibrous structure of ECM allows for easy matrix expansion.
- Porosity in PDMS provides room for vascular, and viscous behavior.
- Small pores in PDMS required so that expansion allows for biodegradation.
- Fibronectin behavior requires flexibility currently more easily accepted by ECM.
- Work on Polymer bio scaffold should focus on adhesion structure of proteins such as fibronectin.

THIS IS A RUBBER ISSUE....