Bio-scaffolding for Regenerative Medicine

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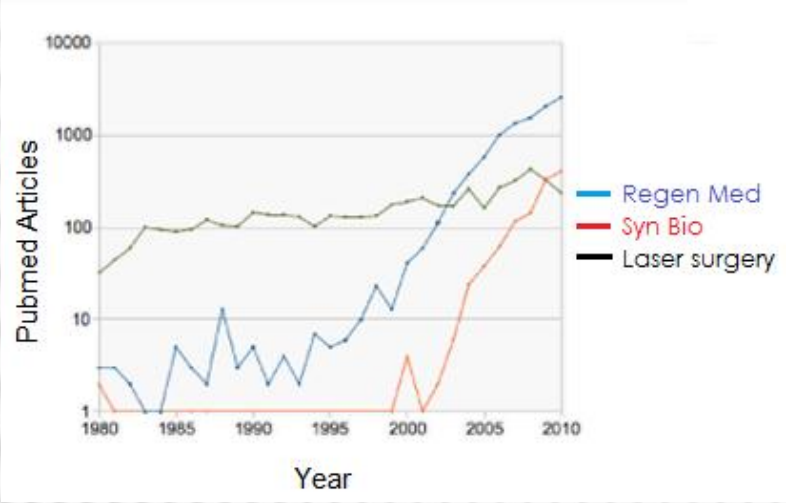
Investigating Scaffolds as a Physicist

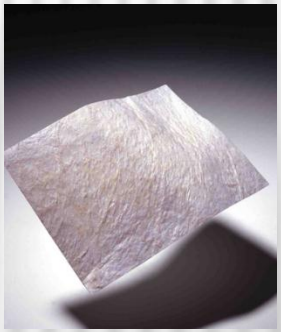
- History of two regenerative organs:
  - Esophagus
  - Bladder
- Material requirements for a bio-scaffold.
- Historical comparison of Synthetic v. Extra Cellular Matrix.
- Material experiments to identify structural requirements of bio-scaffolds.

The Rise and Promise of Regenerative Scaffolding



Exponential Growth

Through the Use of Two Primary Bio Scaffolds

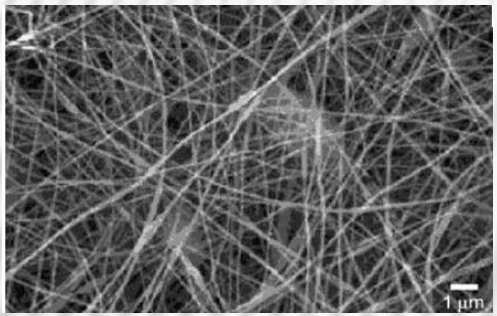


Extracellular Matrix

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.



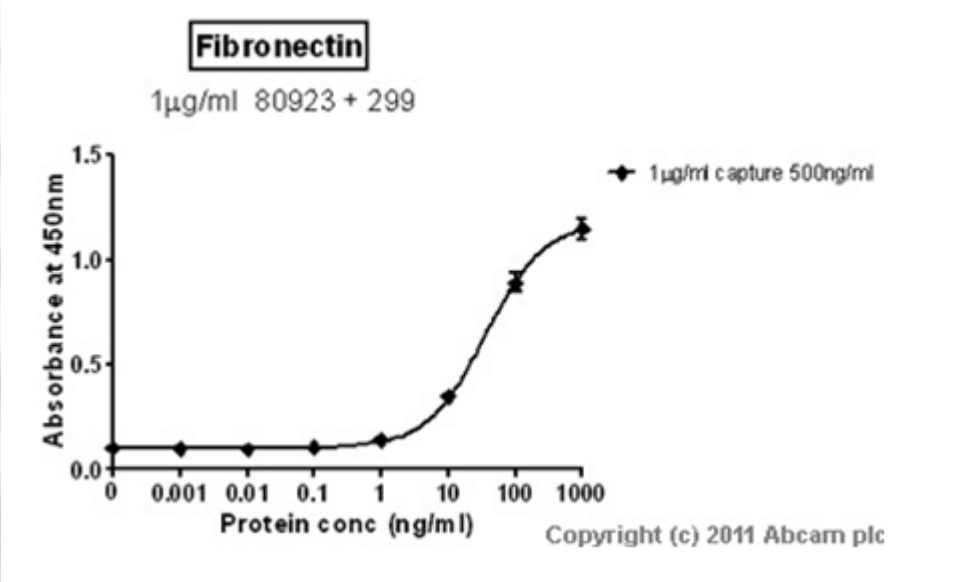
Felt-like microstructure

Can be conditioned in a bio–reactor, and shaped

For example to form an Esophagus

Fibronectin

- A Gycoprotein 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

Fundamental Scaffolds for Organs

- Both dense and loose connective tissue are derived from cells called fibroblasts which secrete the extracellular matrix.

New native Epithelial layer of the esophagus is formed

ECM Scaffold biodegrades

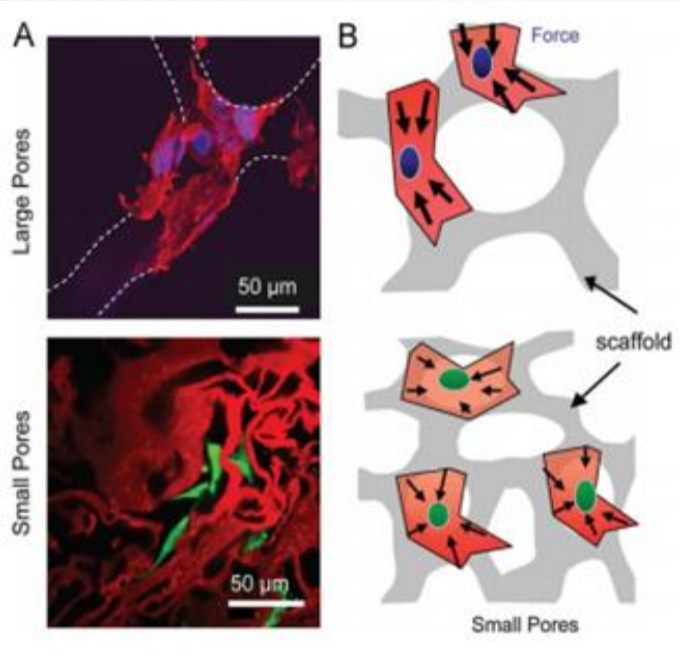
Covalent bonds weakens as ECM Scaffold biodegrades

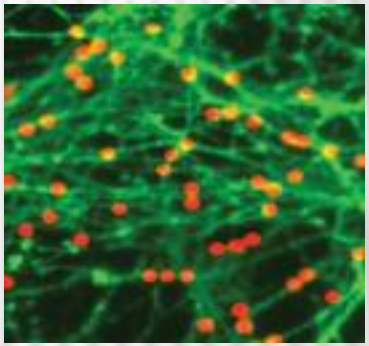
Fibronectin forms a new ECM in the form of the scaffold

Fibronectin forms covalent bonds with the ECM seeping through the felt-like structure

Fibronectin from stem cells are seeded

Mechanism for ECM Cell Binding





Fibronectin on ECM Surface

Reilly et. al 2009

Mechanical Properties of ECM

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

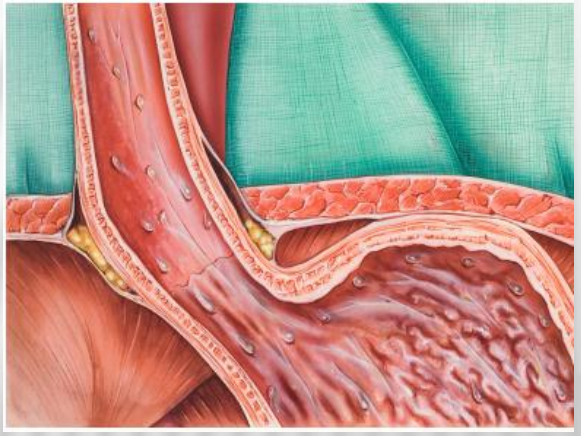
$$\xi = v_{2,s}^{-1/3} l C_n^{1/2} n^{1/2}$$

% PEGDM[b]	q[c]	Compressive Modulus (kPa)	Mesh Size (Å)	q (w/cells)[c]	Compressive Modulus (kPa) (w/cells)
10	9.3 ± 1.0	34 ± 3	140	12.6 ± 0.2	30 ± 1
20	5.2 ± 0.1	360 ± 14	60	6.4 ± 0.05	260 ± 30
30	4.5 ± 0.1	940 ± 60	50	5.2 ± 0.2	400 ± 100
40	4.2 ± 0.1	1370 ± 20	40	—	—

S.J. Bryant et. al 2001

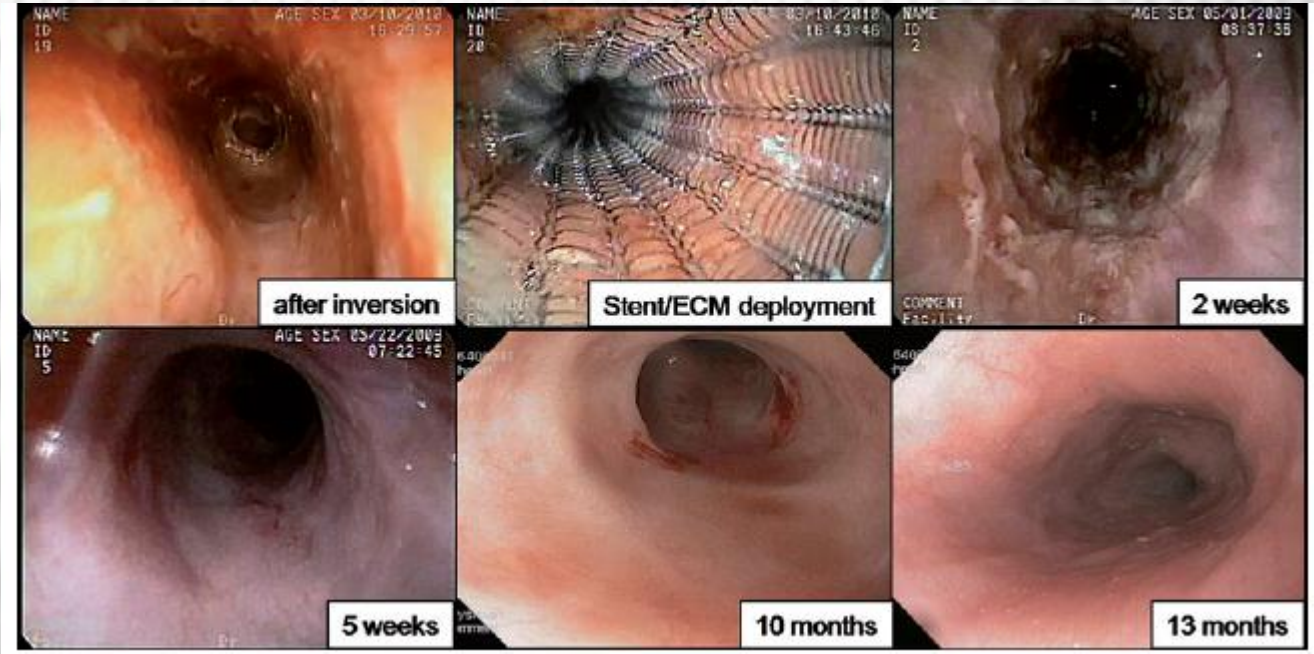
Fundamental Scaffolds for Organ

- 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.



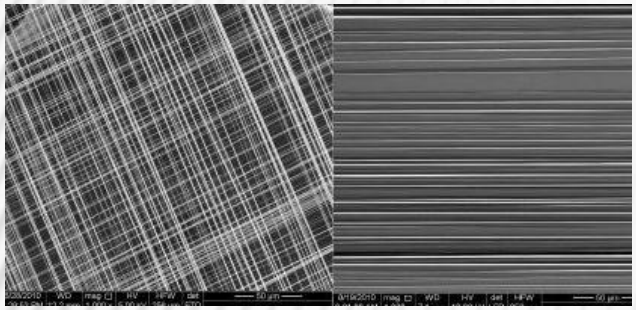
Uses for Epithelial Scaffold Replacement

- 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 Fibers

+

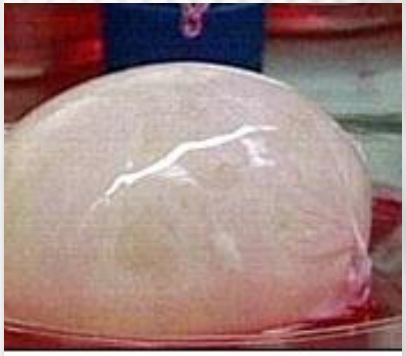
Porous Silicone Rubber-
Polydimethylsiloxane

- 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)

From

To



Lab Grown Bladder

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:

$a_{pore} = n*\pi*d*l*t$

a_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)

π = pi

d = average pore diameter

l = membrane thickness

t = pore tortuosity factor

Thus:

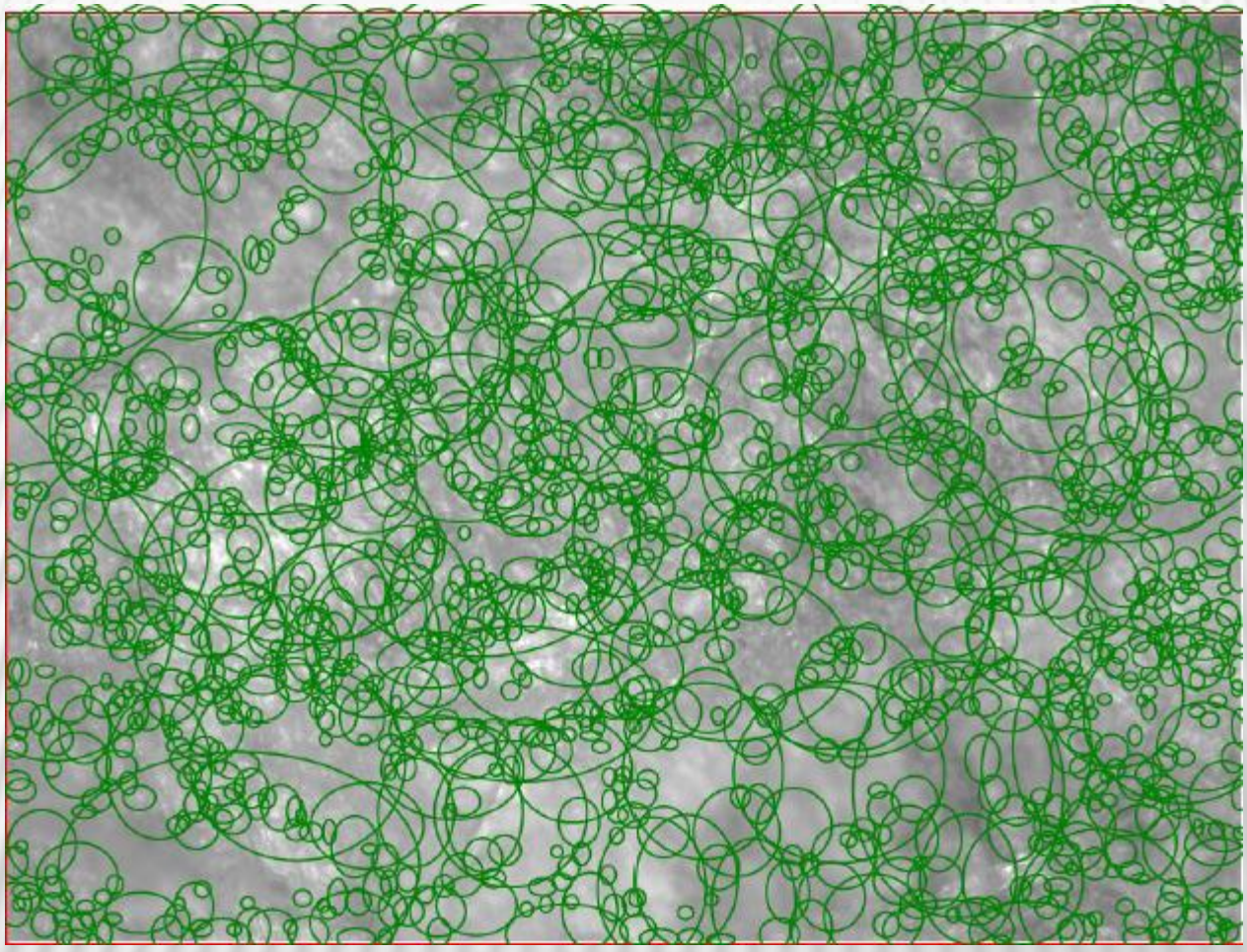
$l_2 = (n*\pi*d*l*t)_1 / (n*\pi*d*t)_2$

Polydimethylsiloxane (PDMS)

All Tests run on at TA instrument Q 800 in rapid compression mode
Frequency 2hz
Temperature 100C
Strain 1%

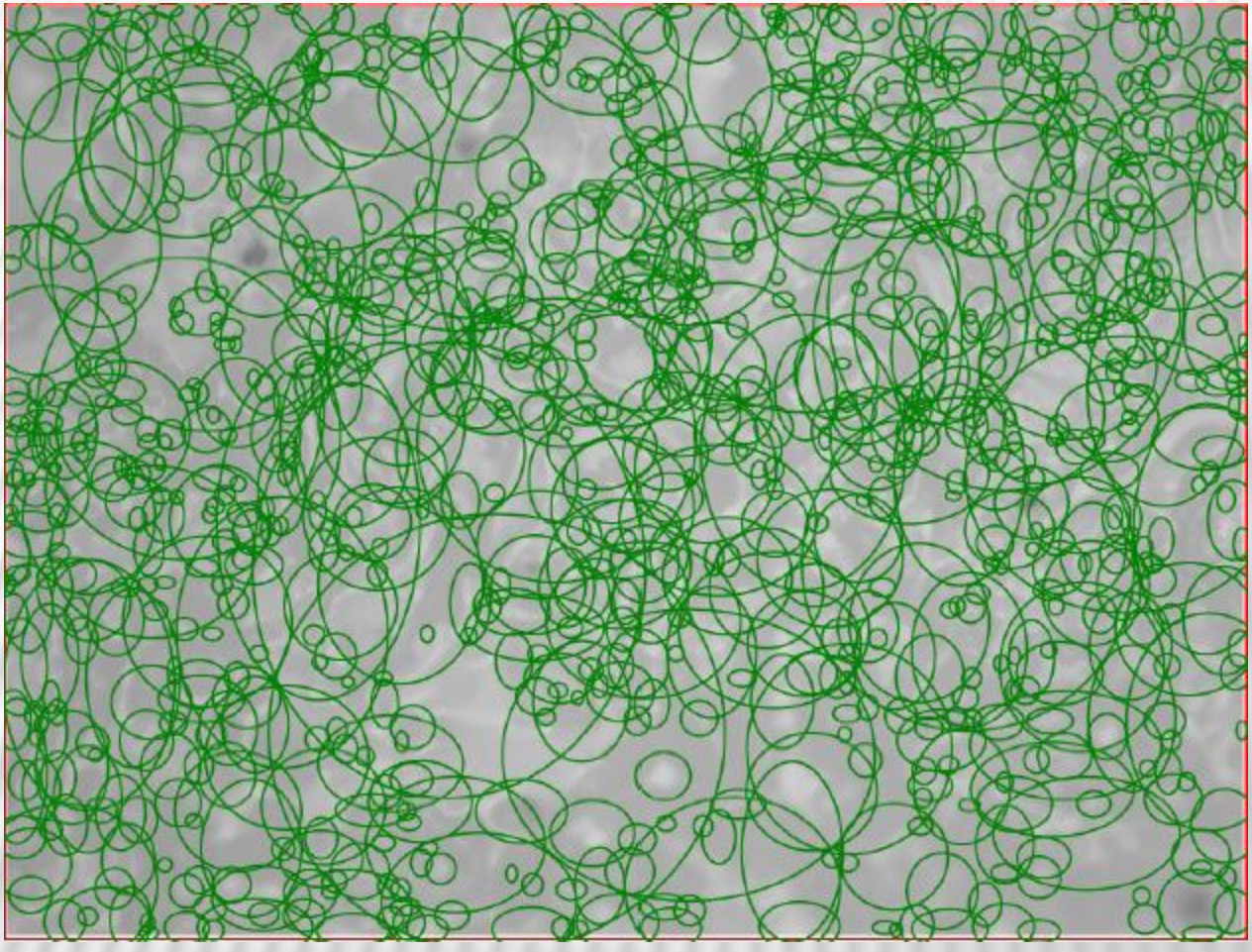
- E’ = 435 kPA
- tan δ = 0.0002
- Chemical structure $CH_3[Si(CH_3)_2O]_nSi(CH_3)_3$
- Hydrophobic, requiring pore structure

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 μm^2
- The total area covered by porosity in the ECM is 1689 μm^2
- Average pore size for PDMS is 1.973227 μm^2
- Average pore size for ECM is 1.140332 μm^2

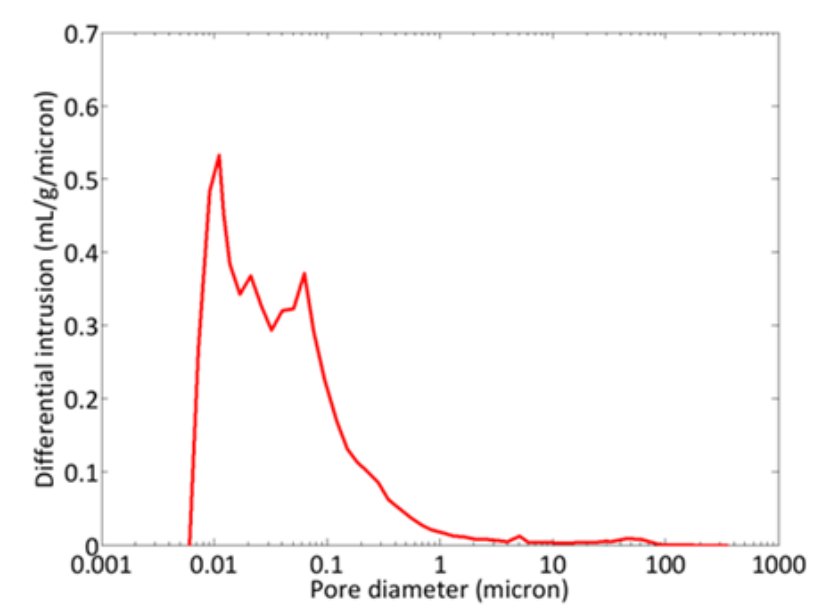
What does this tell us?

Results

The total area of porosity can be reduced in PDMS if the porosity size is appropriately decreased.

Importance of Porosity

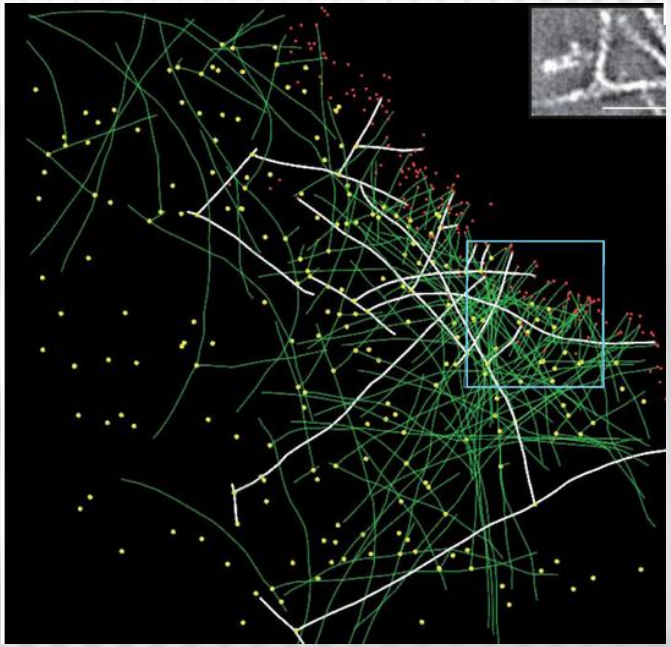
- Porosity provides expansion space.
- Porosity provides binding sites.
- Porosity aids in bio destruction for degradability.
- Small pore size for optimization to avoid shear collapse.



Mike Adams et al.

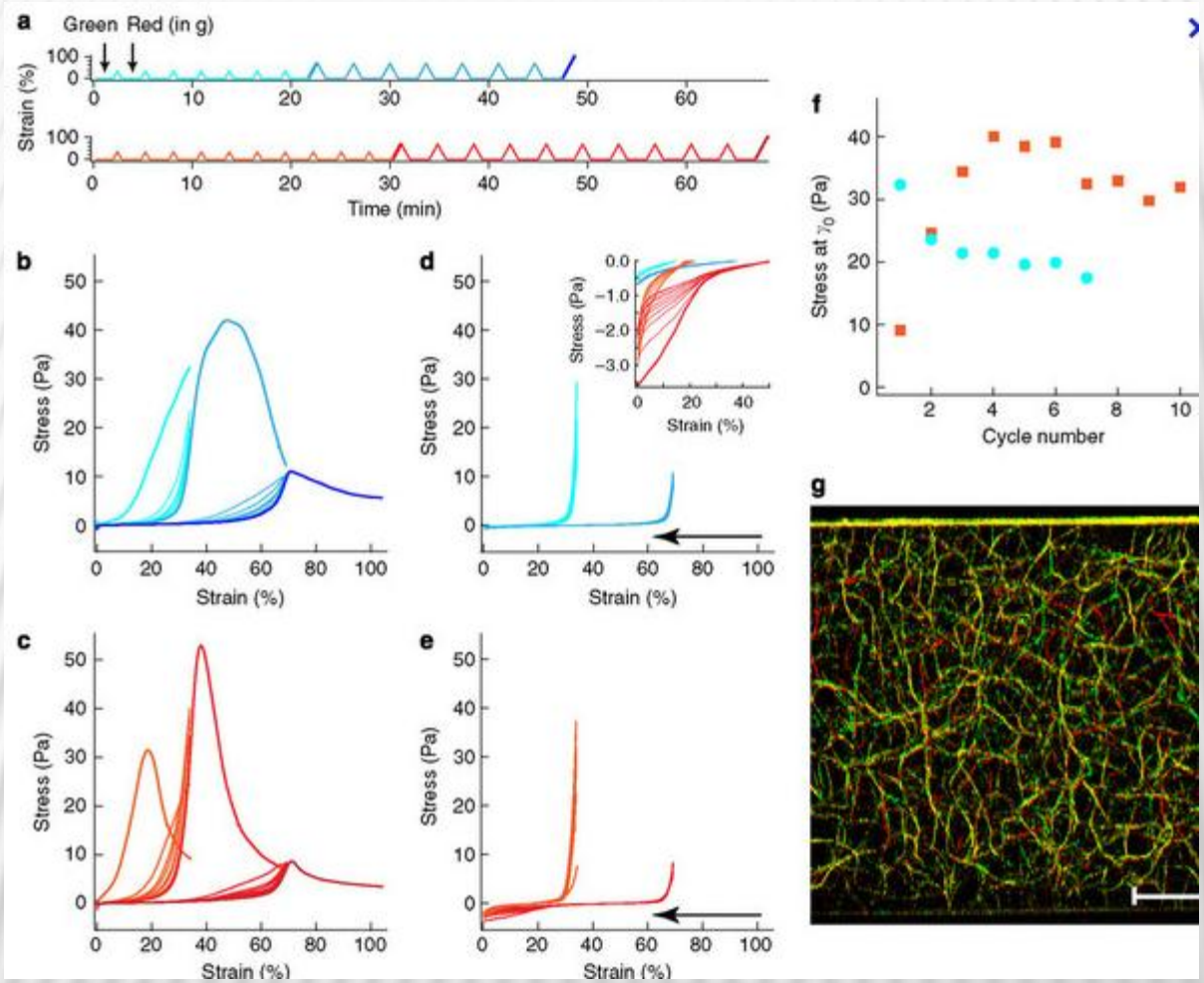
Fibronectic Behavior

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



Daniel Fletcher UC Santa Barbara.

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 biodegredation.
- Fibronectin behavior requires flexibility currently more easy accepted by ECM.
- Work on Polymer bio scaffold should focus on adhesion structure of proteins such as fibronectin.

THIS IS A RUBBER ISSUE….