Lecture 14, Tissue Engineering Scaffolds, 3.054

Tissue engineering scaffolds

- Goal of tissue engineering is to regenerate diseased or damaged tissues
- In the body, cells attach to the extracellular matrix (ECM)
- Composition of ECM depends on the tissue, but typically involves:
  - structural proteins such as collagen, elastin
  - adhesive proteins such as fibronectin, laminin
  - proteogylcons → protein-polysaccharide complexes in which sugars are added to core protein; sugars typically glycosaninoglycans (GAGS) e.g. chondroitin sulfate, dermatin sulfate, heparan sulfate
  - E.g. cartilage — collagen, GAG, hyaluronic acid (HA - proleoglycin)
    bone — collagen and hydroxyapatite
    skin — collagen, elastin, proteoglycans
- Cells have to be attached to ECM, or to other cells, to function (e.g. proliferate, migrate, differentiate...)

Extracellular matrix

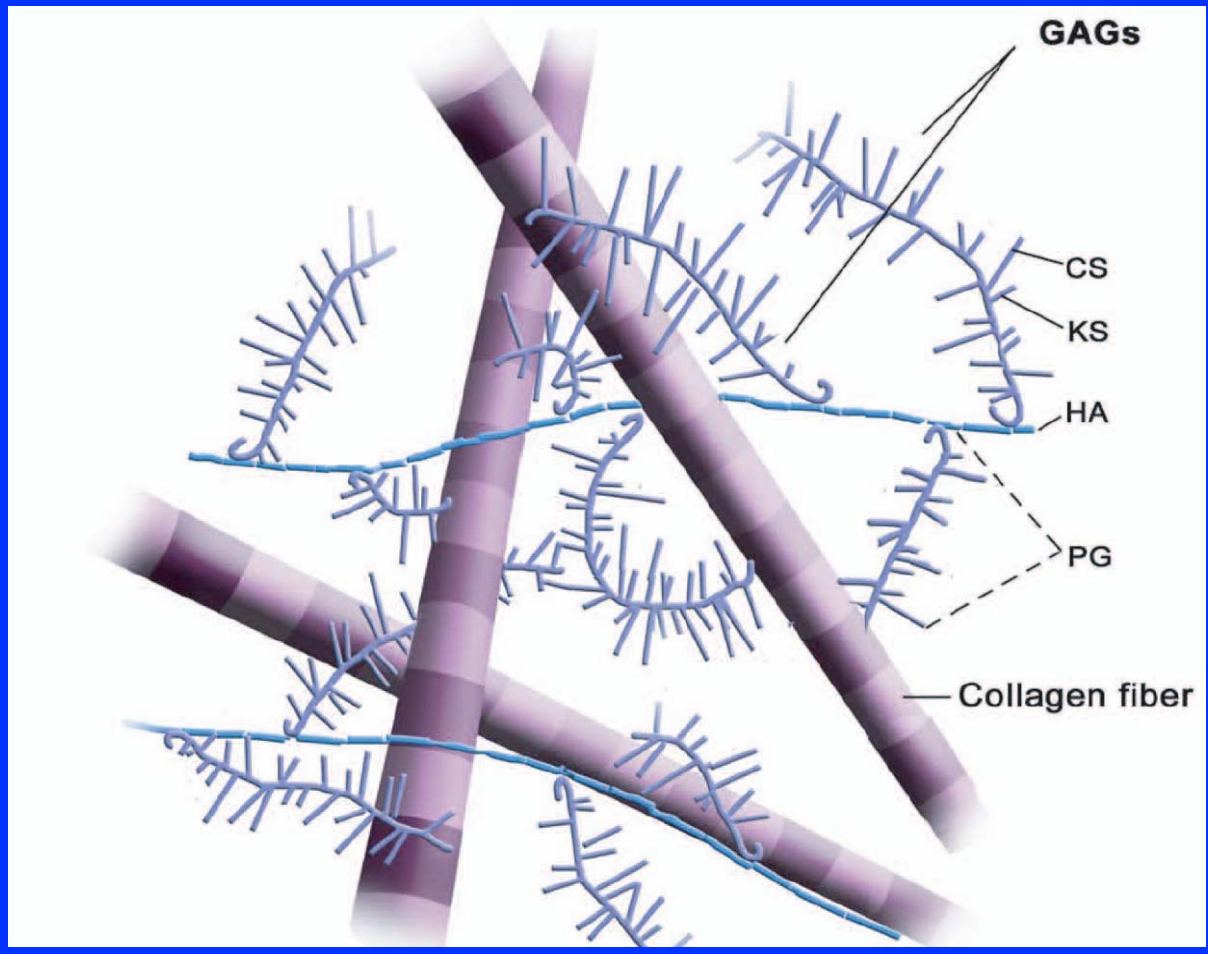


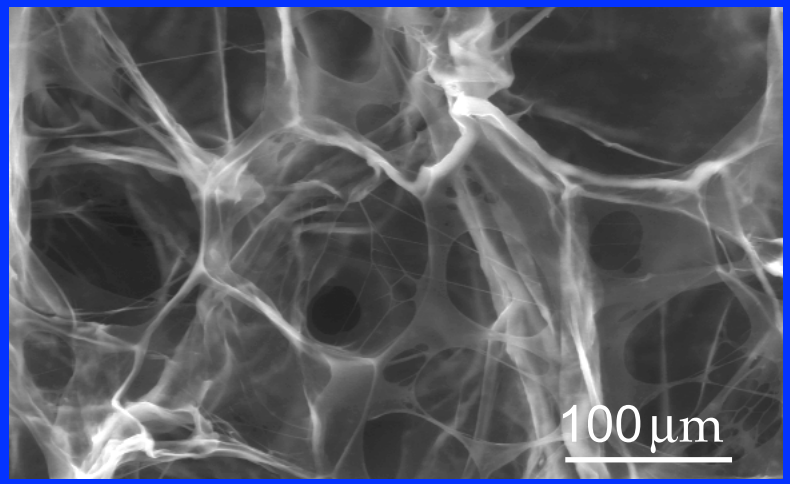
Image by MIT OpenCourseWare. After Ricci.

- Tissue engineering — provide porous scaffold that mimics body's ECM
- Scaffolds for regenerating skin in burn patients have been clinically available for 15 years
- Research on scaffolds for orthopedic, cardiovascular, nervous, gastrointestinal, urogenital tissues ongoing
- At MIT: Bob Langer, Linda Griffith, Sangeeta Bhatia, Al Grodzinsky, Yannas
- In body, cells resorb and deposit new ECM (e.g. bone)
- Tissue engineering scaffolds designed to degrade in the body (from enzymes secreted by cells) and be replaced by natural ECM produced by the cells

Design requirements for scaffolds

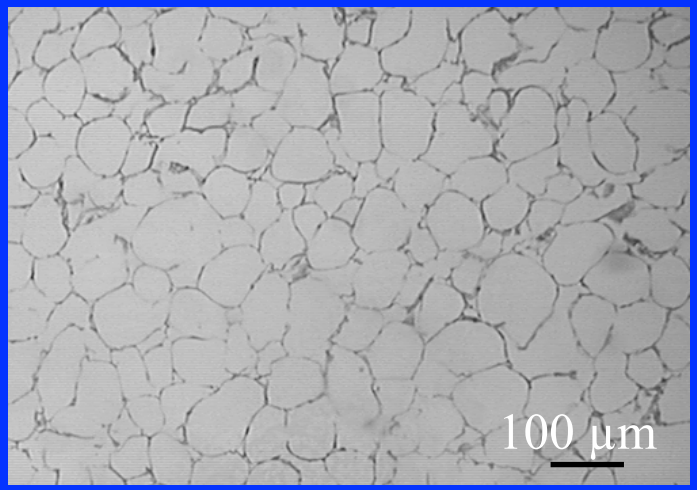
Solid
- must be bio-compatible
- must promote cell attachment, proliferation, migration differentiation, and production of native ECM
- must degrade into non-toxic components that can be eliminated from the body

CG Scaffold: Microstructure



Pek et al., 2004

Fig. 1: Pek, Y. S., M. Spector, et al. *Biomaterials* 25 (2004): 473 82. Courtesy of Elsevier. Used with permission.
http://www.sciencedirect.com/science/article/pii/S0142961203005416



96 µm

O' Brien, Harley et al., 2004

Fig. 4: O'Brien, F. J., B. A. Harley, et al. *Biomaterials* 25, (2004): 1077 86. Courtesy of Elsevier. Used with permission.
http://www.sciencedirect.com/science/article/pii/S0142961203006306

Relative density = 0.005

Design requirements for scaffolds: cellular structure

- Must have large volume fraction of interconnected pores to facilitate cell migration and transport of nutrients and regulatory factors (e.g. growth factors, hormones) $\Rightarrow$ typical porosities $> 90\%$
- Pore size must be within a critical range
  - lower bound controlled by cell size
  - upper bound controlled by density of binding sites available for cell attachment (depends on specific surface area)
  - e.g. skin $20\mu\text{m} < d < 150\mu\text{m}$
    bone $100\mu\text{m} < d < 500\mu\text{m}$
- Pore geometry should be conductive to cell morphology

  E.g. elongated pores for nerve cells

Design requirements for scaffolds

- Sufficient mechanical integrity for handling during surgery, for cell differentiation
- Has to degrade at controllable rate, so that as tissue becomes fully termed, through cell deposition of native ECCM, the scaffold is completely resorbed

Materials

- Natural polymers e.g. collagen, GAGs, alginate, chitosan
- Collagen:
  - major component of ECM in a number of tissues (e.g. skin, bone, cartilage, tendon, ligament)
  - has surface binding sites (ligonds) and is an excellent substrate for cell attachment and proliferation
  - has low Young's modulus (E $\sim$ 0.8 GPa), but can be increased with cross-linking
  - in acetic acid, forms coprecipitate with glycosaminoglycons
  - freeze drying produces porous scaffold
  - can also be used in conjunction with synthetic polymers to get increased E
- Synthetic biopolymers
  - typically use those for resorbable sutures

PGA: polyglycolic acid
PLA: polylactic acid
PLGA: poly (lactic-co-glycolic) acid $\rightarrow$ poly (and capralone)

Degradation rate and mechanical properties can be controlled by controlling ratio of PGA and PLA (as well as molecular weight of each)

- Hydrogels
  - produced by cross-linking water-soluble polymer chains to form insoluble networks
  - used for soft tissues (have high water content and resemble hydrogels)
    e.g. **PEG** polyethylene glycol
    PVA polyvinyl alcohol
    PAA polyacrylic acid
- Synthetic polymers — many processing techniques available
- But don't have cell binding sites — typically have to functionalize (coat surface with adhesive proteins)
- Also — degradation products of synthetic polymers may be cyto toxic or cause inflammatory response (even if polymer itself is not toxic)

Materials

- Scaffolds for regenerating bone typically have a calcium phosphate (e.g. hydroxyapatite, octacalcium phosphate) in a composite with collagen or a synthetic polymer
- A cellular scaffold also used:
  - native ECM from which all cell matter removed
  - decellularization done by combination of physical (e.g. freezing, agitation) and chemical (alkaline, acid treatments) and enzymatic (e.g. trypsin) methods

Processing

- Numerous techniques described in literature, will describe a few

Freeze-drying (Yannas)

- Freeze-dried collagen scaffolds used for skin regeneration
- Microfibrillar type I collagen mixed with acetic acid
- The acid swells the collagen and destroys its periodic bonding removing immunological markers, reducing host immune response

Collagen-GAG Freeze-dried

Salt leaching

Selective laser sintering

Acellular elastin scaffold from porcine heart tissue

Foaming

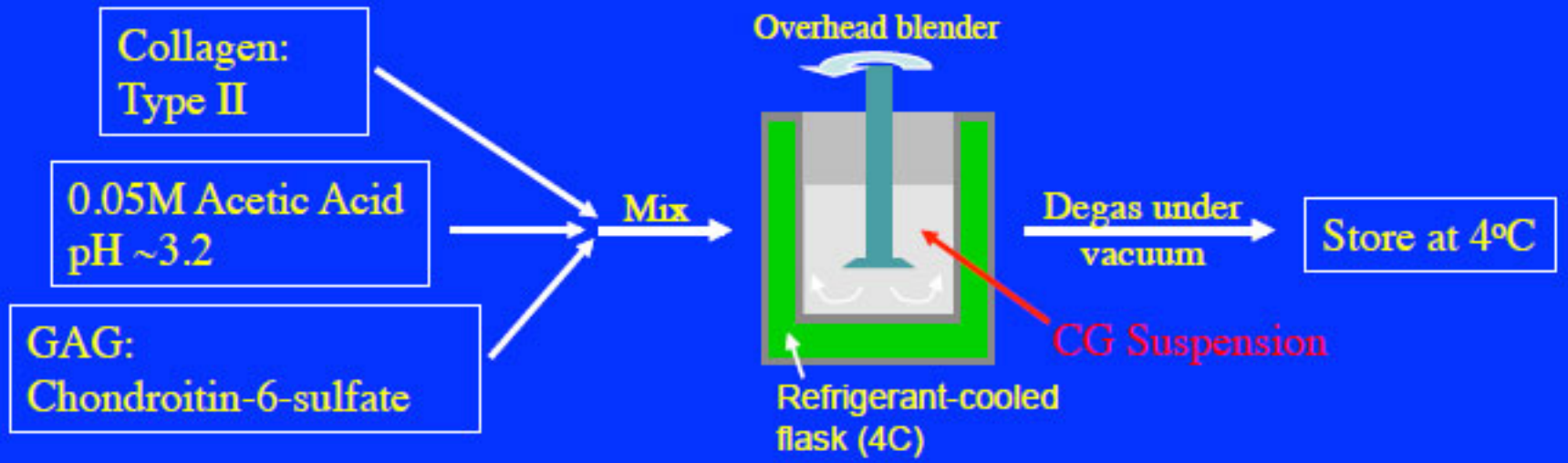
Electrospun

Images removed due to copyright restrictions.

Sources in Cellular Materials in Nature and Medicine

Collagen-GAG Scaffold: Fabrication

Production of CG Suspension



Yannas

CG Scaffold: Fabrication

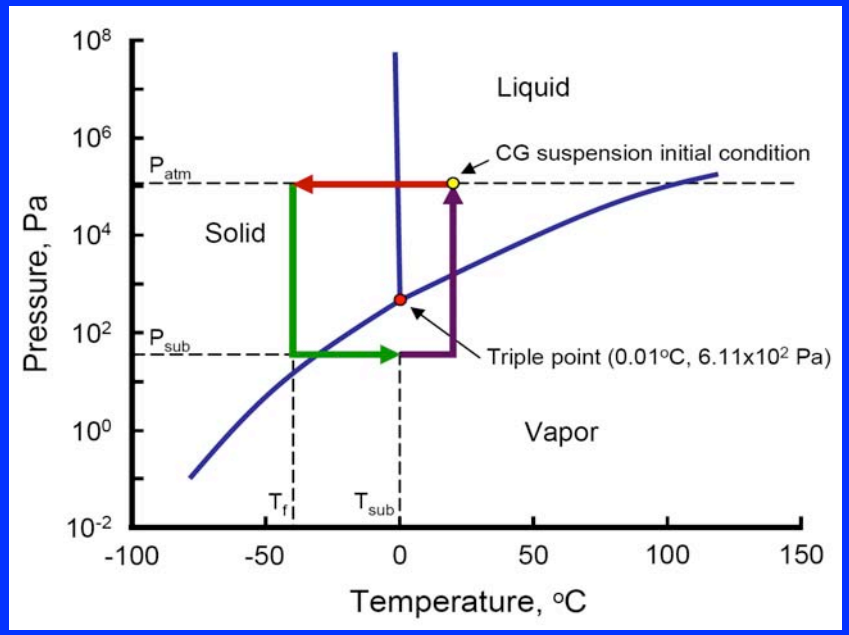
Place CG suspension into stainless steel pan (12.5 x 12.5 cm)

Freeze: Freeze-dryer

Ice crystals surrounded by collagen and GAG fibers

Sublimation: P=75mTorr, T=0°C Removes ice content

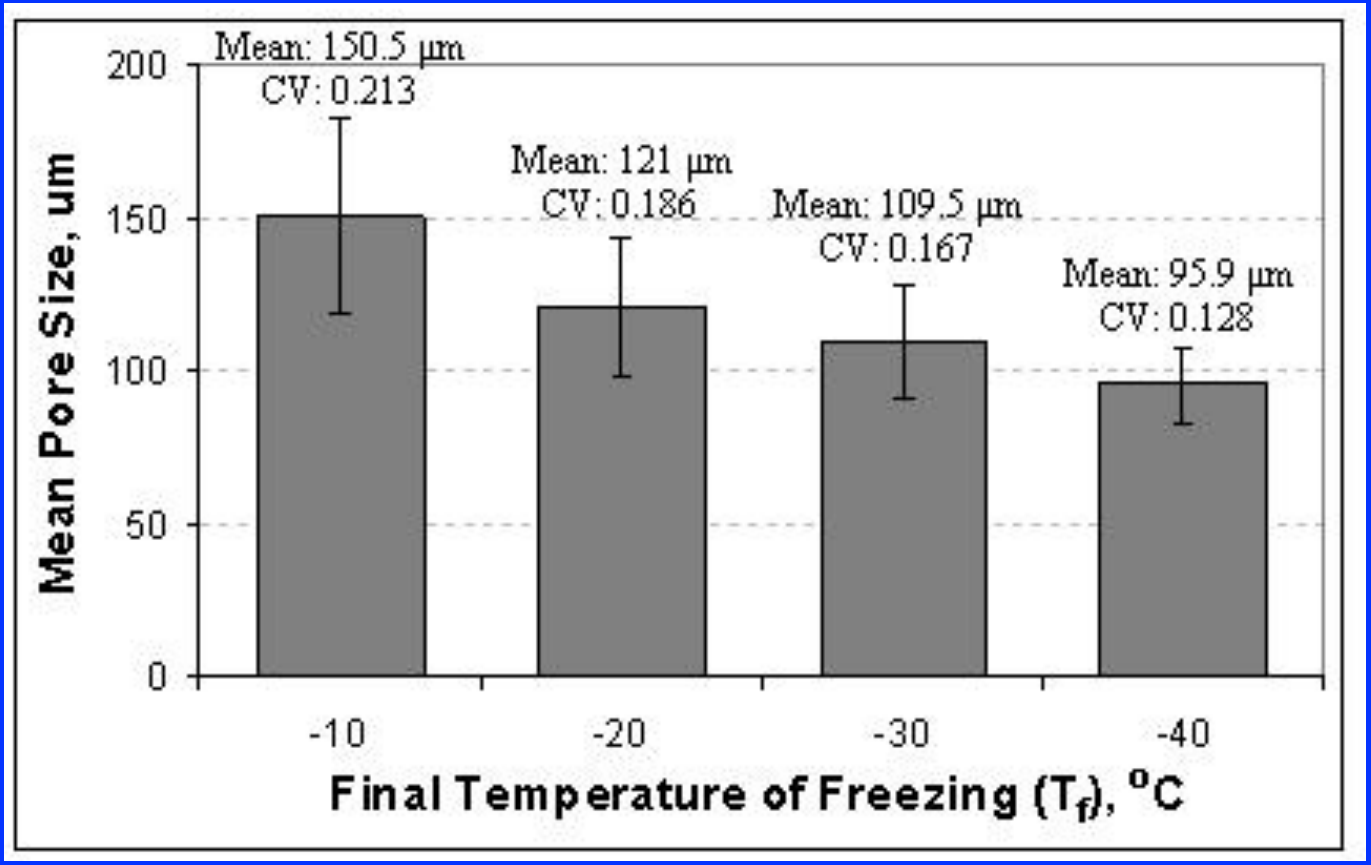
Porous, CG scaffold



Yannas, Harley

Courtesy of Brendan Harley. Used with permission.

CG Scaffold: Pore Size



O'Brien, B. A. Harley, I. V. Yannas, et al. *Biomaterials* 26 (2005): 433 41. Courtesy of Elsevier. Used with permission.
http://www.sciencedirect.com/science/article/pii/S0142961204002017

Harley, O'Brien

- Then, add chondroitin-6-sulfate (GAG) which cross-links with the collagen, forming a precipitate out of the solution
- Freeze-drying gives porous scaffold
- $\rho^*/\rho_0 = 0.005$
- Pore sizes $\sim\ 100 - 150\,\mu$m
- For nerve regeneration — directional cooling — elongated pores

Foaming

- Hydrogel can be foamed by bubbling CO_2
- Can use strainer to act as filter to control bubble size (e.g. cell culture strainer)

Leaching a fugitive phase

- Can use salt or paraffin wax as fugitive phase
- Combine powder of polymer and salt, heat to bind powder, leach out salt
- Control density by volume fraction of fugitive phase
- Control pore size by particle size of fugitive phase

Electrospinning

- Fibers produces from a polymer solution extruded through thin nozzle
- Apply high voltage electric field to spin fibers
- Obtain interconnected network of micron-scale fibers

Rapid prototyping

- Build up successive layers of solid, one layer at a time
- 3D printing; selective laser sintering; stereolithography of photosensitive polymer
- Computer control allows fabrication of complex geometries

Mechanical behavior of scaffolds

- Consider behavior of collagen-GAG scaffold
- Compression $\sigma - \epsilon$ curve: 3 typical regimes

$$E^*/E_s = (\rho^*/\rho_s)^2 \quad \text{(bending)} \qquad \sigma^*_{\text{el}} = 0.05\, E_s\, (\rho^*/\rho_s)^2 \quad \text{(buckling)}$$

- E_s measured by removing a single strut ($l \approx 80\mu m$), bonding one end to a glass slide and performing a bending test using an AFM

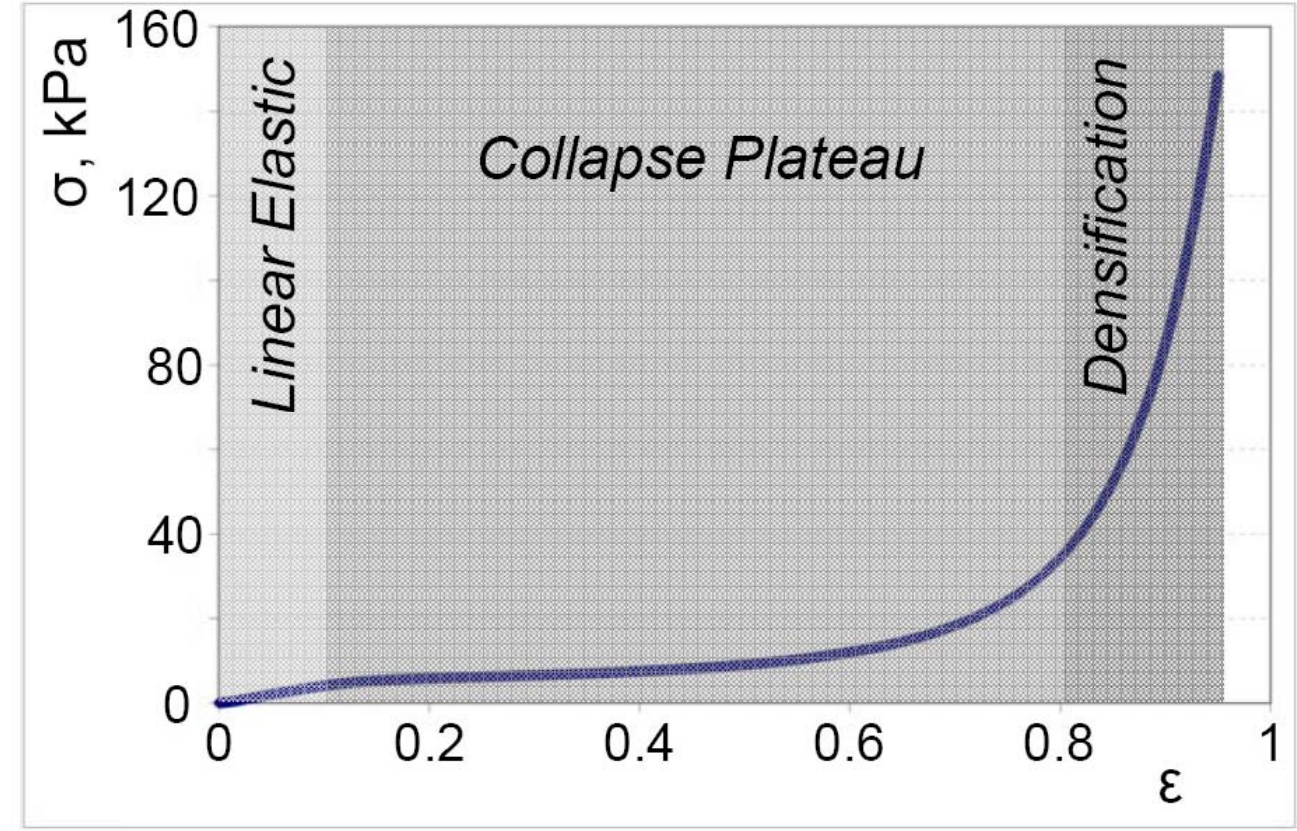
  $E_s = 762$ MPa (dry)

- For $\rho^*/\rho_s = 0.0058, \quad 121\mu$m pore size:

	E^* (Pa)	σ^*_{el} (Pa)	
Measured	30,000	5150	
Calculated	25,600	5120	(using $C_2 = 0.2$, based on $\epsilon^*_{\text{el}} = 0.2$ - measured)

- Tests on higher density ($\rho^*/\rho_s = 0.009, 0.012, 0.018$) – $E^*, \sigma^* \propto (\rho^*/\rho_s)$ (linear)
- Increasing density increased viscosity of collagen-GAG suspension prior to freezing — harder to get homogeneous mix
- Higher density scaffolds had heterogeneities (e.g. large voids), reducing mechanical properties
- Also increases cross-link density $\Rightarrow$ $E^* \uparrow, \quad \sigma^*_{\text{el}} \uparrow$
- Also varied pore size $\Rightarrow$ $E^*, \sigma^*_{\text{el}}$ constant, as expected

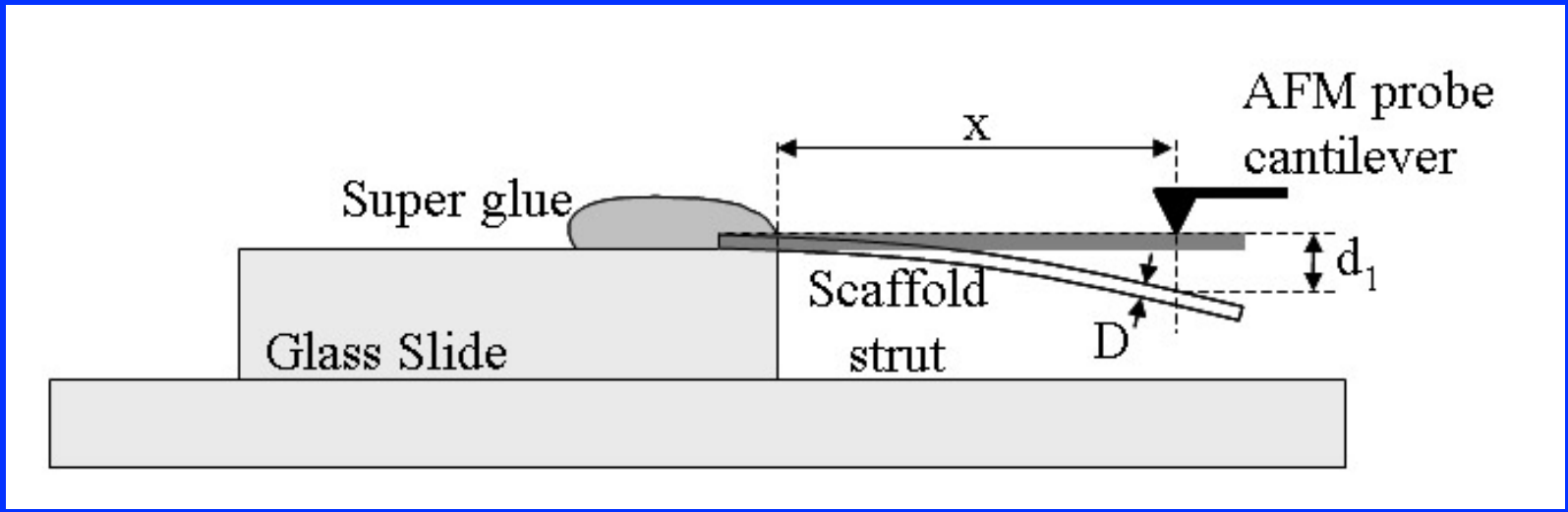
CG Scaffold: Compression (Dry)



Source: Harley, B. A., et al. *Acta Biomaterialia* 3 (2007): 463 74. Courtesy of Elsevier. Used with permission.
http://www.sciencedirect.com/science/article/pii/S1742706107000025

Harley et al., 2007

Solid Strut Modulus



$E_s = 762$ MPa (dry)

$E_s = 5.28$ MPa (wet)

Source: Harley, B. A., et al. *Acta Biomaterialia* 3 (2007): 463 74. Courtesy of Elsevier. Used with permission.
http://www.sciencedirect.com/science/article/pii/S1742706107000025

Harley, Silva

Mechanical properties of honeycomb-like scaffolds

- Honeycomb-like scaffolds have also been proposed

Sangeeta Bhatia
- Hexagonal honeycomb — designed to increase diffuse nutrient transport to hepatocyles for liver regeneration

George Engelmeyer
- Scaffolds with rectangular pores of varying aspect ratio and diamond shaped pores used to study effect of pore geometry on fibroblast orientation

Bob Langer
- Accordion-like honeycomb is designed to match anisotropy in the mechanical properties of cardiac tissue; like hexagonal honeycomb, but vertical walls corrugated

Models:

- Triangulated hexagonal honeycomb: stretch dominated; expect $E^* \propto E_s(\rho^*/\rho_s)$
- Rectangular cell: loading along struts $E^* \propto E_s(\rho^*/\rho_s)$

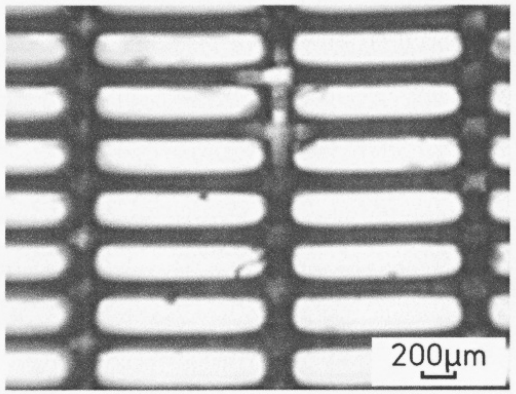
loading at θ° to struts $E^* \propto E_s(\rho^*/\rho_s)^3$

- Diamond cells: equivalent to hexagonal honeycomb $h = 0,\ \theta = 45^\circ$

$h = 0$
$\theta = 45^\circ$

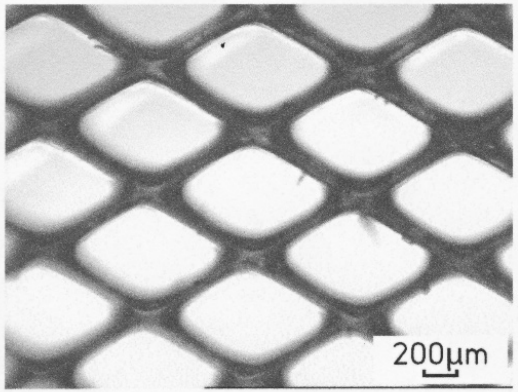
Tsang
et al. 2007

Figure removed due to copyright restrictions. See Figure 4: Tsang, V. L., et al. *FASEB Journal* 21, no. 3 (2007): 790-801. http://www.fasebj.org/content/21/3/790

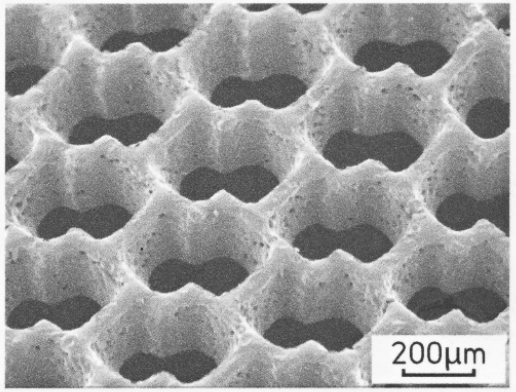


Source: Engelmayr, George C., Jr., et al. "Guidance of Engineered Tissue Collagen Orientation by Large-scale Scaffold Microstructures." *Journal of Biomechanics* 39 (2006): 1819–31. Courtesy of Elsevier. Used with permission.

Engelmayer
et al., 2006



Engelmayer
et al., 2006



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Source: Jean, A., and G. C. Engelmyr Jr. "Finite Element Analysis of an Accordion-like Honeycomb Scaffold for Cardiac Tissue Engineering." *Journal of Biomechanics* 43 (2010): 3035-43. Courtesy of Elsevier. Used with permission.

3.054 / 3.36 Cellular Solids: Structure, Properties and Applications
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