

## 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
  - proteoglycans → protein-polysaccharide complexes in which sugars are added to core protein; sugars typically glycosaminoglycans (GAGs)

e.g. chondroitin sulfate, dermatin sulfate, heparan sulfate

- e.g. cartilage - collagen, GAG, hyaluronic acid (HA-proteoglycan)
- bone - collagen + 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

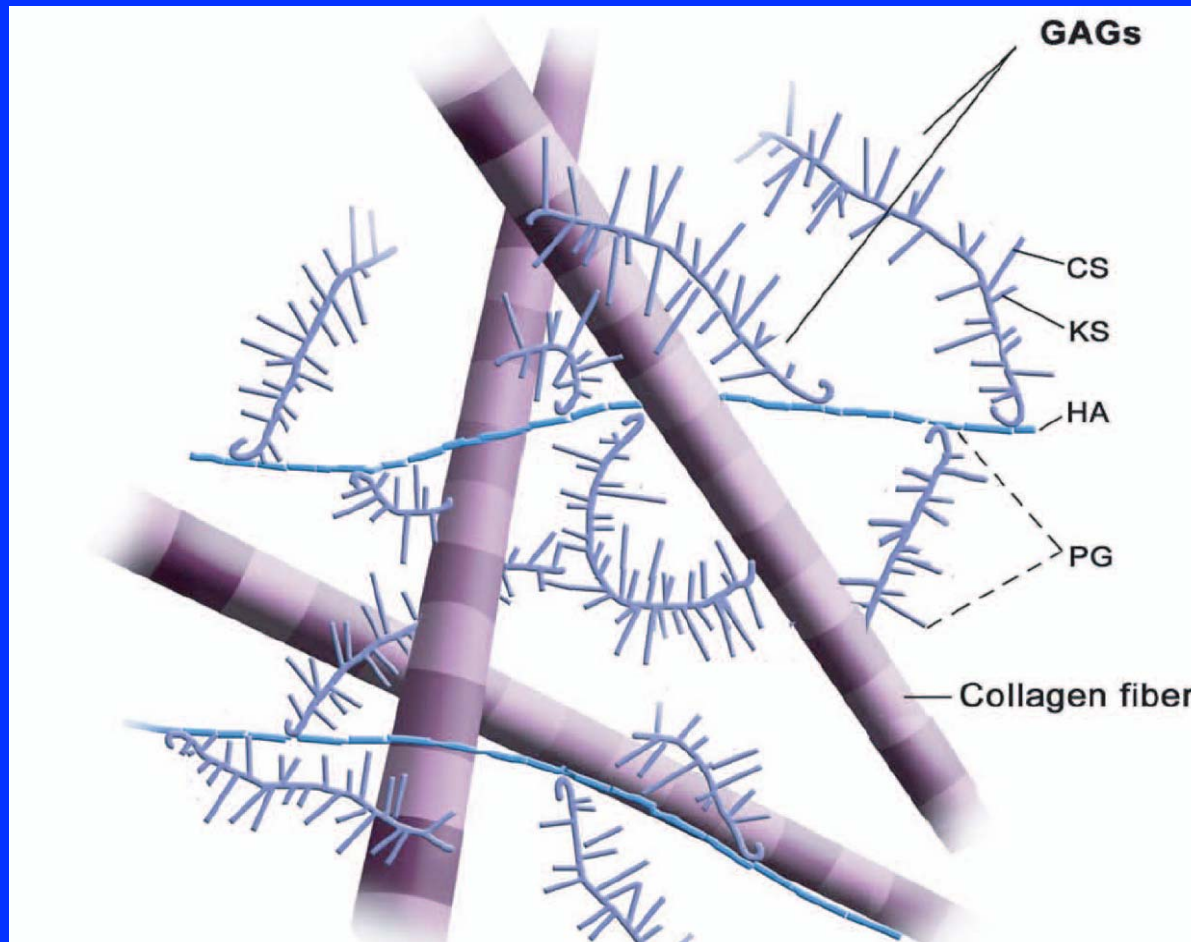


Image by MIT OpenCourseWare. After Ricci.

- 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 orthopaedic, cardiovascular, nervous, gastrointestinal, urogenital tissues ongoing
  - at MIT: Bob Langer, Linda Griffith, Sangeeta Bhatia, Al Grodzinsky, Yamas
  - in body, cells resorb + deposit new ECM (eg. bone)
- 

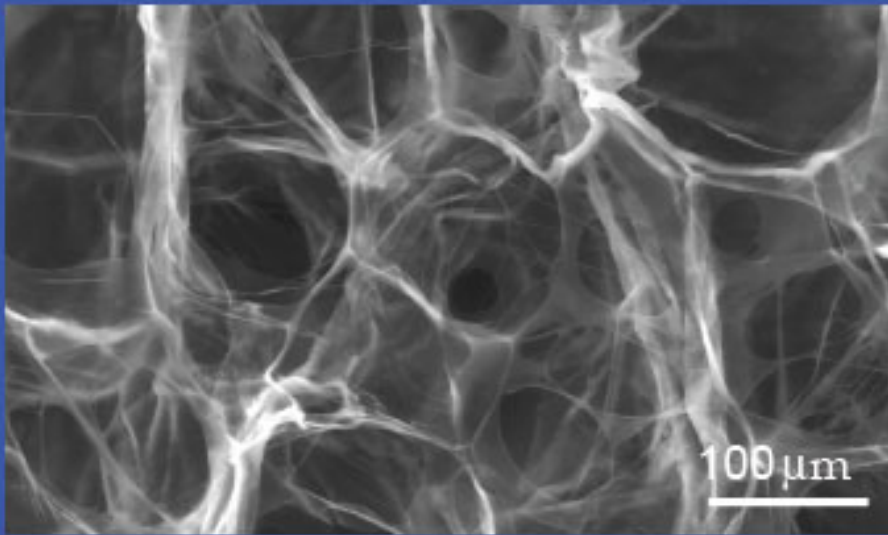
- tissue engineering scaffolds designed to degrade in the body (from enzymes secreted by cells) + be replaced by natural ECM produced by the cells

### Design requirements for scaffolds

Solid - must be biocompatible

- must promote cell attachment, proliferation, migration, differentiation + 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 + transport of nutrients + regulatory factors (e.g. growth factors, hormones) => typical porosities > 90%
- pore size must be within a critical range
  - lower bound controlled by cell size
  - upper bound " " density of binding sites available for cell attachment (depends on specific surface area)

- eq. skin  $20\mu m < d < 150\mu m$
- bone  $100\mu m < d < 500\mu m$

- pore geometry should be conducive to cell morphology  
eg. 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 formed, through cell deposition of native ECM, the scaffold is completely resorbed

## Materials

- natural polymers eg. collagen, GAGs, alginate, chitosan
  - collagen
    - major component of ECM in a number of tissues (eg. skin, bone, cartilage, tendon, ligament)
    - has surface binding sites (ligands) + is an excellent substrate for cell attachment + proliferation
    - has low Young's modulus ( $E \sim 0.8 \text{ GPa}$ ) but can be increased with cross-linking
    - in acetic acid, forms coprecipitate with glycosaminoglycans

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  - freeze drying produces porous scaffold
  - can also be used in conjunction with synthetic polymers to get incr. E
  - Synthetic biopolymers
    - typically use those for resorbable sutures
      - PGA : polyglycolic acid
      - PLA : polylactic acid
      - PLGA : poly(lactic-co-glycolic) acid
      - poly( $\epsilon$  caprolone)
- degradation rate + mech. prop. can be controlled by controlling ratio of PGA + PLA (as well as molecular weight of each)

## • hydrogels

- produced by crosslinking water soluble polymer chains to form insoluble networks
- used for soft tissues (have high water content + 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 cytotoxic or cause inflammatory response (even if polymer itself is not toxic)



## Materials

- scaffolds for regenerating bone typically have a calcium phosphate (eg. hydroxyapatite, octacalcium phosphate) in a composite with collagen or a synthetic polymer
  - acellular scaffolds also used
    - native ECM from which all cell matter removed
    - decellularization done by combination of physical (eg. freezing, agitation) + chemical (alkaline, acid treatments) + enzymatic (eg. 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 + destroys its periodic banding removing immunological markers, reducing host immune response



Collagen-GAG  
Freeze-dried

Salt leaching

Selective laser  
sintering

Acellular  
elastin scaffold  
from porcine  
heart tissue

Foaming

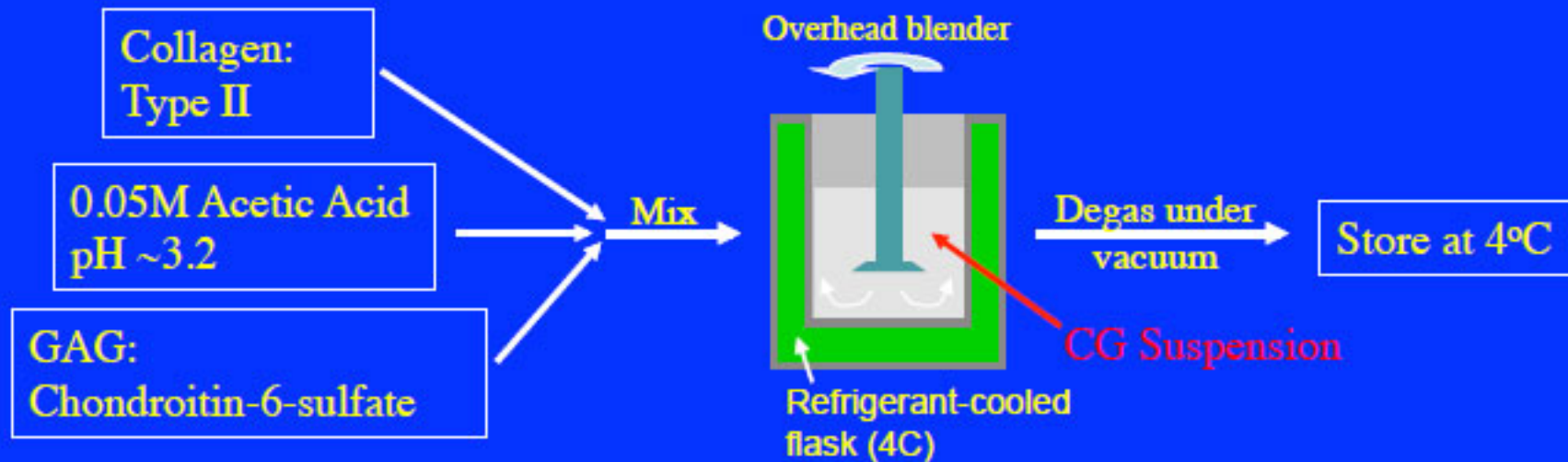
Electrospun

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Sources in Cellular  
Materials in Nature and  
Medicine

# Collagen-GAG Scaffold: Fabrication

## Production of CG Suspension



Yannas

# CG Scaffold: Fabrication

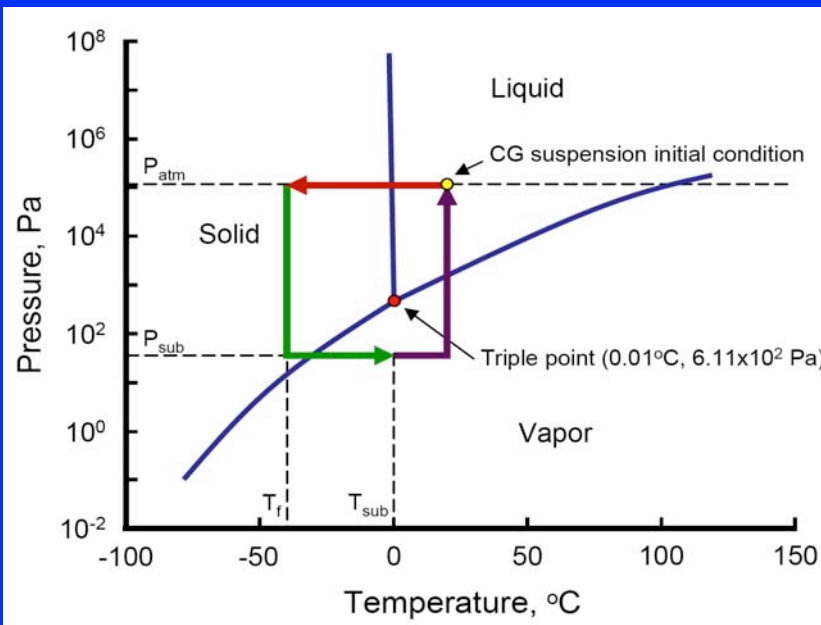
Place CG suspension into stainless steel pan (12.5 x 12.5 cm)

Freeze:  
Freeze-dryer

Ice crystals surrounded by collagen and GAG fibers

Sublimation:  
 $P=75\text{mTorr}$ ,  
 $T=0^\circ\text{C}$   
Removes ice content

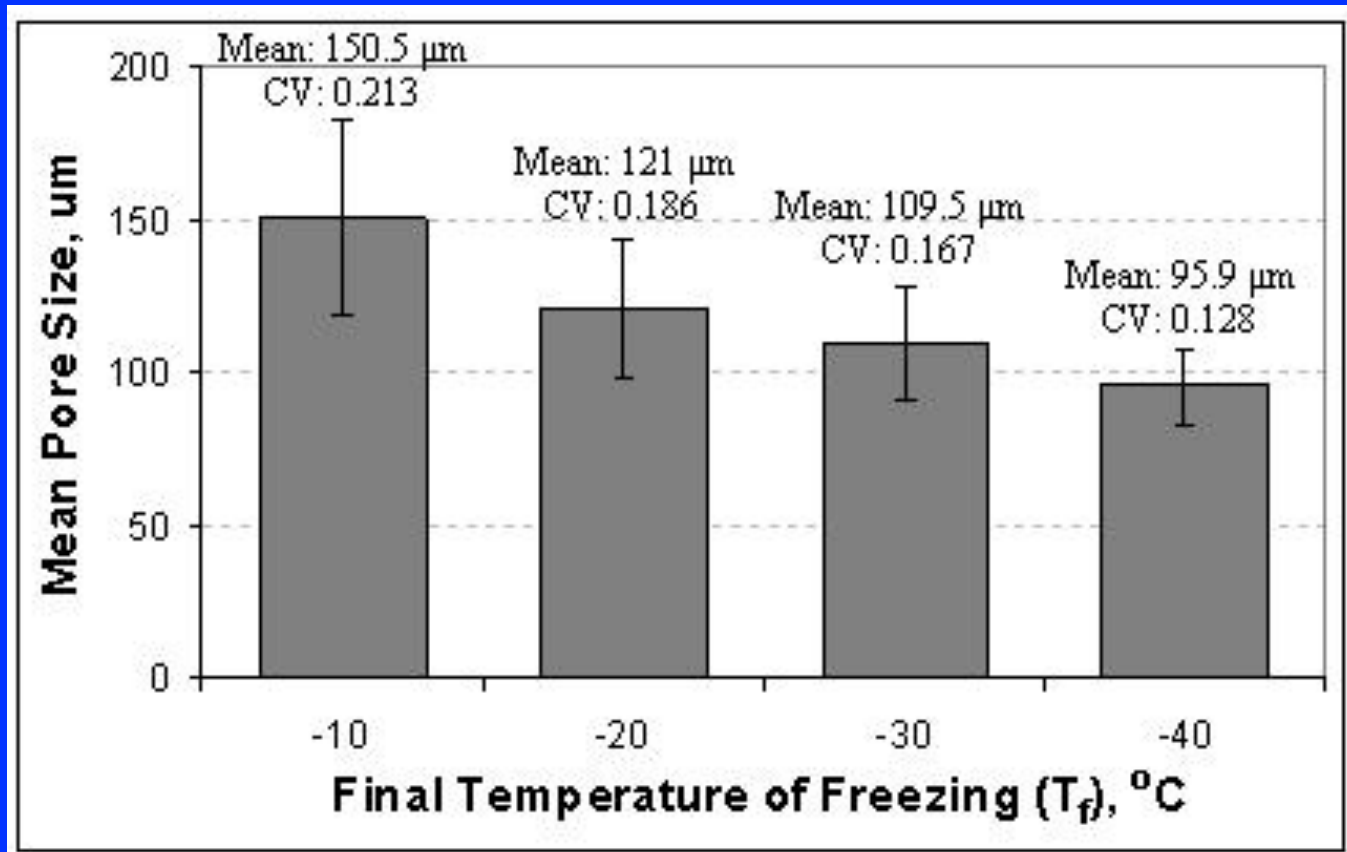
Porous, CG scaffold



Yannas, Harley



# 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 <sup>with</sup> the collagen, forming a precipitate out of the solution
- freeze-drying gives porous scaffold
- $\rho^* l_p = 0.005$
- pore sizes  $\sim 100-150 \mu\text{m}$
- for nerve regeneration - directional cooling - elongated pores

### Foaming

- hydrogel can be foamed by bubbling  $\text{CO}_2$
- can use strainer to act as filter to control bubble size (eg. cell culture strainer)

### Leaching a fugitive phase

- can use salt or paraffin wax as fugitive phase
- combine powder of polymer + 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 produced from a polymer solution extruded through thin nozzle
- apply high voltage electric field to spin fibers
- obtain interconnected network of micron-scale  $\phi$  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 behaviour of scaffolds

- consider behaviour of collagen-GAG scaffold
- compression  $\sigma$ - $\epsilon$  curve: 3 typical regimes

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

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

$$E_s = 762 \text{ MPa (dry)}$$



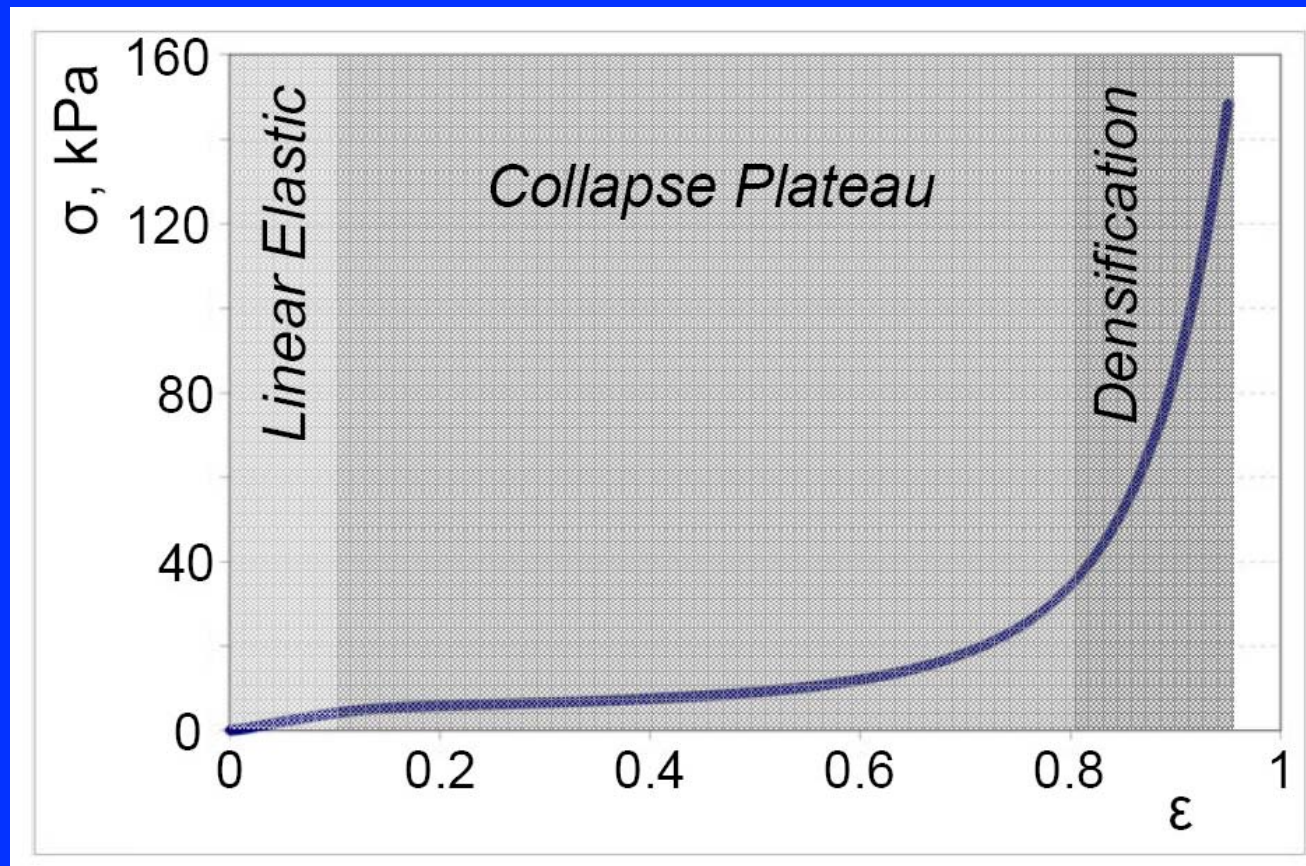
- for  $\rho^*/\rho_s = 0.0058$ , 121  $\mu\text{m}$  pore size:

|            | $E^*$ (Pa) | $\sigma_{el}^*$ (Pa)   |
|------------|------------|--|
| measured   | 30,000     | 5150   |
| calculated | 25,600     | 5120 (using $C_2 = 0.2$ , based on $\epsilon_{el}^* = 0.2$ <sup>measured</sup> ) |

- tests on higher density ( $\rho^*/\rho_s = 0.009, .012, .018$ ) -  $E^*, \sigma^* \propto (\rho^*/\rho_s)$  (linear)
- increasing density increased viscosity of collagen-GAG suspension prior to freezing - harder to get homogeneous mix

- higher density scaffolds had heterogeneities (eg. large voids), reducing mechanical properties
- also increased cross-link density  $\Rightarrow E^* \uparrow \sigma_{el}^* \uparrow$
- also varied pore size  $\Rightarrow E^*, \sigma_{el}^*$  constant, as expected

# CG Scaffold: Compression (Dry)



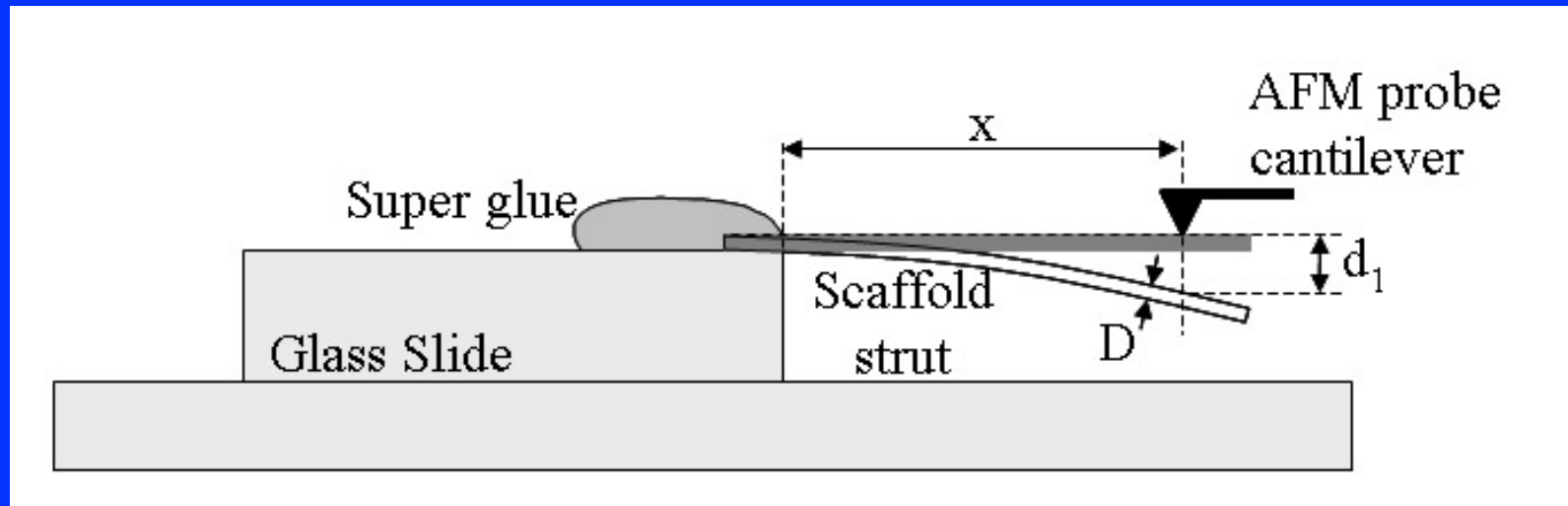
Source: Harley, B. A., et al. *Acta Biomaterialia* 3 (2007):

463-74. Courtesy of Elsevier. Used with permission.

<http://www.sciencedirect.com/science/article/pii/S1742706107000025>

Harley et al., 2007

# Solid Strut Modulus



$E_s = 762 \text{ MPa}$   
(dry)

$E_s = 5.28 \text{ MPa}$   
(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 behaviour of honeycomb-like scaffolds

- honeycomb-like scaffolds have also been proposed
- hexagonal honeycomb - designed to increase diffuse nutrient transport to hepatocytes for liver regeneration
- scaffolds with rectangular pores of varying aspect ratio + diamond shaped pores used to study effect of pore geometry on fibroblast orientation

Sangeeta Bhatia

George Engelmeier

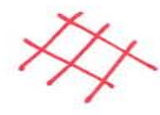
Bob Langer

- accordion-like honeycomb - designed to match anisotropy in the mechanical properties of cardiac tissue; like hex. honeycomb but vertical walls corrugated

- triangulated hex. honeycomb: - stretch dominated; expect  $E^* \propto E_s (\rho^*/\rho_s)$
- rectangular cells: loading along struts  $E^* \propto E_s (\rho^*/\rho_s)$   
 at  $\theta = 0^\circ$  to struts  $E^* \propto E_s (\rho^*/\rho_s)^3$



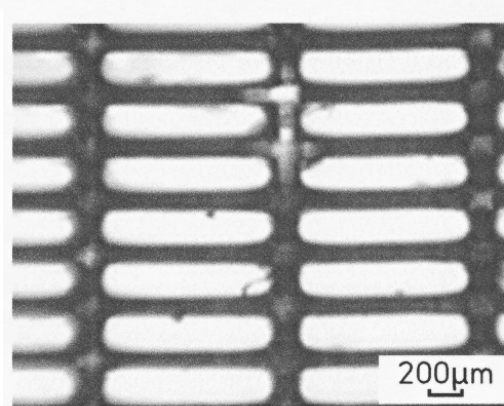
- diamond cells: equivalent to hex. honeycomb  $\theta_0 = 0 \quad \theta = 45^\circ$



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

Tsang  
et al. 2007

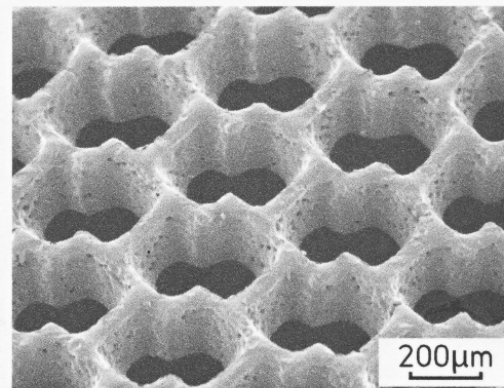
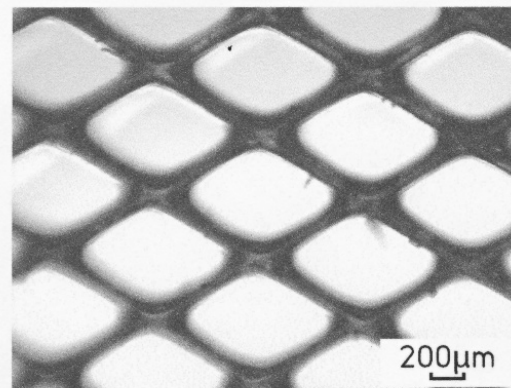
Figure removed due to copyright restrictions. See Figure 4: Tsang, V. L., et al. *FASEB Journal* 21, no. 3 (2007): 790-801. <http://www.fasebj.org/content/21/3/790>



Source: Engelmayer, 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

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Source: Jean, A., and G. C. Engelmayer 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.

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