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)

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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

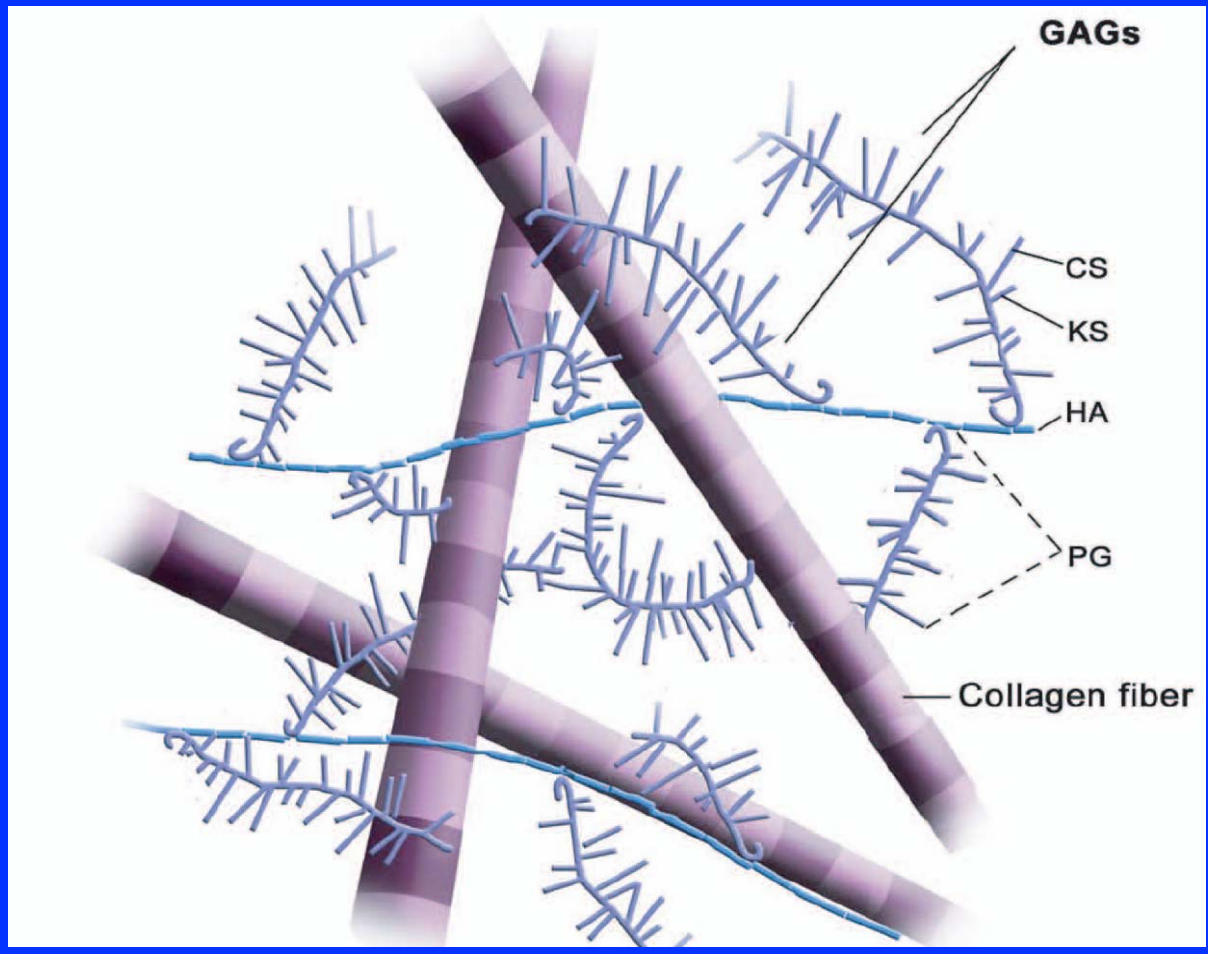


Image by MIT OpenCourseWare. After Ricci.

- 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, Yannas
- in body, cells resorb + deposit new ECM (eg. bone)

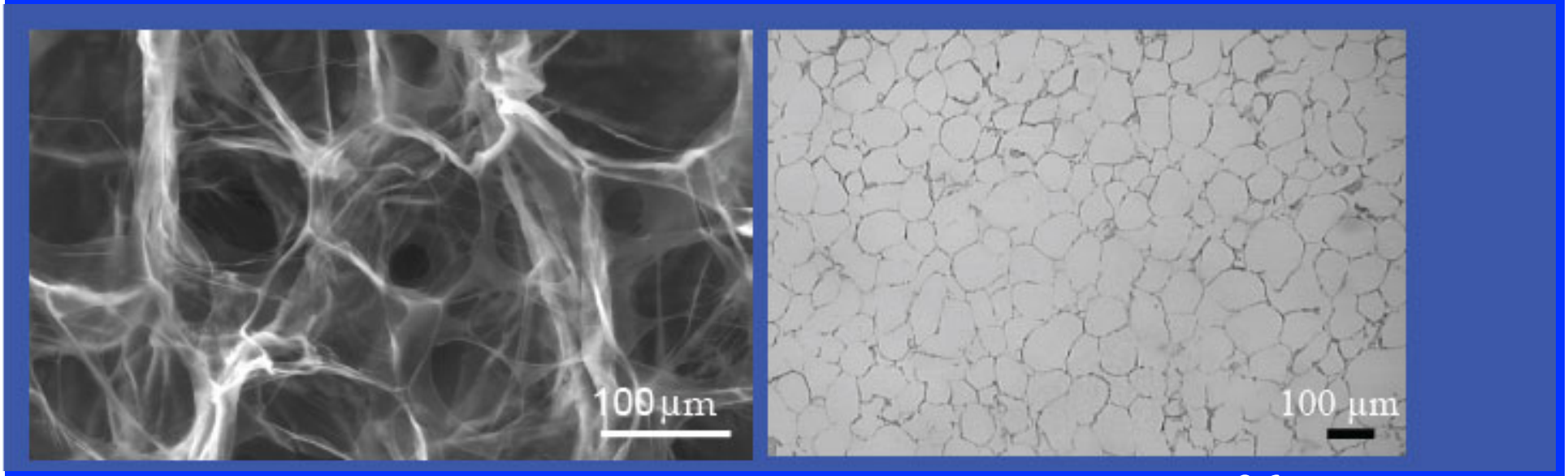
- tissue engineering scaffolds designed to degrade in the body (from enzymes secreted by cells) + be replaced by natural ECM produced by the cells

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



96 µm

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

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)

  - eg. skin $20\,\mu m < d < 150\,\mu m$
    bone $100\,\mu m < d < 500\,\mu m$
- pore geometry should be conducive 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 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 ($E \sim 0.8$ 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 → degradation rate + mech. prop. can be controlled by controlling ratio of PGA + PLA (as well as molecular weight of each)

poly (ε capralone)

- 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

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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

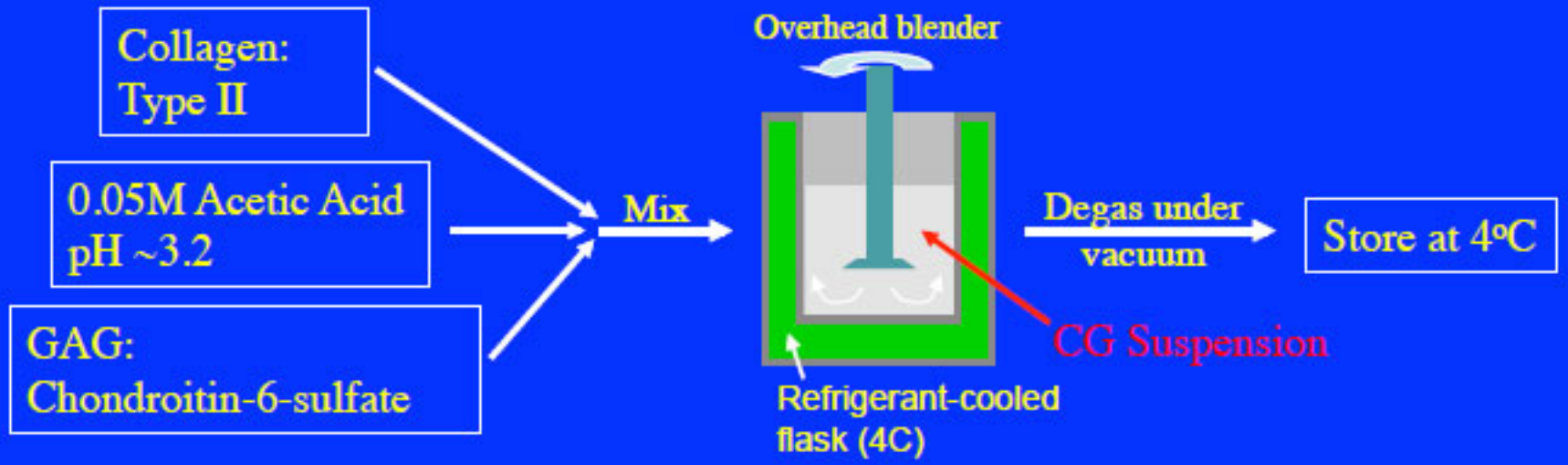
Electrospun

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

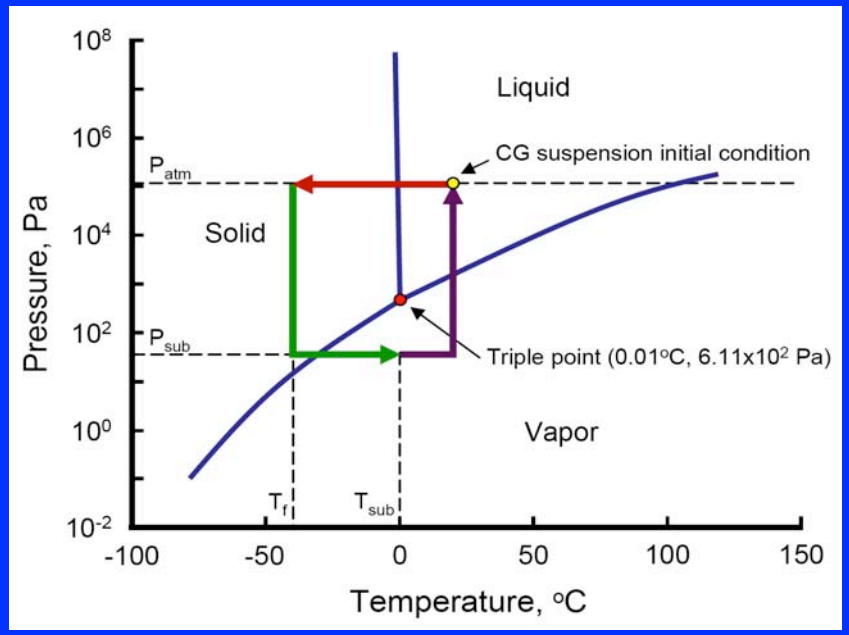
Place CG suspension into stainless steel pan (12.5 x 12.5 cm)

→ Freeze: Freeze-dryer →

Ice crystals surrounded by collagen and GAG fibers

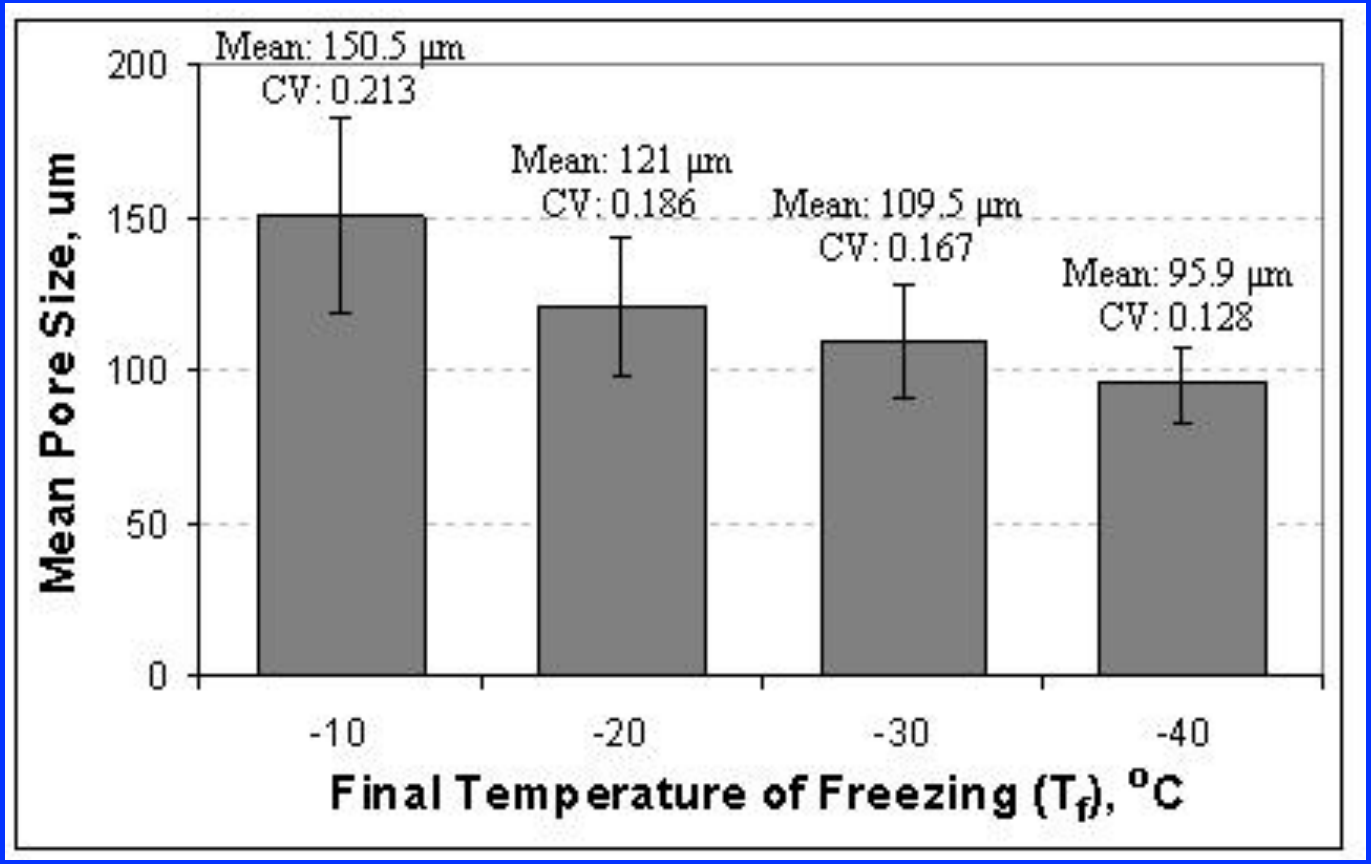
→ Sublimation: P=75mTorr, T=0°C Removes ice content →

Porous, CG scaffold



Yannas, Harley

CG Scaffold: Pore Size



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 ~ 100-150 μm
- for nerve regeneration - directional cooling - elongated pores

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Foaming

- hydrogel can be foamed by bubbling 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 parafin 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 ϕ fibers.

Rapid prototyping

- build up successive layers of solid, one layer at a time
- 3D printing; selective laser sinking; stereolithography of photosensitive polymer
- computer control allows fabrication of complex geometries.

Mechanical behaviour of scaffolds

- consider behaviour of collagen-GAG scaffold
- compression σ-ϵ curve: 3 typical regimes

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

- E_s measured by removing a single strut ($l \approx 80\mu m$), 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 μm pore size:

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

- 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

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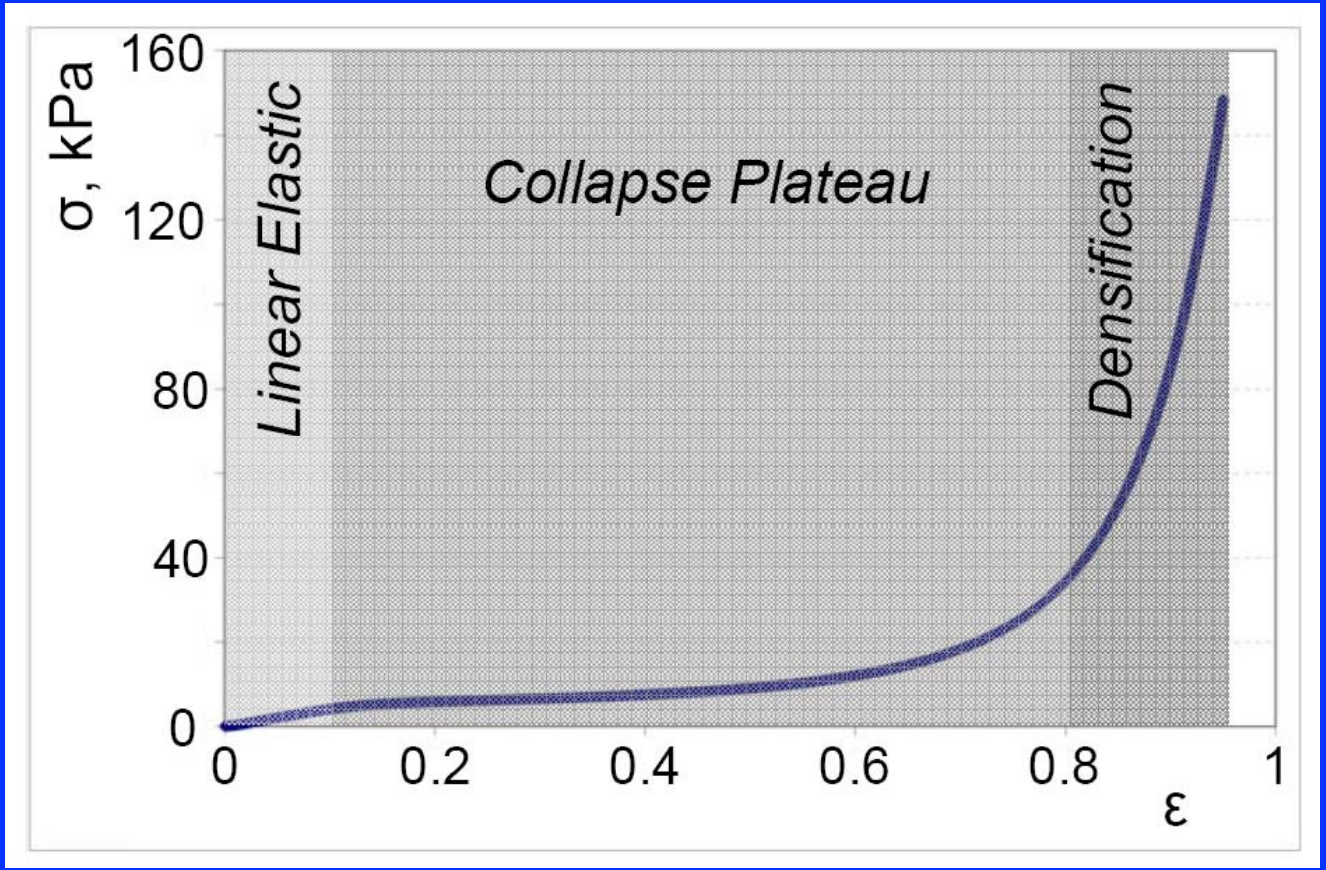
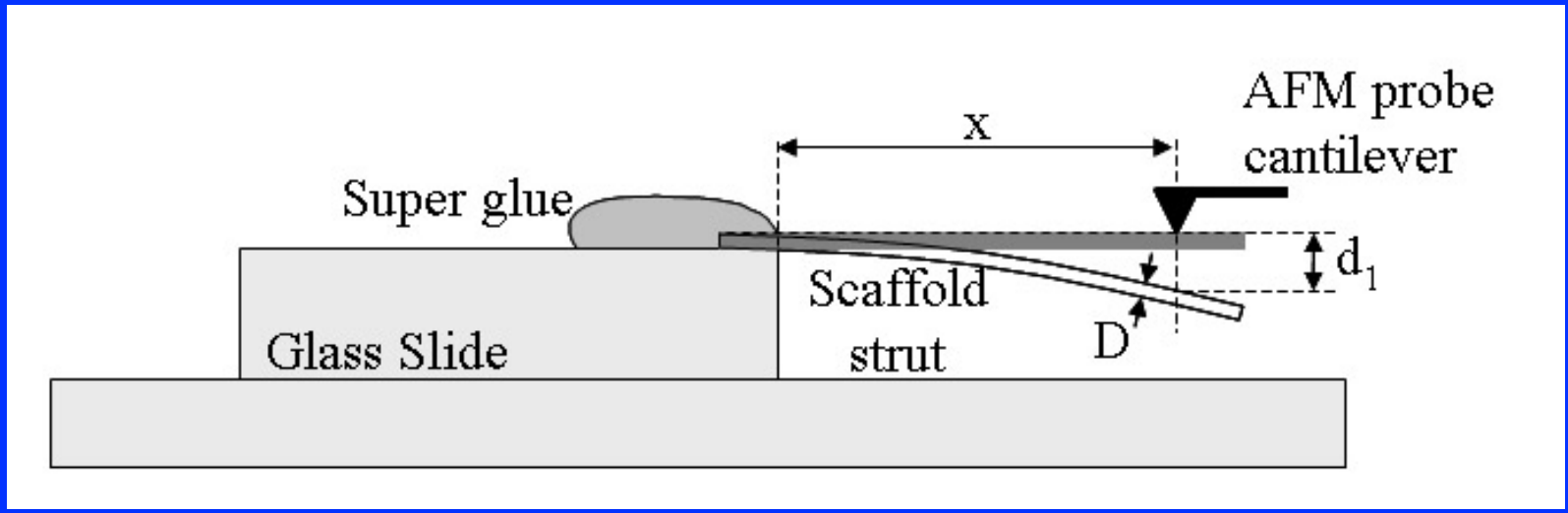
- higher density scaffolds had heterogeneities (eg. large voids), reducing mechanical properties
- also increased cross-link density ⇒ $E^* \uparrow$ $\sigma^*_{el} \uparrow$
- also varied pore size ⇒ E^*, σ^*_{el} constant, as expected

CG Scaffold: Compression (Dry)



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 behaviour of honeycomb-like scaffolds

- honeycomb-like scaffolds have also been proposed

Sangeeta Bhatia
- hexagonal honeycomb - designed to increase diffuse nutrient transport to hepatocytes for liver regeneration

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

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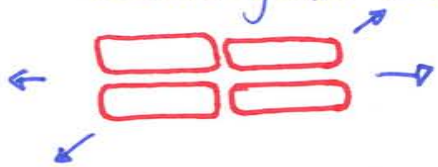
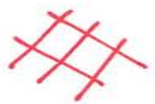
Bob Langer
- accordion-like honeycomb - designed to match anisotropy in the mechanical properties of cardiac tissue; like hex. honeycomb but vertical walls corrugated

Models.

- 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 $\theta°$ to struts $E^* \propto E_s (\rho^*/\rho_s)^3$



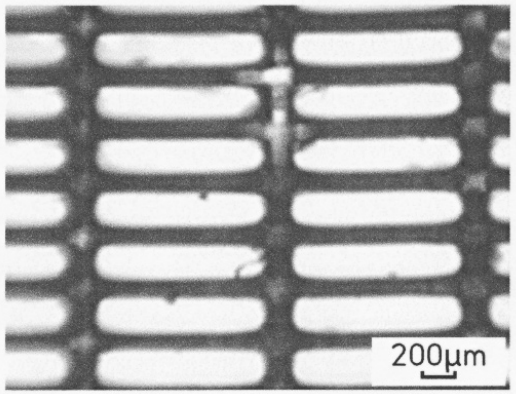


$h = 0$
$\theta = 45°$

- diamond cells: equivalent to hex. honeycomb $h/l = 0$ $\theta = 45°$

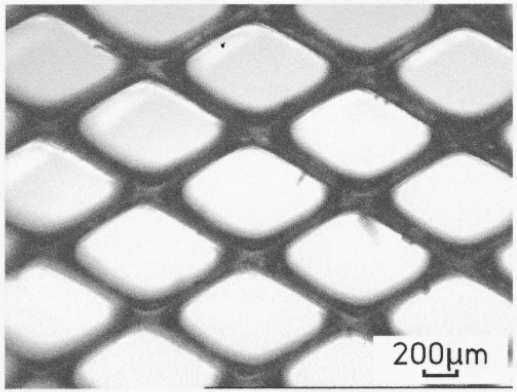
Tsang
et al. 2007

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



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Engelmayer
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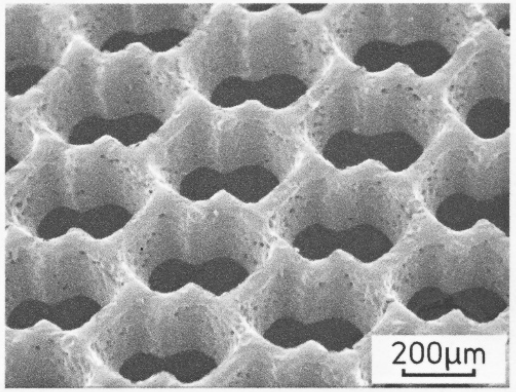

Source: Jean, A., and G. C. Engelmyr Jr. "Finite Element Analysis of an Accordion-like Honeycomb Scaffold for Cardiac Tissue Engineering." *Journal of Biomechanics* 43 (2010): 3035-43. Courtesy of Elsevier. Used with permission.

3.054 / 3.36 Cellular Solids: Structure, Properties and Applications
Spring 2015