

# Fabrication, Microstructure and Mechanical Properties of an Osteochondral Scaffold

Lorna J. Gibson

Materials Science and Engineering

MIT

# Collaborators

- MIT: IV Yannas, BA Harley, S Vickers, B Kanungo, Z Wissner-Gross
- BWH: M Spector, H-P Hsu
- Cambridge: W Bonfield, AK Lynn, S Best, RE Cameron

# Outline

- Cartilage and Current Treatments
- Design Considerations for Osteochondral Scaffolds
- Collagen-GAG Scaffold
- Mineralized Collagen-GAG Scaffold
- Osteochondral Scaffold: Fabrication, Microstructure, Animal Studies

# Articular Cartilage

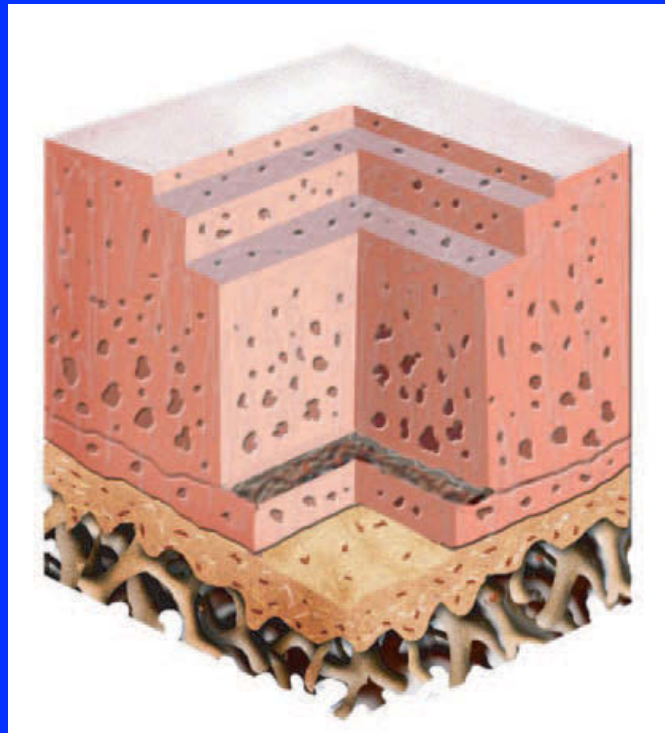


Image by MIT OpenCourseWare.

Superficial and transition cartilage zones

Radial (deep) cartilage zone

Tidemark

Calcified cartilage zone  
Subchondral bone

Trabecular bone

Bone: Type I collagen  
Cartilage: Type II collagen

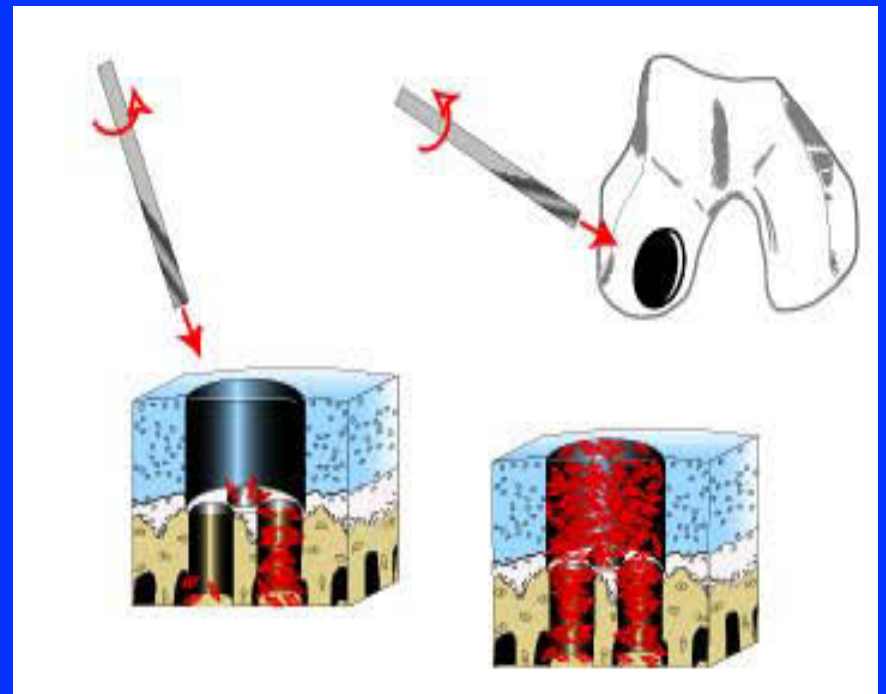
Greene WB (2006)  
Netter's Orthopaedics

# Articular Cartilage

- Avascular: no blood supply
- Low density of chondrocytes
- Damage: sports injuries, osteoarthritis
- Poor capacity for self-repair

# Current Treatments: Marrow Stimulation

- Subchondral bone plate punctured to induce bleeding from marrow cavity
- Marrow derived stem cells entrapped in blood clot
- Stem cells form repair tissue throughout defect
- > 75,000 procedures in the US/year



Courtesy of Andrew Lynn. Used with permission.

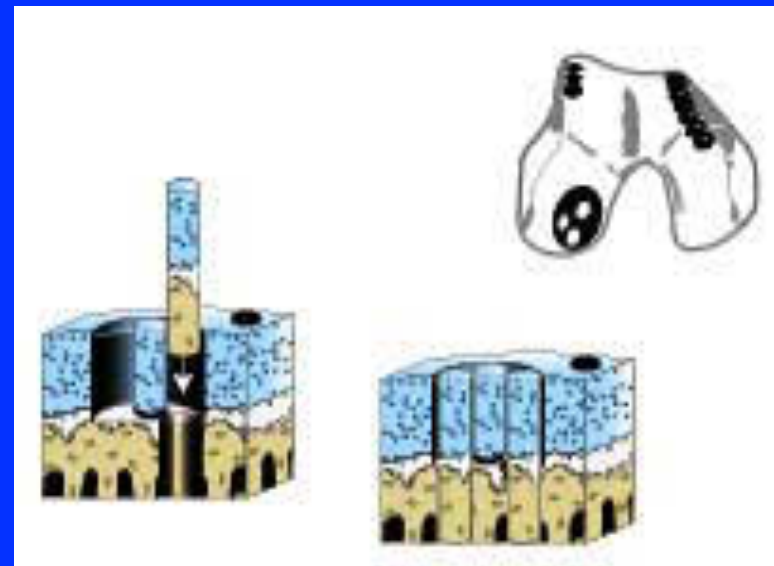
POOR QUALITY OF REPAIR

Lynn (2005)

# Current Treatments: Osteochondral Autograft

- Plugs of bone and cartilage harvested from non load-bearing sites
- Plugs used to fill defect site in a mosaic pattern (mosaicplasty)
- < 2,000 procedures in the US/year

## DONOR SITE MORBIDITY



Courtesy of Andrew Lynn. Used with permission.

Lynn (2005)

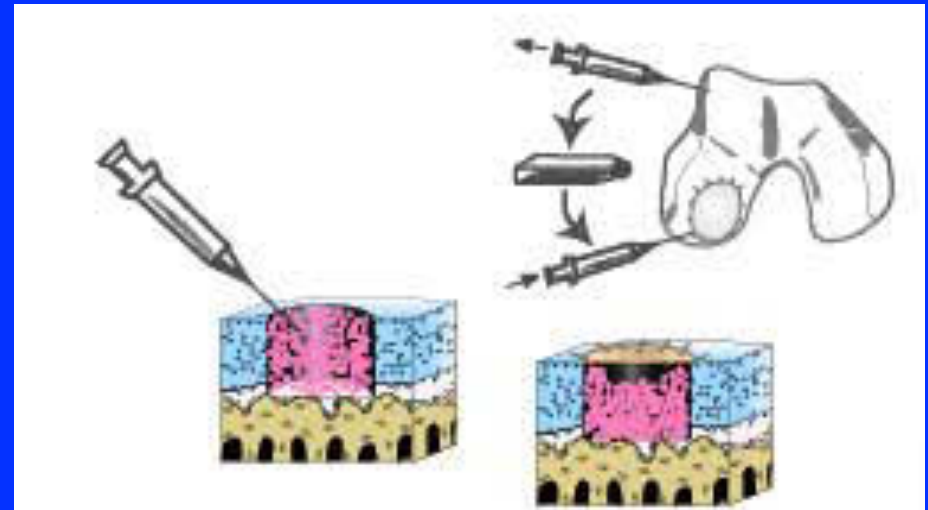
# Current Treatments: Autologous Chondrocyte Implantation

- Cartilage cells harvested from non load-bearing site
- Cells isolated and cultured 2-3 weeks
- Cells re-implanted
- < 2,000 procedures in the US/year

TWO SURGERIES,

COST OF CELL CULTURE

(Dara Torres age 45; Olympic swimmer 84, 88, 92, 00, 08 – 3 silvers)



Courtesy of Andrew Lynn. Used with permission.

Lynn (2005)



# Osteochondral Scaffolds: Design Considerations

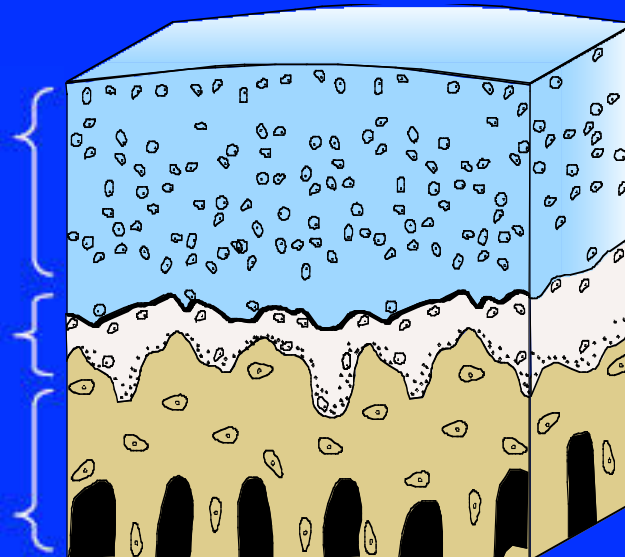
- Use healthy articular joint as a model for scaffold structure and composition
- Bone layer allows access to mesenchymal stem cells
- Control of scaffold parameters (e.g. mineral content, pore size)
- Use materials appropriate for rapid regulatory compliance

# Osteochondral Scaffold

Unmineralized type II collagen

Interdiffusion region

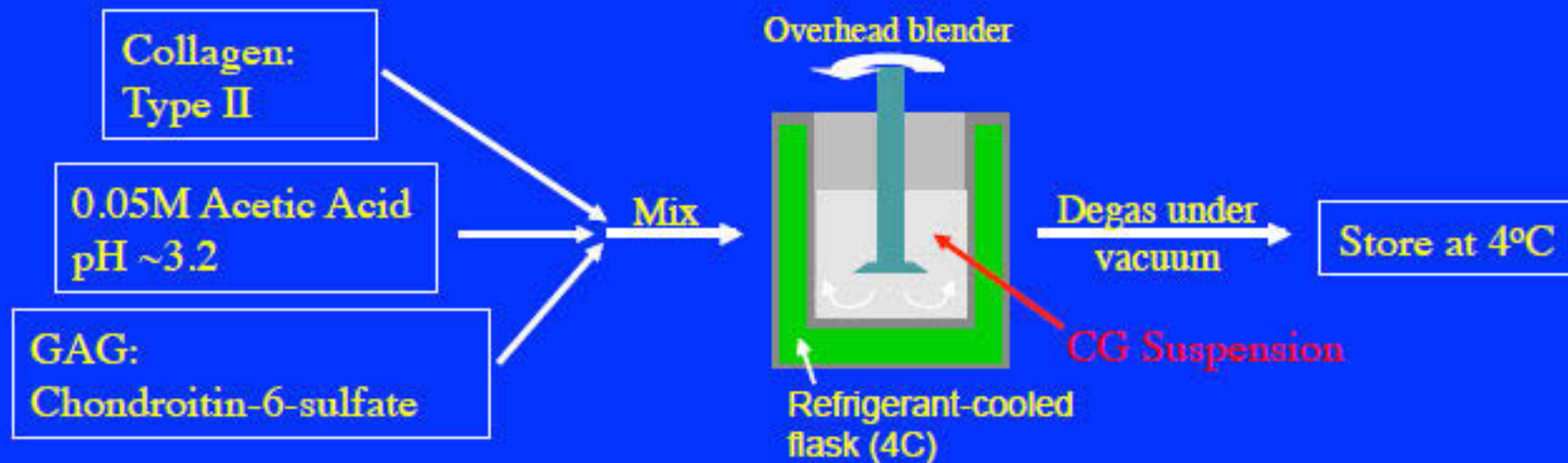
Mineralized type I collagen



Courtesy of Andrew Lynn. Used with permission.

# Collagen-GAG Scaffold: Fabrication

## Production of CG Suspension



Courtesy of Brendan Harley. Used with permission.

# CG Scaffold: Fabrication

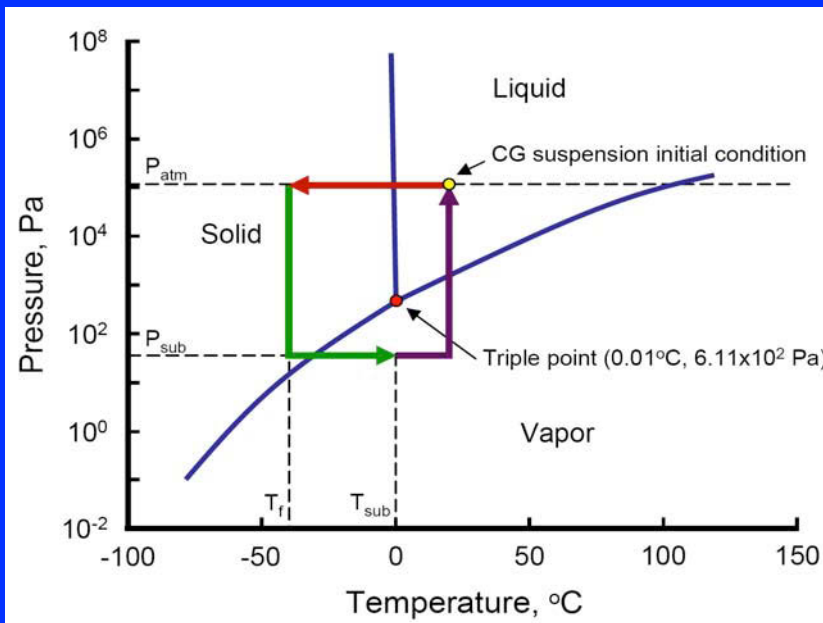
Place CG suspension into stainless steel pan (12.5 x 12.5 cm)

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

12

Courtesy of Brendan Harley. Used with permission.

# CG Scaffold: Microstructure

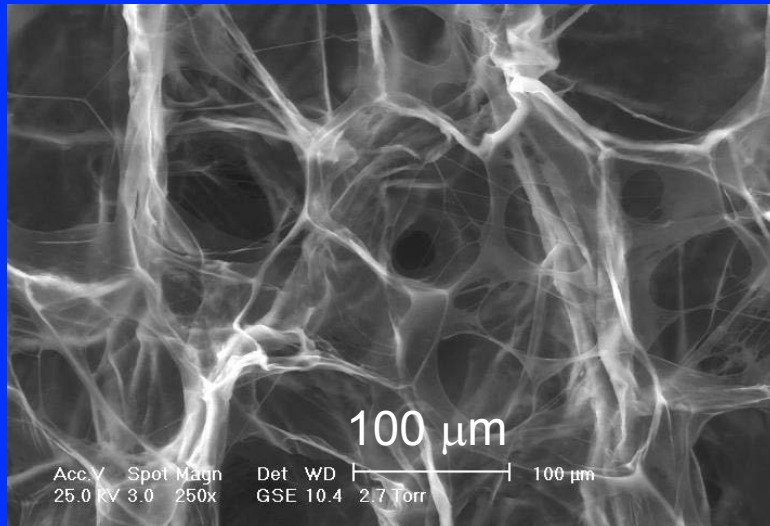


Fig. 1: Pek, Y. S., M. Spector, et al. *Biomaterials* 25 (2004): 473-82. Courtesy of Elsevier. Used with permission.

<http://www.sciencedirect.com/science/article/pii/S0142961203005416>

Pek et al., 2004

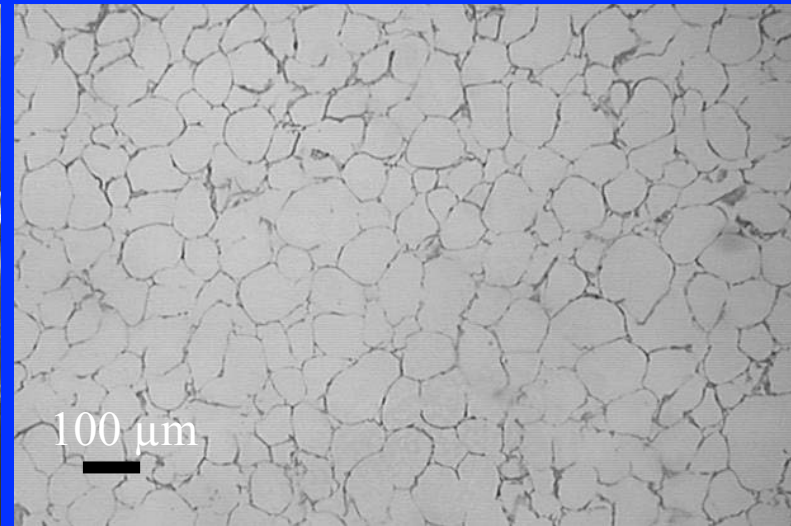


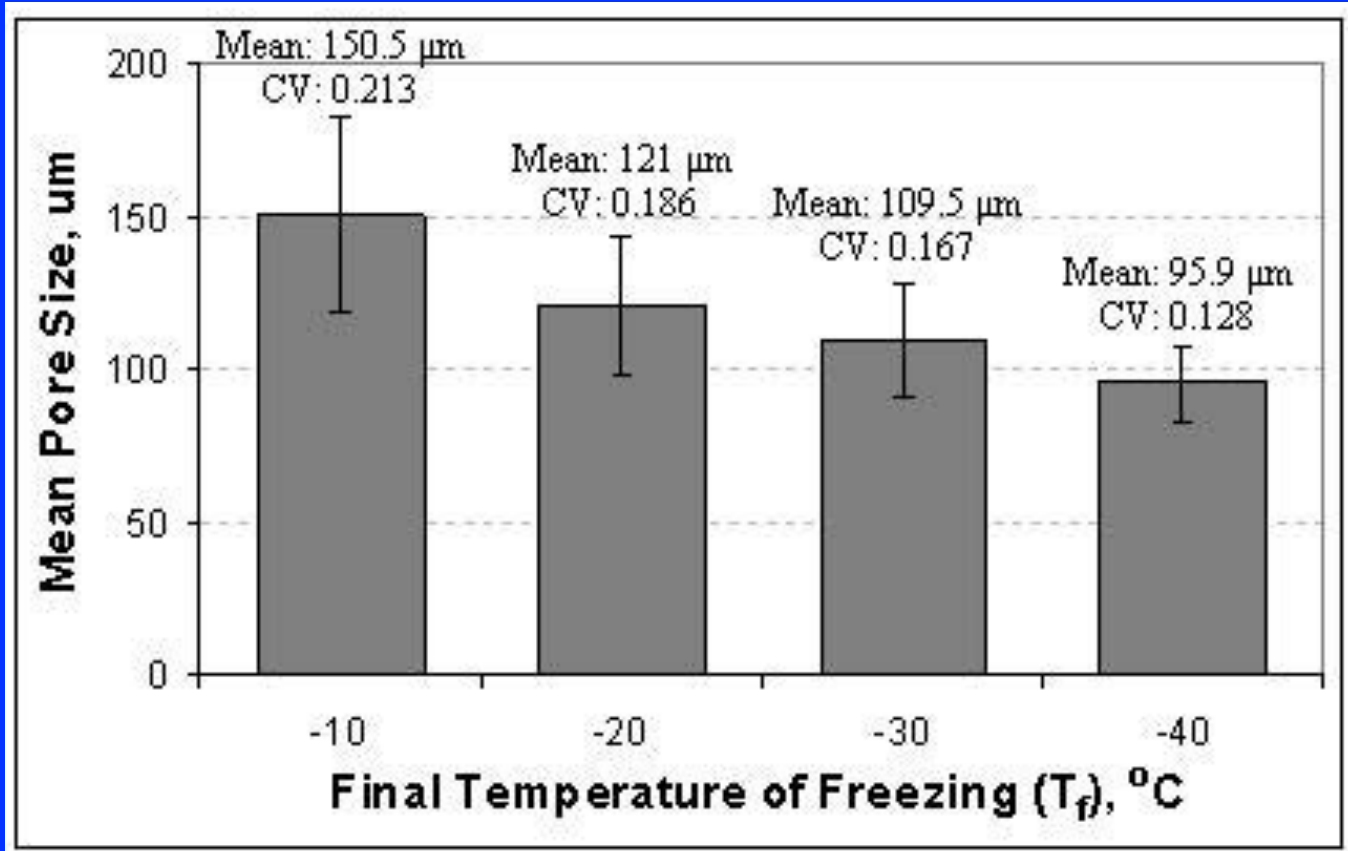
Fig. 4: F. J. O'Brien, B. A. Harley, et al. *Biomaterials* 25 (2004): 1077-86. Courtesy of Elsevier. Used with permission.

<http://www.sciencedirect.com/science/article/pii/S0142961203006306>

O' Brien, Harley et al., 2004

Relative density = 0.005

# CG Scaffold: Pore Size



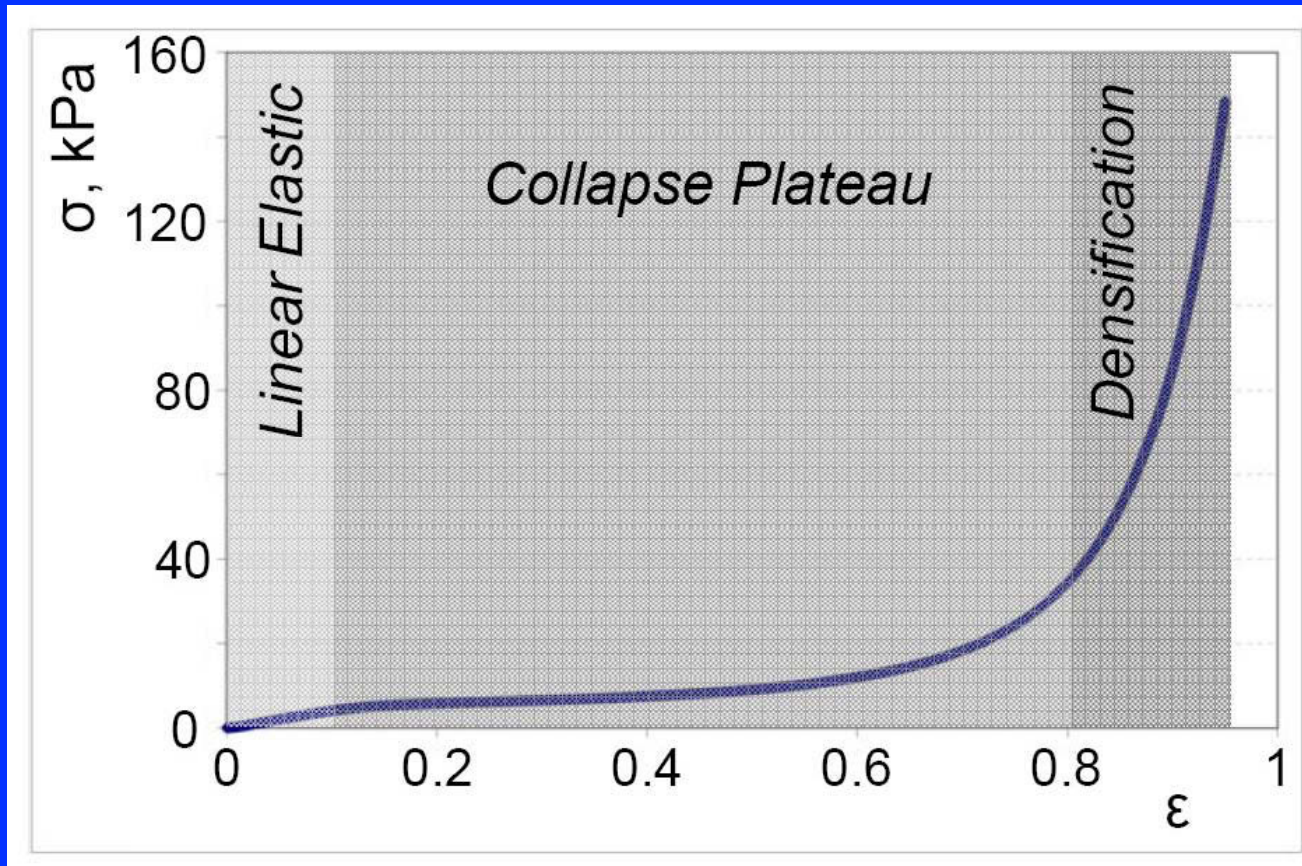
O'Brien, B. A. Harley, I. V. Yannas, et al. *Biomaterials* 26 (2005): 433-41. Courtesy of Elsevier. Used with permission.

<http://www.sciencedirect.com/science/article/pii/S0142961204002017>

Harley and Flemings: solidification model



# CG Scaffold: Compression (Dry)



Harley et al., 2007

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>

# CG Scaffold: Mechanical Properties

	$E^*$ (Pa)	$\sigma_{el}^*$ (Pa)
Dry	30,000	5,150
Wet	208	21



# Mineralized CG Scaffolds: Fabrication



Courtesy of Brendan Harley. Used with permission.

Harley, Lynn, Kanungo

# Mineralized CG Scaffolds: Fabrication

- Mineral: brushite ( $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ )
- Can control mass fraction of brushite between 0 and 80 wt% by controlling the molar ratio of the calcium nitrate hydrate and calcium hydroxide used and the molarity of the phosphoric acid
- Brushite then converted to octacalcium phosphate and then to apatite by hydrolytic conversion

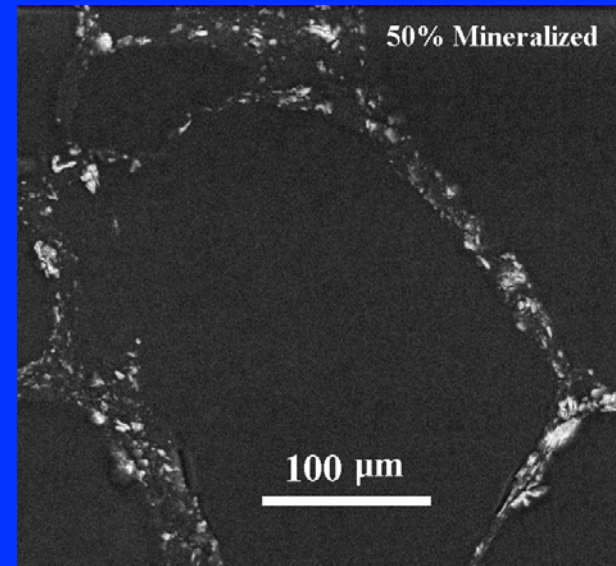
# Mineralized CG Scaffold: Microstructure

Figures removed due to copyright restrictions. See Figure 1b: Harley, B. A., et al. *Journal of Biomedical Materials Research* 92A (2010): 1066-77.  
<http://onlinelibrary.wiley.com/enhanced/doi/10.1002/jbm.a.32361/>

Harley et al., 2010

Pore size 50-1000  $\mu\text{m}$  depending on freezing conditions

Pore size for bone regeneration: 100-500  $\mu\text{m}$



Kanungo et al, 2008

Kanungo, B. P., et al. "Characterization of Mineralized Collagen-glycosaminoglycan Scaffolds for Bone Regeneration." *Acta Biomaterialia* 4, no.3 (2008): 490-503. Courtesy of Elsevier. Used with permission.

# Mineralized CG Scaffold: $\mu$ CT

Figures removed due to copyright restrictions. See Figure 2: Harley, B. A., et al. *Journal of Biomedical Materials Research* 92A (2010): 1066-77.  
<http://onlinelibrary.wiley.com/enhanced/doi/10.1002/jbm.a.32361/>

Uniform distribution of mineral

Harley et al., 2010

# Mineralized CG Scaffold: EDX

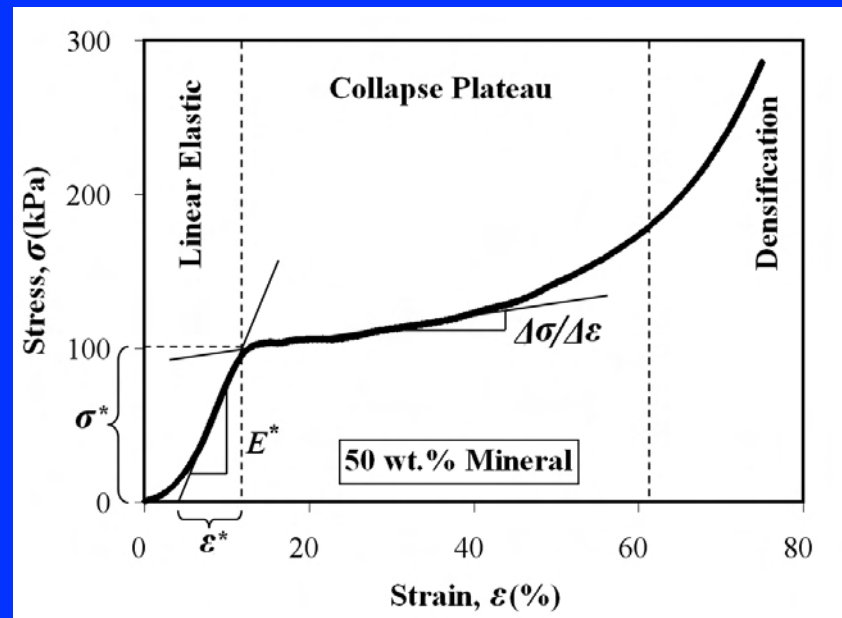
Figures removed due to copyright restrictions. See Figure 3: Harley, B. A., et al. *Journal of Biomedical Materials Research* 92A (2010): 1066-77.  
<http://onlinelibrary.wiley.com/enhanced/doi/10.1002/jbm.a.32361/>

1mm

Uniform distribution of mineral

Harley et al., 2010

# Mineralized CG Scaffold: Compression



Harley et al., 2008

Scaffold	$E^*$ (kPa)	$\sigma^*$ (kPa)	$\epsilon^*$ (%)
Dry	762	85	11
Wet	4.12	0.29	0.07

# Mineralized CG Scaffold: Compression

- Mineralized scaffold can be manually compressed
- On unloading and hydration, recovers all deformation
- Interested in increasing mechanical properties of the mineralized scaffold for improved handling during surgery

# Mineralized CG Scaffold: Compression

- Cross-linked collagen of osteoid has a hydrated modulus in the range of 25-40 kPa
- Engler et al. (2006) show that differentiation of MSCs depends on substrate stiffness
- Substrate stiffness similar to collagen of osteoid leads to differentiation to osteoblast-like morphology
- Interest in increasing stiffness of mineralized scaffold to mimic osteoid stiffness



# Cellular Solids Modelling

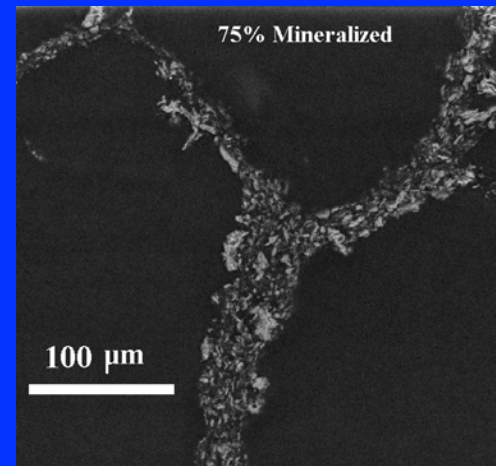
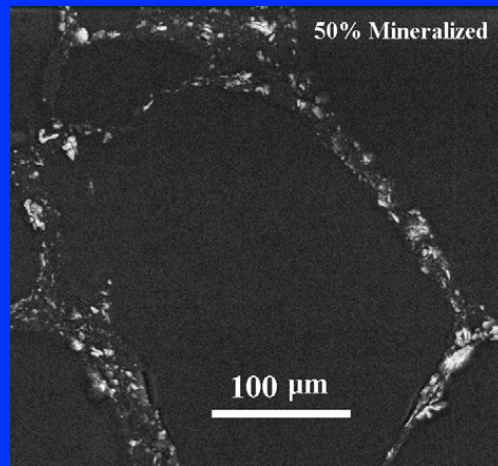
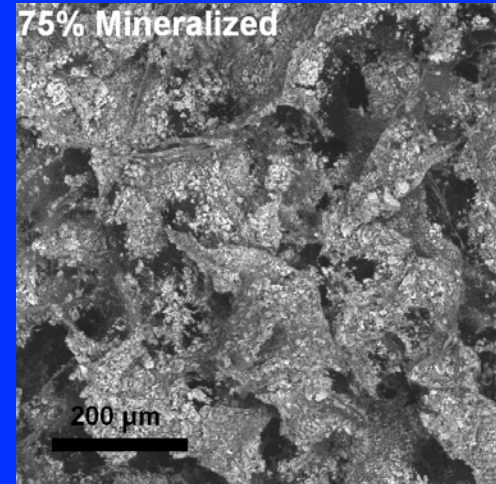
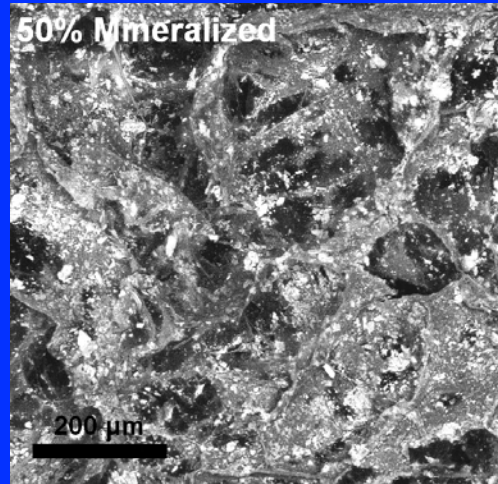
$$E^*/E_s = \left(\rho^*/\rho_s\right)^2$$

$$\sigma^*/\sigma_s = 0.3\left(\rho^*/\rho_s\right)^{3/2}$$

Foam properties depend on:

- solid properties:  $E_s, \sigma_s$
- relative density:  $\rho^*/\rho_s$
- geometrical factor: 1, 0.3

# Increase Mineral Content



50 wt%

75 wt%

Kanungo et al., 2008

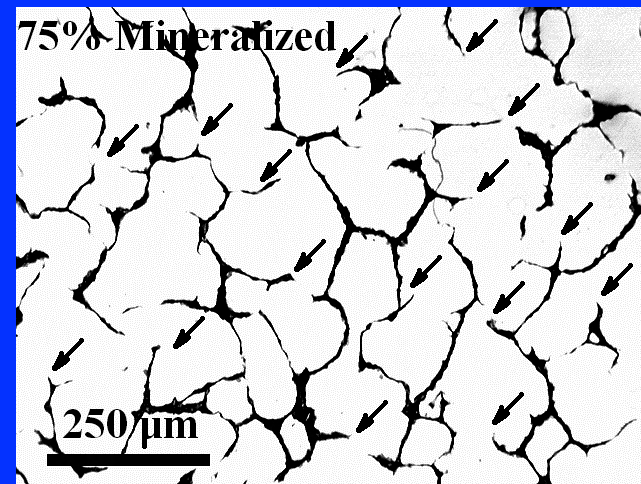
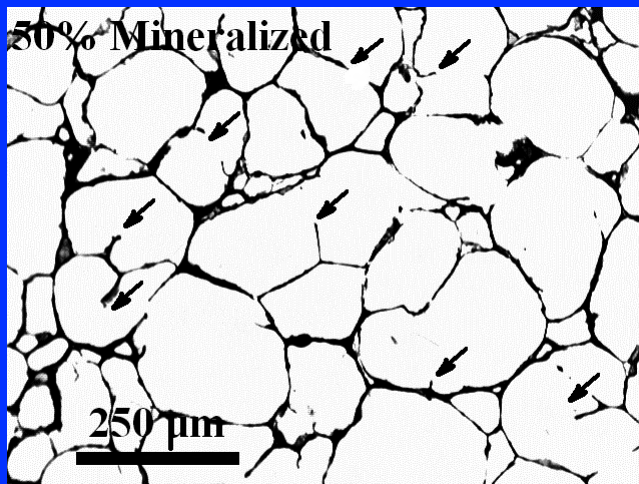
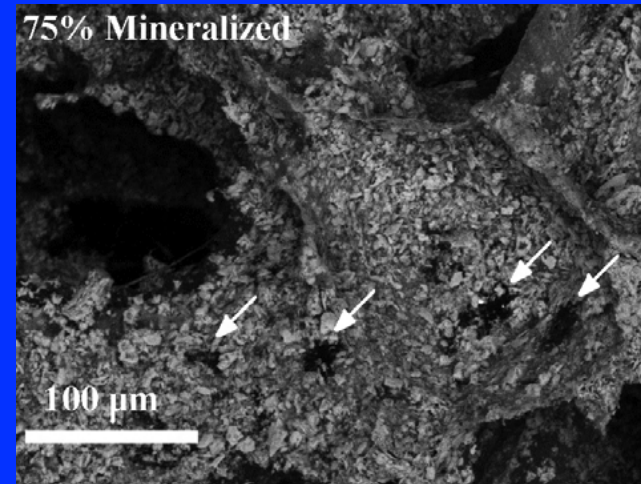
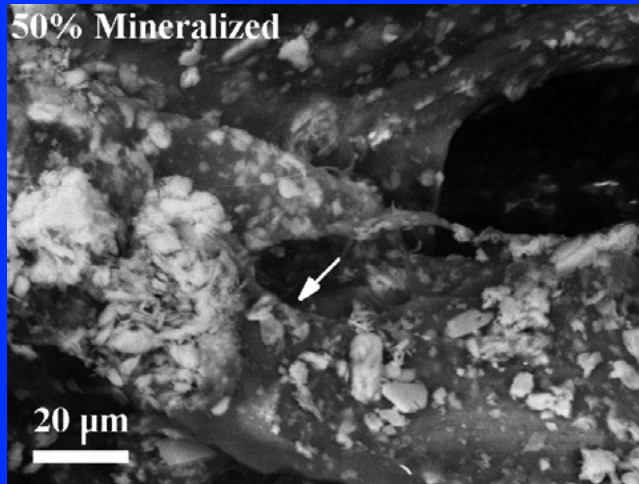
Kanungo, B. P., et al. "Characterization of Mineralized Collagen-glycosaminoglycan Scaffolds for Bone Regeneration." *Acta Biomaterialia* 4, no.3 (2008): 490-503. Courtesy of Elsevier. Used with permission.

# Increase Mineral Content

Scaffold	$E^*$ (kPa)	$\sigma^*$ (kPa)	$\epsilon^*$ (%)
50 wt.%	780	90	12
75 wt.%	370	35	10

Scaffolds of higher mineral content have increased defects: e.g. voids in cell walls, disconnected walls

# Increase Mineral Content



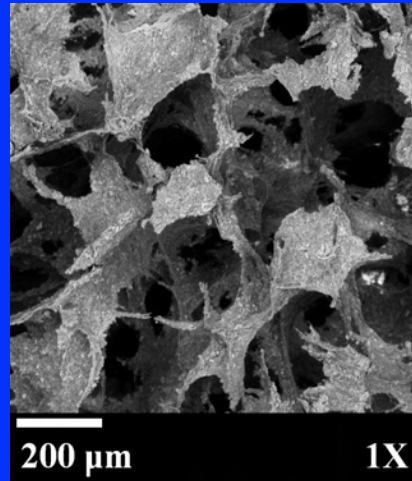
Kanungo, B. P., et al. "Characterization of Mineralized Collagen-glycosaminoglycan Scaffolds for Bone Regeneration." *Acta Biomaterialia* 4, no.3 (2008): 490-503. Courtesy of Elsevier. Used with permission.



# Increase Relative Density

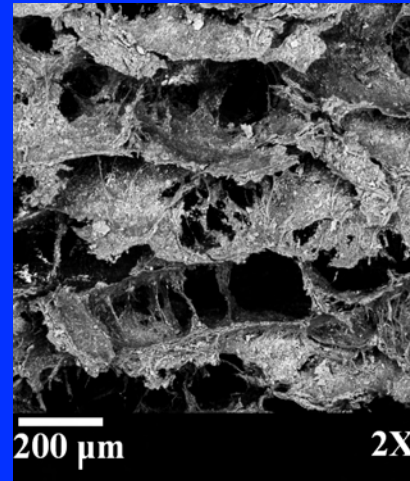
$$\rho/\rho_s = 0.045$$

$$d = 311 \mu\text{m}$$



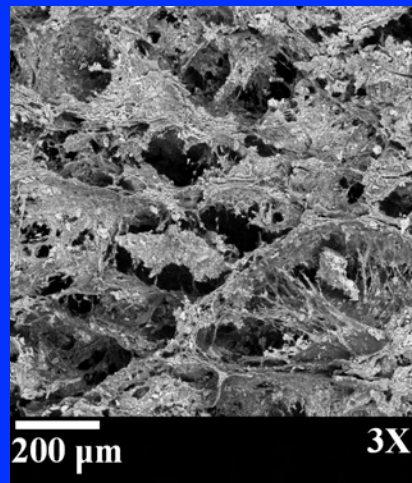
$$\rho/\rho_s = 0.098$$

$$d = 196 \mu\text{m}$$



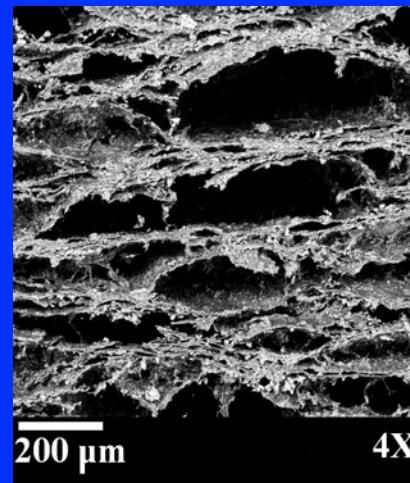
$$\rho/\rho_s = 0.137$$

$$d = 159 \mu\text{m}$$



$$\rho/\rho_s = 0.187$$

$$d = 136 \mu\text{m}$$



Kanungo and Gibson, 2009

Kanungo, B. P., and L. J. Gibson. *Acta Biomaterialia* 5 (2009): 1006-18. Courtesy of Elsevier. Used with permission.

<http://www.sciencedirect.com/science/article/pii/S1742706108003796>

# Increase Relative Density

$\rho^*/\rho_s$ (-)	E (dry) (kPa)	E(wet) (kPa)	$\sigma^*$ (dry) (kPa)	$\sigma^*$ (wet) (kPa)
0.045	780	6.44	39	0.55
0.098	3156	17.8	132	0.83
0.137	6500	34.8	242	2.12
0.187	3660	38.8	275	1.79

# Increase Cross-linking

( $\rho^*/\rho_s = 0.137$ , wet)

	$E^*$ (kPa)	$\sigma^*$ (kPa)
Non-cross-linked	34.8	2.12
DHT	56.0	3.26
EDAC	91.7	4.15

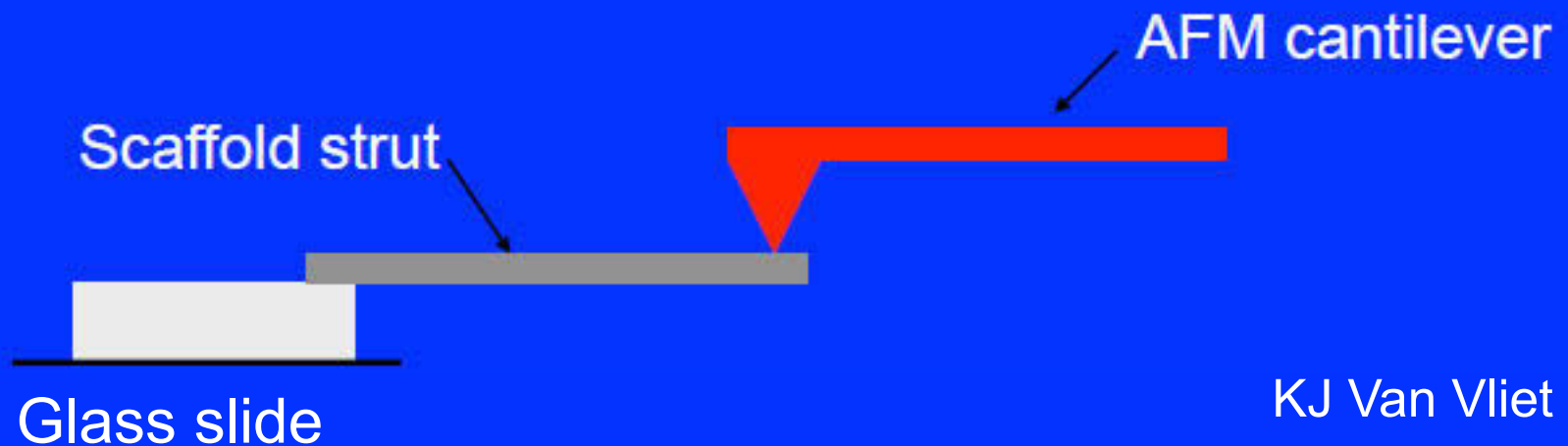
# Increase Relative Density

$\rho^*/\rho_s$ (-)	E (kPa) (no x- link)	E (kPa) (DHT)	E (kPa) (EDAC)
0.045	6.44		
0.098	17.8		
0.137	34.8	56.0	91.7
<i>0.187</i>	<i>38.8</i>		

Can obtain E (wet) in range of 25-40kPa for MSC differentiation to osteoblast-like cells



# Mineralized CG Scaffold: Strut Properties



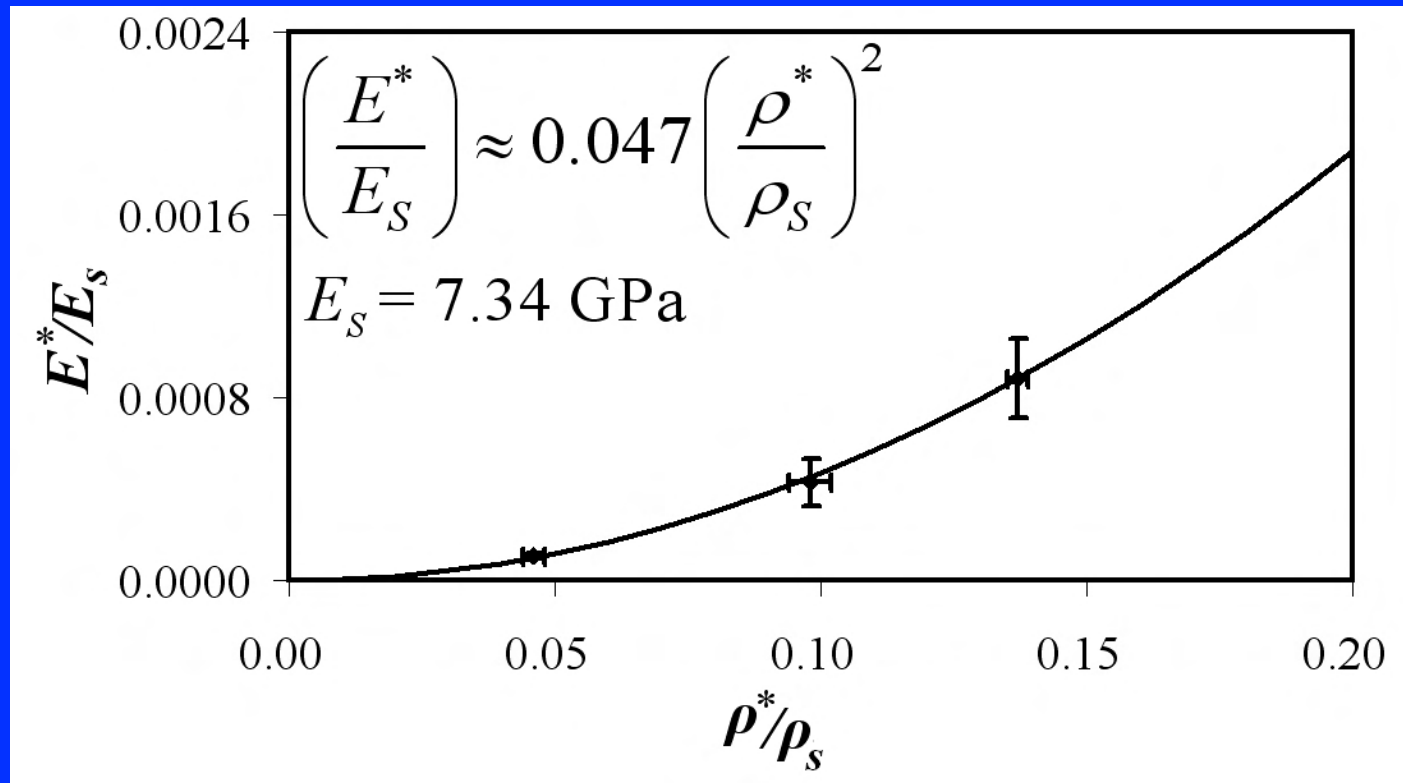
KJ Van Vliet

MCG scaffold:  $E_s = 7.34 + 3.73$  GPa (dry)

Nanoindentation:  $\sigma_s = 201 + 52$  MPa (dry)

Trabecular bone:  $E_s = 18$  GPa,  $\sigma_s = 182$  MPa

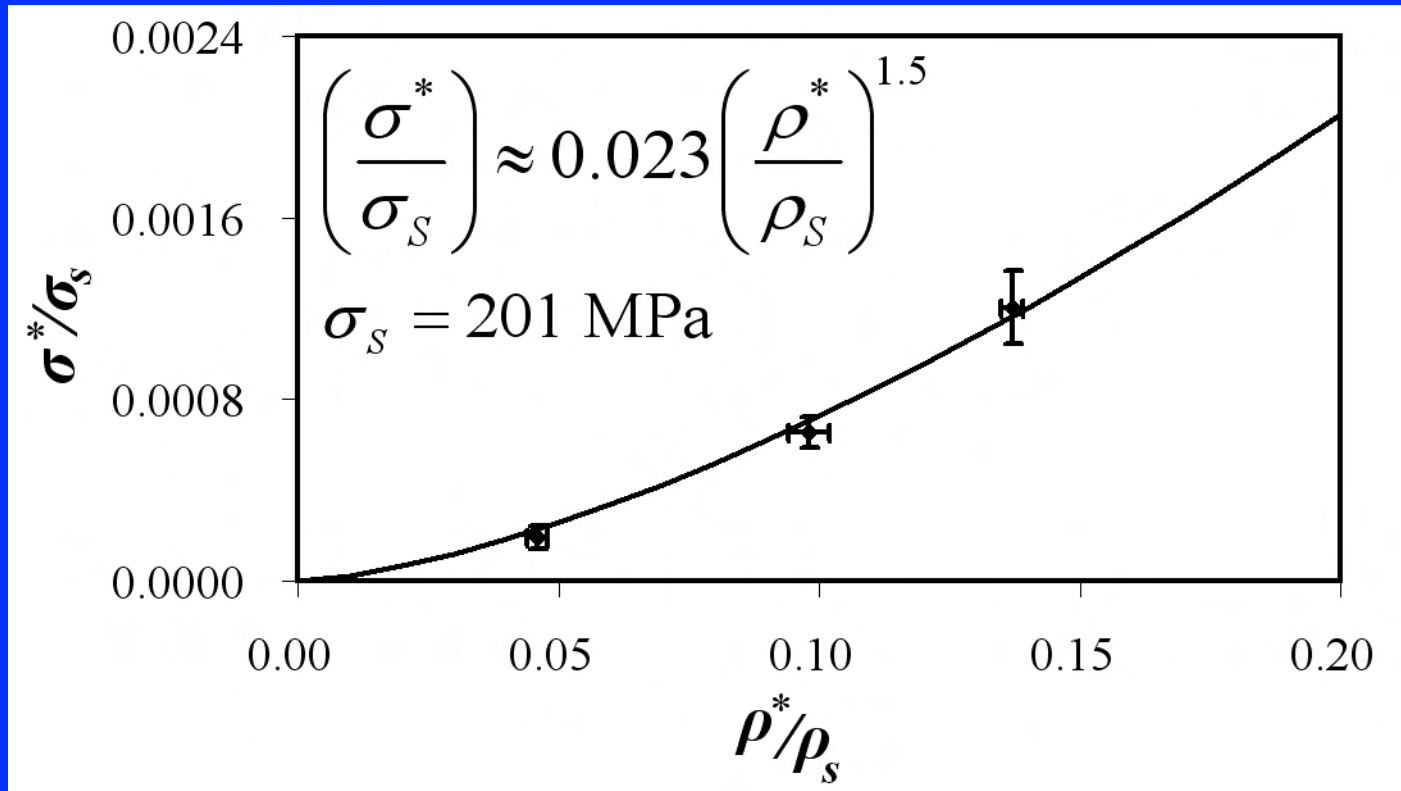
# Cellular Solids Models



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# Cellular Solids Models



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<http://www.sciencedirect.com/science/article/pii/S1742706108003796>

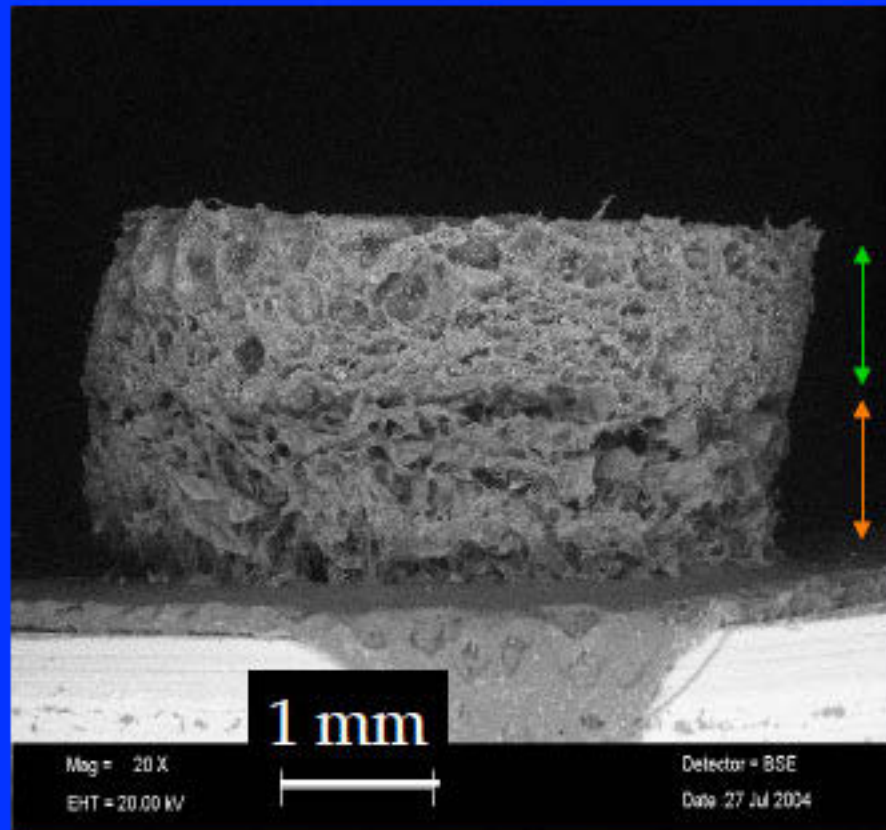
# Osteochondral Scaffolds: Design Considerations

- Use healthy articular joint as a model for scaffold structure and composition
- Control of scaffold parameters (e.g. mineral content, pore size)
- Use materials appropriate for rapid regulatory compliance

# Osteochondral Scaffold: Fabrication

- Liquid-phase co-synthesis
- Pour mineralized CG slurry into mold, then CG slurry into mold
- Allow the two slurries to interdiffuse for 30 minutes at room temperature
- Place the mold containing the slurries into the freeze drier
- Cross-link with EDAC

# Osteochondral Scaffold



Mineralized CG  
scaffold

Type II collagen  
scaffold

# Osteochondral Scaffold: Micro-CT

Figures removed due to copyright restrictions. See: Harley, B. A., et al.  
*Journal of Biomedical Materials Research* 92A (2010): 1078–93.  
<http://onlinelibrary.wiley.com/doi/10.1002/jbm.a.32387/abstract>

# Osteochondral Scaffold: Microstructure

Scaffold	Porosity	Pore size ( $\mu\text{m}$ )
Collagen- GAG	98.3%	653
Mineralized CG	95.5%	419



# Osteochondral Scaffold: Gradual Interface

Collagen-GAG

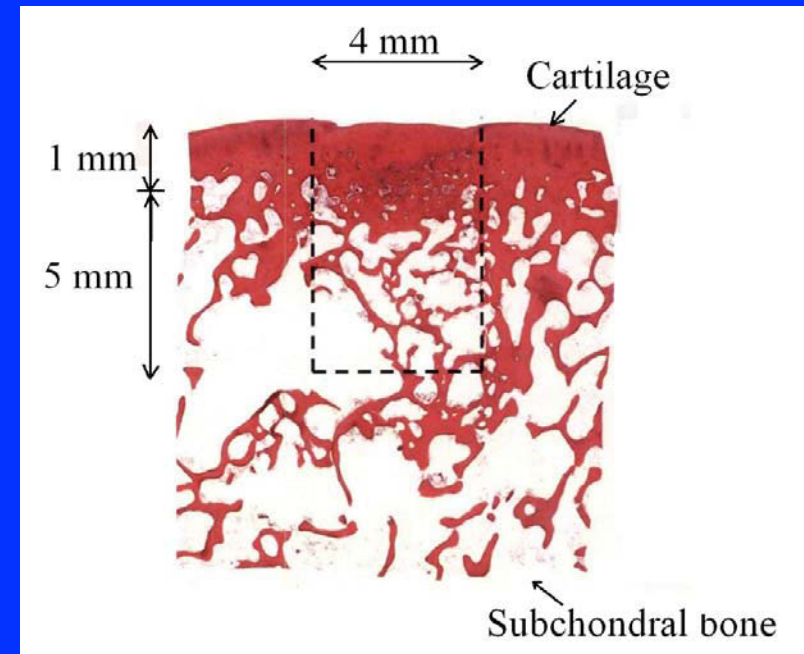
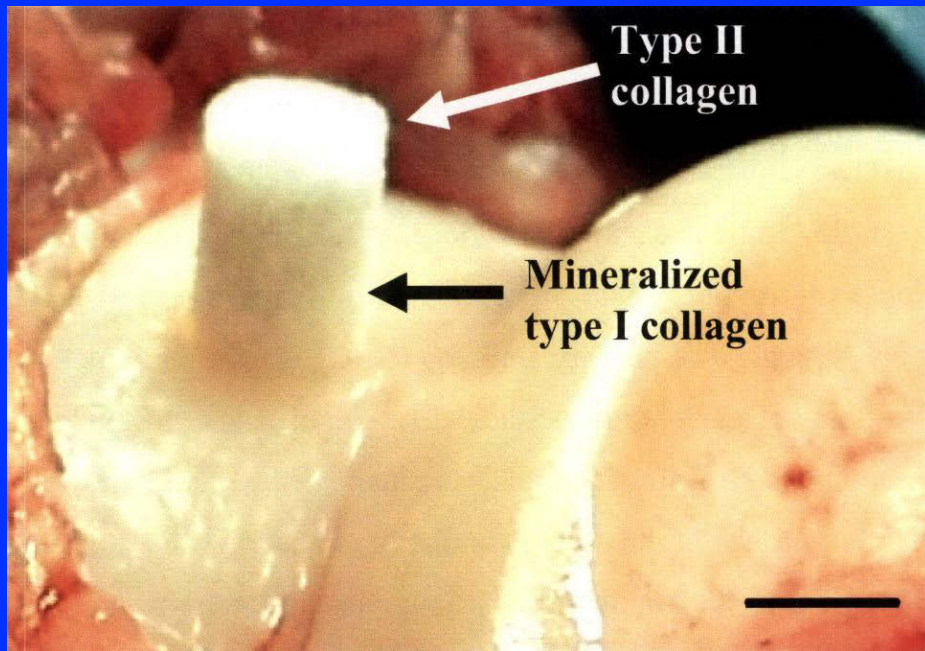
Mineralized  
Collagen-GAG

Figures removed due to copyright restrictions. See: Harley, B. A., et al.  
*Journal of Biomedical Materials Research 92A* (2010): 1078–93.  
<http://onlinelibrary.wiley.com/doi/10.1002/jbm.a.32387/abstract>

Energy-dispersive X-ray (EDX)

Harley et al., 2010

# Osteochondral Scaffold: Goat Model



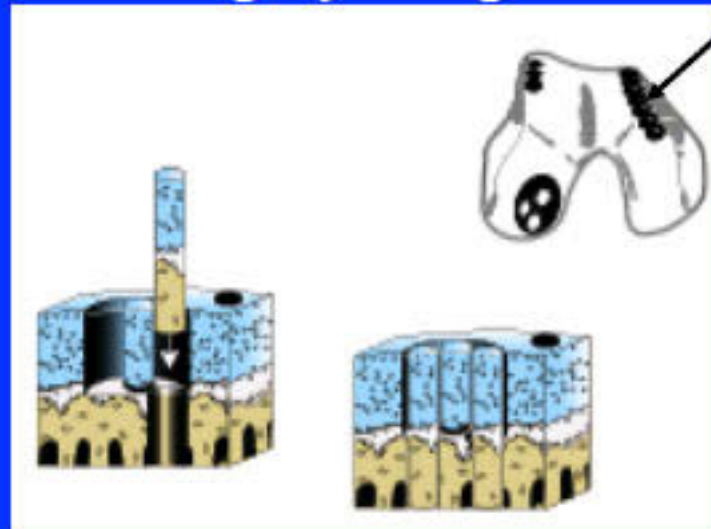
Source: Vickers, S. "Cell-seeded Type II Collagen Scaffolds for Articular Cartilage Tissue Engineering." Ph.D. Thesis. MIT, Department of Mechanical Engineering, 2007.

Vickers, 2007

- Initial animal studies indicate bone and cartilage regeneration (Vickers, 2007)
- Longer term (52 week) animal studies ongoing (Lynn)

# Osteochondral Scaffold: Clinical Use

- CE Mark approval for clinical use in Europe obtained January 2009
- First clinical use February 2009 in backfill of mosaicplasty donor site
- Currently using for primary sites
- As of April 2012: roughly 200 patients treated



Courtesy of Andrew Lynn. Used with permission.

# Conclusions

- Fabricated bilayer osteochondral scaffold with gradient interface
- Structure and composition mimic osteochondral tissues
- Range of mineral content, porosity, pore sizes possible by control of the process
- Used materials already approved for medical devices

# Acknowledgements

- Cambridge-MIT Institute
- Matoula S. Salapatras Professorship (LJG)
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- Universities UK, Cambridge Commonwealth Trust, St. John's College Cambridge (AKL)

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3.054 / 3.36 Cellular Solids: Structure, Properties and Applications  
Spring 2014

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