Fabrication, Microstructure and Mechanical Properties of an Osteochondral Scaffold

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Collaborators

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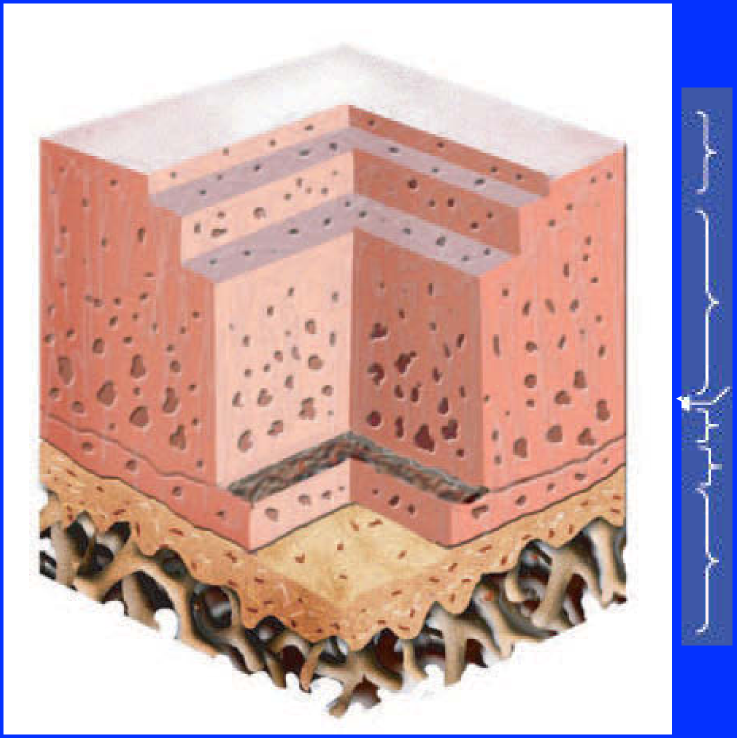
- BWH: M Spector, H-P Hsu
- Cambridge: W Bonfield, AK Lynn, S Best, RE Cameron

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


Superficial and transition cartilage zones

Radial (deep) cartilage zone

Tidemark

Calcified cartilage zone

Subchondral bone

Trabecular bone

Image by MIT OpenCourseWare.

Bone: Type I collagen
Cartilage: Type II collagen

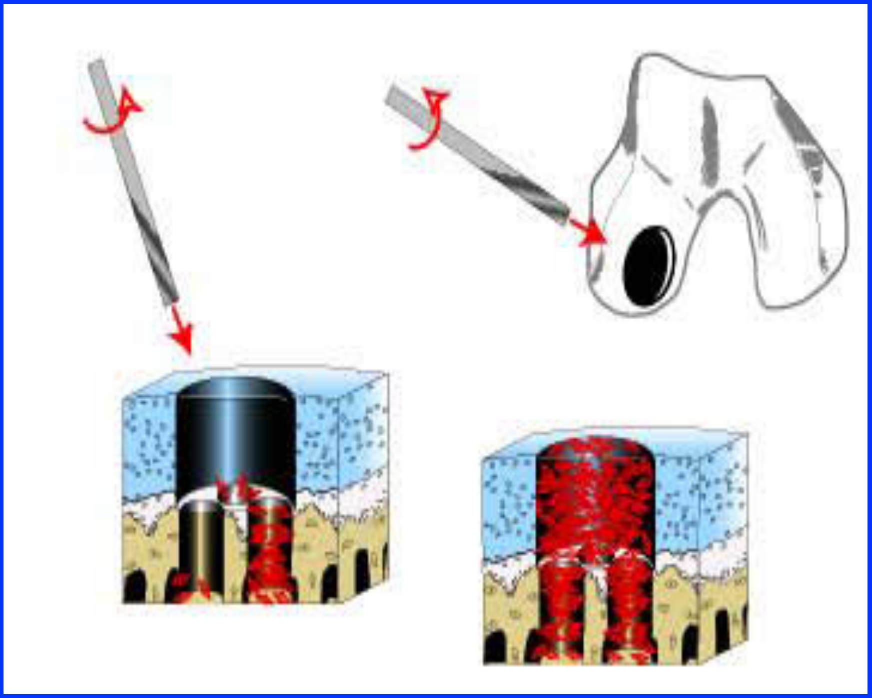
Greene WB (2006)
Netter’s Orthopaedics

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.

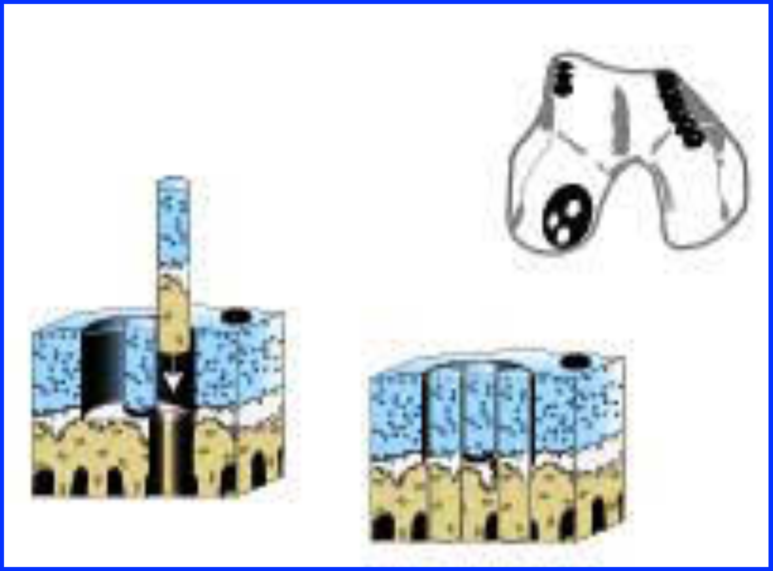
POOR QUALITY OF REPAIR

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

<u>DONOR SITE MORBIDITY</u>



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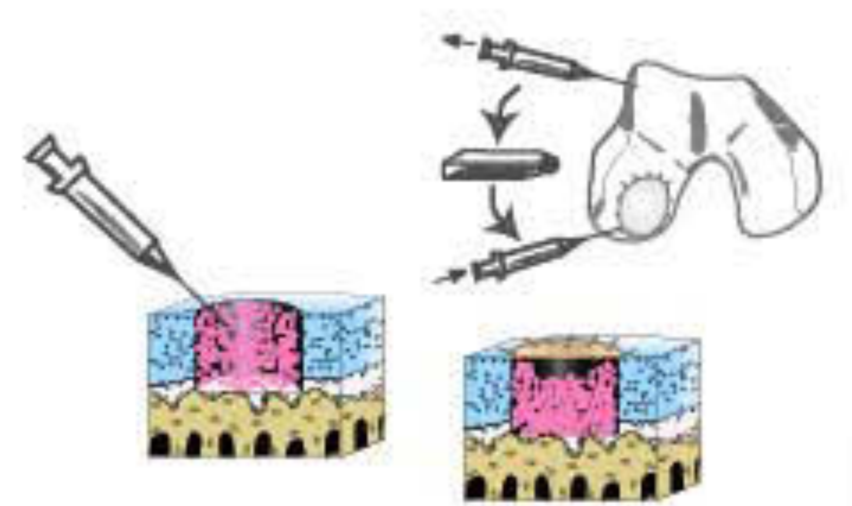
Lynn (2005)

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



Courtesy of Andrew Lynn. Used with permission.

Lynn (2005)

<u>TWO SURGERIES,</u>

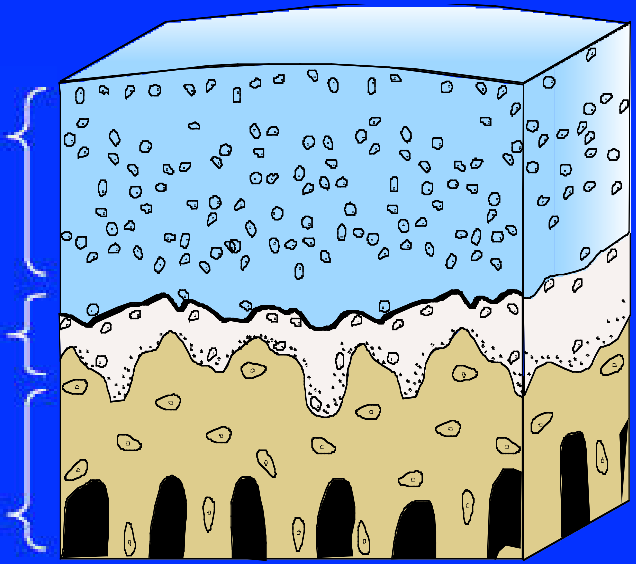
<u>COST OF CELL CULTURE</u>

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

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

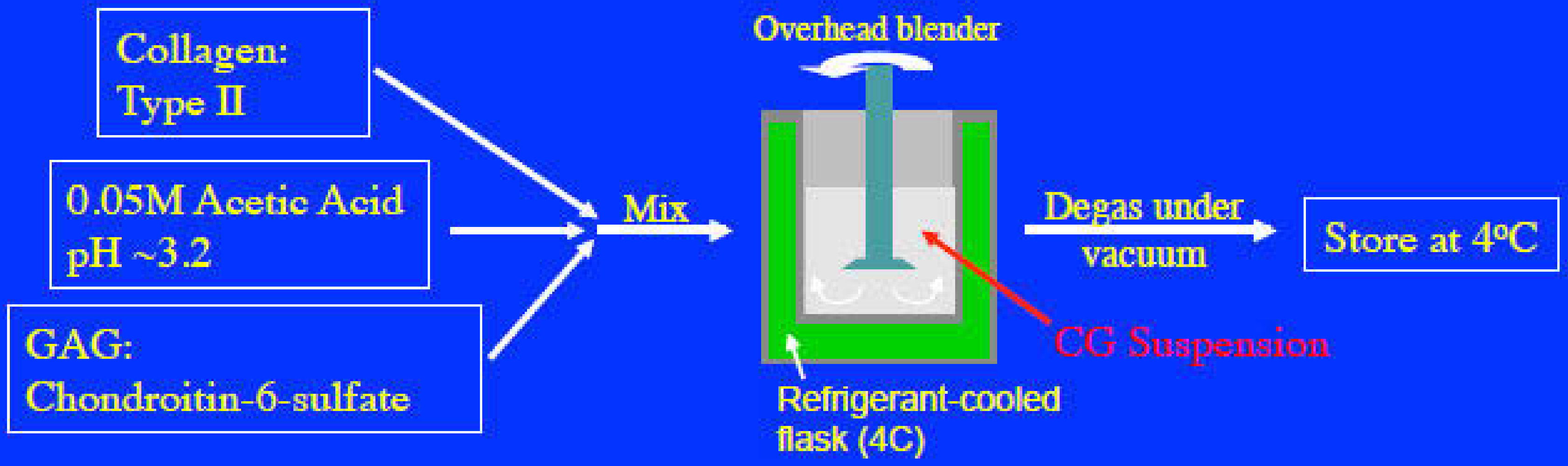
Interdiffusion region

Mineralized type I collagen

Courtesy of Andrew Lynn. Used with permission.

Collagen-GAG Scaffold: Fabrication

Production of CG Suspension



Collagen:
Type II

0.05M Acetic Acid
pH ~3.2

GAG:
Chondroitin-6-sulfate

Mix

Overhead blender

Refrigerant-cooled flask (4C)

CG Suspension

Degas under vacuum

Store at 4°C

Courtesy of Brendan Harley. Used with permission.

Yannas

CG Scaffold: Fabrication

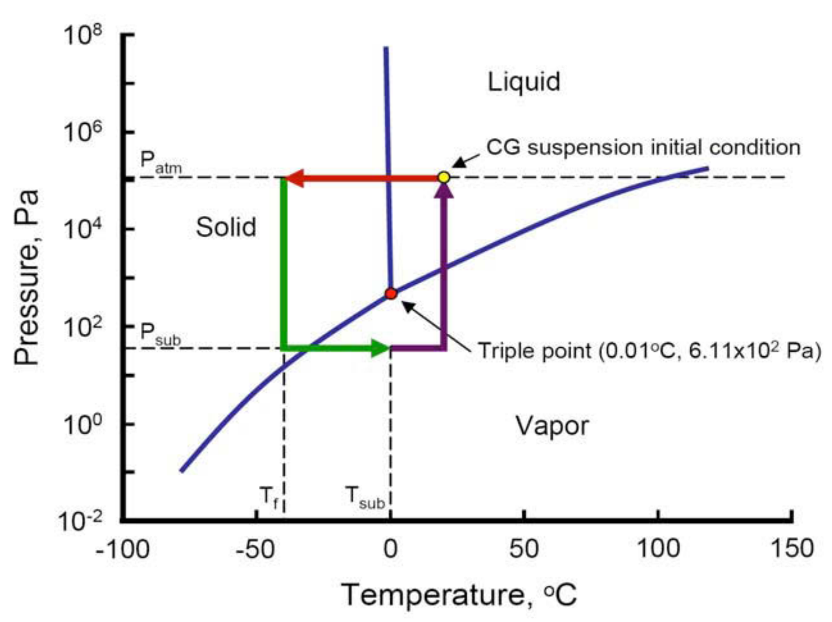
Place CG suspension into stainless steel pan (12.5 x 12.5 cm)

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: Microstructure

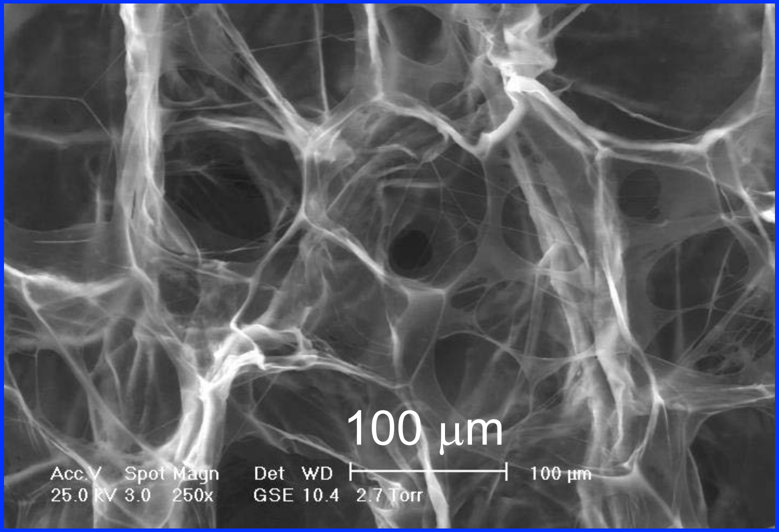
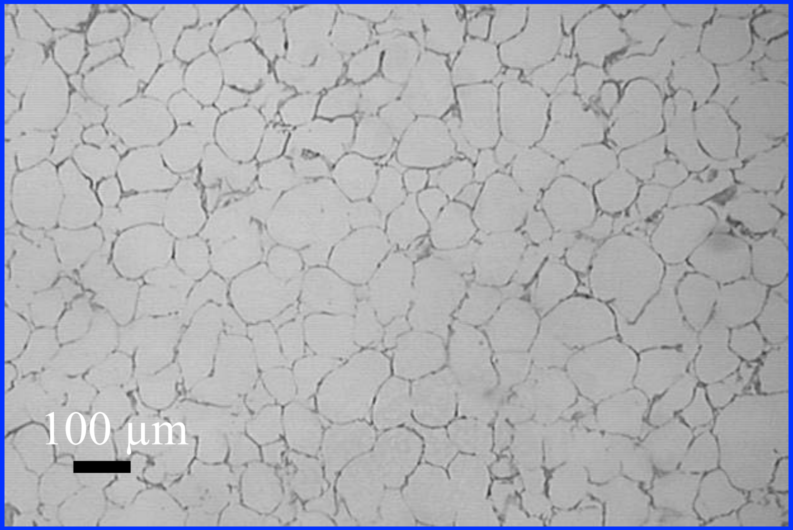


Fig. 1: Pek, Y. S., M. Spector, et al. *Biomaterials* 25 (2004): 473-82. Courtesy of Elsevier. Used with permission.
http://www.sciencedirect.com/science/article/pii/S0142961203005416



$96\ \mu m$

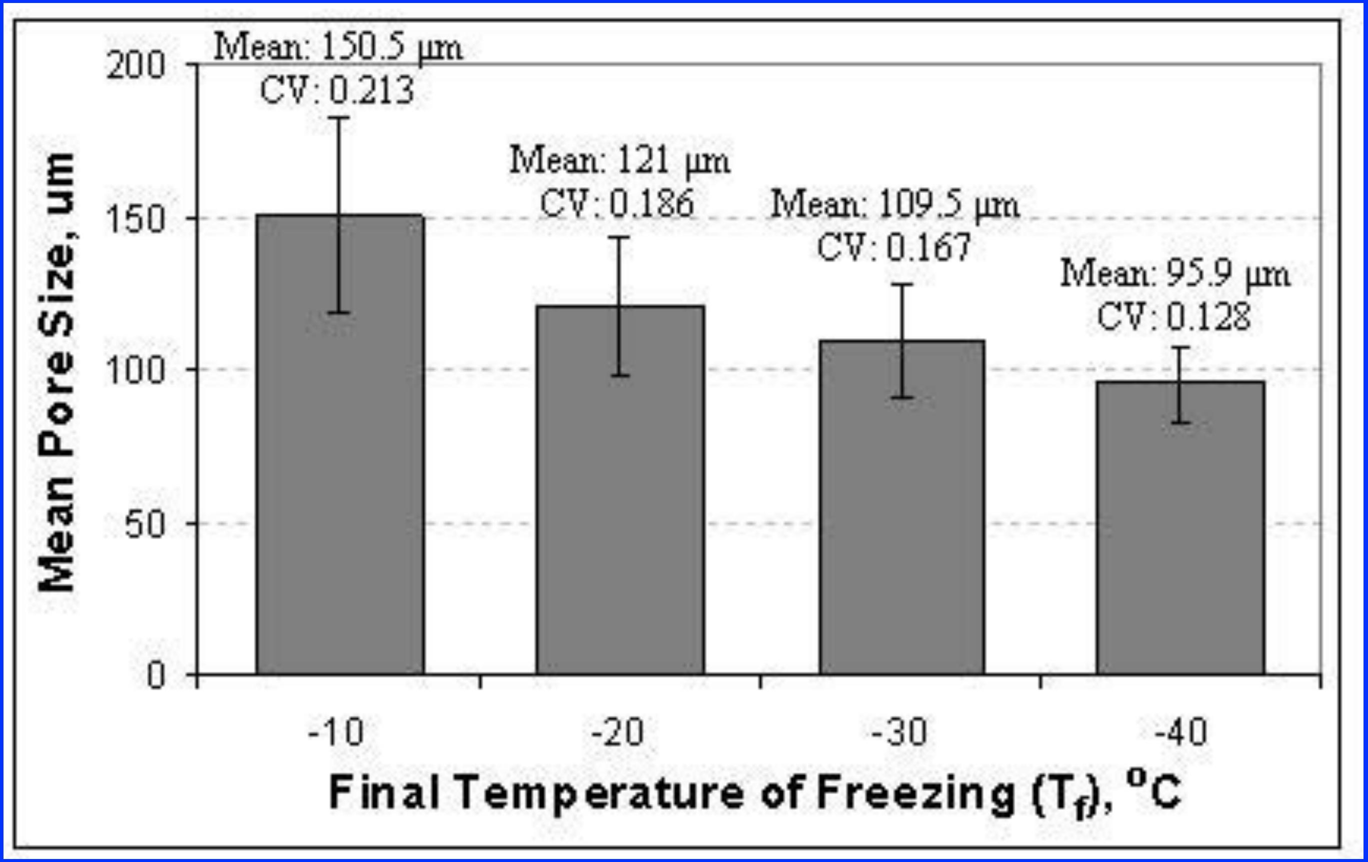
Fig. 4: F. J. O'Brien, B. A. Harley, et al. *Biomaterials* 25 (2004): 1077-86. Courtesy of Elsevier. Used with permission.
http://www.sciencedirect.com/science/article/pii/S0142961203006306

Pek et al., 2004

O' Brien, Harley et al., 2004

Relative density = 0.005

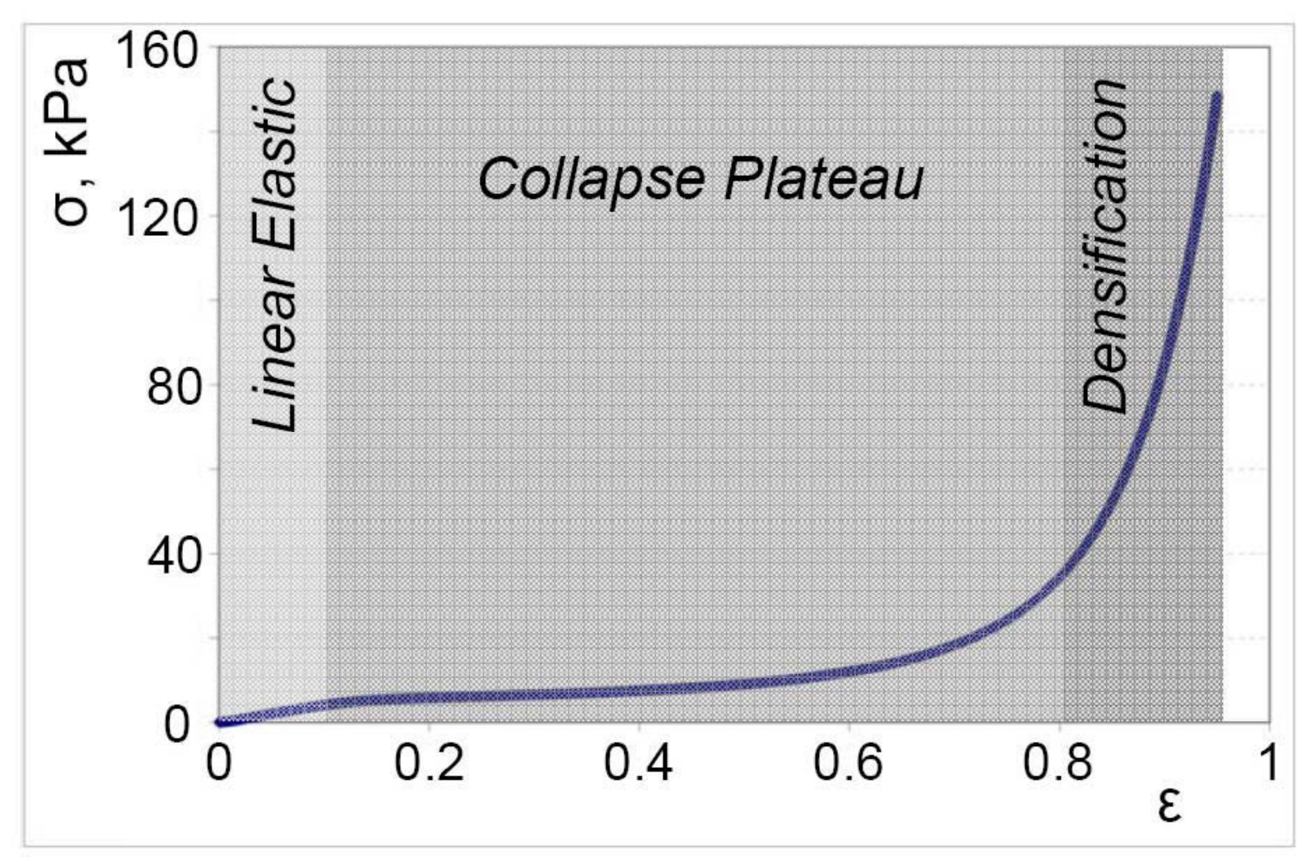
CG Scaffold: Pore Size



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)



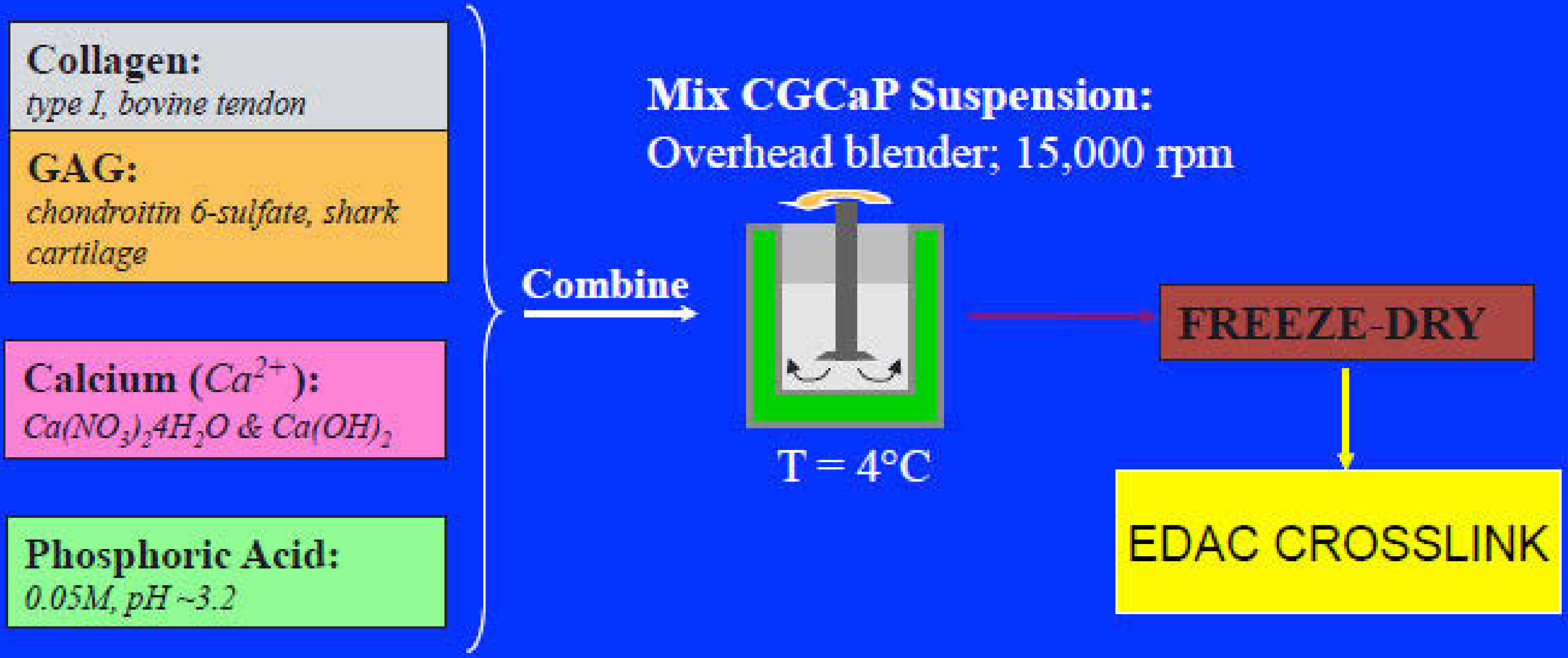
Harley et al., 2007

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)	σ^*_{el} (Pa)
Dry	30,000	5,150
Wet	208	21

Mineralized CG Scaffolds: Fabrication



Collagen:
type I, bovine tendon

GAG:
chondroitin 6-sulfate, shark cartilage

Calcium (Ca^{2+}):
$Ca(NO_3)_2 4H_2O$ & $Ca(OH)_2$

Phosphoric Acid:
0.05M, pH ~3.2

Combine

Mix CGCaP Suspension:
Overhead blender; 15,000 rpm

T = 4°C

FREEZE-DRY

EDAC CROSSLINK

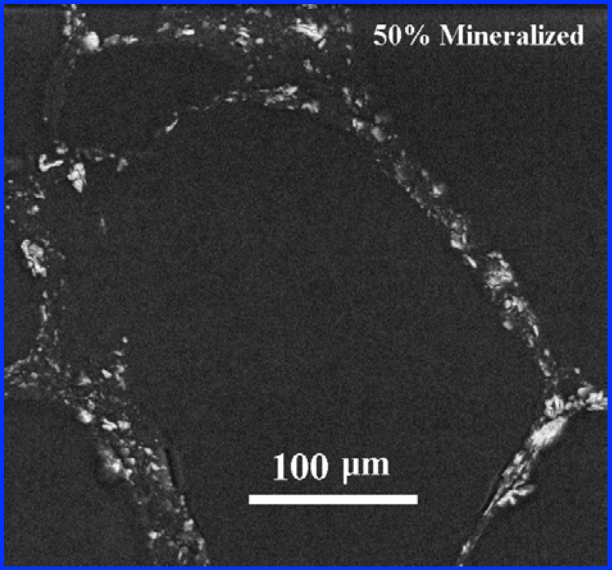
Courtesy of Brendan Harley. Used with permission.

Harley, Lynn, Kanungo

Mineralized CG Scaffolds: Fabrication

- Mineral: brushite ($CaHPO_4.2H_2O$)
- 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



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.

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 µm depending on freezing conditions

Pore size for bone regeneration: 100-500 µm

Mineralized CG Scaffold: μCT

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

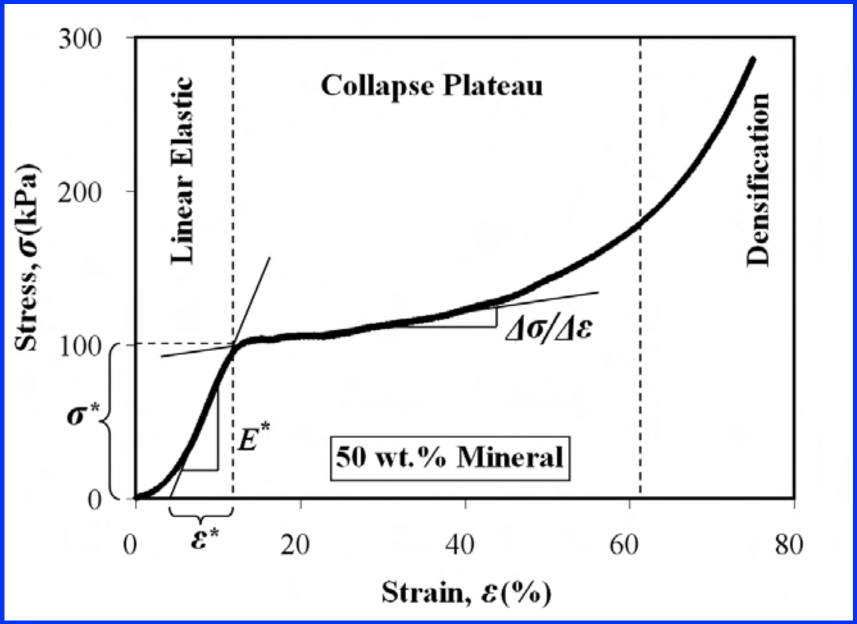
Figures removed due to copyright restrictions. See Figure 3: Harley, B. A., et al. *Journal of Biomedical Materials Research* 92A (2010): 1066-77. http://onlinelibrary.wiley.com/enhanced/doi/10.1002/jbm.a.32361/

1mm

Uniform distribution of mineral

Harley et al., 2010

Mineralized CG Scaffold: Compression



Harley et al., 2008

Scaffold	E^* (kPa)	σ^* (kPa)	ε^* (%)
Dry	762	85	11
Wet	4.12	0.29	0.07

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: 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

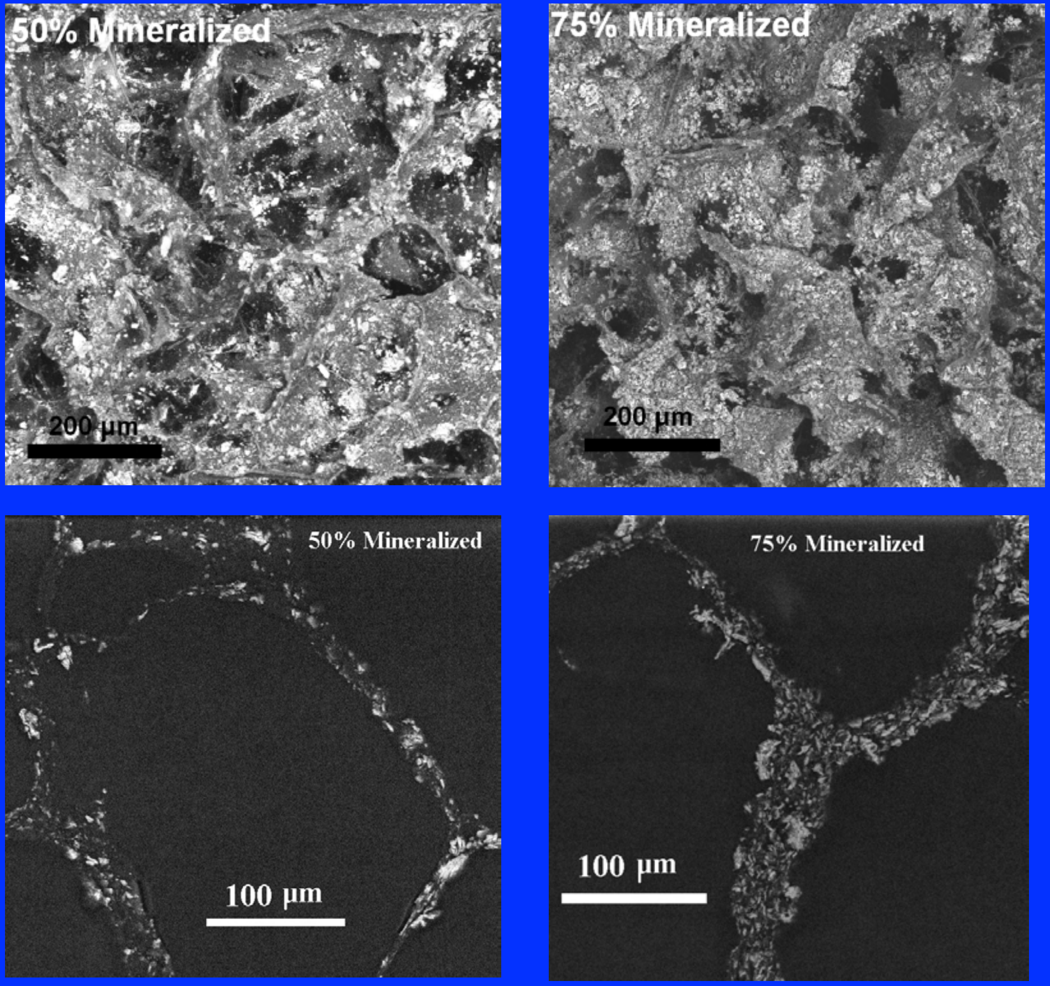
$$E^*/E_s = \left(\rho^*/\rho_s\right)^2$$

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

Foam properties depend on:

- solid properties: E_s, σ_s
- relative density: ρ^*/ρ_s
- geometrical factor: 1, 0.3

Increase Mineral Content

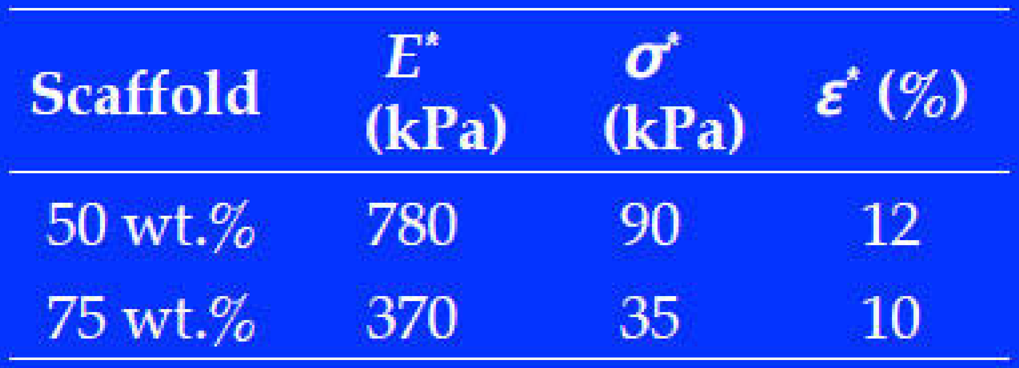


50 wt%　　75 wt%

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

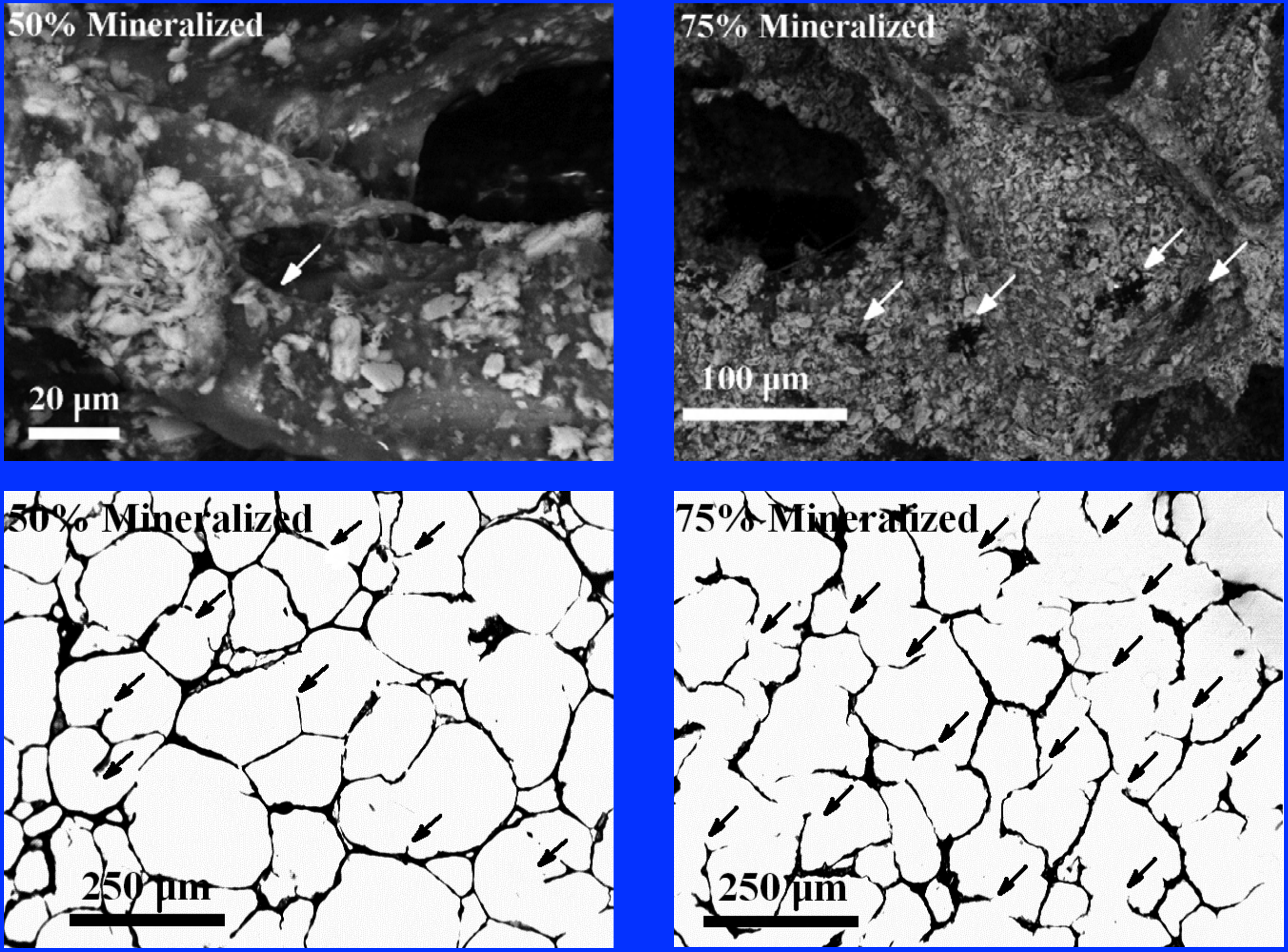


Scaffold	E^* (kPa)	σ^* (kPa)	ε^* (%)
50 wt.%	780	90	12
75 wt.%	370	35	10

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

Kanungo et al., 2008

Increase Mineral Content

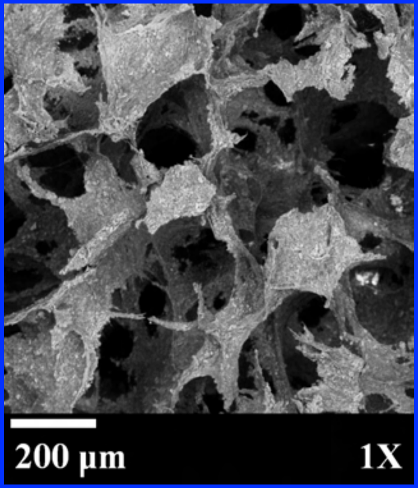


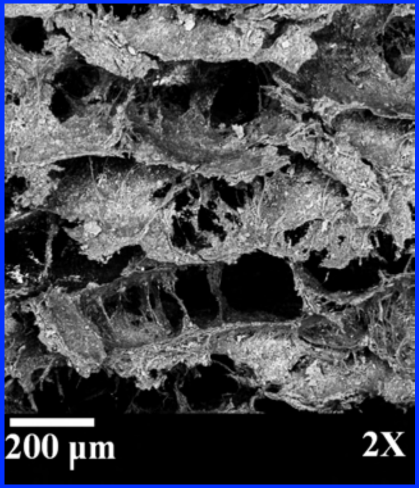
Kanungo, B. P., et al. "Characterization of Mineralized Collagen–glycosaminoglycan Scaffolds for Bone Regeneration." *Acta Biomaterialia* 4, no.3 (2008): 490-503. Courtesy of Elsevier. Used with permission.

Increase Relative Density

$\rho/\rho_s = 0.045$

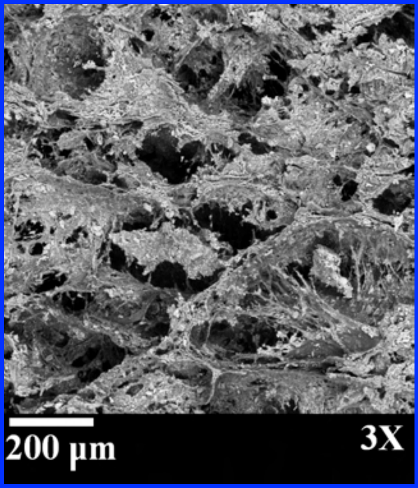
$d = 311\ \mu m$

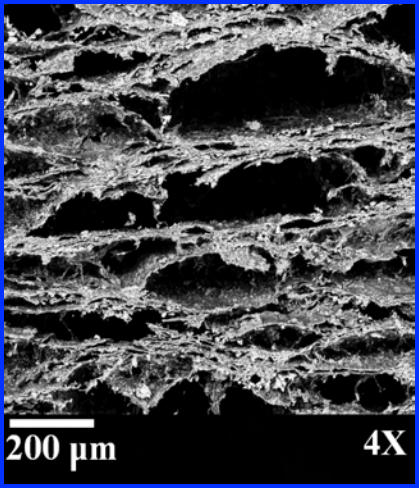




$\rho/\rho_s = 0.098$
$d = 196\ \mu m$

$\rho/\rho_s = 0.137$
$d = 159\ \mu m$





$\rho/\rho_s = 0.187$
$d = 136\ \mu m$

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

ρ^*/ρ_s (-)	E (dry) (kPa)	E(wet) (kPa)	σ^* (dry) (kPa)	σ^* (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 (ρ^*/ρ_s = 0.137, wet)

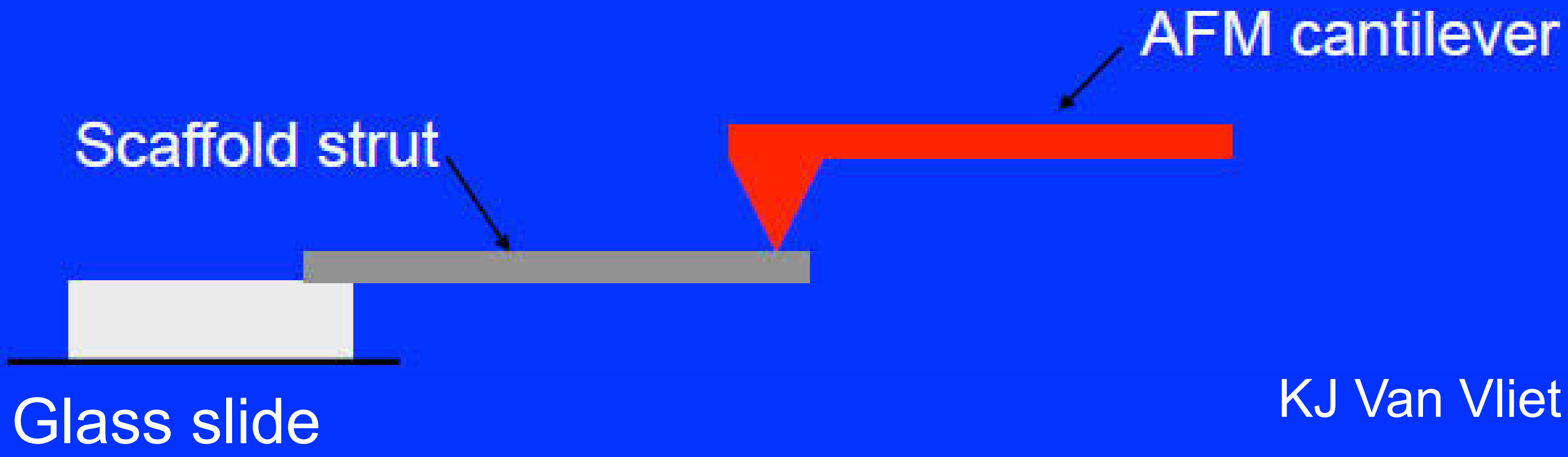
	E^* (kPa)	σ^* (kPa)
Non-cross-linked	34.8	2.12
DHT	56.0	3.26
EDAC	91.7	4.15

Increase Relative Density

ρ^*/ρ_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
0.187	*38.8*		

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

Mineralized CG Scaffold: Strut Properties



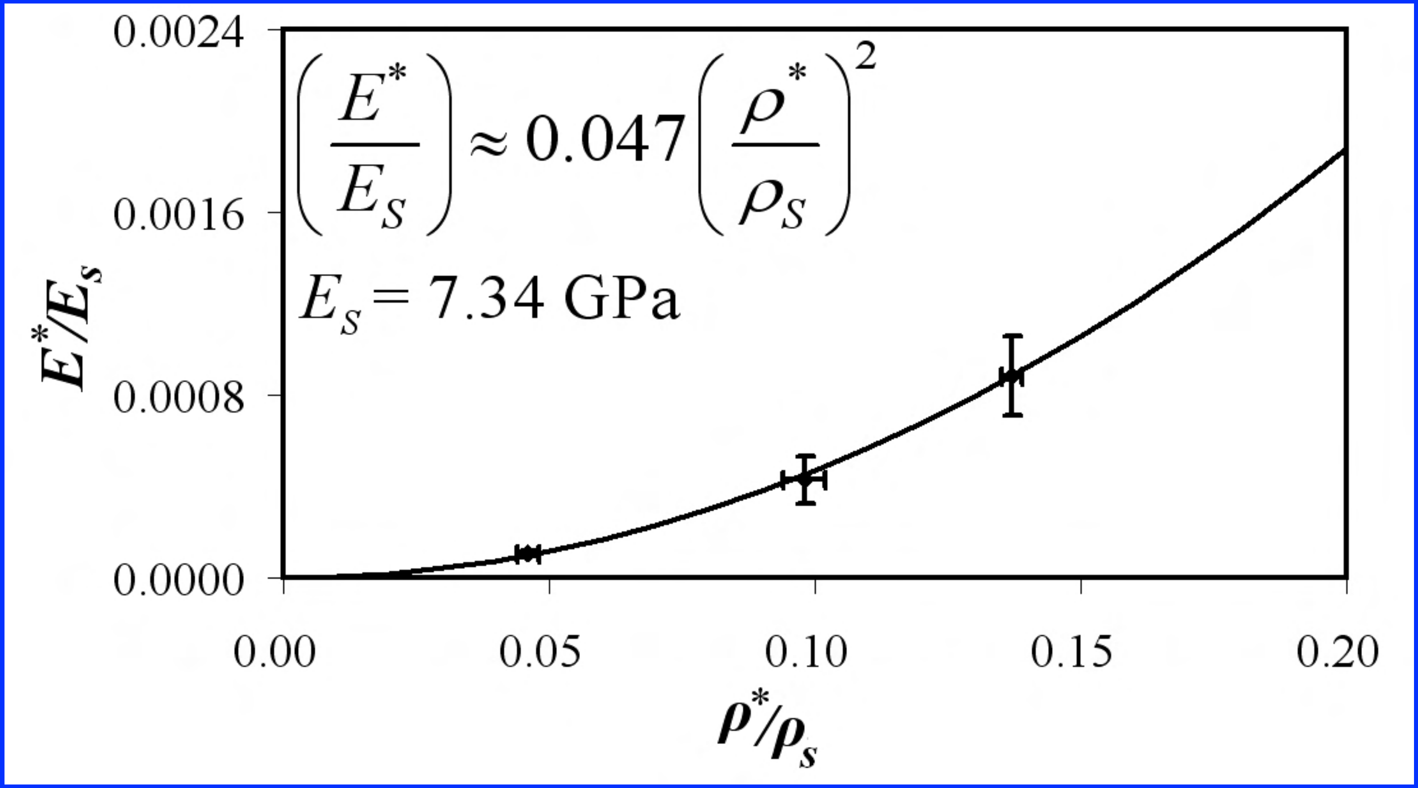
KJ Van Vliet

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

Nanoindentation: σ_s = 201 + 52 MPa (dry)

Trabecular bone: E_s = 18 GPa, σ_s = 182 MPa

Cellular Solids Models



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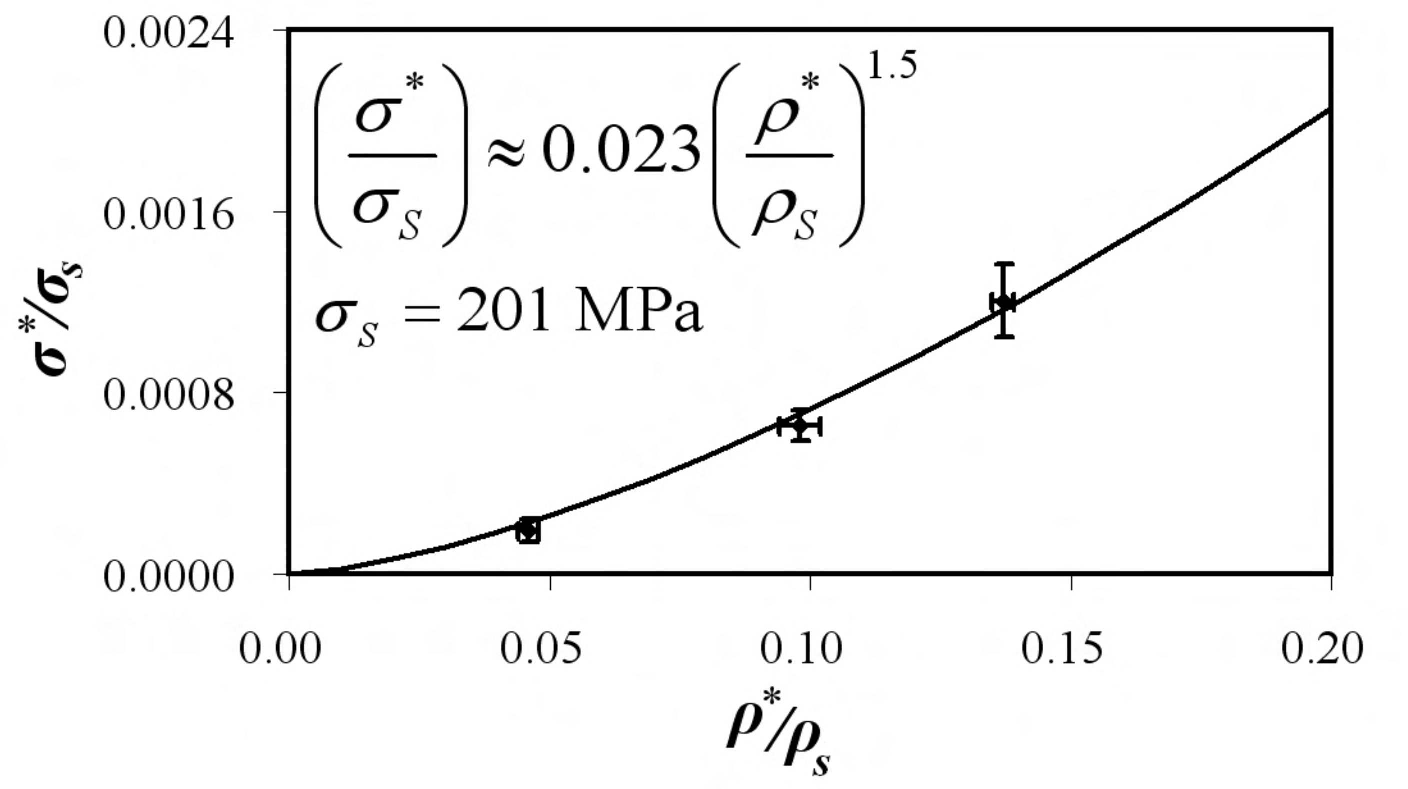
http://www.sciencedirect.com/science/article/pii/S1742706108003796

Kanungo and Gibson, 2009

Cellular Solids Models



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

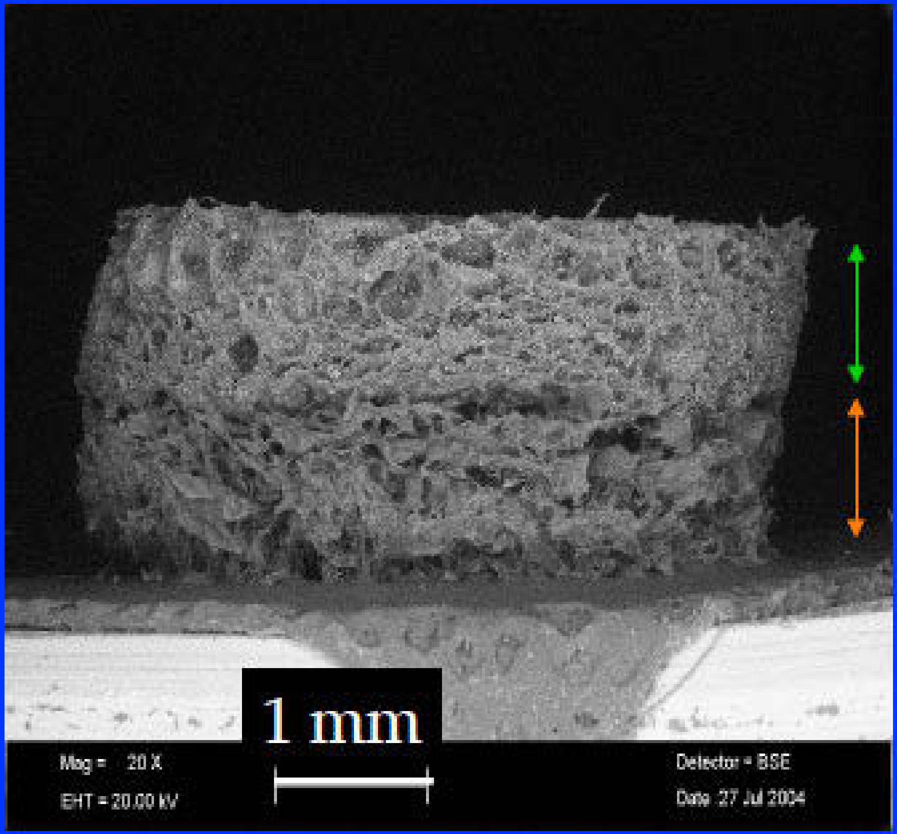
Osteochondral Scaffolds: Design Considerations

- 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 (μm)
Collagen-GAG	98.3%	653
Mineralized CG	95.5%	419

Osteochondral Scaffold: Gradual Interface

Collagen-GAG

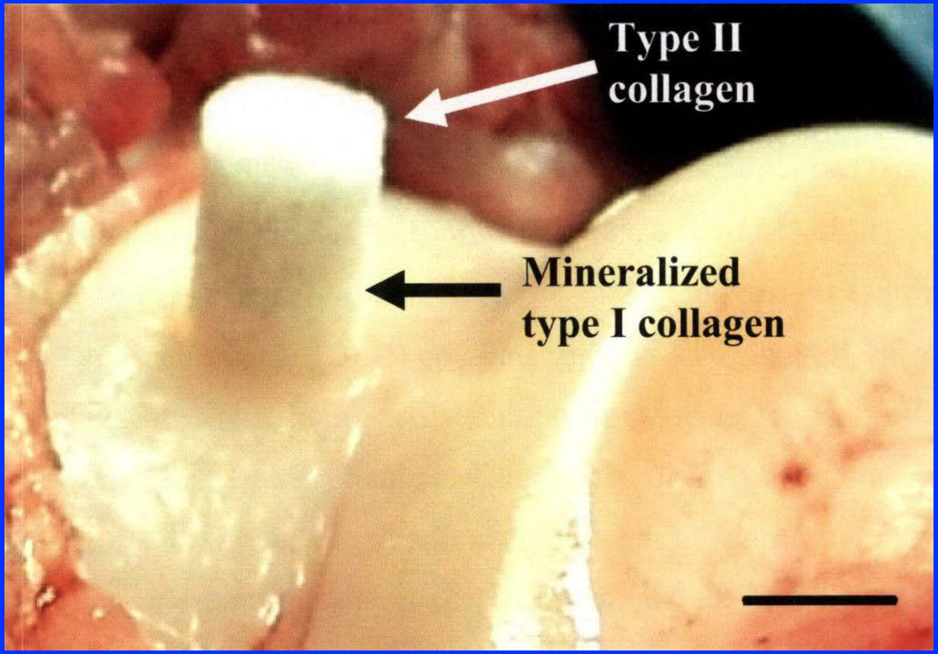
Mineralized
Collagen-GAG

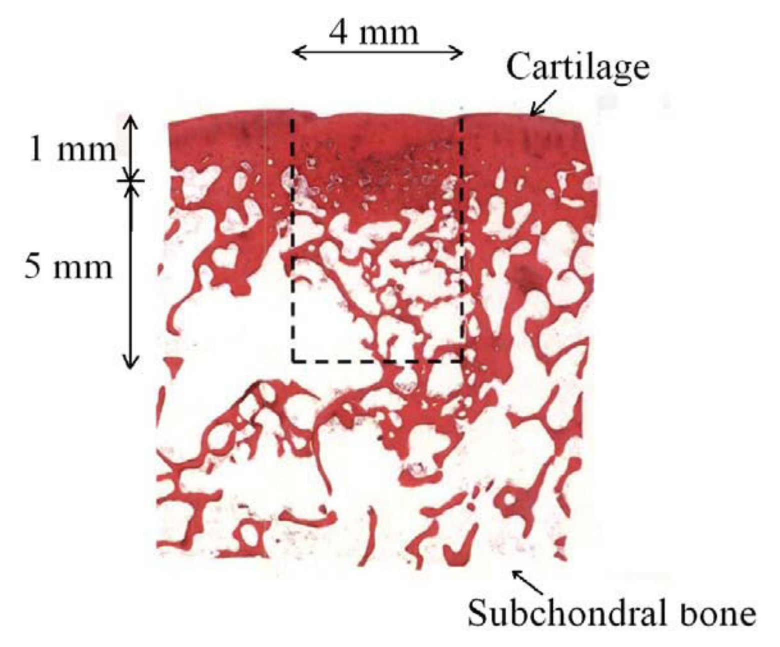
Figures removed due to copyright restrictions. See: Harley, B. A., et al. *Journal of Biomedical Materials Research* 92A (2010): 1078–93.
http://onlinelibrary.wiley.com/doi/10.1002/jbm.a.32387/abstract

Energy-dispersive X-ray (EDX)

Harley et al., 2010

Osteochondral Scaffold: Goat Model





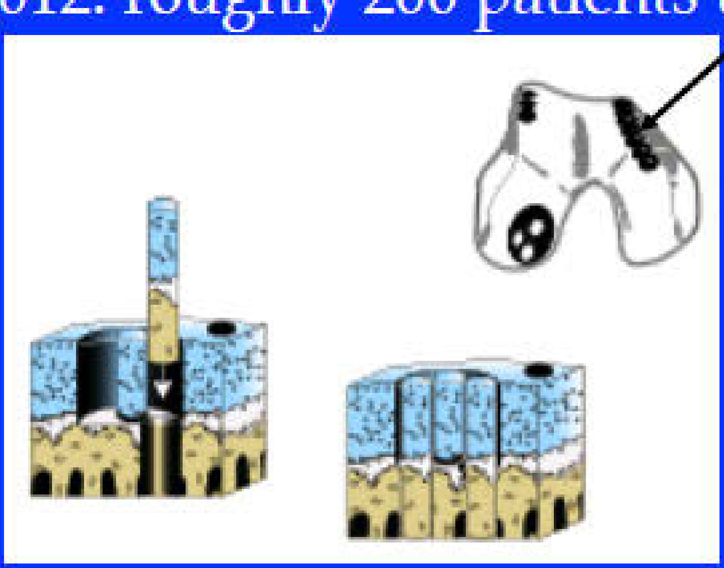
Source: Vickers, S. "Cell-seeded Type II Collagen Scaffolds for Articular Cartilage Tissue Engineering." Ph.D. Thesis. MIT, Department of Mechanical Engineering, 2007.

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



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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

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- Universities UK, Cambridge Commonwealth Trust, St. John’s College Cambridge (AKL)

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