University of Virginia

2016 UVA Orthopaedic Research Retreat

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University of Virginia

Clinical and Research Challenges Associated with Spinal Fusion

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Clinical Problem

- Over 400,000 spinal fusions / year in U.S. alone

- Iliac Crest Autograft Harvest
  - 30% complication rate
    - Chronic pain - Scar
    - Infection - Bleeding
  - Limited availability
    - Revision surgery
    - Large reconstructions
  - ? Variable osteogenic activity between individuals
    - ? Smokers
    - ? Elderly

Properties of Ideal Graft Material

1. Osteoconductive matrix
   - Scaffold or framework into which bone growth occur

2. Osteoinductive factors
   - Growth factors such as BMP, TGF-B – promote bone formation

3. Osteogenic cells
   - Mesenchymal cells, osteoblasts, and osteocytes

Bone Graft Options for Spine Fusion

- Autograft
- Allograft
  - Cortical bone
  - Cancellous bone
- Bone graft substitutes
  - Natural materials
  - Synthetic polymers
- Growth factors
  - BMPs
- Stem cells
  - Bone marrow aspirates
  - Allograft from bone marrow or fat tissue

Bone Morphogenetic Protein (BMP)

- Part of the TGF-beta superfamily
- Naturally occurring protein in human bone
- Signaling molecule
- Must be placed on carrier in order to stay in region of repair and influence skeletal formation
- Over 20 structurally related BMP and BMP-receptors identified and described
  - rhBMP2- Infuse
  - rhBMP7- OP1

How Do They Compare?

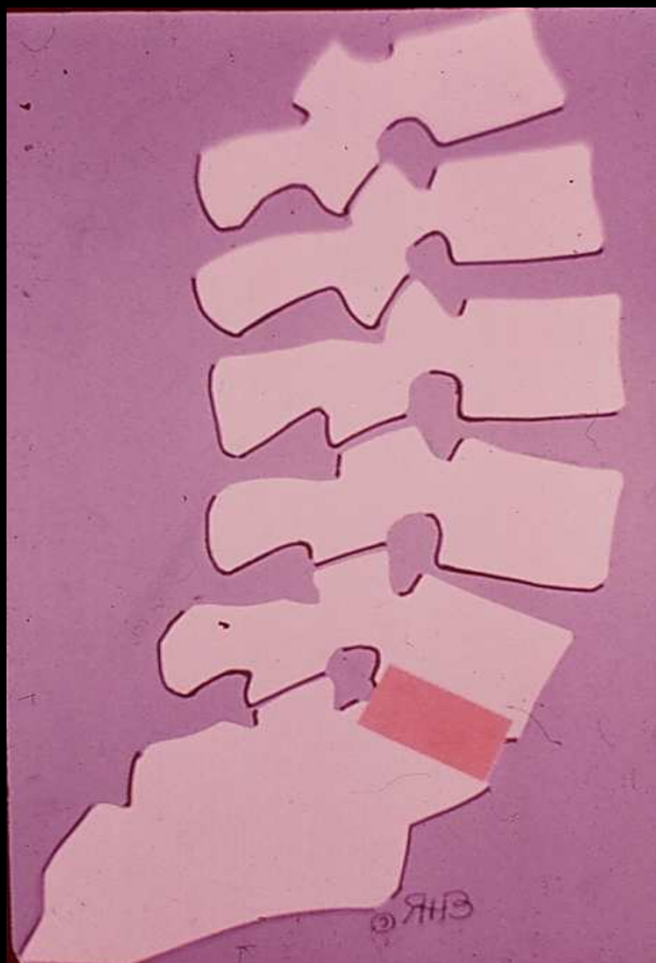
Material	Osteogenic	Osteoinductive	Osteoconductive	Structural Support
Autogenous cancellous bone	+	–	+	–
Autogenous cortical bone	+	–	±	
Allograft cortical	–	–	±	+
Allograft cancellous	–		+	–
Demineralized bone matrix	–	+	+	–
Ceramic	–	–	+	±
BMP	–	+	–	–

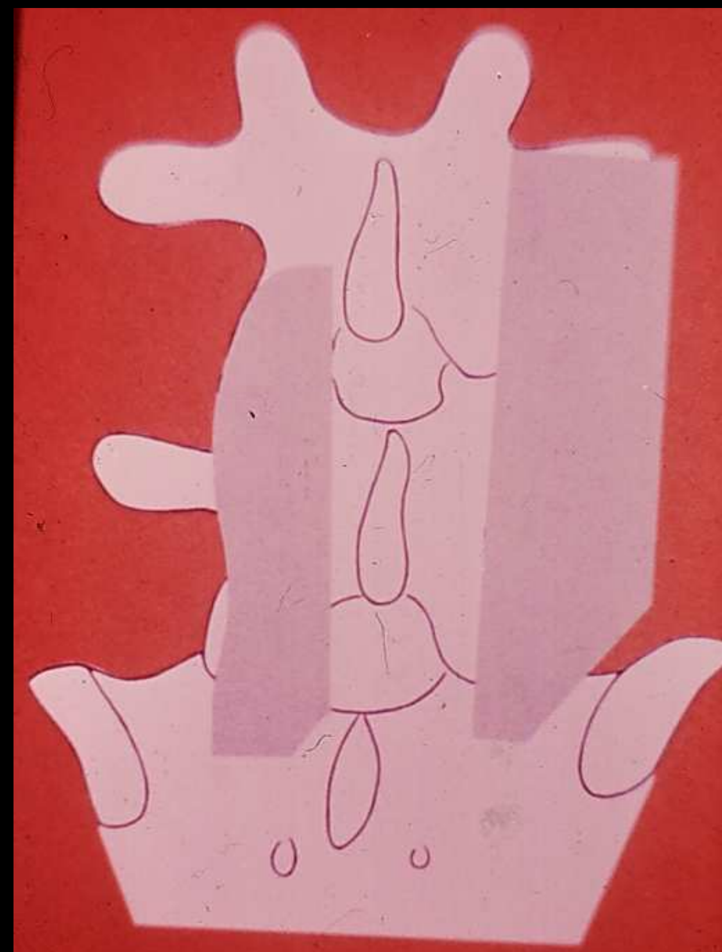
Allografts

- Fresh
  - Increased antigenicity
- Fresh-Frozen
  - less immunogenic
  - BMP preserved
- Freeze Dried
  - Loses structural integrity
  - Depletes BMP
  - Least immunogenic
  - Purely osteoconductive

- Cortical Bone Graft
  - Slow incorporation
  - Remodeling of existing Haversian systems
  - via resorption followed by deposition of new bone
  - Weak during resorption phase (fatigue Fracture)
- **Cancellous Bone Graft**
  - Revascularizes quickly
  - Osteoblasts lay down new bone on old trabeculae
  - CREEPING SUBSTITUTION

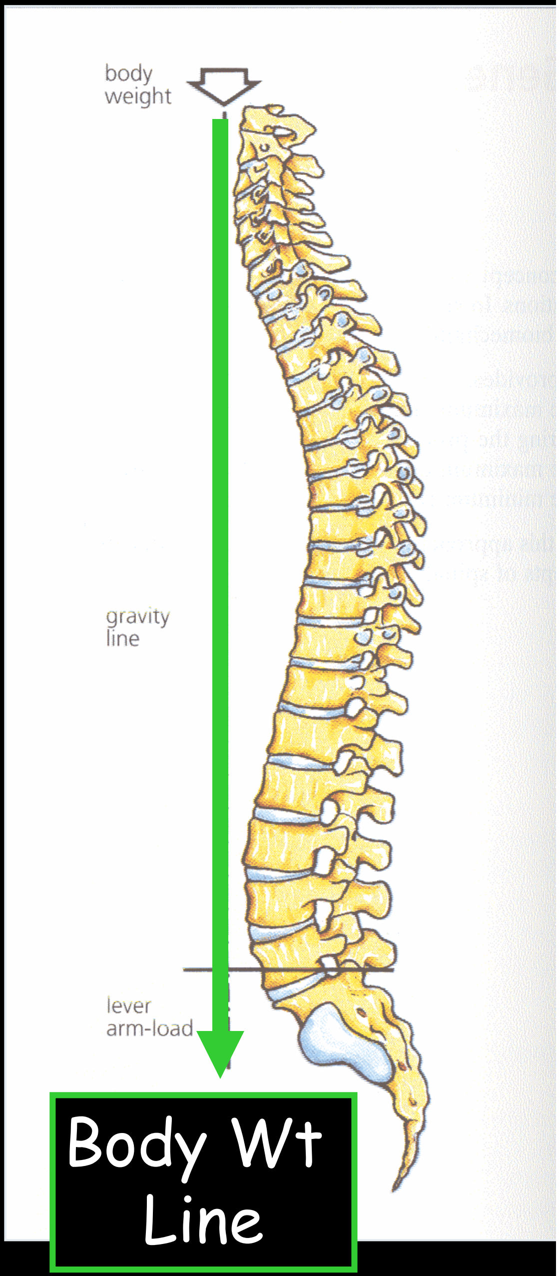
Anterior vs. Posterior Spinal Fusions



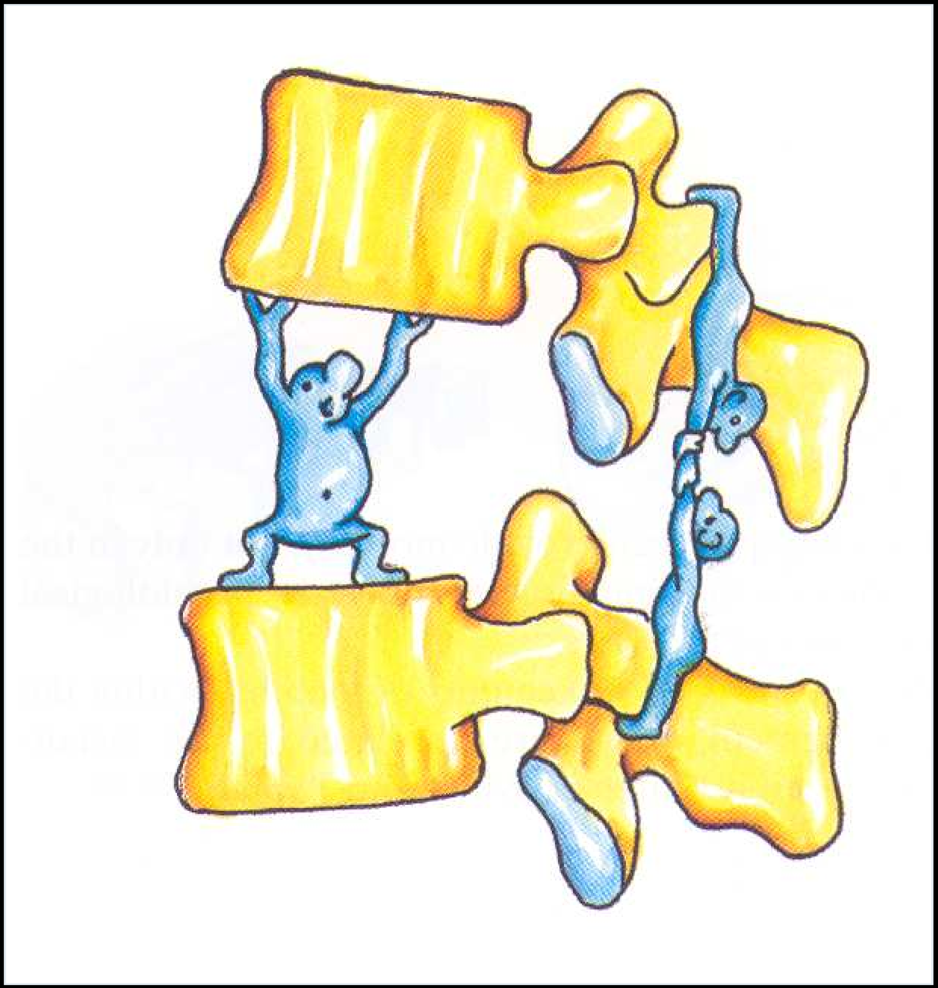


Spinal Biomechanics

- Body weight line is anterior to spinal column
- Vertebral body
  - Anterior column support
- Posterior elements
  - Tension band



Spine Biomechanics



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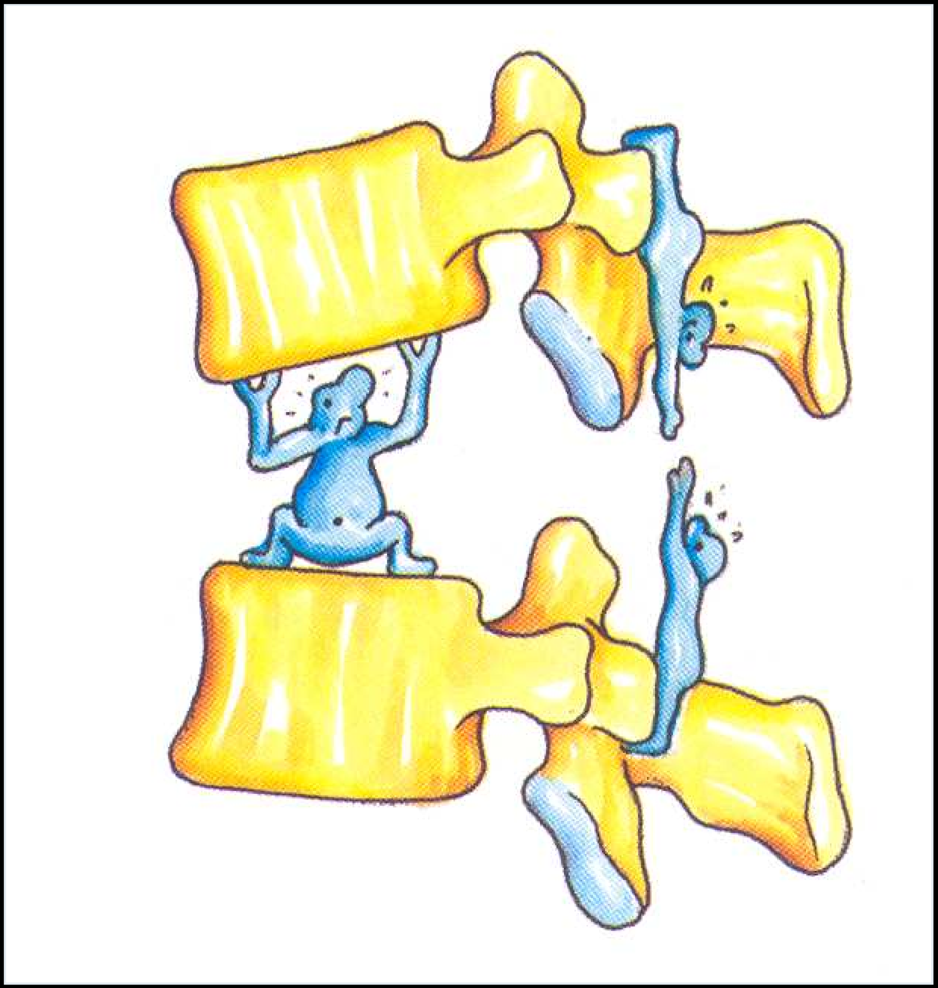
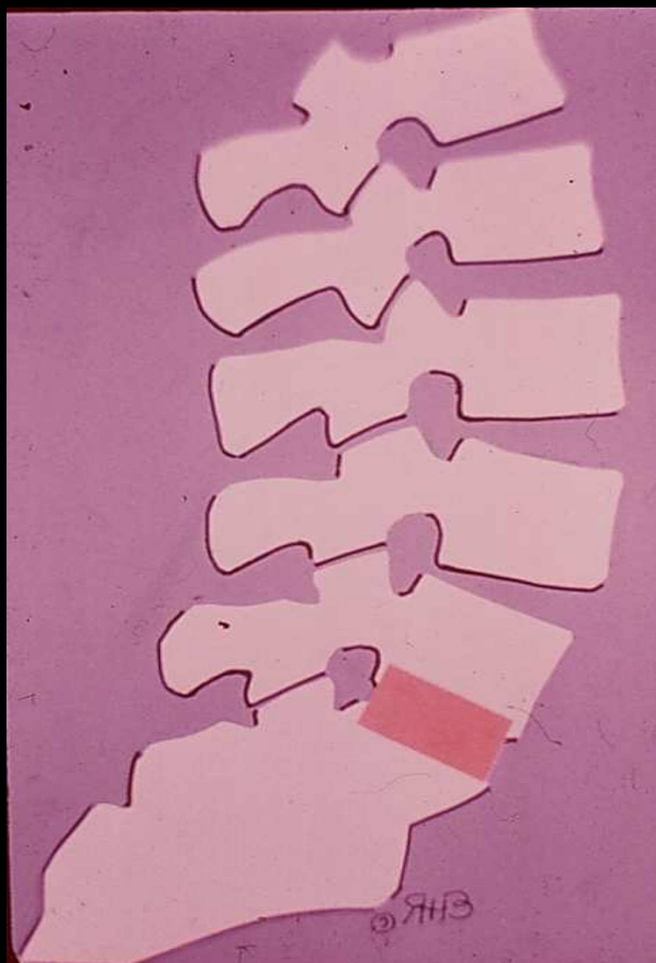
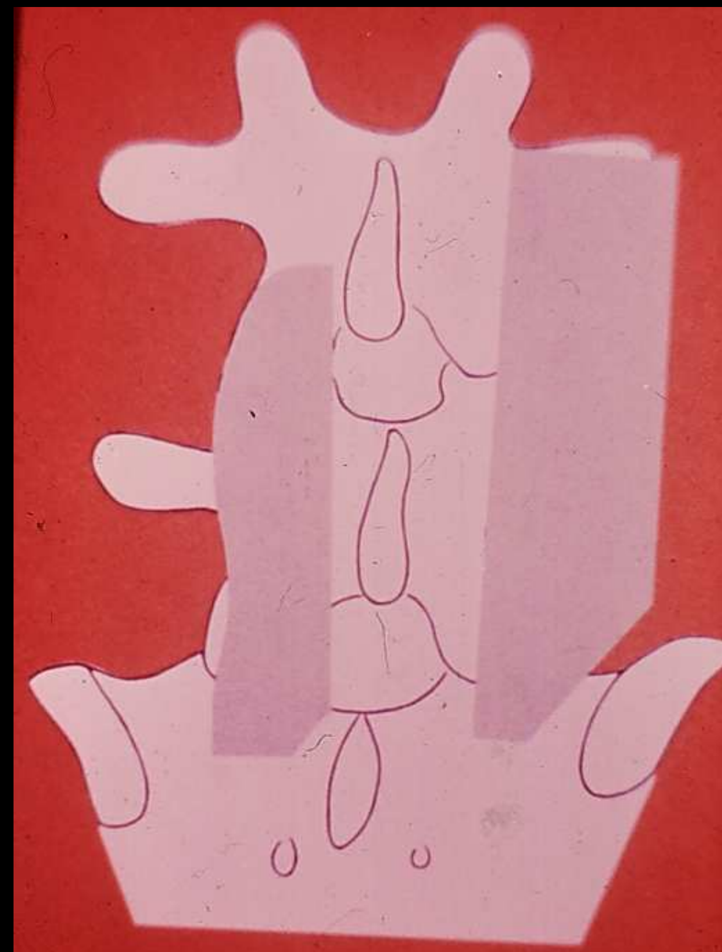
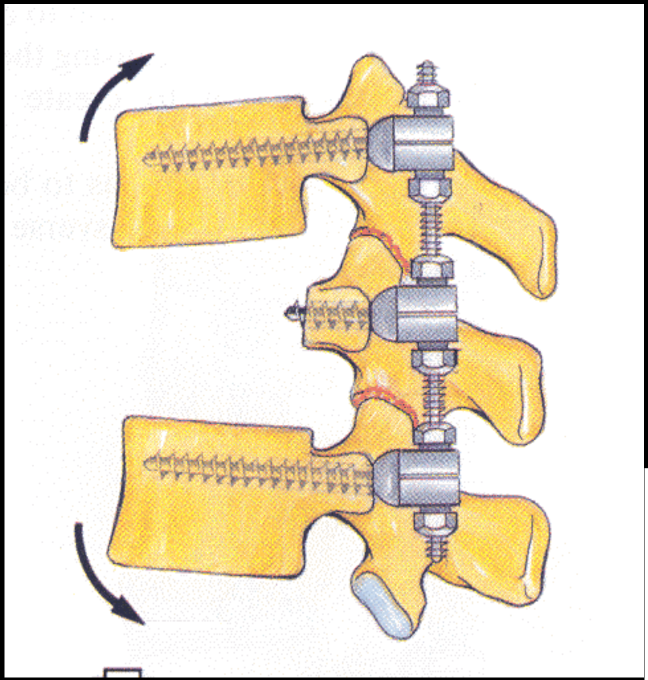


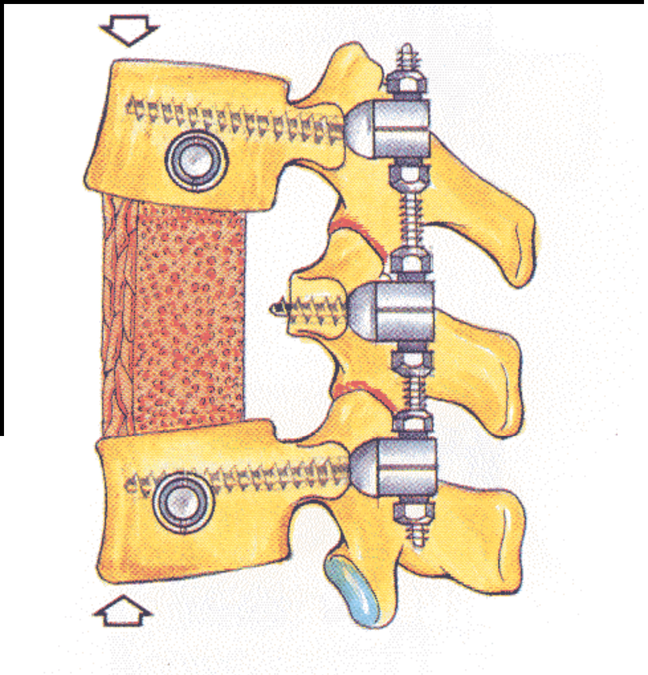
Anterior vs. Posterior Spinal Fusions

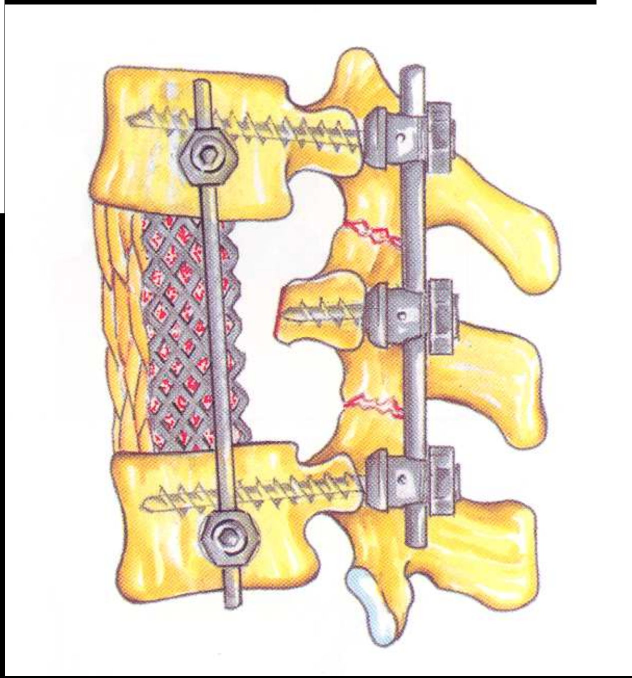




Spine Biomechanics







One Solution

Tissue Engineering Principles

- Repair, Restore, or Regenerate Tissue

1. Cells
2. Factors
3. Biomaterials

The Spine Journal 6 (2006) 615–623

2006 Outstanding Paper Award: Basic Science

Osteogenic differentiation of adipose-derived stromal cells treated with GDF-5 cultured on a novel three-dimensional sintered microsphere matrix

Francis H. Shen, MD[a,b,*], Qing Zeng, MD[b], Qing Lv, MS[b], Luke Choi, MD[a], Gary Balian, PhD[b,c], Xudong Li, MD, PhD[b], Cato T. Laurencin, MD, PhD[a,b,d,e]

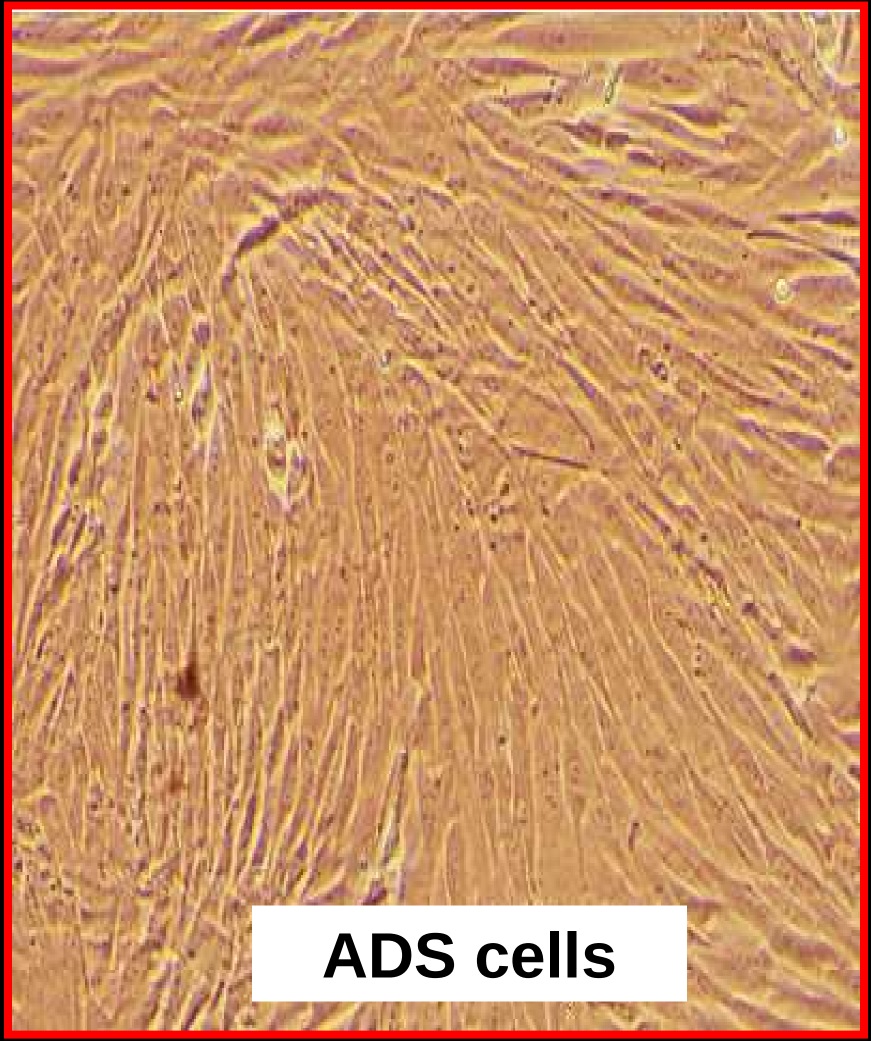
Goals: Bone Graft Substitute

Osteogenic cells	→	Multipotential Adipose-derived stromal (ADS) cell
Osteoinductive protein	→	Growth and Differentiation Factor-5 (GDF-5)
Osteoconductive scaffold	→	Sintered Microsphere Matrix (SMM)

Shen FH, et al. Spine Journal 2006

Method: Cell isolation and Culture

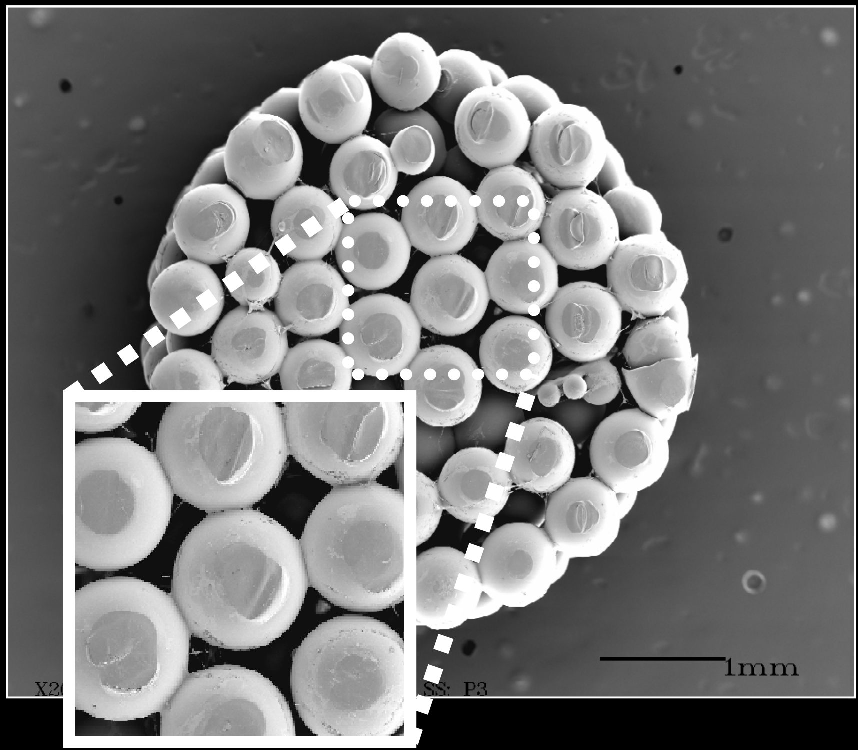
- Multipotential cells
  - Rat inguinal region
  - Isolated and passaged
  - Treated with differentiation media
    - Vitamin D3
    - glyerophosphate
    - ascorbate-2-phosphate
  - GDF-5 100 ng/mL



Shen FH, et al. Spine Journal 2006

Method: Sintered Microsphere Matrix (SMM)

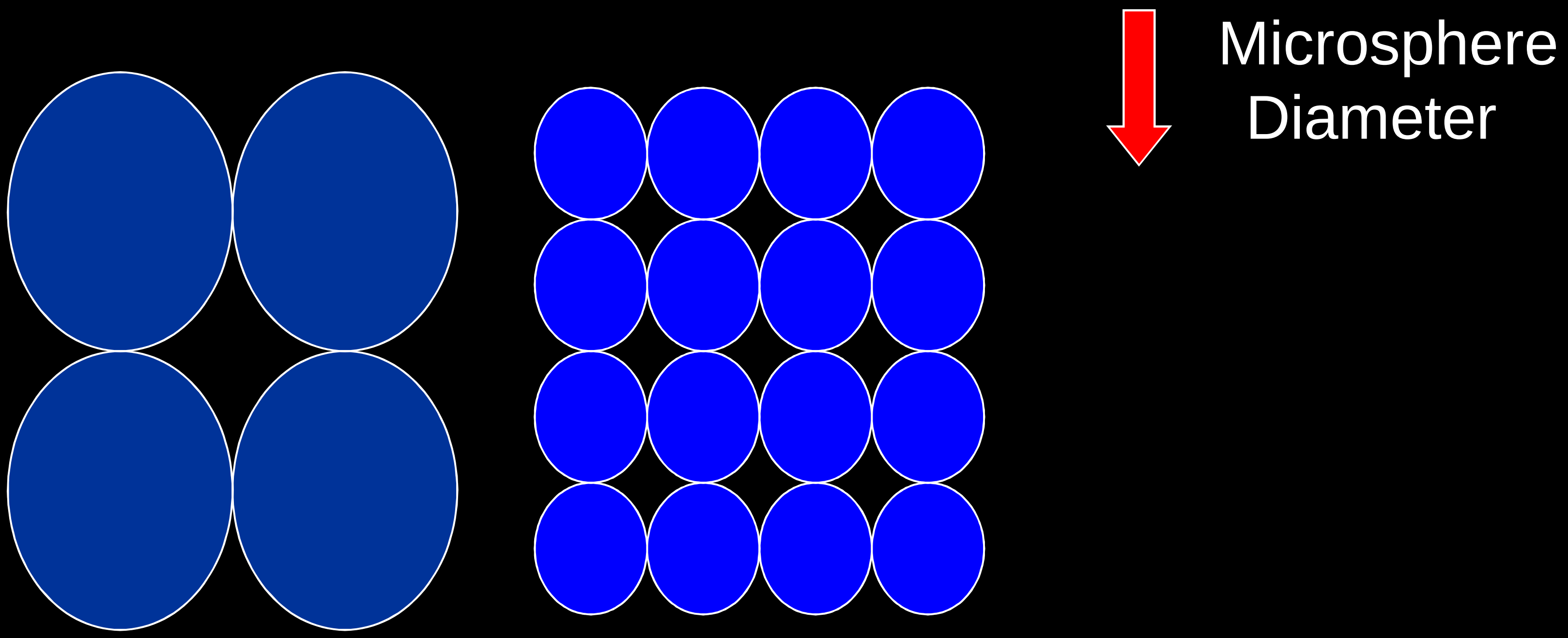
- Poly(lactide-co-glycolide) [85:15] polymer
- Solvent evaporation technique
- Micron sieve
  - Microsphere diameters
    - 500 - 600 µm
  - Optimal pore size
    - 100 - 250µm
- PLAGA spheres packed and heated
  - 80 °C for 3 hrs



Scanning Electron Microscopy

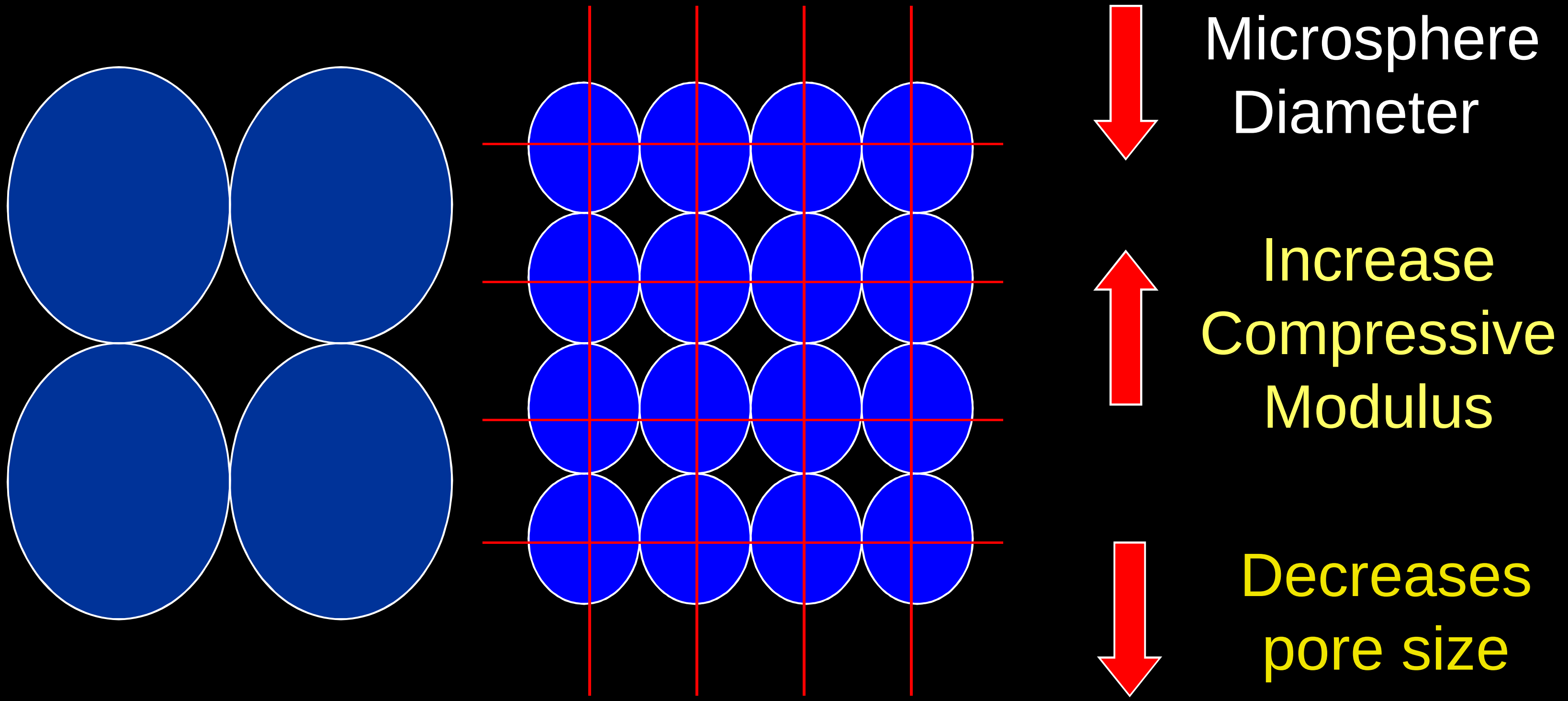
Shen FH, et al. Spine Journal 2006

Sintered Microsphere Matrix (SMM)



Borden M, Attawia M, Laurencin CT. The SMM for bone tissue engineering. J Biomed Mater Res 61:421-429, 2002.

Sintered Microsphere Matrix (SMM)



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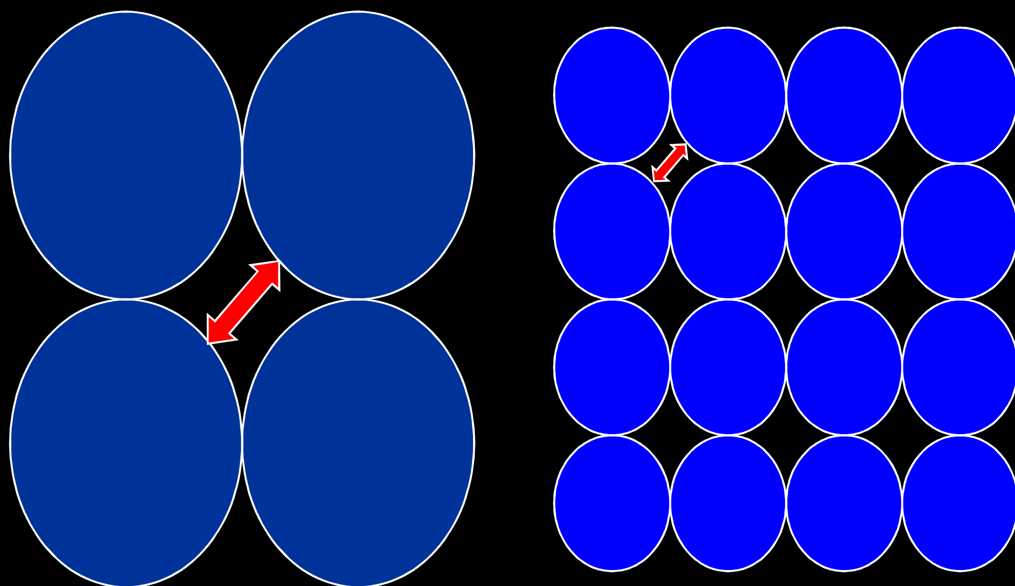


Microsphere Diameter

Increase Compressive Modulus

Decreases pore size

Borden M, Attawia M, Laurencin CT. The SMM for bone tissue engineering. J Biomed Mater Res 61:421-429, 2002.

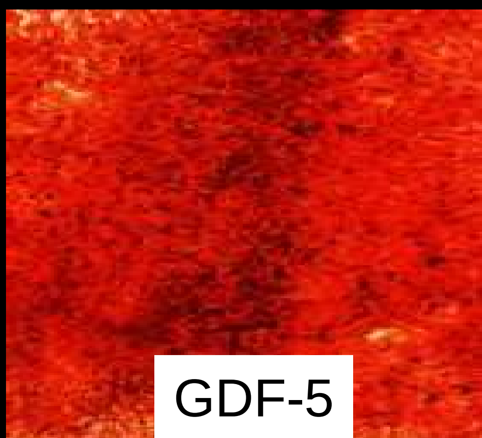
Methods

- Total 78 SMM
  - Seeded with 50,000 cells/matrix
  - Evaluated at 7, 14, 28 days

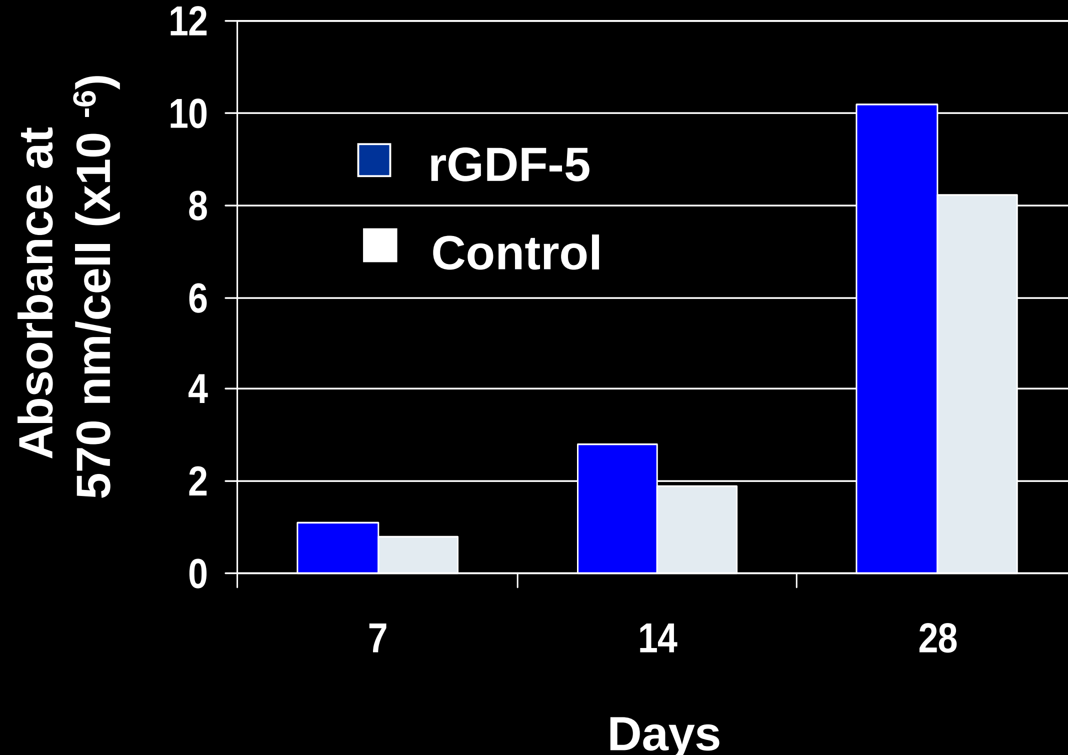
- Alizarin Red Calcium Quantification - 18
- Gene Expression - 18
  - Cbfa1
  - Osteocalcin
  - Alkaline Phosphatase
- Colorimetric Assay (MTS) - 18
- Scanning electron microscopy (SEM) - 6

Results: Mineralization / Calcium Deposition

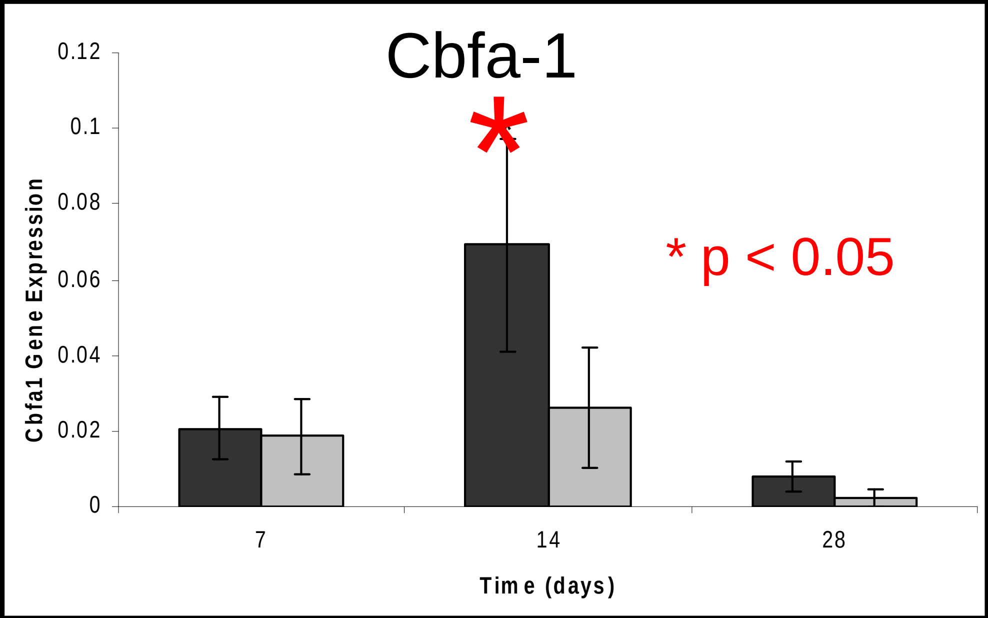
von Kossa staining
for mineralization

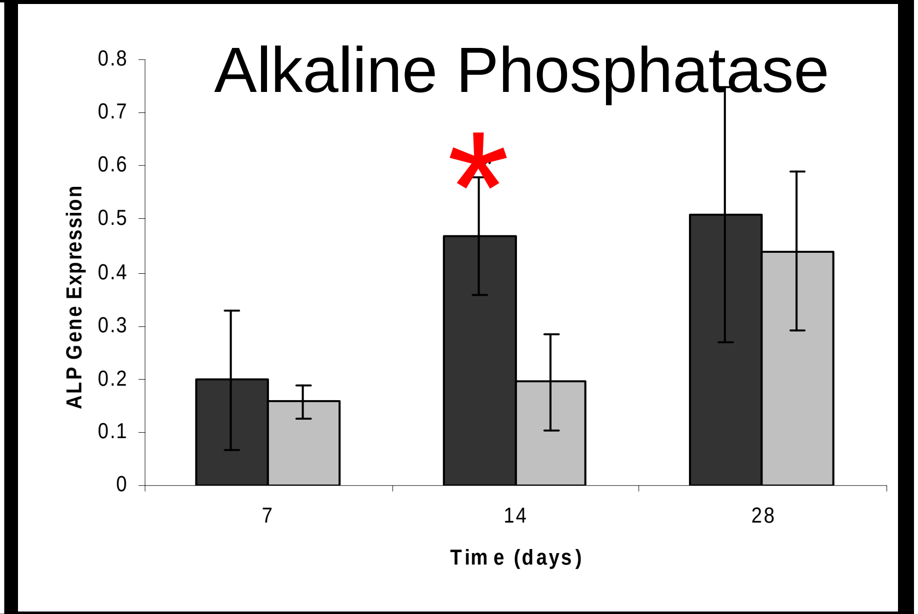


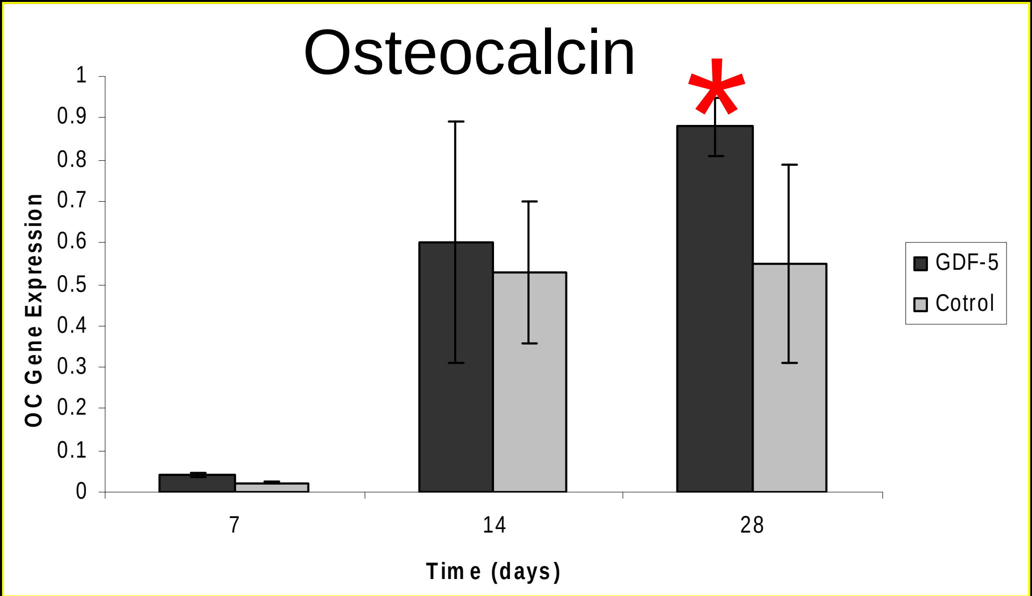
Alizarin Red Staining
for calcium deposition



Results: Gene expression







Multipotential ADS maintain the ability to undergo osteogenic differentiation

Results: MTS Assay

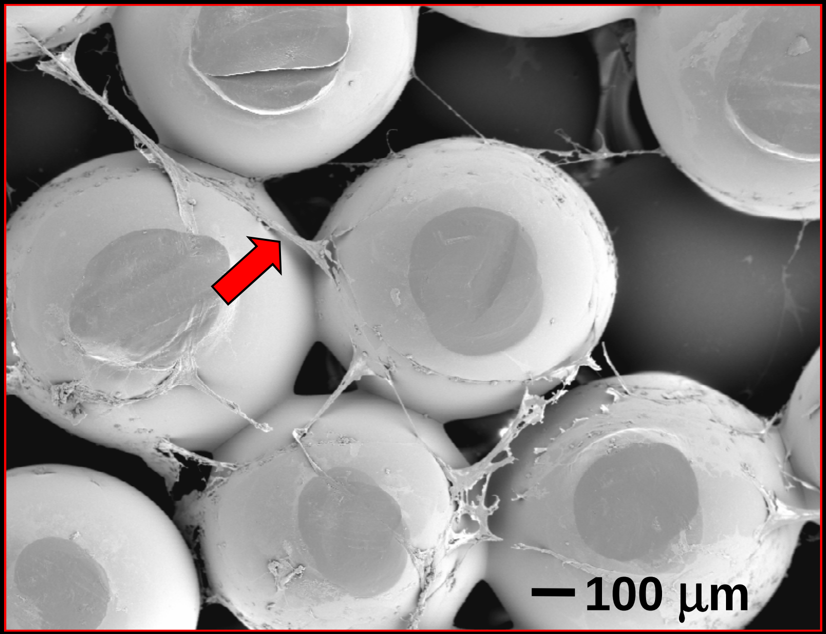
Time (days)	Number of cells per scaffold (1×10^4)
7	2.14 $\pm$ 0.18
14	2.18 $\pm$ 0.15
28	1.94 $\pm$ 0.21

$p > 0.05$ not statistically significant

ADS cells are capable of adhering on 3-dimensional SMM and remain viable

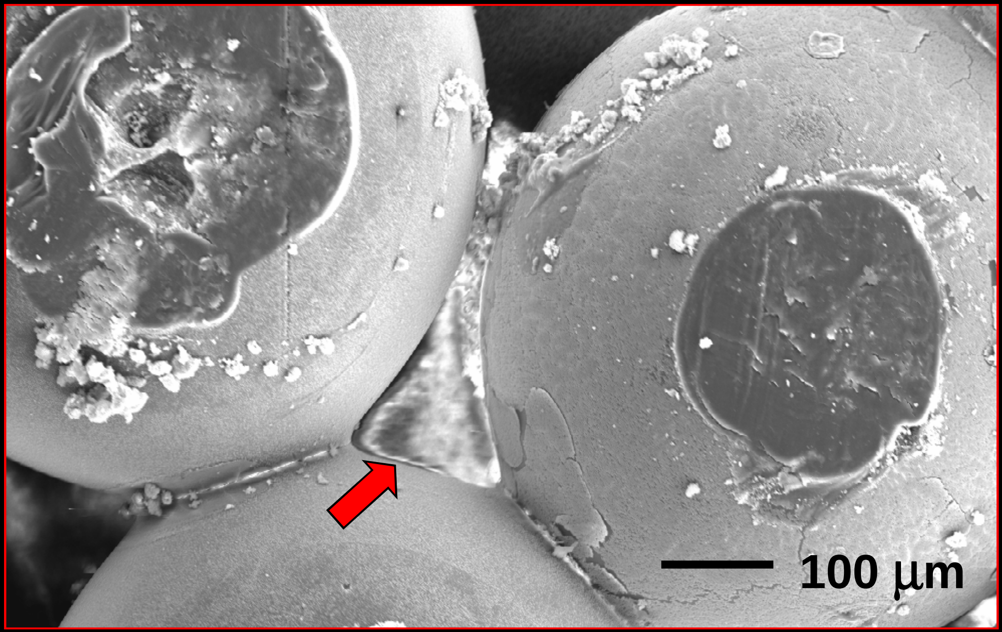
Results: SEM

7 days



Cytoplasmic Extensions

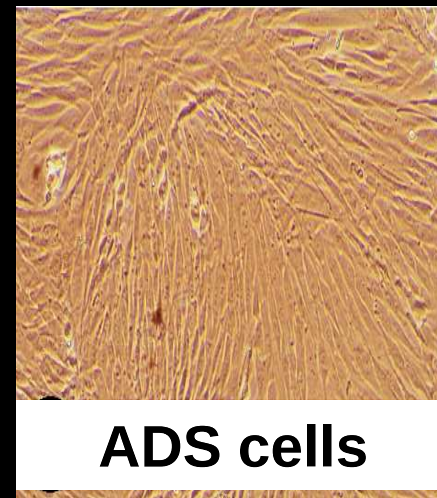
14 days



Cellular Proliferation with elimination of pore space

Discussion

- ADS cells are a viable alternative
  - Increased availability, accessible, less morbidity
  - Capable of osteogenic differentiation in culture
- rGDF-5
  - Potentiates osteogenic differentiation



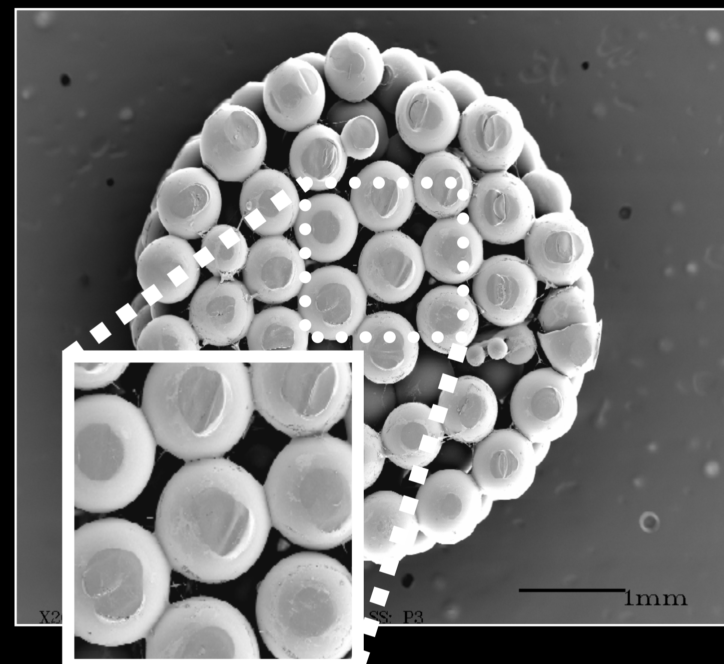
ADS cells

rGDF-5 ↓

Von Kossa

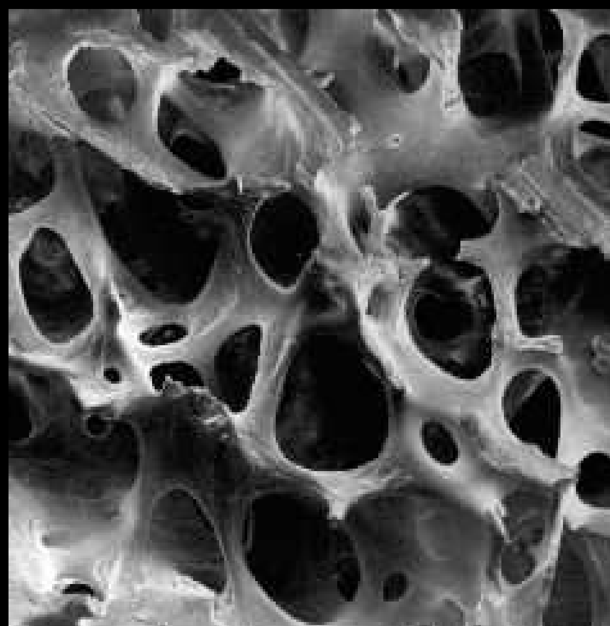
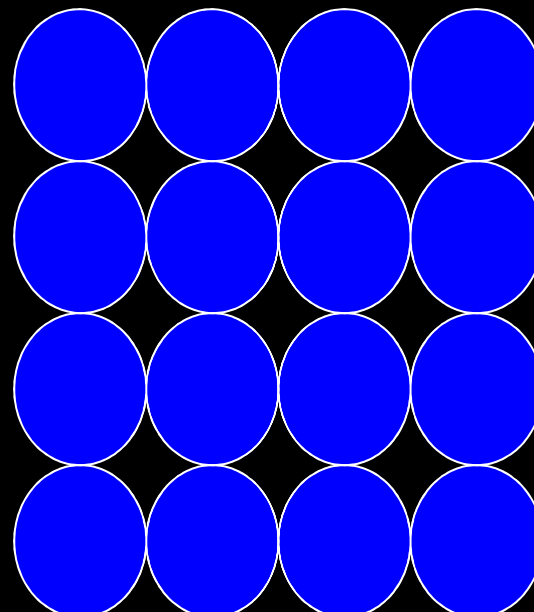
Discussion: Sintered Microsphere Matrix (SMM)

- 3-dimensional bioengineered, biodegradable
  - Structurally and mechanically
    - Similar to trabecular bone
  - ADS cells
    - Adhering to surface
    - Proliferate and penetrate
    - Remain viable
    - Undergo osteogenic differentiation



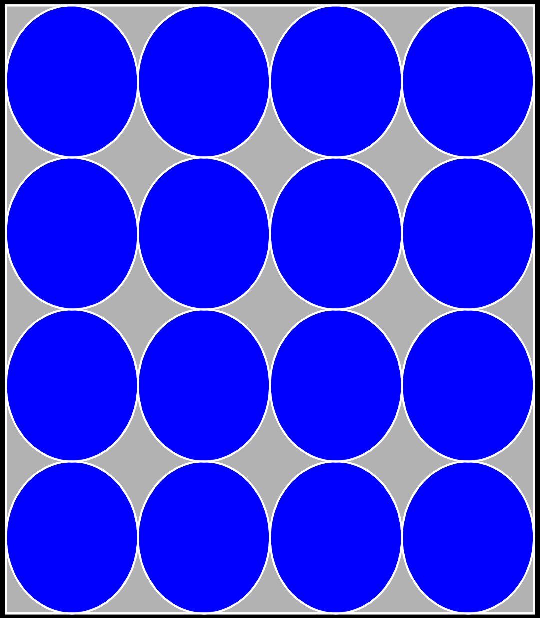
Shen FH, et al. Spine Journal 2006

Sintered Microsphere Matrix (SMM)

70 % porous   30% porous

Sintered Microsphere Matrix (SMM)



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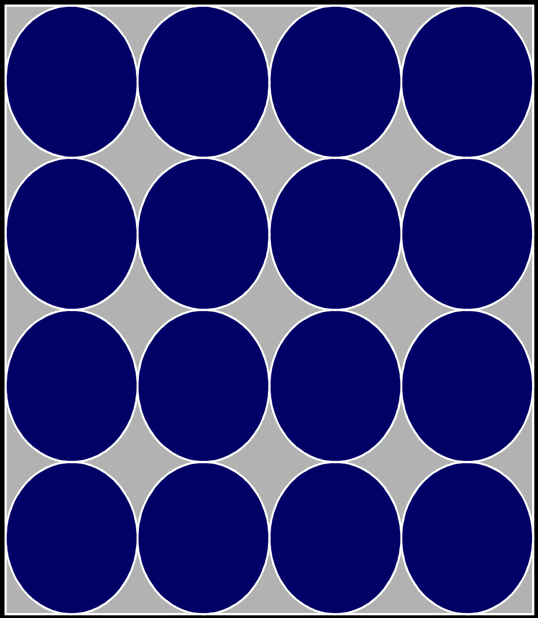
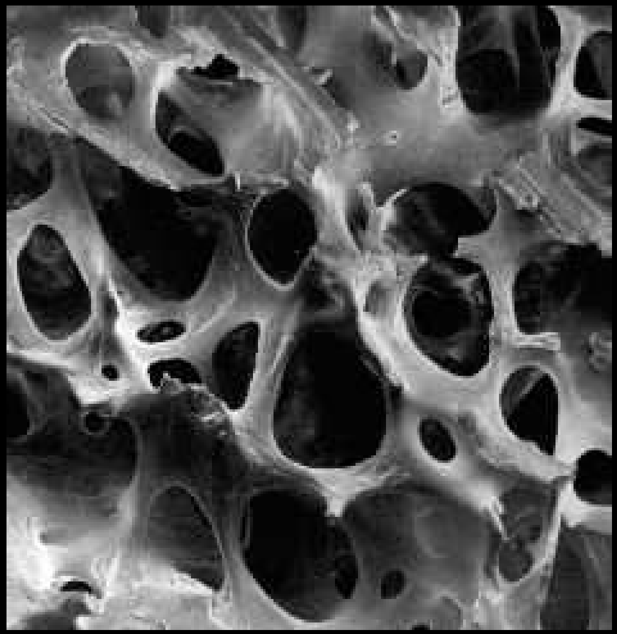
Sintered Microsphere Matrix (SMM)

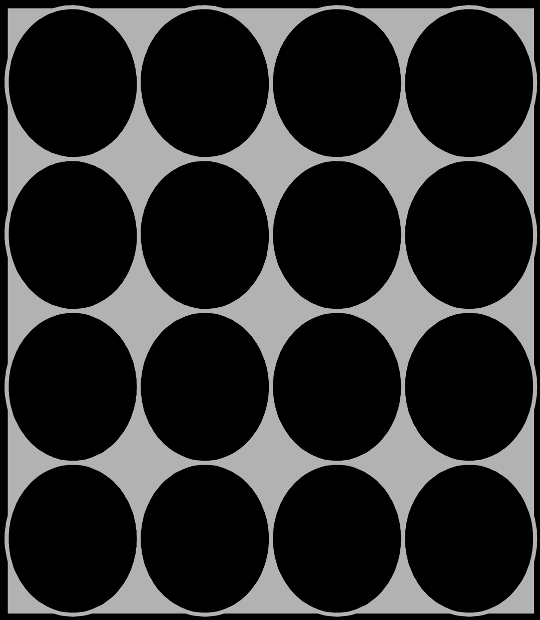
70 % porous



30% porous

Sintered Microsphere Matrix (SMM)





70 % porous   70% porous

ELSEVIER

The Spine Journal 6 (2006) 615–623

THE SPINE JOURNAL

2006 Outstanding Paper Award: Basic Science

Osteogenic differentiation of adipose-derived stromal cells treated with GDF-5 cultured on a novel three-dimensional sintered microsphere matrix

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[b]Orthopaedic Research Laboratories, P.O. Box 800374, University of Virginia, Charlottesville, VA 22908-0374, USA
[c]Departments of Biochemistry and Molecular Genetics
[d]Biomedical Engineering, P.O. Box 800759
[e]Chemical Engineering, P.O. Box 400741, University of Virginia, Charlottesville, VA 22908-0759, USA

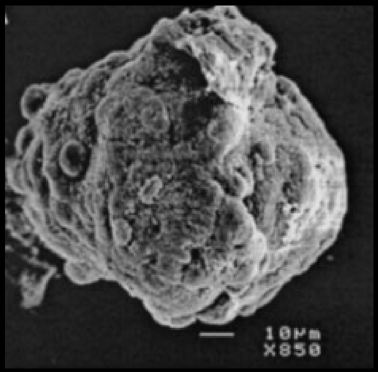
Received 12 January 2006; accepted 12 March 2006

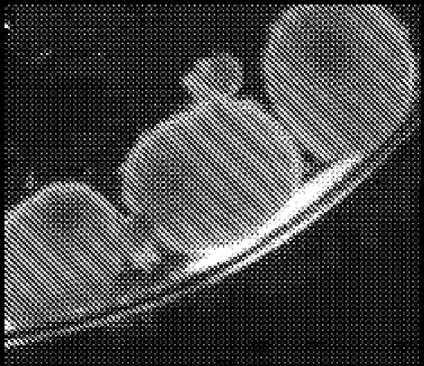
University of Virginia

Current Stem Cell Research:

3D Culture Systems

University of Virginia





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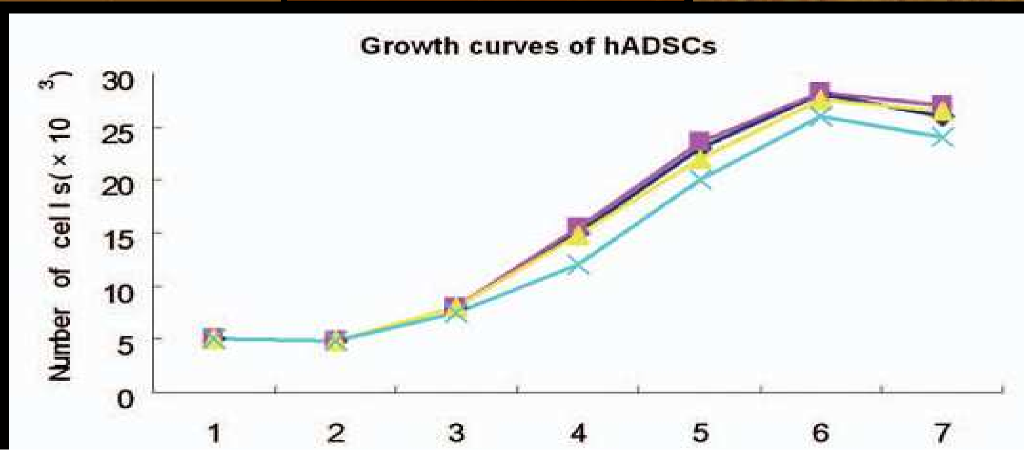
Cellular Growth In Vitro

Adherent monolayers

Cell density limits cell number

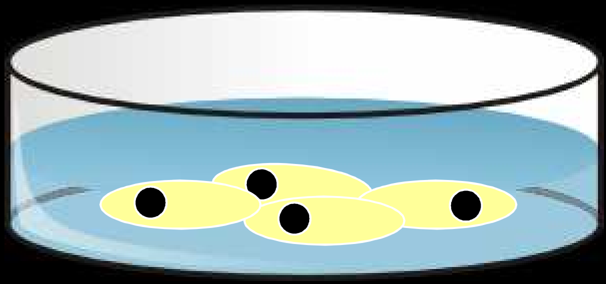
Contact inhibition

2D cultures



Cellular Growth In Vitro

- Cells grown to confluence
- Number of cells lifted
- Placed in set volume



Limitation of Monolayer Culture

- Mammalian cells normally exist in 3D micro-milieu

- Cells cultured in adherent monolayers need to be lifted into suspension

- Disrupts ECM milieu that was established

- ECM critical for tissue repair
  - Serves as a scaffold
  - Modulator of cell function
  - Growth factor functionality

What's the answer?

- Goal
  - 3D spherical culture systems
  - Homogenous sized spheroids
  - Utilize cells natural disposition to attach to each other without the need for artificial scaffolds.
- Limitations
  - Inadequacy of standard techniques
  - Current nonadherent surfaces
  - Delicate aggregates
  - Irregular geometry

What's the answer?

- Hanging drop technique
  - Harrison 1907
  - Carrel 1992
- Recently used by tumor biologist
  - Last 25 years
  - More accurately reproduces 3D environment
    - Nutrient and signal gradient
    - Cell-cell contact interaction
    - Cell-ECM interaction

Modeling

- Simple geometry
  - Modeling growth
  - Modeling oxygen, nutrient, metabolite transport
  - Model effect of anti-cancer treatment

Sphericity of Aggregates

$$\Phi = \frac{\pi \times \sqrt{\frac{4A}{\pi}}}{P}$$

Volume of the Aggregates

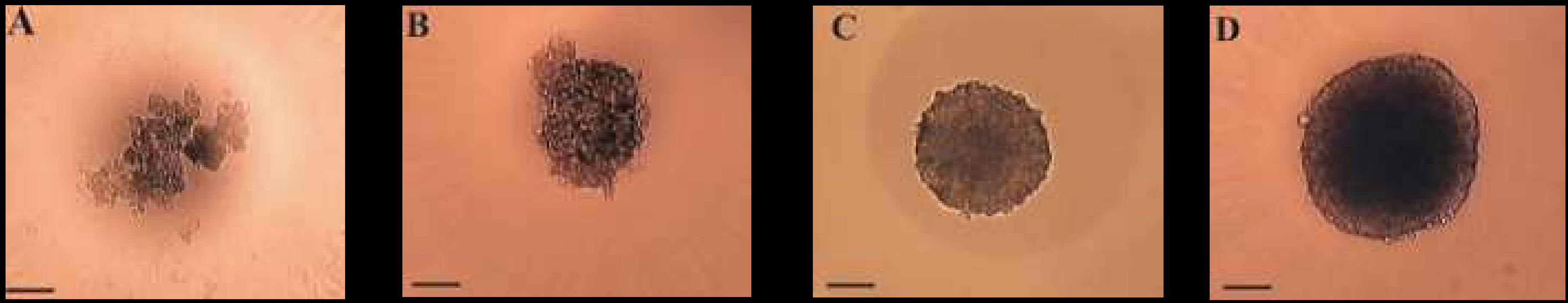
$$V = \frac{4\pi}{3}\left(\frac{P}{\pi}\right)^3$$

Shape factor corrected volume, V’

$$V' = \Phi V$$

3D Spheroid Cultures

- Advantage for tumor biologist?
  - Captures complexity of solid tumor
  - Concentric arrangement of heterogeneous cells
  - Mimic initial avasular stages of solid tumor in vivo
    - Not yet vascularized micrometastatic foci



Day 1 | Day 4 | Day 7 | Day 10

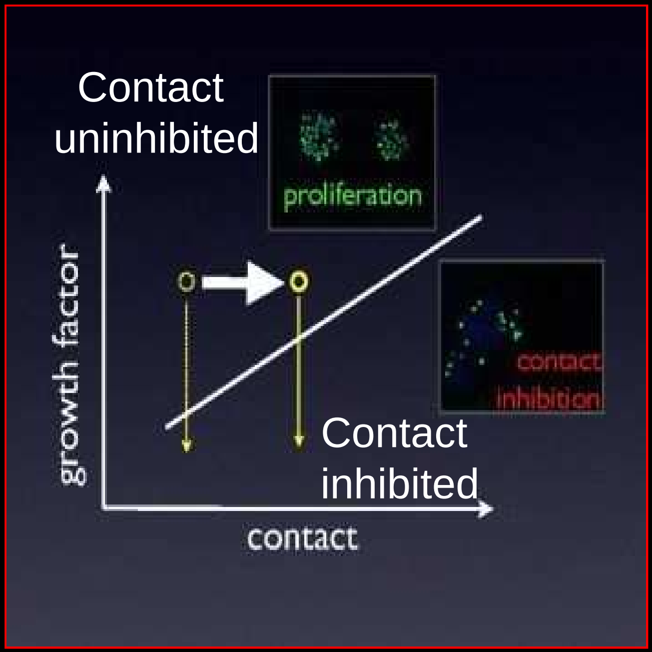
Kelm et al. Homogeneous multicellular tumor spheroids. Biotech Bioeng. 2003

MA (Multicellular Aggregate)

- Cells should therefore be delivered as a 3D multicellular aggregates (MAs)

- Modify contact inhibition

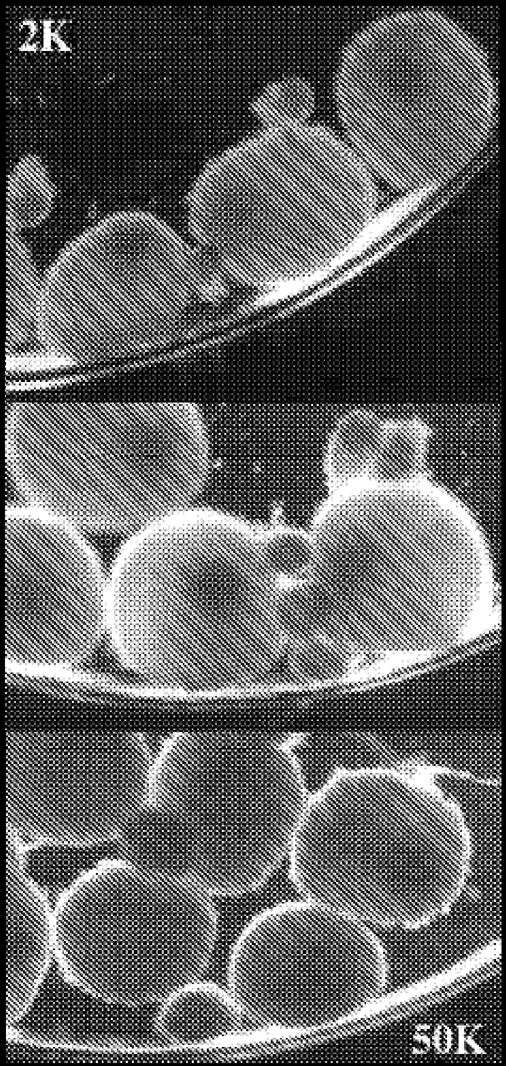
- Maintain ECM



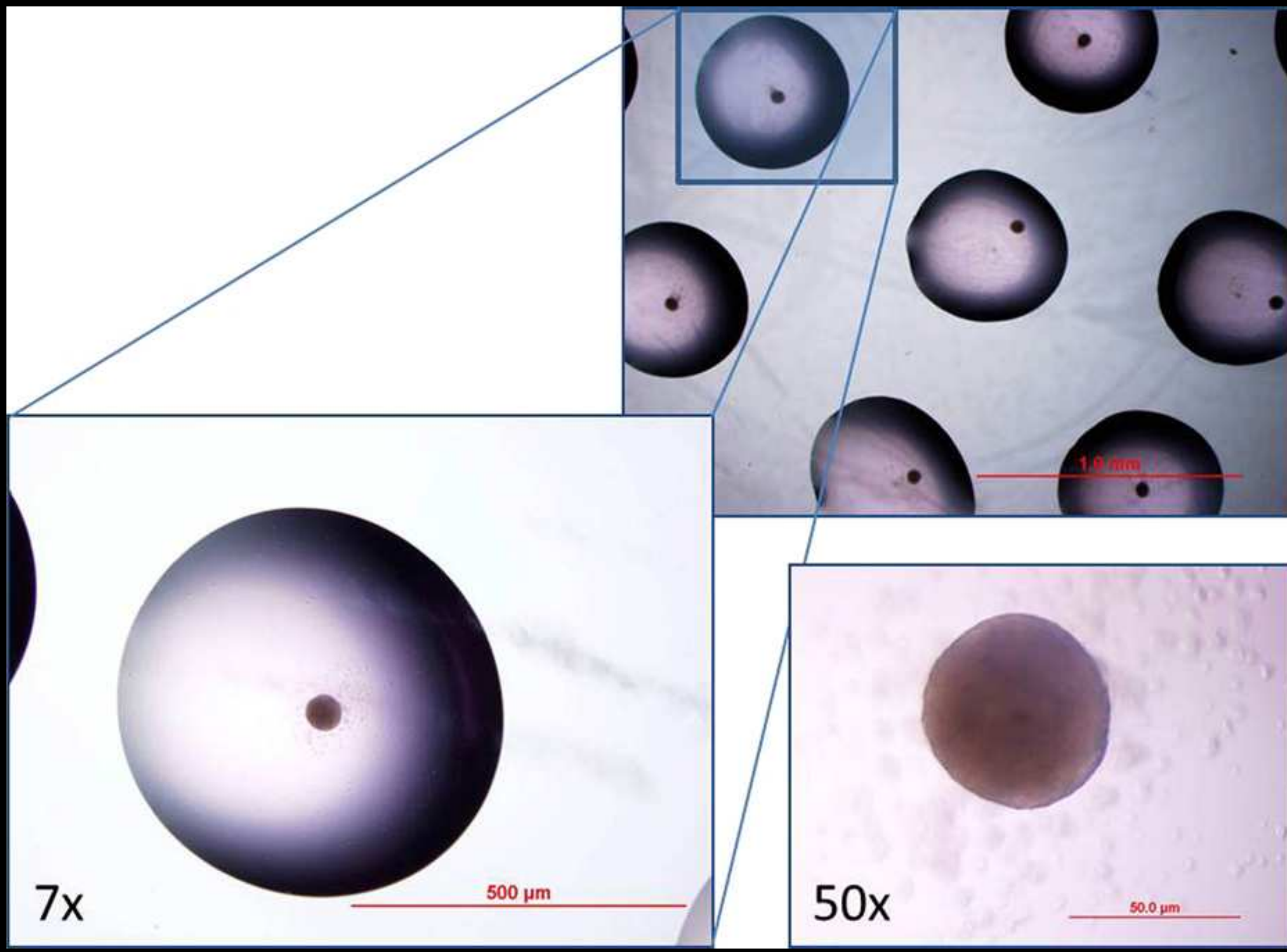
PNAS/Jin-Hong Kim,
Keiichiro Kushiro, Nicholas Graham,
Anand Asthagiri

Hanging Drop Technique

- Cell suspensions dispensed into wells
- Wells are inverted
- Hanging drops are held in place by surface tension
- Cells accumulate at free liquid-air interface
- Method forms significantly larger and more homogeneous spheroids

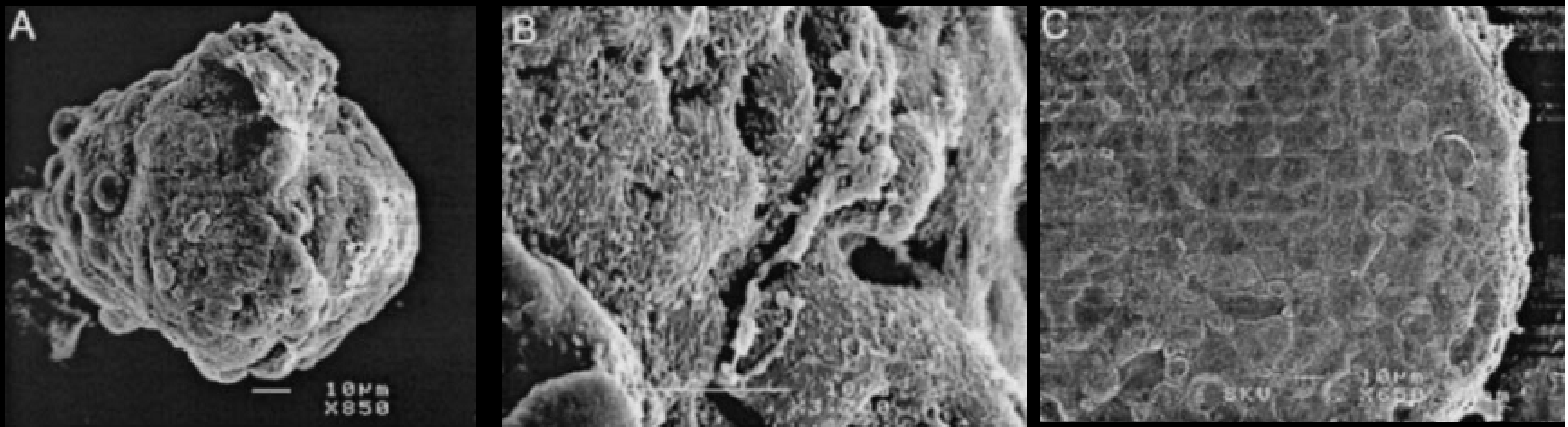


Katz A. Patent
USA2009/0304643



Shen FH, et al. Spine Journal 2012

Scanning Electron Microscopy



SEM of 5 day spheroid
Extensive ECM
Cells barely distinguishable

Kelm et al. Homogeneous multicellular tumor spheroids. Biotech Bioeng. 2003

Multicellular Aggregates

- Upon plating on standard tissue culture
- MA readily adhered
- Gave rise to confluent monolayers of cells
- Plasticity towards adipose, cartilage, and bone phenotype

Shen FH, et al. Spine Journal 2012

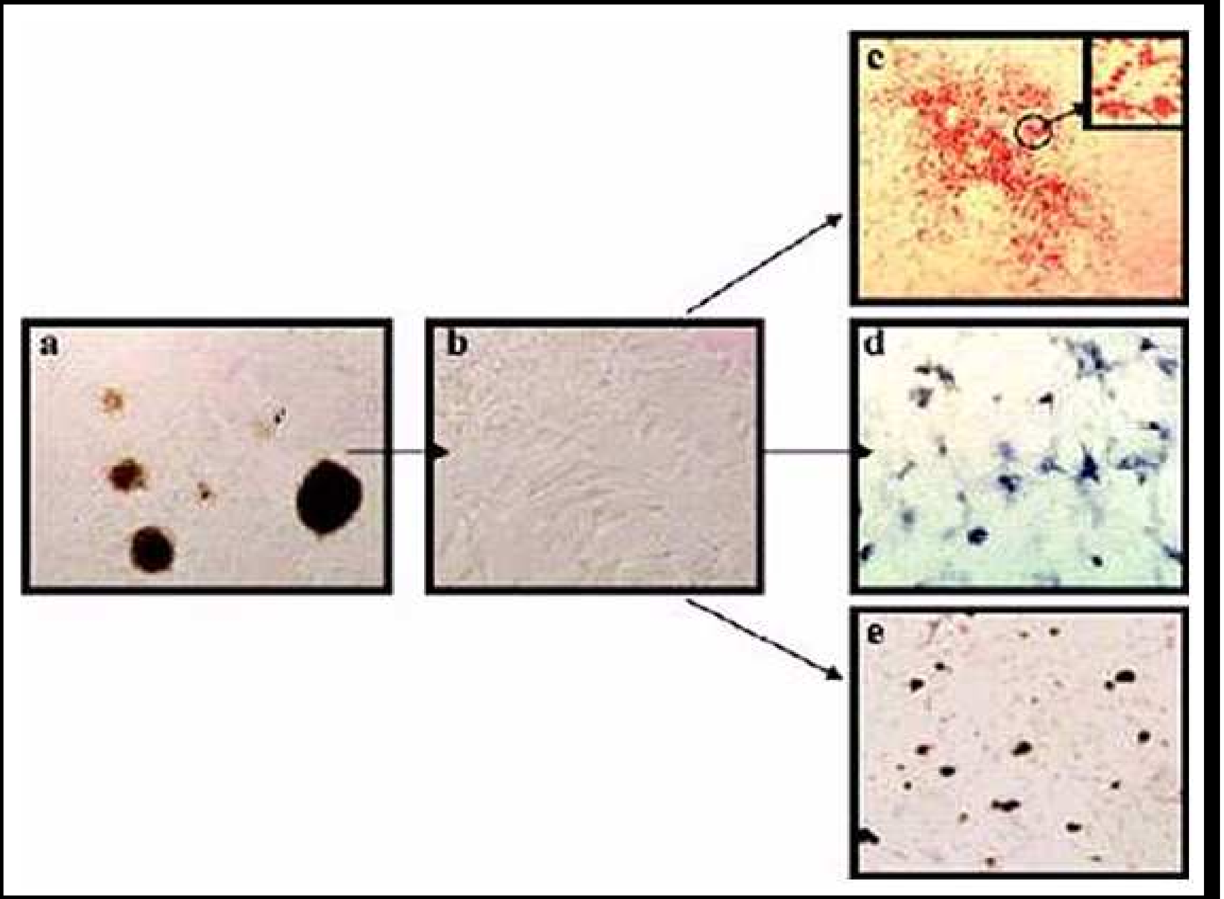
a) BM stromal cell hanging droplet

b) Organoid bodies confluent monolayer

c) Adipogenic

d) Chondrogenic

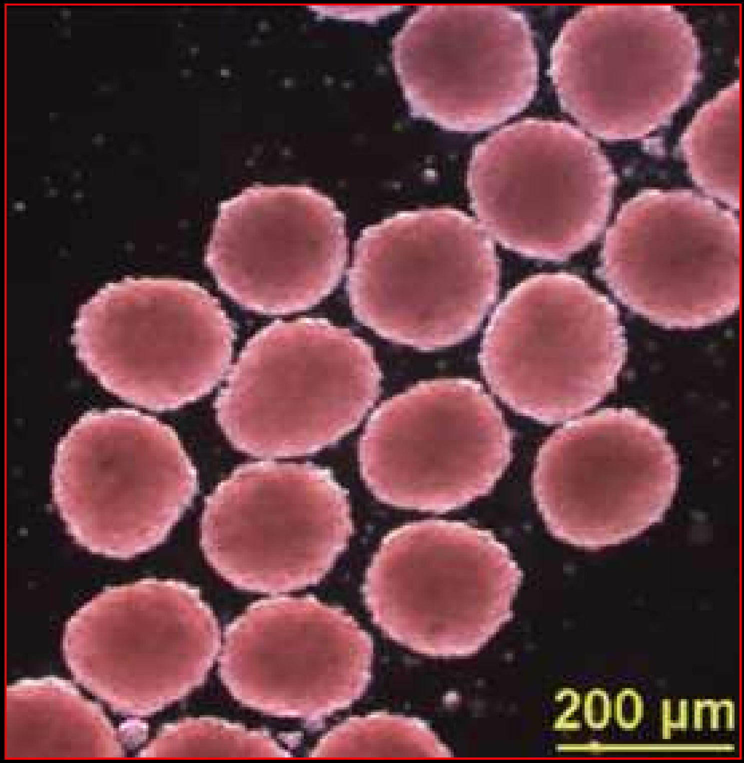
e) Osteogenic



Banerjee et al. Application hanging drop technique for stem cell differentiation and cytotoxicity studies. Cytotech 2006

hADS Cells

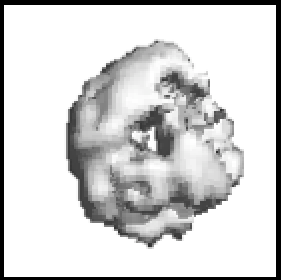
- MA form with little variability in aggregate size
- More resistant to mechanical dissociation than monolayer
- Enzymatic strategies necessary for dissociation



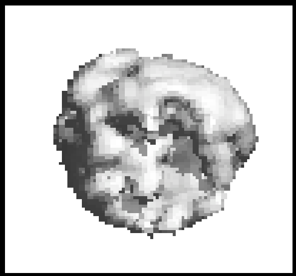
Katz AJ et al. Tissue Engin A 2010

Human Adipose Cells – 3DMA – 50k cells/pellet

Control

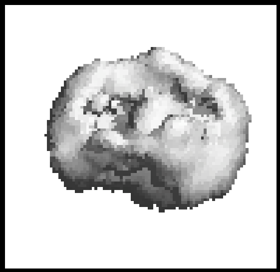


Osteogenic Medium

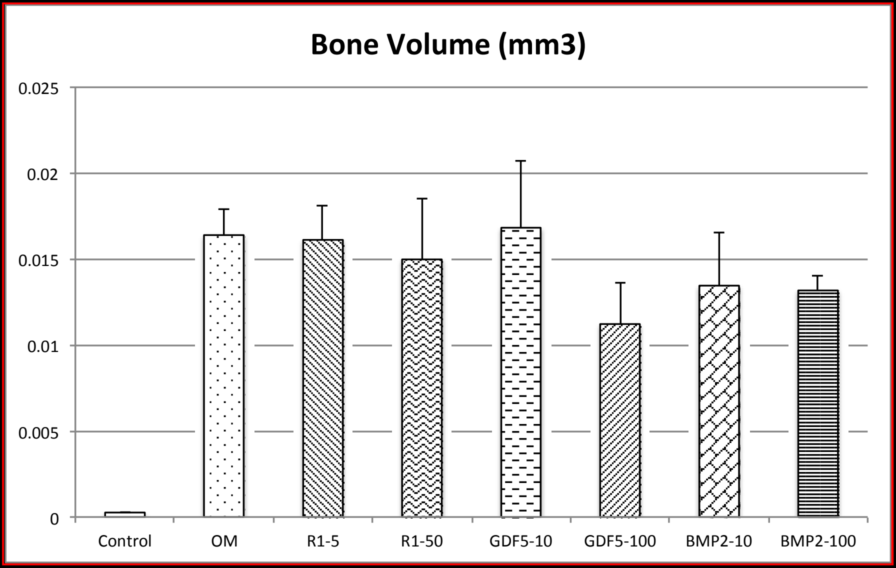


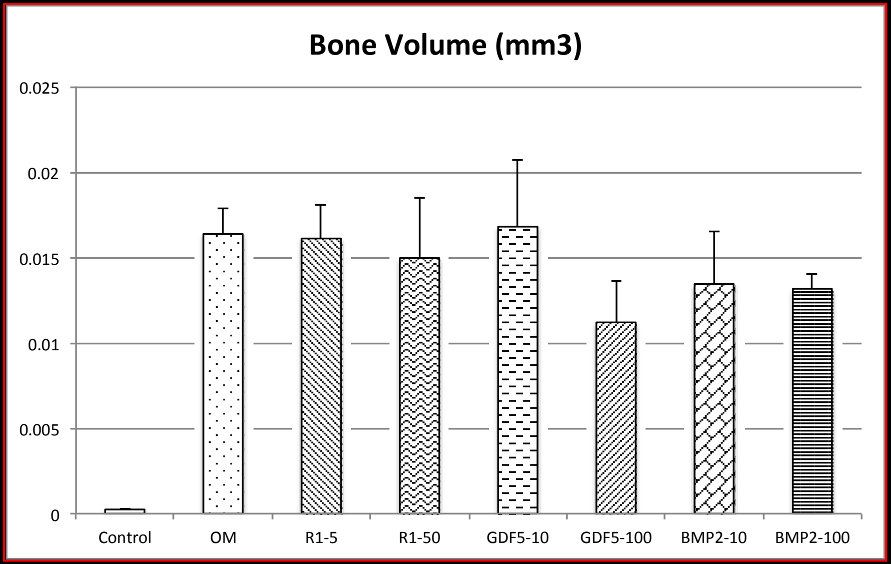
R1 Peptide (50 nM)

GDF-5 (100 nM)

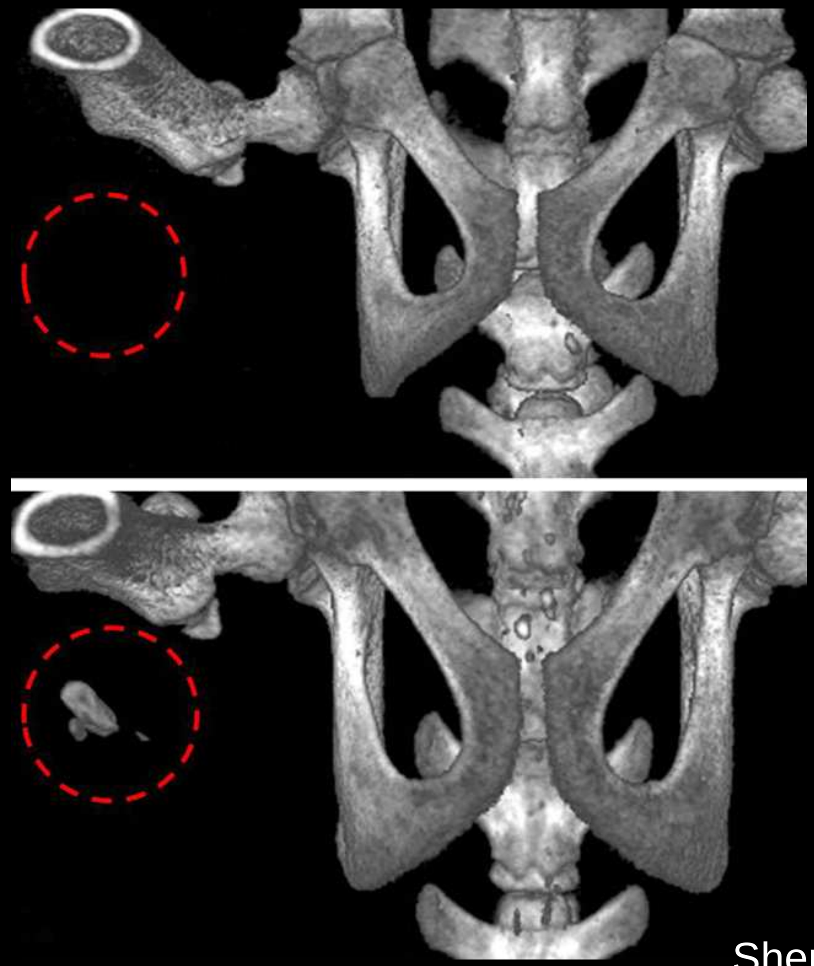


BMP-2 (100 nM)

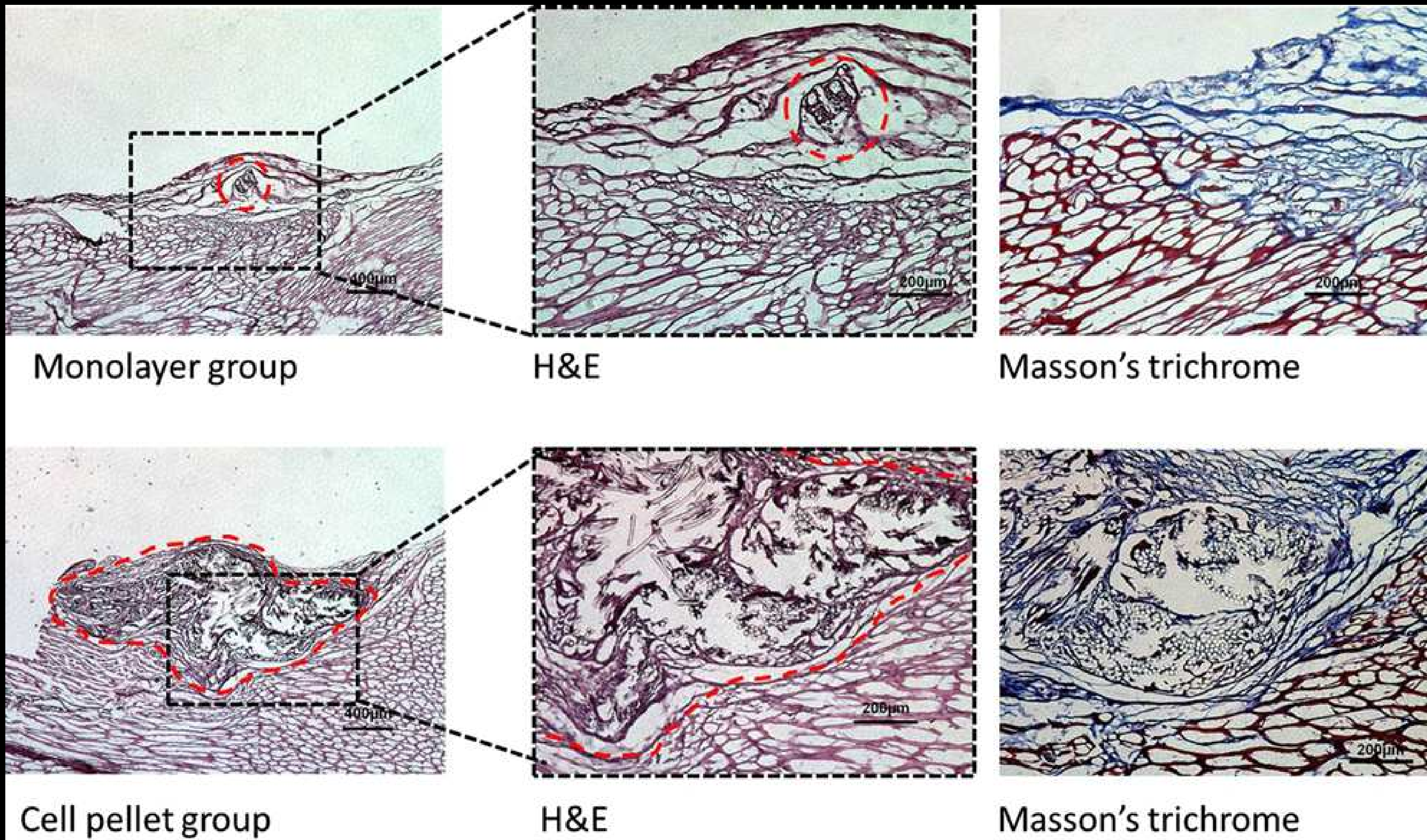




In Vitro Muscle Pouch Study



Shen FH. Spine Journal 2012



Shen FH. Spine Journal 2012

Conclusion

- 3D culture systems have great advantage
- Creates organelle system
  - Advantage for tumor biologists
- MA retain cellular plasticity
  - Osteogenesis
- Has potential role for other specialties
  - Increased handling capabilities
  - Maintains extracellular matrix
  - More easily modeled

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2012 Outstanding Paper: Runner-up

Implications of adipose-derived stromal cells in a 3D culture system for osteogenic differentiation: an in vitro and in vivo investigation

Francis H. Shen, MD[a,*], Brian C. Werner, MD[a], Haixiang Liang, MD[a], Hulan Shang, MS[b], Ning Yang, PhD[b], Xudong Li, MD, PhD[a], Adam L. Shimer, MD[a], Gary Balian, PhD[a,c], Adam J. Katz, MD, FACS[b,d,e]

Future?

- Future is unknown
- Patient population is aging
- Number of primary and revision spine surgeries continues to increase
- Use of biologics alone has not resolved the problem
- We need to continue to have collaborative projects that work to find translational answers for our patients.

Properties of Ideal Graft Material

1. Osteoconductive matrix
   - Scaffold or framework into which bone growth occur

2. Osteoinductive factors
   - Growth factors such as BMP, TGF-B – promote bone formation

3. Osteogenic cells
   - Mesenchymal cells, osteoblasts, and osteocytes

THANK

YOU!