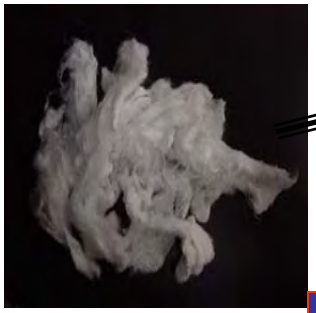
In Vitro Analysis of Cell/Scaffold Medical Products

In vitro Characterization of Hard Tissue Constructs with Structural Role (bone, ligaments, tendons, cartilage)

Kaplan Lab - Tufts University

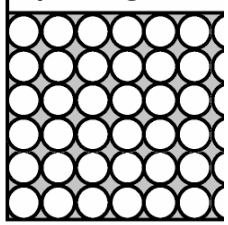
Tissue Engineered Bone
3D Porous Silk Scaffolds

fiber



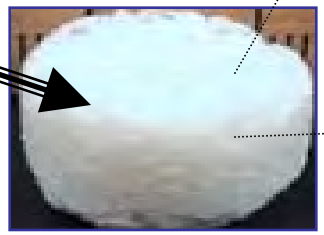
solution

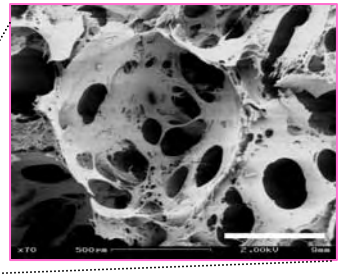
porogen



A. Scaffold Processing

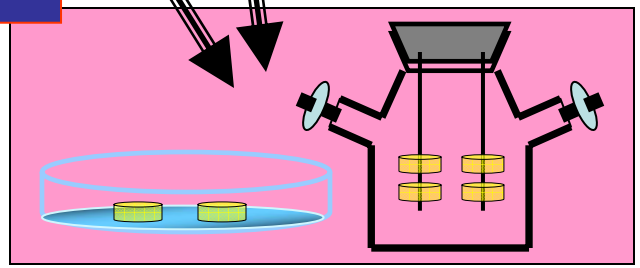
scaffold



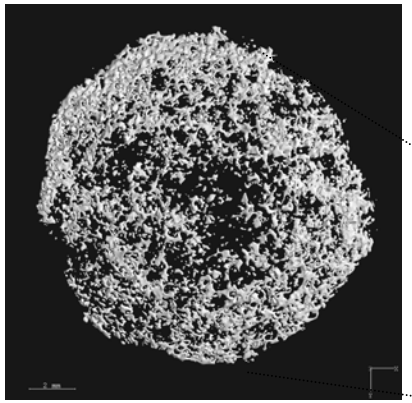


SEM

B. Cell Seeding & Bioreactors, *in vitro*



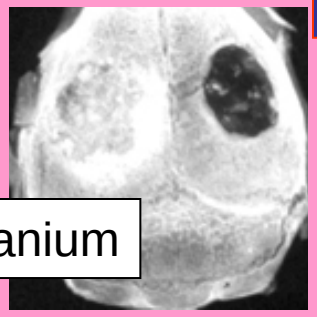
bone formation



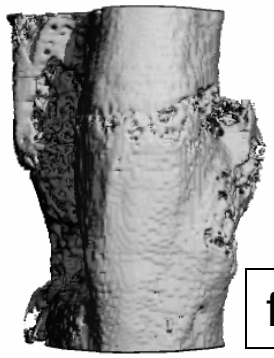
microCT

C. Bone Repairs *In vivo*

cranium

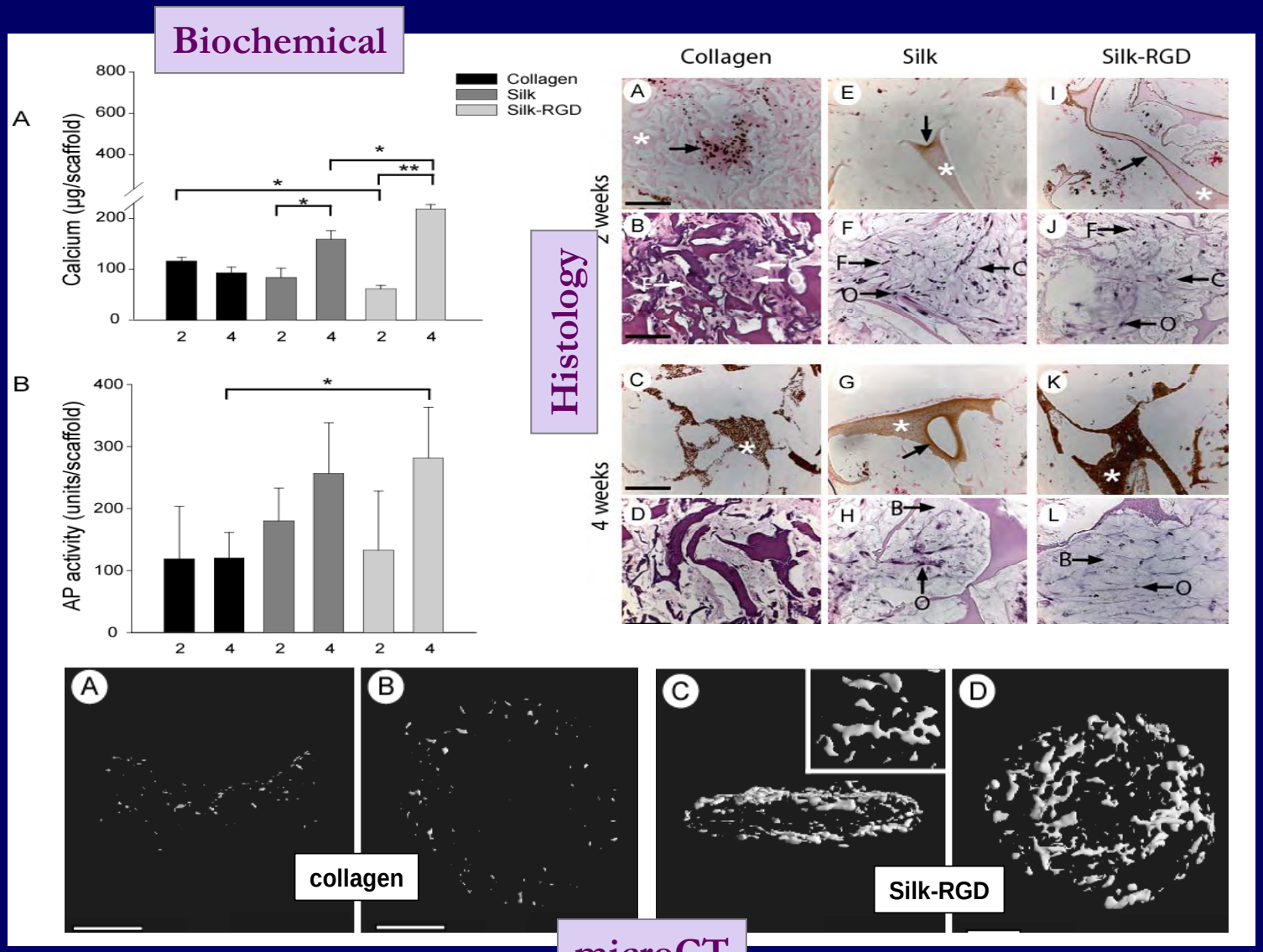


femur



Wang et al., Matls Today, 2007

Bone Formation on 3D Protein Scaffolds



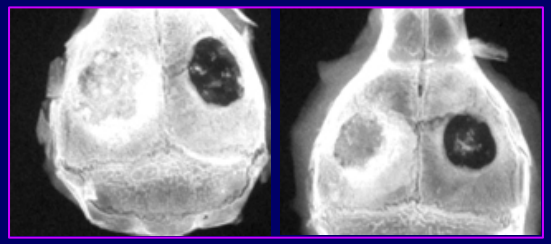
- hMSCs
- 2 & 4 wks
- Static
- In vitro

Histology
Von Kossa – A,C,E,G,I,K
H&E – B,D,F,H,J,L
Calcification
* Polymer
O osteoblast-like cell
F fibroblast-like cell
B collagen
Bars = 70 u

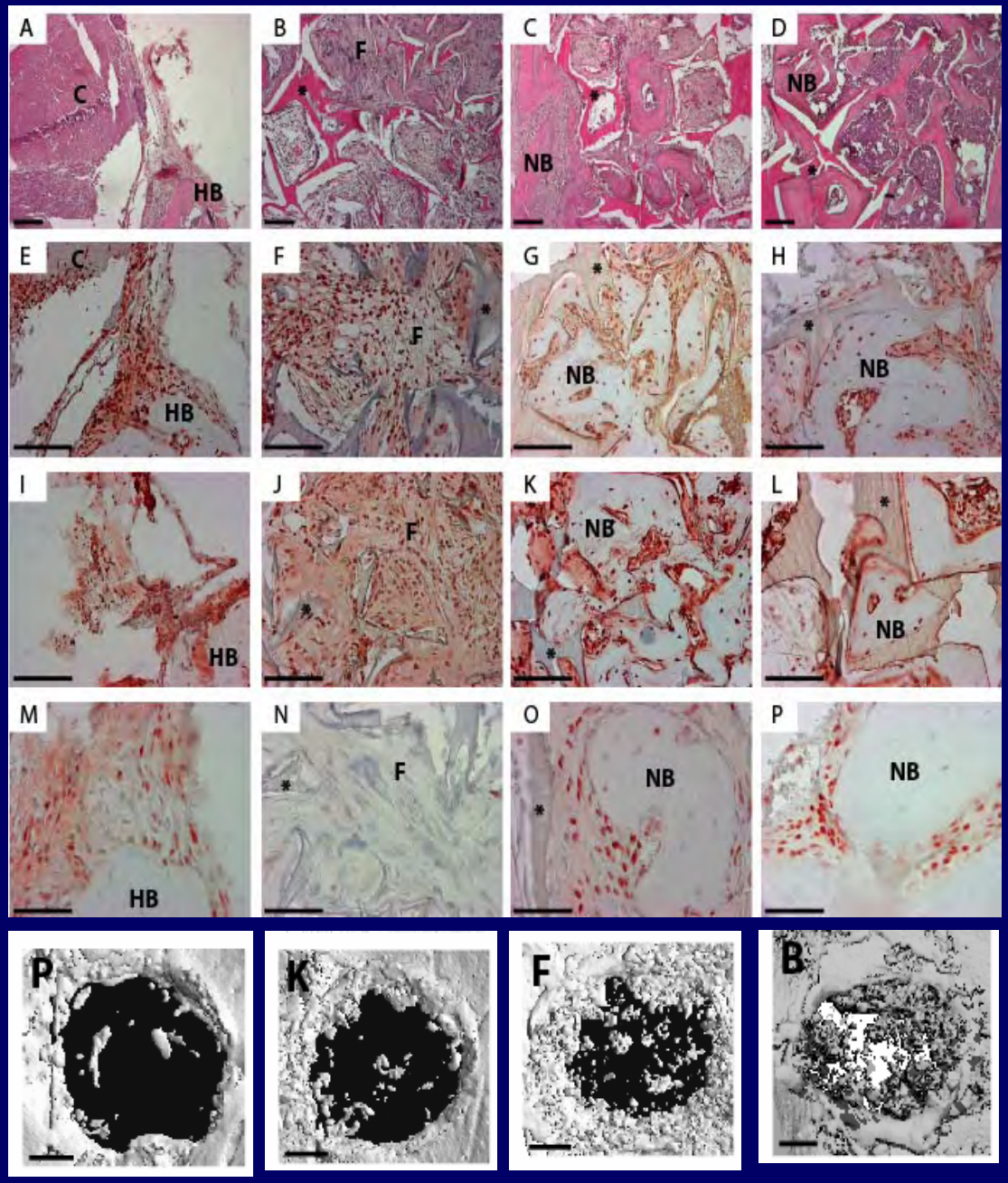
Meinel et al., JMBR, 2004

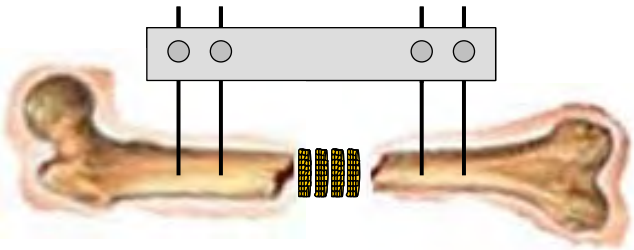
Bone Repair
in vitro & in vivo

- Calvarial defect (4 mm)
- Nude mice
- 4 weeks
- silk scaffolds



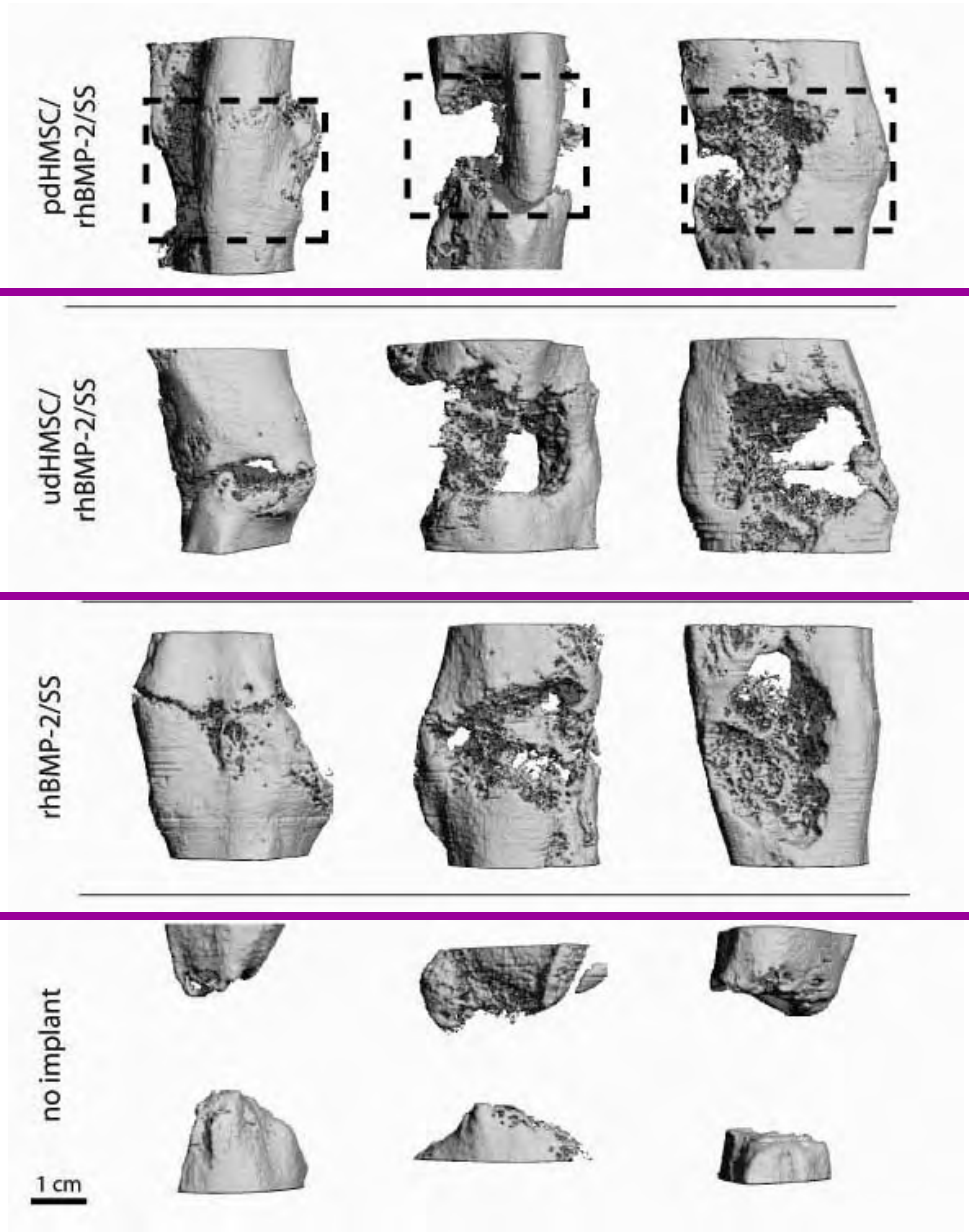
Meinel et al. Bone, 2005





Micro-CT

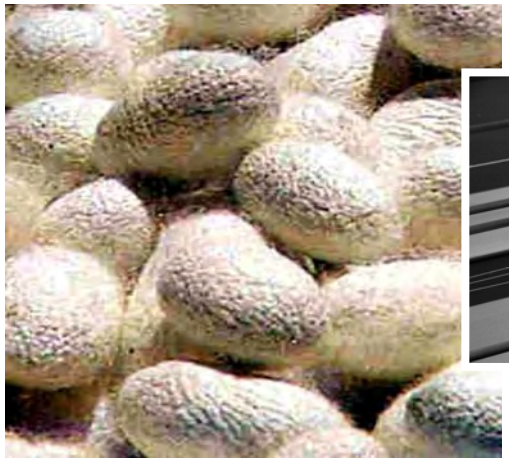
- rat critical size femoral defects (5 mm)
- 8 weeks
- silk scaffolds



Hofmann et al., Bone, 2006; Kirker-Head et al., Bone, 2007

A

Silk cocoons



Silk yarn

Wire ropes

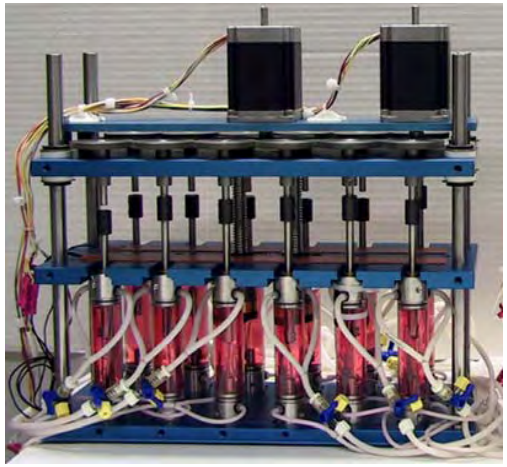
Stem cells

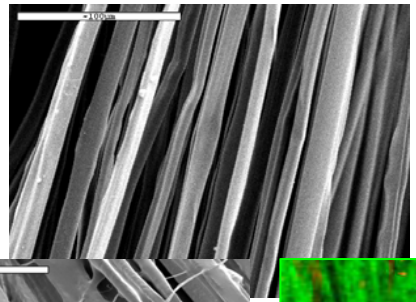
Bioreactor

Fibrous Protein Matrix Engineering

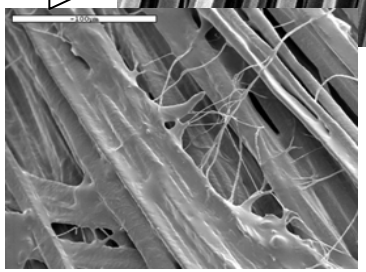
B

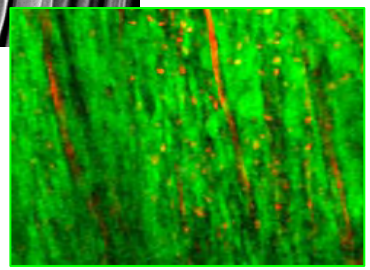
Complex Mechanical Signaling





Ligament Outcomes





Mechanical Forces & Functional Ligament Tissue

Wang et al., Matls Today, 2007

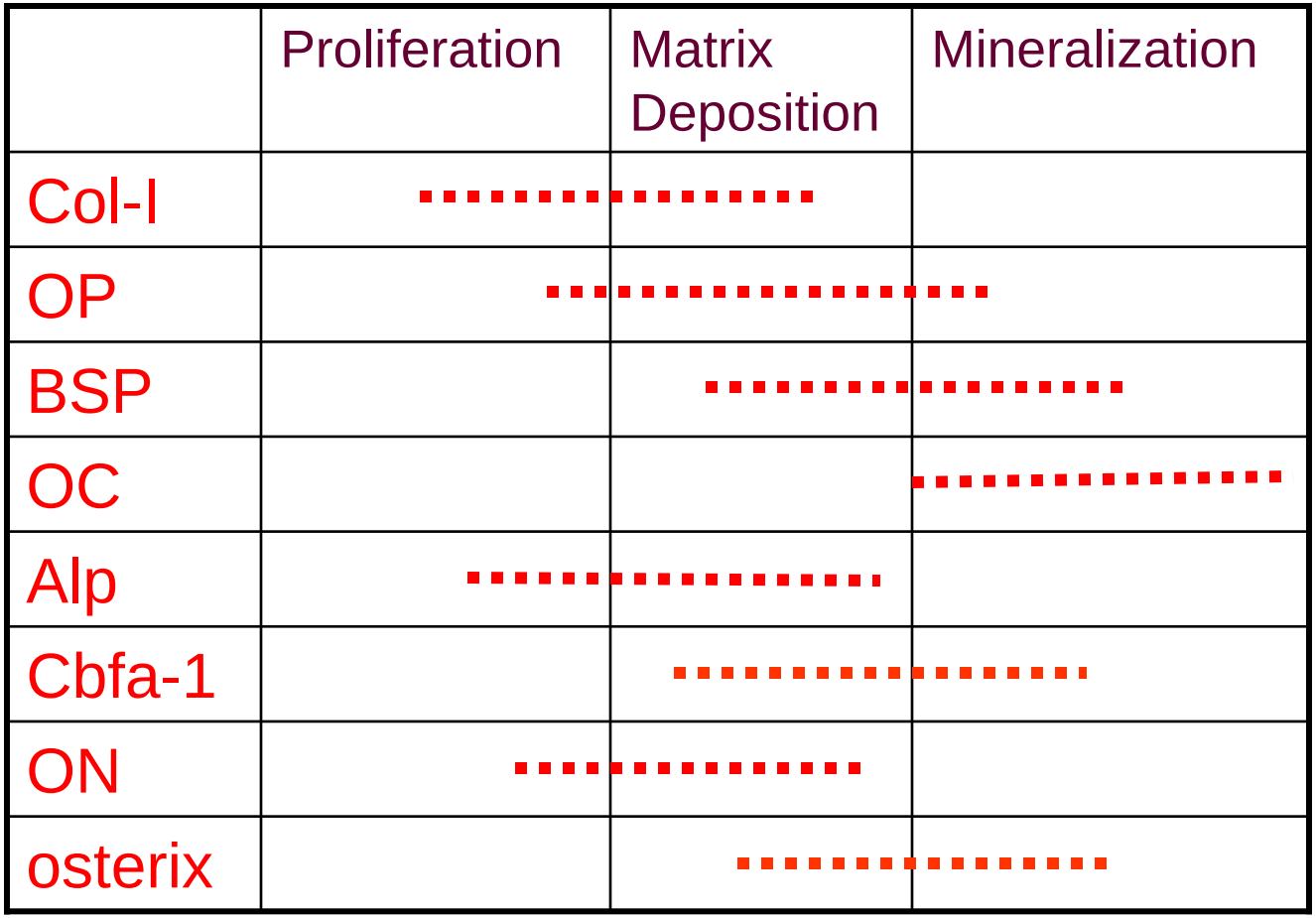
Outcomes – In Vitro

- Biochemistry and Structure - immunohistochemistry and staining - ECM composition, organization, distribution…….
- Genetics - markers for tissue type…….
- Cell Biology - density, types, distribution…….
- Mechanical Properties – tension, compression…….

Challenges (many!)

- Scaffold source material – impact cell signaling, outcomes
- Scaffold features (morphology, structure, chemistry) – different outcomes
- Matching degn rate to tissue remodeling (in vitro vs. in vivo, tissue sp.)
- Cells – immume cells, co-cultures w/ECs…….
- Markers – time-dependent outcomes, when to measure, how often…..
- Cultivation conditions – serum, growth factors (conc., time…….)
- Mechanics – complex forces, shear…….
- Tissue size – transport issues, vascularization in vitro…….

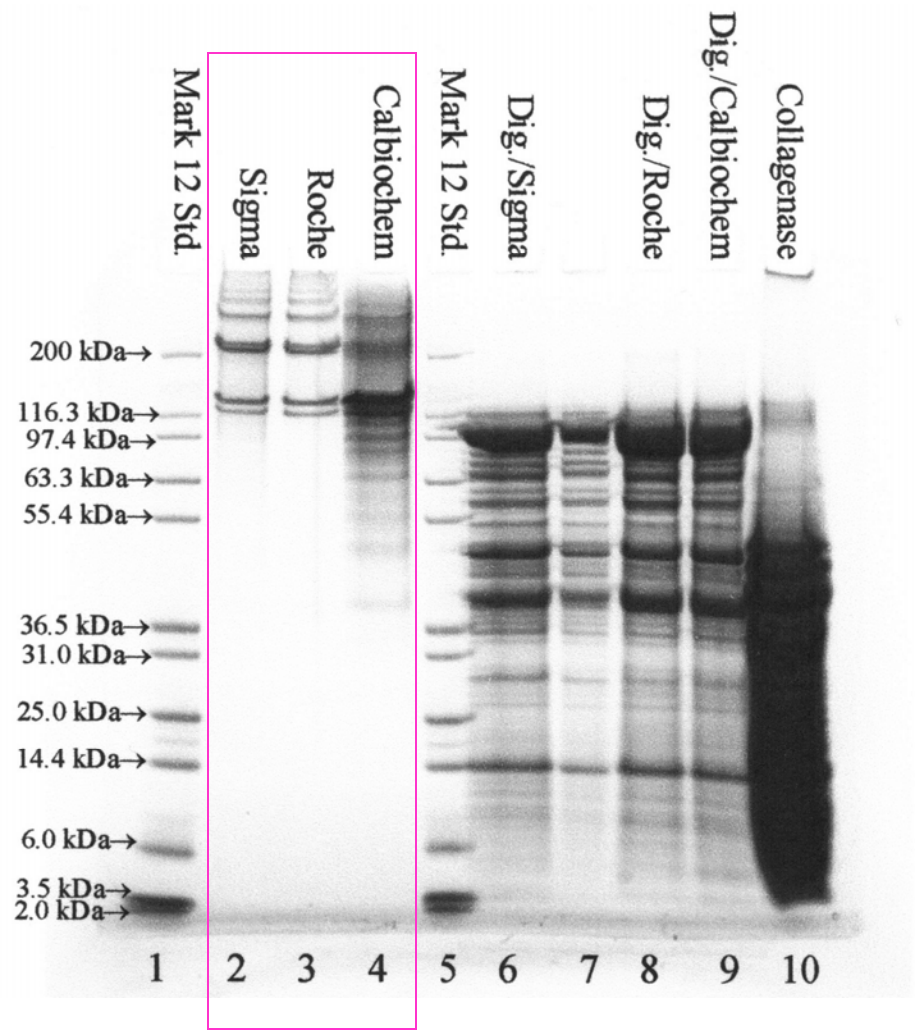
Gene Expression (temporal patterns) During Osteogenic Differentiation



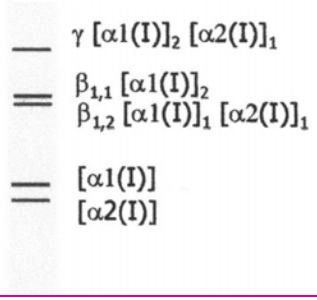
Early - Mid - Late - Stage Markers

Col-1=collagen type I; OP=osteopontin; BSP=bone sialoprotein; OC=osteocalcin; Alp=alkaline phosphatase; Cbfa-1=core binding factor a1

Comparing Commerical Collagen Sources – SDS PAGE



chain locations



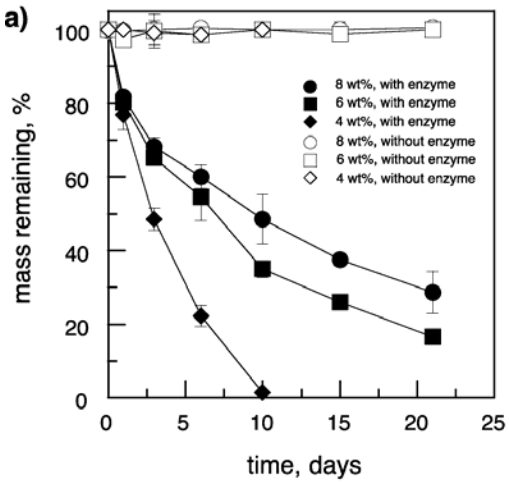
1 - Mark 12 Standards
2 - Sigma Bovine Collagen
3 - Roche Rat Tail Collagen
4 - Calbiochem Human Placental Collagen
5 - Mark 12 Standard
6 - Collagenase Digested Sigma Bovine Collagen
7 - Collagenase Digested Sigma Bovine Collagen
8 - Collagenase Digested Roche Rat Tail Collagen
9 - Collagenase Digested Calbiochem Human Placental Collagen
10 - Collagenase

- Sigma and Roche relatively pure and non-degraded
- Calbiochem digested

Abraham et al., JBMR, 2007

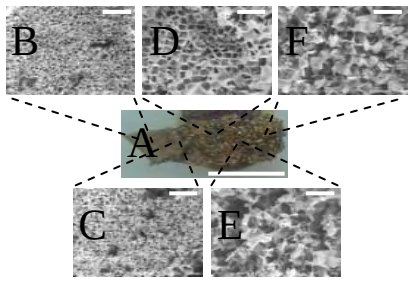
3D Porous Silk Fibroin Matrices - Processing Phase Diagrams

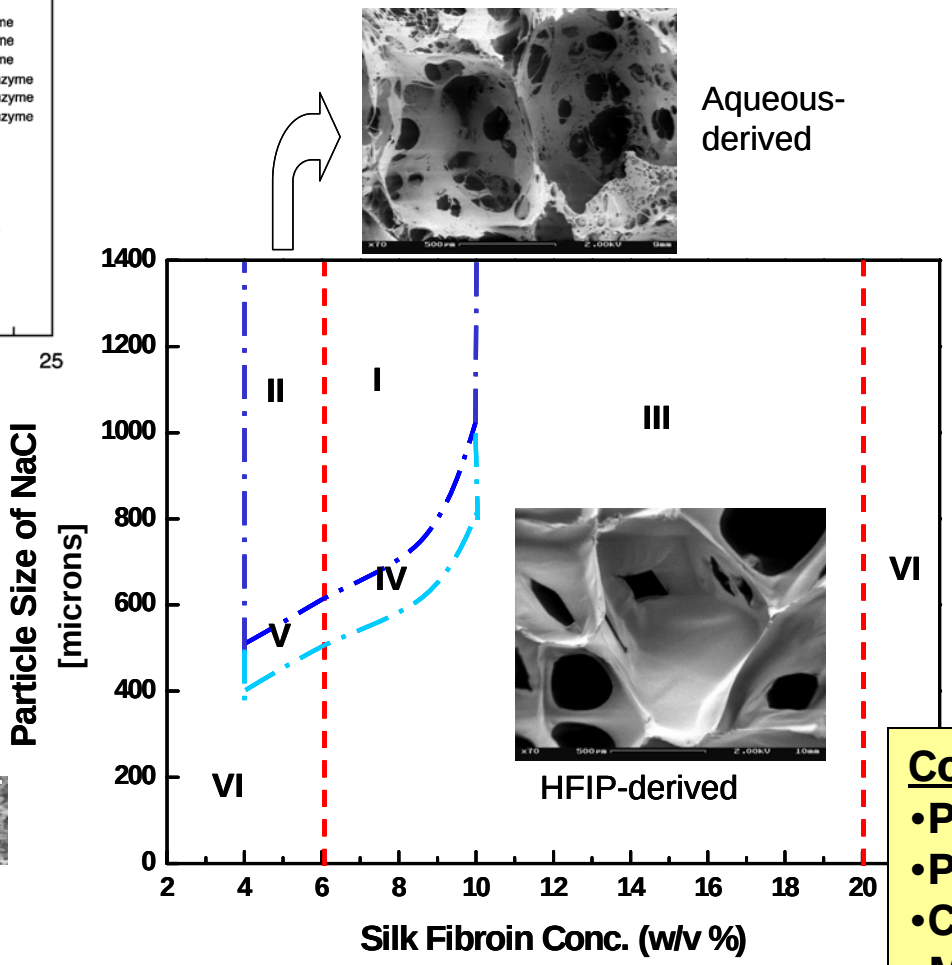
[control of structure & morphology via processing]



degradation

gradients





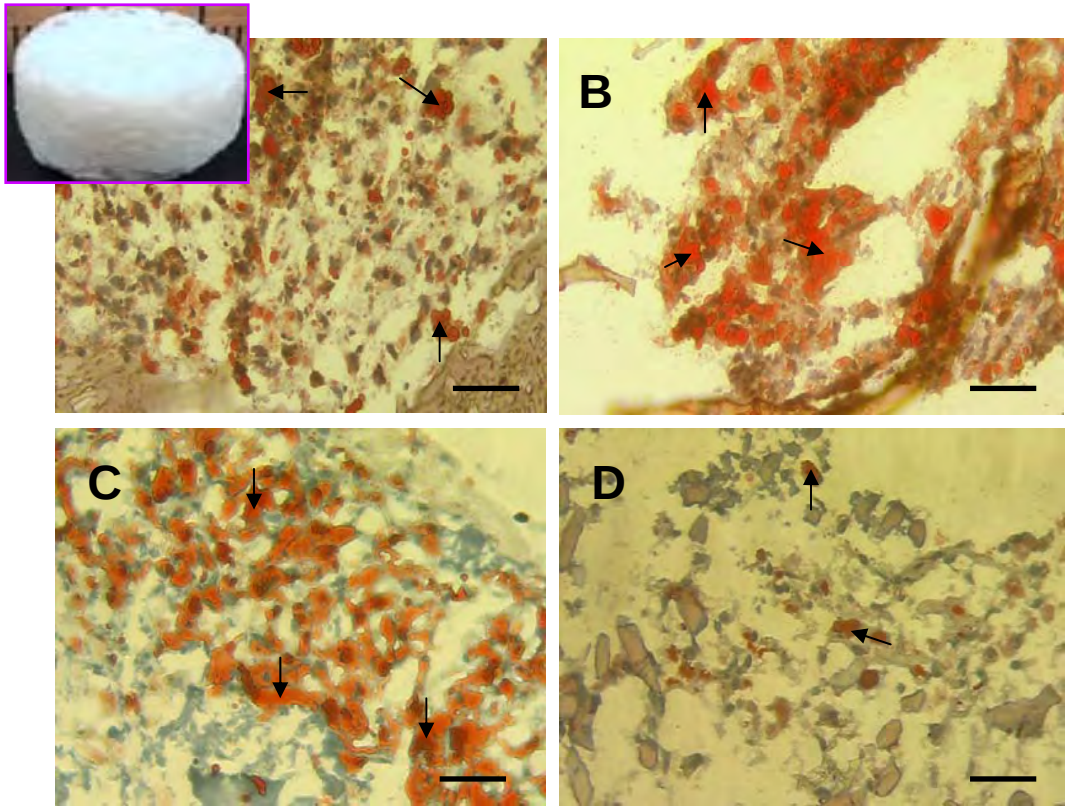
	Aqueous system	HFIP system
I	O	O
II	O	Δ
III	X	O
IV	Δ	O
V	Δ	Δ
VI	X	X

O : homogeneous
Δ : mixed
X : no scaffold

Control Points:

- **Pore size**
- **Porosity**
- **Chemical decoration**
- **Monolithic / gradient**
- **Degradability**

Kim et al., Aust. J. Chemistry, 2005



Soft Tissue Engineering

→hMSCs vs hASCs

→Scaffold type

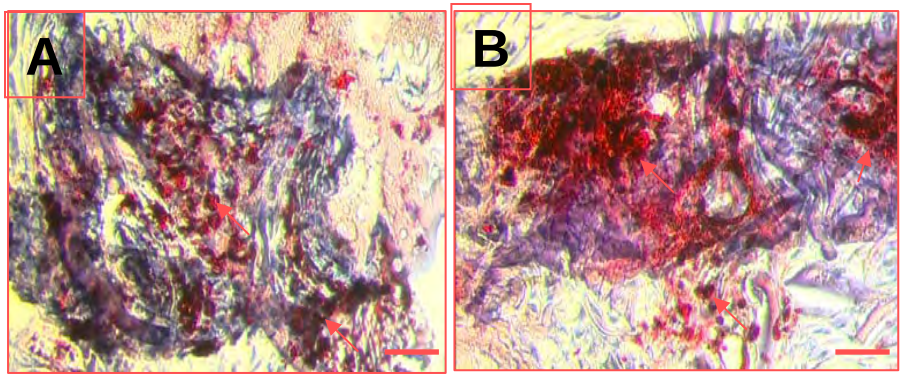
***In vitro* - Oil Red-O - ASC-seeded scaffolds.**
(A) aqueous silk, (B) HFIP silk, (C) collagen, (D) PLA, 21 days.
Scale bar = 50 μm

→silk water-based (AB), silk-HFIP (HF), collagen (COL), poly-lactic acid (PLA), cultivated 21 days before implantation - 4 weeks in mice

→ COL scaffolds and PLA scaffolds were irretrievable

Mauney et al., Biomaterials, 2007

In Vivo Responses



A= hASCs, B= hMSCs

Scale = 50 um

Hard Tissue Constructs with Structural Role

Distinguishing Feature - tissues that transmit mechanical loads during 'normal' activity

General Goal for Treatment Strategies via Tissue Engineering

- **Improve existing treatments (equal/better than current standard of care):**
  - **Faster recovery time**
  - **Better short-term/long-term function (e.g., pain, mechanical support)**
  - **Improved delay in disease progression**
  - **Delay future need for more aggressive options**
  - **Little/no morbidity or side effects**

Additional Criteria

- **Implantable and retained under appropriate mechanical loading conditions**
- **Meet/exceed current 'best' treatment for that tissue (in appropriate animal model)**
- **Viability (cellular) after implantation**
- **Safe**
- **Functionally integrated into/replaced by host tissue**

Butler et al., Evaluation criteria for musculoskeletal and craniofacial tissue engineering constructs: Conference Rpt. in review Tissue Engineering, 2008.

Bone

- Needs - large segmental defects, bone-soft tissue interfaces, spine fusion, fracture nonunions
- Control - autograft or allograft, BMP2/collagen sponge, normal bone
- Outcomes
  (a) restoration of full mechanical function
  (b) integration – morphology (CT, micro-CT), biology (revascularization – histology, osteoclast/osteoblast remodeling)
  (c) physiological (Ca/P by XPS/FTIR), mechanics (torsion, correlation of 3D bone volume/distribution with integration strength)

Intervertebral Disc

- Needs - disc degeneration
- Control – PT, anesthetics, fusion
- Outcomes
  (a) pain free motion
  (b) restoration of physical/biochemical properties – comparison to normal disc and fusion
  (c) structural integrity (MRI, at least 90% of disc ht)
  (d) biochemistry (ECM ratios, cytokine levels), inhibition of innervation and vascularization into the NP)
  (e) biomechanics (initial fixation under functional load, in vitro strength, concentric range of motion, restoration of normal pressure-volume)

Butler et al., Evaluation criteria for musculoskeletal and craniofacial tissue engineering constructs: Conference Rpt. in review, Tissue Engineering, 2008.

Meniscus

→ Needs - repair in avascular zone, partial meniscectomy, premature OA
→ Control - ?
→ Outcomes
(a) structure/morphology (imaging, integration, histology)
(b) biochemistry
(c) mechanics (contact pressure, extrusion under compression)
(d) articular surface (histology, biochemical, mechanical)

ACL

→ Needs - traumatic rupture
→ Control - autologous patelar tendon/hamstring tendon, allograft tissues
→Outcomes
(a) Mechanical (limp, activity monitoring, joint motion, joint laxity vs. time, stiffness and failure from load-displacement tests)
(b) biological (gross inspection of cartilage, synovium, effusion), microscopic examination of bone-ligament interface, inflammatory cells, vascularization, 3,6,12 mo post surgery

Butler et al., Evaluation criteria for musculoskeletal and craniofacial tissue engineering constructs: Conference Rpt. in review, Tissue Engineering, 2008.

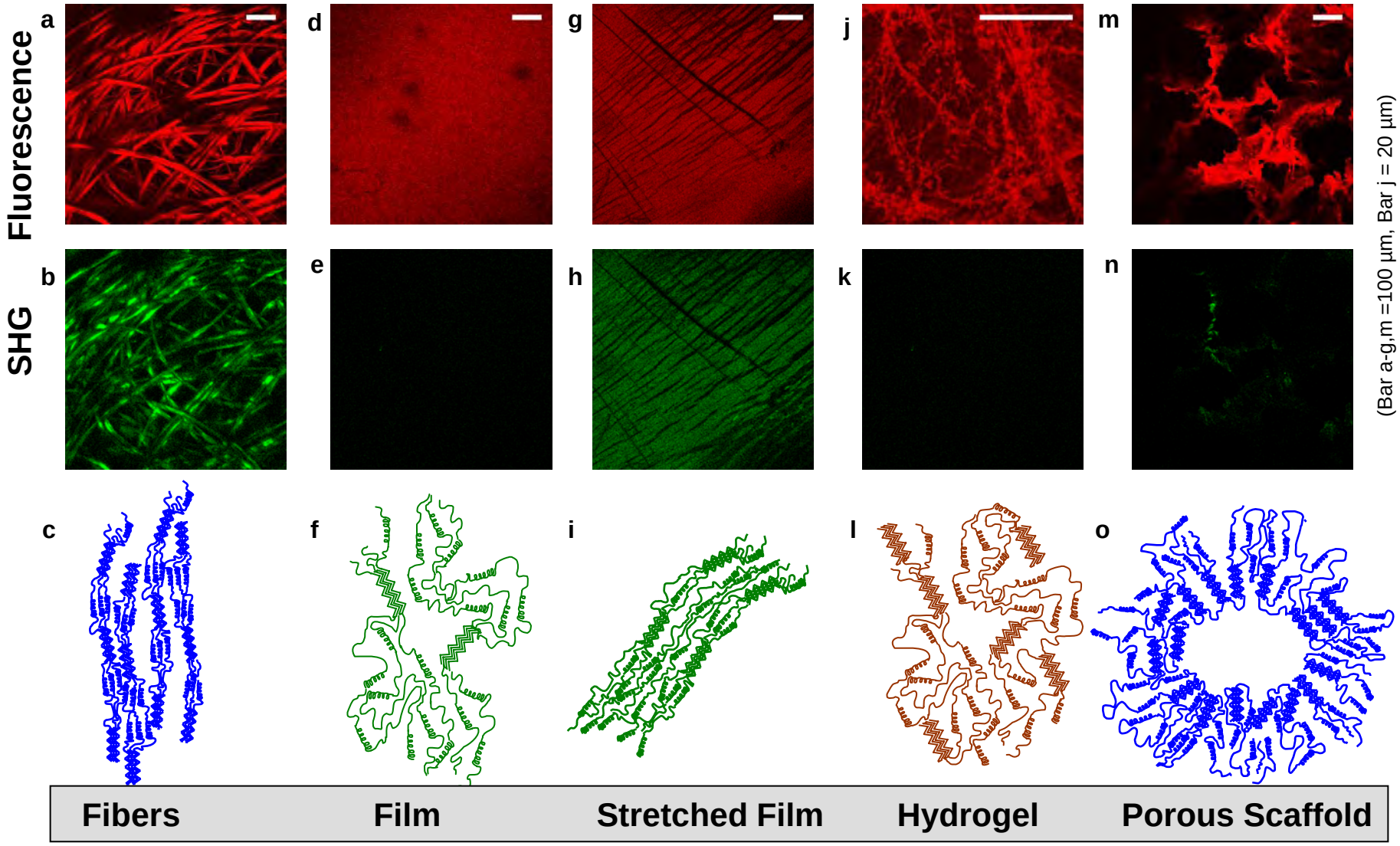
Specific Research Needs to Support Clinical Goals

- validated animal models (normal, disease, repair, maturity/dev't)
- in vitro indicators of long-term in vivo outcomes
- quantitative behavior measures of pain in large animals
- non-invasive assessments (imaging)
- functional assessment measures
- rehabilitation programs
- biomimetic systems as predictors of in vivo (pre-clinical) outcomes (acute and chronic), disease, nutrition, development/regeneration

Butler et al., Evaluation criteria for musculoskeletal and craniofacial tissue engineering constructs: Conference Rpt. in review, Tissue Engineering, 2008.

Imaging – Silk Biomaterials (w/ I. Georgakoudi)

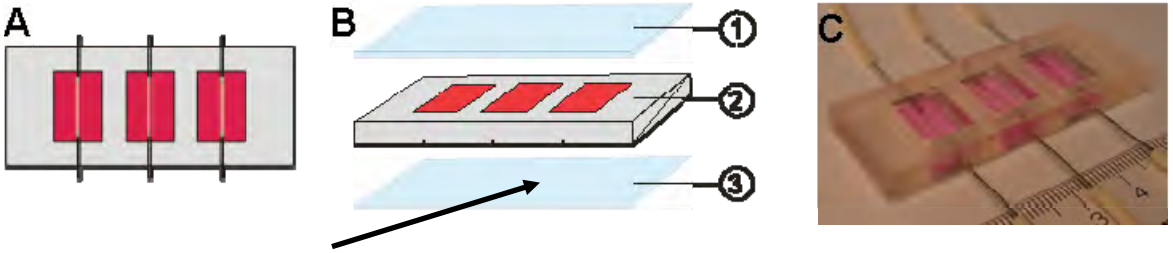
Two Photon Excited Fluorescence & Second Harmonic Generation



- 800 nm excitation, 20x (0.7NA) objective
- Fluorescence collected through 525 nm filter with a 25nm band pass
- SHG collected in forward direction through 410 nm filter with a 20nm band pass.

Rice et al., 2007

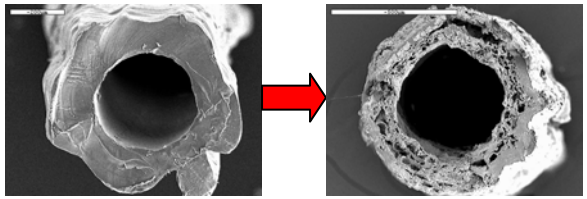
Single-Channel Vascular Diffusion System



cover glass bottom allows for real-time imaging

Three bioreactors –
1 cm x 1.5 cm x 0.5 cm

Perfused by needles spanned by silk microtubes (500 μm ID)



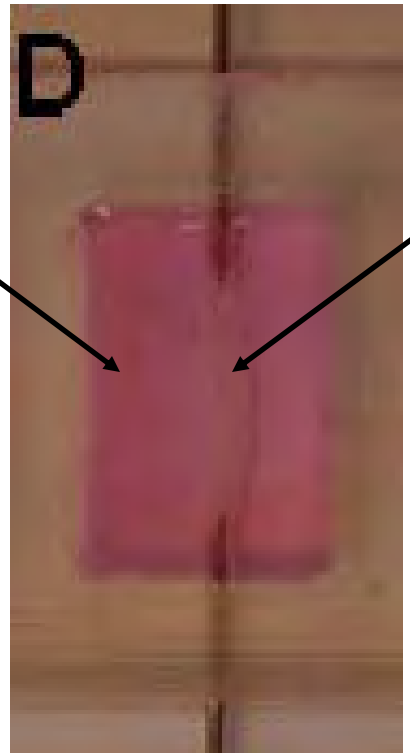
100% silk **80% silk/20% PEO**

Cell-seeded hydrogel

collagen I

hMSCs

growth factors



Vascular-like perfusion

silk microtube

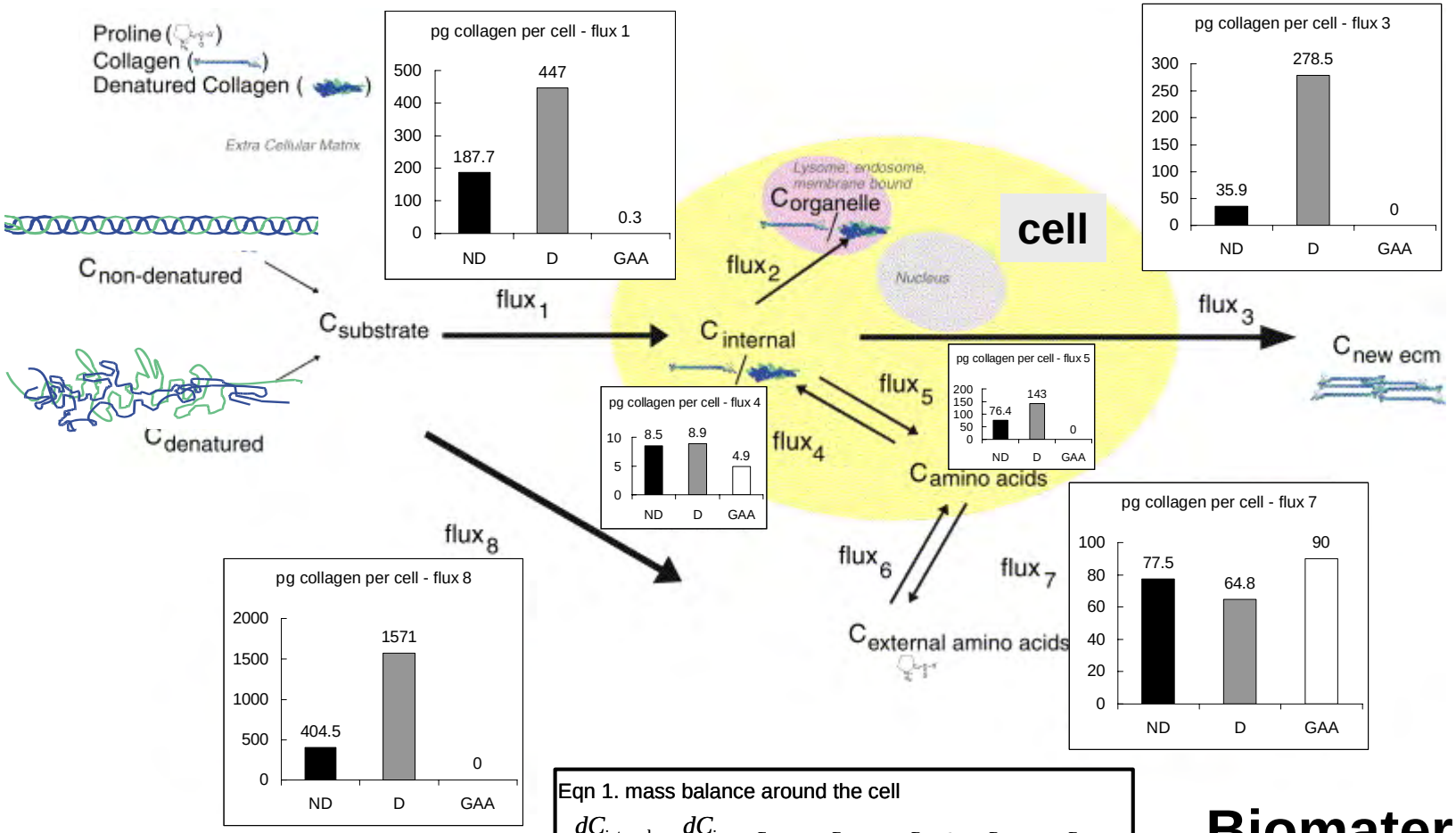
endothelial cells

controllable porosity

Control of specific parameters

Ability to measure and model oxygen diffusion

Lovett et al., Biomaterials 2007



ND = native collagen

D = denatured collagen

GAA = control

Abraham et al., Biomaterials, 2007 & Expt. Cell Res, 2007

Eqn 1. mass balance around the cell

$$\frac{dC_{\text{internal}}}{dt} = \frac{dC_{\text{i}}}{dt} = flux1 - flux2 - flux3 - flux4 + flux5$$

Eqn 2. mass balance around the organelles

$$\frac{dC_{\text{organelle}}}{dt} = \frac{dC_{\text{o}}}{dt} = flux2$$

Eqn 3. mass balance around the internal pool of amino acids

$$\frac{dC_{\text{Amino Acids}}}{dt} = \frac{dC_{\text{AA}}}{dt} = flux5 + flux6 - flux4 - flux7$$

Eqn 4. kinetics of new ECM production

$$if \ \frac{dC_{\text{i}}}{dt} \neq 0, \qquad flux3 = \text{rate of } C_{in} \text{ to } C_{newECM} = kC_{in}^{\ n}$$

Biomaterial Matrix Remodeling

Quantitative Flux analysis