Massachusetts Institute of Technology
Harvard Medical School
Brigham and Women's Hospital
VA Boston Healthcare System

2.79J/3.96J/20.441/HST522J

REGENERATION OF JOINT TISSUES
Bone

M. Spector, Ph.D.

CONFLICT OF INTEREST STATEMENT

Prof Spector derives royalty income from certain products referred to as Bio-Oss, from Geistlich Pharma (Wolhusen, Switzerland).

TISSUES COMPRISING JOINTS

	Permanent Prosthesis	Regeneration Scaffold
Bone	Yes	Yes
Articular cartilage	No	Yes*
Meniscus	No	Yes*
Ligaments	No	Yes*
Synovium	No	No

* In the process of being developed

TYPES OF TISSUES
Which Tissues Can Regenerate Spontaneously?

	Yes	No
Connective Tissues		
• Bone	√	
• Articular Cartilage, Ligament, Intervertebral Disc, Others		√
Epithelia (e.g., epidermis)	√	
Muscle		
• Cardiac, Skeletal		√
• Smooth	√	
Nerve		√

FACTORS THAT CAN PREVENT REGENERATION

- Size of defect
  - *e.g.*, bone does not regenerate in large defects
  - Solution: fill defect with osteoconductive particles that adapt to the cavity or a form-filling absorbable "cement"
- Collapse of surrounding tissue into the defect
  - *e.g.*, periodontal defects
  - Solution: membranes for guided tissue regeneration (GTR)
- Excessive strains in the reparative tissue
  - *e.g.*, unstable fractures
  - Solution: fracture fixation apparatus
- Disease

ELEMENTS OF TISSUE ENGINEERING/ REGENERATIVE MEDICINE

- SCAFFOLD
  - Porous, absorbable synthetic (*e.g*., polyglycolic acid) and natural (*e.g*., collagen) biomaterials
- CELLS (Autologous or Allogeneic)
  - Differentiated cells of same type as tissue
  - Stem cells (*e.g*., bone marrow-derived)
  - Other cell types (*e.g*., dermal cells)
- REGULATORS
  - Growth factors or their genes
  - Mechanical loading
  - Static versus dynamic culture ("bioreactor")

* Used individually or in combination, but often with a scaffold)

CASE STUDY

Problem

- 56-year-old man received ablative tumor surgery 8 years previously in the form of a subtotal mandibulectomy.
- 7 cm had been bridged with a titanium reconstruction plate since initial surgery.
- Head and neck region had been further compromised by radiation treatment.
- Because he had been given Warfarin for an aortic valve replacement bony defects had to be kept to a minimum to avoid major postoperative bleeding.

PH Warmke, *et al.*, Lancet 364:766 (2004)

Image of patient's skull and mandible implant removed due to copyright restrictions.

How to regenerate the mandible?

- **Wound healing compromised by radiation treatment**
- **Limited blood supply to the area due to radiation treatment**
- **Inability to harvest bone for grafting, due to Warfarin treatment**

Image of patient's skull and mandible implant removed due to copyright restrictions.

Scaffold ?

Cells ?

Regulators ?

How to regenerate the mandible?

- **Wound healing compromised by radiation treatment**
- **Inability to harvest bone for grafting**
- **Limited blood supply to the area**

CASE STUDY

Solution

- Grow a subtotal replacement mandible inside the latissimus muscle with full bony continuity.
- Provide an adequate vascular network to allow for subsequent transplantation of a viable graft into the defect.
- Ensure that the replacement is shaped to the defect, thus improving the chances of adequate postoperative function and a satisfactory esthetic result.

PH Warmke, *et al.*, Lancet 364:766 (2004)

CASE STUDY

Methodology

- 3D CT of the patient's head to design a virtual replacement of the missing part of the mandible with computer-aided design.
- A titanium mesh scaffold was then formed onto the model, which was subsequently removed.
- The titanium mesh cage was filled with ten bone mineral blocks which were coated with 7 mg recombinant human BMP-7 embedded in 1 g bovine type 1 collagen.
- 20 mL bone marrow was aspirated from the right iliac crest to provide undifferentiated precursor cells as a target for recombinant human BMP-7.
- Bone marrow was mixed with 5 g natural bone mineral of bovine origin (particle size 0·5–1·0 mm) and this mixture was used to fill the gaps among the blocks inside the cage.
- The titanium mesh cage was then implanted into a pouch of the patient's right latissimus dorsi muscle.

CASE STUDY

Methodology

- 7 weeks postop, transplantation of the mandibular replacement.
- The replacement was harvested along with an adjoining part of the latissimus dorsi muscle containing the thoracodorsal artery and vein that had supplied blood for the entire transplant.
- This pedicled bone-muscle flap was then transplanted into the defect site via an extraoral approach.
- Minor bone overgrowth on the ends of the replacement was curetted to fit the transplant easily into the defect.
- After the old titanium reconstruction plate was removed, the mandibular transplant was fixed onto the original mandible stumps with titanium screws, returning the contour of the patient’s jaw line to roughly that present before the mandibulectomy.
- The vessel pedicle was then anastomosed onto the external carotid artery and cephalic vein by microsurgical techniques.

Lancet 2004; 364: 766–70

Several slides containing images from the Lancet paper removed due to copyright restrictions.

INCUBATION OF TISSUE ENGINEERING CONSTRUCTS IN ECTOPIC SITES

- Allows for implantation of a mature, functional tissue engineered implant immediately upon excision of the lesion/tumor
  - Use of autologous cells
- Allows for development of the construct in an *in vivo* (autologous) environment
  - Exposed to host cells and regulatory molecules
  - Not exposed to mechanical loading during development
  - Development can be monitored
  - At the appropriate stage of development the vascularized construct can be transplanted to the target defect

Slide content removed due to copyright restrictions.
Text and images describing INFUSE® Bone Graft, a recombinant human bone morphogenetic protein (rhBMP-2) in an absorbable collagen sponge.

www.sofamordanek.com

ROLES OF THE BIOMATERIALS/ SCAFFOLDS (MATRICES)

1) the scaffold serves as a framework to support cell migration into the defect from surrounding tissues; especially important when a fibrin clot is absent.
2) serves as a delivery vehicle for exogenous cells, growth factors, and genes; large surface area.
3) before it is absorbed a scaffold can serve as a matrix for cell adhesion to facilitate/"regulate" certain unit cell processes (*e.g.*, mitosis, synthesis, migration) of cells *in vivo* or for cells seeded *in vitro*.
    a) the biomaterial may have ligands for cell receptors (integrins)
    b) the biomaterial may selectively adsorb adhesion proteins to which cells can bind
4) may structurally reinforce the defect to maintain the shape of the defect and prevent distortion of surrounding tissue.
5) serves as a barrier to prevent the infiltration of surrounding tissue that may impede the process of regeneration.

SCAFFOLDS: PRINCIPLES

- Chemical Composition
- Pore Structure/ Architecture
- Degradation Rate
- Mechanical Properties

SCAFFOLDS: PRINCIPLES

Mechanical Properties

- Strength
  - high enough to resist fragmentation before the cells synthesize their own extracellular matrix.
- Modulus of elasticity (stiffness)
  - high enough to resist compressive forces that would collapse the pores.
  - transmit stress (strain) in the physiological range to surrounding tissues; prevent concentrated loading and “stress shielding.”

Composition

- For synthetic polymers; blending polymers with different mechanical properties and by absorbable reinforcing fibers and particles.
- For natural polymers (*viz.*, collagen) by cross-linking and reinforcing with mineral (or by mineralization processes).
- Use of absorbable calcium phosphate materials, including natural bone mineral.

COMPRESSIVE PROPERTIES

	Ultimate Comp. Str. (MPa)	Modulus of Elasticity (GPa)
Cortical Bone	140 - 200	14 - 20
Cancellous Bone	5 - 60	0.7 - 1.5
Synthetic HA*	200 - 900	34 - 100
Bone Mineral	25	6

(anorganic bone)

* Hydroxyapatite

SCAFFOLD (MATRIX) MATERIALS
Calcium Compounds

- Natural
  - Bone mineral (treated bone; xenogeneic)
- Synthetic
  - Hydroxyapatite
  - Calcium carbonate
  - Calcium phosphate
  - Calcium sulfate
  - Others

BONE GRAFTS AND GRAFT SUBSTITUTES
(Scaffolds for Bone Tissue Engineering)

Bone	Components of Bone	Calcium Phosphate Ceramics
Autograft Allograft* Xenograft	Mineral Alone (Anorganic Bone) or Organic Matrix Alone (Demineralized Bone)	Hydroxyapatite (Including Sintered Bone) Tricalcium Phosphate **Other Calcium Compounds** Calcium Sulfate Calcium Carbonate

* Works well; potential problems of transmission of disease and low grade immune reaction

BONE MINERAL VERSUS SYNTHETIC HYDROXYAPATITE

	Bone Mineral	Synthetic Calcium Phosphates
Chemical	Calcium-deficient carbonate apatite and other calcium phosphate phases	Hydroxyapatite Whitlockite (TCP)
Crystalline	Small crystalline size; noncrystalline phase	Large crystallites; high crystallinity
Mechanical	Lower strength; lower modulus	Dense; higher strength; higher modulus

DE-ORGANIFIED BOVINE TRABECULAR BONE

Natural Bone Mineral

Image removed due to copyright restrictions.
Millimeter-structure view of bone.

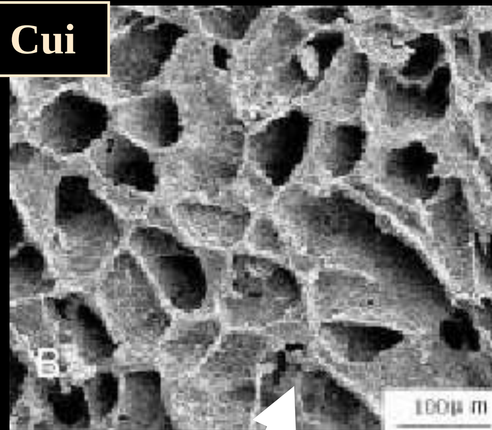
Scaffold Structures

Langer and Freed

Image removed due to copyright restrictions.

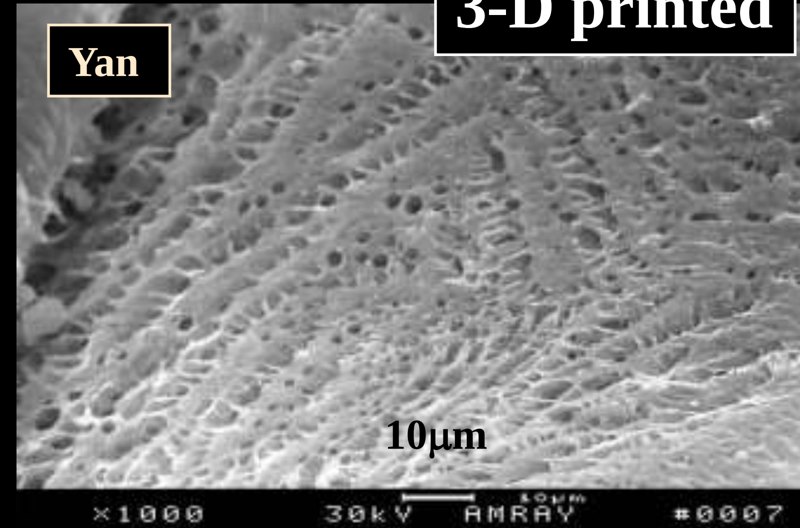
Fiber mesh; PLA-PGA

Cui



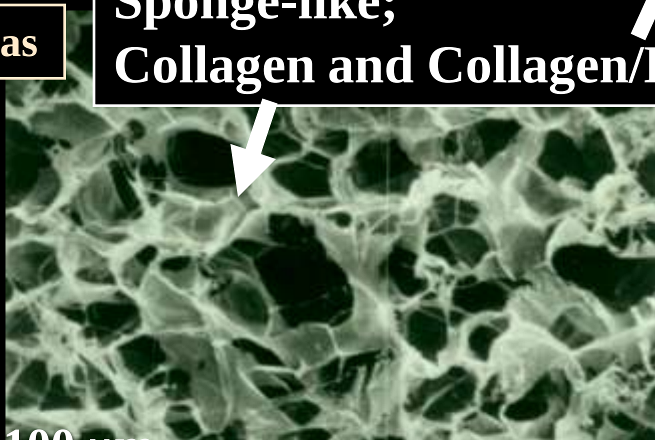
3-D printed

Yan



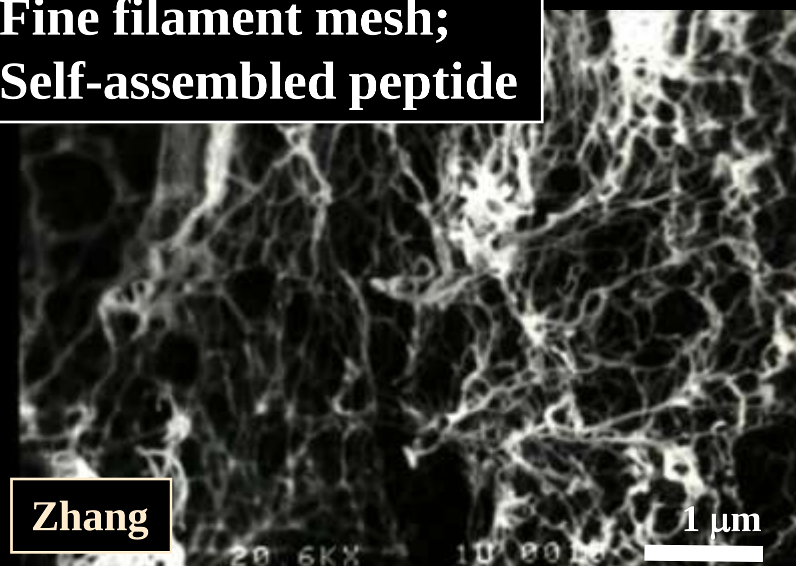
Yannas

Sponge-like; Collagen and Collagen/HA



Fine filament mesh; Self-assembled peptide

Zhang



BIOMATERIALS FOR BONE TISSUE ENGINEERING

Biomimetics

Synthesize scaffold materials using principles and processes underlying biomineralization.

Biomineralized Materials as Biomaterial Scaffolds

Use biomineralized structures as they naturally occur or after treatments for modification.

Cortical Bone (compact bone)

Bone

Photo removed due to copyright restrictions. Bone structure.

Image removed due to copyright restrictions. Medical illustration of bone structure

Photo removed due to copyright restrictions. Bone structure.

Cancellous Bone (spongiosa; trabecular bone)

Trabecular Bone; Scanning Electron Micrographs

Osteoblast

Trabecula covered by osteoblasts

Photos removed due to copyright restrictions.

Orthop. Basic Sci. AAOS, 2000

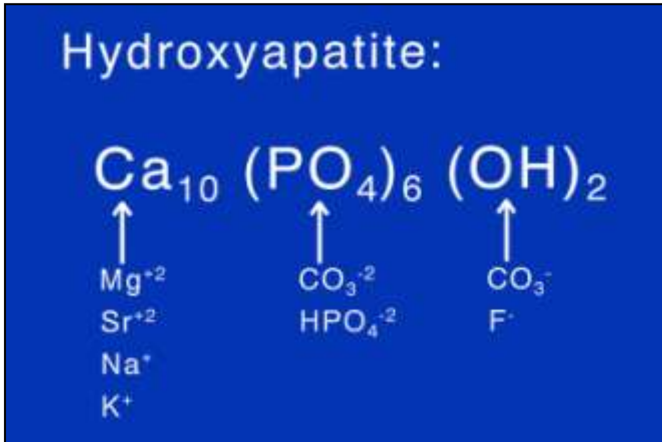
Mineralization of Collagen in Bone

Collagen Molecule

Diagrams removed due to copyright restrictions.

Collagen Fibril

How do the crystallites bond to one another?



Lee DD and Glimcher M, J. Mol. Bio. 217:487, 1991
Lee DD and Glimcher M,. Conn. Tiss. Res. 21:247, 1989

Image removed due to copyright restrictions.

TEM of Unstained Sections of Bone

Image removed due to copyright restrictions.

M. Spector, J Microscopy 1975;103:55

Transmission Electron Microscopy; unstained sections

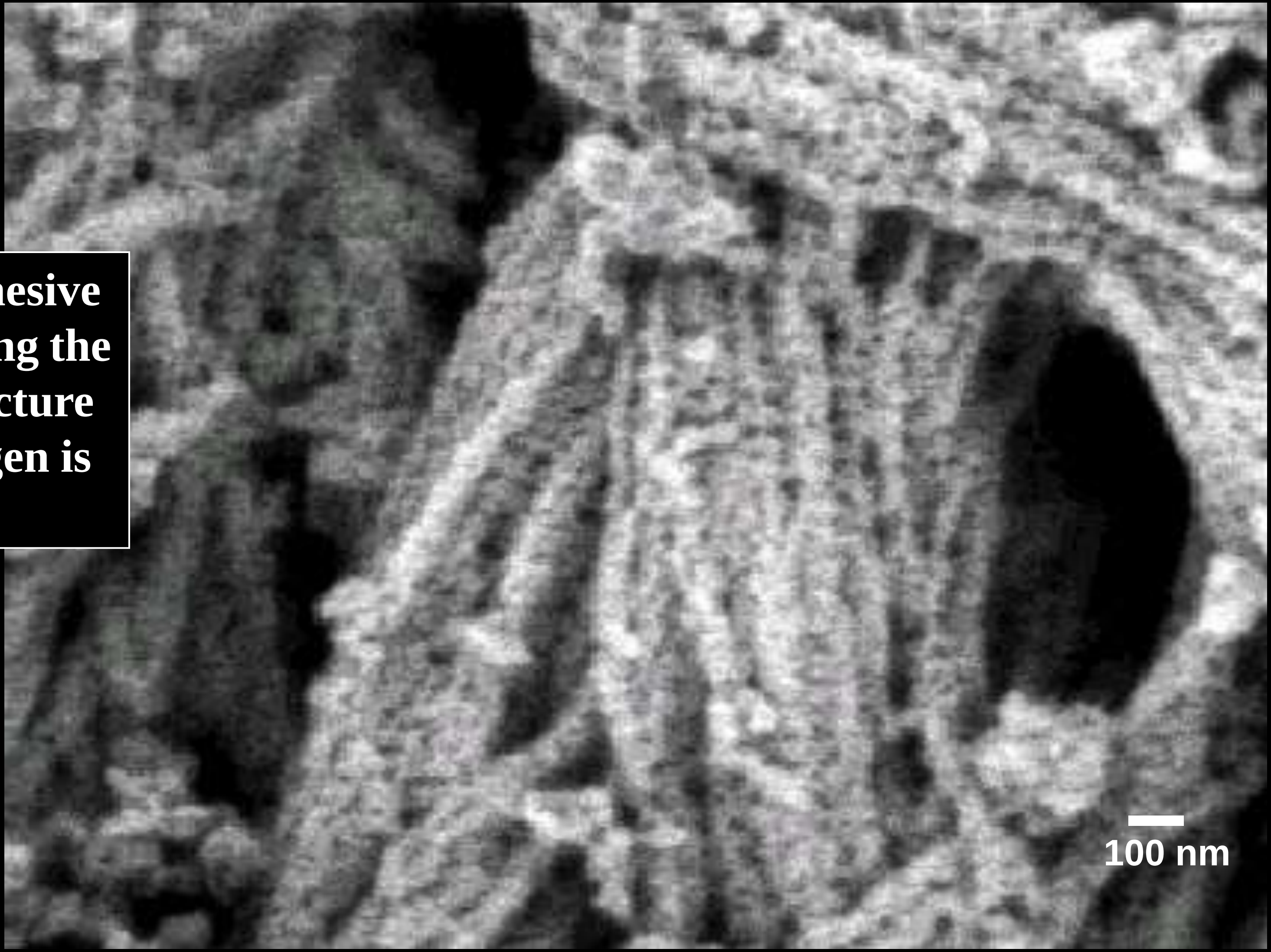
Two images removed due to copyright restrictions.
See Fig 4b and c in Benezra Rosen, V., et al. *Biomat. 23:921 (2002).*

- **Bovine bone from which all the organic matter was removed; anorganic bovine bone; Bio Oss.**
- **The crystalline architecture is retained even after removing the organic (collagen) template.**

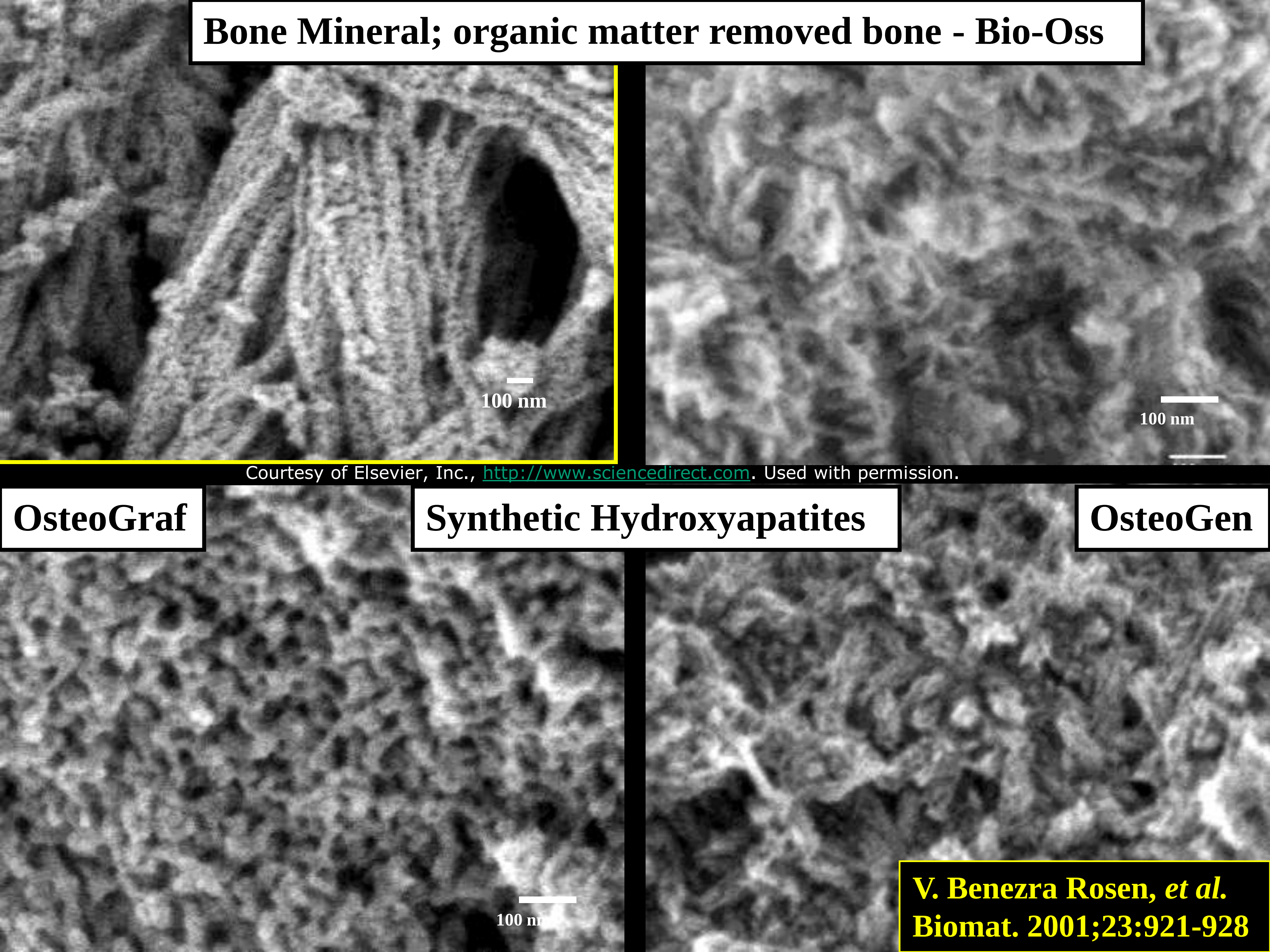
V. Benezra Rosen, *et al.*, Biomat. 243:921 (2002)

The collagen fibril structure (diameter and periodic pattern) is reflected in the organization of the apatite crystallite structure.

What is the cohesive force maintaining the crystallite structure after the collagen is removed?



V. Benezra Rosen, *et al.*, Biomat. 243:921 (2002)


Bone Mineral; organic matter removed bone - Bio-Oss
100 nm
100 nm
OsteoGraf
Synthetic Hydroxyapatites
OsteoGen
100 nm
V. Benezra Rosen, *et al.*
Biomat. 2001;23:921-928

ISSUES RELATED TO PERFORMANCE OF BONE GRAFT SUBSTITUTE MATERIALS (Scaffolds for Bone Tissue Engineering)

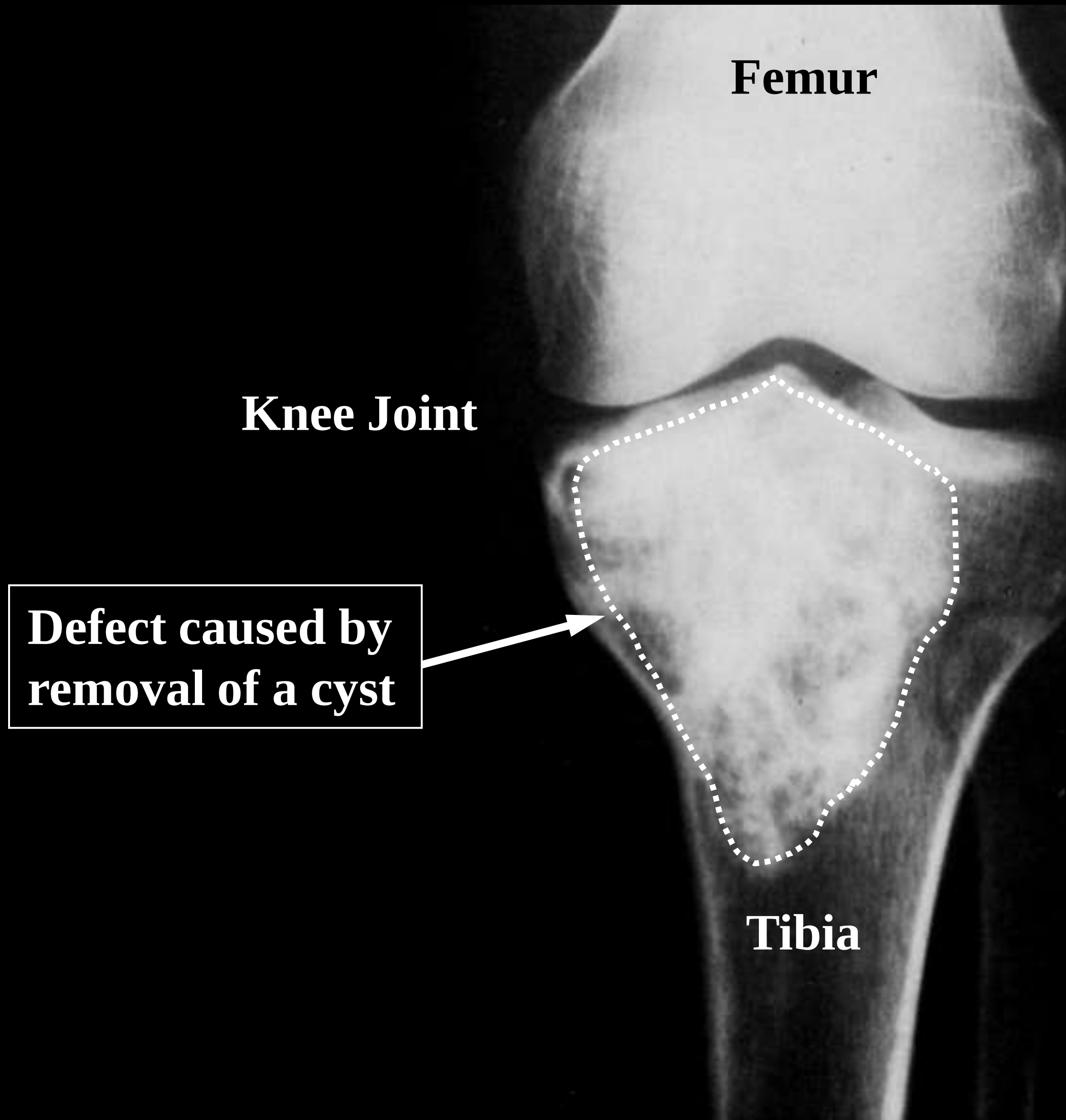
- **Incorporation** of the graft into host bone (to stabilize the graft material) by bone formation on the surface of the graft material (osteoconduction).
- **Osteoclastic resorption** of the graft (vs. dissolution) may be important because osteoclasts release regulators of osteoblast function.
- **Modulus matching** of the graft material to host bone to prevent stress shielding.

Synthetic Hydroxyapatite Particles Implanted in a Periodontal Defect (Prof. Brion-Paris)

Photo removed due to copyright restrictions.

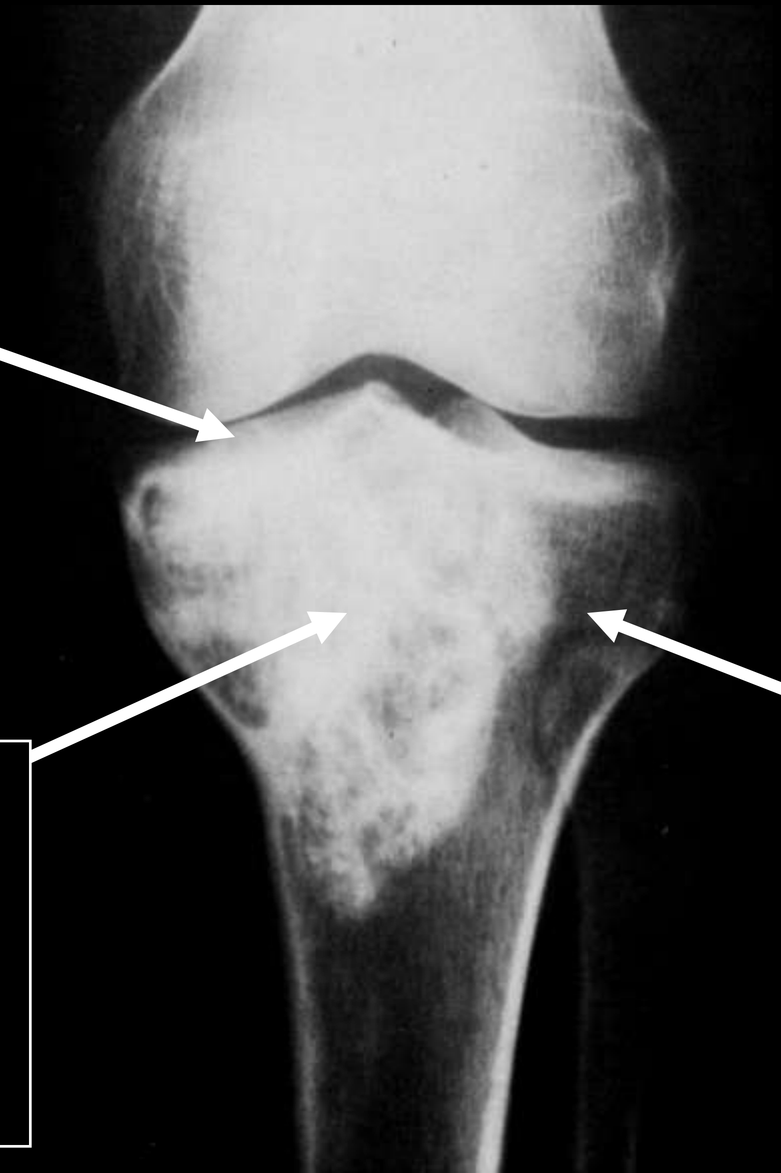
Failure to Incorporate:
Migration of synthetic hydroxyapatite particles from the periodontal defect in which they were implanted.

Defect in the Proximal Tibia Filled with Particles of Synthetic Hydroxyapatite, 1yr f-u



- Bone can regenerate, but full regeneration will not occur in defects this large.
- Not enough autologous bone can be obtained to fill the large defect.
- Problem with allograft is transmission of disease and immune response.
- Need to implant a scaffold material.
- In this case particles of synthetic hydroxyapatite were used as the scaffold material.

Defect in the Proximal Tibia Filled with Particles of Synthetic Hydroxyapatite, 1yr f-u Failure Due to Lack of Modulus Matching

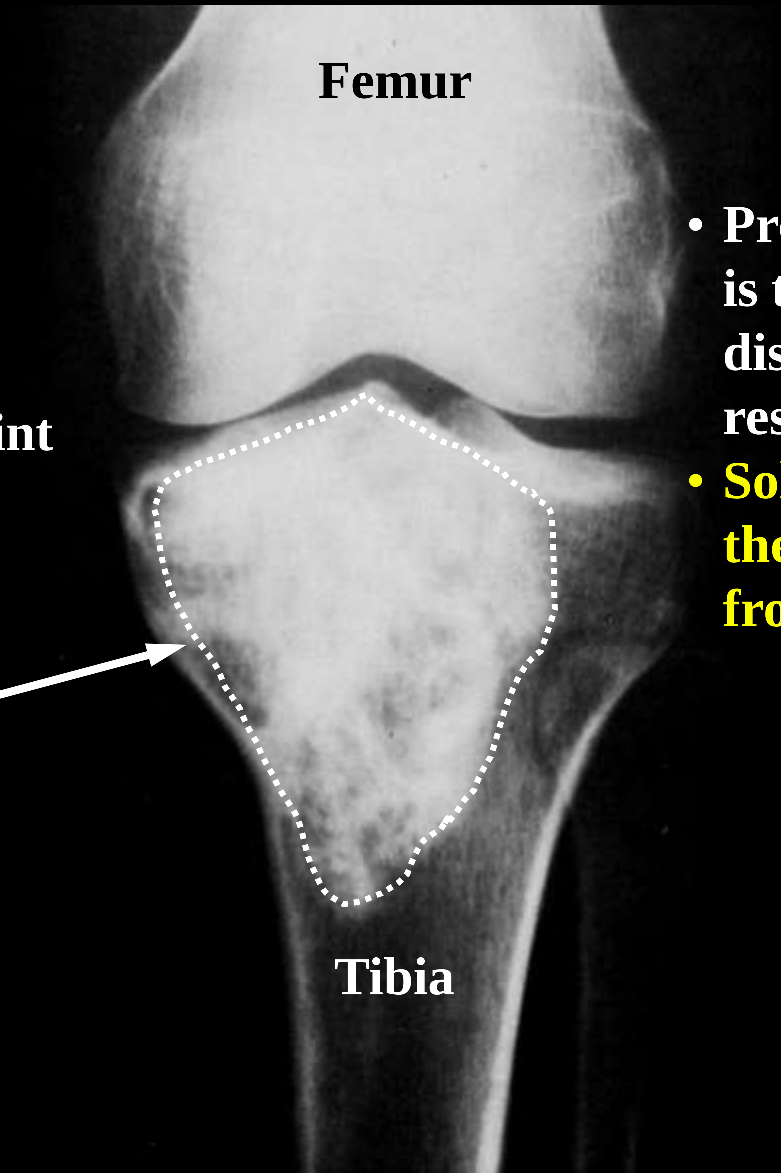


Potential for breakdown of the overlying art. cart. due to high stiffness of the subchondral bone?

Region of high density and stiffness (cannot be drilled or sawn)

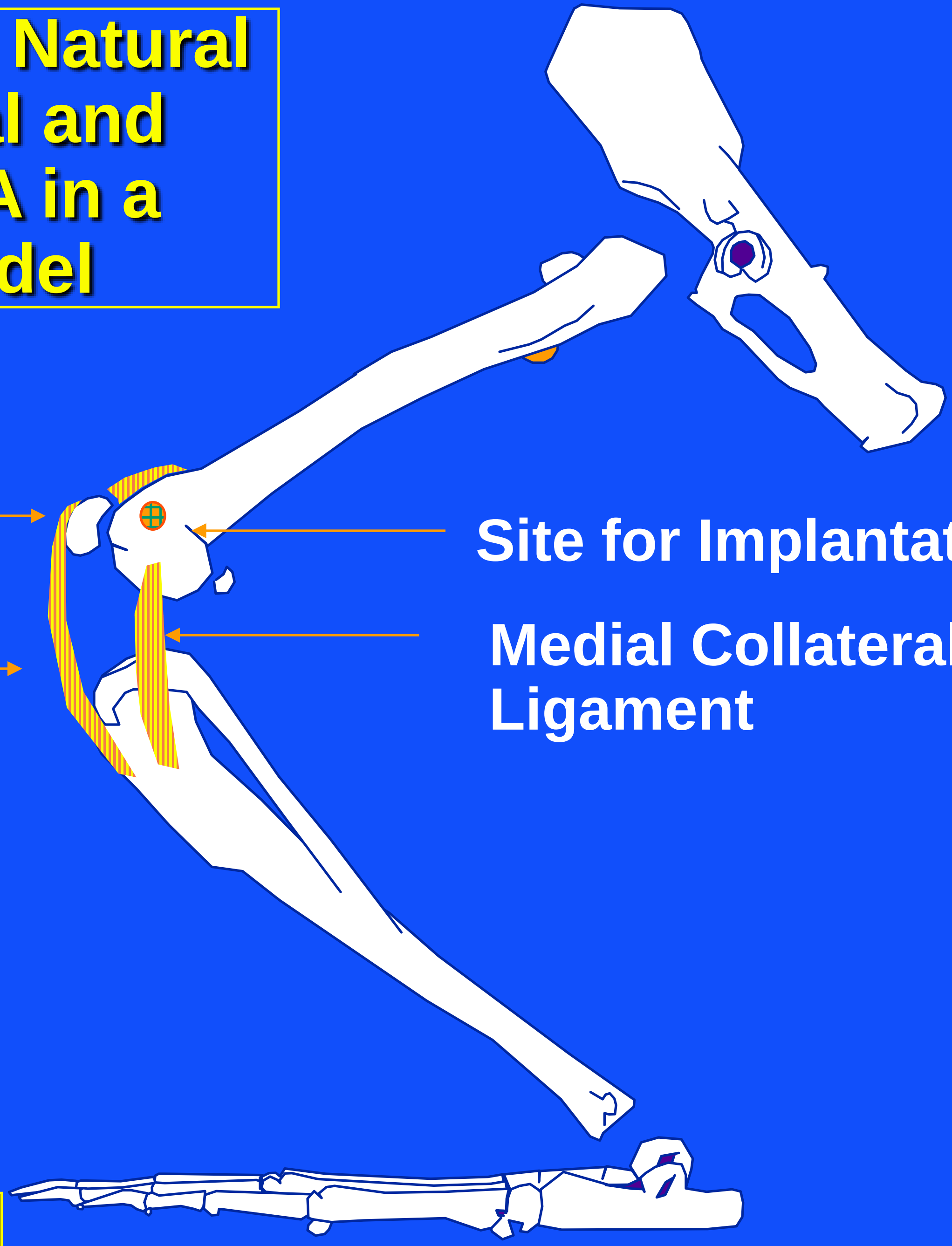
Bone loss due to stress-shielding?

Defect in the Proximal Tibia Filled with Particles of Synthetic Hydroxyapatite, 1yr f-u



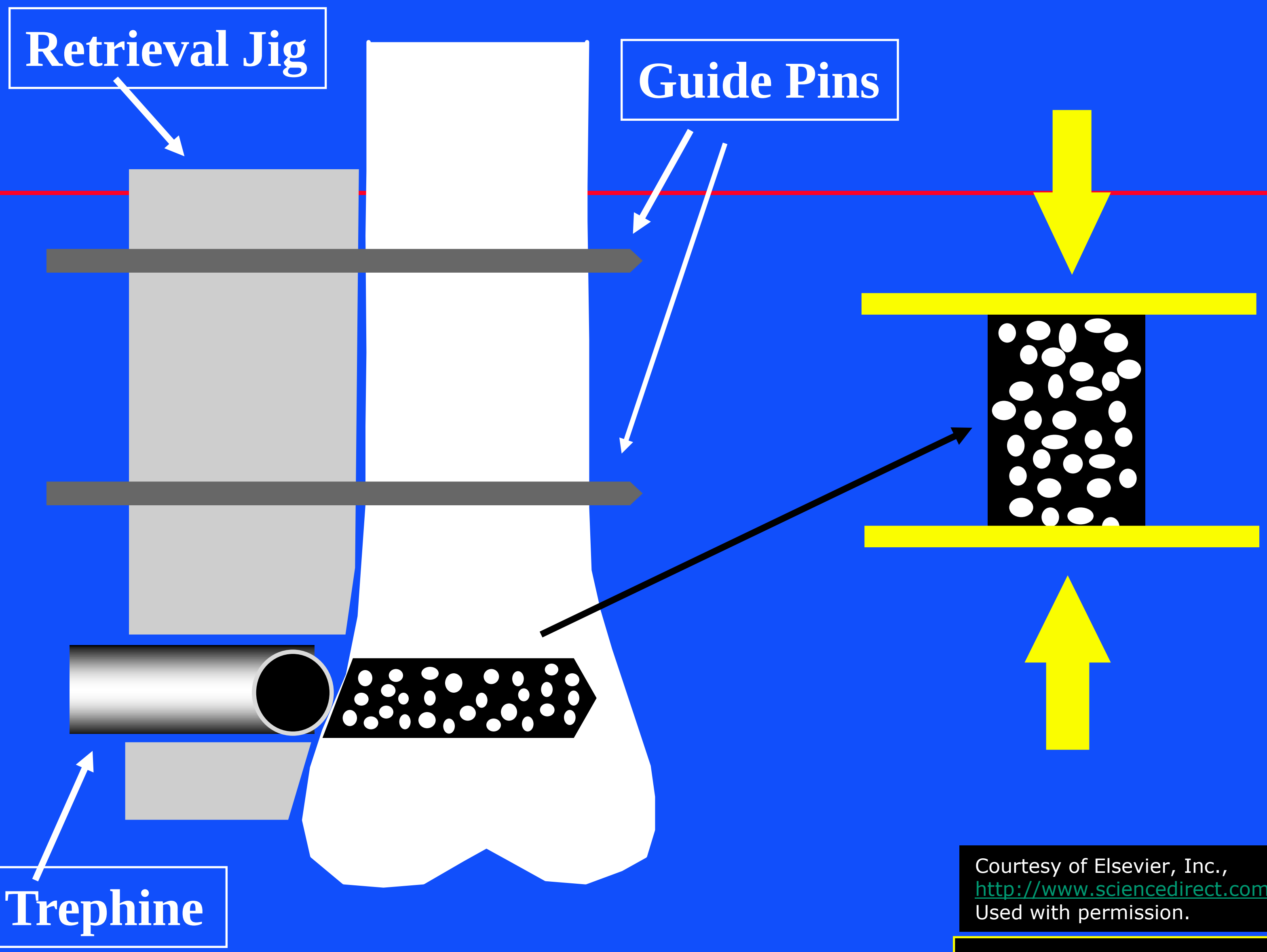
- **Problem with allograft is transmission of disease and immune response.**
- **Solution is to remove the organic matter from bone.**

Comparison of Natural Bone Mineral and Synthetic HA in a Rabbit Model



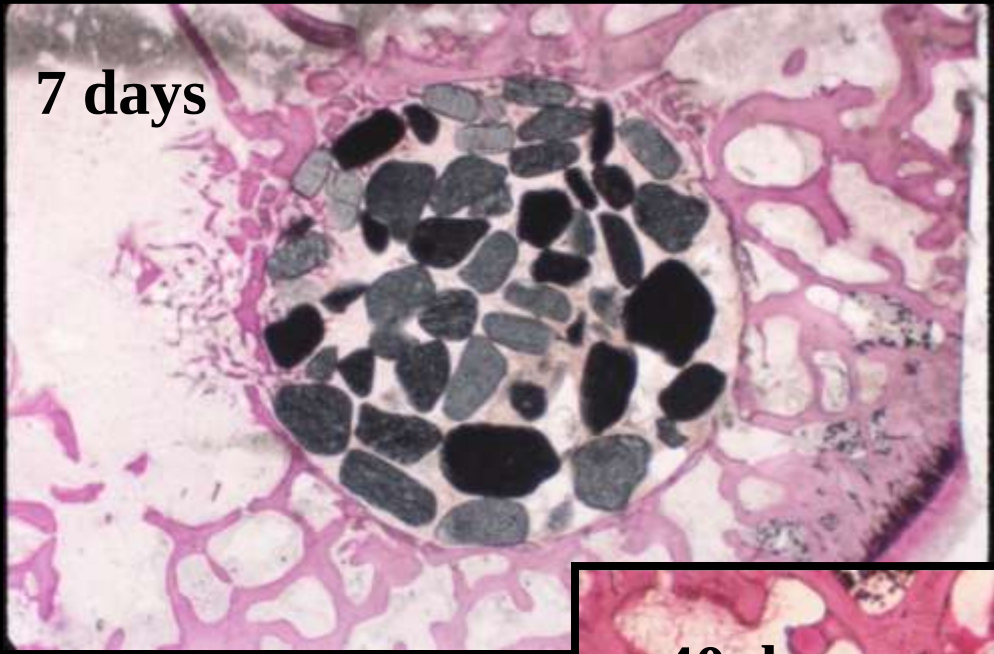
T. Orr, *et al.*
Biomat. 2001;22:1953 1959

RABBIT MODEL

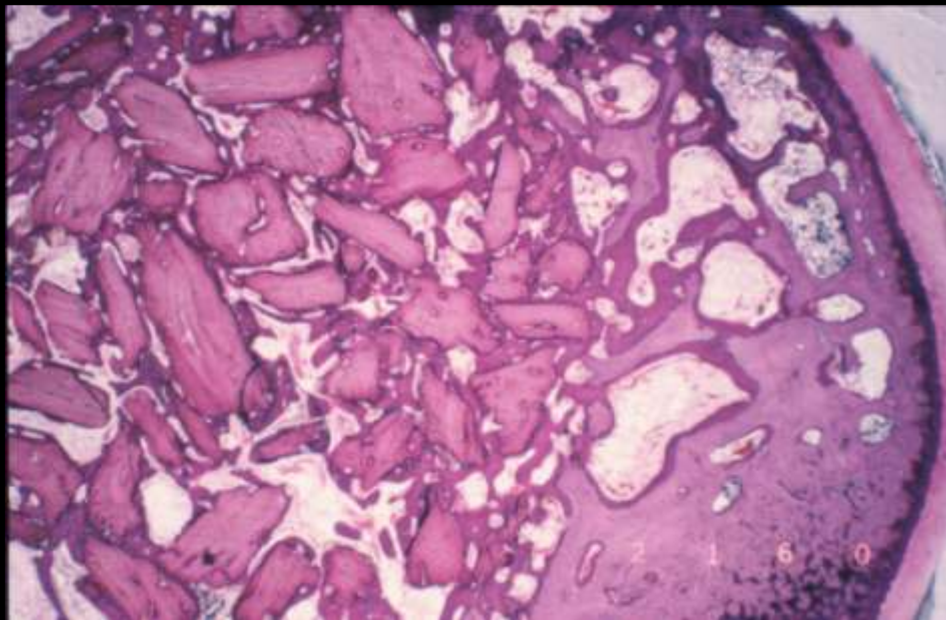


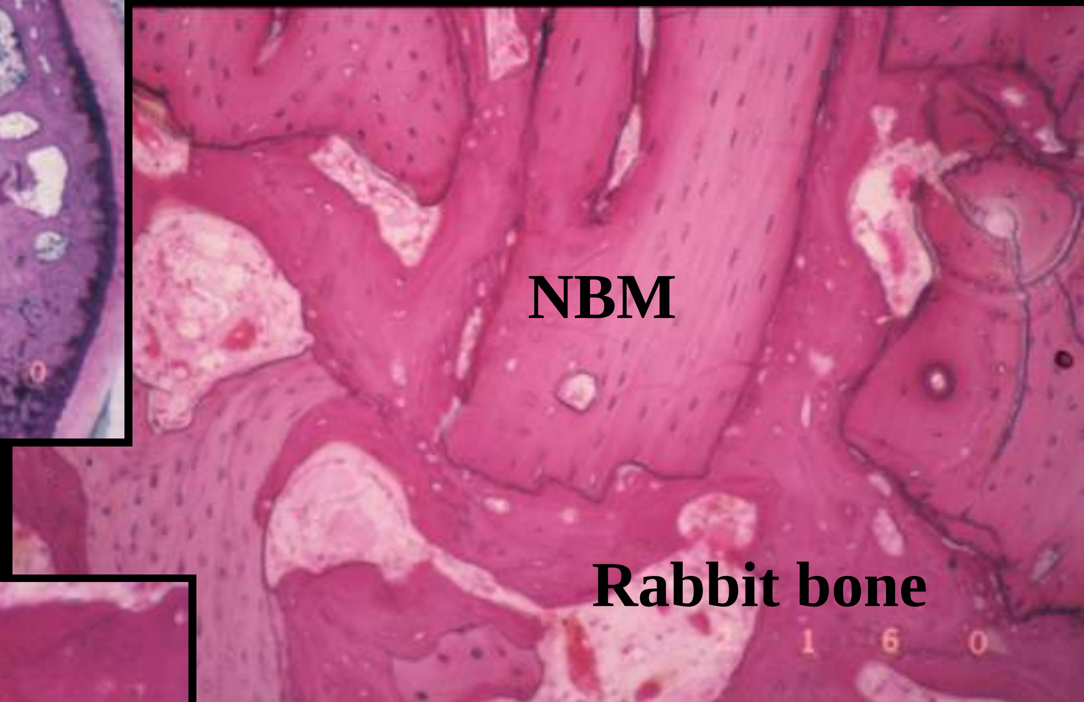
T. Orr, *et al.*
Biomat. 2001;22:1953 1959

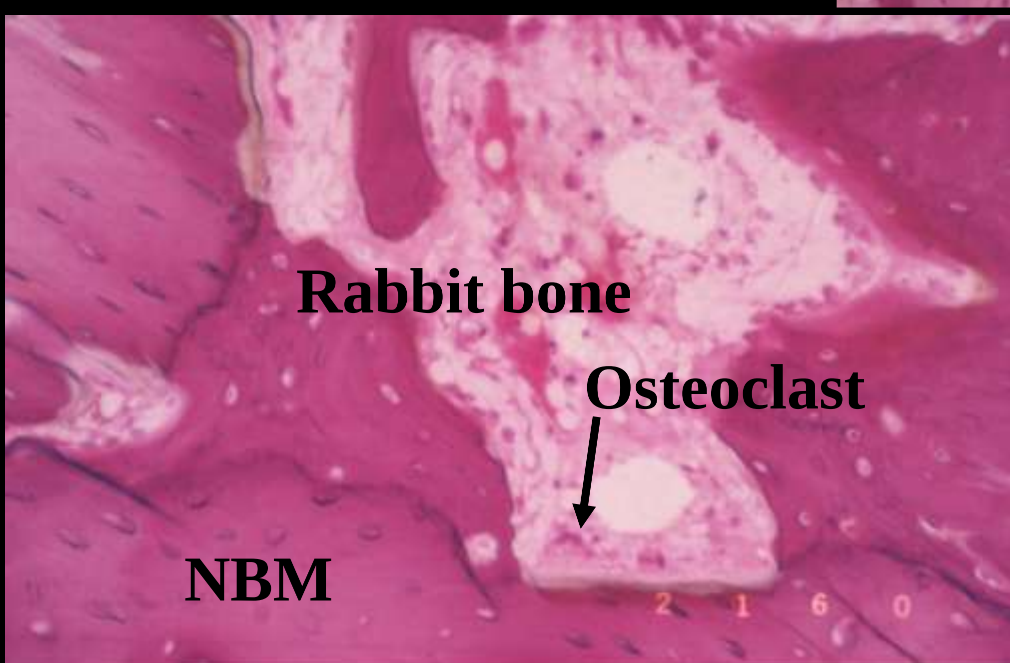
Synthetic Hydroxyapatite

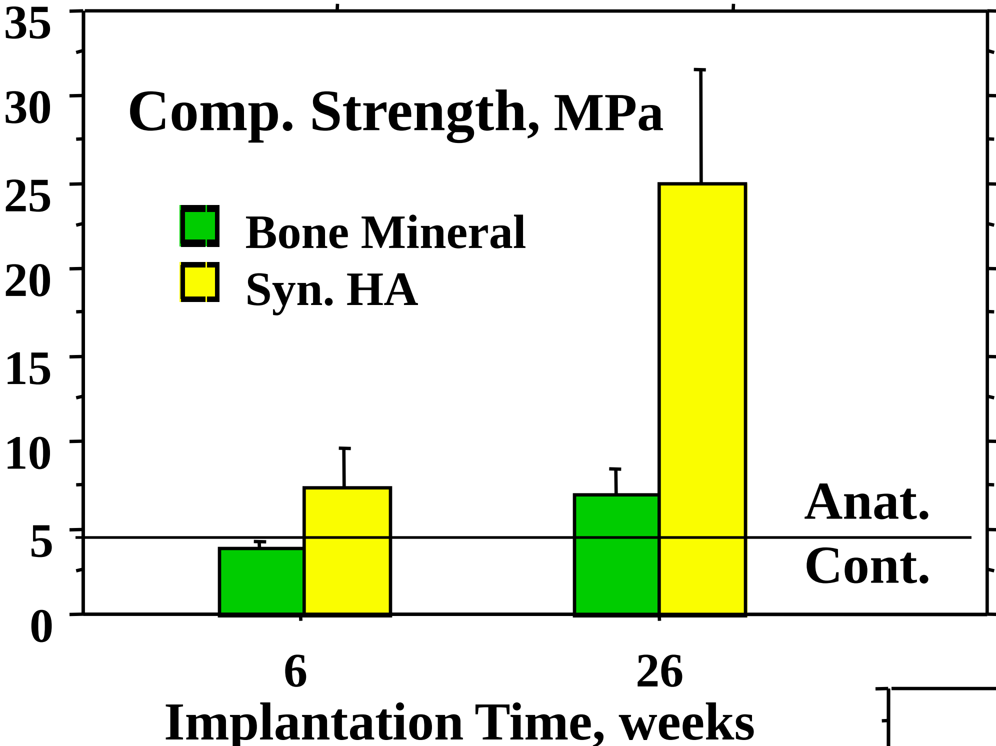


Natural Bone Mineral
40 days

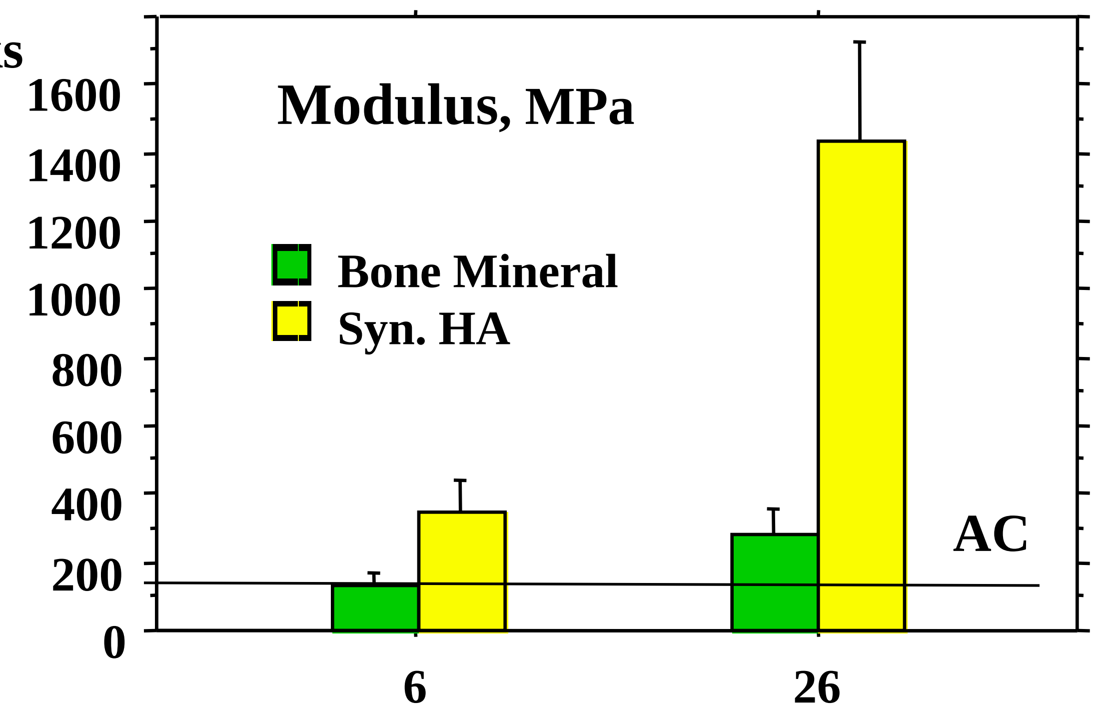




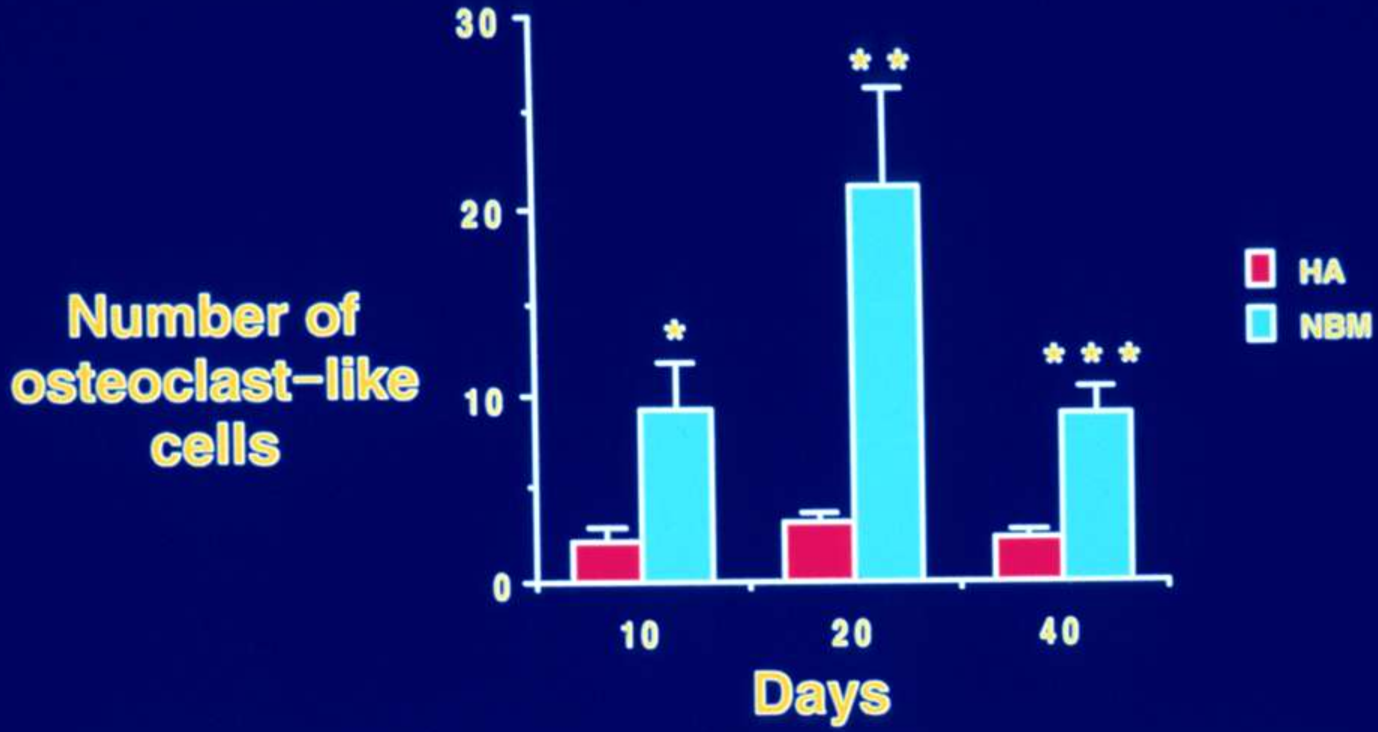




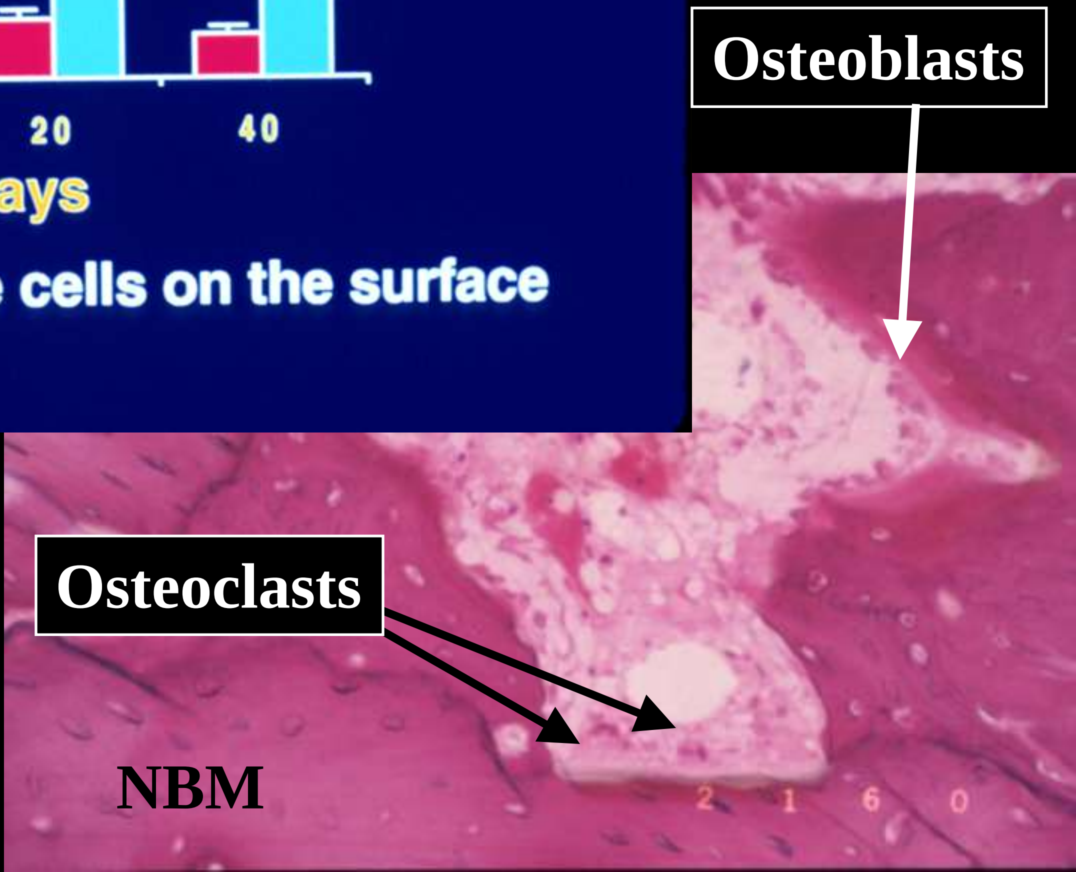
The strength of the site implanted with synthetic hydroxyapatite is high but so to is the modulus (stiffness).

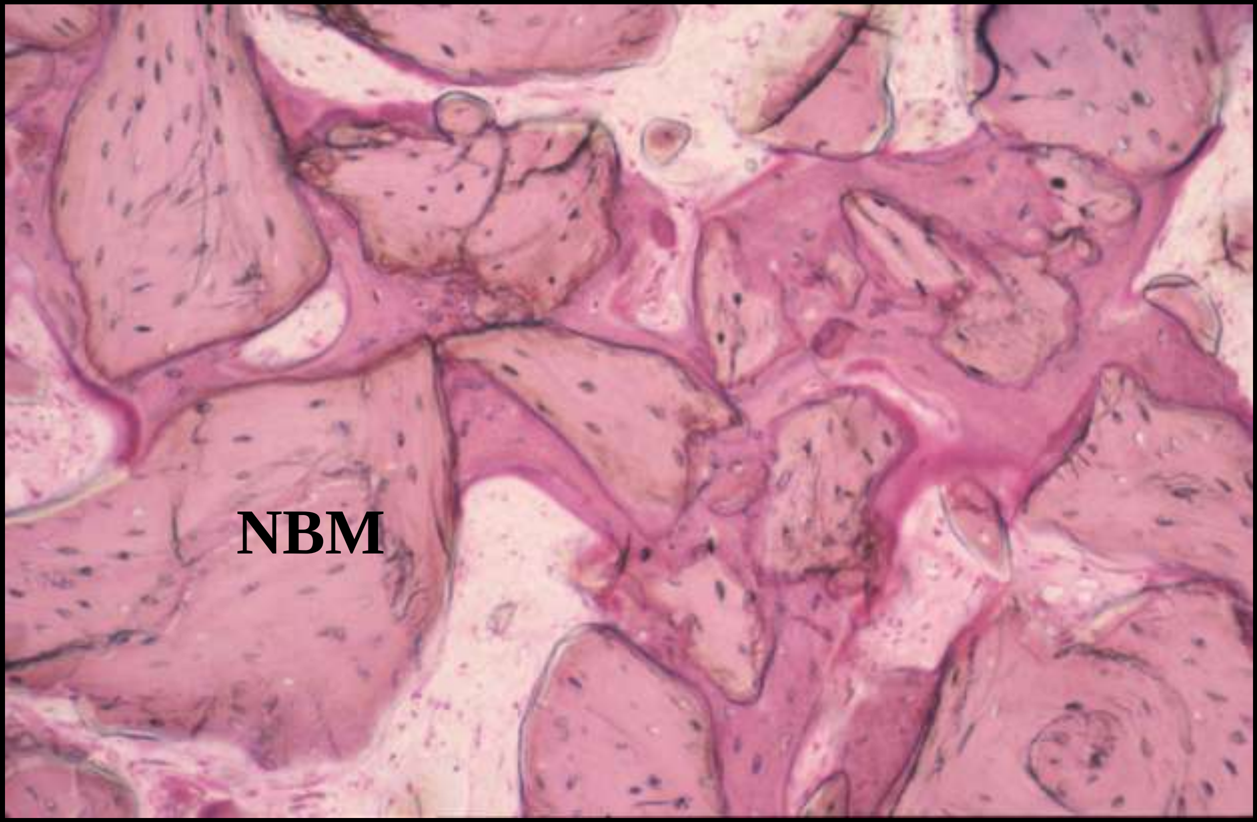


T. Orr, *et al*.
Biomat. 2001;22:1953 1959

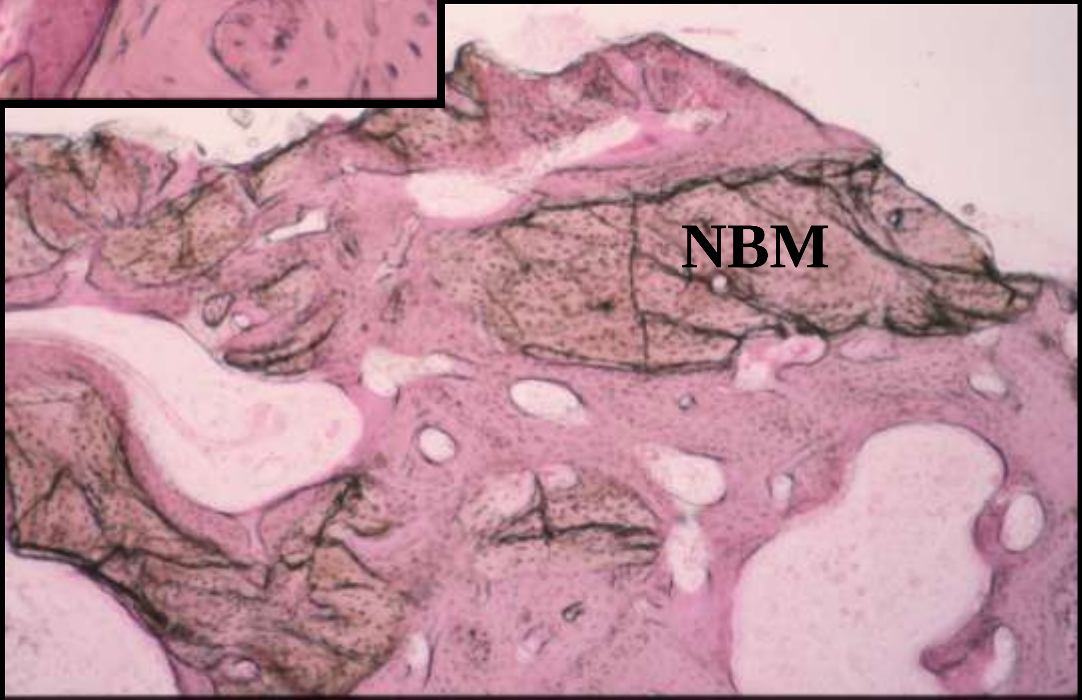


Number of osteoclast-like cells on the surface of the particles.





Biopsy from ankle fusion patient implanted with particles of natural bone mineral, 6 mo.



BONE GRAFT MATERIALS (Scaffolds for Bone Tissue Engineering)

- Allograft bone remains a valuable substance for grafting; care must be taken with respect to the transmission of disease.
- Many off-the-shelf bone graft substitute materials are now available and should be of value for many applications.
- Need to be aware of how the increase in stiffness caused by certain materials will affect the surrounding tissues so that we do not cause greater problems than we are trying to solve.

BIOMATERIALS FOR BONE TISSUE ENGINEERING

Biomimetics

Synthesize scaffold materials using principles and processes underlying biomineralization.

Biomineralized Materials as Biomaterial Scaffolds

Use biomineralized structures as they naturally occur or after treatments for modification.

BIOMIMETIC BONE SCAFFOLD

- Produce a type I collagen sponge-like scaffold first, and then immerse it in a mineralizing solution
- Produce the type I collagen scaffold in a mineralizing solution

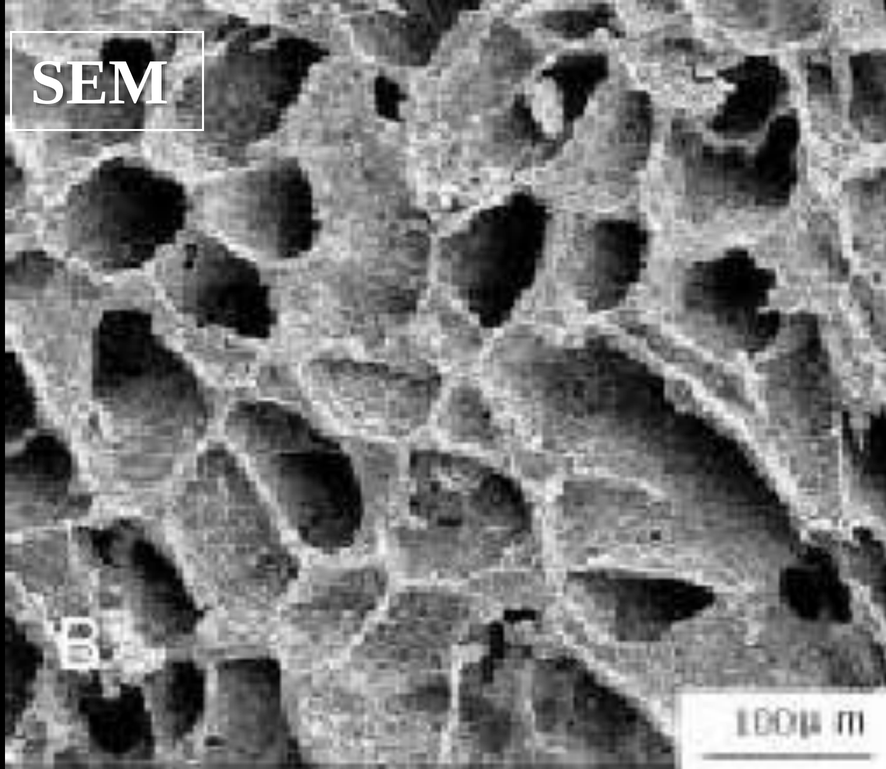
BIOMIMETIC BONE SCAFFOLD

Nano-HAp/collagen (nHAC) composite was developed by producing a type I collagen scaffold in a solution of calcium phosphate.

HA crystals and collagen molecules self-assembled into a hierarchical structure through chemical interaction, which resembled the natural process of mineralization of collagen fibers.

Du C, Cui FZ, Zhang W, *et al.*, J BIOMED MATER RES 50:518 (2000)
Zhang W, Liao SS, Cui FZ, CHEM MATER 15:3221 (2003)

Porous Composite nHAC/PLA



HRTEM of mineralized collagen fibers

Image removed due to copyright restrictions.
See Fig. 3 in Zhang, W., S. S. Liao, and F. Z. Cui. "Hierarchical Self assembly of Nano fibrils in Mineralized Collagen." *Chemistry of Materials* 15, no. 16 (Aug 12, 2003): 3221 3226.

BIOMANUFACTURING

BIOMANUFACTURING:

A US-CHINA NATIONAL SCIENCE FOUNDATION-SPONSORED WORKSHOP

June 29-July 1, 2005, Tsinghua University, Beijing, China

W. Sun, Y. Yan, F. Lin, and M. Spector

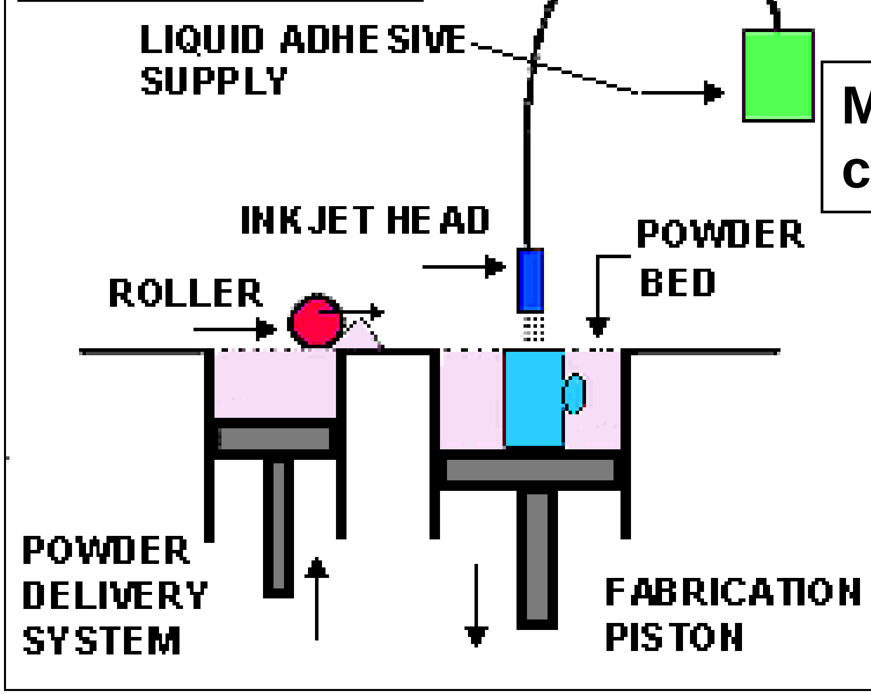
New technologies for producing scaffolds with *precision (computer-controlled) multi- scale control of material, architecture, and cells.*

www.mem.drexel.edu/biomanufacturing/index.htm

Tiss. Engr., In Press

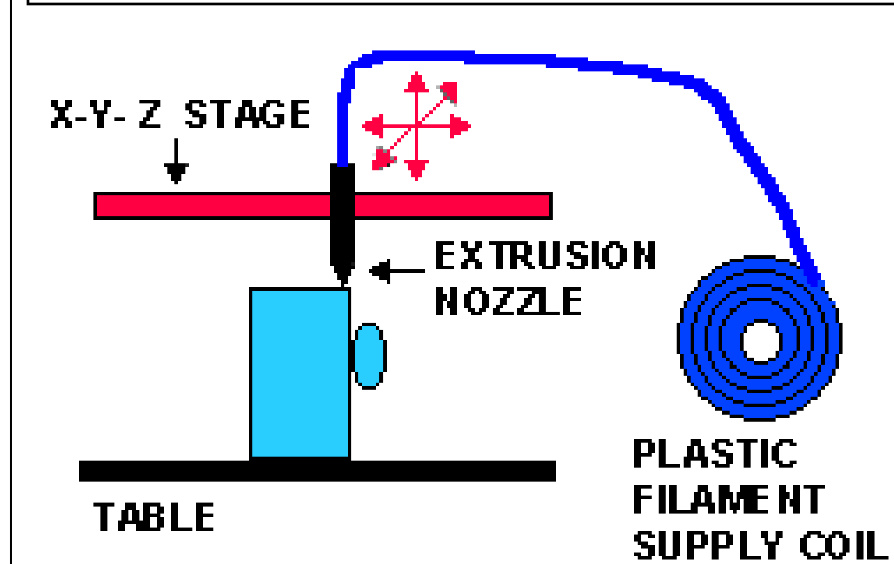
Solid Free-Form Fabrication Technologies

3-D Printing



Multiple inkjet heads; print cells as cells aggregates or individual cells

Fused Deposition Modeling



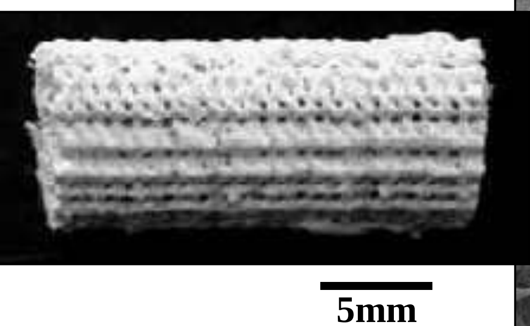
Materials Letters 57 (2003) 2623–2628

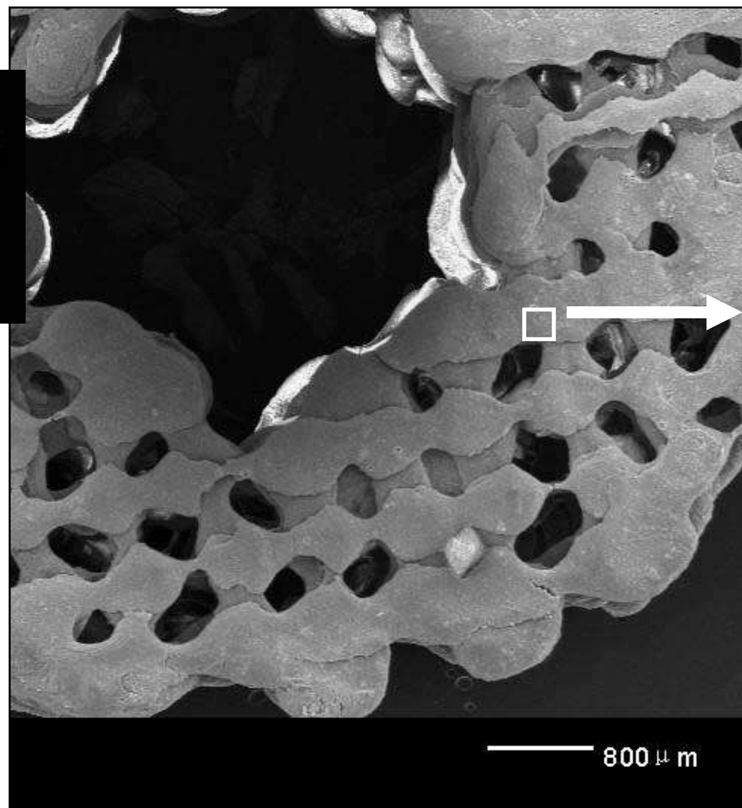
Layered manufacturing of tissue engineering scaffolds via multi-nozzle deposition

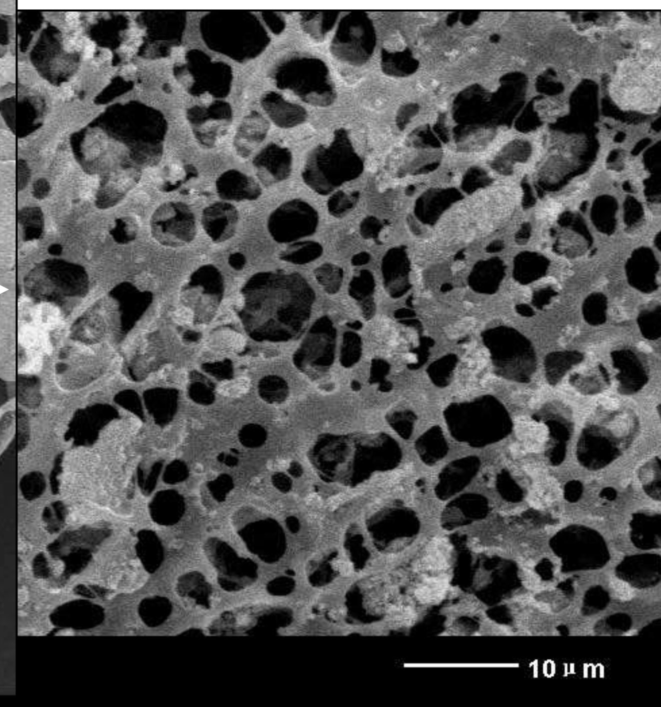
Yongnian Yan[a], Zhuo Xiong[a,*], Yunyu Hu[b], Shenguo Wang[c], Renji Zhang[a], Chao Zhang[b]

[a] Department of Mechanical Engineering, Tsinghua University, Beijing 100084, China
[b] Fourth Military Medical University, Xi'an 710032, China
[c] Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080, China

Single-nozzle deposition using polylactic acid and tricalcium phosphate.







Biomaterials 26 (2005) 5864–5871

Fabrication of viable tissue-engineered constructs with 3D cell-assembly technique

Yongnian Yan[a,b], Xiaohong Wang[a,b,*], Yuqiong Pan[a,b], Haixia Liu[a,b], Jie Cheng[a,b], Zhuo Xiong[a,b], Feng Lin[a,b], Rendong Wu[a,b], Renji Zhang[a,b], Qingping Lu[a,b]

[a] *Center of Organism Manufacturing, Department of Mechanical Engineering, Tsinghua University, Beijing 100084, PR China*

[b] *Institute of Life Science & Medicine, Tsinghua University, Beijing 100084, PR China*

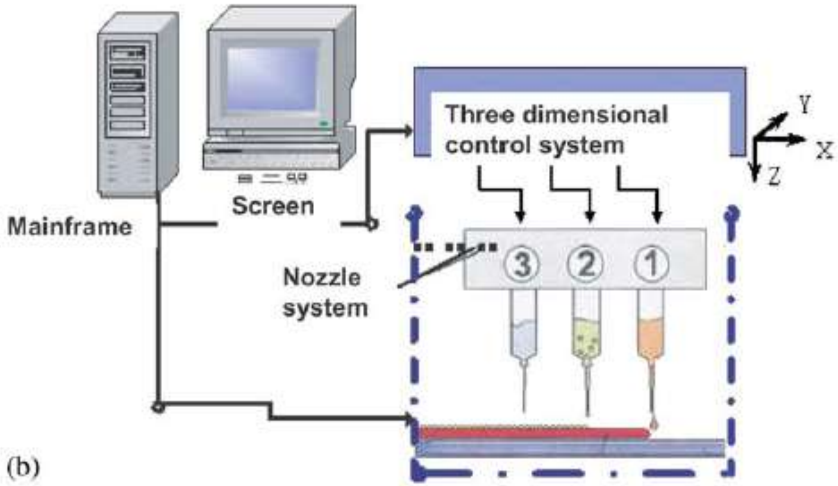
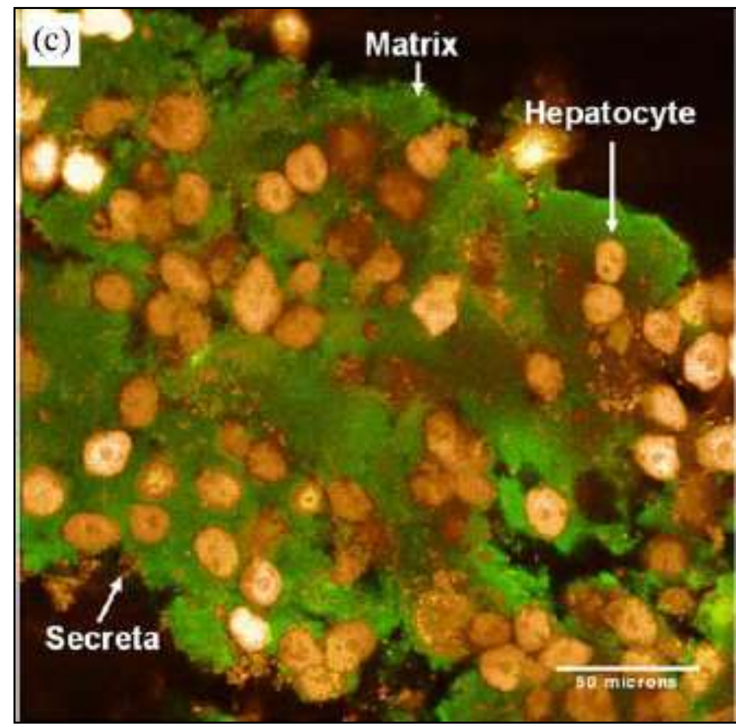
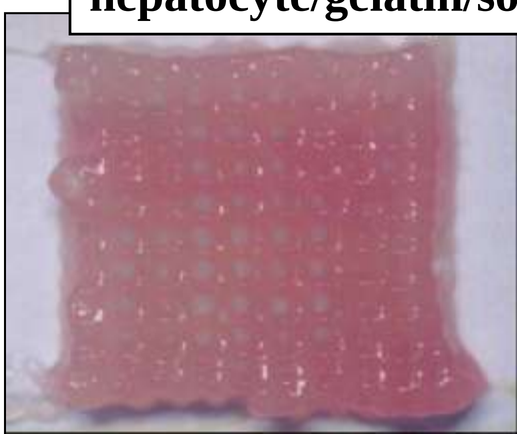
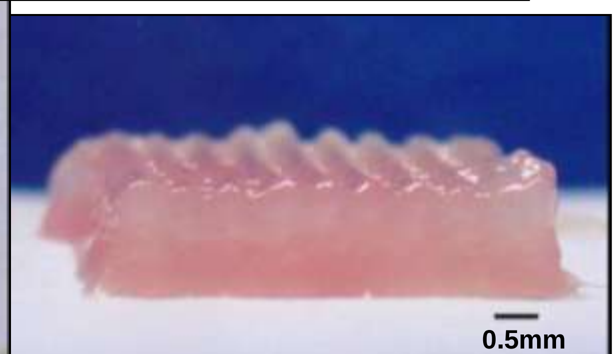


Fig. 1. A cell assembling machine and another cell assembling system.



hepatocyte/gelatin/sodium alginate construct





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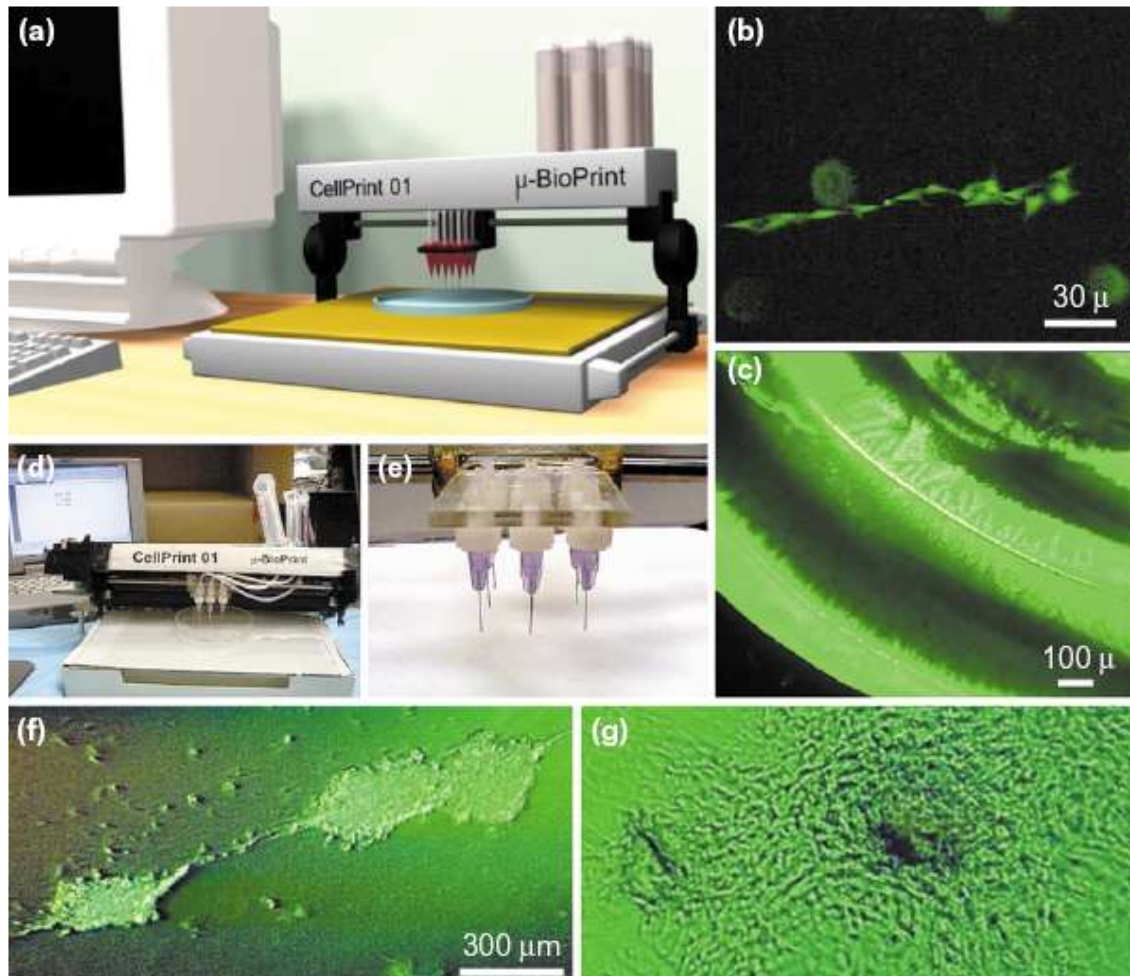
TRENDS in Biotechnology Vol.21 No.4 April 2003

Organ printing: computer-aided jet-based 3D tissue engineering

Vladimir Mironov[1], Thomas Boland[2], Thomas Trusk[1], Gabor Forgacs[3] and Roger R. Markwald[1]

[1]Department of Cell Biology and Anatomy, Medical University of South Carolina, Charleston, SC 29425, USA
[2]Department of Bioengineering, Clemson University, Clemson, SC USA
[3]Departments of Physics and Biology, University of Missouri, Columbia, MO, USA

Printing single cells, cell aggregates and the supportive, biodegradable, thermosensitive gel according to a computer generated template.



b) bovine aortic endothelial cells printed in 50 mm diam. drop in a line. After 72 hrs. the cells attached to the Matrigel support and maintained their positions,
f) endothelial cell aggregates printed on collagen,
g) fusion of cells in (f).

Image-based design and solid freeform fabrication to produce biphasic composite scaffolds

– Taboas, J.M., *et al*.. Biomat 24:181; 2003

Cartilage: Porous polylactic acid (seeded with fully differentiated porcine chondrocytes) bonded to porous hydroxyapatite (HA)

Bone: Porous HA seeded with human primary fibroblasts transduced with an adenovirus expressing BMP-7

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Fig. 4a in Schek, R. M., et al. "Tissue Engineering Osteochondral Implants for Temporomandibular Joint Repair." *Orthodontics & Craniofacial Research* 8 (2005): 313-319.

Fig. 3a and 5b in Schek, Rachel M., et al. "Engineered Osteochondral Grafts Using Biphasic Composite Solid Free-Form Fabricated Scaffolds." *Tissue Engineering* 10 (2004): 1376-1385.

Biphasic scaffolds promoted the simultaneous growth of bone, cartilage, and mineralized interface tissue; young nude mice, 4 wks post-op

Schek, R.M.,, *et al*., Tissue Eng 10:1376;2004