Massachusetts Institute of Technology
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2.782J/3.961J/BEH.451J/HST524J

BONE SUBSTITUTE MATERIALS AND TISSUE ENGINEERING

M. Spector, Ph.D.

INCUBATION OF TISSUE ENGINEERING CONSTRUCTS IN ECTOPIC SITES

- Allows for additional development for 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 construct can be transplanted to the target defect

INCUBATION OF TISSUE ENGINEERING CONSTRUCTS IN ECTOPIC SITES

- Nude (Immune Deficient) Mouse Experimental Model
  - Subcutaneous site used as the *in vivo* environment to investigate the development of tissue induced by xenogeneic (human) cells

CONSIDERATIONS IN TISSUE ENGINEERING

- Clearly define the specific clinical problem to be solved.
- Implement the simplest procedure for treating the problem to achieve a meaningful clinical benefit.
  - Benefit-Risk Ratio (e.g., risks of using cells: cell transformation, morbidity of a 2nd surgical procedure)
  - Cost
- Need to evaluate tissue engineering products in animal models that relate to human problems to be treated.

CLINICAL PROBLEMS REQUIRING BONE TISSUE ENGINEERING

- Fusion
  - Spine fusion
- Defects resulting from the excision of tumors or cysts.
- Incomplete fracture healing
- Bone loss associated with prostheses
  - Particle-induced osteolysis
  - Stress shielding

ELEMENTS OF TISSUE ENGINEERING/ REGENERATIVE MEDICINE

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

TISSUE ENGINEERING ENDPOINTS

- Morphological/Histological/Biochemical
  - Match the composition and architecture of the tissue.
  - Problem: A complete analysis is difficult and no clear relationships yet with functional and clinical endpoints.
- Functional (Functional Tissue Engineering)
  - Achieve certain functions; display certain properties (*e.g.*, mechanical properties).
  - Problem: Difficult to measure all properties; Which mechanical properties are the most important?
- Clinical
  - Pain relief.
  - Problems: Can only be evaluated in human subjects and the mechanisms (including the placebo effect) and kinetics of pain relief (*e.g.*, how long it will last) are unknown.

SCAFFOLDS

Chemical Composition

- Collagen-GAG (Yannas)
- Polyglycolic/polylactic acid (Langer and Freed)
- Self-assemblying proteins (Zhang)
- Nano-Ap/Collagen Composite-self assembly (Cui)
- Chitin/chitosan (Xu and others)

Structure/Architecture

- Fiber mesh, like noodles (Langer and Freed)
- Free Form Fabrication-3-D printing (Yan)
- Sponge-like (Yannas and Cui)
- Fine filament mesh (Zhang)

Scaffold Structures

Fiber mesh, like noodles

Photos removed due to copyright restrictions.

PRINCIPLES AND PRACTICE OF TISSUE ENGINEERING

Principles

- Scaffolds can regulate cell function by their chemical make-up
- Scaffolds can regulate cell function by their structure/architecture

Practice

- Methods for producing scaffolds with selected chemical composition and structure

PRINCIPLES

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

PRINCIPLES

Chemical Composition

- Scaffolds can regulate cell function by their chemical make-up
  - Affects cell attachment through integrin binding, or absence of attachment in the case of hydrogels
  - Affects cell behavior through interactions with integrins
- Degradation rate and mechanical properties are dependent on the chemical make-up

PRINCIPLES

Pore Structure/Architecture

- Percentage porosity
  - number of cells that can be contained
  - strength of the material
- Pore diameter
  - surface area and the number of adherent cells
  - ability of cells to infiltrate the pores
- Interconnecting pore diameter
- Orientation of pores
  - can direct cell growth
- Overall shape of the device needs to fit the defect

PRINCIPLES

Degradation Rate

- Too rapid does not allow for the proper regenerative processes.
- Too slow interferes with remodeling.
- For synthetic polymers regulated by blending polymers with different degradation rates (*e.g.*, PLA and PGA).
- For natural polymers (*viz.*, collagen) by cross-linking.

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.
- For synthetic polymers regulated by 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) or synthetic polymers (*e.g.*, PLA).

PRACTICE

Methods for Producing Scaffolds*

- Fibers (non-woven and woven)
- Freeze-drying
- Self-assembly
- Free-form manufacturing

* Need to consider the advantages and disadvantages with respect to the production of scaffolds with selected chemical composition and structure

BIOMINERALIZATION AND SCAFFOLDS FOR BONE TISSUE ENGINEERING

Biomineralization

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.

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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 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

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 (anorganic bone)	6

Mineralization of Collagen in Bone

Schematic of collagen structure
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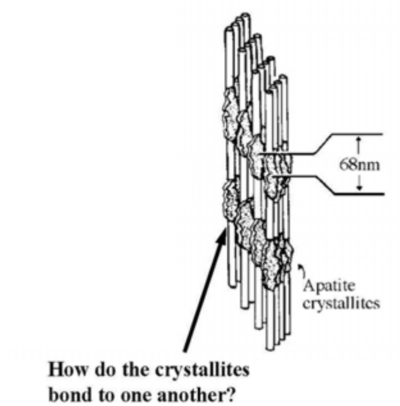


Hydroxyapatite:

$$Ca_{10}\,(PO_4)_6\,(OH)_2$$

Ca_{10} ↑	$(PO_4)_6$ ↑	$(OH)_2$ ↑
Mg^{+2}	CO_3^{-2}	CO_3^{-}
Sr^{+2}	HPO_4^{-2}	F^{-}
Na^{+}		
K^{+}		

Photos removed due to copyright restrictions.

Photos removed due to copyright restrictions.

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.

Photos removed due to copyright restrictions.

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

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

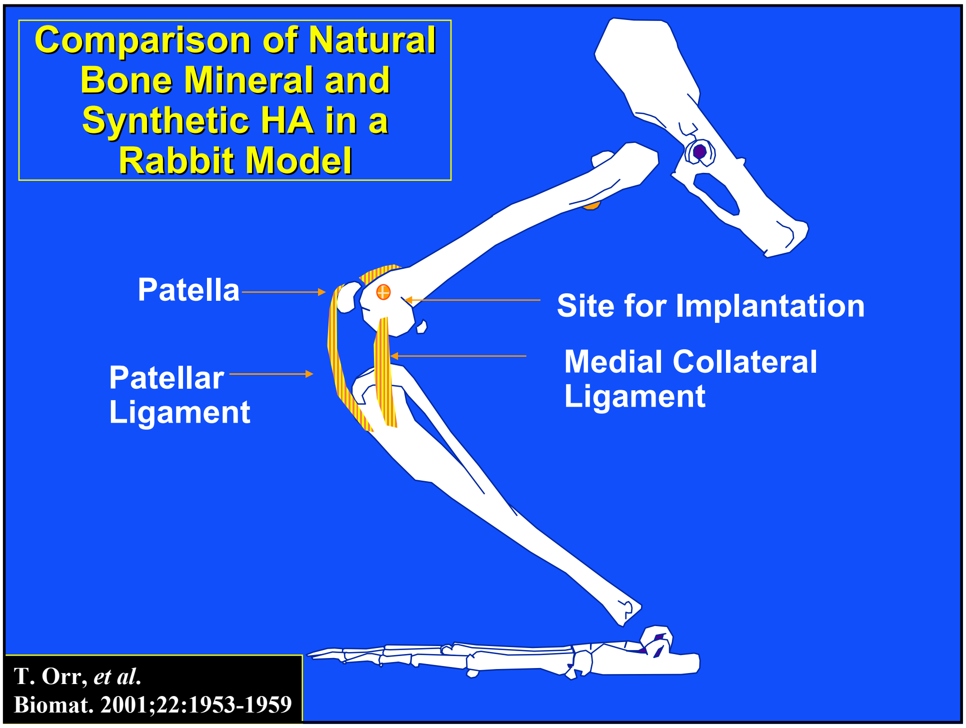
- **Incorporation** of the graft into host bone (to stabilize the graft material) by bone formation on the surface of the graft material (osteoconduction).
- **Osteoclastic resorption** of the graft (vs. dissolution) may be important because osteoclasts release regulators of osteoblast function.
- **Modulus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

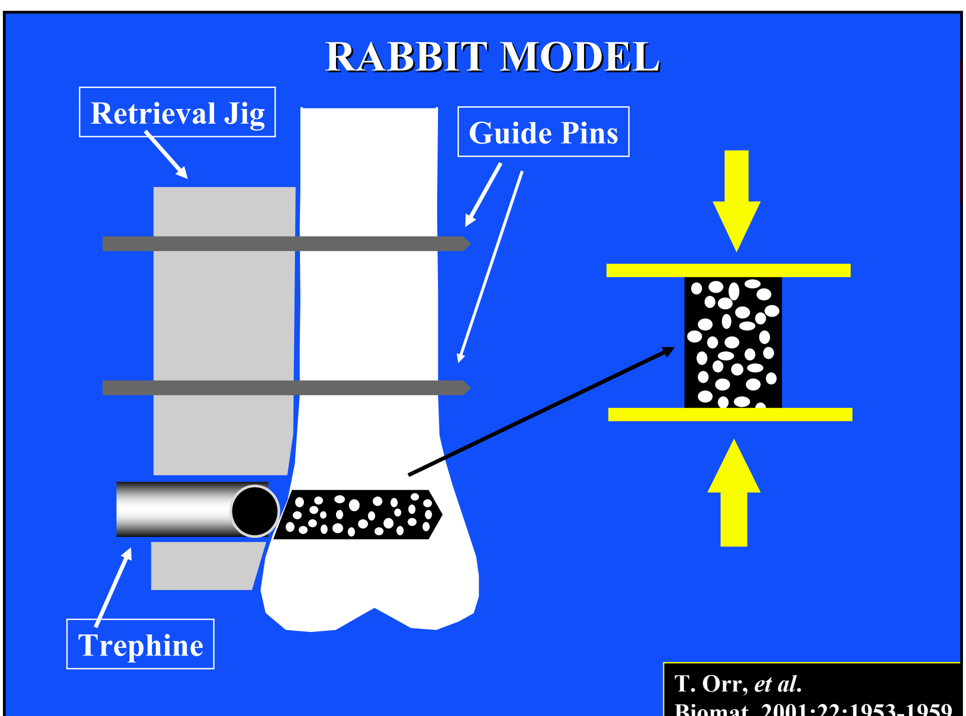
Synthetic Hydroxyapatite Particles Implanted in a Periodontal Defect (Prof. Brion-Paris)

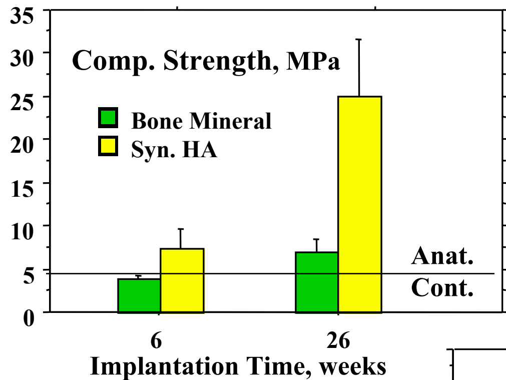
Photo removed due to copyright restrictions.

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

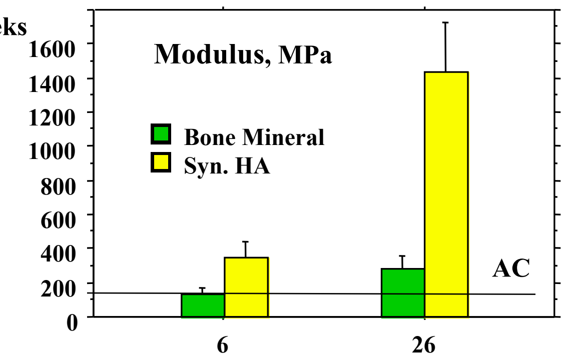
Photo removed due to copyright restrictions.


Comparison of Natural Bone Mineral and Synthetic HA in a Rabbit Model
Patella
Site for Implantation
Medial Collateral Ligament
Patellar Ligament
T. Orr, *et al.*
Biomat. 2001;22:1953-1959


RABBIT MODEL
Retrieval Jig
Guide Pins
Trephine
T. Orr, *et al.*
Biomat. 2001;22:1953-1959



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

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.

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

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Porous Composite nHAC/PLA

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BIOMIMETIC BONE SCAFFOLD

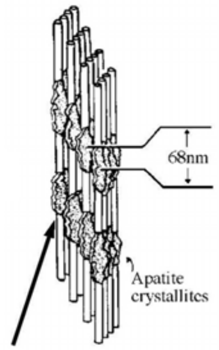
Nano-HAp/collagen (nHAC) composite was developed by mineralizing type I collagen in a solution of calcium phosphate.

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)

Mineralization of Collagen in Bone

Schematic of collagen structure removed due to copyright restrictions.

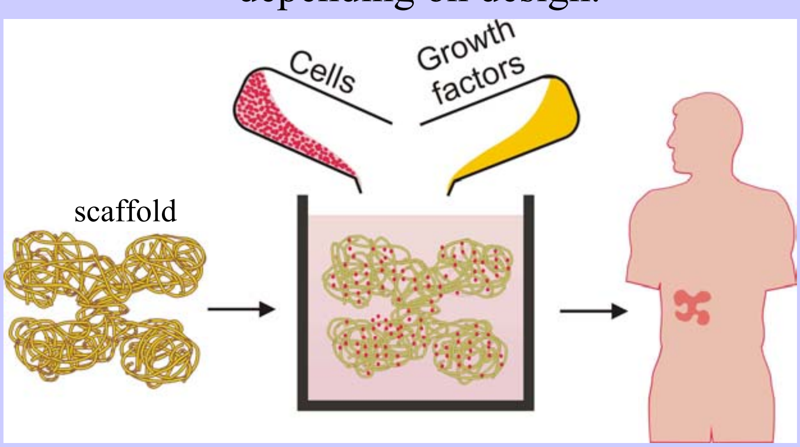


Hydroxyapatite:

$Ca_{10}\,(PO_4)_6\,(OH)_2$

Ca_{10} ↑	$(PO_4)_6$ ↑	$(OH)_2$ ↑
Mg^{+2}	CO_3^{-2}	CO_3^{-}
Sr^{+2}	HPO_4^{-2}	F^{-}
Na^{+}		
K^{+}		

In Tissue Engineering, growth factors and Cells are definite, but scaffold may be various depending on design.



F-Z Cui, Tsinghua Univ., Beijing

- Thus, our object is to manufacture the scaffold with similarity to the special natural ECM, in composition and hierarchical structure, as well as in physiology.
- However, this object is not easy. In one hand, the exact structure and fabrication process are still obscure for many ECMs, from the opinion of Materials Engineering. In another hand, the available technology has not be able to fabricating the known hierarchical structure of ECM.
- We had made our effort in biomimetic fabrication of scaffold in bone, liver and brain. Here, is the example of bone scaffold.

Structure model of natural bone on molecule level

Image removed due to copyright restrictions.
See Landis, W. J., et. al. *J Structural Biology* 110, no. 39 (2003).

- The formation of a two-dimensional array of molecules as detailed by Hodge and Petruska
- A three-dimensional array leading to the creation of extensive channels or gaps throughout the assemblage.
- A number of cylindrically shaped molecules 300 nm in length and 1.23 nm in width aggregated in parallel.

- Our aim is to make it in lab.

Biomimetic Fabrication of Artificial Bone

◆The nano-HAp/collagen (nHAC) composite has been developed for the first time by mineralizing the type I collagen in the solution of calcium phosphate.
HA crystals and collagen molecules self-assembled into a hierarchical structure through chemical interaction, which resembles the natural process of mineralization of collagen fibers

Du chang et al, JBMR 1999

zhang wei et al, Chem Mater 2003

Photo removed due to copyright restrictions.
See 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.

HRTEM of mineralized collagen fibers.

Photo removed due to copyright restrictions.
See Liao, S. S., F. Z. Cui, W. Zhang, et. al.
"Hierarchically Biomimetic Bone Scaffold Materials: Nano-HA/collagen/PLA Composite."
Journal of Biomedical Materials Research Part B: Applied Biomaterials 69B, no. 2 (May 15, 2004): 158-165.

SEM morphology of the porous composite nHAC/PLA.

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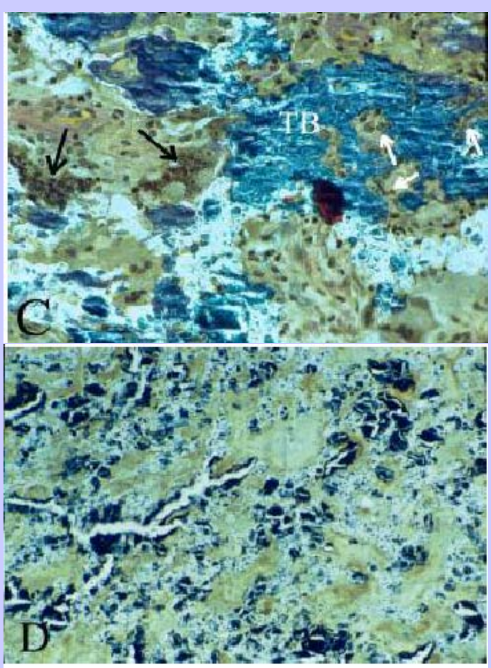
See Liao, S. S., F. Z. Cui, W. Zhang, et. al.
"Hierarchically Biomimetic Bone Scaffold Materials: Nano-HA/collagen/PLA Composite."
Journal of Biomedical Materials Research Part B: Applied Biomaterials 69B,
no. 2 (May 15, 2004): 158-165.

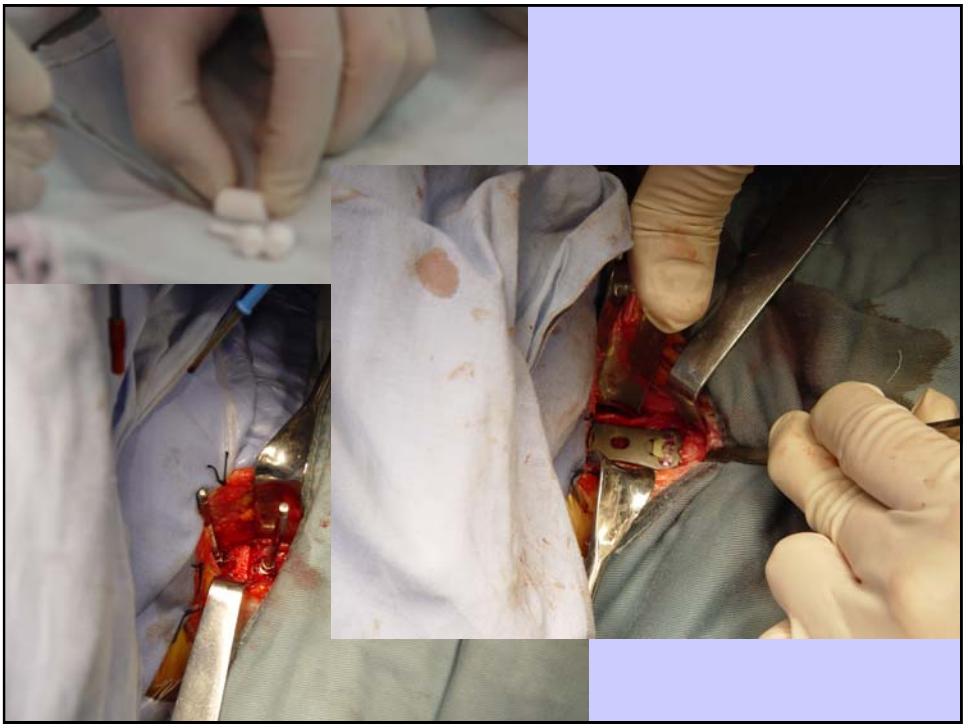
TEM micrographs of nHAC/PLA material; insert is selected area of the electron pattern of the central part of the image.

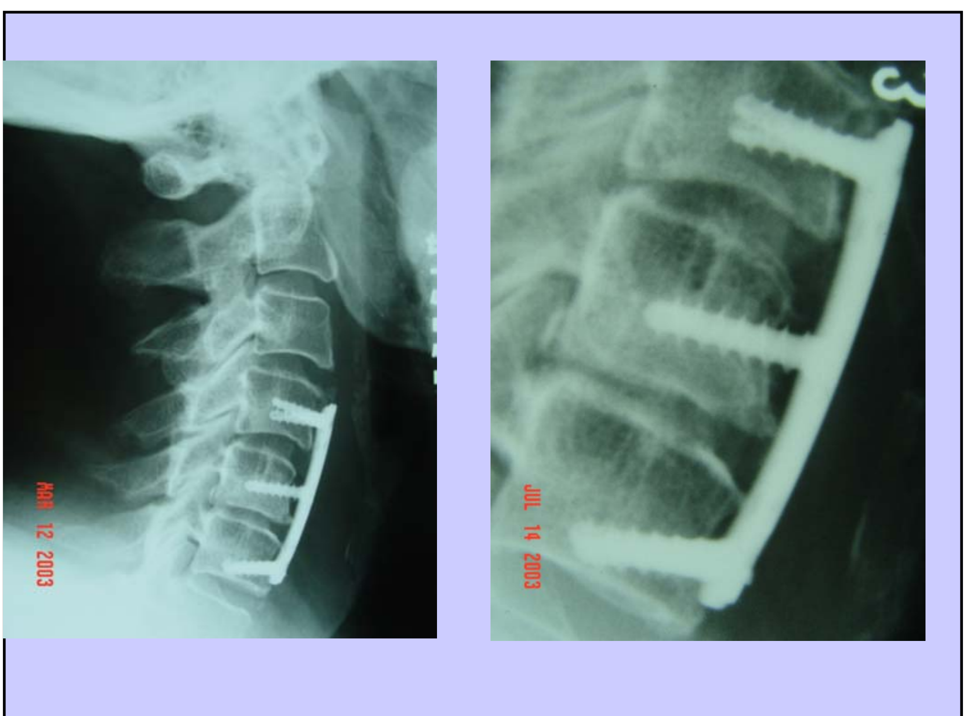


(C) (D)
Group nHAC/PLA+rhBMP-2 4 weeks,

the new trabecular bone (TB) distribute in the whole fusion mass area, the mature bone matrix were shown as particle to block, 200× and 100×. The white arrows refer to the osteoblast and osteocyte around the new trabecular bone.

Image removed due to copyright restrictions.
Diagram of "Lumbar Intertransverse Spinal Fusion of Rabbits."





Slides courtesy of Prof. Fu-Zhai Cui. Used with permission.