Bionanotechnology: The Use of Nanotechnology for Biomedical Applications

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http://www.nanotechcongress.com

Definitions

Nanotechnology: The use of materials whose components exhibit significantly changed properties by gaining control of structures at the atomic, molecular, and supramolecular levels.

Tissue Engineering: The creation, repair, and/or replacement of tissues and organs by using a combination of cells, biomaterials, and/or biologically active molecules.

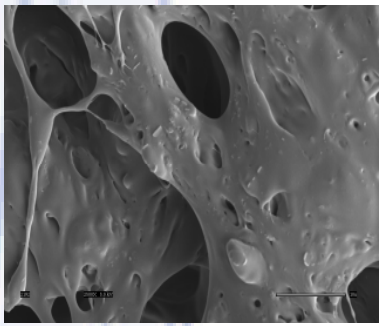
So what do

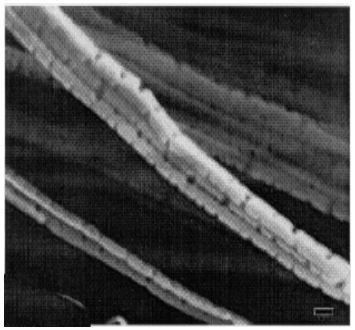
Nanotechnology and

Tissue Engineering

have in common ?

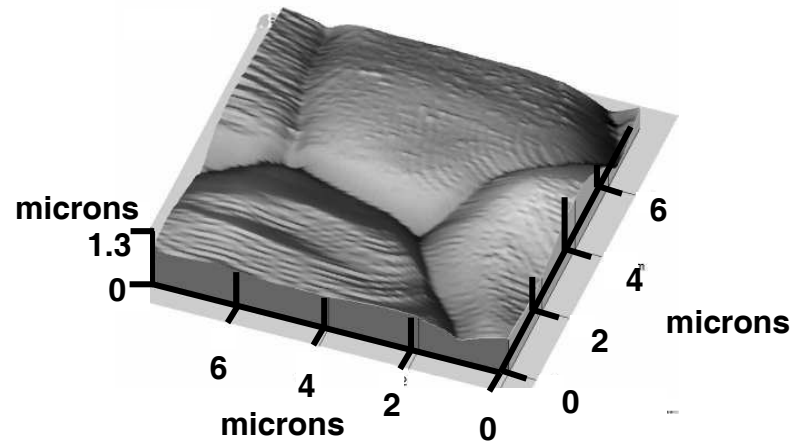
Closer Look at Tissue Engineering: Successful Tissue Engineering Materials



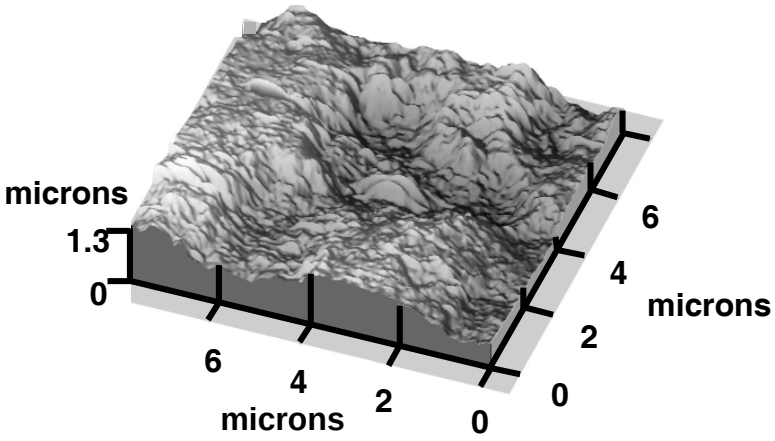


- Formation and maintenance of viable tissue closely apposed to the surface of biomaterials is essential for their clinical success.
- Novel materials are needed which possess properties to support cell adhesion leading to new tissue growth.
- This is true for any tissue engineering application.

Closer Look at Nano-technology in Biomedical Applications



Conventional Grain Size



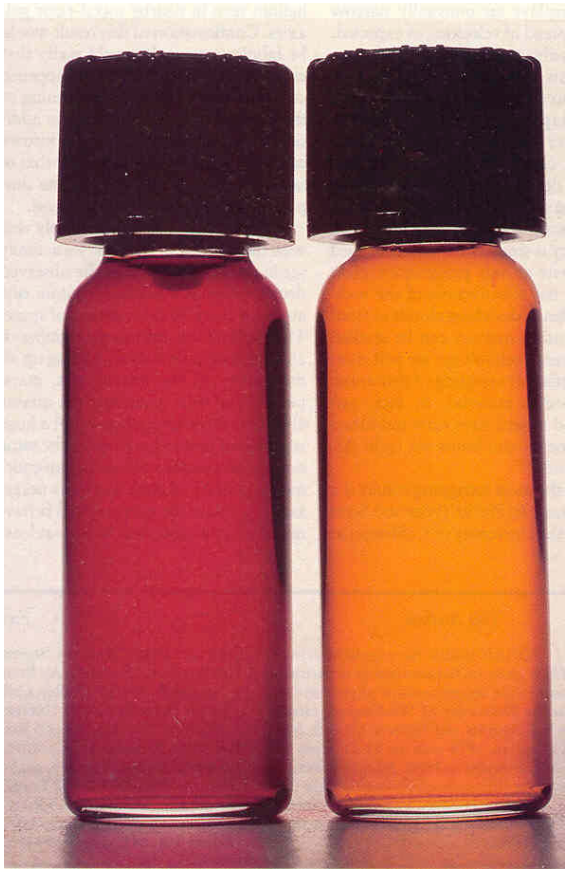
Nanophase Grain Size

Compared to conventional grain size materials, nanophase materials possess enhanced:

- processing,
- catalytic,
- optical,
- mechanical,
- electrical, and
- surface

properties that may improve existing biomedical implant applications.

Special Optical Properties of Nanophase Materials

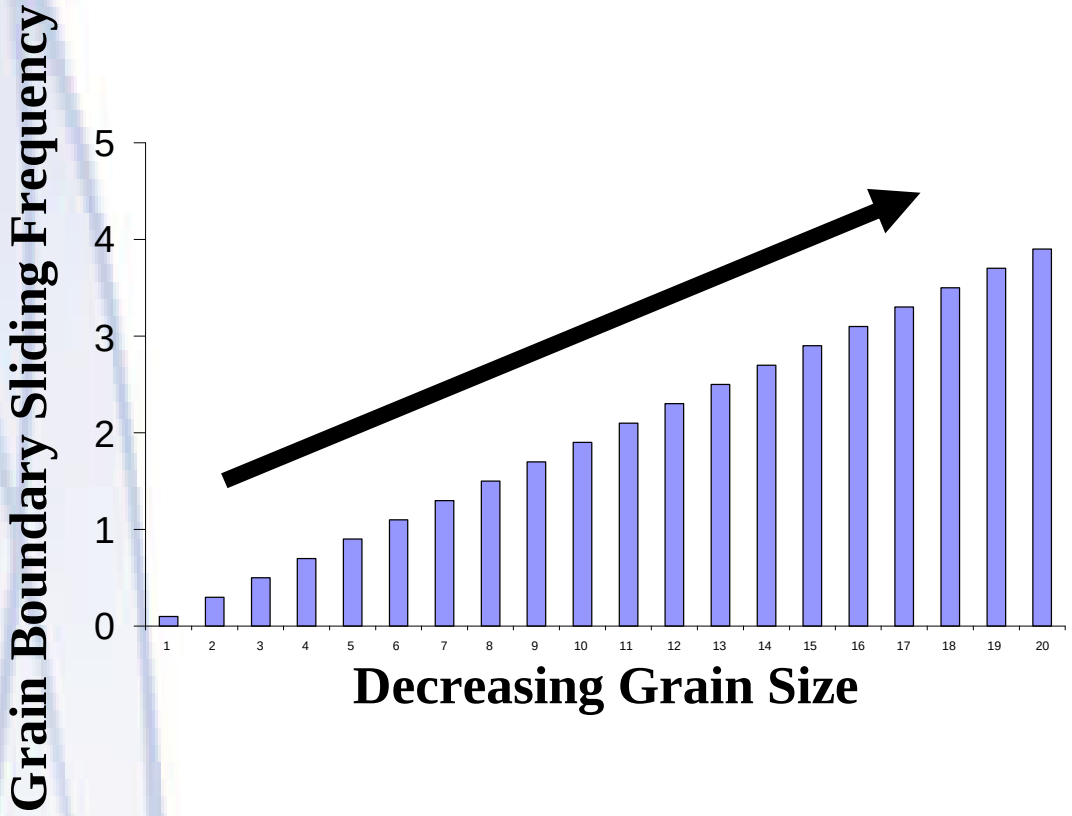


- Compared to conventional grain size materials, nanophase materials have unique optical properties largely unexplored in biomedical applications.

- We can now synthesize UV and visible light transparent ceramics that may enhance existing biomedical implant applications.

From Siegel RW, Scientific American 1996, *275*:121.

Special Mechanical Properties of Nanophase Materials

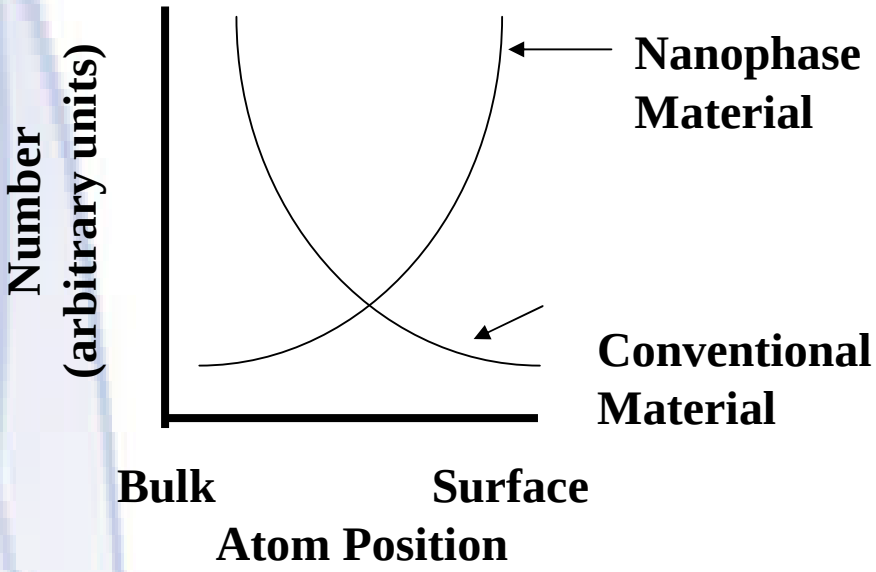


- **Compared to conventional grain size ceramics, nanophase ceramics have increased grain boundary sliding which may be useful in biomedical implant applications.**

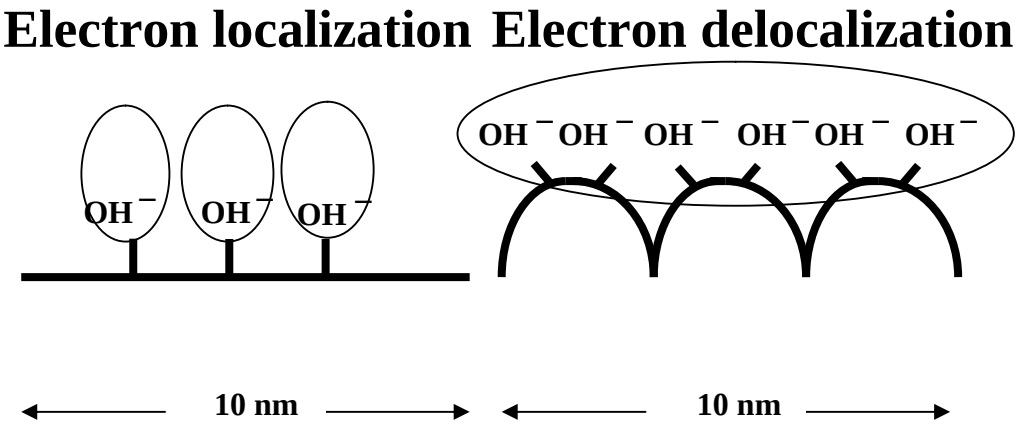
T. J. Webster, in Advances in Chemical Engineering Vol. 27, Academic Press, NY, pgs. 125-166, 2001.

But, today, we will concentrate on special surface properties of nanophase materials important for tissue engineering applications.

Special Surface Properties of Nanophase Materials



Higher number of atoms at the surface for nanophase compared to conventional materials.



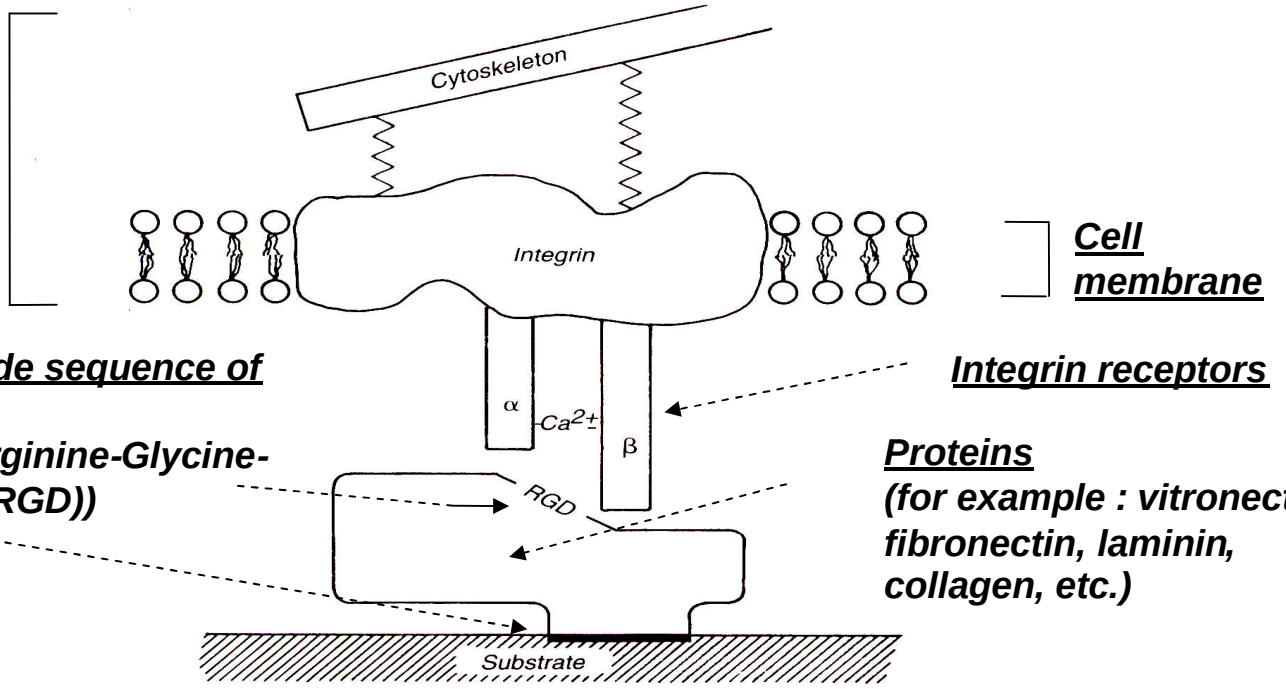
(a) Conventional (grain size: > 100 nm)

(b) Nanophase (grain size: < 100 nm)

Nanophase materials have higher surface areas, possess greater numbers of atoms at the surface, altered electron delocalization, etc*.

T. J. Webster, in Advances in Chemical Engineering Vol. 27, Academic Press, NY, pgs. 125-166, 2001.

Successful Tissue Engineering Materials Depend on Optimal Surface Properties for Cell Function



Cell

Cell membrane

Adhesive peptide sequence of protein
(for example Arginine-Glycine-Aspartic Acid (RGD))

Integrin receptors

Proteins
(for example : vitronectin, fibronectin, laminin, collagen, etc.)

Surface properties affecting protein conformation/bioactivity:
Wettability; topography; etc.

T. J. Webster, in Advances in Chemical Engineering Vol. 27, Academic Press, NY, pgs. 125-166, 2001.

Why Nanophase Materials for Tissue Engineering ?

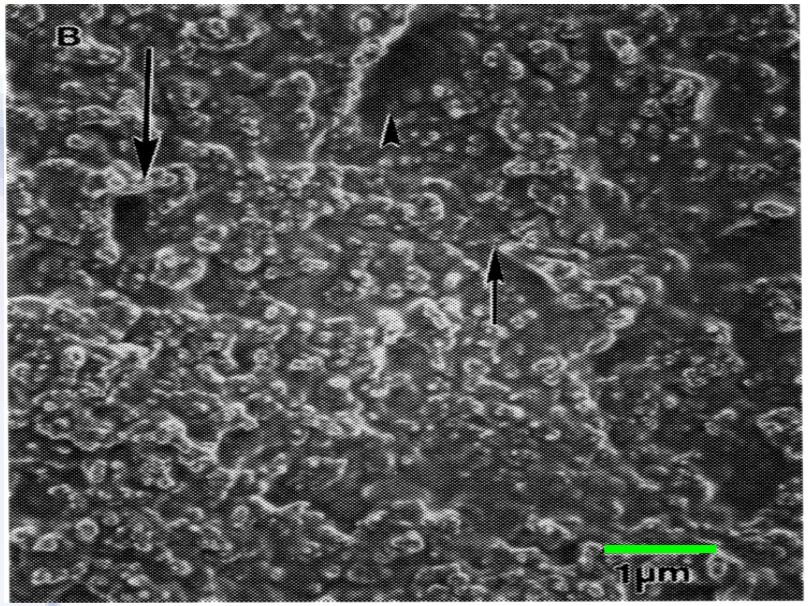
- **Proteins contained in extracellular matrices are nano-structured, thus, cells in our bodies are accustomed to interacting with nanophase materials.**

Characteristic Nano-dimensions of Proteins in Natural Tissue*

Protein	Characteristic Dimensions
Fibronectin	Dimer of two identical subunits; 60-70 nm long; 2-3 nm wide.
Vitronectin	Linear molecule 15 nm long.
Laminin	Cruciform configuration with one 50 nm long arm and two 35 nm long short arms; total length 50 nm; total width 70 nm.
Collagen	Triple helical linear protein consisting of 2 α(1) chains and one α(2); 300 nm long; 0.5 nm wide; 67 nm periodicity.

***Data obtained from Ayad *et al.*, 1994.**

Why Nanophase Materials for Tissue Engineering ?



Cast Replica of Vascular Tissue Demonstrating Nanometer Roughness *

— Bar = 1µm

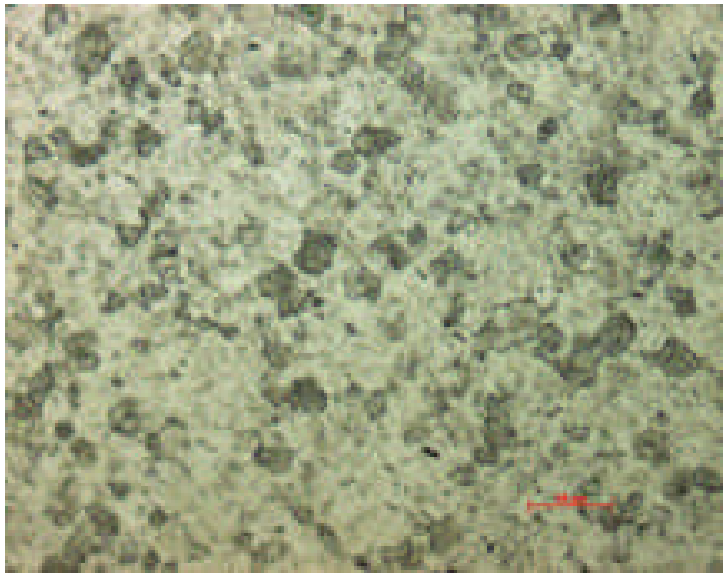
- **Due to the presence of numerous nano-structures (i.e., proteins) in the body, cells are accustomed to interacting with surfaces that have a large degree of nanometer roughness.**
- **Despite this fact, current synthetic materials used as tissue engineering scaffolds possess conventional surface features only.**

*Goodman S.L. et al., Biomaterials. 1996 Nov;17(21):2087-95.

Non-biologically Inspired Surface Roughness of Conventional Implants

- **It is believed that one reason why current orthopedic implants only have a 15 year lifetime is due to non-biologically-inspired surface roughness.**

- **Such surface roughness does not promote sufficient new bone growth for long term implant integration into surrounding bone.**



Conventional (Rolled) Ti Sheet:
ASTM grain size number, 7.5; ave. grain diameter, 50 µm; bar = 100 µm.

Objective

- **The objective of the studies to be presented was to determine whether *in vitro* cell functions and *in vivo* responses can be increased on biologically-inspired nano-structured surfaces.**

Ways to Synthesize Nanophase Materials

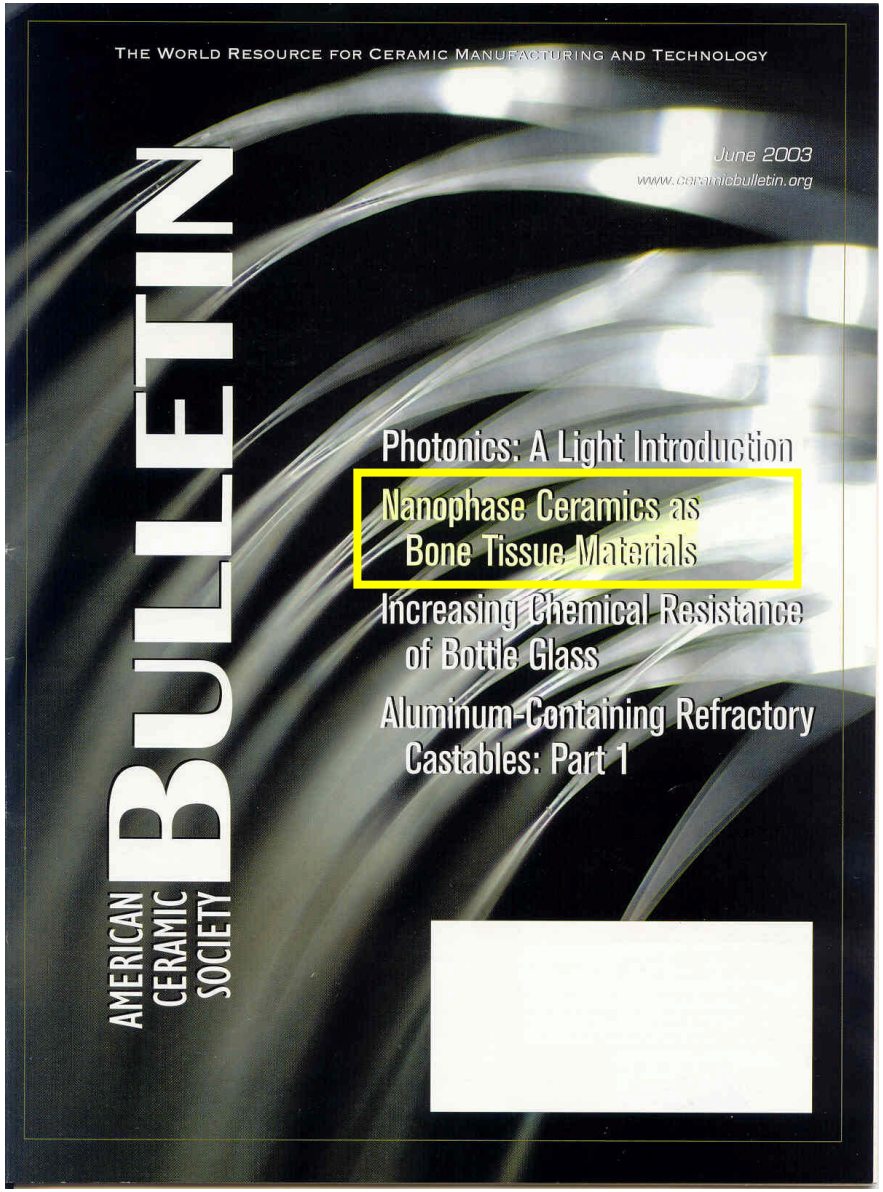
- There are many techniques to synthesize nanophase materials (or nano-structured surface roughness):
  - Physical Vapor Synthesis,
  - Electro-explosion,
  - Chemical Vapor Deposition (CVD),
  - Sol-gel,
  - Nanolithography,
  - Chemical Etching, and
  - etc.
- However, altered cell behavior seems to be independent on the methods used and as long as a nanostructured roughness is created, increased tissue regeneration results.

Targeted Applications

- **Increased tissue regeneration has been demonstrated on nanophase compared to conventional materials for:**
  - **bone,**
  - **cartilage,**
  - **vascular,**
  - **bladder, and**
  - **neural**

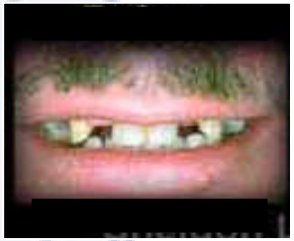
applications.

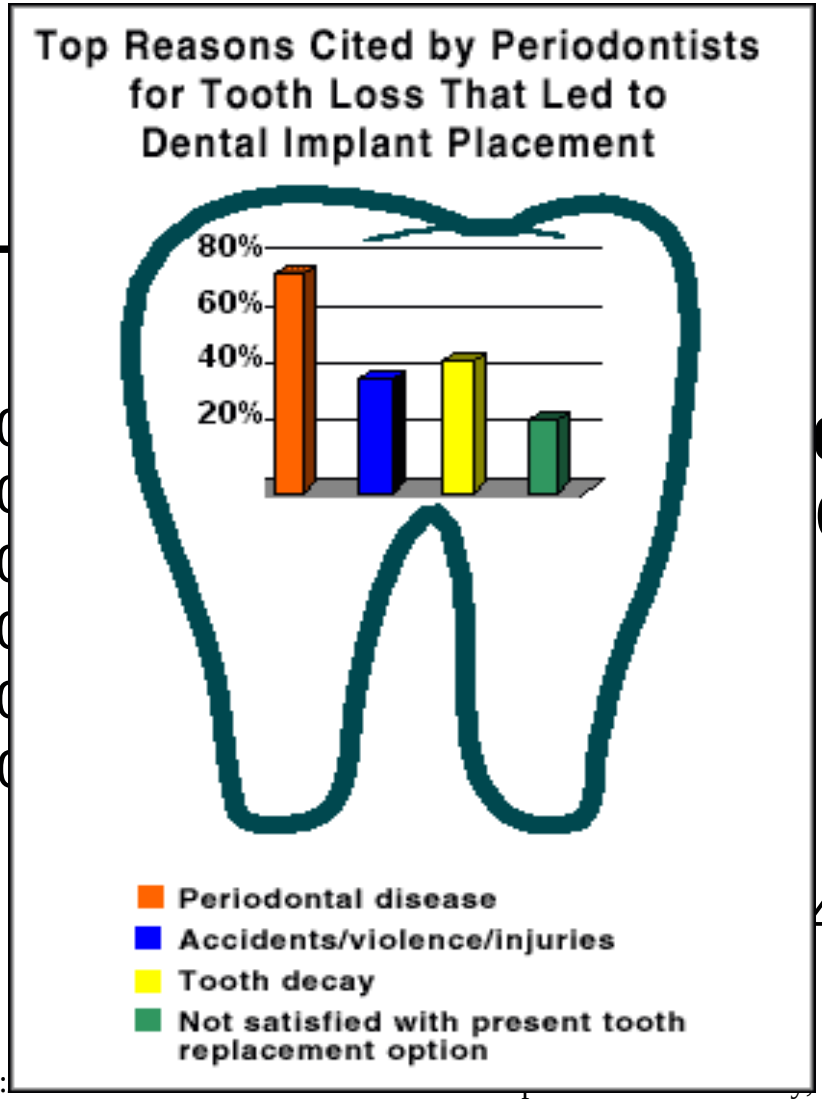
PART I BONE:
Nanospherical Ceramics



***American Ceramic Society Bulletin*, 82(6): pp. 1 – 8, 2003.**

The Problem:
Current Orthopedic Implant Failures



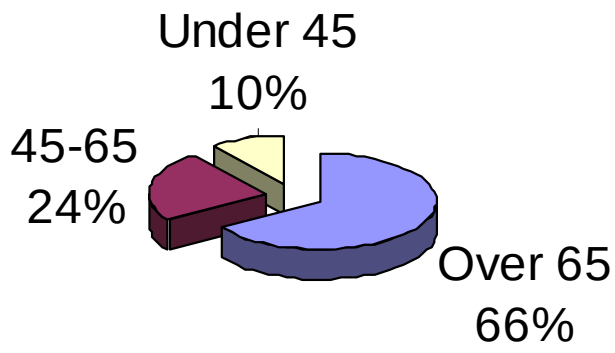


12.8% of the hip arthroplasties performed were revision surgeries

An estimated 300,000 dental implants have been used in the United States

http://www.aaos.org/wordhtml/press/arthropl.htm; http:[illegible] *et al.* The Journal of Contemporary Dental Practice 2003; 4(2):035-050.
http://www.perio.org/consumer/implants.survey.htm; http://www.asmileawaitsyou.com/missng.htm

The Problem:
Current Orthopedic Implant Failures

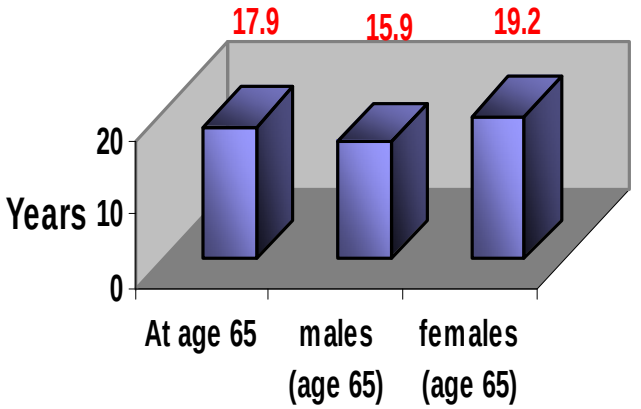


Avg. Implant Lifetime ≈ 12-15 years

Dowson D. Proceedings of the Institution of Mechanical Engineers. Part H- Journal of Engineering in Medicine 2001; 215(4): 335-358.

25% failure rate for dental implants after 15 years

Many patients receiving the implants are 35-54 years old



http://www.aaos.org/wordhtml/press/joinrepl.htm; http://www.aaos.org/wordhtml/press/hip_knee.htm; Minino AM, MPH, and Smith BL. National Vital Statistics Reports 2001; 49(12); http://www.cdc.gov/nchs/fastats/lifexpec.htm; http://www.oxmed.com/docs/datafiles/hip%20replacement%20cemented%20or%20uncemented.html

The Problem:
Current Orthopedic Implant Failures

Inflammation

Fibrous Tissue Encapsulation → Wear Particles →

Cell Adhesion → Interfacial Stresses and Strains →

Direct Bone Apposition → Microdamage to Surrounding Bone →

Osteolysis
Loosening
Pain

FAILURE!!

More Fully Integrated Interface

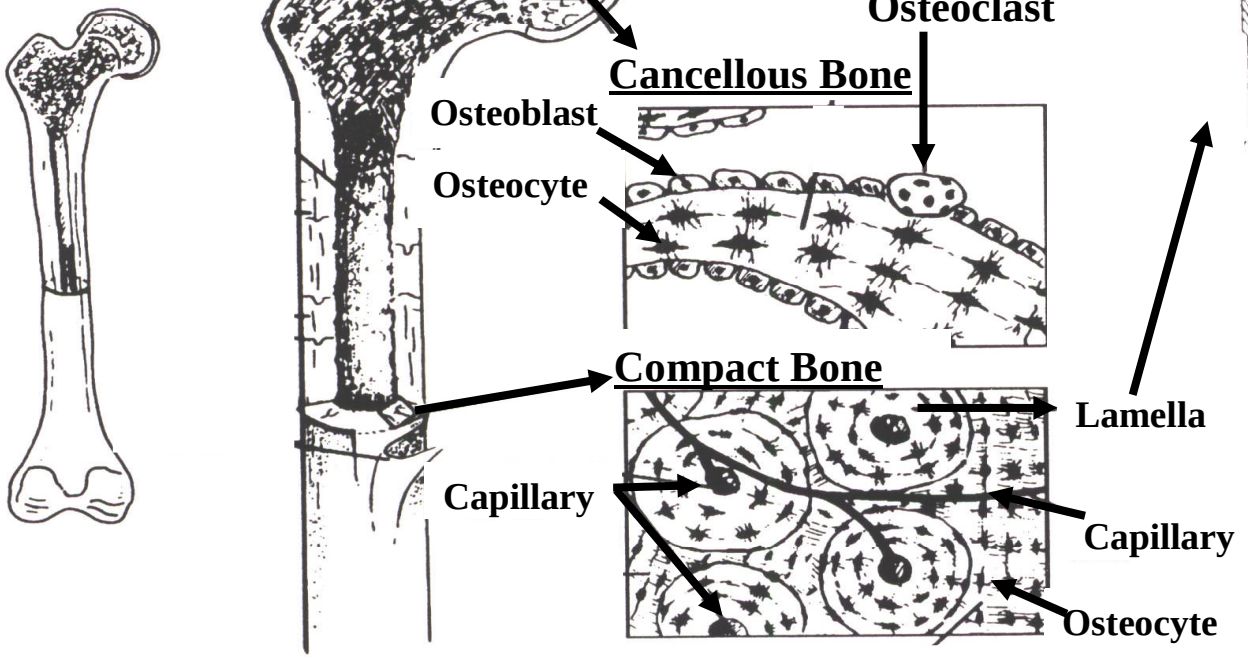
Anderson JM *et al.* Host reactions to biomaterials and their evaluation. In: Biomaterials science: An introduction to materials in medicine. San Diego: Academic Press, Inc., 1996. p. 165-214.

Dowson D. Proceedings of the Institution of Mechanical Engineers. Part H- Journal of Engineering in Medicine 2001; 215(4): 335-358.

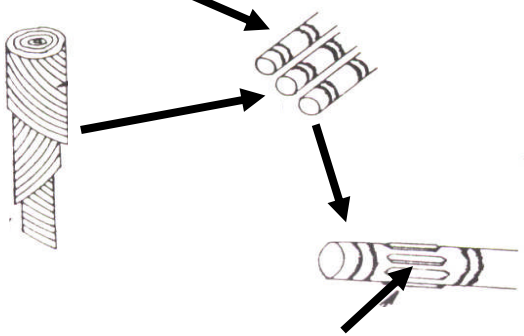
Huiskes R and Boeklagen R. Biomat 1989; 22: 793-804.

http://www.dental-implants.com/implant_systems/system_types.html

Bone is a Nanophase Material



Collagen Fibers: composed of Type I collagen which is a triple helix 300 nm in length; 0.5 nm in width; and periodicity of 67 nm

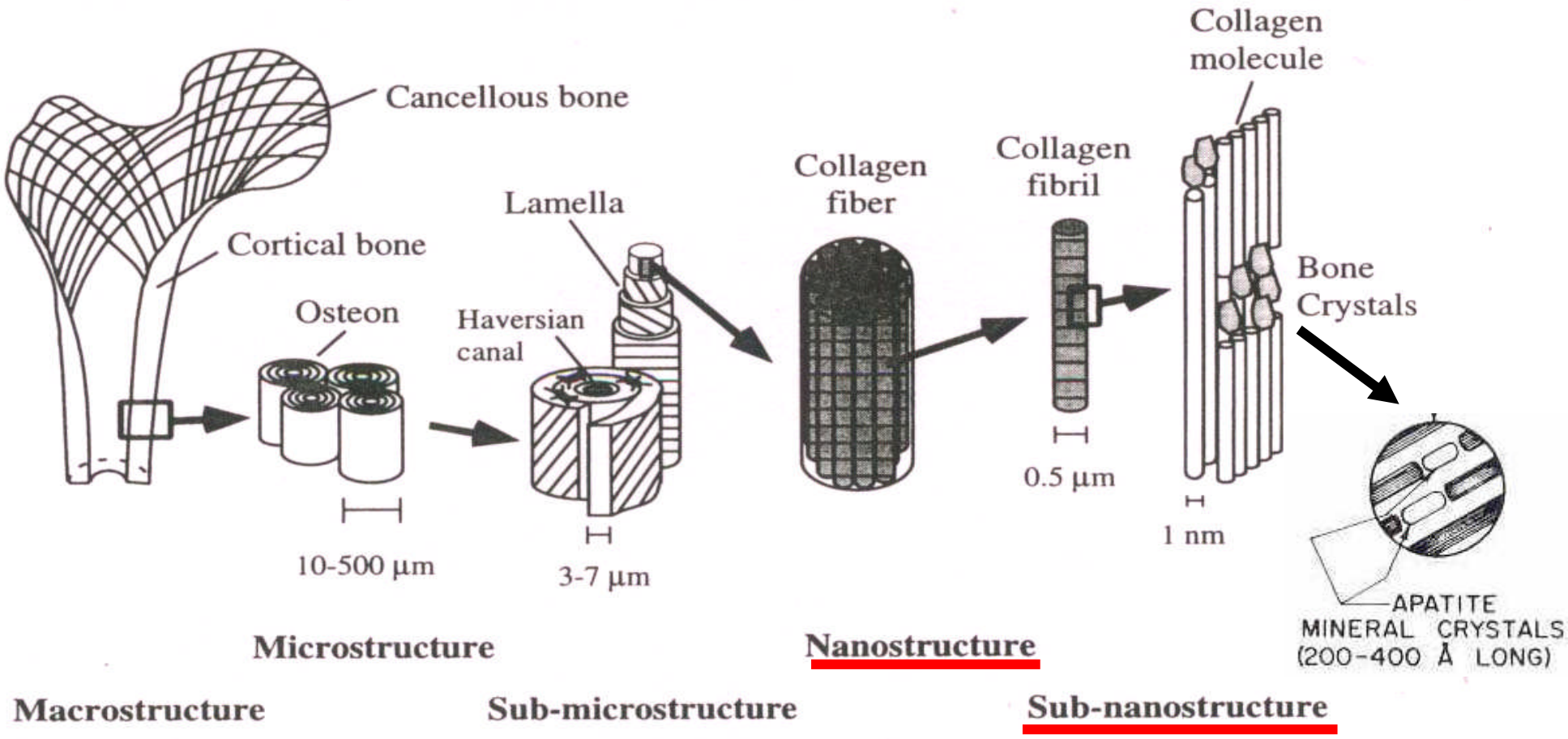


Hydroxyapatite Crystals: less than 50 nm in length and 5 nm in diameter

Redrawn and adapted from Fung Biomechanics: Mechanical Properties of Living Tissue, Springer-Verlag, New York, 1993 and Keaveny and Hayes, Bone 7:285, 1993.

Hierarchical Level of Bone Structure

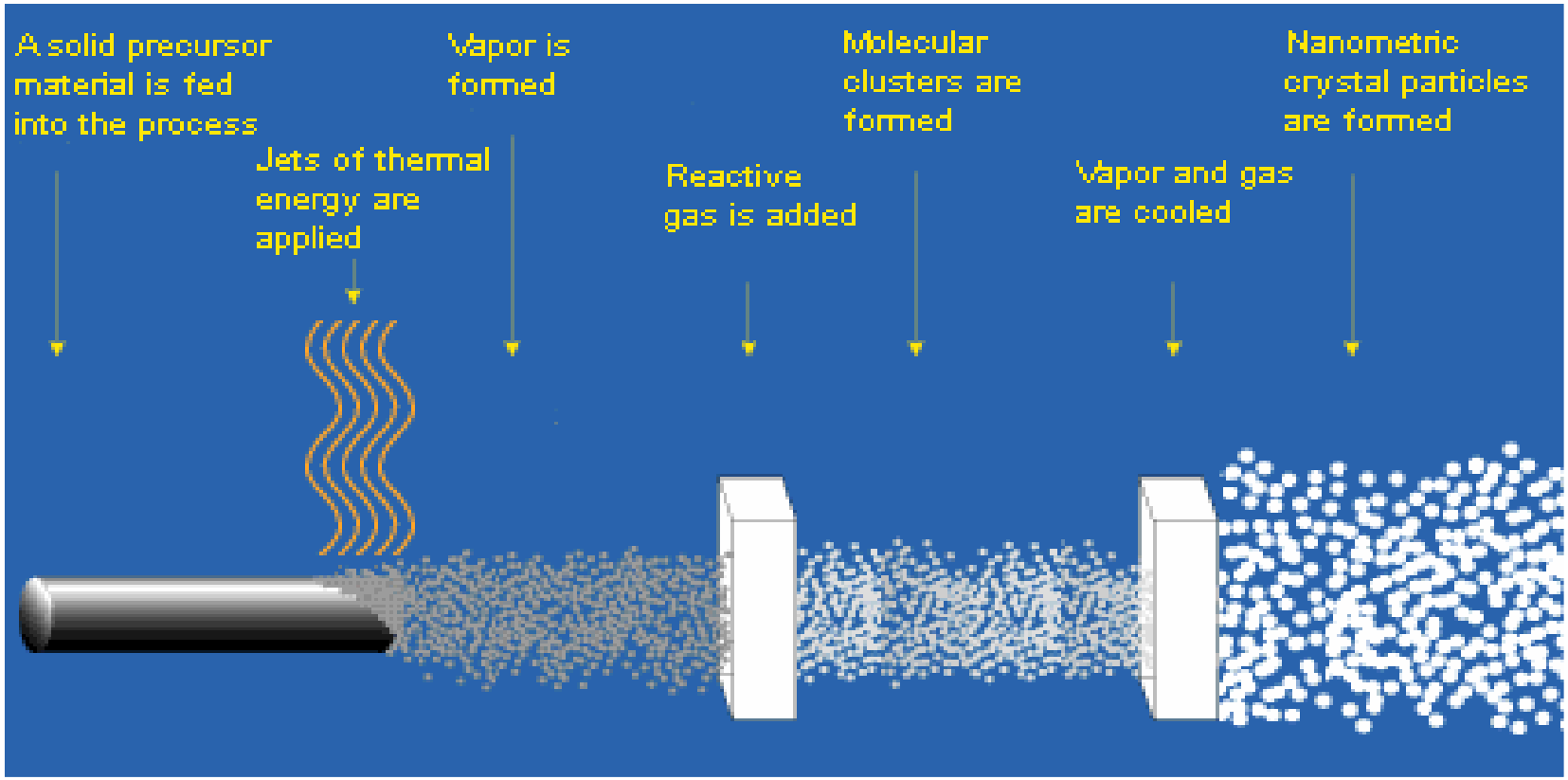
Cells interact with nanostructures & sub-nanostructures



Cowin et al., (1987) Handbook of bioengineering. McGraw Hill: New York

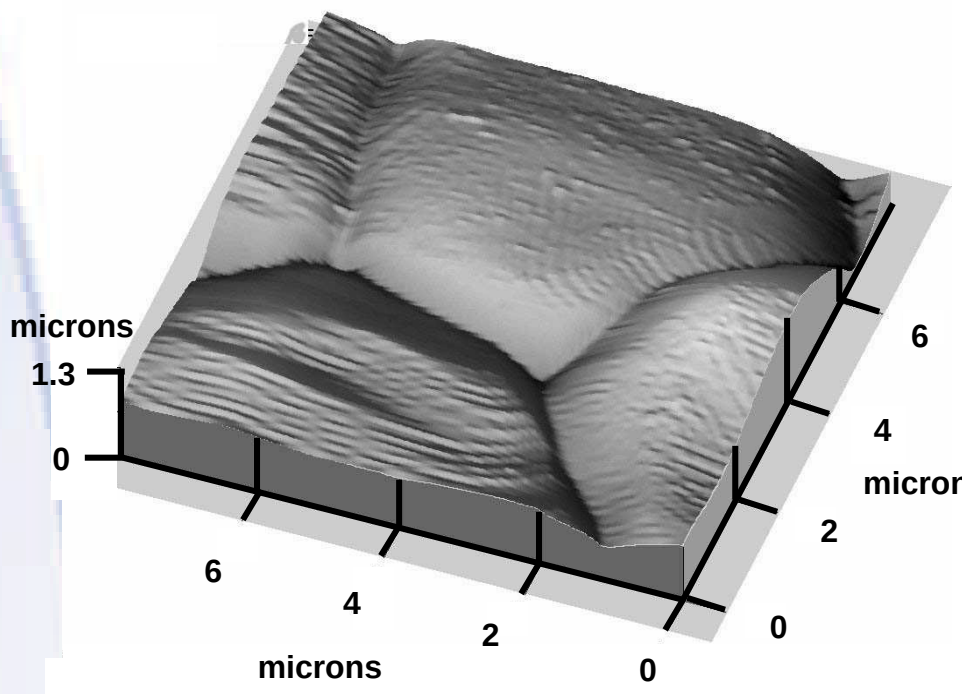
Nanospherical Ceramic Synthesis

- **Physical Vapor Synthesis was used:**
  - **Arc energy applied to solid metal which creates a vapor at high temperature.**
  - **A reactant gas is added and cooled at a controlled rate.**
  - **The vapor condenses to form nanoparticles with a defined crystallinity.**

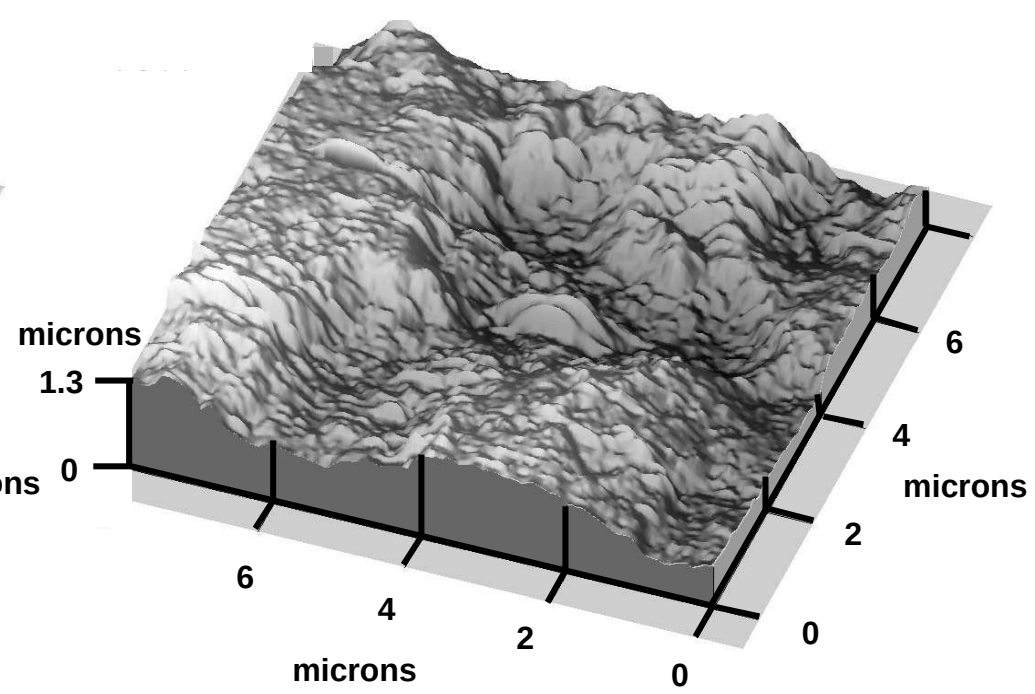


From T. J. Webster, in Advances in Biochemical Engineering/Biotechnology (K. Lee and D.L. Kaplan, editors), Springer-Verlag, in press, 2005.

Nanospherical Ceramic Synthesis



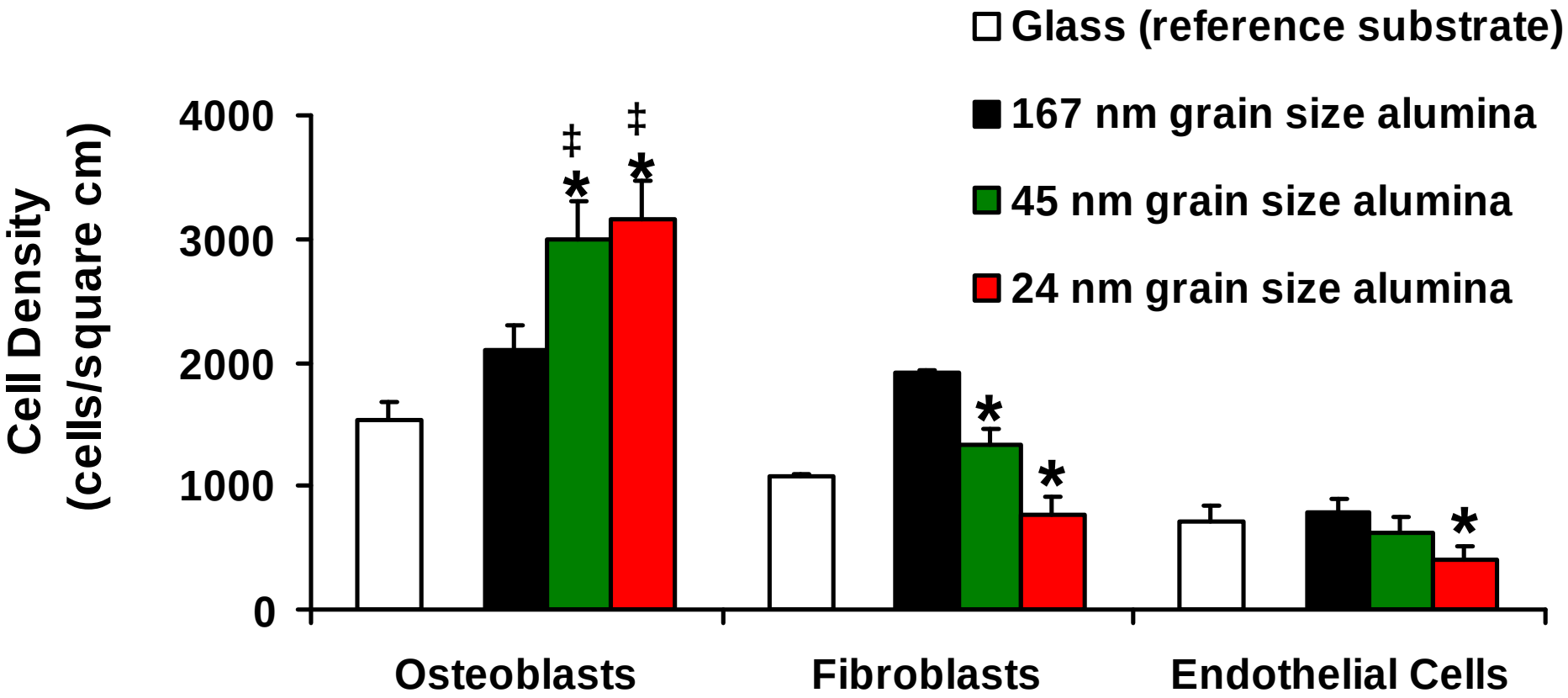
4,520 nm (conventional) Titania



39 nm (nanophase) Titania

Webster TJ, Siegel RW, Bizios R, *Biomaterials* 20:1221, 1999.

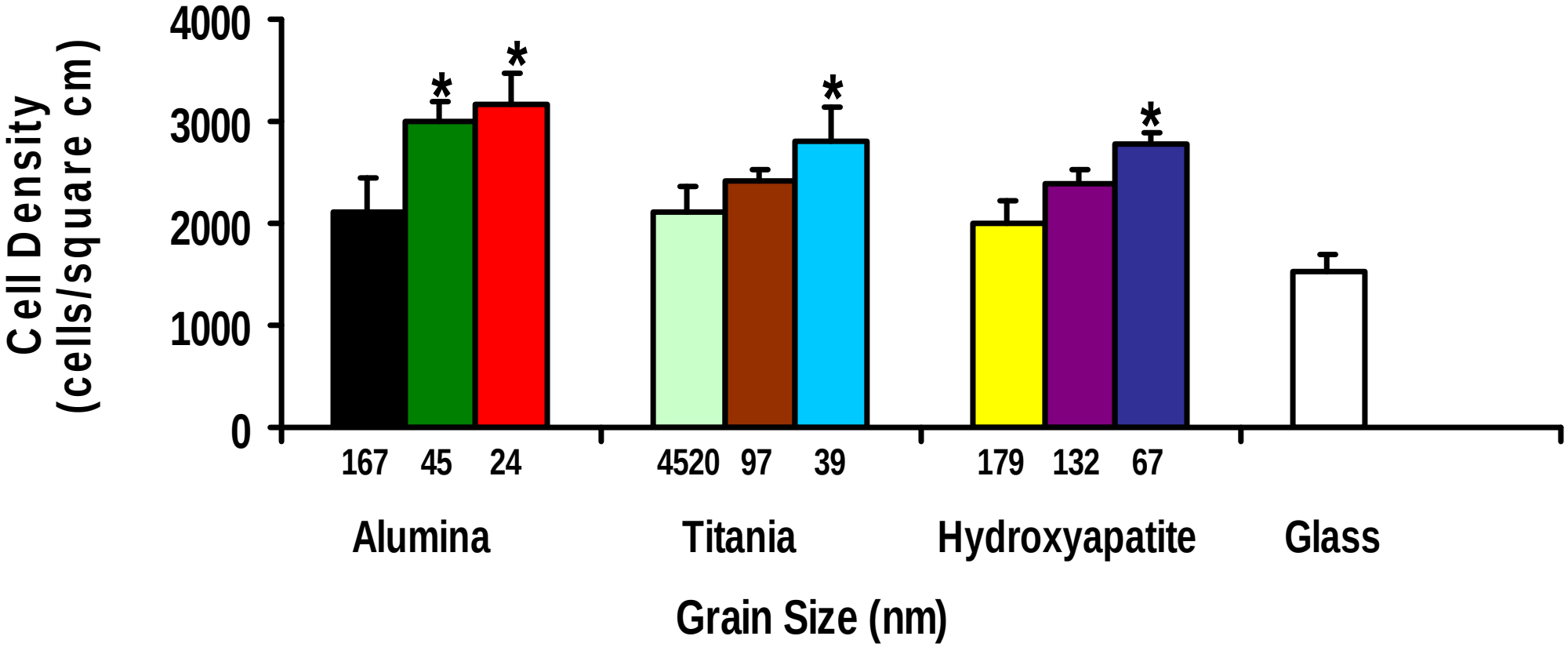
Comparison of Cell Adhesion on Nanophase Alumina



Culture medium = DMEM supplemented with 10% fetal bovine serum. Adhesion time = 4 hours. Values are mean +/- SEM; n = 3; * $p < 0.01$ (compared to 167 nm grain size alumina); ‡ $p < 0.01$ (compared to fibroblast and endothelial cell adhesion on respective grain size alumina).

T. J. Webster, C. Ergun, R. H. Doremus, R.W. Siegel, and R. Bizios, "Specific proteins mediate enhanced osteoblast adhesion on nanophase ceramics," *Journal of Biomedical Materials Research* 51:475-483 (2000).

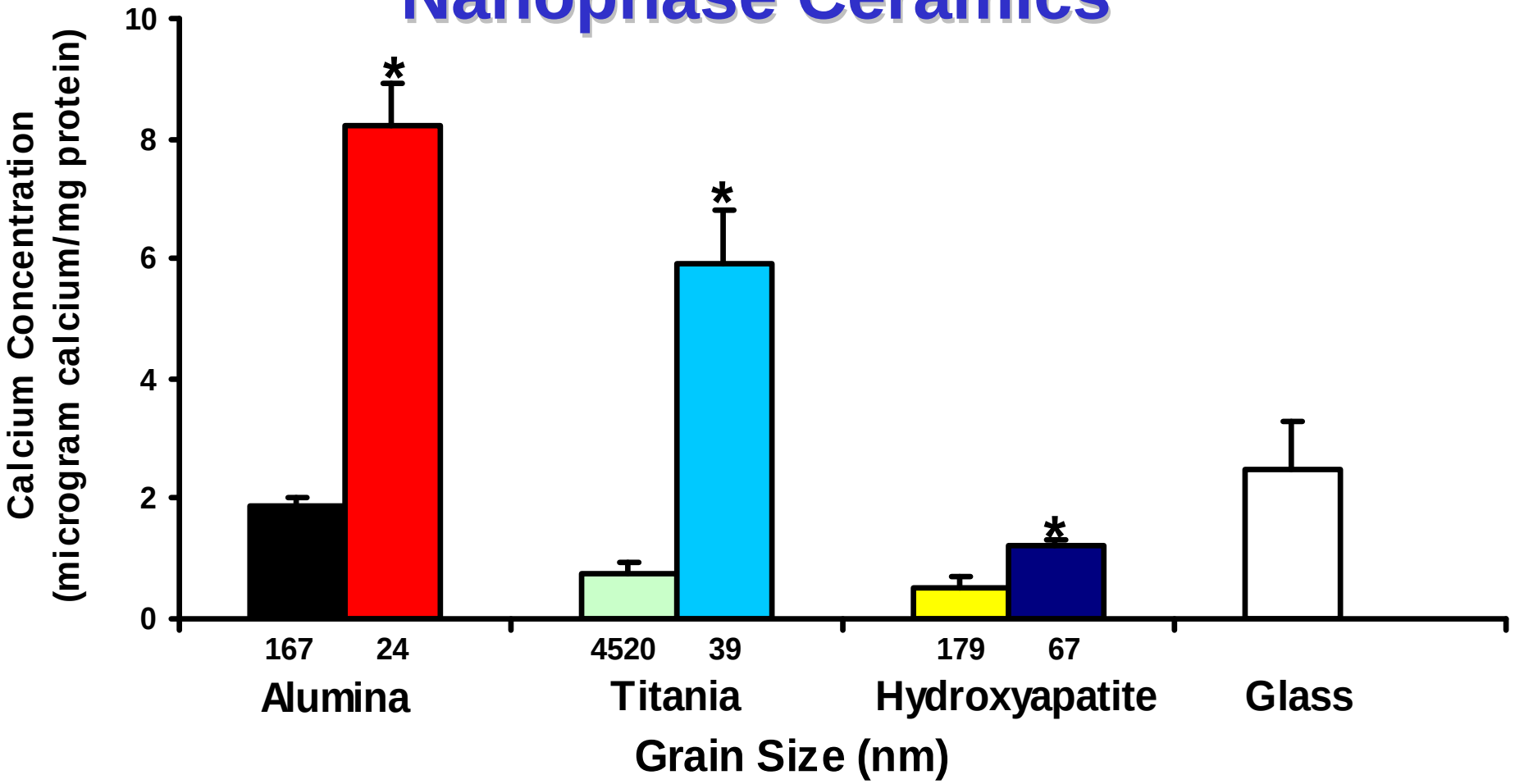
Enhanced Osteoblast Adhesion on Nanophase Ceramics



Culture media = DMEM supplemented with 10% fetal bovine serum. Adhesion time = 4 hours. Values are mean +/- SEM; n = 3; * $p < 0.01$ (compared to respective conventional grain size ceramic).

T. J. Webster, C. Ergun, R. H. Doremus, R.W. Siegel, and R. Bizios, "Specific proteins mediate enhanced osteoblast adhesion on nanophase ceramics," *Journal of Biomedical Materials Research* 51:475-483 (2000).

Enhanced Calcium Deposition on Nanophase Ceramics

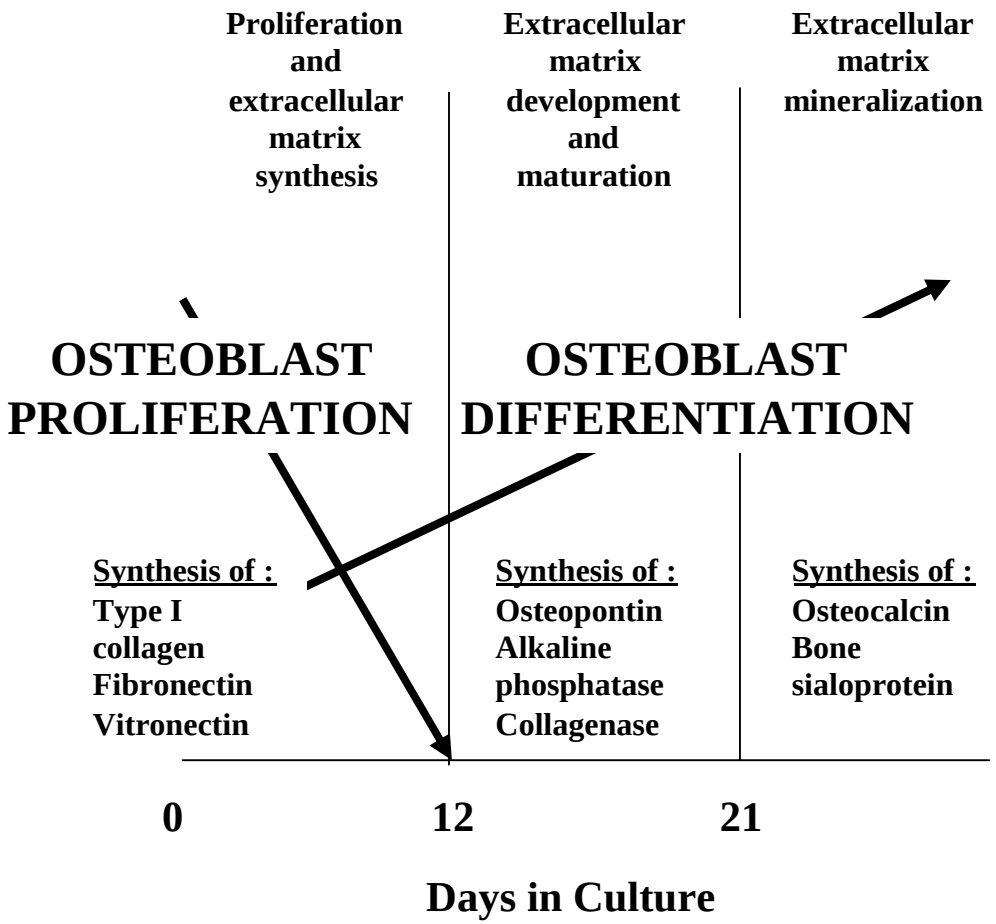


Culture medium = DMEM supplemented with 10% fetal bovine serum, 50 micrograms/mL L-ascorbate and 10 mM β-glycerophosphate. Culture time = 28 days. Values are mean +/- SEM; n = 3; * $p < 0.01$ (compared to respective conventional grain size ceramic).

T. J. Webster, R. W. Siegel, and R. Bizios, "Enhanced functions of osteoblasts on nanophase ceramics," *Biomaterials* 21:1803-1810 (2000).

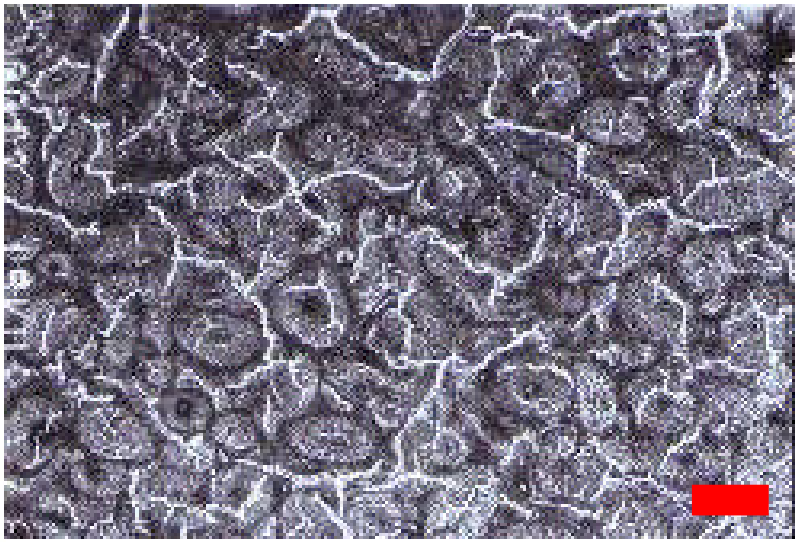
Enhanced Adhesion Translates into Increased Subsequent Functions

Stages of Osteoblast Differentiation

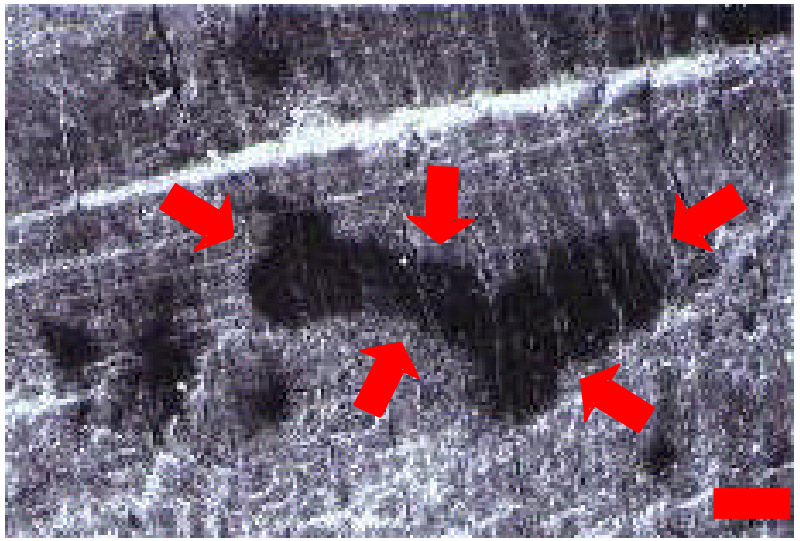


T. J. Webster, in Advances in Chemical Engineering Vol. 27, Academic Press, NY, pgs. 125-166, 2001.

Scanning Electron Micrographs of Resorption Pits on Devitalized Bone



Presence of calcitonin

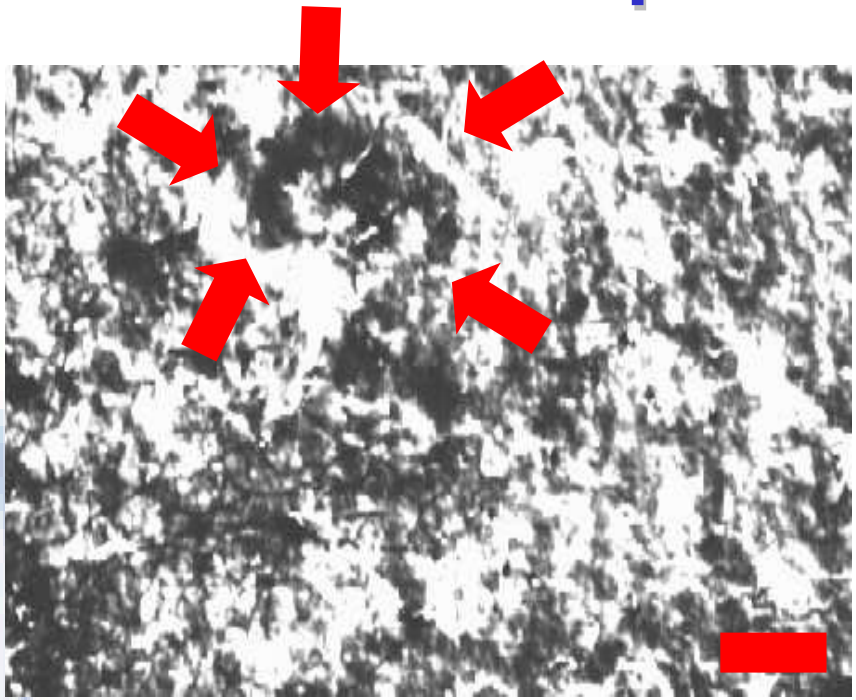


Absence of calcitonin

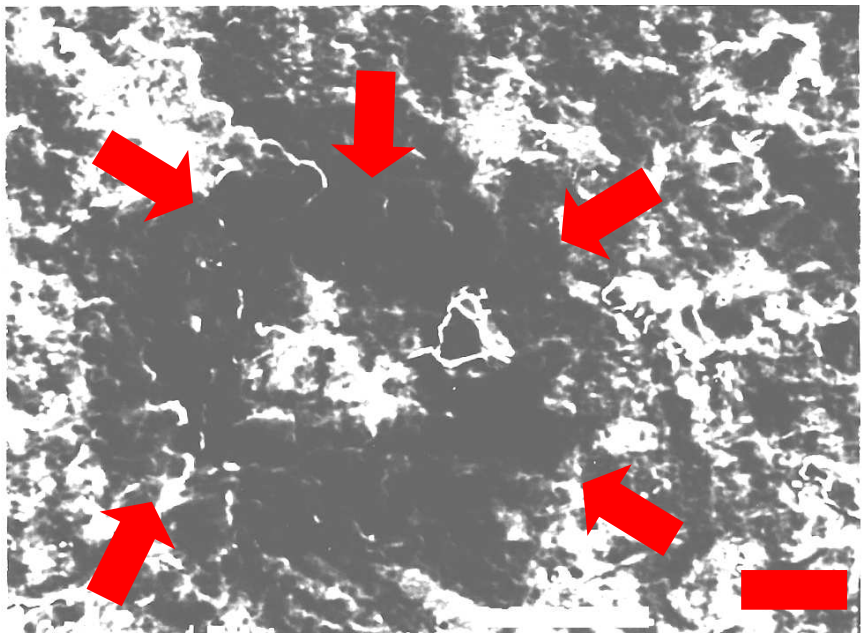
Note: cracks present on surface of devitalized bone occurred during sample preparation for scanning electron microscopy after the cell-culture experiments. **Culture time = 13 days; bar = 100 microns.**

T. J. Webster, C. Ergun, R. H. Doremus, R. W. Siegel, and R. Bizios,
***Biomaterials* 22: 1327-1333 (2001).**

Scanning Electron Micrographs of Resorption Pits on Alumina



167 nm grain size (conventional) alumina

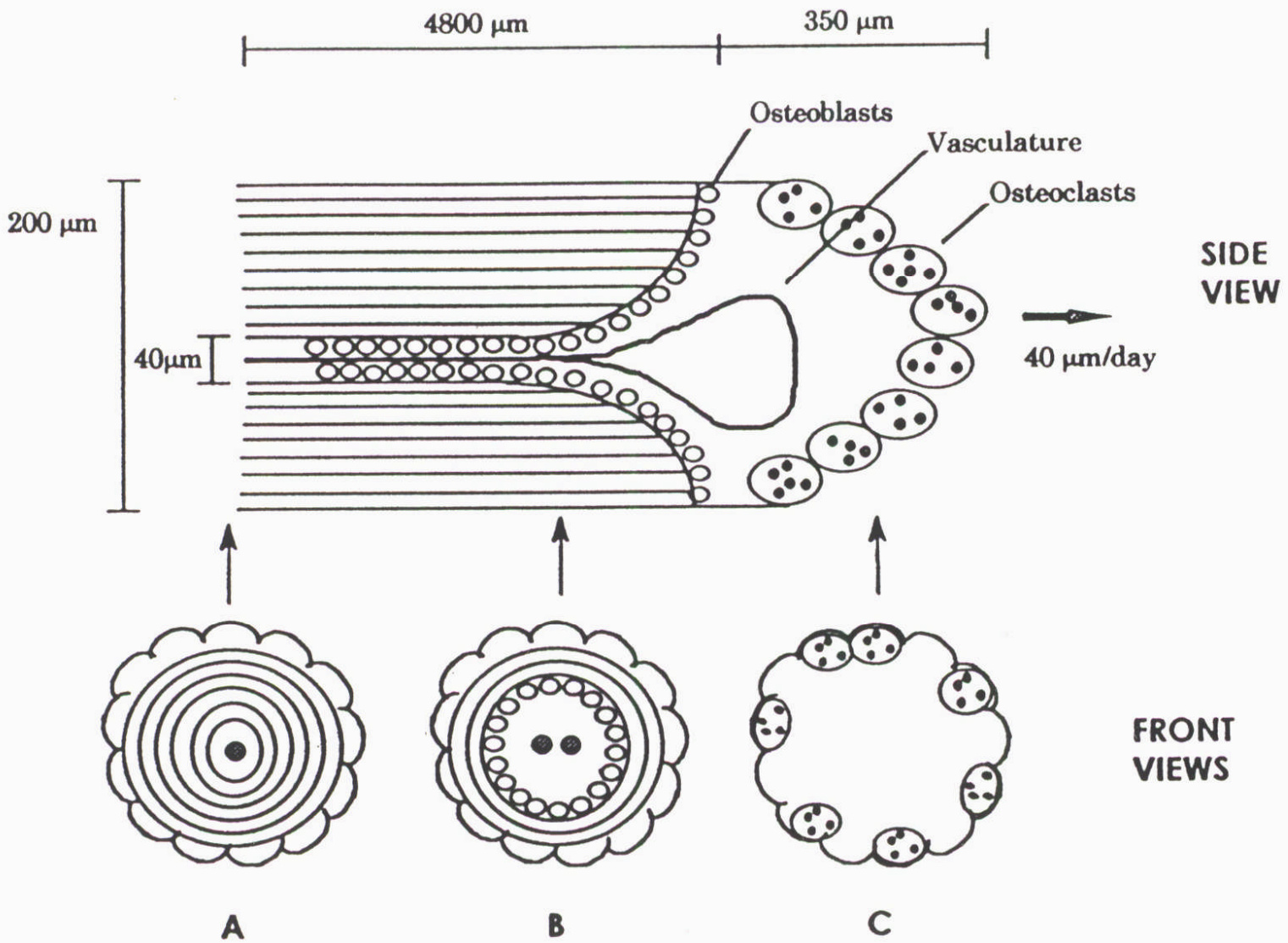


24 nm grain size (nanophase) alumina

Culture medium = DMEM supplemented with 10% fetal bovine serum, 1% antibiotic/antimycotic, and 10^{-8} M Vitamin D_3; culture time = 13 days; bar = 100 microns.

T. J. Webster, C. Ergun, R. H. Doremus, R. W. Siegel, and R. Bizios,
***Biomaterials* 22: 1327-1333 (2001).**

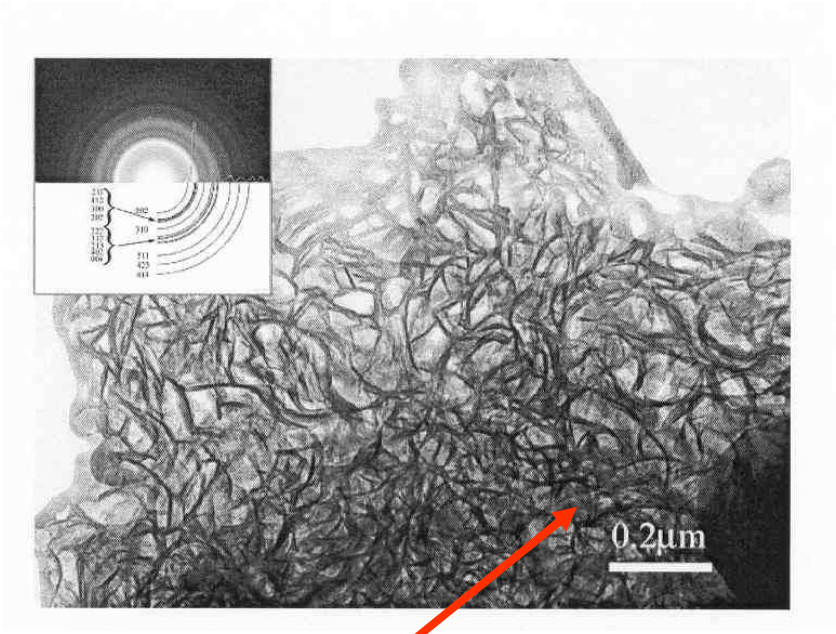
Possible Enhanced Coordinated Functions of Osteoclasts and Osteoblasts on Nanophase Ceramics

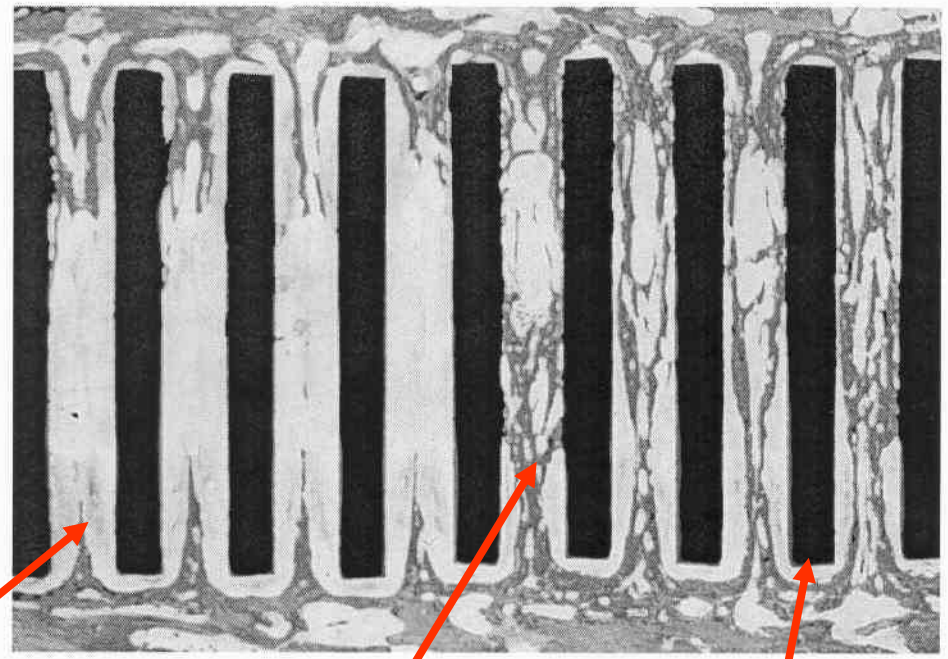


Adapted and redrawn from Martin, B.R. and Burr, D.B., *Structure, Function and Adaptation of Compact Bone*, Raven Press, New York, 1989.

in vitro Results Translate into *in vivo* Results

Novel Nanostructured Apatite Coating Increases *in vivo* Bone Growth





Nano-apatite 100 – 200 nm crystal sizes

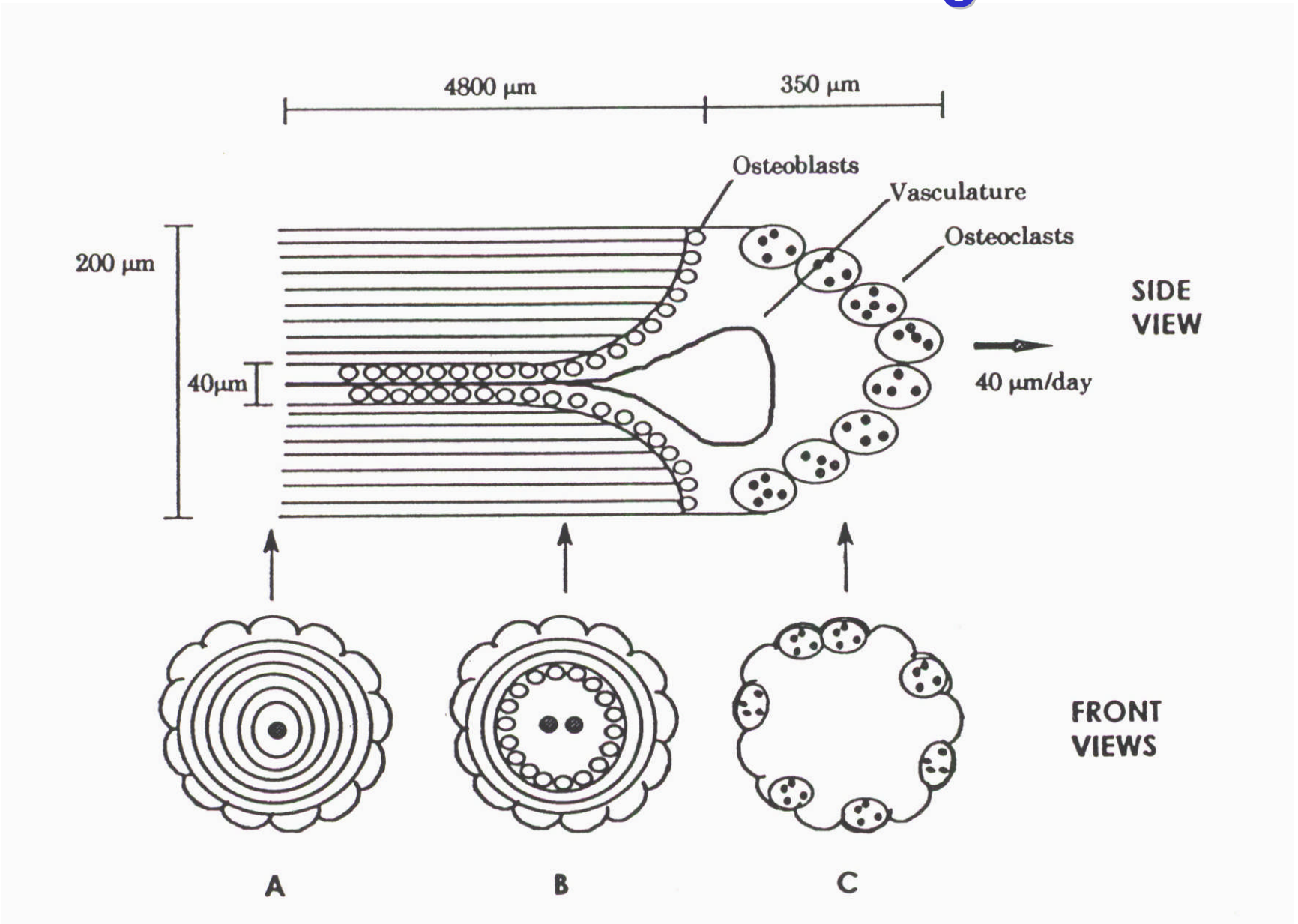
Non-coated Ti

More bone growth

Nano-apatite coated Ti

Li, Journal of Biomedical Materials Research 66A:79-85, 2003.

Function of the Bone-Modeling Unit



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