Bio-nanotechnology: Implications for Designing More Effective Tissue Engineering Materials

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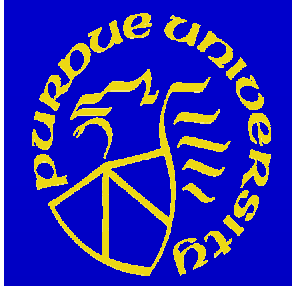
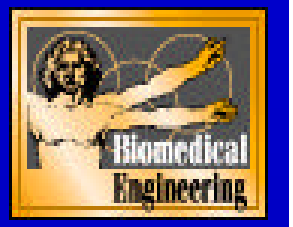
Definitions

Nanotechnology: The use of materials and systems whose structures and components exhibit novel and significantly changed physical and chemical 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.





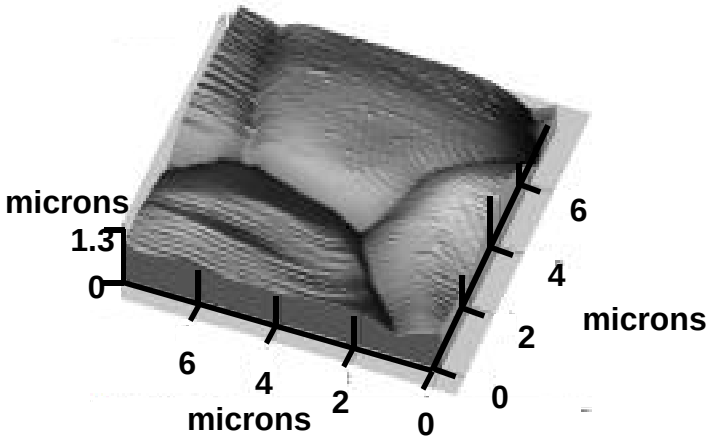
So what do

Nanotechnology and

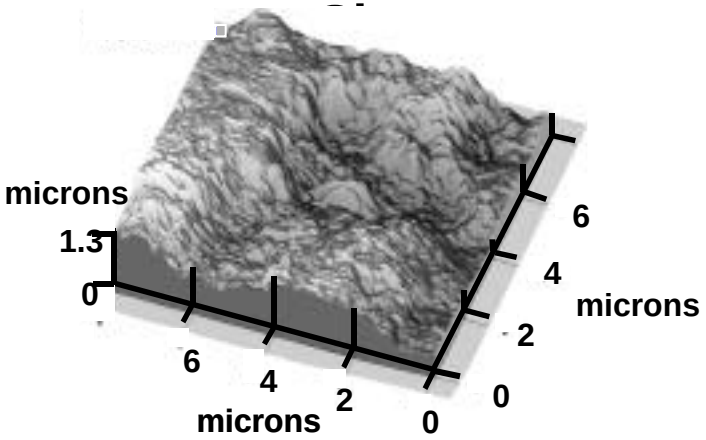
Tissue Engineering

have in common ?

Closer Look at Nano-technology in Biomedical Applications



Conventional Grain



Nanophase Grain Size

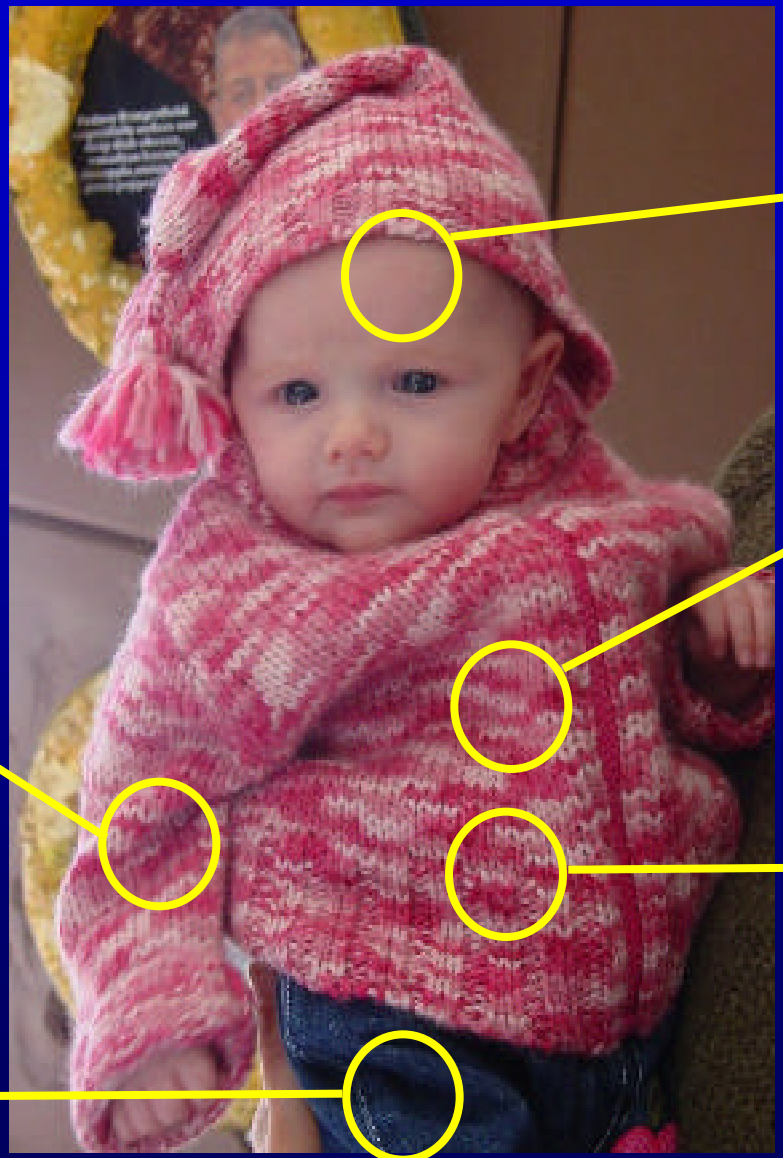
Compared to conventional grain size materials, nanophase materials possess enhanced:

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

properties that may enhance existing biomedical implant applications.

Closer Look at Tissue Engineering

Closer Look at Tissue Engineering



Neural bridges

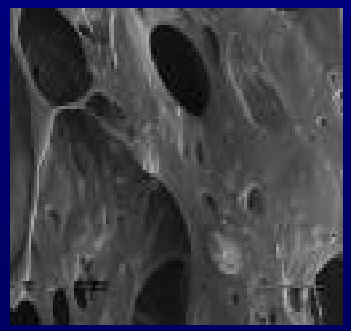
Vascular grafts

Cartilage

Bladder

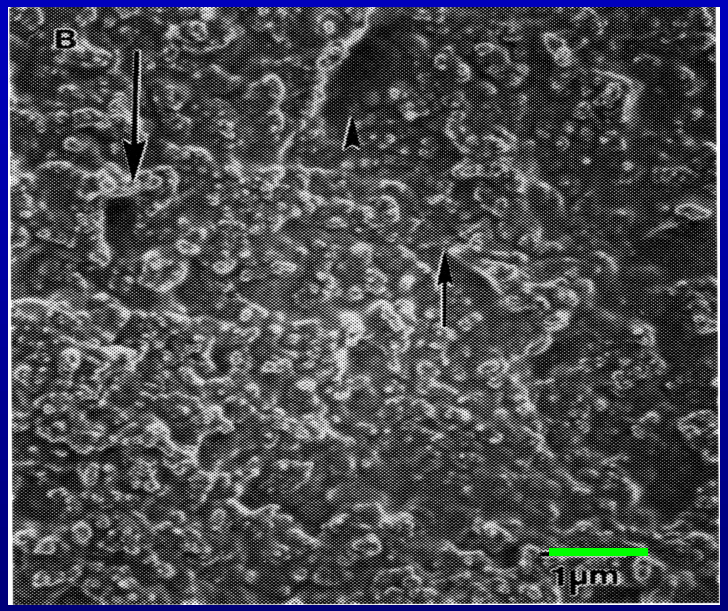
Orthopedic

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.

Special Surface Properties of Nano-structured Materials Important for Tissue Engineering



Cast Replica of Vascular Tissue Demonstrating Nanometer Roughness *

—Bar = 1µm

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

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

Special Surface Properties of Nano-structured Materials Important for Tissue Engineering

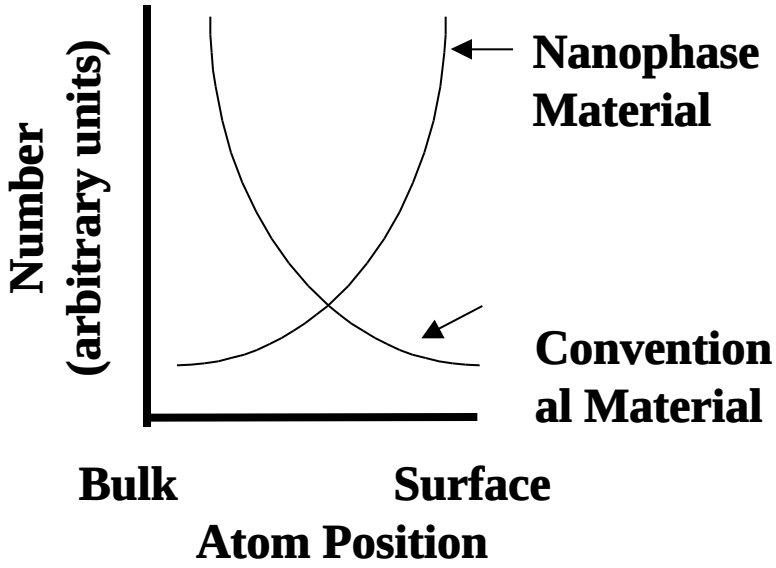
- **Proteins contained in extracellular matrices are nano-structured.**

Characteristic Nano-dimensions of Proteins in Natural Tissue*

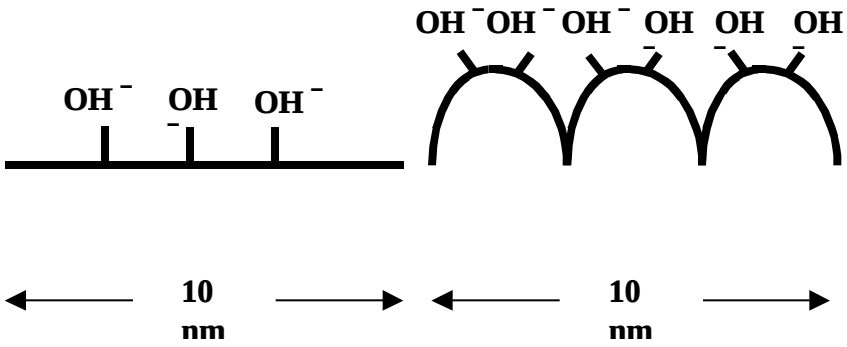
Protein	Characteristic Dimensions
Fibronectin	Dimer of two identical subunits; 60-70 nm long; 2-3 nm wide.
Vitronectin	Linear molecule 15 nm long.
Laminin	Cruciform configuration with one 50 nm long arm and two 35 nm long short arms; total length 50 nm; total width 70 nm.
Collagen	Triple helical linear 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.**

Special Surface Properties of Nano-structured Materials Important for Tissue Engineering



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



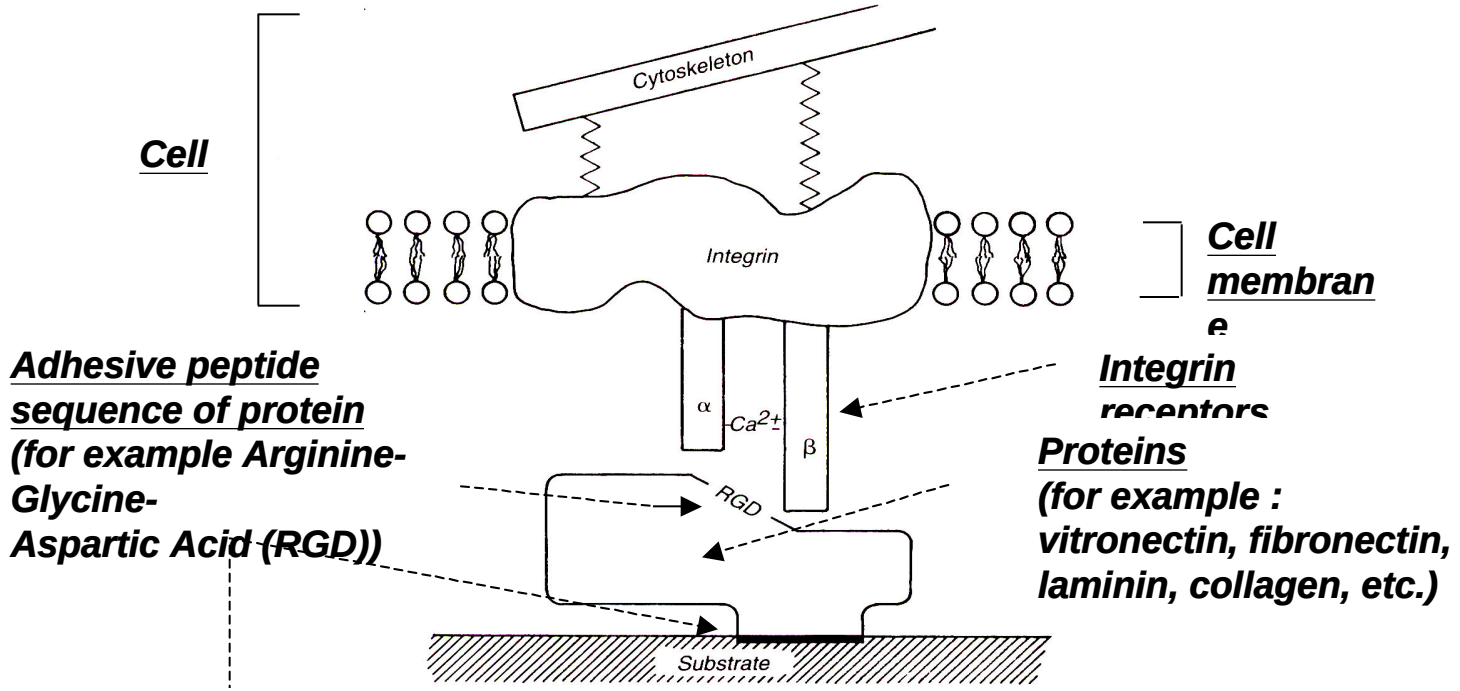
(a) Conventional (grain size: > 100 nm)

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

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

* redrawn and adapted from Klabunde, K.J.; et al., J. Phys. Chem. 1996, *100*:12141; T. J. Webster, in Advances in Chemical Engineering Vol. 27, Academic Press, NY, pgs. 125-166, 2

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



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

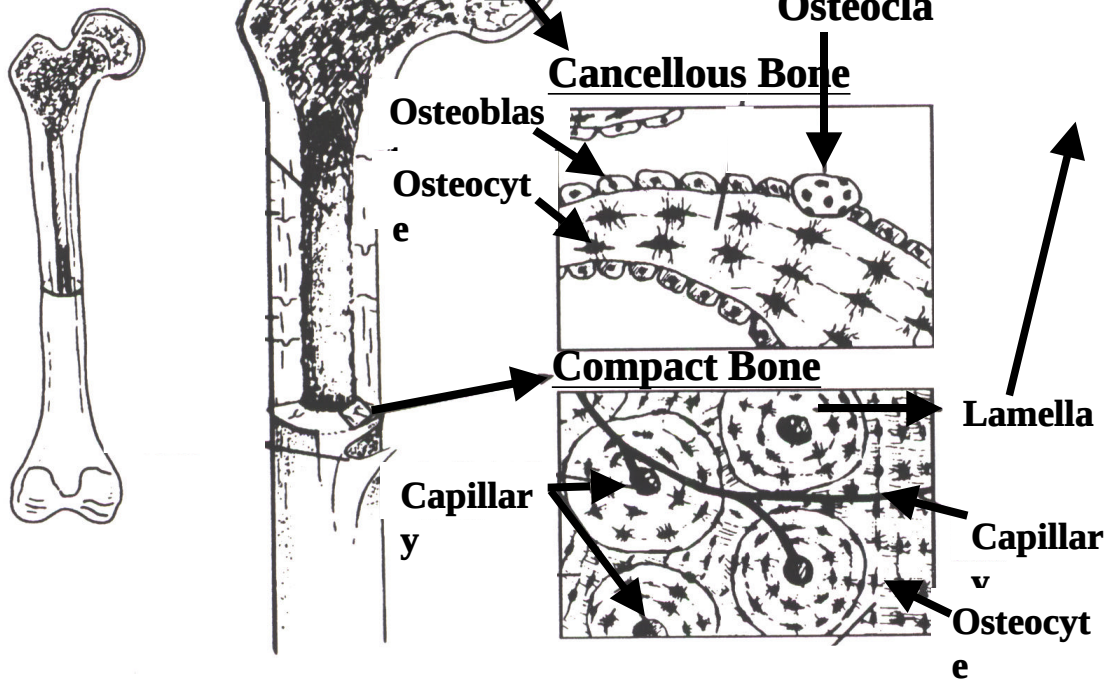
Adapted and redrawn from Schakenraad, J.M . pp. 140-141, in *Biomaterial Science (B. Ratner et al., eds.),* Academic Press, Inc., San Diego, CA, 1996; T. J. Webster, in Advances in Chemical Engineering Vol. 27, Academic Press, NY, pgs. 125-166, 2001.

Objective

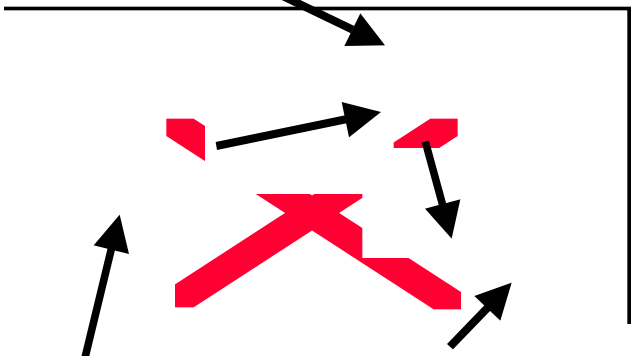
- **The objective of the studies to be presented was to determine whether cell functions can be increased on biologically-inspired nano-structured surfaces.**

BONE PART I:
NANO-SPHERICAL CERAMICS

Bone is a Nanophase Material



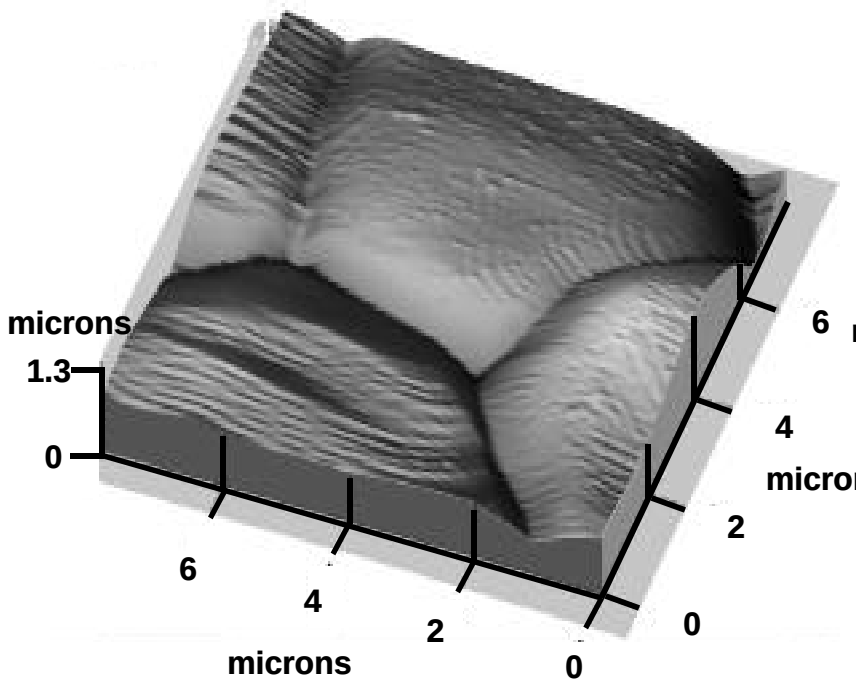
Collagen Fibers: composed of Type I collagen which is a triple helix 300 nm in length; 0.5 nm in width; and periodicity of 67 nm



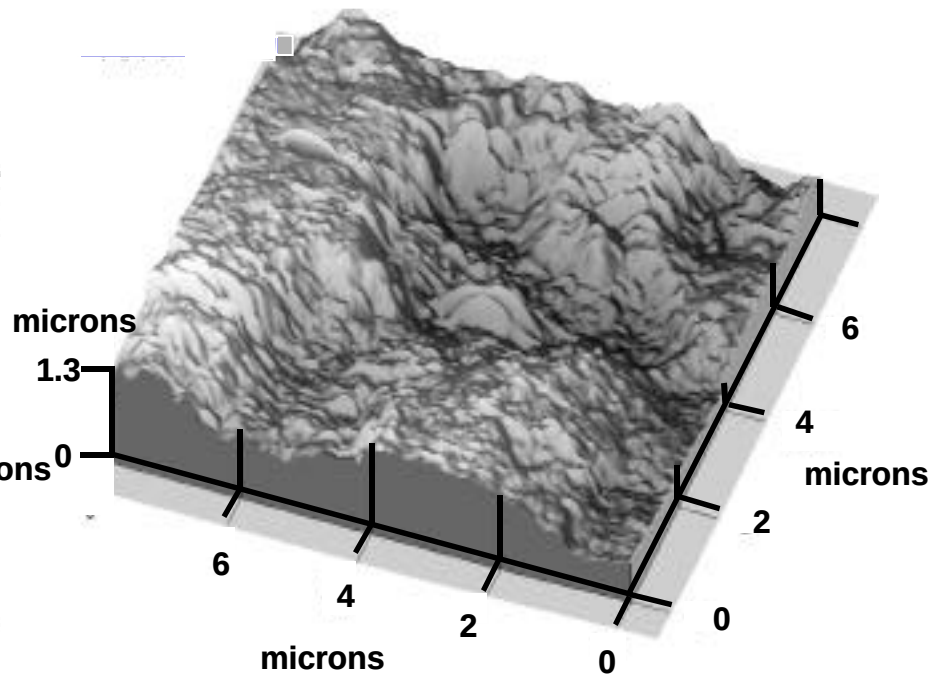
– **Hydroxyapatite Crystals:** less than 50 nm in length and 5 nm in diameter

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

Atomic Force Micrographs of Nanophase and Conventional Titania



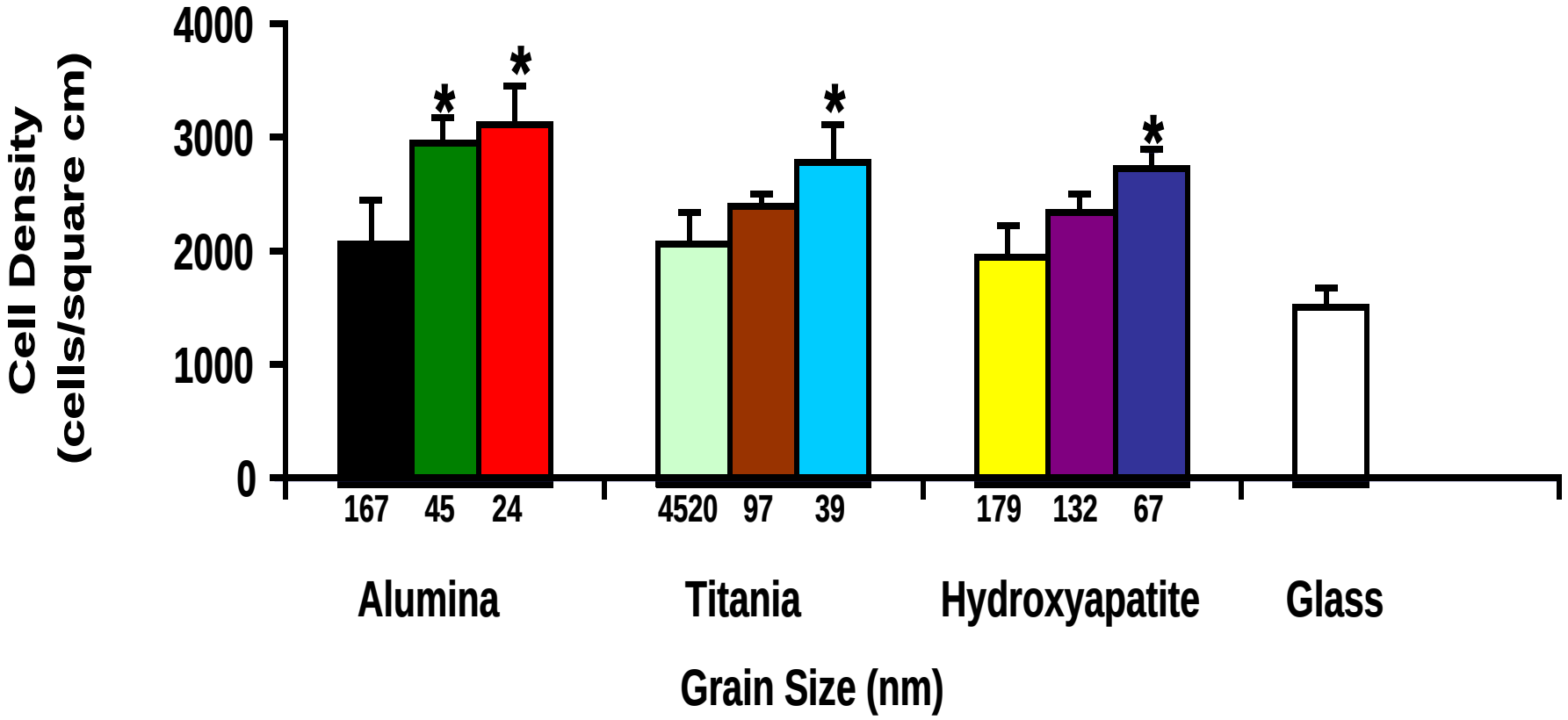
4,520 nm (conventional) Titania



39 nm (nanophase) Titania

Webster TJ, Siegel RW, Bizios R, "Osteoblast adhesion on nanophase ceramics," *Biomaterials* 20:1221, 1999.

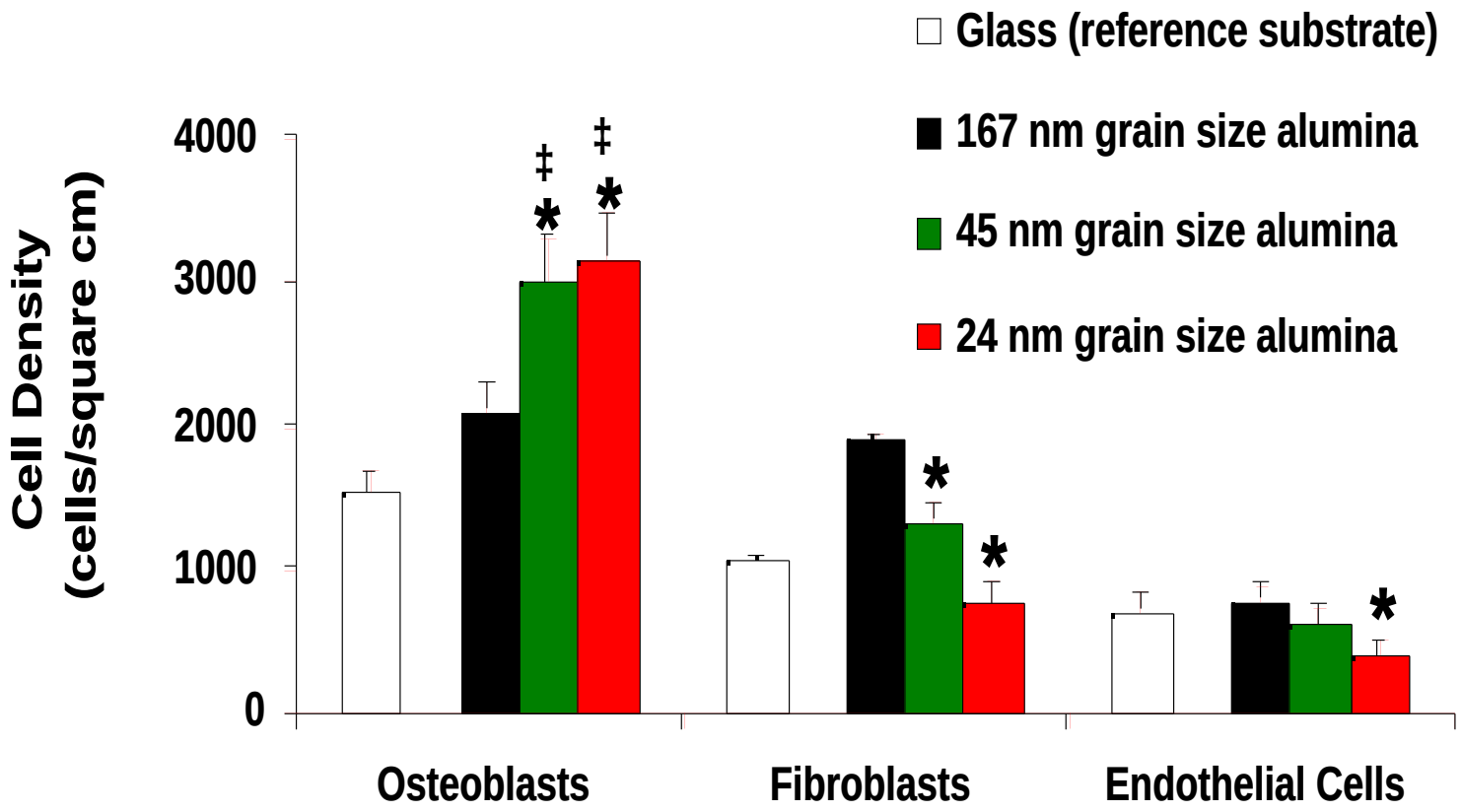
Enhanced Osteoblast Adhesion on Nanophase Ceramics



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

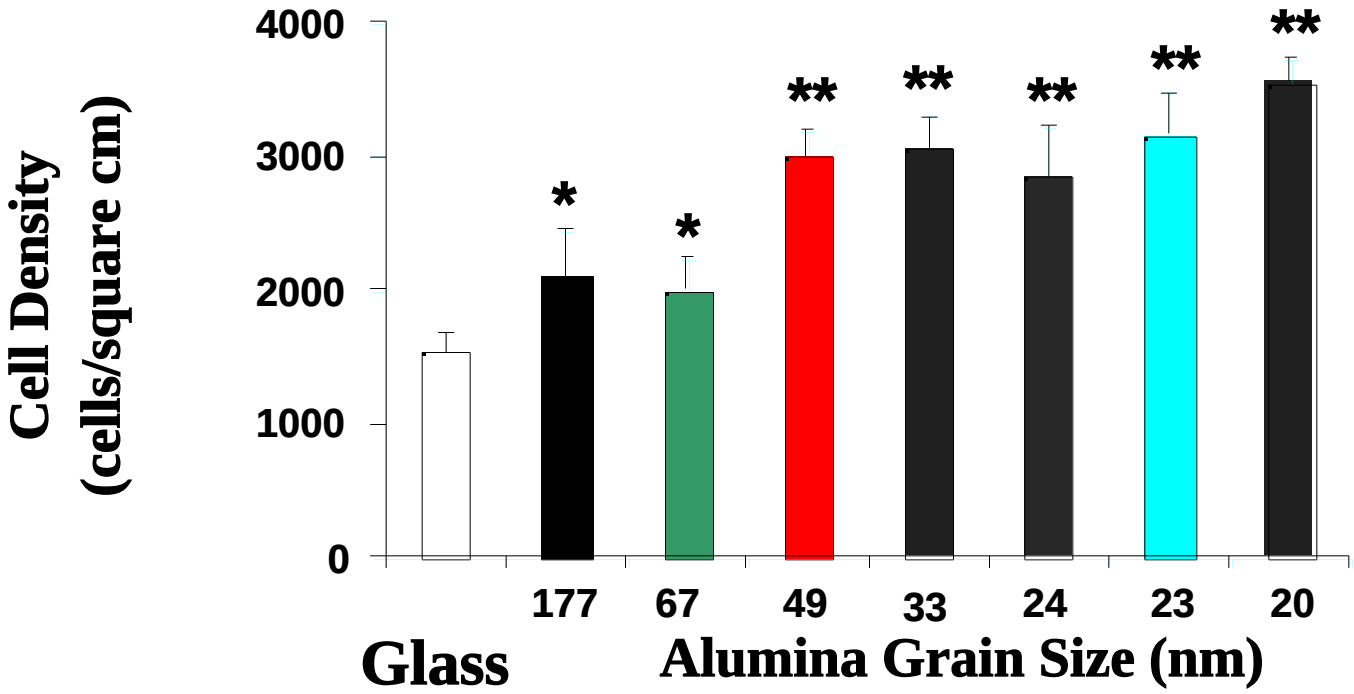
Comparison of Cell Adhesion on Nanophase Alumina



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).

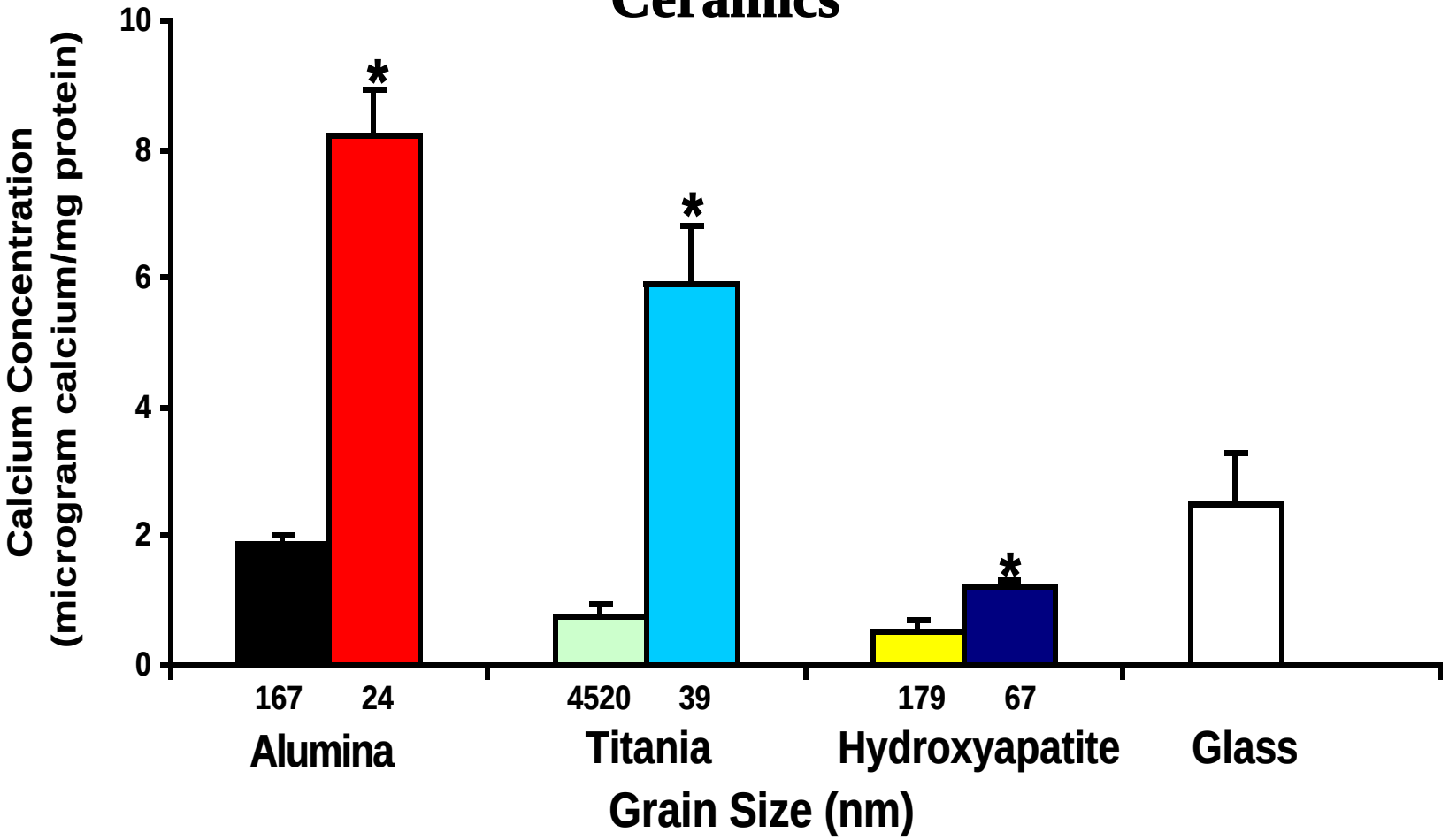
T. J. Webster, C. Ergun, R. H. Doremus, R.W. Siegel, and R. Bizios, "Specific proteins mediate enhanced osteoblast adhesion on nanophase ceramics," *Journal of Biomedical Materials Research* 51:475-483

Osteoblast Adhesion on Alumina of Various Grain Sizes



Culture media = DMEM supplemented with 10% fetal bovine serum. Adhesion time = 4 hours. Values are mean +/- SEM; n=3; * $p < 0.05$ (compared to reference substrate); ** $p < 0.01$(compared to 177 nm grain size alumina).

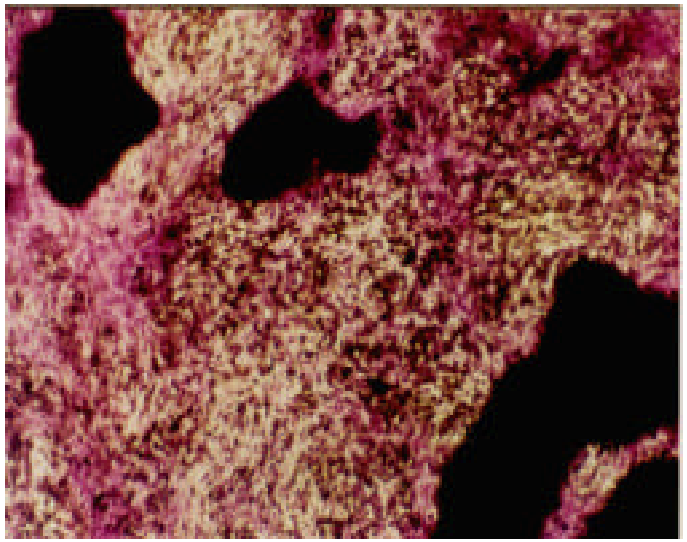
Enhanced Calcium Deposition on Nanophase Ceramics



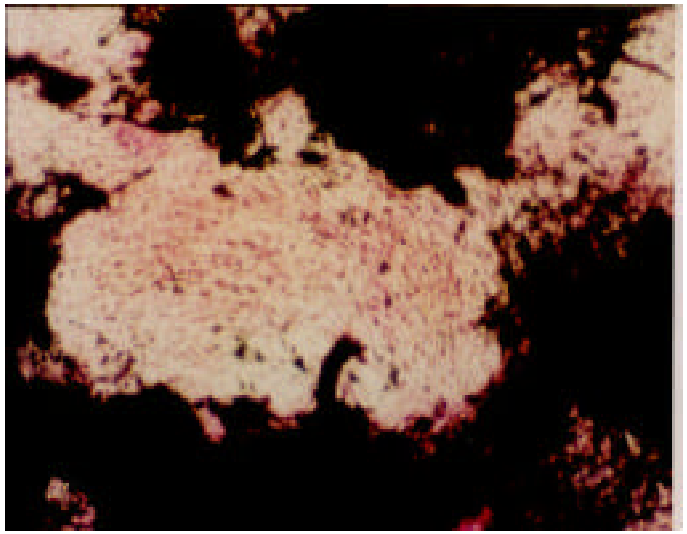
Culture medium = DMEM supplemented with 10% fetal bovine serum, 50 micrograms/mL L-ascorbate and 10 mM β-glycerophosphate. Culture time = 28 days. Values are mean +/- SEM; n = 3; * $p < 0.01$ (compared to respective conventional grain size ceramic).

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

Enhanced Deposition of Mineral by Osteoblasts Cultured on Nanophase Ceramics



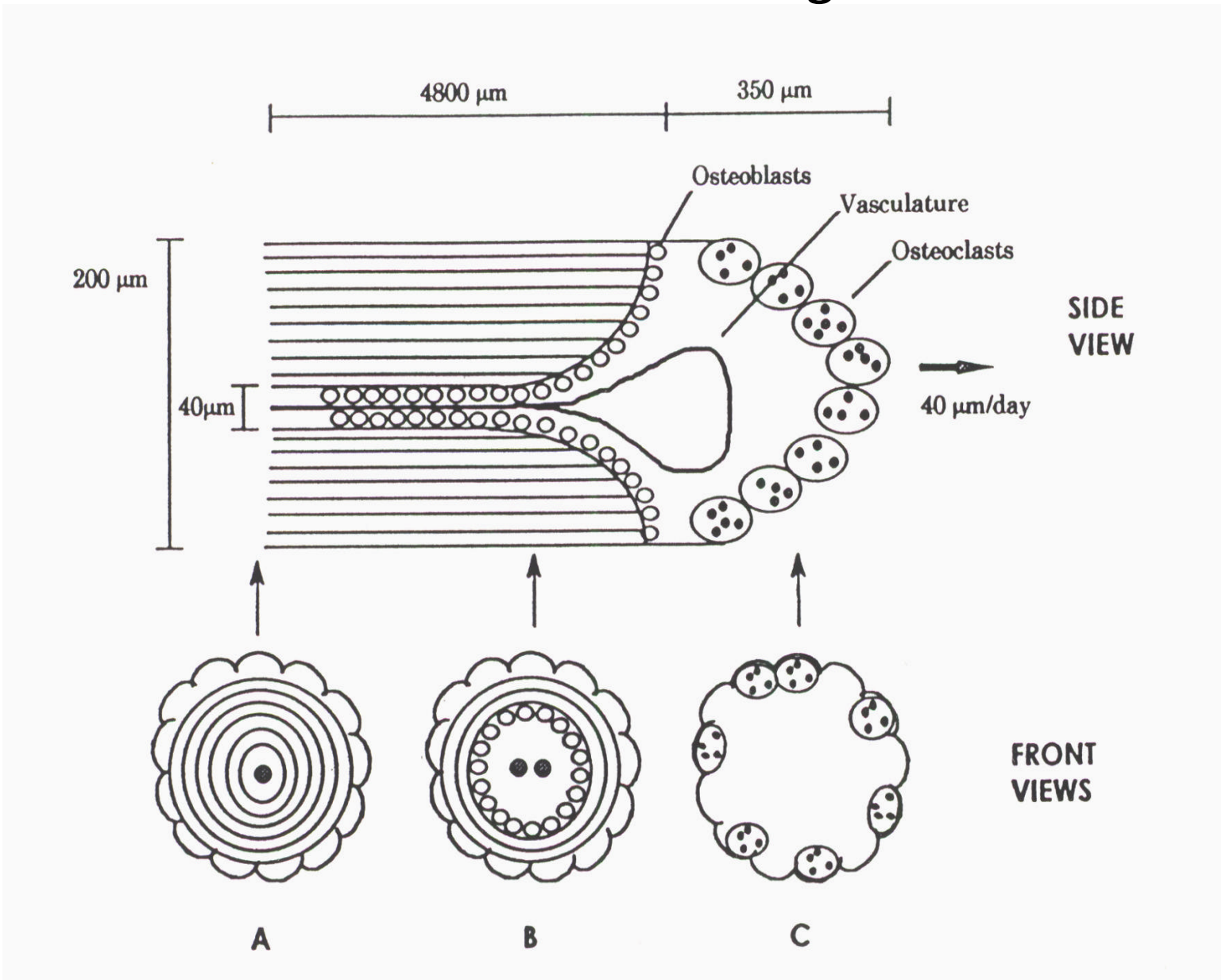
Conventional hydroxyapatite after 28 days



Nanophase hydroxyapatite after 28 days

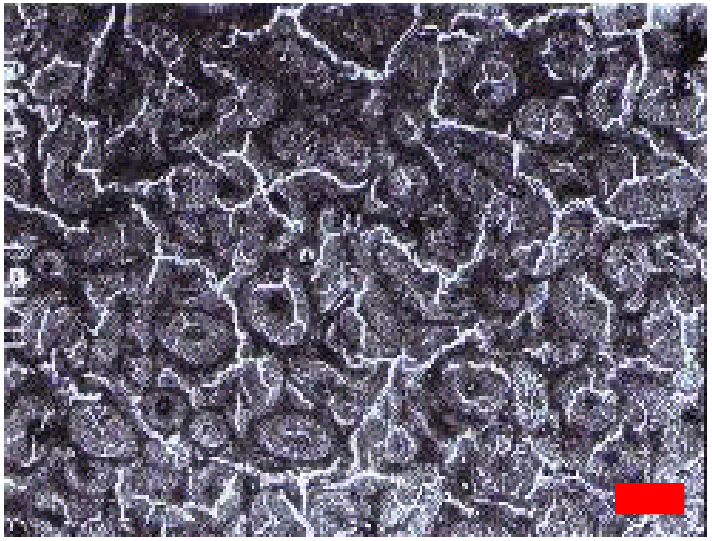
Culture medium = DMEM supplemented with 10% fetal bovine serum, 50 μg/mL ascorbic acid, and 10 mM β-glycerophosphate.

Function of the Bone-Modeling Unit

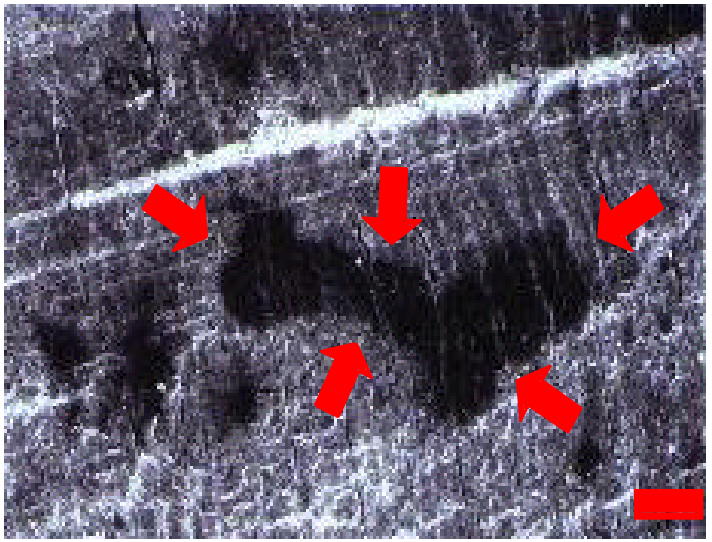


Adapted and redrawn from Martin, B.R. and Burr, D.B., *Structure, Function and Adaptation of Compact Bone*, Raven Press,

Scanning Electron Micrographs of Resorption Pits on Devitalized Bone



Presence of calcitonin

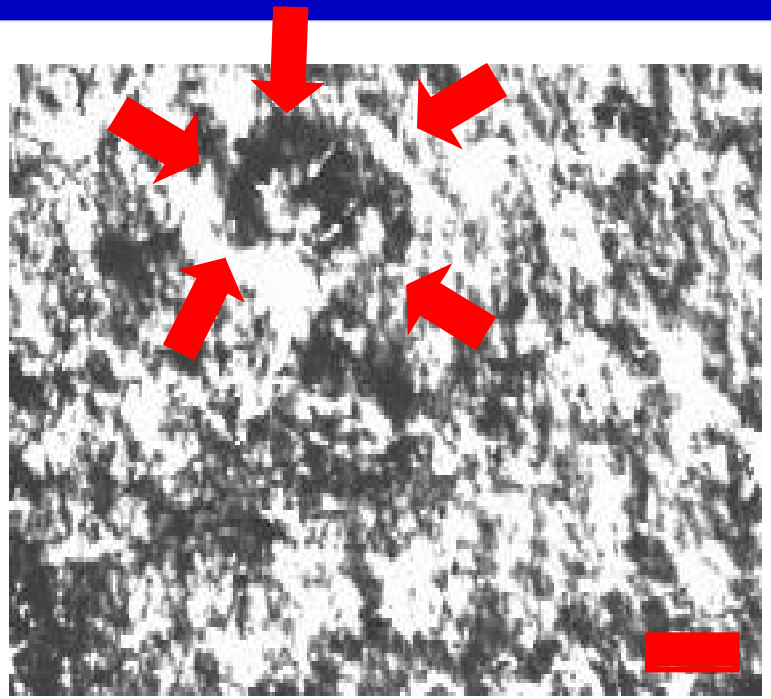


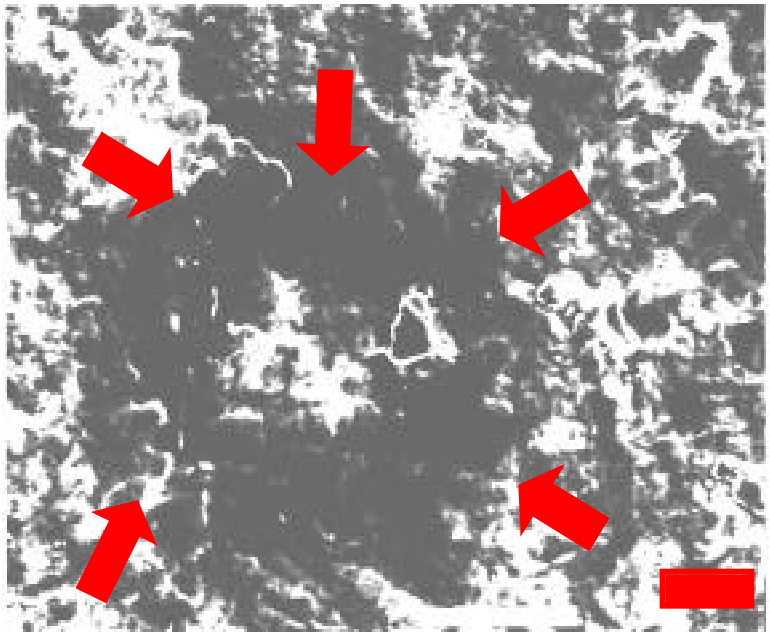
Absence of calcitonin

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, "Enhanced functions of osteoclast-like cells on nanophase ceramics," ***Biomaterials*** **22: 1327-1333 (2001).**

Scanning Electron Micrographs of Resorption Pits on Alumina





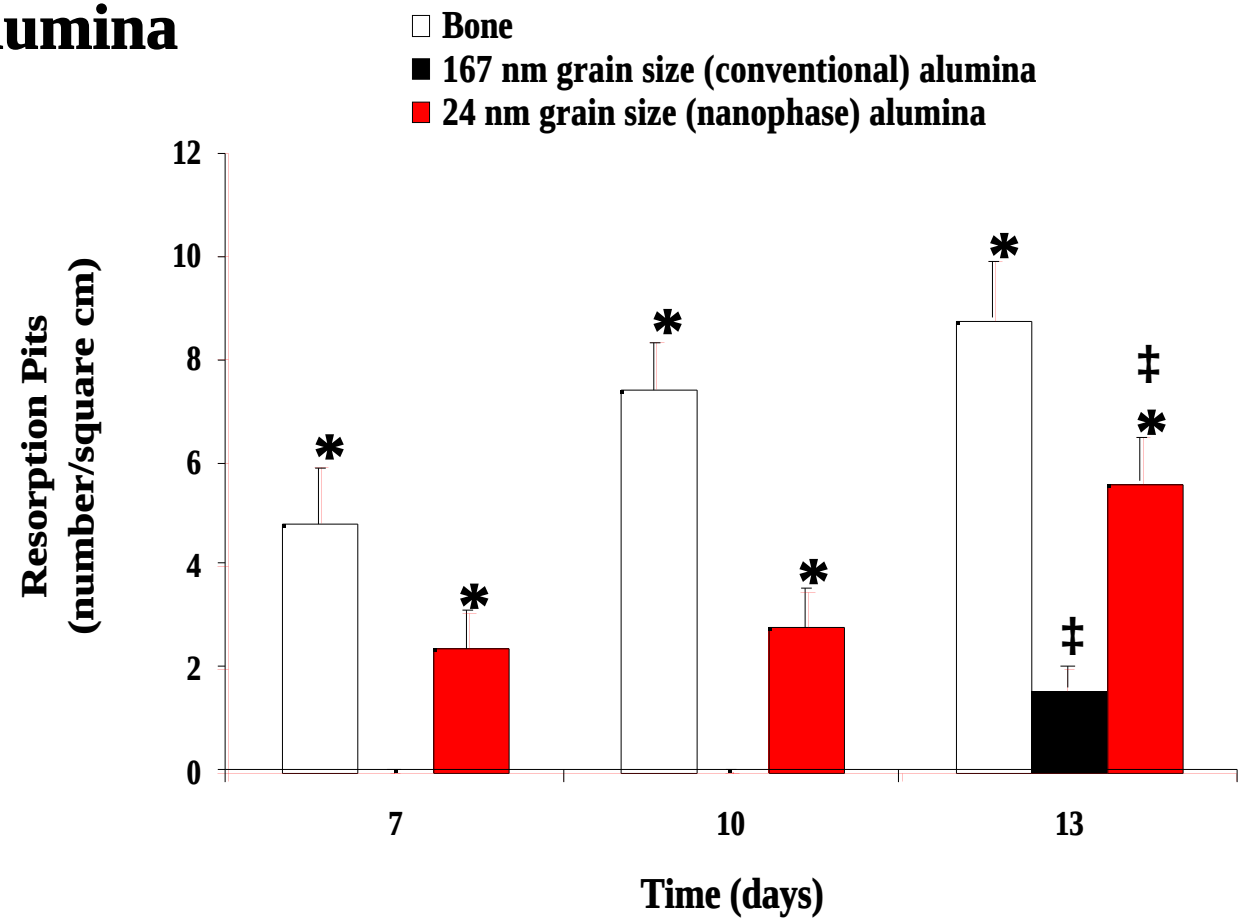
167 nm grain size (conventional) alumina

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.

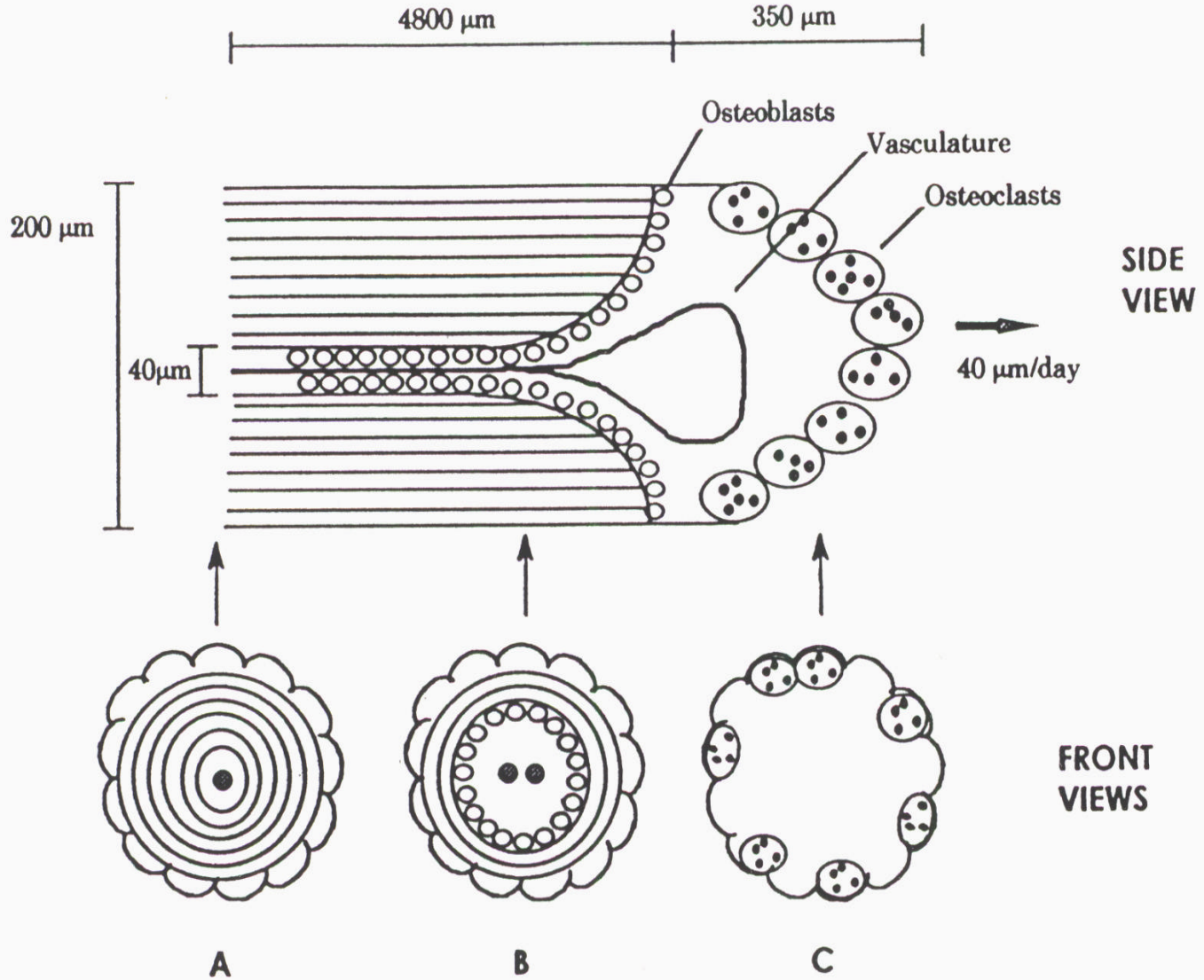
T. J. Webster, C. Ergun, R. H. Doremus, R. W. Siegel, and R. Bizios, "Enhanced functions of osteoclast-like cells o nanophase ceramics," *Biomaterials* 22: 1327-1333 (2001).

Enhanced Formation of Resorption Pits on Nanophase Alumina



Culture medium = DMEM supplemented with 10% fetal bovine serum, 1% antibiotic/antimycotic, 10^{-8} M Vitamin D_3 ; values are mean +/- SEM; n = 3; * $p < 0.01$ (compared to respective conventional grain size ceramic); ‡ $p < 0.01$ (compared to respective substrates after 7 days of culture).

Possible Enhanced Coordinated Functions of Osteoclasts and Osteoblasts on Nanophase Ceramics

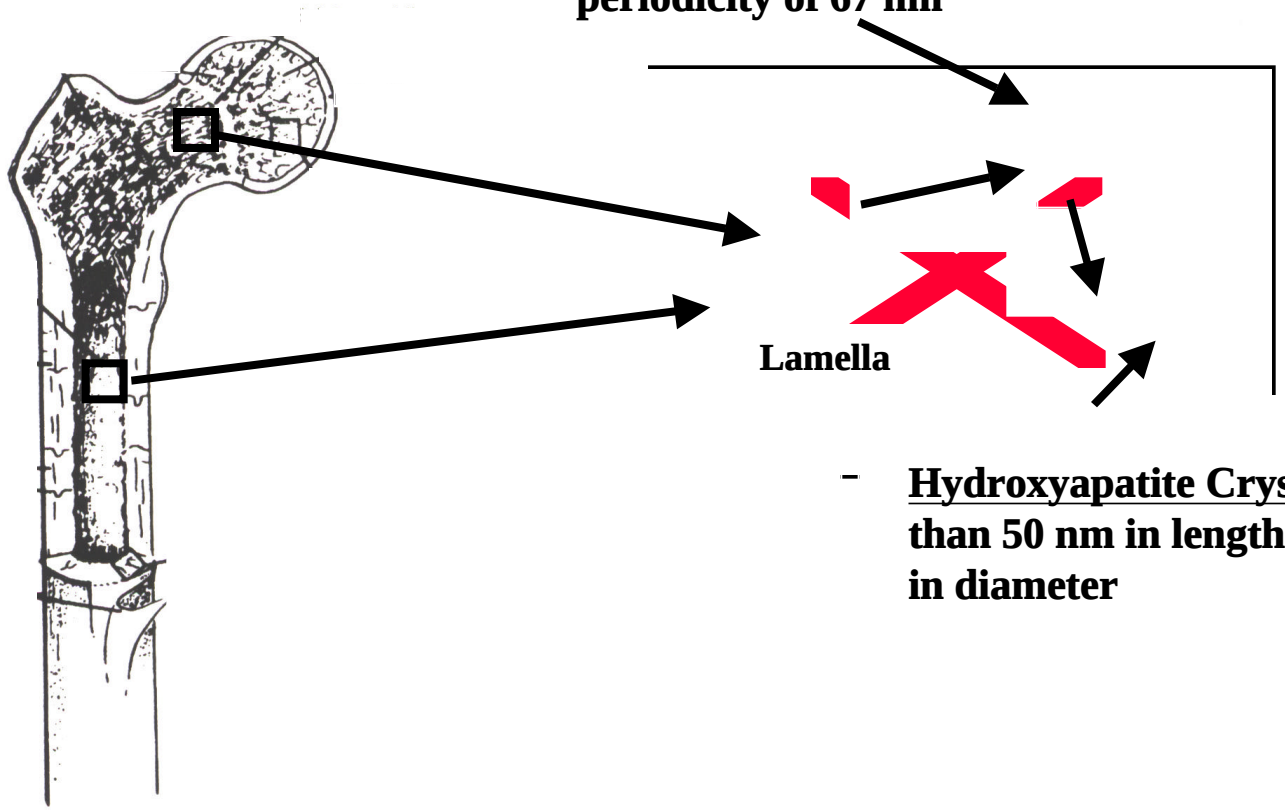


Adapted and redrawn from Martin, B.R. and Burr, D.B., *Structure, Function and Adaptation of Compact Bone*, Raven Press, New York, 1989.

BONE PART II:
NANO-FIBER CERAMICS

Bone is a Nano-fibered Material

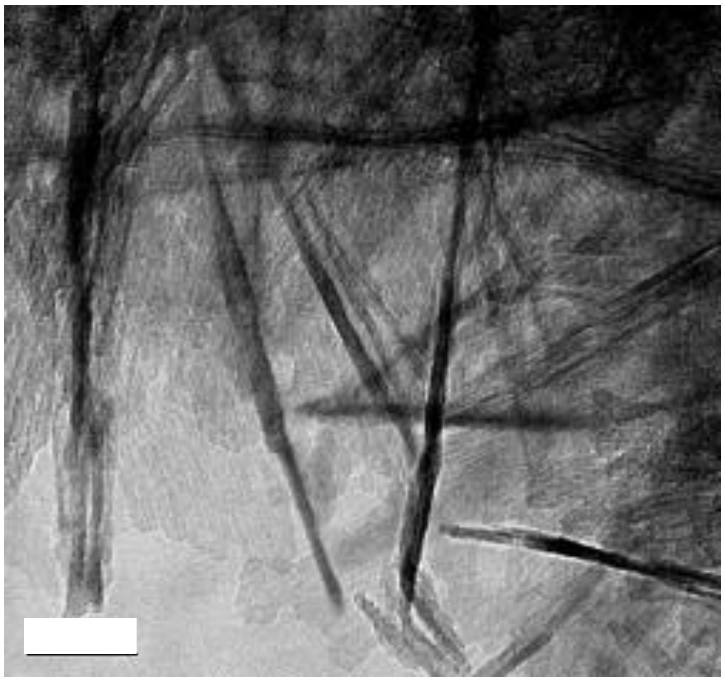
Collagen Fibers: composed of Type I collagen which is a triple helix 300 nm in length; 0.5 nm in width; and periodicity of 67 nm



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

Structure of Bone

Design of Nano-fiber Ceramics for Orthopedic Implants

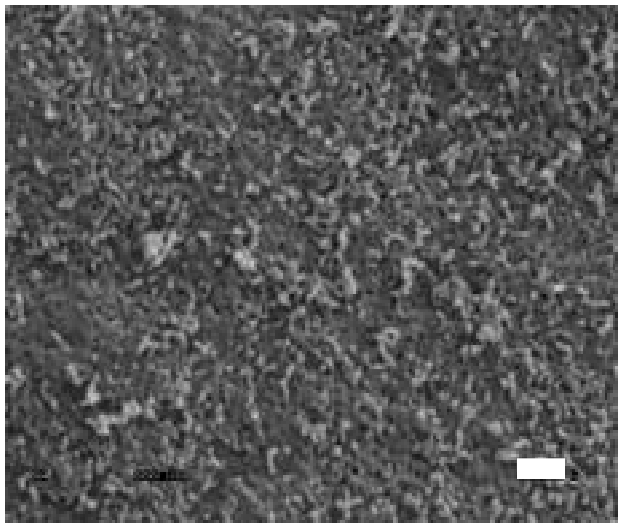


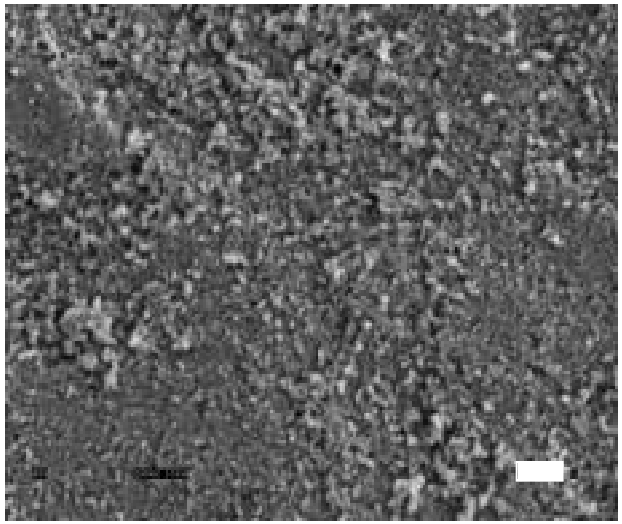
TEM of Individual Alumina Nano-dimensional Fibers

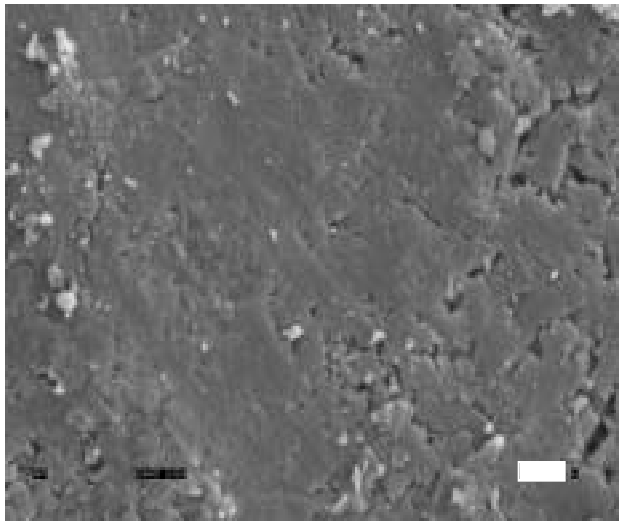
Bar = 20 nm.

L. G. Gutwein, F. Tepper, and T. J. Webster, "Increased osteoblast function on nanofibered alumina," ***American Ceramic Society 26th Annual Meeting Conference Proceedings*****, in press (2003).**

High Magnification Scanning Electron Micrographs of Alumina Substrates





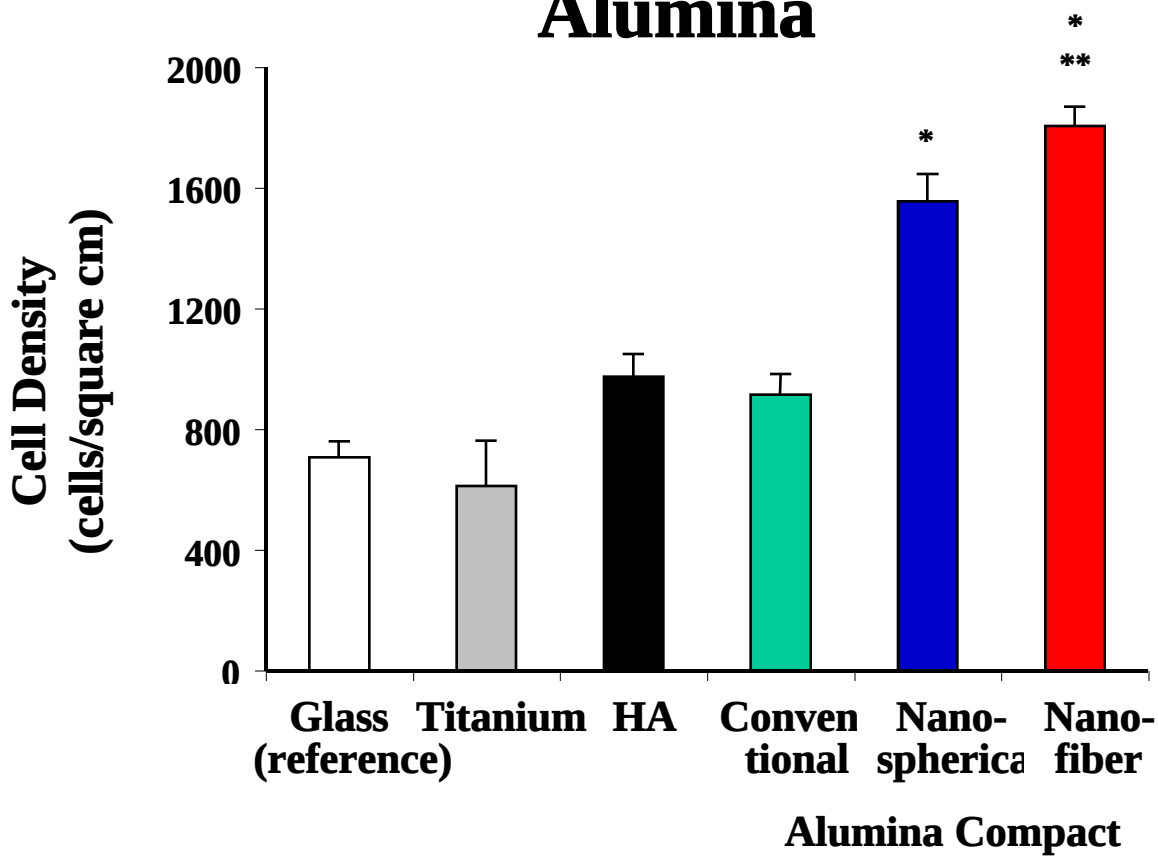


Conventional Alumina **Nano-spherical Alumina** **Nano-fiber Alumina**

Original Magnification = 10,000X; Bar = 1 μm.

L. G. Gutwein, F. Tepper, and T. J. Webster, "Increased osteoblast function on nanofibered alumina," ***American Ceramic Society 26th Annual Meeting Conference Proceedings*****, in press (2003).**

Enhanced Osteoblast Adhesion on Nano-fiber Alumina



Culture medium = DMEM supplemented with 10% fetal bovine serum. Seeding density = 2,500 cells/cm^2. Culture time = 2 hrs. Values are mean +/- SEM; n = 3; * $p < 0.01$ (compared to titanium); ** $p < 0.05$ (compared to nano-spherical alumina).

L. G. Gutwein, F. Tepper, and T. J. Webster, "Increased osteoblast function on nanofibered alumina," ***American Ceramic Society 26th Annual Meeting Conference Proceedings*****, in press (2003).**

BONE PART III:
CARBON NANOFIBERS

Design of Carbon Nanofibers for Orthopedic Implants

Fiber	Fiber Diameter (nm)	Surface Energy
Nanophase w/ pyrolytic outer core	100	Low
Conventional w/ pyrolytic outer core	200	Low
Nanophase w/o pyrolytic outer core	60	High
Conventional w/o pyrolytic outer core	125	High

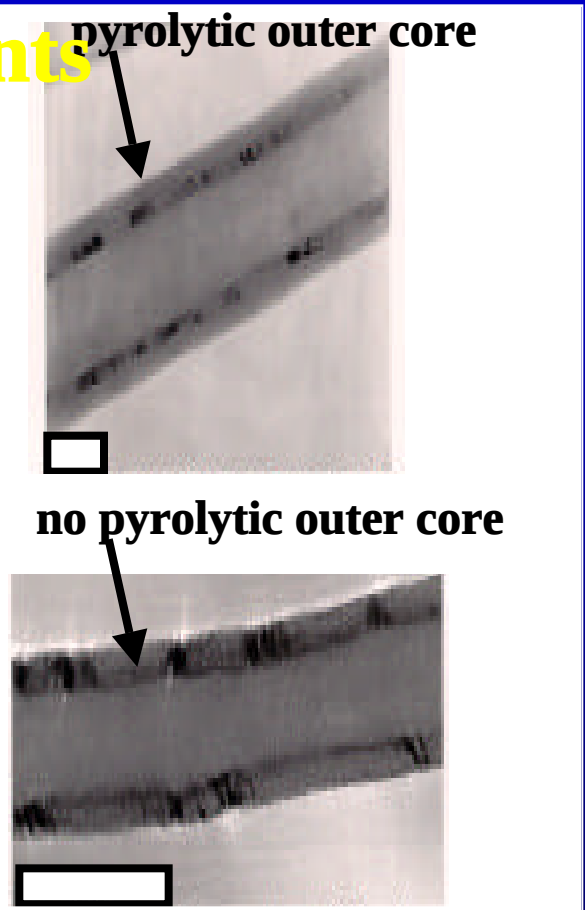
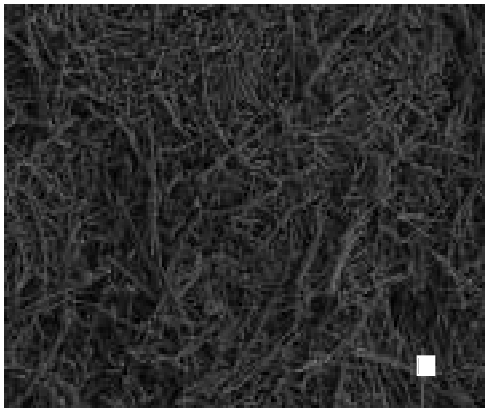


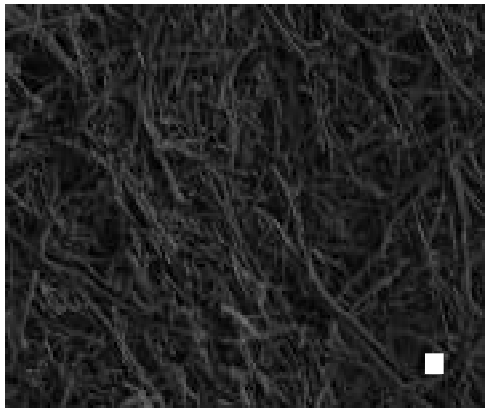
Figure: TEM of Individual Carbon Nano-dimensional Fibers
Bar = 100 nm.

K. L. Elias, R. L. Price, and T. J. Webster, *Biomaterials* 23: 3279-3287 (2002).

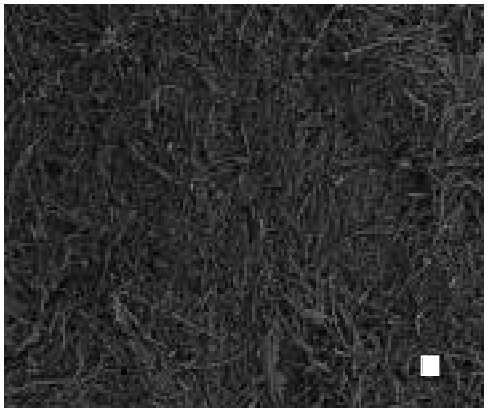
Scanning Electron Micrographs of Carbon Fiber Compacts



Nanophase w/Low Surface Energy

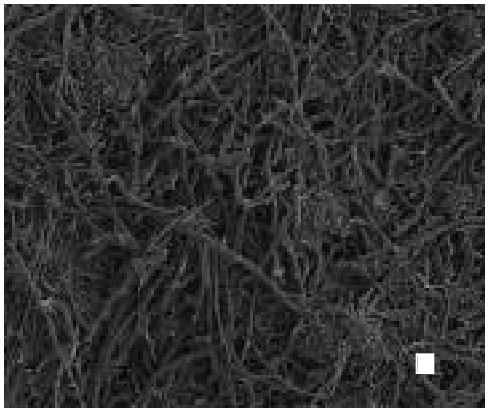


Conventional w/Low Surface Energy



Nanophase w/High Surface Energy

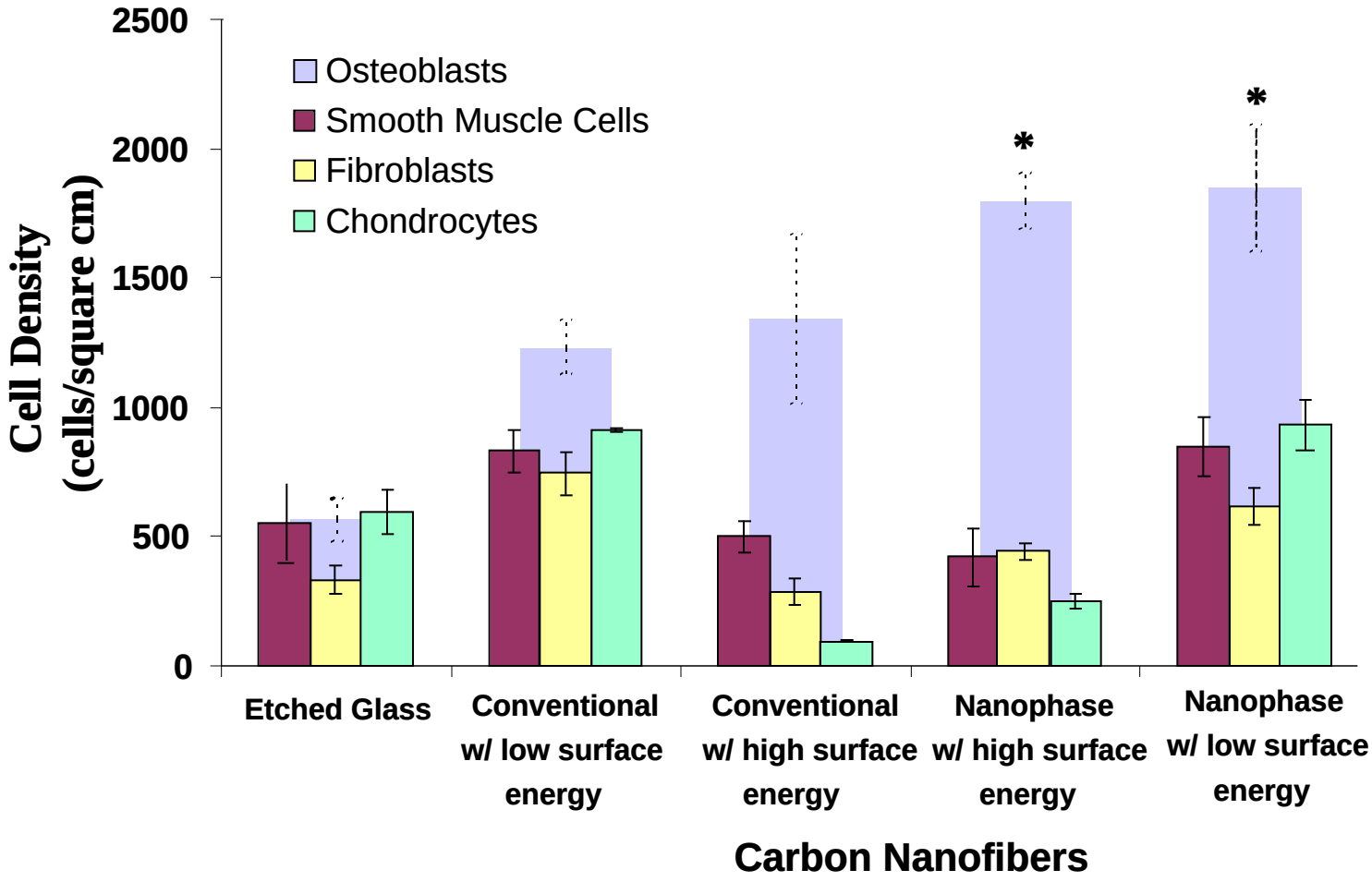
Bar = 1 μm.



Conventional w/High Surface Energy

R. L. Price, M. C. Waid, K. M. Haberstroh, and T. J. Webster, "Increased, select bone cell adhesion on formul containing carbon nanofibers," *Biomaterials*, 24(11): 1877 – 1887, 2003.

Enhanced, Select Osteoblast Adhesion on Carbon Nanofibers

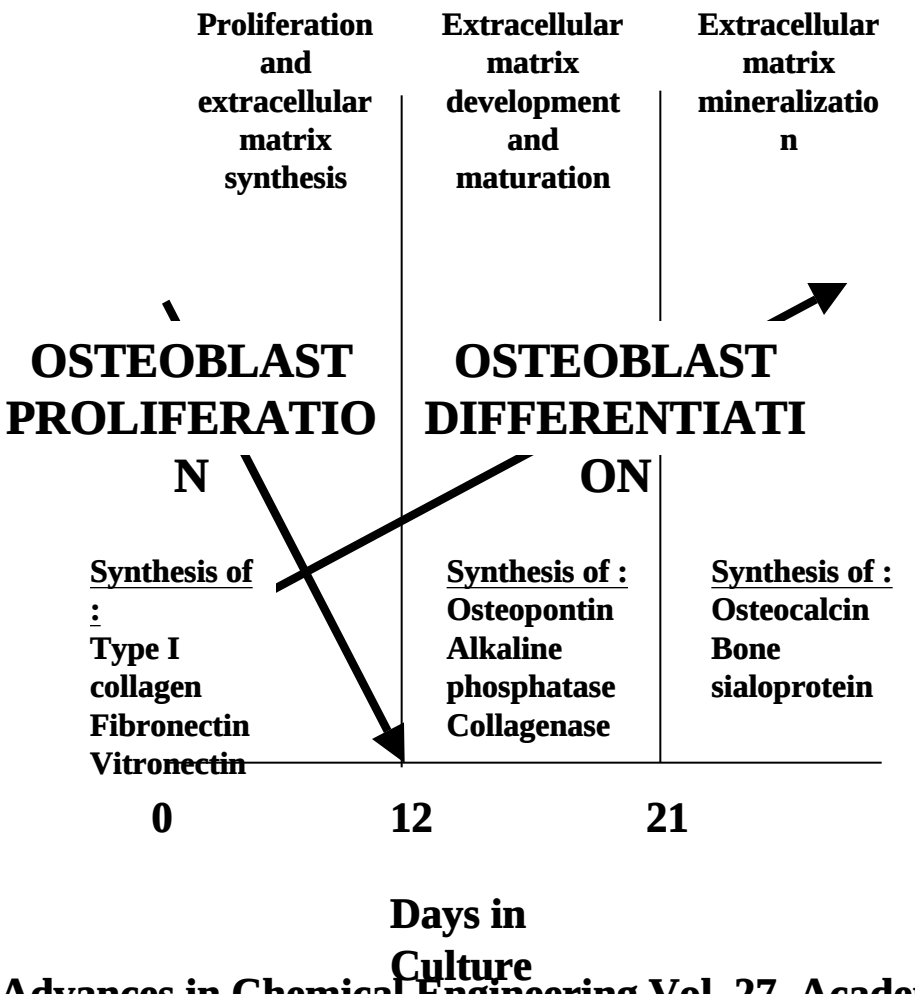


Values are mean +/-SEM; n=3; * $p<0.10$ (compared to conventional counterpart).

R. L. Price, M. C. Waid, K. M. Haberstroh, and T. J. Webster, "Increased, select bone cell adhesion on formul containing carbon nanofibers," *Biomaterials*, 24(11): 1877 – 1887, 2003.

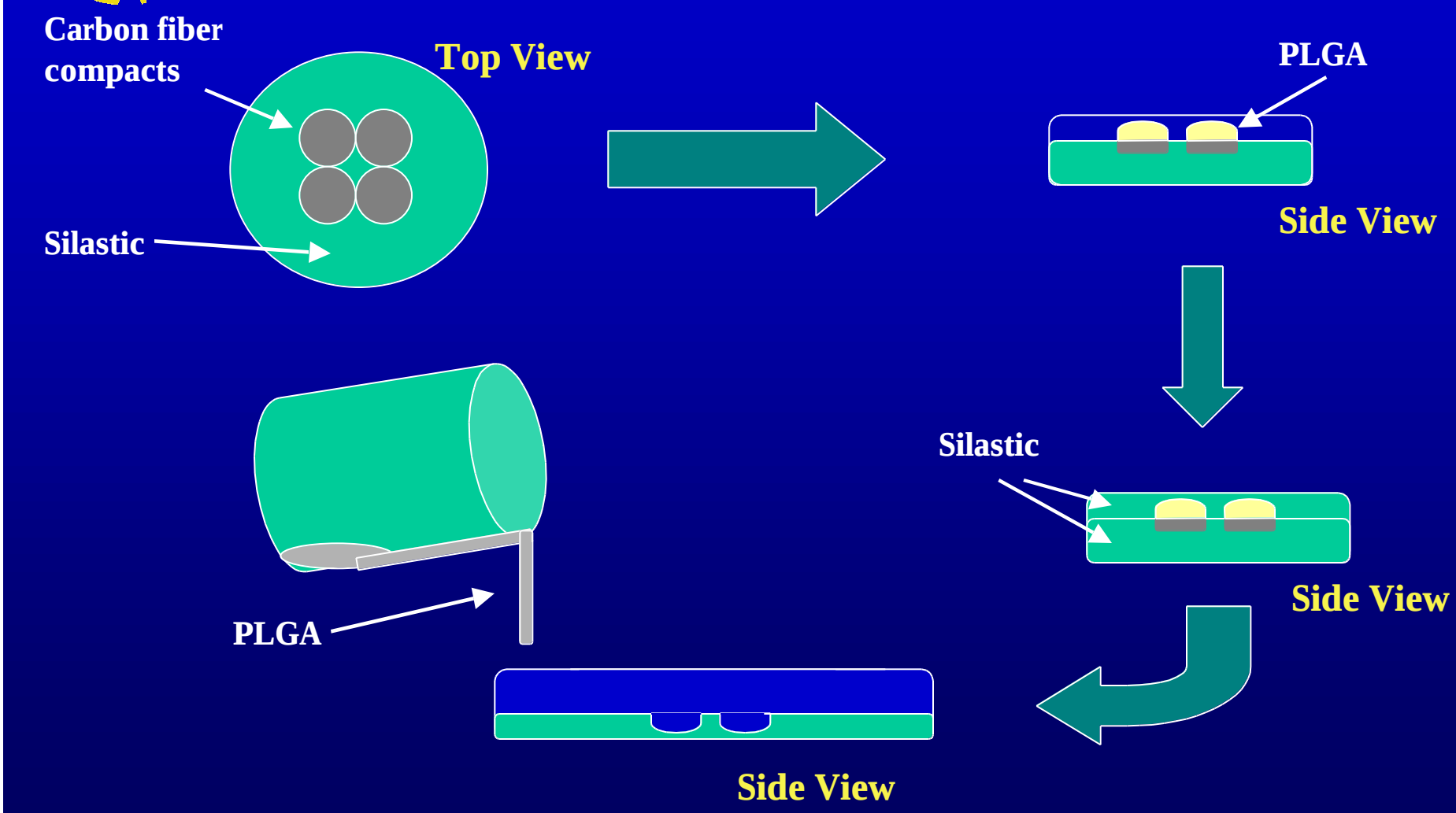
Enhanced Adhesion Translates into Increased Subsequent Functions

Stages of Osteoblast Differentiation

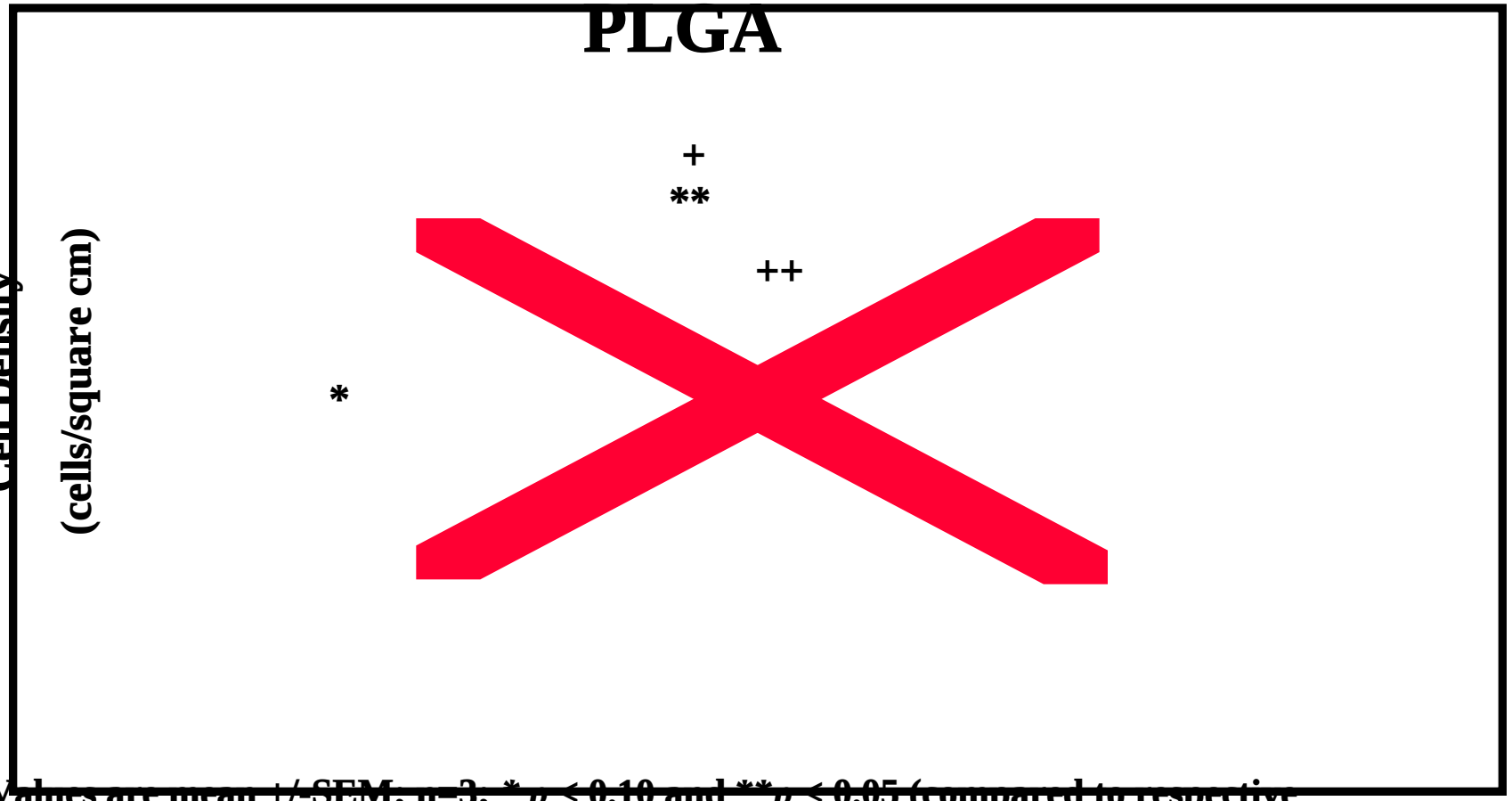


T. J. Webster, in Advances in Chemical Engineering Vol. 27, Academic Press, NY, pgs. 125-166, 2

Transfer of Carbon Nanofiber Topography



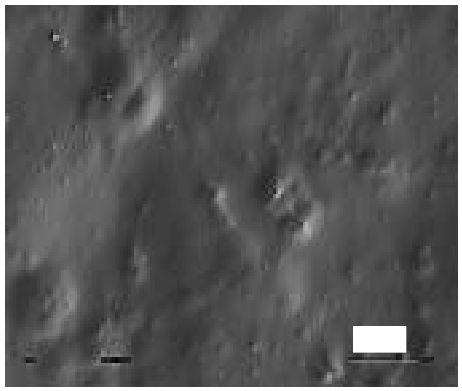
Enhanced Osteoblast Adhesion on Carbon Nanofiber Topography Transferred to PLGA



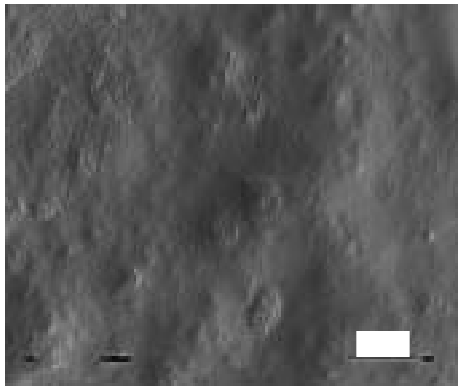
Values are mean +/-SEM, n=3; * $p < 0.10$ and ** $p < 0.05$ (compared to respective conventional form); + $p < 0.1$ and ++ $p < 0.11$ (compared to PLGA nanophase).

BONE PART IV: NANOSTRUCTURED CERAMIC/POLYMER COMPOSITES

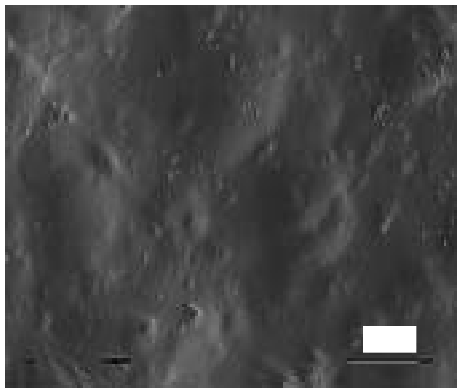
Scanning Electron Micrographs of PLGA/Titania Composites



Conventional PLGA/Conventional Titania

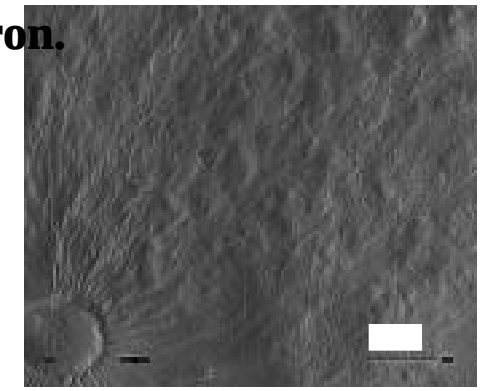


Conventional PLGA/Nanophase Titania



Nano-structured PLGA/Conventional Titania

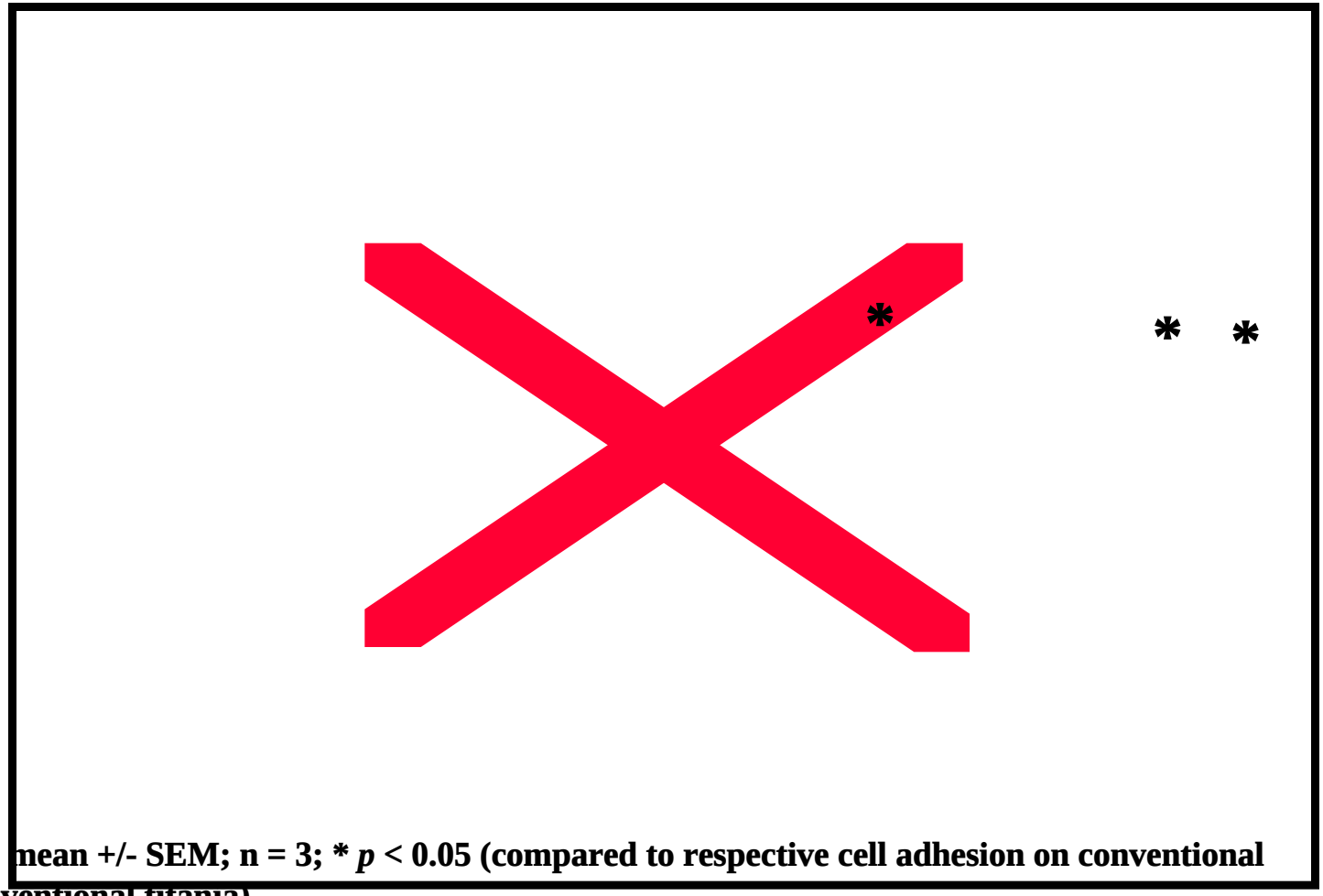
Scale bar = 1 micron.



Nano-structured PLGA/Nanophase Tit

S. Kay, A. Thapa, K. M. Haberstroh, and T. J. Webster, "Nanostructured polymer:nanophase ceramic composites en osteoblast and chondrocyte adhesion," *Tissue Engineering*, 8(5): 753-761 (2002).

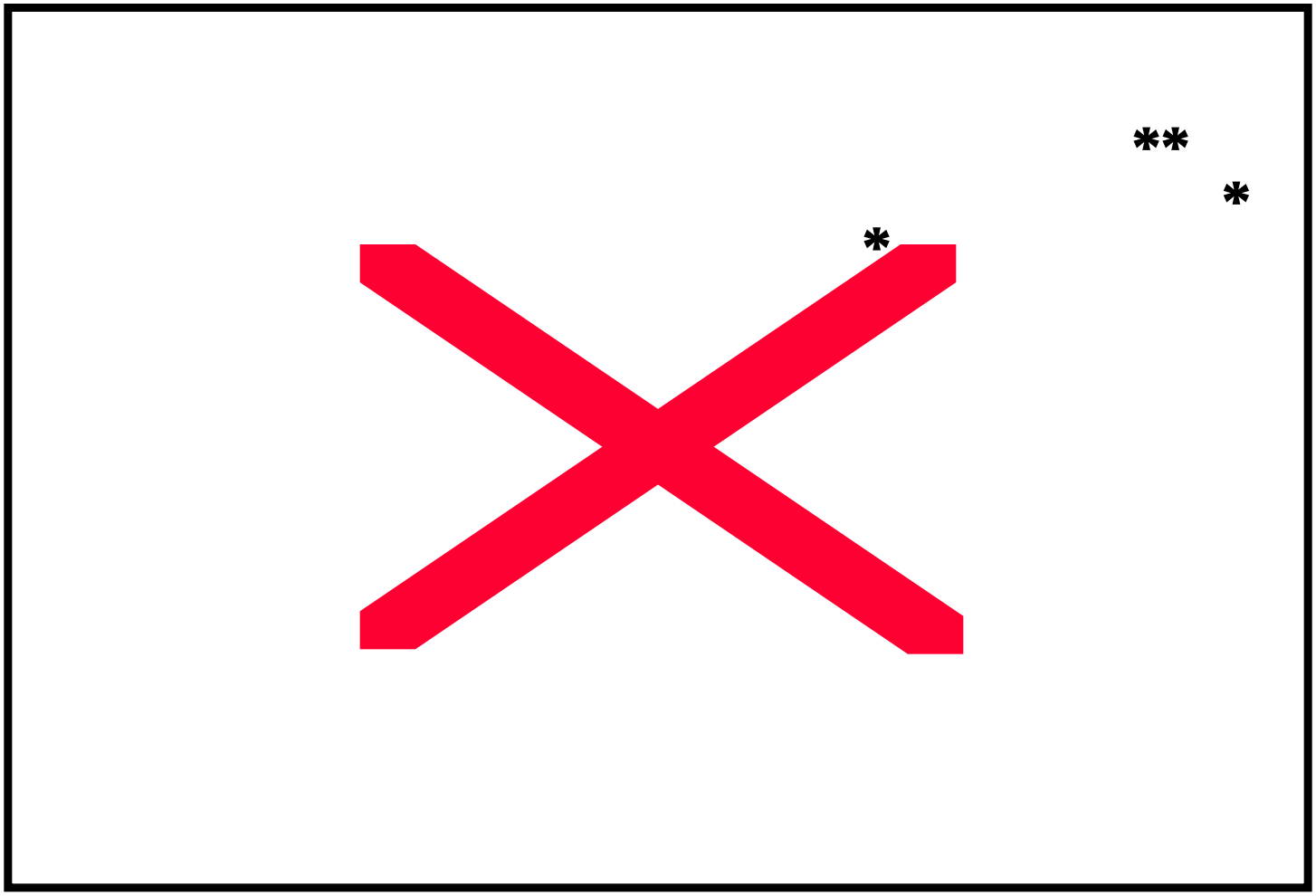
Enhanced Adhesion on Composites of Nanophase PLGA and Conventional Titania



Values are mean +/- SEM; n = 3; * $p < 0.05$ (compared to respective cell adhesion on conventional PLGA/conventional titania).

S. Kay, A. Thapa, K. M. Haberstroh, and T. J. Webster, "Nanostructured polymer:nanophase ceramic composites en osteoblast and chondrocyte adhesion," *Tissue Engineering*, 8(5): 753-761 (2002).

Enhanced Adhesion on Composites of Nanophase PLGA and Nanophase Titania

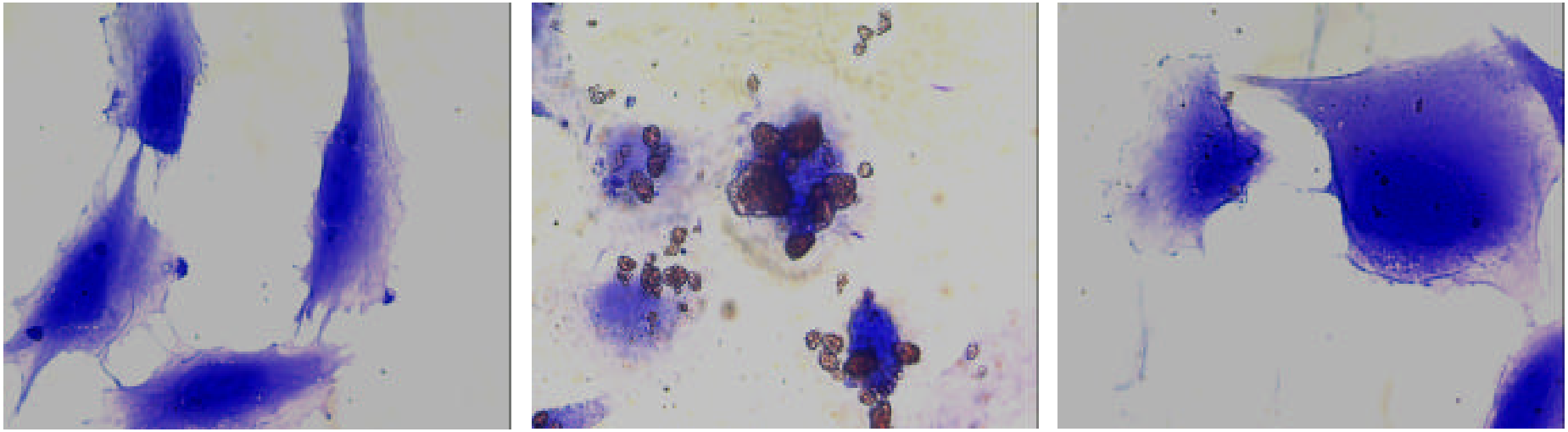


Values ar
PLGA/nanophase titania).

S. Kay, A. Thapa, K. M. Haberstroh, and T. J. Webster, "Nanostructured polymer:nanophase ceramic composites en
osteoblast and chondrocyte adhesion," *Tissue Engineering*, 8(5): 753-761 (2002).

BONE PART V:
NANO-STRUCTURED WEAR DEBRIS

Osteoblast Morphology in the Presence of Alumina Particle Wear Debris



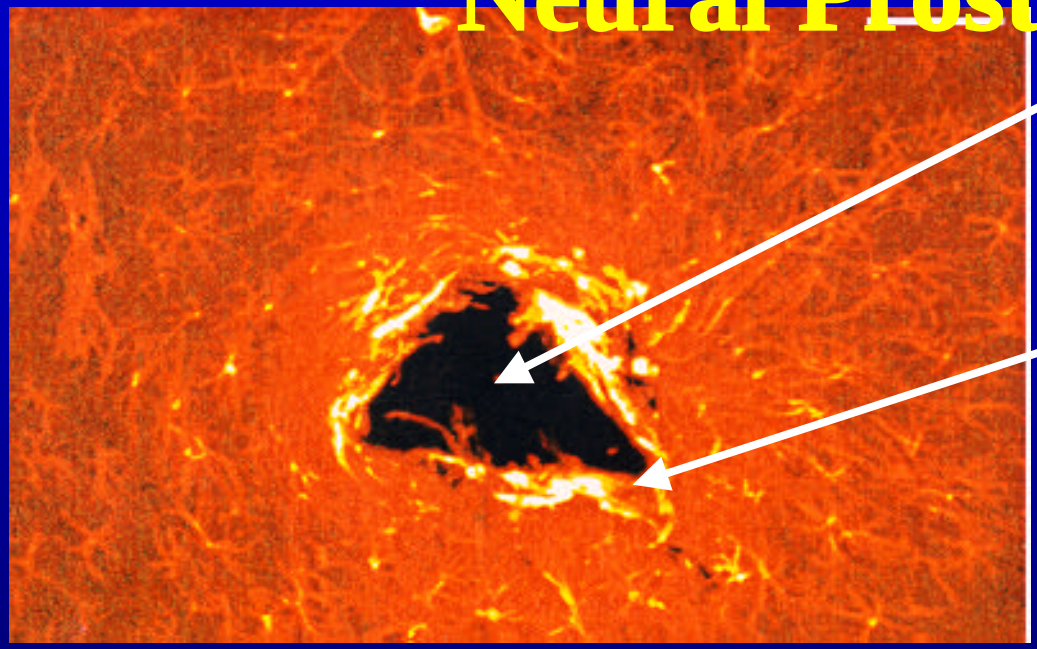
(a) **(b)** **(c)**

Osteoblasts in the presence of **(a)** no particulates (control), **(b)** conventional, and **(c)** nanophase alum particles (100 μg/ml) for 2 hrs. Original magnification = 40X.

L. G. Gutwein and T. J. Webster, "Osteoblast and chrondrocyte proliferation in the presence of alumina and titania nanoparticles," *Journal of Nanoparticle Research*, 4:231-238 (2002).

OTHER TISSUES

Design of Carbon Nanofibers for Neural Prostheses



- Implants of the nervous system must support and guide nerve-cell regeneration over gaps of damaged nerve tissue.
- Material formulations that possess electrical properties which transmit and/or receive electrical signals from nerve cells without signal deterioration over time may guide axon regeneration to enhance prostheses efficacy.

Design of Carbon Nanofibers for Neural Implants

- Decreased functions of astrocytes on smaller dimension carbon nanofibers.
- Decreased functions of astrocytes on polymer composites containing increased amounts of carbon nanofibers.

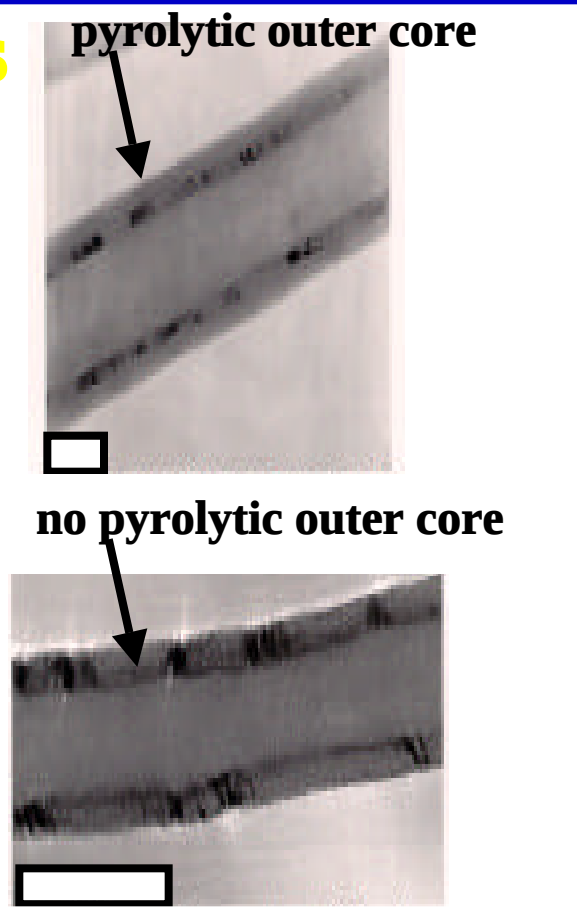
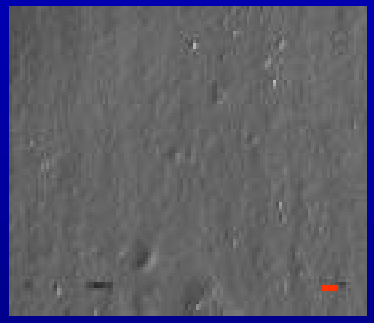


Figure: TEM of Individual Carbon Nano-dimensional Fibers
Bar = 100 nm.

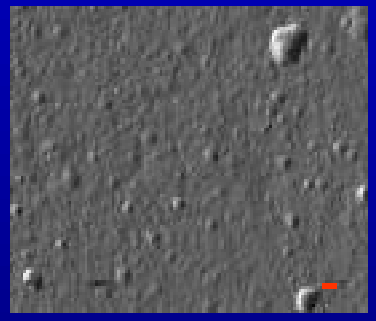
J. L. McKenzie, M. C. Waid, R. Shi, T. J. Webster, "Cytocompatibility of astrocytes on carbon nanofibers," submitted to *Biomaterials* (2003).

Design of Nano-structured Polymer for Vascular/Bladder Applications

- Increased functions of vascular and bladder cells on nano-structured polymer films.

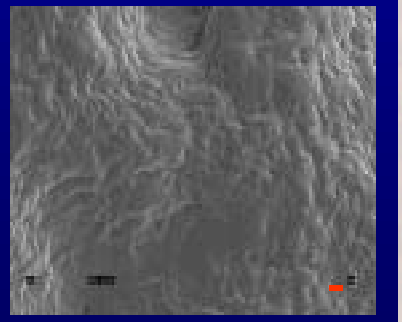


Conventional PLGA



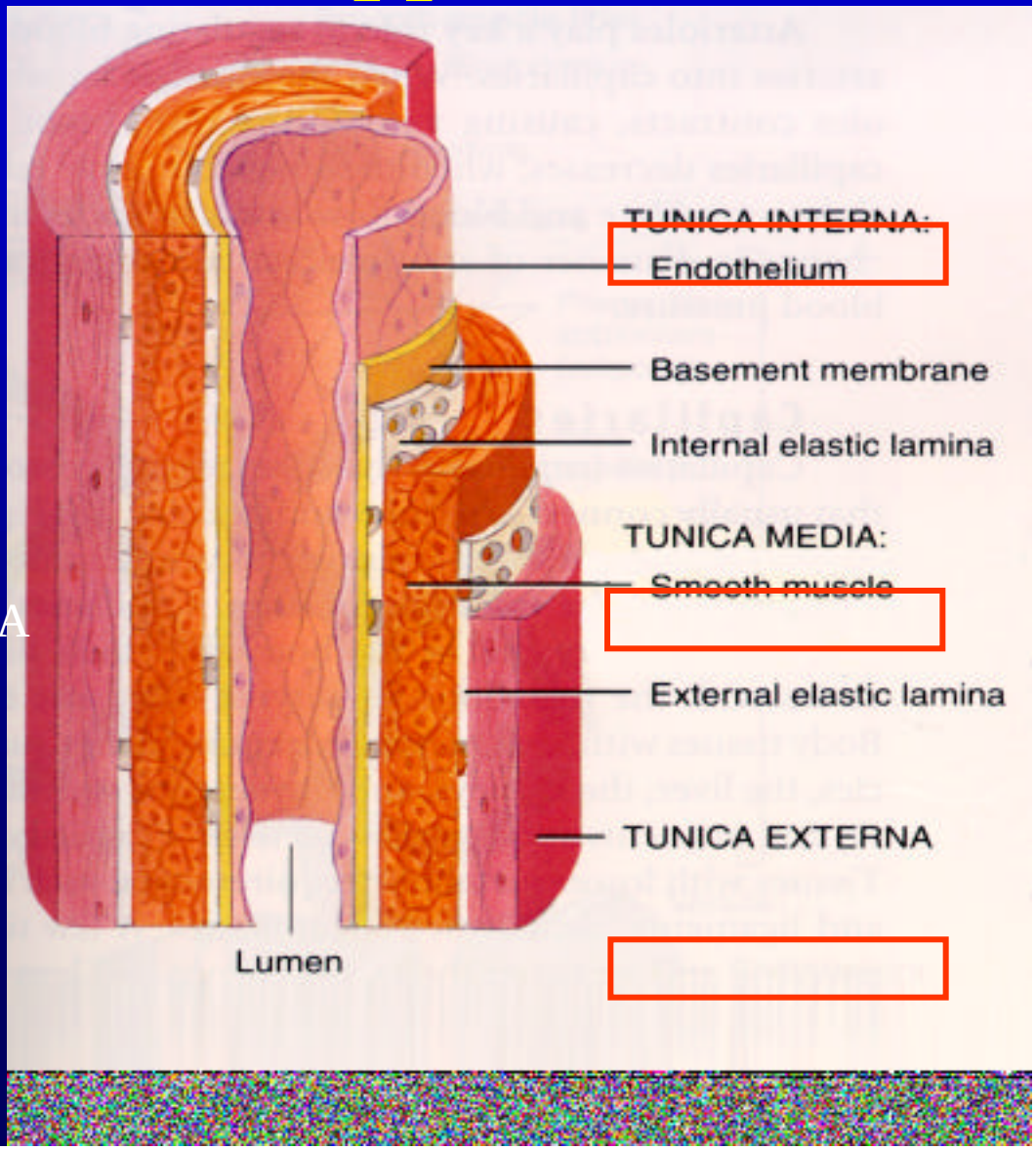
Nano-structured PLGA

Conventional PU



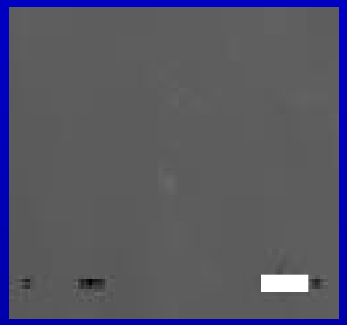
Nano-structured PU

Bar = 10μm

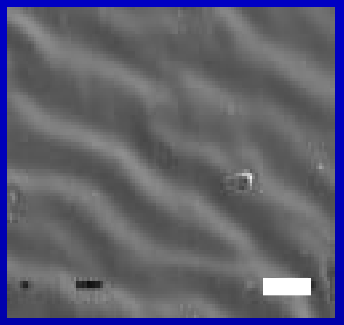


Design of Nano-structured Polymer for Articular Cartilage Repair

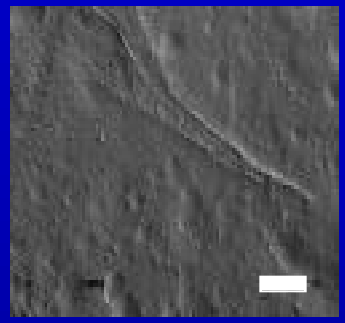
- Increased functions of chondrocytes on aligned and nano-structured polymer films.



Conventional PLGA

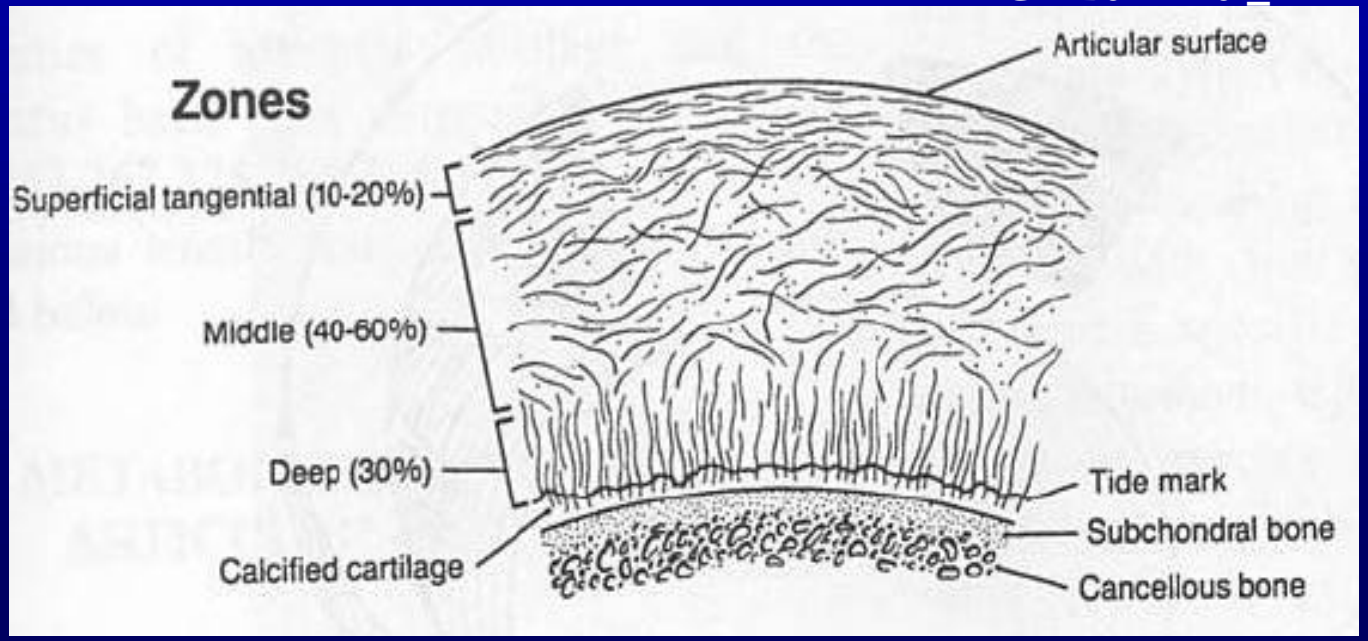


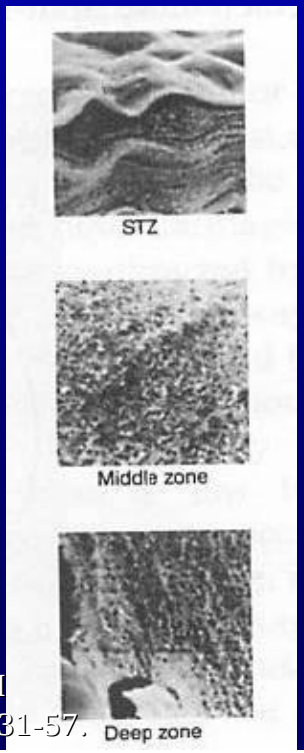
Aligned PLGA



Nano-structured

PLGA Bar = 10 _m





Courtesy of Mow VC, Proctor CS, Kelly MA: Biomechanics of articular cartilage, in Nordin M, Frankel VH (eds): Basic Biomechanics of the Musculoskeletal System, ed 2. Philadelphia, Lea & Febiger, 1989, pp 31-57.

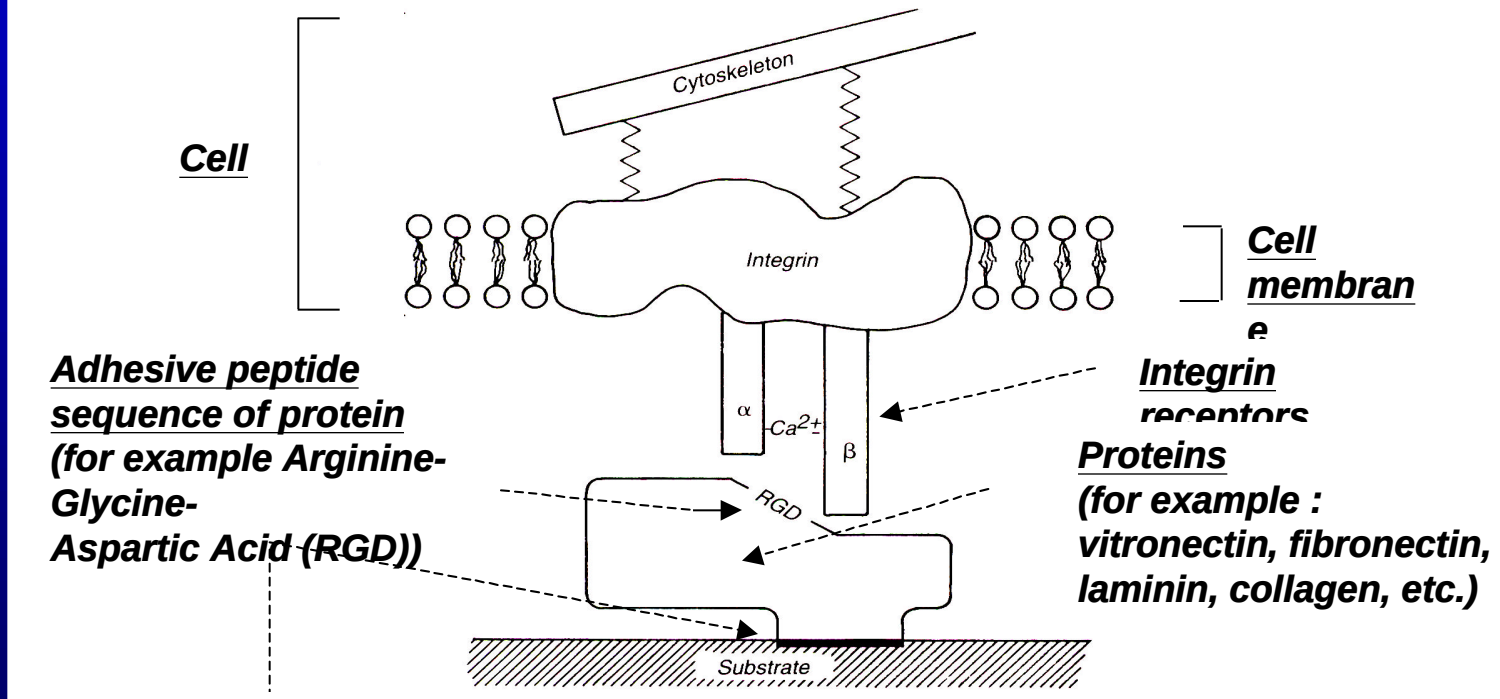
Discussion

Other research groups have observed altered cell functions on nanometer compared to conventional topographies:

- **Increased endothelial cell spreading on 13, 35, and 95 nm islands created by polymer demixing of polystyrene and poly(4-bromostyrene)[1].**
- **Increased neuron axon extension on quartz with surface features less than 100 nm[2].**
- **Increased alignment of epithelial cells on 130 nm-wide and 9 micron-deep grooves using holographic photolithography[3].**

Nano-structured materials possess higher percentages of atoms at the surface, increased portions of surface defects, and greater numbers of material boundaries at the surface that may be influencing protein interactions important for cell function.

Nanophase Materials Enhance Availability o Cell-adhesive Domains of Proteins to Promote Subsequent 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.

Adapted and redrawn from Schakenraad, J.M . pp. 140-141, in *Biomaterial Science (B. Ratner et al., eds.),*,Academic Press, Inc., San Diego, CA, 1996.

Conclusions

- Compared to conventional materials, nano-spherical ceramics, nano-fibered ceramics, carbon nanofibers, nano-structured PLGA, nanophase ceramic/PLGA composites enhanced osteoblast function.

- Due to their ability to biomimic fundamental constituent dimensions of natural tissue, nano-structured materials have the potential to become the next-generation of hard and soft implant materials with increased efficacy.

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Angstrom Medica and Spire Biomedical

Argonide Corp.

Nano-fibered Alumina Project

Applied Sciences, Inc.

Carbon Nanofiber Project

Nanophase Technologies, Corp.

Nano-spherical Ceramic Project

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Elliott Slamovich, Sherry Voytik-Harbin

Dr. Jeremiah Ejiofor

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Grace Park

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Nate Pitner

Rachel Price

Michiko Sato

Anil Thapa