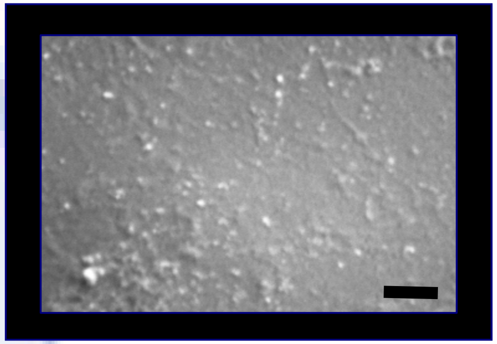
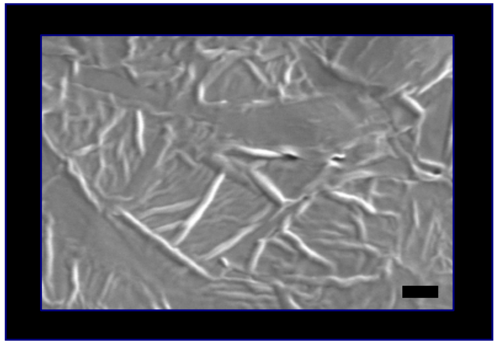
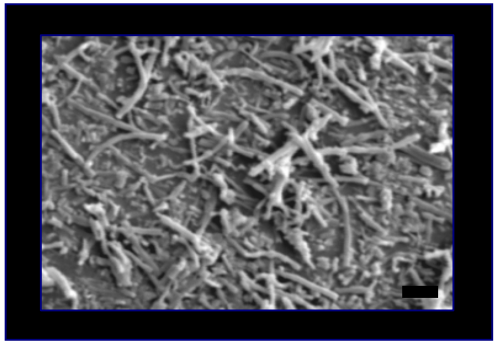
Poly-carbonate Urethane : Carbon Nanofibers (Wt%) Composites



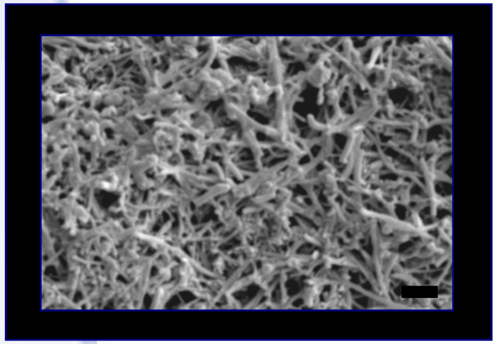
100:0



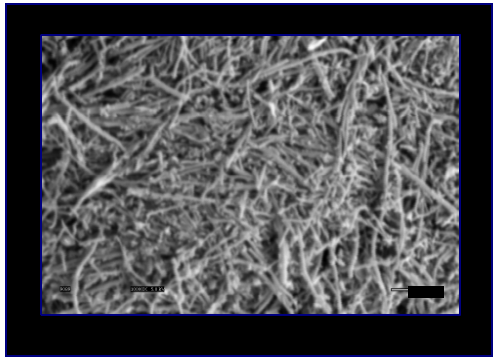
98:2



90:10



75:25

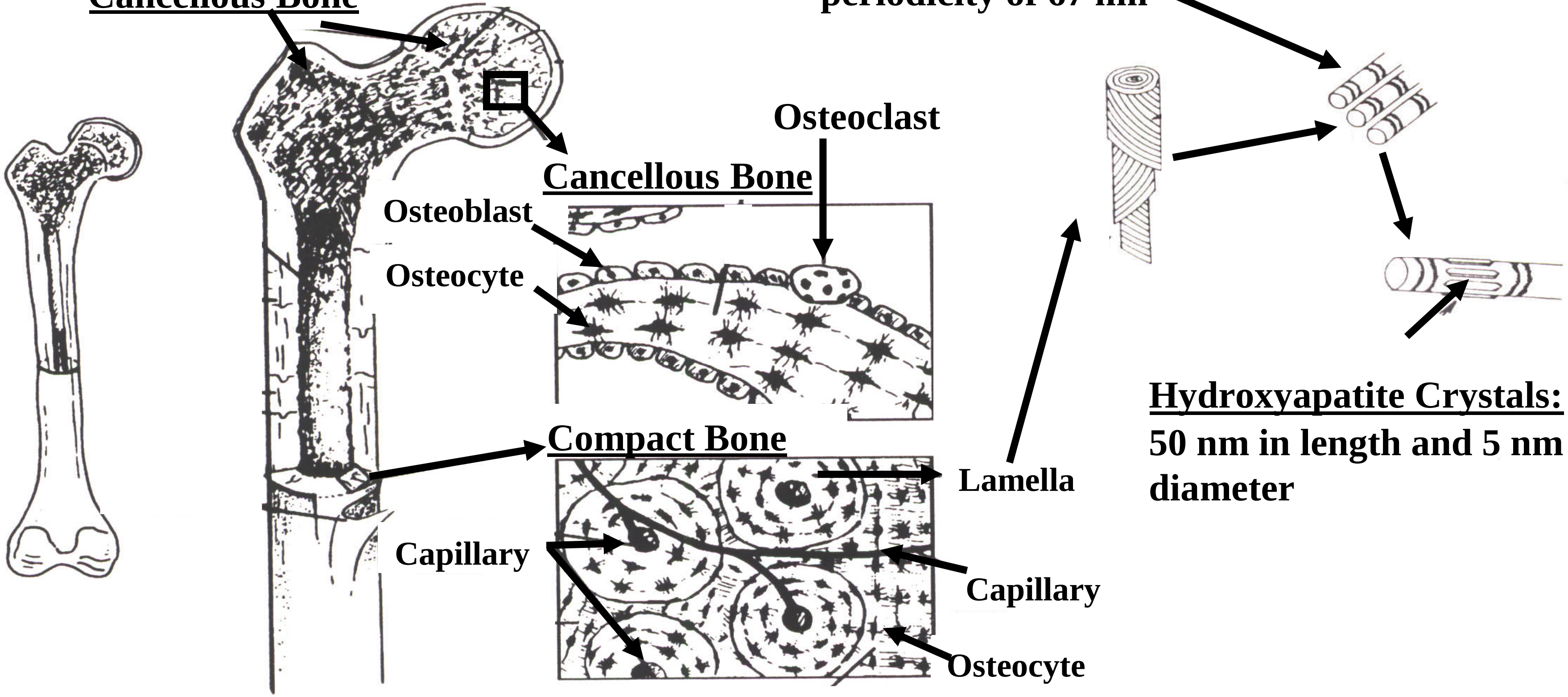


0:100

Scale bar = 1 μm.

R. L. Price, M. C. Waid, K. M. Haberstroh, and T. J. Webster,
***Biomaterials*, 24(11): 1877 – 1887, 2003.**

Bone is an Aligned 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

Cancellous Bone

Osteoclast

Cancellous Bone

Osteoblast

Osteocyte

Compact Bone

Lamella

Capillary

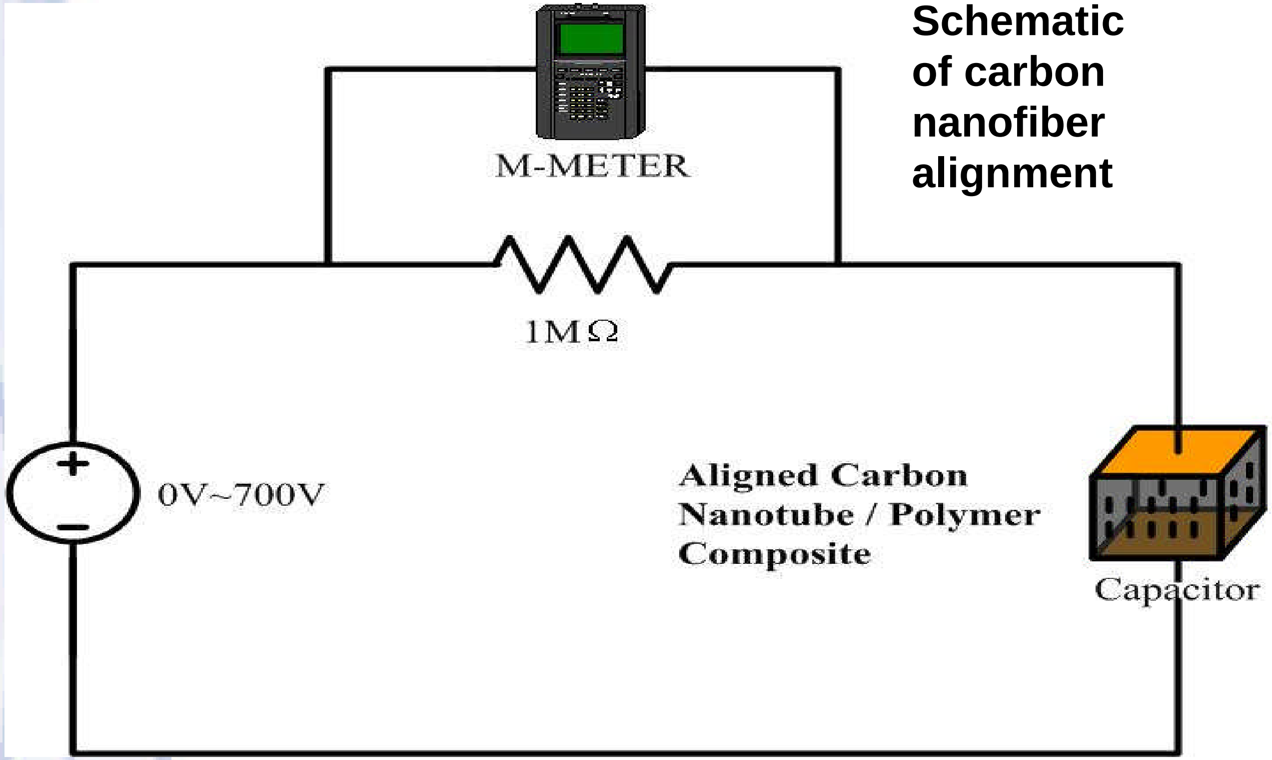
Capillary

Osteocyte

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

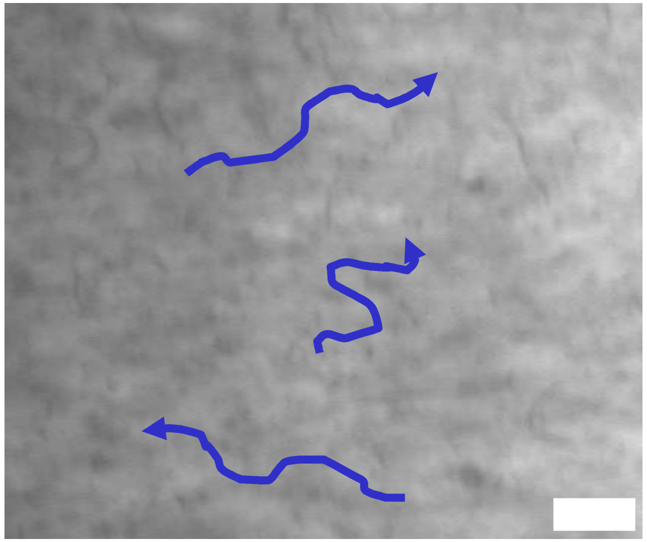
Redrawn and adapted from Fung Biomechanics: Mechanical Properties of Living Tissue, Springer-Verlag, New York, 1993 and Keaveny and Hayes, Bone 7:285, 1993.

Carbon Nanofiber Alignment in Poly-urethane Composites

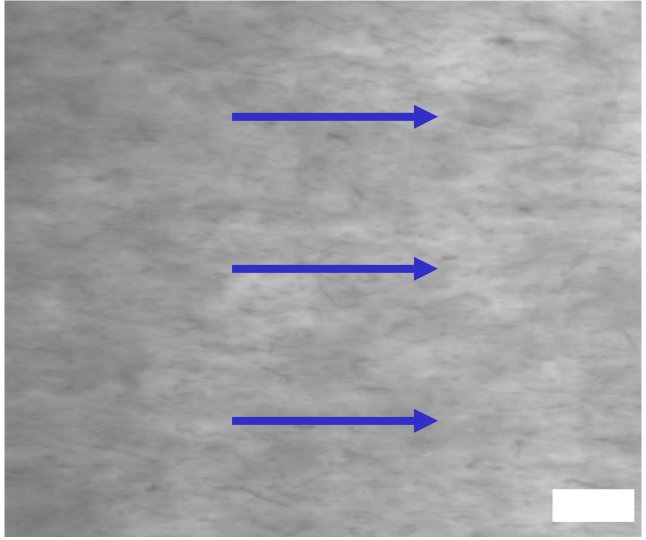


Schematic of carbon nanofiber alignment

0 volts



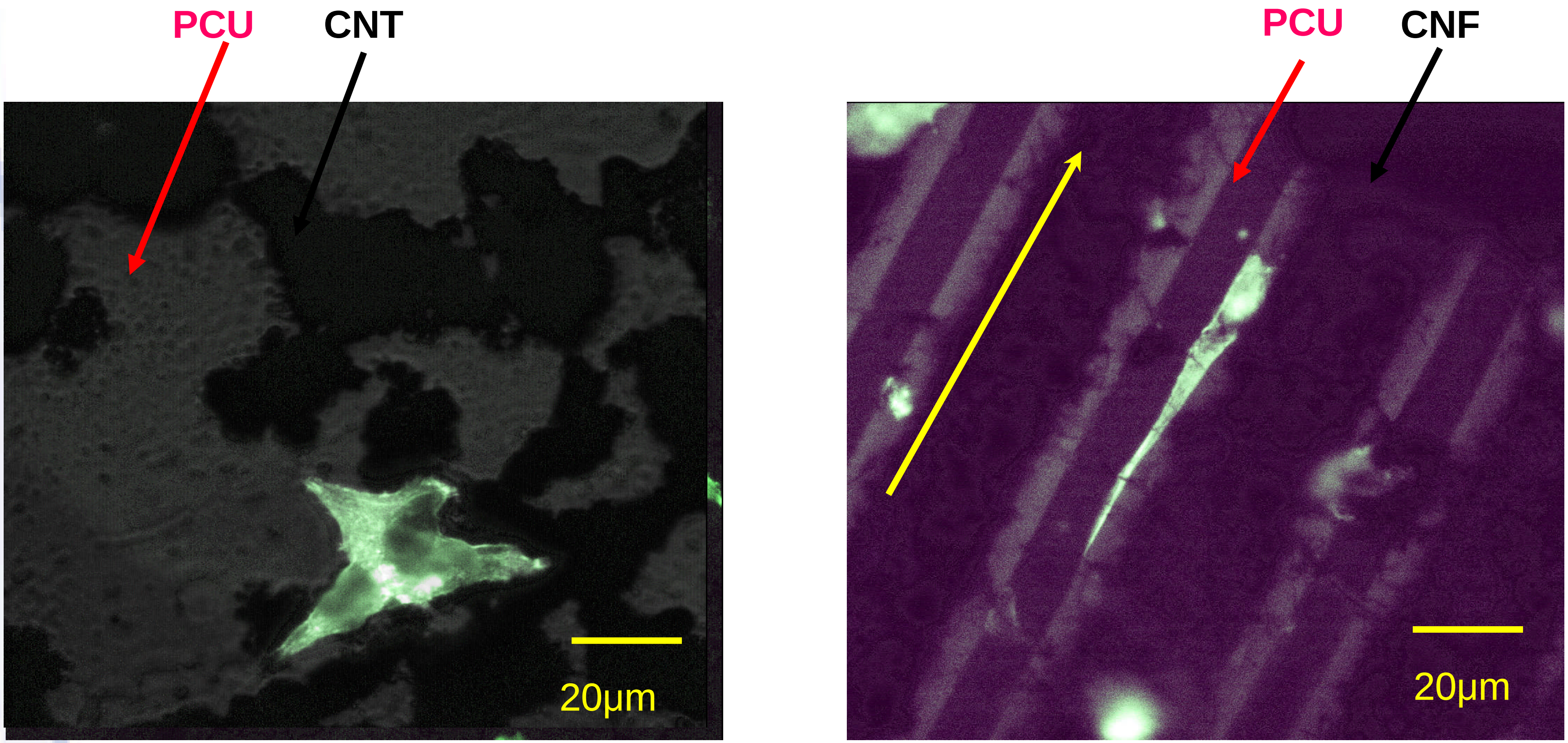
500 volts



J. U. Ejiofor, M. C. Waid, J. L. McKenzie, R. L. Price, and T. J. Webster, "Nano-biotechnology: carbon nanofibers as improved neural and orthopedic implants," *Nanotechnology* 15:48-54 (2004).

Scale bar = 10 µm

Carbon Nanofiber Surface Alignment in Poly-urethane Composites

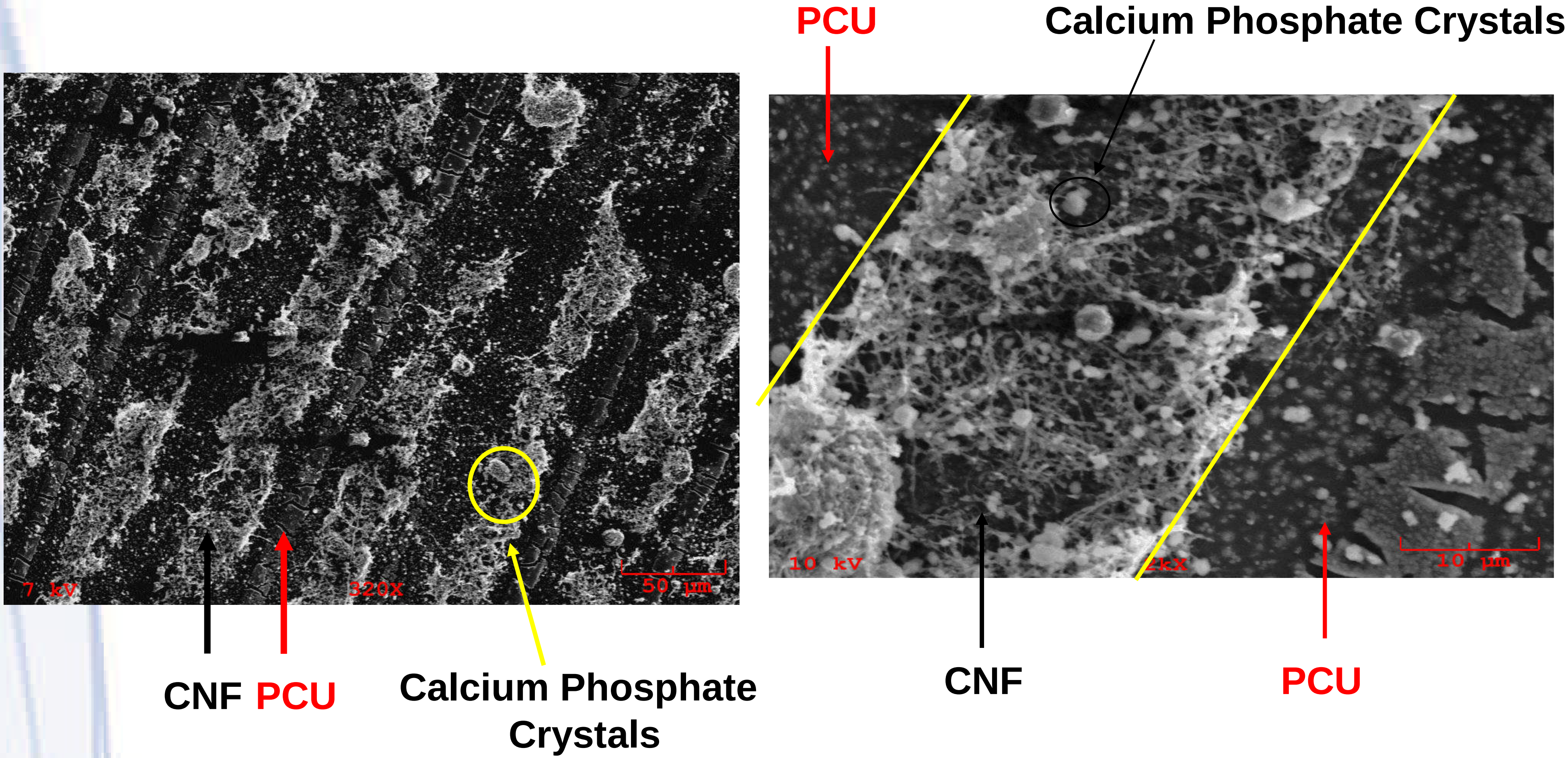


Non-Aligned (CNF/PCU)

Aligned (CNT/PCU)

Fluorescent microscopy images of osteoblast (after 2 days of culture)

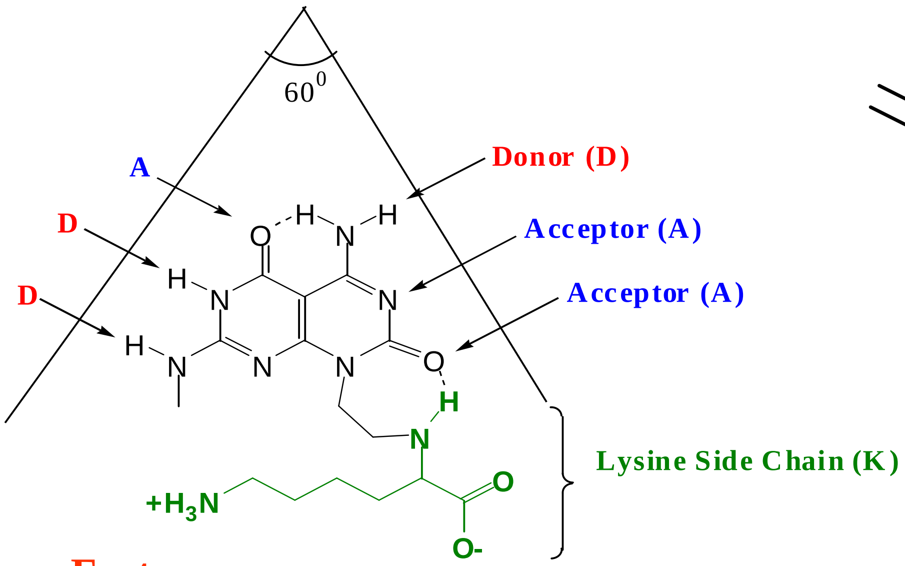
Carbon Nanofiber Surface Alignment Controls Osteoblast Mineral Deposition



Scanning Electron Microscopy images of osteoblasts after 21 days of culture.

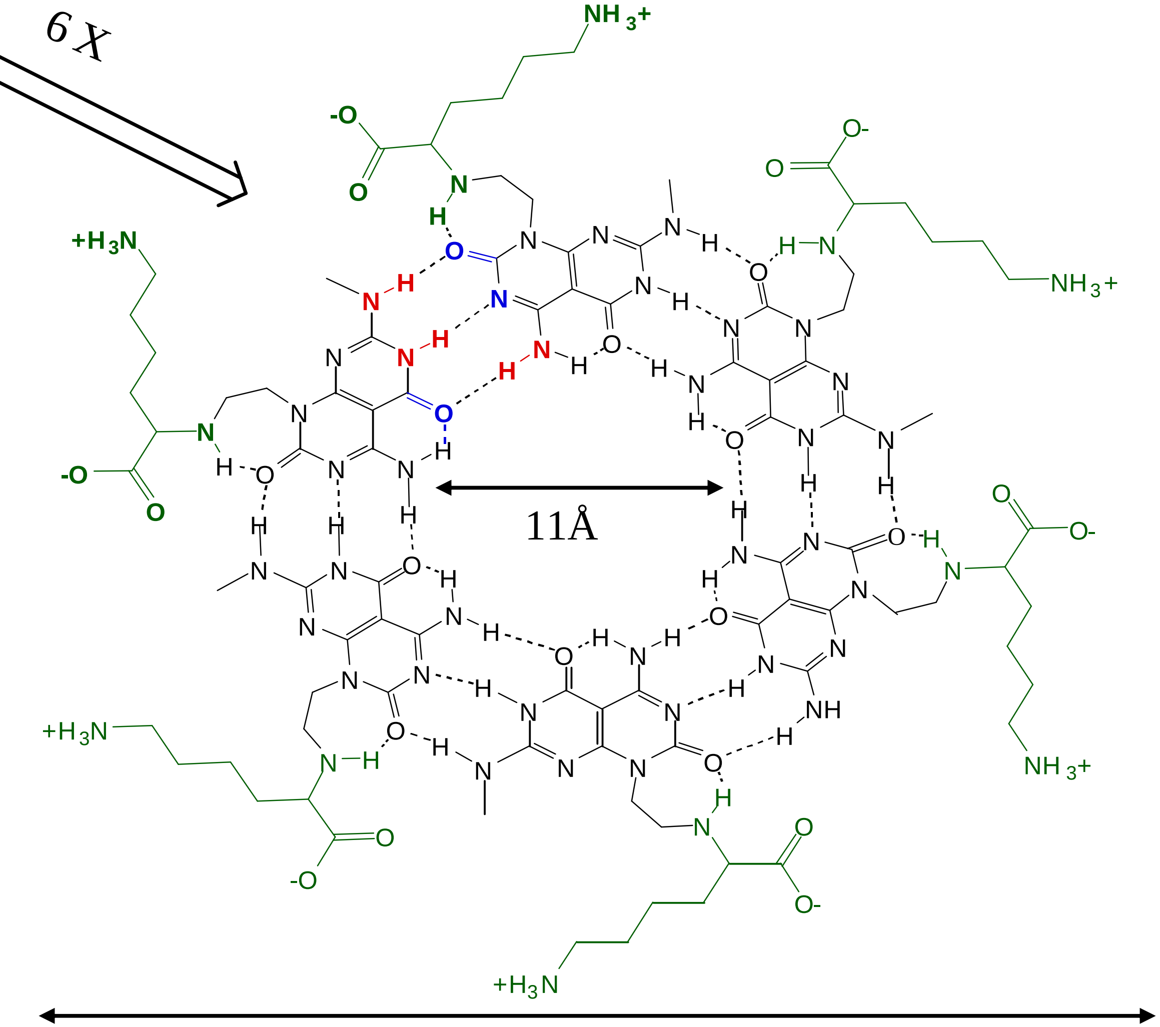
PART I (cont.)
BONE: Other Novel Nanotube Structures

Helical Rosette Nanotubes (HRN)



Features:

- **Guanine DDA array**
- **Cytosine AAD array**
- **Side chain moiety dictates supramolecular chirality & surface chemistry**
- **Ethylene spacer unit linking base to chiral center allowing intramolecular H bond**
- **CH_3 group minimize peripheral access of water**

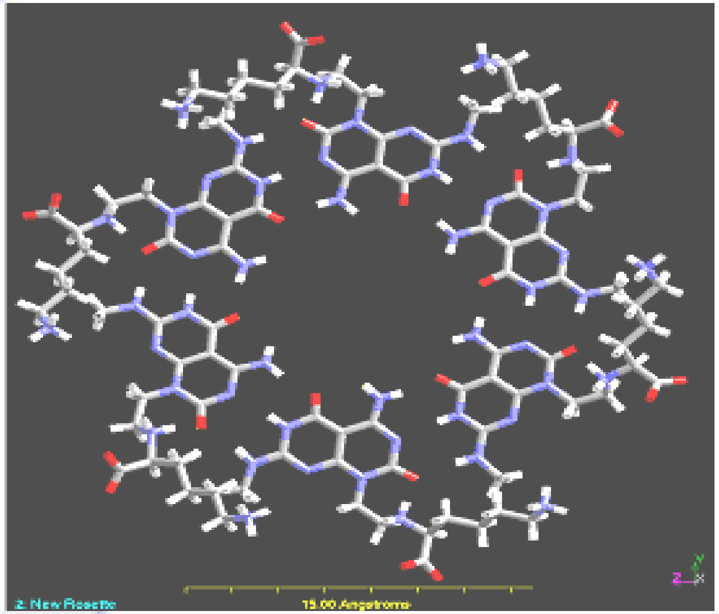


35Å

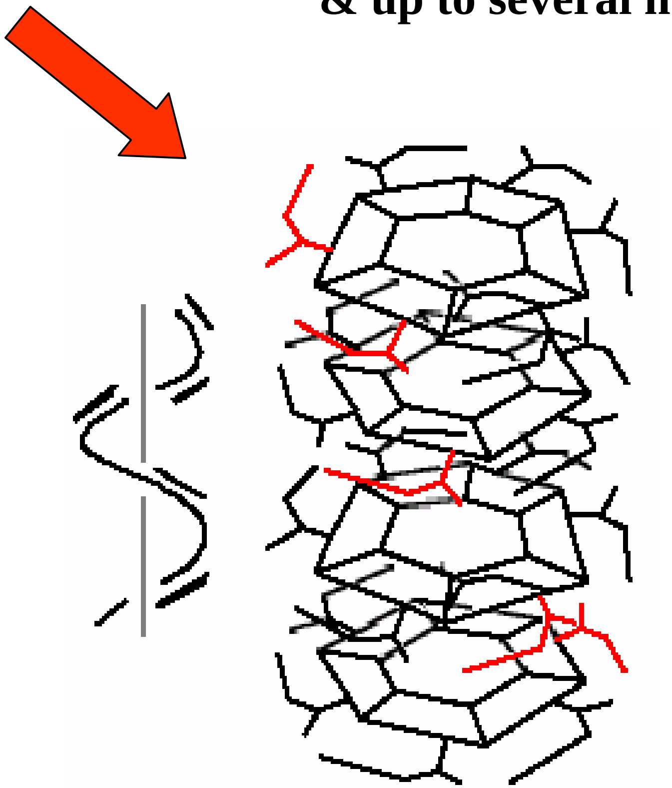
Six-membered supermacrocycle = "Rosette"

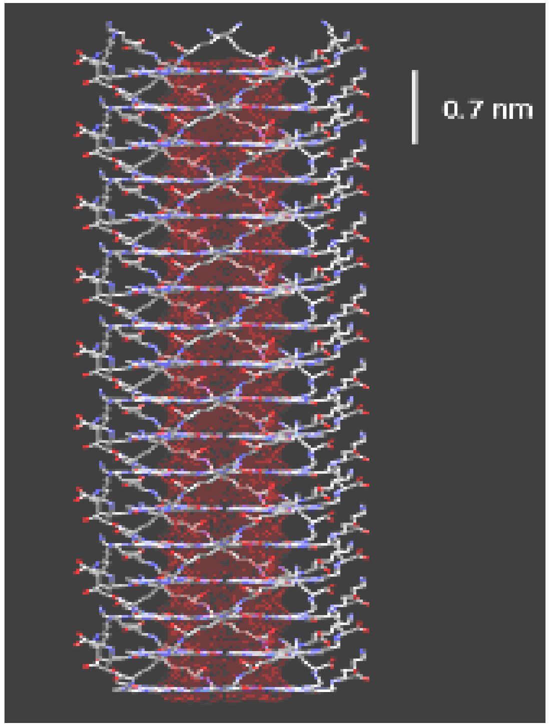
Fenniri H. et. al. Helical Rosette Nanotubes: Design, Self-Assembly, & Characterization. *J. Am. Chem. Soc.* **2001**, *123*, 3854-3855

HRN: From Supermacrocycle to Supramolecule



Several rosettes stack up to form a nanotube with a hollow core 11Å across & up to several millimeters long.





Fenniri H. et. al. Helical Rosette Nanotubes: Design, Self-Assembly, & Characterization. *J. Am. Chem. Soc.* **2001**, *123*, 3854-3855

Heating HRN-K for Novel Bone Tissue Engineering Scaffolds

HRN-K
powder

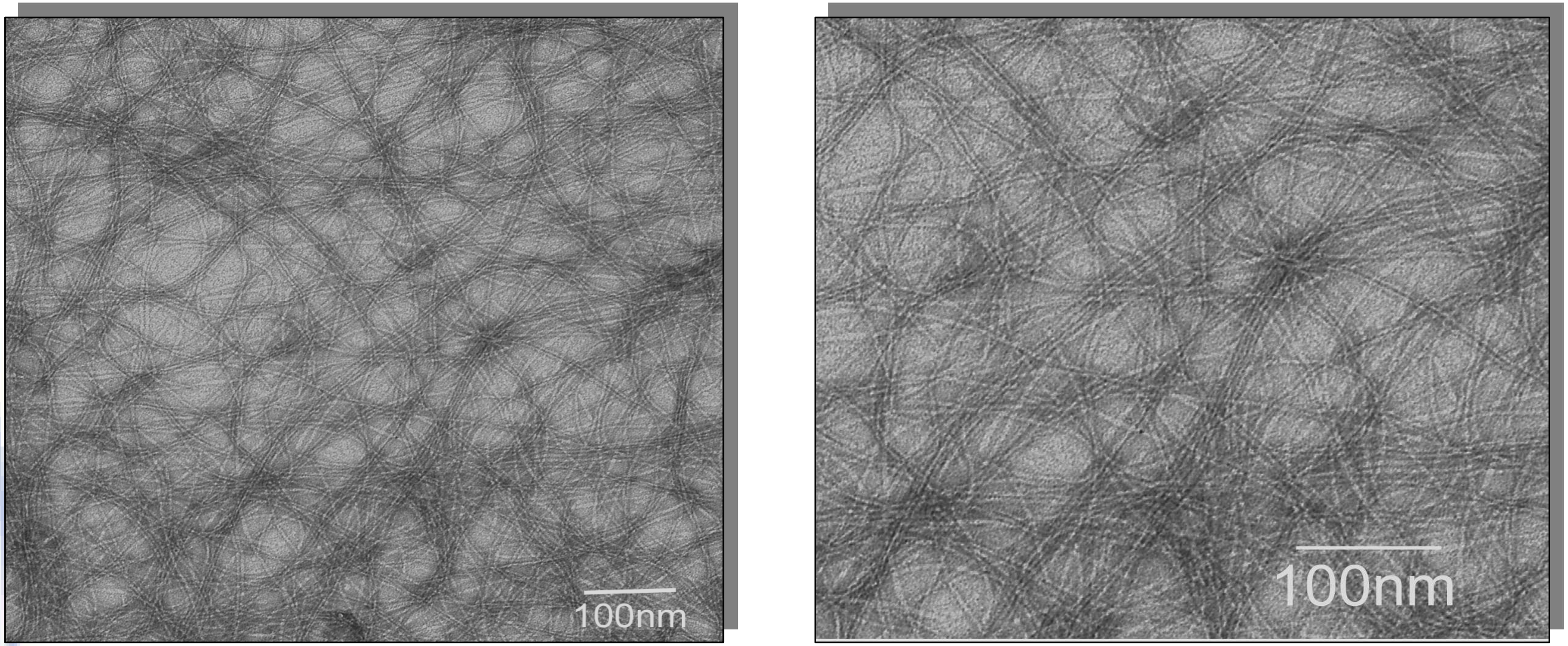
1mg/ml

HRN-K in H_2O

Heat 60°C (± 5°C) in a water bath for 10 mins

Imaged with TEM for aggregation behavior

Results: Novel Scaffold for Increased Bone Tissue Regeneration

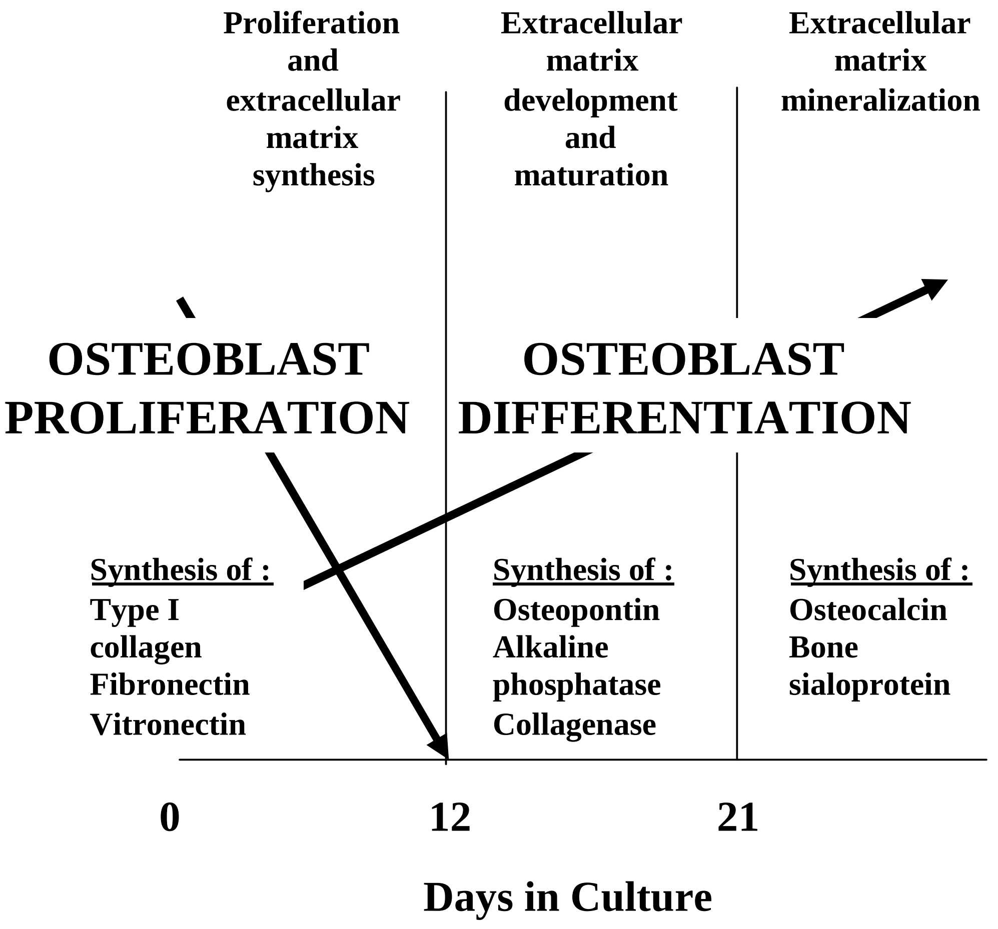


TEM micrographs of a sample of heated 1mg/ml HRN-K. HRN outer diameter was determined from NIH Image to be 4.6 ± 0.09 nm.

HRN-K form multiple layers of densely packed bundles of nanotubes upon heating. This is in agreement with the observed increase in viscosity and implicates potential use as a tissue engineering scaffold material.

Enhanced Adhesion Translates into Increased Subsequent Functions

Stages of Osteoblast Differentiation



Proliferation and extracellular matrix synthesis

Extracellular matrix development and maturation

Extracellular matrix mineralization

OSTEOBLAST PROLIFERATION

OSTEOBLAST DIFFERENTIATION

Synthesis of :
Type I collagen
Fibronectin
Vitronectin

Synthesis of :
Osteopontin
Alkaline phosphatase
Collagenase

Synthesis of :
Osteocalcin
Bone sialoprotein

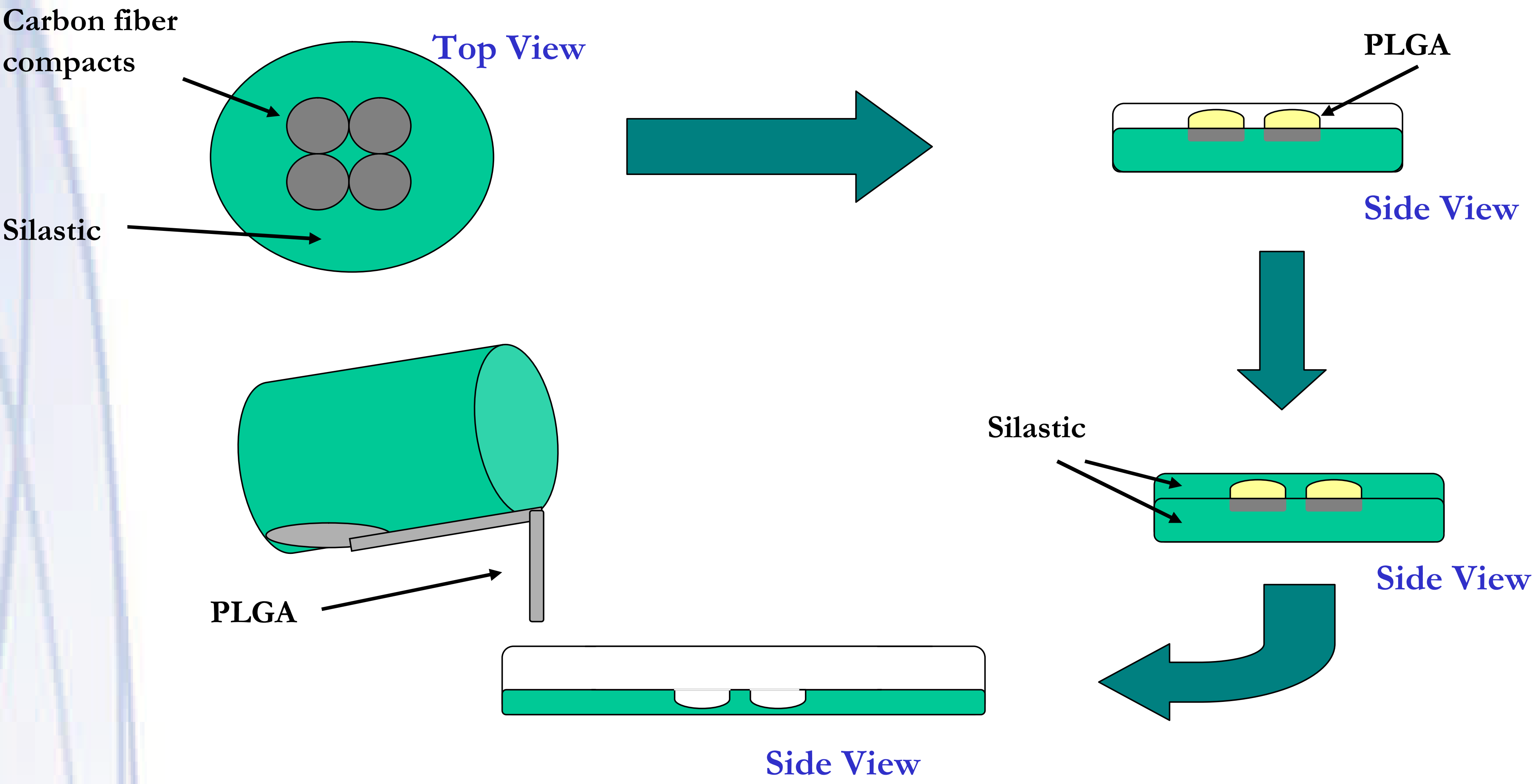
0 12 21

Days in Culture

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

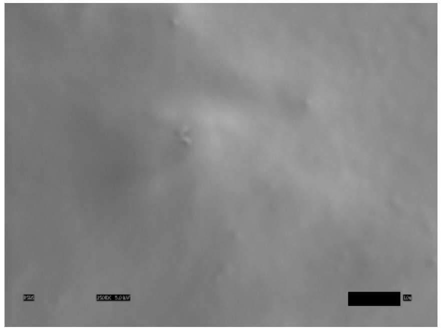
PART I (cont.)
BONE: Polymers

Transfer of Carbon Nanofiber Topography to Polymers

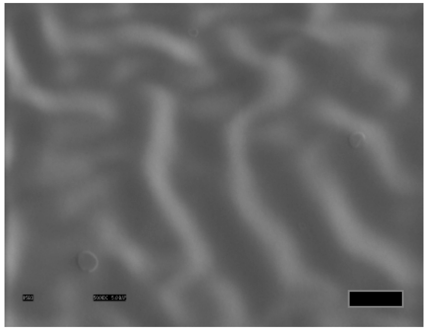


K. Ellison, R.L. Price, T.J. Webster, *Journal of Biomedical Materials Research*, in press (2005).

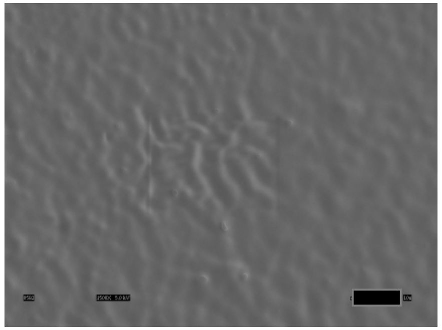
Scanning Electron Micrographs of PLGA Casts of Carbon Fiber Compacts



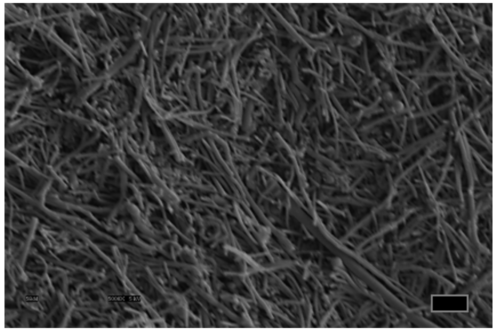
Unaltered PLGA



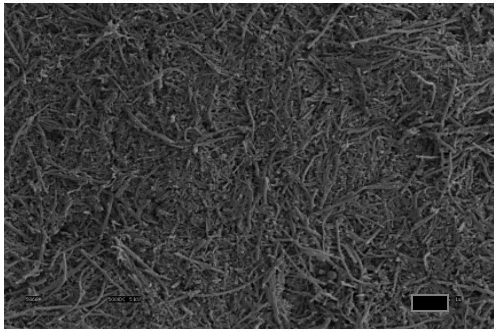
PLGA from Conventional Fibers



PLGA from Nanophase Fibers



Conventional Carbon Fibers

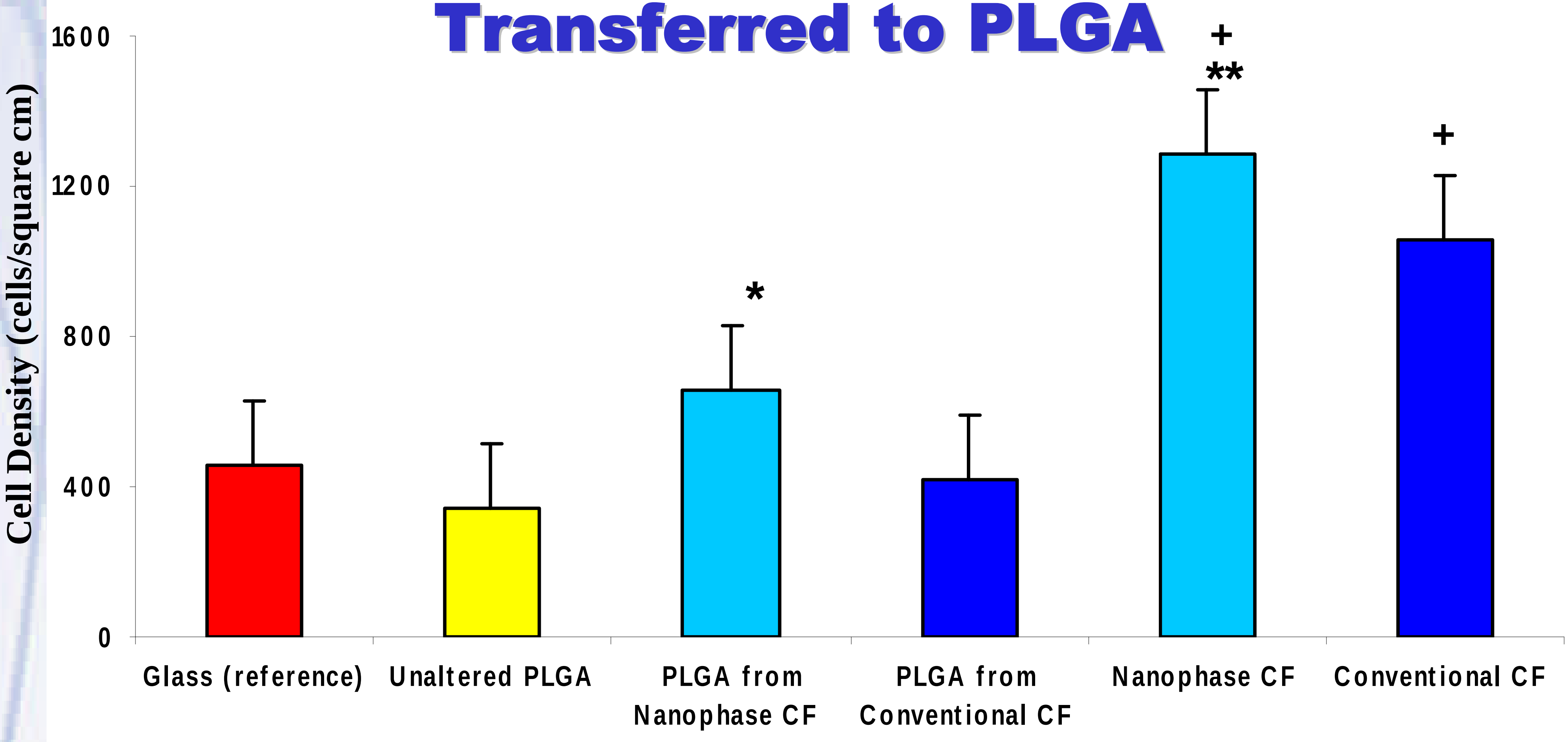


Nanophase Carbon Fibers

Bar =1 μm.

K. Ellison, R.L. Price, T.J. Webster, *Journal of Biomedical Materials Research,* in press (2005).

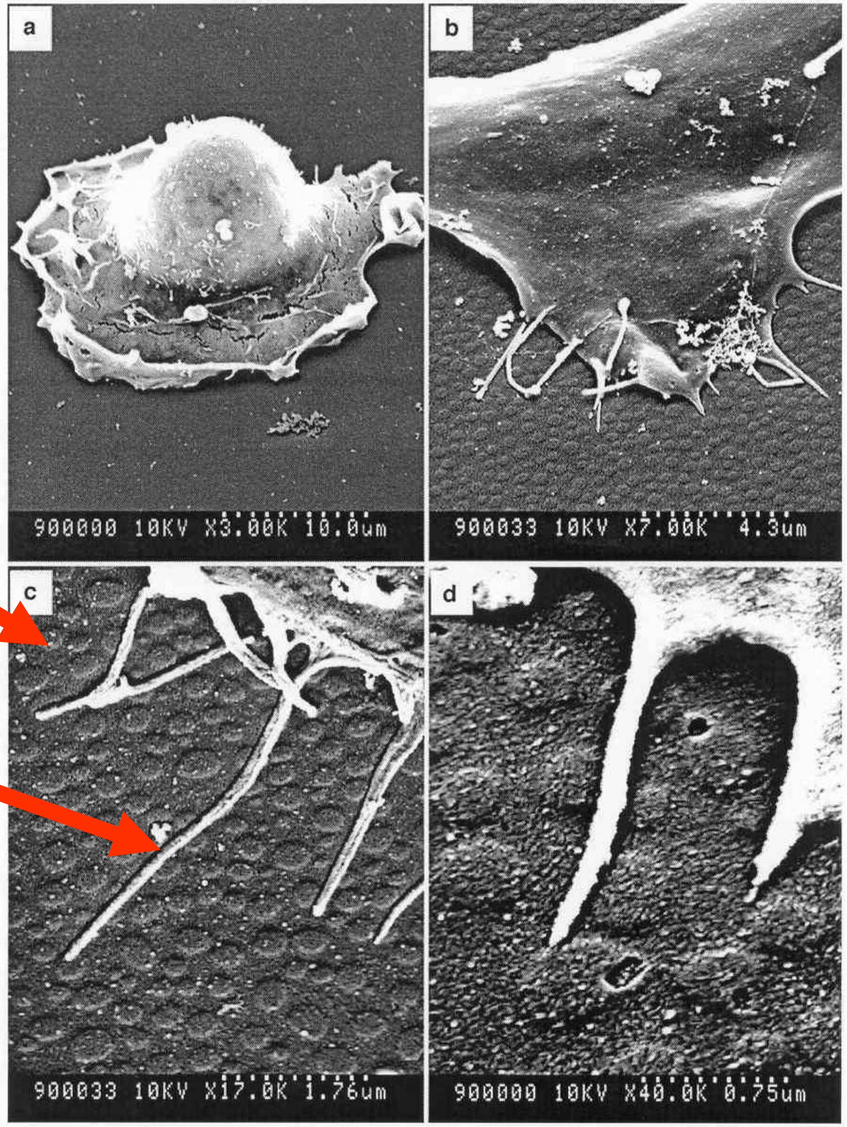
Enhanced Osteoblast Adhesion on Carbon Nanofiber Topography Transferred to PLGA



Values are mean +/-SEM; n=3; Significantly (* $p < 0.10$ and ** $p < 0.05$) greater compared to respective large CNF counterpart; Significantly (+ $p < 0.10$) greater compared to PLGA.

K. Ellison, R.L. Price, T.J. Webster, *Journal of Biomedical Materials Research*, in press (2005).

Other Novel Nano-structured Polymers



Polymer Demixed Nanoislands

Fibroblast Filopodia

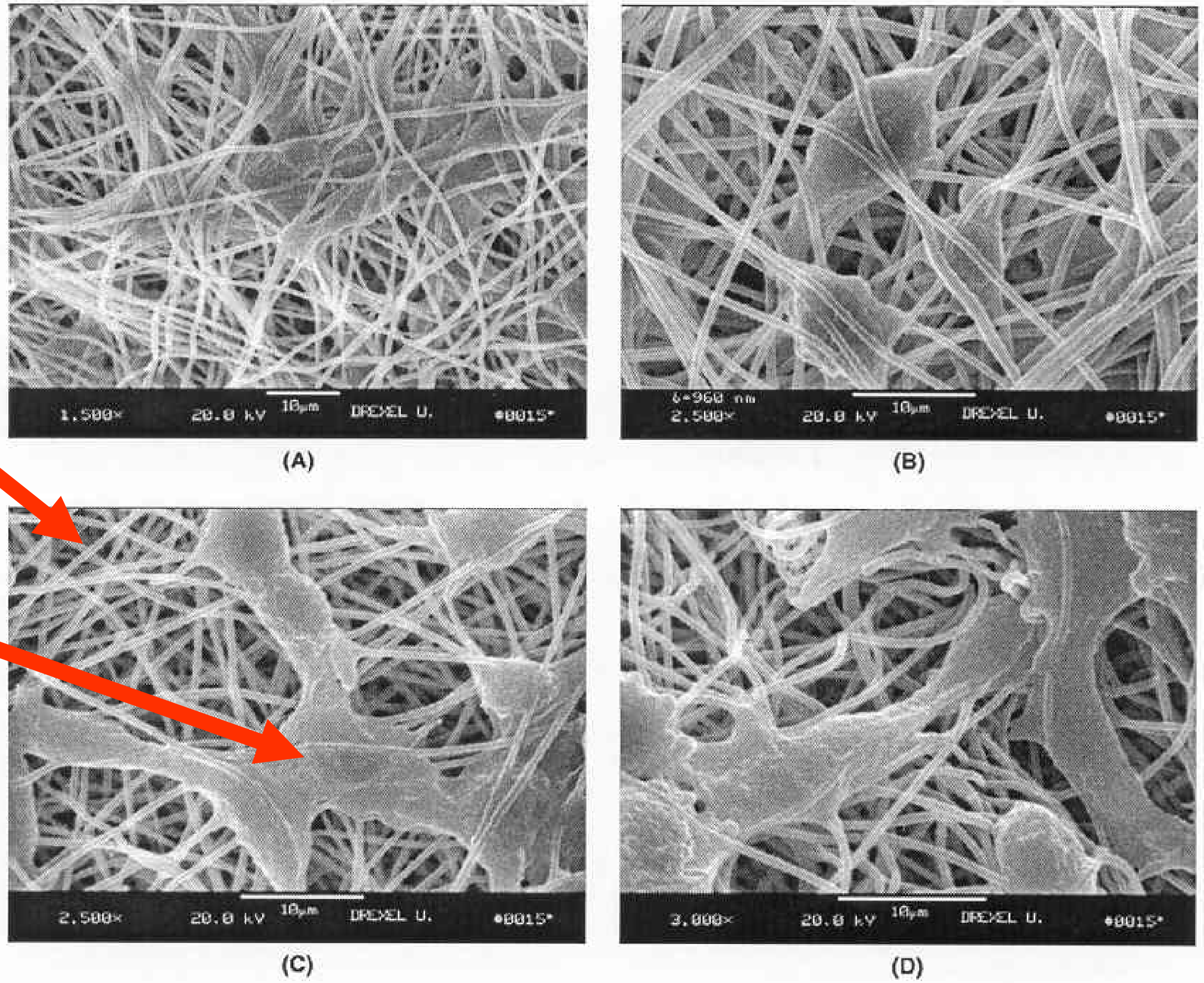
Nanoislands Created by Demixing Polystyrene and Polybromo-sytrene

Li et al., Journal of Biomedical Research 60:613-621, 2002.

Other Novel Nanofibrous Scaffolds

Electrospun Nanofibrous PLGA

Fibroblasts



(A) (B) (C) (D)

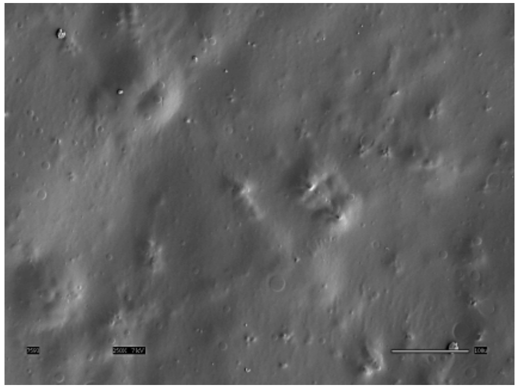
Li et al., Journal of Biomedical Research 60:613-621, 2002

PART I (cont.)

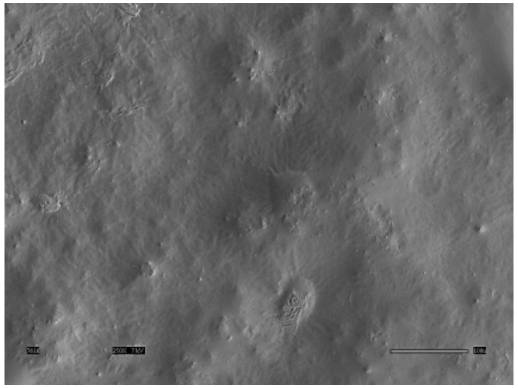
BONE:

Nanophase Ceramics/Polymer Composites

Scanning Electron Micrographs of PLGA/Titania Composites

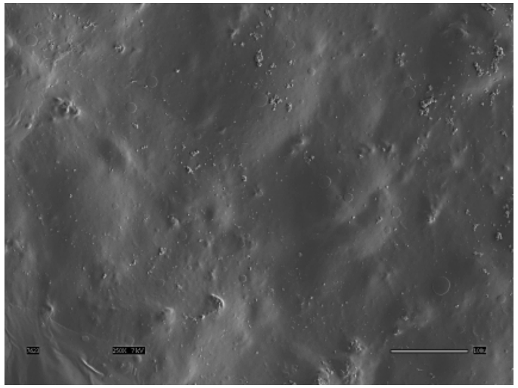


Conventional PLGA/Conventional Titania

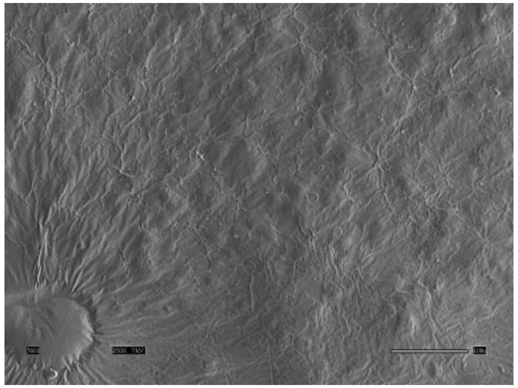


Conventional PLGA/Nanophase Titania

Scale bar = 1 micron.



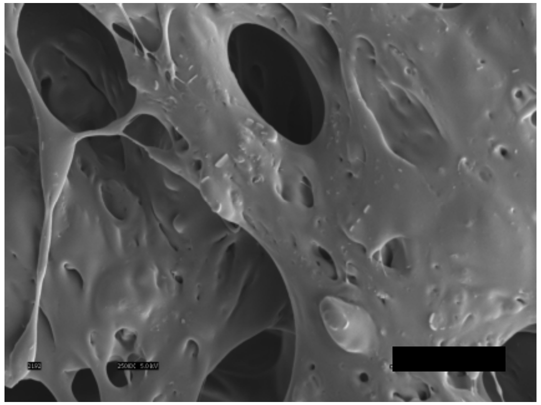
Nano-structured PLGA/Conventional Titania



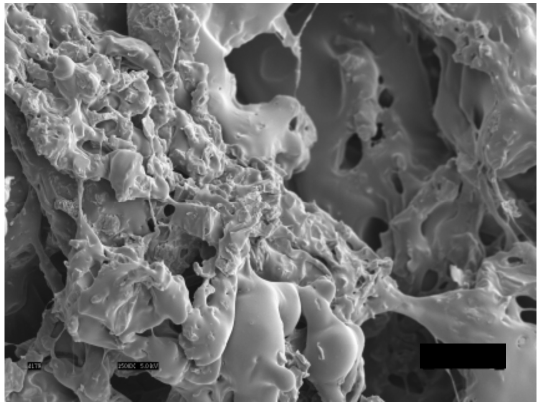
Nano-structured PLGA/Nanophase Titania

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

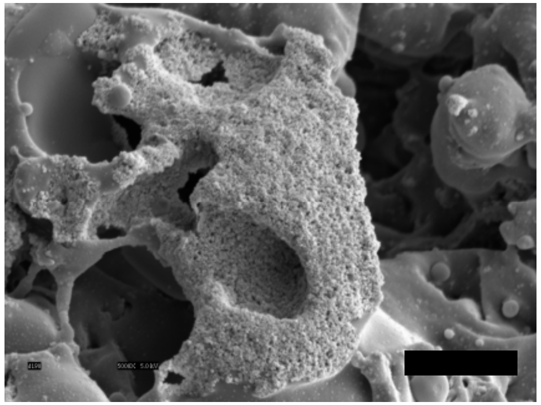
However,
Our Tissues are Three-dimensional



Plain PLGA



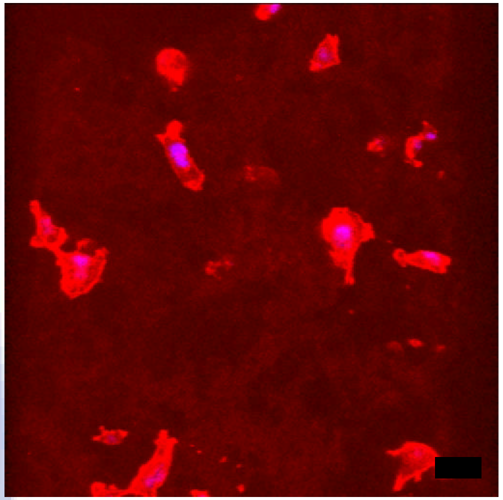
PLGA:Conventional TiO_2
70:30 wt.%



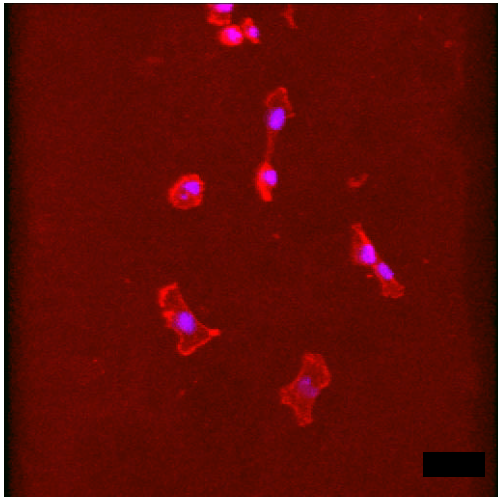
PLGA:Nanophase TiO_2
70:30 wt.%

Scanning electron micrographs (SEM) of PLGA: TiO_2 composites. Scale bar = 10 microns.

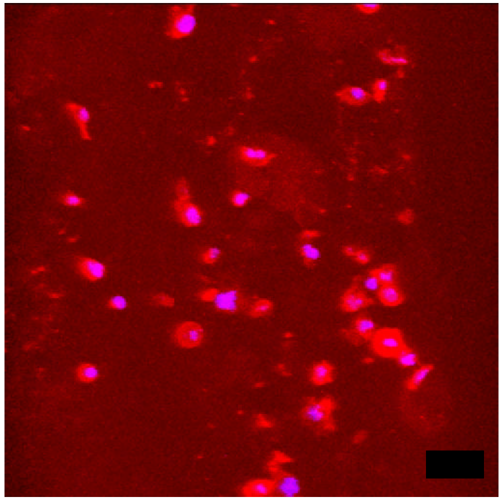
Increased Osteoblast Function on Nanophase TiO_2:PLGA Composites



PLGA:Conventional TiO_2
70:30 wt.%



Plain PLGA

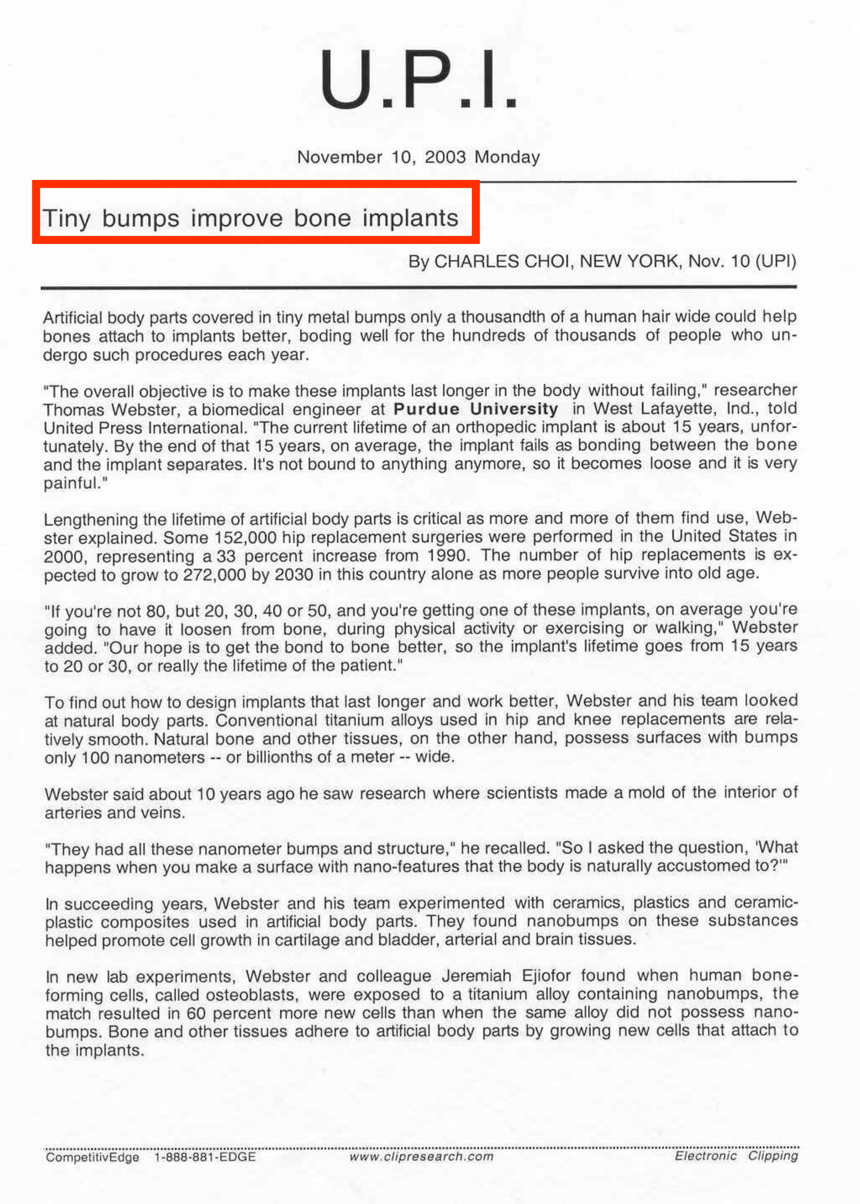


PLGA:Nanophase TiO_2
70:30 wt.%

Confocal microscope images of osteoblasts in PLGA: TiO_2 composites. Scale bar = 50 microns.

PART I (cont.)

BONE: Nano-structured Metals



U.P.I.

November 10, 2003 Monday

Tiny bumps improve bone implants

By CHARLES CHOI, NEW YORK, Nov. 10 (UPI)

Artificial body parts covered in tiny metal bumps only a thousandth of a human hair wide could help bones attach to implants better, boding well for the hundreds of thousands of people who undergo such procedures each year.

"The overall objective is to make these implants last longer in the body without failing," researcher Thomas Webster, a biomedical engineer at **Purdue University** in West Lafayette, Ind., told United Press International. "The current lifetime of an orthopedic implant is about 15 years, unfortunately. By the end of that 15 years, on average, the implant fails as bonding between the bone and the implant separates. It's not bound to anything anymore, so it becomes loose and it is very painful."

Lengthening the lifetime of artificial body parts is critical as more and more of them find use, Webster explained. Some 152,000 hip replacement surgeries were performed in the United States in 2000, representing a 33 percent increase from 1990. The number of hip replacements is expected to grow to 272,000 by 2030 in this country alone as more people survive into old age.

"If you're not 80, but 20, 30, 40 or 50, and you're getting one of these implants, on average you're going to have it loosen from bone, during physical activity or exercising or walking," Webster added. "Our hope is to get the bond to bone better, so the implant's lifetime goes from 15 years to 20 or 30, or really the lifetime of the patient."

To find out how to design implants that last longer and work better, Webster and his team looked at natural body parts. Conventional titanium alloys used in hip and knee replacements are relatively smooth. Natural bone and other tissues, on the other hand, possess surfaces with bumps only 100 nanometers -- or billionths of a meter -- wide.

Webster said about 10 years ago he saw research where scientists made a mold of the interior of arteries and veins.

"They had all these nanometer bumps and structure," he recalled. "So I asked the question, 'What happens when you make a surface with nano-features that the body is naturally accustomed to?'"

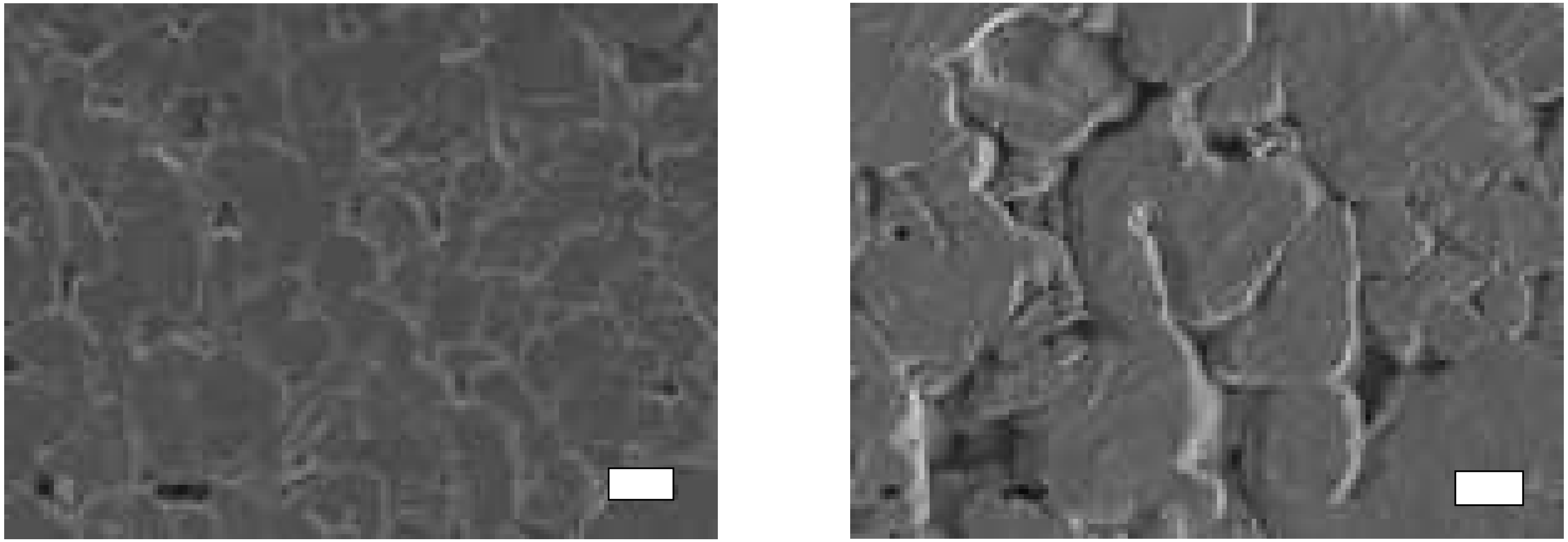
In succeeding years, Webster and his team experimented with ceramics, plastics and ceramic-plastic composites used in artificial body parts. They found nanobumps on these substances helped promote cell growth in cartilage and bladder, arterial and brain tissues.

In new lab experiments, Webster and colleague Jeremiah Ejiofor found when human bone-forming cells, called osteoblasts, were exposed to a titanium alloy containing nanobumps, the match resulted in 60 percent more new cells than when the same alloy did not possess nanobumps. Bone and other tissues adhere to artificial body parts by growing new cells that attach to the implants.

Constituent Nanoparticle Metals

Material	Category	ASTM designation	Particle size (µm)	Particle shape
Ti	Nanophase	F-67; G2	0.5 - 2.4	Spongy
Ti	Conventional	F-67; G2	> 10.5	Spongy
Ti6Al4V (prealloyed)	Nanophase	F-136	0.5 - 1.4	Spongy (Ti); irregular (Al /V)
Ti6Al4V (prealloyed)	Conventional	F-136	> 7.5 (Ti); ≤ 44	Spongy (Ti); irregular (Al /V)
Co28Cr6Mo (blend elemental)	Nanophase	F-75; F-799	0.2 – 0.4	Spherical and irregular mix
Co28Cr6Mo (blend elemental)	Conventional	F-75; F-799	14 - 26	Spherical and irregular mix

Ti Compacts



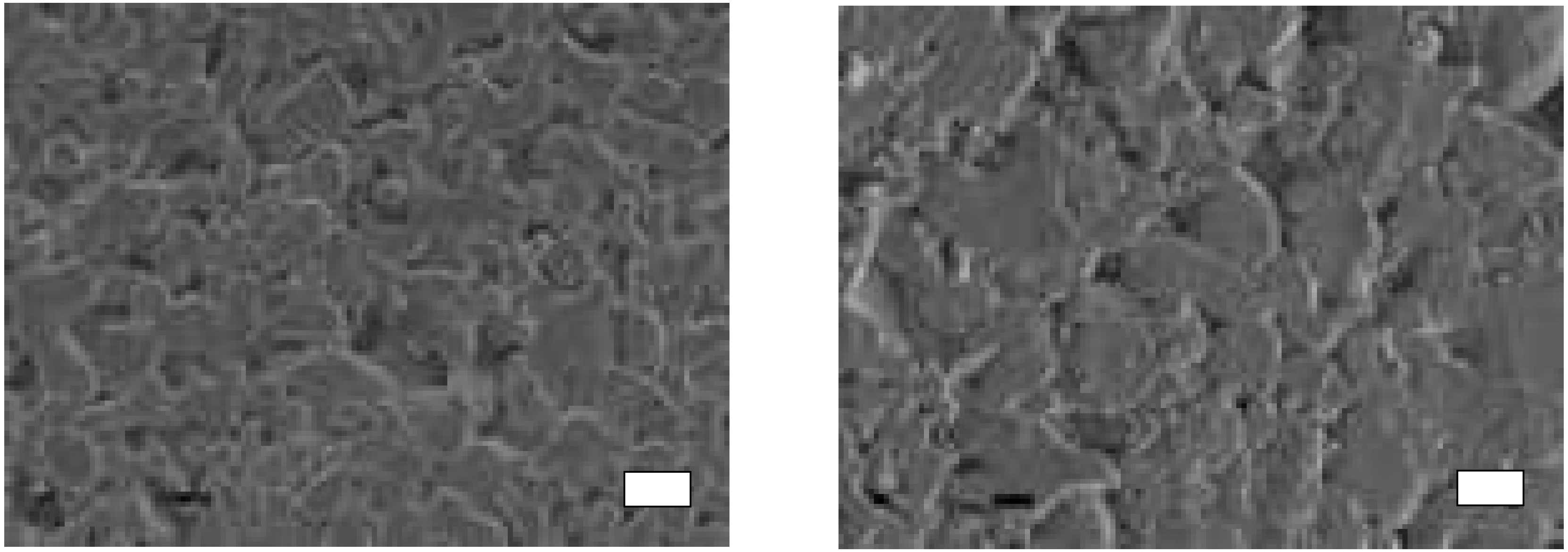
Ti (nanophase)

Ti (conventional)

Scanning electron micrographs (SEM). Scale bar = 1 micron for nanophase Ti and 10 microns for conventional Ti.

T.J. Webster and J. Ejiofor, Biomaterials, in press, 2005.

Ti6Al4V Compacts



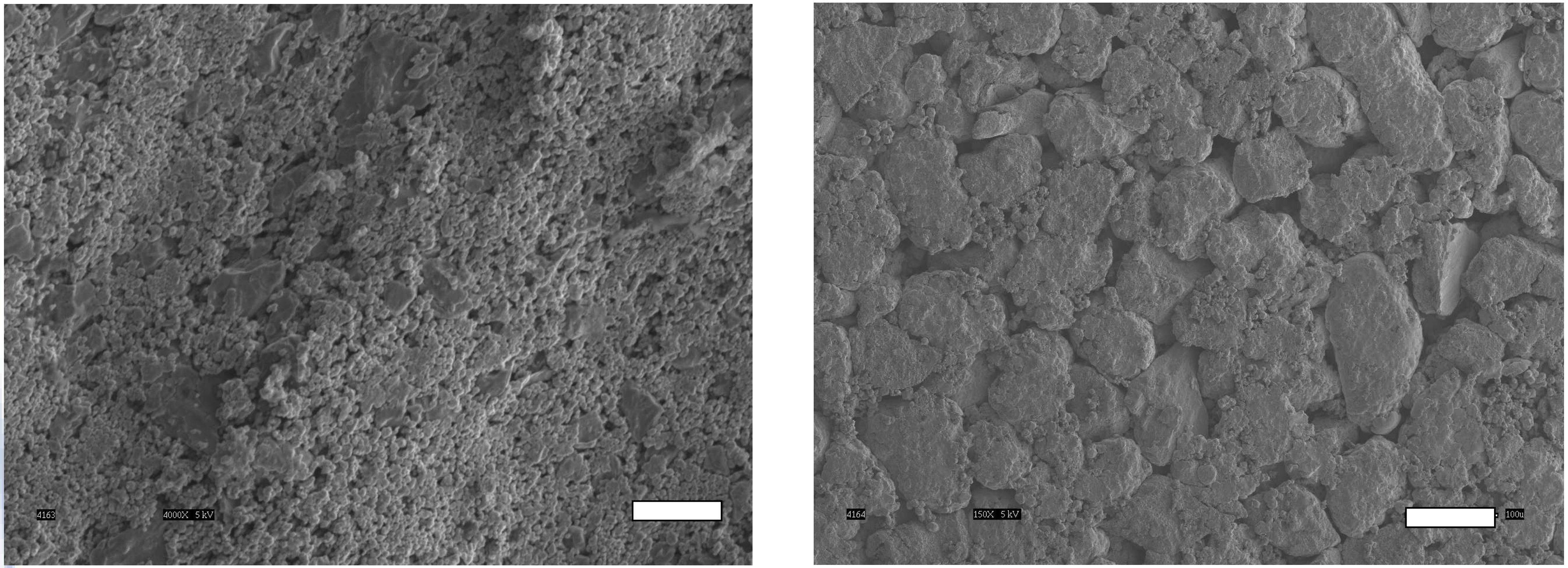
Ti6Al4V
(nanophase)

Ti6Al4V
(conventional)

Scanning electron micrographs (SEM). Scale bar = 1 micron for nanophase Ti6Al4V and 10 microns for conventional Ti6Al4V.

T.J. Webster and J. Ejiofor, Biomaterials, in press, 2005.

CoCrMo Compacts



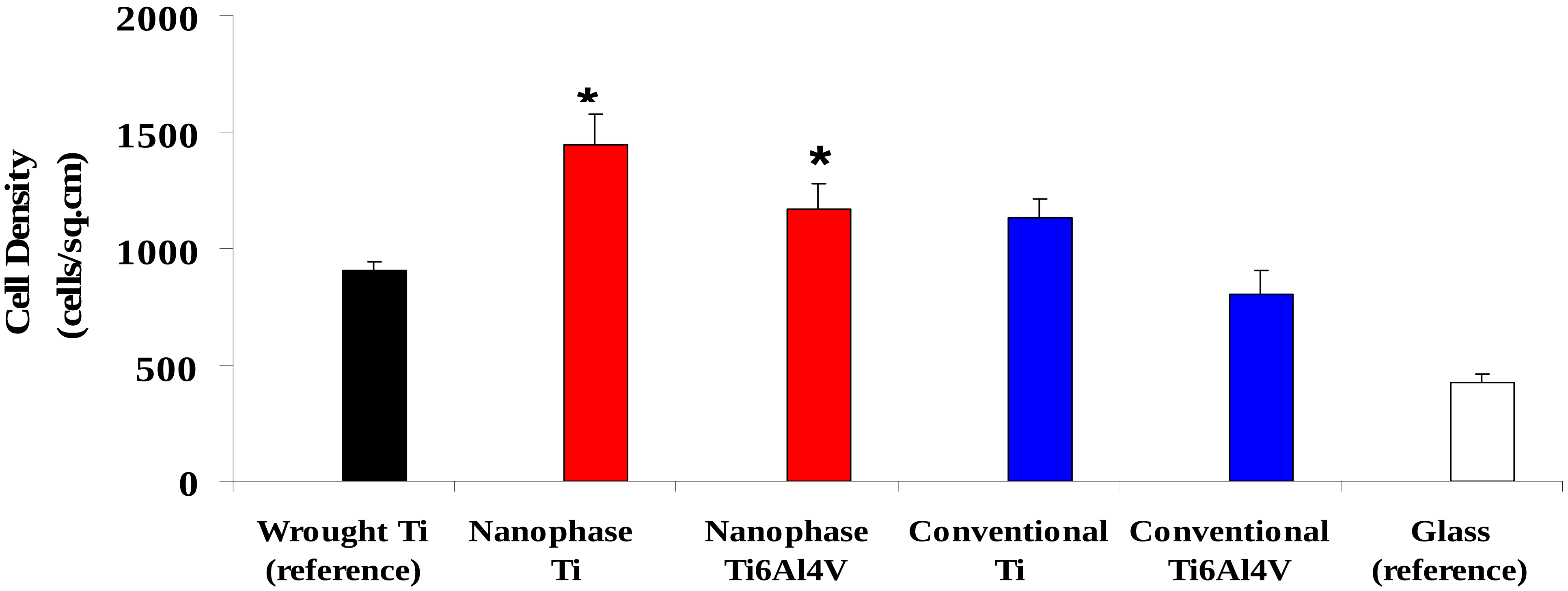
CoCrMo
(nanophase)

CoCrMo
(conventional)

Scanning electron micrographs (SEM). Scale bar = 10 microns.

T.J. Webster and J. Ejiofor, Biomaterials, in press, 2005.

Enhanced Osteoblast Adhesion on Nanophase Ti and Ti6Al4V



Time = 1 hour.

Values are mean +/- SEM; n = 3; * $p < 0.1$ (compared to respective cell adhesion on conventional Ti or Ti6Al4V) .