NANOPHASE CERAMICS: THE FUTURE ORTHOPEDIC AND DENTAL IMPLANT MATERIAL

Thomas J. Webster

Department of Biomedical Engineering, Purdue University, West Lafayette, Indiana 47907

Traditional materials utilized for orthopedic and dental applications have been selected based on their mechanical properties and ability to remain inert in vivo; this selection process has provided materials that satifisfy physiological loading conditions but do not

ADVANCES IN CHEMICAL ENGINEERING, VOL. 27 0065-2377/01 $35.00

duplicate the mechanical, chemical, and architectural properties of bone. The less than optimal surface properties of conventional materials have resulted in clinical complications that necessitate surgical removal of many such failed bone implants due to insufficient bonding to juxtaposed bone. Sufficient bonding of an implant to juxtaposed bone (i.e., osseointegration) is needed to minimize motion-induced damage to surrounding tissues and support physiological loading conditions, criteria crucial for implant success. Insufficient osseointegration can be caused by biomaterial surface properties that do not support new bone synthesis and/or mechanical properties that do not match those of surrounding bone; mismatch of mechanical properties between an implant and surrounding bone may lead to stress and strain imbalances that cause implant loosening and eventual failure. Clearly, the next generation of biomaterials for orthopedic and dental implant applications must possess both biocompatible surface properties that promote bonding of juxtaposed bone and mechanical properties similar to those of physiological bone. Due to unique surface and mechanical properties, as well as the ability to simulate the three-dimensional architecture of physiological bone, one possible consideration for the next generation of orthopedic and dental implants with improved efficacy are nanophase materials. This chapter presents reports of the design, synthesis, and evaluation of nanophase materials for increased orthopedic and dental implant efficacy.

I. Introduction

An estimated 11 million people in the United States have received at least one medical implant device; orthopedic implants (including fracture, fixation, and artificial joint devices) accounted for 51.3% of these implants in 1992 (Praemer *et al.,* 1992). Among joint-replacement procedures, hip and knee surgeries represented 90% of the total and in 1988 were performed 310,000 times in the United States alone (Praemer *et al.,* 1992). Implanting an orthopedic or dental implant can be a costly procedure (due to surgery, hospital-provided care, physical therapy costs, and recuperation time) and involves considerable patient discomfort. Both patient discomfort and cost can increase if surgical revision becomes necessary after implantation when an orthopedic or dental implant is rejected by host tissue, is insufficiently integrated into juxtaposed bone, and/or fails under physiological loading conditions. Careful design of implants with improved properties (such as

biocompatibility, ability to enhance integration into surrounding bone, and mechanical properties similar to physiological bone) could increase biomaterial success rate and, therefore, decrease patient discomfort as well as surgical costs associated with device retrieval and implantation of another orthopedic and dental prostheses.

Successful biomaterials must integrate into surrounding tissue by eliciting timely and desirable responses from surrounding cells. Orthopedic and dental biomaterials of the future must promote swift deposition of new bone on the surface of implants and support bonding of juxtaposed bone (that is, osseointegrate) to stabilize prostheses *in situ* and thus minimize motion-induced damage to surrounding tissues. In addition, and equally as important, biomaterials of the future must possess mechanical properties similar to those of surrounding bone; mismatch between mechanical properties of implants and surrounding bone could cause imbalances in stress and strain distribution, thus, leading to bone resorption and eventual implant loosening or failure (Kaplan *et al.,* 1994; Brunski, 1991; Lehman *et al.,* 1994).

Conventional orthopedic and dental materials (such as commercially pure titanium, Ti–6Al–4V, and Co–Cr–Mo alloys) meet requirements for mechanical loads in the physiological range, but their less than optimal surface properties (leading to insufficient osseointegration) have resulted in clinical complications and necessitated surgical removal of many such failed bone implants (Kaplan *et al.,* 1994). In contrast, ceramics, which have exceptional biocompatibility and surface properties with bone cells and tissues but are brittle under loading, have experienced limited use in biomedical applications. The next generation of biomaterials for orthopedic and dental implant applications must possess both biocompatible surface properties that promote bonding of juxtaposed bone and mechanical properties similar to those of physiological bone.

One possible consideration for the next generation of orthopedic and dental implants with improved efficacy are nanophase materials. Nanophase materials are new material formulations that possess grains composed of the same atoms, but the atoms are fewer (less than tens of thousands) and smaller (less than 100 nm in diameter) than in conventional materials (which contain several billions of atoms and grain sizes of micrometers to millimeters) (Siegel, 1996). Control of the size of the constituent clusters and the manner in which these clusters are assembled in nanostructures has produced new materials with unique, custom-made mechanical, electrical, chemical, magnetic, and optical structures and properties (Siegel, 1996). Despite their great promise, investigations of nanophase materials as orthopedic and dental implants have been close to nonexsistent.

This chapter will discuss, in detail, properties of bone and of nanophase ceramics that promise increased orthopedic and dental implant efficacy.

Limited reports in the literature that discuss the design, synthesis, and evaluation of nanophase ceramics for orthopedic and dental implants will also be discussed.

II. Mechanical Properties of Bone

Bone is a well-organized tissue whose primary function is to support mechanical loading and protect vital organs in the body (Martin and Burr, 1989). A unique living tissue, bone possesses the ability to regenerate itself and adapt its geometry to accommodate local stress and strain from the surrounding physiological milieu. In the course of normal, daily activities, mechanical loads are applied to bone; for example, human jaws sustain 0.25 kN during chewing, and human hip joints are exposed to 3 to 5 kN and 0.75 kN during walking and standing, respectively (Kaplan *et al.,* 1994; Park and Lakes, 1992). Mechanical properties of bone change with architecture (i.e., cortical or trabecular), porosity (for example, 30% and 50 to 90% porosity for cortical and trabecular bone, respectively), anatomical differences (for example, the modulus of elasticity varies by 10% in bone from the human hip and tibia), and age (e.g., the modulus of elasticity varies by up to 20% in bone from 20- to 90-year-old humans) (Kaplan *et al.,* 1994; Fung, 1993).

The mechanical properties of cortical bone (specifically, the femur, tibia, humerus, and radius) of various species (specifically, horse, cattle, pig, and human) in tension, compression, and torsion are listed in Table I. It should be noted, for example, that human femur tensile strength (namely, 124 MPa) (Yamada, 1970) is in the same order of magnitude to that of cast iron (170 MPa) (Beer and Johnston, 1981) but, surprisingly, low in weight (Kaplan *et al.,* 1994; Fung, 1993). These unique properties of bone are a direct consequence of the synergy of its molecular, cellular, and tissue arrangement.

III. Bone Physiology

A. Microarchitecture

At the microscopic level, bone consists of two structures: woven and lamellar (Fig. 1a). Woven bone (with an average mineral grain size of 10 to 50 nm) is the immature, or primitive, form of bone and is normally found in the metaphyseal region of growing bone as well as in fracture callus (Kaplan *et al.,* 1994; Park and Lakes, 1992). Woven bone is coarse-fibered and

TABLE I
MECHANICAL PROPERTIES OF CORTICAL BONE IN TENSION, COMPRESSION, AND TORSION[a]

Bone type	Horses	Cattle	Pigs	Humans (20–39 years)
		Ultimate tensile strength (MPa)		
Femur	121 ± 1.8	113 ± 2.1	88 ± 1.5	124 ± 1.1
Tibia	113	132 ± 2.8	108 ± 3.9	174 ± 1.2
Humerus	102 ± 1.3	101 ± 0.7	88 ± 7.3	125 ± 0.8
Radius	120	135 ± 1.6	100 ± 3.4	152 ± 1.4
		Ultimate percent elongation (%)		
Femur	0.75 ± 0.01	0.88 ± 0.02	0.68 ± 0.01	1.41
Tibia	0.70	0.78 ± 0.01	0.76 ± 0.03	1.50
Humerus	0.65 ± 0.01	0.76 ± 0.01	0.70 ± 0.03	1.43
Radius	0.71	0.79 ± 0.01	0.73 ± 0.03	1.50
		Modulus of elasticity in tension (GPa)		
Femur	25.5	25.0	14.9	17.6
Tibia	23.8	24.5	17.2	18.4
Humerus	17.8	18.3	14.6	17.5
Radius	22.8	25.9	15.8	18.9
		Ultimate compressive strength (MPa)		
Femur	145 ± 1.6	147 ± 1.1	100 ± 0.7	170 ± 4.3
Tibia	163	149 ± 1.4	106 ± 1.1	ND[b]
Humerus	154	144 ± 1.3	102 ± 1.6	ND
Radius	156	152 ± 1.5	107 ± 1.6	ND
		Ultimate percent contraction (%)		
Femur	2.4	1.7 ± 0.02	1.9 ± 0.02	1.85 ± 0.04
Tibia	2.2	1.8 ± 0.02	1.9 ± 0.02	ND
Humerus	2.0	1.8 ± 0.02	1.9 ± 0.02	ND
Radius	2.3	1.8 ± 0.02	1.9 ± 0.02	ND
		Modulus of elasticity in comoression (GPa)		
Femur	9.4 ± 0.47	8.7	4.9	ND
Tibia	8.5	ND	5.1	ND
Humerus	9.0	ND	5.0	ND
Radius	8.4	ND	5.3	ND
		Ultimate shear strength (MPa)		
Femur	99 ± 1.5	91 ± 1.6	65 ± 1.9	5.4 ± 0.6
Tibia	89 ± 2.7	95 ± 2.0	71 ± 2.8	ND
Humerus	90 ± 1.7	86 ± 1.1	59 ± 2.0	ND
Radius	94 ± 3.3	93 ± 1.8	64 ± 3.2	ND
		Torsional modulus of elasticity (GPa)		
Femur	16.3	16.8	13.5	3.2
Tibia	19.1	17.1	15.7	ND
Humerus	23.5	14.9	15.0	ND
Radius	15.8	14.3	8.4	ND

[a] Data are mean ±S.E.M. (Adapted from Yamada, 1970.)
[b] ND = no data.

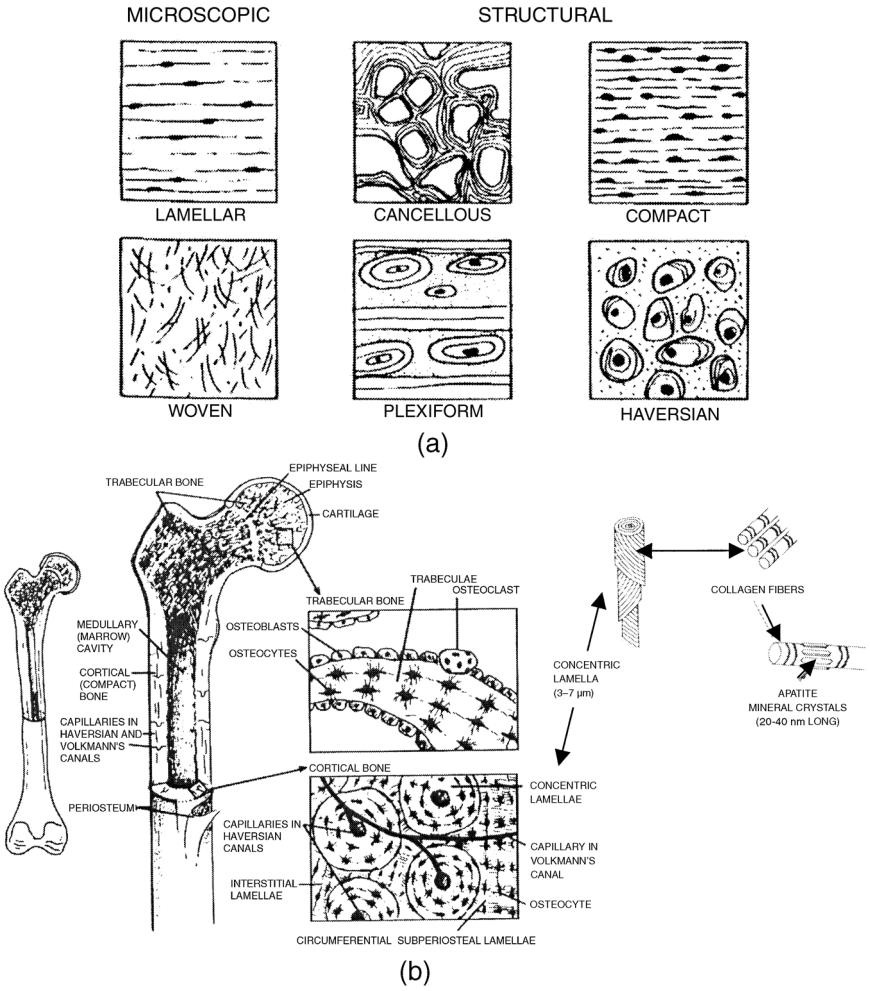


FIG. 1. Microarchitecture and structural classifications of physiological bone. (a) Schematic of microscopic and structural classifications of bone. (Redrawn and adapted from Kaplan *et al.,* 1994.) (b) Schematc of the microarchitecture of the femur. (Redrawn and adapted from Keaveny and Hayes, 1993, and Fung, 1993.)

contains no uniform orientation of collagen fibers; the isotropic mechanical characteristics (i.e., mechanical behavior independent of orientation of applied force) of woven bone are a consequence of its unoriented, nonuniform collagen fibers (Fung, 1993).

The other microarchitectural form of bone, lamellar bone, actively replaces maturing woven bone and, consequently, contains up to 100 times more mineralized matrix or hydroxyapatite; these hydroxyapatite crystals

are 20 to 80 nm long and 2 to 5 nm thick in the human femur (Kaplan *et al.,* 1994; Park and Lakes, 1992). Lamellar bone is highly organized and contains stress-oriented collagen fibers; such orientation results in anisotropic mechanical properties (i.e., mechanical behavior dependent on the orientation of applied force) with greatest strength parallel to the longitudinal axis of the collagen fibers (Fung, 1993).

B. Structural Organization of the Bone Microarchitecture

Woven bone and lamellar bone are structurally organized into either trabecular (spongy or cancellous) or cortical (dense or compact) bone (Kaplan *et al.,* 1994; Park and Lakes, 1992). Trabecular bone, characterized by 50 and 90% porosity, contains large pores (up to several millimeters in diameter (Kaplan *et al,* 1994)). Trabecular bone, found primarily at the metaphyses and epiphyses of both long and cuboidal bones, is organized into a three-dimensional branching lattice with spicules of trabeculi oriented in the direction of principal stress; for trabecular bone, compression is the dominant force under physiological loading conditions (Fung, 1993).

Cortical bone, characterized by less than 30% porosity and composed of small pores (up to 1 mm in diameter), is classified as *haversian* bone and contains Volkmann's canals (canals in which capillaries reside) (Fig. 1b), lacunae, and canaliculi (Keaveny and Hayes, 1993; Fung, 1993). Cortical bone is found at the diaphyses of long bones and as circular envelopes in cuboidal bone (Keaveny and Hayes, 1993; Fung, 1993). In compact bone vascular channels are randomly oriented, in plexiform bone the vasculature is located in layers of woven bone dispersed within the lamellar bone, and in haversian bone (the most complex type of cortical bone) vascular channels circumferentially surround lamellar bone (Fig. 1a) (Kaplan *et al.,* 1994). The circular arrangements of bone around vascular channels found in haversian bone are called *osteons*. Osteons are usually oriented along the long axis of bone; the central canal of the osteon (called the *haversian canal*) contains hematopoietic cells, capillaries, and, occasionally, nerves (Keaveny and Hayes, 1993; Fung, 1993). The capillaries in the haversian canal are derived from the principal nutrient arteries of the epiphyseal and metaphyseal arteries of cortical bone and supply oxygen and nutrients necessary to maintain bone homeostasis (Park and Lakes, 1992).

C. Chemical Composition of the Bone Matrix

The human femur is a composite material; approximately 70% of its matrix is inorganic hydroxyapatite, 20% is organic, and 10% is water (Kaplan

et al., 1994; Fung, 1993). The detailed composition of bone depends on species, age, anatomical location, dietary history, and either the absence or presence of disease (Kaplan *et al.,* 1994; Park and Lakes, 1992).

1. Inorganic Phase

The inorganic component of bone is primarily platelike (20 to 80 nm long and 2 to 5 nm thick) crystalline hydroxyapatite, $Ca_5(PO_4)_3(OH)$ or HA (Kaplan *et al.,* 1994; Park and Lakes, 1992). Small amounts of impurities may be present in the mineralized HA matrix; for example, carbonate may replace phosphate groups, whereas chloride and fluoride may replace hydroxyl groups. Because release of ions from the mineral bone matrix controls cell-mediated functions, the presence of impurities may impact important biological aspects (and, subsequently, affect chemical and mechanical properties of bone) that are critical to normal bone function; for example, impurities present in the mineralized matrix may affect cellular function(s) that influence new bone formation (Kaplan *et al.,* 1994; Park and Lakes, 1992).

2. Organic Phase

Approximately 90% of the organic phase of bone is Type I collagen; the remaining 10% consists of noncollagenous proteins, such as lipids and other macromolecules (i.e., growth factors, cytokines, and adhesive proteins) (Kaplan *et al.,* 1994; Fung, 1993; Park and Lakes, 1992). Growth factors and cytokines (such as insulinlike growth factors and osteogenic proteins), proteins contained in serum, bone-inductive proteins (such as osteonectin, osteopontin, and osteocalcin), and extracellular matrix compounds (such as bone sialoprotein, bone proteoglycans, and other phosphoproteins as well as proteolipids) are present in the mineralized matrix and may mediate bone–cell function such as formation of new bone by osteoblasts and bone resorption by osteoclasts (Kaplan *et al.,* 1994).

a. *Collagen.* Distribution of the various types of collagen in the human body is tissue specific (Ayad *et al.,* 1994). Collagen (mainly Type I) found in bone is synthesized by osteoblasts (the bone-forming cells) and is secreted as triple helical procollagen into the extracellular matrix, where collagen molecules are stabilized by cross-linking of reactive aldehydes among the collagen chains. Each of the 12 types of collagen found in the body consists of 3 polypeptide chains composed of approximately 1400 amino acids. For example, Type I collagen (molecular weight 139,000) possesses 2 identical $\alpha 1$(I) chains and 1 unique $\alpha 2$ chain; this configuration results in a

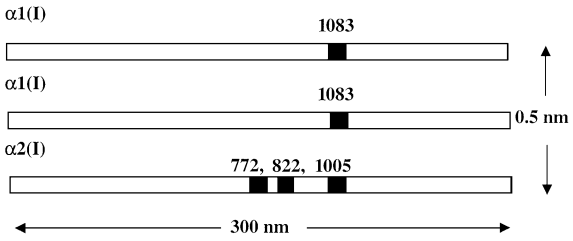


FIG. 2. Schematic representation (not to scale) of the amino acid sequence of a Type I collagen. A Type I collagen consists of two identical of α1(I) and one unique α2(I) chains whose combination produces a triple helical structure 300 nm long and 0.5 nm thick, with a periodicity of 67 nm. Arginine–glycine–aspartic acid adhesive peptides (■) start at peptide sequence 1083 in the α1(I) chain as well as at 772, 822, and 1005 in the α2(I) chain. (The schematic was redrawn using information obtained from the following references: Ayad *et al.,* 1994; Mathews and van Holde, 1990; and Darnell *et al.,* 1990.)

linear molecule that is 300 nm long (Fig. 2) (Ayad *et al.,* 1994; Mathews and van Holde, 1990). The linear molecules (or fibers) of Type I collagen are grouped in triple helical bundles having a periodicity of 67 nm, with gaps (called *hole-zones*) between the ends of the molecules and pores between sides of parallel molecules. During new bone formation, noncollagenous proteins and mineral are synthesized by osteoblasts and deposited into the hole-zones and pores of the collagen matrix. Type I collagen has cell-adhesive properties, particularly for osteoblasts (the bone-forming cells) (Steele *et al.,* 1993), due to the presence of the adhesive peptide arginine–glycine–aspartic acid (RGD) starting at amino acid sequence 1083 in the α1(I) chain and at sequences 777, 822, and 1005 in the α2(I) chain.

b. *Noncollagenous Proteins.* Numerous noncollagenous proteins are found in the bone matrix; osteocalcin, osteonectin, alkaline phosphatase, osteopontin, and bone sialoprotein (discussed in the section "Osteoblasts: The Bone-Forming Cells") are synthesized by osteoblasts. Other proteins, such as laminin, fibronectin, and vitronectin (discussed in the following sections) are found in blood serum as well as in the extracellular matrix of fibrous tissue and bone.

(i) *Laminin.* Laminin is a family of large glycoproteins that are distributed ubiquitously in the basement membrane of tissues; laminin has been shown to perform key roles in the development, differentiation, and migration of cells (primarily endothelial cells, cells that line the vasculature of the body, and neuronal cells, cells of the central nervous system) (Ayad *et al.,* 1994). There are four isomeric forms of laminin, specifically, A–S–B2, M–B1–B2,

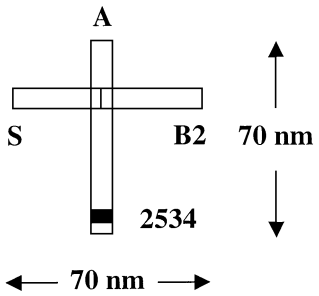


FIG. 3. Schematic representation (not to scale) of the amino acid sequence of the A–S–B2 isomer of laminin. Laminin is composed of one long arm (A) and two short arms (S and B2) arranged in a cruciform configuration 70 nm long and 70 nm wide. The arginine–glycine–aspartic acid adhesive peptide (■) starts at peptide sequence 2534 in chain A. (The schematic was redrawn using information obtained from the following references: Ayad *et al.,* 1994; Mathews and van Holde, 1990; and Darnell *et al.,* 1990.)

and M–S–B2; a truncated form of the B2, B2t chain was recently discovered (Ayad *et al.,* 1994). Distribution of these isomeric forms of laminin in the body is associated with developmental stages and is tissue specific. The laminin (molecular weight of 820,000) molecule has a cruciform configuration with one long arm (approximately 70 nm long) and two short arms (each approximately 35 nm long; Fig. 3) (Darnell *et al.,* 1990). Laminin has cell-adhesive properties, particularly for endothelial cells (Graf *et al.,* 1987), due to the presence of the following adhesive domains: peptides arginine–glycine–aspartic acid and isoleucine–lysine–valine–alanine–valine (IKVAV), which are located in chain A and start at peptide sequences 2534 and 2116, respectively; peptides leucine–glycine–threonine–isoleucine–proline–glycine (LGTIPG), arginine–tyrosine–valine–valine–leucine–proline–arginine (RYVVLPR), proline–aspartic acid–glycine–serine–glycine–arginine (PDGSGR), and tyrosine–isoleucine–serine–arginine (YIGSR), which are located in chain B1 and start at peptide sequences 463, 662, 923, and 950, respectively; and peptide leucine–arginine–glutamic acid (LRE), which is located in chain S and starts at sequence 1705 (Ayad *et al.,* 1994).

(ii) *Fibronectin.* Fibronectin (molecular weight 273,715) is a widely distributed glycoprotein present at high concentrations in bone matrix, plasma (for example, 300 μg/ml of human plasma), and in other body fluids and tissues (Ayad *et al.,* 1994). The principal functions of fibronectin are to mediate cell migration during development and wound healing, regulate cell growth and differentiation, and participate in haemostasis and thrombosis (Ayad *et al.,* 1994). Body-fluid fibronectin is a dimer of two identical subunits (each 60 to 70 nm long and 2 to 3 nm wide) covalently linked near the carboxyl termini by a pair of disulfide bonds (Fig. 4) (Darnell *et al.,* 1990).

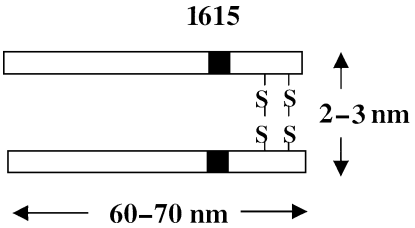


FIG. 4. Schematic representation (not to scale) of the amino acid sequence of fibronectin. Fibronectin is a dimer of two identical subunits (60 to 70 nm long and 2 to 3 nm long) covalently linked by a pair of disulfide bonds. The arginine–glycine–aspartic acid adhesive peptide (■) starts at peptide sequence 1615. (The schematic was redrawn using information obtained from the following references: Ayad *et al.,* 1994; Mathews and van Holde, 1990; and Darnell *et al.,* 1990.)

Fibronectin is an important adhesive protein (primarily for fibroblasts, cells that contribute to callus formation and fibrous encapsulation events that lead to orthopedic and dental implant loosening and eventual failure, but also for osteoblasts, the bone-forming cells (Thomas *et al.,* 1997) and various other cells) due to the presence of a number of bioactive domains such as arginine–glycine–aspartic acid, isoleucine–aspartic acid–alanine–proline–serine (IDAPS), leucine–aspartic acid–valine (LDV), arginine–glutamic acid–arginine valine (REDV), which start at peptide sequences 1615, 1994, 2102, and 2182, respectively (Ayad *et al.,* 1994).

(iii) *Vitronectin.* Vitronectin, found in both plasma and the extracellular matrix of bone, participates in a variety of physiological processes, including hemostasis, phagocytosis, tissue repair, and immune response. Vitronectin exists in two forms: a single chain (molecular weight of 75,000) and an endogenously clipped, two-chain form held together by disulfide bonds (molecular weights of approximately 65,000 and 10,000, respectively) (Ayad *et al.,* 1994). Structurally, vitronectin is asymmetrically shaped (total length approximately 15 nm; Fig. 5) with a large content of β-pleated sheets (Ayad *et al.,* 1994). Vitronectin plays an important role in adhesion and migration

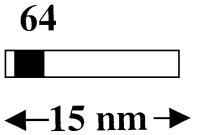


FIG. 5. Schematic representation (not to scale) of the amino acid sequence of vitronectin. Vitronectin is 15 nm long and possesses the arginine–glycine–aspartic acid adhesive peptide (■) starting at peptide sequence 64. (The schematic was redrawn using information obtained from the following references: Ayad *et al.,* 1994; Mathews and van Holde, 1990; and Darnell *et al.,* 1990.)

of various cells, particularly osteoblasts, the bone-forming cells (Steele *et al.,* 1993; Healy *et al.,* 1994). The vitronectin molecule contains the arginine–glycine–aspartic acid peptide starting at sequence 64 (Ayad *et al.,* 1994).

D. Cells of the Bone Tissue

1. Osteoblasts: The Bone-Forming Cells

It is well established that osteoblasts and, to a lesser extent, osteocytes (mature osteoblasts) contribute to new bone synthesis (Keaveny and Hayes, 1993; Kaplan *et al.,* 1994). The principal difference between osteoblasts and osteocytes is their relative location in bone. Osteoblasts are located on the periosteal and endosteal surfaces of bone (Fig. 1(b)). Once an osteoblast becomes surrounded by a mineralized matrix, however, the cell is characterized by a higher nucleus–to–cell cytoplasm ratio and becomes known as an osteocyte (Kaplan *et al.,* 1994). Osteocytes are arranged concentrically around the central lumen of an osteon and in between lamellae (Fig. 1(b)). Osteocytes possess extensive cell processes with which they establish contacts with adjacent osteocytes through small channels, or *canaliculi,* present in bone. Due to their three-dimensional distribution and interconnecting cell processes, osteocytes are believed to play a pivotal role in communicating physiological stress and strain signals in bone tissue (Kaplan *et al.,* 1994). Osteocytes regulate new bone formation by modulating osteoblast function through secretion of growth factors such as the insulinlike growth factor I and the tissue growth factor β (Kaplan *et al.,* 1994; Trippel, 1998). These growth factors mediate the differentiation of osteoblasts from immature, non-calcium-depositing cells to mature osteoblasts that deposit calcium-containing mineral into the extracellular matrix of bone. Phenotypic markers of the differentiation of non-calcium-depositing osteoblasts to osteoblasts that deposit calcium-containing mineral *in vitro* have been well studied (Stein and Lian, 1993; Stein *et al.,* 1990); these studies have provided much evidence concerning *in vivo* functions of osteoblasts leading to the synthesis and deposition of bone on newly implanted prostheses.

Three distinct periods of osteoblast differentiation at the genetic level have been identified during *in vitro* examination of developing osteoblasts after initial adhesion to a surface: (1) cell proliferation and extracellular matrix synthesis, (2) extracellular matrix development and maturation, and (3) extracellular matrix mineralization (Stein and Lian, 1993). A schematic of the time course of osteoblast function and synthesis of extracellular matrix proteins on a newly implanted biomaterial is shown in Fig. 6.

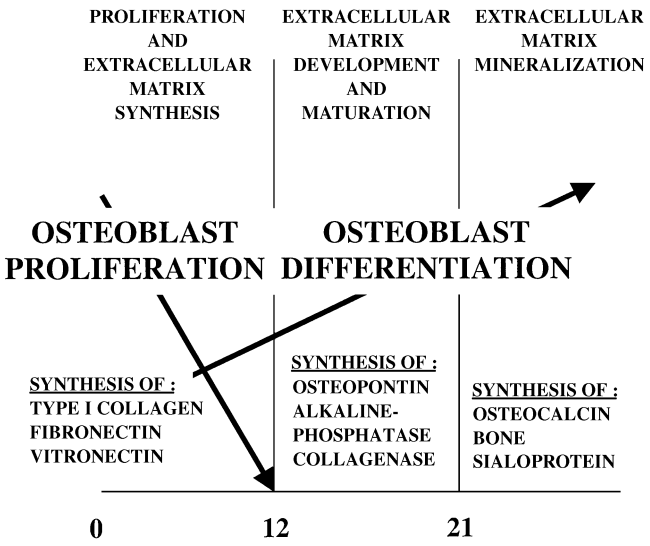


FIG. 6. Time course of osteoblast function and synthesis of extracellular matrix proteins on a newly implanted biomaterial. After initial adhesion of osteoblasts to a newly implanted biomaterial, three distinct phases of osteoblast differentation occur : (1) proliferation and extracellular matrix synthesis, (2) extracellular matrix development and maturation, and (3) extracellular matrix mineralization. (Adapted and redrawn from Stein and Lian, 1993.)

After initial adhesion to a biomaterial device, osteoblasts actively proliferate and express genes for Type I collagen, fibronectin, and vitronectin (Stein *et al.,* 1990). Osteoblasts also express genes for osteopontin, an acidic glycoprotein that possesses several calcium-binding sites and mediates adhesive interactions of these cells with the extracellular matrix (Stein and Lian, 1993). Genes for osteopontin synthesis are expressed minimally during the proliferative stages but in higher quantities late in the extracellular matrix development and maturation stages of osteoblast development (Stein *et al.,* 1990).

As the proliferative phase ends and the extracellular matrix development and maturation phase begins, alkaline phosphatase activity (expressed by numerous cells, including osteoblasts, fibroblasts, and leukocytes as well as by bone marrow reticular cells (Gehron-Robey, 1989)) and mRNA expression for proteins (such as osteocalcin, bone sialoprotein, and collagenase) associated with the osteoblastic phenotype are increased tenfold (Stein and Lian, 1993); for example, expression of the collagenase (an enzyme that aids in collagen turnover during reorganization and maturation of the extracellular

matrix) gene is upregulated as the proliferative phase ends and the extracellular matrix mineralization phase commences (Stein and Lian, 1993).

Bone sialoprotein, osteopontin, and osteocalcin are synthesized and deposited as the mineralization process begins and mineral nodules form (Stein and Lian, 1993). Bone sialoprotein contains the cell-adhesive arginine–glycine–aspartic acid peptide sequence and may thus mediate osteoblast adhesion on the extracellular matrix (Gehron-Robey, 1989). Osteocalcin, a calcium-binding protein, interacts with hydroxyapatite and is thought to mediate coupling of bone resorption (by osteoclasts) and bone formation (by osteoblasts and/or osteocytes) (Stein and Lian, 1993).

2. Osteoclasts: The Bone-Resorbing Cells

Osteoclasts, cells primarily responsible for resorption of bone, are distinguished by their large (20 to 100 μm in diameter), multinuclear morphology (Fig. 1(b)). Osteoclasts are derived from pluripotent (i.e., capable of differentiating into various cells, including monocytes and macrophages) cells of the bone marrow (Kaplan *et al.,* 1994; Park and Lakes, 1992). Osteoclasts resorb bone by forming ruffled cell membrane edges (thereby increasing their surface area of attachment onto bone surfaces), lowering the pH of the local environment by producing hydrogen ions through the carbonic anhydrase system (thus increasing the solubility of hydroxyapatite crystals, the major inorganic component of bone), and, lastly, by removing organic components of the matrix via acidic proteolytic digestion that results in the formation of bone resorption pits termed *Howship's lacunae* (Kaplan *et al.,* 1994; Park and Lakes, 1992). The bone-resorbing activity of osteoclasts is believed to instigate the formation of new bone by osteoblasts, as discussed in the section "Bone Remodeling" (Rifkin and Gay, 1992; Stein and Lian, 1993; Heegard, 1993).

3. Fibroblasts

Fibroblasts are derived from mesenchymal cells of the bone marrow (Kaplan *et al.,* 1994; Park and Lakes, 1992), are found unbiquitously in various tissues (such as skin, vasculature, lungs, and bone), and are primilarily responsible for the formation of fibrous, connective tissue (Darnell *et al.,* 1990). Fibroblasts participate in maintaining the mechanical integrity of bone by synthesizing and secreting collagen into the extracellular matrix (Kaplan *et al.,* 1994). However, excessive secretion of fibrous tissue and/or callus formation during new bone formation may compromise the mechanical properties (such as bending) of bone. Moreover, fibrous encapsulation and callus formation around newly implanted orthopedic or dental prostheses

are the most frequent causes of incomplete osseointegration between an implant and juxtposed bone (Brunski, 1991); incomplete osseointegration may lead to implant loosening and eventual failure. For these reasons, functions (such as adhesion, synthesis and secretion of fibrous tissue) of fibroblasts should be minimized at the surface of a newly implanted orthopedic or dental device.

E. Bone Remodeling

Both cortical and trabecular bone are continuously remodeled through the formation of a bone-modeling unit (BMU), or *cutter-cone;* this process involves activation of osteoclasts, leading to resorption of bone by osteoclasts and formation of new bone by osteoblasts on the site of the "old," resorbed bone (Fig. 7) (Martin and Burr, 1989). Under normal physiological conditions (i.e., in the absence of either growth or disease) the dynamics of bone remodeling maintain bone homeostasis throughout a person's lifetime.

Nutrients necessary for bone remodeling, mesenchymal stem cells (pluripotent cells capable of differentiating into various cells such as osteoblasts, endothelial cells, and fibroblasts), and hematopoeitic cells (including cells

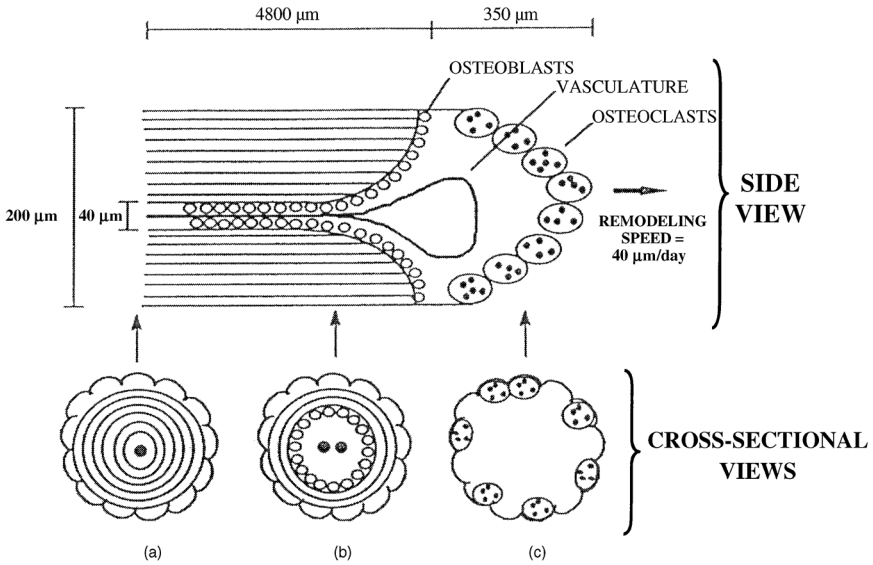


Fig. 7. Schematic diagram of a bone modeling unit, or cutter-cone. Cross-sectional views ((a), (b), and (c)) match respective side view sections of a bone modeling unit. (Adapted and redrawn from Martin and Burr, 1989.)

from the monocyte or macrophage cell line capable of differentiating into osteoclasts) are supplied to bone through the vascular network of the bone marrow. Osteoclasts (either on or in the bone as well as those supplied by the vascular network) are activated by growth factors, cytokines, and proteins present in the bone matrix to resorb old bone (Martin and Burr, 1989). Osteoblasts are then activated by growth factors (such as insulin-like growth factors I and II) secreted by osteoclasts to deposit calcium-containing mineral (Kaplan *et al.,* 1994). Mineral accretion in skeletal tissue arises by (1) nucleation or initial recruitment and deposition of precipitating ions (such as calcium, phosphate, and other ions) into pores and/or *hole zones* of the extracellular collagen matrix that had been secreted by osteoblasts, (2) hydroxyapatite (HA) crystal growth promoted by noncollagenous proteins (such as phosphoproteins, osteonectin–collagen complexes, and proteolipids), and (3) secondary nucleation, in which new crystals of hydroxyapatite are deposited on nuclei of existing hydroxyapatite (Kaplan *et al.,* 1994; Park and Lakes, 1992). Extracellular matrix proteins, growth factors, and cytokines are also believed to control the size (20 to 80 nm in diameter) as well as extent of HA crystal growth by preventing further deposition of mineral by osteoblasts according to mechanisms that have yet to be fully understood (Kaplan *et al.,* 1994; Keaveny and Hayes, 1993).

The extent of bone remodeling is influenced by a number of factors, including but not limited to dietary history, exercise frequency, age, injury, and/or the presence of a prosthetic, manufactured device (Kaplan *et al.,* 1994; Park and Lakes, 1992). More importantly, the extent of bone remodeling that occurs at an implant surface will determine the fate of the prosthetic device; for example, loosening and failure of the orthopedic or dental implant may result from either (1) little or no remodeling in the bone surrounding an implant, which may lead to malnourished juxtaposed bone, or (2) enhanced remodeling in the bone surrounding an implant, which may lead to excessive bone resorption, or osteolysis (Brunski, 1991). Events that occur at the tissue–implant interface will, clearly, control the extent of bone remodeling around the prostheses and, therefore, integration of the biomaterial into surrounding bone.

IV. The Tissue–Implant Interface

Implantation of a synthetic material into mammalian living tissue causes a number of biological host responses, including rejection by the body, encapsulation in newly formed fibrous tissue, and successful incorporation into surrounding tissues. The fate of an implanted device is determined by cellular

or molecular events at tissue–implant interfaces; these events are mediated by the wound-healing process of bone in response to surface properties of the prostheses.

A. Wound-Healing Response of Bone

Implanting or introducing a biomaterial into the body by surgical procedures inevitably causes damage to surrounding tissues, and, consequently, initiates a series of host responses. Inflammation and the wound-healing process involve recruitment of a variety of cell types, body fluids, and proteins to the tissue–implant interface (Anderson, 1993; Hench and Ethridge, 1975). For orthopedic and dental implants, select osteoblast (bone-forming cells) recruitment to the implant-material surface is imperative for subsequent formation of new bone, leading to successful osseointegration. Bonding of orthopedic and dental implants to surrounding bone provides mechanical stability to the prostheses *in situ* and minimizes motion-induced trauma to surrounding tissues; formation of callus (instead of bony) tissue at these implantation sites decreases implant efficacy and may eventually result in clinical failure (Brunski, 1991).

B. Protein Interactions with Biomaterial Surfaces

Specific domains of proteins (for example, those mentioned in the section "Organic Phase") adsorbed to biomaterial surfaces interact with select cell membrane receptors (Fig. 8); accessibility of adhesive domains (such as specific amino acid sequences) of select adsorbed proteins may either enhance or inhibit subsequent cell (such as osteoblast) attachment (Schakenraad, 1996). Several studies have provided evidence that properties (such as chemistry, charge, and topography) of biomaterial surfaces dictate select interactions (such as type, concentration, and conformation or bioactivity) of plasma proteins (Sinha and Tuan, 1996; Horbett, 1993; Horbett, 1996; Brunette, 1988; Davies, 1988; Luck *et al.,* 1998; Curtis and Wilkinson, 1997). Albumin has been the protein of choice in protein-adsorption investigations because of availability, low cost (compared to other proteins contained in serum), and, most importantly, well-documented conformation or bioactive structure (Horbett, 1993); recently, however, a number of research groups have started to examine protein (such as fibronectin and vitronectin) interactions with material surfaces that are more pertinent to subsequent cell adhesion (Luck *et al.,* 1998; Degasne *et al.,* 1999; Dalton *et al.,* 1995; Lopes *et al.,* 1999).

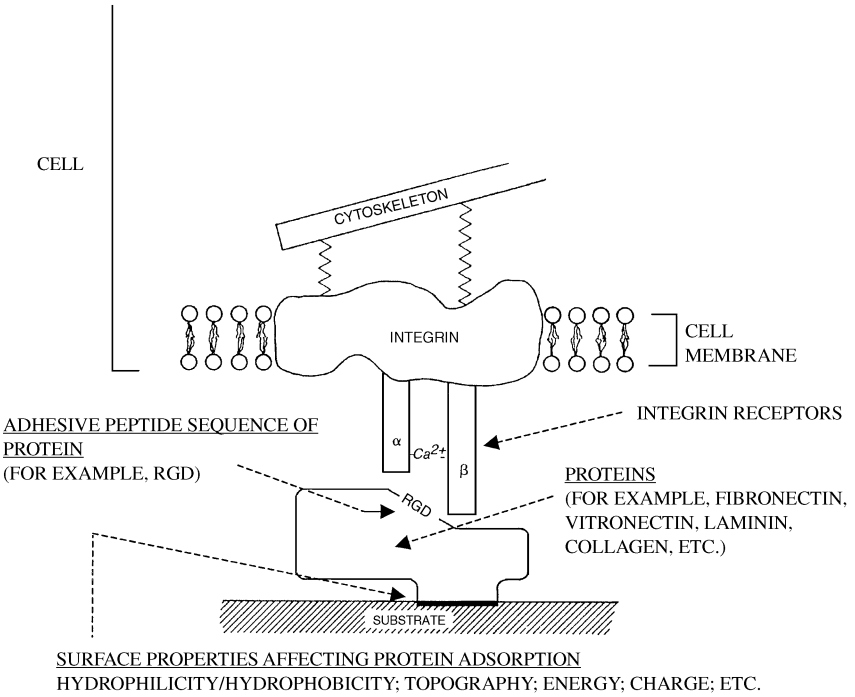


FIG. 8. Schematic representation of protein-mediated cell adhesion on biomaterial surfaces. Biomaterial surface properties (such as hydrophilicity/hydrophobicity, topography, energy, and charge) affect subsequent interactions of adsorbed proteins; these interactions include but are not limited to adsorbed protein type, concentration, and conformation. Changes in protein–surface interactions may alter accessibility of adhesive domains (such as the peptide sequence arginine–glycine–aspartic acid) to cells (such as osteoblasts, fibroblasts, or endothelial cells) and thus modulate cellular adhesion. (Adapted and redrawn from Schakenraad, 1996.)

It has been reported in the literature that changes in the type and concentration of protein [specifically, albumin (Luck *et al.,* 1998), fibronectin (Degasne *et al.,* 1999), and vitronectin (Dalton *et al.,* 1995; Lopes *et al.,* 1999)] adsorption on material surfaces depends on a material's surface properties, such as chemistry (i.e., either polymer, metal, or ceramic), hydrophilicity and hydrophobicity, roughness, and surface energy. Specifically, maximum vitronectin (Lopes *et al.,* 1999), fibronectin (Degasne *et al.,* 1999) and albumin (Horbett, 1993; Luck *et al.,* 1998) adsorption was noted on hydrophilic surfaces with high surface roughness and/or energies.

Recent studies have attempted to further elucidate mechanisms of protein adsorption on biomaterial surfaces. For example, Ellingsen (1991) reported that adsorption of calcium on titanium surfaces subsequently enhanced binding of select proteins. In contrast, adsorption of other ions (such as

magnesium) contained in physiological fluids on titanium surfaces did not affect subsequent select protein adsorption (Ellingsen, 1991).

C. Protein-Mediated Cell Adhesion on Biomaterial Surfaces

Select proteins that mediate adhesion of specific anchorage-dependent cells (such as osteoblasts, fibroblasts, and endothelial cells) on substrate surfaces have been identified (Underwood and Bennett, 1989; Thomas *et al.,* 1997; Ayad *et al.,* 1994). For example, adsorption of fibronectin and vitronectin on tissue-culture polystryene subsequently enhanced osteoblast, fibroblast, and endothelial cell adhesion (Underwood and Bennett, 1989). More importantly, fibronectin and vitronectin adsorption on borosilicate glass, in a competitive environment, maximized fibroblast and osteoblast adhesion, respectively (Thomas *et al.,* 1997). Ayad *et al.* (1994) reported that enhanced adsorption of laminin on tissue-culture polystyrene promoted subsequent endothelial cell adhesion. These studies provided evidence that adsorption of specific protein(s) can, subsequently, control select cell adhesion on material surfaces.

Cells interact with their external environment through signals (such as chemical, electrical, and mechanical) transmitted through the cell membrane. For this reason, understanding cellular interactions with a biomaterial surface requires elucidation of molecular processes that occur at the cell membrane–biomaterial interface. For example, cellular adhesion (a crucial prerequiste function for anchorage-dependent cells such as osteoblasts) has been well examined at the molecular level. Cell-binding regions of extracellular matrix proteins (such as the arginine–glycine–aspartic acid peptide sequence present in vitronectin, fibronectin, collagen and laminin, as discussed in the section "Organic Phase") and respective cell-membrane-intercalated receptors (i.e., the integrins) have been identified as being among the most important mechanisms for cell adhesion to substrates, including borosilicate glass and tissue-culture polystyrene (Kramer *et al.,* 1993).

Integrins are a family of transmembrane heterodimeric glycoproteins that are receptors for specific epitopes of extracellular matrix proteins and for other cell-surface molecules (Kramer *et al.,* 1993). Integrins exist as a dimer complex composed of an α-subunit (120–180 kD) noncovalently associated with a β-subunit (90–110 kD) (Hynes, 1992). At least 8 β-subunits and 14 α-units have been identified and are concentrated at loci, called focal adhesion sites, of close proximity between cells and extracellular matrices on substrates (Hynes, 1992). Focal adhesion sites are points of aggregation of, and are physically associated with, intracellular cytoskeletal molecules that control, direct, and modulate cell function in response to extracellular signals (Schwartz, 1992).

A number of integrins bind to the RGD sequence of extracellular matrix proteins; for example, integrins $\alpha_v\beta_1$ and $\alpha_v\beta_3$ bind to the RGD epitope (ligand) of vitronectin, fibronectin, and Type I collagen (Cox *et al.,* 1994). Expression of integrins on human osteoblasts is mediated by surface properties (such as topography and composition) of substrates on which these cells adhere (Gronowicz and McCarthy, 1996; Schneider and Burridge, 1994; Sinha and Tuan, 1996); specifically, expression of the $\alpha_v\beta_5$ and $\alpha_v\beta_6$ integrins was enhanced when the cells adhered on surfaces of increased roughness (e.g., sandblasted titanium substrates) and on smooth surfaces (e.g., grit-polished titanium substrates), respectively (Sinha and Tuan, 1996). Because the $\alpha_v\beta_5$ integrin has been exclusively associated with binding to the RGD protein ligand of vitronectin, it can be suggested that vitronectin plays a critical role in the adhesion of osteoblasts on rough surfaces. Furthermore, $\alpha_v\beta_6$ has been exclusively associated with binding to the RGD protein ligand of fibronectin; for this reason, it can also be suggested that fibronectin is an important protein for osteoblast adhesion on smooth surfaces.

In addition to mediating cell adhesion, it has been demonstrated that integrin expression by osteoblasts determines their phenotypic expression (see the section "Osteoblasts: The Bone-Forming Cells"). For example, addition of either soluble concentrations of RGD (Moursi *et al.,* 1996) or antibodies of the integrin pair $\alpha_v\beta_6$ (Moursi *et al.,* 1997) to confluent osteoblast cultures, blocked initiation and formation of mineral nodules; these results provided evidence that integrin–fibronectin interactions leading to extracellular matrix development play a crucial role in osteoblast function (Moursi *et al.,* 1996, 1997).

However, integrin–RGD interactions are not the only mechanisms by which osteoblasts adhere. Several articles suggested that *in vivo* (Nakamura and Ozawa, 1994) and *in vitro* (Puleo and Bizios, 1992; Dalton *et al.,* 1995) osteoblasts attach to an implanted material through cell membrane heparan sulfate proteoglycan interactions with, for example, heparin-binding sites on fibronectin and collagen. Nakamura and Ozawa (1994) immunohistochemically detected heparan sulfate on the membranes of osteoblasts attached to bone matrix. In addition, by blocking heparin-binding sites of fibronectin (with platelet factor IV), osteoblast adhesion on fibronectin was inhibited by 45% (Puleo and Bizios, 1992). In this manner, in addition to integrin–RGD interactions, it has been demonstrated that osteoblast adhesion is also mediated by cell membrane heparan sulfate (Laterra *et al.,* 1983; Izzard *et al.,* 1986).

Reports found in the literature suggested that the peptide sequence lysine–arginine–serine–arginine (KRSR) selectively enhanced osteoblast adhesion by possibly binding to heparan sulfate on the membranes of osteoblasts (Dee *et al.,* 1996). Compared to unmodified glass, Dee (1996) demonstrated enhanced osteoblast, fibroblast, and endothelial cell adhesion

on borosilicate glass modified with the immobilized integrin-binding RGD peptide. In contrast, compared to unmodified borosilicate glass, osteoblast adhesion was significantly greater, whereas fibroblast and endothelial cell adhesion were similar on borosilicate glass modified with the immobilized heparan–sulfate-binding KRSR peptide (Dee, 1996; Dee *et al.,* 1996). For this reason, Dee *et al.* (1996) suggested that proactive orthopedic and dental implants must enhance osteoblast adhesion by both integrin and heparan sulfate mechanisms; this could be accomplished by modifying the surface of a biomaterial with both integrin-binding peptides containing the RGD sequence and heparan sulfate-binding peptides containing the KRSR sequence (Dee, 1996; Dee *et al.,* 1996).

V. Materials Currently Used as Orthopedic and Dental Implants

Metals (such as commercially pure titanium, titanium alloys, stainless steel, and Co–Cr alloys), ceramics (such as alumina, hydroxyapatite, and bioglass), and polymers (such as polymethyl methacrylate (PMMA) and ultrahigh-molecular-weight polyethyene) have been used as either single or multicomponent orthopedic and dental implants (Park and Lakes, 1992). Traditionally, materials (such as metals, ceramics, and polymers) currently used for orthopedic and dental implant applications were selected by trial-and-error processes; to date, titanium and titanium alloys have been the orthopedic and dental material of choice due to their superior mechanical properties (such as low-weight–high-strength ratio), excellent resistance to corrosion, and ability to remain inert *in vivo* (Lausmaa *et al.,* 1990). Although satisfying (but not matching; see Table II) mechanical requirements of bone, titanium and titanium alloys have failed clinically, often due to insufficient bonding to juxtaposed bone—that is, incomplete osseointegration (Kaplan *et al.,* 1994).

Bioceramics, such as alumina or aluminum oxide (Al_2O_3), hydroxyapatite ($Ca_5(PO_4)_3(OH)$), calcium phosphates, bioglass–ceramics, and carbon-based alloys, possess exceptional biocompatability with bone cells and tissues; mechanical properties (such as low ductility), however, have limited their wide use as orthopedic and dental implant materials (Table II) (Doremus, 1992; Grenoble *et al.,* 1972). Once implanted into bone (usually as coatings for traditional orthopedic or dental implant metals), various bioceramics (such as hydroxyapatite, bioactive glass, and calcium phosphate) physiochemically bonded to bone and, in select cases, promoted new bone formation, leading to implant osseointegration (Jarcho *et al.,* 1977; Garcia and Doremus, 1992; Hench, 1993; Ducheyne, 1994, 1999). Direct chemical bonding of hydroxyapatite-coated titanium implants to adjacent bone

TABLE II
COMPARISON OF MECHANICAL PROPERTIES OF SELECT ORTHOPEDIC AND DENTAL MATERIALS AND BONE

Material	Modulus of elasticity (GPa)
Bone[a]	
Cortical bone	17
Trabecular bone	100
Metals[b]	
Titanium	110
Steel alloy	21
Aluminum alloy	7
Ceramics[c]	
Calcium phosphate	40–117
Alumina	380
Polymers[b]	
Polymethyl methacrylate (PMMA)	2

Data obtained from:
[a] (Fung, 1993).
[b] (Kaplan *et al.,* 1994).
[c] (Park and Lakes, 1992).

has been reported (Garcia and Doremus, 1992); the coordination between negatively charged carboxalate groups in collagen present in bone and calcium ions present on apatite surfaces has been proposed as the most likely ionic-bonding mechanism (Garcia and Doremus, 1992). Surface properties (such as chemical composition, specific surface area, crystal structure, and porosity) of bioceramics determine the rate, formation, and chemical composition of apatites synthesized on these material surfaces (Jarcho *et al.,* 1977; Garcia and Doremus, 1992; Hench, 1993; Radin and Ducheyne, 1993; Ducheyne, 1994; Pereira *et al.,* 1994; Yubao *et al.,* 1994). Deposition of calcium-deficient, carbonate-containing, bonelike apatite on the surface of bioceramics is thought to be the critical step in their osseointegration. Several studies have reported formation of apatite layers *in vitro* in the presence of acellular conditions that simulated the physiological milieu (that is, buffered saline solutions containing protein and ion concentrations similar to that of human plasma) (Radin and Ducheyne, 1993; Pereira *et al.,* 1994; Li *et al.,* 1994). In contrast to these studies, numerous reports in the literature demonstrated insufficient formation of bone on orthopedic and dental implant materials consisting of ceramics (such as calcium phosphates and hydroxyapatite) (Doremus, 1992). To date, the source of inconsistency in the efficacy of bioceramics remains largely unclear.

A. Novel Surface Modifications of Conventional Orthopedic and Dental Implants

Several attempts have been made to increase the cytocompatibility of conventional materials for orthopedic and dental applications using *in vitro* cellular models. Anchorage-dependent cells (such as osteoblasts, fibroblasts and endothelial cells) must first adhere to a surface in order to perform normal, subsequent functions (such as spreading and proliferation). Although adhesion is a critical cell process, subsequent functions (such as the deposition of calcium-containing mineral) by osteoblasts on orthopedic and dental implants are of tantamount importance. *In vitro* functions of osteoblasts have been well documented on conventional orthopedic and dental materials composed of metals (such as commercially pure titanium (Degasne *et al.,* 1999; Keller *et al.,* 1994; Wen *et al.,* 1996)), metal alloys (for example, Ti–6Al–4V (Puleo *et al.,* 1993), Co–Cr–Mo (Garvey and Bizios, 1995), and 316L stainless steel (Garvey and Bizios, 1995)) and ceramics (such as hydroxyapatite (Malik *et al.,* 1992) and bioglasses (Davies and Matsuda, 1994)). Reports in the literature demonstrated increased osteoblast adhesion, cell spreading, proliferation, synthesis of extracellular matrix proteins (such as alkaline phosphatase), and deposition of extracellular matrix calcium on conventional titanium surfaces with increased microsize roughness (i.e., sandblasted, heated treated, acid-etched, and machined surfaces) (Degasne *et al.,* 1999; Keller *et al.,* 1994; Wen *et al.,* 1996; Curtis and Wilkinson, 1997; Brunette, 1988). Besides promoting functions of osteoblasts, increased microsurface roughness of conventional ceramic surfaces (such as acid-etched hydroxyapatite) enhanced synthesis of tartrate–resistant–acid phosphatase (TRAP) and bone-resorption activity of osteoclast cells (Gomi *et al.,* 1993; Matsunaga *et al.,* 1999).

In addition to increased surface roughness, chemical modifications of materials currently used for orthopedic and dental implants have enhanced *in vitro* osteoblast functions such as adhesion, proliferation, synthesis of extracllular matrix proteins, and deposition of calcium-containing mineral (Healy *et al.,* 1994; Dee, 1996; Dee *et al.,* 1996). By immobilizing bioactive chemical compounds on functional groups of a silane-coated orthopedic or dental biomaterial, a traditional biomaterial may be transformed into one that can elicit specific responses from surrounding living cells and tissues. To date, enzymes (i.e., glucose oxidases, or glutamate oxidase (Tiller *et al.,* 1999)), antibodies (such as antimouse immunoglobulin G (Turkova, 1999)), and specific peptide sequences (such as arginine–glycine–aspartic acid–serine and lysine–arginine–serine–arginine (Dee, 1996; Dee *et al.,* 1996)) have been immobilized on various materials (i.e., glass, polymers, and metal oxides) to enhance osteoblast functions. It is not clear whether, upon

implantation, the bioactivity of these immobilized peptides may be affected because of the interactions of macromolecules from physiological fluids and tissues; osseointegration of juxtaposed bone and biomaterial surfaces modified with immobilized specific bioactive groups remains to be proven *in vivo*. Clearly, new material formulations must be designed that retain their bioactivity *in vivo* to promote osseointegration with surrounding bone.

VI. Next Generation of Orthopedic and Dental Implants: Nanophase Ceramics

Traditional materials for orthopedic and dental applications have been selected based on their mechanical properties and ability to remain inert *in vivo;* this selection process has provided materials that satisfied physiological loading conditions but did not duplicate the mechanical, chemical, and architectural properties of bone. Most importantly, to date, failure of conventional orthopedic and dental implant materials is often due to insufficient bonding to juxtaposed bone (that is, insufficient osseointegration).

Biomaterial scientists and engineers are currently investigating novel formulations and modifications of existing materials that elicit specific, timely, and desirable responses from surrounding cells and tissues to support the osseointegration of the next generation of orthopedic and dental biomaterials (Ratner, 1992). Enhanced deposition of mineralized matrix at the bone–implant interface provides crucial mechanical stability to implants. Proactive orthopedic and dental biomaterials could consist of novel formulations that selectively enhance osteoblast function (such as adhesion, proliferation and formation of calcium-containing mineral) while, at the same time, minimize other cell (such as fibroblast) functions that may decrease implant efficacy (e.g., fibroblast participation in callus formation and fibrous encapsulation of implants *in vivo*).

Nanophase materials are new formulations of materials that are composed of grains of the same atoms but with fewer (less than tens of thousands) and smaller (less than 100 nm in diameter) atoms than in conventional forms (which contain several billion atoms and have grain sizes of micrometers to millimeters in diameter) (Siegel, 1996). Nanocrystalline materials exhibit enhanced magnetic, catalytic, electrical and optical properties when compared to conventional formulations of the same material (Siegel and Fougere, 1994, 1995a,b; Siegel, 1996, 1994). Moreover, nanophase ceramics can be synthesized so they possess similar grain size, geometry, and microarchitecture as that of healthy, physiological bone (see the sections "Microarchitecture" and "Structural Organization of the Bone Microarchitecture"). Properties

(specifically, mechanical and surface) of nanophase materials pertaining to the design and synthesis of orthopedic and dental implants of increased efficacy are expanded upon in the sections that follow.

A. Surface Properties of Nanophase Ceramics for Enhanced Orthopedic and Dental Implant Efficacy

1. Rationale

Orthopedic and dental implants with surface properties that promote cell and tissue interactions that lead to implant osseointegration are needed. Surface properties (such as area, charge, and topography) depend on the grain size of a material; in this respect, nanophase materials, which, by their very nature, possess higher surface area with increased portions of surface defects (such as edge–corner sites) and grain boundaries (Klabunde *et al.,* 1996), have an advantage that currently remains largely unexplored for biomedical applications. The increased surface reactivity of nanomaterials has been utilized for catalytic applications (Klabunde *et al.,* 1996); for example, compared to conventional (greater than a 100-nm average grain size) magnesium oxide (MgO), nanophase (i.e., a 4-nm average grain size) materials, such as magnesium oxide (MgO) and aluminum nitride, possessed higher surface area (100 to 160 m^2/g compared to 200 to 500 m^2/g for MgO, respectively), less acidic OH^- groups (due to a much higher proportion of edge sites for the nanophase MgO to cause delocalization of electrons; Fig. 9), increased adsorption of acidic species (such as SO_2^- and CO_2^-), and increased destructive adsorption of organophosphorous and of chlorocarbons (Klabunde *et al.,* 1996; Baraton *et al.,* 1997). It is extremely attractive to ponder if and

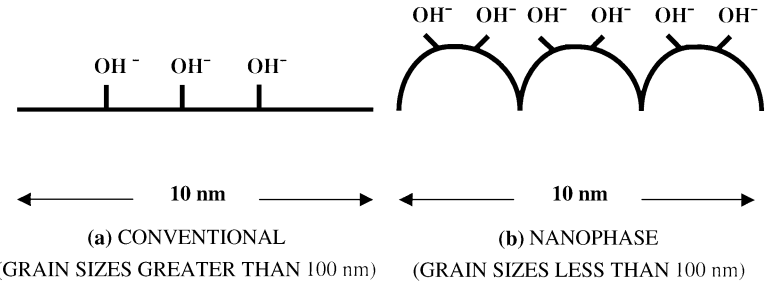


Fig. 9. Theoretical predictions of changes in surface properties of nanophase materials that affect the hydroxide layer. Compared to (a) conventional materials, (b) nanophase materials possess higher surface area and less acidic OH^- groups (due to an increase in electron delocalization) in the hydroxide layer. (Adapted and redrawn from Klabunde *et al.,* 1996.)

how these enhanced surface properties (such as increased surface area and charge, as well as ability to alter adsorption of chemical species) could be used to promote bonding of juxtaposed bone to an orthopedic or dental implant composed of nanophase ceramics.

2. Experimental Evidence

Clearly, some of the best examples illustrating the potential advantages of nanoceramic surface properties in advancing orthopedic and dental implant applications are calcium phosphate and/or calcium phosphate derivatives (such as hydroxyapatite ceramics, tricalcium phosphates, calcium carbonates, and bioactive glasses). As the main inorganic component of bone, hydroxyapatite (with a physiological platelike 25×3.5-nm geometry (Muller-Mai *et al.,* 1995)) has been an attractive material for hard-tissue repair over the last three decades. When implanted, these materials (sometimes polygonal coarse particles less than 100 nm in diameter (Muller-Mai *et al.,* 1995)) are nontoxic and antigenically inactive, do not induce cancer, and can, in some cases, bond directly with bone without any intervening fibrous–connective tissue layer.

Recent studies on ectopic bone formation (osteoinduction or material-induced osteogenesis) of calcium phosphate biomaterials (with grain sizes less than 100 nm) showed that osteoinduction might be an intrinsic property of calcium phosphate biomaterials. Ripamonti (1991, 1996) reported bone formation in coral-derived hydroxyapatite implanted in muscles of baboons, rabbits, and dogs. Vargervik (1992) reported ectopic bone formation in porous hydroxyapatite ceramics in monkeys; Yamasaki and Saki (1992) found bone formation induced by hydroxyapatite ceramics in dogs; Toth *et al.* (1993) found bone formation in dogs induced by hydroxyapatite–tricalcium phosphate as well as α- and β-calcium pyrophosphate; Klein *et al.* (1994) observed bone tissue in calcium phosphate ceramic in soft tissues of dogs; and Yang *et al.* (1996, 1997) and Yuan *et al.* (1997a–e) reported hydroxyapatite–tricalcium phosphate ceramic-induced osteogenesis in dogs, pigs, and rabbits.

In spite of these investigations, many reports in the literature demonstrate that these nanoapatite ceramics are not always osteoinductive and, furthermore, do not possess mechanical properties similar enough to bone for sustained osseointegration (Muller-Mai *et al.,* 1995; Doremus, 1992; Du *et al.,* 1999; Weng *et al.,* 1997), criteria necessary for increased orthopedic and dental implant efficacy. Moreover, mechanisms of osteoinduction of calcium phosphate ceramics are not clear and seem to depend on specific nanoapatite material properties (such as surface properties and crystallinity) and the animal tested (i.e., dog versus rabbit). Undoubtedly, the incidental cases of calcium phosphate biomaterial-induced osteogenesis indicate promise in

the development of nanoapatites with instrinsic osteoinductive properties, but surface properties of these novel prostheses that induce bone growth must obviously be understood.

Surface roughness (as determined by macro- and microporosity, grain size, etc.) crystallinity, and wettability (or hydrophobicity) of calcium phosphate–derived ceramics have all been shown to influence osteoinductivity. For example, hydroxyapatite substrates with high amounts of surface microporosity induced bone formation under the skin of dogs (Yamaski and Saki, 1992). Yuan *et al.* (1999) showed that surfaces with microporosity promoted calcium phosphate ceramic-induced osteogenesis in dorsal muscles of dogs by increasing surface area and subsequent adsorption of proteins and growth factors that stimulated osteoblastic activity. Even the size of macropores influenced osteoinduction; for example, pore diameters of 100 to 600 μm on the surface of hydroxyapatite and calcium phosphate ceramics enhanced bone ingrowth in dogs, rabbits, and humans (Inoue *et al.,* 1992; Kawamura *et al.,* 1987; Flately *et al.,* 1983; Passuti *et al.,* 1989). It has also been suggested that apatite crystals sintered at low temperatures, which possess a low degree of crystallinity, were more active in bone formation (de Bruijn *et al.,* 1994).

In contrast to reports demonstrating the effect of surface properties on the extent of osseointegration of calcium phosphate with juxtaposed bone, few studies have addressed the mechanisms of enhanced osteoblastic activity on these nanoapatites. One set of *in vitro* studies pinpoints grain size in the nanometer regime as the major parameter for enhancing ceramic cytocompatability. For example, compared to respective conventional, larger grain size, ceramic formulations, enhanced adhesion of osteoblasts (the bone-forming cells) and decreased adhesion of fibroblasts (cells that contribute to fibrous encapsulation and callus formation events that may lead to implant loosening and failure) have been observed on nanophase alumina, titania, and HA (Webster *et al.,* 1998, 1999a,b, 2000a). In fact, decreasing alumina grain size from 167 to 24 nm increased osteoblast adhesion 51% and at the same time decreased fibroblast adhesion 235% after 4 hr (Webster *et al.,* 2000a).

Investigations of the underlying mechanism(s) revealed that the concentration, conformation, and bioactivity of vitronectin (a protein contained in serum that is known to mediate osteoblast adhesion ((Thomas *et al.,* 1997); see the section "Vitronectin") was responsible for the select, enhanced adhesion (a crucial prerequisite for subsequent, anchorage-dependent-cell function) of osteoblasts on these novel nanoceramic formulations. Specifically, of the proteins (such as albumin, laminin, fibronectin, collagen, and vitronectin) tested, vitronectin adsorbed in the highest concentration on nanophase alumina after 4 hr; moreover, competitive adsorption of vitronectin was 10% greater on nanophase compared to conventional alumina (Webster *et al.,*

2001a). Furthermore, and in contrast to protein adsorption to conventional ceramics, vitronectin adsorption to nanophase ceramics was controlled by calcium-mediated mechanisms (Webster *et al.,* 2001a). Calcium mediation affected the conformation of vitronectin subsequently adsorbed on nanophase ceramics to promote osteoblast adhesion. Specifically, a novel adaptation of the standard surface-enhanced Raman scattering (SERS) technique provided evidence of increased unfolding of vitronectin adsorbed on nanophase ceramics (Webster *et al.,* 2001a). Protein conformation plays a critical role in mediating subsequent cell interactions (such as cell adhesion on material surfaces; see the section "Protein-Mediated Cell Adhesion on Biomaterial Surfaces"). For example, vitronectin unfolding promoted availability of specific cell-adhesive epitopes (such as arginine–glycine–aspartic acid–serine) for subsequent enhanced osteoblast adhesion; evidence supporting this claim was provided by competitive inhibition studies (Webster *et al.,* 2001a).

Webster *et al.* (2000a) suggested that the topography (such as roughness dictated by nanometer grain size and nm pore size) of nanophase ceramics influenced interactions (such as adsorption and/or configuration or bioactivity) of select proteins that affected subsequent cell adhesion. For example, because of protein stereochemical structure and ceramic pore dimensions, vitronectin (a linear protein 15 nm in length (Ayad *et al.,* 1994); see the section "Vitronectin") preferentially adsorbed to the small (e.g., 0.69, 0.98, and 0.66 nm for alumina, titania, and hydroxyapatite formulations, respectively) pores present in nanophase ceramics, whereas larger proteins that do not promote osteoblast adhesion (such as laminin with a cruciform configuration 70 nm both in length and width (Ayad *et al.,* 1994); see the section "Laminin") adsorbed to the large (e.g., 2.94, 23.3, and 3.1 nm for alumina, titania, and hydroxyapatite, respectively) pores present in conventional ceramics. In addition, variations in ceramic surface topography (such as nanophase and conventional titania with average surface roughness values of 32 nm and 16 nm, respectively (Webster *et al.,* 2000a)) on the same order of magnitude as the size of proteins may have affected adsorbed protein configuration and, thus, availability of bioactive domains (i.e., specific amino acid sequences) that mediate subsequent osteoblast adhesion. Due to protein dimensions in the nanometer regime (see the section "Organic Phase"), through the use of nanoceramics, engineers can now modify a surface to control and manipulate adsorbed protein configuration or bioactivity for increased bone cell interactions; this is, most likely, the largest uninvestigated and promising potential of nanophase ceramics in biomedical applications.

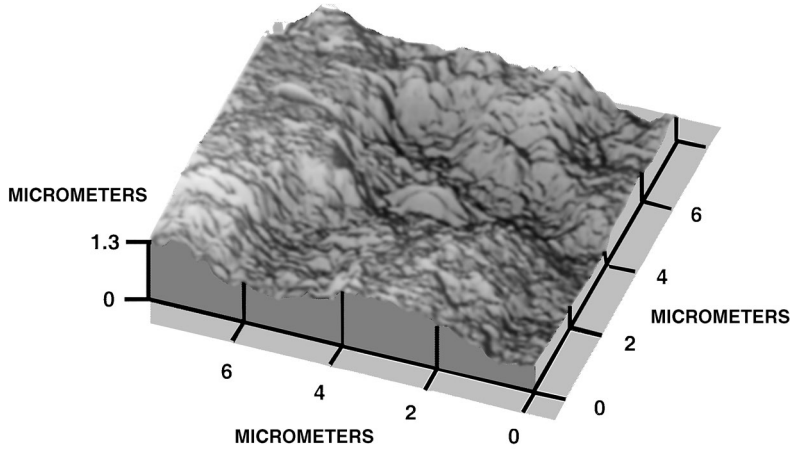
Adhesion of osteoblasts to ceramic surfaces alone, however, is not adequate to achieve long-term osseointegration of orthopedic and dental implants; subsequent osteoblast functions (such as proliferation, synthesis of

extracellular matrix proteins, and deposition of extracellular calcium) are required. *In vitro* studies conducted by Webster *et al.* (2000b) also provided the first evidence of enhanced osteoblast proliferation, alkaline phosphatase synthesis, and concentration of extracellular matrix calcium on ceramics of decreased grain size (specifically on nanophase alumina, titania, and hydroxyapatite).

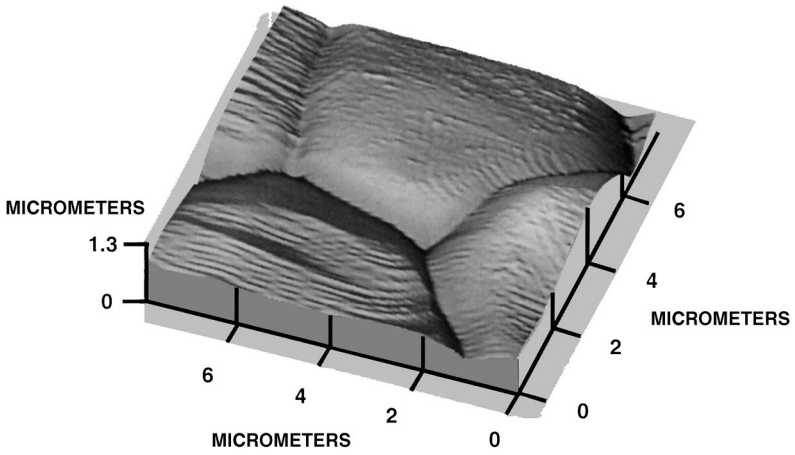
However, other cell functions must also be investigated on proposed biomaterials; this is true because maintainence of healthy bone juxtaposed to an implanted surface requires the formation of a bone-remodeling unit (consisting of activation of bone cells by the action of growth factors such as insulinlike growth factors I and II (Kaplan *et al.,* 1994), resorption of bone by osteoclasts, and formation of new bone by osteoblasts on the site of the old resorbed bone; see the section "Bone Remodeling"). For this reason, Webster *et al.* (2001b) investigated osteoclastlike cell function on nanophase ceramics and demonstrated enhanced synthesis of tartrate-resistant acid phosphatase (TRAP) and subsequent increased formation of resorption pits when ceramic grain size was reduced into the nanometer regime. Such enhanced corresponding events between osteoclasts and osteoblasts may provide a plausible explanation for the observed, improved osseointegration between nanoapatites and juxtaposed bone *in vivo* (Doremus, 1992).

It is imperative to determine properties of nanoceramics that enhance osteoblast and osteoclast functions; Webster *et al.* (2000a,b, 2001a,b) proposed the following:

1. Enhanced osteoblast and osteoclast functions on nanophase ceramics due to increased nanosize surface roughness (Fig. 10). Compared to conventional ceramics, nanophase ceramics possess increased surface roughness (by 35 to 50%) resulting from both decreased grain size and decreased diameter of surface pores (Webster *et al.,* 1999b). These results confirm those obtained by other research groups who reported enhanced functions of osteoblasts and osteoclasts on surfaces with increased roughness (specifically, surfaces with increased roughness values achieved by sandblast, heat treating, acid etching, and machining) (Curtis and Wilkinson, 1997; Degasne *et al.,* 1999; Keller *et al.,* 1994; Gomi *et al.,* 1993; Matsunaga *et al.,* 1999) but are among the first to demonstrate that surface grain sizes of ceramic formulations in the nanometer regime result in nanosize roughness values that selectively promote bone cell functions.
2. Enhanced osteoblast and osteoclast functions on nanophase ceramics due to increased surface wettability. Compared to conventional ceramics, nanophase ceramics exhibit enhanced surface wettability [evidenced, for example, by aqueous contact angles three times smaller



(a) NANOPHASE (39-nm GRAIN SIZE) TITANIA



(b) CONVENTIONAL (4520-nm GRAIN SIZE) TITANIA

FIG. 10. Representative topography of nanophase and conventional titania. Representative atomic force micrographs of (a) nanophase titania with 39-nm grain sizes and of (b) conventional titania with 4520-nm grain sizes, illustrating the different topographies of nanophase compared to conventional grain size ceramics.

when alumina grain size was decreased from 167 to 24 nm (Webster *et al.,* 1999b)] due to surface roughness and/or greater numbers of grain boundaries on their surface. These studies confirm results obtained by other research groups who correlated increased adsorption of vitronectin [a protein that promotes osteoblast adhesion (Thomas *et al.,* 1997)] on material surfaces with greater surface wettability (Luck

et al., 1998) but are the first to demonstrate that surface grain size of ceramic formulations in the nanometer regime increases the number of grain boundaries at the surface to enhance bioceramic surface wettability for vitronectin adsorption which, subsequently, promotes select bone cell function (Fig. 11).

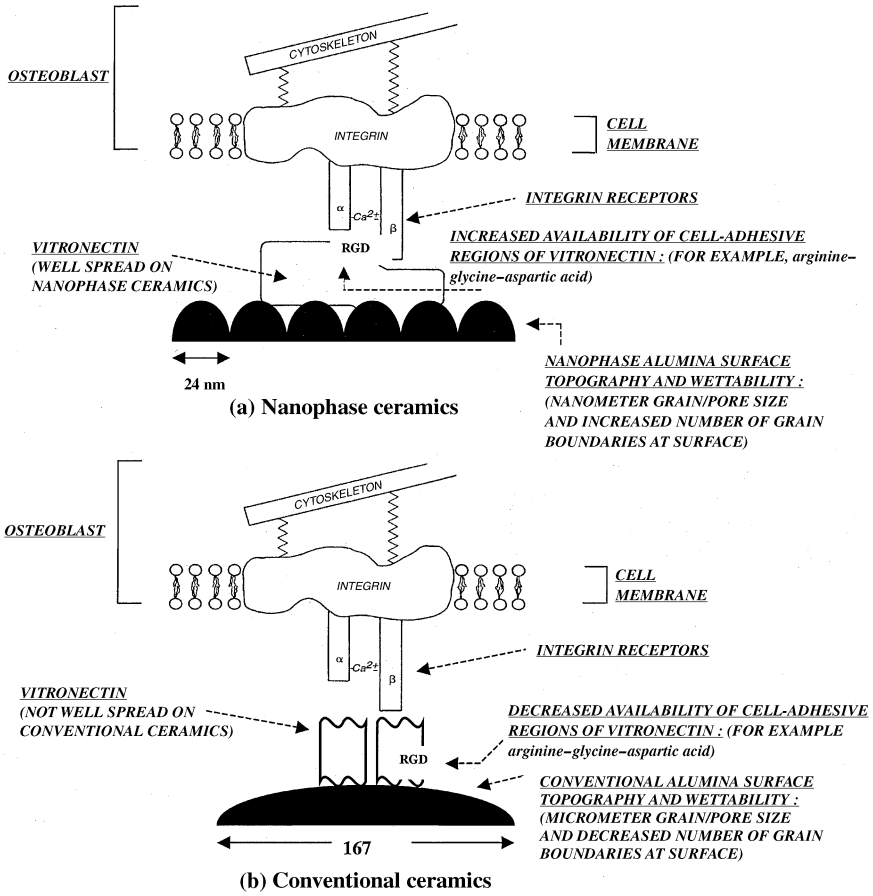


FIG. 11. Unfolding of vitronectin exposes epitopes for osteoblast adhesion on nanophase ceramics. Schematic representation (not in scale) of a possible mechanism for enhanced osteoblast adhesion on (a) nanophase, compared to (b) conventional, ceramics, which involves unfolding of the vitronectin macromolecule to expose select cell-adhesive epitopes (such as arginine–glycine–aspartic acid) for osteoblast adhesion. Increased exposure of cell-adhesive epitopes of vitronectin for enhanced osteoblast adhesion on nanophase ceramics may be due to nanometer surface topography and/or increased wettability due to the greater number of grain boundaries at the surface.

Due to their ability to selectively promote both osteoblast and osteoclast function, nanophase ceramics provide a preferable alternative to conventional orthopedic and dental implants that fail to integrate with surrounding bone; it is undoubtedly highly desirable to minimize, if not avoid, clinical complications that necessitate removal of failed implants as a result of poor surface properties that lead to insufficient osseointegration. These results provide evidence that nanoceramics may be synthesized to match surface properties of bone and, thus, demonstrate strong promise and potential for their use in orthopedic and dental applications.

B. Mechanical Properties of Nanophase Ceramics for Enhanced Orthopedic/Dental Implant Efficacy

1. Rationale

The next generation of orthopedic and dental implants must possess mechanical properties that are similar to those of the surrounding bone. Nanophase ceramics may be synthesized to possess hardness, bending and compressive and tensile strengths that are different than properties of conventional ceramics but similar to those of physiological bone. Indeed, greater mechanical properties (such as hardness, ductility, and enhanced strain to failure) have been reported for ceramics with a reduction in grain size into the nanometer range (Bohn *et al.,* 1991; Mayo *et al.,* 1990, 1992). Mechanical deformation theory indicates that the high-volume fraction of interfacial regions compared to bulk material leads to increased deformation by grain-boundary sliding in nanocrystalline ceramics (Coble, 1963). Similarly, it has been suggested that increased grain-boundary sliding, accompanied by short-range diffusion-healing events as grain size is reduced, results in increased ductility for strongly ionic (that is, ceramic) and covalently bonded nanomaterials (Fig. 12) (Siegel, 1994).

Nanocrystalline materials have been shown to possess increased (by a factor of 2 to 5 for nanocrystalline copper, palladium, and silver (Nieman, 1991; Nieman *et al.,* 1989, 1991a,b)) hardness, to deform at faster (e.g., 34 times faster for 13-nm versus 300-nm zirconia (Ciftcioglu and Mayo, 1990)) superplastic forming rates, to exhibit higher strain rates but without fracture (for example, 40-nm titania deformed without fracture at strains exceeding 0.6 for strain rates as high as 10^3 s^{-1} (Hahn and Averback, 1991; Carry and Mocellin, 1987)), and to bend at lower temperatures than their large-grained (conventional) counterparts (Weertman *et al.,* 1999). For example, Ciftcioglu and Mayo (1990) demonstrated a fourfold reduction in grain size of yittria-stabilized zirconia-accelerated strain rates close to the deformation stress and strain rates typically used for formation of metals via superplastic techniques.

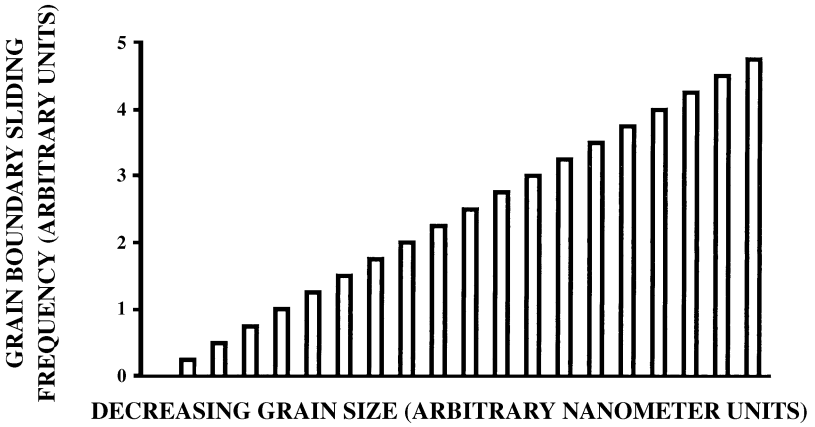


FIG. 12. Schematic deformation properties of nanophase ceramics. For ceramics, decreasing the grain size into the nanometer regime corresponds to an increase in grain boundary sliding, resulting in increased ductility of these materials. (Adapted and redrawn from Siegel, 1994.)

Extended strain to failure has been well documented for submicrometer and nanocrystalline ceramics; elongations to failure of 100 to 800% have been reported for nanophase ceramics whose conventional grain-size counterparts normally fail at 0 to 2% elongation (Maehara and Langdon, 1990; Nieh *et al.,* 1991). Weertman *et al.* (1999) reported plastic deformations of about 100% in compression of single calcium fluoride crystals at temperature conditions under which conventional materials would fail in the elastic regime. True compressive creep testing of nanoceramic materials has substantiated enhanced plasticity; compressive creep tests conducted at moderate temperatures on 99% dense nanophase TiO_2 showed extensive deformation without crack formation (Hahn and Averback, 1991). Nanophase TiO_2 also exhibited enhanced plasticity in tension; Cui and Hahn (1992) subjected 40-nm grain size TiO_2 to biaxial bulge tests at temperatures between 700 and 800°C and reported ductile tensile behavior, without formation of any cracks, as the samples deformed up to true strain levels of 0.1 (Cui and Hahn, 1992). Considering that the brittle nature of conventional, larger-grain-size, ceramics inhibits their widespread use as orthopedic or dental materials, it is interesting to ponder if and how the enhanced mechanical properties of nanophase ceramics could be incorporated into the next generation of biomaterials with improved osseointegrative capabilities.

2. Experimental Evidence

To date, few research groups have incorporated the enhanced mechanical properties of nanophase ceramics into orthopedic and dental applications.

One example in the literature demonstrates that nanophase (10–15 nm average grain size) diamond-coated (via microwave plasma chemical vapor deposition) titanium implants exhibited improved fracture toughness and adhesion compared to conventional coatings (Catledge and Vohra, 1999). Toprani *et al.* (2000) reported levels of strain more indicative of ductile coatings than ceramic during indentation tests. The authors note that the observed increased fracture toughness comes at the expense of decreased hardness, which may be improved by synthesizing layers of nanocrystalline diamond on layers of high phase purity microcrystalline diamond (Catledge *et al.,* 2000); by controlling placement of nano- and microcrystalline layers, one can obtain a compositelike film whose toughness, hardness, and surface roughness properties can be tailored as desired. For this reason, future biomaterials consisting of nanophase ceramics can be tailored to meet clinical requirements associated with anatomical differences or patient age; such requirements arise because, for example, the modulus of elasticity varies by 10% in bone from the human hip and tibia and by up to 10% in bone from 10- and 90-year-old human (Kaplan *et al.,* 1994). Conventional orthopedic and dental implants are not synthesized to simulate differences in mechanical properties of bone based on anatomical differences or patient age; instead, a "one-size-fits-all" model is currently used.

Another example in the literature demonstrating how mechanical properties of nanophase ceramics could enhance orthopedic and dental implants is by Webster *et al.* (1999a), who demonstrated that compared to respective conventional ceramics, bending properties (specifically, bending modulus, bending strength, flexural rigidity, and bending structural stiffness) of nanophase alumina, titania, and hydroxyapatite ceramics are closer to those of human femur bone; for example, the average bending modulus of nanophase alumina (namely, 35 GPa) was 1.8 times greater, whereas conventional alumina was 2.7 times greater (specifically, 52 GPa) than the respective value of human femur bone (in the range of 19 GPa) (Fung, 1993). Moreover, compared to values of the bending strength for conventional hydroxyapatite (in the range 38–113 MPa), Ahn *et al.* (2000) also showed bending strengths of nanocrystalline hydroxyapatite (182 MPa) closer to those of physiological bone (160 MPa).

Undoubtedly, changes in porosity (such as bulk and surface porosity and diameter of individual pores) of nanophase ceramic formulated by Webster *et al.* (1999) provide an explanation for the observed differences in mechanical properties of respective nanophase and conventional ceramic formulations; for example, individual surface pores four times smaller were achieved in nanophase (67-nm grain size) compared to conventional (179-nm grain size) HA formulations (Webster *et al.,* 2000a). Compared to conventional formulations, therefore, the bending modulus of nanophase ceramics

may be the result of the combined effects of (1) increased grain-boundary sliding mechanisms due to a larger number of grain boundaries present in nanophase ceramics, and/or (2) smaller crack initiation sites due to smaller diameters of individual surface pores (i.e., fewer and less surface flaws) present on nanophase ceramics. For these reasons, nanophase ceramics provide a preferable alternative to conventional orthopedic and dental implants that fail due to crack initiation and propagation during *in vivo* loading; it is, undoubtedly, highly desirable to minimize, if not avoid, clinical complications that necessitate removal of failed implants as a result of poor mechanical properties. These results provided evidence that nanophase ceramics may be synthesized to match bending properties of bone and thus demonstrated strong promise and potential for their use in orthopedic and dental implant applications.

VII. Conclusions

Nanostructured ceramics provide alternatives not yet fully explored for orthopedic and dental implant applications; the improved mechanical properties of these novel ceramic formulations, in addition to their established exceptional biocompatibility, constitute characteristics that promise improved orthopedic and dental efficacy. Requirements applicable for the design of nanophase ceramics for orthopedic and dental applications include the following:

1. Mechanical properties (such as bending, hardness, and compressive and tensile strength) of bioceramics similar to human bone can be obtained by decreasing the grain size of ceramic formulations into the nanometer regime. Such mechanical properties must be incorporated into bioceramics for orthopedic and dental applications; mechanical properties similar to those of physiological bone are needed in order to minimize imbalances in stress and strain distributions at the tissue–implant interface, which often lead to bone resorption (i.e., osteolysis) and eventual implant loosening and failure.
2. Surface properties (such as topography and wettability) of bioceramics similar to human bone can be obtained by decreasing the grain size of ceramic formulations into the nanometer regime. Such surface properties must be incorporated into proactive bioceramics for orthopedic and dental applications; surface properties similar to those of physiological bone are needed in order to promote select cell interactions that lead to sufficient osseointegration between an orthopedic or

dental implant and juxtaposed bone. Sufficient bonding of juxtaposed bone to an implanted surface stabilizes the prostheses *in situ,* minimizes motion-induced damage to surrounding tissues, and is crucial to the clinical success of orthopedic and dental implants.

A proactive orthopedic or dental bioceramic should be designed to promote the adsorption of vitronectin, a protein that optimizes subsequent adhesion of osteoblasts. Besides adsorption, proactive bioceramics for orthopedic and dental applications should promote unfolding and thus enhance the bioactivity of vitronectin by exposing epitopes (such as integrin and heparan sulfate-binding sites) necessary for select osteoblast adhesion. Unfolding and increased bioactivity of vitronectin can be accomplished by nanophase ceramic topography (as controlled by nanometer surface grain size) and increased wettability (due to higher numbers of grain boundaries at the surface).

As the disciplines of cell–tissue engineering and nanophase material science develop and mature, the preceding design criteria will be expanded and refined. Undoubtedly, nanophase ceramics have great potential to become the next generation of choice proactive biomaterials for innovative biotechnology and biomedical applications that could have profound clinical impact.

REFERENCES

Ahn, E., Gleason, N. J., Nakahira, A., and Ying, J. Y., Properties of nanostructured hydroxyapatite-based bioceramics. *Proc. Sixth World Biomaterials Congress.* 643 (2000).

Anderson, J. M., Mechanisms of inflammation and infection with implanted devices. *Cardiovasc. Pathol.* **2,** 33S–41S (1993).

Ayad, S., Boot-Handford, R., Humphries, M. J., Kadler, K. E., and Shuttleworth, A., "The Extracellular Matrix Factsbook." Academic Press, San Diego, 1994, pp. 29–149.

Baraton, M. I., Chen, X., and Gonsalves, K. E., FTIR study of nanostructured aluminum nitride powder surface: determination of the acidic/basic sites by CO, CO_2 and acetic acid adsorptions. *Nanostructured Materials* **8** (4), 435–445 (1997).

Beer, F., and Johnston, E. R., "Mechanics of Materials." McGraw-Hill Book Company, New York, 1981, p. 585.

Bohn, R., Haubold, R., Birringer, R., and Gleiter, H., Nanocrystalline intermetallic compounds—An approach to ductility. *Scripta Metall. Mater.* **25,** 811 (1991).

Brunette, P. M., The effect of surface topography on cell migration and adhesion, *in* "Surface Characterization of Biomaterials: Progress in Biomedical Engineering, Volume 6" (B. D. Ratner, Ed.), pp. 203–217. Elsevier, New York, 1988.

Brunski, J. B., Influence of biomechanical factors at the bone-biomaterial interface, *in* "The Bone-Biomaterial Interface" (J. E. Davies, Ed.), pp. 391–404, University of Toronto Press, Toronto, 1991.

Carry, C., and Mocellin, A., Structural superplasticity in single phase crystalline ceramics *Ceramics International* **13** (2), 89–98 (1987).

Catledge, S., Baker, P., Tarvin, J., and Vohra, Y., Multilayer nanocrystalline/microcrystalline diamond films studied by laser reflectance interferometry, *in* Diamond and Related Mater., **9** (8), 1512–1517 (2000).

Catledge, S., and Vohra, Y., Effect of nitrogen addition on the microstructure and mechanical properties of diamond films grown using high-methane concentrations. *J. App. Phys.* **86** (1), 698–700 (1999).

Ciftcioglu, M., and Mayo, M. J., Processing of nanocrystalline ceramics, *in* "Superplasticity in Metals, Ceramics and Intermetallics Symposium Proceedings" (M. J. Mayo, M. Kobayashi, J. Wadsworth, Eds.), pp. 77–86. Materials Research Society, Pittsburgh (1990).

Coble, R. L., Development of microstructure in ceramic systems. *J. of App. Phy.* **34,** 1679 (1963).

Cox, D., Aoki, T., Seki, J., Motoyama, Y., and Yoshida, K., The pharmacology of the integrins. *Medical Research Reviews* **14,** 195–228 (1994).

Cui, Z., and Hahn, H., Tensile deformation of nanostructured TiO_2 at low temperatures. *Nanostructured Materials* **1,** 419 (1992).

Curtis, A., and Wilkinson, C., Review: Topographical control of cells. *Biomaterials* **18** (24), 1573–1583 (1997).

Dalton, B. A., McFarland, C. D., Gengenbach, T. R., Griesser, H. J., and Steele, J. G., Polymer surface chemistry and bone cell migration. *J. Biomat. Sci. Polym. Ed.* **9** (8), 781–799 (1995).

Darnell, J., Lodish, H., and Baltimore, D., "Molecular Cell Biology." W.H. Freeman and Company, New York, 1990.

Davies, J. E., The importance and measurement of surface charge species in cell behaviour at the biomaterial interface, *in* "Surface Characterization of Biomaterials: Progress in Biomedical Engineering, Volume 6" (B. D. Ratner, Ed.), pp. 219–234. Elsevier, New York, 1988.

Davies, J. E., and Matsuda, T., Extracellular matrix production by osteoblasts on bioactive substrates *in vitro. Scanning Microscopy* **2,** 1445–1452 (1994).

de Bruijn, J., Bovell, Y. P., and van Blitterswijk, C., Osteoblast and osteoclast responses to calcium phosphates. *Bioceramics* **7,** 293–298 (1994).

Dee, K. C., Considerations for the design of proactive dental/orthopaedic implant biomaterials, Ph.D. thesis, Rensselaer Polytechnic Institute, 1996.

Dee, K. C., Andersen, T. T., Rueger, D. C., and Bizios, R., Conditions which promote mineralization at the bone/implant interface: a model *in vitro* study. *Biomaterials* **17,** 209–215 (1996).

Degasne, I., Basle, M. F., Demais, V., Hure, G., Lesourd, M., Grolleau, B., Mercier, L., and Chappard, D., Effects of roughness, fibronectin and vitronectin on attachment, spreading, and proliferation of human osteoblast-like cells (Saos-2) on titanium surfaces. *Calcified Tissue International* **64** (6), 499–507 (1999).

Doremus, R. H., Review: bioceramics. *J. Mat. Sci.* **27,** 285–297 (1992).

Du, C., Cui, F. Z., Zhu, X. D., and de Groot, K., Three-dimensional nano-Hap/collagen matrix loading with osteogenic cells in organ culture. *J. Biomed. Mat. Res.* **44,** 407–415 (1999).

Ducheyne, P., Bioactive ceramics. *J. Bone Joint Surg.* **76B,** 861–862 (1994).

Ducheyne, P., Stimulations of biological function with bioactive glass. *MRS Bull.* **23** (11)**,** 43–49 (1999).

Ellingsen, J. E., A study on the mechanism of protein adsorption to TiO_2. *Biomaterials* **12** (6), 593 (1991).

Flately, T. J., Lynch, K. L., and Benson, M., Tissue response to implants of calcium phosphate ceramics in the rabbit spine. *Clinical Orthop.* **179,** 246–252 (1983).

Fung, Y. C., "Biomechanics Mechanical Properties of Living Tissues Second Edition." Springer-Verlag, New York, 1993, pp. 500–538.

Garcia, R., and Doremus, R. H., Electron microscopy of the bone-hydroxyapatite interface from a human dental implant. *J. Mat. Sci.: Materials in Medicine* **3,** 154–156 (1992).

Garvey, B. T., and Bizios, R., A transmission electron microscopy examination of the interface between osteoblasts and metal biomaterials. *J. Biomed. Mat. Res.* **29** (8), 987–992 (1995).

Gehron-Robey, P., The biochemistry of bone. *Endocrinology and Metabolism Clinics of North America* **18,** 859–902 (1989).

Gomi, K., Lowenberg, B., Shapiro, G., and Davies, J. E., Resorption of sintered hydroxyapatite by osteoclasts *in vitro*. *Biomaterials* **14** (2), 91–96 (1993).

Graf, J., Ogle, R. C., Robey, F. A., Sasaki, M., Martin, G. R., Yamada, Y., and Kleinman, H. K., A pentapeptide from the laminin B1 chain mediates cell adhesion and binds the 67000 laminin receptor. *Biochemistry* **26,** 6896 (1987).

Grenoble, D. E., Katz, J. L., Dunn, K. L., Gilmore, R. S., and Murty, K. L., The elastic properties of hard tissues and apatites. *J. Biomed. Mat. Res.* **6,** 221–233 (1972).

Gronowicz, G., and McCarthy, M. B., Response of human osteoblasts to implant materials: integrin-mediated adhesion. *J. Orthop. Res.* **14** (6), 878–887 (1996).

Hahn, H. J., and Averback, R. S., Low-temperature creep of nanocystalline titanium (IV) oxide. *J. Amer. Ceramic Soc.* **74** (11), 2918–2921 (1991).

Healy, K. E., Lom, B., and Hockberger, P. E., Spatial distribution of mammalian cells dictated by material surface chemistry. *Biotech. Bioeng.* **43,** 792–800 (1994).

Heegard, A., Structure and molecular regulation of bone matrix proteins. *J. Bone Mineral Res.* **8,** S843–S847 (1993).

Hench, L. L., Bioceramics: from concept to clinic. *Amer. Ceramic Soc. Bull.* **72,** 93–98 (1993).

Hench, L. L., and Ethridge, E. C., Biomaterial—The interfacial problem. *Adv. Biomed. Eng.* **5,** 35–150 (1975).

Horbett, T. A., Principles underlying the role of adsorbed plasma proteins in blood interactions with foreign materials. *Cardiovasc. Pathol.* **2,** 137S–148S (1993).

Horbett, T. A., Proteins: Structure, Properties and Adsorption to Surfaces. In "Biomaterials Science: An Introduction to Materials in Medicine," (B. D. Ratner, A. S. Hoffman, A. S. Schoen, and J. E. Lemons, Eds.), pp. 133–140. Academic Press, New York, 1996.

Hynes, R. O., Integrins: versatility, modulation, and signaling in cell adhesion. *Cell* **69,** 11–25 (1992).

Inoue, O., Shimabukura, H., Shingaki, Y., and Ibaraki, K., Our application of high porosity hydroxyapatite cubes for the treatment of non-cystic benign tumors. *Bioceramics* **5,** 411–418 (1992).

Izzard, C. S., Radinsky, R., and Culp, L. A., Substratum contacts and cytoskeletal reorganization of BALB/c3T3 cells on a cell-binding fragment and heparin-binding fragments of plasma fibronectin. *Experimental Cell Research* **165,** 320–336 (1986).

Jarcho, M., Kay, J. F., Gumaer, K. I., Doremus, R. H., and Drobeck, H. P., Tissue, cellular and subcellular events at a bone-ceramic hydroxylapatite interface. *J. Bioeng.* **1,** 79–92 (1977).

Kaplan, F. S., Hayes, W. C., Keaveny, T. M., Boskey, A., Einhorn, T. A., and Iannotti, J. P., Form and function of bone, *in* "Orthopaedic Basic Science" (S. P. Simon, Ed.), pp. 127–185. American Academy of Orthopaedic Surgeons, Columbus, Ohio, 1994.

Kasemo, B., and Lausmaa, J., Surface science aspects of inorganic biomaterials. *C.R.C. Critical Rev. in Biocompatibility* **2,** 335–380 (1986).

Kawamura, M., Iwata, H., and Miura, T., Chondroosteogenic response to crude bone matrix proteins bound to hydroxyapatite. *Clinical Orthop.* **217,** 281–292 (1987).

Keaveny, T. M., and Hayes, W. C., Mechanical properties of cortical and trabecular bone. *Bone* **7,** 285–344 (1993).

Keller, J. C., Stanford, C. M., Wightsman, J. P., Draughn, R. A., and Zaharias, R., Characterization of titanium implant surfaces. III. *J. Biomed. Mat. Res.* **28,** 939–946 (1994).

Klabunde, K. J., Stark, J., Koper, O., Mohs, C., Park, D., Decker, S., Jiang, Y., Lagadic, I., and Zhang, D., Nanocrystals as stoichiometric reagents with unique surface chemistry. *J. Phys. Chem.* **100** (30), 12142–12153 (1996).

Klein, C., de Groot, K., Chen, W., Li, Y., and Zhang, X., Osseous substance formation in porous calcium phosphate ceramics in soft tissues. *Biomaterials* **15,** 31–34 (1994).

Kramer, R. H., Enenstein, J., and Waleh, N. S., Integrin structure and ligand specificity in cell-matrix interactions, *in* "Molecular and Cellular Aspects of Basement Membranes" (D. H. Rohrbach and R. Timpl, Eds.), pp. 239–258. Academic Press, New York, 1993.

Laterra, J., Silbert, J. E., and Culp, L. A., Cell surface heparan sulfate mediates some adhesive responses to glycosaminoglycan-binding matrices including fibronectin. *J. Cell Biol.* **96,** 112–121 (1983).

Lausmaa, J., Kasemo, B., Matsson, H., and Odelius, H., Multi-technique surface characterizations of oxide films on electropolished and anodically oxidized titanium *App. Surf. Sci.* **45,** 189–200 (1990).

Lehman, W. B., Strongwater, A. B., Tunc, D., Kummer, F., Atar, D., Grant, A. D., Kramer, M., and Rohovsky, M. W., Internal fixation with biodegradable plate and screw in dogs. *J. Pediatric. Orth.,* Part B **3,** 190–193 (1994).

Li, P., Kanasniemi, I., and de Groot, K., Bonelike hydroxyapatite induction by a gel-derived titania on a titanium substrate. *J. Am. Ceram. Soc.* **77,** 1307–1312 (1994).

Lopes, M. A., Monteiro, F. J., Santos, J. D., Serro, A. P., and Saramago, B., Hydrophobicity, surface tension, and zeta potential measurements of glass-reinforced hydroxyapatite composites. *J. Biomed. Mater. Res.* **45** (4), 370–375 (1999).

Luck, M., Paulke, B.-R., Schroder, W., Blunk, T., and Muller, R. H., Analysis of plasma protein adsorption on polymeric nanoparticles with different surface characteristics. *J. Biomed. Mat. Res.* **39,** 478–485 (1998).

Maehara, Y., and Langdon, T. G., Superplasticity in ceramics. *J. Mater. Sci.* **25,** 2275 (1990).

Malik, M. A., Puleo, D. A., Bizios, R., and Doremus, R. H., Osteoblasts on hydroxyapatite, alumina and bone surfaces *in vitro:* Morphology during the first 2 h of attachment *Biomaterials* **13** (2), 123–128 (1992).

Martin, B. R., and Burr, D. B., "Structure Function and Adaptation of Compact Bone." Raven Press, New York, 1989.

Mathews, C. K., and van Holde, K. E., "Biochemistry." Benjamin/Cummings, Redwood City, CA, 1990.

Matsunaga, T., Inoue, H., Kojo, T., Hatano, K., Tsujisawa, T., Uchiyama, C., and Uchida, Y., Disaggregated osteoclasts increase in resorption activity in response to roughness of bone surface. *J. Biomed. Mat. Res.* **48** (4), 417–423 (1999).

Mayo, M., Siegel, R. W., Liao, Y. X., and Nix, W. D., Nanoindentation of nanocrystalline ZnO. *J. Mat. Res.* **7,** 973 (1992).

Mayo, M., Siegel, R. W., Narayanasamy, A., and Nix, W. D., Mechanical properties of TiO_2 as determined by nanoindentation. *J. Mat. Res.* **5,** 1073 (1990).

Moursi, A. M., Damsky, C. H., Lull, J., Zimmerman, D., Doty, S. B., Aota, S-I., and Globus, R. K., Fibronectin regulates calvarial osteoblast differentiation. *J. Cell Sci.* **109,** 1369–1380 (1996).

Moursi, A. M., Globus, R. K., and Damsky, C. H., Interactions between integrin receptors and fibronectin are required for calvarial osteoblast differentiation *in vitro*. *J. Cell Sci.* **110,** 2187–2196 (1997).

Muller-Mai, C. M., Stupp, S. I., Voigt, C., and Gross, U., Nanoapatite and organoapatite implants in bone: Histology and ultrastructure of the interface. *J. Biomed. Mater. Res.* **29,** 9–18 (1995).

Nakamura, H., and Ozawa, H., Immunohistochemical localization of heparan sulfate proteoglycan in rat tibiae. *J. Bone Mineral Res.* **9,** 1289–1299 (1994).

Nieh, T. G., Wadsworth, J., and Wkai, F., Recent advances in superplastic ceramics and ceramic composites. *Int. Mater. Rev.* **36,** 146 (1991).

Nieman, G. W., Processing and mechanical behavior of nanocrystalline Cu, Pd and Ag., Ph.D. Thesis, Northwestern University, 1991.

Nieman, G. W., Weertman, J. R., and Siegel, R. W., Microhardness of nanocrystalline palladium and copper produced by inert gas condensation. *Scripta Metallurgica* **23,** 2013 (1989).

Nieman, G. W., Weertman, J. R., and Siegel, R. W., Mechanical behavior of nanocrystalline metals. *J. Mat. Res.* **6,** 1012 (1991a).

Nieman, G. W., Weertman, J. R., and Siegel, R. W., Mechanical behavior of Nanocrystalline Cu and Pd, *in* "Microcomposites and Nanophase Materials" (D. C. Van Aken, Ed.), p. 15. TMS, Warrendale, 1991b.

Park, J. B., and Lakes, R. S., "Biomaterials: an Introduction Second Edition," Plenum Press, New York, 1992, pp. 79–244.

Passuti, N., Daculsi, G., Rogez, J. M., Martin, S., and Bainvel, J. V., Macroporous calcium phosphate ceramic performance in human spine fusion. *Clinical Orthop.* **248,** 169–176 (1989).

Pereira, M. M., Clark, A. E., and Hench, L. L., Calcium phosphate formation on sol–gel-derived bioactive glasses *in vitro*. *J. Biomed. Mater. Res.* **28,** 693–698 (1994).

Praemer, A., Furner, S., and Rice, S. D., "Musculoskeletal Conditions in the United States." American Academy of Orthopaedic Surgery, Park Ridge, IL, 1992.

Puleo, D. A., and Bizios, R., Mechanisms of fibronectin-mediated attachment of osteoblasts to substrates *in vitro*. *Bone and Mineral* **18,** 215–226 (1992).

Puleo, D. A., Preston, K. E., Shaffer, J. B., and Bizios, R., Examination of osteoblast-orthopaedic biomaterials interactions using molecular techniques. *Biomaterials* **14,** 111–114 (1993).

Radin, S. R., and Ducheyne, P., The effect of calcium phosphate ceramic composition and structure on *in vitro* behavior. II. Precipitation. *J. Bone and Mineral Res.* **27,** 35–45 (1993).

Ratner, B., New ideas in biomaterials science—A path to engineered biomaterials. Society for Biomaterials 1992 Presidential Address. *J. Biomed. Mat. Res.* **27,** 837–850 (1992).

Rifkin, B. R., and Gay, C. V., "Biology and Physiology of the Osteoclast." Academic Press, Boca Raton, FL, 1992.

Ripamonti, U., The morphologenisis of bone in replicas of porous hydroxapatite obtained from conversion of calcium carbonate exoskeletons of coral. *J. Bone Jt. Surg.* **73A,** 692–703 (1991).

Ripamonti, U., Osteoinduction in porous hydroxyapatite implanted in heterotopic sites of different animal models. *Biomaterials* **17,** 31–35 (1996).

Schakenraad, J. M., Cells: their surface and interactions with materials, *in* "Biomaterials Science: An Introduction to Materials in Medicine" (B. D. Ratner, A. S. Hoffman, A. S. Schoen, and J. E. Lemons, Eds.), pp. 141–146. Academic Press, New York, 1996.

Schneider, G., and Burridge, K., Formation of focal adhesions by osteoblasts adhering to different substrata. *Exp. Cell. Res.* **214** (1), 264–269 (1994).

Schwartz, M. A., Transmembrane signaling by integrins. *Trends Cell Biol.* **2,** 304–308 (1992).

Siegel, R. W., Nanophase materials, *in* "Encyclopaedia of Applied Physics," Vol. 11., 173–199, VCH Publishers, New York, 1994.

Siegel, R. W., Creating nanophase materials. *Sci. Amer.* **275,** 42–47 (1996).

Siegel, R. W., and Fougere, G. E., Mechanical properties of nanophase materials, *in* "Nanophase Materials: Synthesis-Properties-Applications" (G. C. Hadjipanayis and R. W. Siegel, Eds.), p. 233. Kulwer, Dordrecht, 1994.

Siegel, R. W., and Fougere, G. E., Mechanical properties of nanophase metals. *Nanostructured Materials* **6,** 205 (1995a).

Siegel, R. W., and Fougere, G. E., Grain size dependent mechanical properties in nanophase materials. *Material Research Society Symposium Proc.* **362,** 219 (1995b).

Sinha, R. K., and Tuan, R. S., Regulation of human osteoblast integrin expression by orthopaedic implant metals. *Bone* **18,** 451–457 (1996).

Steele, J. G., McFarland, C., Dalton, B. A., Johnson, G., Evans, M. D. M., Howlett, C. R., and Underwood, P. A., Attachment of human derived bone cells to tissue culture polystyrene and to unmodified polystyrene: The effect of surface chemistry upon initial cell attachment. *J. Biomat. Sci. Polymer Ed.* **5,** 245–257 (1993).

Stein, G. S., and Lian, J. B., Molecular mechanisms mediating proliferation/differentiation interrelationships during progressive development of the osteoblast phenotype. *Endocrine Reviews* **14,** 424–442 (1993).

Stein, G. S., Lian, J. B., and Owen, T. A., Relationship of cell growth to the regulation of tissue-specific gene expression gene expression during osteoblast differentiation. *FASEB J.* **4,** 3111–3123 (1990).

Thomas, C. H., McFarland, C. D., Jenkins, M. L., Rezania, A., Steele, J. G., and Healy, K. E., The role of vitronectin in the attachment and spatial distribution of bone derived cells on materials with patterned surface chemistry. *J. Biomed. Mater. Res.* **37,** 81–93 (1997).

Tiller, J., Berlin, P., and Klemm, D., A novel efficient enzyme-immobilization reaction on NH_2 polymers by means of L-ascorbic acid. *Biotechnol. Appl. Biochem.* **30** (2), 155–162 (1999).

Toprani, N., Catledge, S., and Vohra, Y., Interfacial adhesion and toughness of nanostructured diamond coatings. *J. Mater. Sci.* **15** (5), 1052–1055 (2000).

Toth, J. M., Lynch, K. L., and Hackbarth, D. A., Ceramic-induced osteogenesis following subcutaneous implantation of calcium phosphates. *Bioceramics* **6,** 9–13 (1993).

Trippel, S. B., Potential role of insulinlike growth factors in fracture healing. *Clinical Orthopaedics* **355S,** S301–313 (1998).

Turkova, J., Oriented immobilization of biologically active proteins as a tool for revealing protein interactions and function. *J. Chromatogr. B Biomed. Sci. Appl.* **722** (1–2), 11–31 (1999).

Underwood, P. A., and Bennett, F. A., A comparison of the biological activities of the cell-adhesive proteins vitronectin and fibronectin. *J. Cell Sci.* **93** (4), 641–649 (1989).

Vargervik, K., Critical sites for new bone formation, *in* "Bone Grafts and Bone Substitutes" (M. B. Habal and A. H. Reddi, Eds.), p. 112–120. W. B. Saunders, Philadelphia, 1992.

Webster, T. J., Siegel, R. W., and Bizios, R., An *in vitro* evaluation of nanophase alumina for orthopaedic/dental applications, *in* "Bioceramics 11: 11th International Symposium on Ceramics in Medicine" (R. Z. LeGeros and J. P. LeGeros, Eds.), p. 273–276. World Scientific, New York, 1998.

Webster, T. J., Siegel, R. W., and Bizios, R., Design and evaluation of nanophase alumina for orthopaedic/dental applications. *Nanostructured Mat.* **12,** 983–986 (1999a).

Webster, T. J., Siegel, R. W., and Bizios, R., Osteoblast adhesion on nanophase ceramics. *Biomaterials* **20,** 1221–1227 (1999b).

Webster, T. J., Ergun, C., Doremus, R. H., Siegel, R. W., and Bizios, R., Specific proteins mediate enhanced osteoblast adhesion on nanophase ceramics. *J. Biomed. Mat. Res.* **51** (3), 475–483 (2000a).

Webster, T. J., Ergun, C., Doremus, R. H., Siegel, R. W., and Bizios, R., Enhanced functions of osteoblasts on nanophase ceramics. *Biomaterials* **21,** 1803–1810 (2000b).

Webster, T. J., Schadler, L. S., Siegel, R. W., and Bizios, R., Mechanisms of enhanced osteoblast adhesion on nanophase alumina involve vitronectin. *Tissue Engineering* **7** (3), 291–302 (2001a).

Webster, T. J., Ergun, C., Doremus, R. H., Siegel, R. W., and Bizios, R., Enhanced functions of osteoclast-like cells on nanophase ceramics. *Biomaterials* **22** (11), 1327–1333 (2001b).

Weertman, J. R., Farkas, D., Hemker, K., Kung, H., Mayo, M., Mitra, R., and van Swygenhoven, H., Structure and mechanical behavior of bulk nanocrystalline materials. *MRS Bulletin* **24** (2)**,** 44–50 (1999).

Wen, X., Wang, X., and Zhang, N., Microrough surface of metallic biomaterials: a literature review. *Biomed. Mat. Eng.* **6** (3), 173–189 (1996).

Weng, J., Liu, Q., Wolke, J. G. C., Zhang, X., and de Groot, K., Formation and characteristics of the apatite layer on plama-sprayed hydroxyapatite coatings in simulated body fluid. *Biomaterials* **18** (15), 1027–1035 (1997).

Yamada, H., *in* "Strength of Biological Materials" (F. G. Evans, Trans.). Williams and Wilkins, Baltimore, MD, 1970.

Yamasaki, H., and Saki, H., Osteogenic response to porous hydroxyapatite ceramics under the skin of dogs. *Biomaterials* **13,** 308–312 (1992).

Yang, Z., Yuan, H., Tong, W., Zou, P., Chen, W., and Zhang, X., Osteogenesis in extraskeletally implanted porous calcium phosphate ceramics: Variability among different kinds of animals. *Biomaterials* **17,** 2131–2137 (1996).

Yang, Z., Yuan, H., Zou, P., Tong, W., Qu, S., and Zhang, X., Osteogenic responses to extraskeletally implanted synthetic calcium phosphate ceramics an early stage histomorphological study in dogs. *J. Mater. Sci. Med.* **8,** 697–701 (1997).

Yuan, H., Yang, Z., Zou, P., Li, Y., and Zhang, X., Rapid osteogenesis in porous biphasic calcium phosphate ceramics implanted in domestic pigs. *Biomed. Eng. Appl. Bas. Com.* **9,** 268–273 (1997a).

Yuan, H., Li, Y., Yang, Z., Feng, J., and Zhang, X., An investigation on the osteoinduction of synthetic porous phase-pure hydroxyapatite ceramic. *Biomed. Eng. Appl. Bas. Com.* **9,** 274–278 (1997b).

Yuan, H., Li, Y., Yang, Z., Feng, J., and Zhang, X., Calcium phosphate ceramic induced osteogenesis in rabbits, *in* "Biomedical Materials Research in the Far East (III)," (X. Zhang and Y. Ikada, Eds.), pp. 228–229. Kobunshi Kankokai, Kyoto, Japan, 1997c.

Yuan, H., Li, Y., Yang, Z., and Zhang, X., Osteoinduction of pure β-TCP ceramic in dogs, *in* "Biomedical Materials Research in the Far East (III)," (X. Zhang and Y. Ikada, Eds.), pp. 188–189. Kobunshi Kankokai, Kyoto, Japan, 1997d.

Yuan, H., Li, Y., Kurashina, K., and Zhang, X., "Host tissue response of calcium phosphate cement," *in* Biomedical Materials Research in the Far East (III). (X. Zhang and Y. Ikada, Eds.), Kobunshi Kankokai, Kyoto, Japan, 1997e, p. 116–117.

Yuan, H., Kurashina, K., de Bruijn, J., Li, Y., de Groot, K., and Zhang, X., A preliminary study on osteoinduction of two kinds of calcium phosphate ceramics. *Biomaterials* **20,** 1799–1806 (1999).

Yubao, L., Klein, C. P. A. T., Xingdong, Z., and de Groot, K., Formation of bone apatite-like layer on the surface of porous hydroxyapatite ceramics. *Biomaterials* **15,** 835–841 (1994).