DOI: 10.1002/adem.201080140

Nanobiomaterials: State of the Art and Future Trends

By *Lei Yang, Lijuan Zhang* and *Thomas J. Webster**

In the past decade, it is clear that the development of biomaterials has entered the ''nanotechnology era.'' The interface between biomaterials and nanotechnology has created enormous opportunities to improve the prevention, diagnosis, and treatment of numerous diseases. Nanobiomaterials, a new term describing biomaterials with constituent or surface feature sizes less than 100 nm (10^{-7} m), provide not only extraordinary materials with unique structures and properties to solve our most traditional biomedical puzzles, but also provide unprecedented knowledge and principles toward understanding biology, medicine, and materials science. At the commencement of the second decade of the new millennium, it is worthwhile to review the current state of the art for the use of nanobiomaterials in medicine as well as possible future trends. Therefore, this paper seeks to summarize the current advances in nanobiomaterials research, spanning a wide range of tissue engineering applications (both soft and hard tissues), drug delivery, disease detection, and disease treatment. In addition, emerging concerns on the safety of manufacturing and using nanobiomaterials (especially toxicological issues) with necessary future research directions from the design of intelligent nanobiomaterials to molecular mechanisms of cell–nanomaterial interactions are also discussed.

1. Introduction

Over the past decade, it was clear that the development of biomaterials has entered the ''nanotechnology era.'' Nanotechnology-derived biomaterials have become some of the fastest emerging and developing arenas at the intersection of materials science and biology, and have resulted in biomaterials with an extraordinary impact on medicine. Specifically, nanotechnology-derived biomaterials refer to those biomaterials whose structures or components exhibit novel and significantly changed properties when their dimensions are at the nanoscale (i.e., 1–100 nm or 10^{-9}–10^{-7} m particle or grain size diameters). Not surprisingly, nanotechnology-derived biomaterials have been widely used in a broad spectrum of biological and biomedical applications, from artificial implants to drug delivery to medical imaging. These nanobiomaterials include (but are not limited to) metals, ceramics, polymers, hydrogels, and novel self-assembled materials with structures from 0-D (e.g., dots and particles) to 3-D (e.g., tissue engineering scaffolds).

Undoubtedly, the rapid development of nanobiomaterials has been creating a new multidisciplinary area across biology, materials science, and nanotechnology. The reason behind the establishment of this new area is that nanotechnology has not only provided novel materials and tools for biological and medical purposes, but it is also reshaping our thinking toward applying these materials in science and technology. For example, the increasing use of materials and systems at the nanoscale has stimulated studies on nanomaterial safety and toxicity for both occupational and research levels. Today, studies on nanostructured biomaterials have evolved into more comprehensive and systematic studies, resulting in the further understanding of mechanisms behind biological

[*] *Dr. T. J. Webster, L. Yang, L. Zhang*
Institute for Molecular and Nanoscale Innovation (IMNI), Brown University Providence, RI 02912, (USA)
E-mail: thomas_webster@brown.edu, yleibrown@gmail.com, lijuan_zhang@brown.edu
Dr. T. J.Webster, L.Yang
School of Engineering, Brown University Providence, RI 02912, (USA)
L.Zhang
Department of Chemistry, Brown University Providence, RI 02912, (USA)
Dr. T. J.Webster
Department of Orthopaedics, Brown University Providence, RI 02912, (USA)

responses to materials and the consequent better design of such materials. It may be too early to assert that nanostructured biomaterials will be widely used in numerous clinical arenas since the final efficacy of nanobiomaterials in human applications is not currently known, thus, the full benefit of nanostructured biomaterials cannot be accurately assessed at this time.

Along these lines, it is clearly worthwhile to review the current state of the art concerning the use of nanobiomaterials in medicine, which have just experienced another accelerated period of development since the start of the new millennium. This paper reviews the recent advances in nanobiomaterials and the new opportunities nanobiomaterials have created, seeking to provide an updated review for this fast developing area. This paper is organized by first discussing the rationale and application of nanobiomaterials in medicine, covering nanobiomaterials used as implants and in regenerative medicine, drug delivery and medical diagnosis. In addition, a brief overview of properties of nanomaterials attractive for medical applications is presented and a section concerning new safety issues related to nanobiomaterial use and manufacturing is also included.

2. An Overview of Nanomaterial Properties and Biological Responses to Nanomaterials

Nanoscale materials are defined as materials with building block size scales (e.g., grains, particles, fibers, tubes, etc.) within 1–100 nm in at least one dimension.[1] Nanomaterials possess numerous unique properties compared to bulk conventional materials (e.g., materials with microstructured features in the micron or larger sizes): (i) much larger surface areas and resulting increased surface reactivity; (ii) greatly enhanced mechanical properties (such as high ductility and high yield strength) due to various mechanisms depending on their chemistry (such as increased grain boundary sliding and short-range diffusion-healing); (iii) exceptional magnetic, optical, and electrical properties due to stacking, alignment, and orientation of nanoscale building blocks (grains, supermolecules, etc.); and (iv) homogeneity and high purity in composition and structure because of reaction or mixing at the molecular and atomic levels.

Specifically, nanoscale materials and structures provide a few other important properties to a biomaterial. First is their chemical and structural similarity to natural tissues or biological systems which have nanoscale hierarchical components. One example is nanophase collagen/calcium phosphates mimicking the nanostructure of bone. Second is the comparable size of nanoscale materials to biomolecules and bio-microstructures, which enables researchers to detect, manipulate and mediate these bio-components. Lastly, nanostructured materials can be readily tailored to reveal extraordinary variations in surface properties.

Compared to the understanding of nanomaterial properties and the ability to control these properties, what occurs at the interface between nanomaterials and biological systems (e.g., cells and tissues) has not been completely understood to date. A general consensus is that there are a sequence of events which occurs at the interface between biomaterials and a cell[2,3]: (i) adsorption of proteins from blood and tissue fluids onto the nanomaterial surface (usually protein desorption also occurs at the same time); (ii) tissue cells and/or inflammatory cells approach the material; (iii) possible targeted release of matrix proteins from the biomaterial and selected adsorption of specific proteins; and (iv) adhesion of cells and commencement of subsequent cell functions (e.g., proliferation, differentiation, phagocytosis, etc.). Besides these host responses toward the nanomaterials, conversely, material responses to the host (like material decomposition and release) also exist at the cell–biomaterial interface.[3] All of these interfacial events are crucial for the success of nano biomaterials, because they are closely related to material cytocompatibility properties and immune or inflammatory host responses that ultimately determine the efficacy and safety of nanobiomaterials.

Although understanding the interfacial interactions between nanomaterials and biological systems is still under progress, mediating cellular or tissue responses toward nanomaterials is not a complete mystery any more. Generally, cells and tissues recognize (subsequent to initial protein interactions) both surface and bulk properties of nanostructured materials *in vivo* and *in vitro*, so cell and tissue responses can be altered or controlled to some extent by manipulating these material properties.[1,4,5] Surface properties often refer to surface chemistry and charge, material topography, and surface energetics, while bulk properties closely relate to cell or tissue responses including inherent chemistry, stiffness, porosity, and so on.[1,4–10] Figure 1 illustrates the nanomaterial–cell interface and the interactions between cells, proteins, and material properties. Nanomaterials have successfully demonstrated the ability to modulate cell and tissue responses *in vitro* and *in vivo* due to their extraordinary properties discussed above, and the rest of this article will review the latest progress on this topic.

3. Nanobiomaterials for Tissue Engineering

3.1. Soft Tissue Engineering Applications

A great number of nanobiomaterials have been used for soft tissue engineering purposes, which are summarized in Table 1. In this section, specific applications using nanobiomaterials are reviewed.

3.1.1. Cardiovascular Applications.

Natural vascular tissue is hierarchically layered with numerous nanoscale features in its extracellular matrix (ECM), which is composed of nano dimensional collagen and elastin. This lesson from nature guides material scientists (and nanotechnologists) to design better biomaterials for treating cardiovascular disease, and significant progress to date in using nanomaterials to mimicking these actual

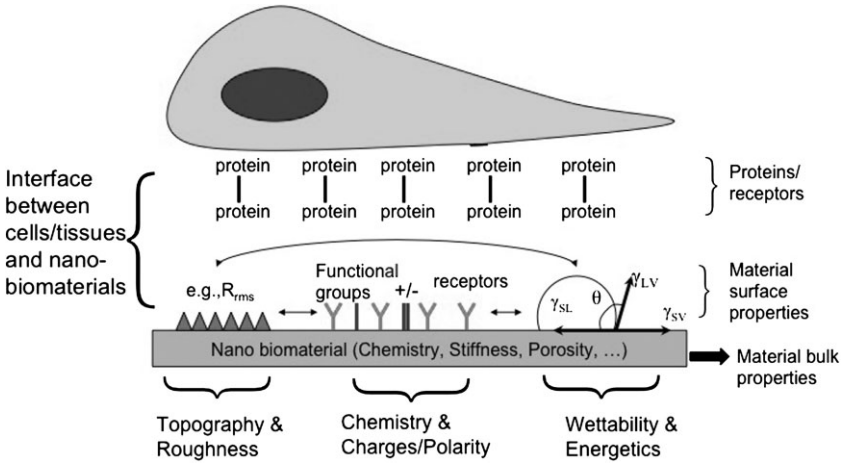

Fig. 1. Schematic illustrating the potential interfacial interactions between a cell (or tissue) and nanobiomaterials. Double arrows indicate interactions among surface properties of nanobiomaterials. In the schematic, examples of nanomaterial surface properties include: root mean square roughness (R_{rms}), surface electrical charges (+/−), contact angle (θ), interfacial tension between solid and vapor phases (γ_{SV}), interfacial tension between solid and liquid phases (γ_{SL}), and interfacial tension between liquid and vapor phases (γ_{LV}).

nanostructures in vascular tissues has occurred (Fig. 2). Specifically, nanomaterials have been designed, fabricated, and modified to improve and mediate vascular cell (e.g., vascular endothelial and smooth muscle cells) functions and also to inhibit inflammation and thrombosis on stents.[11]

A number of recent studies indicate that nanostructured surfaces could increase vascular cell functions (including the adhesion and total collagen and elastin synthesis by endothelial cells) on titanium,[12,13] CoCrMo,[13] NiTi,[14] poly(lactic-*co*-glycolic) acid (PLGA),[15] and poly(dimethylsiloxane) (PDMS).[16] More interestingly, in several recent studies, greater competitive endothelial cell functions compared to smooth muscle cell functions were observed on nanostructured titanium surfaces, indicating enhanced endothelial cell functions over that of smooth muscle cells to perhaps limit vascular restenosis.[12] Another *in vivo* study implanted nano-cylindrical poly(ε-caprolactone) (PCL) with surface features 160 nm in height and 100 nm in diameter and a natural polyester with nanopits 100 nm in depth and 120 nm in diameter into rats and observed higher vascular cell densities (number of microvessels in a mm^2 unit area) and decreased inflammation near the nanocylindrical PCL.[17]

Despite such promises, thrombotic responses to nanobiomaterials have not been clearly elucidated to date. However, Ferraz *et al*. investigated platelet responses to nanoporous alumina membranes with pore diameters of 20 and 200 nm and revealed that the 20 nm membrane could increase platelet growth, and spreading and P-selectin (a cell adhesion molecule on the surfaces of activated endothelial cells) expression, while the 200 nm membrane showed the least platelet responses.[18]

In addition to nanofeatured surfaces mostly used for vascular stent applications, numerous nanostructured grafts or 3-D nanoscaffolds for cardiovascular tissue engineering applications have been developed by techniques such as electrospinning and self-assembly.[19,20] A main advantage of these fabrication techniques is that highly ordered nanoarchitectures can be readily manufactured to mimic the oriented multilayer structure of blood vessels. Punshon *et al*. developed a novel nanocomposite vascular graft by directly attaching polyhedral oligomeric silsesquioxane onto urethane segments, and *in vitro* tests revealed that confluent endothelial cell layers formed on the nanocomposites after 14 days and remained viable and confluent for up to 35 days.[21] Lee *et al*. prepared nanofibrous scaffolds by electrospinning collagen, elastin, and poly(L-lactic acid) (PLLA), and showed extensive smooth muscle cell infiltration into the nanoscaffolds.[22] Poly(L-lactic-*co*-ε-caprolactone) (P(LLA-CL)) copolymer scaffolds with aligned nanofibers produced by electrospinning significantly improved the adhesion and proliferation of smooth muscle cells compared to plane polymer films.[23] This study also observed oriented attachment and migration of smooth muscle cells along the axis of the aligned nanofibers, indicating a possibility of engineering vascular cells into ordered patterns as found in native vessels. Nanoscaffolds of self-assembled peptides (AcN–RADARADARADARADA–$CONH_2$) developed by direct solid phase synthesis enhanced not only the formation of confluent endothelial cell monolayers but also nitric oxide (a key substance in the vasorelaxation process) and laminin 1 (a main component of the basement membrane) release and collagen IV deposition.[24] *In vivo* tests also demonstrated promising properties of nanoscaffolds as just described *in vitro*. Tacrolimuseluting biodegradable nanofibers (TEBN) implanted in a rat model also showed enhanced endothelialization and reduced intimal hyperplasia, suggesting the prevention of venous anastomosis on the nanostructures.[25]

In summary, current studies on nanobiomaterials for cardiovascular tissue engineering applications focus on creating bio-inspired nanoscale roughness or architectures on numerous metals, ceramics, and polymers. Thus, adjustable vascular cell functions *in vitro* and *in vivo* have been achieved by using nanobiomaterials, indicating enormous promises to promote the efficacy of cardiovascular implants or scaffolds for tissue regeneration without changing base material chemistry.

3.1.2. Neural Tissue Engineering.

Repairing damaged nerves and recovering the full function of the nervous system are probably the most challenging tasks in neural tissue engineering. Nanobiomaterials may also provide possibilities to heal damaged nerves faster through their exceptional cytocompatibility and electrical properties.[11] For example, nanosized ZnO has been observed to

Table 1. Nanobiomaterials for soft tissue engineering.

Material category	Nanobiomaterials	Structural feature	Applications
Self-assembly structures	Peptide-amphiphiles (PA)	Branched-PA self-assembling coatings	Promoting initial adhesion of primary human bladder smooth muscle cells[209]
		Self-assembling hydrogel scaffolds	Fosters chondrocyte extracellular matrix production and cell division[210]
		3-D network of nanofibers	Enhancing MSC attachment, proliferation, and osteogenic differentiation[211]
		Nanofiber scaffolds	Enhancing the formation of confluent cell monolayers of human aortic endothelial cells (HAEC)[24]
	Polyelectrolyte multilayer assemblies	Nanoscale porous multilayers [poly(allylamine hydrochloride) (PAH), poly(acrylic acid) (PAA)]	Significantly promoting corneal epithelial cell proliferation and migration speeds[212]
Polymers	Polystyrene	Electrospun scaffolds	Significantly increasing smooth muscle cell attachment[44]
	Poly(lactic-*co*-glycolic acid) (PLGA)	Nanostructured film	Enhancing endothelial, smooth muscle cell, and bladder smooth muscle cell attachment[41,213]
		Nanostructured PLGA scaffolds	Enhancing cell adhesion and growth, promoting elastin and collagen production[214]
		NaOH-treated PLGA scaffolds	Increasing chondrocyte attachment, total intracellular protein, and extracellular matrix synthesis[34]
		PLGA/nano-hydroxyapatite hybrid scaffolds	Increasing MSC attachment, viability, and proliferation[215]
		Tacrolimus-eluting nanoscale fiber	Reducing intimal hyperplasia and preserving endothelialization[25]
	Poly(ether urethane) (PU)	Nanostructured films	Increasing bladder smooth muscle cell attachment[41]
	Poly(ε-caprolactone) (PCL)	Electrospun nanofibrous scaffold	Carrier for MSC transplantation[216]
		Honeycomb-patterned film	Enhancing cell survival and yield of rat small hepatocytes[217]
	Poly(dimethylsiloxane) (PDMS)	Nanorough film	Increasing endothelial cell adhesion and elongation[16]
	Poly(L-lactic acid) (PLLA)	Nanofibrous scaffolds coupled with laminin	Enhancing axonal extension of PC12 cells[30]
	Chitosan	Nano-/microfibrous 3-D scaffolds	Facilitating chondrocyte attachment and proliferation[36]
Ceramics	Alumina	Nanoporous membranes	Decreasing platelet adhesion[18]
Metals	Titanium	Nanotubular anodized titanium	Enhancing chondrocyte adhesion[218]
	Gold	Thin films with nanoscale roughness	Increasing embryonic stem-cell-derived neural precursors adhesion and differentiation[27]
Semiconductors	Silicon	Nano-island silicon	Increasing insulinoma cell adhesion and insulin secretion[215]
		Silica nanoparticle-modified surfaces	Enhancing osteogenic differentiation of human mesenchymal progenitor cells[219]
Carbon nanostructures	Carbon nanofiber (CNF)	Polycarbonate urethane/CNF composite	Decreasing adhesion of astrocytes,[29] increasing neural functions[58]
	Carbon nanotube (CNT)	Functionalized multiwalled CNT	Enhancing embryonic rat-brain neuron responses[28]
	Diamond	Ultra-nanocrystalline diamond films	Induction and regulation of differentiation of neural stem cells[32]

increase neuron excitability by a possible mechanism of activating voltage-gated Na^+ channels in neurons.[26] Another study revealed that embryonic stem cell-derived neural precursors adhered the best and differentiated the fastest on nanorough gold thin films (root mean square surface roughness of 21 nm) compared to planar gold surfaces, and axonal outgrowth of embryonic stem cells could be directed by a combination of micron scale grooves and nanoscale surface features.[27] Carbon nanotubes (CNTs) or carbon nanofibers (CNF) are also strong candidates for repairing nerves due to their excellent electrical conductivity and mechanical properties. Mattson *et al*. first reported that embryonic rat-brain neurons can grow on multiwalled CNT, providing the necessary cytocompatibility data for

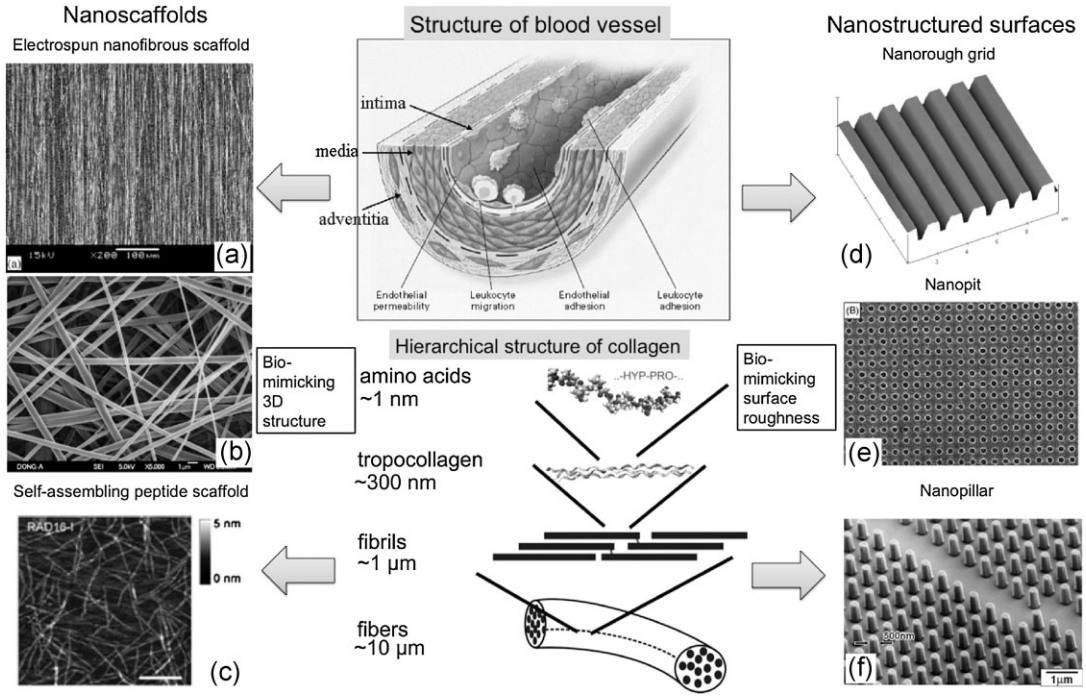

Fig. 2. Illustration of nanobiomaterials for cardiovascular tissue engineering. The structure of blood vessels and the hierarchical structure of nanoscale collagen are demonstrated. Examples of nanobiomaterials: (a) PLLA aligned nanofibers, (b) PLGA nanofiber scaffold, (c) self-assembly of peptides RAD16-I, (d) titanium grids with nanorough surfaces, (e) silicon nanopits, and (f) polystyrene (PS) nanopillar surfaces (images adapted and redraw from ref.[200–207]).

the use of CNT in neural tissue engineering applications.[28] Further studies of neurons on CNT modified with 4-hydroxynonenal showed that neurons exhibited multiple neurites with extensive branching,[28] More importantly, nanostructured carbon materials revealed an inhibitory effect on astrocyte (characteristic star-shaped glial cells in the brain and spinal cord) functions, leading to a possible decrease in glial scar tissue formation. Studies on CNF/polycarbonate urethane (PCU) composites showed that the presence of CNF can decrease astrocyte adhesion and proliferation.[29]

In addition, nanobiomaterials have been studied or designed as biomimetic scaffolds to support neural stem cells or Schwann cells for nerve repair. For example, rat adrenal phenochromocytoma cell (PC12 cell) viability and neurite axonal extensions on laminin/PLLA nanofibrous scaffolds were enhanced compared to unfunctionalized scaffolds.[30] Neuronal cell attachment and differentiation as well as extensive neurite outgrowth were observed on nanoscaffolds self-assembled from peptides (AcN–RADARADARADARADA–CNH_2 and AcN–RARADADA-RARADADA–CNH_2).[31] Primary rat neurons also formed active synapses on such scaffold surfaces in situ. A recent study showed that hydrogen-terminated ultrananocrystalline diamond (UNCD) could spontaneously induce neuronal differentiation on neural stem cells, suggesting the potential of UNCD as a biomaterial for central nervous system tissue engineering.[32]

Undoubtedly, tissue regeneration for the nervous system (especially the central nervous system) is extremely challenging. However, there is also no doubt that the emergence of nanobiomaterials has paved the way for possible solutions to repair damaged nervous system tissue. Although current studies are still preliminary, they have demonstrated the use of various nanoscale materials with extraordinary electrical properties (e.g., CNT and piezoelectric ZnO) as potential breakthroughs in neural tissue engineering.

3.1.3. Cartilage Tissue Engineering.

Cartilage tissue is composed of a small percentage of chondrocytes (cartilage synthesizing cells) and a dense nanostructured ECM rich in collagen fibers, proteoglycans and elastin fibers.[11] Recovery or regeneration of cartilage is extremely difficult due to limited chondrocyte mobility and an absence of progenitor cells in the dense ECM as well as a lack of an efficient vascular network structure for cartilage growth.[33]

For the reasons stated before, nanobiomaterials can create a biomimetic micro- and nano-environment (or interface) for improving chondrocyte functions and differentiation from progenitor cells. For example, greater chondrocyte functions (adhesion, proliferation, and/or ECM synthesis) were observed on NaOH-treated nanostructured PLGA scaffolds and nanorough anodized titanium compared to conventional untreated materials, indicating the possible promotion of cartilage growth.[34] Therefore, nanostructured self-assembled scaffolds were devised to encapsulate chondrocytes and/or progenitor cells for reconstructing cartilage tissue. For instance, hydrogel nanoscaffolds fabricated from self-assembling peptides KLD-12 (sequence AcN–KLDLKLDLK-LDL–CNH_2) were encapsulated with chondrocytes, and

4-week *in vitro* tests showed promoted chondrocyte differentiation and improved synthesis of a true cartilage-like ECM rich in proteoglycans and type II collagen.[35] Similarly, Shim *et al*.[36] fabricated nano-/microfibrous 3-D scaffolds by electrospinning chitosan nanofibers onto a predefined microfibrous mesh, and *in vitro* culture tests showed that this nano-/microfibrous 3-D matrix could promote chondrocyte proliferation and glycosaminoglycan synthesis compared to a microfibrous 3-D matrix. Another recent study fabricated a nanofibrous PCL scaffold by electrospinning, and mesenchymal stem cells (MSCs) cultured in the nanoscaffolds showed a chondrocytic phenotype differentiation comparable to that observed for MSC maintained as cell aggregates or pellets.[37] Recently, a novel 3-D PLGA/nano-HA scaffold infiltrated with MSC was implanted into osteochondral defects in rat knees, and the results showed that the defects were filled with smooth and hyaline-like cartilage abundant in glycosaminoglycan and collagen deposition.[38]

Nanomaterials can improve the mechanical properties of cartilage tissue engineering scaffolds, which is also an important factor affecting the lifetime and performance of the scaffolds. For example, hydroxyapatite (HA) nanoparticles were incorporated into a poly(vinyl alcohol) (PVA) gel by an in situ precipitation method, and the resultant nano-HA/PVA gel composites showed similar mechanical properties as that of natural articular cartilage.[39]

The study of cartilage regeneration using nanobiomaterials is still in its early stage. Constructing hierarchical structures through the use of nanobiomaterials to resemble the complicated assembly of human cartilage as well as mediating chondrocyte and progenitor viability and functions still remain key questions, which should be the focus of future studies. In addition, a recent study implied a promising strategy of cartilage regeneration which may be of great interest to nanobiomaterial researchers.

Hori *et al*. investigated the chondrogenic potential of magnetically labeled synovium-derived cells (M-SDCs) and demonstrated the regeneration of the articular cartilage after delivering the M-SDCs to the lesion and treating with an intra-articular magnet.[40] Obviously, magnetic nanoparticles are possibly better candidates for magnetic labeling and, thus, are worth further study.

3.1.4. Bladder Tissue Engineering.

Bladder tissue regeneration after cystectomy is a newly emerging area in soft tissue engineering, but nanomaterials have already exhibited exceptional promise as a bladder tissue replacement due to their biologically inspired roughness and increased surface energy or selectivity toward protein adsorption.[11] For example, Thapa *et al*. found that PLGA and polyurethane (PU) films with nanometer surface features enhanced bladder smooth muscle cell (BSMC) functions compared to conventional nanosmooth surfaces, and they attributed the enhancement to the increased nanometer surface roughness that mimics the nanometer topography of native bladder tissue.[41] Pattison *et al*.[42] used similar polymers (PLGA and PU) to construct 3-D nanostructured scaffolds using a solvent casting and salt leaching process. *In vitro* results indicated that these scaffolds enhanced human BSMC adhesion, proliferation, and the production of ECM proteins (specifically, elastin, and collagen).[42] A preliminary *in vivo* study provided evidence of little to no calcium stone formation in augmented nanostructured PLGA and PU rat bladders, indicating a promising solution to the common problem of calcium stone formation on currently used bladder replacement materials (whether synthetic or natural).[42] In addition to PLGA and PU, Harrington *et al*.[43] developed fiber-bonded poly(glycolic acid) (PGA) scaffolds coated with self-assembled branched peptide-amphiphiles (PA) and *in vitro* tests revealed greater initial adhesion of primary human BSMC on PA-functionalized scaffolds than uncoated scaffolds. Of course, electrospun polymeric nanofibers can also mimic the oriented collagen nanostructures in the bladder ECM. A 3-D electrospun polystyrene scaffold with aligned nanofibers (200 nm in diameter to resemble collagen fiber alignment in bladder tissue) guided the organization of BSMC actin filaments in a way similar to the native tissue.[44]

In addition, recent studies have also demonstrated that PU:CNF composites (weight ratios from 1:4 to 4:1) promoted healthy human bladder urothelial cell growth while inhibiting urothelial carcinoma cell and ScaBER cell (a human bladder cancer cell line) viability and secretion of growth factors (unpublished work). These studies, together with the examples above, highlight the great potential nanobiomaterials can offer in this new area of repairing bladder tissue while fighting bladder cancer.

3.2. Hard Tissue Engineering Applications

Representative nanobiomaterials for hard tissue engineering applications are listed in Table 2. Specific applications of some of these nanobiomaterials are highlighted below.

3.2.1. Orthopedic Applications.

3.2.1.1. Joint Prosthesis: Orthopedic implants (including hip, knee, shoulder, and elbow implants) have been widely used to treat injured or diseased bones for more than half of a century, but finding better implant materials is always challenging. First, due to the hard tissues they will replace, the implant materials need to possess extraordinary mechanical properties (such as high strength, proper toughness, and high wear-resistance), not usually required in the aforementioned soft tissue implant applications. Second, the implant materials need to be biocompatible and, moreover, osteoconductive in order to form robust biological bonding with host bone tissues. Weak osseointegration on the implant can result in implant failure. Third, the implant materials need to survive over a very long time (e.g., from years to tens of years) under severe conditions like motion, impact, and corrosion after implantation. Before the emergence of nanostructured biomaterials, few materials could meet all the requirements and the current available implant materials

Table 2. Nanobiomaterials for hard tissue engineering.

Material category	Chemical or structural feature	Applications
Metals and alloys	Nanophase Ti and Ti6Al4V	Increasing osteoblast adhesion,[48] calcium and phosphorus deposition[52]
		Decreasing fibroblast and increased osteoblast functions[220]
	Nanophase CoCrMo	Increasing osteoblast adhesion,[48] calcium and phosphorus deposition[52]
	Nanostructured selenium	Increasing osteoblast adhesion[49]
	Anodized nanotubular Ti	Enhancing osteoblast adhesion, proliferation, functions[64,65,67,68]
	Nanocrystalline silver	Antibacterial materials for dental implants[111]
	Colloidal platinum nanoparticles (CPNs)	Dental adhesive material[113]
Hydroxyapatite (HA)	Nanophase HA	Increasing osteoblast functions[54]
	HA/collagen nanocomposite	Conducting osteoblasts to form new bone[100]
	HA/chitosan nanofibers	Increasing bone formation[101]
	Adhesive containing HA nanorods	Increasing the bulk mechanical properties of the adhesive and its micro-shear bond strength to dentin[114]
Other ceramics	Zirconia	Enhancing osseointegration[109]
	Alumina (Al_2O_3)	Increasing osteoblast adhesion, proliferation, synthesis of alkaline phosphatase (ALP) and deposition of calcium-containing mineral[53,54]
	Titania (TiO_2)	Increasing osteoblast adhesion, proliferation, synthesis of alkaline phosphatase (ALP) and deposition of calcium-containing mineral[53,54,63]
Polymers	Poly(lactic-*co*-glycolic) acid (PLGA)/nanophase Ti composites	Increasing osteoblast adhesion, synthesis of alkaline phosphatase (ALP), and deposition of calcium-containing mineral[55,98]
	Nanostructured PLGA-coated nanostructured Ti	Increasing osteoblast cell density[57]
	Carbon nanofiber (CNF)/polyurethane (PU) composite	Increasing osteoblast functions[58]
	NaOH-treated PU	Decreasing fibroblast cell density[97]
	NaOH-treated poly(ε-caprolactone) (PCL)	Decreasing fibroblast cell density[97]
	Poly(methyl methacrylate) (PMMA)-grafted nanoclay	A dentin bonding system with higher shear bond strength[116]
	Nano/microfibrous chitosan 3-D scaffolds	Facilitating chondrocyte attachment and proliferation[36]
Self-assembled structures	Helical rosette nanotubes (HRNs) hydrogel scaffolds	Enhancing osteoblast functions[62,105]
	3-D network of peptide-amphiphile (PA) nanofibers	Increasing MSC attachment, proliferation, and osteogenic differentiation[104]
Carbon nanostructures	Nanocrystallinity diamond (NCD)	Enhancing osteoblast functions[61]
	Carbon nanotube (CNT) scaffolds	Retaining electrical properties necessary for secretory activities[83]
	Chemically modified CNT scaffold	Increasing bone cell proliferation[84]
	Poly(lactic acid) (PLLA/CNT composite	Increasing osteoblast proliferation, extracellular calcium deposition, and upregulating mRNA expression for collagen type-I[88]
	Multiwall CNT/ultrahigh molecular weight polyethylene (UHMWPE) composite	Increasing cytocompatibility of osteoblast-like cells[95]
	Vertically aligned multi-walled CNT	Enhancing adhesion and proliferation of osteoblast-like cells[90]
	CNF/ polycarbonate urethane (PCU) composite	Promoting selective adhesion and alignment of osteoblasts[91]
	CNF/PLGA composite	Enhancing select osteoblast adhesion[221]

(e.g., zirconia, Ti and its alloys, CoCrMo, and several types of stainless steel) constantly experience problems of insufficient osseointegration over the long term.[45] The development of nanobiomaterials has brought countless opportunities and approaches to tackle these difficulties faced by today's implant materials, offering a tremendous amount of success for nanostructured biomaterials in a variety of bone implant applications.

Creating nanorough (i.e., nanoscale) surface features using various surface modification techniques has emerged as an effective approach to promote osseointegration of orthopedic implants. Numerous studies have shown that nanometer surface roughness improves initial protein adsorption (such as vitronectin and fibronectin), subsequent osteoblast attachment, and eventual osseointegration over micron rough surfaces.[46,47] Promoted osteoblast responses (including orientation and shape, adhesion, proliferation, differentiation or mineral deposition, etc.) on nanorough surfaces compared to conventional surfaces of pure metals,[48–50] alloys,[48,50–52] ceramics,[50,53,54] polymers,[50,55–57] carbon materials (diamond and CNTs, etc.),[58–61] and hydrogels,[62] highlight how this trend is independent of material chemistry.

Although the underlying mechanisms of promoted biological responses on nanoscale compared to micron-scale roughness is not completely known to date, it has been speculated that the increased surface area and surface grain boundaries, higher surface energy and hydrophilicity, and structural or dimensional similarity to natural tissues play crucial roles in understanding nanomaterial superiority.[48,53,63]

Guided by this important information, numerous surface modification methods have been developed to produce nanobiomaterials with desired surface properties. For example, a direct chemical modification method known as anodization can create uniform nanotubular titania (TiO_2) structures with diameters less than 100 nm by directly anodizing Ti orthopedic implants in an electrochemical cell which uses Ti as an anode.[64,65] Theoretically, anodization can be applied to any metal that is stable to oxidation to fabricate nanoscale tubular or porous surfaces. Importantly, anodized Ti (more strictly, titania) with nanoscale porous or tubular surfaces has demonstrated enhanced bone cell adhesion, proliferation, and differentiation (including mineral deposition) compared to unmodified conventional Ti.[65–68] Nanorough or nanoporous surfaces created by chemical etching can also enhance the adhesion, proliferation, mineral precipitation, and gene expression of osteogenic cells.[69] Different chemical etching methods with acids,[70–72] bases,[73] and oxidants[74,75] have also been used to fabricate nanometer or submicron scale nanotopographies, rendering the control of surface roughness, wettability, and eventual bone cell responses possible.[69] For example, nanometer pits with 20 nm diameters in the titania layer on Ti or its alloys can be reproducibly fabricated by sulfuric acid-hydrogen peroxide etching,[76,77] and in addition, topography, roughness, wettability of the titania nanostructures, and density of hydroxyl groups on the surfaces can be controlled by altering etching time or the electrolyte solution.[76,78]. More importantly, *in vitro* studies showed that these nanopit surfaces can promote the activity and bone-related gene expression of osteoblasts while inhibiting that of fibroblasts (connective or granulation tissue forming cells).[69,79]

Besides direct chemical modification, other fabrication approaches such as physical vapor deposition (PVD) and chemical vapor deposition (CVD) are commonly applied to yield nanostructured implant coatings which ultimately improve not only osseointegration but also wear resistance at the bone–implant interface. For example, nanostructured diamond-like carbon (DLC) films that can be coated on metallic implant surfaces have lower frication coefficients and better bone cell attachment compared to conventional DLC films.[80] Recently, nanocrystalline diamond (NCD) coatings (CVD-deposited polycrystalline diamond films) with grain sizes in the nanometer scale, demonstrated potential as orthopedic implant coatings to extend implant service lifetime.[59] NCD coatings have low friction coefficients, ultra-low wear rates and exhibit many other superior properties [such as high chemical resistance, high fracture toughness, and high bonding strength to various implant materials (such as Ti alloys and stainless steels)] attractive for orthopedic implant applications.[81,82] More importantly, the feasibility of controlling osteoblast functions (i.e., adhesion, proliferation, and differentiation) by adjusting surface properties of NCD provides for the design of improved orthopedic implants to promote biological interactions.[59,61] In this case, NCD with spherical grains less than 100 nm significantly enhanced osteoblast functions (adhesion, proliferation, and differentiation) while NCD with greater grain sizes (200–1000 nm) prohibited osteoblast activities. Together with the excellent anti-wear properties of both NCD coatings, these *in vitro* results enable a better orthopedic implant coating by meeting device requirements in different regions, some of which demand greater osseointegration while others need to be highly wear-resistant.

In addition, many studies have shown that CNTs and CNFs are also promising coating materials for bone implantation due to their excellent electrical conductivity, greater mechanical strength, and unique chemical–biological properties.[58,83–86] Since it has been speculated that bone regenerates under electrical conduction,[87] researchers have utilized the increased conductivity of CNT to promote bone cell proliferation and calcium deposition,[83,84,88] or to upregulate gene expression of various bone-growth related factors.[88,89] In addition, the shape and orientation of osteoblasts could be significantly affected by altering the periodicity and alignment of CNT arrays.[90] Similarly, osteoblasts also elicit greater adhesion on CNF coated PCU[91] and CNF-PLGA compacts[92] compared to plain PCU and conventional carbon fiber-PLGA compacts, respectively. Moreover, due to its extremely high tensile strength and stiffness, CNT-based nanocomposites have also been used to improve fracture toughness[93] and anti-wear properties[94,95] of implant coatings, while maintaining excellent bioactivity to osteoblasts.

3.2.1.2. Bone Tissue Engineering: Developing biodegradable nanostructured scaffolds is another highly active research area for the use of nanobiomaterials in orthopedic applications, especially bone tissue engineering. These developments have mainly focused on two different directions: bone scaffolds with structural functions for the treatment of segmental defects and scaffolds for the treatment of cavitary defects (such as injectable scaffolds). Both kinds of scaffolds are discussed as below.

Synthetic and natural polymers [such as PLLA, PGA, PLGA, PCL, PU, gelatin, collagen, chitosan, silk, etc.) have been engineered into nanostructures with adequate structural properties via electrospinning, phase separation, particulate leaching, chemical etching, and 3-D printing techniques (Table 2).[11] Osseointegration studies on these polymeric nanostructures revealed enhanced adsorption of bone cell adhesive proteins (fibronectin and vitronectin), promoted bone cell attachment and osteogenic functions, and even inhibited functions of undesired cells. For example, it has been reported that 3-D PLLA nanofibrous scaffolds enhanced the selective adsorption of fibronectin and vitronectin from blood,

leading to increased osteoblast functions,[96] but in contrast, also decreased fibroblast adhesion on PLGA, PU, and PCL of the same surface chemistry but surface roughness values in the nanoscale regime from 50 to 100 nm.[97]

Nanocomposites containing polymers and ceramics (e.g., calcium phosphates such as HA and tricalcium phosphate (TCP) which are the inorganic components of human bone) or carbon materials (CNT or CNF) are probably more versatile for coating bone tissue engineering scaffolds, since the advantages of both materials can be optimized to best mimic the hierarchical structure, microenvironment, and mechanical properties of natural bone. Nanophase titania/PLGA composites mimicking the nanometer roughness of natural bone were reported to promote osteoblast adhesion, alkaline phosphatase (ALP) activity and mineralization compared to only PLGA.[98] Similar *in vitro* or *in vivo* results have been found on numerous nanocomposites (such as PCL/HA/gelatin nanofibrous scaffolds,[99] HA/collagen nanocomposites,[100] HA/chitosan nanofibers,[101] PLGA/TCP nanocomposites,[102] and PLA/CNT composites[88]). Recently, magnetic scaffolds modified by dip-coating HA/collagen scaffolds in iron oxide nanoparticles (IONs, 200 nm) have been developed.[103] The study proposed to use such magnetic scaffolds which can attract and adsorb growth factors, stem cells or other bio-agents via magnetic guiding to treat bone defects. Their preliminary results also indicated that such magnetic scaffolds supported the adhesion and proliferation of human bone marrow stem cells.

Moreover, several novel self-assembled nanostructures are also promising for bone tissue engineering applications. For example, self-assembled 3-D peptide-amphiphilic (PA) nanofibers invented by Hosseinkhani *et al*. were used as effective scaffolds for enhancing the osteogenic differentiation of MSC.[104] Another osteogenic nanoscaffold known as helical rosette nanotubes (HRNs) prepared through the self-assembly of DNA base pairs also revealed significantly improved osteoblast functions.[62] One promise of nano self-assembled materials is that they can be readily modified by small molecules, like protein segments or peptides. PA modified by arginine–glycine–aspartic acid (RGD, or Arg-Gly-Arp) and HRN modified by RGD, lysine or KRSR (Lys-Arg-Ser-Arg) showed even better osteogenic responses (such as enhanced ALP activity, osteocalcin synthesis, or calcium deposition) from MSC or osteoblasts than respective unmodified materials.[62,104,105]

In addition, the self-assembling technique has also been used to prepare injectable nanoscaffolds or hydrogels which are usually infiltrated with drugs or proteins and used to treat cavitary defects in bone. An injectable PA nanofibrous scaffold carrying BMP-2 was developed to promote bone regeneration *in vivo* though the prolonged release of the protein for up to 24 days.[106] In another study, an injectable nanohydrogel was made by adding RGD- or lysine-conjugated HRN (HRN-RGD-K) to polymerized 2-hydroxyethyl methacrylate (pHEMA), and *in vitro* results indicated enhanced fibronectin adsorption followed by osteoblast adhesion on HRN-RGD-K.[107]

In summary, the extensive use of nanobiomaterials in orthopedic applications has been shown time and time again to be an innovative and effective approach to create biomimetically inspired surfaces for osseointegration or bone regeneration. Exciting *in vitro* and *in vivo* results indicate the extraordinary opportunities that nanobiomaterials may bring to the orthopedic community. Although many problems remain unsolved (e.g., safety issues for the manufacturing of nanoparticles) for eventual clinical practices, the potential of nanobiomaterials in medicine is immense and, thus, should be further studied.

3.2.2. Dental Applications.

Due to the similarity between bone and teeth, restoration and reconstruction of teeth share many common characteristics and principles as those discussed for orthopedic prosthetics. Hence, it is not surprising that nanobiomaterials have been widely designed, studied, and modified for dental applications. Generally, there are two major uses of nanomaterials: dental implants and dental adhesives, which are briefly reviewed here due to space. Similar to bone implants, fast osseointegration is widely accepted in dentistry as the basis for dental implant success.[108] In this sense, most orthopedic nanostructured biomaterials reviewed in the previous section are also suitable for dental implant applications. In fact, Ti has been widely used for dental implants due to similar mechanical and biocompatibility properties described for bone implants. Ti with biologically inspired nanoscale roughness has been demonstrated as an excellent dental implant material.[108] Other nanomaterials under consideration include titania (TiO_2) coatings,[108] calcium phosphate and derived coatings,[108] calcium phosphate coated zirconia,[109] gelatin nanogold coatings,[110] and silver compound coatings.[111] Svanborg *et al*.[112] analyzed 12 types of commercially available oral implants and reported that many of them had nanostructures on the implant surfaces (Table 3).

In contrast to dental implant materials, dental adhesive materials emphasize different requirements such as the durability of the bond between teeth and adhesive as well as esthetic issues like color.[113] Recently, nanotechnology-engineered dental adhesives improved bond performance of the adhesives in tooth-colored restorations. Specifically, Nagano *et al*. used colloidal platinum nanoparticles (CPNs) to reinforce an adhesive resin cement and observed double the micro tensile bond strength in CPN treated cements compared to non-treated cements.[113] In another study, nanostructured silver coordinated polymers were found to be light-stable and have antibacterial properties.[111] Moreover, recently, HA nanoparticles,[114] nanofilled resin-modified glass-ionomers,[115] and poly(methyl methacrylate) (PMMA)-grafted nanoclays[116] have also been studied for dental adhesive purposes.

Clearly, novel properties of nanobiomaterials have provided new and improved functions for dental implants and

Table 3. Commercially available oral implants with nanorough surfaces.[112]

Product name	Manufacturer	Mean surface roughness at a nanometer level [μm]	Mean surface roughness at a micrometer level [μm]
Lifecore turned	Lifecore Biomedical	0.012	0.22
3i Nanotite	3i Biomet	0.023	0.28
3i Prevail	3i Biomet	0.023	0.29
3i Osseotite polished part	3i Biomet	0.021	0.40
Astra Tech Tioblast	Astra Tech AB	0.016	0.64
Lifecore RBM	Lifecore Biomedical	0.018	0.66
3i Osseotite etched part	3i Biomet	0.020	0.68
Dentatus machined	Dentatus AB	0.043	0.90
Nobel Biocare TiUnite	Nobel Biocare AB	0.033	1.19
Astra Tech Osseospeed	Astra Tech AB	0.021	1.32
Southern implant	Southern implant	0.032	1.34
Straumann SLA	Straumann	0.049	1.53
Dentatus blasted	Dentatus AB	0.073	1.61

adhesive materials, creating new choices for dental restoration. The exciting commercialization status of many nanomaterial-based oral implants also demonstrates the extraordinary opportunities that nanobiomaterials will bring to the medical field in the near future.

3.3. Anti-Infection and Anti-Carcinogenic Nanobiomaterials

Bacterial infection is a common and serious problem associated with various implantation procedures, causing pertinent adverse complications in host tissues, failure of implants and even death of patients. Although antibiotics are widely used, problems of toxicity, antibiotic resistance, adverse responses of patients, effective time, and range of use always demand better approaches to prevent infection. Therefore, a new area of anti-infection nanobiomaterials has emerged in order to utilize the extraordinary surface properties of nanomaterials (such as ultrasmall grain sizes, increased surface area and roughness, increased grain boundaries, etc.) for antibiotic purposes. A number of nanomaterials have been studied to date, and many results are promising. For example, Puckett *et al*. examined the *in vitro* adhesion of *Staphylococcus aureus, Staphylococcus epidermidis* (*S. epidermidis*), and *Pseudomonas aeruginosa* on conventional Ti, nanorough Ti (produced by electron beam evaporation), and nanotubular and nanotextured Ti (produced by two different anodization processes).[117] The results indicated that the nanorough Ti surfaces decreased the adhesion of all of the aforementioned bacteria the most compared to conventional Ti (micron sized grains), demonstrating promise for fabricating anti-infection implants through nanotechnology.[117] Colon *et al*. tested bacterial adhesion on nanophase (grain size of 23 nm) and microphase (grain size of 4.9 μm) ZnO as well as nanophase (grain size of 23 nm) and microphase (4.1 μm grain size) TiO_2, and revealed decreased *S. epidermidis* adhesion on nanophase ZnO and TiO_2 compared to respective microphase materials.[118] In addition, superparamagnetic IONs were also shown to prevent biofilm (an aggregate of bacteria in which cells adhere to each other and/or to a surface) formation. Decreased *S. epidermidis* numbers were observed when exposed to 100 $\mu g \cdot mL^{-1}$ of SPION for up to 48 h, and prevention of colony assembly (a prerequisite to biofilm formation) was also observed at lower SPION dosages of 10 $\mu g \cdot mL^{-1}$ after 12 h.[119] However, not all nanomaterials exhibit antibacterial effects.

A recent study on the adhesion of non-motile bacteria *Streptococci* and motile *P. fluorescens* on micro- and nanopatterned substrates (Ti, Cu, and Au) showed that initial bacterial adhesion was significantly less on micro-patterned surfaces than that on nanopatterned surfaces.[120] The mixed results of bacterial adhesion on nanostructured Ti suggests that a lot more studies are required to uncover bacterial responses to nanobiomaterials, and a variety of complexities (such as bacterial type, material chemistry, randomness of material topography, etc.) need to be considered.

However, the antibacterial advantage of several nanomaterials has demonstrated a high possibility of clinical applications. For example, silver nanoparticles (diameters 3–18 nm) have been coated on the surface of plastic catheters (coating thickness $\approx$ 100 nm) and coated catheters showed significant *in vitro* antimicrobial activity and inhibited biofilm formation (as tested by *Escherichia coli*, *Enterococcus*, *S. aureus*, coagulase-negative *Staphylococci*, *P. aeruginosa*, and *Candida albicans*). These results suggest several applications of silver nanoparticles in reducing the risk of infection in patients with indwelling catheters.[121] Another study developed fabric and porous ceramic filters using nanophase HA powder and revealed an excellent bacterial filtration capability.[122] The porous HA filter was further loaded with silver ions and *in vitro* tests showed effective antibacterial and antiviral properties.[123]

Anti-carcinogenic nanomaterials have also received increasing interest from researchers because, instead of using drugs and harmful radiation, inherent anticancer properties of nanomaterials have less side effects as well as toxicity but may have acceptable biocompatibility properties to host tissues. For example, Tran *et al*. created nanostructured surfaces to

promote healthy bone tissue growth and prevent cancerous bone cell functions by incorporating nanophase selenium (Se) on Ti and stainless steel implants through an in situ colloidal technique.[124,125] *In vitro* tests of non-cancerous osteoblasts revealed greater adhesion and proliferation on Se-coated Ti and stainless steel than that on uncoated Ti and stainless steel, but more importantly, the functions of cancerous osteoblasts on these Se coated surfaces were significantly inhibited compared to results obtained on non-Se coated implant materials. Zhang *et al*. also found that highly ordered spherical PLGA nanotopographies of different sizes (190, 300, 400, and 530 nm) can alter lung carcinoma epithelial cell adhesion density, and this information may be used to design appropriate material surfaces for inhibiting cancer cell functions for a wide range of applications.[126] Although the mechanism of cancer-inhibiting properties of nanophase materials is unknown, the promises of anti-carcinogenic but biocompatible nanomaterials (without the use of drugs) are exceptional.

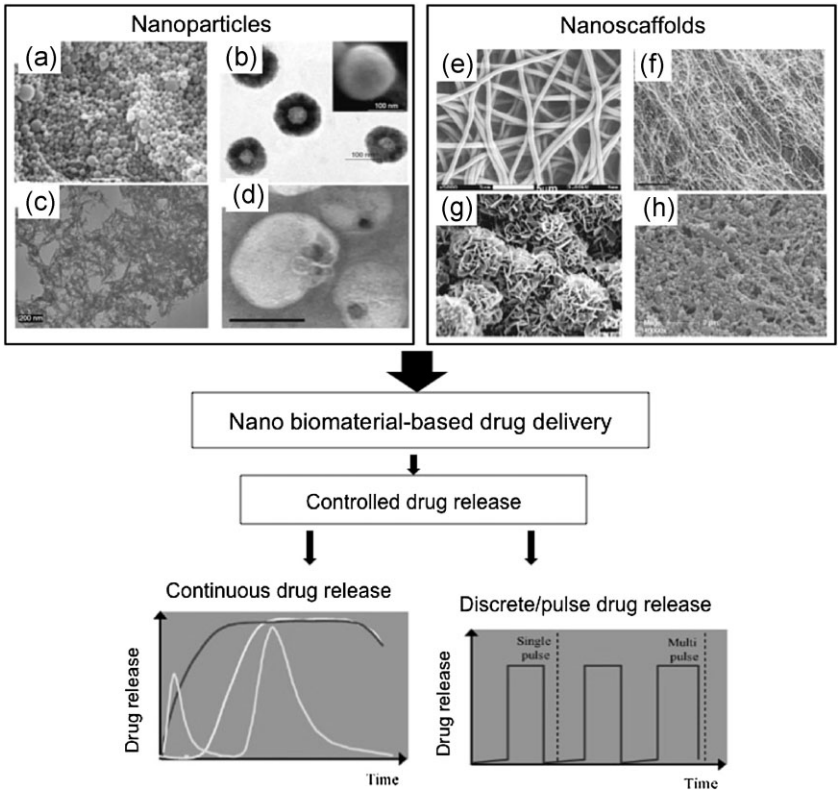

Fig. 3. Illustration of nanobiomaterials for drug delivery. Different drug release strategies are shown. Examples of nanobiomaterials: (a) PLGA nanoparticles, (b) hollow calcium phosphate nanospheres, (c) calcium phosphate coated γ-Fe_2O_3, (d) magnetic liposomes, (e) electrospun silk scaffold, (f) self-assembled PA nanofiber networks, (g) nanocrystalline apatite modified poly(lactide-co-glycolide) (PLAGA) microsphere scaffolds, and (d) PLLA nanofibrous scaffolds incorporated with PLGA nanospheres (images and redraw from refs.[130,208).

4. Nanobiomaterials for Drug Delivery Applications

The use of nanomaterials in drug delivery for treating various diseases is widespread. In particular, nanomaterials used as drug delivery carriers span a wide range of forms, chemistries, and geometries. Almost all of the materials (including, but not limited to, polymers, metals, ceramics, semiconductors, sol–gels, and self-assembled molecular complexes) from zero-dimensions (dots, particles) to three-dimensions have been used to deliver small molecular drugs and various classes of biomolecules with specific release kinetics and biodistribution.[127] Here, we review nanobiomaterials for drug delivery purposes based on their architectural differences, which can be placed into two general categories: nanoparticles and nanoscaffolds (Fig. 3).

4.1. Nanoparticles

Compared to scaffolds, particulate drug carriers possess a number of advantages (such as being less invasive, higher payload-to-mass ratio, faster circulation, and ease of production). Most importantly, nanoscale drug carrying particles can enhance endocytosis of drugs by target cells, and thus facilitate deeper penetration into capillaries and through fenestrations to, ultimately, enhance cellular uptake.[127] Studies have shown that, following systemic administration, nanoparticles with sizes from 10 to 70 nm in diameter can penetrate capillaries and those with sizes 70–200 nm have the most prolonged circulation time compared to other sizes.[127] Therefore, nanoparticles are probably the most common drug carriers today.

4.1.1. Polymeric Nanoparticles.

Polymeric nanoparticles are more frequently used as drug carriers than other kinds of materials, and one reason is that the release profiles of drugs can be adjusted by tailoring the (bio) degradation properties of the polymers. For example, the density of reactive carboxyl groups on PLGA nanoparticles have been controlled by adjusting the combination of high molecular weight (MW) encapped and low MW non-encapped PLGA in order to prolong celecoxib (a drug for treating arthritis) release.[128] Another study demonstrated that paclitaxel (model drug)-loaded PVA-*g*-PLGA nanoparticles decreased initial drug release from 80 to 38% when the PLGA-to-PVA mass ratio increased from 15 to 30.[129]

In addition, drug loading and trafficking can be controlled by altering surface hydrophilicity/charge and generating free functional groups on polymeric nanoparticles (surface modifications).[130] Generally, surface modification methods include emulsification of oil phase polymers in hydrophilic aqueous phases, coupling agent modification of polymeric particles, and micelle formation of copoly-

mers.[131] These methods have been applied to a large number of polymeric nanoparticles (such as PLGA, PCL, PLA, PEG, chitosan, PMMA, etc.) to achieve prolonged release, long circulation, targeted drug/protein/cell/gene delivery, and non-invasive drug delivery (like oral delivery).[131] A novel nanomicelle structure assembled by Tetronic-PCL-heparin copolymer chains was fabricated to deliver both basic fibroblast growth factor (bFGF, mediating the formation of new blood vessels) and indomethacin (IMC, a drug for treating arthritis and gout). *In vitro* drug release tests showed that IMC released over 3 weeks while bFGF released over 2 months in a controlled manner.[132] Similar micelle structures (sizes about 50–100 nm) with a hydrophobic core and hydrophilic outer shell can be used to deliver drugs (such as antiphlogistic and anticancer drugs), DNA or other proteins.[133,135]

Co-polymerization using PEG (also known as PEGylation) can increase blood circulation times of the drug carrier and mask bound biomolecules (proteins, DNA, etc.) to achieve a longer drug release.[136] For example, bovine serum albumin (BSA) loaded PEG-PLGA nanoparticles extended the half-life of BSA twenty times compared to BSA loaded in PLGA alone *in vivo*.[137] Another exemplary study designed 60-nm hybrid nanoparticles consisting of a PLLA core loaded with conjugates of paclitaxel, a lipid interface, and a PEG corona. The nanoparticles revealed prolonged drug release over $\approx$ 12 days, and also inhibited human aortic smooth muscle cell proliferation *in vitro* and showed greater *in vivo* vascular retention during percutaneous angioplasty over non-targeted controls.[138] PEGylated nanoparticles can be further modified for targeted delivery. For instance, copolymer PLA-PEG-COOH nanoparticles were covalently conjugated to amine-modified RNA aptamers that bind to a prostate-specific membrane antigen, and these bioconjugates can efficiently target and become engulfed by prostate LNCaP epithelial cells.[139] A number of PEGylated copolymers [such as methoxy poly(ethylene glycol)/PCL (MPEG/PCL), amine-terminated MPEG/PCL and PEG-cationized gelatin] have also been used as non-viral gene delivery carriers.[140,141]

Lastly, modified polymeric nanoparticles are also potential carriers for non-invasive drug delivery. For example, nanoparticles made of PLGA (PLLA/PGA = 50:50) were evaluated for their potential suitability for oral delivery of insulin-phospholipid complexes for up to 12 h.[142]

Despite the progress made in the design and fabrication of polymeric nanoparticles for drug delivery purposes, a number of hurdles hinder the ultimate application of these polymeric nanoparticles. For example, better control over degradation and bioavailability of nanoscale drug carriers has not been fully realized. Also, the fate of these nanoparticles after drug delivery, especially their possible toxicological impacts on the host and exit mechanisms, has not been completely understood or elucidated. Therefore, further investigation beyond current *in vitro* and *in vivo* studies is imperative.

4.1.2. Inorganic Nanoparticles.

Compared to polymeric nanoparticles, inorganic nanoparticles possess a few unique *in vivo* properties such as long biodegradation times (or even non-degradable), less swelling or porosity changes during use, as well as chemical and structural similarity to host tissues like bone.[143] These properties impart promises for drug delivery including long retention times for drugs, high stability to temperature and pH changes, and increased biocompatibility. In addition, inorganic nanoparticles also possess some electrical, mechanical (e.g., piezoelectric properties, ultrahigh hardness, etc.), magnetic (e.g., superparamagnetic properties), and optical (e.g., photothermal effects, electroluminescence, etc.) properties rarely seen in polymeric nanoparticles.[143]

Due to their bioactivity and adjustable bio-absorbability, calcium phosphate nanoparticles have been widely studied as novel delivery carriers for antibiotics (e.g., gentamicin sulfate, flomoxef sodium, tetracycline, etc.), anti-inflammatory agents (e.g., salicylic acid, IMC, etc.), analgesic and anticancer drugs (e.g., mercaptopurine, estradiol, etc.), growth factors [e.g., bone morphological proteins (BMPs), transforming growth factors β (TGF-β), etc.], proteins (e.g., collagen I and osteocalcin), and genes (e.g., DNA).[144,145] Recent studies indicated that grain size, surface area, calcium to phosphorus ratios, and the structure of calcium phosphate nanoparticles can be easily adjusted to control drug release kinetics.[143] For example, HA-like hollow nanospheres (sizes 145 ± 20 nm) that can collapse into pin-shaped nanocrystallites under ultrasonic waves have been fabricated to achieve on-off delivery (pulse or discrete release) of drugs.[146]

Besides on-off delivery, hollow nanostructured drug carriers can also achieve a time-delayed release behavior with a high drug-loading capacity. Hollow silica nanospheres have a time-delayed multiple-stage release profile and a capability of entrapping an eightfold increased quantity of drugs compared to solid ones.[147,148] Hollow gold nanocages are also promising drug delivery carriers since drug release can be controlled externally via the opening and closing of pores in the nanocages.[149]

CNT is also a popular hollow platform for drug delivery. A recent animal study (involving implantation of particles into the mouse skull subperiosteum) revealed that multi-walled CNT conjugated with collagen and recombinant human bone morphological protein-2 (rhBMP-2) accelerated bone formation after implantation in a mouse muscle.[150] Other hollow novel ceramic nanostructures for drug delivery purposes include alumina and calcium carbonate nanoshells, which were reviewed in a recent review paper.[143]

More interestingly, inorganic nanoparticles such as iron oxide, silica, and gold have been developed as multifunctional platforms for targeted delivery, imaging, diagnostic, and therapeutic purposes. Magnetic IONs (including hematite γ-Fe_2O_3, magnetite Fe_3O_4, and associated compounds) have extraordinary magnetic properties for magnetic resonance imaging (MRI), and these properties can be coupled with

other properties like acceptable biocompatibility, ease of functionalization and magnetic-thermal effects for the purposes of imaging, sensing, diagnosing, and hyperthermia therapy.[151,152] Drug-conjugated ION can also be delivered to target sites by applying external forces (e.g., external magnetic field) and this technique has reached clinical trials such as a cancer therapy.[153] In addition, gold nanoparticles (including both solid and hollow structures) conjugated with a specific antibody or molecule can target and then destroy specific tumor cells by a photothermal effect.[149] A novel multi-functional drug delivery platform based on CNT was recently developed by covalently attaching multiple copies of tumor-specific monoclonal antibodies, radiometal-ion chelates, and fluorescent probes to the sidewalls of CNT.[154] This platform can selectively target and image tumors with prototype-radiolabeled or fluorescent-labeled antibodies *in vivo* and *in vitro*, and lastly, deliver drugs to the tumor site. Another novel material currently being explored for various medical applications are gold layer over silica core nanoshells.[155,156] The thickness of the gold layer can be precisely tuned, so that the nanoshell can be selectively activated through tissue irradiation with near-infrared light to perform localized therapeutic thermal ablation. The approach was recently used to eradicate transmissible venereal tumors in mice.[156]

The integration of unique chemical and physical properties of inorganic nanoparticles enables these multi-functional platforms to complete multiples tasks besides drug delivery (such as molecular imaging) and, thus, this has become a popular research direction over the past few years.

4.1.3. Other Nanoparticles.

Composite nanoparticles also integrate distinct properties from different materials into a single drug delivery system, enabling a synergic, and multi-purpose therapy for treating a variety of diseases. For example, nanomagnetic liposomes fabricated by combining magnetic moieties with phospholipids are excellent carriers for rhBMP-2 and this composite drug delivery system promoted new bone formation in a rat bone-defect model by combining appropriate amounts of rhBMP-2 and magnetic induction.[157] In another study, IONs incorporated with poly(*N*-isopropylacrylamide) can soak up model drugs (doxorubicin, DNA-interacting anticancer drug) at a temperature greater than 32–37 °C and then can be directed into the rabbit liver by a magnetic field.[158] Another thermosensitive nanocomposite, poly(*N*-isopropylacrylamide-*co*-propyl acrylic acid) nanogels conjugated with an RGD-containing peptide and transferrin, had dual targeting ability to tumor cells and could release doxorubicin in response to temperature changes above 34.4 °C.[159] A similar rationale of achieving desirable properties by combining several nanomaterials into one composite nanomaterial has been widely studied and other examples are not reviewed here due to space.

In summary, nanoparticles have boosted drug delivery applications by providing for better control and/or programmable release kinetics, high drug loading and trafficking efficacy, targeted delivery, and multi-purpose delivery. The advantages of nanoparticles (such as their small size, controllable shape and structure, and facile surface modification) have revealed great potential for the next generation of drug delivery applications.

4.2. Nanostructured Scaffolds

As drug delivery platforms, nanostructured scaffolds not only transport drugs and control drug release, but they also serve as mechanical and/or structural support for cellular function and tissue regeneration. In scaffold-based drug delivery, controlled drug release is realized by tailoring bulk or surface chemistries (such as biodegradability, functional groups, composites, etc.), architecture (alignment, assembly, etc.), and porosity of nanoscaffolds.[77] For bio-mimicking purposes, drug-eluting nanoscaffolds are usually 3-D (in some cases, 2-D coatings or films) and their structures are often fabricated into ordered or hierarchical architectures resembling native biological systems (like bone or vessels) or the natural ECM.

A number of existing implants or scaffolds have been modified by nanotechnology to carry and deliver specific drugs. For example, nanotubular anodized titania has been used to load bone growth factors (e.g., BMP-2)[160] and antibiotics or anti-infection agents (e.g., penicillin G and gentamicin)[161,162] to promote bone growth and suppress bacterial infection after implantation. Nanotubular titania coated with a drug loaded calcium phosphate material revealed a prolonged release of penicillin G for up to 21 days.[162] In addition, CNT are excellent drug delivery carriers and have been successfully coated or patterned on titanium, anodized nanotubular titanium and PCU, imparting drug delivery capability to these implant materials.[163,164] Similarly, nanocrystalline apatite precipitated on PLGA microsphere sintered scaffolds enhanced drug adsorption and slowed drug release (as tested by chicken egg ovalbumin as a model drug).[165] Besides simply prolonging drug release, nanoscaffolds can also release drugs over a multi-step process with complicated release profiles. For example, a novel system of incorporating PLGA nanospheres into prefabricated PLLA nanofibrous scaffolds could release BMP-7 in a temporarily controlled manner, exhibiting tunable release phases (especially, a controlled initial burst release) depending on the chemical and degradation properties of the nanospheres.[166]

Novel self-assembled material coatings can add additional promise for the controlled release of drugs to implants. An example is HRN which have demonstrated exceptional potential to enhance osteoblast functions when either coated on titanium or incorporated into HA.[105] Drugs like dexamethasone (an anti-inflammatory and immunosuppressant) can be loaded into the long (up to several microns) interior of the HRN revealing prolonged drug release for up to 28 days.[167] Therefore, HRN scaffolds are promising multi-functional platforms for treating bone diseases and supporting bone regeneration.

There are a large number of studies using electrospinning to prepare porous or fibrous nanoscaffolds for delivering drugs or specific molecules. For example, electrospun PCL nanoscaffolds loaded with the drug simvastain (a lipid-lowering drug) significantly improved osseous integration and better *in vivo* bone mineralization compared to PCL scaffolds alone in the reconstruction of cranial bone defects in a rat model.[168] Natural polymers like silk have also been electrospun into scaffolds and loaded with molecules such as BMP-2 to promote bone regeneration *in vivo* and *in vitro*.[169] As an example of an electrospun ceramic nanoscaffold, calcium phosphate precursors mixed with polyvinyl chloride (PVC) were electrospun and then sintered into highly interconnected nanofibrous networks to be used for drug delivery and treating bone defects.[170]

Drug eluted nanoscaffolds serve both purposes of tissue regeneration (or prosthetic restoration) and drug delivery, and thus are very attractive for orthopedic, cardiovascular, and neural medical applications. However, loading drugs in nanoscale structures is sometimes extremely challenging. In addition, the controlled release of drugs in nanoscaffolds is not as optimal as many nanoparticle platforms. Therefore, further studies in this promising area are highly desirable.

5. Nanobiomaterials for Detection and Diagnosis

5.1. Nanobiomaterials in Medical Imaging

Medical imaging heavily relies on the development of sophisticated probes to detect biological processes on the cellular and molecular level.[171] Nanoscale probes have shown exceptional advantages over single molecule-based contrast agents. These advantages include producing better contrast, a capability to integrate multiple properties (such as several types of contrast generating materials), lengthy circulation times, and the possibility to include high drug payloads.[171] Thus, various exciting imaging agents and new imaging systems have been developed using nanomaterials (Table 4).

The largest imaging nanomaterial category includes the family of IONs from Fe_2O_3 (hematite) to Fe_3O_4 (magnetite). ION are sensitive contrast agents to induce negative contrast or signal loss as well as homogenous signals, and, thus, are widely studied as contrast agents in MRI for numerous tissues.[172,173] For example, superparamagnetic iron oxide was found to be a safe contrast agent to increase tumor-to-liver contrast-to-noise (C/N) ratios and improve the detection of liver metastases.[174] In another study, MSC labeled with superparamagnetic IONs were injected into rat brains and MRI could readily monitor the delivery of magnetically labeled MSC to brain tissue.[175] A number of studies also showed the efficacy of ferumoxtran-10 (superparamagnetic agents which can be taken up by normal nodes following intravenous or subcutaneous injection) in metastatic lymph node imaging,[173] allowing the detection of small and otherwise undetectable lymph-node metastases in patients with prostate cancer.[176] Hyafil *et al*. evaluated and demonstrated the ability of ferumoxtran-10-enhanced MRI to quantify local macrophage infiltration in the aortic wall of hypercholesterolemic rabbits.[177] Because of their long blood half-life and T1-shortening effect which yields optimal contrast between the vessel and the adjacent tissue, IONs could be better blood pool agents for evaluating cerebral perfusion, myocardial or renal perfusion, angiography, or detection of hepatic vascular lesions.[173,178] For example, Corot *et al*. compared different classes of blood pool agents in a rabbit model and reported better imaging results in IONs than the macromolecular agent P792 (a rapid clearance MRI contrast agent).[178] In addition, due to its excellent drug delivery capacity stated in the previous section, IONs are designed to achieve both imaging and therapeutic purposes. A dual-purpose probe of superparamagnetic IONs was developed for transferring siRNA (small interfering RNA) and simultaneous imaging of its accumulation in tumors. *In vivo* results revealed that uptake of these probes in tumors could be monitored by MRI and the delivery of siRNA achieved substantial silencing in tumors.[179]

Table 4. Nanobiomaterials for bioimaging and sensing purposes.

Material category	Chemical or structural features	Examples of applications
Ceramics	Iron oxide nanoparticles (IONs)	MRI agents for a large variety of imaging and sensing purposes, such as *in vitro* location and pathway imaging, *in vivo* cancer detection and diagnosis, drug/cell/gene tracking, sentinel lymph nodes (SLN) imaging[172–179]
	Dye-doped silica nanoparticles	Low-photobleaching, high-stable imaging agent[222]
	Nanoporous ZrO_2/chitosan composite	Glucose detection[191]
Quantum dots	Cd/Se/Te-based quantum dots	Imaging cancer cells, for example, SLN imaging[182]
	CdSe/ZnS	*in vitro* imaging[223]
Metals	Gold nanoparticles	Cancer detection, imaging and diagnosis[224]
	Silver nanoparticles, nanofilms, etc.	Fluorescence enhancing agents, cancer detection and diagnosis[189,225]
Other nanomaterials	Single fluorescent nanodiamond	Low-photobleaching labeling agent[181]
	Perfluorocarbon	MRI contrast agent for fibrin clots[184]
	Fluorescent polystyrene nanobeads	Visualizing SLN[183]
	Carbon nanotubes (CNTs)	Protein detection,[195] antigen and DNA detection[225]
	Si nanowires	Streptavidin detection[193]

Besides iron oxides, there are many other nanoscale materials being studied for medical imaging purposes. Gold nanocages as contrast enhancement agents for both optical coherence tomography and photoacoustic tomography showed an improved performance since the localized surface plasmon resonance of the Au nanocages can be tuned into the near-infrared (where the attenuation of light by blood and soft tissue is greatly reduced).[180] In a recent study, the fluorescence of single fluorescent nanodiamond was found to be much brighter than that of a single dye molecule under the same excitation conditions, and the nanodiamond showed no signs of photobleaching even after 5 min of continuous excitation and no fluorescence blinking within a time resolution of 1 ms.[181] Many semi-conductor quantum dots are also widely used for fluorescent imaging since they have wide excitation windows, narrow emission windows and high fluorescence efficiency.[171] Hikage *et al*. prepared fluorescent quantum dots (Cd/Se/Te-based quantum dots) to visualize a high-risk area in sentinel lymph nodes (SLN) for lymph node metastasis with a high degree of accuracy.[182] They further conjugated the quantum dots with tumor-specific cellular markers to visualize cancer cells (or other specific cells) in SLN, which could eventually increase the detection rate of cancer metastasis in SLN. In another study, Nakajima *et al*. used fluorescent polystyrene nanobeads with uniform sizes to efficiently visualize SLN by a laser scanning fluorescence detection system and determined the most appropriate size for SLN imaging in rats was 40 nm.[183]

Conjugation with specific functional groups makes nanomaterials more promising for imaging purposes. For example, lipid-encapsulated perfluorocarbon nanoparticles were surface-modified by numerous Gd-diethylenetriaminepentaacetic acid (Gd-DTPA, a type of contrast agent) complexes to yield an effective targeted contrast agent for MRI-detection of fibrin clots, showing a dramatic increase in the detectability of fibrin clots compared to the test without using targeted contrast agents.[184] Tang *et al*. prepared novel fluorescent nanoparticles via self-assembly of water-soluble conjugated polymers (CP) on Ag/SiO_2 (core–shell) nanoparticles. The fluorescence intensity of CP assembled on Ag/SiO_2 nanoparticles was enhanced 1.3-fold compared to that of CP assembled on silica nanoparticles only.[185] In another example, Amemiya *et al*. developed a system for streptavidin detection by conjugating biotin to nanosized bacterial magnetic particles (BMP) which were used as magnetic markers for magnetic force microscopy (MFM) imaging. The sensitivity of streptavidin detection increased 100 times more than a traditional fluorescent detection system as the minimum streptavidin detection limit is 1 $pg \cdot mL^{-1}$, indicating its applications in highly sensitive immunoassays and DNA detection.[186]

Along these lines, nanoparticles with bio-imaging capabilities have been innovatively coupled with other functionalities such as drug delivery and tissue regeneration, which was mentioned in the previous section. However, similar to drug delivery nanoparticles, the exit strategy, or the destiny of these nanoparticles after severing their function remains unclear; it is an imperative to seek such answers in the near future.

5.2. Biosensors Based on Nanobiomaterials

The development of nanobiomaterials have also resulted in unprecedented progress in novel screening and detection approaches for biological systems. As compared to earlier catalyst system-based biosensors, the next generation affinity biosensors deliver real-time information about antibody to antigen attachment, cell receptors to their ligands, and DNA and RNA to nucleic acids with a complimentary sequence.[187] To date, nanomaterials have demonstrated a capability to offer greater opportunities for creating the next generation of affinity biosensors by imparting biosensors with more stable, direct, accurate, and reproducible detectability (Table 4). For example, cells (after the uptake of gold nanoparticles (30–50 nm)) revealed increased signal strength from surface-enhanced Raman (SER) signatures, and parallel transmission electron microscopy (TEM) studies indicated the formation of nano-aggregates providing optimum SERS enhancement for ultrasensitive probing inside the endosomal compartment of cells.[188] Silver nano-islands were modified as SER gene probes to detect cancer genes, which exhibited high sensitivity and selectivity in detecting DNA targets without the use of radioactive labels.[189]

Based on these extraordinary opportunities from nanomaterials, a number of novel biosensors have been developed. Maxwell *et al*. developed a new class of nanobiosensors that is able to recognize and detect specific DNA sequences and single-base mutations in a homogeneous format by using colloidal gold nanoparticles.[190] Yang *et al*. developed a glucose biosensor using a surface-treated nanoporous ZrO_2/chitosan composite and the biosensor retained about 75.2% of its original response to glucose even after one-month storage in a phosphate buffer saline.[191] Gaster *et al*. designed a simple and sensitive nanosensor-based immunoassay by combining high density arrays of giant magnetoresistive nanosensors and magnetic nanoparticles, enabling rapid and high-throughput identification of the precise cause of aberrant or cross-reactive binding events.[192] In addition, highly sensitive and real-time electrically-based sensors to detect streptavidin were invented using biotin-modified silicon nanowires, the sensitivity of this nanosensor was at least in the picomolar concentration range.[193]

Besides detecting molecules, nanomaterial-based biosensors have also revealed a great potential to detect larger cellular biological systems like bacteria and growing bone tissue. For example, Park *et al*. showed higher bacterial adhesion and greater select bacterial metabolism on nanophase (grain size of 32 nm) than conventional titania, which indicated a possible application in biosensors for bacteria detection based on nanophase titania.[194] In another study, multi-walled CNT growth on nanotubular anodized titania were found to enhance the redox reaction of proteins

synthesized by osteoblasts, suggesting a possible biosensing material to generate an electrical signal which can be interpreted later as information to indicate bone growth onto implants.[195] As one can see, the field of nanobiomaterials for biosensing is very young but attractive. Nanobiomaterials have many extraordinary properties that can facilitate the development of biosensor in many aspects, from creating better detecting molecules to increasing the bioavailability of the sensor.

6. Future Trends in Nanobiomaterials

In a relatively short time period, nanobiomaterials have exhibited tremendous progress in medical applications and extraordinary promises to advance medicine. On one hand, after years of development, nanobiomaterials have entered a new era characterized by novel or multiple functionalities, such as smart or intelligent biomaterials.[196] On the other hand, a better understanding of the mechanisms and risks behind interactions between nanobiomaterials and biological systems (from all aspects of chemistry, physics, cell biology, and materials science) is urgent. These incentives indicate several possible developing trends of nanobiomaterials in the near future.

First of all, emphasis is given to the increasing concern for the safety and toxicity of nanomaterials. Both *in vitro* and *in vivo* evidence has supported the importance of these concerns and, therefore, they should not be overlooked in terms of biomaterial fabrication and clinical use. Toxic responses to nanoparticles generated from the degradation of implant materials, wear debris from artificial joints, and residue from nanomaterial processing, need to be understood.[11] Preliminary progress in the area of toxicity of nanoparticles has generated a number of important findings on how and why nanomaterials reveal toxic effects to the human body. For example, in most cases, the toxicity of nanoparticles is largely dependent on size, dosage, and surface properties (such as surface charge, hydrophilicity and surface area, etc.) of nanoparticles delivered to the host tissue. In addition, cellular uptake and internalization is an important pathway of nanoparticles causing damage to cells and tissues, and such damage is directly related to the reactive oxygen species (ROS, e.g., peroxides) generated by the host biological system responding to nanomaterials. However, the influence of nanobiomaterials on general human health and the environment is not well understood, and current studies often report contradictory results. Undoubtedly, it is necessary to further investigate the health and environmental impacts of nanobiomaterials before ultimately using and manufacturing these materials for human applications.

Second, developing smart or intelligent nanobiomaterials with the ability to respond to environmental changes is another promising direction. As previously described, several nanomaterials have been devised and fabricated to achieve specific functions in response to magnetic, electrical, temperature, light, and ultrasound signals. Responsive and fully automated nanobiomaterials may become a reality in the future.

Third, multi-functional biomaterials will better serve medical needs. Instead of nanomaterials designed for single purposes or realizing limited functions, multi-functional nanobiomaterials or nanoscale systems are desirable. For example, the next generation of nanobiomaterials for bone regeneration should simultaneously enhance tissue regeneration while minimizing immune responses and inhibit infection, and it would be even better if the implant materials themselves can indicate whether bone growth is occurring.[4] Integration of biomaterials with electronic devices, like micro- and nano-electromechanical systems (MEMS and NEMS, respectively) and nanochips, will revolutionize current biomedical applications.

Forth, there is a compelling need to understand the mechanisms of the interactions between nanobiomaterials and biological systems, which provide important information for designing and fabricating better materials. Future investigations will emphasize this understanding at the molecular level and submicron or nanoscale interactions as well as the impact of nanomaterial structures and properties. In addition, this area has been actively studied by mathematical and computational models and simulations, which are becoming an essential part of the experimental toolkit to understand the cellular processes (such as actin-based filopodial and lamellipodial extensions) on nanomaterials.[197]

Lastly, computer simulation and finite elements modeling have also been used to understand and predict the properties of highly hierarchical biological nanostructures.[198,199] These computational approaches have provided a better vision of how nanoscale materials and structures serve biological purposes. Therefore, it is believed that the development of such computer-aided tools is another popular future direction focusing on facilitating the choice of proper nanomaterials and the design and creation of novel nanobiomaterials.

Received: November 19, 2010
Final Version: January 26, 2011

[1] T. J. Webster, *Nanotechnology for the Regeneration of Hard and Soft Tissues*, World Scientific, Hackensack, N.J. London **2007**.
[2] D. A. Puleo, A. Nanci, *Biomaterials* **1999**, *20*, 2311.
[3] C. J. Wilson, R. E. Clegg, D. I. Leavesley, M. J. Pearcy, *Tissue Eng.* **2005**, *11*, 1.
[4] D. Khang, J. Carpenter, Y. W. Chun, R. Pareta, T. J. Webster, *Biomed. Microdevices* **2010**, *12*, 575.
[5] A. J. Dulgar-Tulloch, R. Bizios, R. W. Siegel, *J. Biomed. Mater. Res. A* **2009**, *90A*, 586.
[6] B. D. Boyan, V. L. Sylvia, Y. H. Liu, R. Sagun, D. L. Cochran, C. H. Lohmann, D. D. Dean, Z. Schwartz, *Biomaterials* **1999**, *20*, 2305.

[7] D. Khang, S. Y. Kim, P. Liu-Snyder, G. T. R. Palmore, S. M. Durbin, T. J. Webster, *Biomaterials* **2007**, *28*, 4756.
[8] D. E. Discher, P. Janmey, Y. L. Wang, *Science* **2005**, *310*, 1139.
[9] T. A. Ulrich, E. M. D. Pardo, S. Kumar, *Cancer Res.* **2009**, *69*, 4167.
[10] S. Itoh, S. Nakamura, M. Nakamura, K. Shinomiya, K. Yamashita, *Biomaterials* **2006**, *27*, 5572.
[11] L. J. Zhang, T. J. Webster, *Nano Today* **2009**, *4*, 66.
[12] S. Choudhary, K. M. Haberstroh, T. J. Webster, *Tissue Eng.* **2007**, *13*, 1421.
[13] S. Choudhary, M. Berhe, K. M. Haberstroh, T. J. Webster, *Int. J. Nanomed.* **2006**, *1*, 41.
[14] H. D. Samaroo, J. Lu, T. J. Webster, *Int. J. Nanomed.* **2008**, *3*, 75.
[15] D. C. Miller, K. M. Haberstroh, T. J. Webster, *J. Biomed. Mater. Res. A* **2007**, *81A*, 678.
[16] A. Ranjan, T. J. Webster, *Nanotechnology* **2009**, *20*, 305102.
[17] G. Giavaresi, M. Tschon, J. H. Daly, J. J. Liggat, D. S. Sutherland, H. Agheli, M. Fini, P. Torricelli, R. Giardino, *J. Biomat. Sci.-Polym. E* **2006**, *17*, 1405.
[18] N. Ferraz, J. Carlsson, J. Hong, M. K. Ott, *J. Mater. Sci.-Mater. M* **2008**, *19*, 3115.
[19] E. R. Kenawy, J. M. Layman, J. R. Watkins, G. L. Bowlin, J. A. Matthews, D. G. Simpson, G. E. Wnek, *Biomaterials* **2003**, *24*, 907.
[20] S. G. Zhang, *Nat. Biotechnol.* **2003**, *21*, 1171.
[21] G. Punshon, K. M. Sales, D. S. Vara, G. Hamilton, A. M. Seifalian, *Cell Proliferat.* **2008**, *41*, 321.
[22] S. J. Lee, J. J. Yoo, G. J. Lim, A. Atala, J. Stitze, *J. Biomed. Mater. Res. A* **2007**, *83A*, 999.
[23] C. Y. Xu, R. Inai, M. Kotaki, S. Ramakrishna, *Biomaterials* **2004**, *25*, 877.
[24] E. Genove, C. Shen, S. G. Zhang, C. E. Semino, *Biomaterials* **2005**, *26*, 3341.
[25] M. Mutsuga, Y. Narita, A. Yamawaki, M. Satake, H. Kaneko, A. Usui, Y. Ueda, *Interact. Cardiovasc. Thorac. Surg.* **2009**, *8*, 402.
[26] J. X. Zhao, L. J. Xu, T. Zhang, G. G. Ren, Z. Yang, *Neurotoxicology* **2009**, *30*, 220.
[27] G. J. Bakeine, J. Ban, G. Grenci, A. Pozzato, S. Dal Zilio, M. Prasciolu, L. Businaro, M. Tormen, M. E. Ruaro, *Microelectron. Eng.* **2009**, *86*, 1435.
[28] M. P. Mattson, R. C. Haddon, A. M. Rao, *J. Mol. Neurosci.* **2000**, *14*, 175.
[29] J. L. McKenzie, M. C. Waid, R. Y. Shi, T. J. Webster, *Biomaterials* **2004**, *25*, 1309.
[30] H. S. Koh, T. Yong, C. K. Chan, S. Ramakrishna, *Biomaterials* **2008**, *29*, 3574.
[31] T. C. Holmes, S. de Lacalle, X. Su, G. S. Liu, A. Rich, S. G. Zhang, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 6728.
[32] Y. C. Chen, D. C. Lee, T. Y. Tsai, C. Y. Hsiao, J. W. Liu, C. Y. Kao, H. K. Lin, H. C. Chen, T. J. Palathinkal, W. F. Pong, N. H. Tai, I. N. Lin, I. M. Chiu, *Biomaterials* **2010**, *31*, 5575.
[33] R. Vasita, D. S. Katti, *Int. J. Nanomed.* **2006**, *1*, 15.
[34] G. E. Park, M. A. Pattison, K. Park, T. J. Webster, *Biomaterials* **2005**, *26*, 3075.
[35] J. D. Kisiday, B. Kurz, M. A. DiMicco, A. J. Grodzinsky, *Tissue Eng.* **2005**, *11*, 141.
[36] I. K. Shim, W. H. Suh, S. Y. Lee, S. H. Lee, S. J. Heo, M. C. Lee, S. J. Lee, *J. Biomed. Mater. Res. A* **2009**, *90A*, 595.
[37] W. J. Li, R. Tuli, X. X. Huang, P. Laquerriere, R. S. Tuan, *Biomaterials* **2005**, *26*, 5158.
[38] D. T. Xue, Q. Zheng, C. Zong, Q. Li, H. Li, S. J. Qian, B. Zhang, L. N. Yu, Z. J. Pan, *J. Biomed. Mater. Res. A* **2010**, *94A*, 259.
[39] Y. S. Pan, D. S. Xiong, *Wear* **2009**, *266*, 699.
[40] J. Hori, M. Deie, T. Kobayashi, Y. Yasunaga, S. Kawamata, M. Ochi, *J. Orthop. Res.* **2011**, *29*, 531.
[41] A. Thapa, D. C. Miller, T. J. Webster, K. M. Haberstroh, *Biomaterials* **2003**, *24*, 2915.
[42] M. Pattison, T. J. Webster, J. Leslie, M. Kaefer, K. M. Haberstroh, *Macromol. Biosci.* **2007**, *7*, 690.
[43] D. A. Harrington, A. K. Sharma, B. A. Erickson, E. Y. Cheng, *World J. Urol.* **2008**, *26*, 315.
[44] S. C. Baker, N. Atkin, P. A. Gunning, N. Granville, K. Wilson, D. Wilson, J. Southgate, *Biomaterials* **2006**, *27*, 3136.
[45] T. J. Webster, *Advances in Chemical Engineering*, Vol. *27*, Academic Press, San Diego, CA **2001**, pp. 125.
[46] H. Liu, T. J. Webster, *Biomaterials* **2007**, *28*, 354.
[47] E. M. Christenson, K. S. Anseth, L. J. J. P. van den Beucken, C. K. Chan, B. Ercan, J. A. Jansen, C. T. Laurencin, W. J. Li, R. Murugan, L. S. Nair, S. Ramakrishna, R. S. Tuan, T. J. Webster, A. G. Mikos, *J. Orthop. Res.* **2007**, *25*, 11.
[48] T. J. Webster, J. U. Ejiofor, *Biomaterials* **2004**, *25*, 4731.
[49] P. Tran, T. J. Webster, *Int. J. Nanomed.* **2008**, *3*, 391.
[50] N. Tran, T. J. Webster, *Wires Nanomed. Nanobi.* **2009**, *1*, 336.
[51] T. K. Monsees, K. Barth, S. Tippelt, K. Heidel, A. Gorbunov, W. Pompe, R. H. W. Funk, *Cells Tissues Organs* **2005**, *180*, 81.
[52] B. C. Ward, T. J. Webster, *Biomaterials* **2006**, *27*, 3064.
[53] T. J. Webster, R. W. Siegel, R. Bizios, *Biomaterials* **1999**, *20*, 1221.
[54] T. J. Webster, C. Ergun, R. H. Doremus, R. W. Siegel, R. Bizios, *Biomaterials* **2000**, *21*, 1803.
[55] T. J. Webster, T. A. Smith, *J. Biomed. Mater. Res. A* **2005**, *74A*, 677.
[56] N. R. Washburn, K. M. Yamada, C. G. Simon, S. B. Kennedy, E. J. Amis, *Biomaterials* **2004**, *25*, 1215.
[57] L. J. Smith, J. S. Swaim, C. Yao, K. M. Haberstroh, E. A. Nauman, T. J. Webster, *Int. J. Nanomed.* **2007**, *2*, 493.
[58] T. J. Webster, M. C. Waid, J. L. McKenzie, R. L. Price, J. U. Ejiofor, *Nanotechnology* **2004**, *15*, 48.
[59] L. Yang, B. W. Sheldon, T. J. Webster, *J. Biomed. Mater. Res. A* **2009**, *91A*, 548.

[60] R. Pareta, L. Yang, A. Kothari, S. Sirinrath, X. C. Xiao, B. W. Sheldon, T. J. Webster, *J. Biomed. Mater. Res. A* **2010**, *95A*, 129.
[61] L. Yang, B. W. Sheldon, T. J. Webster, *Biomaterials* **2009**, *30*, 3458.
[62] L. J. Zhang, S. Ramsaywack, H. Fenniri, T. J. Webster, *Tissue Eng. Pt A* **2008**, *14*, 1353.
[63] X. L. Zhu, J. Chen, L. Scheideler, R. Reichl, J. Geis-Gerstorfer, *Biomaterials* **2004**, *25*, 4087.
[64] K.-H. Kim, T.-Y. Kwon, S.-Y. Kim, I.-K. Kang, S. Kim, Y. Yang, J. L. Ong, *J. Oral Implantol.* **2006**, *32*, 8.
[65] C. Yao, T. J. Webster, *J. Nanosci. Nanotechnol.* **2006**, *6*, 2682.
[66] H. H. Huang, S. J. Pan, Y. J. Lai, T. H. Lee, C. C. Chen, F. H. Lu, *Scripta Mater.* **2004**, *51*, 1017.
[67] C. Yao, V. Perla, J. L. McKenzie, E. B. Slamovich, T. J. Webster, *J. Biomed. Nanotechnol.* **2005**, *1*, 68.
[68] K. Das, S. Bose, A. Bandyopadhyay, *Acta Biomater.* **2007**, *3*, 573.
[69] F. Variola, F. Vetrone, L. Richert, P. Jedrzejowski, J. H. Yi, S. Zalzal, S. Clair, A. Sarkissian, D. F. Perepichka, J. D. Wuest, F. Rosei, A. Nanci, *Small* **2009**, *5*, 996.
[70] S. Ban, Y. Iwaya, H. Kono, H. Sato, *Dent. Mater.* **2006**, *22*, 1115.
[71] M. S. Sader, A. Balduino, A. Soares Gde, R. Borojevic, *Clin. Oral Implants Res.* **2005**, *16*, 667.
[72] M. Takemoto, S. Fujibayashi, M. Neo, J. Suzuki, T. Matsushita, T. Kokubo, T. Nakamura, *Biomaterials* **2006**, *27*, 2682.
[73] W. Xue, X. Liu, X. Zheng, C. Ding, *Biomaterials* **2005**, *26*, 3029.
[74] D. E. MacDonald, B. E. Rapuano, N. Deo, M. Stranick, P. Somasundaran, A. L. Boskey, *Biomaterials* **2004**, *25*, 3135.
[75] S. R. Sousa, M. M. Bras, P. Moradas-Ferreira, M. A. Barbosa, *Langmuir* **2007**, *23*, 7046.
[76] J. H. Yi, C. Bernard, F. Variola, S. F. Zalzal, J. D. Wuest, F. Rosei, A. Nanci, *Surf. Sci.* **2006**, *600*, 4613.
[77] F. Variola, J. H. Yi, L. Richert, J. D. Wuest, F. Rosei, A. Nanci, *Biomaterials* **2008**, *29*, 1285.
[78] X. Lu, Y. B. Wang, X. D. Yang, Q. Y. Zhang, Z. F. Zhao, L. T. Weng, Y. Leng, *J. Biomed. Mater. Res. A* **2008**, *84A*, 523.
[79] F. Variola, J. H. Yi, L. Richert, J. D. Wuest, F. Rosei, A. Nanci, *Biomaterials* **2008**, *29*, 1285.
[80] N. Yasumaru, K. Miyazaki, J. Kiuchi, *Appl. Surf. Sci.* **2008**, *254*, 2364.
[81] R. Lappalainen, M. Selenius, A. Anttila, Y. T. Konttinen, S. S. Santavirta, *J. Biomed. Mater. Res. B* **2003**, *66B*, 410.
[82] D. M. Gruen, *Annu. Rev. Mater. Sci.* **1999**, *29*, 211.
[83] L. P. Zanello, *Micro. Nano Lett.* **2006**, *1*, 19.
[84] L. P. Zanello, B. Zhao, H. Hu, R. C. Haddon, *Nano Lett.* **2006**, *6*, 562.
[85] W. Wei, A. Sethuraman, C. Jin, N. A. Monteiro-Riviere, R. J. Narayan, *J. Nanosci. Nanotechnol.* **2007**, *7*, 1284.
[86] B. S. Harrison, A. Atala, *Biomaterials* **2007**, *28*, 344.
[87] D. M. Ciombor, R. K. Aaron, *Foot Ankle Clin.* **2005**, *10*, 579.
[88] P. R. Supronowicz, P. M. Ajayan, K. R. Ullmann, B. P. Arulanandam, D. W. Metzger, R. Bizios, *J. Biomed. Mater. Res.* **2002**, *59*, 499.
[89] N. Tsuchiya, Y. Sato, N. Aoki, A. Yokoyama, F. Watari, K. Motomiya, B. Jeyadevan, K. Tohji, *AIP Conf. Proc.* **2007**, *898*, 166.
[90] S. Giannona, I. Firkowska, J. Rojas-Chapana, M. Giersig, *J. Nanosci. Nanotechnol.* **2007**, *7*, 1679.
[91] D. Khang, M. Sato, R. L. Price, A. E. Ribbe, T. J. Webster, *Int. J. Nanomed.* **2006**, *1*, 65.
[92] R. L. Price, K. Ellison, K. M. Haberstroh, T. J. Webster, *J. Biomed. Mater. Res. A* **2004**, *70*, 129.
[93] K. Balani, R. Anderson, T. Laha, M. Andara, J. Tercero, E. Crumpler, A. Agarwal, *Biomaterials* **2007**, *28*, 618.
[94] Y. Chen, T. H. Zhang, C. H. Gan, G. Yu, *Carbon* **2007**, *45*, 998.
[95] J. Reis, S. Kanagaraj, A. Fonseca, M. T. Mathew, F. Capela-Silva, J. Potes, A. Pereira, M. S. A. Oliveira, J. A. Simoes, *Braz. J. Med. Biol. Res.* **2010**, *43*, 476.
[96] K. M. Woo, V. J. Chen, P. X. Ma, *J. Biomed. Mater. Res. A* **2003**, *67A*, 531.
[97] R. J. Vance, D. C. Miller, A. Thapa, K. M. Haberstroh, T. J. Webster, *Biomaterials* **2004**, *25*, 2095.
[98] H. Liu, E. B. Slamovich, T. J. Webster, *J. Biomed. Mater. Res. A* **2006**, *78A*, 798.
[99] J. R. Venugopal, S. Low, A. T. Choon, A. B. Kumar, S. Ramakrishna, *Artif. Organs* **2008**, *32*, 388.
[100] M. Kikuchi, S. Itoh, S. Ichinose, K. Shinomiya, J. Tanaka, *Biomaterials* **2001**, *22*, 1705.
[101] Y. Zhang, J. R. Venugopal, A. El-Turki, S. Ramakrishna, B. Su, C. T. Lim, *Biomaterials* **2008**, *29*, 4314.
[102] O. D. Schneider, F. Weber, T. J. Brunner, S. Loher, M. Ehrbar, P. R. Schmidlin, W. J. Stark, *Acta Biomater.* **2009**, *5*, 1775.
[103] N. Bock, A. Riminucci, C. Dionigi, A. Russo, A. Tampieri, E. Landi, V. A. Goranov, M. Marcacci, V. Dediu, *Acta Biomater.* **2010**, *6*, 786.
[104] H. Hosseinkhani, M. Hosseinkhani, F. Tian, H. Kobayashi, Y. Tabata, *Biomaterials* **2006**, *27*, 4079.
[105] L. J. Zhang, Y. P. Chen, J. Rodriguez, H. Fenniri, T. J. Webster, *Int. J. Nanomed.* **2008**, *3*, 323.
[106] H. Hosseinkhani, M. Hosseinkhani, A. Khademhosseini, H. Kobayashi, *J. Control Release* **2007**, *117*, 380.
[107] L. Zhang, F. Rakotondradany, A. J. Myles, H. Fenniri, T. J. Webster, *Biomaterials* **2009**, *30*, 1309.
[108] G. Mendonca, D. B. S. Mendonca, F. J. L. Aragao, L. F. Cooper, *Biomaterials* **2008**, *29*, 3822.
[109] J. Lee, J. H. Sieweke, N. A. Rodriguez, P. Schupbach, H. Lindstrom, C. Susin, U. M. E. Wikesjo, *J. Clin. Periodontol.* **2009**, *36*, 610.
[110] Y. H. Lee, G. Bhattarai, S. Aryal, N. H. Lee, M. H. Lee, T. G. Kim, E. C. Jhee, H. Y. Kim, H. K. Yi, *Appl. Surf. Sci.* **2010**, *256*, 5882.

[111] T. V. Slenters, I. Hauser-Gerspach, A. U. Daniels, K. M. Fromm, *J. Mater. Chem.* **2008**, *18*, 5359.
[112] L. M. Svanborg, M. Andersson, A. Wennerberg, *J. Biomed. Mater. Res. B* **2010**, *92B*, 462.
[113] F. Nagano, D. Selimovic, M. Noda, T. Ikeda, T. Tanaka, Y. Miyamoto, K. Koshiro, H. Sano, *Bio.-Med. Mater. Eng.* **2009**, *19*, 249.
[114] M. Sadat-Shojai, M. Atai, A. Nodehi, L. N. Khanlar, *Dent. Mater.* **2010**, *26*, 471.
[115] E. Coutinho, M. V. Cardoso, J. De Munck, A. A. Neves, K. L. Van Landuyt, A. Poitevin, M. Peumans, P. Lambrechts, B. Van Meerbeek, *Dent. Mater.* **2009**, *25*, 1347.
[116] M. Atai, L. Solhi, A. Nodehi, S. M. Mirabedini, S. Kasraei, K. Akbari, S. Babanzadeh, *Dent. Mater.* **2009**, *25*, 339.
[117] S. D. Puckett, E. Taylor, T. Raimondo, T. J. Webster, *Biomaterials* **2010**, *31*, 706.
[118] G. Colon, B. C. Ward, T. J. Webster, *J. Biomed. Mater. Res. A* **2006**, *78A*, 595.
[119] E. N. Taylor, T. J. Webster, *Int. J. Nanomed.* **2009**, *4*, 145.
[120] C. Díaz, M. C. Cortizo, P. L. Schilardi, S. G. Gd. Saravia, M. A. F. Ld. Mele, *Mater. Res.* **2007**, *10*, 11.
[121] D. Roe, B. Karandikar, N. Bonn-Savage, B. Gibbins, J. B. Roullet, *J. Antimicrob. Chemoth.* **2008**, *61*, 869.
[122] L. Yang, X. S. Ning, K. X. Chen, H. P. Zhou, *Ceram. Int.* **2007**, *33*, 483.
[123] L. Yang, X. S. Ning, Q. F. Xiao, K. X. Chen, H. P. Zhou, *J. Biomed. Mater. Res. B* **2007**, *81B*, 50.
[124] P. A. Tran, L. Sarin, R. H. Hurt, T. J. Webster, *J. Biomed. Mater. Res. A* **2010**, *93A*, 1417.
[125] P. A. Tran, L. Sarin, R. H. Hurt, T. J. Webster, *J. Mater. Chem.* **2009**, *19*, 2653.
[126] L. Zhang, Y. W. Chun, T. J. Webster, *Int. J. Nanomed.* **2010**, *5*, 269.
[127] M. Goldberg, R. Langer, X. Q. Jia, *J. Biomat. Sci.-Polym. E* **2007**, *18*, 241.
[128] P. A. McCarron, W. M. Marouf, R. F. Donnelly, C. Scott, *J. Biomed. Mater. Res. A* **2008**, *87A*, 873.
[129] U. Westedt, M. Kalinowski, M. Wittmar, T. Merdan, F. Unger, J. Fuchs, S. Schaller, U. Bakowsky, T. Kissel, *J. Control Release* **2007**, *119*, 41.
[130] L. Yang, T. J. Webster, *Expert Opin. Drug Del.* **2009**, *6*, 851.
[131] S. W. Choi, W. S. Kim, J. H. Kim, *J. Disper. Sci. Technol.* **2003**, *24*, 475.
[132] J. S. Lee, J. W. Bae, Y. K. Joung, S. J. Lee, D. K. Han, K. D. Park, *Int. J. Pharm* **2008**, *346*, 57.
[133] K. Kataoka, A. Harada, Y. Nagasaki, *Adv. Drug Deliver Rev.* **2001**, *47*, 113.
[134] H. B. Liu, S. Farrell, K. Uhrich, *J. Control Release* **2000**, *68*, 167.
[135] G. Riess, *Prog. Polym. Sci.* **2003**, *28*, 1107.
[136] D. Putnam, *Nature Mater.* **2006**, *5*, 439.
[137] Y.-P. Li, Y.-Y. Pei, X.-Y. Zhang, Z.-H. Gu, Z.-H. Zhou, W.-F. Yuan, J.-J. Zhou, J.-H. Zhu, X.-J. Gao, *J. Control Release* **2001**, *71*, 203.
[138] J. M. Chan, L. F. Zhang, R. Tong, D. Ghosh, W. W. Gao, G. Liao, K. P. Yuet, D. Gray, J. W. Rhee, J. J. Cheng, G. Golomb, P. Libby, R. Langer, O. C. Farokhzad, *P. Natl. Acad. Sci. USA* **2010**, *107*, 2213.
[139] O. C. Farokhzad, S. Jon, A. Khademhosseini, T. N. Tran, D. A. Lavan, R. Langer, *Cancer Res.* **2004**, *64*, 7668.
[140] J. S. Jang, S. Y. Kim, S. B. Lee, K. O. Kim, J. S. Han, Y. M. Lee, *J. Control Release* **2006**, *113*, 173.
[141] T. Kushibiki, Y. Tabata, *J. Biomat. Sci.-Polym. E* **2005**, *16*, 1447.
[142] F. Cui, K. Shi, L. Zhang, A. Tao, Y. Kawashima, *J. Control Release* **2006**, *114*, 242.
[143] L. Yang, B. W. Sheldon, T. J. Webster, *Am. Ceram. Soc. Bull.* **2010**, *89*, 24.
[144] M. P. Ginebra, T. Traykova, J. A. Planell, *J. Control Release* **2006**, *113*, 102.
[145] K. Kim, J. P. Fisher, *J. Drug Target* **2007**, *15*, 241.
[146] Y. R. Cai, H. H. Pan, X. R. Xu, Q. H. Hu, L. Li, R. K. Tang, *Chem. Mater.* **2007**, *19*, 3081.
[147] J. Wang, X. Gao, X. Yang, Y. Gan, W. Weng, Z. Gou, *Nanoscale Res. Lett.* **2009**, *4*, 1171.
[148] J. F. Chen, H. M. Ding, J. X. Wang, L. Shao, *Biomaterials* **2004**, *25*, 723.
[149] C. M. Cobley, L. Au, J. Y. Chen, Y. N. Xia, *Expert Opin. Drug Del.* **2010**, *7*, 577.
[150] Y. Usui, K. Aoki, N. Narita, N. Murakami, I. Nakamura, K. Nakamura, N. Ishigaki, H. Yamazaki, H. Horiuchi, H. Kato, S. Taruta, Y. A. Kim, M. Endo, N. Saito, *Small* **2008**, *4*, 240.
[151] C. C. Berry, A. S. G. Curtis, *J. Phys. D-Appl. Phys.* **2003**, *36*, R198.
[152] C. J. Xu, S. H. Sun, *Polym. Int.* **2007**, *56*, 821.
[153] V. I. Shubayev, T. R. Pisanic, S. H. Jin, *Adv. Drug Deliver Rev.* **2009**, *61*, 467.
[154] M. R. McDevitt, D. Chattopadhyay, B. J. Kappel, J. S. Jaggi, S. R. Schiffman, C. Antczak, J. T. Njardarson, R. Brentjens, D. A. Scheinberg, *J. Nucl. Med.* **2007**, *48*, 1180.
[155] L. R. Hirsch, J. B. Jackson, A. Lee, N. J. Halas, J. West, *Anal. Chem.* **2003**, *75*, 2377.
[156] L. R. Hirsch, R. J. Stafford, J. A. Bankson, S. R. Sershen, B. Rivera, R. E. Price, J. D. Hazle, N. J. Halas, J. L. West, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 13549.
[157] T. Matsuo, T. Sugita, T. Kubo, Y. Yasunaga, M. Ochi, T. Murakami, *J. Biomed. Mater. Res. A* **2003**, *66A*, 747.
[158] Y. H. Deng, C. C. Wang, X. Z. Shen, W. L. Yang, L. An, H. Gao, S. K. Fu, *Chem.-Eur. J* **2005**, *11*, 6006.
[159] C. Y. Quan, C. Chang, H. Wei, C. S. Chen, X. D. Xu, S. X. Cheng, X. Z. Zhang, R. X. Zhuo, *Nanotechnology* **2009**, *20*, 335101.
[160] G. Balasundaram, C. Yao, T. J. Webster, *J. Biomed. Mater. Res. A* **2008**, *84A*, 447.
[161] K. C. Popat, M. Eltgroth, T. J. LaTempa, C. A. Grimes, T. A. Desai, *Biomaterials* **2007**, *28*, 4880.
[162] C. Yao, T. J. Webster, *J. Biomed. Mater. Res. B* **2009**, *91B*, 587.

[163] S. Sirivisoot, C. Yao, X. Xiao, B. W. Sheldon, T. J. Webster, *Nanotechnology* **2007**, *18*, 365102.
[164] J. Y. Kim, D. Khang, J. E. Lee, T. J. Webster, *J. Biomed. Mater. Res. A* **2009**, *88A*, 419.
[165] E. Jabbarzadeh, L. S. Nair, Y. M. Khan, M. Deng, C. T. Laurencin, *J. Biomat. Sci.-Polym. E* **2007**, *18*, 1141.
[166] G. Wei, Q. Jin, W. V. Giannobile, P. X. Ma, *Biomaterials* **2007**, *28*, 2087.
[167] Y. Chen, *Self-assembled Nanotubes for Drug Delivery and Tissue Regeneration*, PhD thesis, *Brown University, Providence* **2010**.
[168] E. Piskin, I. A. Isoglu, N. Bolgen, I. Vargel, S. Griffiths, T. Cavusoglu, P. Korkusuz, E. Guzel, S. Cartmell, *J. Biomed. Mater. Res. A* **2009**, *90A*, 1137.
[169] C. M. Li, C. Vepari, H. J. Jin, H. J. Kim, D. L. Kaplan, *Biomaterials* **2006**, *27*, 3115.
[170] X. S. Dai, S. Shivkumar, *Mat. Sci. Eng. C-Bio. S* **2008**, *28*, 336.
[171] D. P. Cormode, T. Skajaa, Z. A. Fayad, W. J. M. Mulder, *Arterioscl. Throm. Vas.* **2009**, *29*, 992.
[172] D. P. Cormode, T. Skajaa, Z. A. Fayad, W. J. Mulder, *Arterioscler. Thromb. Vasc. Biol.* **2009**, *29*, 992.
[173] C. Corot, P. Robert, J. M. Idee, M. Port, *Adv. Drug Deliver Rev.* **2006**, *58*, 1471.
[174] M. F. Bellin, S. Zaim, E. Auberton, G. Sarfati, J. J. Duron, D. Khayat, J. Grellet, *Radiology* **1994**, *193*, 657.
[175] P. Walczak, J. Zhang, A. A. Gilad, D. A. Kedziorek, J. Ruiz-Cabello, R. G. Young, M. F. Pittenger, P. C. M. Van Zijl, J. Huang, J. W. M. Bulte, *Stroke* **2008**, *39*, 1569.
[176] M. G. Harisinghani, J. Barentsz, P. F. Hahn, W. M. Deserno, S. Tabatabaei, C. H. van de Kaa, J. de la Rosette, R. Weissleder, *New Engl. J. Med.* **2003**, *348*, 2491.
[177] F. Hyafil, J. P. Laissy, M. Mazighi, D. Tchetche, L. Louedec, H. Adle-Biassette, S. Chillon, D. Henin, M. P. Jacob, D. Letourneur, L. J. Feldman, *Arterioscl. Throm. Vas.* **2006**, *26*, 176.
[178] C. Corot, X. Violas, P. Robert, G. Gagneur, M. Port, *Invest. Radiol.* **2003**, *38*, 311.
[179] Z. Medarova, W. Pham, C. Farrar, V. Petkova, A. Moore, *Nat. Med.* **2007**, *13*, 372.
[180] S. E. Skrabalak, J. Y. Chen, Y. G. Sun, X. M. Lu, L. Au, C. M. Cobley, Y. N. Xia, *Accounts Chem. Res.* **2008**, *41*, 1587.
[181] C. C. Fu, H. Y. Lee, K. Chen, T. S. Lim, H. Y. Wu, P. K. Lin, P. K. Wei, P. H. Tsao, H. C. Chang, W. Fann, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 727.
[182] M. Hikage, K. Gonda, M. Takeda, T. Kamei, M. Kobayashi, M. Kumasaka, M. Watanabe, S. Satomi, N. Ohuchi, *Nanotechnology* **2010**, *21*, 185103.
[183] M. Nakajima, M. Takeda, M. Kobayashi, S. Suzuki, N. Ohuchi, *Cancer Sci.* **2005**, *96*, 353.
[184] X. Yu, S. K. Song, J. J. Chen, M. J. Scott, R. J. Fuhrhop, C. S. Hall, P. J. Gaffney, S. A. Wickline, G. M. Lanza, *Magn. Reson. Med.* **2000**, *44*, 867.
[185] F. Tang, F. He, H. C. Cheng, L. D. Li, *Langmuir* **2010**, *26*, 11774.
[186] Y. Amemiya, T. Tanaka, B. Yoza, T. Matsunaga, *J. Biotechnol.* **2005**, *120*, 308.
[187] A. Vaseashta, D. Dimova-Malinovska, *Sci. Technol. Adv. Mat.* **2005**, *6*, 312.
[188] J. Kneipp, H. Kneipp, B. Wittig, K. Kneipp, *Nanomed.-Nanotechnol.* **2010**, *6*, 214.
[189] T. Vo-Dinh, L. R. Allain, D. L. Stokes, *J. Raman Spectrosc.* **2002**, *33*, 511.
[190] D. J. Maxwell, J. R. Taylor, S. M. Nie, *J. Am. Chem. Soc.* **2002**, *124*, 9606.
[191] Y. H. Yang, H. F. Yang, M. H. Yang, Y. L. Liu, G. L. Shen, R. Q. Yu, *Anal. Chim. Acta* **2004**, *525*, 213.
[192] R. S. Gaster, D. A. Hall, S. X. Wang, *Nano Lett.* **2011**, (E-pub ahead of print, DOI: 10.1021/nl1026056.).
[193] Y. Cui, Q. Q. Wei, H. K. Park, C. M. Lieber, *Science* **2001**, *293*, 1289.
[194] M. R. Park, M. K. Banks, B. Applegate, T. J. Webster, *Int. J. Nanomed.* **2008**, *3*, 497.
[195] S. Sirivisoot, T. J. Webster, *Nanotechnology* **2008**, *19*, 295101.
[196] D. G. Anderson, J. A. Burdick, R. Langer, *Science* **2004**, *305*, 1923.
[197] T. D. Pollard, J. Berro, *J. Biol. Chem.* **2009**, *284*, 5433.
[198] H. J. Gao, B. H. Ji, I. L. Jager, E. Arzt, P. Fratzl, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 5597.
[199] T. E. Karakasidis, C. A. Charitidis, *Mat. Sci. Eng. C-Bio. S* **2007**, *27*, 1082.
[200] M. J. Buehler, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 12285.
[201] a) M. J. Buehler, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 12285; b) M. J. Dalby, N. Gadegaard, M. O. Riehle, C. D. W. Wilkinson, A. S. G. Curtis, *Int. J. Biochem. Cell B* **2004**, *36*, 2005.
[202] E. Genove, C. Shen, S. Zhang, C. E. Semino, *Biomaterials* **2005**, *26*, 3341.
[203] J. Lu, M. P. Rao, N. C. MacDonald, D. Khang, T. J. Webster, *Acta Biomater.* **2008**, *4*, 192.
[204] R. Ross, *N. Engl. J. Med.* **1999**, *340*, 115.
[205] H. J. Shin, C. H. Lee, I. H. Cho, Y. J. Kim, Y. J. Lee, I. A. Kim, K. D. Park, N. Yui, J. W. Shin, *J. Biomater. Sci. Polym. Ed.* **2006**, *17*, 103.
[206] F. Yang, R. Murugan, S. Wang, S. Ramakrishna, *Biomaterials* **2005**, *26*, 2603.
[207] S. Nomura, H. Kojima, Y. Ohyabu, K. Kuwabara, A. Miyauchi, T. Uemura, *Jpn. J. Appl. Phys. 2* **2005**, *44*, L1184.
[208] M. Biondi, F. Ungaro, F. Quaglia, P. A. Netti, *Adv. Drug Deliver Rev.* **2008**, *60*, 229.
[209] D. A. Harrington, E. Y. Cheng, M. O. Guler, L. K. Lee, J. L. Donovan, R. C. Claussen, S. I. Stupp, *J. Biomed. Mater. Res. A* **2006**, *78A*, 157.

[210] J. Kisiday, M. Jin, B. Kurz, H. Hung, C. Semino, S. Zhang, A. J. Grodzinsky, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 9996.
[211] H. Hosseinkhani, M. Hosseinkhani, H. Kobayashi, *J. Bioact. Compat. Pol.* **2006**, *21*, 277.
[212] C. S. Hajicharalambous, J. Lichter, W. T. Hix, M. Swierczewska, M. F. Rubner, P. Rajagopalan, *Biomaterials* **2009**, *30*, 4029.
[213] D. C. Miller, A. Thapa, K. M. Haberstroh, T. J. Webster, *Biomaterials* **2004**, *25*, 53.
[214] M. A. Pattison, S. Wurster, T. J. Webster, K. M. Haberstroh, *Biomaterials* **2005**, *26*, 2491.
[215] G. J. Bakeine, A. Bertolotti, C. Zennaro, G. Grenci, A. Pozzato, S. Dal Zilio, M. Prasciolu, M. Carraro, L. Businaro, M. Tormen, M. Alessiani, R. Nano, P. Dionigi, *Microelectron. Eng.* **2009**, *86*, 1468.
[216] W. J. Li, R. Tuli, C. Okafor, A. Derfoul, K. G. Danielson, D. J. Hall, R. S. Tuan, *Biomaterials* **2005**, *26*, 599.
[217] S. Tsukiyama, M. Matsushita, M. Tanaka, H. Tamura, S. Todo, S. Yamamoto, M. Shimomura, *Jpn. J. Appl. Phys.* **2008**, *47*, 1429.
[218] K. Burns, C. Yao, T. J. Webster, *J. Biomed. Mater. Res. A* **2009**, *88A*, 561.
[219] A. M. Lipski, C. J. Pino, F. R. Haselton, I. W. Chen, V. P. Shastri, *Biomaterials* **2008**, *29*, 3836.
[220] A. Cohen, P. Liu-Synder, D. Storey, T. J. Webster, *Nanoscale Res. Lett.* **2007**, *2*, 385.
[221] R. L. Price, K. Ellison, K. M. Haberstroh, T. J. Webster, *J. Biomed. Mater. Res. A* **2004**, *70A*, 129.
[222] P. Sharma, S. Brown, G. Walter, S. Santra, B. Moudgil, *Adv. Colloid Interface Sci.* **2006**, *123–126*, 471.
[223] J. M. Garguilo, B. A. Davis, M. Buddie, F. A. M. Kock, R. J. Nemanich, *Diam. Relat. Mater.* **2004**, *13*, 595.
[224] P. K. Jain, I. H. El-Sayed, M. A. El-Sayed, *Nano Today* **2007**, *2*, 18.
[225] W. W. Zhong, *Anal. Bioanal. Chem.* **2009**, *394*, 47.

