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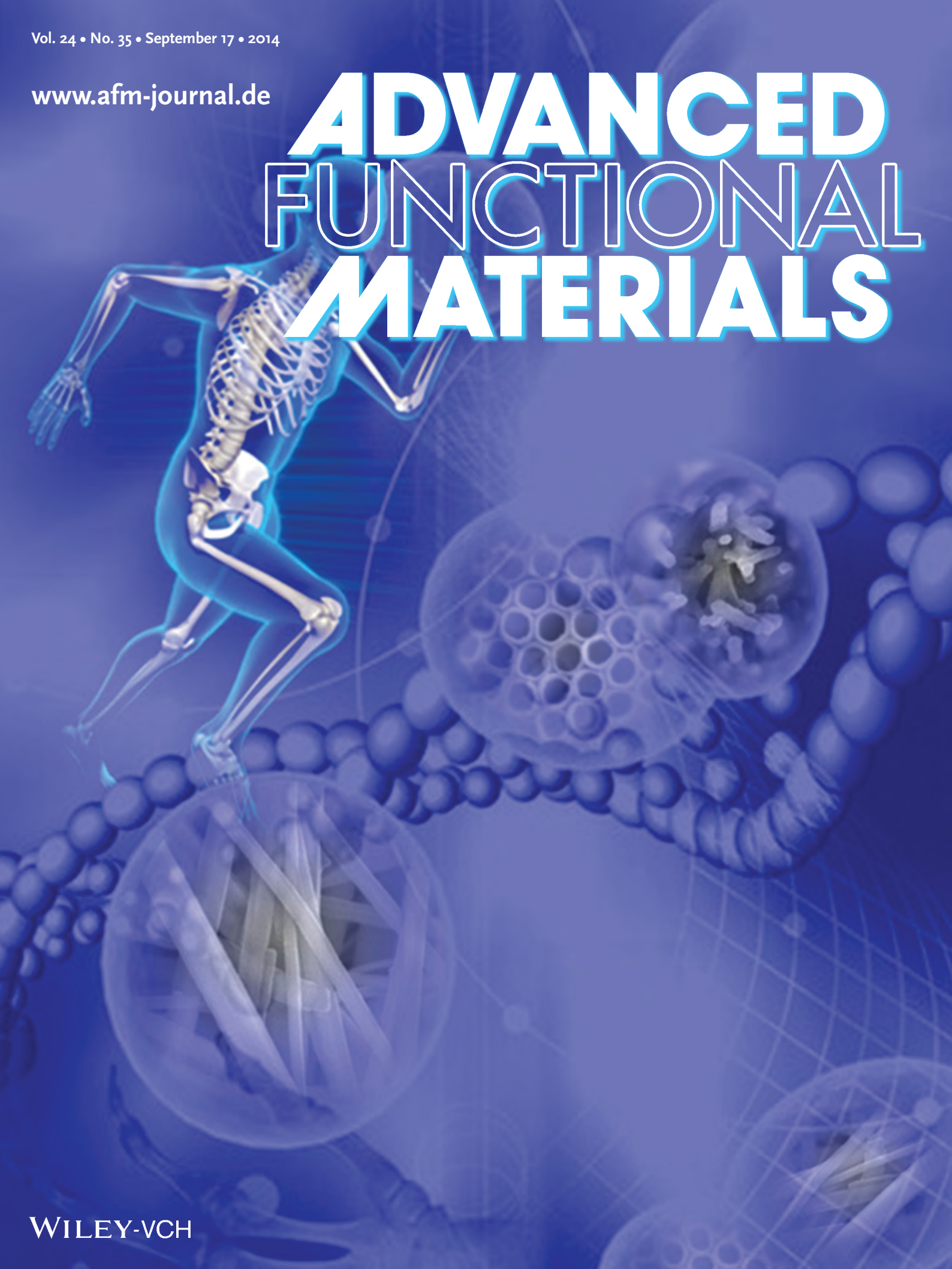

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Functionalized TiO_2 Based Nanomaterials for Biomedical Applications

Shuilin Wu, Zhengyang Weng, Xiangmei Liu, K.W. K. Yeung, and Paul. K. Chu**

As baby boomers age, diabetes mellitus, cancer, osteoarthritis, cardiovascular diseases, and orthopedic disorders are more widespread and the demand for better biomedical devices and functional biomaterials is increasing rapidly. Owing to the good biocompatibility, chemical stability, catalytic efficiency, plasticity, mechanical properties, as well as strength-to-weight ratio, titanium dioxide (TiO_2) based nanostructured materials are playing important roles in tissue reconstruction and diagnosis of these diseases. Here, recent advance in the research of nanostructured TiO_2 based biomaterials pertaining to bone tissue engineering, intravascular stents, drug delivery systems, and biosensors is described.

1. Introduction

Titanium dioxide (TiO_2) has attracted much attention since the discovery of its excellent photocatalytic performance in water splitting when illuminated by ultraviolet (UV) light.[1–3] It has been followed by extensive research on the fabrication, structure, and applications of nanostructured TiO_2 based materials.[4–6] As a result, nanostructured TiO_2 materials with various morphologies such as nanoparticles, nanorods, nanowires, nanotubes, and other hierarchical nanostructures have been produced by different techniques including hydrothermal/solvothermal processes,[7–11] sol-gel method,[12–14] anode oxidation,[15–18] vapor deposition,[19,20] microwave,[21] sonochemistry,[22] and so on. The materials have been applied in a myriad of areas involving energy and environmental research as well as biomedical engineering owing to their unique characteristics, comprising low density, large strength-to-weight ratio, photochemical stability, high catalytic efficiency, excellent biocompatibility, good corrosion resistance, as well as excellent mechanical properties.[23–31]

Functionalized TiO_2 based nanostructured materials have positive effects in many biomedical applications such as bone scaffolds, vascular stents, drug delivery systems, and biosensors. For example, nano-TiO_2 scaffolds accelerate the rate of apatite formation and enhance osteoblast adhesion, proliferation, and differentiation.[32–36] Possessing good blood compatibility and anti-coagulation characteristics, TiO_2 nanotube arrays are promising for vascular implants, and nanostructured TiO_2 has been widely reported as drug carriers as well.[37,38] In particular, TiO_2 nanotubes have been shown to be a superior platform for local drug delivery due to their excellent biocompatibility, controllable dimensions, surface chemistry, and large surface-to-volume ratio.[39–42] By changing the nanotube diameter, wall thickness, and length, the release kinetics of specific drugs can be tailored to achieve stable and sustained release.[43] Generally, the requirements for biosensors are good reproducibility and sensitivity to specific chemical and biochemical compounds, and owing to its high sensitivity to glucose, hydrogen peroxide, and cancer cells, nano-TiO_2 has been extensively studied in biosensing applications, for example, detection of blood glucose in diabetes mellitus patients and early monitoring of cancer.[44–46]

As a result of the increasing population of aging baby boomers, diseases such as diabetes mellitus, cancer, osteoarthritis, cardiovascular diseases, and orthopedic disorders are increasing, and therefore biomaterials with better performance are demanded and the unique properties of nanostructured titania are attractive to many biomedical applications. The objective of this article is to review recent advance and development of TiO_2 based nanomaterials in biomedical applications with emphasis on bone tissue engineering, intravascular stents, drug delivery systems, and biosensors.

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2. Bone Reconstruction

Bone tissue engineering offers an alternative approach for the repair and regeneration of lost bones,[47] and bone scaffolds must satisfy three requirements, namely good mechanical support, sufficient space for bone seed cells, and delivery of growth factors. The architecture determines the ultimate shape of the newly formed bone tissues.[48,49] An ideal scaffold for bone engineering should fulfil the following number of criteria:[50,51] a) biocompatibility to enable cell attachment, differentiation, and proliferation; b) osteoconduction and osteoinduction; c) biodegradability at a rate matching the rate of new tissue formation; d) mechanical properties (for example, the strength of the scaffolds should be sufficient to provide mechanical stability in load bearing sites prior to regeneration of new tissues), and e) interconnected porous structure with a porosity of over 90% and a pore size between 300 and 500 μm for optimal cell penetration, tissue growth, and vascularization.

2.1. Nanostructured TiO_2 Based Bone Implant Materials

TiO_2 makes a good scaffold because it can satisfy many of the aforementioned demands. For example, TiO_2 is biocompatible,[52] enhances ingrowth of bone and vascular tissues,[53] possesses antibacterial properties,[54,55] and delivers good osteoconductive performance.[56,57] Osteoconductivity is important for scaffolds as this property affects the integration between the scaffold and bone tissues.[58,59] In this respect, synthetic TiO_2 scaffolds show a high porosity, excellent interconnectivity, and sufficient mechanical strength boding well for load-bearing orthopedic and dental applications.[60,61] Owing to the inherently high compressive strength of ceramic TiO_2 in comparison to other osteoconductive materials such as calcium phosphate ceramics (CaP), bioactive glass, and CaP/polymer composites, TiO_2 can provide better mechanical strength to the scaffold even at high porosity. A compressive strength of 2.5 MPa has been observed for TiO_2 scaffolds with a porosity of 85%, and the strength can be retained after implantation due to the non-resorbable nature of TiO_2.[61] In contrast, the values obtained for CaP and CaP/polymer composite scaffolds with a similar porosity are generally in the range of 0.1–1 MPa[62–64] and below 2 MPa, which is the minimum value of trabecular bone.[65]

Nanostructured materials play a fundamental role in orthopedic research because natural bone has a structural hierarchy on the nanometer scale and is composed of nanostructured collagen fibrils and apatite nanocrystals.[66–68] Hence, incorporation of nano-topographical features that mimic the organization of bone is a burgeoning research area in tissue engineering,[69] and TiO_2 based nanomaterials have been used as hierarchical structures in scaffolds to foster bone regeneration.[70–73] A hierarchical structure composed of micro- and nanoscale constituents offers a suitable surface topography for cell functions as the natural extracellular matrix is mimicked.[71] There have been attempts to fabricate micro/nanostructures for bone reconstruction. For example, in comparison with untreated titanium, better adhesion and spreading of osteoblasts can be achieved on the hierarchically micro/nanotextured titanium surface of TiO_2 nanotubes with diameters ranging from 15 to 80 nm.[71]

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The intracellular total protein content on the acid-etched (5 V) anodized surface and the alkaline phosphatase (ALP) activity on the acid-etched (20 V) anodized surface increase by about 30% relative to the smooth surface, and collagen secretion goes up to about 115 and 125% on the 5 and 20 V anodized samples, respectively. Enhancement of multiple cell functions is expected to lead to faster bone maturation around the implants without compromising the bone mass. In addition, the hierarchical

micro/nanotextured surfaces can retain the mechanical interlocking ability of the microtopography, thereby strengthening the bonding between the implants and surrounding tissues.[71] One dimensional nanotitanate wires and belts have been shown to grow almost perpendicularly to the metallic substrate, thus mimicking the lowest level of hierarchical organization of natural bone. The resulting surface displays superhydrophilicity, favors deposition of hydroxyapatite, and accelerates cell attachment and proliferation.[72] A recent study reveals that the diameter of the TiO_2 nanotubes of the nanostructured titanium surface influences the cell behavior.[73] Even though all nanotextured surfaces are observed to induce the osteogenic differentiation of mesenchymal stem cells (MSCs) in the absence of osteogenic supplements, hierarchical hybrid micropits and nanotubes with an inner diameter of about 80 nm can better promote MSC proliferation and osteogenic differentiation simultaneously.[73]

Nanostructured TiO_2 has been combined with inorganic materials, such as SiO_2 and Al_2O_3-SiO_2,[74,75] and organic materials, such as different kinds of polymers,[76–80] to produce bone scaffolds that have excellent biocompatibility, good mechanical properties, and enhanced osteoconductivity. For example, SiO_2-TiO_2 nanofibers with different SiO_2 contents have been prepared by Wang et al.,[74] and their findings suggest that a larger diameter, degree of crystallization, and titania content enhance osteodifferentiation of MSCs.[74] Furthermore, scaffolds composed of TiO_2 based ternary composites, such as Al_2O_3-SiO_2-TiO_2, have been prepared by Naga et al.[75] In comparison with materials without scaffolds, in vitro studies show that MG-63 osteoblast-like cells attach and spread well on the new scaffold and the ALP activity is higher in the serum of the implanted animals. In vivo studies disclose that the TiO_2 based scaffolds implanted into bone defects are highly bioactive. The histological analysis shows that after 5 months the femur defects are reconstructed by newly formed bone tissues. This scaffold also possesses a higher bending strength of 7.1 MPa even at a higher porosity of 66%. The excellent properties including bioactivity, bone conductivity, and mechanical stability are ascribed to the addition of small amounts of titania and silica into the scaffolds.[75] Some TiO_2/polymer composites, such as TiO_2/polylactic acid (PLA), TiO_2/poly(lactic-co-glycolic acid) (PLGA), and TiO_2/poly (ether-ether ketone) (PEEK) show controllable swelling and degradation compared to the pure polymeric scaffolds, and in vitro tests indicate that osteoblasts attach well to the exposed area and grow into the inner pores of the scaffolds.[77–80]

2.2. Modulation of Cell and Tissue Behavior

The two types of cells that orthopedic implants are in contact with are osteoblast and osteo-progenitor cells (MSCs). Osteoblast cells are mature adult cells specific to the bone tissue and responsible for building bone and depositing minerals to make up the bone matrix. MSCs are bone marrow derived pluripotent cells with the capacity to differentiate into different cell types including osteoblasts, chondrocytes, and adipocytes.[31] In terms of the effects of nanostructured materials on the behavior of osteoblast cells and MSCs, it has been shown that TiO_2 bone scaffolds with different nanostructures exhibit enhanced effects on the growth rate and bone forming ability.[31,81–95] **Table 1** highlights the results obtained from some recent studies revealing the different influence of the TiO_2 nanostructures on osteoblasts and MSCs. In addition to the dimensions of the nanostructures, surface features such as the geometry, spacing, pattern, and symmetry have been shown to affect the cell response on the biomaterials.[77–80,86–95]

Increased osteoblast adhesion on nanograined materials was first reported in 1999, especially for TiO_2 with grain sizes between 32 and 56 nm, as shown in **Figure 1**.[81] In fact, like nanostructured TiO_2, other nanophases with similar size and structures to nanostructured titania can also present similar effects. For example, osteoblast adhesion was significantly greater on alumina with grain sizes in the range of 23–49 nm than on alumina with grain sizes in the range of 67–177 nm.[81] However, it is widely accepted that aluminum causes neurotoxicity and can induce Alzheimer's disease, which blocks the possible biomedical application of nanostructured alumina. Furthermore, titanium based alloys have been widely applied as orthopedic and dental implant materials, and as a native oxide, TiO_2 on the surface of these metallic implants has been widely used since the 1970s due to its excellent biocompatibility.[43] Since then, the cytotoxicity of TiO_2 nanomaterials has been systematically investigated from cellular to molecular levels.[87] A high concentration of TiO_2 nanoparticles (0.10 and 0.20 mg mL^{-1}) adversely affects the viability of MSCs. After culturing for 3 days in a medium with TiO_2 nanoparticles 196 nm in size, the relative cell viability decreases to 51.73% compared to the control group (without TiO_2 nanoparticles) and it is further reduced to 36.39% and 34.92% after culturing for 7 and 14 days, respectively. The average amount of vinculin in the MSCs treated with 196 nm TiO_2 nanoparticles decreases to 63.4% and 51.3% of the control level after culturing for 12 and 24 h, respectively. In addition, after 14 days the MSCs treated with 14 nm TiO_2 nanoparticles display significantly higher ALP activity than those modified with 108 and 196 nm titania nanoparticles. The results confirm that TiO_2 nanoparticles have a negative impact on the viability, adhesion, migration, proliferation, and differentiation of MSCs in a size-dependent and dose-dependent manner.[87]

Recent research demonstrates that modification of TiO_2 fibers benefits adhesion and proliferation of MG63 cells on the surface of titanium.[95] Nanofibers with a diameter of 200 nm have the best apatite formation ability and osteoblast compatibility, indicating that the surface bioactivity of titanium can be regulated by the TiO_2 fiber diameter. Other studies confirm that TiO_2 nanofibers lead to faster bone bonding between the implants and surrounding tissues in vivo and this is crucial to early bone formation on metallic implants.[96]

The cell behavior on titanium implants can be modulated by controlling the nanostructure of the titania formed on the substrate, for instance, flat TiO_2, nanotubes (NTs), and nanowires (NWs).[89] As shown in **Figure 2**, the cells attached to flat TiO_2 have a round morphology, whereas those attached to the TiO_2 NTs and NWs have a polygonal shape with extended lamellipodia. In comparison with TiO_2 nanotubes, pronounced protrusion of filopodia and increased cell attachment are observed for TiO_2 NWs. These findings suggest that TiO_2 NWs can provide a

Table 1. Summary of in vitro studies of osteoblasts and MSCs on various TiO_2 based nanomaterials.

Types of nanostructure	Methods	Cell tapes	Assessment of cell behavior	Ref.
Chitin-chitosan/nanostructured TiO_2 composites	Lyophilization technique	MG-63, L929, hMSCs	Enhanced cell-seeding.	[77]
Alginate with TiO_2 nanoneedles	Lyophilization technique	MG-63, hMSCs	Enhanced cell adhesion and proliferation.	[78]
TiO_2/PEEK nanocomposites	Codispersion using an electronic blender	MG-63	Promoted cell attachment and improved osteoblast cell spreading.	[79]
Nanophase TiO_2/PLGA composites	Solvent evaporation	Osteoblasts	Better osteoblast adhesion ability.	[80]
TiO_2 nanonodular	Sputter deposition	Osteoblasts	Enhanced attachment, spread, adhesion, proliferation, and differentiation of osteoblasts.	[86]
TiO_2 nanoparticles	Hydrothermal synthesis	MSCs	The size of nanoparticle negatively affected osteogenic differentiation.	[87]
TiO_2 nanowires	Thermal oxidation	Human osteosarcoma (HOS) cells	Increased cell adhesion and proliferation.	[88]
TiO_2 nanowires	Anodization	MG-63	Pronounced protrusion of filopodia and enhanced cell attachment, proliferation and differentiation.	[89]
TiO_2 nanofibers	Electrospinning	MC3T3-E1 cells	Facilitated a higher cellular differentiation capacity.	[90]
TiO_2 nanotubes	Anodization	Osteoblast, MSCs	30 nm diameter promoted adhesion whereas 70 to 100 nm diameter elicited stem cell elongation, induced cytoskeletal stress and selective differentiation.	[91]
TiO_2 nanotubes	Anodization	Preosteoblast (MC3T3-E1)	Cell adhesion on the diameter of 20–70 nm was better; but severely impaired on 100–120 nm. The proliferation rates increased with increasing the diameter from 20 to 120 nm.	[92]
TiO_2 nanopillars	Anodization through alumina mask	Mesenchymal stem cells (hMSCs)	15 nm high topography features resulted in the greatest cell response with bone matrix nodule forming.	[93]
TiO_2 nanofiber arrays	Electrospinning	MG-63	Higher expression of ALP on the flat side, higher osteocalcin level on the patterned side.	[94]
TiO_2 nanofiber arrays	Electrospinning	MG-63	Diameter of 200 nm improved apatite formation and cell proliferation.	[95]

favorable rough and porous surface for osteoblast cell attachment and spreading.[89] A micropit and nanonodule hybrid topography of TiO_2 on titanium implants has been prepared by Kubo et al.[86] For rat bone marrow-derived osteoblasts cultured on titanium disks, the micro-nano-hybrid topography enhances osteoblast differentiation and proliferation. These biological effects are most pronounced when the titania nanonodules in the micropits have a diameter of 300 nm.[86] Rani et al.[97] have described a simple hydrothermal method to fabricate non-periodical TiO_2 nanostructures (nanotubes, nanoleaves, and nanoneedles) on titanium implants, and these nanostructures have been evaluated for in vitro cellular response as well as in vivo osteointegration. The results show that nanoleaves (vertically aligned, non-periodical leaf-like structures with a thickness on the nanoscale) show a distinct increase in osteoblast cell proliferation, ALP activity, and collagen synthesis compared to other types of nanomorphology such as nanotubes and nanoneedles.[97]

As one of the most common nanostructures of titania, TiO_2 nanotubes have been widely used to improve the bone cell behavior.[98–102] Park et al. have shown that the vitality, proliferation, and motility of MSCs and the subsequent differentiation into bone-forming cells are influenced by the diameter of the tubular nano-TiO_2.[100] As shown in **Figure 3**, TiO_2 nanotubes with a diameter of 15 nm exhibit the best adhesion, proliferation, migration, and differentiation of MSCs, while the behavior impairs gradually as the diameter increases, and death of MSCs is observed when the value reaches 100 nm.[100] In order to clarify whether this high sensitivity of cell response is a specific phenomenon of stem cells or reflects a universal cell behavior, Park et el. have explored the nanoscale response of two main bone cells: osteoblasts and osteoclasts.[101] Their results show that the response of both bone-forming/resorbing cells and stem cells is sensitive to the nanoscale surface topography, and 15 nm is the universal geometric constant to support cell adhesion and differentiation.[101] However, there is still controversy on the effects of the titania nanotube size on cell behavior. As shown in **Figure 4**,[102] Brammer et al. have obtained results contrary to those of Park et al.[100,101] Increasing the nanotube diameter leads to increased elongation/stretching of the cell bodies, enhanced levels of alkaline phosphatase, and greater bone-forming ability. In particular, large (100 nm diameter) nanotubes have the greatest potential as bone implant materials because they induce more osteoblast elongation (aspect ratio of 11:1) and a higher up-regulated level of the ALP activity than the smaller (30–70 nm diameter) nanotubes.[102]

The crystalline structure of nano-TiO_2 influences the cell behavior, and osteoblasts show better adhesion and proliferation on the nanotopographical anatase phase than on the rutile or

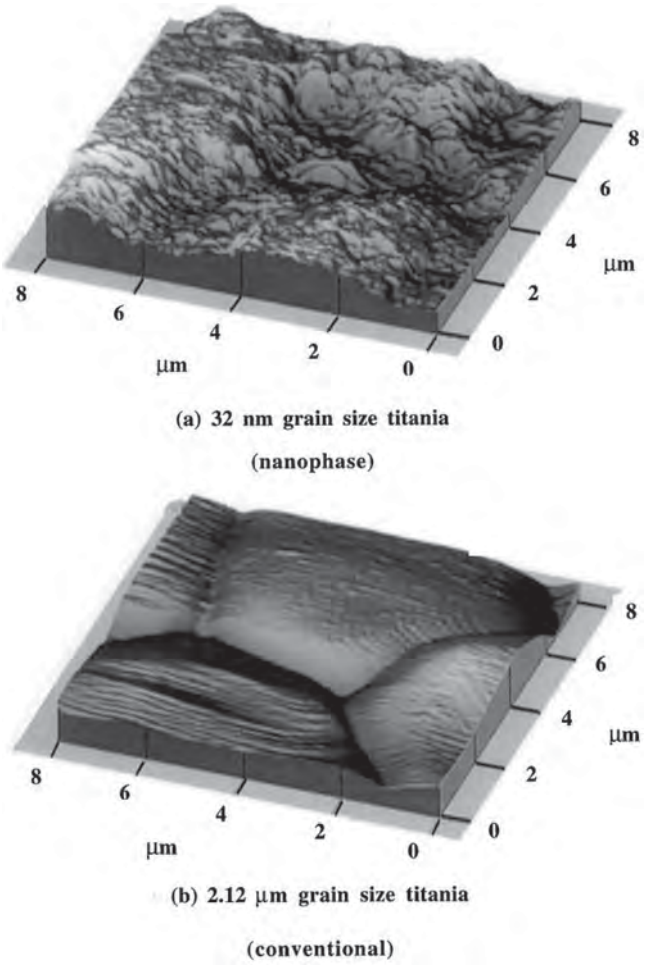

Figure 1. Atomic force micrographs: a) nanophase and b) conventional titania. Note the unique surface properties of the nanophase materials which enhance bone cell functions. Reproduced with permission.[81] Copyright 1999, Elsevier.

amorphous one.[103,104] As shown in **Figure 5**, proliferation and mineralization of preosteoblasts cultured on anatase or anatase/rutile nanotube layers are significantly higher than on smooth and amorphous nanotube layers, providing evidence that the crystal structure can override chemical effects, thus playing a big role in cell proliferation and mineralization.[104]

To summarize, TiO_2 with different morphology and micro/nanostructure affects the behavior of bone cells differently although the exact mechanism is still debatable. Research activities have heretofore focused on the biological behavior of bone cells on different TiO_2 nanostructures, but researchers are making efforts to unravel the underlying mechanism, while realization of the ideal nanostructured TiO_2 for orthopedic and dental applications may be distant in the future.

3. Intravascular Stents

Thrombosis and restenosis are two major and common complications associated with vascular prosthetics. Thrombosis is caused by inadequate migration and proliferation of endothelial cells (ECs) which exist inside the blood vessels, and restenosis is caused by proliferation of vascular smooth muscle cells (VSMCs) which surround the EC layer.[105] Current strategies to mitigate these problems, such as drug-eluting stents, focus on reducing VSMC proliferation. However, the therapy is often concomitant with a high risk of late thrombosis,[106] because drug-eluting coatings also inhibit EC function, migration, and proliferation leading to poor re-endothelialization of the lumen.[107,108] ECs prevent not only blood coagulation but also VSMC proliferation.[109] Therefore, rapid re-endothelialization and normal EC function are crucial to vascular implants, and in order to minimize complications an ideal stent should spur EC migration, proliferation, and function while reducing VSMC proliferation.[110]

Recent studies suggest that TiO_2 nanotube arrays are promising for vascular implants.[111–116] Experiments conducted on vascular cells show that the nanotubes may enhance the EC

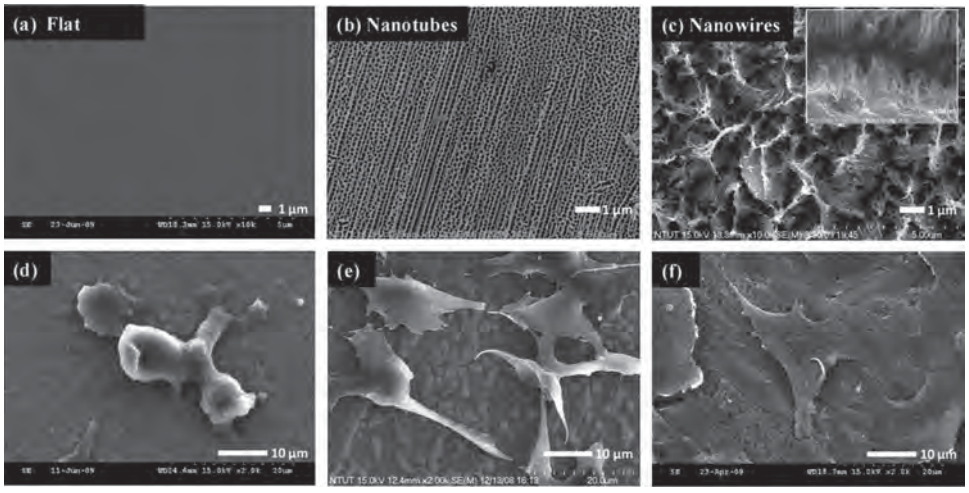

Figure 2. SEM images of three different kinds of TiO_2 surfaces: a) flat, b) NTs and, c) NWs; cell (osteoblast) morphology: d) flat, e) NTs, and f) NWs. Reproduced with permission.[89]

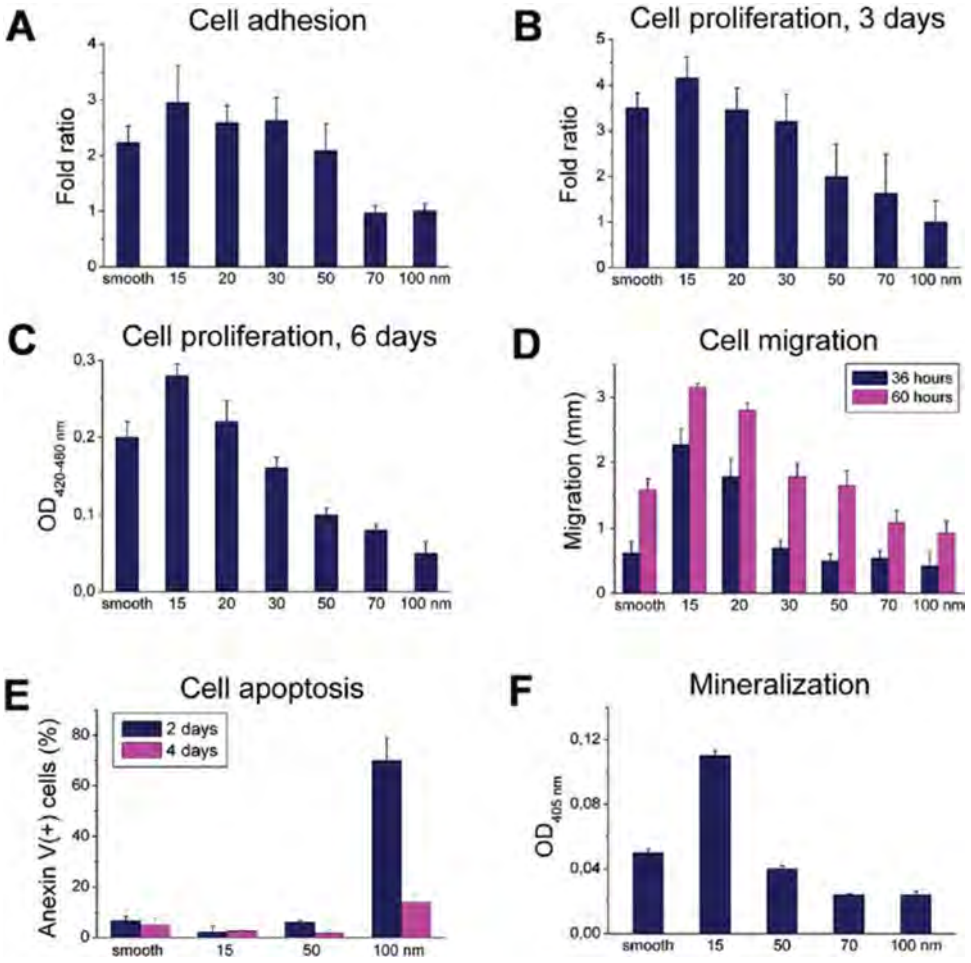

Figure 3. Relationship between cellular response and nanoscale distances. The green fluorescent protein (GFP)-labeled rat mesenchymal stem cells in the culture medium containing 2% fetal calf serum are plated on the titanium chips coated with TiO_2 nanotubes with six different diameters with the polished TiO_2-coated chips as the control ("smooth"). A) For the measurement of cell adhesion, the samples are washed 1 h after cell plating and adherent cells are counted by a fluorescence microscope. The cell proliferation rates are determined by counting the adherent cells after 3 days (B) and by a colorimetric assay (WST assay) 6 days after cell plating (C). D) For the measurement of cell migration in a wounding assay, the cells are plated at a density of 50 000 cells cm^{-2}, and 3 h later a track 3.4 mm wide is created in the confluent cell layer. The cell motility is evaluated by measuring the remaining width after 36 and 60 h. E) Apoptosis is analyzed by staining cells with Annexin V-FITC 2 and 4 days after plating (cell density of 5000 cm^{-2}). The surface-labeled cells are counted using a fluorescence-activated cell sorter. F) Osteogenic differentiation of stem cells after 2 weeks of cell culture in the osteogenic differentiation medium is assessed by staining the calcium phosphate mineral deposition with alizarin red and colorimetric analysis of the dye. Reproduced with permission.[100] Copyright 2007, American Chemical Society.

motility, proliferation, and function, while decreasing the VSMC proliferation.[113,114] TiO_2 nanotubes are biocompatible and can be produced with precise dimensions to enhance the bioactive properties to reduce both the thrombotic and restenotic risk. The effects of nanotubular TiO_2 on the response of vascular cells, ECs, and VSMCs have been studied by Peng et al.[114] To determine how nanotube arrays affect the morphology of ECs and VSMCs, bovine aortic endothelial cells (BAECs) and mouse vascular smooth muscle cells (MOVASs) are cultured on both nanotubular and flat surfaces. As shown in **Figure 6**, besides enhancing the expression of smooth muscle α-actin, the nanotubular surface improves EC proliferation and inhibits VSMC proliferation.[114] The data suggest that engineering of the proper titania nano-topographical cues influences both the EC and VSMC behavior, and the technique may be useful for stents or in other vascular applications. The diameter of the titania nanotubes plays an important role in stimulating the differentiation of MSCs into ECs and VSMCs.[115] For example, 15 nm titania nanotubes produce a substantially stronger stimulation to differentiation of mesenchymal cells into ECs and VSMCs than those with dimensions of 70–100 nm, while high rates of apoptosis can only be observed for the 100 nm nanotubes. Moreover, EC adhesion, proliferation, and motility are several fold higher on the 15 nm than on the 100 nm nanotubes.[115] Further research indicates a clear dominance of the nanoscale geometry on EC behavior over surface chemistry and crystallinity of the TiO_2 nanotubes. Hence, fine-tuning of the TiO_2 surface on the nanoscale is critical to optimize the EC and VSMC response on vascular implants.[115]

Since the ideal vascular implant should prevent VSMC proliferation and favor EC proliferation, migration, and quiescence, TiO_2 nanotube arrays may be one of the promising candidates

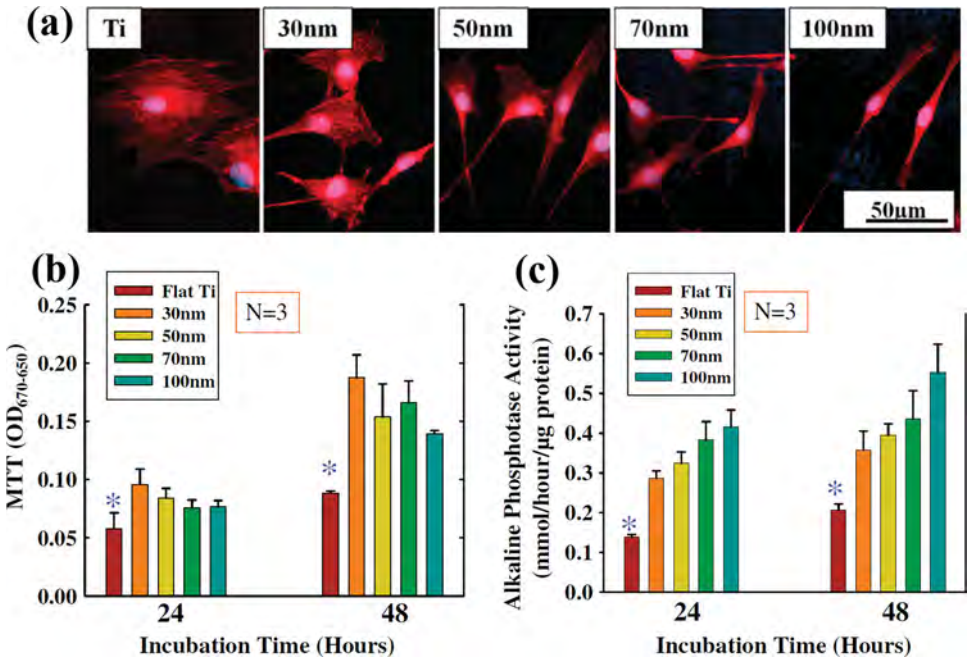

Figure 4. a) Immunofluorescent images of cytoskeletal actin (red) and nucleus-staining diamidino-2-phenylindole (DAPI, blue) for osteoblasts on Ti and 30, 50, 70, 100 nm diameter TiO_2 nanotube surfaces after 24 h of culture incubation; b) MTT assay data showing the optical density (OD) of the reaction product of the MTT working solution; c) ALP activity of osteoblast cells cultured on flat Ti and TiO_2 nanotubes with various dimensions after incubation for 24 and 48 h. Reproduced with permission.[102] Copyright 2009, Elsevier.

for next-generation stents or vascular devices because of the tailored response of ECs and VSMCs on the materials.

4. Drug Delivery Systems

Medical devices such as orthopedic implants, dental implants, and vascular stents may require subsequent drug therapy to prevent infection or minimize inflammation. Drugs released directly from the implant surface rather than systemically can reduce unnecessary side effects. The aim of controlled drug delivery is to (i) administer the appropriate amount of drug to the relevant sites in the human body and (ii) regulate the drug delivery profile in order to optimize the therapeutic benefits.[117] TiO_2 nanomaterials are suitable for drug-eluting implants and their drug delivery properties spur potential applications in the biomedical field.[117] Various types of nanostructured TiO_2 have been studied as drug carriers, for instance, nanoporous TiO_2,[117,118]

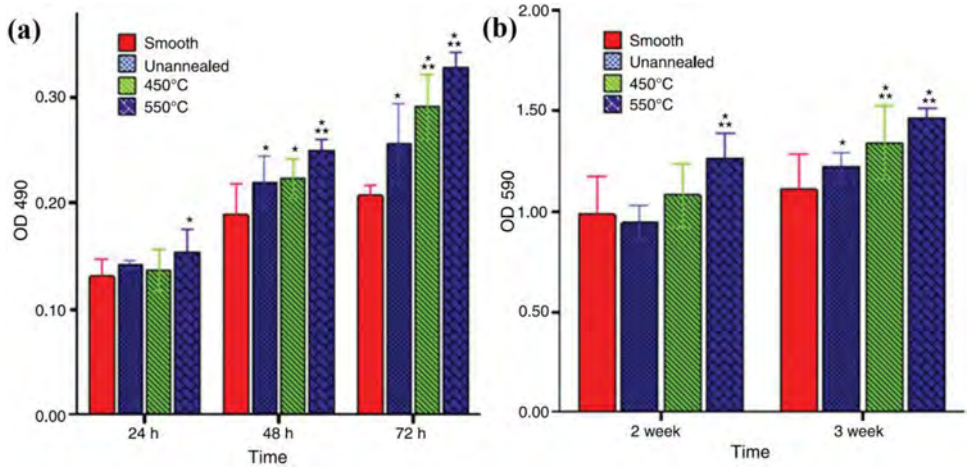

Figure 5. a) MTT assay data showing the viability and proliferation of osteoblasts cultured on smooth titania layers, unannealed nanotube layers, and annealed nanotube layers (450 and 550 °C) after incubation for 24, 48, and 72 h. b) Mineralization of MC3T3-E1 preosteoblasts cultured on smooth titania layers, unannealed nanotube layers, and annealed nanotube layers (450 and 550 °C) after 2 and 3 weeks. The titania nanotubes have an amorphous structure after formation and converted to anatase and anatase/rutile after annealing at 450 and 550 °C, respectively. The error bars represent the standard deviations for the three samples for each data point. *$P < 0.05$ compared for the smooth sample and **$P < 0.05$ compared to the unannealed nanotubes. Reproduced with permission.[104]

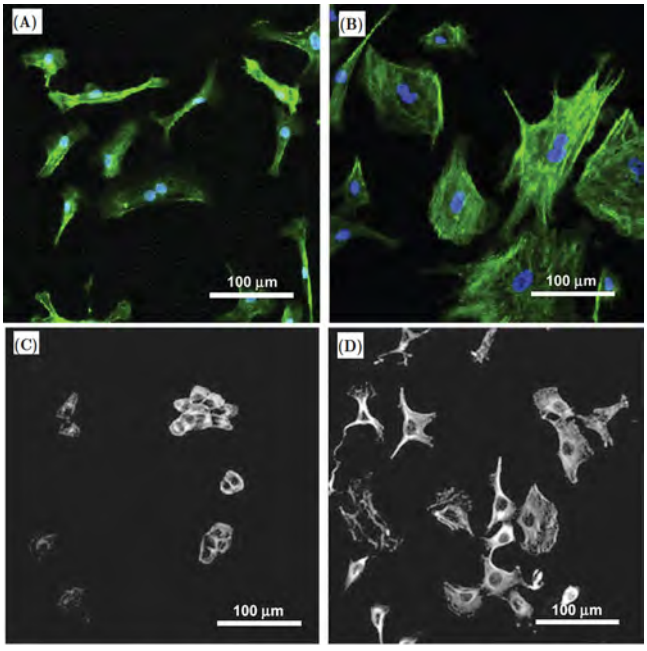

Figure 6. F-actin (green) and nuclear (blue) stains of BAEC grown on A) nanotubular TiO_2 versus B) flat surfaces for 24 h. F-actin staining of MOVASs grown on C) nanotubular TiO_2 versus D) flat surfaces after 24 h. Reproduced with permission.[114] Copyright 2009, Elsevier.

mesoporous TiO_2 nanospheres,[119] and TiO_2 nanotubes.[120–122] Owing to the large open volume, tubular nano-titania has great potential as carriers for drug loading and release.[123]

Different kinds of drugs such as antibacterial agents, anticancer drugs, and genes can be delivered by nanostructured TiO_2. After an initially high release rate, a nearly constant release rate can be obtained over a long period of time. The new drug delivery systems composed of TiO_2 nanomaterials have the following advantages: high delivery efficiency, precise control of the dose, prolonged exposure to drugs up to months, and reduced toxicity.

4.1. Antibacterial Agent Delivery

Bacterial infection is one of the common complications in orthopedics and patients are often subjected to an antibiotic drug therapy after the initial surgery. Systemic antibiotics delivery has some drawbacks, such as systemic toxicity and limited bioavailability, and so a local antibiotics therapy is the preferred way of drug administration. In this respect, nano-TiO_2 has received much attention due to its unique properties including good stability, environmental friendliness, safety, and broad-spectrum antibiosis.[124] Local delivery of antibiotics from implants comprising TiO_2 nanomaterials at the surgical site has been studied.

4.1.1. Carrier of Inorganic Antimicrobial Agents

Metals such as silver (Ag), copper (Cu), and zinc (Zn) are well known inorganic antimicrobial agents which have been widely used in vitro and in vivo,[125–128] and favorable effects have been shown.[129–131] Recently, Ag nanoparticles as one of the broad spectrum antibiotics have attracted considerable interest.[132–138] Owing to the high efficiency of TiO_2 as an antibiotics carrier, Ag/TiO_2 shows high antibacterial activity and Ag has been incorporated into titania in various structures, for example, Ag doped mesoporous TiO_2,[133,134] nanostructured Ag-TiO_2 thin films,[135] nano-Ag/TiO_2 nanotubes,[136] nanostructured Ag-TiO_2,[137] and TiO_2@C/Ag core-shell composites.[138] The high antibacterial activity is due to the large specific surface area, and the amount of released Ag^+ ions can be modulated by immobilizing them inside the pores in order to obtain sustained antibacterial effects.[137]

The antibacterial properties of Cu embedded TiO_2 have also been studied.[139–142] Hang et al. have used magnetron sputtering to fabricate TiCu films and produced Cu-Ti-O nanotubes (NTs) by anodization.[139] The Cu-Ti-O NTs with 1 at% Cu show desirable antibacterial activity and cytocompatibility.[139] The optimal Cu content is 3.5 at% from the perspective of antibacterial performance against *S. aureus*.[140,141] This nanocomposite antibacterial system exhibits good antibacterial activity even in the absence of light, suggesting that the Cu/TiO_2 nanocomposite may be used in vivo.[142]

The antibacterial properties of Zn-doped TiO_2 nanomaterials have also been investigated in recent years.[143–146] Zn-doped Ti-based nanofibers promote antimicrobial effects against *Staphylococcus aureus* and *Escherichia coli* due to cell membrane disruption and cytoplasma leakage.[143] The antibacterial activity of $ZnCl_2/TiO_2$, $Zn(Ac)_2/TiO_2$, $Zn(NO_3)_2/TiO_2$, and $ZnSO_4/TiO_2$ follows the subsequent order: $ZnSO_4 > ZnCl_2 > Zn(NO_3)_2 > Zn(Ac)_2$, and the highest antibacterial activity observed for $ZnSO_4/TiO_2$ is possibly ascribed to the improved surface acidity.[144] It has also been reported that zinc can be incorporated into TiO_2 coatings to achieve a good bacterial inhibition ability, and the better antibacterial activity of Zn-incorporated TiO_2 coatings may be attributed to the fact that Zn ions can be slowly and constantly released from the coatings.[145]

4.1.2. Carrier of Organic Antimicrobial Agents

In addition to metals and associated cations, many organic antimicrobial agents can be immobilized on TiO_2 nanomaterials or embedded in TiO_2 nanostructures to mitigate bacterial infection and inflammation. For example, vancomycin, penicillin, gentamicin, antimicrobial peptides, and indomethacin have been used as target drugs because they are commonly used to diminish inflammation and inhibit the growth of bacteria.[147–152]

Vancomycin is an effective drug for staphylococcal infection and can be used to modify titanium surfaces to determine the effect of surface morphology on the drug loading and release profiles.[147] Sustained drug release using vancomycin as the model molecule from mesoporous TiO_2 coatings with a wormhole-like architecture has been studied.[148] The materials exhibit an improved therapeutic performance and have potential applications in orthopedics, dentistry, and drug delivery.[148]

Nanotubular TiO_2 has been loaded with penicillin by Yao et al. using co-precipitation of the drug and calcium phosphate crystals on nanostructured titania.[149] This delivery system shows a delayed release of drugs for up to 3 weeks. In addition, contrary to conventional thinking that penicillin-based drug release should

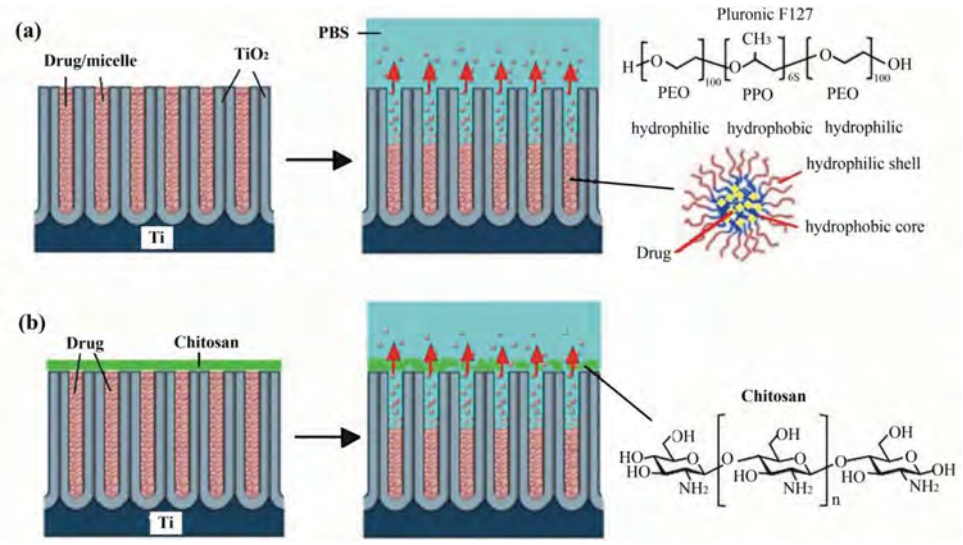

Figure 7. Schematic diagrams of two approaches used for extended drug release from titania nanotube arrays: a) Pluronic F127 polymeric micelle as nanocarrier used for encapsulating the water insoluble drug (indomethacin) and b) chitosan polymer layer coated on top of the TNTs used to control drug release from the nanotubes. PBS = phosphate buffered saline. Reproduced with permission.[152] Copyright 2011, Scientific Research.

decrease cell functions, similar osteoblast adhesion between the control and and drug loaded materials is observed.[149]

Gentamicin-loaded TiO_2 nanotubes have been fabricated to prevent bacterial infection after surgery, and the drug-eluting nanotubes reduce bacteria adhesion on the surface and enhance osteoblast differentiation.[150] Ma et al. have reported local delivery of antimicrobial peptides (AMPs) through nanotubular titania on titanium.[151] The antimicrobial activity tests against *Gram-positive bacterium* and *Staphylococcus aureus* demonstrate that the AMP-loaded nanotubular surface can effectively kill the bacteria (99.9% efficacy) and reduce the total bacteria number on the surface after culturing for 4 h.[151]

Two approaches to extend drug release of water insoluble drugs by integrating TiO_2 nanotube arrays (TNTs) with polymeric micelles (Pluronic F127) and biopolymer (chitosan) coatings have been proposed by Aw et al.[152] The water insoluble and anti-inflammatory drug indomethacin is used as the model drug, as shown in **Figure 7**. Significant improvement in the drug release characteristics, reduced burst release (from 77% to 39%), and extended overall release from 9 days to more than 28 days are observed, suggesting the great potential of TNT based antibacterial systems for sustained drug delivery to combat chronic infection and inflammation after surgery.[152]

4.2. Anticancer Drug Delivery

Cancer is a major health problem and common treatments include surgical removal of tumors, radiotherapy, and chemotherapy.[153,154] In chemotherapy, the treatment dose to balance effective anticancer activity and toxicity is crucial.[155] However, one of the major problems associated with chemotherapy is that it damages the surrounding healthy organs and tissues because many anticancer drugs are designed simply to destroy cells. The threat of severe side effects caused by the random distribution of the drugs throughout the body limits the maximum dosage.[156,157] The concept of hiding drugs with nanoparticle systems is a promising method to decrease the toxicity.[158,159] In many cases, nano-TiO_2 is effective in treating cancer due to its versatility and unique properties.[160–166]

A smart pH-responsive drug delivery system (DDS) based on TiO_2 nanoparticles has been developed by Zhang et al.[160] This DDS has the ability of controlled release while enhancing the chemotherapeutic efficiency of daunorubicin (DNR). The average pH in humans is 7.4 at which the drug can stay within the body for a long time without harming healthy cells. The extracellular pH of cancer tumors is about 6.0 which is more acidic than that of healthy blood and normal tissues and the pH of the endosomes within the cancer cells is even more acidic (pH = 5.0). DNR can be released from the DDS much more rapidly at the pH of 5.0 and 6.0, as shown in **Figure 8**,

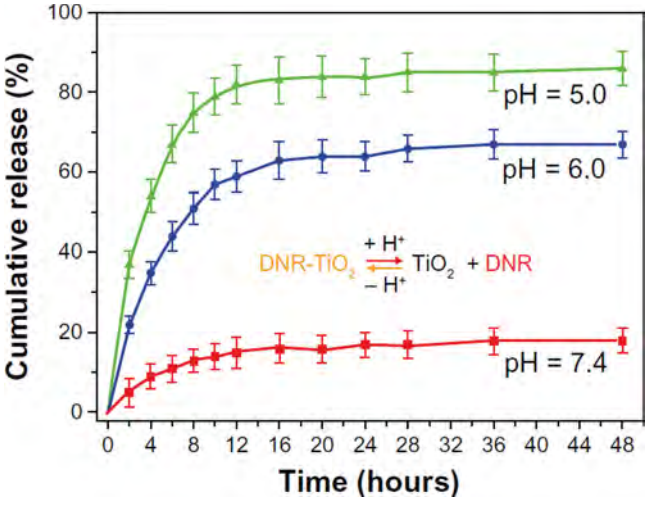

Figure 8. In vitro daunorubicin release behavior at the pH of 7.4, 6.0, and 5.0 (DNR, daunorubicin; DNR-TiO_2, daunorubicin-TiO_2 nanocomposites). Reproduced with permission.[160] Copyright 2012, Dove Medical Press.

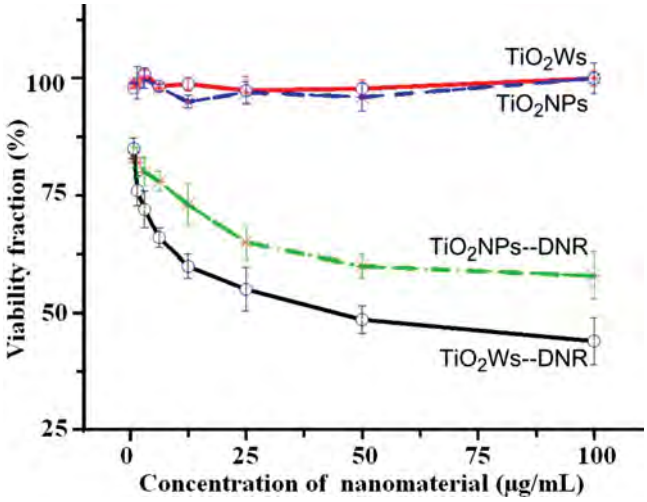

Figure 9. MTT assay: cell viability of SMMC-7721 cells treated for 48 h with TiO_2 Ws alone (red line, filled circles), TiO_2 NPs alone (blue dash line, filled stars), or combined with 1.5×10^{-5} mg mL^{-1} DNR. Reproduced with permission.[165] Copyright 2009, Elsevier.

and this is a desirable characteristic of tumor-targeting drug delivery.[160]

In order to reduce the serious side effects of doxorubicin (DOX), a study has been carried out to study the complex formed between DOX and TiO_2 nanoparticles (NPs) in the drug delivery system.[164] The adsorbed DOX is released controllably by external glutathione (GSH) and both in vitro and in vivo monitoring of DOX can be achieved in real time using TiO_2 NPs as the platform.[164] The DOX-TiO_2 nanotubes (DOX-TiO2 NT) system has been reported by Wang et al. and their results show that in a neutral pH environment (pH = 7), almost no DOX is released from the DOX-TiO_2 NT whereas under acidic conditions, the release rate can be as high as 75%.[162]

Li et al. have explored the drug delivery and anti-tumor function by establishing a system composed of one-dimensional TiO_2 whiskers (TiO_2 Ws) and daunorubicin.[165] In the case of human hepatocarcinoma cells (SMMC-7721 cells), TiO_2 Ws can obviously increase the intracellular concentration of DNR and enhance the potential anti-tumor efficiency, as shown in **Figure 9**, indicating that TiO_2 Ws can be used as an efficient drug carrier to introduce DNR to target cells. **Figure 10** illustrates the possible rationale for TiO_2 Ws being an efficient drug carrier. This is mainly due to the unique large-area properties of TiO_2 Ws, and the specific electrostatic interaction endows TiO_2 Ws with the ability of effective drug delivery. Therefore, TiO_2 Ws can incorporate more DNR molecules than TiO_2 NPs and sequently carry them into cells to increase the intracellular concentration of DNR to inhibit proliferation of the targeted cells leading to apoptosis.[165]

TiO_2/polymer composite nanoparticles have been utilized as drug carriers in cancer therapy. For example, FA-PEG-TiO_2 synthesized by grafting folic acid (FA) onto polyethylene glycole (PEG)ylated TiO_2 nanoparticles can be used for targeted delivery of paclitaxel, an anticancer drug.[166] The FA-PEG-TiO_2 system has the ability to target cancer cells and is also capable of evading the reticuloendothelial system. The TiO_2 nanocarriers possess a higher adsorption capability, and the in vitro release profile of paclitaxel from the FA-PEG-TiO_2 nanoparticles shows fast release initially followed by a sustained release phase.[166]

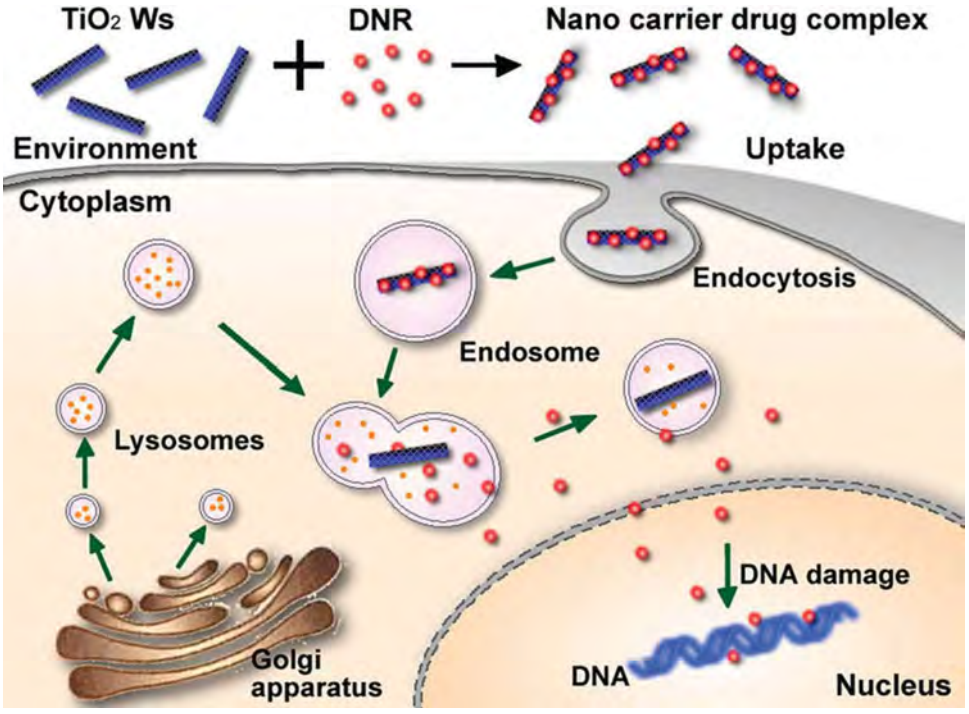

Figure 10. Illustration of the possible mechanism accounting for the enhanced uptake of DNR into SMMC-7721 cells via TiO_2 Ws drug delivery. Reproduced with permission.[165] Copyright 2009, Elsevier.

4.3. Gene Therapy Carriers

Organisms often encounter unwanted foreign or mutated DNA that may negatively affect their health. Traditional modes of diagnosis are often unable to detect the presence of deleterious DNA. In addition, conventional treatments do not address the underlying cause of the diseases and do not discriminate well between the target and healthy cells, thereby resulting in low therapeutic efficacy. Hence, both imaging and elimination of unwanted genes are major goals in molecular biology.[167–169] On the heels of rapid development in genetic engineering, it is possible to directly alter a genome and insert or remove a chunk of DNA, and nano-titania holds great promise in gene therapy by facilitating the targeted delivery of DNA into tissues and cells.

Preparation of TiO_2-DNA nanocomposites has been reported.[170–172] Paunesku et al. have synthesized TiO_2-DNA nanocomposites composed of DNA oligonucleotides covalently attached to 4.5 nm TiO_2 nanoparticles.[170,171] The TiO_2-DNA nanocomposites not only retain the intrinsic photocatalytic capacity of TiO_2 and bioactivity of the oligonucleotide DNA, but also possess the unique properties of a light-inducible nucleic acid endonuclease suitable for gene therapy.[170] These TiO_2-DNA nanocomposites can be specifically retained in the mitochondria or nucleoli.[171] Brown et al. have developed a peptide nucleic acid (PNA)-TiO_2 nanoconjugate.[172] PNAs are used to synthesize DNA analogs resistant to degradation by cellular enzymes. The PNA-TiO_2 nanoconjugates can be hybridized to target single-stranded DNA (ssDNA), double-stranded DNA (dsDNA), and supercoiled plasmid DNA under simulated physiological ionic and temperature conditions, thus enabling rapid, inexpensive, sequence-specific concentration of nucleic acids in vitro. After the addition of imaging agents or peptides, the PNA-TiO_2 nanoconjugates can enhance the hybridization capability boding well for genetic engineering.[172]

Compared to traditional drug delivery, TiO_2 nanostructured materials can control drug release to improve the therapeutic efficacy and reduce the side effects as well as the pain caused by multiple doses. In spite of the positive effects, the use of nanostructured TiO_2 in drug delivery systems still has hurdles such as low drug loading, few loadable drug species, and lack of control of the release rate. Therefore, this field still requires further exploration.

5. Biosensors

A biosensor is a device incorporating a biological sensing element either closely connected to or integrated to a transducer. Specific molecular recognition is a fundamental prerequisite on the basis of the affinity between complementary structures such as enzyme-substrate, antibody-antigen, and receptor-hormone, and this property is often used to generate the concentration dependent signals. The sensitivity and specificity depend on the biological recognition systems.[173,174] Since the development of enzyme-based sensors for glucose by Clark and Lyons,[175] improvements have been made in terms of the sensitivity, selectivity, and reproducibility as a result of rapid developments in nanotechnology and nanomaterials. Nanomaterial-based biosensors, which represent the integration of materials science, molecular engineering, chemistry, and biotechnology, improve the sensitivity and specificity of biomolecule detection and have great potential in biomolecule recognition and pathogenic diagnosis.[176,177]

In order to improve the performance of biosensors, TiO_2 nanomaterials such as nanoparticles,[178] nanotubes,[179,180] nanofibers,[181] gold nanoparticle encapsulated TiO_2 nanoclusters,[182] and TiO_2/SiO_2 nanocomposites[183,184] have been used in biosensing devices for enzymes, antibodies, microorganisms, and DNA. Nanostructured TiO_2 based biosensors are sensitive, selective, fast, and reproducible for the detection of various chemical and biochemical compounds such as glucose, hydrogen peroxide, and cancer cells because of their superior properties including nontoxicity, large surface area, high adsorptivity, good uniformity, and excellent biocompatibility. In fact, TiO_2 based biosensors have been proposed to be a prospective interface for the immobilization of biomolecules.[185,186]

5.1. Enzymatic TiO_2 Based Biosensors

Since Cosnier and coworkers reported an amperometric glucose biosensor based on mesoporous TiO_2 films in 1997,[187] the biomedical applications of nanostructured TiO_2 for enzyme immobilization and biosensing have attracted considerable attention. Enzyme immobilization is a key step in the fabrication of a sensitive and stable biosensor. Generally, in an enzymatic biosensor, in order to avoid the loss of enzymes, they are immobilized to the surface of the sensor either by cross-linking with glutaraldehyde[188] or by being protected by a thin gel or polymer layer of Nafion, for example.[189,190] TiO_2 nanomaterials are excellent materials to immobilize enzymes on a conducting surface forming an electrochemical enzyme biosensor.[191–193] In the device, the electrode needs to meet the following requirements: a) high electrochemical activity, b) high enzyme loading capacity, and c) fast sorption and reaction kinetics.[194] It has been reported that TiO_2 modified electrodes can retain enzyme bioactivity and provide long-term stability of enzymes.[195] **Table 2** shows the common TiO_2 based electrochemical enzymatic biosensors and their corresponding performance in terms of sensitivity and detection limits reported in recent years. Various types of enzymes such as glucose oxidase (GOD), horseradish peroxidase (HRP), urease, cytochrome C (cyt. c), and glutamate dehydrogenase can be immobilized on TiO_2 nanomaterials.[192,196–213]

Enzymatic biosensors are used for the detection of H_2O_2 which is used as a signaling molecule to regulate many different cellular processes, blood glucose, lactic acid, vitamin C, uric acid, urea, glutamic acid, and transaminase. As shown in **Figure 11**, TiO_2 nanorods (TNR) are prepared on a titanium electrode by a hydrothermal route and further employed as a supporting matrix for the immobilization of Nafion-coated horseradish peroxidase (HRP) as well as the fabrication of the H_2O_2 biosensor.[192] The TiO_2 nanorod film facilitates direct electron transfer between the enzyme and Ti electrode and the electroactive HRP gives rise to efficient enzyme loading on the TNR/Ti electrode. This biosensor exhibits a

Table 2. TiO_2 based enzymatic electrochemical biosensors.

Type of TiO_2 nanomaterials	Biocomponents	Detection technique	Sensitivity/detection limit	Response time	Ref.
Porous TiO_2	GOD	Amperometric	0.3 μA mM^{-1} (glucose)	Less than 10 s	[196]
Ag-TiO_2 nanotubes	GOD	Amperometric	0.39 μA mM^{-1} cm^{-2} (glucose)	—	[197]
Unhybridized TiO_2 nanotube arrays	GOD	Amperometric	199.6 μA mM^{-1} cm^{-2} (glucose)	—	[198]
TiO_2 nanotube arrays	GOD	Amperometric	36 μA mM^{-1}/5 μM (glucose)	Within 10 s	[199]
TiO_2 nanotube array	GOD	Potentiostatic	45.5 μA mM^{-1} cm^{-2} /2.0×10^{-3} mM (glucose)	Below 5.6 s	[200]
3D macroporous TiO_2	GOD	Amperometric	151 μA cm^{-2} mM^{-1}/0.02 μM (glucose)	—	[201]
Mesoporous TiO_2	GOD	Amperometric	4 mA M^{-1} cm^{-2} (glucose)	—	[202]
Nanotubular Au/TiO_2	GOD	Potentiostatic	Rather low (glucose)	—	[203]
Colloidal Pt/TiO_2	GOD	Amperometric	0.25 μM (glucose)	—	[204]
Mesoporous TiO_2/SnO_2	HRP	Amperometric	1.07 AM^{-1} cm^{-2}/1 μM (H_2O_2)	—	[205]
TiO_2 nanotubes	HRP	Amperometric	1.2×10^{-6} M (H_2O_2)	—	[206]
Au/TiO_2 nanocomposite	HRP	Amperometric	5.9×10^{-6} mol L^{-1} (H_2O_2)	3 s	[207]
TiO_2 nanowires	HRP	Amperometric	124 μA cm^{-2} mM^{-1}/0.32 μM (H_2O_2)	—	[208]
TiO_2 nanorods	HRP	Amperometric	416.9 μA mM^{-1} (H_2O_2)	—	[191]
Au/TiO_2 nanotube arrays	HRP	Amperometric	2×10^{-6} mol L^{-1} (H_2O_2)	—	[209]
TiO_2 thin films	GOD and HRP	Amperometric	≈10^{-6} M (glucose and H_2O_2)	7 s for GOD and 6 s for HRP	[210]
Au/TiO_2 nanotubes	Cyt. c	Amperometric	1.21×10^{-6} mol L^{-1} (H_2O_2)	—	[211]
Au/TiO_2 nanoneedle film	Cyt. c	Amperometric	49.4 mA cm^{-2} M^{-1}/ 4.5×10^{-8} M (H_2O_2)	—	[212]
TiO_2–CeO_2 nanocomposite	Urease and glutamate dehydrogenase	Amperometric	0.9165 μA cm^{-2} mM^{-1}/0.166 μM (urea)	10 s	[213]

fast response, high sensitivity (416.9 μA mM^{-1}), wide linear response range (2.5 to 0.46 mM), and a detection limit as low as 12 nM.[192]

A GOD/Ag/TiO_2 glucose biosensor has been prepared by photo-reducing silver nanoparticles on TiO_2 nanotube arrays (NTAs) followed by adsorption of GOD.[197] The nanotubular structure of the TiO_2/NTAs substrate and the highly dispersed Ag nanoparticles provide a hole-rich structure for the steady immobilization of GOD. The typical current response can be measured by amperometry as shown in **Figure 12**. Figure 12 (i) reveals an excellent electrochemical behavior and Figure 12 (ii) shows a high sensitivity of 0.390 μA mM^{-1} cm^{-2} obtained from the GOD/Ag/TiO_2 NTAs sensor. This can be attributed to the uniform distribution of Ag nanoparticles and synergistic

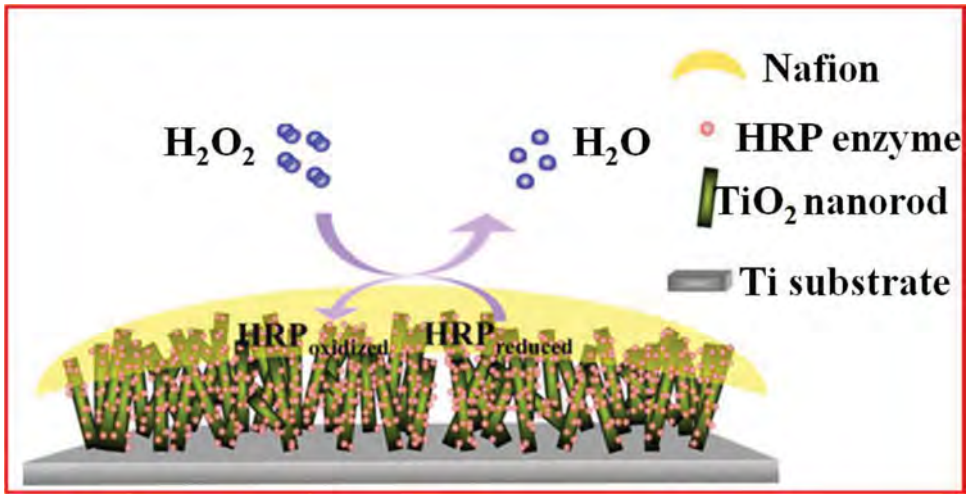

Figure 11. Schematic representation of the H_2O_2 biosensor based on a Nafion/HRP/TNR/Ti electrode. Reproduced with permission.[192] Copyright 2013, Springer.

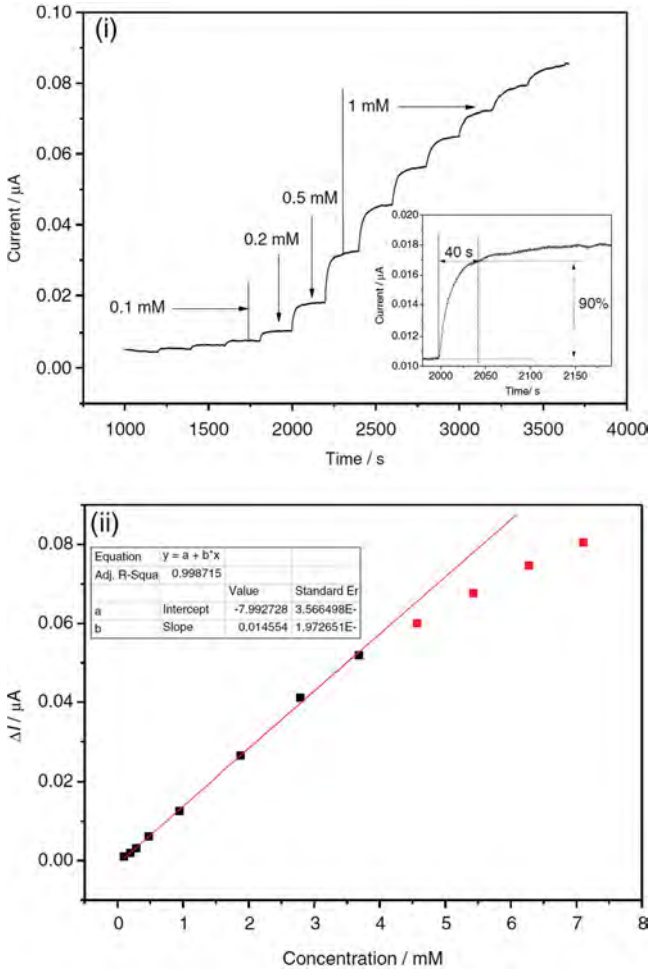

Figure 12. Amperometric detection of glucose by the GOx/Ag/TiO_2 NTAs electrode: i) chronoamperogram of the GOx/Ag/TiO_2 NTAs electrode for successive addition of glucose in 50 mM PBS. The insert shows the response time when achieving 90% of steady current response. ii) Calibration curve for glucose concentrations between 0.1 and 8 Mm. Reproduced with permission.[197] Copyright 2014, Springer.

effects of the conductive Ag nanoparticles and biocompatible TiO_2 nanotubes.[197]

A biosensor for glucose has been developed by immobilization of glucose oxidase onto unhybridized TiO_2 nanotube arrays by an optimized cross-linking technique.[198] The sensitivity can be as high as 199.61 μA mM^{-1} cm^{-2}, which is about 12 and 713 times higher than that on the TiO_2 film (16.91 μA mM^{-1} cm^{-2}) and Ti sheet (0.28 μA mM^{-1} cm^{-2}) based enzymatic electrodes, respectively. In addition, it has a low detection limit of 3.8 μM and signal-to-noise ratio of 3, as shown in **Figure 13**.[198] When the two enzymes GOD and HRP are co-immobilized on the TiO_2 based nanostructured surfaces, direct electron transfer between enzyme and electrodes is significantly enhanced due to the nanostructured environment of the TiO_2 based layers.[210] These biosensors have a good sensitivity, low detection limit (≈10^{-6} M), and fast time response (few seconds) making them promising in low-cost, miniaturized multi-functional biosensors.[210] The amperometric response of third-generation GOD and HRP

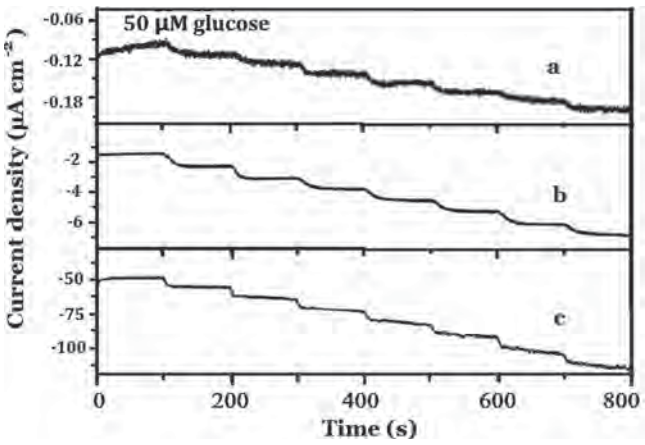

Figure 13. Current-time response of the three enzyme electrodes after the successive addition of 50 μM glucose into the 0.2 M phosphate buffer solution (pH 6.8) at an applied potential of –0.5 V (vs. SCE): a) current-time response of the Ti sheet enzyme electrode; b) current-time response of the TiO_2 film enzyme electrode; c) current-time response of the TiO_2 nanotube array enzyme electrode. Reproduced with permission.[198] Copyright 2013, Springer.

biosensors based on functionalized TiO_2–Si electrodes is shown in **Figure 14**.

5.2. Non-Enzymatic TiO_2 Based Biosensors

Besides enzymes and glucose, TiO_2 nanomaterials constitute an excellent matrix for the immobilization of other biological components such as hemoglobin, antibodies, antigen, cells, and DNA. The semiconducting nature of TiO_2 facilitates direct electron transfer between the biological components and electrode. **Table 3** shows different biosensors fabricated with various TiO_2 nanomaterials and biological components (except enzymes).[214–223]

TiO_2 nanoparticles mixed with carbon nanotubes (TiO_2/CNT) prepared on carbon paper have been utilized to enhance the detection sensitivity of biomolecules.[220] The electrochemical signals from the modified electrodes covered with cancer cells are significantly larger than those from the bare carbon paper. Different kinds of leukemia cells, such as K562/ADM and K562/BW cells, can be recognized because of the different electrochemical behavior and hydrophilic/hydrophobic nature of the modified electrode, arising from the specific components of the plasma membranes of the target cells. It is thus possible to develop biocompatible and multi-functional biosensors for early diagnosis of cancer.[220]

An et al. have employed Au-doped TiO_2 nanotube arrays to prepare a photoelectrochemical immunosensor for the detection of α-Synuclein (a-SYN), which is a very important neuronal protein associated with Parkinson's disease.[219] The immunosensor has a high sensitivity, stability, and reproducibility in protein detection. The currents are proportional to the a-SYN concentrations and the linear range spans from 50 pg mL^{-1} to 100 ng mL^{-1} with a detection limit of 34 pg mL^{-1}.[219] Nanostructured Au-TiO_2 particles have also been used to construct a label-free amperometric immunosensor for

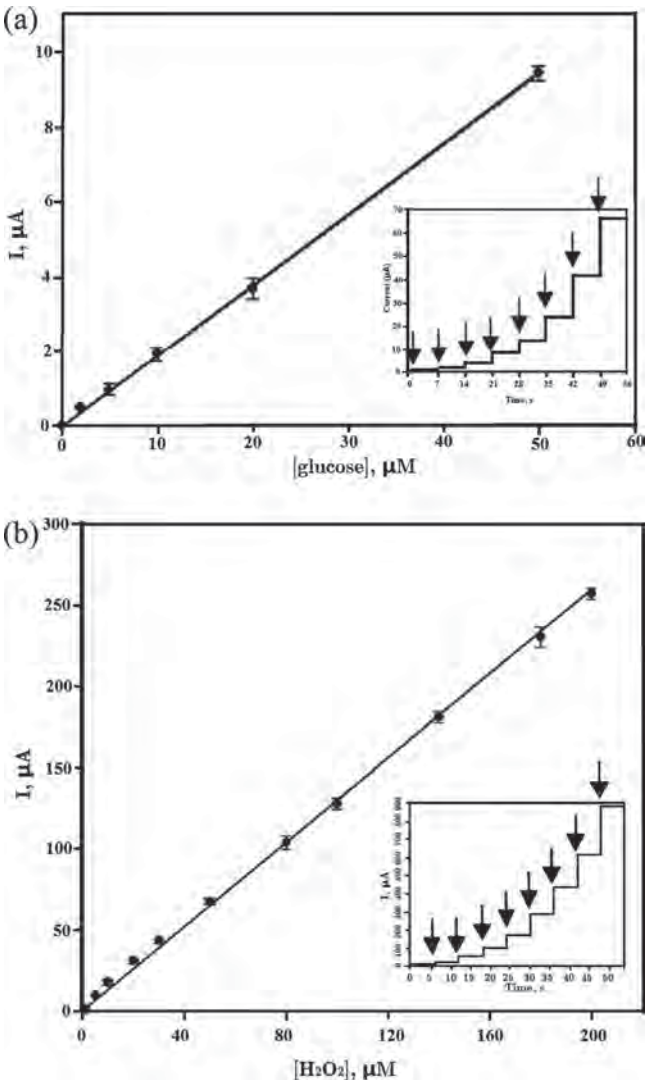

Figure 14. Calibration curves: a) glucose biosensor based on TiO_2 modified Si electrodes and b) H_2O_2 biosensor based on TiO_2 modified Si electrodes. The inset graphs show the typical amperometric response of the biosensors to successive injection of glucose and H_2O_2 into the stirred 0.1 M phosphate buffer solution at pH of 7.0. Reproduced with permission.[210] Copyright 2006, Elsevier.

carcinoembryonic antigen (CEA) determination.[221] These spherical Au-TiO_2 nanoparticles with a large surface-to-volume ratio, film-forming ability, and high stability provide a good microenvironment for the immobilization of biomolecules, consequently improving the surface coverage of proteins. The immunosensor exhibits a good linearity in the concentration range between 0.2 and 160.0 ng mL^{-1} towards CEA with a coefficient of 0.9965 and a detection limit of 0.06 ng mL^{-1}. It also shows a good selectivity, reproducibility, and high sensitivity and therefore great potential in clinical immunoassays.[221]

In comparison with traditional biosensors, nanostructured TiO_2 based biosensors offer many advantages, for instance, enhanced absorption of biological components, more active surface sites, good reaction micro-environment, faster electron transfer, shorter response time, better sensitivity, and so on. However, the best sensitivity of nano-biosensors can be achieved only under optimized conditions in the laboratory and in vivo experiments are still lacking. Hence, it is essential to better understand the nanomaterials-biomarkers interactions in different biological micro-environments, and more research is needed to develop next-generation TiO_2 based biosensors that can satisfy various clinical requirements.

6. Conclusion and Outlook

Recent advances pertaining to nanostructured TiO_2 in four important areas are reviewed, namely bone scaffolds, intravascular stents, drug delivery systems, and biosensors. Because of their unique geometrical characteristics as well as good biocompatibility, nanostructured TiO_2 has immense potential in the biomedical field. Although the benefits of TiO_2 nanomaterials have been extensively reported, more thorough understanding of the underlying mechanisms is crucial for further development. As bone scaffolds, nanostructured TiO_2 accelerates the rate of apatite formation and enhances adhesion, proliferation, and differentiation of osteoblasts. Nevertheless, how the dimensions, geometry, and surface features of the TiO_2 nanostructures affect cell response is still controversial. With regard to TiO_2 nanotube intravascular stents, desirable effects are observed for two major cell types and so they are promising for next-generation vascular devices, even though a better understanding of the major processes associated with vascular stents

Table 3. TiO_2 biosensors based on other biological components.

Type of TiO_2 nanomaterial	Biocomponents	Detection technique	Sensitivity/detection limit (μM)	Ref.
Chitosan–TiO_2 nanorods	Hemoglobin	Amperometric	0.72 μM (H_2O_2)	[214]
DS-doped TiO_2 film	Hemoglobin	Amperometric	1.0 μM (H_2O_2)	[215]
TiO_2 nanotubes	Hemoglobin	Amperometric	1.5×10^{-6} M (H_2O_2)	[216]
TiO_2–PTATB composite film	Hemoglobin	Amperometric	3.7×10^{-7} M (H_2O_2)	[217]
TiO_2 nanoparticles	α-1-fetoprotein antibody	Amperometric	0.1 ng mL^{-1} (α-1-fetoprotein)	[218]
Au-doped TiO_2 nanotubes	Antibody	Photoelectrochemical	34 pg mL^{-1} (α-synuclein)	[219]
TiO_2/CNT nanocomposites	Cancer cells	Amperometric	Leukemia cells	[220]
Au-TiO_2 hybrid nanocomposite film	Carcinoembryonic antigen	Amperometric	0.06 ng mL^{-1} (antigen-antibody)	[221]
TiO_2 nanowire bundle	Monoclonal antibodies	EIS	102 cfu mL^{-1} (Listeria monocytogenes)	[222]
TiO_2/DNA thionin nanocomposite	DNA	Amperometric	0.05 mM (H_2O_2)	[223]

and topography modulation is necessary. As drug delivery systems, although nanostructured TiO_2 can carry different types of drugs, the mechanism for sustained release must be improved to address the ever increasing clinical needs. In biosensing applications, novel nanostructures that can immobilize more enzymes on the surface of TiO_2 and accelerate electron transfer between the biological components and electrode continue to be researched extensively.

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