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REVIEW

Biomedical Applications of TiO_2 Nanostructures: Recent Advances

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Abstract: Titanium dioxide (TiO_2) nanostructures are one of the most plentiful compounds that have emerged in various fields of technology such as medicine, energy and biosensing. Various TiO_2 nanostructures (nanotubes [NTs] and nanowires) have been employed in photoelectrochemical (PEC) biosensing applications, greatly enhancing the detection of targets. TiO_2 nanostructures, used as reinforced material or coatings for the bare surface of titanium implants, are excellent additive materials to compensate titanium implants deficiencies—like poor surface interaction with surrounding tissues—by providing nanoporous surfaces and hierarchical structures. These nanostructures can also be loaded by diversified drugs—like osteoporosis drugs, anticancer and antibiotics—and used as local drug delivery systems. Furthermore, TiO_2 nanostructures and their derivatives are new emerging antimicrobial agents to overcome human pathogenic microorganisms. However, like all other nanomaterials, toxicity and biocompatibility of TiO_2 nanostructures must be considered. This review highlights recent advances, along with the properties and numerous applications of TiO_2-based nanostructure compounds in nano biosensing, medical implants, drug delivery and antibacterial fields. Moreover, in the present study, some recent advances accomplished on the pharmaceutical applications of TiO_2 nanostructures, as well as its toxicity and biocompatibility, are presented.

Keywords: titanium dioxide nanomaterial, drug release, antibacterial, implants, biosensors, nanotoxicity

Introduction

Nanotechnology, as one of the most interesting areas of science, gives scientists the ability to create, control and use materials in the nanometer scale.[1–4] Biotechnology has helped to transfer the knowledge of biological systems to industry. Furthermore, the distinctive physicochemical and biological properties of nanomaterials resulted in the use of nanotechnology in different health care areas.[4–9] Nanobiotechnology is one of the latest emerging fields of science, which is the interface between biology and nanotechnology, analyzing and creating novel functionalized nano biosystems.[8] This multidisciplinary research field has great potential in the development of improved medical engineering.[1,10]

Several nanostructures with unique properties have been fabricated by nanotechnology for biotechnological applications,[1,2,6,8,9,11-14] among which, nano-sized titanium dioxide (TiO_2) has been widely used. The element of titanium was first discovered in 1791 by William Gregor. TiO_2 is a white and poorly soluble material with numerous applications in biomedical fields like cosmetics, medicines and pharmaceutical products.[5,15,16] It consists of two crystalline forms, Rutile and

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Anatase, which have important industrial applications. It has also been proven that mixed polymorphs of TiO_2 (for example, Anatase 80% and Rutile 20%) are more efficient for biomedical use compared to the presence of one crystal. Anatase form of TiO_2 is more active than the Rutile form in regard to photocatalytic and cytotoxic properties.[16] However, most of the metal-oxide nanoparticles (NPs) are costly for industrial scale-up, and some are toxic.[7,13] TiO_2 is not only cost-effective but is mostly a non-toxic substance,[17] which has been approved by the American Food and Drug Administration (FDA) for utilization in food and drug-related products.[18]

Nanostructured TiO_2 has broad potential applications due to their nanosized features, low toxicity, good biocompatibility, intrinsic properties and versatile fabrication techniques.[11,12]

For example, nanostructured arrays composed of TiO_2 nanotube wall, including Ag_2O and Cu NPs, are ideal candidates for biomedical applications such as antibacterial surface coating. Additionally, they have good cytocompatibility, and are useful for osteoblast cell spreading, proliferation and differentiation.[19,20] Furthermore, the drug delivery system, bone/drug implant, self-cleaning substrate and antibacterial agent, for example, are wide spectrum applications of TiO_2 nanostructures.

In recent years, various types of nanostructured systems have been developed for delivery of chemotherapeutic agents in an effort to destroy tumor cells effectively, yet most of these delivery systems are still not able to target tumor cells specifically, and their drug release process is poorly controlled, leading to serious side effects.[21,22] Thus, the ability to obtain specific accumulation of drug at the tumor site is still an important challenge. Therefore, focusing on design of an ideal drug delivery system, which has combination of abilities for stimuli-triggered drug release and cancer cell targeting, is required. Porous TiO_2 nanostructures offer the potential to solve this problem, owing to their remarkable properties, such as high functional surface and photocatalytic activity.[23]

TiO_2 nanostructures, like titania nanotubes (NTs), are desirable structures to be used for surface coating of implants, and compared to polymer based implants, TiO_2 NTs have good stability against disintegration and swelling. More importantly, TiO_2 nanostructures are able to be used as a smart delivery system to control drug release in implants. These aspects of TiO_2 nanostructures address the most important challenges of the previously devised implant.[24]

On the other hand, the porous structure of TiO_2 NTs enhances bone regeneration and repair, which is favorable in bone tissue growth and implant biological fixation.[12,25-29] Nowadays, the increasing risk of device-related infections in medicine result in a serious health problem. Furthermore, the spread of antibiotic resistant bacteria in biomedical devices is considered a large threats to human health. Therefore, much attention is focused on newly developed anti-microbial strategies for medical implements used in clinic. Among different strategies, the idea of coating device surfaces with antimicrobial active metals are considered one of the essential strategies. However, the bimetallic corrosion is unavoidable during the service. In this regard, photocatalytic TiO_2 nanomaterials with anatase form provide more advantages for antimicrobial purposes.[30] Titania photocatalytic activity of TiO2 under the UV irradiation exposure results in disinfecting properties, mostly related to the generation of reactive oxygen species (ROS).[31]

Sensitive and accurate detection of biological analytes at low concentrations is another applicability of TiO_2 nanostructures that is very beneficial for biomedical research, as well as clinical diagnosis. In recent years, there has been considerable interest in the sensing application of TiO_2 for use in biosensors.[12,27]

According to literature, nanostructured TiO_2 appears to be an inert and safe material when exposed to human body. A deep understanding of nanoscale phenomena will pave the way for fabrication of novel biomedical devices and constructing the antibacterial surfaces, implants devices and more. As TiO_2 nanomaterials have received remarkable attention in different fields, this review focuses on the recent advances of its biomedical application, discussing its nanotoxicity and most important factors affecting its biocompatibility, as well as future challenges in these regards.

TiO_2-Based Drug Delivery Systems for Cancer Therapy

Nowadays, nanotechnology in cancer treatment and diagnosis is more popular as a result of the severe side effects of traditional chemotherapeutic agents due to their cytotoxicity on normal cells.[1,6,13,14,32] The most challenging aim of nanotechnology research in cancer therapy is discovering nanostructures for delivery and release of drugs in a way that enhances the therapeutic effect and decreases the side effects.[9] Targeted drug delivery and controlled drug release are the main strategies to achieve this aim. TiO_2 nanostructures—due to the high biocompatibility, tunable drug releasing ability and low toxicity—are recognized as an appropriate candidate to increase

the clinical therapeutic effect of conventional chemotherapeutic agents through targeted delivery and controlled release.

TiO_2-based nanostructures are potent systems for both targeted delivery and controlled release of cytotoxic anticancer agents. In the following, we review the important studies of TiO_2 nanostructures in targeted drug delivery and different controlled release systems.[33]

Targeted Drug Delivery

Several lines of studies have shown that targeted drug delivery of cytotoxic chemo agents loaded on TiO_2-based nanostructures could increase the therapeutic efficiency of agents in cancer treatment. For the first time, Li et al investigated the potential application of one-dimensional TiO_2 whiskers for drug delivery in cancer therapy and its synergistic effect on internalization and accumulation of daunorubicin in SMMC-7721 cells.[34] Likewise, it has been reported that conjugation of doxorubicin (DOX) to TiO_2 NPs caused synergistic response in breast cancer cell lines.[35]

In a recent study, ZnS quantum dots were deposited onto TiO_2 nanotube surface, then the drug release profile was studied using 5-fluorouracil (5-FU), indicating that TiO_2/ZnS NTsare of value as drug delivery systems.[36] Targeted drug delivery using surface-modified NPs increases the performance of the anticancer drugs by specific delivery of them to cancer cells, minimizing the toxicity of anticancer drugs.[1,13,32] In 2013, Venkatasubbu et al observed the anticancer activity of paclitaxel attached to modified hydroxyapatite (Hap) and TiO_2 NPs in diethylnitrosamine (DEN)-induced hepatocarcinoma in animal models. Surface modification of NPs conducted by polyethylene glycol (PEG) as a non-immunogenic polymer and folic acid as a tumor marker. The hematological and biochemical results of research showed the higher anticancer activity of the surface-modified paclitaxel attached to the Hap and TiO_2 NPs in comparison to pure paclitaxel.[37] In this manner, the treatment of ovarian cancer cell line with cisplatin loaded on hyaluronic acid-TiO_2 NPs enhanced drug accumulation in cancer cells compared to free cisplatin.[38] In addition to the mentioned advantages, nanostructure mediated targeted drug delivery in cancer cells can enhance bioavailability and release time of drug.[1,13,14,37]

Controlled Drug Release in Cancer Therapy

Among new strategies for cancer treatment, light is one of interesting external stimulus for releasing of a drug from delivery system due to its capability for providing a spatial and temporal control over the release of anticancer agents. TiO_2 nanostructures also attract great attention as photoactive drug delivery carriers. In addition to photoactivity, TiO_2-based nanostructures have wide range of valuable properties including high surface area, stability, availability, and possibility for surface modification making them as a suitable carrier for attachment of various drugs.[39] In 2014, Wang et al reported a new multifunctional porous TiO_2 NPs loaded with paclitaxel, which had the potential of ultraviolet (UV)-triggered drug release. They modified TiO_2 NPs with polyethyleneimine (PEI) as a cancer-targeting agent. The authors believed that this development could be more informative for light-controlled drug release and skin cancer treatment.[23]

In another study, a mesoporous TiO_2 shell for Near-Infrared-Triggered drug delivery was prepared by loading DOXto prepare a porous TiO_2 shell core-shell structures, and HA capping was performed then the drug release was studied. The results indicated that the cell viability decreased clearly at lower drug doses, making it a promising method for cancer therapy.[40] In a recentstudy, colloidal TiO_2 NPs were used as carrier for light-controlled delivery of ruthenium complex (metallo-drug) to the melanoma cancer cell line. This system showed fast release profile upon UV light compared with visible light illumination. Besides, the cell death increased by UV light as compared to red light. The authors proposed that both components of system are photosensitizer and could induce cell death by generating ROS.[39]

It has been reported that the elevated level of oxidative stress by exogenous agents, due to the elevation of ROS in tumor environment, can be more destructive in cancer cells.[41] Prior studies have shown that induction of ROS production in a tumor environment triggers apoptosis, which is the appropriate type of cell death in cancers.[42] Thus, the exposure of cancer cells to increased level of ROS can be a new potential treatment to selectively induce cell death in cancer cells without affecting normal cells.[41]

Similar to light, ultrasound is another external stimuli for induction of drug release in TiO_2-based nanomaterials. Several studies have confirmed that ultrasound is more effective than UV-light due to the deeper penetration in tumor cells. Besides that, ultrasound causes no harm to the host cells. In this procedure, ultrasound stimulates TiO_2 as a sonosensitizer and generates ROS to kill tumor cells. TiO_2 NPs have indicated enhanced antitumor activity against different cancer cell lines after stimulation with ultrasonic.[33] In an investigation conducted on hydrophilized TiO_2 (H TiO_2) NPs in sonodynamictherapy, $HTiO_2$ NPs showed long circulating time and more resistance against degradation.[43]

Radiofrequency is an excellent noninvasive strategy, which can be used to trigger drug release in TiO_2 NT based-delivery systems. AuNPs are one of the most efficient thermal transducers for transferring radiofrequency energy to induce drug release from TiO_2 NTs.[44] This approach has demonstrated significant potential in the inhibition of hepatocellular and human gastrointestinal cancer cells.[45]

TiO_2 NT platforms can be used as the most promising carriers for controlled drug release of anticancer agents in local drug delivery systems. It has been shown that dimensions and surface functionalization of TNTs plays important role in controlling drug release from TiO_2 NTs. In a study completed involving TiO_2 NTs with different lengths and sizes, higher lengths of tubes provided higher total drug elution of paclitaxel.[46]

Stimuli-Controlled Drug Release

In recent years, new developed cancer therapy systems pose both function of controlled release and targeted delivery of drug. In a study, multifunctional porous TiO_2 NPs were functionalized with PEI to provide photocatalytic property for TiO_2, in order to become a UV- controlled drug release system. In such a system, the loaded antitumor agent was not released in the blood and normal tissues during circulation due to the blocking role of PEI. Additionally, to further target cancer cells, which overexpress folate receptor, folic acid was conjugated covalently to the surface of TiO_2 NPs.[23]

In another study, DOX loaded TiO_2 NPs, which were coated with polymeric phenyboronic acid (pPBA) through boronic ester bond, demonstrated high tumor targeting capability due to the specific interaction of PBA with sialylated epitope of tumor cells. On the other hand, ultrasound irradiation through ROS generation could release DOX from NP via cleavage of boronic ester bonding (Figure 1).[47]

TiO_2-Based Antibacterial Devices for Prevention and Treatment of Infections

With the advent of nanotechnology, materials, such as metals and metal oxides, have been noticed as effective microbicide agents, which have the advantage of improved safety and stability compared to organic antimicrobial agents.[17,48,49] A large number of studies show the inhibitory activity of TiO_2 because of photocatalytic action, which is due to ROS —such as O_2, OH and H_2O_2—being generated.[31] ROS is the product of redox reactions occurring between an adsorbent (water or oxygen) and electrons of TiO_2 when illuminated by UV light at wavelength of shorter than 385 nm.[31,50] Figure 2 shows the schematic diagram of bacterial destruction by illuminated TiO_2. The most important drawback to TiO_2 is its necessity for UV light irradiation for activation of photocatalyst. However, recent studies have solved this problem by using visible light-activated photocatalysts, like Ag/AgBr/TiO_2, and doped-TiO_2 nanostructures.[51–53]

The first use of TiO_2 as a photocatalytic micro biocide was reported by Matsunaga et al (1985), when they investigated TiO_2 antibacterial activity towards *Lactobacillus acidophilus, Saccharomyces cerevisiae* and *E. coli*.[54] Various studies have explained that the photocatalytic activity of TiO_2 in water is efficient against broad range of organisms, such as bacteria (Gram-negative and Gram-positive), fungi, viruses, algae, protozoa and bacterial toxins.[31] TiO_2 thin films are as potent as the suspended form, and a major advantage of them is easy separation of catalyst from liquid at the end of process. The pioneering study by Matsunaga et al proved that TiO_2-Pt thin film inhibits microbial cell viability in water and in exposure of UV-light.[54]

Nowadays, controlling inhabitant bacterial species on medical devices and implants is a serious problem in biomedicine due to the high prevalence of antimicrobial resistance and biofilm generation. Increasing microorganism resistance is one of the most important challenges in many industries. In this regard, capability of TiO_2 as an antimicrobial agent has been studied in several biomedical researches. This part of the review aims to focus on newly developed research studies in biomedical application of TiO_2 antibacterial properties. Table 1 summarizes the antibacterial applications of different TiO_2 nanostructures.

Antibacterial Application in Dentistry

Titanium is widely applied in dental implants because of its suitable chemical and mechanical properties. However, infection of Ti-based dental implants still creates additional problems that can result in implant failure. Controlling infection and acquiring another technique for placing implants into bones are prominent for long-term dental implant success. Modifying implant surface is one of the effective ways to enhance the Ti implants properties. In this case, TiO_2 NTs not only provide antibacterial activity, but also could enhance osteogenic activity of implants.[56,57] This approach has been used by Liu et al to confirm that TiO_2 NTs, along with Zn, increase implant efficacy due to cytocompatibility and antibacterial functions at a proper concentration. This study depicted a novel

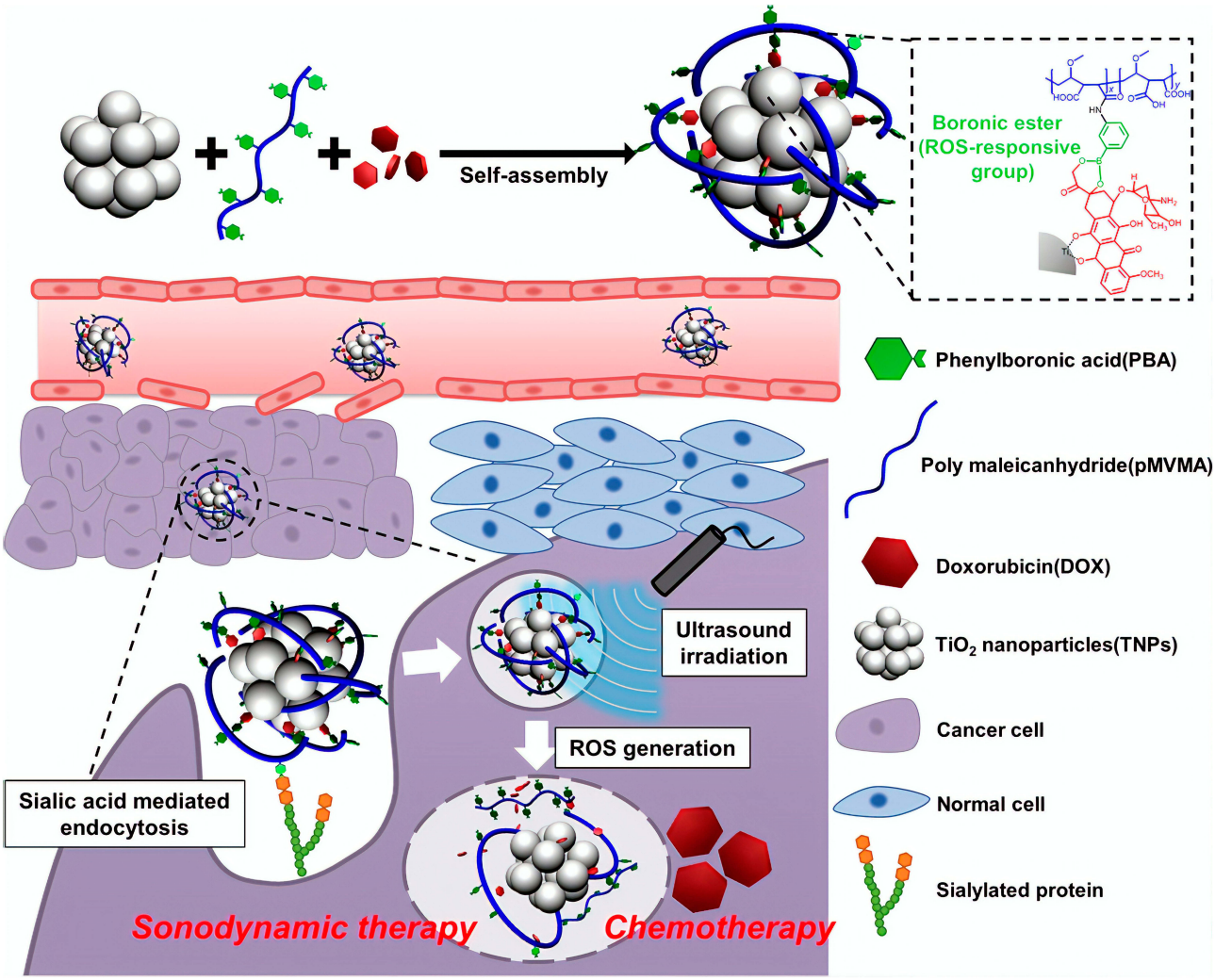

Figure 1 A schematic image indicates a TiO_2 based-targeted delivery of an anticancer agent and controlled drug release induced by ultrasound irradiation.
Notes: Reprinted from: Kim S, Im S, Park EY, et al. Drug-loaded titanium dioxide nanoparticle coated with tumor targeting polymer as a sonodynamic chemotherapeutic agent for anti-cancer therapy. *Nanomedicine*. 2020;24:102110. Copyright © 2019 Elsevier Inc. All rights reserved. With permission from Elsevier.[47]

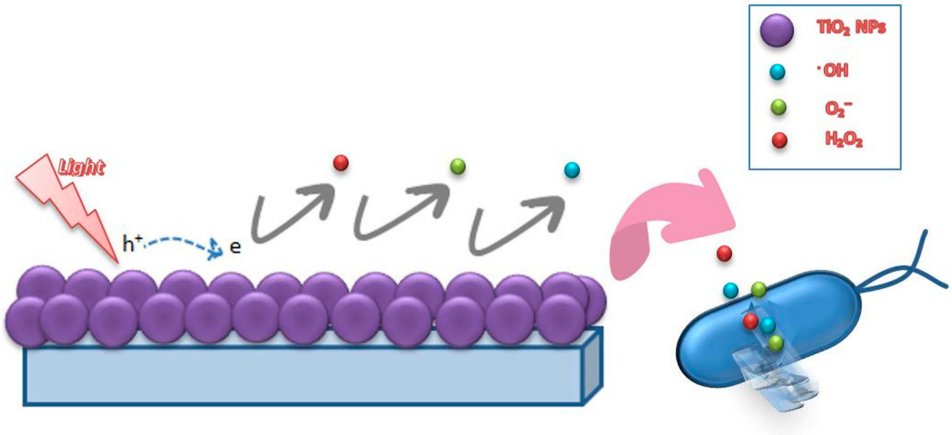

Figure 2 The bacterial destruction by UV-illuminated TiO_2 NPs.

Table 1 Antibacterial Properties of TiO_2 Nanostructures in Biomedical Applications

Application	Component	Additional Properties	Antibacterial Activity	Condition	Ref
Dentistry Implants	TiO_2 NTs+ Zn	Cytocompatible	*S. mutans, Porphyromonas gingivalis*	–	58
	Heat(NH400NTs (NH400)	Osteoblast viability	*Porphyromonas gingivalis*	–	63
	Glass ionomer cement (GIC) + TiO_2 NPs	Long-lasting restoration	*S. mutans*	Direct contact with GIC + TiO_2 NPs	58
	TiO_2+ PMMA/PEEK nanocomposite	Cytocompatible	*E. coli, S. aureus*	Under dark condition	60
	Ag-doped TiO_2 NTs(TNT)	–	*S. aureus*	-	70
Orthopedic Implants	TNTs decorated with TiO_2 NPs	Stem cell osteogenic	*S. mutans, Porphyromonas gingivalis,*	Direct contact	58
	Ti-160	Osteoblast proliferation	*S. aureus, P. aeruginosa* Amp-resistant *E. coli*	Direct contact (16 hours at 37° C) on to the material surface	74
	TiO2 +micro-arc oxidation (TM) composites coated on Mg alloy	in vivo degradation behavior and cytocompatible	*E.coli*	Direct contact	71
Medical and Hospital Devices	PLA-TiO_2 nanocomposite films	Water absorption properties	*K. pneumoniae, S. aureus*	Direct contact	55
	Sulfur-doped TiO_2	–	*E.coli*	Hospital lighting conditions.	79
	TiO_2-coated silicon catheters	–	*E. coli* K-12	Exposure of UV light	78
	TiO_2-coated catheters	–	*E. coli*	Exposure of UV light	76

surface modification in dental implant applications.[58] Furthermore, it has been found that modification of Ti-based dental implants with multilayer TiO_2 nano-network can increase cell adhesion, proliferation and mobility.[59]

Peri-implantitis is the devastating inflammation affecting the soft and hard tissues around dental implants, a process that results in bone loss. Therefore, inhibition of initial adhesion of bacteria on the implant is essential to avoid implant-associated infections.[60–62] In a recently published research from 2017, the antibacterial activity and osteoblast viability of heat and plasma treated TiO_2 NTs are evaluated. The study was conducted in four groups—including the polished titanium, TiO_2 NTs, heat-treated (at 300ºC) TiO_2 NT and heat-treated (at 400ºC)—TiO_2 NT and also in four groups based on plasma treatment. The results showed that plasma treatment reduces the adhesion of *Porphyromonas gingivalis*, but it does not affect osteoblast activity. Overall, the heat-treated group at 400°C was chosen as the most suitable for dental implants because of optimum osteoblast and antibacterial activity.[63] In another study, hydrothermal treatment of nano-structures on Ti implants at 225ºC for 5 hours presented a new topography with nanoflower structure that showed antibacterial effect against methicillin resistant *Staphylococcus aureus*. Besides that, these Ti implants did not exhibit cytotoxicity against mammalian cells during the 14 days and improved calcium deposition from osteoblast.[64] All the evidence shows that changing treatment parameters—such as pressure, temperature and time—can result in different crystalline phase and topographies.

Tooth decay or dental caries are a common problem that occurs when acid-producing bacteria in the mouth dissolves the outer layers of the teeth. To avoid this, dental disinfectants based on metal oxide nanostructures

have shown beneficial in compared to conventional disinfectant such as chlorhexidine.[65] According to a previously published study, TiO_2-containing nanomaterials have demonstrated antibacterial effect toward the oral pathogenic species of *Streptococcus* mutants.[66] Moreover, the use of TiO_2 NPs in dental composites, cement, sealants, bases, liners and adhesive materials yielded a strong antibacterial effect. In a research conducted by Garcia-Contreras et al, it was proposed that glass ionomer cement (GIC) incorporated with TiO_2 NPs is a promising dental material for its antibacterial capability and long-lasting restoration against mastication force.[67]

Antibacterial Application in Orthopedic Implants

A bone infection, which is also known as osteomyelitis, is a consequence of bacteria or fungi invasion. Bone infections are mostly caused by the *S. aureus, Staphylococcus epidermidis* (*S. epidermidis), Enterobacteriaceae* and *Pseudomonas aeruginosa* bacteria.[68] Typically, osteomyelitis occurs after bone surgery or bone fracture repaired by implants. In this condition, a prolonged and specific antibiotic treatment is necessary. Therefore, preventing wound and bone infection by self-sterilized implants would be helpful. As mentioned previously in this report, modification of implant surfaces with TiO_2 nanostructures is a new approach in this regard.[69]

Hou et al studied Ag-doped TiO_2 NTs to reveal that surface modification of Ti-based implants provides antibacterial properties for TiO_2.[70] This strategy decreased bacterial adhesion onto the surface of TiO_2 NTs as compared to bare Ti surfaces and unloaded TiO_2 NTs. In recentwork, TiO_2 incorporated micro-arc oxidation (TM) composites coated on Mg alloy introduced as a bioactive material, which possesses antibacterial properties against *E.coli*, in vivo degradation behavior and cytocompatibility for orthopedic applications.[71]

In orthopedic fractures, metal pins and wires are employed as a fixation device, and these devices are susceptible to bacterial colonization of *S. aureus, S. epidermidis* and *E.coli*.[72] It has been suggested that coating a pin or wire with a TiO_2-containing material is effective, since photo-killing activity of TiO_2 will influence the bacteria more than the host tissue. However, more cytotoxic observation is required before clinical application.

In addition to antibacterial properties of TiO_2 modified surfaces, being non-poisonous and environmentally safe are the most important priority for modification of implant surfaces by TiO_2 nanostructures.[58]

Furthermore, TiO_2 NPs are able to promote osteogenic differentiation.[73] In this manner, for the first time, Liu et al simultaneously investigated antibacterial and stem cell osteogenic properties of TiO_2 NTs decorated with TiO_2 NPs. This investigation proved that applying TiO_2 NPs on TiO_2 NTs increases surface area and photocatalysis action of TiO_2 NTs. This phenomenon lowered the numbers of *S. mutans* and *Porphyromonas gingivalis* on the TiO_2 NTs-TiO_2 NPs as compared to pure Ti and TiO_2 NTs after the first seven days.[58] The current research applied the electrophoretic deposition method to produce two distinct surface nanotopographies, Ti-160 and Ti-120. Surfaces treated with Ti-160 showed significant reduction of *S. aureus* (95.6%), *Pseudomonas aeruginosa* (*P. aeruginosa*) (90.2%) and ampicillin-resistant *E. coli* (81.1%), which was active against both Gram-negative and Gram-positive bacteria. Ti-120 treated surface also decreased the bacterial colonization and, further, caused osteoblast proliferation for up to 5 days.[74]

Applying TiO_2 NTs arrays as a carrier for drugs—such as antibiotics—is a new approach to overcome infection-related problems, which is discussed in next section.[68] Another vital aspect of TiO_2 is its biocompatibility, which is clinically proven. This property has also been discussed further in the following section. Furthermore, as demonstrated in Figure 3, the most important aspect in which these nanobiomaterials are used for implant are high surface area, good wettability, resistance to corrosion, homeostasis and low toxicity.

Antibacterial Application in Medical and Hospital Devices

Microbial biofilms are one of the most serious challenges in the field of medical devices. *S. epidermidis* and *S. aureus* are the most common bacteria to form a biofilm on medical devices and cause an infection. Antibacterial properties of TiO_2-coated surfaces could be employed in the hospital industry because traditional approaches of disinfection methods are not as effective as photocatalytic methods.[75] Bonetta et al reported the application of TiO_2 in the coating of Petri dishes and ceramic tiles surfaces to show that TiO_2 coated surface is the best in deactivation of *E. coli, S. aureus, Pseudomonas putida* and *Listeria innocua* in exposure of UV-light.[18]

TiO_2-coated catheters are another promising antibacterial application of TiO_2 in biomedicine due to their safety and potential of light-induced disinfection for clinical use.

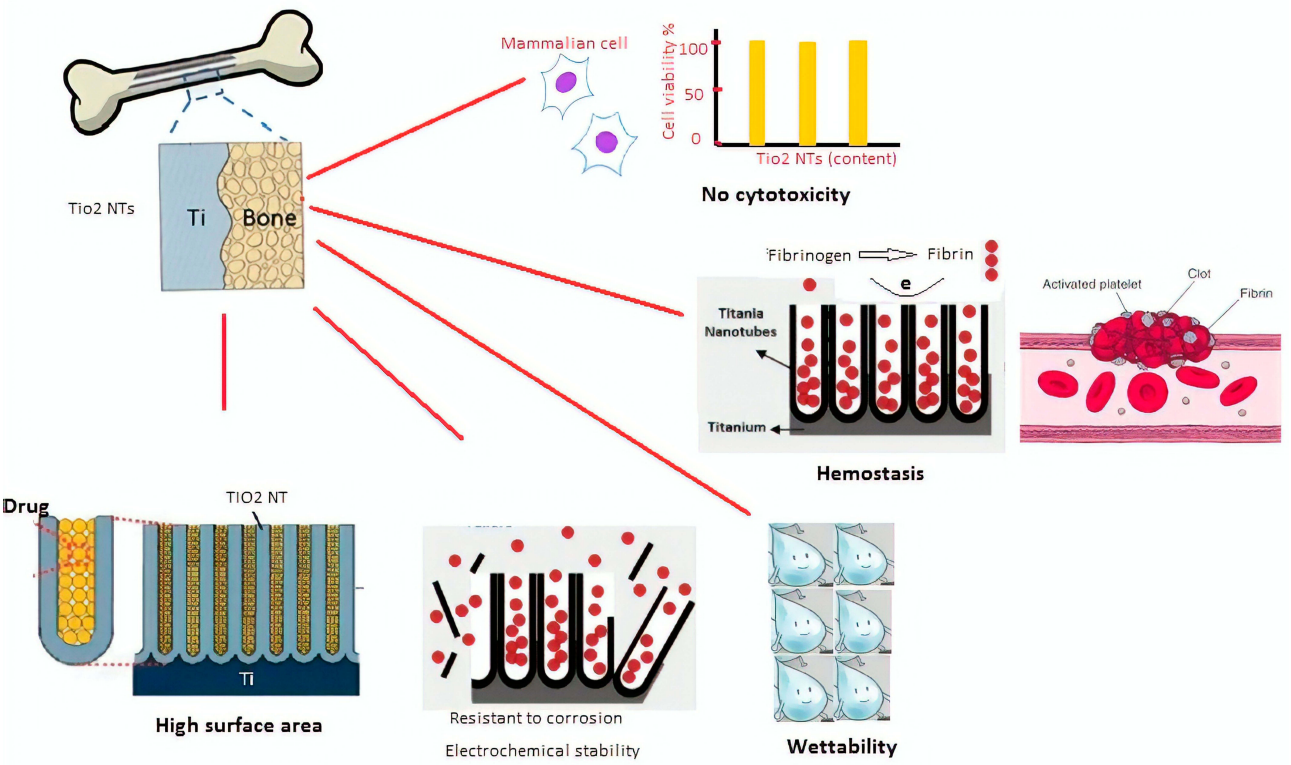

Figure 3 The main properties of TiO_2 nanostructure make it suitable for implant and other in vivo applications.

Sekiguchi et al developed TiO_2-coated catheters for clean intermittent catheterization (CIC) and improved the antibacterial activity of the TiO_2 catheter against *E. coli*. As a result, the number of *E. coli* declined to an insignificant level within 60 min exposure to UV light.[76] Besides, TiO_2-coated silicone catheters and medical tubes showed bactericidal effect on *E. coli* cells.[77]

In clinical and medical devices, sterile needles, such as lancets, are demanded and applied as a self-monitoring device for blood glucose (SMBG) in diabetes. In the progression of needles, the safe-sterilizing capability is one of the serious issues. Up until now, different sterilizing methods have been suggested, among which Gamma-ray irradiation is widely employed. The main problem regarding γ-ray irradiation is equipment costs and γ-ray leakage. Therefore, the antibacterial properties of TiO_2—due to its photocatalytic activity—have been examined. Nakamura et al developed a self-sterilizing lancets coated with an annealed TiO_2 layer. Their observation determined that these coated lancets had antibacterial properties against *E. coli* K-12 suspension under UV-illumination.[78] Besides, sulfur-doped TiO_2 could also be applied for antimicrobial purposes on the surfaces of medical devices under hospital lighting conditions. This study performed by Dunnill et al confirmed that 99.5% of *E. coli* death occurs after 24 hours of irradiation.[79]

In addition, Li et al,[80] fabricated a multifunctional textiles with biocidal activity (composite of TiO_2 and Ag NPs) against common Gram-positive and Gram-negative bacteria, along with proposed antibacterial activity (Figure 4). The risk of microbial infection is one of the important factors in producing antimicrobial wound healing materials. On the other hand, uncontrolled bleeding from wound is the main cause of infection in most of accident and surgery cases. Therefore, developing effective materials with hemostatic and antimicrobial property is required.

Several lines of studies have demonstrated that the combination of metal oxides, such as TiO_2, with natural polymers can be a promising approach for the control of infection and hemorrhage.[47] In this regard, nanocomposites based on TiO_2 and chitosan NPs have mostly studied. In 2013, Archana et al developed nanocomposited films consisting of TiO_2 NPs loaded with chitosan-pectin, which have high antibacterial activity against a broad range of bacteria and improved hemostatic ability due to the presence of TiO_2. Moreover, the introduced nanocomposite showed no toxicity in in vivo and in vivo studies.[81] The most important application of these findings

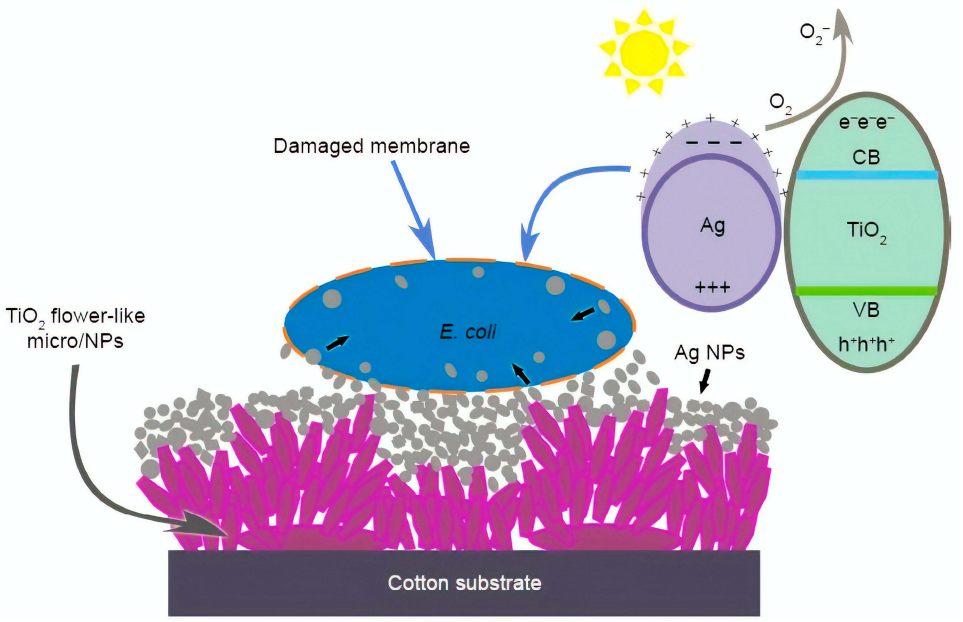

Figure 4 The suggested antibacterial activity mechanism of flower-like nanostructures on cotton surface coated with TiO_2 and Ag NPs.
Notes: Reproduced with permission from Li S, Zhu T, Huang J, Guo Q, Chen G, Lai Y. Durable antibacterial and UV-protective Ag/TiO_2@fabrics for sustainable biomedical application. *Int J Nanomedicine*. 2017;12:2593-2606. © 2017 Li et al; Creative Commons Attribution – Non Commercial (unported, v3.0) License (http://creativecommons.org/licenses/by-nc/3.0/).[80]

could be the design of wound healing bandages, which protect wound from microbial infection and hemorrhage.

Other research evaluated the effects of TiO_2 doped films into polyester and polyethylene to inactivate and kill bacteria. The TEM images of damaged *E. coli* reveal that, after 120 min, outer layers of bacterial cell wall demonstrate discontinuities in some regions.[82] In turn, cell bacteria inactivation leads to changes in cell morphology. As the cell membrane regulates the material flow and osmotic pressure into and out of the cell, leakage of internal components of cell caused by the cell wall damage grow on the TiO_2 nanostructured film.

TiO_2-Based Implants

Due to the nanotopographical characteristics of TiO_2 nanostructures, they have successfully been incorporated into medical applications as implants to cover some of the titanium implants' deficiencies. Here, the application of these valuable nanostructures in bone, dental and drug release implants are going to be discussed.

Bone Implants

Bone is one of the most common transplant tissues.[83] There are three main approaches to overcome bone defects. The first is an autologous bone graft, in which bone is harvested from the patient's own body. Although this method is the gold standard, it suffers from some downsides—like short-term instability, insignificant defects, and limited supply. Bone allografts, or bone transplanted from a donor, is the second method that partly compensates for the drawbacks of the first method. However, the clinical failure of this method is overwhelming (30–60% over ten years). The third consists of employing synthetic implants and engineering of new bone to replace damaged bone; this method is exponentially growing to overcome the limits of previous methods. However, this method also faces some deficiencies, like insufficient mechanical strength necessary to carry load.[83,84]

There are some key factors regarding the integration of implants and tissue engineering materials with living bone, such as surface properties—micro and nanotopography—and composition. To this end, a bioactive, cell-friendly and artificial implant material was fabricated by researchers.[85] In this study, nanotubular structure (from titanate materials) enhanced cellular behaviors and apatite formation ability of cells on this substrate. They claim that these nanostructures made from titanate biomaterials are promising candidates for biomedical application and bone implants.

Fabrication of material surfaces that provide osseointegration and support an implant for our body is one of the key

challenges in orthopedic biomaterials. A critical principle to be considered in both bone and all tissue engineering is to provide cell support and guide tissue formation through using 3D, and usually porous, scaffolds. Among metals—such as stainless steel, cobalt alloys, titanium alloys, etc.—nanostructured TiO_2 continues to be considered one of the most interesting materials due to their outstanding features, such as low toxicity, flexibility, high corrosion resistance and high tensile strength.[86] A qualified scaffold must also possess some essential characteristics, including being biocompatible[87] and porous with interconnection porosity to transfer nutrient and metabolic waste. In addition, it must have a unique surface pattern for cell adhesion, growth and proliferation in all types of tissues; in the case of bone-implants, it should stimulate osteogenesis.[83,87] Lastly, it is also important that mechanical properties of scaffold be similar to host tissue and that its degradation rate is as fast as the tissue formation rate.

Bone is a natural inorganic-organic composite, including an organic matrix composed of collagen fibrils and an inorganic reinforcement made of Hap nanocrystals.[88] As shown in Figure 5, natural bone gains its unique mechanical properties from hierarchical structures assembled in a highly organized way, expanded from nanoscale (collagen fibers) to macroscopic dimensions (dense cortical bone and a macroporous network of bone).[83,87] This hierarchical structure of bone provides not only tensile strength but also the toughness of bone. Thus, a successful bone scaffold must simulate the hierarchical structure of bone by bringing together multiple length scale materials with various mechanical properties. Also, the important role of surface characteristics in the interaction of extracellular matrix with the implant and osseointegration is indispensable.[29,83]

Osseointegration is defined as a direct structural and functional connection between ordered, living bone and the surface of a load-carrying implant. In other words, osseointegration is a development of newly formed bone around the artificial implant. In this regard, the interaction between tissue and implant at the interface is the crucial factor of implant success or failure. Nanotopography of the scaffold, which promotes cell function, is one of the most important criteria in osseointegration. In this regards, titania nanostructures have attracted much attention.[29]

Titanium and its different alloys have been used as the main material in bone implants because of their high specific strength, satisfactory stability, low elastic modules that perfectly match with elastic modules of tissue, and ability to form a thin and stable oxide (TiO_2) layer on the surface that is resistant to corrosion. However, disadvantages include reduced interaction with surrounding tissue and their bioactivity.[88] One of the approaches to overcome these problems is to coat the surface of this metal family with biocompatible compounds.[89]

In 2015, the Chung group coated the Ti surface with hydroxyl apatite-TiO_2 (HAp-TiO_2) and immobilized BMP-2 on the surface to construct a uniform nanoporous structure. BMP-2 (bone morphogenetic protein-2) is the group of growth factors that are unique because of their influence on cell differentiation of osteoblasts. Furthermore, HAp-TiO_2, with a proper porous structure, encourages cell growth and adhesion. All of these features lead to a reduction in bone healing time at a bone-implant surface.[90]

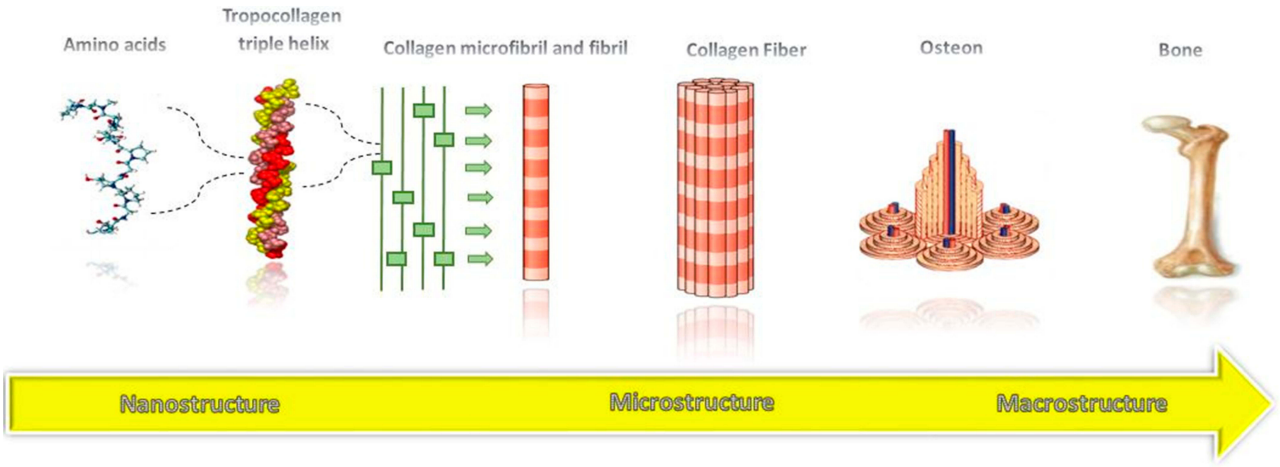

Figure 5 The hierarchical structure of natural bone.

Mohammadi et al completed an experiment to compare the impact of SiO_2, TiO_2 and SiC on enhancement of setting time, mechanical strength and hydraulic reaction of calcium phosphate bone cement. Their results indicated that TiO_2 NPs are an excellent addition to enhance mechanical strength of calcium phosphate cement in the short-term.[91] The nanometric thickness increased surface area and porous structure of TiO_2 NTs coatings, accelerating cell adhesion and improving bone capabilities.[92]

Even the diameter of TiO_2 NTs is one of the important factors in cell adhesion and osseointegration. In 2013, the influence of different diameters prepared by anodization TiO_2 NTs on the adsorption of collagen type I was investigated.[93] Collagen type I (Col-I) was adsorbed with a higher outcome and faster speed on TiO_2 NTs with diameter around 100 nm compared to 30 nm, in which the molecular dynamics simulations confirmed that Van der Waals forces and the hydrogen bond between Col-I molecules and TiO_2 NTs were the main driving force in adsorption mechanism. Thus, not only does TiO_2 NTs dimension have an essential role in the regulation of biological functions of cells, but also—as this research demonstrated—it influences the adsorption capacity of collagen on this family of nanostructures.[93]

In a recently developed study, an efficient method was described to enhance the adhesion of TiO_2 NTs. This utilized high-pressure torsion treatment to encourage grain refinement on the basic Ti layer. This process also improved the osseointegration and biocompatibility of TiO_2 NTs due to the increased surface elastic modulus. In this research, it has been shown that TiO_2 NTs with length of 0.4 μm, because of less interfacial stress, have high adhesion strength as compared to the longer NTs with length of 2 μm.[94]

Employing an innovative method, Alves et al managed to fabricate a collection of 50–90 nm diameter TiO_2 NTs, which simulated the morphology of micron structure of natural bone, and filled them with bioactive elements (calcium and phosphorous) to construct a more efficient bio-functional implant surface. The results demonstrated an enhancement in osteoblastic cell function and adhesion at the interface, along with displaying an improved corrosion behavior for TiO_2 NTs.[95] Not only do they participate in the bone-implant interface, but TiO_2 NPs could also play an important role in improving the quality of implants. Polymethylmethacrylate (PMMA) is bone cement with the primary function of transferring forces from bone to prosthesis. PMMA has been used in surgical fixation of artificial joints for a long time.[96] Khaled et al studied the physical and mechanical properties of PMMA bone cement that was reinforced with nano-sized titania fiber fillers (0–2%). The results revealed significant improvement in fracture toughness (63%), flexural strength (20%) and modulus (20%), and the optimal composition contained 1% wt nanostructured TiO_2 fibers compared to control cement.[97]

Dental Implants

Organ loss due to disorders, disease or injuries leads to the need for organ restoration—for example, joints or teeth—which is conventionally performed by replacement with artificial material. These artificial materials should be connected to surrounding tissues via fibrous connective tissues in order to efficiently perform their biological functions.[98,99] Like other organ replacements, the long-term success of dental implants largely depends on rapid healing with safe integration into the jaw bone and surface. Among the few characteristics that define a dental implant system, the surface treatment, topography, macro design and geometry of implant are the main parameters for both short- and long-term durability of dental implants.[100,101]

As a significant effective factor on osseointegration, implant surfaces—which are critical for implant stability—have been studied and developed further in recent decades to provide a faster and more adequate osseointegration process in newly formed bone. Surface characteristics of the dental implant are defined at the macro-, micro-, and nano-scale. The most common form of nano features encountered in dental implant surfaces is nanoroughness.[91,102] In other words, along with chemistry of dental implants, surface topography is crucial to achieving suitable osseointegration. The porous surface has a crucial role in this area. A porous surface provides more substantial space for drug loading and, with better biocompatibility, TiO_2 NTs arrays are desirable candidates to cover the surface of the dental implant. A study in which TiO_2 NTs were grown on dental implant surfaces by anodic oxidation method confirmed abovementioned fact. TiO_2 NTs with a diameter of 60 nm and length of 10 μm that were loaded with BMP-2 showed improved osseointegration.[103]

The interaction between implants and blood play a vital role in peri-implant healing. In some cases, immunological interaction due to the exposure of implantable titanium causes inflammation or fibrosis, leading to implant failure. It has been demonstrated that TiO_2 NTs have good blood-compatibility, indicating their high potential for surface modification of blood-contacting implants.[46] Even immobilizing some biological molecules—like Gly-Arg-Gly-Asp-Ser peptide—on TiNTs may improve osseointegration in dental implants.[104]

A 20–160 nm mixed nano-/submicron-scale TiO_2 network layer successfully enhanced human bone marrow stem cell adhesion on titanium dental implants. The result was significantly positive.[105] TiO_2 mesh layer on a polished Ti surface with averaged lateral mesh size (between 34 and 93 nm) showed a better blood response, since size scale of the nano-mesh TiO_2 layer was similar to that of blood proteins. All these actions led to promoted cell growth in the application of dental implants.[106] Also, the increment in blood compatibility is associated with minimizing the activation of both blood platelets and blood clotting cascade on the implant surface in the plasma. Huang et al investigated the effects of different constructions of the TiO_2 NT on blood platelet behaviors and adhesion. The results of their research suggested that surface chemistry, structure, wettability and crystalline phase had a collegial effect on the platelet-rich plasma responses, where amorphous TiO_2 NTs with larger diameter could reduce platelet adhesion and activation. Additionally, it has been reported that combination of several properties of TiO_2 NTs—such as wettability, surface topography and chemistry—can improve cell adhesion, migration, proliferation and differentiation. In this case, development of high contrast wettability patterns on surface of TiO_2 nanostructures have been a promising approach to achieve more applicable biomedical applications, such as implant scaffolds.[107,108]

Several lines of studies have demonstrated that crystalline phase plays a key role in cell behaviors and blood compatibility of TiO_2 NTs. TiO_2 NTs with mixed crystalline phases could cause a decline in platelet adhesion and fibrin network formation phenomena.[109] It has been proposed that rutile TiO2 NTs provide highest protein adsorption of collagen and fibronectin. While crystalline phase of TiO2 NTs has no significant effect on cell proliferation and differentiation.[110]

In conclusion, the nanotopography of dental implant surface results in better osseointegration, cell adhesion and blood response of dental implants, which are very important in the success of the implant.

Like bone implants, incorporation of some biological compounds can promote TiO_2 biocompatibility in dental implants, too. Biocompatibility of TiO_2/HA nanocomposites with surroundings tissue has been evaluated by Youn et al. They coated the surface of commercial titanium implants with nano TiO_2/HA composite bioceramic utilizing sol-gel route. In order to examine the nerve regeneration, cultured Schwann cells were used. SEM, MTT assay, total protein content, leakage of cytosolic lactate dehydrogenase (LDH) activity and measurement of the amount of brain-derived neurotrophic factor (BDNF) secreted by Schwann by enzyme-linked immunosorbent assay (ELISA) was performed to evaluate the response of the cells to the coating. The observed results emphasized the good biocompatibility of TiO_2/HA nanocomposite bioceramic coating with Schwann cells for nerve regeneration around dental implants.[111] A novel multiform TiO_2 nanonetwork was introduced for dental implants by Yang and Huang.[112] The authors highlighted that the hydrophilicity and protein adsorption ability were improved on nanonetwork structures leading to enhance cell adhesion, mobility, proliferation and osteogenic differentiation.

Drug-Releasing Implants

After implant surgery, the patient receives drugs—such as antibiotics, anti-inflammatories and growth factors—to prevent infection, inflammation and induce suitable integration of implant with natural tissue, respectively. These medicines are delivered by four different delivery routes, namely oral, intravenous, intramuscular and topical. The uptake of antibiotics is only provided by intravenous, intramuscular and topical routes because this group of drugs travels to the liver, where it is then inactivated. Although growth factors are often delivered intravenously and topically, systematic growth factor delivery is not effective enough, as the drug cannot reach the interface of implant and tissue. Systemic doses increase is not a solution to this problem because it leads to cellular toxicity.[113] Therefore, the need for development of a more suitable directed drug delivery system to the site of implantation is unavoidable. This strategy has the advantage of minimizing the side effects of systematic administration by decreasing the drug concentration.[114] In the following, two novel local drug release systems based on TiNTs, and TiO_2 mesoporous films is introduced.[114]

TiNTs arrays, fabricated by electrochemical method with pore diameter of 10 to 300 nm, thickness of 0.5 to 500 μm, high pore volume of more than 20 m^3/g, high surface area of 180 to 250 m^2/g, high aspect ratio of ~1000–2000 and NT density of ~1010 NTs/cm^2, have been investigated for application in local drug delivery. The system can be described as a layer of tightly packed, vertically aligned, ordered NT structures with hexagonal arrangement that grows perpendicularly to the Ti surface. TiNTs have also attracted considerable attention because of their NT structure, high surface area and loading capacity, tunable drug release capability, tailorable surface chemistry, both chemical stability and

mechanical rigidity, terrific biocompatibility, and also the ability to grow on the surface of current implants. Logic and his research group have done fabulous work in this area and provided most of the comprehensive reviews on this topic, so it will not be covered further in this work.[68]

Mesoporous TiO_2 films not only provide a chemically inert mesoporous surface to combine with either active low molecular weight hydrophobic or hydrophilic substance, but also they promote osseointegration as they supply nanotopography on the surface. A 200 nm-thick mesoporous TiO_2 thin film with 6 nm-sized pores has been developed and applied to the surface of titanium implants. Then, it was loaded with renowned osteoporosis drugs, Alendronate (ALN) and Raloxifene (RLX). The drug-loaded implant was compared to the implant without drugs regarding implant fixation and *de novo* bone growth. Early bone-implant fixation by both drugs has been improved by the ex vitro evaluation, and locally delivered RLX got the most surprising results.[114]

The mesoporous TiO_2 thin film also can be loaded by antibacterial agents, as they can provide a high surface area in order to load high doses of drugs. In 2014, a mesoporous thin film fabricated using the previously mentioned method was loaded by four different antibacterial agents, and their antibacterial effect was evaluated against five different bacteria species. Additionally, silver NPs and minocycline—as two antibacterial agents—were loaded together to be evaluated for their synergic bactericidal effect. The combination of these two antibacterial agents affected all five types of bacteria. Because of the high capability of releasing the antibacterial agent, this device could be applied to the field of dental and orthopedic implants to prevent bacterial infections.[115]

Osteoporosis drugs and antibiotics are not the only agents able to be loaded on nanoTiO_2 coated surfaces. Magnesium (Mg), an important element in proliferation and metabolism of osteoblast cells in the human body, can also be incorporated onto these surfaces. It is a critical element for protein formation and growth factor expression, while also aiding bone mineral deposition on the surface of implant.[116,117] Mesoporous TiO_2 thin films loaded with Mg have been used to evaluate the impact of Mg on osseointegration, osteoconductivity and osteoblast differentiation. The results demonstrated that local release of Mg from mesoporous TiO_2 thin film coating causes higher expression of OC, RUNX2 and IGF1 genes, which are involved in osteoblast differentiation and bone formation.[118] Also, new bone formation occurred around all surfaces, and there was an observation of a tendency toward more bone-to-implant contact.[116] The newly formed bone additionally displayed a similar bone mineral density to that natural bone.[118]

Strontium (Sr) is a promising mineral that can take part in decreasing bone fracture risk in osteoporotic patients. It has attracted much attention after the development of an anti-osteoporotic drug, Strontium Ranelate (SR).[119] An increase in osteoblast replication and mineralization of bone matrix result of Sr is achieved via the calcium-sensing receptor (CaR) dependent mechanism. In 2013, an experiment conducted by Zhao et al examined the influence of Sr on osteogenic effects and properties. They loaded different amounts of Sr in TiO_2 NTs with different diameters. The results determined the positive effects of NT_{10}-Sr_3 in inducing differentiation of osteogenesis, bone morphogenetic protein, mesenchymal stem cells and adsorption of favorable proteins for cell function.[119]

Polyetheretherketone (PEEK) is an FDA approved thermoplastic biopolymer that has been used extensively in orthopedic applications because of excellent mechanical, chemical and biocompatible properties. The inert nature of PEEK is the most critical impediment. Lately, it has been demonstrated that titanium film improves this property as a bioactive coating and could be directly used onto PEEK implants by e-beam evaporation. In a study, Roy et al increased *in vitro* biocompatibility of PEEK by designing nanoporous TiO_2 surface that allowed in vivo osteoconductivity of implant by enhancing immobilization and delivery of BMP-2.[72]

TiO_2 coatings on implant surfaces not only deliver a drug locally to the target but also reduce organ toxicity. TiO_2 NTs have a high capability to be applied to the Ti implants, because they increase biocompatibility and surface area of the implant, however, uncontrolled drug release and poor mechanical properties are two crucial disadvantages with TiO_2 NTs. From this aspect of view, Jia and Kerr engineered a drug carrier for ibuprofen by using biocompatible PLGA (polylactic-co-glycolic acid)/TiO_2 NTs instead of pure TiO_2 NTs. Ibuprofen is an anti-inflammatory drug with very short plasma half-life (1–3 h). Developing polymer/TiO_2 NTs increased the time of ibuprofen drug release up to 5 days for low-molecular-weight PLGA and nine days for high-molecular-weight PLGA.[120]

Moreover, Gulati et al attempted to enhance drug release by coating TiO_2 NTs with different thicknesses of chitosan or PLGA. TiO_2 NTs were produced through a two-step

anodization procedure. The result revealed that thin PLGA coatings through one dip-coating cycle yielded 57 ± 1% burst drug release during 6 hours, which is faster than thin chitosan, yielding 40 ± 2%. In a contrary manner, thin PLGA and chitosan coatings obtained after five dip-coating cycles released 12 ± 2% and 35± 2% of the drug during 6 hours, respectively. These results confirmed that the type and thickness of polymer are two critical factors to control drug release quantity. Furthermore, the result of this study confirmed that polymer-coated TiO_2 NTs exhibit higher osteoblast adhesion and proliferation compared to non-coated TiO_2 NTs.[121] Likewise, another study presented chitosan coated TiO_2 NTs, which were used for controlled drug release of gentamicin. These NTs demonstrated simultaneous antibacterial properties and osteoblast adhesion.[122] Development of such strategies can overcome essential problems in orthopedic implants related with bacterial infections and bone cell promotion.

Designing a sustained drug releasing system for veterinary medicine is another area of research that significantly decreases the harmful side effect and improves the therapeutic effect for the animal disease. The Enrofloxacin Hydrochloride (Enro) is a veterinary antibiotic with the lowest minimum inhibitory concentrations (MICs) mostly used in poultry. Lai et al developed a long-term sustained releasing system for Enro drug. This group used hydrothermal reaction method for concocting a drug by TiO_2 NTs, which have a high specific area (246.784 m^2/g) and desirable controlled release ability. in vivo drug delivery of Enro-TiNTs showed drug release rate of 32.65% in the PBS solution, while the desired release profile was achieved in low temperatures.[123]

Biosensors Based on TiO_2

In recent years, nanotechnology has made considerable progress in the development of biosensors.[6,14] Due to their unique properties (e.g., small size and big surface area-to-volume ratio), nanomaterial-based biosensors provide sensitive and rapid biological detection. Although different kinds of nanomaterials have been used in medical biosensors, this section of the paper is exclusively confined to nanobiosensors based on TiO_2 nanostructures.[124–126]

Recent interest in novel hybrid systems based on biomolecules and TiO_2 nanostructures has led to considerable success in the fabrication of bio-nano hybrid devices, such as biomolecule-sensitized solar cells (BSSCs) and photoelectrochemical cells (PECs).[127] Apart from these above-mentioned devices, interfacing of biomolecules and TiO_2 nanostructured thin film has opened up a route for further developments in this area, including biosensors. Sensitive detection of biological analytes provides opportunities for further investigation into the process involved in monitoring patient response to medical or surgical therapy. To be commercially viable, a biosensor should be inexpensive, user-friendly, sensitive and accurate, fast, and easily manufactured with high select rates.[25,128,129]

Furthermore, TiO_2 nanostructures have been employed to construct various detecting devices, such as humidity, oxygen and hydrogen sensors. These nano-sized semiconductors have proven to be an excellent electrode material in biosensors due to their special characteristics, such as porous structure, large specific surface area and excellent biocompatibility. TiO_2 can be used as immobilizing matrix, which reacts with the amine and carboxyl groups of enzymes and maintains their biocatalytic activity.[130,131]

TiO_2-based nanocomposites have attracted considerable interest for use in the biosensor. In 2015, Wang et al used DNA functionalized nanocomposite containing electro-reduced graphene oxide, TiO_2 nanowires and chitosan to detect the specific title gene sequence from *Vibrio parahaemolyticus*. The presence of TiO_2 nanowires increases the interface area of nanocomposite.[132]

TiO_2-based biosensing of target analytes is commonly carried out by electrochemical (e.g., amperometric and potentiometric process) or PEC techniques. Electrochemical biosensors are usually based on amperometric and potentiometric detection. When the bio-recognition elements—such as antibodies, enzymes, aptamers or cells—bind to or react with target molecules, the conductive properties of a medium between electrodes changes, resulting in a detectable current signal (amperometric), a detectable potential or charge accumulation (potentiometric).

Among various electrochemistry-based biosensors developed so far, the PEC biosensors that are based on the photoelectric phenomena have attracted a great deal of attention. The PEC assay has the advantage of enabling both optical and electrochemical detection. Because of the significant scientific and technological interest in recent years, here, we focus on PEC biosensors.

Depending on the intended manner of biosensing, the construction of biosensors can be different.[12] In general, a typical TiO_2-based PEC biosensor comprises three main components: a nanostructured TiO_2 film deposited onto a conductive substrate as the working electrode (WE), which is responsible for generating and transporting electrons and holes under illumination; a counter electrode (CE)

coated with some electrocatalyst, and an electrolyte placed between the two electrodes to shuttle the holes to the CE.

The PEC biosensors are based on the PEC process in which photon-to-electricity conversion occurs. The general mechanism of the PEC process involves photon absorption by the semiconductor, generation and separation of electron (e^-)-hole (h^+) pairs, and transfer of electrons to the WEand holes to the electrolyte. The presence of target analyte changes the photocurrent generated by the system; this change in photocurrent is proportional to concentration of target in the sample (Figure 6).

Various biomolecules, such as enzymes and DNA, have been used as bio-recognition elements. For instance, in the case that DNA is conjugated to TiO_2, the presence of target DNA leads to formation or cleavage of double-stranded DNA resulting in change in light-harvesting performance of the WE directly or indirectly. So, this causes a change in charge separation and, subsequently, the generated photocurrent.[133] The material used as a transducer in TiO_2-based PEC biosensors can be made of (a) TiO_2 alone, (b) TiO_2 hybrid with some inorganic semiconductors, or (c) composite of TiO_2 with other materials.

Narrow-band gaps quantum dots can sensitize TiO_2 NPs and achieve energy band modulation. CdS NPs with a bandgap as narrow as 2.4 eV and a broad excitation spectrum are ideal sensitizing materials. Excitation of the CdS causes charge separation and prompts transfer of conduction band (CB) electrons to that of TiO_2.

Recently, CdS QDs/TiO_2 NTs hybrid has been used for sensitive detection of prostate-specific antigen (PSA), which is important as a tumor marker for prostate cancer.[134] The CdS QDs loaded TiO_2 NTs were used as transducers. The sensitization of TiO_2 with CdS can expand the wavelength range of excitation and improve the photoelectric performance of TiO_2 electrodes. Also, the coupling of CdS and TiO_2 can reduce the recombination of the photo-induced electrons and holes; so, these phenomena result in higher conversion efficiency.[135]

In the presence of PSA, through an immune-sandwich assembly, immune-gold labeled alkaline phosphatase (ALP) was attached to the CdS QDs/TiO_2 NTs electrode (Figure 7). ALP is able to catalyze ascorbic acid 2-phosphate (AAP) hydrolysis in situ in order to generate AA for efficient electron-donating. Results indicate that increased PSA concentration caused improved photocurrent responding.

Recently, a PEC biosensor based on CdS sensitized Fe-doped TiO_2 has been reported by Fan et al for the detection of squamous cell carcinoma antigen. Iron doping of TiO_2 improves the absorption of composite in visible region. In addition, rough surfaces of Fe-TiO_2 NPs are suitable for sufficient binding of carboxyl functionalized CdS NPs.[136] PEC biosensors can be integrated with other detection methods to create biosensors with desired properties and behaviors. For example, Bernacka-Wojcik et al developed a disposable biosensor by combining dye-sensitized solar cells (DSSCs), in which TiO_2 nanoparticulate films were used as a photoanode and colorimetric DNA detection method for sensing specific DNA sequence from *Mycobacterium tuberculosis*.[137]

The mechanism of the biosensor is described as follows. A source of light with certain wavelength is adjusted to

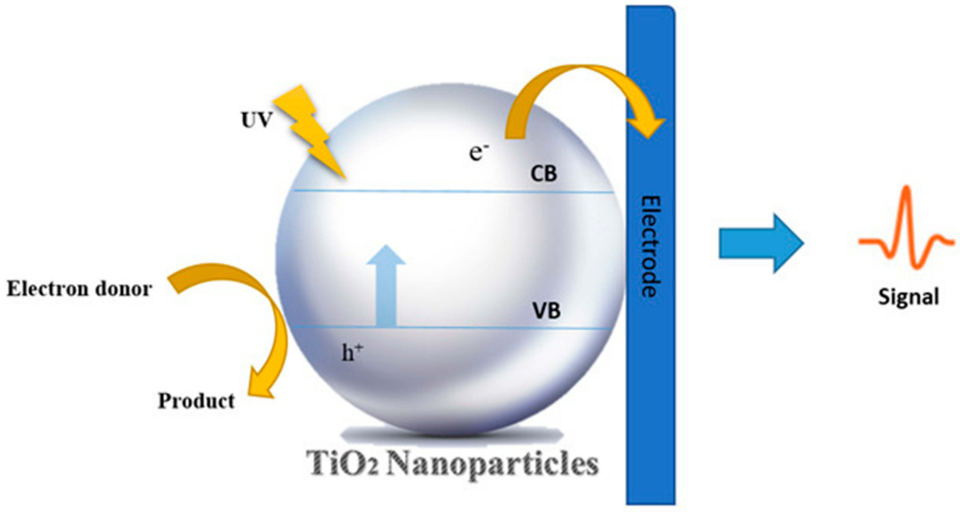

Figure 6 The schematic diagram of the biosensor PEC process.

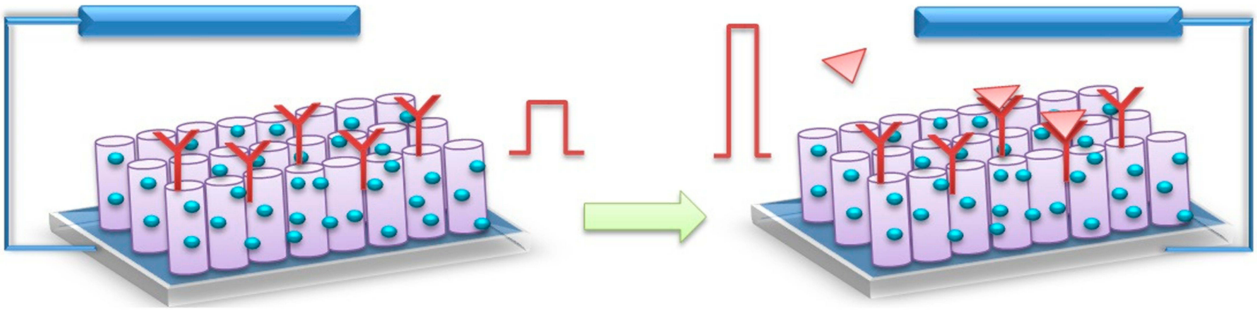

Figure 7 Schematic demonstration of PEC biosensors based on TiO_2 NTs. The signal is enhanced in the presence of target molecules.

shine directly on the solution containing DNA functionalized gold NPs and passes through it. Finally, a DSSC, placed after the solution, absorbs passed light and generates photocurrent. Since the dye adsorbed on the surface of TiO_2 nanoparticulate film has the maximum absorbance at the wavelength of shined light, any obstacle in the absorption of light at this wavelength leads to a decline in photocurrent.

Target DNA can bind to complementary strands of target DNA attached to the Au NPs, leading to their aggregation. Monodispersed and aggregated Au NPs have different absorbance peaks. In the presence of target DNA in Au NPs solution, the absorbance peak of solution at the wavelength similar to those of the shined light decreases, so the DSSC can capture more light at this wavelength to increase generated photo-current. The biosensor was able to detect the aggregation of Au NPs concentrations as low as 1.0 nM.

In another work, a nanocomposite of g-C3N4 and TiO_2 nanosheets was used to fabricate a PEC biosensor that was able to detect glucose within a detection limit of only 0.01 mM.[138] Two-dimensional TiO_2 nanosheets with a high specific surface area showed promising potential to accommodate a substantial load of glucose oxidase. Besides, in order to excite PEC biosensor by visible irradiation and avoid possible deactivation by UV light, g-C3N4 was used to minimize the bandgap of the nanocomposite.

In conclusion, nanobiosensors based on TiO2 nanostructures (such as NPs, nanorods, and NTs) are developed for sensing of wide range of biomarkers (including DNA, RNA, proteins, lipids, metabolites, etc.).[139] In recent years, PEC biosensors based on TiO2 nanostructures have attracted much attention due to their desirable sensitivity and great analytical performance.[140,141] However, further research is needed to provide more sensitive and selective TiO2-based biosensors to be used in clinical applications.

Toxicity and Biocompatibility of TiO_2 Nanostructures

In recent years, nanoscience has been more widely is used as an interdisciplinary field of medical science and engineering.[1,13] Nanomaterials with unique physicochemical properties, such as large surface area to volume ratios, led to being enormous reactive compounds compared with bulk materials and, thus, can be potentially hazardous.[142,143] Undoubtedly, considering the wide range of nanomaterial applications throughout our daily lives—like health, food, medicine, industry, cosmetics, agriculture, etc.—the nanotoxicity evaluation and safety assessment of nanomaterials is an important area, given the limited information available in this regard.[144–146]

Understanding the toxicity mechanism of nanomaterials will be useful for predicting the potential toxicity of nano-sized particles on human health. In this regard, several in vivo and *in vitro* studies have been completed to assess ROS generation, changes in activity of antioxidant enzymes, lipid peroxidation, damaged DNA bases, protein carbonyl content, total glutathione concentration, superoxide dismutase activity, catalase activity and total antioxidant potential.[143,145,147]

Nano-sized materials that are directly related to human health must have no allergic or inflammation effects. Therefore, design and fabrication of nontoxic materials that have no hazardous potential for human health are one of the nanotoxicology research aims.[145] NPs can interact with macromolecules and cell organs for a long time in different ways, such as circulation, extravasation, diffusing, binding, internalizing, degrading, releasing, reacting, killing, regulation of expression, remodeling and normalizing (Figure 8).[148] Considering the small size of nanomaterials, these particles can penetrate and undergo uptake into human cells and tissue through ingestion, skin penetration, injections and inhalation.[149] Besides, nanostructures toxicity commonly demonstrate effects through membrane damage, dysfunction

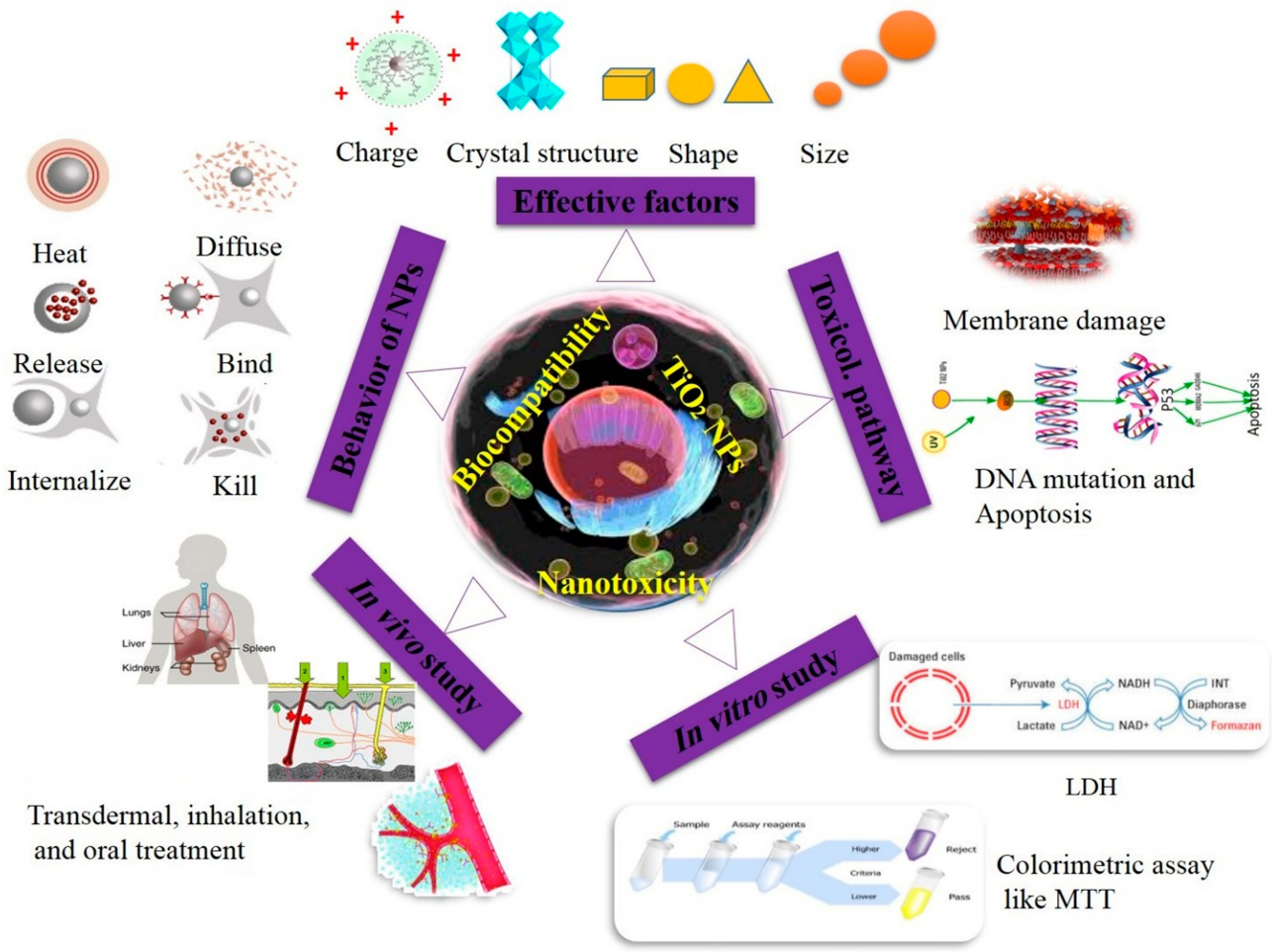

Figure 8 Key parameters affecting the toxicity of TiO_2 nanostructures and methods of assessing their toxicity.

of protein, DNA mutation, cell apoptosis, proliferation behavior and more (Figure 8).

The primary mechanism of NP toxicity is ROS and subsequent production of oxidative stress. The dangerous aspect of this mechanism eventually ends up in amyotrophic lateral sclerosis, cardiovascular, pulmonary, neurodegenerative, arthritis, renal or cancer diseases.[147,149] ROS production is one of the main mechanisms of nanotoxicity, which could trigger a series of oxidative stress that affects some physiological redox-regulated functions and leads to cell disruption. Therefore, it causes several toxic mainstream events, including occurrence of apoptosis, genotoxicity, uncontrolled cell signaling, change in cell motility, cytotoxicity and cancer initiation.[13,32] In addition, oxidative stress at a higher level could lead to intracellular Ca^{2+} release and change in mitochondrial membrane structure.[147,150] Rallo et al recommended that oxidative stress induced by NPs is associated with cell signaling changes in three stages named low (*nrf2* transcription factor defense against oxidative stress),[151] high (activation of inflammation signaling pathway via *NFκB*) and very high level (activation of cell self-destruction pathways and necrosis) of oxidative stress.[152]

As mentioned, TiO_2 NPs are one of the most commonly used nanomaterials with medical, industrial, and commercial applications.[146,147,153,154] The aim of drug carrier systems is to enhance efficacy and decrease adverse reactions of drug molecules.[144] Nevertheless, the TiO_2 particles in the nanometer-scale have unknown hazardous effects.[153] Therefore, before using each NPs—especially TiO_2, we need to know about its biocompatibility and toxicity in advance. Generally speaking, toxicity of a nano-sized material is mostly dependent on the NP type, concentration, time of exposure, physico-chemical properties of the particles (size, shape, particle surface, surface ± charge and surface-containing groups), aggregation, mode of interaction with cells, NPs ion release, dissolution, crystal structure (Ru or

An), hydrophobicity, bandgap, pH of medium, surface coating group and functionalization. These properties of NPs can affect cellular uptake and generate adverse results (Figure 8).[147,148,155,156] For instance, accumulation of NPs with high aspect ratios (NPs shape) in macrophages is slower than ones with low aspect ratios. This, in turn, leads to reducing the time of clearance and efficiency of removal from the blood.[148] As described, characteristic properties of nanostructures influence their biological interaction with surrounding molecules.[149] It should be mentioned that cells can only uptake drug nanocarriers smaller than 300 nm. Therefore, size is a key factor of toxicity for a drug carrier system to be easily uptaken by cells and tissues.[1,2,7,13,154]

On one hand, as previously mentioned, size is one of the significant determinants that affect interaction of NPs with cells and proteins and is a key factor in evaluation of toxicity of NPs. On the other hand, size and surface area to volume ratio affect the ability of nano-sized particles to penetrate cell membranes and other biological barriers, leading to several side effects.[144,156] Palaniappan et al pointed out that the structural conformation of proteins is dependent upon particle size of TiO_2.[142] Apart from size, the particle shapes—such as rode, spherical, fibers or tubes—are a crucial factor to determine the toxicity and have different toxicity potential because of easier or faster endocytosis uptake, or perhaps different contact area with the cell membrane.

Surface charge is one of the other most important factors of NPs toxicity, as it influences the hydrophobicity of NPs, adsorption of ions, interaction of NPs with physiological environments, can affect the NPs and protein interactions, all of which may change bio-distribution of NPs. However, toxicity and undesirable effects of NPs can be reduced by coating, which affects the stability of NPs and inhibits their agglomeration.[149,156] Crystalline structure is one of the other factors of ultrafine particle toxicity. The TiO_2 crystal structure effect (*Anatase*: TiO_2–An with <25 nm and *Rutile*: TiO_2–Ru with<100 nm) was evaluated on the genotoxicity responses in human hepatoma *HepG2* cells. The results showed that the genotoxicity potential of TiO_2 NPs varies based on crystal structure, so that genotoxicity of TiO_2–An crystal structure (with smaller size) is more inducing than TiO_2–Ru crystal structure, whereas both crystal structures caused ROS production, DNA damage and up-regulated mRNA expression of p53. This study recommended that not only size but also crystalline structure influence the NP toxicity.[157]

Suh et al studied toxicity by several different metal oxide NPs using cell membrane damage assays, and among the metal oxides, TiO_2 tends to be less hazardous to organisms than other nanomaterials (TiO_2<Al_2O_3<SiO_2).[158] Titanium oxide is a harmless metal oxide and is non-toxic; therefore, regarding their good biocompatibility, titanium NTs can be used as drug carriers.[143,153,154] Shokuhfar et al introduced a biocompatible nanocarrier in 2013 for anti-inflammatory drug molecules delivery based on TiO_2 NTs and reported that this system can be applied to many other drug systems.[154] Wang et al developed a pH-controlled antitumor drug release system of TiO_2–NTs. They reported that TiO_2–NTs carriers have good biocompatibility and excellent drug delivery properties.[159] A comparative study of ZnO and TiO_2 NPs nanotoxicity was conducted by De Angelis et al (based on NPs size, number of particles, surface area, surface charge, agglomeration state, impurities, chemical composition and ion-release; results showed that ZnO NPs produced more significant cytotoxicity than TiO_2. They found that TiO_2 NPs were not cytotoxic, but both ZnO and TiO_2 induced ROS generation. In addition, they suggested that due to spherical shape of these TiO_2 NPs, higher cell interaction of TiO_2 was observed in comparison to ZnO NPs, which consequently leads to higher cellular uptake. Also, the ROS production was lower after 24 h exposure to TiO_2 NPs than that observed after 6 h. For this reason, they suggested that maintaining antioxidant potential of cells after 24 h TiO_2 NPs exposure is the reason for low level ROS production.[160]

One of the most important issues which must be considered to select NPs type in medicine and nanomaterials-based products is toxicity or safety of nanomaterials. Toxicity of TiO2-based nanostructures have been widely evaluated as in previous researches. These nanostructures are safe (or at least less toxic than other types of NPs), user-, and eco-friendly than other nanostructures.

Summary and Perspectives

TiO_2 nanostructures—including NPs, NTs and nanorods—and their composites have attracted further attention in the field of medicine due to their unique properties, such as non-toxicity, biocompatibility and affordability.[1,11,12,161,162] Biomedical applications of these fascinating nanomaterials can be categorized into four main groups: biosensing, drug delivery, antibacterial activity and implant applications. The potential application of TiO_2 in electrochemical and PEC biosensors for detection of various analytes has been widely investigated in recent years. Possessing the two methods for excitation (light) and detection (current), PEC biosensors are

considered to be very sensitive technique with low background signals and good analytical performance. However, this method also faces some deficiencies. TiO_2, with a wide bandgap (3.2 eV for anatase, 3.0 eV for rutile phase), absorbs only a small portion of the solar spectrum in the UVregion.[163,164] In addition, the photogenerated holes formed at illuminated TiO_2 can have a destructive effect on biomolecules.[165] It is very important to investigate toxicity of special nanostructures used in biomedical applications. We reviewed toxicity of TiO_2 nanostructures in various manners, such as drug delivery, implant application and more.

Moreover, the effective factors in toxicity, toxicological pathways and different in vivo and in vivostudies are mentioned. A growing body of research demonstrates that TiO_2 nanostructures are safer than many other nanomaterials for biomedical applications. However, much more information and careful evaluation of toxicity and clinical outcome of TiO_2 is required before applying it to clinical practice. The development of bacterial resistance to common antibacterial agents and necessity to perform self-sterilizing conditions highlights the need for robust materials to control bacterial infections. It has been demonstrated that TiO_2 NPs can fulfill this role effectively.

Despite powerful antibacterial activity of nano-sized TiO_2, its light dependent property has been reported as its drawback. However, this property has made TiO_2 a smart nanomaterial for both antibacterial and drug delivery uses. Recent advances have also confirmed that TiO_2 NPs can have light-control drug release. This phenomenon can be a compelling aspect for most cancer therapy strategies, such as radiotherapy of skin cancers. Besides, modifying chemotherapy agents by TiO_2 NPs improve bioavailability and release time of the drug for clinical applications.

The unique properties of TiO_2 nanomaterials, like nontoxicity and nanotopographical characteristics, have attracted more and more attention to the fabrication of implants. Some of the titanium implants' deficiencies can be covered by Titania. The literature has investigated the potential of TiO_2 as bone, dental and drug release implants. Considering all of these aspects, it can be stated that nanomaterials based on TiO_2 offer the potential to develop valuable materials for biomedical applications. Finally, in order to properly develop and use TiO_2 nanomaterials in medicine, further studies will undoubtedly be necessary.

Disclosure

The authors report no conflicts of interest in this work.

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