TiO_2 Used for Better Performance as Orthopedic Implants

Subjects: Orthopedics

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Titanium dioxide (TiO_2) is the native oxide layer of Ti which has good biocompatibility as well as enriched physical, chemical, electronic, and photocatalytic properties. The formed nanostructures during fabrication and the enriched properties of TiO_2 have enabled various functionalization methods to combat the micro-organisms and enhance the osteogenesis of Ti implants.

TiO2 Ti implants antibacterial properties osteogenesis

1. Important Facts about Orthopedic Implant

1.1. Implant Failure

The success of bone surgical operations mainly depends on the quality of implantable biomaterials. Implant success is mainly halted by the infections caused by post-operative complications. Certain factors may lead to bacterial infections or even failure, including extensive damage to local tissues, improper fixation, smoking, diabetes, chemotherapy, irradiation, and inappropriate surgical techniques [1]. The implants may get an infection from surgery equipment, medical staff, room atmosphere, or bacteria in the patient's blood. The outcome of these microbial infections sometimes becomes grave, leading to a second surgery, amputation, or even death [2]. Implant infections are mostly initiated by *Staphylococcus epidermidis* (*S. epidermidis*), *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*), and *Enterobacteriaceae* [3].

Implant failure may occur at early or late stage [4]. Lack of osseointegration may lead to early implant failure, whereas in late implant failures, osseointegration works well at the beginning but decreases later due to disease and biochemical overload [5]. Researchers have identified various reasons for implant failures, which include infectious and physical damage [6]. Implant failures can be minimized by maintaining hygienic measures, caring for physical damage, and regular review of implants.

Progressive bone loss occurs due to inflammatory lesions in the soft tissues associated with the implants [4] and peri-implant disease [7]. Poor hygienic measures, unmanaged diseases such as diabetes, and the use of corticosteroids in immune-compromised individuals may all lead to that situation [8][9]. Despite taking all necessary hygienic measures, bacterial infections may still occur. Studies have suggested that joint infections may take place in 1% of primary and 3–7% of multiple surgeries [10][11]. Patients with multiple surgeries have a higher risk of

mortality and infection [10]. Implant infections and failures are a large economic burden on the health system. In the US, it costs more than $8.6 billion annually [10][12].

1.2. Fundamental Requirements of Orthopedic Implants

Bone is naturally composed of organic, inorganic, and collagen fibrils. The nano-hierarchical structures give shape and mechanical strength to bones [13]. The structures include small molecular amino acids forming tropocollagen helixes and nanoscale collagen fibers forming a microporous network of bones. There is a crucial interaction between surface characteristics and the extracellular matrix for osteointegration [14]. Bone mesenchymal stem cells (BMSC) in the bone marrow are known to typically respond to metallic implants with the production of soft tissue rather than bone, which causes implants to fail [15][16]. Guiding stem cell differentiation to a desired specific line on the surface of the material is a key factor in the success of implants [17][18]. Osteoblasts are mature bone cells, whereas osteoprogenitor cells are pluripotent cells having the capacity to differentiate into different kinds of cells. Osteoblasts and osteoprogenitor cells are in direct contact with the implants.

For better outcomes, the hierarchical structures of the bone must be simulated by the implant with surface nanostructures to support bone tissue regeneration. Apart from the surface nanostructures, other modifications, including nanoparticles, may help further. For example, bismuth oxide (Bi_2O_3) has features including electrochemical stability, high biocompatibility, and a medium band gap [19][20]. The contact of Bi_2O_3 nanoparticles and TiO_2 nanocones resulted in a heterojunction that formed a built-in electric field and promoted the osteogenesis of BMSC on the basis of TiO_2 nanostructures [21].

2. Functionalization Approaches of TiO_2 for Better Antibacterial and Osteogenesis Property

2.1. Topological Influence of the TiO_2 Nanostructures

Topological modification is among the proposed methods to achieve surface functionalization. Studies have shown that surface nanostructure and topography may affect the migration, elongation, proliferation, and differentiation of stem cells [22][23][24]. In fact, cells and tissues in vivo will experience many topographic features ranging from nanoscale to microscale [25]. Thus, building a surface nanostructure on implants is an important research direction in the fields of artificial bones, joints, and dental implants [26][27][28]. The regulation of cell fate by surface topography is carried out by direct contact with adhering cells.

It has been widely accepted to form TiO_2 nanotubes on Ti surfaces by doing anode oxidation, and the annealing after anodization enhances the nanotube's roughness and osseointegration capability [29][30]. Cell behavior is affected by the diameter of TiO_2 nanotubes [29]. For instance, small nanotubes (30 nm in diameter) have been shown to promote BMSC adherence without significant differentiation, while larger nanotubes (70–100 nm in diameter) cause a dramatic lengthening of stem cells, which induces cytoskeletal stress and selective differentiation into osteoblast-like cells [31]. A diameter of 70 nm is the optimum size of TiO_2 nanotubes for

osteogenic differentiation of stem cells derived from human adiposity [32]. The diameters of TiO_2 nanotubes are crucial for surface roughness and hydrophilicity. Several studies have shown that increasing diameter can increase antibacterial characteristics [33][34]. Ercan et al. found that nanotubes with a diameter of 80 nm had more antibacterial properties than the 30 nm diameter nanotubes against various strains of *S. aureus* due to higher hydrophobicity [35]. Other factors apart from the diameter, including the length, the gap between walls, and crystal forms, also influence the TiO_2 nanotubes. Nano-engineered Ti prepared from hydrothermal etching has also been reported to be effective against gram-negative bacteria, *E. coli* [36].

TiO_2 nanorod, another TiO_2 nanostructure, also significantly influences the BMSC behavior [32]. The TiO_2 nanorod array surface is very effective in regulating the differentiation of BMSC towards osteoblasts. In another study, TiO_2 ceramics were synthesized and TiO_2 nanorods were used to compare the BMSC cellular adhesion and self-renewal characteristics when commercial culture plates were used as the control group [37]. All samples demonstrated good biocompatibility from day 2 to day 8, suggesting that TiO_2 ceramic promotes cell adhesion, renewal, and cellular morphology).

Increasing the average surface roughness of the implant promotes osteointegration and is another topology-based surface modification [38]. The surface roughness enhances protein adsorption and osteoblastic functions [39]. The inorganic coating may include calcium phosphate/hydroxyapatite and certain peptides [40]. However, a thick layer of calcium phosphate coating has poor stability [41]. To address this issue, biomimetic strategies were devised, which have shown good versatility [38][42]. This coating has great osteoconductive potential in vivo [43].

2.2. Drug Loading and Release Based on the TiO_2 Nanostructures

Antibiotics are very effective at killing bacteria, but antibiotics taken by oral or muscular injection have very low efficiency in treating infections in the bone. Localized drug release from the implant surface can solve the problem. TiO_2 nanostructures such as nanotubes and nanopores are highly facilitated to do drug-loading [38][39]. TiO_2 nanotubes are especially favored because of their larger surface area and one-end open feature [44]. The drug delivery of the nanotubes is significantly affected by the fabrication conditions. It is also found that drug release was promoted by increasing the dimensions (length, width, and diameter) of nanotubes [45]. Loading into the nanotubes with infection-reducing drugs, such as penicillin and streptomycin, largely improves the performance of titanium implants [46][47].

By increasing the dimensions of the nanotubes, drug release was promoted, but drug loss also increased during the rinsing process. To overcome this problem, periodic structures in the nanotubes are prevented, which demonstrated a significant improvement in the drug release control; the periodic structures largely reduced drug burst release from 77% to 50% and extended overall release from 4 days to more than 17 days [28].

2.3. Element Incorporation

Apart from biotics, the antibacterial property can also be promoted by introducing antibacterial ions, such as silver (Ag), zinc (Zn), and magnesium (Mg) [48][49][50][51][52]. Jia et al. reported a method to incorporate Ag nanoparticles

into TiO_2 microporous coatings using polydopamine [48]. A sustained release of Ag^+ ions for up to 28 days was observed, which endowed the Ti implant with long-term antibacterial ability. An additional trap-killing of the bacteria was enabled with these Ag nanoparticles. Negatively charged bacteria were attracted toward the positively charged Ag nanoparticles and killed with more efficiency. More Ag doping to TiO_2 for better antibacterial properties can be found in the literature [53][54][55].

Zn is an important trace element in the human body, and it has a pivotal role in DNA synthesis, enzymatic activities, biomineralization, hormonal activities, and antibacterial characteristics [56][57][58][59][60]. Zn doping in TiO_2-based biomaterial has also been found to possess excellent antibacterial activities and better cell-material interactions [61][62]. The bacterial killing was due to the penetration of Zn^{2+} in the bacterial surface membranes [63].

Mg is a microelement in the body and contributes to numerous cellular functions including enzymatic reactions, proteins, and nucleic acid synthesis; it is also effective in reducing inflammation and bone loss [64][65]. The incorporation of Mg can inhibit bacterial infection and osteolysis. Yang Y et al. designed a surface with Mg incorporated into the TiO_2 nanotubes [66]. The surface demonstrated remarkable antibacterial properties, enhanced cytocompatibility, and inhibited osteoclast genesis, both *in vitro* and *in vivo*. The nanostructures and alkaline microenvironment during degradation were responsible for the antimicrobial ability. The continuous release of Mg^{2+} suppressed the osteolysis via down-regulation of NF-κB/NFATc1 signaling. Mg doping has multiple therapeutic effects; however, an alkaline environment may pose a serious challenge in clinical use. Controlled release of Mg is the possible solution but needs further exploration [67]. Many other studies support that Mg incorporation can enhance the antibacterial and osteogenesis property of the implants [67][68].

2.4. Electron Transfer

In recent years, an antibacterial theory based on the electron transfer between the material surface and the microbes has been proposed. Electron transfer is a common event in the photochemical modulation of materials, as well as a fundamental event for the energy generation of organisms [69]. A group of microbes can do extracellular electron transfer spontaneously by transferring the electron outside the cells to environmental minerals [70]. However, using the electron transfer approach to inhibit implant infection is a quite new topic [71].

Vecitis et al. found that the antibacterial properties of single-arm carbon nanotubes are closely related to their electronic state. With the same diameter and length, metallic carbon nanotubes can cause severe deformation and collapse of the bacterial cells, while those in a semi-conductive state have no antibacterial properties [72]. Faria et al. found that the composite structure of Ag nanoparticles and graphene lamellae has a strong bactericidal ability, but graphene lamellae itself does not, suggesting that the electronic interactions between the substrate and the modified materials have a dominant impact on the antibacterial property [73].

TiO_2 also has complex interactions with the bacteria and osteoblasts via electron transfer. TiO_2 is a semiconductor, and biological cells can also be regarded as semiconductors [74]. Once contacted, they form heterojunctions, which may involve electron transfer. Therefore, functionalization based on the electron transfer property also influences

the performance of TiO_2 as an orthopedic implant. Au and Ag nanoparticles or graphene sheets deposited on the TiO_2 surface can endow TiO_2 with antibacterial properties [74][75][76][77][78][79]. On the Ag@TiO_2 surface, electrons were stored on the Ag nanoparticles, and induced valence-band hole (h+) accumulation, which caused cytosolic content leakage of the bacteria [75]. On the Au@TiO_2 surface, electron transfer was due to the plasmon effect of Au nanoparticles, which captured the electrons in the respiratory chain on the living bacterial cell membrane and transferred them to the TiO_2 substrate. Au@TiO_2 formed the Schottky barrier, which prevented the return of electrons, causing continued electron loss in the bacteria until death [77][79]. Similarly, graphene coating resulted in a large increase in the electrical conductivity of TiO_2 because of the combination of the unpaired π electrons of graphene and the Ti atoms [80]. The enhanced electron transfer from the bacterial cell membrane to the graphene-TiO_2 interface leads to bacterial death.

Electron transfer also works for osteogenesis. Zhou et al. fabricated a SnO_2–TiO_2 heterojunction and hierarchical structure on the surface of the Ti implant [81]. The electron transfer among the hierarchical Schottky barrier significantly improved the osteogenic function of the cells around the implant both in vitro and in vivo. In another work, they constructed a layered double hydroxide (LDHs)–TiO_2 heterojunction, which promoted the transfer of holes in materials to the physiological environment, enhancing the antibacterial effect of the implant [82]. Ning et al. generated a microscale electrostatic field (MEF) by doing patterned NT (rutile) and IT (anatase) surface modifications on Ti [83]. The electron transfer between NT and IT zones formed a sustained built-in MEF, which polarized the BMSC and activated the expression of osteogenic genes. The MEF greatly promoted bone regeneration around the implant.

Apart from TiO_2, the Ti surface can also make electron transfer-based interactions with the bacteria. In a study by Wang et al., Ag was implanted on the Ti surface using plasma technology, and this modification changed the Ti surface from non-antibacterial to antibacterial [79]. The bacteria-killing was not due to Ag^+ ion release, but due to the micro galvanic reaction at the nano interface between Ag nanoparticles and Ti substrate. The reaction disturbed the process of electron transfer in the bacteria respiratory chain and produced a large number of reactive oxygen species (ROS) in the bacterial cells, resulting in their death.

2.5. Electrical Functionalization

Based on the electron transfer mechanism of the above studies, researchers have further developed an innovative method to make the TiO_2 surface obtain antibacterial properties through electrical tuning. In the beginning, it was found that an alternating current (AC) of about ±2 μA applied to the ZnO nanowires in a physiological solution could significantly improve the antibacterial property of ZnO after the current was removed. The “sustained bacteria sterilization” was different from the “instant bacteria sterilization” because the latter was due to electroporation when AC was applied to the nanowires, but the former was due to surface functionalization by the electrical tuning [84]. After that, a 2 V low-voltage direct current (DC) power supply was used to conduct electrical treatment on the Ti plate with a TiO_2 layer in the culture medium for 20 min. This DC tuning also changed the TiO_2 surface from non-antibacterial to highly antibacterial [85]. After the electric tuning, TiO_2 gained a strong ability to kill various bacteria and showed strong inhibition of biofilm formation. Meanwhile, the DC-tuned TiO_2 surface had no negative effect on

the osteoblast. The adhesion and proliferation of the cells were found to be as effective as those on the control TiO_2 surface.

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