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REVIEW

TiO_2 nanotube platforms for smart drug delivery: a review

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Abstract: Titania nanotube (TNT) arrays are recognized as promising materials for localized drug delivery implants because of their excellent properties and facile preparation process. This review highlights the concept of localized drug delivery systems based on TNTs, considering their outstanding biocompatibility in a series of ex vivo and in vivo studies. Considering the safety of TNT implants in the host body, studies of the biocompatibility present significant importance for the clinical application of TNT implants. Toward smart TNT platforms for sustainable drug delivery, several advanced approaches were presented in this review, including controlled release triggered by temperature, light, radiofrequency magnetism, and ultrasonic stimulation. Moreover, TNT implants used in medical therapy have been demonstrated by various examples including dentistry, orthopedic implants, cardiovascular stents, and so on. Finally, a future perspective of TNTs for clinical applications is provided.

Keywords: TiO_2 nanotubes, anodization, drug delivery, orthopedic implant

Introduction

In the conventional systemic drug delivery administration, drugs are typically delivered by oral, parenteral, and inhalation routes and so on, where drugs are distributed to the whole body and not to the specific site of interest.[1] The inherent limitations of conventional therapies could be addressed on the basis of developing more efficient and rational drug delivery systems. In this regard, strong interdisciplinary research strategies combining the efforts of material scientists, engineers, medical scientists, biologists, and clinicians have recently demonstrated promising results.[2] The localized drug delivery systems are recognized as the most promising methods for controlling drug release since drug-releasing implants possess persistent and controlled drug release.[3] A recent review by Raliya et al provides a summary on the widespread use of nanostructured or nanocomposite materials for disease diagnostics, drug delivery and biomedical applications, as well as various nanoparticle synthesis routes, characterization, and functionalization methodologies for biomedical applications.[4] The mesoporous silica prepared with a simple electrochemical process based on organic synthesis and porous silicon has been investigated extensively in recent years.[5–9] Owing to their unique properties, much research interest has been focused on the electrochemical preparation of nanopores or nanotube materials from transition metal oxides.[10–15] In particular, titania nanotubes (TNTs) and nanoporous anodic alumina are the most significant examples.[16–20]

Fabrication of TNTs is based on a low-cost, facile process, with controllable nanotube structures, due to self-ordering electrochemical anodization process. The TiO_2 material possesses excellent biocompatibility and has demonstrated tunable

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drug-releasing performance. More importantly, it can be generated on the surface of the existing medical implants.[21,22] The dimensions of the TNTs could be controlled by changing the electrochemical anodization parameters including anodized voltage, time, electrolyte, and so on.[23–25] Compared with previously devised polymer based on implants, TNTs hold good stability to address problems such as implant swelling or disintegration. However, drug release will be uncontrollable when TNT implants are embedded into the living body.[26–29] Thus, smart drug delivery strategies are urgently needed to control the drug release by tuning the diffusion mechanism.

To address the problems depicted earlier, this review aims at reporting the most recent advances on drug-releasing implants based on TNTs for localized drug delivery. The fabrication, properties, and biocompatibility of TNTs are briefly introduced. Some concepts for drug release controlled by externally triggered stimuli are also reviewed, and the advantages and inherent limitations of these systems are discussed in detail. In addition, the recent progress in the practical application of TNTs related to bone therapies, dentistry, cardiovascular stents, and brain tumors is described. Finally, the review is concluded with the prospective outlook on the TNTs implants used in clinical application.

Self-ordered nanotubular implants based on TNTs

TNTs prepared by anodizing metallic titanium (Ti) in an electrolyte are recognized as one of the most outstanding drug-releasing implants in drug delivery systems.[30,31] Some researchers have explored the optimization in anodization parameters to achieve a high degree of self-ordering in the grown TNTs.[32–41] TNT arrays with controllable nanotube diameters and hexagonal arrangement fabricated by electrochemical anodization based on Ti surface with highly ordered nanotube structures are schematically shown in Figure 1.[34,42,43] TNT fabrication is a unique electrochemical process termed self-assembling anodization,[44] which is based on inexpensive materials and equipment. The dimension of TNTs could be adjusted by electrochemical anodization parameters including the anodization voltage, time, the composition of electrolyte, and so on.[44–49] TNTs can be fabricated on various structures including three dimensional nonplanar and curved surfaces such as thin, long surgical wires and needles for bone fixture. Considering this versatility, TNTs are clinically used as implants or surgical supports in orthopedics because of their excellent malleability and biocompatibility in the host body.

As described earlier, TNTs fabricated by electrochemical anodization have attracted notable attention in the past few years due to various excellent properties of TNTs. In general, the typical electrochemical setup involves two electrodes comprising Ti (working electrode) and an alternate metal (counter electrode) that are immersed in electrolyte and are connected to an external voltage as shown in Figure 1A. The metal dissolution and then the oxide formation on the Ti substrate occur in the initial electrochemical process, and the dissolution of TiO_2 layer is initiated with the formation of self-ordered and vertically aligned porous or tubular oxide

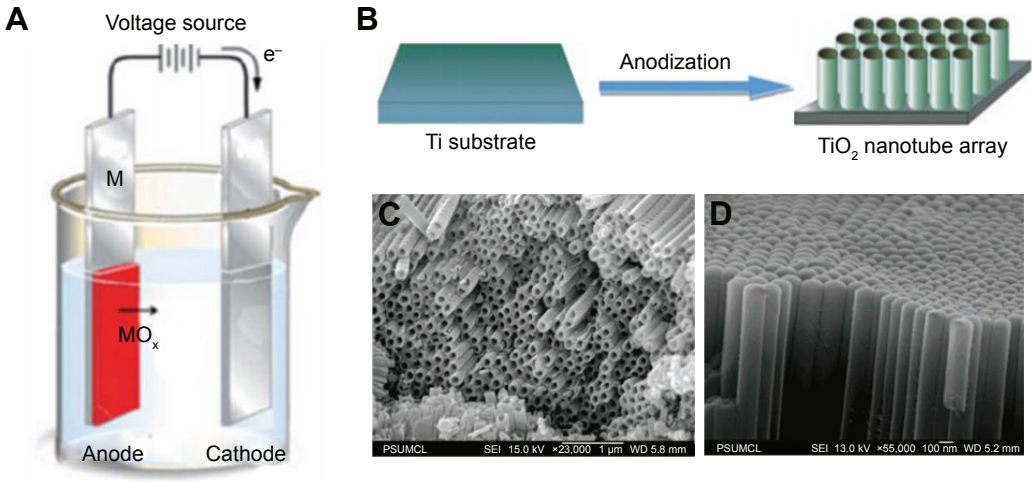

Figure 1 The formation and structure of TNTs.

Notes: (**A**) and (**B**) Electrochemical cell and anodization process for the formation of TNT layer on Ti substrate. SEM images of TNTs for (**C**) bottom and side surface and (**D**) top surface. (**A**) Reprinted *Curr Opin Solid State Mater Sci*, 11, Macak JM, Tsuchiya H, Ghicov A, et al, TiO_2 nanotubes: self-organized electrochemical formation, properties and applications, 3–18,[34] copyright 2007, with permission from Elsevier. (**B**) Reproduced from Ge MZ, Cao CY, Li SH, et al. In situ plasmonic Ag nanoparticle anchored TiO_2 nanotube arrays as visible-light-driven photocatalysts for enhanced water splitting. *Nanoscale*. 2016;8:5226–5234,[43] with permission of The Royal Society of Chemistry. (**C**, **D**) Reprinted with permission from Paulose M, Shankar K, Yoriya S, et al. Anodic growth of highly ordered TiO_2 nanotube arrays to 134 µm in length. *J Phys Chem B*. 2006;110:16179–16184.[20] Copyright 2006 American Chemical Society.

Abbreviations: SEM, scanning electron microscope; TNT, titania nanotube.

nanostructures following the establishment of electrochemical equilibrium between these two reactions.

Biocompatibility of TNTs: in vitro and in vivo studies

The application of TNTs is a promising alternative to develop various medical implants and devices because of their excellent biocompatibility, mechanical strength, and chemical resistivity; Ti and its alloys have been applied in orthopedic and dental implants for many years.[49–54] Most biocompatibility studies of TNTs focused on their significant application in dentistry, orthopedics, and cardiovascular surgery; especially TNT implants presented a great affinity for bone cell adhesion and differentiation. In initial studies, it was demonstrated that the TNT surface could provide an excellent template for bone cell growth and osteoblast activity based on their better cell growth performance than Ti surface.[55–58] Figure 2A and B reveals that bovine aortic endothelial cells (ECs) on Ti surfaces are more spread out and cover greater surface areas, whereas bovine aortic ECs on TNTs displayed elongated morphologies, which results in bovine aortic ECs on TNTs cover most of the average area occupied by the control cells.[55] Popat et al demonstrated that TNT surface could be used as a favorable template for marrow stromal cell growth and differentiation and provided the evidence that the osteoblast activity can be greatly improved by controlling nanotopographies, as shown in Figure 2C and D.[57]

TNTs are recognized as promising implants for enhanced bone osseointegration, vascular stents with better control hemorrhage, and excellent blood-contacting devices, which results from TNTs possessing favorable surface for stem cell proliferation, hydroxyapatite formation, and the outstanding biochemistry inertness.[59–61] Kim et al carried out the biocompatibility research on cellular responses of human osteosarcoma cells to TNTs,[62] and Advincula et al investigated the cell responses of osteoblast-like MC3T3-E1 cells to TNTs.[63] From these results, it is demonstrated that the cells on the TNTs proliferated more actively and that the alkaline phosphatase activity expressed to a higher degree compared with those on pure Ti. Other biocompatibility researches further demonstrated a higher alkaline phosphatase activity based on better cell growth on TNTs in vivo conditions.[64–66] Oh et al

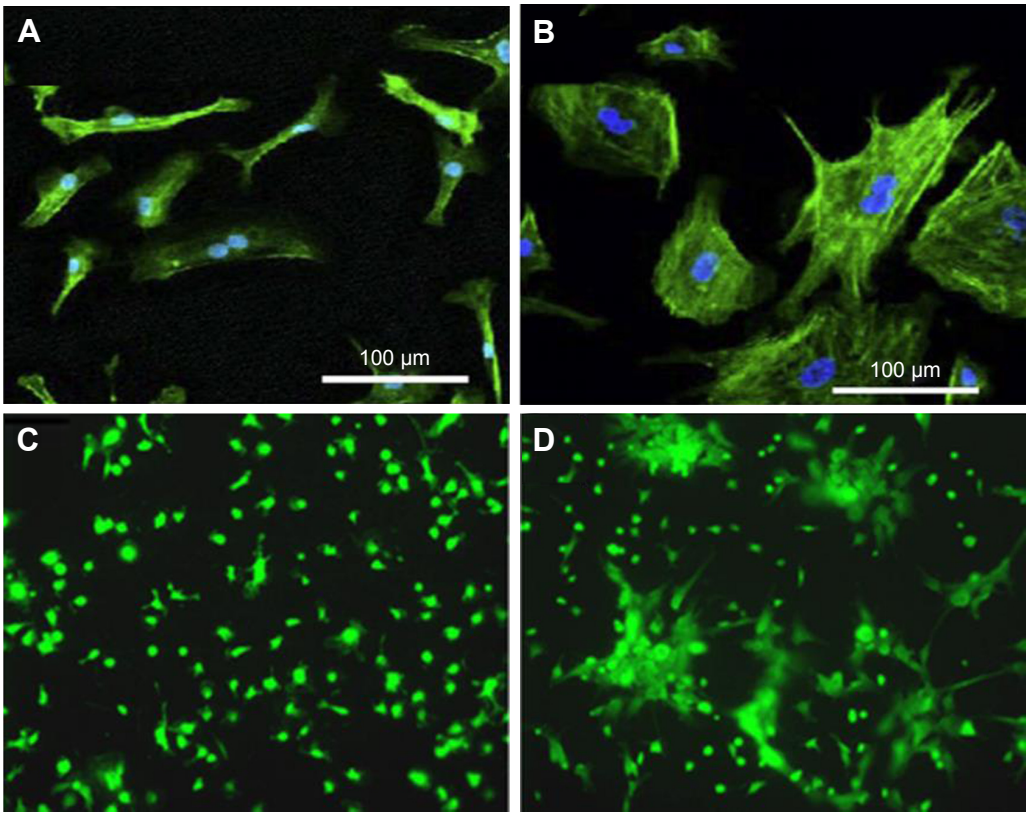

Figure 2 F-actin and nuclear stains of BAEC.
Notes: F-actin (green) and nuclear (blue) stains of BAEC grown on (**A**) TNTs versus (**B**) flat surfaces for 24 hours. Fluorescence microscopy images (×10) of live marrow stromal cells stained with calcein on (**C**) Ti and (**D**) TNT surfaces. (**A** and **B**) Reprinted from *Biomaterials*, 30, Peng L, Eltgroth ML, LaTempa TJ, Grimes CA, Desai TA, The effect of TiO_2 nanotubes on endothelial function and smooth muscle proliferation, 1268–1272,[55] Copyright (2009), with permission from Elsevier. (**C** and **D**) Reprinted from *Biomaterials*, 28, Popat KC, Leoni L, Grimes CA, Desai TA, Influence of engineered titania nanotubular surfaces on bone cells, 3188–3197,[57] Copyright (2007), with permission from Elsevier.
Abbreviations: BAEC, bovine aortic endothelial cell; TNT, titania nanotube.

reported a remarkable adhesion and reproduce of osteoblasts on TNTs, with the filopodia entering the nanotubes and forming an interlocked cell structure.[67,68] Furthermore, TNTs also promote the development of drug-releasing implants for the treatment of bone-related diseases including cancer and post-implantation infections. Feschet-Chassot et al predicted the toxicity of TNT layer in biological systems using a protozoaic cell model and concluded that TNTs did not affect undefined esterases activity and cell growth rate.[69]

Human and rat mesenchymal stem cells (MSCs), their adhesion, propagation, and differentiation are directly related to the diameters of TNTs, which was described as an exceptional discovery in stem cell studies.[70–72] Hu et al fabricated bone morphogenetic protein 2 (BMP2)-loaded TNTs and found that cells grew well on BMP2-loaded TNTs as schematically shown in Figure 3.[71] MSCs adhered to bare TNTs array, which displayed round or narrow spreading morphologies, are shown in Figure 3A. By contrast, MSCs presented well-spreading morphologies when adhered to multilayer-coated TNTs as demonstrated in Figure 3C. Compared to the morphologies of MSCs cultured onto TNTs, MSCs adhered to BMP2-loaded TNTs have no obvious difference during the growth process as shown in Figure 3B. Figure 3E schematically illustrated the preparation of BMP2-loaded TNTs, the process of cell adhesion to multilayer-coated and BMP2-loaded TNTs and cellular responses. Bauer et al found that monolayers from octadecylphosphonic acid (OPDA) could significantly enhance the attachment of MSCs to TNT surfaces and demonstrated that the surface wettability was also an important parameter for cells adhesion.[73] Furthermore, for the effects of TNT geometry on osteogenesis of MSCs, lots of studies have been carried out to investigate the effects of TNT geometry on osteogenesis of MSCs, such as crystal structure of TNTs, surface chemistry, and other differentiation approaches on the response of various stem cells.

For in vivo biocompatibility studies, von Wilmowsky et al investigated the effect of a TNT layer on bone formation by placing TNT implants into pigs. The immune-histological analysis was used to explore the bone implant.[74] In this study, the effects of these implants were evaluated on the basis of the peri-implant bone formation, bone-implant contact, and immunohistochemistry, demonstrating that TNT coatings enhance osteoblast functions and resist shearing forces evoked by implant insertion, displaying positive outcome of bone formation characteristics of TNTs as compared to commercially available pure Ti implants. Another study performed by Park et al showed that the capability of improving osseointegration for protein-loaded TNT implants based on

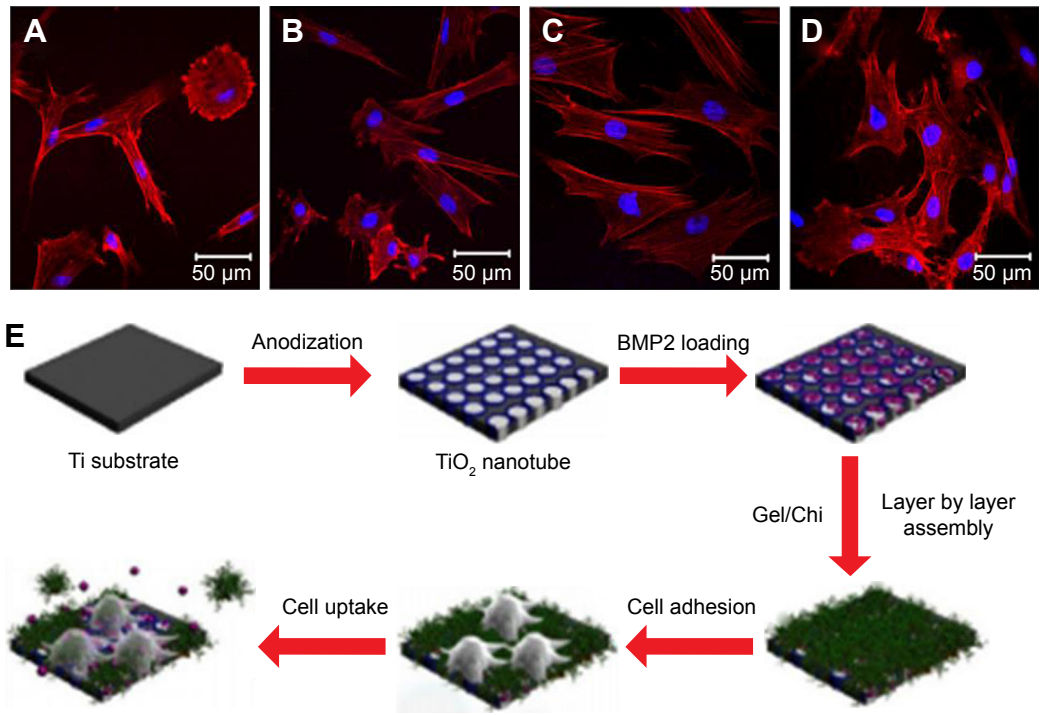

Figure 3 CLSM observations of MSCs adhered to different substrates after culture for 1 day.
Notes: (**A**) Bare TNTs; (**B**) BMP2-loaded TNTs; (**C**) Gel/Chi multilayer-coated TNTs; (**D**) Gel/Chi multilayer-coated and BMP2-loaded TNTs; and (**E**) schematic illustration of the fabrication of BMP2-loaded TNTs and cellular responses. Reprinted from *Acta Biomater*, 8, Hu Y, Cai K, Luo Z, et al, TiO_2 nanotubes as drug nanoreservoirs for the regulation of mobility and differentiation of mesenchymal stem cells, 439–448,[71] Copyright (2012), with permission from Elsevier.
Abbreviations: BMP2, bone morphogenetic protein 2; TNT, titania nanotube; MSCs, mesenchymal stem cells; CLSM, confocal laser scanning microscope.

loading fibroblast growth factor (FGF) and human fibronectin fragment (hFNIII9-10) fusion protein inside TNTs on Ti implants inserted in rabbit tibia for 3 months.[75] It is worthwhile stressing that these experiments were carried out over a period of 2–3 months, and more studies should be carried out to investigate longer healing periods of TNT implants for clinical therapies. Popat et al observed greatly increased chondrocyte adhesion on TNT surfaces compared with bare Ti and no existence of chronic inflammation or fibrosis in vivo biocompatibility study performed by implanting TNT surfaces subcutaneously in rats and performing histological analysis during 4 weeks.[57] The results indicated that calcium and phosphorous concentrations were higher on the TNT surface, which suggested that matrix deposition was created on the nanotubular surface. In addition, Bjursten et al performed an in vivo study on TNT implants to verify that TNTs significantly enhanced bone bonding strength to the ambient tissues after 4 weeks of experimental implantation in rabbit tibias.[76]

All the earlier studies confirmed the safety of TNTs as biocompatible implants and thus demonstrated their safe usage in the in vivo and ex vivo contexts. However, more in vitro and in vivo studies are needed to resolve many other problems in the future, specifically in vivo studies are needed to explore the effect of the surface modification of TNTs on their biocompatibility and osseointegration properties.

External stimuli for responsive and on-demand drug delivery

It has been demonstrated that TNT structure is a suitable platform to develop drug-releasing implants due to its ability of loading different payloads of therapeutics, and TNT-based implants are recognized as one of the most excellent nanomaterials to address many disadvantages of traditional drug administration.[3] It is well known that different drug release strategies need to be designed for different therapies, disease conditions, and specific parts of the host body. The drug release strategies may vary from short to long release, rapid on-demand release, or time-programmed release with single or multiple drug loadings. Therefore, TNT-based drug-releasing systems must be designed with flexible drug release capabilities and optimized parameters in order to fulfill the requirements of different therapies. Considering that, increasing studies are focused on exploring different strategies in TNT-based drug-releasing implants in order to design and advance their drug-releasing performances for specific drugs and therapies. Several typical concepts of stimulated drug release from TNTs with external triggers were described in this work.

pH- and temperature-sensitive drug delivery

TNTs have been demonstrated to be a promising platform for controlled local drug delivery, while the drug release from pure TNTs is very quick. Thus, pH-responsive polymers were applied to overcome the problem of too rapid drug release from TNTs. Jia et al reported that poly(lactic-co-glycolic acid) (PLGA) added into TNTs could improve the drug release profile.[77] In their study, carprofen and lidocaine were used as the model drugs to investigate the drug release profile using PLGA/TNTs with different types of drugs. To investigate drug release from PLGA/TNTs under different pH conditions, lidocaine and carprofen releases were studied in sodium acetate buffer with pH 3.5, phosphate-buffered saline with pH 7.4, and phosphate buffer with pH 10.5, respectively, under the constant temperature of 37°C. The degree of TNT swelling was found to vary with pH values. The drug can easily diffuse into the medium through the swelling polymer during the critical time t_1 as shown in Figure 4, followed the t_2 time region, where the remaining drug completely

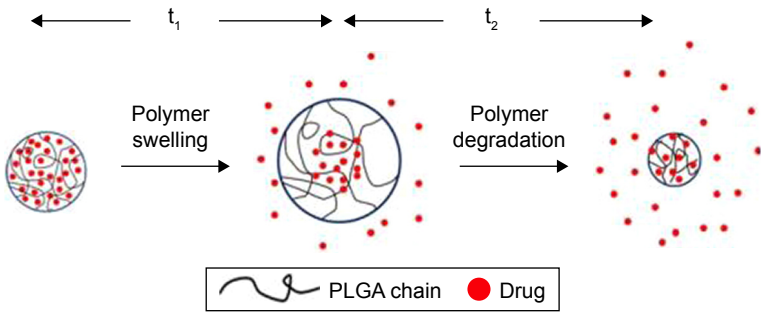

Figure 4 Schematic diagram explaining pH-dependent drug release shape.
Note: Reprinted from Jia H, Kerr LL. Kinetics of drug release from drug carrier of polymer/TiO_2 nanotubes composite-pH dependent study. *J Appl Polym Sci*. 2015;132:41750,[77] with permission from John Wiley and Sons.
Abbreviation: PLGA, poly(lactic-co-glycolic acid).

releases as a result of polymer degradation.[77] Furthermore, in order to reveal the mechanism and potential of lidocaine and carprofen release from TNTs, the drug release profiles were investigated on the basis of drug-loaded pure TNTs and drug-loaded PLGA/TNTs; the former was used as the control sample.[77]

For temperature-sensitive drug delivery based on TNTs, temperature-responsive polymers were explored to decorate TNT implants. Cai et al fabricated a temperature-sensitive drug delivery system based on TNTs by using vitamin B_2 as a model drug and explored kinetics of controlled drug release from TNTs with temperature-responsive trigger.[78] In their study, the hydrogel layer formed from poly(N-isopropylacrylamide) (PNIPAAm) and poly(acrylamide) (PAAm) was used as a cap that was coated on the surface of TNT layers for sealing the open nanotubes. The PNIPAAm/PAAm composite hydrogel presents a highly water swollen state that prevents the drug release from TNTs when the surrounding temperature is below its lower critical solution temperature, whereas the composite hydrogel is in a collapsing state to allow drug releasing from TNTs once the temperature is higher than the lower critical solution temperature of the composite hydrogel, as shown in Figure 5A. The drug release profile of pure TNTs at 25°C is higher than that of hydrogel-coated TNTs under the same condition, since there is no barrier to block the drug release at all. When the temperature increases to ~38°C due to the inflammatory reaction, hydrogel-coated TNTs show much higher release profiles than that of hydrogel-coated TNTs at 25°C as shown in Figure 5B. This is because 38°C is higher than the lower critical solution temperature of composite hydrogel; hence, the composite hydrogel was in a collapsing state to allow drug release from TNTs.

Based on the existing research, temperature-sensitive drug delivery based on TNTs has promising potential for practical applications. However, it has to be cautioned that although interesting, the clinical relevance of such a device is still mainly hypothetical or that the composite hydrogel used needs to be adapted. In the same vein of thought, it is difficult to obtain in vivo pH control under normal conditions, and therefore, the actual value of a pH-sensitive drug delivery also seems quite hypothetical at the moment.

Light-sensitive drug delivery

For in-depth study of the controlled release of drugs or therapeutics using TNTs, light-sensitive release is also a promising strategy. Song et al reported that amphiphilic TNTs were used to provide a highly controllable drug release system based on a hydrophobic cap (monolayer of OPDA) on the top of TNTs, which can be removed by ultraviolet (UV)-induced chain scission. In this work, different drug-loading approaches were used to study hydrophilic drug release from the amphiphilic TNTs as outlined in Figure 6A.[16] For reference, unmodified TNTs were used to load horseradish peroxidase (HRP) by simple immersion (Figure 6A, case I), which leads to physisorbed HRP molecules within TNTs. In the second case, TNTs capped with OPDA were immersed in HRP (Figure 6A, case II), which also leads to physisorbed drug molecules in the lower part of TNTs but remains trapped by OPDA after evaporation of the surfactant, dimethyl sulfoxide. TNTs capped without OPDA were used for HRP grafting by a 3-aminopropyltriethoxysilane/vitamin C monolayer linker covalently attached (Figure 6A, case III). TNTs capped with OPDA were attached to the HRP in the lower part of TNT wall as in case III (Figure 6A, case IV). For different loading approaches, the HRP release characteristics are presented in Figure 6B–E. From these results, it was demonstrated that UV light could make chain scission and then induce drug release from TNTs, thus opening potential perspectives for the drug delivery systems based on light control.

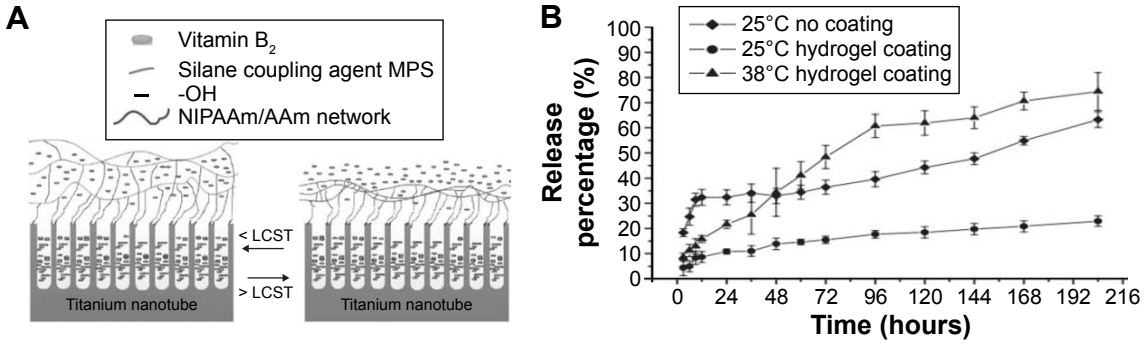

Figure 5 Schematic illustration of temperature-controlled stimulus to trigger drug release based on TNTs.
Notes: (**A**) Overview of drug-loaded TNTs with hydrogel coating; (**B**) drug release profiles from TNTs. Reprinted from Cai K, Jiang F, Luo Z, Chen X. Temperature-responsive controlled drug delivery system based on titanium nanotubes. *Adv Eng Mater*. 2010;12:B565–B570,[78] with permission from John Wiley and Sons.
Abbreviations: AAm, acrylamide; LCST, lower critical solution temperature; NIPAAm, N-isopropylacrylamide; TNT, titania nanotube.

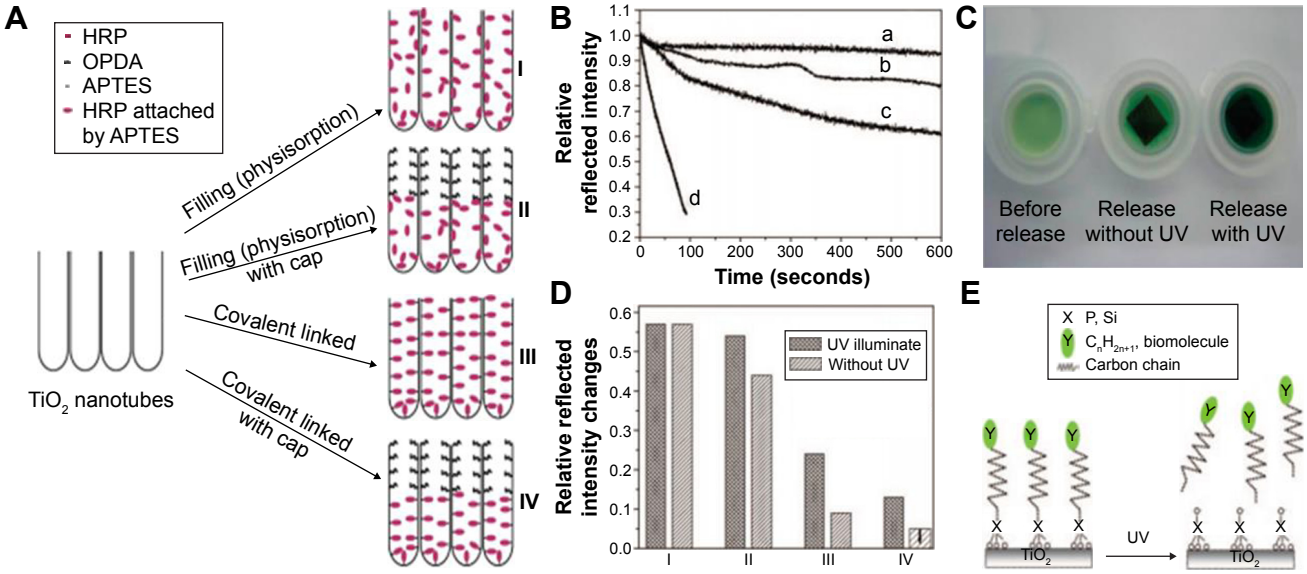

Figure 6 Schematic illustration of four methods for drug loading and profiles of drug release from TNTs.
Notes: (**A**) Four approaches for drug loading using HRP as a model drug; (**B**) relative intensity of reflected light (wavelength 550 nm) as a function of time after exposure of HRP-loaded amphiphilic TNTs to PBS without illumination (curve a), 50% UV illumination (curve b), full UV illumination (curve c) and the release of HRP in TNTs without any surface modification (curve d); (**C**) optical images of the solution containing indicator substrate (ABTS) and H_2O_2 before HRP release (left) and after HRP release without (middle) and with UV illumination for 40 minutes (right); (**D**) relative reflected intensity changes for the four different types of nanotubes used in this study (according to the scheme of **A**) with and without UV illumination; (**E**) schematic illustration of the HRP release under UV illumination. Reprinted with permission from Song YY, Schmidt-Stein F, Bauer S, Schmuki P. Amphiphilic TiO_2 nanotube arrays: an actively controllable drug delivery system. *J Am Chem Soc*. 2009;131:4230–4232.[16] Copyright (2009) American Chemical Society.
Abbreviations: APTES, 3-aminopropyltriethoxysilane; HRP, horseradish peroxidase; OPDA, octadecylphosphonic acid; PBS, phosphate-buffered saline; TNT, titania nanotube; UV, ultraviolet.

In addition, chain scission-induced drug release from TNTs can also be triggered by suitable infrared (IR) laser irradiation. Moon et al carried out the study by immobilizing gold nanorods (GNRs) onto the surface of TNTs through a grafting technique and investigating the drug release from TNTs triggered by near-IR laser irradiation.[15] The antimicrobial activity was detected to evaluate the effectiveness of the controlled drug release from GNR-grafted TNTs by IR laser irradiation. These results demonstrated that the photothermal activity of GNR could allow remote control of on–off drug elution by IR laser irradiation. GNRs are known to have much potential for mediating photodynamic and photothermal effects under near IR light.[79–84] As such, GNR has been found to be as a photosensitizer for promising application in the field of cancer treatment. Since IR light can overcome the limitation of visible light that cannot penetrate deep into the skin, GNR-grafted TNTs can be expected to overcome the limitation of TiO_2 showing photocatalytic activity within the UV to IR region, thereby facilitating the development of novel implantation materials.

Radiofrequency-sensitive drug delivery

Radiofrequency (RF)-responsive release is an excellent strategy for satisfying some therapies that require the use of noninvasive external stimuli. This concept has been introduced into the drug release based on TNT implants as shown in Figure 7A.[85] In this drug delivery system, RF was used as an external stimuli to trigger the release of polymeric micelles and drugs from TNT deposited gold nanoparticles (AuNPs). Compared to release profiles under no-trigger conditions, the polymeric micelles displayed an abrupt in vitro release under RF triggering as shown in Figure 7B and C. From the results, it is demonstrated that near complete (90%–100%) release of all three samples was achieved within 1–3 hours after RF-induced heating of the AuNPs loaded inside TNTs. In addition, from the graphs, we also find that the drug release from AuNP-loaded TNTs is faster than drug-micelles release from AuNP-loaded TNTs, and drug-micelles without AuNPs achieved the lowest release. The release rate increases with extending the RF exposure time from 5 to 10 minutes, whereas there is no effect on drug-micelles release from TNTs without AuNP loading. Thus, it can be verified that AuNPs are good thermal transducers that transfer RF energy to trigger drug release from TNTs.

In addition, single-walled carbon nanotubes, gold silica nanoshells, and water-soluble derivatives of C60 fullerenes were used as stimulants in the RF field to investigate the impact of RF.[86–92] Raoof et al reported that noninvasive RF field-induced heating of metal nanoparticles owns outstanding properties over others in the treatment of hepatocellular cancer by summarizing recent approaches for delivering noninvasive RF field-mediated hyperthermia to malignant cells

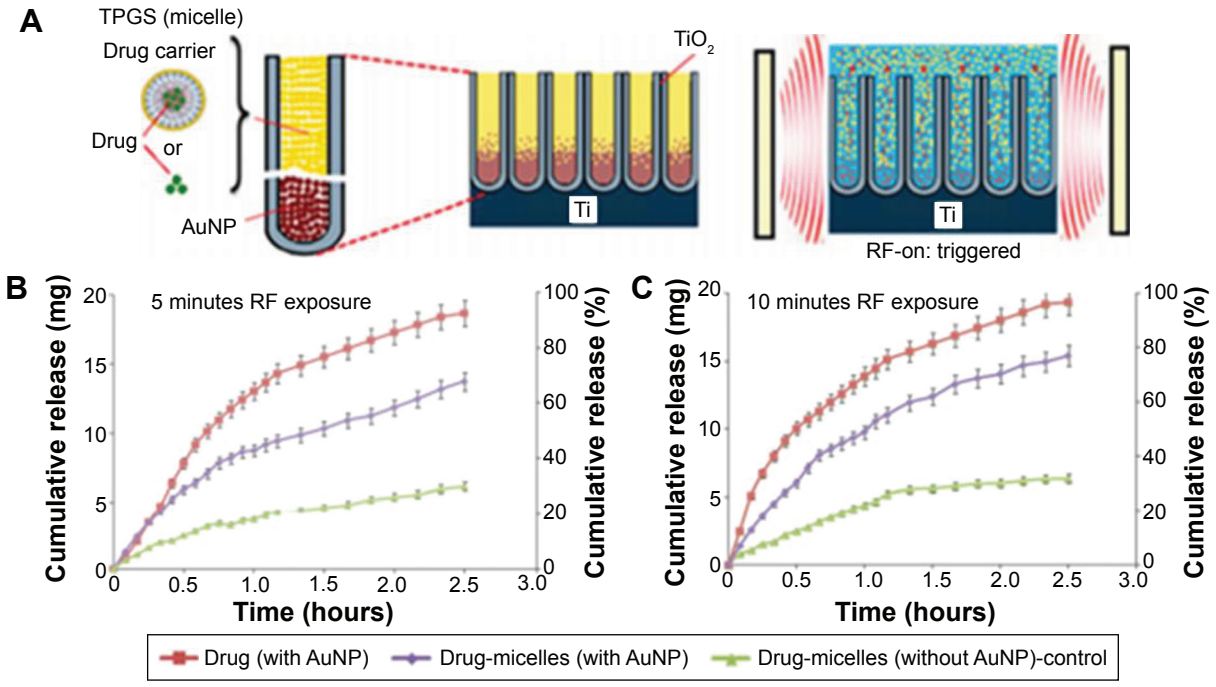

Figure 7 Schematic representation of a model drug release from TNTs implants for in vitro release studies with the RF trigger.
Notes: (**A**) Noninvasive and on-demand triggered release from drug-eluting TNTs using RF and AuNPs. (**B**) Profiles of RF-triggered release of drug (indomethacin-encapsulated TPGS) from TNTs with and without AuNPs as energy transducer in comparison with the control (nontrigger) sample. Release profiles for different exposure times: (**B**) 5 minutes and (**C**) 10 minutes, respectively. Reproduced from Aw MS, Kurian M, Losic D. Non-eroding drug-releasing implants with ordered nanoporous and nanotubular structures: concepts for controlling drug release. *Biomater Sci*. 2014;2:10–34, with permission of The Royal Society of Chemistry.[85]
Abbreviations: AuNP, gold nanoparticle; RF, radiofrequency; TNT, titania nanotube.

and its application to hepatocellular cancer.[93,94] During the process of RF-induced drug release, the power of RF did not change or damage the micelle structure and TNTs implants. Therefore, RF-sensitive drug delivery possesses significant potential to ablate cancer cells at the implant location.

Magnetic-sensitive drug delivery

Magnetic-sensitive drug delivery is a new concept of drug encapsulated in nanomagnetic structures that possess excellent possibilities for magnetic field-triggered drug release. Aw et al designed drug-releasing implants assisted by external magnetic field based on magnetic nanoparticles (MNPs) loaded inside TNTs.[95] In this study, TNTs were loaded with three types of amphiphilic micelles at the top acting as drug carriers and MNPs at the bottom of the nanotubes. Meanwhile, dopamine modification of iron oxide MNPs (DOPA-Fe_3O_4) with soft interfacial cushion were used to improve the biocompatibility of the MNPs, as shown in Figure 8. For the drug-release profiles, it was confirmed that immediate 100% release of all three drug carriers was achieved within 1–1.5 hours upon the application of the magnetic field. Although this strategy also presents some limitations such as uncontrolled release triggered by existing magnetic fields from the environment, it is still particularly valuable

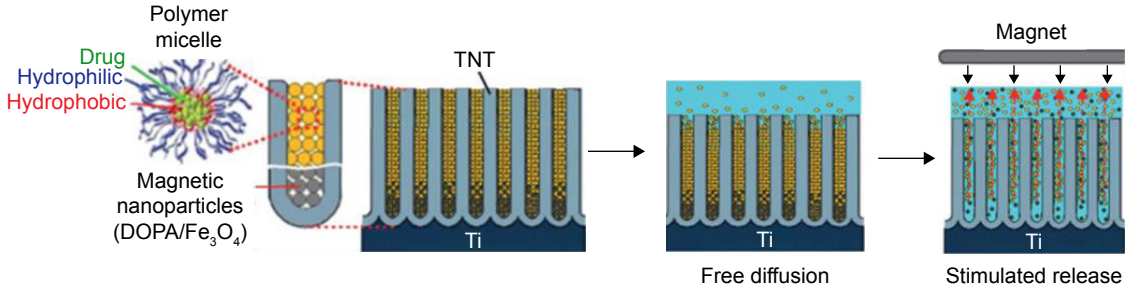

Figure 8 Schematic representation of the magnetic stimuli-responsive drug release from TNTs which integrates polymer micelles as drug carriers incorporated with poorly soluble drugs and magnetic nanoparticles loaded at the bottom of the nanotubular structures.
Notes: The release is achieved by applying an external magnetic field. Reproduced from Aw MS, Addai-Mensah J, Losic D. Magnetic-responsive delivery of drugcarriers using titania nanotube arrays. *J Mater Chem*. 2012;22:6561–6563,[95] with permission of The Royal Society of Chemistry.
Abbreviation: TNT, titania nanotube.

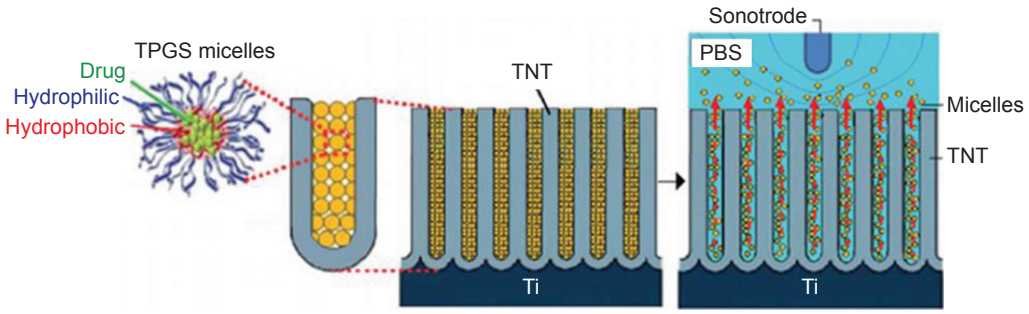

Figure 9 Schematic illustration of ultrasound-stimulated DD based on TNT implants and polymeric micelles as drug carriers. Reprinted from *Int J Pharm*, 443, Aw MS, Losic D, Ultrasound enhanced release of therapeutics from drug-releasing implants based on titania nanotube arrays, 154–162,[97] Copyright (2013), with permission from Elsevier.
Abbreviations: DD, drug delivery; PBS, phosphate-buffered saline; TNT, titania nanotube.

for drug-releasing implants in orthopedics and bone surgery where on-demand release is needed under emergency situation. Moreover, Shrestha et al used TNTs filled with MNPs to achieve magnetic- and photocatalytic-guided release of drugs.[96] The release concept is based on the fact that the UV-induced hole generation in the valence band of the TiO_2 will lead to chain-scission of a monolayer attached to TiO_2.

Ultrasound-sensitive drug delivery

The ultrasound-sensitive drug delivery based on applying the local ultrasonic field is expected to be a more reliable method compared with the magnetic-sensitive drug delivery that is uncontrollable in drug release when triggered by magnetic fields. Aw et al reported the application of local ultrasonic external field for triggering drug release from TNTs.[97] In this study, ultrasonic waves were used as the trigger for stimulus-responsive local drug delivery system combining TNT implants as shown in Figure 9. The ultrasound-mediated drug-micelles release based on exerting oscillating pressure waves from a probe inserted in phosphate buffered saline (pH 7.2) close to the drug-micelle-loaded TNTs and a nonsteroidal anti-inflammatory drug (Indomethacin) was used as the model for water insoluble drug encapsulated in polymer micelles. With regard to the application of this concept, it can be applied for bone therapies, local drug delivery, and implantable drug delivery systems including stents and brain drug delivery. Of course, more studies of ex vivo or in vivo models using different drug-releasing implants and drugs are needed to achieve significant understandings based on the concept.

These studies demonstrated that the drug release from TNT implants can be remotely triggered by external stimuli, thus it is expected to be applied in some medical therapeutics such as bone therapy, local chemotherapy, systemic chemotherapy, and so on. However, the development of these concepts are still at a preliminary stage, and further in vitro and in vivo studies are required to confirm their feasibility for practical applications in the living body.

TNT implants for clinical application

Although studies of TNT implants used for controlled drug delivery are still at their preliminary stage, they have demonstrated remarkable capabilities in terms of versatility in various clinical applications. Some typical examples of these are bone implants, dentistry, cardiovascular stents, and brain tumors, in which localized drug delivery devices based on TNTs are regarded as a promising alternative to overcome limitations of the conventional drug delivery systems.

Bone therapy and dentistry

Conventional therapies based on systemic administration of drugs for treating bone-related diseases present inherent limitations and associated side effects, whereas localized drug administration based on TNTs could offer outstanding potential advantages for treating bone-related diseases. Bone infections are associated with bone implants coming into contact with the living tissue. Considering the inflammatory response results from bone infections, Aninwene et al used penicillin/streptomycin- (anti-infection drugs) and dexamethasone- (an anti-inflammatory drug) loaded TNTs. The physical adsorption of the drugs could promote the anti-inflammatory properties of the TNTs, and the drug-eluting technique could be extended with enhanced osteoblast adhesion.[98] Gulati and Aw et al reported water-insoluble indomethacin-loaded TNTs for anti-inflammation and anti-infection.[99–101] In their works, in vitro studies were performed to demonstrate the potential applicability of TNTs for loading and releasing water-insoluble drugs using planar and wire implants, which provides the foundation for TNTs to be applied in delivering therapeutics to prevent inflammations in bone-related diseases. Moreover, Popat et al investigated the drug release kinetics of gentamicin-loaded TNTs and its effect on reducing bacterial adhesion on the surface.[102]

To enhance the osseointegration capability of orthopedic implants within the microenvironment surrounding the

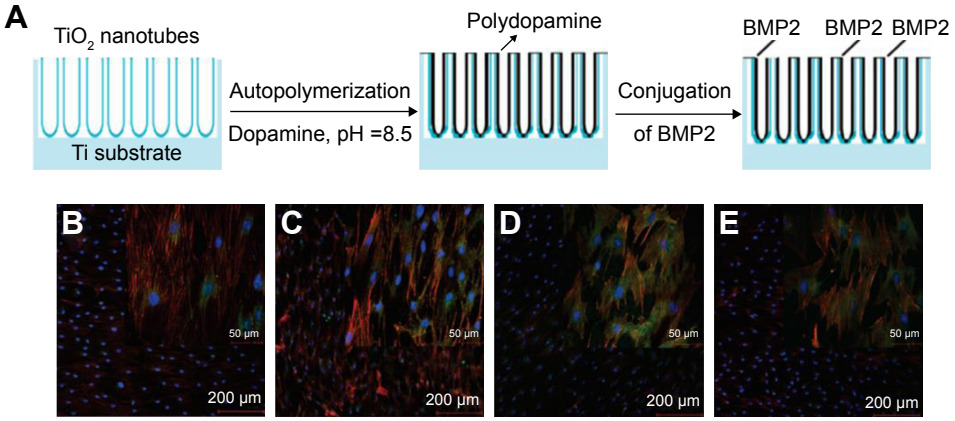

Figure 10 The surface functionalization of TNTs with BMP2 for cell proliferation and differentiation.
Notes: (**A**) Schematic illustration of the conjugation of BMP2 onto TNTs. Fluorescence images of MSCs adhered to (**B**) Ti, (**C**) BMP2-PDOP-Ti, (**D**) 30 nm TNTs, (**E**) BMP2-PDOP-30 nm TNTs. Cells were stained with actin filaments (red), cell nuclei (blue), and vinculin (green) in this study. Reprinted with permission from Lai M, Cai K, Zhao L, Chen X, Hou Y, Yang Z. Surface functionalization of TiO_2 nanotubes with bone morphogenetic protein 2 and its synergistic effect on the differentiation of mesenchymal stem cells. *Biomacromolecules*. 2011;12:1097–1105.[104] Copyright (2011) American Chemical Society.
Abbreviations: BMP2, bone morphogenetic protein 2; TNT, titania nanotube; MSCs, mesenchymal stem cells; PDOP, polydopamine.

bone, the implant surface must promote the functions of different cell species, including osteoblasts and stem cells, as well as enhance bone healing.[103] Lai et al reported BMP2 loaded inside TNTs could promote MSC proliferation and differentiation, extending the scope of stem cell engineering and cell-based therapies.[104] To investigate the adhesion and proliferation of MSCs, the cytoskeleton morphology of MSCs was visualized with a triple staining of actin, vinculin, and nucleus by immunocytochemistry as shown in Figure 10. The result suggested that the conjugation of BMP2 onto TNTs promoted cell proliferation (Figure 10B–E). The diameters of TNTs were able to affect the adhesion, spreading, and differentiation of MSCs, and specifically, larger diameters could promote the protein adsorption.[104–108] Therefore, the physical dimension of TNTs is an important factor for modulating biological functions in bone cells and tissue engineering.

The implant surface is another important factor in terms of integration within the host body, given that it acts as an interface between artificial element and biological environment. Bovan et al highlighted the role of material surfaces in regulating cell response to implants, and the result demonstrated that the surface roughness and chemistry of bare orthopedic implants have a significant effect on osteoblasts and chondrocytes growth.[109] Kunze et al reported that annealed TNT coatings with anatase phase are good precursors for the formation of calcium hydroxyapatite ceramic.[110] In their study, more nuclei were formed on the TNT surface than on flat compact TiO_2 in the initial stages of apatite growth, which demonstrated that TNTs are more beneficial to osseointegration than flat compact TiO_2. Furthermore, Shim et al used Ti-anodized implants coated with FGF-2-loaded poly(lactide-co-glycolide) nanoparticles shown in Figure 11A for enhancing bone regeneration by an approach of the electrospray deposition, and the obtained results indicated that the factor-releasing particles fabricate stable and the releasing performance was extended over

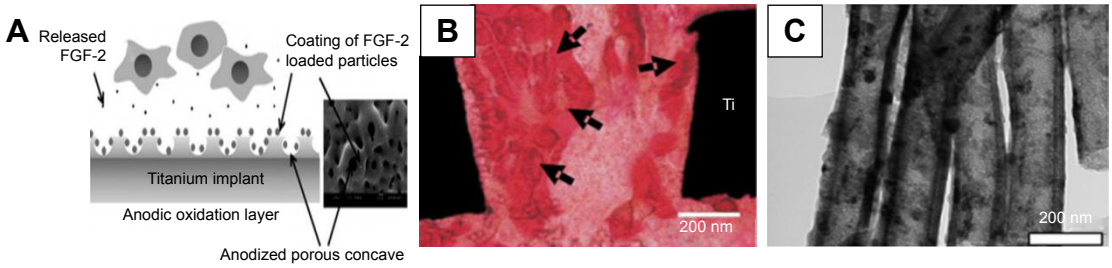

Figure 11 The modification of Ti substrate or TiO_2 nanostructured layer for improving their functionality.
Notes: (**A**) The surface modification of Ti disc implants by growth factor-releasing particles to enhance osseointegration. (**B**) TGF-β1-loaded Ti led to increases in bone-to-implant contact and bone volume within 1 mm macropores. (**C**) TEM image of antibacterial AgNPs incorporated within TNTs. (**A**) Reprinted from Shim IK, Chung HJ, Jung MR, et al. Biofunctional porous anodized titanium implants for enhanced bone regeneration. *J Biomed Mater Res A*. 2014;102A:3639–3648,[111] with permission from John Wiley and Sons. (**B**) Reprinted from *Adv Drug Deliv Rev*, 59, Moioli EK, Clark PA, Xin X, Lal S, Mao JJ, Matrices and scaffolds for drug delivery in dental, oral and craniofacial tissue engineering, 308–324,[113] Copyright (2007), with permission from Elsevier. (**C**) Reprinted from *Biomaterials*, 32, Zhao L, Wang H, Huo K, et al, Antibacterial nanostructured titania coating incorporated with silver nanoparticles 5706–5716,[114] Copyright (2011), with permission from Elsevier.
Abbreviations: AgNP, silver nanoparticle; TEM, transmission electron microscopy; TGF-β1, transforming growth factor-β1; TNT, titania nanotube.

2 weeks, thus enhancing the proliferation of bone tissues.[111] It is known that some biologically active substances such as FGF, platelet-derived growth factor (PDGF), transforming growth factor β and insulin-like growth factor could break inflammatory cell release.[112] Moioli et al demonstrated that incorporation of growth factor β (transforming growth factor β) in TNTs led to significantly enhanced bone-to-implant contact and increased bone volume within the 1 mm macropores as compared to placebo controls as shown in Figure 11B.[113] TNTs loaded with Ag nanoparticles (AgNPs) possess a relatively long-term antibacterial ability and simultaneously promote cell functions, and AgNPs attached to the inner wall of the TNTs have a diameter of ~10–20 nm as shown in Figure 11C.[114]

For dentistry diseases, typically for dental caries caused by bacteria, fluoride is a great effective agent in the therapeutic treatment and could prevent caries and enhance remineralization of enamel lesions.[115–117] Therefore, some fluoride-releasing devices were designed to provide controlled fluoride release without accelerating the fluoride concentration in serum, thereby prevent caries formation. Ti and its alloys are well-accepted candidates for dental implants.[21] The electrochemical stability of TNTs in artificial saliva was evaluated by Pirvu et al.[118] Their study indicated a slight preference in terms of a human gingival fibroblast (HGF) survival and adhesion for TNTs with a more hydrophilic character, and the electrochemical date revealed that TNTs possess excellent stability in artificial saliva. HGFs were cultured on the Ag/FGF-2 immobilized TNTs for cytocompatibility determination.[119] The result indicated that Ag/FGF-2-decorated TNTs has an excellent cytocompatibility compared to pure Ti, and the immobilized FGF-2 could enhance HGF cell attachment and proliferation. Furthermore, Bhattarai et al examined the feasibility of chitosan-gold nanoparticles conjugated with plasmid DNA/c-myb (Ch-GNPs/c-myb)-coated Ti implants and inserted it into rat mandibles to determine its in vivo effect.[120] The obtained results support the view that c-myb can serve as a potent molecule in promoting tissue regeneration around dental implants. In short, these studies demonstrate that TNTs can provide more efficient and rational clinical treatments for dental diseases, with a great promising perspective for real clinical applications.

Cardiovascular stents

Coronary stents could improve immediate and late results of balloon angioplasty by tacking up dissections and preventing wall recoil,[121] thus avoiding the heart occlusion in the treatment of coronary heart disease. However, traditional coronary stents present some inherent clinical complications such as restenosis, the need for further revascularization, and other various problems emerged after the cardiovascular surgery,[122] which still remains as a crucial challenge in cardiology. To solve these problems, the concept of local drug delivery can be achieved by drug-eluting stents coated with polymer surfaces used for controlled drug release. However, several polymer coatings have shown an induction of inflammatory response and increased neointima formation, thus limited the clinical applicability of these alternative coronary stents. To avoid the side effect, new materials have been tested to overcome inherent drawbacks of drug-releasing cardiovascular stents coated with polymers.

In this regard, Wieneke et al investigated the effect of a new inorganic ceramic nanoporous aluminum oxide coating on neointima proliferation and its suitability as a carrier for the immunosuppressive drug tacrolimus.[123] These cardiovascular stents were prepared as stainless steel stents coated with aluminum, which demonstrated the potential applicability of drug-releasing cardiovascular stents coated with nanoporous inorganic materials. The durability and biocompatibility of ceramic coatings are significantly important for drug-releasing coronary stents. Recent in vivo studies have demonstrated that the shedding of particle debris released from the nanoporous coatings produces a great increment of neointimal hyperplasia.[124] Fine et al reported that drug-releasing coronary stents based on Ti promote better interactions with ECs.[125] In this study, a new material called rosette nanotubes without drugs was coated on Ti stent surfaces, aiming to enhance the EC adhesion and proliferation. The obtained results indicated that rosette nanotube-coated Ti allows the growth of a uniform endothelium on their surface and prevents the stent from becoming loose and being dislodged after implantation and therefore prevent particle debris release from the implant surface. In addition, heart valves coated with ceramic TiO_2 have been shown to exert profound antithrombogenic and break resistant properties.[126,127]

Although recent studies suggest that TNT arrays may be a well-accepted candidate for developing vascular implants, the effects of TNTs on vascular cells should be investigated carefully. With regard to this, Peng et al studied the response of primary human ECs and vascular smooth muscle cells (VSMCs) to TNT arrays through gene expression analysis.[128] In their study, microarrays revealed that TNTs can enhance EC proliferation and motility, prevent VSMC proliferation, and decrease expression of molecules related to coagulation and inflammation in ECs and VSMCs. The work thus suggested that TNTs could be a promising candidate for

the next-generation vascular device coating because of the divergent response of ECs and VSMCs. Nevertheless, it should be emphasized that more in vivo studies are required to evaluate their feasibility for the living body because the aforementioned drug-releasing stent experiments are far from real-life clinical conditions.

Brain tumors

For the treatment of brain tumors, systemic chemotherapy has some inherent drawbacks that significantly limit the clinical effectiveness. To address these limitations, a localized chemotherapy is desirable to treat malignant glioma. In this regard, drug-releasing implants based on nanotechnological approaches can provide alternative ways to satisfy the requirement. López et al prepared an implant based on nanostructured TiO_2 loaded with an antiepileptic model drug to overcome the conventional systemic administration channel blocked by the blood–brain barrier.[129] This study confirms that the device shows excellent biocompatibility to the brain tissue, which proves the viability of its safe implantation in the brain and its therapeutic effectiveness to treat epilepsy. Gulati et al reported a new alternative to treat brain-related diseases based on the drug-releasing implant prepared with anodizing Ti sheets to obtain TNTs on their surface for drug delivery application.[130] The obtained in vitro results demonstrated that TNTs loaded with anticancer drugs can provide a controlled drug release to effectively destroy cancer cells and thus possess the outstanding capacity for localized treatment of brain-related diseases.

The biocompatibility of the as-prepared TNTs was studied by observing the growth of osteosarcoma (MG-63) cells on the TNT surface, and TNTs loaded with the antineoplastic drug were investigated by using MG-63 cells as targets and cisplatin as the model drug.[131] Their results demonstrated that TNTs show excellent biocompatibility and can be used as a drug delivery vehicle, with efficient drug loading and releasing ability to significantly suppress the growth of MG-63 cells. Moreover, Kalbacova et al demonstrated that TNTs can be used as a photocatalyst to kill cancer cells.[132] In this work, it was reported that a focused UV light or X-ray excitation to trigger a photocatalytic reaction on TiO_2 administrated locally to a tumor. From these studies, it is suggested that TNTs have the potential to be used for localized cancer therapy, and more advanced systems are expected to be designed for exploring these concepts in the future.

Conclusion and future perspectives

This work reviewed the recent advances in the research of drug-releasing implants based on TNTs. It is demonstrated that the application of TNTs is a promising alternative to develop various localized drug delivery systems that possess the capability to overcome the limitations of systemic drug therapies. TNT layers can be used as orthopedic and stent implants without limitations in their shapes and forms based on the remarkable properties of TNTs such as excellent biocompatibility and mechanical and thermal stability, thereby promoting bone cell adhesion, differentiation and proliferation, hydroxyapatite formation, osseointegration, and hemocompatibility.

It is confirmed that several smart strategies including pH, thermal, light, RF, magnetic, and ultrasound were used as triggers for drug release from TNTs, which show outstanding features offering great perspectives and opportunities for TNT applications. Although still at initial stage, these external stimulus strategies can be described as promising triggers for controlling drug release from TNT implants. A series of studies involving ex vivo and in vivo animal models have been performed to prove the excellent biocompatibility of the TNT implants. These concepts may be applied for human clinical trials after long-term toxicity assay and tolerability studies carried out on animals to evaluate the safety. Therefore, more in vivo studies should be performed for the localized drug delivery systems applied in clinical trials.

Finally, the drug-releasing TNT implants are used for localized drug delivery in the field of medical applications, including bone therapies, dentistry, cardiovascular stents, treatment of localized cancer, and other therapies that required an implantable device. These multifaceted localized drug delivery systems based on TNTs are used to control the drug release with optimized concentration for a range of time scales, which is expected to facilitate their smoother translation into clinical practice in the future.

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Disclosure

The authors report no conflicts of interest in this work.

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