Recent advances on smart TiO_2 nanotube platforms for sustainable drug delivery applications

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Abstract: To address the limitations of traditional drug delivery, TiO_2 nanotubes (TNTs) are recognized as a promising material for localized drug delivery systems. With regard to the excellent biocompatibility and physicochemical properties, TNTs prepared by a facile electrochemical anodizing process have been used to fabricate new drug-releasing implants for localized drug delivery. This review discusses the development of TNTs applied in localized drug delivery systems, focusing on several approaches to control drug release, including the regulation of the dimensions of TNTs, modification of internal chemical characteristics, adjusting pore openings by biopolymer coatings, and employing polymeric micelles as drug nanocarriers. Furthermore, rational strategies on external conditions-triggered stimuli-responsive drug release for localized drug delivery systems are highlighted. Finally, the review concludes with the recent advances on TNTs for controlled drug delivery and corresponding prospects in the future.

Keywords: TiO_2 nanotubes, electrochemical anodization, modification, stimulated drug delivery, drug-releasing implant

Introduction

To address the limitations of conventional drug therapies related to restricted drug solubility, short circulating time, lack of selectivity, side effects, and unfavorable pharmacodynamics, considerable studies have been carried out in past years toward the development of more efficient drug delivery systems.¹-⁴ Approximately 90% of drugs that are used currently on the market are hydrophobic and poorly water-insoluble, and this restricts their pharmacological efficiency and particularly challenges the systemic delivery route.⁵,⁶ Another inherent limitation of the therapies is that various drugs are inactivated or removed by the gastrointestinal system and other body organs including kidney and liver, and only <1% of administered drug molecules reach the site of interest, where higher dosages of drugs are required to achieve an optimized local concentration.⁷,⁸ Therefore, innovative drug delivery approaches are urgently needed to address the disadvantages of traditional drug delivery.

The inherent limitations of conventional therapies could be addressed on the basis of developing more efficient and rational drug delivery systems, in which two concepts involved in targeting drug delivery and localized drug delivery systems are verified to be the most perspective strategies. The utilization of nanotechnology to medicine is an emerging field with significant potential for localized drug delivery systems. Nanotechnology has boosted the development of various new nanomaterials and drug carriers for drug release applications, including polymer micelles, liposomes vesicle, multifunctional dendritic polymers, nanocapsules, nanospheres, TiO_2 nanotubes (TNTs),...
and so on, as shown in Figure 1. For these nanoporous or nanotube carriers, a special niche in drug delivery technology has been guaranteed to correspond with them because of their simple preparation, controlled nanoporous or nanotube formation, mechanical rigidity, chemical resistivity, high loading capability, high surface area, and so on. TNT arrays formed on Ti surface by a facile electrochemical anodizing process are recognized as a desirable candidate to promote clinical therapeutic effect of medical implants.

This paper aimed at reviewing TNTs used as carriers for controlled drug release and compiling the most recent advances on TNT-based drug-releasing implants for localized and smart drug delivery applications. Various methods designed to control sustained drug release from TNT implants are discussed, which include controlling TNT morphologies and chemical modification. Additionally, some advanced strategies on externally triggered stimuli-responsive drug release are discussed, and these sources hold significant potential of producing alternative drug release pathways that could overcome the limitations of the traditional diffusion mechanism. Finally, this review concludes a general overview on the future trends, challenges, and the prospective outlook for the interesting and promising research field.

**Development of TNTs by electrochemical anodization**

With the development of TNTs constructed by electrochemical anodizing, more and more attention is paid to achieve higher nanotube growth rates, improve controllable dimensions and nanotubes ordering. In order to improve the technology of TNT fabrication, various electrochemical protocols were addressed to support it, which involve aqueous and organic electrolytes with different chemical compositions and electrochemical conditions. The electrochemical anodization process is carried out usually in electrolytes containing some fluoride ions to fabricate TNT layers. In electrolytes with acid, salt, or alkali solution, short tube lengths were formed (from 400 nm to 2 μm) during anodization process, and these TNT arrays lacked organized structure and uniformity, which are described as the first generation of TNTs. Then TNTs of longer lengths (5–7 μm) were fabricated in a pH-controlled electrolyte containing small amounts of fluoride ions, which are considered as the second generation of TNTs. The third generation of TNT arrays with further longer length (1,000 μm) was fabricated in nonaqueous polar organic electrolytes in the presence of fluoride species by electrochemical anodization. Recently,
non-fluoride-based synthesis of TNTs has been reported, which are addressed as the fourth generation, showing significant improvement in the growth rate and the quality of fabricated TNTs.

For TNT arrays formed in fluoride-based electrolyte, an appropriate external voltage and low electrolyte acidity are needed for yielding TNT layers and growing long tubes. At a controlled anodization voltage of 80–120 V, electrochemical anodization of Ti results in vertically oriented and hexagonally closely packed TNT structures with high growth rate, optimal geometry, and stable mechanical characteristics. In addition, some other TiO₂ morphologies were reported, such as nanopore branched, bamboo-type nanotubes, inner tubes, spongy, nanolace, and multilayer nanotubes were fabricated by controlling the voltage during the process of nanotube preparation. Thereby the study of TNT fabrication is a dynamic and active research area, and the development of novel strategies and synthesis methods is expected to be extended in the future.

**Strategies to control drug delivery from TNTs**

Drug delivery from nanotubes is dependent on the diffusion process when TNTs are implanted into the host body with physiological milieu. This diffusion process can be defined by Fick’s first law, which is influenced by various factors such as molecular size of the drugs, charge, dissolution rate and diffusion coefficient, dimensions of nanotubes, charge and surface chemistry, and interfacial interaction of drug molecules and TNT surface. Depending on these conditions, different drug release profiles were obtained and different strategies have been implemented into TNT-based systems in order to provide a controlled drug release for a broad range of clinical therapies. It is known that different drug release strategies need to be considered for different therapies, thus 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. It is worthwhile stressing that zero-order type release is the most satisfactory release strategy for drug-releasing implants, which results in the drug being released at a uniform and constant rate independent of concentration and time. 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. A schematic diagram summarizing these strategies aimed at controlling the release of drugs from TNTs is presented in Figure 2.

In this schematic diagram, a single nanotube was subjected to various modifications for controlling drug release, including A) structural modifications of diameter and length of TNTs, B) surface modifications, C) adjusting pore openings of TNTs with polymer deposition, D) biodegradable polymer coatings, E) polymeric micelles as drug nanocarriers, and F) stimulated drug release strategies by external sources.

**Dimensions of TNTs and surface functionalization**

According to Fick’s law, the diffusion rate of drug molecules from nanopores depends on their dimensions as these confined nanochannels can be controlled precisely at nanoscale. To investigate the influence of pore size on the drug release profiles, studies from several groups confirmed that the drug release is considerably related to the dimensions of TNTs. Aw et al studied the relationship between the length of the TNTs and anodization time, and their results demonstrated that length of TNTs is controlled by anodization time and that the drug release time was extended with an increase in the nanotube length based on drug loaded in TNT implants of different nanotube lengths (25–100 μm) and a fixed pore diameter of 110 nm. The drug release from TNTs can be extended by shrinking their lengths and diameters, which results from the fact that drug molecules encapsulated deeper inside TNTs needed a longer time to diffuse out of nanotubes because of the capillary force. In addition, Hamlekhan et al studied that anodization condition (voltage and duration) influences the release profiles of TNT groups based on the dimensions of TNTs influenced by anodization conditions. As demonstrated by various studies, length and diameter of TNTs are increased with the increasing anodization voltage. Moreover, the amount of drug loaded in TNTs increases as the anodization duration is increased based on comparing the profiles with the TNT dimensions specified in all TNT groups, as presented in Figure 3.

The next strategy for controlling drug release from TNTs was based on the surface functionalization of the nanotubes. The aim of this strategy is to dynamically change the interaction between drug molecules and inner walls of the nanotubes for altering the drug release kinetics. This approach was previously demonstrated on porous silica particles and was successfully translated into TNTs by using polymers and self-assembled monolayers with excellent stability and flexibility for surface modification. Organic silanes (ie, 3-aminopropyl triethoxysilane [APTES], penta-fluorophenyldimethylchlorosilan [PFPTES]) and phosphonic acids (ie, 2-carboxyethyl-phosphonic acid and 16-phosphono-hexadecanoic acid) were used to modify...
TNTs, which demonstrate that this approach could change drug loading and release characteristics of TNTs for hydrophilic and hydrophobic drugs, as shown in Figure 4. It is worthwhile stressing that APTES holds the silane group hydrophilic that can impart different properties onto the surface of TNTs. Moreover, APTES protonates can give similar properties of drugs in phosphate-buffered saline (PBS) with neutral pH.

Based on the results presented above, it is demonstrated that drug loading and releasing features are significantly influenced by surface charge and chemical and interfacial properties. Specific surface modification strategy is useful for rational designing implants with splendid properties for optimized application, whereas this strategy is still limited to achieve a sustained release of drugs from TNTs for a longer duration.

**Plasma polymerized biopolymer coating modifications**

In order to overcome the problem that a long and sustained drug release cannot be realized by surface modification of TNTs, a new strategy using plasma polymer coatings on the top surface of TNTs to reduce the opening of nanopores, which confirmed that drugs release from TNTs is possible to follow the zero-order release kinetics. Various drugs with different size and properties have been successfully performed on TNTs based on this concept, and these drugs...
Figure 3 Concentration of drug released from TNTs anodized at (A) 60 V, (B) 70 V, (C) 90 V, and (D) 120 V.

Notes: The area of less than 30 min corresponds to active release stage. During this stage, most of the loaded drug is released from nanotubes into aqueous environment. Some groups of TNTs release the overall amount of the loaded drug in less than 15 min, while the other groups prolong release to about 1 h (marked by vertical dash line). Error bars show variance for n = 3. Copyright IOP Publishing. Reproduced with permission. All rights reserved. Hamlekhan A, Sinha-Ray S, Takoudis C, et al. Fabrication of drug eluting implants: study of drug release mechanism from titanium dioxide nanotubes. J Phys D Appl Phys. 2015;48:275401. Published 10 June 2015.

Abbreviations: h, hours; min, minutes; d, days; TNT, TiO$_2$ nanotube.

Figure 4 Schemes showing the concept of chemical modification.

Notes: (A) Modification on TNTs by phosphonic acid using 2-carboxyethyl-phosphonic acid (2-phos) and 16-phosphono-hexadecanoic acid (16-phos); (B) drug release from 2-phos, 16-phos-modified TNTs and the control sample (unmodified, bare TNTs). 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, http://dx.doi.org/10.1039/C3BM60196J.

Abbreviations: h, hours; TNT, TiO$_2$ nanotube.
include anti-inflammatory drugs, anticancer drugs, functionalized metal oxide nanospheres, bovine serum albumin, and bone morphogenetic proteins (BMPs) drug micelles. It is worthwhile noticing that plasma deposition method holds a capability of modifying the top surface of TNT layers, and the modified TNTs are imparted to desirable properties including antibacterial activity, implant rejection, anti-biofouling, and better integration to reduce sensing capabilities. With the plasma deposition method being widely used in medical application, the disadvantages of this technology are also presented, such as the calibration requirement of plasma conditions, its high cost, and some unavailable technical knowledge.

Considering these limitations of the plasma deposition, a significantly simpler method with low cost was explored based on coating TNT opening. Poly (lactic-co-glycolic acid) (PLGA) and chitosan were selected for dip-coating, which result from two polymers with biocompatible and biodegradable possibilities and are known to have some beneficial properties including antibacterial and osseo-integration and are also used for drug delivery systems. PLGA or chitosan was coated on drug-loaded TNTs by dip-coating for controlling drug release and improving antibacterial and bone integration of TNTs, as schematically shown in Figure 5.

![Figure 5](https://www.dovepress.com/)

**Figure 5** Schematic diagram of TNTs implants loaded with drugs where the nanotubes were covered with ultrathin film of biodegradable polymer (PLGA or chitosan) using a simple dip-coating process.

**Notes:** Reprinted from Acta Biomater, Volume 8, Gulati K, Ramakrishnan S, Aw MS, Atkins GJ, Findlay DM, Losic D. Biocompatible polymer coating of titania nanotube arrays for improved drug elution and osteoblast adhesion, pages 449–456, Copyright 2012, with permission from Elsevier.

**Abbreviations:** PLGA, poly (lactic-co-glycolic acid); TNT, TiO₂ nanotube.

Significant changes in drug release profiles were observed because of coating a polymer film on openings of the nanotubes as shown in Figure 6. From the results, it was demonstrated that drug release profiles were significantly influenced by polymers’ chemical composition and the thickness of polymer layer. Meanwhile, the burst release occurred at the first 6 hours is presented in release process regardless of a polymer film exited or not, which can be explained that the initial release is from drug loaded on TNTs’ top surface, and the high concentration gradient through nanotubes allows drugs’ rapid release at the beginning of release duration. In addition, it was also concluded that TNT arrays coated with a thin PLGA polymer layer shows an extended release duration with a higher level of burst release and that a thin chitosan layer coated on TNTs could provide a shorter release duration with a lower level of burst release.

Form these results, it was demonstrated that the drug release can extend to several months with zero-ordered kinetics by controlling the thickness of the biopolymer film coated on TNTs. This design of TNT implants is focused on its local drug delivery with several weeks releasing, which has been performed by a study based on post-surgical implant surgeries, and its result indicates that systemically delivered gentamicin has fewer side effects in promoting bone healing. The adhesion and prolifera-
tension of human osteoblastic cells on TNT implants were investigated to confirm polymer-coated TNTs, especially chitosan coating could enhance osteoblast integration to implants.\textsuperscript{2} TNTs with dip-coating modification are believed to be achievable in a clinical environment because of their facile fabrication process and no specific technology requirements.

**Advanced TNT drug systems with multi-drug and stimulated drug release**

**Multi-drug delivery with micelles as drug carriers from TNTs**

Considering the treatment of some complex diseases that require more than one kind of drug, a new concept of using polymeric micelles for loading drugs was addressed, especially multi-drug nanocarriers were integrated into TNTs for designing implants with advanced multi-drug releasing. This advanced drug release strategy holds the capability of delivering several drugs for a pre-programmed time to permit a later release, as schematically presented in Figure 7A–D.\textsuperscript{58} As the most desired candidates for this concept, hydrophobic and hydrophilic properties of drug carriers provide a desired structure of polymer layers in TNTs without inter-mixing, and a unique successive release pattern from TNTs can be achieved based on this multiple-drug delivery system. In terms of drug release profiles based on TNTs loaded with two layers drug carries, Figure 7E shows the sequential releasing of regular micelles (d-\textalpha-tocopheryl polyethylene glycol 1000 succina [TPGS]) encapsulating two hydrophobic drugs (indomethacin and itraconazole) loaded at the top, followed by the releasing of inverted micelles (PEGylated phospholipids [DGP]) encapsulating hydrophilic drugs (gentamicin) loaded at the bottom.\textsuperscript{58}

Compared with conventional drug carriers, polymeric micelles can enhance drug delivery system because of the prolonged therapeutic effects of drugs in targeted organs or tissues.\textsuperscript{82} Considering the candidates of polymers, poly(ethylene oxide) (PEO)-poly(propylene oxide) (PPO)-PEO consisted of hydrophilic polar head (PEO) and copolymers-lipophilic alkyl tail (PPO) was used as drug carriers due to its organic chains with different number of tri-block monomers.\textsuperscript{83} TPGS, a vitamin E derivative, can significantly improve drug encapsulation efficiency and increase the bioavailability of anticancer drugs, thus was selected as a drug carrier.\textsuperscript{84,85} Moreover, the PEGylated phospholipids

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**Figure 6** Comparative drug release graphs of anti-inflammatory drug (indomethacin) from polymer-coated TNTs/Ti.

**Notes:** (A, B) Overall and burst drug release from uncoated TNTs/Ti and TNTs/Ti coated with thin and thick chitosan layers; (C, D) overall and burst drug release from uncoated TNTs/Ti and TNTs/Ti coated with thin and thick PLGA layers. Reprinted from Acta Biomater, Volume 8, Gulati K, Ramakrishnan S, Aw MS, Atkins GJ, Findlay DM, Losic D. Biocompatible polymer coating of titania nanotube arrays for improved drug elution and osteoblast adhesion, pages 449–456, Copyright 2012, with permission from Elsevier.\textsuperscript{80}

**Abbreviations:** min, minutes; PLGA, poly (lactic-co-glycolic acid); TNT, TiO\textsubscript{2} nanotube.
(DGP 2000 and 5000) are also used in drug delivery system in broad commercial applications.

The multi-drug delivery system used in various local drug therapies presents sequential release of drug carriers by varying types of drug-loaded carriers and positions of carries in TNTs. Release profiles of this multi-drug delivery system can be controlled by adjusting the length and pore diameters of TNTs, surface properties of micelles and their loading conditions. Furthermore, this multi-drug delivery system fully satisfies complex requirements for bone therapies required over long periods to prevent inflammation and improve implant integration.

Stimulated drug delivery from TNTs with external trigger
Extended drug release for long-term therapies are not satisfied in critical situations such as unexpected onset of inflammation, sudden viral attack, osteomyelitis, and so on, where high concentrations of drug are immediately required. To settle these emergency conditions, a concept of stimulated drug delivery system with external trigger based on TNTs is put forward to achieve therapeutic efficacy.

Extensive investigations were explored to increase the treatment effect in the required time period, which sometimes needed high dosage with precise schedule or only for a short time frame. This concept allows for precise control and delivery of drugs according to the specific needs of the patient, thereby improving the efficacy of drug delivery systems. This approach offers a promising method for targeted and effective drug delivery in the treatment of various diseases.

Figure 7 Scheme depicting the concept for controlling multiple drug release from TNTs.
Notes: (A) TNTs loaded with two types of polymer micelles, a regular micelle (TPGS) encapsulated with hydrophobic and an inverted micelle (DGP 2000) encapsulated with hydrophilic drug; (B) scheme of sequential drug release with layered drug carriers with details of two-step drug release in (C) and (D); (E) sequential and multiple release of drug carriers loaded with three drugs from TNTs. Reproduced from Aw MS, Addai-Mensah J, Losic D. A multi-drug delivery system with sequential release using titania nanotube arrays. Chem Commun. 2012;48:3348–3350, with permission of The Royal Society of Chemistry, http://dx.doi.org/10.1039/C2CC17690D.
Abbreviations: TNT, TiO$_2$ nanotube; TPGS, d-α-tocopheryl polyethylene glycol 1000 succina; DGP, PEGylated phospholipids.
period to treat urgent diseases. For satisfying the demand of delivering pre-determined amount of drugs required by pharmacokinetic parameters, several concepts are reported, including magnetic, ultrasound, and voltage.

Magnetic-sensitive drug delivery
A concept of drug encapsulated in nanomagnetic structures was proposed, which focused on designing triggered drug delivery systems because the nanomagnetic structures possess exciting possibilities for magnetic field triggered drug release. Regarding this concept, Shrestha et al reported on using TNTs filled with magnetic nanoparticles (MNPs) in order to achieve magnetic- and photocatalytic-guided release of drugs. In this study, a model drug was attached to magnetic TNTs by using a silane coupling agent as a cross-linker, then ultraviolet (UV) irradiation was provided for inducing chain scission of this agent monolayer attached to TNTs, thus the release of the fluorescent molecule takes place at the anchoring siloxane groups as schematically outlined in Figure 8A. It was demonstrated by the phenomenon that the release of the fluorescent marker into the electrolyte was clearly visible after few seconds of UV irradiation imparted to dye-functionalized TNTs, as presented in Figure 8B and C. From these results, it is shown that the approach reported here can realize a temporally and spatially photoduced drug release based on magnetic-sensitive drug delivery system.

In addition, a new concept was addressed, aiming to design drug-releasing implants being assisted by MNPs loaded inside TNTs. In this study, iron oxide MNPs (DOPA-FeO) with dopamine modification was used for improving the biocompatibility of the MNPs and their loading inside the TNTs. Considering drug carriers, three types of amphiphilic micelles including Pluronic F127, TPGS, and PEO-PPO-PEO were explored to study the concept of magnetic-sensitive drug delivery system. For the drug-release profiles, it is confirmed that cumulative release of the three drug carriers reaches ~100% within 1–1.5 hours under the application of the magnetic field. Although this strategy also presents some limitations of uncontrolled release triggered by external magnetic fields from the environment, it is still particularly valuable for drug-releasing implants in orthopedics and bone surgery where on-demand release is required under emergency situation.

Figure 8 Schematic representation of the model drug release from TNTs.
Notes: (A) The release principle of active molecules (model drug) from the functionalized magnetic TNTs upon irradiation with UV light. The release of the fluorescent dye into the surrounding system by the dye-functionalized magnetic TNTs with UV light was “off” (B) and “on” (C). The movement of the tube layers in water was guided by a permanent magnet underneath the petri dish. Reproduced from Shrestha NK, Macak JM, Schmidt-Stein F, et al. Magnetically guided titania nanotubes for site-selective photocatalysis and drug release. Angew Chem Int Ed. 2009;48:969–972. Copyright © 2009 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.
Abbreviations: TNT, TiO nanotube; UV, ultraviolet.
Ultrasound-sensitive drug delivery

In order to overcome the drawbacks of magnetic field-stimulated release, the drug-releasing system based on ultrasound-mediated drug and nanocarrier release from TNTs was explored. Aw et al reported the application of local ultrasonic external field for triggering drug release from TNTs. It is the first time that ultrasound-mediated drug micelles are selected for local drug delivery system, and Figure 9A shows this concept of using ultrasonic waves (USW) as a trigger combined with TNT implants. For controlling drug-micelles release from TNTs, several USW parameters were explored, including pulse length, amplitude, pulsation time, and power intensity. The USW power intensity controlled by various distance between probe and sample has a significant effect on the profile of drug release from TNTs as shown in Figure 9B. In this work, drug release profiles vary as the distance between the probe and sample is changed, for example, when the distance is set as 2.0, 1.5, 1.0, and 0.5 cm, it corresponds to the power of 25, 50, 75, and 100 W, as presented in Figure 9C. It is indicated that the distance between the probe and sample is shorter, the USW power intensity is greater, and the force of the impact becomes stronger. These effects may result from the fact the wave energy could propagate directly without much hindrance in the medium.

With regard to the mechanism of drug-micelles release from TNTs by USW, it is likely involved that a combination of thermal and cavitation processes caused by mechanical vibration result from forces produced by the ultrasound waves in interaction with buffer and TNT implants. The application of this strategy can be involved in bone therapies and local delivery systems including stents or brain drug delivery. However, more ex vivo or in vivo studies based on various drugs loaded inside drug-released TNT implants are required to demonstrate the feasibility of this concept.

Voltage-sensitive drug delivery

Among various stimuli-responsive drug delivery system approaches, the voltage-sensitive release is another attractive strategy for its beneficial properties. Impartation of voltage could induce the chain scission based on TNTs grafted with...
octadecylphosphonic acid for wettability or attached to an enzyme of horseradish peroxidase, as reported by Song et al. In this study, a reaction of OH with terephthalic acid (TA) to form 2-hydroxyterephthalic acid (TAOH) results in a blue fluorescence at different voltages for identical duration as shown in Figure 10A. The results indicate that the strong blue fluorescence is visible when TNTs are held at 5 V, whereas virtually no fluorescence can be seen at 1.5 V or no voltage. For these reasons, it is possible that generated valence-band holes can react with their environment in a similar manner as photogenerated holes in TNTs at a potential of 5 V. Figure 10B schematically shows voltage-induced OH-radical formation and payload release, which demonstrates voltage-induced pseudo photocatalytic processes, and in particular the valence band ionization mechanism may result in the chain scission reactions from TNTs.

In addition, Sirivisoot et al reported an approach that was used to trigger drug release by an electrical field. In their study, drugs were encapsulated into multi-walled carbon nanotubes (MWCNTs) grown out of TNTs, where drugs release from TNTs under the control of electrical field. Their research achievement could be applied on treating bone repair and other more serious bone diseases. Furthermore, Sirivisoot et al carried out an experiment by doping polypyrrole with antibiotics (penicillin and streptomycin) and an anti-inflammatory drug (dexamethasone); their loading by electrodeposition inside MWCNTs grown on TNTs was considered as the further advancement of voltage-sensitive drug delivery. Although the development of this strategy is in its primary stage, it has an enormous potential for advanced developments in drug-released TNT implants.

**Ex vivo and in vivo studies of drug release characteristics**

Most of the aforementioned studies on drug release therapies of TNTs were performed through in vitro experiments using PBS as eluting medium. This situation is significantly different from real clinical circumstances that possess the real bone tissues and real biological environment, thereby many challenges are presented for in vivo applications, especially for how to accurately monitor the distribution of drug molecules from TNTs to the bone tissue. For this challenge, implantable TNT-Ti wires were selected to be inserted into the bone for providing extended drug release as reported by Aw et al. In this study, a TNT-Ti wire was inserted into a hole drilled through each bone core, fitting tightly into the bone core’s center, as shown in Figure 11A, and trabecular bone core with TNT-Ti wire inside the bone chamber was connected to perfusion pump that provides culture media to keep bone cells alive as shown in Figure 11B. Biomimicry images were captured at different times for the 5-day experiment on drug-release studies as presented in Figure 11C–F. From these results, it is demonstrated that the model drug’s concentration in 3D bone matrix is increasing and changes in the concentration exist across all directions from TNT-Ti implants. In brief, the TNT-Ti wire can be considered as a safe drug-releasing implant used in the local drug delivery system for bone therapies such as bone infection, bone inflammation, and even bone cancer.

A suitable in vivo performance must be provided before any biomaterial is used in a real clinical application, thus TNTs have to integrate within the bone tissue and survive the stresses experienced during surgical insertion inside the animal model. As described in the previous section, von Wilmowsky et al used pigs for studying the in vivo performance of TNT-Ti implants. In this study, investigations on peri-implant bone formation, bone–implant contact, and immunohistochemistry were performed for evaluating the effects of these implants, demonstrating that TNT coatings can enhance osteoblast functions, suppress shearing forces caused by implant insertion, and promote bone formation when compared to commercially available pure Ti implants. Another study reported by Park et al demonstrated that bone–implant contact results showed the
capability of improving osseointegration for protein-loaded TNT implants based on loading fibroblast growth factor and human fibronectin fragment (hFNIII9–10) fusion protein inside TNTs on Ti implants which were inserted in rabbit tibia, followed up for 3 months. 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 TNTs implants for clinical therapies.

Apparently, these studies help establishing future databases consisting of detailed information on the degree of toxicity on the nanoscale, which would help to clarify the division of toxic effects of nanoscale materials, including TNTs. Furthermore, extensive studies on the interaction between cells/tissues from different organs and parts of the body with TNTs are also required.

**Conclusion and future perspectives**

Recent advances of drug-releasing TNT implants are reviewed in this work, and it is indicated that the application of TNTs is a promising alternative to develop various localized drug delivery systems that possess the capability to overcome limitations of systemic drug therapies. TNTs present beneficial properties for drug delivery application, including controllable nanotube dimensions, tunable geometries and surface chemistry, high surface area, high and versatile drug-loading capacity for several drugs, ability to modulate drug release kinetics, and so forth.

In this review, it is confirmed that TNT implants have a significant potential in clinical therapeutics, and capabilities of this implant can be realized by tuning their drug-releasing characteristics and providing multi-drug release of different drugs in different fashions. These approaches aim to optimize drug dosage, release rate, and time needed for a broad range of specific therapies, which have been presented in detail in this review. For these purposes, several strategies including magnetic, electromagnetic, and ultrasonic were used as triggers to release drugs from TNTs, which present outstanding features offering great perspectives and opportunities for TNT applications. Although still at initial stage, these external stimulus strategies are considered as promising applications in drug-releasing implants for developing smart clinical therapies.

Regarding the excellent biocompatibility of TNTs, numerous studies based on cells, ex vivo or in vivo animal models have been performed to prove their excellent biocompatibility. It is indicated that long-term toxicity assay and tolerability studies are needed to be performed on animals to evaluate the safety of blank TNTs and drug-loaded TNTs before proceeding with human clinical trials, thereby more in vivo studies are urgently required before these localized drug delivery systems can be applied in clinical trials.

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The authors report no conflicts of interest in this work.

References
1. Losic D, Simovic S. Self-ordered nanopore and nanotube platforms for drug delivery applications. Expert Opin Drug Deliv. 2009;6:1363–1381.
2. 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.
3. Mainardes RM, Silva LP. Drug delivery systems: past, present, and future. Curr Drug Targets. 2004;5:449–455.
4. Fähr A, Liu X. Drug delivery strategies for poorly water-soluble drugs. Expert Opin Drug Deliv. 2007;4:403–416.
5. Wolinski JB, Colson YL, Grinstaff MW. Local drug delivery strategies for cancer treatment: gels, nanoparticles, polymeric films, rods, and wafers. J Control Release. 2012;159:14–26.
6. Prakash S, Malhotra M, Shao W, Tomaro-Duchesneau C, Abbasi S. Polymeric nano-hybrids and functionalized carbon nanotubes as drug delivery carriers for cancer therapy. Adv Drug Deliv Rev. 2011;63:1340–1351.
7. Losic D, Aw MS, Santos A, Gulati K, Bariana M. Titania nanotube arrays for local drug delivery: recent advances and perspectives. Expert Opin Drug Deliv. 2015;12:103–127.
8. Santos A, Aw MS, Bariana M, Kumeria T, Wang Y, Losic D. Drug-releasing implants: current progress, challenges and perspectives. J Mater Chem B. 2014;2:6157–6182.
9. Van D, McGuire T, Langer R. Small scale systems for in vivo drug delivery. Nat Biotechnol. 2003;21:1184–1191.
10. Duncan R. Nanomedicine gets clinical. Nanotoday. 2005;8:16–17.
11. Amiji MM. Nanotechnology for targeted drug and gene delivery. Nanomedicine. 2006;2:299–300.
12. Kayser O, Lemke A, Trejo NH. The impact of nanotechnology on the development of new drug delivery systems. Curr Pharm Biotechnol. 2005;6:3–5.
13. Wang S. Ordered mesoporous materials for drug delivery. Micropor Mesopor Mat. 2009;117:1–9.
14. Wang G, Otoone AN, Blair EA, Denton K, Tao Z, Asefa T. Functionalized mesoporous materials for adsorption and release of different drug molecules: a comparative study. J Solid State Chem. 2009;182:1649–1660.
15. Son SJ, Bai X, Lee SB. Inorganic hollow nanoparticles and nanotubes in nanomedicine: part 1. Drug/gene delivery applications. Drug Deliv. 2007;12:650–656.
16. Bianco A, Kostarelos K, Prato M. Applications of carbon nanotubes in drug delivery. Curr Opin Chem Biol. 2005;9:674–679.
17. Klumpp C, Kostarelos K, Prato M, Bianco A. Functionalized carbon nanotubes as emerging nanovehicles for the delivery of therapeutics. Biochim Biophys Acta. 2006;1758:404–412.
18. Lai YK, Lin LX, Pan F, et al. Bioinspired patterning with extreme wetability contrast on TiO2 nanotube array surface: a versatile platform for biomedical applications. Small. 2013;9:2945–2953.
19. Martin CR, Kohli P. The emerging field of nanotube biotechnology. Nat Rev Drug Discov. 2003;2:29–37.
20. Son SJ, Bai X, Nan A, Ghandehari H, Lee SB. Template synthesis of multifunctional nanotubes for controlled release. J Control Release. 2006;114:143–152.
21. Ge MZ, Cao CY, Li SH, et al. In-situ plasmonic Ag nanoparticles anchored TiO2 nanotube arrays as visible-light-driven photocatalysts for enhanced water splitting. Nanoscale. 2016;8:5226–5234.
22. Gulati K, Kant K, Findlay D, Losic D. Periodically tailored titania nanotubes for enhanced drug loading and releasing performances. J Mater Chem B. 2015;3:2553–2559.
23. Ge MZ, Cao CY, Huang JY, et al. Synthesis, modification and photo/photocatalytic degradation applications of TiO2 nanotube arrays: a review. Nanotechnol Rev. 2016;5:75–112.
24. Moon KS, Bae JM, Jin S, Oh S. Infrared-mediated drug elution activity of gold nanorod-grafted TiO2 nanotubes. J Nanomater. 2014;2014:750813.
25. Song YY, Schmidt-Stein F, Bauer S, Schmuki P. Amphiphilic TiO2 nanotube arrays: an actively controllable drug delivery system. J Am Chem Soc. 2009;131:4230–4232.
26. Lai YK, Huang JY, Cui ZQ, et al. Recent advances in TiO2-based nanostructures with controllable wettability and adhesion. Small. 2016;12:2203–2224.
27. Ge MZ, Cao CY, Huang JY, et al. A review of one-dimensional TiO2 nanostructures for environmental and energy applications. J Mater Chem A. 2016;4:6772–6801.
28. Zhang YY, Jiang ZL, Huang JY, et al. Titane and titania nanostructured materials for environmental and energy applications: a review. RSC Adv. 2015;5:79479–79510.
29. Ge MZ, Li QS, Cao CY, et al. One-dimensional TiO2 nanotube photocatalysts for solar water splitting. Advanced Science. 2016;3: article 1600152.
30. Jia H, Kerr LL. Kinetics of drug release from drug carrier of polymer/TiO2 nanotubes composite-pH dependent study. J Appl Polym Sci. 2015;132:41750.
31. Li HQ, Lai YK, Huang JY, et al. Multifunctional wettability patterns prepared by laser processing on superhydrophobic TiO2 nanostructured surfaces. Journal of Materials Chemistry B. 2015;3:342–347.
32. Zwillinger D, Barrientos-Cetina M, Forrester PA, Catanese E, Farrant P, Cipollone G, Furst T, Williams K, Parent A, Butler R, et al. Photoresponsive surfaces for biomedical applications. J Phys Chem C. 2009;113:2932–2938.
33. Grimes CA. Synthesis and application of highly ordered arrays of TiO2 nanotubes. J Mater Chem. 2006;17:1451–1457.
34. Mor GK, Varghese OK, Paulose M, Shankar K, Grimes CA. A review on highly ordered, vertically oriented TiO2 nanotube arrays: fabrication, material properties, and solar energy applications. Sol Energ Mat Sol C. 2006;90:2011–2075.
35. Lai YK, Gao XF, Zhanhong HF, Huang JY, Lin CJ, Jiang JL. Designing superhydrophobic nanostructures with tunable water adhesion. Adv Mater. 2010;21:3799–3803.
36. Cai Q, Paulose M, Varghese OK, Grimes CA. The effect of electrolyte composition on the fabrication of self-organized titanium oxide nanotube arrays by anodic oxidation. J Mater Res. 2005;20:230–236.
37. Cheong YL, Lam FK, Ng SW, Hassan Z, Ng SS, Low IM. Fabrication of titanium dioxide nanotubes in fluoride-free electrolyte via rapid breakdown anodization. J Porous Mater. 2015;22:1437–1444.
38. Prakashan HE, Shankar K, Paulose M, Varghese OK, Grimes A. A new benchmark for TiO2 nanotube array growth by anodization. J Phys Chem Lett. 2007;11:7235–7241.
39. Syrek K, Kapusta-Kolodziejska J, Jarosz M, Sulkia GD. Effect of electrolyte agitation on anodic titanium dioxide (ATO) growth and its photoelectrochemical properties. Electrochim Acta. 2015;180:801–810.
40. Ge M, Cao C, Li S, et al. Enhanced photocatalytic performances of n-TiO2 nanotubes by uniform creation of p-n heterojunctions with p-BiO2 quantum dots. Nanoscale. 2015;7:11552–11560.
41. Lai Y, Pan F, Xu C, Fuchs H, Chi L. In situ surface-modification-induced superhydrophobic patterns with reversible wettability and adhesion. Adv Mater. 2013;25:1682–1686.
42. Roy P, Berger S, Schmuki P. TiO2 nanotubes: synthesis and applications. Angew Chem Int Ed. 2011;50:2904–2939.
43. Paulose M, Shankar K, Yoriya S, et al. Anodic growth of highly ordered TiO2 nanotube arrays to 134 μm in length. J Phys Chem B. 2006;110:16179–16184.
44. Paulosee M, Peng LL, Popat KC, et al. Fabrication of mechanically robust, large area, polycrystalline nanotubular/porous TiO2 membranes. J Membr Sci. 2008;319:199–205.
45. Zhang Y, Fu W, Yang H, et al. Synthesis and characterization of TiO2 nanotubes for humidity sensing. Appl Surf Sci. 2008;254:5545–5547.
46. Alamm NK, Grimes CA. Formation of vertically oriented TiO2 nanotube arrays using a fluoride free HCl aqueous electrolyte. J Phys Chem Lett. 2007;11:13028–13032.
47. Bauer S, Kleber S, Schmuki P. TiO2 nanotubes: tailoring the geometry in H2PO4/HF electrolytes. Electrochem Commun. 2006;8:1321–1319.
48. Albu SP, Ghicov A, Alldabergerowa S, et al. Formation of double-walled TiO2 nanotubes and robust anatase membranes. Adv Mater. 2008;20:4135–4139.
49. Chen B, Lu K. Hierarchically branched titania nanotubes with tailored diameters and branch numbers. Langmuir. 2012;28:2937–2943.
50. Jia H, Kerr LL. Sustained ibuprofen release using composite poly (lactic-co-glycolic acid)/titanium dioxide nanotubes from Ti implant surface. J Pharm Sci. 2013;102:2341–2348.
51. Brammer KS, Kim H, Koh N, et al. Highly bioactive 8 nm hydrolytic TiO2 nanotubes elicit enhanced bone cell response. Adv Eng Mater. 2011;13:B88–B94.
52. Simchi A, Tamjid E, Pishbin F, Boccaccini AR. Recent progress in inorganic and composite coatings with bactericidal capability for orthopaedic applications. Nanomedicine. 2011;7:22–39.
53. Anunvene GE, Yao C, Webster TJ. Enhanced osteoblast adhesion to drug-coated anodized nanotubular titanium surfaces. Int J Nanomed. 2008;3:257–264.
54. Moseke C, Hage F, Vornadren E, Gbureck U. TiO2 nanotube arrays deposited on Ti substrate by anodic oxidation and their potential as a long-term drug delivery system for antimicrobial agents. Appl Surf Sci. 2012;258:5399–5404.
55. Ticu E-L, Vercaigne-Marko D, Froidevaux R, Huma A, Artenie V, Klein MO. Early implant healing: promotion of platelet activation and cytokine release by topographical, chemical and biomimetic titania surface modifications in vitro. Clin Oral Impl Res. 2012;23:504–510.
56. Williams EH, Davydov AV, Motayed A, et al. Immobilization of streptavidin on 4H-SiC for biosensor development. Appl Surf Sci. 2012;258:6056–6063.
57. Losic D, Cole MA, Dommann B, Vasilev K, Grieser HH. Surface modification of nanoporous alumina membranes by plasma polymerization. Nanotechnology. 2008;19:245704.
58. Simovic S, Losic D, Vasilev K. Controlled delivery drug from porous materials by plasma polymer deposition. Chem Commun. 2010;46:1317–1319.
59. Simovic S, Losic D, Vasilev K. Controlled release from porous platforms. Pharm Technol. 2011;35:68–71.
60. Noh K, Brammer KS, Choi C, Kim SH, Frandsen CJ, Jin S. A new nanoporous TiO2 platform for drug release via nanotubular aluminum oxide. J Biomate Nanotechnol. 2011;2:226–233.
61. Schmid G. Materials in nanoporous alumina. J Mater Chem. 2002;12:1231–1238.
62. Nair LS, Laurencin CT. Polymers as biomaterials for tissue engineering and controlled drug delivery. Adv Biochem Eng Biotechnol. 2006;102:47–90.
63. Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, Langer R. Nanocarriers as an emerging platform for cancer therapy. Nat Nanotechnol. 2007;2:751–760.
64. Galiati K, Atkins GJ, Findlay DM, Losic D. Nano-engineered titanium for enhanced bone therapy. Clin Oral Impl Res. 2012;340315.
65. Slowing II, Vivero-Escoto JL, Wu CW, Lin VSY. Mesoporous silica nanoparticles as controlled release drug delivery and gene transfection carriers. Adv Drug Delivery Rev. 2008;60:1278–1288.
66. Lin CX, Qiao SZ, Yu CZ, Ismadji S, Lu GQ. Periodic mesoporous silica and organosilica with controlled morphologies as carrier for drug release. Micropor Mesopor Mat. 2009;117:213–219.
67. Paulose M, Peng LL, Popat KC, et al. Fabrication of mechanically robust, large area, polycrystalline nanotubular/porous TiO2 membranes. J Membr Sci. 2008;319:199–205.
68. Cai KY, Jiang F, Luo Z, Chen XY. Temperature-responsive controlled drug delivery system based on titanium nanotubes. Adv Eng Mater. 2010;12:B565–B570.
69. Caliskan N, Bayram C, Erdal E, Karahaliloglu Z, Denkbas EB. Titania nanotubes with adjustable dimensions for drug reservoir sites and enhanced cell adhesion. Mater Sci Eng C. 2014;35:100–105.
70. Wen LX, Ding HM, Wang JX, Chen JF. Porous hollow silica nanoparticles as carriers for controlled delivery of ibuprofen to small intestine. J Nanosci Nanotechnol. 2006;6:3139–3144.
71. Ajami E, Aguey-Zinsou FK. Functionalization of electropolished titanium surfaces with silane-based self-assembled monolayers and their application in drug delivery. J Colloid Interface Sci. 2012;385:258–267.
72. Simovic S, Addai-Mensah J, Losic D. Polymer micelles in drug delivery devices and materials. Adv Drug Delivery Rev. 2008;60:1266–1277.
73. Simovic S, Losic D, Vasilev K. Controlled drug release from porous materials by plasma polymer deposition. Chem Commun. 2010;46:1317–1319.
74. Simovic S, Losic D, Vasilev K. Controlled release from porous platforms. Pharm Technol. 2011;35:68–71.
75. Noh K, Brammer KS, Choi C, Kim SH, Frandsen CJ, Jin S. A new nanoporous TiO2 platform for drug release via nanotubular aluminum oxide. J Biomate Nanotechnol. 2011;2:226–233.
76. Schmid G. Materials in nanoporous alumina. J Mater Chem. 2002;12:1231–1238.
77. Nair LS, Laurencin CT. Polymers as biomaterials for tissue engineering and controlled drug delivery. Adv Biochem Eng Biotechnol. 2006;102:47–90.
78. Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, Langer R. Nanocarriers as an emerging platform for cancer therapy. Nat Nanotechnol. 2007;2:751–760.
79. Galiati K, Atkins GJ, Findlay DM, Losic D. Nano-engineered titanium for enhanced bone therapy. Proc SPIE. 2013;8812:1–6.
80. Galiati K, Ramakrishnan S, Aw MS, Atkins GJ, Findlay DM, Losic D. Biocompatible polymer coating of titania nanotube arrays for improved drug elution and osteoblast adhesion. Acta Biomater. 2012;8:449–456.
86. Sawant RM, Hurley JP, Salmaso S, et al. “SMART” drug delivery systems: double-targeted pH-responsive pharmaceutical nanocarriers. *Bioconjug Chem*. 2006;17:943–949.

87. Savonitto S, Urbano MD, Caracciolo M, et al. Urgent surgery in patients with a recently implanted coronary drug-eluting stent: a phase II study of “bridging” antiplatelet therapy with tiroliban during temporary withdrawal of clopidogrel. *Br J Anaesth.* 2010;104:285–291.

88. Ho HH, Lau TW, Leung F, Tse H-F, Siu C-W. Peri-operative management of anti-platelet agents and anti-thrombotic agents in geriatric patients undergoing semi-urgent hip fracture surgery. *Osteoporosis Int.* 2010;21:573–577.

89. Shrestha NK, Macak JM, Schmidt-Stein F, et al. Magnetically guided titania nanotubes for site-selective photocatalysis and drug release. *Angew Chem Int Edit.* 2009;48:969–972.

90. Aw MS, Addai-Mensah J, Losic D. Magnetic-responsive delivery of drug carriers using titania nanotube arrays. *J Mater Chem.* 2012;22:6561–6563.

91. Aw MS, Losic D. Ultrasound enhanced release of therapeutics from drug-releasing implants based on titania nanotube arrays. *Int J Pharm.* 2013;443:154–162.

92. Song YY, Roy P, Paramasivam I, Schmuki P. Voltage-induced payload release and wettability control on TiO$_2$ and TiO$_2$ nanotubes. *Angew Chem Int Ed.* 2010;49:351–354.

93. Sirivisoot S, Pareta R, Webster TJ. A conductive nanostructured polymer electrodeposited on titanium as a controllable, local drug delivery platform. *J Biomed Mater Res A.* 2011;99A:586–597.

94. Sirivisoot S, Pareta R, Webster TJ. Electrically controlled drug release from nanstructured polypyrrole coated on titanium. *Nanotechnology.* 2011;22:085101.

95. Sirivisoot S, Yao C, Xiao X, Sheldon BW, Webster TJ. Greater osteoblast functions on multiwalled carbon nanotubes grown from anodized nanotubular titanium for orthopedic applications. *Nanotechnology.* 2007;18:365102.

96. Sirivisoot S, Webster TJ. Multiwalled carbon nanotubes enhance electrochemical properties of titanium to determine in situ bone formation. *Nanotechnology.* 2008;19:2123–2131.

97. Aw MS, Khalid KA, Gulati K, et al. Characterization of drug-release kinetics in trabecular bone from titania nanotube implants. *Int J Nanomed.* 2012;7:4883–4892.

98. von Wilmowsky C, Bauer S, Lutz R, et al. In vivo evaluation of anodic TiO$_2$ nanotubes: an experimental study in the pig. *J Biomed Mater Res B.* 2009;89B:165–171.

99. Park JM, Koak JY, Jang JH, Han CH, Kim SK, Heo SJ. Osseointegration of anodized titanium implants coated with fibroblast growth factor-fibronectin (FGF-FN) fusion protein. *Int J Oral Maxillofac Implants.* 2006;21:859–866.

100. Li X, Wang L, Fan Y, Feng Q, Cui F. Biocompatibility and toxicity of nanoparticles and nanotubes. *J Nanomater.* 2012;2012:548389.