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Local drug delivery to the bone by drug-releasing implants: perspectives of nano-engineered titania nanotube arrays

Titania nanotube (TNT) arrays fabricated by electrochemical anodization of titanium are currently one of the most attractive nanomaterials due to their remarkable properties. In this review, we highlight recent research activities that are focused on the application of the TNT arrays for local drug delivery, specifically for addressing problems associated with orthopedic implants. The advantages of drug-releasing implants based on TNT arrays for local delivery of therapeutics in bone related to these challenging problems including inflammation, infection and osseointegration are discussed. An overview of recent research to advance the drug-releasing performance of TNT arrays and the potential of their future applications and development are presented.

Bones are remarkable, playing a key role in critical functions in human physiology including movement, protection and support for other organs, blood cell production, storage of minerals, fats and growth factors, calcium homeostasis, blood acid–base regulation and housing multiple progenitor cells (mesenchymal and hemopoietic) [1,2]. The bone is made up of compact (or cortical) bone and spongy (or cancellous) bone, depending on structural and biomechanical requirements, and contains three predominant cell types: osteoblasts, osteoclasts and osteocytes [1]. The extracellular matrix of bone is composed of an organic matrix (collagen) that is highly mineralized with calcium phosphate in the form of hydroxyapatite (HAP) [1]. Normal development and maintenance of the skeleton requires bones to be constantly resorbed by osteoclasts and rebuilt by osteoblasts. In healthy individuals, this balance is well coordinated, with the bone mass and microstructural integrity maintained in a steady state. However, disturbances of this equilibrium are seen in numerous bone conditions, such as osteoporosis, osteoarthritis, Paget's disease, bone infections, and primary and secondary bone cancers [3,4]. Bone metastases are a common complication of cancer diseases, with 65–80% of patients with metastatic breast and prostate cancers suffering this complication, and are a major cause of death in such patients [5].

The impact of bone diseases and traumatic injury on quality of life and health expenditure is staggering. Collectively, disease conditions of the skeleton represent at least 10% of annual healthcare expenditure, with enormous impact

on quality of life and on society in general [6,7]. These conditions bring with them an increasing need for orthopedic surgery including total joint replacement, spine fusion and fracture repair [6,7]. Total hip and knee joint replacements are now the most common major orthopedic surgical procedures, with several millions performed worldwide annually [7]. Infection of the bone, most commonly associated with trauma or bone implants, is a serious complication in orthopedics requiring prolonged hospitalization, complex revision procedures, implant failure and fracture non-union. It causes substantial suffering and even the death of patients, and is expensive to treat [7]. Improved methods are needed to treat infections in bones, the ideal being the maintenance of high concentrations of antibiotic at the infection site without high systemic levels.

Local drug delivery in bone using drug-releasing implants

A number of therapeutic approaches have been developed to treat bone diseases, primarily based on systemic drug administration (orally or intravenously) [8,9]. However, the delivery of drugs to specific skeletal sites is a challenge [9]. There are many limitations of conventional systemic drug therapy in bone, such as low efficacy, poor bioavailability and biodistribution, long duration or repetitive hospitalization, drug overdose, lack of selectivity and toxicity. Ideally, in order to increase the effectiveness and to reduce systemic side effects, drugs (e.g., growth factors, antibiotics, antiresorptives and anti-tumor agents) should be applied focally and

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Key Terms

Local drug delivery: Method of administering a pharmaceutical compound directly to the desired sites without the need to expose the entire body to it.

Titania nanotube array: Titanium oxide layer composed of self-organized and vertically aligned nanotubes formed by electrochemical anodization of titanium.

specifically to bone sites requiring therapy at optimal concentrations and over appropriate time periods [10,11]. Moreover, systemic drug dosage may not effectively reach bone that is poorly perfused as a result of a disease or trauma [9]. To overcome the limitations of conventional therapy, **local drug delivery** to bone has been recognized as a promising solution and the concept of a skeletal drug-delivery system was introduced by Buchholz *et al.* in the 1970s [12]. Local delivery of drugs offers many advantages, leaving other sites unaffected and preventing serious side effects, obtaining locally optimal concentrations of often expensive drugs without diluting them across the entire body, and **optimizing bioavailability by avoiding rapid breakdown and clearance of drugs**, particularly by the liver [13,14]. Therefore, methods to deliver drug to local bone sites has become one of the main focuses in bone therapy. There are several reviews on this topic showing recent trends on the development and problems of bone implants and drug delivery to the bone [15–18].

Various inorganic and organic–inorganic composites have been proposed as bone implant coatings to provide improved and better interacting interfaces with anti-infection characteristics including biodegradable polymers poly(methyl methacrylate), poly(lactic acid) or poly(glycolic acid), collagen, hyaluronan, chitosan, fibrin, silk, HAP, ceramics and injectable calcium phosphate cements have been tested as implants for the delivery of bone active agents [14,19–25]. The materials have been molded into different geometries and configurations; for example, membranes, granules, matrices, implant coatings, microparticles, hydrogels, fibers, sponges and foams [19–25]. Some of these implants have been used clinically to deliver bone-healing agents or anti-inflammatory agents for bone repair or tissue-engineering applications [26,27]. The main limitation of these implants is their inadequate ability to provide controlled and sustained drug release over a long period of time. These materials are mostly amorphous, with large variation of porosity and non-reproducible preparation, which in turn makes bone therapy unpredictable [19,26–28]. In addition, the implants are usually not applied inside the bone, which considerably limits the availability of drugs. In most cases these implants were designed for the delivery of specific drugs and do not have the flexibility to be applied to a wide range of therapeutic agents, such as water-insoluble drugs, drug carriers or labile agents (proteins and genes).

Advances in nanoscience and nano-engineering have led to the development of several novel drug-delivery platforms that addresses challenges associated with the bone implantable drug-delivery systems. Using these technologies, the size, size distribution, porosity, geometry and surface functionality can be controlled at the nanoscale. As a result, several approaches for surface modification by nanostructuring of existing titanium (Ti) implants, commonly used in orthopedics, have been applied and the development of new implants with **drug-releasing functions** have been introduced [19,29–33]. Among these approaches, self-organized TiO₂ or **titania nanotube (TNT) arrays** generated on Ti surfaces by electrochemical anodization are recognized as a promising solution [34]. The fabrication of TNT layers can easily be integrated into existing implant technology to add new properties, such as a improved biocompatibility, osseointegration, large surface area including drug loading/releasing function [34]. This integration with existing Ti implants to create implantable drug-delivery devices is an emerging new trend in implantable therapeutics, as a strategy to overcome several clinical problems and complications associated with conventional implants. The concept of drug-delivery devices for controlled and localized delivery of therapeutics have already found applications in various conditions such as cardiovascular disease, diabetes and cancer [17,18,35].

This review describes recent advances in the development of nano-engineered approaches for the local delivery of drugs into bones; with particular focus on TNT arrays engineered on Ti surfaces. Initially, basic information about TNT arrays including the description of their fabrication, structure and properties is presented. In the following sections the various examples on how additional functionalities can be included in Ti implants by synthesizing TNT arrays on them are discussed. Finally, we present an overview of the progress on the development of TNT arrays with advanced drug-releasing properties including: controlled/enhanced drug release, multidrug delivery, stimuli-responsive release and ‘smart’ biosensing bone implants.

TNT arrays: fabrication, structure & properties

TNT arrays fabricated by a self-ordering process with electrochemical anodization have attracted remarkable attention in recent years as one of the most popular nanomaterials [36–39].

This is due to their unique combination of wide band gap semiconductor and photocatalytic properties, nanotube geometry, biocompatibility and large surface area [36–39]. TNT arrays have been used for diverse applications, including photocatalysis for hydrogen production, solar cells, energy storage, catalysis, membranes, water purification, sensors, biosensing, cell growth and drug delivery [34,36,38,40,41]. The fabrication process and structure of TNT arrays are shown in **FIGURE 1**. TNT arrays are fabricated on the Ti surface by simple cost-effective electrochemical anodization processes as represented in **FIGURE 1A**. TNT arrays are composed of vertically oriented, highly ordered nanotubes with hexagonal arrangements and controllable nanotube diameters (10–300 nm) and thickness (0.5–300 μm), presented in **FIGURE 1B**. The lengths of the nanotubes correlate with the efficiency of film formation, with the longest nanotubes and the highest efficiency being found for nanotubes formed under controlled voltage. Typical SEM images of TNT arrays are shown in **FIGURE 1C**, with the top and the cross-sectional SEM images showing that the nanotubes are separated into individual entities and closed at the bottom. The TNT layer is composed of millions of ordered, densely packed, vertically aligned nanotubes, which have the capacity to accommodate and release a considerable amount of therapeutic agent (1–2 mg/cm^2), including insoluble drugs,

antibiotics, proteins, genes and drug carriers (nanoparticles [NPs] and micelles).

The electrochemical fabrication of self-ordered titania nanostructures was introduced in 1999 by Zwilling *et al.* by the anodization of Ti in a fluoride electrolyte [42]. This early work represents the first generation of TNT arrays with the length only 0.5 μm as result of extensive dissolution. Since then, a number of anodization approaches, mainly focused on finding the optimal electrolyte and anodization conditions have been explored to achieve a self-ordering regimen for titania nanotube growth with longer and controllable nanotube dimensions. Several reviews that have been published provide more details on the development of TNT materials [36–41]. The second generation of TNT array synthesis, with a length of approximately 5–7 μm , was reported by Macak *et al.* and Cai *et al.* by using an aqueous buffered electrolyte and by proper control of the anodization electrolyte pH thereby reducing chemical dissolution of the Ti dioxide during anodization [43–45]. These and following studies demonstrated that structural parameters of TNT arrays, including inner diameter, wall thickness, length and TNT crystallinity can be controlled by adjusting electrochemical conditions such as the composition of Ti substrate, electrolyte, pH, temperature, anodization voltage, current and anodization time. In general, it was shown that anodic TNT layers can be formed in aqueous and non-aqueous electrolytes containing

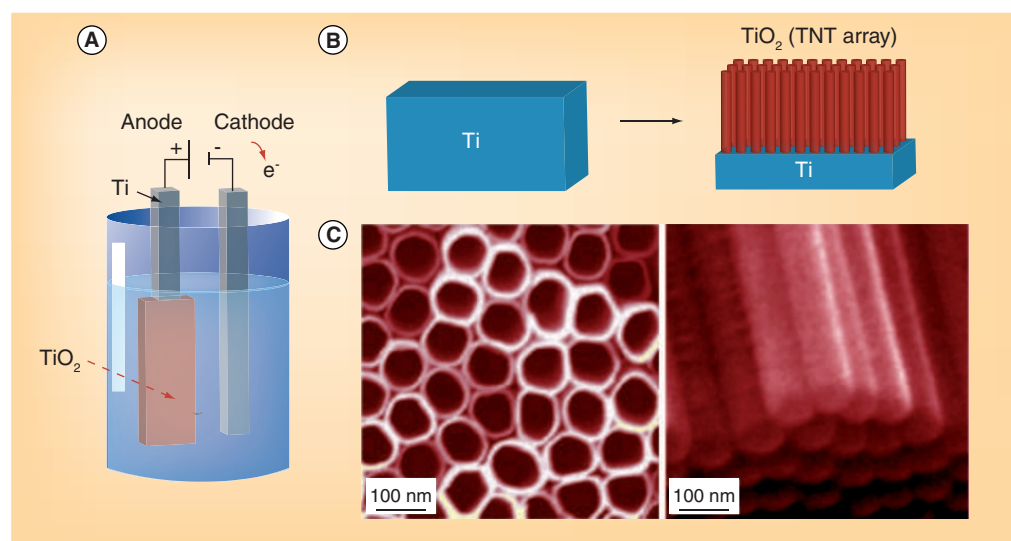


Figure 1. Titania nanotube arrays formation and structure. (A & B) Electrochemical cell and anodization process for formation of oxide layer on Ti with self-organized and vertically aligned arrays of nanotube structures. **(C)** Scanning electron microscopy images of the top and bottom surface show typical morphology of TNT structures. TNT: Titania nanotube array.

small amounts of fluoride ions (hydrofluoric acid [HF], sodium fluoride [NaF] or ammonium fluoride [NH₄F]) [36–41]. The third generation of TNT array synthesis, with nanotube lengths up to approximately 1000 µm, was achieved using a non-aqueous, polar organic electrolyte such as formamide, dimethyl sulfoxide, ethylene glycol or diethylene glycol [46–51]. To keep dissolution low and grow long tubes, low electrolyte acidity and low fluoride concentration are desirable conditions. The greatest tubes were obtained in organic viscous electrolytes using anodization voltage of 80–120 V, which yields hexagonal, vertically oriented and uniformly close-packed TNT structures. Recently, nonfluoride electrolyte-based synthesis of TNT arrays has been reported, which may be considered as the fourth synthesis generation [52]. New self-ordering titania morphologies, such as bamboo-type nanotubes, nanolace, branched tubes, inner tubes and multilayer nanotubes have been also produced by altering the voltage during the formation of nanotubes [53,54]. Although there is considerable research focused on surface modification of similar materials, such as porous silicon and nanoporous anodized aluminium oxide, surprisingly the surface modifications of TNT nanotubes to improve their drug-releasing properties have not yet been widely explored [55–57].

Biocompatibility of TNT arrays

Biocompatibility is the prerequisite for application of new biomaterials and it is defined in terms of cellular response and tissue integration of implantable biomaterials. Ti and its alloys (e.g., Ti-6Al-4V) have been proven as benchmark biocompatible materials and clinically used as orthopedic and dental implants for more than 50 years [58]. The biocompatibility of these materials is further improved by surface roughening, chemical modifications and HAP and composite polymer coating [59,60]. The engineering of TNT arrays on Ti surfaces not only improves biocompatibility of the Ti-based implants, but also provides additional therapeutic functions by enabling localized drug delivery. The majority of biocompatibility studies on TNT surfaces have been focused on their potential applications for tissue implant engineering, vascular implants and stem cells, where the importance of nanometric-scale topography and surface modifications (wettability) were studied [34].

Several studies have demonstrated that the TNT surface is a favorable platform for bone cell growth and differentiation, which provides

clear evidence that osteoblast activity can be significantly enhanced using controlled nanotubular topographies [60–63]. Park *et al.* have demonstrated that mesenchymal stem cell adhesion, spreading, growth and differentiation is significantly influenced by the TNT diameter with optimal diameter at 15 nm and continuous reduction with increasing diameters [61]. This effect is observed not only for mesenchymal stem cells but also for hematopoietic stem cells, endothelial cells, osteoblasts and osteoclasts. The explanation is that the effect is caused by integrin clustering in the cell membranes, leading to a focal adhesion complex, which is approximately 10 nm in diameter and an excellent geometrical fit into the tube opening of 15 nm [61]. The TNT array surface has higher cell adhesion, proliferation and viability for up to 7 days of culture, when compared with plain Ti surfaces, as shown by Popat *et al.* [64]. Cells cultured on TNT arrays surface also showed a higher alkaline phosphatase (ALP) activity without causing adverse immune response under *in vivo* conditions. The ability of TNT to enhance short- and long-term osseointegration *in vitro* is also demonstrated by Popat *et al.* [65]. The calcium and phosphorous concentrations were 50% higher on these surfaces suggesting that bone matrix deposition was unregulated on nanotubular surfaces. Furthermore, *in vivo* biocompatibility was proven by implanting TNT arrays discs in rats and performing histological analysis during 4 weeks; chronic inflammation or fibrosis was absent [65]. Excellent biocompatibility of prepared TNT arrays (pore diameters 100 nm) was confirmed by the increased growth of osteosarcoma (MG-63) cells [66]. A study by Burns *et al.* showed increased chondrocyte adhesion on TNT arrays in comparison to normal Ti surfaces (particularly suited for cartilage-related applications) [67]. The precalcification of TNT arrays, by soaking in Na₂HPO₄ solution overnight and then in saturated Ca(OH)₂ solution, has been shown to further enhance their osteoblast biocompatibility [68].

An *in vivo* study of TNT arrays relevant to their orthopedic applications was conducted by von Wilmsky *et al.*, where the TNT/Ti implant was implanted in a pig skull bone [69]. Bone-implant contact and immunohistochemistry analyses were performed at regular time intervals up to 90 days. It was found that the bone implant interface of TNT (diameter 30 nm)-modified Ti implants had significantly increased type-I collagen expression compared

with commercially pure Ti implants. This *in vivo* study suggests that TNT arrays provide a superior interface than bare Ti, with the capability to accelerate bone cell growth and hence bone healing. Although solid Ti and Ti-based alloys, including other implantable biomaterials are nontoxic, foreign-body reactions in the form of inflammation, thrombosis, fibrosis and infection can be triggered by metal particulates. Hence, hemocompatibility is a key consideration for the long-term success of blood contacting biomaterials to understand the physiological response elicited from blood–nano-biomaterial interactions. Smith *et al.* performed comprehensive hemocompatibility studies on TNT arrays and showed that blood serum protein adsorption, platelet adhesion/activation, and blood clotting kinetics on TNT arrays is increased [70]. However, the hemocompatibility can be altered by changing the size parameters of TNT arrays, which can be controlled by the fabrication conditions. These biocompatibility studies reinforce the fact that TNT arrays are remarkable implantable biomaterials for bone growth and maintenance with considerably improved characteristics such as cell adhesion, osseointegration properties and hemocompatibility.

TNT arrays for addressing the challenges of bone implants

Ti and its alloys have been widely applied as a major material for orthopedic implants owing to their excellent biocompatibility, corrosion resistance and favorable mechanical properties; however, the failure of bone implants still exists

and is often caused by inflammatory responses, bacterial infections and poor osseointegration [31,58]. An extensive post-surgical systematic drug therapy is regularly applied to address these problems, which are not always successful. However, in order to avoid implant failure and resurgery, all these clinical practices compromising patient compliance and causing enormous costs need to be revised or replaced urgently. As a result current implant technology is applying nanomaterials in combination with biomolecules to modulate such biological responses in order to improve implant integration, minimize inflammation and avoid any bacterial invasion [29–32]. One of the newest approaches is to deliver drugs locally from the implant surface that have both prosthetic, as well as therapeutic functions. Therefore, the engineering of existing Ti-based implants with TNT surface, or the design of new TNT implantable devices, are recognized as the most promising solutions toward achieving these goals. These perspectives of TNT arrays are summarized in **FIGURE 2**.

■ Inflammatory reaction

When an implant comes in contact with the living tissue, it generates an inflammatory response. This response is also initiated by the injury to the tissue or organ during surgical insertion of the implant. These reactions cause tissue and blood/interstitial fluid proteins to adhere onto the implant surface. These proteins activate platelets and immune cells which collectively act like a catalyst to the coagulation process resulting in the formation of a transient provisional

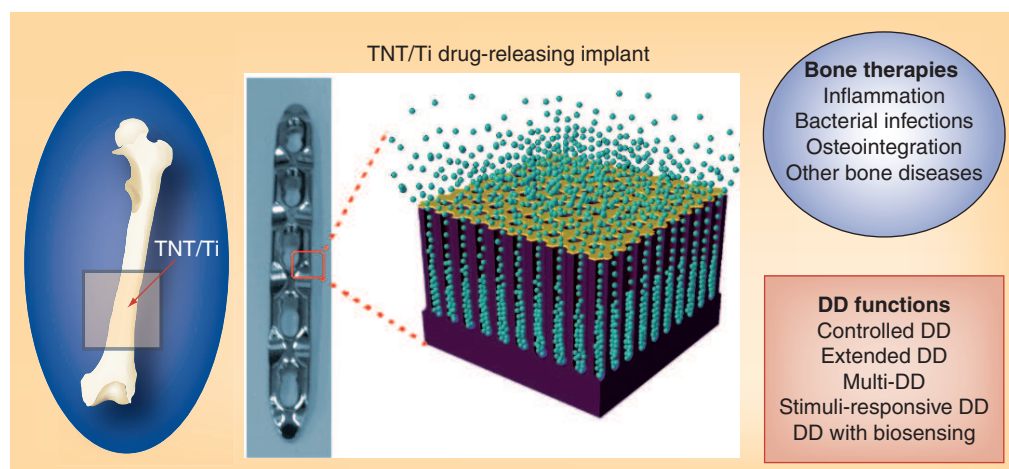


Figure 2. An overview of the applications of titania nanotubes as drug-releasing implants.

DD: Drug delivery; TNT: Titania nanotube.

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matrix [71]. Treatments for inflammatory conditions in bone currently require systemic administration of anti-inflammatory drugs. Ainslie *et al.* investigated difference in inflammatory responses between TNT arrays and control Ti surface in order to justify the role of nanostructuring in modulating immune responses [72]. The viability/morphology of monocytes, inflammatory cytokines and reactive oxygen species production as an indication of immune reactions were evaluated showing that TNT arrays significantly reduced inflammation response compared with the Ti surfaces. To improve anti-inflammatory properties of TNT arrays Aninwene *et al.* investigated the use of anti-inflammatory and immunosuppressant drug dexamethasone on TNT arrays (60-nm diameter) loaded on TNT arrays by physical adsorption and deposition from simulated body fluid (SBF) [73]. Results showed improved drug-elution characteristics (for up to 3 days) along with enhanced osteoblast adhesion for SBF, in contrast with physical adsorption, which is explained to the formation of a highly cytocompatible surface for bone cells when SBF was used.

Indomethacin is a nonsteroidal anti-inflammatory drug, commonly used for postsurgical treatment of bone inflammation and to improve bone healing after implant surgery [74,75]. Our group performed a study to show the application of TNT arrays for the drug loading and release of water insoluble indomethacin [76,77]. This provided proof-of-principle of the potential application of TNT arrays as bone implants for the delivery of therapeutics to prevent inflammation. In this case, TNT arrays with pore diameters of 70–90 nm and a length of 40 μm were prepared and their *in vitro* drug-release characterization showed a two-phase release, with a burst release during the first 6 h, followed by slow release over 2 weeks. These results demonstrated that a large amount of drug (1–2 mg/cm^2) can be loaded inside TNT arrays and the release behavior observed was suitable for the implant therapeutic applications.

To improve drug-release characteristics of anti-inflammatory drugs from TNT arrays and to achieve an extended release with optimized drug-release kinetics, several new strategies were recently demonstrated by our group, based on structural and chemical modifications of TNT structures [77–81]. One strategy is based on polymer coating of the top surface of drug-loaded TNT arrays and the second on the encapsulation of drug into polymer micelles as drug

carrier loaded into TNT arrays. Controlled and extended drug release over 6–8 weeks with zero-order release kinetics and reduced burst release could be achieved by polymer coating of the implants and this is a particular advantage of the TNT arrays drug-releasing platforms, in comparison with bone cements and polymer gels, which display unpredictable and uncontrolled release pattern with profound burst release at the initial stage [79–81]. In addition to their improved drug release, these modified TNT arrays showed increased osteoblast adhesion and cell proliferation as compared with uncoated TNT arrays [76]. Further studies are required to investigate the release kinetics of these drugs inside the bone tissue environment.

■ Bacterial infection

Bacterial infection of bone (osteomyelitis) is a devastating complication, which can occur after open fractures or after orthopedic implant surgery [82]. The treatment of bone infection requires a high-dose prolonged systemic antibiotic treatment. In the case of infected implants, a typical treatment usually involves several stages, including the removal of the implant and insertion of antibiotic-laden cement into the site. These treatments have a high morbidity and are not always successful as the bacteria can form biofilms on tissues or implants, which evade the host immune system and are difficult to treat with conventional antibiotic therapy. The most common microorganisms linked with implant infections are: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterobacteriaceae* and *Pseudomonas aeruginosa* [83]. To reduce the chances of serious complications, antibiotic therapy is prescribed for 6–8 weeks following implant surgery, which is sometimes not able to eradicate bacteria protected by the biofilm and can not access infected bone sites. Clearly, new approaches need to be explored to find strategies that can prevent biofilm formation and combat bacterial infection. Local delivery of an antibiotic would have the advantages of lowering the systemic dosage of toxic drug, fewer adverse and side effects, more efficient therapeutic performance and effective treatment. To address these infection-related problems of orthopedic implants, several approaches have been explored using TNT arrays loaded with antibiotics, silver NPs (AgNPs) and antimicrobial peptides [64,84–88].

Popat *et al.* demonstrated a local antibiotic therapy from the surface of Ti for reducing *Staphylococcus epidermidis* adhesion [64]. TNT

arrays of 80-nm diameter and 400-nm length were fabricated on Ti and loaded with 200, 400 and 600 µg of gentamicin sulfate. Significantly reduced bacterial adhesion was observed on the TNT surface (in comparison with Ti surface and unloaded nanotubes), while normal osteoblast adhesion and proliferation were retained, indicating a vital development of a prophylactic approach for the prevention of bacterial infection. Yao *et al.* demonstrated an improved method for effective drug loading of penicillin-based antibiotics in TNT arrays [84]. TNT arrays with 80-nm tube diameters and 200-nm lengths were fabricated and loaded with penicillin/streptomycin mixtures in SBF (with various ratios) in order to precipitate with calcium phosphate crystals into TNT arrays. This study demonstrated for the first time that co-precipitated drug coatings on TNT arrays could delay the drug release for up to 3 weeks. Not only the diffusion-dependent first-order kinetics of drug elution were obtained, but also the osteoblast activity for TNT arrays was retained for both drug-coated and -uncoated samples. Furthermore, it was shown that the loading and release characteristics of TNT arrays can easily be tailored by nanotube dimensions to meet specific therapeutic dosage. More specifically, Ercan *et al.* proved that heat treatments on different diameters of TNT arrays (20, 40, 60 and 80 nm) had varied antibacterial responses of *S. epidermidis* and *S. aureus* [88]. It was found that heat treatment (500°C for 2 h) reduced live and dead bacteria adhesion (for both types of bacteria), particularly for TNTs with a diameter 80 nm.

In addition to antibiotics, Zhao *et al.* have reported that the antibacterial effects of TNT arrays can be achieved by loading them with AgNPs using a simple process of AgNO₃ immersion and UV irradiation [86]. AgNPs that were already known to possess antibacterial properties were found to adhere to the inner walls of TNT arrays. It was shown that the size and amount of the AgNPs inside TNT arrays can be controlled by immersion time and the concentration of Ag solution. Ag-incorporated TNT arrays were capable of destroying all the initially present planktonic bacteria in the culture medium during the first several days and preventing bacterial adhesion for 30 days. AgNPs presented some cytotoxicity, which can further be reduced by controlling their release rate from the nanotubes.

Another approach specifically aimed to prevent the infection of antibiotic-resistant bacteria using TNT arrays has been reported [87]. Antimicrobial peptides with wide spectrum

antibiotic capability [HHC-36] were loaded inside TNT arrays and tested against *S. aureus*. The results demonstrated that TNT arrays were able to kill 99.9% of bacteria and the release lasted for over 7 days. Interestingly, the anatase type of TNT arrays used in the study was found to have higher release compared with amorphous TNT arrays. This approach was significant as it demonstrated new possibilities to treat infection problems of implants, particularly those caused by bacterial resistance from *S. aureus*, which was almost impossible to treat by conventional antibiotic drug administration.

While studies using TNT arrays implants for solving infection problems are encouraging, they are mainly based on *in vitro* investigations. *In vivo* studies of these new methods are required to further strengthen the concept of loading antibacterial agents inside TNT arrays.

■ Poor osseointegration

Poor implant osseointegration due to non-interacting implant surface is one of the most common reasons for the implant failure. For implant integration with the surrounding bone microenvironment, it is important that the implant surface controls the behavior of different cell types in bone, including osteoblasts, osteoclasts and stem cells and promote cell functions to achieve enhanced bone healing [89]. This will ensure a long-term stable anchoring and at the same time minimize any risk of implant loosening, fibrous tissue formation and micromotion at the bone implant interface. To improve osseointegrating properties of Ti implants, several surface roughening approaches have been explored using mechanical methods (machining, grinding and blasting), chemical methods (acid/alkali etching) and electrochemical anodization [59,60]. As it was previously described, recent studies have indicated that nanostructures (nanotube and nanopore morphologies) are more suitable than microstructures for cell-adhesion and cell-proliferation functionalities, which indicates TNT arrays as an excellent support for short- and long-term integration [90]. Chemical modifications of implants with a variety of bioactive molecules such as proteins, enzymes, peptides and calcium phosphate/HAP have been also applied to improve osseointegration [91]. Antibiotic-loaded TNT arrays were evaluated for their osteoblast adhesion characteristics by Popat *et al.* [64]. Preosteoblastic cell lines (MC3T3-E1) were cultured on TNT arrays and increased osteoblast cell adhesion and proliferation were observed for up to 7 days in comparison

Key Term

Drug-releasing implants:

Medical implants with loaded drugs that can be released for therapeutic purpose.

to bare Ti surfaces. Furthermore, the cells cultured on these nanoarchitected surfaces showed 50% enhanced properties for both ALP activity and calcium concentration, indicating TNT arrays ability to upregulate bone cell functions and matrix deposition.

In vivo testing of TNT modified Ti surfaces have been carried out by subcutaneous implantation in male Lewis rats and subsequent histology analysis after 4 weeks [65]. It was shown that the TNT surfaces promote growth and maintenance of bone-forming cells. Increased adhesion, proliferation, ALP activity and deposition of bone matrix was observed after cell culturing of osteoblasts on TNT arrays as compared with normal Ti surfaces. Also no sign of chronic inflammation or fibrosis was found for TNT arrays. These studies suggest that nanoarchitected Ti implants with TNT arrays can serve to enhance implant bonding with the bone microenvironment and promote the bone-healing process.

One of the key factors for implant integration in bone is the fast kinetics of HAP formation on the implant surface from body fluid. A number of studies have shown that HAP formation is accelerated by TNT arrays as compared with the flat Ti and TiO₂ surfaces, confirming a strong size effect [14–16,92]. A 3D structure is optimal for embedding precursors for HAP formation that promote HAP nucleation. Improved osteoblast functions and integration properties of TNT implants with an HAP layer was observed during *in vitro* studies. *In vivo* experiments with pigs demonstrated that TNT surface can enhance collagen Type 1 and BMP-2 expression and that higher implant contact can be established if the implant has a TNT layer [69]. Chemical modification of TNT arrays by biopolymers such as chitosan and PLGA have been shown to increase osteoblast functions such as cell adhesion and proliferation as compared with bare TNT arrays and normal Ti surfaces [80]. Neupane *et al.* reported a unique method of promoting growth and attachment of bone cells by loading gelatin-stabilized gold NPs (G-Au NPs) into TNT arrays using lipophilization technique [93]. Osteoblast attachment and spread was significantly enhanced for G-Au NPs/TNT arrays, producing an interlocked cell structure by the movement of filopodia from growing cells inside the nanopores. Also the cytotoxicity of G-Au NPs/TNT arrays surfaces was considerably reduced as compared with other substrates.

TNT arrays for advanced drug-releasing implants

Many studies exploring TNT arrays for drug-delivery applications indicated their potential for the development of **drug-releasing implants** and local drug delivery to the bone. However, there are some disadvantages that need to be addressed before they can be used. One limitation of the proposed drug-delivery systems on TNT arrays is that their drug-release pattern is directed by diffusion of drug molecules from the nanotube structure that can be described by Fick's first law. The diffusion is dependent on the TNT dimensions and geometry, which can be used to control drug-release kinetics, but it possesses certain limitations. Previous studies suggested that at a size scale of 100 nm and larger, diffusion of drug molecules is largely insensitive to tube diameter and the total drug release is dependent only on the tube length [94]. The drug-release pattern exhibits a fast drug release (burst) at the initial stage of release as a result of faster diffusion of drugs loaded near to the top open end of TNT arrays. This high burst release can be beneficial for some applications (e.g., preventing bone infection) but might exceed optimal therapeutic dosage and have a negative impact on the bone environment, as well as cause unnecessary wastage of the loaded drug. The time of drug release from TNT arrays is a function of the TNT length, which also controls the amount of loaded drug inside nanotubes. In the case of TNT arrays with lengths of a few microns this release time is very short – from hours to few days to a maximum of 1 week. This short drug release is not favorable for bone therapies where long treatment times are required, such as the treatment of infections. Therefore, the development of TNT arrays with sustained and extended delivery of drugs with zero-ordered drug-release kinetics is important for many applications. There may also be situations where a combination of factors is required, which means that the therapy with TNT arrays using several different drugs is the preferred solution. Finally, to treat emergency conditions, when immediate release of drug from the implant is required, the incorporation of sensing or stimuli-responsive properties into TNT arrays for drug-delivery systems is highly desirable. To address some of these limitations, several new approaches were recently reported to advance the properties of TNT arrays and extend their potential for future applications as drug-releasing implants and devices [95,96].

■ Extended drug release

To improve drug-releasing characteristics of TNT arrays and achieve extended release of several weeks and months with optimized drug-release kinetics, several new strategies, based on combined structural and chemical modifications of the nanotube structures were recently demonstrated [76,78–81]. A novel method to control the drug release from TNT arrays was introduced by capping the top surface of drug loaded TNT arrays with plasma polymers, which resulted in extended drug release over 6–8 weeks with zero-order release kinetics [78–81]. The release rate of the drug is controlled by the plasma deposition time (0–300 s), which generates different thicknesses of the plasma polymer film (allylamine polymer) on top of the nanotubes [78–81]. The concept has been proven for water-insoluble inflammatory drugs, such as indomethacin and also for antibiotics such as gentamicin, vancomycin, levofloxacin and proteins [97]. Recently, this method was further improved by using simpler and less expensive polymer-coating techniques, such as dip and spin coating of biopolymers

poly(DL-lactide-co-glycolide (PLGA) and chitosan (**FIGURE 3A & B**). Using this technique, the drug-release behavior of TNT arrays is controlled by the thickness and biodegradability of coated polymer film. Controllable and zero-order release kinetics with reduced burst release (from >60% to <30%) is achieved by these polymer coatings, with a significant improvement compared with bone cements and polymer gels, which display unpredictable and uncontrolled burst release (**FIGURE 3C & D**). This release pattern is especially useful in bone implant therapies that require a reasonably large initial dose, followed by a prolonged maintenance dose over a few weeks. The use of a biodegradable and antibacterial polymer, such as chitosan, provides favorable cell-adhesion properties and has the additional advantage of enhancing osseointegration (**FIGURE 3E**). These results demonstrated the ability to design TNT arrays with advanced and multifunctional properties.

Polymeric micelles with lipophilic or hydrophilic cores have been reported as exceptional drug-carriers with the ability to store/carry more than one drug, shield sensitive drugs and support

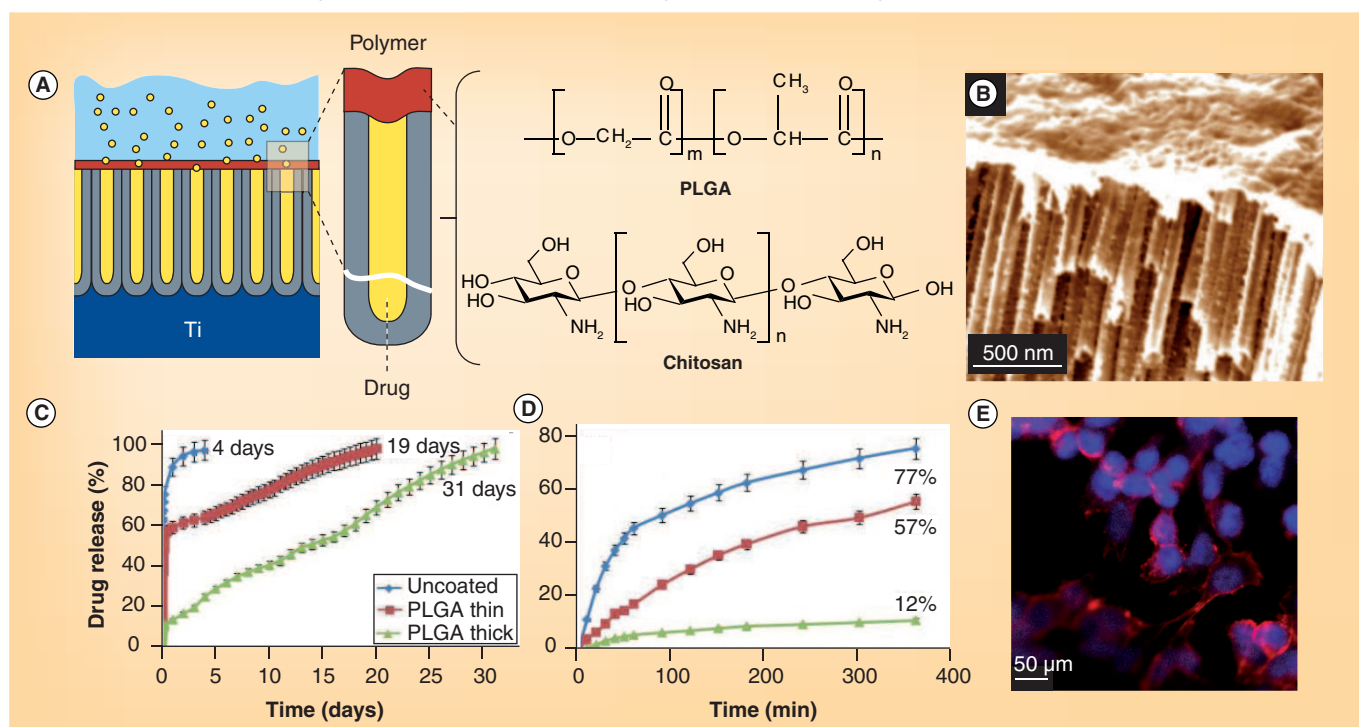


Figure 3. Titania nanotubes with extended drug release. (A) Modification of titania nanotube (TNT) arrays/Ti with biopolymers (PLGA and chitosan) to achieve extended drug release; (B) scanning electron microscope image of the top TNT arrays/Ti surface loaded with indomethacin and coated with chitosan layer; (C & D) comparative drug-release graphs of anti-inflammatory drug (indomethacin) from PLGA-coated TNT arrays/Ti, showing overall and burst release from thin and thick coating; (E) spreading of human osteoblastic cells imaged by confocal microscopy on: TNT arrays/Ti coated with chitosan. PLGA: Poly(DL-lactide-co-glycolide).

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Key Term

Stimuli-responsive drug delivery:

Drug release generated by external stimulants such as magnetic and electric field, light, temperature, pH, ultrasound, lasers and radiofrequency.

delayed release with favorable kinetics [98,99]. It was shown that large amounts of water-insoluble drug indomethacin can be encapsulated into polymeric micelles (i.e. Pluronic®, TPGS and PEO–PPO–PEO) prior to loading inside TNT arrays and an extended release of more than 4–8 weeks with zero order kinetics was achieved [80,81]. The system is very flexible as the release characteristics can be controlled by diameters and nanotube length, their surface chemistry, the size and interfacial properties of polymer micelles. Polymer micelles as drug carriers with encapsulated drugs were not only used to improve drug-release kinetics of TNT arrays, but also to develop some new functions, including extended drug release with zero-order kinetics, sequential multidrug release and time-delayed drug release that will be discussed in the following sections.

■ Multidrug loading & delivery

For further development of the drug-delivery capability of TNT arrays, our group harnessed the unique properties of polymer micelles as drug carriers to demonstrate their applications for delayed, sequential and multiple drug release [100,101]. Polymer micelles with lipophilic or hydrophilic cargo space are proven as excellent drug carriers with the ability for passive targeting, evade recognition by the reticuloendothelial system, protect sensitive drugs, increase drug dissolution and provide the loading of several drugs with slowed drug release when in contact with the cell environment [98,102]. In the first case, where delayed drug release was required, polymer micelles as drug carriers encapsulated with drug were loaded at the bottom of the TNT arrays structures and their delayed release was obtained by loading blank micelles (without drug) on the top. Delayed and time-controlled drug release was successfully achieved by controlling the ratio of blank and drug-loaded micelles [101]. The concept was demonstrated using four different polymer micelles (regular and inverted) loaded with water-insoluble (indomethacin) and water-soluble drugs (gentamicin). The delayed drug release from TNT arrays can be tuned from 1 to 7 days by the amount of blank micelles loaded on the upper layer of the TNT arrays. This strategy demonstrates the feasibility of designing TNT implants for treatments that require the release of drugs at desirable postponed time, for example after surgery, to suppress bacterial/viral infections or enhance bone integration.

A multidrug delivery and sequential drug release by incorporating polymer micelles as drug-carriers encapsulated with several drugs and loaded inside TNT arrays has recently been demonstrated [100–101]. The rationale is built on the combination of **unique geometrical features** of TNT and polymer micelles specifically arranged in a layered fashion inside the nanotube structures. The idea is based on the formation of two or more **immiscible layers of drug carriers** to create a series of sequential releases in a time-controlled manner. Regular micelles to encapsulate hydrophobic and inverted micelles for hydrophilic drugs that **have opposite interfacial properties** (hydrophilic and hydrophobic) are selected to make these layers without intermixing. The immiscible layers of drug carriers generate a **unique release pattern from nanotubes** in successive and independent steps where the numbers of released drugs, their quantity, release time and release order can be controlled by loading conditions, TNT lengths, pore diameters and surface properties of micelles (**FIGURE 4A**). The system is generic and can be applied to diverse local drug therapies including both insoluble or/and water soluble drugs with immediate, delayed and sustained therapeutic actions. Specifically, the system possesses essential attributes to simultaneously address the complex requirements for bone therapies where multidrug treatment is required over long periods to suppress inflammation and improve implant integration or tissue regeneration. Although the primary purpose behind this approach was to address challenges for bone implants, it can be applied to a varied number of other local drug-delivery applications (e.g., coronary stents).

■ Stimuli-responsive drug delivery

Another way to regulate drug delivery from TNT implants is to develop **stimuli-responsive drug delivery**. Stimulants that could be used are as diverse as magnetic fields together with magnetic NPs, heat (temperature), light, ultrasound, lasers, radiofrequency, pH and hypoxia [103–104]. The emphasis is on minimizing intrusiveness and eliminating the invasiveness of the triggering device. As mentioned previously, for conventional porous materials, drug release is typically sustained such that its kinetics can be expressed in terms of diffusion of adsorbed molecules. But for certain conditions, such as severe bacterial infection or osteomyelitis, it is important to have fast local-drug dosage at specific concentrations. As a result, TNT arrays in regards to their structures

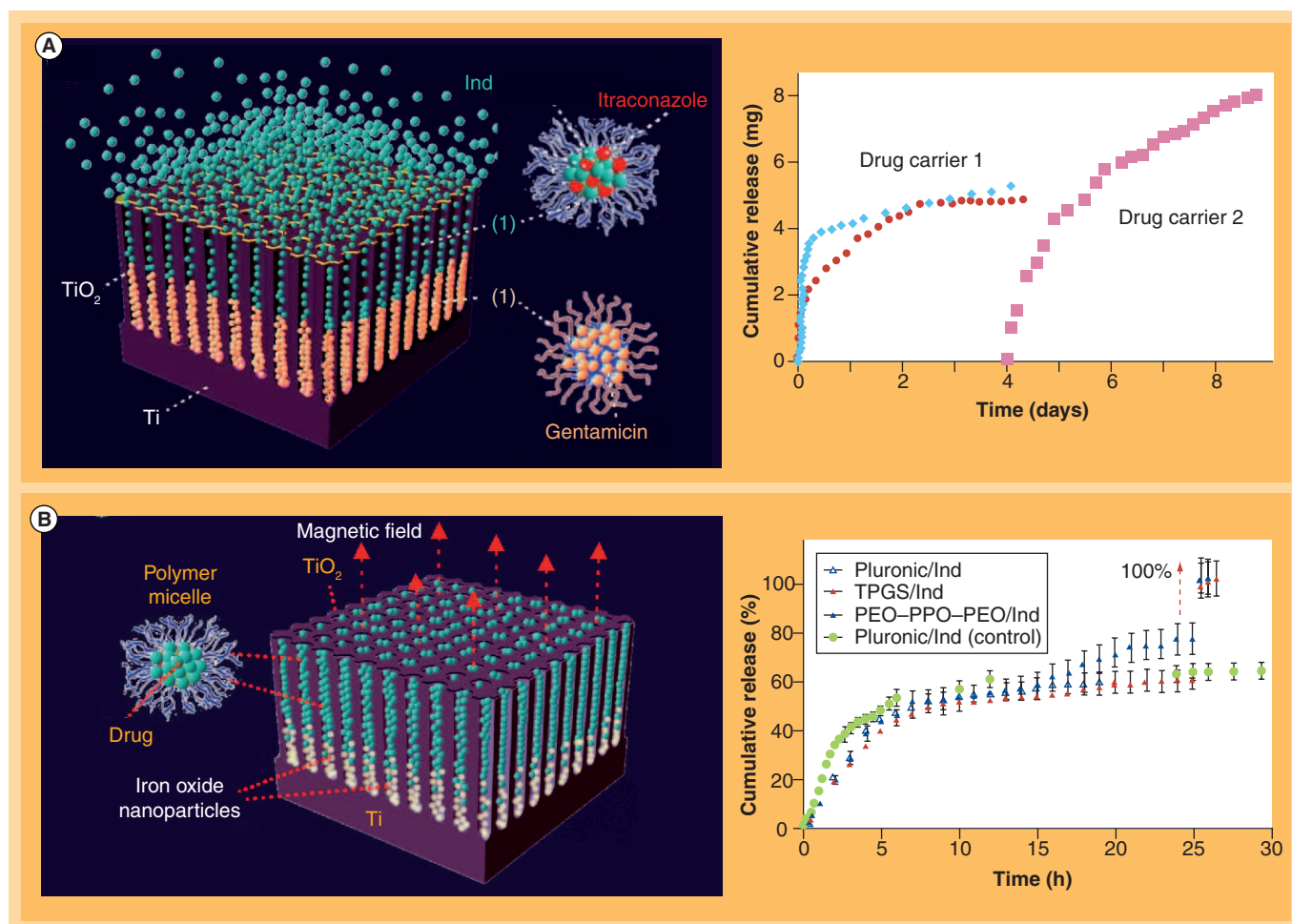


Figure 4. Titania nanotubes with multidrug delivery and stimuli-responsive release. (A) Multidrug release using titania nanotube (TNT) arrays with polymer micelles as drug carriers. TNT arrays were loaded with two types of polymer micelles, a regular micelle (TPGS) encapsulated with two hydrophobic anti-inflammatory drugs (indomethacin and itraconazole) and an inverted micelle (DGP 2000) encapsulated with a hydrophilic antibiotic (gentamicin). The drug-release graph shows sequential and multiple release of drug carriers loaded with three drugs, showing the first release from the top layer with indomethacin and itraconazole followed by the second release after 5 days from the gentamicin. **(B)** Magnetic stimuli-responsive drug release from TNT arrays, which integrates polymer micelles as drug carriers incorporated with poorly soluble drugs and magnetic nanoparticles loaded on the bottom of nanotubular structures. The graph shows the release profile of magnetic field-triggered release of drug (indomethacin) encapsulated polymer micelles (TPGS, Pluronic® and PEO-PPO-PEO) 1 day after loading. Nontriggered release with Pluronic-indo was used as a control.

Ind: Indomethacin; PEO: Poly(ethylene) oxide; PPO: Poly(propylene) oxide; TPGS: D- α -tocopheryl polyethylene glycol succinate 1000.

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(B) Reproduced with permission from [106,202] © The Royal Society of Chemistry (2012).

are excellent drug-releasing platforms to design stimulus-responsive drug-delivery system.

Shrestha *et al.* reported the first external-field drug release from titania nanotubes, which takes advantages of the fact that long molecules attached to TNT surface can be released photocatalytically [105]. Such tubes can be loaded with drug molecules that are attached by suitable linker molecules. Drug release is not limited to UV reactions but also can be triggered by x-rays or by electrically (voltage induced) catalysis. Fluorescence microscopy images demonstrated that UV-induced photocatalytic activity of the

magnetic TNT nanotubes may be used to kill cancer cells.

A more practical strategy for triggered drug release from TNT arrays using magnetic NPs was presented by our group [106]. Magnetic NPs made of iron oxide with a size of 18 nm, were used as the triggering device under an induced magnetic field to release polymer micelles loaded with drug. Dopamine was linked to magnetite iron(II,III) oxide NPs ($\text{DOPA}/\text{Fe}_2\text{O}_3/\text{Fe}_3\text{O}_4$), as it forms a robust cationic anchor on their surface and its primary amine can be used as the reaction site to covalently immobilize biomolecules of

interest [107]. Indomethacin was encapsulated in the lipophilic cores of micelles. The preparation was carried out in such a way that the magnetite NPs was loaded first at the bottom of the TNT arrays, followed by drug nanocarrier loading. The drug is thus released upon the induction of magnetic field, through the DOPA-modified magnetite NPs (**FIGURE 4B**). This approach can be applied for several situations where urgent therapeutics are required, such as for the bone marrow system to validate tumor responses [108], and for bone diseases such as septic arthritis or prosthetic joint infection, which necessitate early attention to prevent irreversible cartilage destruction [109]. Moreover, osteomyelitis often complicates diabetic foot infection with ulceration and is rarely cured by antibiotics alone; early surgical intervention, for instance, with implantable devices coupled with stimulated drug delivery may achieve a better outcome.

These 'smart' and strategic systems have not yet been implemented for orthopedics but may well prove beneficial for treating bone diseases and stimulating new bone growth. Another recent attempt towards this approach is incorporating drugs into multiwalled carbon nanotubes (MWCNTs) grown out of TNT and controlling drug release from these tubes through an electrical field [109]. Yang *et al.* have suggested that multiple deliveries in a single TNT system could be an optimal solution for treating bone diseases and regulating tissue generation at various stages of bone repair [109]. Another potential advance is suggested by a study by Sirvisoot *et al.*, which demonstrated polypyrrole doped separately with antibiotics (penicillin and streptomycin) and an anti-inflammatory drug (dexamethasone) electrodeposited on a substrate having MWCNTs grown out of TNT arrays [110,111]. It was observed that approximately 80% of the drug was released when an external voltage was applied, thereby acting like a trigger-enabled drug-eluting bone implant. Although such an 'on-demand drug-delivery' technology is in its infancy, it has a huge potential for versatile developments in drug delivery using TNT arrays.

■ Drug delivery with biosensing function

Further advances in the implant technology would require an in-built sensing functionality that would aid in monitoring the implant integration and bone condition. As compared with conventional radiology techniques, a sensing implant would give a more accurate and live

feedback as it will be present inside the environment we would like to examine. This will be beneficial to study the commonly encountered bone infection or inflammation associated with orthopedic implants. Sirvisoot *et al.* have shown that MWCNTs grown out of TNT arrays enhance osteoblast functions and aid in new bone formation [112]. They suggested electrochemical sensing applications to monitor bone formation by fabricating MWCNTs on TNT/Ti based on the ability of MWCNT to enhance direct electron transfer. The implant system was proposed to sense components of osteoblast extracellular matrix by detecting their redox reaction profiles. These results suggest that a MWCNT modified Ti implant surface can serve as an electrochemical electrode to monitor infection, inflammation or bone growth from the implant surface. In the future, this technology can prove tremendously beneficial in implant-related diagnostics.

■ Fabrication of TNT array implants

Most drug-delivery studies using TNT arrays have been performed on TNT arrays generated on commercial Ti foils or Ti plates used as model substrates. However, it should be pointed that an oxide layer with nanotube structures is possible to prepare on clinically used orthopedic implants, which are made from Ti, Ti alloys or other materials (Al, Ta, Hf, W and stainless steel) [36,37]. Because of the self-organizing nature of TNT arrays formation, even complex-shaped surfaces, such as dental implant surfaces or hip implants can be coated with TNT layers. It includes different shapes such as plates, needles, screws and pins commonly used in bone-correction surgeries. Surprisingly, there are no reports on the fabrications of TNTs on these clinically proven orthopedic implants and the study of their practical applications. Instead of using orthopedic implants our group recently demonstrated the fabrication of TNT arrays on Ti wire (0.25-mm and on 0.75-mm diameters), which was proposed as a simple implant for surgical-free implantation [85,113]. These prepared wire implants with TNT layers were loaded with the antibacterial drug gentamicin and a drug release of over more than 10 days was achieved. The TNT/Ti wire implants could replace Kirschner wires (stainless steel pins or K-wires) for fixing wrist fractures or hand injuries in order to reduce bacterial infection (or 'pin-tract infection'), which is frequently observed in the case of K-wires [114]. A more significant application is to insert this drug-releasing implant directly inside

a bone to treat other bone diseases, including primary bone cancer (osteosarcoma) [113].

Future perspective

In the last decade there has been tremendous progress in the development of new and advanced drug-delivery systems and devices based on multidisciplinary approaches that combines nanotechnology, materials science, biomedical engineering and medicine. This review presents the recent progress on the various applications of TNT arrays generated by electrochemical anodization on Ti surface for local drug-delivery applications. **This new and remarkable nano-material** was shown to have many advantageous and favorable characteristics, including excellent chemical, mechanical and osseointegration properties, as well as biocompatibility, high surface area and controllable nanotube dimensions that can mimic the dimensions of constituent components of natural bone that is inexpensive to make and easy to integrate into existing implant technology. These properties are specifically suited for drug delivery in bone and the development of new drug-releasing implants or devices to address existing problems of systematic drug delivery and the failure of bone implants. Therefore it is not surprising that research on the applications of TNT arrays as drug-releasing therapeutic implant devices has been considerably increasing in the past several years.

Although still in the early stages, a number of *in vitro* studies clearly shows the potential of TNT arrays for drug-delivery applications, which include numerous examples from anti-inflammatory drugs, antibiotics, proteins and growth factors that are important to address the most critical problems of orthopedic implants (inflammation,

infection and poor osseointegration). These studies have suggested that TNT implants are able to release therapeutics locally for extended periods, which makes this technology potentially applicable for promoting new bone growth, eliminating bone infection and treating local inflammation or even treatment of skeletal malignancies. Unfortunately only a limited number of *in vivo* studies have been carried out, which confirmed biocompatibility/therapeutic ability of TNT arrays; these studies are required in the future before this technology moves into clinical trial stage.

Future research regarding the development of TNT arrays as drug-releasing implants potentially has multiple directions that include:

- Increasing biocompatibility and therapeutic ability by chemical immobilization of drug molecules on implant surfaces; for example, antibacterial and anti-inflammatory drugs;
- Achieving controlled and sustained drug release over a longer period of the time (>4 months) using specific surface modifications, structural design of pores and combining biodegradable polymers;
- New developments on external stimuli-responsive drug-delivery systems with the capability to release specific drug when required;
- Development of 'smart' drug-releasing systems with multifunctional properties and biosensing capability;
- Implementation of TNT arrays into micro-fabrication devices for creating chip-based drug-delivery platforms;
- Extensive *ex vivo* and *in vivo* studies before moving into clinical trials.

Executive summary

Titania nanotube arrays: fabrication, structures & properties

- The fabrication methods of titania nanotube (TNT) arrays have been considerably improved in recent years.
- The TNT arrays with controllable nanotube dimensions and geometries were fabricated.

Biocompatibility

- TNT arrays have been proven as a biocompatible material suitable for local drug delivery and bone-therapy applications.

Drug delivery applications of TNT arrays

- Successful loading and delivery of anti-inflammatory drugs, antibiotics, proteins and growth factors have been reported.
- Drug-releasing characteristics for controlled and extended drug release were improved by polymer coating and using drug carriers.
- Several new concepts including multidrug delivery, delayed and sequential release and stimulus-controlled release were demonstrated.

Future perspective

- Considerable potential for the development of a new generation of drug-releasing implant, to revolutionize existing bone-implant technology.
- More *ex vivo* and *in vivo* studies using animal models are required in the near future before clinical investigation.

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