Nanobiomaterials in Clinical Dentistry, Second Edition, shows how a variety of nanomaterials are being used to solve problems in clinical dentistry today. New nanomaterials are leading to a range of emerging dental treatments that utilize more biomimetic materials that more closely duplicate natural tooth structure (or bone, in the case of implants). The chapters discuss the advantages and challenges of using a variety of nanomaterials, and also include case studies to illustrate how a variety of materials are best used. This makes for a detailed examination of how nanobiomaterials are used in clinical dentistry that will be a good guide to researchers and practitioners alike.

This book brings together an international team of experts from the fields of materials science, nanotechnology, and dentistry, to explain these new materials and their applications for the restoration, fixation, replacement, or regeneration of hard and soft tissues in and about the oral cavity and craniofacial region.

The main topics covered include applications in dental specialties (orthodontics, endodontics, pediatric dentistry, periodontics, prosthodontics, and implant dentistry), salivary diagnostics using bioMEMS/NEMS systems, nanochips for oral cancer diagnosis, biomimetic nanomaterials, and nanotechnology for tooth repair and regeneration. For this second, expanded edition, each chapter is substantially updated with recent advances in nanomaterials. New chapters, including chapters focusing on the use of chlorhexidine nanoparticles as a coating on orthodontic elastomers to prevent white spot lesions and advances in stem cell therapy for dental applications, have also been added.

Features of This Book
• Prepared by an interdisciplinary and international group of scientists and practitioners in the fields of nanomaterials, dental implants, medical devices, and clinical practice
• Comprehensive professional reference for the subject covering materials fabrication and use of materials for all major diagnostic and therapeutic dental applications—repair, restoration, regeneration, implants, and prevention
• Complements the editors’ previous book on nanotechnology applications for dentistry

About the Editor
Karthikeyan Subramani is an assistant professor of dental medicine at Roseman University of Health Sciences, United States. He was the recipient of the prestigious 2006 Andre Schroeder Research Prize from Straumann (Switzerland) for his innovative research findings during his biomedical nanotechnology degree program in the United Kingdom. Dr. Subramani was involved in the International Team for Implantology (ITI) funded research projects in Switzerland, the Netherlands, and in the United States. He has authored numerous peer-reviewed research papers and review manuscripts, and has authored numerous book chapters.

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Nanobiomaterials in Clinical Dentistry

Second Edition

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Bioactive inorganic-ion-doped titania nanotube coatings on bone implants with enhanced osteogenic activity and antibacterial properties

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17.1 INTRODUCTION

Osteoporosis and bone fracture have become a worldwide problem with an aging global population and the increase in average life expectancy. New artificial implantable devices with enhanced mechanical and biological performance are required in clinical surgery. Titanium (Ti) and its alloys are widely used as bone implant materials and are expected to be the next generation of artificial orthopedic biomaterials due to their high strength, low modulus close to bone (4—30 GPa), good biocompatibility, and high corrosion resistance [1]. However, the natural titanium oxide film formed on the titanium surface makes it bio-inert and difficult to bond with bone tissues. Furthermore, bacterial infection occurs occasionally even in sterile circumstances. Implant failures with undesirable bone integration, poor bone quality, or infection have been observed in clinics.

To improve the osseointegration of Ti implants, much attention has been focused on the modification of implant surfaces. Considering that bone tissue is a complex system with nano-/microcollagen fibers and nutrient chemical ions, it is an effective way to develop nano-/microtopographical features doped with inorganic ions on Ti surfaces for enhancing the bioactivity of Ti implants. Hence, titania nanotubes (NTs) with controllable nanoscale properties have been developed on the Ti surface by anodization. The nanotubular topography not only mimics the dimension of collagen fibril in bones to some extent [2,3], but also is an
excellent drug-delivery platform, especially for inorganic ions [4]. Firstly, inorganic ions are much smaller molecules than growth factors and antibiotics and function at very low doses. Long-lasting activity can be achieved by increasing the loaded amounts and controlling the release rate appropriately. Secondly, these agents are stable due to their inorganic nature, thereby facilitating the use of loading processes and loading methods that tend to have harsh conditions. Thirdly, the stable properties of the agents may also permit relatively long storage after fabrication of the implants and it is important for commercial adoption.

Many inorganic ions involved in bone metabolism, such as Sr, Zn, Mg, and Ca, have been doped into NTs. The coatings of inorganic-ion-doped NTs have been found to foster the growth of nano-structured hydroxyapatite in simulated body fluids [5,6], enhance extracellular matrix secretion, mineralization, and other functions of osteoblasts, and even induce the commitment of mesenchymal stem cells (MSCs) toward bone lineage in the absence of extraosteoegenic supplements (OS) [7,8]. Emerging in vivo evidence also suggests the ability of NT coatings to enhance osseointegration [9–12].

Implant-associated infection, which is another issue impairing the normal function of bone implants, is usually difficult to treat and sometimes requires implant removal and repeated revision surgeries [13]. Various means, such as thorough sterilization and stringent aseptic surgical protocols, have been proposed to mitigate bacterial contamination. However, bacterial invasion usually occurs after surgery and complications can arise from infection of nearby tissues or a hematogenous source at a later time. Infections associated with bone implants are characterized by bacterial colonization and biofilm formation on the implanted device and infection of the adjacent tissues (peri-implantitis). Bacteria in the biofilm are far more resistant to antibiotics, resulting in persistent infection despite aggressive antibiotic therapy [14]. As emerging antibiotic resistance becomes more challenging, developing novel implants or surface modification methods with dual functions of excellent bone bonding ability and long-lasting antibacterial ability through a procedure ready for industrial production and clinical application is the need of the hour in implant dentistry. Silver ions or nanoparticles are good antibacterial agents and have been doped into NTs. The antibacterial ability in vitro and in vivo of silver-doped NT coatings has been thoroughly studied [15,16].

In this chapter, we summarize the latest progress on coatings of inorganic-ion-doped TiO$_2$ NTs and review the fabrication methods, the effects of the coatings on bone cell functions in vitro, osseointegration in vivo, and antibacterial ability.

### 17.2 Fabrication of Inorganic-Ion-Doped TiO$_2$ Nanotubes

Inorganic-ion-doped TiO$_2$ NTs are commonly fabricated through electronic anodization and following a hydrothermal treatment process. The fabrication methods and mechanisms are detailed as follows.
17.2.1 **ANODIZATION FOR DEVELOPING TIO$_2$ NANOTUBES ON TI**

Uniform and highly ordered NT arrays can be readily fabricated by anodization of a Ti foil in F$^-$_containing electrolytes. The as-anodized NTs, which grow in situ on the Ti substrate, are highly oriented perpendicular to the Ti surface [17]. The NTs are generally fabricated in the aqueous hydrofluoric acid electrolyte in a two-electrode electrochemical cell at a constant potential between 5 and 35 V [5,18,19]. At a low anodization voltage of 5 V, the morphology of the anodized film is sponge-like, with a typical pore size of 25 nm. At 20 V, hollow and cylindrical tube-like features with an inner diameter of 80 nm form (Fig. 17.1). Besides the HF/H$_2$O electrolyte, many other aqueous electrolytes have also been developed to fabricate NTs, for example, H$_2$SO$_4$/NaF/H$_2$O [3], NaH$_2$PO$_4$/HF [6], and Na$_2$SO$_4$/HF [6]. The diameter of the NTs can be regulated to vary from 15 to 140 nm and the length can range from 200 to 1000 nm by adjusting the electrolyte content and anodization voltage [17]. When using polar organic electrolytes, much longer NTs of hundreds of micrometers can be fabricated [20]. Ethylene glycol (EG) is the most commonly used organic solvent to fabricate NTs. The typical NTs formed by anodization of a titanium foil in an EG solution with 0.5 wt.% NH$_4$F, 5 vol.% CH$_3$OH, and 5 vol.% H$_2$O at 10 V for 1 hour and 40 V

![Figure 17.1](image)

(A) and (B) NTs formed at 5 and 20 V in 0.5 wt.% aqueous hydrofluoric acid solution with tube diameters of 25 and 80 nm, respectively. (C) and (D), NTs formed at 10 and 40 V in an EG solution with 0.5 wt.% NH$_4$F, 5 vol.% CH$_3$OH, and 5 vol.% H$_2$O with tube diameters of 30 and 80 nm, respectively. *NT*, Nanotube; *EG*, ethylene glycol.

(A) and (B) Reprinted with permission from L.Z. Zhao, S.L. Mei, W. Wang, P.K. Chu, Z.F. Wu, Y.M. Zhang, The role of sterilization in the cytocompatibility of titania nanotubes, Biomaterials 31 (2010) 2055–2063 [18].
for 40 minutes are shown in Fig. 17.1C and D, respectively. The two NTs with diameters of 30 and 80 nm have been used to incorporate various inorganic ions [21,22].

17.2.2 HYDROTHERMAL TREATMENT FOR DOPING INORGANIC IONS

Many divalent inorganic ions (such as Sr, Zn, Ba, etc.) are incorporated into crystalline/amorphous TiO$_2$ NTs through a hydrothermal treatment method [9,11,23]. The composites exist eventually in the form of perovskite-type titanates MTiO$_3$ (M = Sr, Zn, Ba, etc.). In the hydrothermal process, pH in the solution is demonstrated to play important roles in the morphology and composition of the hydrothermal products [24]. Fig. 17.2 shows the morphology changes of amorphous TiO$_2$ NTs after hydrothermal treatment at 200°C for 6 hours in solutions with different pH. In HCl solution (pH = 3), nanorods are composed of compact nanoparticles with a large diameter the same as previously formed NTs. In DI water (pH = 6.5), some small NPs with a diameter of 40 nm are formed in nanorods. By further increasing the pH to 11, smaller NPs of 20–30 nm in diameter are

**FIGURE 17.2**

Top and cross-sectional FE-SEM micrographs of amorphous TiO$_2$ NTs after hydrothermal treatment at 200 °C for 6 h in water with different pH values: (A) pH = 3, (B) pH = 6.5, and (C) pH = 11. (D) The corresponding XRD pattern of (B). (E) and (F) TEM and HR-TEM images of an NR and NP in (B). The pH value is adjusted with 1 M HCl and 1 M NaOH and the inset scale bar is 500 nm. NT, nanotube.

observed on the surface. At higher pH (pH = 12.2, 0.02 M NaOH), the nanorods are dissolved and nanosheets begin to form.

The principle of perovskite-type titanates MTiO$_3$ formation with the hydrothermal reaction involves a hydroxy radical-induced dissolution and recrystallization with a combination of metal ions. The amorphous TiO$_2$ NTs are not stable in water and the unstable TiO$_6^{2-}$ absorb water molecules via the surface hydroxyl groups to form soluble species of Ti(OH)$_6^{2-}$, which can be further dehydrated and precipitated to form crystal anatase TiO$_2$ nanoparticles or MTiO$_3$ nanoparticles when M$^{2+}$ exists. The overall reaction is described as follows [25]:

$$\text{TiO}_x + 4\text{H}_2\text{O} + \left(1 - \frac{x}{2}\right)\text{O}_2 \rightarrow \text{Ti(OH)}_6^{2-} + 2\text{H}^+ (1 < x < 2)$$  \hspace{1cm} (17.1)

$$\text{Ti(OH)}_6^{2-} + 2\text{H}^+ \rightarrow \text{TiO}_2 \downarrow + \text{H}_2\text{O}$$  \hspace{1cm} (17.2)

or

$$\text{Ti(OH)}_6^{2-} + \text{M}^{2+} \rightarrow \text{MTiO}_3 \downarrow + 3\text{H}_2\text{O}$$  \hspace{1cm} (17.3)

where M is the metal source. In order to form MTiO$_3$ precipitates, process (17.2) should be controlled, as reactions (17.2) and (17.3) are competitive processes. Zhang et al. propose acetates of metals as reaction precursors and fabricated ZnTiO$_3$, CoTiO$_3$, and NiTiO$_3$ NTs by hydrothermal treatment [24]. In the reaction process, weak acid radicals supplied in these solutions consume H$^+$ and restricted process (17.2). Therefore, process (17.3) is prompted for facilitating the production of MTiO$_3$ precipitates.

17.3 OSTEOINDUCTIVE ACTIVITY OF INORGANIC-ION-DOPED TiO$_2$ NTS

17.3.1 STRONTIUM-DOPED TiO$_2$ NANOTUBES

Strontium (Sr), of which 98% in the human body can be found in the skeleton, plays an important role in bone metabolism [26]. Strontium ranelate is a clinical drug for treating and preventing osteoporosis in postmenopausal women [27]. Many experimental studies in vitro and in vivo have proved that strontium ranelate can stimulate osteoblast differentiation, inhibit osteoclast formation, and rebalance differentiation of bone marrow MSCs [28–31]. Therefore, strontium has been widely incorporated into bone and dental implants, including bioceramics, cement, and metals, to improve the osteoinductive activity of scaffolds [32–34].

As mentioned in the Section 17.1, TiO$_2$ NTs are ideal carriers for inorganic ions. Huo and his cooperators have fabricated a series of Sr-doped TiO$_2$ NT surfaces and explored their bioactivity and osteointegration ability [12,22]. By controlling voltage and anodization time in the anodization process and the
concentration of Sr(OH)$_2$ in the hydrothermal process, NTs with diameters of 30 nm (NT10) and 80 nm (NT40) and different contents of Sr are fabricated (Fig. 17.3) [22]. Initial adherent cell numbers and cell cytotoxicity characterized by lactate dehydrogenase on different surfaces show no significant difference. This demonstrates that Sr incorporation has no obvious influence on cell adhesion and cell cytotoxicity. NT40 and NT40-Sr induce more protein deposition but the proteins distribute unevenly compared to NT10 series. This is because the top ends of the NT wall of NT10 and NT10-Sr are smoother than in the NT40 series. Cell morphology and cell migration are closely related with surface topography. It is demonstrated that a distance smaller than 50–70 nm between two neighboring integrins is favorable for integrin clustering and focal adhesion formation for further cell spreading or migration [35–37]. Therefore, cell motility and cell spreading on NT40-Sr are both better than NT40 due to the proper size of nanotopography improved by protein distribution (Fig. 17.4). The influence of NT
surfaces on osteogenic differentiation of MSCs is a most important indicator to evaluate the bioactivity of NTs. The mRNA expression levels of osteogenic-related genes of MSCs on NT-Sr surfaces, including the runt-related transcription factor 2 (RUNX2, a key transcript factor for bone formation), alkaline phosphatase (ALP, an early marker for osteogenic differentiation), osteocalcin (OCN, a late marker for osteogenic differentiation), type I collagen (Col-I, main content of bone ECM), and bone morphogenic protein 2 (BMP-2), are significantly upregulated in the absence of osteogenic serum. The ALP staining and extracellular matrix mineralization are also detected to evaluate the osteogenic differentiation of MSCs (Fig. 17.5). Sr-doped NTs show more ALP staining and calcium nodules indicating stronger osteogenic differentiation of MSCs than NTs.

### 17.3.2 Zinc-Doped TiO$_2$ Nanotubes

Zinc is an essential trace element in the human body and its weight is about 1.4–2.3 g on the biochemical level [26]. The zinc content of bone is about...
0.015–0.025 wt.% of the total amount, which is relatively high compared to other tissues. Zinc has been known to play important roles in many physiological behaviors, including normal growth, immune functions, and neurodevelopment. Zinc is involved in various cellular signaling pathways through 1400 zinc-finger proteins accounting for a large part of the transcription regulatory proteins [38]. In bone, zinc participates in bone matrix calcification, stimulates bone apposition by osteoblasts, and inhibits osteoclastic resorption [39–41]. In addition, zinc possesses effective antibacterial ability [42,43]. Considering the dual functions of zinc, it has been incorporated into various bone implants to enhance the osteogenic activity and antibacterial ability of implants [44].

Zinc-doped TiO₂ NTs with diameters of 30 nm (NT10-Zn) and 80 nm (NT40-Zn) have been fabricated and their osteogenic and antibacterial function have been detected by Huo et al. [21]. The loading amount of zinc on NTs in the reports ranges from 1.2 to 60.2 μg/cm², which is safe for the human body. As excessive zinc uptake brings risks of anemia, leucopenia, and neutropenia [45], and so the total loading amount is an important factor to be considered. Antibacterial ability of NT-Zn samples toward adherent bacteria and planktonic bacteria are shown in Fig. 17.6. The NT-Zn samples show higher antibacterial
efficiency compared to NTs with the general trend of NT40-Zn3 > NT10-Zn3 > NT40-Zn1 > NT10-Zn1. The antibacterial ability of NT-Zn samples is related to the loading and release contents of Zn. The antibacterial ability of NT-Zn samples decreases gradually with time and Zn loading content, which is the same as the trend of Zn release kinetics. The antibacterial mechanism of Zn is considered to be related to the production of reactive oxygen species [44].

Besides antibacterial ability, the osteogenic abilities of NT-Zn samples have also been investigated. In the absence of exogenous osteoinductive serum, most NT-Zn samples enhance the osteogenic differentiation of MSCs, characterized with more dense nodular ALP areas and higher ECM mineralized nodule formation [21]. MC3T3-E1 mouse preosteoblasts are also used to detect the osteogenic ability of NT-Zn samples [9]. Except the above characterization, the expression levels of osteogenesis-related genes, including ALP, OCN, OPG, and Col-1, were estimated by qRT-PCR assay. NT-Zn samples exhibit higher mRNA levels for all osteogenic-related genes (Fig. 17.7).

ERK1/2 signaling is believed to play an important role in the osteogenic differentiation of MSCs and found to be modulated by different biomaterials [46]. NT-Zn samples show a higher ERK expression degree compared to NT samples (Fig. 17.8). The biological effects of NT-Zn samples are ascribed to the combined effects of the topographical cues and Zn release. As discussed in the above section on NT-Sr, the NTs within the proper range of diameters facilitate the formation of focal adhesions. Evidence collected from other tissues and cells indicates that Zn can initiate ERK1/2 signaling [47], even though there is still no evidence from MSCs.

In vivo experiment for evaluating the osteointegration of NT-Zn samples fabricated by anodization at 40 V for 40 minutes and hydrothermal treatment for 1 or
3 hours has been performed by Li et al. [9]. Ti samples are inserted into the tibiae of Sprague–Dawley rats aged 1 month. Four weeks later, the rats are sacrificed for micro-CT evaluation. The transverse 3D images shown in Fig. 17.9 illustrate that more trabeculae are observed around the NT implants, especially around the zinc-loaded materials. The quantitative analysis of micro-CT shows that all the zinc-loaded implants resulted in increased BV/TV, Tb. N, and Conn.D, and decreased Tb. Sp compared with TiO2-NTs (Table 17.1). Unlike in vitro cellular experiments, the differences between various Zn loading capacities are not statistically significant. The authors test the biomechanical ability through a push-out test. NT-Zn implants exhibit significantly increased strength of fixation compared to Ti and TiO2-NT (Table 17.2), indicating better osteointegration ability than Ti and TiO2-NT.

![Figure 17.7](image)

**FIGURE 17.7**
Relative expressions of (a) ALP, (b) OCN, (c) OPG, and (d) Col-1 by MC3T3-E1 cells cultured on different substrates for 2 weeks, all values normalized to β-ACTIN. *P < .05, **P < .01 compared with Ti, #P < .05, ##P < .01 compared with TiO2-NTs, &P < .05 compared with TiO2-Zn1h. The data are expressed as mean ± SD (n = 4). ALP, Alkaline phosphatase, OCN, osteocalcin.

FIGURE 17.8
Western blot of pERK1/2 and ERK1/2 levels in the MSCs cultured on the different samples for 48 h. β-Actin is used as a control for equal loading. MSC, Mesenchymal stem cell.

FIGURE 17.9
Micro-CT transverse 3D images of the proximal tibiae with implants after 4 weeks.
17.4 ANTIBACTERIAL ACTIVITY OF SILVER-DOPED TiO$_2$ NANOTUBES

Silver (Ag) has attracted a lot of attention as one of the strongest bactericides. Silver shows many advantages compared to traditional antibiotics, such as broad antibacterial spectrum, effective antibacterial activity to most bacteria including antibiotic-resistant bacteria, noncytotoxicity at low doses, and low risk of producing resistant strains [48–50].

Silver nanoparticles have been controllably incorporated into NTs and their antibacterial capability has been investigated [51–53]. The NT-Ag surfaces show excellent antibacterial ability for both planktonic and adhesive Staphylococcus aureus bacteria throughout 30 days, which effectively prevents biofilm formation (Figs. 17.10 and 17.11) [51].

### Table 17.1 Quantitative Results of the Femora With Implants by Micro-CT Within Volume of Interest 4 Weeks After Implantation

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ti</td>
</tr>
<tr>
<td>BV/TV (%)</td>
<td>13.31 ± 2.74</td>
</tr>
<tr>
<td>Tb. N (mm$^{-1}$)</td>
<td>1.63 ± 0.21</td>
</tr>
<tr>
<td>Tb. Th (μm)</td>
<td>70.34 ± 6.04</td>
</tr>
<tr>
<td>Tb. Sp (mm)</td>
<td>0.93 ± 0.13</td>
</tr>
<tr>
<td>Conn.D. (mm$^{-3}$)</td>
<td>23.71 ± 2.20</td>
</tr>
</tbody>
</table>

BV/TV, Ratio of bone tissue volume to total tissue volume; Tb. N, the mean trabecular number; Tb. Th, the mean trabecular thickness; Tb. Sp, the mean trabecular separation; Conn.D, the mean connectivity density.

*P < .05 and **P < .01 compared with Ti, #P < .05 compared with TiO$_2$-NTs. The data are expressed as mean ± SD (n = 16).


### Table 17.2 Results of the Biomechanical Push-Out Test 12 Weeks After Implantation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ti</td>
</tr>
<tr>
<td>Maximal push-out force (N)</td>
<td>21.5 ± 3.2</td>
</tr>
</tbody>
</table>

*P < .05 and **P < .01 compared with Ti, #P < .05 compared with TiO$_2$–NTs. The data are expressed as mean ± SD (n = 16).

In addition, Ag$_2$O nanoparticles have also been doped into NTs through TiAg magnetron sputtering and anodization [54]. The surfaces exhibit long-term antibacterial activity against both *S. aureus* and *Escherichia coli* up to 28 days and show no significant difference in osteogenic induction for MC3T3-E1 cells after 14 days coculture (Fig. 17.12).

The antibacterial activity of NT-Ag surfaces has also been detected in vivo through an infected rat model by Cheng et al. [16]. Bacteria are injected into rats and NT-Ag-coated Ti implants are inserted simultaneously. X-ray examination, histological analysis, and immunohistochemistry (Fig. 17.13) are used to analyze the infection degree in rats. Histological analysis (H&E staining) shows that Ti and NT implants present acute pyogenic infection with an abundant neutrophilic exudate (white arrow) at 2 weeks, chronic inflammatory cell infiltration (black

**FIGURE 17.10**

(A) Noncumulative silver release profiles from NT-Ag into PBS, (B) antibacterial rates against planktonic bacteria in the medium (Rp), and (C) antibacterial rates against adherent bacteria on the specimen (Ra). The antibacterial assays data are expressed as means ± standard deviations (*n* = 3). One-way ANOVA followed by SNK post hoc test is utilized to determine the level of significance. *P*<.05 and **P**<.01.

arrow) at 3 weeks, and intramedullary necrosis including abscess and osteonecrosis (red arrow) at 4 weeks postsurgery. In contrast, an NT-Ag implant at 2 weeks displays a small amount of neutrophilic exudate, which is a normal inflammatory response because of implantation of the metal rod, and no neutrophilic exudate can be observed at 3 and 4 weeks. Immunohistochemical analysis proves the existence of bacteria in Ti and NTs at 2, 3, and 4 weeks, respectively, but no bacterial survival in NT-Ag group.

Silver ion release is believed to be a key factor for controlling the duration time of antibacterial activity. Polymers are good adhesives for holding Ag nanoparticles and mitigate the release rate of silver ion. Polydopamine, known as a reductive agent for noble metals and self-polymerization ability, has been applied to fabricate a composite film of PDA/Ag [55,56]. The film shows a relatively slower release rate and a longer-term antibacterial ability towards *E. coli* and *S. aureus* than NT-Ag surfaces. In addition, the antibacterial activity of PDA/Ag under visible light is higher than that in the dark [56].

The in vitro cell culture assay and in vivo assay have tested the bioactivity of NT-Ag surfaces [57]. NT-Ag samples are incubated with epithelial cells and fibroblasts to assess the degree of fibrosis. Cells on NT-Ag surfaces show spheri-cal shape, exhibit dense filopodia, and secrete abundant extracellular matrix,

**FIGURE 17.11**
Representative images showing viability of the bacteria on samples after 7 days of incubation displayed by acridine orange and ethidium bromide fluorescence staining. The live bacteria appear green, while dead bacteria are orange.

indicating that NT-Ag surfaces are inclined to fibrosis formation (Fig. 17.14). The in vivo assays are performed to evaluate foreign-body reactions. As shown in Fig. 17.15A, the hematoxylin and eosin staining results show that the reactive capsule thickness of different samples accord with the trend of PT > NT-Ag-0.5 > NT ≈ NT-Ag-1.0. Meanwhile, the inflammation reaction indicated by cd68-positive macrophage cells shows the inflammatory trend of NT-Ag-0.5 > PT > NT ≈ NT-Ag-1.0 (Fig. 17.15B). The results imply that the nanotubular sample with high-dose superficial Ag is more inflammatory than PT in vivo.
FIGURE 17.13
Histological and IHC analysis of three group of rats contaminating different implants at 2, 3, and 4 weeks postsurgery. Yellow scale bar = 1000 μm.

A common view for the antibacterial effect of Ag nanoparticles to planktonic bacteria is the released Ag\(^+\) into medium. Silver ions can act as a bridging agent between thiols forming chains that lead to irreversible aggregation of thiol-bearing molecules and following denaturation of DNA or peptides [58]. ROS is another important pathway for the antibacterial activity of silver. Ag\(^+\) ions will disrupt the balance of ROS system, increase intracellular ROS concentration, and eventually cause cellular death [59]. This is the reason for enhanced antibacterial effect of PDA/Ag under visible light because more ROS are produced under visible light irradiation [56]. A recent research reveals that electron transfer between Ag nanoparticles and metallic substrate can increase intracellular oxidative stress,
FIGURE 17.15

In vivo biocompatibility of the samples: (A) Light micrographs of hematoxylin and eosin-stained soft tissues previously in contact with the samples at 12 days postimplantation noting the difference in thickness for the fibrous capsules elicited by various samples. Scale bars for insets = 50 mm. (B) Confocal micrographs of cd68-positive cells and counterstained cells from soft tissues previously in contact with the samples at 12 days postimplantation noting the abundance of cd68-positive cells from soft tissues previously in contact with PT and NT-Ag-0.5. Scale bars for insets = 50 mm. (C) Thickness of fibrous capsules elicited and the ratio of cd68-positive cells recruited by various samples. One-way ANOVA followed by SNK post hoc test is utilized to determine the level of significance, *P<.05, **P<.01.

which enhances the antibacterial activity of Ag nanoparticles [60]. Electron transfer take place when bacteria are in contact with electrically conductive materials and ROS is believed to be controlled and consumed sequentially in the electron transfer process. As shown in Fig. 17.16, in the Ag-NPs@Ti systems, the electrons produced by Ti may go to the intracellular part of the bacteria through the Ag-NPs to disturb the electron transfer process to induce abnormal intracellular production of ROS [60]. Furthermore, NT-Ag surfaces have been found to enhance the bactericidal efficiency of antibiotics against various bacteria, even methicillin-resistant S. aureus [15]. The enhancement of bactericidal activity is considered to derive from various synergistic effects of Ag and antibiotics (Fig. 17.17). Ag and vancomycin can both lyze the bacteria cell wall for penetration of bactericidal agents into the bacterium. The cell wall destruction caused by Ag effectively helps other antibiotics to enter the bacteria for further reaction with DNA. Gentamicin and Ag can both denature the 30 S ribosomal subunit of bacteria to stop protein translation for bacteria survival.

**FIGURE 17.16**

Generation of oxidative stress in the surrounding solution and within the bacteria.

There are various inorganic ions in bone tissue, therefore effective strategies incorporate dual or multi-ions to synergistically enhance osteointegration of Ti implants. Many trials have been performed to prove this view. Huang et al. fabricated Sr- and Si-loaded NTs (Si-Sr-NTs) through anodization and hydrothermal treatment in the solution of SiO₂ and Sr(OH)₂ \[11\]. The ALP production of MC3T3-E1 osteoblastic cells is higher on Si-Sr-NTs than NTs. It is worth noting that the Si-Sr-NTs exhibit better corrosion resistance than NT samples. Si-Sr-NT samples possess lower \( i_{corr} \) values in the Tafel polarization curves and larger capacitive loops in the Nyquist plots, suggesting that Si-Sr-NTs display superior corrosion resistance ability (Fig. 17.18).

**FIGURE 17.17**

The potential mechanism of synergistic effect between antibiotic and Ag: (1) lyzing the cell wall; (2) stimulating the penetration of nano-Ag into cell; (3) inactivating RNA polymerase; (4) denaturing 30 S ribosomal subunit; (5) preventing DNA unwinding; and (6) inactivating DNA gyrase.


### 17.5 DUAL FUNCTIONS OF MULTIION-DOPED TIO₂ NANOTUBES

There are various inorganic ions in bone tissue, therefore effective strategies incorporate dual or multi-ions to synergistically enhance osteointegration of Ti implants. Many trials have been performed to prove this view. Huang et al. fabricated Sr- and Si-loaded NTs (Si-Sr-NTs) through anodization and hydrothermal treatment in the solution of SiO₂ and Sr(OH)₂ \[11\]. The ALP production of MC3T3-E1 osteoblastic cells is higher on Si-Sr-NTs than NTs. It is worth noting that the Si-Sr-NTs exhibit better corrosion resistance than NT samples. Si-Sr-NT samples possess lower \( i_{corr} \) values in the Tafel polarization curves and larger capacitive loops in the Nyquist plots, suggesting that Si-Sr-NTs display superior corrosion resistance ability (Fig. 17.18).
Dual-functional NT surfaces on Ti implants with both antibacterial activity and osteoinductive ability also have been fabricated. Cheng et al. fabricated Sr- and Ag-loaded NTs (NT-Ag/Sr) and detected the antibacterial activity and osteoinductive ability in vitro and in vivo [10]. The SEM and ZOI test results shown in Fig. 17.19 show that the antibacterial ability of NT-Ag/Sr towards E. coli (ATCC25922) and S. aureus (ATCC25925 and ATCC43300) is better than Ti and NTs and the antibacterial ability increased with an increase of Ag loading content. The evaluation of bone regeneration has been performed in an osteoporotic animal model, namely ovariectomized rats. The qualitative micro-CT evaluation shown in Fig. 17.20 displayed the information on the peri-implant trabecular microstructure and the implant osteointegration. More trabecular microstructures are developed around NT implants, especially in Sr-loaded samples. NT-Ag/Sr samples display higher Tb. N, Conn. D, and BV/TV, and lower Tb. Sp than NTs, indicating better osteointegration of NT-Ag/Sr in vivo. Zhang et al. fabricated Sr/ZnO-loaded NTs grafted with octadecylphosphonic acid (OPDA)-toluene [61]. The surface exhibits multibiofunctions, including the osteoinductivity ascribing to Sr and self-antibacterial ability contributing to the synergistic effects of ZnO and hydrophobic OPDA.
Antiadhesive, antibacterial assay and zone of inhibition test. (A) The antiadhesive effect of the sample surface was verified by SEM. Shrinkage of bacteria (black arrows) can be detected on the four silver-containing samples; (B) the four silver-loaded samples present a visible inhibition zone on the Mueller–Hinton plates spread with the three bacterial strains. The TiO$_2$-NT samples and pure Ti samples showed no evidence of an inhibition zone. The diameter of the ZOI was around 1.8 cm for the NT40-Ag$_{2.0}$Sr$_3$, NT10-Ag$_{2.0}$Sr$_3$ samples and approximately equal to 1.7 cm for the NT10-Ag$_{1.5}$Sr$_3$, NT40-Ag$_{1.5}$Sr$_3$ samples on the Mueller–Hinton plates spread with ATCC25922. On the ATCC25923 Mueller–Hinton plates, the ZOI diameter was 1.9 cm for the NT40-Ag$_{2.0}$Sr$_3$, NT10-Ag$_{2.0}$Sr$_3$ samples and 1.6 cm for the NT10-Ag$_{1.5}$Sr$_3$ and NT40-Ag$_{1.5}$Sr$_3$ samples.

Reprinted with permission from H. Cheng, W. Xiong, Z. Fang, H. Guan, W. Wu, Y. Li, et al., Strontium (Sr) and silver (Ag) loaded nanotubular structures with combined osteoinductive and antimicrobial activities, Acta Biomater. 31 (2016) 388–400.
Micro-CT evaluation in a bone osteoporosis model (female rats, 6 weeks old). (A) Transverse 3D images of the implant osseointegration and peri-implant trabecular microstructure 5 mm below the tibial plateau; (B) compared with Ti, all the nanotube-coated implants showed increased BV/TV (a, the bone volume per total volume), Conn.D (b, the mean connective density), and Tb. N (d, the mean trabecular number), decreased Tb. Sp (e, the mean trabecular separation), and no obvious change in Tb. Th (c, the mean trabecular thickness). Compared with TiO2-NTs, the Sr-loaded implants resulted in increased BV/TV, Tb. N, and Conn. D and decreased Tb. Sp. The largest BV/TV, Tb. N, and Conn. D were observed for NT40-Ag1.5Sr3 and NT40-Ag2.0Sr3 rod samples, together with the smallest Tb. Sp. In comparison, the NT10-Ag1.5Sr3 and NT10-Ag2.0Sr3 rod samples showed a smaller increase in BV/TV, Tb. N, and Conn. D. *P<.05 vs Ti, #P<.05 vs TiO2-NTs, and ▲ P<.05 vs NT10-Ag2.0Sr3.

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17.6 CONCLUSIONS

The osteointegration ability and antibacterial ability of Ti implants are two major problems in clinical application. The NT surfaces, especially inorganic ion-doped NTs, have been demonstrated to effectively improve the bioactivity of Ti implants by delivering bioactive or antibacterial inorganic ions. These biofunctionalized surfaces with nanotopography have huge promise in fabricating orthopedic implants with better clinical performance.

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REFERENCES


