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ARTICLE

Antibacterial Activities of TiO_2 Nanotubes on Porphyromonas gingivalis

Cite this: DOI: 10.1039/xoxxooooox

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Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/xoxxooooox

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Titanium-based nanomaterials have been widely used as the dental implant for the advantages of antiseptic and nano-interface effects. Specially, their application as surface decontamination biocompatible materials attracts more and more attention. In this study, TiO_2 nanotube-based antibacterial system has been fabricated by anodic oxidation method and its morphology, crystalline phase and hydrophilic property were characterized. The effects of TiO_2 nanotubes on bacterial growth inhibition as well as bacteria cell fate were also investigated. The results indicated that TiO_2 nanotubes with mixed phases showed excellent antibacterial performance under Ultraviolet light irradiation and their antibacterial ability could be attributed to the oxidative stress induced by the TiO_2 nanotubes. The antibacterial performance of TiO_2 nanotube coating can be manipulated with the photocatalytic activity as well as the geometry characteristic. Our study firstly unravel that interaction with TiO_2 surface may increase the potential survive chance of *Porphyromonas gingivalis* when exposed to the antibacterial drug.

1. Introduction

Dental implant is one of the most important methods in treating missing teeth for its excellent stability and biocompatibility.[1,2] However, dental implant has low mechanical strength and may cause periimplantitis. Specially, the periimplantitis occur in the functional bone joint surface, leading to the inflammatory process and failure of transplantation. Thus, the adverse effects need to be considered in order to full achieve their application in biomedical fields. Several factors could attribute to periimplantitis. Firstly, the low biocompatibility of implant leads to little blood vessels existing at the implant and bone layer interface, which inhibit antibody reaching to the surface of the implant and eventually cause the reduced resistance around the local host.[3] In addition, microbial adhesions and their toxic products could lead to epithelial separation and form periodontal pocket when implant exposed to oral environment without the protection of the periodontal membrane and vascular plexus, resulting in loss of supporting bones and reducing the mechanical stability at bone-implant interface.[4] Besides, bacteria and other microorganisms could adhere to the implant surface, forming the bacteria plaque biofilm which disable the immune system and antibacterial agent [5-7] as well as leading to the failure of the implanting. Thus, to develop dental implant materials with enhanced biocompatibility and antibacterial performance is under urgent for full achieving their application in biomedical field.[7, 8]

Generally, the dental implant materials include: metals and alloys, ceramics materials, carbon materials, polymer materials, composite materials. [9] Compared with other implant materials, metal alloys such as ZrO_2, Ti and TiO_2 have been widely used in the field of stomatology and orthopaedics for their advantages of excellent corrosion resistance and appropriate mechanical properties. Among these alloys materials, TiO_2 nanomaterials attract great attention due to its great stability and biocompatibility. However, few articles investigate such functional nanomaterials potential to be used as dental implant, thus, to evaluate the potential application of TiO_2 as dental implant materials is under urgent need.[10,11] Because of the absence of antibacterial properties, titanium based materials need to be modified to achieve excellent surface decontamination activity. Nanotechnologies have been demonstrated as great methods to fabricate functional materials with specific properties include self-clean properties.[11-16] Specially, TiO_2 coated Titanium structures could reduce the bacteria adhesion and display excellent antibacterial property which could be attributed to the nanoscale surface roughness and high surface energy.[17-19] TiO_2 have ability to absorb the light which wavelength is below 400nm and generate electron (e^-) and hole (h^+) pair which can produce active hydroxyl free radical ($\cdot OH$) and superoxide anion radical (O^{-2}). The reactive oxygen species could interact with the cell walls, lead to bacterial cell membrane rupture or killing of the germs.[20,21] In addition, such reactive oxygen species can break the basic group of DNA strand, disrupt the duplication process of DNA

in microbial cells and ultimately cause cell death. Thus, TiO_2 nanotube (TNT) has been applied as excellent candidate in the field of antibacterial coating [22-24] and bone transplantation [25,26] because of its highly ordered tubular structure, high specific surface area, high surface roughness and capacity for drug loading.[22]

For orthopedic applications, TiO_2 nanotubes have ability to selectively adsorb vitronectin and fibronectin and therefore promote to the association with bone reconstruction involving vitro cell adhesion, proliferation and differentiation.[27,28] TiO_2 nanotubes with smaller diameter (30 nm) could improve the adhesion of osteoblast at the greatest extent, while such effects were not observed for TiO_2 with large diameter (70 nm). What's more, bacterial behaviour also exhibited size and topography dependent manner when cultured on TiO_2 nanotubes surface. It was observed that the number of bacteria cultured on relatively rough TiO_2 nanotubes surface are significantly reduced, compared with the smooth surface of the Ti. Specially, bacteria cultured on nanotubes with diameter of 40-60 nm [29] and 200 nm [30] showed most reduced number. The stress response of germs to the nanotubes could lead to the rupture of bacteria cell membrane [31] and cause cell death. The nano-interface properties of TiO_2 can inhibit adhesion and proliferation of certain bacteria. However, it was noted that the interaction between nanomaterials and bacteria will increase some bacterial drug resistance via increasing of horizontal transfer between resistance genes of bacteria, which may induce potential threat to global public health. For example, nano alumina can promote RP4 plasmid from Escherichia coli and salmonella joint and lead to the horizontal transfer drug resistance genes.[32] Therefore, the potential risk assessment of nano-effect should arouse great concerns. *Porphyromonas gingivalis* can generate virulence factors which could result in the loss of periodontal attachment level. Thus, in this study, we use *P. gingivalis 381* to investigate the bacteria responses to the change of calibers and related the drug resistance under different reagent concentration. Consequently, we could evaluate the antibacterial property and potential threaten of TiO_2 based functional nanomaterials. Our work could provide guideline for the development of novel dental implant materials.

2. Experiments

2.1 Preparation and characterization of TiO_2 nanotubes

TiO_2 nanotubes were fabricated using commercially pure titanium sheets (Φ32*1.2 mm). The titanium sheets were mechanical polished then clean with acetone, ethanol and deionized water separately, followed by drying in air. These are defined as "conventional Ti". A two-step anodic oxidation method was used to prepare the TNT coatings. The cleaned titanium sheets were oxidized in the glycol containing 0.3%wt ammonium fluoride at 60 V for 5 h. A platinum sheet was used as the cathode. Both platinum and Ti were connected to a DC power supply (SKD-1105A, SAKO) through copper wires. The samples were cleaned with ethanol and deionized water and dried in air. Further, the samples were oxidized in the glycol containing 0.3%wt ammonium fluoride at varied voltage (20-60 V) and time (6-30 h). Finally, cleaned Ti sheet and TNT were annealed at 450°C for 4 h (Brother vacuum tube furnace, China) with heating/cooling rate of 2°C/min. The surface morphology of the prepared TNT was observed by the field emission scanning electron microscope (FE-SEM, ULTRA PLUS, Zeiss, Germany). Specially, the samples where bacteria were cultured on were washed by the phosphate buffered saline (PBS) and immersed with glutaraldehyde for 12 h at 4°C. Then the samples were dehydrated with ethanol and dried in air. At last, the morphology bacteria on the treated TNT were observed using FE-SEM. The crystalline characteristic and phase formation of the prepared TNT were analyzed by the X-ray diffraction analysis (XRD, Philips X'Pert PRO, USA) with the Cu-Kα as radiation source. The scan rage was set from 20° to 90°. The water contact angles of the TNT with different diameters were measured by the image collection and analysis systems (Dataphysics OCA20, Germany). The water contact angle measurements were performed at room temperature with distilled water as the determining medium. Each experiment was repeated five times and data is expressed as mean ± standard deviation.

2.2 Bacterial culture

P. gingivalis 381 (China Medical University Hospital of Stomatology) were inoculated onto BHI blood agar plates (Brain heart infusion, OXOID, UK) and revived (80%N_2, 10%H_2, and 10% CO_2, anaerobic condition) in constant temperature incubator (Shanghai Jing macro laboratory equipment co., LTD) for 5 days. Then the bacteria were inoculated onto BHI broth (OXOID, UK) and cultured under anaerobic condition for 48h. The samples were collected after centrifuge at 4°C for 5 min with 3000 r/min. (1-15 k, SIGMA, Germany). Then collected samples were suspended in the BHI liquid medium and bacteria solutions of $1*10^9$CFU/L were prepared by McFarland nephelometry.

2.3 Bacteria cultured on different substrates with metronidazole

Prior to inoculation, the conventional Ti and TNT were sterilized for 6h under an UV light with a power of 20W. Metronidazole with different concentrations (Group A and B) were used to study the effect of TNT on *P. gingivalis*. The Metronidazole (China Pharmaceutical and Biological Products) solution was diluted with BHI broth and mixed with bacteria solution for preparing the bacteria samples. Prior to the experiment, the minimum inhibitory concentration (MIC) of the metronidazole for *P. gingivalis* was first determined using classic drug susceptibility testing method (agar dilution method). The MIC_{90} was about 10mg/L which was set as the lower limit of the drug concentration in the experiment. The final concentration of metronidazole in Group A and B were 10mg/L and 15mg/L, respectively. TNT samples with different diameters and titanium sheet (total 6) were placed into six well plates (Corning, USA) for each group. The bacteria solution with metronidazole concentration of 10mg/L (group A) or 15mg/L (group B) was added to each six-well plates (GIISON, French). The bacteria were cultured (80%N_2, 10%H_2, 10%CO_2, anaerobic environment) in constant temperature (37°C) for 48 h. 2ml bacteria solution was took from each six-well plates and measured under UV spectrophotometer (UV765, Shanghai Precision and Scientific Instrument Co., Ltd.) to determine the corresponding bacteria density. Each experiment was repeated three times and data is expressed as mean ± standard deviation.

3. Results

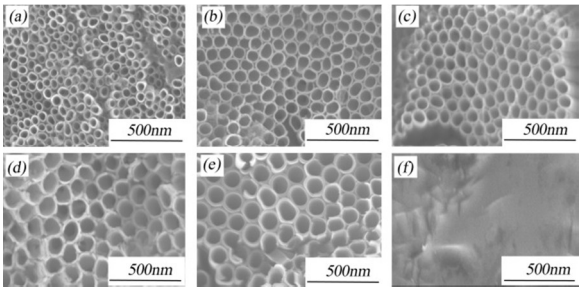

Fig. 1 FE-SEM images of TNT with different diameters: (a) 30 nm, (b) 50 nm, (c) 60 nm, (d) 80 nm, (e) 100 nm, (f) titanium sheet.

The obtained morphology of TNT (annealed) was shown in Fig. 1. The nanotubes with different calibres could be achieved by altering parameters such as oxidation time and anodizing voltage. TNT with different diameters (30 nm, 50 nm, 60 nm, 80 nm, 100 nm, respectively) was fabricated. Annealing treatment made little difference to the diameter of the nanotube.

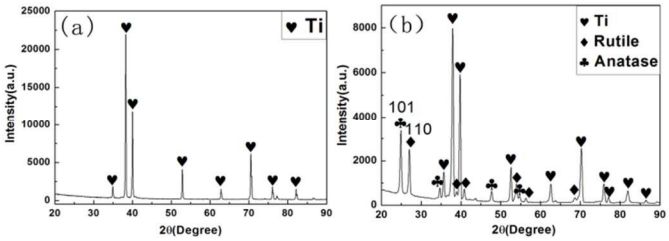

Fig. 2 XRD Patterns of (a) unannealed TNT and (b) annealed TNT.

The XRD pattern of TNT was shown in Fig 2. Without heat treatment, the nanotubes had amorphous structure, and only diffraction peak of Ti could be observed in the XRD spectrum (as shown in Fig. 2a). Whereas in the XRD pattern of annealed TNT, the characteristic peaks of 101 and 110 revealed the existence of anatase and rutile (as shown in Fig. 2b).

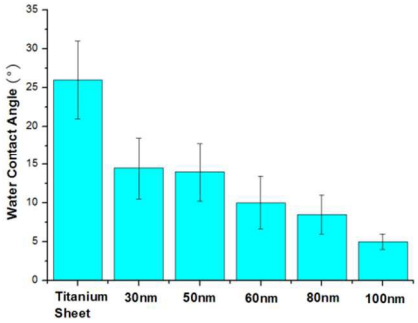

Fig. 3 The water contact angles of Titanium sheet and TiO_2 nanotubes with different diameters.

The water contact angle data of TNT and titanium sheet were shown in Fig. 3. Water contact angle is an important indicator to assess hydrophobic and hydrophilic of the surface. Titanium sheet exhibited hydrophobic compared with the TiO_2 nanotubes. In addition, nanotubes exhibited obvious hydrophilic property with the increase of caliber and the lowest contact angle was obtained for the nanotubes with 100nm diameter.

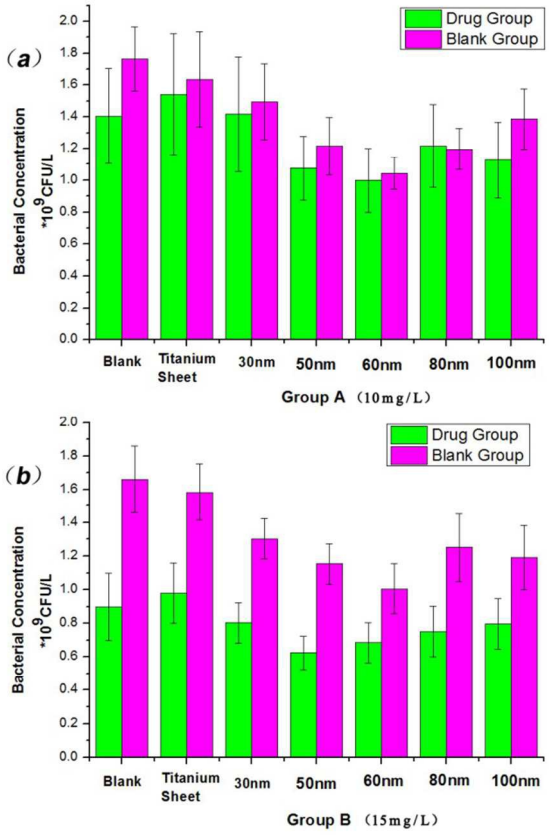

Fig. 4 Bacterial density of different samples: (a) Group A (metronidazole: 10 mg/L), (b) Group B (metronidazole: 15 mg/L).

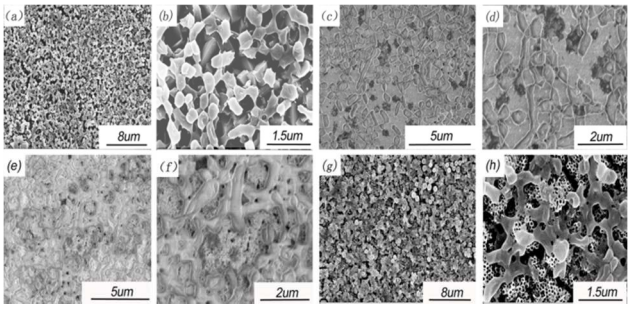

Fig. 5 FE - SEM image of bacteria on different substrates in Group A. a-b: titanium sheet, c-d: 30 nm, e-f: 60 nm, g-h: 100 nm.

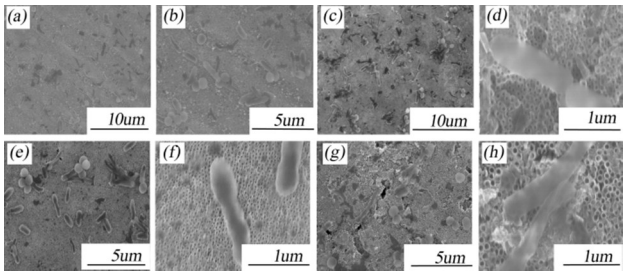

Fig. 6 FE - SEM image of bacteria on different substrates in Group B. a-b: titanium sheet, c-d: 30 nm, e-f: 60 nm, g-h: 100 nm.

Bacterial densities on various culture substrates of group A and group B were shown in Fig. 4, respectively. The bacteria cultured on the Ti sheet or TiO_2 nanotubes showed reduced

number compared with blank sample for both groups. Specially, for bacteria cultured on the TNT, firstly the number of bacteria decreases with the increase of TNT's diameter and the lowest density was achieved when diameter was 60nm. And it's interesting to note that TNT with diameter of 60 nm seemed to be an inflexion. The number of survived bacteria increased when cell cultured on the TNT with diameter 60-100 nm. Fig. 5 and Fig. 6 showed SEM image of bacteria in group A and B (Drug Group). For group A, in which there was lower concentration of metronidazole, more bacteria adhesion and fusion was found on the nanotubes. However, in group B which contained higher concentration of metronidazole, the growth of bacteria was significantly inhibited. Moreover, it's interesting to note that bacteria cultured on TNT with diameter 60 nm showed shrinking morphology, which were significant different from others.

4. Discussion

The TNT could increase the surface area and roughness of the titanium surface, promoting the adhesion of some cells.[33] However, for bacteria, it was proved that TNT could induce significantly reduction on number of cells adhered to its surface. [18] It is known that TiO_2 nanotubes have great surface energy than the unmodified Ti.[17] By analyzing wetting angle experimental results, we can conclude that the water contact angel of TiO_2 nanotubes increase, indicating enhanced hydrophilia of samples, which is corresponding with increased TiO_2 nanotubes diameter. Currently, opinion on how materials' hydrophilia/free regulate cellular behavior of bacterial is not consistent. Previously, some reports demonstrate that the enhanced hydrophilic surface could promote bacterial adhesion and proliferation and the bacterial were expected to growth with the increased TiO_2 nanotubes diameter.[34,35] However, in our experiment, with increased TiO_2 nanotubes diameter, the number of bacterial decreased first then increase, which indicated that there are complex factors to regulate bacterial behavior at the biocompatible interface. In this study, the TNT nanotubes exhibited obviously different antibacterial performance when compared with blank sample and titanium sheet sample, which could be attributed to the sterilization and their nanoscale effects. The nanotubes were sterilized by the ultraviolet irradiation and produce high oxidative photo-generated holes (h+) which could react with H_2O and O_2, and further generate active groups with strong oxidization ability including hydroxyl radicals (·OH) and super oxide free radical (O^{2-}).[36] These active groups can interact protein, nucleic acid and cell membrane of bacteria, causing damage to molecular structures.[37, 38] In addition, the reactive oxygen generated by TiO_2 could increase lipid peroxidation and lactate dehydrogenase, causing the damage to DNA and lead to cell death.[39] In this process, the unsaturated fatty acids in the cell membrane can also be oxidized by ·OH group, which would affect the cell membrane fluidity and structure. Subsequently, the thickness of cell membrane would decrease, and the cell apoptosis occur.[40,41] Thus, the interactions between TNT nanotubes and bacteria would eventually lead to cell death with generation of reactive oxidation group under UV light excitation, and it is worth noting that the mixed crystalline (anatase and rutile) have enhanced ability to produce photo-generated holes (h+), resulting in stronger antibacterial effect than amorphous TiO_2. In a word, generation of oxidative stress under UV light irradiation of TiO_2 has contributed to their antibacterial effect and nanotoxicity.

The response behaviours of bacteria to TNT with different geometry characteristics were also investigated. Previous studies demonstrated that the antibacterial property of TNT was mainly affected with the number of active groups, which were correlated with the specific surface area of the functional materials. The antibacterial materials with increase of specific surface area displayed enhanced decontamination ability.[42] Specially, the specific surface area of nanotubes is correlated with the diameter [42], thus nanotubes with the increased pipe diameters are expected to have improved bacterial growth inhibition effect. It was worth to note that the bacteria survive on TNT decreased corresponding with the increase in diameters ranging from 40 nm to 60 nm. Specially, TNT with diameter of 60 nm has higher density and thinner nanotube wall which is beneficial to inhibit hole electron recombination and displayed improved photocatalytic ability than TNT with diameter of 30 nm and 50 nm nanotubes, [30] thus the most significant antibacterial effect was expected to be observed. [43] However, for bacteria cultured on TNT with diameter from 60 nm to 100 nm, the number of bacteria began to increase. The observed improved growth behaviour of bacteria suggested that survival rate of bacteria on TNT is determined by multi factors. Previous studies indicated materials at nanoscale have specific effects which can trigger series biological behavior changes on cells.[43-45] In this study, *P. gingivalis* on TNT also exhibit nanoscale dependence behavior. TNT with diameter ranging from 30nm - 60nm could cause physical punch on cell via mechanical contact to the cell membrane because of the cylindrical shape and high aspect ratio.[46,47] The physical interaction could induce cell member ruptures, cause the cytoplasm outflow and eventually lead to cell apoptosis. However, the continuous increase in the diameter of TNT (i.e. 60-100 nm) could reduce directly mechanical contact effects of cell and show decreased inhibition on growth of bacteria.[48] Thus, the survival rate of bacteria was dominated by the photocatalytic oxidation ability of TNT and nano geometry characteristic dependence of bacteria. It was interesting to note that the concentration of bacterial cultured on TNT with 80 nm in drug group A was slightly higher than that of the control group. The possible reason could be that the drug inhibition effect on bacteria in Group A was weak due to relatively low drug concentration in group A (close to MIC_{90}) and the potential impact of TNT on metronidazole (discussed below). In addition, TNT with larger diameter (80nm) could weaken the physical punch effect and resulted in the increase of bacteria. The exact mechanism will be investigated in subsequent experiments.

The potential disadvantage on the drug resistance caused by nanotubes was also discussed in this paper. Bacteria could obtain exogenous resistant genes mainly via three ways: transformation, transduction and joint. The joint is the most effective and common route of horizontal transfer which means DNA transfer between cells with the aid of plasmid.[49] Previous studies found that nano alumina could promote the conjugational transfer of multi-resistant plasmid RP4 from *Escherichia coli* to *Salmonella* joint (200 times of the untreated cells). On some condition, nano materials could promote the horizontal transmission of resistance gene in the level of different strains, and leads to the increase of bacterial drug resistance.[32] Thus, in order to evaluate the effect of TNT on bacterial drug resistance property, we tested antibacterial properties of TNT nanotubes to bacteria treated with drug in different concentrations. The results suggested that when exposed to drug with low concentration, the antibacterial

effects of TNT nanotubes were significantly lower than the blank group and most of them were lower than the control group. Under relatively high drug concentration exposure, the antibacterial effect of all samples had a certain improvement. However, the antibacterial effects of TNT coatings were still lower than blank group and control group. The good antibacterial effects of TNT coatings with 50 nm might be attributed to the nanometer size effect. Oxidative stress induced by the TNT coating could change the *P. gingivalis* cell membrane's fluidity and structure [41] thus promoting the plasmid containing resistance genes transfer and transmit between cells,[50,51] eventually result in the increase of bacterial drug resistance performance. There might be two reasons for the relatively low antibacterial effect of metronidazole on the TNT coating. First of all, it had been reported that the photo catalytic ability of TiO_2 could be used to degrade the metronidazole under the visible light.[52] It reduced the concentration of metronidazole in a certain extent which would weaken the drug effect. In addition, oxidative stress of nanotubes can restrain the sterilization effect of metronidazole. Metronidazole entered into bacteria cell and its nitro can be easily reduced to amino by electron transfer protein under the anaerobic environment. It can prevent the synthesis of bacterial DNA and degrade the synthesized DNA to achieve the antibacterial effect.[53] While TNT produced photo-generated holes (h+) have strong oxidation ability under illumination, could restrain the reduction process by the production of catatonic amino. Therefore, TNT coating might weak the inhibition effect of metronidazole on bacteria growth. However, for further validating the hypothesis, related investigations should be implemented in our future studies.

5. Conclusions

In this study，the two-step method was used to prepare TNT coatings with different diameters and the antibacterial properties of different TNT coatings and its impact on bacterial resistance were studied. The biological behaviours of bacteria on the surface of the TNT coating seem to be affected by both oxidative stress and nanometer size effect. In addition, the nanotubes could be inclined to attenuate the antibacterial effect of metronidazole. The argument would be bolstered with the further investigation.

Acknowledgements

Support for this work by the National Natural Science Foundation of China (50872019, 51002027), post-doctoral foundation of China (2013M530930), scientific foundation of educational department of Liaoning Province (L2012084), the state key laboratory of metal matrix composite, Shanghai Jiaotong University (no. mmckf1411), Basic scientific Research Foundation of Central College (N130402001).

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1 T. Albrektsson, G. Zarb, P. Worthington and A. Eriksson, *Int Joral Max Impl.*, 1986, **1**, 11.

2 M. Esposito, J.M. Hirsch, U. Lekholm and P. Thomsen, *Int Joral Max Impl.*, 1999, **4**, 473.

3 K.S. Brammer, S, Oh, C.J. Cobb, L.M. Bjursten, H. van der Heyde. and S. Jin, *Acta Bimater.*, 2009, **5**, 3215.

4 T. Nakamura, A. Amano, I. Nakagawa, S. Hamada, *FEMS Microbiol Lett.*, 1999, **175**, 267.

5 M.M. Danser, A.J. vanWinjelhoff, U. vanderVelden, *J Priodontol.*, 1997, 68, 209.

6 S. Kotsovilis, I.K. Karoussis, M. Trianti and I. Fourmousis, *J clin periodontol.*, 2008, **35**, 621.

7 G. Tawil, R. Younan, P. Azar and G. Sleilati, *Int Joral Max Impl.*, 2008, **23**, 744.

8 B.H. Lee, Y.D. Kim, J.H. Shin and K.H. Lee, *J biomed Mater Res.*, 2002, **61**, 466.

9 Z. Wang, Y. Shen, M. Haapasalo, *Dent Mater*, 2014, **30**, E1-E16.

10 X. Liu, P.K. Chu and C. Ding, *Mater Sci Eng R.*, 2004, **47**, 49.

11 T.N. Kim, A. Balakrishnan, B. Lee, W. Kim, B. Dvorankova, K. Smetana, et al, *J Mater Sci-Mater M.*, 2008, **19**, 553.

12 C.B. Dong, Z.J. Yan, J. Kokx, D.B. Chrisey, C.Z. Dinu, *Appl Surf Sci.*, 2012, **258**, 9218.

13 A.S. Campbell, C.B. Dong, F.K. Meng, J. Hardinger, G. Perhinschi, N.Q. Wu, C.Z. Dinu, *ACS Appl Mater Inter.*, 2014, **6**, 5393.

14 Q. Xu, M.T. Li, J.B. Niu, Z.H. Xia, *Langmuir*, 2013, **29**, 13743.

15 C. Liu, A. Tian, H. Yang, Q. Xu, X. Xue, *Appl Surf Sci*, 2013, **287**, 218–222.

16 A. Tian, X. Qin, A. Wu, H. Zhang, Q. Xu, D. Xing, H. Yang, B. Qiu, X. Xue, D. Zhang, C. Dong, *Int J nanomed*, 2015, **10**, 1-16.

17 S.D. Puckett, E. Taylor, T. Raimondo and T.J. Webster, *Biomaterials*, 2010, **31**, 706.

18 G. Colon, B.C. Ward and T.J. Webster, *J Biomed Mater Res A.*, 2006, **78**, 595.

19 V.K. Truong, R. Lapovok, Y.S. Estrin, S. Rundell, J.Y. Wang, C.J. Fluke, et al, *Biomaterials*, 2010, **31**, 3674.

20 T. Saito, T. Iwase, J. Horie and T. Morioka, *J Photoch Photobio B*, 1992, **14**, 369.

21 H. Aita, N. Hori, M. Takeuchi, T. Suzuki, M. Yamada, M. Anpo, et al, *Biomaterials*, 2009, **30**, 1015.

22 K.M. Kummer, E.N. Taylor, N.G. Durmas, K.M. Tarquinio, B. Ercan and T.J. Webster, *J Biomed Mater Res B.*, 2013, **101**, 677.

23 A. Roguska, A. Belcarz, T. Piersiak, M. Pisarek, G. Ginalska and M. Lewandowska, *Eur J Inorg Chem.*, 2012, **32**, 5199.

24 L. Zhao, H. Wang, K. Huo, L. Cui, W. Zhang, H. Ni, et al, Antibacterial nano-structured titania coating incorporated with silver nanoparticles, *Biomaterials*, 2011, **32**, 5706-5716.

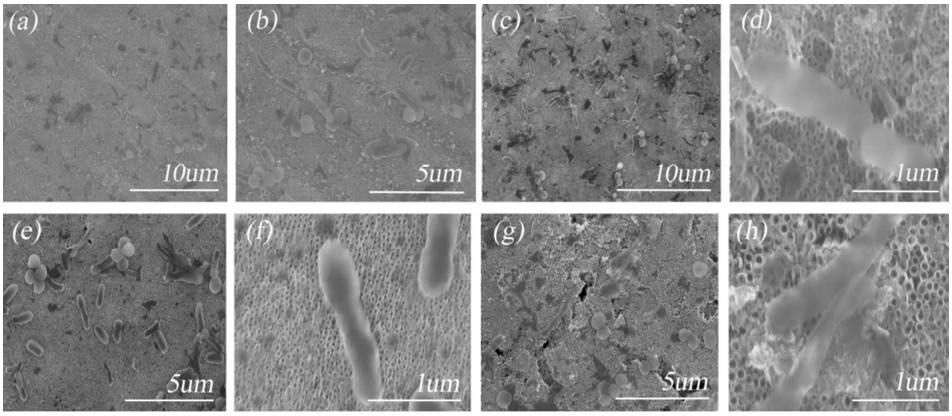
25 T.J. Webster, J.U. Ejiofor, *Biomaterials*, 2004, **25**, 4731.

26 A. Tian, X.F. Qin, A.H. Wu, H.Z. Zhang, Q. Xu, D.G. Xing, H. Yang, B. Qiu, X.X. Xue, D.Y. Zhang, C.B. Dong, *Int. J. Nanomed.*, 2015, in proof.

27 S. Oh, C. Daraio, L.H. Chen, T.R. Pisanic, R.R. Finones and S. Jin, *J Biomed Mater Res A.*, 2006, **78**, 97.

28 L.M. Bjursten, L. Rasmusson, S. Oh, G.C. Smith, K.S. Brammer and S. Jin, Titanium dioxide nanotubes enhance bone bonding in vivo, *J Biomed Mater Res A*., 2010, **92**, 1218-1224.
29 B. Ercan, E. Taylor, E. Alpaslan and T.J. Webster, *Nanotechnology*, 2011, **22**, DOI: 10.1088/0957-4484/22/29/295102.
30 H.R. Li, Q. Cui, B. Feng, J. Wang, X. Lu and J. Weng, *Appl Surf Sci.*, 2013, **284**, 179.
31 S. Kang, M. Herzberg, D.F. Rodrigues and M. Elimelech, *Langmuir*, 2008, **24**, 6409.
32 Z. Qiu, Y. Yu, Z. Chen, M. Jin, D. Yang, Z. Zhao, et al, *Pnatl Acad Sci USA*., 2012, **109**, 4944.
33 H. Zhou, X. Gan, J. Wang, X.L. Zhu and G.X. Li, *Anal Chem.*, 2005, 77, 6102.
34 J. Ji, W. Zhang, *J Biomed Mater Res*, 2009, **88**, 448-453.
35 J.H. Lee, S.J. Lee, G. Khang, H.B. Lee, *J Biomater Sci Polym Ed*, 1999, **10**, 283-294.
36 G. Storz, J.A. Imlayt, *Curr Opin Microbiol.*, 1999, **2**, 188.
37 R. Kohen, A. Nyska, *Toxicol pathol.*, 2002, **30**, 620.
38 A.M. Gallardo-Moreno, M.A. Pacha-Olivenza, M.C. Fernández-Calderón, C. Perez-Giraldo, J.M. Bruque and M.L. Gonz lez-martin, *Biomaterials*, 2010, **31**, 5159.
39 K.J. Davies, M. Delsignore and S. Lin, *J Biol Chem.*, 1987, **262**, 9902.
40 Q. Xiao, L.Ouyang, *J Phys Chem Solids.*, 2011, **72**, 39.
41 S.S. Soni, G.S. Dave, M.J. Henderson and A. Gibaud, *Thin Solid Films*, 2013, **531**, 559.
42 Q. Cui, B. Feng, W. Chen, J.X. Wang, X. Lu and J. Weng, *J Inorg Mater*, 2010, **25**, 916.
43 I. Demetrescu, C. Pirvu and V. Mitran, *Bioeletrochemistry*, 2010, **79**, 122.
44 K.K. Sethi, I.V. Yannas, V. Mudera, M. Eastwood, C. Mcfarland and R.A. Brown, *Wound Repair Regen*, 2002, **10**, 397.
45 K.M. Woo, V.J. Chen and P.X. Ma, *J Biomed Mater Res A.*, 2003, **67**, 531.
46 S. Kang, M. Pinault, L.D. Pfefferle and M. Elimelech, *Langmuir*, 2007, **23**, 8670.
47 Y.J. Tang, J.M. Ashcroft, D. Chen, G. Min, C.H. Kim, B. Murkhejee, et al, *Nano Lett.*, 2007, **7**, 754.
48 S. Grigorescu, C. Ungureanu, R. Kirchgeorg, P. Schmuki and I. Demetrescu, *Appl Surf Sci.*, 2013, **270**, 190.
49 S.H. So, K. Miyahara and Y. Ohshima, *Genes Cells*, 2011, **16**, 639.
50 J.W. Beaber, B. Hochhut, M.K. Waldor, *Nature*, 2004, **427**, 472.
51 E. Maiques, C. Ubeda, S. Campoy, N. Salvador, I. Lasa, R.P. Novick, et al, *J Bacteriol.*, 2006, **188** , 2726.
52 S.S. Boxi, S. Paria, *RSC Adv.*, 2014, **4**, 37752.
53 P. Poulet, D. Duffaut, J.P. Lodter, *J Clin Periodontol.*, 1999, **26**, 261.

Graphical Abstract



Porphyromonas gingivalis were seeded on the TiO_2 nanotube array coatings to evaluate the potential impact of the TiO_2 nanotubes on bacteria's growth and drug resistance. We discovered that the antibacterial performance of TiO_2 nanotube coating can be manipulated with the photocatalytic activity as well as the geometry characteristic and the interaction with TiO_2 surface may increase the potential survive chance of *Porphyromonas gingivalis* when exposed to the antibacterial drug.