Hindawi Publishing Corporation
Journal of Nanomaterials
Volume 2015, Article ID 818565, 9 pages
http://dx.doi.org/10.1155/2015/818565

Research Article

The Promotion of Antibacterial Effects of Ti6Al4V Alloy Modified with TiO_2 Nanotubes Using a Superoxidized Solution

Ernesto Beltrán-Partida,[1,2] Benjamin Valdez-Salas,[2] Alan Escamilla,[2] Aldo Moreno-Ulloa,[3,4] Larysa Burtseva,[2] Ernesto Valdez-Salas,[5] Mario Curiel Alvarez,[2] and Nicola Nedev[2]

[1]*Facultad de Odontología Mexicali, Universidad Autónoma de Baja California, Avenida Zotoluca y Chinampas, s/n, 21040 Mexicali, BC, Mexico*
[2]*Instituto de Ingeniería, Universidad Autónoma de Baja California, Boulevard B. Juárez y Calle de la Normal s/n, 21280 Mexicali, BC, Mexico*
[3]*University of California San Diego, School of Medicine, 9500 Gilman Drive, La Jolla, CA 92093, USA*
[4]*Sección de Estudios de Posgrado e Investigación, Escuela Superior de Medicina, Instituto Politécnico Nacional, Plan de San Luis y Díaz Mirón, 11340 México, DF, Mexico*
[5]*Centro Medico Ixchel, Bravo y Obregón, 21000 Mexicali, BC, Mexico*

Correspondence should be addressed to Benjamin Valdez-Salas; benval@uabc.edu.mx

Received 18 February 2015; Revised 9 April 2015; Accepted 20 April 2015

Academic Editor: P. Davide Cozzoli

The purpose of the present study was to synthetize 80 nm diameter TiO2 nanotubes (NTs) on Ti6Al4V alloy using a commercially superoxidized water (SOW) enriched with fluoride to reduce anodization time and promote the antibacterial efficacy against *Staphylococcus aureus* (*S. aureus*). The alloy discs were anodized for 5 min and as a result, NTs of approximately 80 nm diameters were obtained with similar morphology as reported in previous studies using longer anodization times (1-2 h). Filed emission-scanning electron microscopy (FE-SEM) and energy dispersive X-ray spectroscopy (EDX) were used to characterize the materials surfaces. The NTs showed significantly decreased *S. aureus* viability after 1, 3, and 5 days of culture in comparison to nonanodized alloy. Likewise, SEM analysis also suggested lower bacterial adhesion on the NTs surface. No differences in bacterial morphology and topography were observed on both materials, as analyzed by SEM and atomic force microscopy (AFM). In conclusion, 80 nm diameter NTs were grown on Ti6Al4V alloy in 5 min by using a SOW solution enriched with fluoride, which resulted in a material with promoted antibacterial efficacy against *S. aureus* for up to 5 days of in vitro culture when compared to nonanodized alloy.

1. Introduction

Despite strict sterilization procedures biomaterials associated infection (BAI) is a common complication associated with any implanted biomaterial [1]. Once infection arises, bacteria tend to aggregate into a polymeric matrix forming a biofilm on the biomaterial surface, which is difficult for the host defense and antimicrobial therapy to destroy [2, 3]. Such BAI may lead to removal of the implant, revision surgery, and even amputation, all of which are associated with high medical costs [4, 5]. One of the most common causes of BAI is related to *S. aureus*, which has become an important protagonist in metallic materials, bone joints, orthopedic implants, and soft tissue infections [6–11]. Therefore, there is the need for biomaterials with improved antibacterial properties in order to increase the success of dental and orthopedic implants.

Bacterial adhesion to the implanted material is known to be the first and most critical step in the pathogenesis of the infection, in part because nonadhered bacteria are rapidly phagocytized by the immune system [12, 13]. Thus, prevention of bacterial adhesion exerts a fundamental role in preventing the implant infection [14]. Currently, bacterial infection has been safeguarded through the chemoprophylactic use of antimicrobial agents before surgery, primarily when they are systematically administered and in some cases even locally released from the biomaterial. Nevertheless, this latter

strategy has important limitations such as the rapid release of the adsorbed antibiotic, antibiotics resistance, or systemic toxicity [12, 13, 15].

Another important strategy for inhibiting the bacterial adhesion and development of biofilm is by means of modifying the material surface properties. Surface modification techniques tailor the surface chemistry and topography of the biomaterial, which affects microbial colonization [13]. Different procedures have been developed to modify the chemical composition and/or the topography of the biomaterial surface such as surface functionalization with antibacterial copolymers, UV irradiation, or peptide immobilization, therefore inhibiting bacterial adhesion in a favorable manner [16–18].

Metal-based biomaterials such as titanium (Ti) and its alloys are widely used for orthopedic and dental applications due to their favorable mechanical properties, corrosion resistance, and biocompatibility [2, 19]. In this regard, the Ti6Al4V alloy offers superior physical and mechanical properties compared to commercially pure Ti (CP-Ti), as well as excellent biocompatibility [20]. Among the surface modification techniques to fabricate nanostructural coatings, anodic oxidation is a well-known process that provides a strong adhered NTs layer to the material surface [21, 22], increasing its corrosion resistance [23, 24], surface area [25], hydrophilicity [26], and more importantly biocompatibility [27]. Nonetheless, previous reports suggest that the anodization process using fluoride with distilled water as an electrolyte to obtain NTs of 80–100 nm diameter requires period of 1 h [28, 29] and perhaps 2 h [21]. Moreover, various studies suggest that Ti alloy surfaces modified with a nanostructured coating could decrease bacterial adhesion; that is, Ercan et al. showed that amorphous NTs treated with electrical stimulation could decrease *S. aureus* after 2 days of culture [30]; Grigorescu et al. suggest that TiZr NTs grown for 2 h by anodization reduce *Escherichia coli* (*E. coli*) after 18 h of incubation [31]. Furthermore, Puckett et al. reported an increased number of dead *S. aureus* bacteria on amorphous NTs after 1 h of cultivation [26], suggesting that a nanostructured surface may provide antibacterial properties decreasing bacterial adhesion [6, 32]. According to this fact, we hypothesize that a metallic biomaterial implant improved with NTs coating fabricated using a SOW solution used for disinfection of medical devices [33, 34] and enriched with fluoride will enhance its antibacterial effects, decreasing BAI.

In this work we synthetized for the first time 80 nm NTs on Ti6AlV4 alloy by anodic oxidation using a commercially available superoxidized water solution enriched with fluoride and ethylene glycol. Moreover, we also used UV and SOW for NTs sterilization and we explored its antibacterial effects comparing those of nonanodized alloy. The materials surface was characterized by FE-SEM and the chemical composition by EDX. The *S. aureus* viability on the materials surface was assessed by colony-forming unit (CFU) calculation. Adhered bacteria and topography were also evaluated by SEM and AFM, respectively.

2. Materials and Methods

2.1. Materials for NTs Synthesis. Ti6Al4V alloy (ASTM F136-02) discs with a 150 mm^2 surface area from Supra Alloys Inc. (Camarillo, California, USA) were used. Microdacyn 60, commercially available superoxidized water with the following composition, sodium (<55 ppm) and chloride (<80 ppm), was obtained from Oculus Technologies (Mexico, Mexico). Ammonium fluoride powder (Sigma-Aldrich, ST. Louis, MO) and ethylene glycol (Sigma-Aldrich, ST. Louis, MO) were purchased and used as received.

2.2. Synthesis of NTs. Ti6Al4V alloy discs were polished using SiC emery paper (100 to 2000 grit) and 1-micron alumina was used for finishing. The samples were subsequently cleaned by sonication in water, acetone, and ethanol for 5 min each [31]. Next, mirror finished disc surfaces were mounted on a flat 125 mL cell and then electrolytically anodized using Microdacyn 60 at pH 6.8, containing 10 mg/L of NH_4F and 20% ethylene glycol. A 20 V potential was applied using a DC power supply for 5 minutes and a platinum mesh as a counter electrode. The process was carried out at 24°C. Finally, discs were cleaned in an ultrasonic bath (Transsonic 570 Elma) at 300 W with distilled water for 5 minutes to eliminate the excess of fluoride salts, rinsed with isopropyl alcohol, and then dried in a desiccator for 12 hours. No thermal treatment was applied after the cleaning process and a nonanodized alloy was used as a control.

2.3. Surface Characterization of the Samples. The structural morphology of anodized and nonanodized Ti6Al4V alloys was characterized using FE-SEM (Tescan LYRA 3, Brno, Czech Republic); the images were taken at a 15 and 20 kV accelerating voltage. The chemical composition of the alloys surface was examined using EDX (JSM-6010LA, JEOL, Tokyo, Japan) with a silicon drift detector, coupled to the SEM.

2.4. Bacterial Cell Culture. For the bacterial growth experiments, *S. aureus* (Strain American Type Culture Collection 25923, American Type Culture Collection, Manassas, VA, USA) which has been shown to be a good biofilm former and capable of producing mature biofilms after only 24 hours was used [6, 30]. For the preparation of the inoculums, the strain was freshly grown overnight on tryptic soy agar (TSA) plates (Beckton Dickinson, Sparks, Maryland, USA). Discrete colonies were obtained from TSA plates and suspended in tryptic soy broth (TSB) to an optical density (O.D.) of 0.3, pH 7.0, assessed using a spectrophotometer (LAMBDA 25, Perkin Elmer, Connecticut, USA). All experimental specimens were sterilized using ultraviolent (UV) irradiation for 30 minutes each side [35] and rinsed with SOW for 1 h.

2.5. Bacterial Viability on the Samples. For the determination of viable cells on the samples, 100 μL of *S. aureus* suspension containing approximately 1×10^7 CFU/mL (O.D. 0.3) plus 100 μL of fresh TSB was used to cover the material's surface. This inoculum was incubated on the specimens for 4 hours at

Table 1: Surface elemental composition by EDX analysis.

Surface	Atomic concentration (%)					
	C	V	Al	Ti	O	F
Non-anodized alloy	5.96	5.67	5.37	83.00	0.00	0.00
NTs	4.00	0.00	5.40	61.91	25.51	3.18

37°C to allow adhesion in a static model. Thereafter, 2 mL of warm TSB was deposited, and the incubation time continues for D1, D3, and D5. After the corresponding incubation days, the specimens were rinsed twice with phosphate saline buffer (PBS; pH 7.0) to remove any unbounded cells. The substrates were transferred into sterile conical tubes (Falcon, BD Biosciences, Franklin Lakes, NJ, USA) with 5 mL of fresh TSB medium. The tubes were placed in an ultrasonic bath and sonicated for 15 minutes at 120 W to release the attached cells from the biomaterial [36, 37]. Next the materials were removed and the remaining suspensions were diluted with PBS (pH 7.0) and cultured at 37°C for 24 hours in TSA plates. Bacterial viability was assessed by CFU counting in TSA plates. The above procedure was performed 20 times for each material [36]. The viability was expressed as $\log_{10}$ CFU/mL ± SD.

2.6. Bacterial Density and Morphology on the Samples by SEM. In order to determine the bacterial density and morphology at the end of each incubation period, the samples were prepared for SEM (JSM-6010LA, JEOL, Tokyo, Japan) analysis according to the following procedure: the discs were rinsed in PBS (pH 7.0) to wash away nonadhered bacteria and then fixed with 2.5% glutaraldehyde (Sigma-Aldrich, ST. Louis, MO) in 0.1 mol/L PBS (pH 7.0) for 2 h at 25°C. Next, samples were washed three times with PBS (10 minutes each wash), dehydrated in a graded series of ethanol solutions (50, 60, 70, 80, 90, and 100% v/v), and finally sputter-coated with 10 nm gold layer for the analysis.

2.7. Bacterial Topography on the Samples by AFM. To assess any changes on bacteria's topography incubated on the materials, we used AFM analysis. However, we only analyzed D1 in order to obtain a more precise analysis of only a subset of bacteria's surface instead of layers of bacteria. Thus, following D1 of bacterial growth, the samples were prepared in the same way as for SEM, as described above [38]. A Quesant Q-Scope 350 AFM (AMBIOS, Agura Hills, California, USA) was used because of its high-resolution probe with an acceptable resolution in the subnanometer range [39]. The analyses were carried out at room temperature using an antiacoustic box to prevent noises, which can affect measurements. The system was operated in the contact mode, using a 40 μm X-Y and 4 μm Z scanner equipped with a silicon tip and 10 nm tip curvature. The scan speed was 0.5 Hz/sec. The experiment scan area was 25 μm^2.

2.8. Statistical Analysis. At least three independent experiments were performed, each in triplicate. Numerical data were analyzed using GraphPad Prism 6 (San Diego, California, USA). One-way analysis of variance (ANOVA) followed by post hoc Tukey's test was used to determine statistical significance. A P value below 0.05 was considered statistically significant.

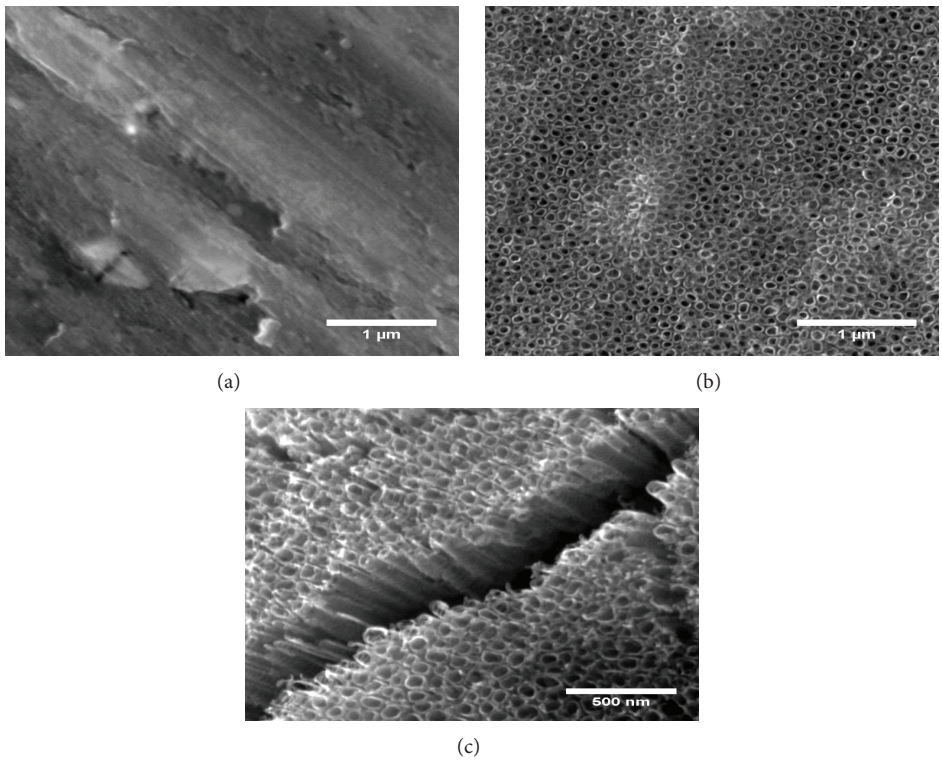
3. Results

3.1. Surface Characterization. Nonanodized Ti6Al4V alloy surface morphology is depicted in Figure 1(a), denoting a smooth texture as expected. Following anodization, a uniform layer of NTs was fabricated over the Ti6Al4V alloy surface, as observed in Figure 1(b). Cross section view is presented in Figure 1(c). The NTs diameter was estimated to be 80 nm and the length 380 nm.

The EDX analyses suggest the formation of NTs based on an increased percentage of oxygen in the chemical composition analysis compared to the nonanodized alloy (see Table 1). Moreover, a trace amount of fluoride can be detected on the NTs surface as shown by an increased percentage of fluoride content (Table 1).

3.2. Bacterial Viability on the Samples by CFU Analysis. The *S. aureus* viability on the samples is shown in Figure 2. At day 1 (D1) of bacterial incubation, the NTs showed 35.4% ($P < 0.05$) decreased viable bacteria when compared to nonanodized material. At day 3 (D3), the number of viable bacteria increased on both materials; nonetheless, a lower bacterial viability was observed on the NTs surface, that is, a 31.5% bacterial growth inhibition ($P < 0.05$) in comparison to the nonanodized material. Finally at day 5 (D5), the NTs surface showed 25% ($P < 0.05$) reduced viable bacteria compared to nonanodized alloy. Also, at D5 no significant changes in bacterial viability were observed compared to D3 on both samples.

3.3. Bacterial Density and Morphology on Samples by SEM. The morphology of the bacterial colonies on the anodized and nonanodized alloys did not vary, as tested by SEM (Figure 3). However, at D1 of incubation we detected an increased cellular colonization and agglomeration on the nonanodized alloy surface (Figure 3(a)) compared to the NTs (Figure 3(b)). At D3, the bacterial colonization increased in both samples (Figures 3(c) and 3(d)), despite being to a lower extent on the NTs surface (Figure 3(d)). Similarly, at D5 adhered bacteria were higher on the nonanodized alloy (Figure 3(e)) when compared to the NTs surface (Figure 3(f)); nevertheless, no apparent increase was found on bacterial density when compared to D3.



(a) (b)

(c)

Figure 1: FE-SEM micrographs of the samples. The nonanodized and anodized Ti6Al4V alloy surfaces were characterized and compared. (a) Nonanodized alloy, (b) NTs, and (c) cross section view of NTs.

3.4. Bacterial Topography. The *S. aureus* topography after D1 of culture on anodized and nonanodized alloys is displayed in Figure 4. There were no apparent bacterial topographic changes on nonanodized (Figure 4(a)) and anodized alloys (Figure 4(b)). Thus, the bacterial topography was not altered by the NTs formed on the anodized alloy.

4. Discussion

Infections of dental and orthopedic devices are strongly associated with bacterial adhesion to the material surface [1, 40]. Once bacteria adhere to the material surface the colony proliferates forming a biofilm [14]. There are two stages involved in the mechanism of the biofilm formation. The first stage comprises the bacterial attachment to the surface by physicochemical interactions [3, 14], while in the second stage, there are cellular and molecular interactions between bacteria that provide an adequate environment for bacterial growth [3, 41]. Thus, according to this fact, by inhibiting the bacterial adhesion one could avoid the biofilm development and therefore reduce the probability of BAI. Previous investigations evaluating changes of bacterial adhesion on the surface of modified commercial implants have shown promising results; that is, Cochis et al. suggested decreased bacterial growth for up to 72 h using Ti surfaces grafted with gallium ions [42]; Jin et al. reported increased in vitro antibacterial performance of Zn/Ag nanoparticles immersed by plasma ion implantation on a Ti surface [43]. In this regard, implants with nanostructured surfaces have been widely investigated for their capacity to enhance the biocompatibility of orthopedic and dental materials, which may also have improved their antibacterial properties [44–47].

In the present study, we built self-organized and ordered NTs (80 nm of diameter) using a SOW containing fluoride by anodization on Ti6Al4V alloy surface, requiring just only 5 min of anodization to obtain similar morphology NTs as reported by Wang et al. who anodized Ti6Al4V for 1 h [28] and Narayanan et al. for 2 h [21] using fluoride and deionized water as electrolyte. This trend could be explained by the presence of the oxidized components that with fluoride synergistically may act promoting a faster oxidation which leads to a nanostructured morphology and significantly impacting the adhesion and colonization of *S. aureus* up to 5 days of incubation. As previously mentioned, the NTs were successfully obtained by the anodization process with traces of fluoride and a decrease in Al due to an increment of oxygen as illustrated by the EDX analysis (Table 1), data which are in accordance with previous reports [3, 26, 48].

Hitherto, the eradication of biofilms-associated infections on orthopedic and dental implants has been an elusive goal [49]. Several approaches have been undertaken to treat those infections, such as the prophylactic and prolonged use of antimicrobial therapy, as well as the use of biomaterials loaded with drugs [50, 51]. However, such options individually possess limitations due to antibiotic-resistance, fast delivery of the immobilized drug, and toxicity or failure that ultimately leads to removal of the implanted biomaterial

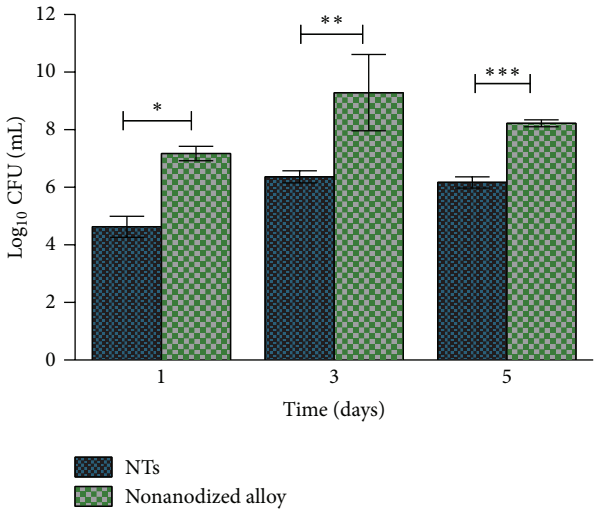


Figure 2: Viability evaluation of *S. aureus* incubated on nonanodized alloy and NTs discs after 1, 3, and 5 days of incubation. The bar graphs show the mean ± SD error bars, $N = 3$, $^{*}P < 0.05$ denotes significance between nonanodized alloy and NTs after 1 day, $^{**}P < 0.05$ indicates significance between nonanodized alloy and NTs after 3 days, and $^{***}P < 0.05$ illustrates significance between nonanodized alloy and NTs after 5 days of incubation.

[11, 52–54]. Therefore, in this study a surface modification on Ti6Al4V alloy was selected to fabricate NTs as an alternative to improve the resistance against bacterial adhesion and on the other hand to promote osteoblast biocompatibility leading to a successful osseointegration process as reported by others [3, 55, 56]. However, these methods require long anodization periods; for instance. Pérez-Jorge et al. described an anodizing method for the fabrication of amorphous NTs that takes a period of 1 h using fluoride as electrolyte in an aqueous solution, showing decreased bacterial adhesion when compared to nonanodized Ti [3]. Similarly, Wang et al. explored an anodization process of 1 h using fluoride dissolved in distilled water, which showed increased osteoblasts-like cells (SaOS2) biocompatibility [56]. Moreover, Portan et al. performed an electrochemical anodization method using distilled water and fluoride and reported enhanced human bone marrow cellular adhesion when compared to nonanodized Ti [55]. Here we report a rapid, simple, and easy anodization process for the synthesis of NTs. This process requires a short period of only 5 min, for fabrication of NTs similar to those obtained in the aforementioned reports. As suggested by the data, the NTs surface denoted a decreased bacterial viability as assessed by CFU counting for up to 5 days of culture as compared to the nonanodized Ti6Al4V alloy. Furthermore, the SEM analysis also suggested a decreased number of adhered cells on the NTs surfaces for the mentioned incubation periods, supporting the viability results. Recently Pérez-Jorge et al. suggested that NTs of 80 nm diameter on Ti limited the bacterial growth of *Staphylococcus epidermidis* (denoted here as *S. epidermidis*) more than Cp-Ti [3]. Moreover, Zhang et al. evaluated *S. aureus* adhesion on NTs and NTs incorporated with vancomycin, showing decreased adhesion after 6 h of culture for both surfaces as compared to Cp-Ti [57]. Possible explanations for such observed effects on the amorphous 80 nm NTs are the increased surface roughness and the decreased water contact angle, where the latter allows the dispersion of bacteria on the surface, thereby limiting its communication, protein deposition, and colonization [3, 13, 26, 30], a property widely observed on amorphous anodized NTs containing fluoride [3, 26, 30].

Previous studies evaluating the NTs surfaces reported an enhanced antibacterial activity after short times: 1 h [26], 4 h [58], and 24 h [30] of incubation with *S. aureus* and *S. epidermidis*. Nonetheless, here we report for the first time a reduction of viable bacteria up to 5 days of culture onto 80 nm NTs compared to nonanodized Ti6AlV4 alloy. The bacterial topography did not account for the observed antibacterial effects, since there were no changes on either bacterial morphology or topography. These results are in agreement with previous reports regarding the *S. aureus* morphology on anodized amorphous NTs [30]. Thus, we hypothesize that the presence of fluoride observed on the NTs may decrease the adhesion forces of *S. aureus*, thereby reducing its attachment without altering its topographical structure as suggested by others [59]. Perhaps, other factors that can contribute to the decrease of the bacterial viability are the contact with electrolyzed components (such as H_2O_2, oxidizing radicals, and chlorine molecules) from the SOW solution which could decrease the bacterial viability [33, 34] or the differences of the surface morphology (between nonanodized Ti6Al4V alloy and NTs) [26, 60]. Furthermore, Pérez-Jorge et al. compared the antibacterial effects of NTs with and without fluoride, suggesting that the presence of fluoride strongly decreased bacterial adhesion [3]. Ercan et al. suggested that anodized amorphous NTs with fluoride could decrease *S. aureus* growth for up to 2 days of bacterial culture when compared to heat-treated anodized NTs without fluoride [30]. In a previous work it was described that 80 nm diameter NTs negatively impact *S. aureus* and *S. epidermidis* viability when compared to nonanodized Ti and smaller diameter NTs (20 or 40 nm) [60]. Similarly, Puckett et al. depicted different bacterial behavior on nanorough, nanotextured, and nanotubular Ti and on nonanodized Ti, reporting an increased number of dead bacteria on anodized nanotubular Ti [26]. The aforementioned evidence clearly indicates that the material surface topography influences bacterial response to a biomaterial, where nanostructured surfaces may offer one of the best options to impede infections, as observed in the current study.

In addition, there are some in vitro reports indicating that NTs fabricated with fluoride could positively impact the biocompatibility, suggesting increased cell proliferation [23, 61], and in vivo osseointegration [62, 63]. Recently, we showed enhanced osteoblast and chondrocyte adhesion and viability on 80 nm NTs when compared to nonanodized alloy [64], thus evidencing that anodized 80 nm NTs fabricated with SOW not only offers excellent antibacterial efficiency but also importantly enhanced biocompatibility in comparison to nonanodized alloy.

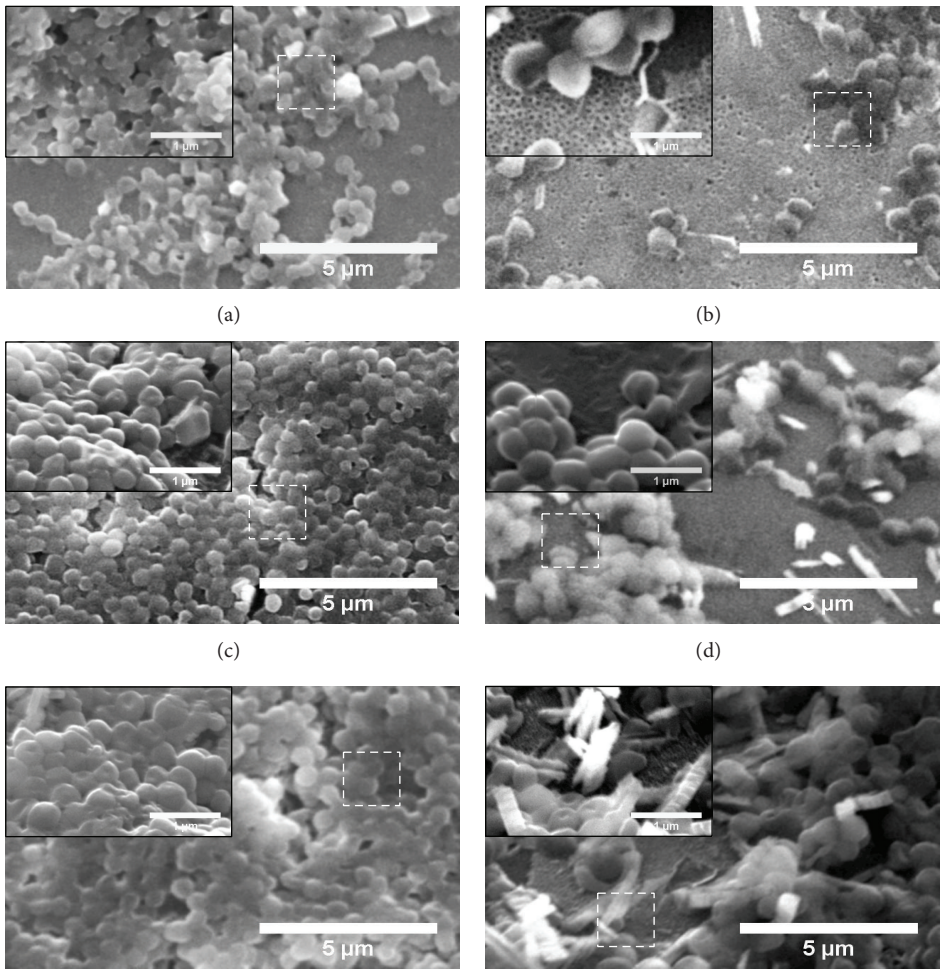


Figure 3: SEM micrographs of *S. aureus* adhered on the experimental substrates. (a) Nonanodized alloy and (b) NTs after D1; (c) nonanodized alloy and (d) NTs after D3; and (e) nonanodized alloy and (f) NTs after D5.

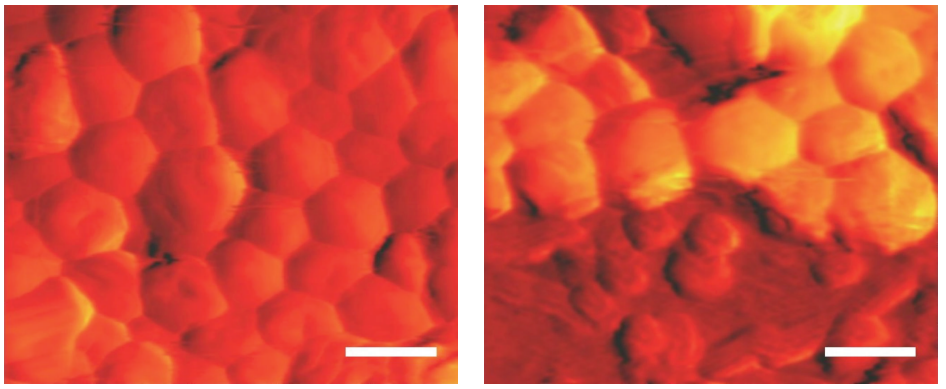


Figure 4: AFM images of *S. aureus* on the surfaces. Differences between bacterial topography were assessed at D1 of culture on the experimental samples. (a) Nonanodized alloy and (b) NTs. Scale bar is 5 μm.

All these results prove that anodization process can be performed using a SOW solution with fluoride as an electrolyte to dramatically reduce the time of anodization and to promote antibacterial properties. Most likely the observed effects are due to the presence of fluoride, the nanostructured surface morphology, and the oxidizing species of the SOW.

5. Conclusions

Surface modification of Ti6Al4V alloy with 80 nm diameter NTs obtained using SOW and fluoride as electrolyte enhances the antibacterial effects of the material against *S. aureus*. We suggest that bacterial inhibition of the NTs can be due to the presence of fluoride derived from the anodization process, modification of the surface morphology, and the oxidized species present in the SOW. The obtained results indicate that NTs fabricated by anodic oxidation using SOW solution enriched with fluoride can be used as a means for the development of biomedical implants with promoted antibacterial properties. However, more investigation regarding the interactions between *S. aureus* and NTs is required in order to corroborate those findings.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Ernesto Beltrán-Partida and Benjamin Valdez-Salas contributed equally to this work.

Acknowledgment

This work was supported by Consejo Nacional de Ciencia y Tecnología (CONACYT-CVU 348737, 114359) for a Ph.D. scholarship.

References

[1] M. Riool, L. de Boer, V. Jaspers et al., "*Staphylococcus epidermidis* originating from titanium implants infects surrounding tissue and immune cells," *Acta Biomaterialia*, vol. 10, no. 12, pp. 5202–5212, 2014.

[2] M.-Y. Lan, C.-P. Liu, H.-H. Huang, and S.-W. Lee, "Both enhanced biocompatibility and antibacterial activity in Ag-decorated TiO_2 nanotubes," *PLoS ONE*, vol. 8, no. 10, Article ID e75364, 2013.

[3] C. Pérez-Jorge, A. Conde, M. A. Arenas et al., "In vitro assessment of Staphylococcus epidermidis and Staphylococcus aureus adhesion on TiO_2 nanotubes on Ti-6Al-4V alloy," *Journal of Biomedical Materials Research Part: A*, vol. 100, no. 7, pp. 1696–1705, 2012.

[4] K. M. D. Merollini, R. W. Crawford, and N. Graves, "Surgical treatment approaches and reimbursement costs of surgical site infections post hip arthroplasty in Australia: a retrospective analysis," *BMC Health Services Research*, vol. 13, article 91, 2013.

[5] K. J. Bozic, S. M. Kurtz, E. Lau et al., "The epidemiology of revision total knee arthroplasty in the united states," *Clinical Orthopaedics and Related Research*, vol. 468, no. 1, pp. 45–51, 2010.

[6] J. Rivadeneira, A. L. Di Virgilio, M. C. Audisio, A. R. Boccaccini, and A. A. Gorustovich, "Evaluation of antibacterial and cytotoxic effects of nano-sized bioactive glass/collagen composites releasing tetracycline hydrochloride," *Journal of Applied Microbiology*, vol. 116, no. 6, pp. 1438–1446, 2014.

[7] C. Soundrapandian, S. Datta, and B. Sa, "Drug-eluting implants for osteomyelitis," *Critical Reviews in Therapeutic Drug Carrier Systems*, vol. 24, no. 6, pp. 493–545, 2007.

[8] M.-T. Tsai, Y.-Y. Chang, H.-L. Huang, J.-T. Hsu, Y.-C. Chen, and A. Y.-J. Wu, "Characterization and antibacterial performance of bioactive Ti–Zn–O coatings deposited on titanium implants," *Thin Solid Films*, vol. 528, pp. 143–150, 2013.

[9] M. Mattioli-Belmonte, S. Cometa, C. Ferretti et al., "Characterization and cytocompatibility of an antibiotic/chitosan/cyclodextrins nanocoating on titanium implants," *Carbohydrate Polymers*, vol. 110, pp. 173–182, 2014.

[10] J. S. Moskowitz, M. R. Blaisse, R. E. Samuel et al., "The effectiveness of the controlled release of gentamicin from polyelectrolyte multilayers in the treatment of *Staphylococcus aureus* infection in a rabbit bone model," *Biomaterials*, vol. 31, no. 23, pp. 6019–6030, 2010.

[11] M. Ma, M. Kazemzadeh-Narbat, Y. Hui et al., "Local delivery of antimicrobial peptides using self-organized TiO_2 nanotube arrays for peri-implant infections," *Journal of Biomedical Materials Research Part A*, vol. 100, no. 2, pp. 278–285, 2012.

[12] J. Esteban and J. Cordero-Ampuero, "Treatment of prosthetic osteoarticular infections," *Expert Opinion on Pharmacotherapy*, vol. 12, no. 6, pp. 899–912, 2011.

[13] M. A. Arenas, C. Pérez-Jorge, A. Conde et al., "Doped TiO_2 anodic layers of enhanced antibacterial properties," *Colloids and Surfaces B: Biointerfaces*, vol. 105, pp. 106–112, 2013.

[14] J. D. Bryers, "Medical biofilms," *Biotechnology and Bioengineering*, vol. 100, no. 1, pp. 1–18, 2008.

[15] D. M. Livermore, "Has the era of untreatable infections arrived?" *Journal of Antimicrobial Chemotherapy*, vol. 64, no. 1, pp. i29–i36, 2009.

[16] A. M. Gallardo-Moreno, M. A. Pacha-Olivenza, L. Saldaña et al., "In vitro biocompatibility and bacterial adhesion of physico-chemically modified Ti6Al4V surface by means of UV irradiation," *Acta Biomaterialia*, vol. 5, no. 1, pp. 181–192, 2009.

[17] L. G. Harris, S. Tosatti, M. Wieland, M. Textor, and R. G. Richards, "*Staphylococcus aureus* adhesion to titanium oxide surfaces coated with non-functionalized and peptide-functionalized poly(l-lysine)-grafted-poly(ethylene glycol) copolymers," *Biomaterials*, vol. 25, no. 18, pp. 4135–4148, 2004.

[18] R. R. Maddikeri, S. Tosatti, M. Schuler et al., "Reduced medical infection related bacterial strains adhesion on bioactive RGD modified titanium surfaces: a first step toward cell selective surfaces," *Journal of Biomedical Materials Research Part: A*, vol. 84, no. 2, pp. 425–435, 2008.

[19] S. Ferraris, A. Venturello, M. Miola, A. Cochis, L. Rimondini, and S. Spriano, "Antibacterial and bioactive nanostructured titanium surfaces for bone integration," *Applied Surface Science*, vol. 31, pp. 279–291, 2014.

[20] C. Giordano, E. Saino, L. Rimondini et al., "Electrochemically induced anatase inhibits bacterial colonization on Titanium Grade 2 and Ti6Al4V alloy for dental and orthopedic devices,"

Colloids and Surfaces B: Biointerfaces, vol. 88, no. 2, pp. 648–655, 2011.

[21] R. Narayanan, H.-J. Lee, T.-Y. Kwon, and K.-H. Kim, "Anodic TiO_2 nanotubes from stirred baths: Hydroxyapatite growth & osteoblast responses," *Materials Chemistry and Physics*, vol. 125, no. 3, pp. 510–517, 2011.

[22] X. Cao, W.-Q. Yu, J. Qiu, Y.-F. Zhao, Y.-L. Zhang, and F.-Q. Zhang, "RGD peptide immobilized on TiO_2 nanotubes for increased bone marrow stromal cells adhesion and osteogenic gene expression," *Journal of Materials Science: Materials in Medicine*, vol. 23, no. 2, pp. 527–536, 2012.

[23] K. Burns, C. Yao, and T. J. Webster, "Increased chondrocyte adhesion on nanotubular anodized titanium," *Journal of Biomedical Materials Research Part A*, vol. 88, no. 3, pp. 561–568, 2009.

[24] R. Narayanan, P. Mukherjee, and S. K. Seshadri, "Synthesis, corrosion and wear of anodic oxide coatings on Ti-6Al-4V," *Journal of Materials Science: Materials in Medicine*, vol. 18, no. 5, pp. 779–786, 2007.

[25] E. M. Christenson, K. S. Anseth, J. J. J. P. van den Beucken et al., "Nanobiomaterial applications in orthopedics," *Journal of Orthopaedic Research*, vol. 25, no. 1, pp. 11–22, 2007.

[26] S. D. Puckett, E. Taylor, T. Raimondo, and T. J. Webster, "The relationship between the nanostructure of titanium surfaces and bacterial attachment," *Biomaterials*, vol. 31, no. 4, pp. 706–713, 2010.

[27] K. Das, S. Bose, and A. Bandyopadhyay, "TiO_2 nanotubes on Ti: influence of nanoscale morphology on bone cell-materials interaction," *Journal of Biomedical Materials Research Part: A*, vol. 90, no. 1, pp. 225–237, 2009.

[28] L. Wang, T.-T. Zhao, Z. Zhang, and G. Li, "Fabrication of highly ordered TiO_2 nanotube arrays via anodization of Ti-6Al-4V alloy sheet," *Journal of Nanoscience and Nanotechnology*, vol. 10, no. 12, pp. 8312–8321, 2010.

[29] C. Yao, E. B. Slamovich, and T. J. Webster, "Enhanced osteoblast functions on anodized titanium with nanotube-like structures," *Journal of Biomedical Materials Research—Part A*, vol. 85, no. 1, pp. 157–166, 2008.

[30] B. Ercan, K. M. Kummer, K. M. Tarquinio, and T. J. Webster, "Decreased *Staphylococcus aureus* biofilm growth on anodized nanotubular titanium and the effect of electrical stimulation," *Acta Biomaterialia*, vol. 7, no. 7, pp. 3003–3012, 2011.

[31] S. Grigorescu, C. Ungureanu, R. Kirchgeorg, P. Schmuki, and I. Demetrescu, "Various sized nanotubes on TiZr for antibacterial surfaces," *Applied Surface Science*, vol. 270, pp. 190–196, 2013.

[32] G. Colon, B. C. Ward, and T. J. Webster, "Increased osteoblast and decreased Staphylococcus epidermidis functions on nanophase ZnO and TiO_2," *Journal of Biomedical Materials Research Part: A*, vol. 78, no. 3, pp. 595–604, 2006.

[33] C. Landa-Solis, D. González-Espinosa, B. Guzmán-Soriano et al., "Microcyn: a novel super-oxidized water with neutral pH and disinfectant activity," *Journal of Hospital Infection*, vol. 61, no. 4, pp. 291–299, 2005.

[34] M. Gunaydin, S. Esen, A. Karadag et al., "In vitro antimicrobial activity of Medilox super-oxidized water," *Annals of Clinical Microbiology and Antimicrobials*, vol. 13, article 29, 2014.

[35] L. Zhao, S. Mei, W. Wang, P. K. Chu, Z. Wu, and Y. Zhang, "The role of sterilization in the cytocompatibility of titania nanotubes," *Biomaterials*, vol. 31, no. 8, pp. 2055–2063, 2010.

[36] I. Yoda, H. Koseki, M. Tomita et al., "Effect of surface roughness of biomaterials on *Staphylococcus epidermidis* adhesion," *BMC Microbiology*, vol. 14, article 234, 2014.

[37] G. Bjerkan, E. Witso, and K. Bergh, "Sonication is superior to scraping for retrieval of bacteria in biofilm on titanium and steel surfaces in vitro," *Acta Orthopaedica*, vol. 80, no. 2, pp. 245–250, 2009.

[38] R. Nicholas, P. Dunton, A. Tatham, and L. Fielding, "The effect of ozone and open air factor on surface-attached and biofilm environmental Listeria monocytogenes," *Journal of Applied Microbiology*, vol. 115, no. 2, pp. 555–564, 2013.

[39] Y.-S. Kim, K. S. Kim, I. Han, M.-H. Kim, M. H. Jung, and H.-K. Park, "Quantitative and qualitative analysis of the antifungal activity of allicin alone and in combination with antifungal drugs," *PLoS ONE*, vol. 7, no. 6, Article ID e38242, 2012.

[40] J. C. M. Souza, P. Ponthiaux, M. Henriques et al., "Corrosion behaviour of titanium in the presence of Streptococcus mutans," *Journal of Dentistry*, vol. 41, no. 6, pp. 528–534, 2013.

[41] Y. H. An and R. J. Friedman, "Concise review of mechanisms of bacterial adhesion to biomaterial surfaces," *Journal of Biomedical Materials Research*, vol. 43, no. 3, pp. 338–348, 1998.

[42] A. Cochis, B. Azzimonti, C. D. Valle, R. Chiesa, C. R. Arciola, and L. Rimondini, "Biofilm formation on titanium implants counteracted by grafting gallium and silver ions," *Journal of Biomedical Materials Research Part A*, vol. 103, no. 3, pp. 1176–1187, 2015.

[43] G. Jin, H. Qin, H. Cao et al., "Synergistic effects of dual Zn/Ag ion implantation in osteogenic activity and antibacterial ability of titanium," *Biomaterials*, vol. 35, no. 27, pp. 7699–7713, 2014.

[44] F. Wang, L. Shi, W.-X. He et al., "Bioinspired micro/nano fabrication on dental implant-bone interface," *Applied Surface Science*, vol. 265, pp. 480–488, 2013.

[45] N. Wang, H. Li, W. Lü et al., "Effects of TiO_2 nanotubes with different diameters on gene expression and osseointegration of implants in minipigs," *Biomaterials*, vol. 32, no. 29, pp. 6900–6911, 2011.

[46] L. M. Bjursten, L. Rasmusson, S. Oh, G. C. Smith, K. S. Brammer, and S. Jin, "Titanium dioxide nanotubes enhance bone bonding in vivo," *Journal of Biomedical Materials Research Part A*, vol. 92, no. 3, pp. 1218–1224, 2010.

[47] C. von Wilmowsky, S. Bauer, R. Lutz et al., "In vivo evaluation of anodic TiO_2 nanotubes: an experimental study in the pig," *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, vol. 89, no. 1, pp. 165–171, 2009.

[48] E. Matykina, J. M. Hernandez-López, A. Conde, C. Domingo, J. J. De Damborenea, and M. A. Arenas, "Morphologies of nanostructured TiO_2 doped with F on Ti–6Al–4V alloy," *Electrochimica Acta*, vol. 56, no. 5, pp. 2221–2229, 2011.

[49] Y. Ma, M. Chen, J. E. Jones, A. C. Ritts, Q. Yu, and H. Sun, "Inhibition of *Staphylococcus epidermidis* biofilm by trimethylsilane plasma coating," *Antimicrobial Agents and Chemotherapy*, vol. 56, no. 11, pp. 5923–5937, 2012.

[50] W. Zimmerli, "Clinical presentation and treatment of orthopaedic implant-associated infection," *Journal of Internal Medicine*, vol. 276, no. 2, pp. 111–119, 2014.

[51] M. D. Taylor and L. M. Napolitano, "Methicillin-resistant Staphylococcus aureus infections in vascular surgery: increasing prevalence," *Surgical Infections*, vol. 5, no. 2, pp. 180–187, 2004.

[52] L. R. Hoffman, D. A. D'Argenio, M. J. MacCoss, Z. Zhang, R. A. Jones, and S. I. Miller, "Aminoglycoside antibiotics induce bacterial biofilm formation," *Nature*, vol. 436, no. 7054, pp. 1171–1175, 2005.

[53] A. Gao, R. Hang, X. Huang et al., "The effects of titania nanotubes with embedded silver oxide nanoparticles on bacteria and osteoblasts," *Biomaterials*, vol. 35, no. 13, pp. 4223–4235, 2014.

[54] S.-Y. Park, Y. P. Chong, H. J. Park et al., "Agr dysfunction and persistent methicillin-resistant Staphylococcus aureus bacteremia in patients with removed eradicable foci," *Infection*, vol. 41, no. 1, pp. 111–119, 2013.

[55] D. V. Portan, A. A. Kroustalli, D. D. Deligianni, and G. C. Papanicolaou, "On the biocompatibility between TiO_2 nanotubes layer and human osteoblasts," *Journal of Biomedical Materials Research Part A*, vol. 100, no. 10, pp. 2546–2553, 2012.

[56] Y. Wang, C. Wen, P. Hodgson, and Y. Li, "Biocompatibility of TiO_2 nanotubes with different topographies," *Journal of Biomedical Materials Research Part A*, vol. 102, no. 3, pp. 743–751, 2014.

[57] H. Z. Zhang, Y. Sun, A. Tian et al., "Improved antibacterial activity and biocompatibility on vancomycin-loaded TiO_2 nanotubes: In vivo and in vitro studies," *International Journal of Nanomedicine*, vol. 8, pp. 4379–4389, 2013.

[58] Z. Peng, J. Ni, K. Zheng et al., "Dual effects and mechanism of TiO_2 nanotube arrays in reducing bacterial colonization and enhancing C3H10T1/2 cell adhesion," *International Journal of Nanomedicine*, vol. 8, pp. 3093–3105, 2013.

[59] P. Loskill, C. Zeitz, S. Grandthyll et al., "Reduced adhesion of oral bacteria on hydroxyapatite by fluoride treatment," *Langmuir*, vol. 29, no. 18, pp. 5528–5533, 2013.

[60] B. Ercan, E. Taylor, E. Alpaslan, and T. J. Webster, "Diameter of titanium nanotubes influences anti-bacterial efficacy," *Nanotechnology*, vol. 22, no. 29, Article ID 295102, 2011.

[61] S. F. Lamolle, M. Monjo, M. Rubert, H. J. Haugen, S. P. Lyngstadaas, and J. E. Ellingsen, "The effect of hydrofluoric acid treatment of titanium surface on nanostructural and chemical changes and the growth of MC3T3-E1 cells," *Biomaterials*, vol. 30, no. 5, pp. 736–742, 2009.

[62] M. Monjo, S. F. Lamolle, S. P. Lyngstadaas, H. J. Rønold, and J. E. Ellingsen, "In vivo expression of osteogenic markers and bone mineral density at the surface of fluoride-modified titanium implants," *Biomaterials*, vol. 29, no. 28, pp. 3771–3780, 2008.

[63] S. F. Lamolle, M. Monjo, S. P. Lyngstadaas, J. E. Ellingsen, and H. J. Haugen, "Titanium implant surface modification by cathodic reduction in hydrofluoric acid: surface characterization and in vivo performance," *Journal of Biomedical Materials Research Part A*, vol. 88, no. 3, pp. 581–588, 2009.

[64] E. Beltrán-Partida, A. Moreno-Ulloa, B. Valdez-Salas et al., "Improved osteoblast and chondrocyte adhesion and viability by surface-modified Ti6Al4V alloy with anodized TiO_2 nanotubes using a super-oxidative solution," *Materials*, vol. 8, no. 3, pp. 867–883, 2015.