Gentamicin-Loaded TiO$_2$ Nanotubes as Improved Antimicrobial Surfaces for Orthopedic Implants

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Titanium oxide nanotube arrays are extremely promising materials for localized drug delivery in orthopedic implants due to their excellent properties and facile preparation. TiO$_2$ nanotubes can act as an effective drug reservoir to prevent bacterial colonization and implant infection and, at the same time, could promote tissue regeneration and effective osseointegration. Here, highly ordered TiO$_2$ nanotubes (NTs) were synthesized by electrochemical anodization of titanium foils and the process parameters were varied in order to obtain a large range of NT diameters to evaluate its influence. The effect of NTs’ diameter on gentamicin loading/release and on osteosarcoma cell and bacterial adhesion was assessed. Anodization was confirmed as an easy and effective method to prepare highly ordered, open top nanotubes with predictable diameter as a function of imposed voltage. A lower amount of bacteria $Staphylococcus Aureus$ adhesion was found on unloaded NTs surfaces at 24 h. When gentamicin was loaded, protracted release and antibacterial action was observed and bacteria adhesion was prevented on all NTs dimension. However, higher cell proliferation and a more favorable cell morphology was observed on smaller nanotubes, to support the indication toward a reduction in NTs diameter for the preparation of effective implant surfaces.

Keywords: nanotubes, titanium oxide, drug delivery, anodization, antibacterial

INTRODUCTION

One of the main challenges in the orthopedic field is the development of implant surfaces that combine a fast and durable fixation with an effective limitation of bacterial adhesion, to prevent implant failure and the incidence of implant-associated osteomyelitis.

Among the large variety of methods aimed at improving the interfacial properties of titanium implants (Giavaresi et al., 2008; De Nardo et al., 2012; LeGeros et al., 2016; Jäger et al., 2017; Zhao et al., 2018; Stewart et al., 2019), the generation of TiO$_2$ nanotubes (NTs) by anodization has recently attracted considerable attention with the objective to evolve from osteoconductive to osteoinductive implant performance (Farid, 2019).

TiO$_2$ nanotubes were first described in the nineties as having a “columnar honeycomb-like lattice,” observed upon addition of fluoride ionic species to the electrolyte in the anodization of Ti and Ti6Al4V (Zwillinger and Darqué-Ceretti, 1997). More recently, titania nanotubes have gained interest as bone contact surfaces mainly for two characteristic properties: a unique topography, to support early osteoblast adhesion and proliferation (Iwata et al., 2017) and the possibility to
incorporate different classes of biologically-active molecules (either with osteogenic or antimicrobial activity) (Liu et al., 2016; Tao et al., 2019; Ion et al., 2020).

In vitro experiments on nanotube surfaces have shown improved cell adhesion, proliferation, and differentiation, as well as enhanced bone-forming abilities (Xia et al., 2012). In vivo experiments with screw- and disk-shaped implants have shown that nanotubes’ surfaces increase direct bone/cell contact and improve osseointegration strength compared to their blasted counterpart (Bjursten et al., 2010; Sul, 2010; Li et al., 2019).

The effectiveness of different bioactive agents differently loaded into nanotubes, which includes the simple adsorption of growth factors (e.g., bone morphogenetic protein-2; Li et al., 2019) or antibiotic compounds, as well as coatings with biopolymers (Huang et al., 2014; Saha et al., 2019), was investigated. Gentamicin-loaded nanotubes, in particular, were used to deliver high doses of antibiotics locally and inhibit bacterial adhesion without systemic toxicity and with no significant effect on osteogenic differentiation (Lin et al., 2014).

Together with the potential benefits in terms of clinical response, the processes for the preparation of nanotubes is simple, scalable, reproducible (Maher et al., 2018), and applicable to relatively complex 3D geometries. TiO2 nanotubes can be effectively grown on any pure titanium surface, and, in principle, to different titanium alloys’ surfaces of implantable orthopedic devices. Sandblasted surfaces, as well as machined surfaces, are eligible substrates for their preparation by anodization. Furthermore, according to specific clinical needs, drug loading can be performed either during the implant production, or, technically, even during surgery.

The array of nanotubes obtained on titania through electrochemical anodization is highly controllable in dimensions by acting on applied voltage and processing time. To date, however, there appears to be no consensus on the optimal nanotube diameter to promote cellular adhesion and proliferation and effective drug delivery. Although nanotubes with an 100-150 nm diameter were shown to promote cell viability (Brammer et al., 2009; Zhang et al., 2015), apoptosis was also reported for diameters larger than 50 nm (Park et al., 2007, 2009; von Wilmowsky et al., 2009; Kulkarni et al., 2016). Similarly, different works (Popat et al., 2007; Lin et al., 2014) have studied the interaction between gentamicin-loaded nanotubes with bacteria, but little attention has been dedicated to the optimization of drug release in time.

Here, highly ordered and open top nanotubes with different diameters were prepared and loaded with gentamicin to evaluate the effect of dimensions on their “reservoir effect,” loading capacity, and release profiles together with the effectiveness in inhibiting bacterial adhesion and growth and on cellular interaction.

MATERIALS AND METHODS

If not otherwise specified, all reagents were purchased from Sigma-Aldrich and used without further purification.
A calibration curve using six known gentamicin concentrations was used to calculate the actual amount of gentamicin released by nanotubes.

**Agar Diffusion Test**

An agar diffusion test (Hudzicki, 2009) was performed to test the ability of nanotubular surfaces to inhibit bacterial growth using *Staphylococcus aureus* (NCTC 10788). Antibiotic-loaded samples were placed on the agar plate seeded with bacteria and incubated overnight at 37°C; the growth inhibition zone was then measured.

**Microbial Adhesion and Proliferation**

A quantitative measure of antibacterial activity of gentamicin-loaded nanotubes samples were obtained by Colonies Forming Units (CFU) counting (Mathur et al., 2006) of *Staphylococcus aureus*. Ti samples were soaked in a suspension of 10⁷ bacteria in 1 mL of trypticase soy broth (TSB) and incubated at 37°C, then both planktonic and adhered cells were evaluated. Bacterial suspension incubated with no material was used as control.

To count planktonic bacteria, 50 μl of supernatant was collected after 7 and 24 h, plated on agar, and incubated at 37°C for 24 h. To count adherent bacteria, titanium disks were washed in PBS to eliminate non-adherent bacteria, inserted in a centrifuge tube with 2 mL of fresh PBS and 3 small glass spheres (∼3 mm diameter), and vortexed for 30 sec to ensure complete detachment of cells.

**Osteosarcoma Cells Adhesion and Proliferation**

Cells’ adhesion and proliferation on both loaded and unloaded NTs was evaluated using a human osteosarcoma cell model (Saos 2) and by evaluating cell adhesion and proliferation on samples after 24 and 72 h (von der Mark et al., 2009; Demetrescu et al., 2010; Pérez-Jorge et al., 2012).

To determine the number of viable and non-viable cells, 50 μl of Hoechst dye and 50 μl propidium iodide were diluted in 900 μl of medium and 60 μl of the resulting solution was added to each culture well. Images of stained samples were acquired under a fluorescence microscope to count live (blue), apoptotic (brighter blue), and dead (red) cells.

Phalloidin staining was used to evaluate cell morphology. Briefly, 80 μl of 3.7% paraformaldehyde solution in PBS was added in each well to fix the samples. After soaking for 5 min in 1% Triton X solution in PBS to permeabilize the cell membrane, 100 μl phalloidin solution (1:100 in PBS) was added and incubated for 1 h at room temperature.

**Statistical Analysis**

All statistical analyses were performed using GraphPad Prism 7. Groups were compared using one-way ANOVA and post-hoc tests after verification of data normality. *P* < 0.05 were considered statistically significant.

**RESULTS**

**Production and Characterization of TiO₂ Nanotubes**

The relation between anodization voltage and nanotube outer (O.D) and inner diameter (I.D.) are illustrated in Figure 1 and were consistent with previous findings (Lin et al., 2014). However, in the chosen conditions, a voltage threshold of 10 V was found, as no formation of nanotubes could be observed below this value.

As can be seen in Figure 2, an array of highly ordered nanotubes with a well-defined and open top morphology is found on the titanium surface.

To build an experimental group on a representatively extended range of diameters, the voltage values in Table 1 were chosen for experimental samples of NTs’ preparation. For each voltage, the corresponding NTs’ diameters, lengths, and the designations used hereafter are indicated. If the length of nanotubes is considered, a reduction in nanotube depth is observed at lower voltages.

**Drug Loading and Release**

The loading efficiency of gentamicin calculated based on the quantity of antibiotic in the rinsing solution is reported in Table 2. As can be observed, similar values were generally found, but bigger nanotubes (NT160) appeared to have a slightly higher loading efficiency compared to smaller ones.

When the *in vitro* release was considered (Figure 3), a significant fraction of loaded gentamicin was found to be released from NTs after 1 h (about 30%). After this initial burst, the antibiotic release decreased slowly to become undetectable after 48 h.

A comparatively higher amount of gentamicin was released from larger nanotubes, but this is only partially attributable to the higher loading efficiency. Release from larger nanotubes was, in fact, notably faster between 1 and 2 h.
**TABLE 1** | TiO$_2$ nanotubes' diameter, length, and sample designation.

| Voltage (V) | I.D (nm) | O.D (nm) | Length (µm) | Designation |
|-------------|----------|----------|-------------|-------------|
| 20          | 31.4 ± 1.2 | 101.9 ± 3.3 | 7.67 ± 0.02 | NT30        |
| 35          | 78.7 ± 2.6 | 168.2 ± 2.1 | 8.56 ± 0.03 | NT80        |
| 70          | 158.2 ± 3.1 | 257.1 ± 3.0 | 10.26 ± 0.04 | NT160       |

**TABLE 2** | Estimated loading efficiency of gentamicin in TiO$_2$ nanotubes.

| Samples       | Loading efficiency (%) |
|---------------|-------------------------|
| NT30/G        | 60.6                    |
| NT80/G        | 61.9                    |
| NT160/G       | 65.1                    |

**FIGURE 2** | Surface morphology of nanotubes’ array obtained at different voltages (A–C) and section view (D). Scale bar = 1 µm.

**FIGURE 3** | Cumulative gentamicin release from loaded NTs.

**FIGURE 4** | Representative images of inhibition zones in Agar diffusion test for gentamicin-loaded samples.

**FIGURE 4** | Agar Diffusion Test

After incubation at 37°C, an inhibition area was observed for all loaded NT samples on agar plates (Figure 4). All gentamicin-loaded disks were characterized by an inhibition zone with a diameter of about 30 mm, surrounding samples, with no statistically significant difference between the three different NTs’ dimensions. As reasonably expected, untreated Ti samples did not show any detectable inhibition zone.

**FIGURE 5** | Number of adhered bacteria on Ti surface after 24 h incubation. * indicates that no bacteria were detected.

**FIGURE 6** | Bacteria number in planktonic form at 7 and 24 h.

**Bacterial Adhesion and Proliferation**

The number of adhered bacteria for gentamicin loaded and unloaded Ti samples after 24 h incubation is shown in Figure 5.
Compared to untreated Ti disks, nanotube samples show a considerable reduction in bacterial adhesion even without gentamicin. When gentamicin was loaded, bacteria could not be detected.

Interestingly, a significant difference in the number of adhered bacteria was found among nanotube diameters size. Specifically, nanotubes with larger diameters were found to allow a lower bacterial adhesion compared to smaller NTs.

When planktonic bacteria were considered (Figure 6), a comparable CFU count was observed for all samples after 7 h. Conversely, after 24 h a statistically significant difference in bacteria number among untreated Ti and all nanotubes samples was observed, with an average 40% reduction. For planktonic bacteria, however, no significant differences were noticed among the different nanotube diameters size.

**Cells Adhesion and Proliferation**

The normalized number of live cells on the surface of NTs samples after 24 and 72 h are reported for unloaded samples in Figure 7. When gentamicin – loaded samples were considered, superimposable results were found (data not shown). In both cases, statistical analysis did not evidence any significant differences in cell number among NTs with different diameter sizes.

Interestingly, initial cell density is notably lower on NTs samples compared to the control (40% less in average), but the number of cells increases comparatively faster on NTs and the number of cells reaches and overcomes the values on control after 72 h.

Differences in cell morphology were instead observed as a result of change in diameter (Figure 8) and phalloidin staining revealed more spindle-shaped cells on nanotubes with a smaller diameter, whereas more rounded cells were observed for larger NTs.
DISCUSSION AND CONCLUSION

To act as an effective drug reservoir and to offer a high number of anchoring sites for cells, TiO₂ nanotube arrays should possess an open top morphology. In this regard, the protocol for NTs preparation optimized in this work appeared extremely effective. In our experience, the balance of water content and fluoride concentration was critical for obtaining nanotubes with an almost 100% open top while containing the process duration. A higher water content, in fact, results in a more open top structure, but generally requires longer treatment times, while time reduction can be obtained by increasing fluoride content in the solution (the morphologies of NTs’ optimization can be found in Supplementary Material).

By changing the anodization voltage, NTs’ diameter can be easily controlled, as a consistent dependence with applied voltage exists and the size of resulting structures is highly predictable. The reservoir capability of NTs was confirmed by the observed release of loaded gentamicin for up to 48 h. After this time interval, the release quantity was too small to be detected by our assay. However, based on the cumulative release, about half the drug loaded is still present in the nanotubes and is potentially effective for a longer time.

In this work we specifically focused on the effect of nanotubes’ diameters and we tried to analyze the largest range possible to evaluate its effect on drug release and cell adhesion. The diameter of nanotubes was confirmed to be a very relevant variable for both drug release and cell/bacteria interaction. Bigger nanotubes (NT160) were capable of accommodating a comparatively higher quantity of antibiotics than smaller ones, but faster release was observed. On the other hand, smaller nanotubes have a higher available surface, are more likely to withhold the antibiotic molecules more effectively, and showed a slightly slower release.

When Ti samples were incubated with bacteria, no adherent cells were found for all gentamicin samples, to indicate that the quantity of loaded antibiotics is sufficient for an effective antibacterial action. Interestingly, a significant reduction of bacterial adhesion was also observed for unloaded samples, but this phenomenon can possibly be explained by the presence of residual fluoride ions (Ercan et al., 2011; Anitha et al., 2015). The number of adhered bacteria was significantly lower for larger nanotubes (160 nm), and this can also be related to the fact that smaller NTs stimulate bacterial cells to produce pili and attach to the surface (Ercan et al., 2011).

When osteosarcoma cells were seeded on the sample surface, although no statistical difference was observed among NTs with different nanotubes, samples with larger nanotubes appeared to induce cellular apoptosis more than smaller ones. A higher cell compatibility of these latter NTs was also confirmed by the spindle-like morphology observed on their surfaces. This more favorable behavior can be explained with the higher number of anchoring site provided (Roy et al., 2011), as small-sized NTs can promote cell growth and differentiation by supporting a higher density of integrin focal contacts (Zhang et al., 2015).

When using gentamicin, many authors report the use of very high doses (up to 20-fold the recommended concentrations) without appreciable side effect on cells in vitro (Schafer et al., 1972; Sigma-Aldrich, 2002; Kovacikanton et al., 2017). To confirm these results, here no difference in the cell viability between loaded and unloaded samples was observed. However, the chosen gentamicin quantity appears adequate to eliminate the bacteria.

Overall, our results confirm that anodization of titanium substrates to prepare TiO₂ nanotubes is an extremely favorable surface treatment and has the potential to both improve osseointegration of orthopedic prosthesis and prevent infection-related failures. Within the tested range, smaller NTs appear as the more promising option for further investigation, as they offer effective antibacterial action together with a more favorable cell interaction.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/Supplementary Material.

AUTHOR CONTRIBUTIONS

RC contributed to the conception and design of the study. LD, VP, and MM performed the experiments and wrote the first draft of the manuscript. MS performed the in vitro tests. All authors contributed to manuscript revision, read and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmats.2020.00233/full#supplementary-material

REFERENCES

Anitha, V. C., Lee, J. H., Lee, J., Narayan Banerjee, A., Woo Joo, S., and Ki Min, B. (2015). Biofilm formation on a TiO₂ nanotube with controlled pore diameter and surface wettability. Nanotechnology 26:5102.

Apolinario, A., Sousa, C. T., Ventura, J., Costa, J. D., Leitao, D. C., Moreira, J. M., et al. (2014). The role of the Ti surface roughness in the self-ordering of TiO₂ nanotubes: a detailed study of the growth mechanism. J. Mater. Chem. 2, 9067–9078. doi: 10.1039/c4ta00871e

Bjursten, L. M., Rasmusson, L., Oh, S., Smith, G. C., Brammer, K. S., and Jin, S. (2010). Titanium dioxide nanotubes enhance bone bonding in vivo. J. Biomed. Mater. Res. Part A. 92, 1218–1224.

Brammer, K. S., Oh, S., Cobb, C. J., Bjursten, L. M., van der Heyde, H., and Jin, S. (2009). Improved bone-forming functionality on diameter-controlled TiO₂ nanotube surface. Acta Biomater. 5, 3215–3223. doi: 10.1016/j.actbio.2009.05.008

De Nardo, L., Altomare, L., Del Curto, B., Cigada, A., and Draghi, L. (2012). Electrochemical surface modifications of titanium and titanium alloys for
biomedical applications. *Coat. Biomed. Appl.* 2012, 106–142. doi: 10.1533/9780857093677.1.106

Demetrescu, I., Pirvu, C., and Mitran, V. (2010). Effect of nano-topographical features of Ti/TiO2 electrode surface on cell response and electrochemical stability in artificial saliva. *Bioelectrochemistry* 79, 122–129. doi: 10.1016/j.bioelechem.2010.02.001

Ercan, B., Taylor, E., Alpaslan, E., and Webster, T. J. (2011). Diameter of titanium nanotubes influences anti-bacterial efficacy. *Nanotechnology* 22:295102, doi: 10.1088/0957-4484/22/29/295102

Farid, S. B. H. (2019). Osteoinduction, osteoconduction, and osseointegration. *Bioencr. Mater. Sci. Engin.* 10, 596–S101.

Giavaresi, G., Chiesa, R., Fini, M., and Sandrini, E. (2008). Effect of a multiphasic anodic spark deposition coating on the improvement of implant osseointegration in the osteotopic trabecular bone of sheep. *Internat. J. Oral Maxillofacial Impl.* 23:659–68.

Huang, P., Wang, J., Lai, S., Liu, F., Ni, N., Cao, Q., et al. (2014). Surface modified titanium nanotubes containing anti-bacterial drugs for controlled delivery nanosystems with high bioactivity. *J. Mater. Chem. B* 2, 8616–8625. doi: 10.1039/c4tb01281

Hudzikci, J. (2009). *Kirby-Bauer disk diffusion susceptibility test protocol.* Washington DC: American Society for Microbiology, 1–14.

Ion, R., Nceula, M. G., Mazzare, A., Mitran, V., Neacșu, P., Schmuki, P., et al. (2020). Drug Delivery Systems Based on Titania Nanotubes and Active Agents for Enhanced Osseointegration of Bone Implants. *Curr. Med. Chem.* 27, 854–902. doi: 10.2174/0929867X2366619072612329

Iwata, N., Nozaki, K., Horisuchi, N., Yamashita, K., Tatsusumi, Y., Miura, H., et al. (2017). Effects of controlled micro-/nanosurfaces on osteoblast proliferation. *J. Biomed. Mater. Res. Part A* 105, 2589–2596. doi: 10.1002/jbm.a.36118

Jäger, M., Jennissen, H., Dittrich, F., Fischer, A., and Köhling, A. (2017). Antimicrobial and osseointegration properties of nanostructured titanium orthopaedic implants. *Materials* 10, 1302. doi: 10.3390/ma10111302

Kovackivá-Taboada, A., Eva, T., Diana, F., Peter, C., Eva, K., Katarina, Z., et al. (2017). In Vitro Assessment of Gentamicin Cytotoxicity on the Selected Mammalian Cell Line (Vero cells). *Adv. Res. Life Sci* 1, 111–116. doi: 10.1515/arsl-2016-0017

Kulkarni, M., Mazzare, A., Schmuki, P., and Iglíć, A. (2016). Influence of anodization parameters on morphology of TiO2 nanostructured surfaces. *Adv. Mater. Lett.* 7, 23–28. doi: 10.5185/amlett.2016.16.6156

LeGeros, R. Z., Coelho, P. G., Holmes, D., Dimaano, F., and LeGeros, J. P. (2016). “Orthopaedic and dental implant surfaces and coatings,” in Biological and Biomedical Coatings Handbook. S. Zhang Florida: CRC Press.

Li, Y., Song, Y., Ma, A., and Li, C. (2019). Surface Immobilization of Titania Nanotubes with Bone Morphogenetic Protein-2 Synergistically Enhances Initial Preosteoblast Adhesion and Osseointegration. *BioMed. Res. Internat.* 2019:5697250.

Lin, W.-T., Tan, H.-L., Duan, Z.-L., Yue, B., Ma, R., He, G., et al. (2014). Inhibited bacterial biofilm formation and improved osteogenic activity on gentamicin-loaded TiO2 nanotubes for improved osteogenic property of Ti6Al4V bone implants. *Mater. Sci. Engin. C* 35, 109982. doi: 10.1016/j.msec.2019.109982

Schräfer, T., Pascale, A., Shimonoski, G., and Came, P. E. (1972). Evaluation of gentamicin for use in virology and tissue culture. *Appl. Microbiol.* 23, 565–570. doi: 10.1128/aem.23.3.565-570.1972

Schneider, C. A., Rasband, W. S., and Eliceiri, K. W. (2012). NIH Image toIJAGE: 25 years of image analysis. *Nat. Methods* 9, 671–675. doi: 10.1038/nmeth.2089

Schneider Frutos, P. C., Díez Peña, E., Barrales-Rienda, J. M., and Frutos, G. (2000). Validation and in vitro characterization of antibiotic-loaded bone cement release. *Int. J. Pharm.* 209, 15–26. doi: 10.1016/s0378-5173(00)00520-2

Sigma-Aldrich (2002). *Sigma-Aldrich.*

Saha, S., Pramanik, K., and Biswas, A. (2019). Silk fibroin coated TiO2 nanotubes with potential for improved osteogenic property of Ti6Al4V bone implants. *Mater. Sci. Engin. C* 97, 1696–1705. doi: 10.1016/j.msec.2019.109982

Zwilling, V., and Darque-Ceretti, E. (1997). Characterization of porous and microstructured oxide films on titanium and Ti-6A1-4V. *Annales de Chimie: Science des Matériaux.* 177, 242–252. doi: 10.1016/s1572-7097(97)80025-7

von der Mark, K., Bauer, S., Park, J., and Schmuki, P. (2009). Another look at ‘Stem cell fate dictated solely by altered nanotube dimension.

von Wilmowsky, C., Bauer, S., Lutz, R., Meisel, M., Neukam, F. W., Toyoshima, T., et al. (2009). In Vivo Evaluation of Anodic TiO2 Nanotubes; An Experimental Study in the Pig. *J. Biomed. Mater. Res. Part B Appl. Biomater* 89, 165–171.

Zhang, R., Wu, H., Ni, J., Zhao, C., Cheng, Y., Zheng, C., et al. (2015). Guided proliferation and bone-forming functionality on highly ordered large diameter TiO2 nanotube arrays, *Mater. Sci. Eng.* 53, 272–279. doi: 10.1016/j.msec.2015.04.046

Zhou, H., Huang, Y., Zhang, W., Guo, Q., Cui, W., Sun, Z., et al. (2018). Mussel-inspired peptide coatings on titanium implant to improve osseointegration in osteoporotic condition. *ACS Biomater. Sci. Engin.* 4, 2505–2515. doi: 10.1021/acsbiomaterials.8b00261

Zwilling, V., and Darque-Ceretti, E. (1997). Characterization of porous and compact oxide films on titanium and Ti-6Al-4V. *Annales de Chimie: Science des Matériaux.* 177, 487–4881. doi: 10.2147/ijan.s24949

Cheng, Y., Zheng, C., et al. (2015). Guided proliferation and bone-forming functionality on highly ordered large diameter TiO2 nanotube arrays, *Mater. Sci. Eng.* 53, 272–279. doi: 10.1016/j.msec.2015.04.046

Zhou, H., Huang, Y., Zhang, W., Guo, Q., Cui, W., Sun, Z., et al. (2018). Mussel-inspired peptide coatings on titanium implant to improve osseointegration in osteoporotic condition. *ACS Biomater. Sci. Engin.* 4, 2505–2515. doi: 10.1021/acsbiomaterials.8b00261

Zwilling, V., and Darque-Ceretti, E. (1997). Characterization of porous and compact oxide films on titanium and Ti-6Al-4V. *Annales de Chimie: Science des Matériaux.* 177, 242–252. doi: 10.1016/s0378-5173(97)80025-7

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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