

Article

Ceftriaxone-Loaded Ti-407 Nanotubular Oxide for In Vitro Inhibition of Bacteria Associated with Postoperative Infections

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Highlights

What are the main findings?

- Successful growth of TiO₂ nanotubes is obtained by anodization on the Ti-407 alloy.
- The nanotube diameter, length, and density can be controlled by the applied anodization voltage.
- Higher antibiotic loading capacity is achieved in TiO₂ nanotubes at higher voltages.
- Antibacterial activity is observed in ceftriaxone-loaded anodized Ti-407 alloy.

What are the implications of the main findings?

- A new functional surface from Ti-407 alloy is obtained by a simple anodization process.
- Nanotube geometry allows estimation of the drug-loading capacity.
- The drug quantity can be increased by increasing the anodization voltage.
- This functional surface can be used as a new platform for drug delivery.

Abstract

Titanium-based implants are widely used in orthopedic and trauma surgery; however, post-operative infections remain a major cause of implant failure due to early bacterial adhesion. Localized antibiotic delivery from surface coatings offers a promising strategy to prevent initial colonization during the critical postoperative period. In this study, a self-organized TiO₂ nanotubular oxide layer was fabricated on Ti-407 by electrochemical anodization in a glycerol/NH₄F electrolyte at 40–60 V. SEM revealed vertically aligned single-walled nanotubes with diameters and lengths of ~80 nm and ~10 µm respectively. XPS analysis verified TiO₂ formation with Al–O, V–O, and fluorine incorporation. Ceftriaxone was successfully loaded into the nanotubular structure, as identified by FT-IR. UV–Vis measurements showed a biphasic release profile consisting of an initial burst followed by sustained release determined by nanotube geometry. In vitro antibacterial activity was evaluated against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* using optical density, CFU quantification, and an agar diffusion assay. Unloaded surfaces showed no inhibition, whereas ceftriaxone-loaded nanotubes significantly reduced bacterial growth up to ~6% and generated clear inhibition zones. These findings demonstrate, for the first time, that TiO₂ nanotubular coatings derived from Ti-407 support drug loading and demonstrate effective in vitro antibacterial activity, highlighting their potential for infection-resistant orthopedic implants.



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Keywords: TiO₂ nanotubes; Ti-407 alloy; ceftriaxone; drug release; antibacterial activity; postoperative infections

1. Introduction

Orthopedic and trauma device-related infections (ODRIs) remain among the most serious complications in musculoskeletal surgery, leading to substantial postoperative morbidity, implant failure, and increased healthcare costs [1,2]. These infections are also an important cause of surgical reintervention and prolonged hospital stays, imposing a significant clinical and economic burden worldwide [3]. Infection rates vary widely depending on the type of procedure and patient-related risk factors, with substantially higher incidences consistently reported in complex trauma cases, open fractures, and resource-limited clinical settings [4–6]. Contamination of the implant surface typically occurs intraoperatively or within the first hours after implantation, a critical period during which the local immune response is transiently suppressed. Opportunistic pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* can readily adhere to metallic substrates and undergo phenotypic transitions that initiate biofilm formation. This process markedly decreases antimicrobial susceptibility, limiting the effectiveness of systemic antibiotic prophylaxis and often necessitating prolonged therapy, repeated debridement, or even implant removal [7–9].

Titanium and its alloys are widely used in orthopedic implants due to their high strength-to-weight ratio, corrosion resistance, and biocompatibility [10]. However, the naturally formed TiO₂ passive layer, under those conditions, is biologically inert and lacks intrinsic antibacterial activity, allowing early bacterial colonization [11]. On the other hand, Ti-6Al-4V remains the current biomedical standard alloy; however, concerns have arisen regarding the potential biological effects of wear or corrosion products containing aluminum and vanadium. In addition, this alloy exhibits high processing costs and limited machinability, restricting its efficiency in manufacturing complex components. These limitations have driven the search for alternative titanium alloys with greater long-term stability, improved biocompatibility, and more cost-effective processing [12–14].

Ti-407 (Ti-3.9V-0.85Al) has recently attracted attention as an advanced titanium alloy developed for applications requiring high ductility, fracture toughness, and fatigue resistance. In addition to its favorable mechanical performance, Ti-407 offers better machinability and lower processing costs than Ti-6Al-4V, making it an appealing candidate for biomedical use [15,16]. Nevertheless, its biomedical properties and surface behavior remain insufficiently explored, and its potential for developing functional coatings to enhance biological performance and reduce bacterial colonization remains largely unexplored [17]. Investigating these aspects could expand its applicability as a next-generation biomaterial that combines mechanical performance with surface-driven functionality.

Among surface-engineering strategies, electrochemical anodization has proven to be an effective and highly controllable method for modifying titanium surfaces to produce ordered TiO₂ nanotubular oxide layer [18–20]. This process, based on field-assisted oxidation and chemical dissolution in electrolytes containing fluoride, allows precise control over nanotube diameter, length, and wall thickness, yielding hollow, uniform structures with a high surface area [21]. The organized morphology resulting from anodization produces a uniform and structurally stable surface that improves coating adhesion to the metallic substrate [22,23]. At the same time, the oxide layer formed increases corrosion resistance and limits the release of potentially cytotoxic metallic ions in physiological environments [24]. The incorporation of fluoride during the process also contributes to

forming a protective barrier against electrochemical degradation, while simultaneously imparting antibacterial effects by interfering with bacterial adhesion and viability at the material interface [25]. Collectively, these structural and chemical characteristics make the TiO₂ nanotubular oxide layer a stable and versatile platform for the design of protective and bioactive coatings [19,26].

Numerous studies have demonstrated the effectiveness of TiO₂ nanotubes as drug-delivery systems for antimicrobial agents. Anodized titanium and Ti-6Al-4V surfaces loaded with antibiotics such as vancomycin and gentamicin have shown significant reductions in bacterial adhesion and biofilm formation in vitro [27–29]. Similarly, the incorporation of antimicrobial metal ions (e.g., silver or zinc) and bioactive molecules such as antimicrobial peptides and catalytic oxides has been shown to enhance bactericidal performance and extend the protective effect of the coatings [30–34]. The release profile, typically consisting of an initial burst followed by sustained release, is a key factor in maintaining therapeutic concentrations at the implantation site during the early postoperative period [35,36].

Given the complex and often polymicrobial nature of postoperative infections, it is essential to employ broad spectrum antibiotics capable of acting against both Gram-positive and Gram-negative pathogens [37,38]. In this regard, ceftriaxone, a third-generation cephalosporin widely used in perioperative prophylaxis for orthopedic and trauma surgery, represents an appropriate model antibiotic for this type of coating [39,40]. It provides broad antimicrobial coverage against the main pathogens implicated in ODRIs. Unlike previous studies that relied on narrow spectrum antibiotic models, the use of ceftriaxone in this work more accurately reflects the clinical reality of implant infections. Localized and sustained release of ceftriaxone from a nanotubular surface could achieve therapeutic concentrations directly at the implant–tissue interface during the critical early postoperative window, minimizing systemic exposure and reducing the risk of antimicrobial resistance [41–43].

Despite advances in the surface functionalization of conventional titanium alloys, only a limited number of studies have addressed the anodization of Ti-407 or explored its ability to generate TiO₂ nanotubes capable of antibiotic loading and release, there is a lack of the understanding of its biomedical potential [17,29,44].

In this context, the results of this work highlight that a controlled growth of TiO₂ nanotubes can be achieved by an anodization process, with the applied voltage directly correlating with the diameter, length, and density of the developed nanotubes. Consequently, these organized TiO₂ nanotubular oxide layers exhibited the capacity to load and release ceftriaxone in a regulated manner, thereby inhibiting the in vitro proliferation of clinically relevant Gram-positive and Gram-negative bacteria associated with ODRIs.

This work represents a first approach to evaluating the feasibility of functionalizing Ti-407 through TiO₂ nanotubular coatings loaded with antibiotics, thereby bridging the gap between emerging titanium alloy development and antimicrobial surface engineering aimed at creating next-generation, infection-resistant implant materials.

2. Materials and Methods

2.1. Processing of Ti-407 Alloy Samples

Flat plates of Ti-407 titanium alloy, previously thermomechanically studied [45], (TIMETAL[®]407, TIMET, Warrensville Heights, OH, USA) were cut into 1 × 1 cm specimens with a thickness of 3 mm using a precision diamond saw (VC-50, LECO Co., St. Joseph, MI, USA). The surfaces were ground using silicon carbide (SiC) abrasive papers up to 2400 grit and subsequently polished with a 3 µm alumina suspension on a microcloth pad until a mirror-like finish was obtained. Samples were ultrasonically cleaned for 10 min in deionized water, acetone, and ethanol (96%), to remove contaminants. Cleaned speci-

mens were air-dried and stored in a vacuum desiccator until anodization. The processing parameters reported in this work were selected from a broader range of conditions evaluated in preliminary studies [17,29,44], with the final parameters chosen to ensure surface homogeneity and reproducible anodization behavior.

2.2. Electrochemical Anodization

A self-organized nanotubular oxide layer was produced on Ti-407 using a two-electrode anodization system with the alloy as the anode (1 cm² exposed area) and a platinum plate as the cathode. Both electrodes were connected to a DC power supply 9110 (BK Precision Co., Yorba Linda, CA, USA) and separated by 20 mm. The electrolyte consisted of 98 mL glycerol, 2 mL distilled water, and 0.1 M NH₄F (total 100 mL, pH 6.4). Anodization was performed at 40, 50, and 60 V for 30 min at room temperature (25°C). After treatment, samples were rinsed with deionized water, acetone, and ethanol; then they were dried at room temperature, and stored in a desiccator until use.

2.3. Structural and Chemical Characterization

Nanotube morphology was examined using a FEI Nova NanoSEMTM 200 (FEI, Eindhoven, The Netherlands) scanning electron microscope. Imaging was performed in high-vacuum mode with a through-lens detector (TLD) at 10.0 kV accelerating voltage, 5.0 mm working distance, 4.0 spot size, and magnifications up to 240,000×. Cross-sectional and rear views were obtained by fracturing anodized samples.

Surface chemical composition was analyzed using a Thermo Scientific K-Alpha XPS (Thermo Fisher Scientific Inc., Waltham, MA, USA) system equipped with an Al K α monochromatic source (1486.6 eV). High-resolution spectra were acquired in CAE mode using a pass energy of 50 eV, an energy step size of 0.10 eV, a 400 μ m spot size, and 10 accumulated scans. Charge correction was applied by setting the C 1s peak to 284.8 eV.

Drug-loaded and unloaded coatings were analyzed by ATR-FTIR (Frontier, diamond/KRS-5 ATR, PerkinElmer, Waltham, MA, USA). Spectra were collected from 4000 to 400 cm⁻¹ with 4 cm⁻¹ resolution and 64 scans per sample under a constant ATR force of 100 N.

2.4. Drug Loading and Release

A ceftriaxone solution (1% *w/v*) was prepared in deionized water. Prior to loading, the anodized Ti-407 samples were rinsed with deionized water and dried at room temperature. For drug loading, 100 μ L of the ceftriaxone solution was applied dropwise onto the anodized TiO₂ nanotubular surface to ensure complete wetting, followed by vacuum drying at a pressure of 2×10^{-3} mbar for 30 min at room temperature.

To achieve a substantial amount of antibiotic loading, the loading–drying cycle was repeated five times under identical conditions, resulting in a total applied volume of 500 μ L per sample. This iterative process looks for drug penetration within the nanotubular structure. After the final drying step, any excess antibiotic remaining on the surface was gently removed using compressed air.

Drug release was evaluated by immersing each loaded sample in 1.5 mL of phosphate-buffered saline (PBS, pH 7.0) and incubating at 37°C under static conditions. At predetermined time intervals ranging from 5 min to 24 h, 2 μ L aliquots of the release medium were withdrawn and replaced with equal volumes of fresh PBS to maintain constant volume. The ceftriaxone concentration in each aliquot was determined by measuring the absorbance at 241 nm using a NanoDropTM 2000 UV–Vis spectrophotometer (Thermo Fisher Scientific Inc., USA). Quantification was performed using a calibration curve prepared in PBS (5–100 μ g/mL), and cumulative release profiles were calculated from the measured concentrations and expressed as the percentage of total drug released over time.

2.5. Antibacterial Evaluation

Antibacterial performance of the unloaded and ceftriaxone-loaded nanotubular Ti-407 surfaces was assessed against *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, and *Escherichia coli* ATCC 25922 by three complementary assays: turbidity measurements, colony-forming unit (CFU) quantification, and an agar diffusion test to determine inhibition zones. Ti-407 specimens of 1.0×1.0 cm and 3.0 mm thick were UV-sterilized at 302 nm for 2.5 h prior to each antibacterial assay to ensure complete surface sterility.

2.5.1. Bacterial Activation

Each strain was activated through daily subculturing in Brain Heart Infusion (BHI) broth for five consecutive days at 37°C prior to testing.

2.5.2. Turbidity Assay (Optical Density Measurement)

Anodized samples (unloaded or ceftriaxone-loaded) were placed in sterile tubes containing 5 mL of BHI broth. Tubes were inoculated with 100 µL of bacterial suspension adjusted to 10^6 CFU/mL (equivalent to a 0.5 McFarland standard prior to dilution). After incubation at 37°C for 24 h, optical density at 635 nm was measured using a Jenway 6320D spectrophotometer (Thermo Fisher Scientific Inc., Madrid, Spain).

The following controls were used: bacteria only, medium only, non-anodized Ti-407, and unloaded nanotubular samples.

2.5.3. Colony-Forming Unit (CFU) Quantification

Following turbidity assessment, 1 mL of culture was transferred into 9 mL of sterile saline (0.85%) and serially diluted from 10^{-1} to 10^{-12} . Aliquots of dilutions 10^{-8} to 10^{-12} were mixed with molten BHI agar maintained at about 45°C and poured in Petri dishes. After incubation at 37°C for 24 h, viable colonies were counted and expressed as CFU/mL.

2.5.4. Agar Diffusion Test

Anodized Ti-407 samples (with or without ceftriaxone) were placed on BHI agar plates previously inoculated with each bacterial strain, standardized at 0.5 McFarland. The plates were incubated at 37°C for 24 h, and the diameter of the inhibition zone (di) was measured using digital calipers.

Bacterial susceptibility was interpreted as follows: $di \leq 12$ mm, resistant; $12 < di < 15$ mm, intermediate; and $di \geq 15$ mm, sensitive [46]. Non-anodized Ti-407 alloy plates (1 cm²) were included as material controls.

2.6. Statistical Analysis

Quantitative data were obtained from three independent experiments, each performed in triplicate, and expressed as mean $\pm$ standard deviation (SD). Statistical significance among groups was determined using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test, with $p < 0.05$ considered statistically significant. All graphs were performed using OriginPro 8.5.

3. Results and Discussion

3.1. Formation and Electrochemical Behavior of the TiO₂ Nanotubular Layer

Figure 1 shows the evolution of current density (Figure 1a) and electrical resistance (Figure 1b) during the anodization of Ti-407 at 40, 50, and 60 V in glycerol/water (98:2 v/v) + 0.1 M NH₄F electrolyte for 30 min at 25°C. Across all applied voltages, the electrochemical response displayed the three stages associated with the formation of self-organized TiO₂

nanotubular layers, indicating that the selected conditions fall within a nanotube-forming regime rather than compact oxide growth [47].

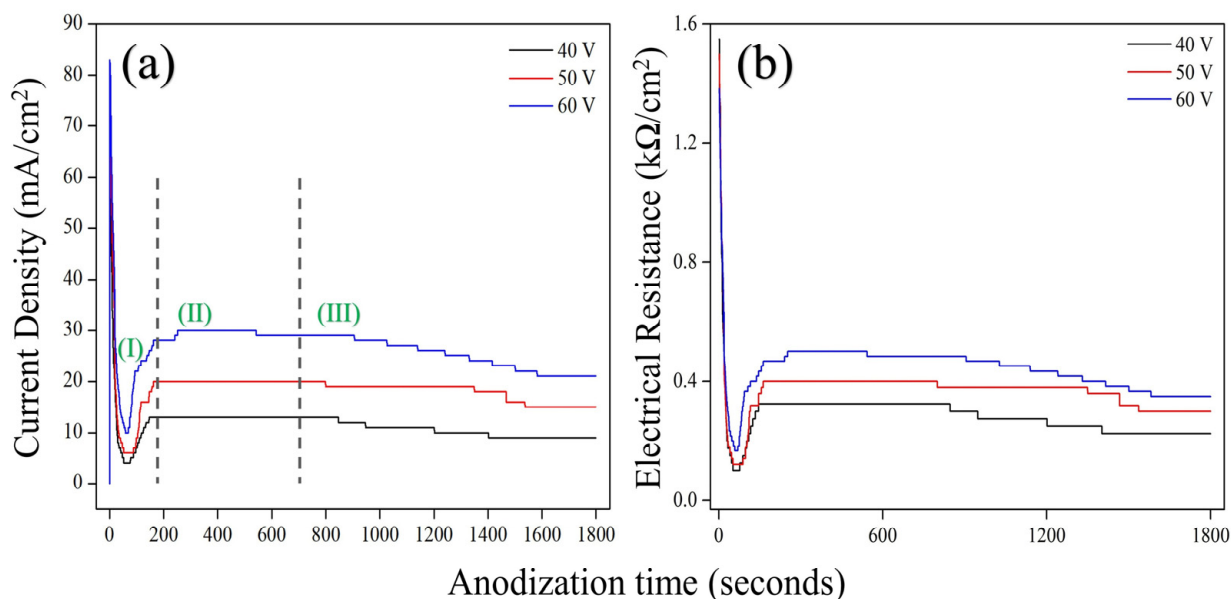


Figure 1. (a) Current density–time curves and (b) electrical resistance evolution recorded during anodization of Ti-407 at applied voltages of 40, 50, and 60 V in glycerol/ NH_4F electrolyte.

At the beginning of anodization (Stage I), a steep decrease in current density is observed in Figure 1a: from 62 to 4 mA/cm^2 at 40 V, from 75 to 6 mA/cm^2 at 50 V, and from 83 to 10 mA/cm^2 at 60 V. This rapid decay corresponds to electrochemical passivation, where titanium at the metal–electrolyte interface reacts with oxygen from the electrolyte to form a dense, insulating TiO_2 barrier layer. The growth of this barrier oxide increases the electrical resistance of the system, restricting ionic and electronic transport and thereby reducing the current flowing through the circuit [47,48]. Comparable current-drop patterns have been reported for anodized commercially pure Ti and Ti–6Al–4V in fluoride-containing media; in the present case, the higher initial current values observed here may reflect enhanced ionic mobility associated with the microstructural composition of Ti-407 [22,49,50]. This suggests that Ti-407 responds efficiently to field-assisted oxidation processes despite the lack of previous anodization studies on this alloy.

As anodization proceeds, Stage II begins, and a gradual increase in current density can be seen in Figure 1a, reaching approximately 13 mA/cm^2 at 40 V, 19 mA/cm^2 at 50 V, and 29 mA/cm^2 at 60 V after about 800 s. This rise in current is associated with the onset of pore nucleation and field-assisted dissolution of the oxide. The continuous migration of fluoride ions towards the oxide/solution interface promotes localized thinning and defect formation within the barrier layer, decreasing its effective electrical resistance and allowing current to increase again [22,50,51]. This process leads to the development and enlargement of pores due to acidification at the pore base and oxidative dissolution of titanium under the applied electric field. The fact that higher voltages produce higher current values in this stage indicates more aggressive dissolution and faster pore deepening, consistent with reports that anodization voltage strongly influences pore growth kinetics and ultimately nanotube dimensions [52,53].

In Stage III, the current–time curves in Figure 1a reach a steady-state regime, with plateau values of approximately 8 mA/cm^2 at 40 V, 15 mA/cm^2 at 50 V, and 21 mA/cm^2 at 60 V. This stabilization reflects a dynamic equilibrium between oxide formation at the metal/oxide interface and chemical dissolution at the pore bottom. Under these condi-

tions, the balance between growth and dissolution sustains the elongation of nanotubular structures rather than further thickening of a compact film [22,52,53]. The higher steady state currents observed at higher voltages suggest more active growth–dissolution kinetics and point to the development of nanotubes with larger internal volume and surface area, aspects that are particularly relevant for drug-loading applications and will be examined in subsequent sections.

The electrical resistance curves in Figure 1b complement these observations and provide additional insight into the evolution of the oxide layer. At the initial stage of anodization (Stage I), the electrical resistance increases sharply due to the rapid formation of a compact TiO₂ barrier layer, consistent with the current decay observed in Figure 1a. As anodization progresses into Stage II, pore nucleation and field-assisted dissolution lead to a partial decrease in the effective resistance, reflecting the development of conductive pathways within the porous oxide layer. Finally, during Stage III, the resistance reaches a quasi-steady regime, in agreement with the stable current plateaus observed in Figure 1a. The fact that higher voltages lead to higher final resistance values and longer stabilization times suggests that thicker or more complex oxide architectures are formed at 60 V than at 40 or 50 V [52,54,55].

Overall, the combined analysis of current density and electrical resistance in Figure 1a,b confirms that Ti-407 can be anodized under the selected conditions to produce TiO₂ nanotubular layers via a fluoride-assisted mechanism analogous to that reported for titanium alloys [19]. The voltage-dependent differences observed in the three electrochemical stages indicate that the applied potential is a key parameter governing the growth kinetics of the oxide layer. In particular, the more dynamic and delayed stabilization behavior at 60 V is consistent with the formation of nanotubular architectures with increased internal volume and accessible surface, features that are expected to enhance antibiotic loading and controlled release which will be explored below.

3.2. Morphological and Dimensional Architecture of the TiO₂ Nanotubular Layer

Figure 2 shows the SEM micrographs of the anodized Ti-407 alloy processed at 40, 50, and 60 V. All anodized specimens exhibited a well-defined nanotubular architecture, confirming that the electrochemical parameters used enabled the formation of self-organized TiO₂ nanotubes on this alloy. The top view images (Figure 2a,d,g) reveal a homogeneous porous oxide surface with circular pore openings (yellow arrows), without evidence of microcracks, peeling, or discontinuities. This morphology aligns with nanotube formation mechanisms previously reported for anodized commercially pure titanium and Ti-6Al-4V under similar fluoride-containing electrolyte conditions [56–58], indicating that the alloying elements in Ti-407 do not hinder nanotube self-organization.

The cross-sectional images (Figure 2b,e,h) display vertically aligned hollow cylindrical nanotubes with continuous single-wall morphology (blue arrows). The tubes maintain uniform inner diameter along their length without collapse or deformation, suggesting a stable balance between field-assisted oxide growth and fluoride-induced dissolution during anodization. This behavior is consistent with established morphological evolution models in anodized titanium oxide systems [59–61].

Rear view images (Figure 2c,f,i) show the shape of the nanotubes extracted from the surface by fracturing the anodized samples. These images confirm the presence of closed-end tube terminations (white circles), which are consistent with those commonly reported in the literature for anodically formed TiO₂ nanotubes [62,63].

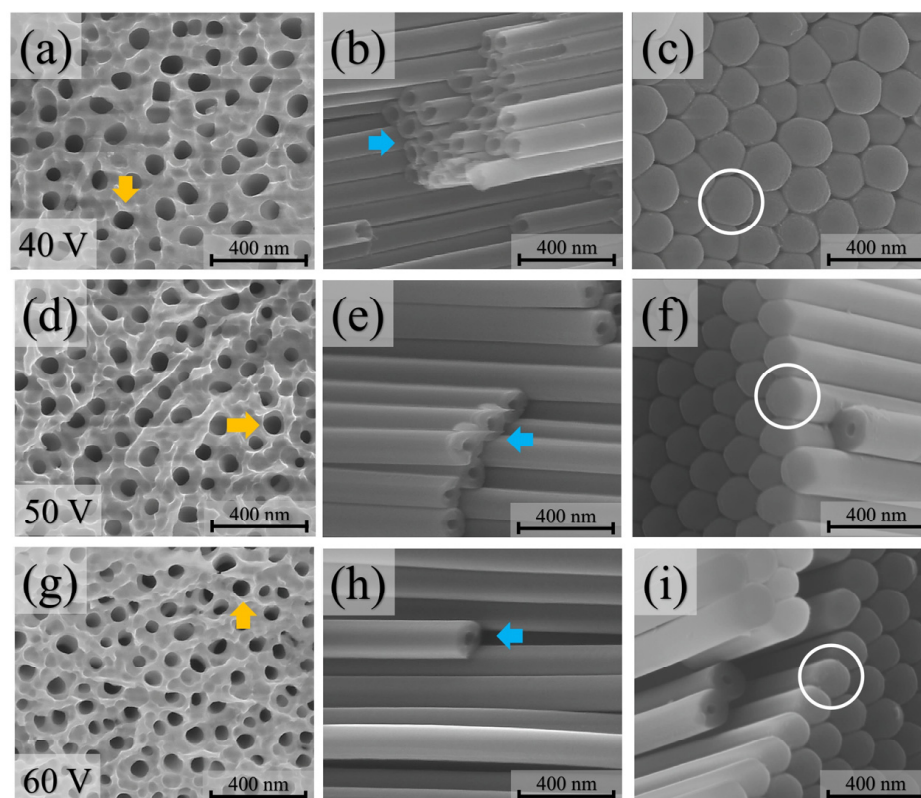


Figure 2. SEM micrographs showing top-view (a,d,g), cross-sectional (b,e,h), and rear-view (c,f,i) morphologies of TiO₂ nanotubular layers formed on the Ti-407 alloy by anodization at room temperature for 30 min under applied voltages of 40, 50, and 60 V, respectively. Yellow arrows indicate the circular pore openings observed on the nanotubular surface. Blue arrows highlight the vertically aligned hollow nanotubes with continuous single-wall morphology in the cross-sectional views. White circles mark the closed-end terminations of the nanotubes observed in the rear-view images.

Quantitative dimensional analysis is presented in Figure 3. Nanotube measurements were performed using calibrated SEM image-processing software (ImageJ, version 1.54g), evaluating at least 100 nanotubes per voltage condition across five non-overlapping regions of each sample. The average nanotube diameter increased slightly with applied voltage, from 81.4 ± 6.5 nm at 40 V to 82.6 ± 7.1 nm at 50 V and 83.1 ± 7.8 nm at 60 V. The observed dispersion in diameter values reflects the inherent heterogeneity of nanotube arrays formed by anodization in viscous glycerol-based electrolytes, where pore nucleation and subsequent growth occur simultaneously across the surface [64,65]. Similarly, the average nanotube length increased from 10.18 ± 1.26 μ m at 40 V to 10.81 ± 2.43 μ m at 60 V. This behavior is consistent with variations in average nanotube length reported for anodic TiO₂ nanotube systems under different anodization potentials [66,67]. Resulting aspect ratios (defined as the length-to-diameter ratio, $AR = L/D$) also showed a voltage-dependent trend. At 40 V, the average AR was 125 ± 18 , increasing to 128 ± 21 at 50 V, and 130 ± 27 at 60 V. These values confirm that even at the lowest voltage condition, the nanotube geometry falls within the high-aspect-ratio regime typically associated with enhanced internal surface area, prolonged diffusion path length, and improved drug-retention behavior in TiO₂ nanotube systems. High-AR architectures (>100) have been reported to delay the outward diffusion of loaded molecules, support gradient-controlled release kinetics, and reduce the probability of uncontrolled burst release compared with shorter structures [20,47]. In practical terms, a higher aspect ratio increases the internal contact surface available for physical adsorption, hydrogen bonding, or electrostatic interaction with antibiotic

molecules, while simultaneously extending the diffusion pathway, contributing to sustained therapeutic release rather than rapid depletion. Therefore, the aspect ratios obtained in this study position the nanotubular structure within the dimensional range recognized as optimal for drug-delivery applications.

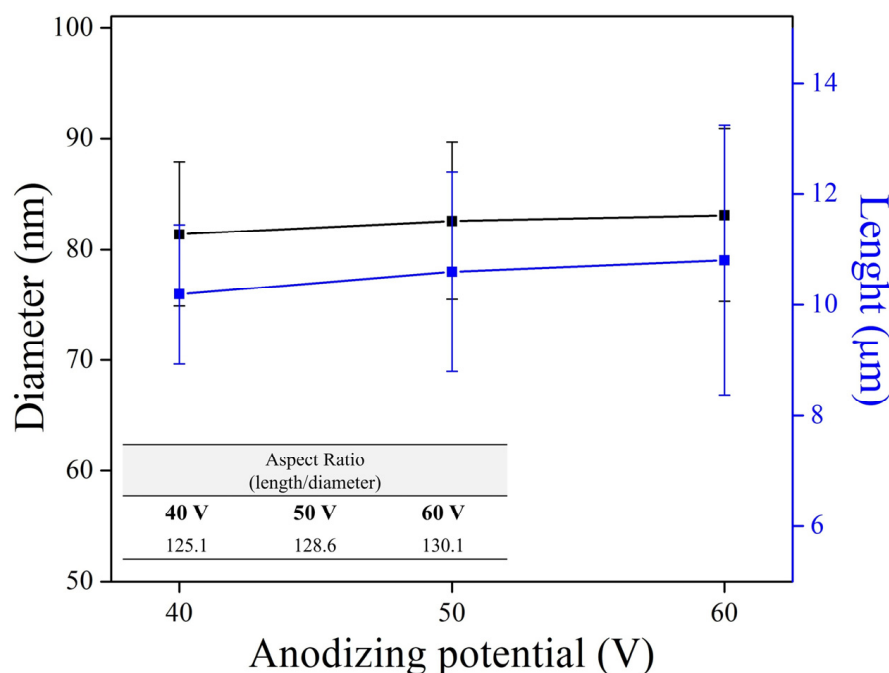


Figure 3. Variation in TiO₂ nanotube length (blue line) and diameter (black line) as a function of anodization voltage. Inset: corresponding aspect ratio (L/D) of Ti-407 nanotubes.

These dimensional trends support the selection of the anodization window used in this study. The voltage range of 40–60 V was chosen because preliminary anodization trials showed that lower voltages (<30 V) resulted in poorly defined or partially developed pore structures, whereas voltages above 70 V led to excessive chemical dissolution, instability of the oxide layer, or collapse of the nanotubular morphology. Previous studies have reported that glycerol-based electrolytes require higher voltage thresholds than aqueous systems to sustain field-assisted oxide growth, due to increased viscosity and reduced ionic mobility [64,65]. Within this processing window, between 40 and 60 V consistently produced ordered and mechanically stable nanotubes with closed-end geometry and dimensions falling within the optimal range reported for drug delivery TiO₂ nanotube systems, where aspect ratios exceeding 100 and diameters between 70 and 110 nm are considered advantageous for sustained release [19,20]. Therefore, this voltage range represents a practical balance between structural stability, reproducibility, and functional performance requirements for antibiotic-loaded biomedical coatings.

Nanotube density and enclosed internal volume trends are shown in Figure 4a. Nanotube density decreased progressively with increasing voltage, from 192 ± 11 tubes/ μm^2 at 40 V, to 187 ± 12 tubes/ μm^2 at 50 V, and 181 ± 14 tubes/ μm^2 at 60 V, reflecting the lateral widening of nanotubes at higher anodization potentials. In contrast, the internal enclosed volume per nanotube increased with voltage, from 0.053 ± 0.006 μm^3 at 40 V, to 0.056 ± 0.007 μm^3 at 50 V, and 0.059 ± 0.008 μm^3 at 60 V, indicating that higher voltages promote larger storage capacity per tube. The opposing trends between nanotube density and enclosed internal volume intersect theoretically near 50 V, suggesting that this value represents a geometrical equilibrium point where drug-loading capacity and release uniformity may be jointly optimized, depending on the desired functional outcome.

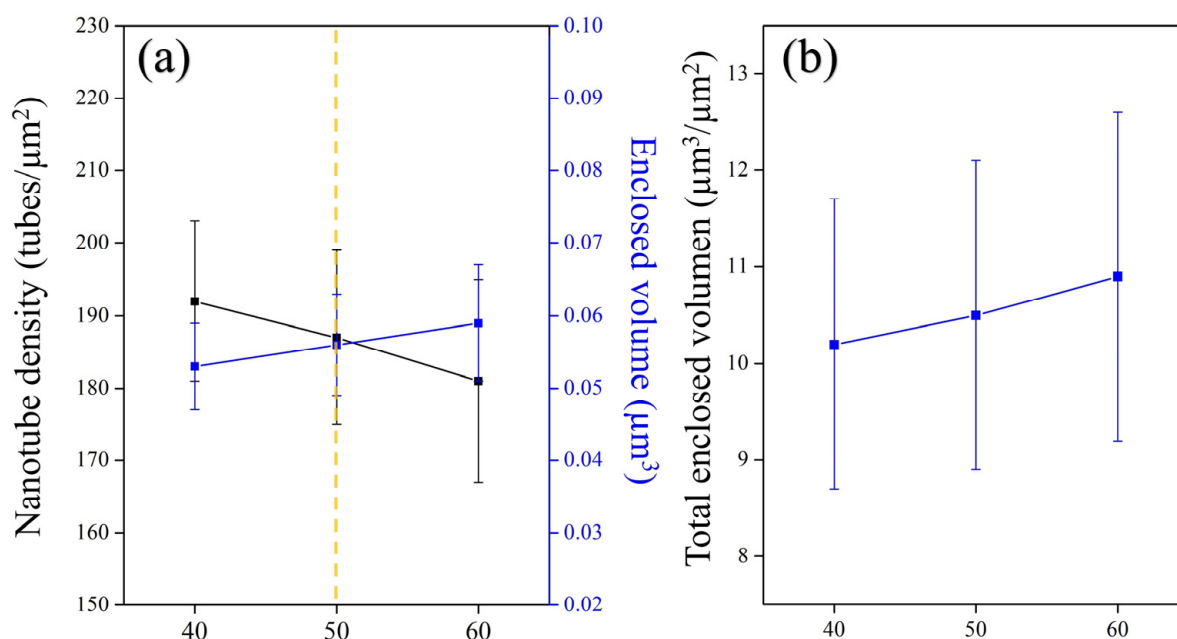


Figure 4. (a) Evolution of nanotube density (black line, left axis) and enclosed volume per individual nanotube (blue line, right axis) as a function of anodization voltage for Ti-407. The dashed line indicates the voltage region where the opposing trends intersect, suggesting a balance between nanotube density and individual tube volume. (b) Total enclosed volume per unit surface area as a function of anodization voltage, highlighting the progressive increase in theoretical drug-loading capacity at higher voltages.

Figure 4b shows that the total enclosed volume per unit surface area increased progressively with voltage, from $10.2 \pm 1.5 \mu\text{m}^3/\mu\text{m}^2$ at 40 V, to $10.5 \pm 1.6 \mu\text{m}^3/\mu\text{m}^2$ at 50 V, and up to $10.9 \pm 1.7 \mu\text{m}^3/\mu\text{m}^2$ at 60 V, demonstrating that samples fabricated at higher potentials provide a larger theoretical drug-loading capacity. Similar relationships between anodization voltage, nanotube volume, and drug-loading potential have been documented for vancomycin, gentamicin, and cefazolin-loaded TiO_2 nanotube systems [27,29,68].

The nanotube dimensions obtained in this study (≈ 81 – 83 nm diameter and ≈ 10 – $11 \mu\text{m}$ length with aspect ratios > 120) fall within the functional range previously reported for drug-delivery TiO_2 nanotubes, where typical performance-optimized geometries span 70–110 nm in diameter and exhibit aspect ratios above 100 [19,20]. Collectively, these observations demonstrate that anodization voltage primarily governs nanotube length and total enclosed volume rather than pore diameter, establishing voltage as a reliable tuning parameter for maximizing drug-loading capacity. This nanotubular architecture forms the structural foundation for ceftriaxone incorporation and release performance, which are examined in the following section.

3.3. Chemical Composition and Drug Confirmation of the TiO_2 Nanotubular Layer

3.3.1. X-Ray Photoelectron Spectroscopy (XPS) Analysis of the TiO_2 Nanotubular Layer

The surface chemical composition and oxidation states of the Ti-407 alloy were analyzed before and after anodization using high-resolution X-ray photoelectron spectroscopy (XPS). Figure 5a–c correspond to the non-anodized Ti-407 alloy (control), while Figure 5d–f correspond to the anodized Ti-407 surface (representative condition at 50 V), as all anodized samples processed in the 40–60 V range exhibited equivalent chemical features. The O 1s regions of the control and anodized surfaces are shown in Figure 5g and h, respectively, whereas Figure 5i presents the F 1s region, detected exclusively after anodization.

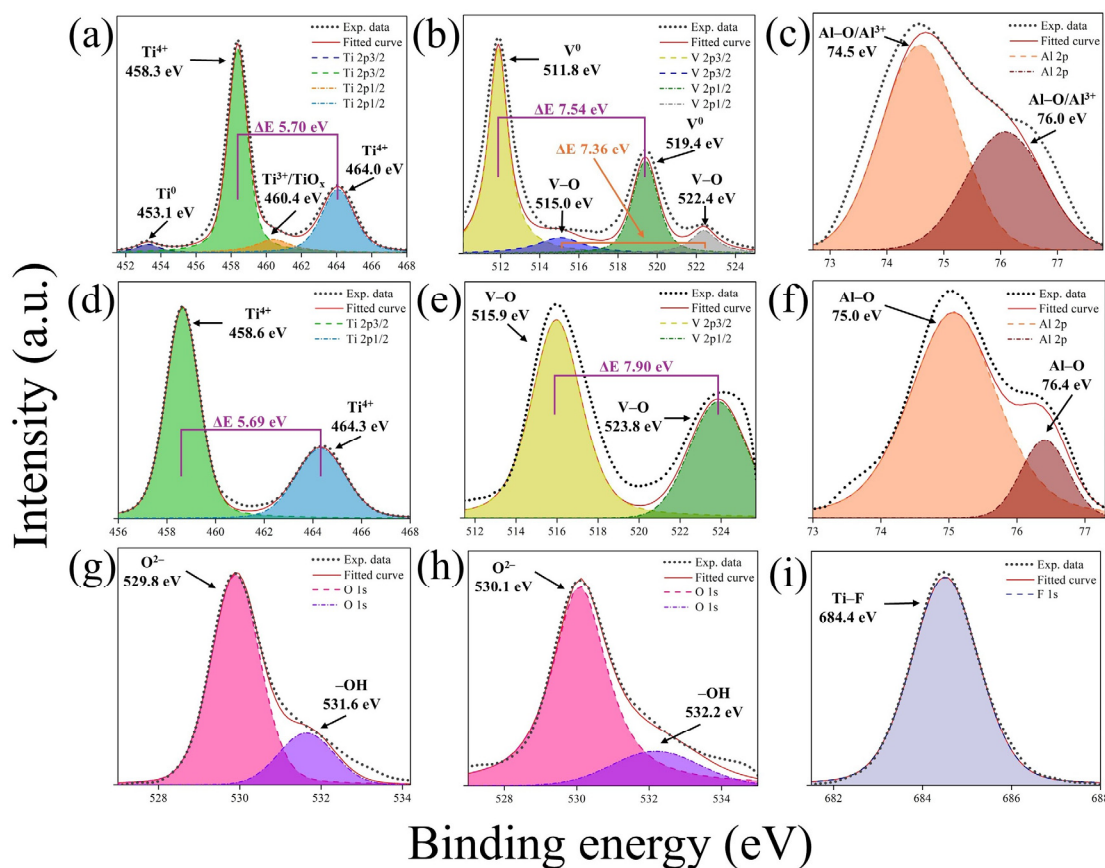


Figure 5. High-resolution XPS spectra of Ti-407 before and after anodization. (a–c) Non-anodized surface: Ti 2p, V 2p, and Al 2p regions showing metallic and oxidized contributions associated with the native oxide. (d–f) Anodized surface (50 V): Ti 2p, V 2p, and Al 2p regions evidencing complete titanium oxidation and strong attenuation of vanadium and aluminum beneath the nanotubular oxide layer. (g,h) O 1s spectra of the non-anodized and anodized surfaces, showing an increased contribution of hydroxylated oxygen species after anodization. (i) F 1s spectrum detected exclusively after anodization, confirming fluoride incorporation from the NH_4F -containing electrolyte. Solid black lines correspond to fitted envelopes.

The Ti 2p spectrum of the non-anodized Ti-407 alloy (Figure 5a) exhibits a multicomponent envelope indicative of a chemically heterogeneous native surface. A low-binding-energy contribution at 453.18 eV is assigned to metallic titanium (Ti^0 , $\text{Ti } 2p_{3/2}$). The dominant oxidized titanium component appears at 458.38 eV ($\text{Ti}^{4+} 2p_{3/2}$), with its spin-orbit counterpart at 464.08 eV ($\text{Ti}^{4+} 2p_{1/2}$), yielding a splitting of $\Delta E \approx 5.70$ eV, characteristic of TiO_2 . In addition, a weak intermediate contribution centered at 460.48 eV is attributed to sub-stoichiometric titanium species ($\text{Ti}^{3+}/\text{TiO}_x$), commonly associated with defect-rich native oxides formed under ambient conditions [62,69]. After anodization (Figure 5d), the Ti 2p region is composed exclusively of the Ti^{4+} doublet, with peaks located at 458.64 eV ($2p_{3/2}$) and 464.33 eV ($2p_{1/2}$), maintaining a spin-orbit splitting of $\Delta E \approx 5.69$ eV. The complete disappearance of Ti^0 and Ti^{3+} contributions confirms full surface oxidation and the formation of a continuous TiO_2 nanotubular oxide layer that fully covers the underlying alloy within the XPS information depth [70–73].

The V 2p spectrum of the non-anodized alloy (Figure 5b) can be described by two spin-orbit doublets, indicating the coexistence of subsurface/metallic and oxidized vanadium contributions beneath the thin native oxide. A low-binding-energy doublet at 511.86 eV ($\text{V } 2p_{3/2}$) and 519.40 eV ($\text{V } 2p_{1/2}$), with $\Delta E \approx 7.54$ eV, is consistent with metallic or subsurface vanadium detectable through the native oxide layer [74,75]. A higher-binding-energy

doublet at 515.05 eV (V 2p_{3/2}) and 522.41 eV (V 2p_{1/2}), with $\Delta E \approx 7.36$ eV, is attributed to oxidized vanadium species at the naturally oxidized alloy surface. Due to the overlap of binding energies among different vanadium oxidation states, these oxidized components are conservatively interpreted as mixed V–O environments rather than a uniquely defined valence state [74,75]. Following anodization (Figure 5e), the V 2p signal is strongly attenuated. Only weak residual peaks at 515.95 eV (V 2p_{3/2}) and 523.85 eV (V 2p_{1/2}) remain detectable, with an apparent $\Delta E \approx 7.90$ eV, slightly broadened due to reduced signal-to-noise ratio and minor charging effects associated with the thick oxide nanostructure [76–78]. The pronounced attenuation of the vanadium signal seems to indicate that vanadium does not form a surface oxide phase within the TiO₂ nanotubular layer. Instead, the residual contribution originates from subsurface vanadium species located beneath the anodic oxide, likely associated with interfacial V–O environments at the metal/oxide interface.

The Al 2p spectrum of the non-anodized Ti-407 alloy (Figure 5c) displays an oxidized aluminum doublet with peaks at 74.57 eV and 76.08 eV, consistent with Al³⁺ species in aluminum oxide or oxyhydroxide environments [79]. After anodization (Figure 5f), the Al 2p signal becomes strongly attenuated, reflecting burial of aluminum beneath the TiO₂ nanotubular layer. Two weak residual components at 75.06 eV and 76.41 eV are detected, corresponding to oxidized aluminum in a modified chemical environment [80]. The higher-binding-energy component may suggest partial interaction with fluoride incorporated during anodization (e.g., Al–O–F or Al–F_x-like environments); however, given the low aluminum content of Ti-407 and the limited probing depth of XPS, the formation of stoichiometric aluminum fluoride phases cannot be unambiguously confirmed [80].

The O 1s spectrum of the non-anodized alloy (Figure 5g) consists of a dominant lattice oxygen component at 529.88 eV, attributed to O^{2−} in metal oxides, and a higher-binding-energy contribution at 531.65 eV associated with surface hydroxyl groups, adsorbed water, and defect-related oxygen species [81]. For the anodized surface (Figure 5h), the lattice oxygen component remains near 530.10 eV, while the higher-binding-energy component increases in relative intensity and shifts to approximately 532.21 eV, reflecting the hydroxyl-rich and defect-dense nature of the TiO₂ nanotubular architecture [81].

A single, well-defined F 1s peak at 684.48 eV is detected exclusively after anodization (Figure 5i). This binding energy is characteristic of fluoride species incorporated into anodic titanium oxides, such as Ti–F or F–Ti–O environments, providing direct chemical evidence of fluoride participation in the nanotube formation process [29,82].

Table 1 summarizes the representative surface elemental composition of the Ti-407 alloy before and after anodization, as determined by X-ray photoelectron spectroscopy (XPS). The anodized composition corresponds to the sample treated at 50 V, which is taken as representative of the surface chemical behavior of anodized Ti-407.

Table 1. Representative surface elemental composition of Ti-407 before and after anodization determined by X-ray photoelectron spectroscopy (XPS) (at.%).

Element	Non-Anodized Ti-407	Anodized Ti-407 *
O 1s	41.5 ± 1.5	36.5 ± 1.5
Ti 2p	14.0 ± 1.0	13.8 ± 1.2
C 1s	28.5 ± 3.5	30.0 ± 4.0
V 2p	10.0 ± 2.0	0.25 ± 0.10
Al 2p	1.2 ± 0.3	0.45 ± 0.15
F 1s	—	4.0 ± 1.0

* The anodized values correspond to the sample anodized at 50 V and are presented as representative of the surface chemical behavior of all anodized Ti-407 samples (40–60 V), which exhibited comparable XPS trends.

For the non-anodized Ti-407 control, the surface is dominated by oxygen (41.5 ± 1.5 at.%) and titanium (14.0 ± 1.0 at.%), confirming the presence of a thin native oxide layer primarily composed of TiO_2 . A substantial carbon contribution (28.5 ± 3.5 at.%) is also detected and attributed to adventitious carbon contamination, which is commonly observed on metallic surfaces exposed to ambient conditions before XPS analysis [83].

Vanadium (10.0 ± 2.0 at.%) and aluminum (1.2 ± 0.3 at.%) are clearly detected on the control surface. This behavior is consistent with the limited thickness of the native oxide layer and the high surface sensitivity of XPS, which allows alloying elements from the substrate to contribute significantly to the detected signal [84].

Following anodization, a pronounced modification of the surface composition is observed, representative of the anodized Ti-407 surfaces. Oxygen remains the most abundant element (36.5 ± 1.5 at.%), consistent with the formation of a TiO_2 nanotubular oxide layer. The titanium content remains essentially unchanged (13.8 ± 1.2 at.%) across all anodized samples, indicating that the near-surface chemistry is dominated by fully oxidized titanium species, in agreement with the absence of metallic Ti contributions in the high-resolution Ti 2p spectra [70–73].

A key observation is the strong attenuation of vanadium and aluminum signals after anodization. Vanadium is reduced to trace levels (0.25 ± 0.10 at.%), while aluminum remains at slightly higher but still minor concentrations (0.45 ± 0.15 at.%). The slightly more pronounced attenuation of vanadium relative to aluminum is consistent with the preferential burial of V beneath the anodic TiO_2 layer and with the tendency of Al to exhibit limited residual surface contributions. This behavior indicates that both elements are covered beneath the anodic oxide, whose thickness exceeds the XPS information depth [19,69,84].

Fluorine is detected exclusively on the anodized samples, with an average surface concentration of 4.0 ± 1.0 at.%. This observation provides chemical evidence of fluoride incorporation from the NH_4F -containing electrolyte during anodization. The F 1s signal is commonly associated with Ti–F and/or F–Ti–O bonding environments reported for anodically grown TiO_2 nanotubes, while the observed variability is consistent with voltage-dependent differences in oxide growth and field-assisted dissolution kinetics during nanotube formation [85,86].

The combined quantitative analysis (Table 1) and high-resolution spectral deconvolution (Figure 5) confirm a clear chemical transformation of the Ti-407 surface induced by anodization. The non-anodized alloy exhibits a chemically heterogeneous surface comprising metallic Ti, TiO_2 , sub-stoichiometric TiO_x species, and oxidized alloying elements, whereas the anodized surfaces are dominated by fully oxidized TiO_2 , with strong attenuation of V and Al signals, increased hydroxylated oxygen contributions, and the exclusive presence of fluorine. The fitted peak positions and characteristic spin–orbit separations for Ti 2p (~ 5.7 eV) and V 2p (~ 7.3 – 7.5 eV) support the physical reliability of the deconvolution and validate the assignment of the observed chemical states. These results confirm the formation of a continuous and chemically stable TiO_2 nanotubular layer that governs the anodized Ti-407 interface and provides a robust platform for subsequent drug-loading and antibacterial functionalization.

3.3.2. ATR-FTIR Confirmation of Ceftriaxone Incorporation

Figure 6 shows the ATR-FTIR spectra of ceftriaxone-loaded TiO_2 nanotubular layers anodized on Ti-407 at applied voltages of 40, 50, and 60 V, recorded in the range of 4000 – 400 cm^{-1} . The spectra exhibit vibrational features associated with both the TiO_2 nanotubular oxide matrix and the incorporated antibiotic, consistent with the behavior reported for anodized titanium-based nanotubular systems used as drug-delivery platforms.

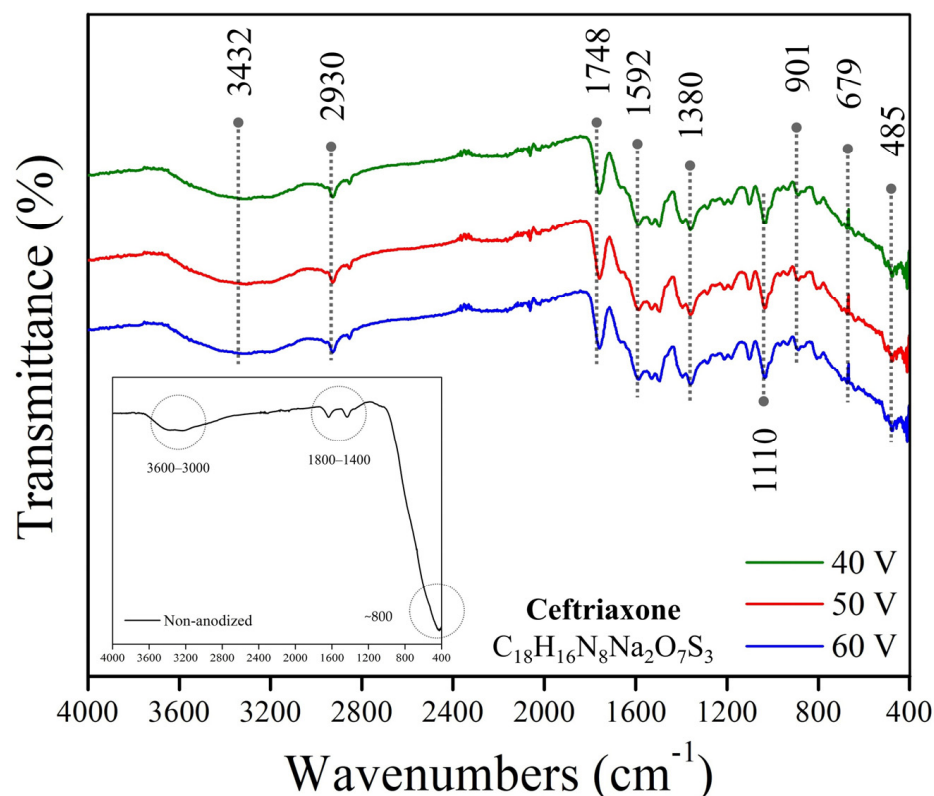


Figure 6. ATR-FTIR spectra of ceftriaxone-loaded TiO_2 nanotubular layers anodized on Ti-407 at 40, 50, and 60 V. Inset: ATR-FTIR spectrum of the anodized Ti-407 surface (50 V), showing TiO_2 -related vibrational modes and the absence of ceftriaxone-related absorption bands.

In the high-wavenumber region, a broad absorption band extending approximately from 3500 to 1600 cm^{-1} is observed for all samples. This band is attributed to O–H stretching and H–O–H bending vibrations associated with surface hydroxyl groups and adsorbed or hydrogen-bonded water molecules [20,87]. The presence of these features is characteristic of anodically formed, non-annealed TiO_2 nanotubular layers synthesized in fluoride-containing electrolytes and reflects the highly hydrophilic and defect-rich nature of the nanostructured oxide surface.

Alongside the oxide related contributions, distinct absorption bands characteristic of ceftriaxone ($\text{C}_{18}\text{H}_{16}\text{N}_8\text{Na}_2\text{O}_7\text{S}_3$) are clearly identified in all loaded samples. The band located at approximately 3432 cm^{-1} is assigned to N–H stretching vibrations of amide groups involved in hydrogen bonding. A pronounced band at $\sim 1748\text{ cm}^{-1}$ corresponds to C=O stretching vibrations of the β -lactam ring, while the absorption observed in the $\sim 1592\text{ cm}^{-1}$ region is attributed to C=N stretching vibrations associated with the oxime functional group of ceftriaxone [88]. The simultaneous presence of these bands provides direct spectroscopic evidence of successful antibiotic incorporation within the TiO_2 nanotubular architecture.

In the mid- and low-wavenumber regions, bands associated with the titanium oxide framework are also observed. Absorptions near $901\text{--}918\text{ cm}^{-1}$ correspond to O–Ti–O vibrational modes, while bands at approximately 679 cm^{-1} and 485 cm^{-1} are attributed to Ti–O and Ti–O–Ti lattice vibrations, respectively. In addition, a band around 1380 cm^{-1} is assigned to flexional vibrations of O–Ti–O bonds [89]. These features are in good agreement with FTIR spectra reported for anodically formed TiO_2 nanotubular layers and confirm that the underlying oxide structure remains intact after drug loading. Minor absorption bands detected near $\sim 2930\text{ cm}^{-1}$ and in the $1100\text{--}1050\text{ cm}^{-1}$ region are attributed to residual species originating from the glycerol/ NH_4F anodization electrolyte and do not interfere with the identification of the antibiotic [89–91].

The overall similarity of the spectra obtained at different anodization voltages indicates that, within the investigated range (40–60 V), the applied voltage does not significantly alter the chemical nature of the TiO₂ nanotubular oxide layer or the vibrational features associated with ceftriaxone. This observation is consistent with the XPS results and supports that anodization voltage primarily governs nanotube morphology rather than the fundamental chemical structure of the oxide or the incorporated drug.

The inset of Figure 6 presents a representative ATR-FTIR spectrum of the anodized Ti-407 nanotubular layer without antibiotic loading (50 V). The spectrum is characterized by a weak and broad O–H-related absorption extending approximately from 3600 to 3000 cm^{−1}, which is attributed to surface hydroxyl groups and physically adsorbed water commonly present on non-annealed TiO₂ nanotubular surfaces. In contrast to the ceftriaxone-loaded samples, no well-defined absorption bands are detected in the 1800–1400 cm^{−1} region that can be attributed to organic functional groups. The bands observed in this range are instead associated with adsorbed molecular water and atmospheric CO₂-derived carbonate species on the hydroxylated TiO₂ surface, confirming the absence of ceftriaxone or other organic residues on the unloaded oxide layer [20,87].

In the low-wavenumber region, a pronounced and broad decrease in transmittance is observed below ~800 cm^{−1}, corresponding to Ti–O and Ti–O–Ti lattice vibrations characteristic of anodically formed, non-annealed TiO₂ [88]. The absence of ceftriaxone-related absorption bands in the inset spectrum demonstrates that the additional vibrational features observed in the loaded samples arise exclusively from the incorporated antibiotic and are not intrinsic to the TiO₂ nanotubular matrix.

The ATR-FTIR analysis corroborates the successful incorporation of ceftriaxone within the TiO₂ nanotubular layers while demonstrating preservation of the oxide framework. The data supports a physical incorporation mechanism without evidence of chemical modification of either the antibiotic or the TiO₂ matrix, providing a spectroscopic foundation for interpreting the subsequent drug release and antibacterial performance.

3.4. Drug Release Profile and Mechanistic Interpretation

To evaluate the drug-release behavior of the TiO₂ nanotubular layers anodized on Ti-407, ceftriaxone release experiments were performed in triplicate for each anodization condition (40, 50, and 60 V). Figure 7 shows the cumulative fraction release of ceftriaxone as a function of immersion time in phosphate-buffered saline (PBS, pH 7.0) at 37 °C. Quantification was carried out by UV–Vis spectroscopy, where all samples exhibited a broad absorption band between 239 and 243 nm with a maximum at 241 nm, consistent with the characteristic absorption of ceftriaxone in aqueous media [90].

For all anodized samples, the release profiles display a biphasic behavior, highlighted in Figure 7 by the yellow and green dashed lines, indicating two distinct release stages. During the initial stage, extending over approximately the first 40 min of immersion, a pronounced burst release is observed. At this time point, anodized samples prepared at 40 and 50 V released 49.8% and 45.2% of the loaded antibiotic, respectively, whereas the samples anodized at 60 V exhibited a lower release fraction of 40.5%. This initial burst is commonly attributed to the rapid dissolution of ceftriaxone molecules weakly adsorbed on the external surface of the nanotubular layer or located near the tube openings, a behavior widely reported for drug-loaded TiO₂ nanotube systems [92]. From a clinical perspective, the combination of an early burst release followed by sustained delivery is consistent with the therapeutic requirements of the immediate postoperative period, where rapid bacterial suppression and prolonged local antibiotic availability are desirable [35,42].

The lower burst release observed for the 60 V samples indicates a more effective retention of the antibiotic within the nanotubular structure during the early release stage. This behavior is consistent with the morphological characteristics discussed in Section 3.2, where nanotubes formed at higher anodization voltages exhibit larger dimensions, higher aspect ratios, and increased total enclosed volume. These features favor deeper drug confinement within the nanotubes and reduce the fraction of the antibiotic readily accessible for rapid desorption.

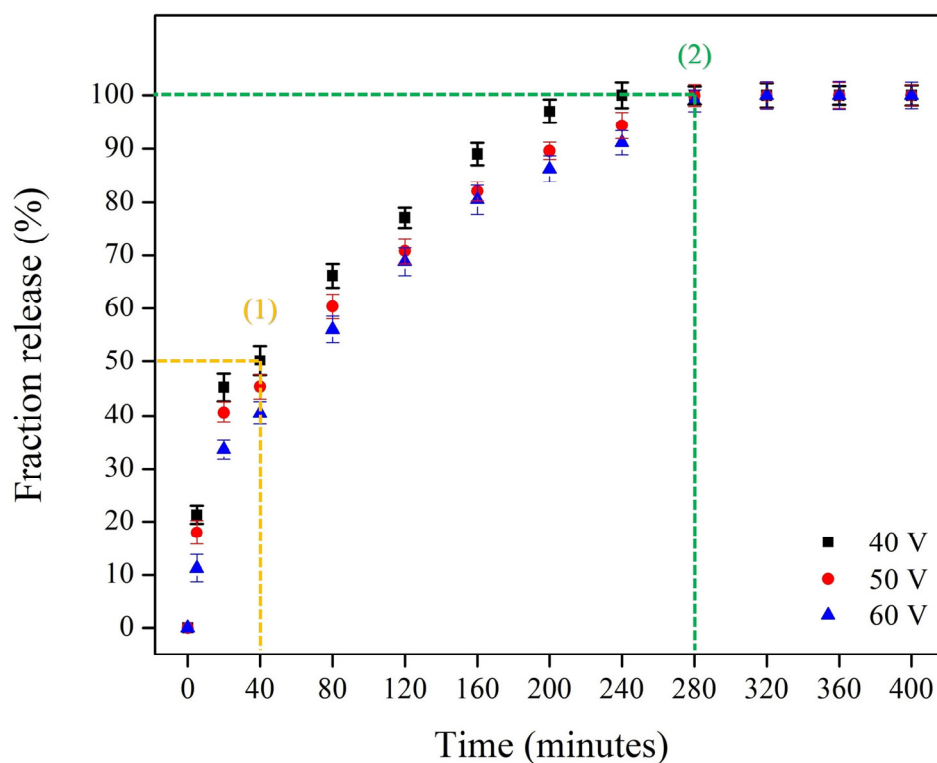


Figure 7. Cumulative ceftriaxone release profiles from TiO₂ nanotubular layers anodized on Ti-407 at 40, 50, and 60 V, measured in PBS (pH 7.0) at 37°C. The yellow dashed lines and label (1) indicate the initial burst-release stage, while the green dashed lines and label (2) denote the subsequent sustained diffusion-controlled release stage.

The second release stage begins immediately after the burst phase and extends up to approximately 280 min, as indicated by the green dashed line in Figure 7. During this period, a sustained and progressively slower release kinetics is observed, ultimately leading to nearly complete antibiotic release for all anodization conditions. This stage is associated with diffusion-controlled release of ceftriaxone molecules confined within the nanotube interior, where the release rate is governed by nanotube geometry, internal volume, and diffusion path length, as well as by the solubility of the antibiotic in the surrounding medium [92,93]. The attainment of complete release without abrupt discontinuities further suggests that the nanotubular architecture remains structurally stable during immersion under the tested conditions.

A clear dependence of the sustained-release behavior on nanotube morphology is evident. As summarized in Table 2, nanotubes synthesized at 60 V exhibit the lowest tube density but the largest total enclosed volume per unit surface area ($\approx 10.6 \mu\text{m}^3 / \mu\text{m}^2$). This structural configuration provides a higher storage capacity and longer diffusion pathways, resulting in a slower and more controlled release profile (Figure 7, blue triangles). In contrast, nanotubes formed at 40 and 50 V, which present higher tube densities and smaller

enclosed volumes, display faster release kinetics during both the burst and sustained-release stages (Figure 7, black squares and red circles, respectively).

Table 2. Morphological parameters of TiO₂ nanotubular layers anodized on Ti-407 at different applied voltages and their relation to ceftriaxone release behavior. Values are reported as mean $\pm$ standard deviation ($n \geq 100$ nanotubes per condition).

Parameter	40 V	50 V	60 V
Length (μm)	10.2 ± 1.3	10.6 ± 2.1	10.8 ± 2.4
Diameter (nm)	81.4 ± 6.5	82.6 ± 7.1	83.1 ± 7.8
Aspect ratio (L/D)	125 ± 18	129 ± 21	130 ± 27
Nanotube density (μm^{-2})	192 ± 11	187 ± 12	181 ± 14
Total enclosed volume ($\mu\text{m}^3/\mu\text{m}^2$)	10.2 ± 1.5	10.5 ± 1.6	10.9 ± 1.7
Ceftriaxone release at 40 min (%)	49.8	45.2	40.5

Taken together, these results demonstrate that nanotube density, length, diameter, and total enclosed volume must be considered collectively to understand and tune drug-release performance. No single morphological parameter alone dictates release behavior; rather, the interplay between nanotube geometry and internal volume determines the balance between initial burst release and prolonged diffusion-controlled release. From a practical perspective, this tunability enables the rational design of TiO₂ nanotubular arrays on Ti-407 with tailored release profiles, depending on whether rapid antibiotic delivery, extended release, or a compromise between both is required.

In general, the release profiles obtained in this study confirm that anodized Ti-407 can support controlled antibiotic delivery through TiO₂ nanotubular layers, with release kinetics directly linked to anodization voltage-dependent morphological features. This release behavior provides the mechanistic framework for interpreting the antibacterial response discussed in the following section.

3.5. Antibacterial Performance of Ceftriaxone-Loaded TiO₂ Nanotubular Layers

The antibacterial performance of unloaded and ceftriaxone-loaded anodized Ti-407 samples was evaluated against *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 29213 using three complementary in vitro assays: turbidity assay (optical density measurement), colony-forming unit (CFU) quantification, and agar diffusion test for inhibition zone determination. Figures 8 and 9, and 10 summarize the results obtained from these assays, respectively. In all experiments, bacterial cultures grown in the absence of material and ceftriaxone were used as the negative control (*E. coli*, E.c-C; *P. aeruginosa*, P.a-C; and *S. aureus*, S.a-C), while a ceftriaxone solution (0.5 $\mu\text{g}/\text{mL}$) was employed as the positive control (CEF-C). Non-anodized Ti-407 samples without ceftriaxone (Ti407-U) and non-anodized Ti-407 samples subjected to the ceftriaxone loading protocol (Ti407-CEF) were included as material controls to decouple the antibacterial effect of the antibiotic from that of the nanotubular architecture. Anodized nanotubular samples obtained at 40, 50, and 60 V were evaluated in both unloaded (40V-U, 50V-U, 60V-U) and ceftriaxone-loaded (40V-CEF, 50V-CEF, 60V-CEF) conditions.

3.5.1. Bacterial Growth Inhibition Assessed by Turbidity (Optical Density)

Figure 8a–c show the percentage of bacterial growth determined by optical density measurements at 635 nm after 24 h of incubation. All bacterial growth values are expressed as percentages relative to the corresponding bacterial growth controls (E.c-C, P.a-C, and S.a-C), which were defined as 100%. Negative controls for *E. coli* (Figure 8a), *P. aeruginosa* (Figure 8b), and *S. aureus* (Figure 8c) showed the expected bacterial proliferation, confirming the viability of the bacterial strains under the experimental conditions.

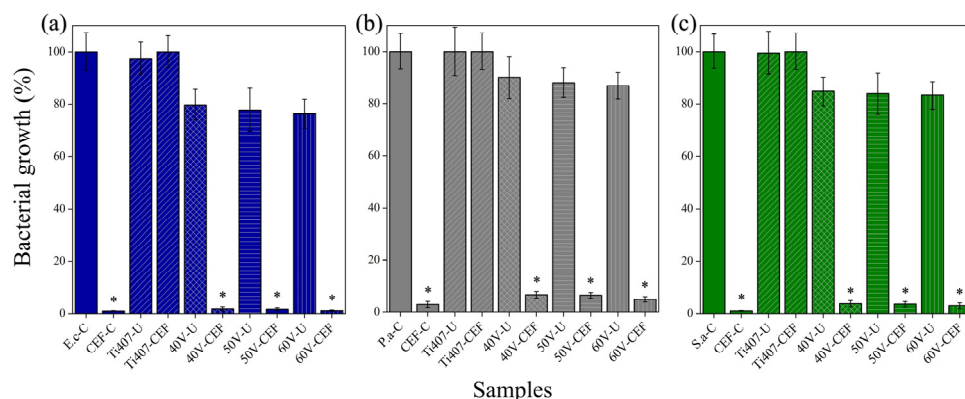


Figure 8. Bacterial growth (%) determined by turbidity assay (OD at 635 nm) after 24 h of incubation for (a) *E. coli* (E.c), (b) *P. aeruginosa* (P.a), and (c) *S. aureus* (S.a) in the presence of the positive control (CEF-Ctrl), non-anodized Ti-407 (Ti407-U and Ti407-CEF), unloaded anodized TiO₂ nanotubular surfaces (40V-U, 50V-U, and 60V-U), and ceftriaxone-loaded anodized TiO₂ nanotubular surfaces (40V-CEF, 50V-CEF, and 60V-CEF). Values are normalized to the corresponding negative controls (E.c-C, P.a-C, and S.a-C; 100%) and reported as mean $\pm$ SD ($n = 3$). * indicates a statistically significant difference ($p < 0.05$).

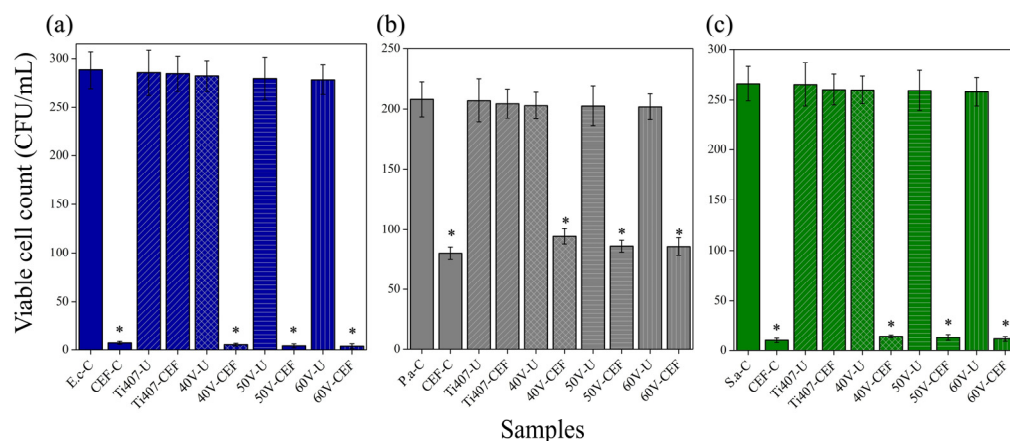


Figure 9. Viable cell count determined by colony-forming unit (CFU/mL) quantification after 24 h of incubation for (a) *E. coli* (E.c), (b) *P. aeruginosa* (P.a), and (c) *S. aureus* (S.a) in the presence of the positive control (CEF-Ctrl), non-anodized Ti-407 (Ti407-U and Ti407-CEF), unloaded anodized TiO₂ nanotubular surfaces (40V-U, 50V-U, and 60V-U), and ceftriaxone-loaded anodized TiO₂ nanotubular surfaces (40V-CEF, 50V-CEF, and 60V-CEF). Data are reported as mean $\pm$ SD ($n = 3$). * indicates a statistically significant difference compared to the corresponding negative controls (E.c-C, P.a-C, and S.a-C) ($p < 0.05$).

Non-anodized Ti-407 samples without ceftriaxone loading (Ti407-U) showed bacterial growth values close to 100% for all tested strains, indicating that the base alloy itself does not exert an inhibitory effect on bacterial proliferation.

On the other hand, non-anodized Ti-407 samples subjected to the ceftriaxone loading protocol (Ti407-CEF) also showed bacterial growth values close to the negative controls, confirming that, in the absence of a nanotubular oxide layer, the alloy is unable to retain ceftriaxone and therefore does not display a measurable antibacterial effect. These two conditions were included as material controls to differentiate the antibacterial effect of the antibiotic from that of the nanotubular architecture.

Similarly, unloaded anodized TiO₂ nanotubular samples (40V-U, 50V-U, and 60V-U) exhibited high bacterial growth values, ranging from approximately 76%–85% depending on the bacterial strain and anodization voltage (40–60 V). These results confirm that the

TiO₂ nanotubular oxide layer itself does not induce a substantial reduction in bacterial growth in the absence of antibiotic loading.

In contrast, all ceftriaxone-loaded nanotubular samples (40V-CEF, 50V-CEF, and 60V-CEF) showed a pronounced and statistically significant reduction in bacterial growth for the three evaluated strains. For *E. coli* (Figure 8a), bacterial growth decreased from approximately 76%–100% in the unloaded samples to 0.9%–1.8% in the ceftriaxone-loaded samples. In the case of *S. aureus* (Figure 8c), growth values were reduced from approximately 83%–100% to 3.0%–3.8%, while for *P. aeruginosa* (Figure 8b), bacterial growth decreased from approximately 87%–100% to 5.0%–6.8%, consistent with the higher intrinsic tolerance of this Gram-negative strain [93]. This overall antibacterial response observed across all ceftriaxone-loaded nanotubular samples is attributed to the localized release of ceftriaxone from the nanotubular architecture (40V-CEF, 50V-CEF, and 60V-CEF), which maintains an effective antibiotic concentration at the bacteria–surface interface. By comparison, unloaded nanotubular (40V-U, 50V-U, and 60V-U) and non-anodized Ti-407 samples (Ti407-U and Ti407-CEF) did not exhibit antibacterial activity, confirming that the observed inhibition is governed by controlled antibiotic delivery rather than by the oxide layer itself [27–29].

Among the anodization conditions, samples anodized at 60 V consistently exhibited the lowest bacterial growth for all three strains, followed by those anodized at 50 and 40 V. This trend correlates with the higher antibiotic loading capacity and the more sustained release behavior associated with nanotubes fabricated at higher anodization voltages, as discussed in Sections 3.2 and 3.4.

3.5.2. Bacterial Viability Evaluation by Colony-Forming Unit (CFU) Quantification

The results of the colony-forming unit (CFU) quantification assay are presented in Figure 9a–c. After incubation, samples were serially diluted (10^{-1} – 10^{-12}) and plated on BHI agar, and CFU/mL was determined from countable plates by applying the corresponding dilution factors.

Negative controls (E.c-C, P.a-C, and S.a-C) and non-anodized Ti-407 without samples of ceftriaxone (Ti407-U) supported substantial bacterial proliferation for *E. coli* (Figure 9a), *P. aeruginosa* (Figure 9b), and *S. aureus* (Figure 9c), yielding high viable cell counts. Similarly, non-anodized Ti-407 samples subjected to the ceftriaxone loading protocol (Ti407-CEF) did not exhibit a significant reduction in CFU values, confirming that, in the absence of a nanotubular oxide layer, the alloy does not retain ceftriaxone at levels sufficient to induce an antibacterial effect. Likewise, unloaded anodized nanotubular samples (40V-U, 50V-U, and 60V-U) did not exhibit a significant reduction in CFU values, confirming that the TiO₂ nanotubular oxide layer itself does not display intrinsic antibacterial activity in the absence of antibiotic loading. In contrast, the positive control (CEF-Ctrl) resulted in a marked and statistically significant reduction in viable cell counts for all three bacterial strains, confirming the expected bactericidal activity of the antibiotic under the experimental conditions.

Consistently, ceftriaxone-loaded nanotubular samples (40V-CEF, 50V-CEF, and 60V-CEF) induced a pronounced reduction in viable cell counts for all three strains. For *E. coli* (Figure 9a), CFU values decreased from approximately 278–288 CFU/mL in the unloaded samples to below 6 CFU/mL in the ceftriaxone-loaded samples, corresponding to a reduction greater than 98%. In the case of *S. aureus* (Figure 9c), viable cell counts were reduced from approximately 258–266 CFU/mL to 11–14 CFU/mL, indicating a reduction on the order of 94%–96%. A less pronounced, yet still significant, reduction was observed for *P. aeruginosa* (Figure 9b), where CFU values decreased from approximately 201–208 CFU/mL in the unloaded samples to 85–94 CFU/mL, consistent with the higher intrinsic tolerance of this Gram-negative strain [93].

Among the anodization conditions, samples prepared at 60 V consistently exhibited the lowest CFU values for all three bacterial strains, followed by those anodized at 50 V and 40 V. This behavior correlates with the higher total enclosed volume and the more sustained release profile of ceftriaxone associated with nanotubes formed at higher anodization voltages, as discussed in Sections 3.2 and 3.4. The strong reduction in CFU values confirms that ceftriaxone remains biologically active after incorporation into and release from the TiO₂ nanotubular structure and that the antibacterial response is governed by controlled local antibiotic delivery rather than by the nanotubular oxide layer itself [27–29].

3.5.3. Bacterial Susceptibility Assessment by Agar Diffusion Test

Figure 10 shows the inhibition zones obtained from the agar diffusion test used to evaluate bacterial susceptibility to ceftriaxone released from the anodized Ti-407 nanotubular surfaces. For all three bacterial strains, unloaded anodized Ti-407 representative samples (50 V) did not produce measurable inhibition zones for *E. coli* (Figure 10a), *P. aeruginosa* (Figure 10e), or *S. aureus* (Figure 10i), indicating that the anodized oxide layer alone does not interfere with bacterial growth or viability under the tested conditions.

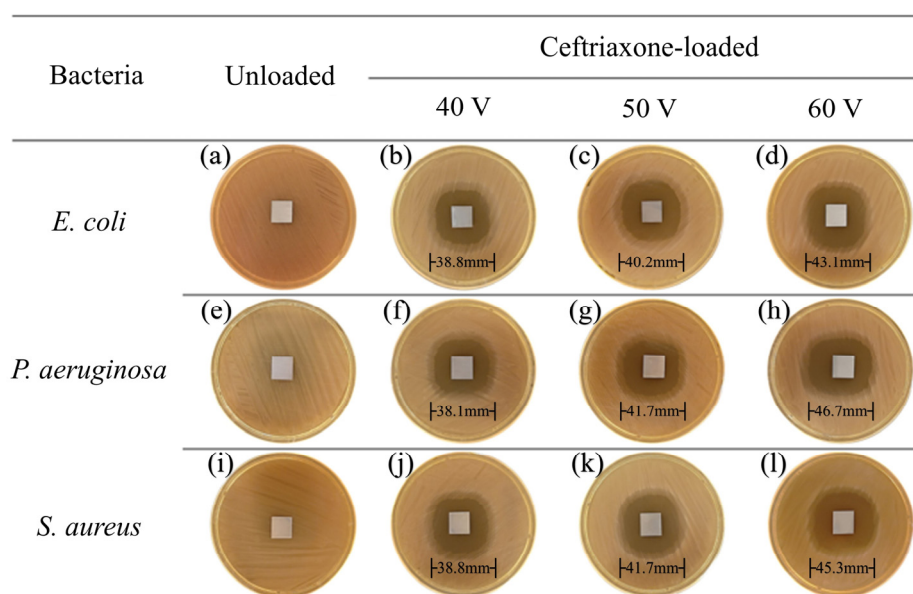


Figure 10. Agar diffusion assay showing inhibition zones produced by ceftriaxone released from anodized TiO₂ nanotubular surfaces fabricated on Ti-407 at 40, 50, and 60 V against (a–d) *E. coli*, (e–h) *P. aeruginosa*, and (i–l) *S. aureus*. The values indicated correspond to the inhibition zone diameter measured across the complete halo.

In contrast, ceftriaxone-loaded anodized samples produced well-defined inhibition zones against *E. coli*, *P. aeruginosa*, and *S. aureus*, as shown in Figure 10b–d, f–h, and j–l, respectively. The diameter of the inhibition halo was measured as the total distance across the zone of bacterial growth inhibition, from one edge of the visible halo to the opposite edge, following the protocol described in Section 2.5.4. Measurements were obtained from three independent specimens per condition, each measured in triplicate ($n = 3$).

For *E. coli* (Figure 10b–d), the inhibition zone diameter increased with anodization voltage, from 38.8 ± 1.2 mm at 40 V, to 40.2 ± 1.4 mm at 50 V, and 43.1 ± 1.0 mm at 60 V. A comparable voltage-dependent trend was observed for *P. aeruginosa* (Figure 10f–h), with inhibition zone diameters of 38.1 ± 1.2 mm, 41.7 ± 1.9 mm, and 46.7 ± 1.3 mm for samples anodized at 40, 50, and 60 V, respectively. Similarly, *S. aureus* (Figure 10j–l) exhibited inhibition zone diameters of 38.8 ± 1.6 mm at 40 V, 41.7 ± 1.8 mm at 50 V, and 45.3 ± 1.2 mm at 60 V.

In general, the progressive increase in inhibition zone diameter with increasing anodization voltage indicates a higher effective antibiotic dose released into the surrounding agar medium. This behavior is consistent with the higher antibiotic loading capacity and sustained release characteristics associated with nanotubes fabricated at higher voltages, as discussed in Sections 3.2 and 3.4. The presence of large and well-defined inhibition zones for all tested strains confirms that ceftriaxone retains its antibacterial activity after incorporation into and release from the TiO₂ nanotubular layer.

It should be noted that, although the present results demonstrate effective antibacterial performance under the tested conditions, the long-term stability and release behavior of ceftriaxone-loaded TiO₂ nanotubular coatings may be influenced by environmental factors such as pH, ionic strength, and coating mass. A systematic evaluation of these parameters was beyond the scope of this study and should be addressed in future work.

3.5.4. Integrated Interpretation of Antibacterial Performance and Release-Driven Mechanism

The combined results obtained from turbidity measurements, CFU quantification, and agar diffusion assays demonstrate a consistent antibacterial response for ceftriaxone-loaded TiO₂ nanotubular coatings fabricated on Ti-407. Across all evaluated strains (*E. coli*, *P. aeruginosa*, and *S. aureus*), unloaded anodized samples showed no measurable antibacterial effect, confirming that the nanotubular TiO₂ oxide layer itself does not exhibit intrinsic bactericidal activity under the tested conditions.

In contrast, ceftriaxone-loaded nanotubular surfaces produced marked reductions in bacterial growth in liquid culture and large inhibition zones in solid media. These effects correlate directly with the release behavior of ceftriaxone from the nanotubular architecture, as described in Section 3.4. Samples anodized at higher voltages exhibited enhanced antibacterial performance, which is consistent with their larger enclosed nanotube volume and higher antibiotic loading capacity.

The antibacterial activity observed against both Gram-negative (*E. coli* and *P. aeruginosa*) and Gram-positive (*S. aureus*) strains is explained by the established mechanism of action of ceftriaxone. The antibiotic inhibits bacterial cell wall synthesis through irreversible binding to penicillin-binding proteins, thereby preventing peptidoglycan cross-linking and ultimately leading to cell lysis [39,40]. The preservation of antibacterial efficacy following incorporation into and release from the TiO₂ nanotubular layer indicates that neither the anodization process nor the loading procedure compromises the biological function of the drug.

Although all three strains were susceptible to ceftriaxone, differences in the magnitude of the antibacterial response reflect both inter-group differences between Gram-negative and Gram-positive bacteria and intra-group variability. Structural features of the bacterial cell envelope contribute to the observed trends, such as the presence of an outer membrane in Gram-negative bacteria, including *E. coli* and *P. aeruginosa*, compared with Gram-positive bacteria such as *S. aureus*. However, the distinct responses observed between *E. coli* and *P. aeruginosa* indicate that additional strain-specific factors, including membrane composition, permeability, and intrinsic resistance mechanisms, also play a role. Together, these factors explain why different levels of antibacterial inhibition were observed among the tested bacterial strains in this study [93]. The antibacterial trends observed in this work are consistent with previous reports on antibiotic-loaded TiO₂ nanotubular systems, including coatings incorporating gentamicin, vancomycin, and cefazolin [27–29,72,80]. In those studies, enhanced antibacterial efficacy was likewise attributed to the combination of high-aspect-ratio nanotube architectures, increased drug-loading capacity, and diffusion-controlled release within the TiO₂ nanotubular architecture. The present results extend these structure–function relationships to ceftriaxone-loaded systems and demonstrate their applicability to Ti-407-based nanotubular coatings, despite the alloy composition.

Overall, the voltage-dependent trends in bacterial growth inhibition and susceptibility reflect a structure–loading–release relationship, in which nanotube geometry governs antibiotic storage capacity and release kinetics, ultimately determining antibacterial performance. These findings highlight the ability of anodized Ti-407 to serve as an effective platform for localized antibiotic delivery, integrating controlled release with robust antibacterial activity.

4. Conclusions

In this study, the Ti-407 alloy was successfully anodized to generate self-organized TiO₂ nanotubular oxide layers capable of functioning as localized antibiotic delivery platforms. Electrochemical anodization in a glycerol/NH₄F electrolyte produced vertically aligned, single-walled, closed-end nanotubes with high aspect ratios and voltage-dependent morphological features, confirming that Ti-407 exhibits anodic behavior analogous to conventional biomedical titanium alloys.

Systematic control of anodization voltage (40–60 V) enabled modulation of nanotube length, density, and total enclosed volume, which directly governed ceftriaxone loading capacity and release behavior. The nanotubular layers exhibited a reproducible biphasic drug-release profile, consisting of an initial burst followed by sustained diffusion-controlled release, with higher anodization voltages yielding more controlled release kinetics due to increased internal storage volume and longer diffusion pathways.

XPS and ATR-FTIR analyses confirmed the formation of a chemically stable TiO₂ nanotubular layer and the successful incorporation of ceftriaxone without alteration of either the oxide framework or the antibiotic structure. Importantly, it seems that vanadium and aluminum originating from the Ti-407 substrate remained covered beneath the nanotubular oxide, indicating effective surface isolation and chemical stability of the anodized layer. However, to elucidate this, a deeper study of the crystal structure and composition of the extracted, fractured nanotubes from the surface is necessary.

In vitro antibacterial evaluation demonstrated that unloaded TiO₂ nanotubular coatings did not exhibit intrinsic antibacterial activity, whereas ceftriaxone-loaded nanotubular surfaces produced pronounced inhibition of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Antibacterial performance scaled consistently with anodization voltage and correlated with the drug-release behavior, confirming a structure–loading–release relationship. The observed strain-dependent responses were consistent with known differences in bacterial cell envelope architecture and antibiotic permeability.

Overall, these findings establish anodized Ti-407 as a functional and effective platform for TiO₂ nanotubular drug-delivery coatings with controlled antibiotic release and robust in vitro antibacterial activity. This work bridges a critical knowledge gap in the surface functionalization of emerging titanium alloys and highlights the potential of ceftriaxone-loaded TiO₂ nanotubular layers for the development of infection-resistant orthopedic implants.

Finally, although the antibacterial efficacy of the ceftriaxone-loaded coatings was clearly demonstrated, further studies are required to evaluate their biological performance, including in vitro cell viability, adhesion, and differentiation assays using osteoblast-like cells.

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References

1. Moriarty, T.F.; Kuehl, R.; Coenye, T.; Metsemakers, W.J.; Morgenstern, M.; Schwarz, E.M.; Riool, M.; Zaat, S.A.J.; Khana, N.; Kates, S.L.; et al. Orthopaedic device-related infection: Current and future interventions for improved prevention and treatment. *EFORT Open Rev.* **2017**, *1*, 89–99. [CrossRef]
2. Stolz, M.; Schmetsdorf, J.; Tomsic, I.; von Lengerke, T.; Chaberny, I.F.; Krauth, C. Costs of surgical site infections in orthopaedic and trauma surgery: A systematic review. *J. Hosp. Infect.* **2025**, *169*, 42–58. [CrossRef]
3. Tande, A.J.; Patel, R. Prosthetic joint infection. *Clin. Microbiol. Rev.* **2014**, *27*, 302–345. [CrossRef]
4. McQuillan, T.J.; Cai, L.Z.; Corcoran-Schwartz, I.; Weiser, T.G.; Forrester, J.D. Surgical site infections after open reduction internal fixation for trauma in low and middle Human Development Index countries: A systematic review. *Surg. Infect.* **2018**, *19*, 254–263. [CrossRef]
5. Whiting, P.S.; Galat, D.D.; Zirkle, L.G.; Shaw, M.K.; Galat, J.D. Risk factors for infection after intramedullary nailing of open tibial shaft fractures in low- and middle-income countries. *J. Orthop. Trauma* **2019**, *33*, e234–e239. [CrossRef]
6. Kortram, K.; Bezstarosti, H.; Metsemakers, W.J.; Raschke, M.J.; Van Lieshout, E.M.M.; Verhofstad, M.H.J. Risk factors for infectious complications after open fractures; a systematic review and meta-analysis. *Int. Orthop.* **2017**, *41*, 1965–1982. [CrossRef]
7. Zimmerli, W.; Trampuz, A.; Ochsner, P.E. Prosthetic-joint infections. *N. Engl. J. Med.* **2004**, *351*, 1645–1654. [CrossRef]
8. Arciola, C.R.; Campoccia, D.; Montanaro, L. Implant infections: Adhesion, biofilm formation and immune evasion. *Nat. Rev. Microbiol.* **2018**, *16*, 397–409. [CrossRef]
9. Costerton, J.W.; Stewart, P.S.; Greenberg, E.P. Bacterial biofilms: A common cause of persistent infections. *Science* **1999**, *284*, 1318–1322. [CrossRef]
10. El-Bassyouni, G.T.; Mouneir, S.M.; El-Shamy, A.M. Advances in surface modifications of titanium and its alloys: Implications for biomedical and pharmaceutical applications. *Multiscale Multidiscip. Model. Exp. Des.* **2025**, *8*, 265. [CrossRef]
11. Li, J.; Zheng, Y.; Yu, Z.; Kankala, R.K.; Lin, Q.; Shi, J.; Chen, C.; Luo, K.; Chen, A.; Zhong, Q. Surface-modified titanium and titanium-based alloys for improved osteogenesis: A critical review. *Heliyon* **2024**, *10*, e23779. [CrossRef]
12. Geetha, M.; Singh, A.K.; Asokamani, R.; Gogia, A.K. Ti based biomaterials, the ultimate choice for orthopaedic implants—A review. *Prog. Mater. Sci.* **2009**, *54*, 397–425. [CrossRef]
13. Willis, J.; Li, S.; Crean, S.J.; Barrak, F.N. Is titanium alloy Ti-6Al-4V cytotoxic to gingival fibroblasts—A systematic review. *Clin. Exp. Dent. Res.* **2021**, *7*, 1037–1044. [CrossRef]
14. Yang, S.M.; Kang, Y.S.; Kim, D.Y.; Park, H.W. Experimental investigation of tool wear and surface integrity using a large pulsed electron beam (LPEB) irradiated end-mill cutting tool for Ti-6Al-4V. *J. Manuf. Process.* **2025**, *139*, 172–181. [CrossRef]
15. TIMET. TIMETAL® 407 Datasheet. Available online: <https://www.timet.com/documents/datasheets/alpha-and-beta-alloys/timet-407.pdf> (accessed on 7 December 2025).
16. Sneddon, S.; Xu, Y.; Dixon, M.; Rugg, D.; Li, P.; Mulvihill, D.M. Sensitivity of material failure to surface roughness: A study on titanium alloys Ti64 and Ti407. *Mater. Des.* **2021**, *200*, 109438. [CrossRef]
17. Tamayo-Jimenez, A.P.; Melendez-Anzures, F.E.; Barron-Gonzalez, M.P.; Lopez-Cuellar, E.M.; Quiñones-Gutierrez, Y.; Garza-Guajardo, J.A.; la Cruz, A.M.-D. Hierarchical Ag-doped hydroxyapatite coatings on TiO₂ nanotubes formed on Ti-407 alloy: Antibacterial evaluation against *Escherichia coli*. *Mater. Proc.* **2025**, *28*, 4. [CrossRef]
18. Gong, D.; Grimes, C.A.; Varghese, O.K.; Hu, W.; Singh, R.S.; Chen, Z.; Dickey, E.C. Titanium oxide nanotube arrays prepared by anodic oxidation. *J. Mater. Res.* **2001**, *16*, 3331–3334. [CrossRef]
19. Roy, P.; Berger, S.; Schmuki, P. TiO₂ nanotubes: Synthesis and applications. *Angew. Chem. Int. Ed.* **2011**, *50*, 2904–2939. [CrossRef]

20. Macak, J.M.; Tsuchiya, H.; Schmuki, P. High-aspect-ratio TiO₂ nanotubes by anodization of titanium. *Angew. Chem. Int. Ed.* **2005**, *44*, 2100–2102. [[CrossRef](#)]
21. Zhuang, Y.; Li, P.; Qin, L.; Zhang, S.; Chen, B.; Zhu, Y.; Wang, B.; Zhu, X. Real role of fluoride ions in the growth of anodic TiO₂ nanotubes. *J. Phys. Chem. C* **2024**, *128*, 5741–5748. [[CrossRef](#)]
22. Regonini, D.; Bowen, C.R.; Jaroenworarluck, A.; Stevens, R. A review of growth mechanism, structure and crystallinity of anodized TiO₂ nanotubes. *Mater. Sci. Eng. R Rep.* **2013**, *74*, 377–406. [[CrossRef](#)]
23. Wang, J.; Lin, Z. Anodic formation of ordered TiO₂ nanotube arrays: Effects of electrolyte temperature and anodization potential. *J. Phys. Chem. C* **2009**, *113*, 4026–4030. [[CrossRef](#)]
24. Sul, Y.T.; Johansson, C.B.; Röser, K.; Albrektsson, T. Qualitative and quantitative observations of bone tissue reactions to anodised implants. *Biomaterials* **2002**, *23*, 1809–1817. [[CrossRef](#)] [[PubMed](#)]
25. Cai, K.; Bossert, J.; Jandt, K.D. Does the nanometre scale topography of titanium influence protein adsorption and cell proliferation? *Colloids Surf. B Biointerfaces* **2006**, *49*, 136–144. [[CrossRef](#)] [[PubMed](#)]
26. Melendez-Anzures, F.E.; Lopez-Cuellar, E.M.; Barron-Gonzalez, M.P.; Zarate-Triviño, D.G.; Martinez-Sanmiguel, J.J.; la Cruz, A.M.-D. Electrodeposited bioactive coating on TiO₂ nanotubes for enhanced in vitro cell viability. In *XLVIII Mexican Conference on Biomedical Engineering. CNIB 2025; IFMBE Proceedings*; Zuñiga-Aguilar, E., Benítez-Mata, B., Reyes-Lagos, J.J., Hernandez Acosta, H.Y., Botello Arredondo, A.I., Bayareh Mancilla, R., Vázquez de la Rosa, J.F., Gutiérrez Valenzuela, C.A., Eds.; Springer: Cham, Switzerland, 2026; Volume 138. [[CrossRef](#)]
27. Ionita, D.; Bajenaru-Georgescu, D.; Totea, G.; Mazare, A.; Schmuki, P.; Demetrescu, I. Activity of vancomycin release from bioinspired coatings of hydroxyapatite or TiO₂ nanotubes. *Int. J. Pharm.* **2017**, *517*, 296–302. [[CrossRef](#)]
28. Gulati, K.; Aw, M.S.; Losic, D. Drug-eluting Ti wires with titania nanotube arrays for bone fixation and reduced bone infection. *Nanoscale Res. Lett.* **2011**, *6*, 571. [[CrossRef](#)]
29. Lopez-Pavon, L.; Dagnino-Acosta, D.; Lopez-Cuellar, E.; Melendez-Anzures, F.; Zarate-Triviño, D.; Barron-González, M.; Moreno-Cortez, I.; Kim, H.Y.; Miyazaki, S. Synthesis of nanotubular oxide on Ti–24Zr–10Nb–2Sn as a drug-releasing system to prevent the growth of *Staphylococcus aureus*. *Chem. Pap.* **2021**, *75*, 2441–2450. [[CrossRef](#)]
30. Mei, S.; Wang, H.; Wang, W.; Tong, L.; Pan, H.; Ruan, C.; Ma, Q.; Liu, M.; Yang, H.; Zhang, L.; et al. Antibacterial effects and biocompatibility of titanium surfaces with graded silver incorporation in titania nanotubes. *Biomaterials* **2014**, *35*, 4255–4265. [[CrossRef](#)]
31. Aw, M.; Gulati, K.; Losic, D. Controlling drug release from titania nanotube arrays using polymer nanocarriers and biopolymer coating. *J. Biomater. Nanobiotechnol.* **2011**, *2*, 477–484. [[CrossRef](#)]
32. Hong, C.; An, S.; Son, M.; Hong, S.S.; Lee, D.H.; Lee, C. In-vitro cell tests using doxorubicin-loaded polymeric TiO₂ nanotubes used for cancer photothermotherapy. *Anti-Cancer Drugs* **2012**, *23*, 553–560. [[CrossRef](#)]
33. Akshaya, S.; Rowlo, P.K.; Dukle, A.; Nathanael, A.J. Antibacterial coatings for titanium implants: Recent trends and future perspectives. *Antibiotics* **2022**, *11*, 1719. [[CrossRef](#)]
34. Ribeiro, B.; Offoiach, R.; Monteiro, C.; Morais, M.R.G.; Martins, M.C.L.; Pêgo, A.P.; Salatin, E.; Fedrizzi, L.; Lekka, M. Electrodeposition of Zn and Cu nanoparticles into TiO₂ nanotubes on Ti6Al4V: Antimicrobial effect against *S. epidermidis* and cytotoxicity assessment. *Micro* **2024**, *4*, 97–116. [[CrossRef](#)]
35. Zilberman, M.; Elsner, J.J. Antibiotic-eluting medical devices for various applications. *J. Control. Release* **2008**, *130*, 202–215. [[CrossRef](#)]
36. Popat, K.C.; Eltgroth, M.; Latempa, T.J.; Grimes, C.A.; Desai, T.A. Decreased *Staphylococcus epidermidis* adhesion and increased osteoblast functionality on antibiotic-loaded titania nanotubes. *Biomaterials* **2007**, *28*, 4880–4888. [[CrossRef](#)]
37. Metsemakers, W.J.; Morgenstern, M.; McNally, M.A.; Moriarty, T.F.; McFadyen, I.; Scarborough, M.; Athanasou, N.A.; Ochsner, P.E.; Kuehl, R.; Raschke, M.; et al. Fracture-related infection: A consensus on definition from an international expert group. *Injury* **2018**, *49*, 505–510. [[CrossRef](#)] [[PubMed](#)]
38. Trampuz, A.; Zimmerli, W. Diagnosis and treatment of infections associated with fracture-fixation devices. *Injury* **2006**, *37*, S59–S66. [[CrossRef](#)]
39. Scholar, E. Ceftriaxone. In *xPharm: The Comprehensive Pharmacology Reference*; Enna, S.J., Bylund, D.B., Eds.; Elsevier: New York, NY, USA, 2007; pp. 1–7. [[CrossRef](#)]
40. Bratzler, D.W.; Dellinger, E.P.; Olsen, K.M.; Perl, T.M.; Auwaerter, P.G.; Bolon, M.K.; Fish, D.N.; Napolitano, L.M.; Sawyer, R.G.; Slain, D.; et al. Clinical practice guidelines for antimicrobial prophylaxis in surgery. *Am. J. Health-Syst. Pharm.* **2013**, *70*, 195–283. [[CrossRef](#)] [[PubMed](#)]
41. Lew, D.P.; Waldvogel, F.A. Osteomyelitis. *Lancet* **2004**, *364*, 369–379. [[CrossRef](#)]
42. Darouiche, R.O. Treatment of infections associated with surgical implants. *N. Engl. J. Med.* **2004**, *350*, 1422–1429. [[CrossRef](#)]
43. Peel, T.N.; Buising, K.L.; Choong, P.F. Prosthetic joint infection: Challenges of diagnosis and treatment. *ANZ J. Surg.* **2011**, *81*, 32–39. [[CrossRef](#)]

44. Melendez Anzures, F.E. Biocompatibilidad de Nanoestructuras Formadas por Anodizado en Aleaciones de Base Titanio Para Aplicaciones Biomédicas. Ph.D. Thesis, Universidad Autónoma de Nuevo León, Monterrey, Mexico, 2023. Available online: <http://eprints.uanl.mx/26463/1/1080312748.pdf> (accessed on 17 December 2025).
45. Barboza, L.; López, E.; Guajardo, H.; Salinas, A. Effect of Initial Microstructure on the Temperature Dependence of the Flow Stress and Deformation Microstructure under Uniaxial Compression of Ti-407. *Metals* **2024**, *14*, 505. [\[CrossRef\]](#)
46. Hudzicki, J. Kirby–Bauer disk diffusion susceptibility test protocol. *Am. Soc. Microbiol.* **2009**, *15*, 1–23. Available online: <https://asm.org/getattachment/2594ce26-bd44-47f6-8287-0657aa9185ad/kirby-bauer-disk-diffusion-susceptibility-test-protocol-pdf.pdf> (accessed on 20 November 2025).
47. Peng, K.; Liu, L.; Zhang, J.; Ma, J.; Liu, Y. The relationship between the growth rate of anodic TiO₂ nanotubes, the fluoride concentration and the electronic current. *Electrochem. Commun.* **2023**, *148*, 107457. [\[CrossRef\]](#)
48. Fornell, J.; Oliveira, N.T.C.; Pellicer, E.; Van Steenberge, N.; Baró, M.D.; Bolfarini, C.; Sort, J. Anodic formation of self-organized Ti(Nb,Sn) oxide nanotube arrays with tuneable aspect ratio and size distribution. *Electrochem. Commun.* **2013**, *33*, 84–87. [\[CrossRef\]](#)
49. Regonini, D.; Satka, A.; Jaroenworarluck, A.; Allsopp, D.W.E.; Bowen, C.R.; Stevens, R. Factors influencing surface morphology of anodized TiO₂ nanotubes. *Electrochim. Acta* **2012**, *74*, 244–253. [\[CrossRef\]](#)
50. Habazaki, H.; Fushimi, K.; Shimizu, K.; Skeldon, P.; Thompson, G.E. Fast migration of fluoride ions in growing anodic titanium oxide. *Electrochem. Commun.* **2007**, *9*, 1222–1227. [\[CrossRef\]](#)
51. Liu, G.; Wang, K.; Hoivik, N.; Jakobsen, H. Progress on free-standing and flow-through TiO₂ nanotube membranes. *Sol. Energy Mater. Sol. Cells* **2012**, *98*, 24–38. [\[CrossRef\]](#)
52. Albu, S.P.; Schmuki, P. Influence of anodization parameters on the expansion factor of TiO₂ nanotubes. *Electrochim. Acta* **2013**, *91*, 90–95. [\[CrossRef\]](#)
53. Neupane, M.P.; Park, I.S.; Bae, T.S.; Yi, H.K.; Watari, F.; Lee, M.H. Biocompatibility of TiO₂ nanotubes fabricated on Ti using different surfactant additives in electrolyte. *Mater. Chem. Phys.* **2012**, *134*, 536–541. [\[CrossRef\]](#)
54. Li, T.; Gulati, K.; Wang, N.; Zhang, Z.; Ivanovski, S. Understanding and augmenting the stability of therapeutic nanotubes on anodized titanium implants. *Mater. Sci. Eng. C* **2018**, *88*, 182–195. [\[CrossRef\]](#)
55. Li, B.; Gao, X.; Zhang, H.C.; Yuan, C. Energy modeling of electrochemical anodization process of titanium dioxide nanotubes. *ACS Sustain. Chem. Eng.* **2014**, *2*, 404–410. [\[CrossRef\]](#)
56. Yu, D.; Zhang, S.; Zhu, X.; Ma, H.; Han, H.; Song, Y. Simulation of anodizing current–time curves and morphology evolution of TiO₂ nanotube arrays. *J. Solid State Electrochem.* **2014**, *18*, 2609–2617. [\[CrossRef\]](#)
57. Zare, H.; Ahmadi, S.; Ghasemi, A.; Ghanbari, M.; Rabiee, N.; Bagherzadeh, M.; Karimi, M.; Webster, T.J.; Hamblin, M.R.; Mostafavi, E. Carbon nanotubes: Smart drug/gene delivery carriers. *Int. J. Nanomed.* **2021**, *16*, 1681–1706. [\[CrossRef\]](#) [\[PubMed\]](#)
58. Jagusiak, A.; Chłopas, K.; Zemanek, G.; Wolski, P.; Panczyk, T. Controlled release of doxorubicin from the drug delivery formulation composed of single-walled carbon nanotubes and Congo red: A molecular dynamics study and dynamic light scattering analysis. *Pharmaceutics* **2020**, *12*, 622. [\[CrossRef\]](#) [\[PubMed\]](#)
59. Li, Y.; Yang, Y.; Li, R.; Tang, X.; Guo, D.; Qing, Y.; Qin, Y. Enhanced antibacterial properties of orthopedic implants by titanium nanotube surface modification: A review of current techniques. *Int. J. Nanomed.* **2019**, *14*, 7217–7236. [\[CrossRef\]](#) [\[PubMed\]](#)
60. Asadi, S.; Mortezaagholi, B.; Hadizadeh, A.; Borisov, V.; Ansari, M.J.; Shaker Majidi, H.; Nishonova, A.; Adelnia, H.; Farasati Far, B.; Chaiyasut, C. Ciprofloxacin-loaded titanium nanotubes coated with chitosan: A promising formulation with sustained release and enhanced antibacterial properties. *Pharmaceutics* **2022**, *14*, 1359. [\[CrossRef\]](#)
61. Wang, T.; Weng, Z.; Liu, X.; Yeung, K.W.K.; Pan, H.; Wu, S. Controlled release and biocompatibility of polymer/titania nanotube array system on titanium implants. *Bioact. Mater.* **2017**, *2*, 44–50. [\[CrossRef\]](#)
62. Filova, E.; Fojt, J.; Kryslova, M.; Moravec, H.; Joska, L.; Bacakova, L. The diameter of nanotubes formed on Ti–6Al–4V alloy controls the adhesion and differentiation of Saos-2 cells. *Int. J. Nanomed.* **2015**, *10*, 7145–7163. [\[CrossRef\]](#)
63. Allam, N.K.; Alamgir, F.; El-Sayed, M.A. Enhanced photoassisted water electrolysis using vertically oriented anodically fabricated Ti–Nb–Zr–O mixed oxide nanotube arrays. *ACS Nano* **2010**, *4*, 5819–5826. [\[CrossRef\]](#)
64. Valota, A.; LeClere, D.J.; Hashimoto, T.; Skeldon, P.; Thompson, G.E.; Berger, S.; Kunze, J.; Schmuki, P. The efficiency of nanotube formation on titanium anodized under voltage and current control in fluoride/glycerol electrolyte. *Nanotechnology* **2008**, *19*, 355701. [\[CrossRef\]](#)
65. Necula, M.G.; Mazare, A.; Ion, R.N.; Ozkan, S.; Park, J.; Schmuki, P.; Cimpean, A. Lateral spacing of TiO₂ nanotubes modulates osteoblast behavior. *Materials* **2019**, *12*, 2956. [\[CrossRef\]](#) [\[PubMed\]](#)
66. Wu, B.; Tang, Y.; Wang, K.; Zhou, X.; Xiang, L. Nanostructured titanium implant surface facilitating osseointegration from protein adsorption to osteogenesis: The example of TiO₂ NTAs. *Int. J. Nanomed.* **2022**, *17*, 1865–1879. [\[CrossRef\]](#) [\[PubMed\]](#)
67. Wang, Q.; Zhou, P.; Liu, S.; Attarilar, S.; Ma, R.L.-W.; Zhong, Y.; Wang, L. Multi-scale surface treatments of titanium implants for rapid osseointegration: A review. *Nanomaterials* **2020**, *10*, 1244. [\[CrossRef\]](#)

68. Simon, A.P.; Ferreira, C.H.; Santos, V.A.Q.; Rodrigues, A.; Santos, J.S.; Trivinho-Strixino, F.; Marques, P.T.; Zorel, H.E.; Sikora, M.S. Multi-step cefazolin sodium release from bioactive TiO₂ nanotubes: Surface and polymer coverage effects. *J. Mater. Res.* **2021**, *36*, 1510–1523. [\[CrossRef\]](#)
69. Sul, Y. Electrochemical growth behavior, surface properties, and enhanced in vivo bone response of TiO₂ nanotubes on microstructured surfaces of blasted, screw-shaped titanium implants. *Int. J. Nanomed.* **2010**, *5*, 87–100. [\[CrossRef\]](#)
70. Capek, D.; Gigandet, M.P.; Masmoudi, M.; Wery, M.; Banakh, O. Long-time anodisation of titanium in sulphuric acid. *Surf. Coat. Technol.* **2008**, *202*, 1379–1384. [\[CrossRef\]](#)
71. Kim, M.G.; Kang, J.M.; Lee, J.E.; Kim, K.S.; Kim, K.H.; Cho, M.; Lee, S.G. Effects of calcination temperature on the phase composition, photocatalytic degradation, and virucidal activities of TiO₂ nanoparticles. *ACS Omega* **2021**, *6*, 10668–10678. [\[CrossRef\]](#)
72. Nawaz, K.; Chia, I.; Huei, G. Glycerol-mediated facile synthesis of colored titania nanoparticles for visible light photodegradation of phenolic compounds. *Nanomaterials* **2019**, *9*, 1586. [\[CrossRef\]](#)
73. Shi, H.; Magaye, R.; Castranova, V.; Zhao, J. Titanium dioxide nanoparticles: A review of current toxicological data. *Part. Fibre Toxicol.* **2013**, *10*, 15. [\[CrossRef\]](#)
74. Ranjan, R.; Mhamane, N.B.; Kolekar, S.K.; Gopinath, C.S. Electronic structure evolution from metallic vanadium to metallic VxOy: A NAPPES study for O₂ + V gas–solid interaction. *J. Phys. Chem. C* **2022**, *126*, 19136–19146. [\[CrossRef\]](#)
75. Hryha, E.; Rutqvist, E.; Nyborg, L. Stoichiometric vanadium oxides studied by XPS. *Surf. Interface Anal.* **2012**, *44*, 1022–1025. [\[CrossRef\]](#)
76. Chunate, H.-T.; Khamwannah, J.; Aliyu, A.A.A.; Tantavisut, S.; Puncreobutr, C.; Khamkongkao, A.; Tongyam, C.; Tumkhanon, K.; Phetrattanarangsi, T.; Chanamuangkon, T.; et al. Titania nanotube architectures synthesized on 3D-printed Ti–6Al–4V implant and assessing vancomycin release protocols. *Materials* **2021**, *14*, 6576. [\[CrossRef\]](#) [\[PubMed\]](#)
77. Osonkie, A.; Lee, V.; Chukwunenye, P.; Cundari, T.; Kelber, J. Plasma modification of vanadium oxynitride surfaces: Characterization by in situ XPS experiments and DFT calculations. *J. Chem. Phys.* **2020**, *153*, 144709. [\[CrossRef\]](#)
78. Silversmit, G.; Depla, D.; Poelman, H.; Marin, G.B.; De Gryse, R. Determination of the V2p XPS binding energies for different vanadium oxidation states (V⁵⁺ to V⁰⁺). *J. Electron Spectrosc. Relat. Phenom.* **2004**, *135*, 167–175. [\[CrossRef\]](#)
79. Klomjit, P.; Buchheit, R.G. Localized corrosion inhibition of AA7075-T6 by calcium sulfate. *Corrosion* **2016**, *72*, 486–499. [\[CrossRef\]](#)
80. Popinciuc, M.; Jonkman, H.T.; Van Wees, B.J. Energy level alignment at Co/AlO_x/pentacene interfaces. *J. Appl. Phys.* **2007**, *101*, 4–9. [\[CrossRef\]](#)
81. Hou, L.; Liang, Q.; Wang, F. Mechanisms that control the adsorption–desorption behavior of phosphate on magnetite nanoparticles: The role of particle size and surface chemistry characteristics. *RSC Adv.* **2020**, *10*, 2378–2388. [\[CrossRef\]](#)
82. Moreno-López, J.C.; Ruano, G.; Ferrón, J.; Ayala, P.; Passeggi, M.C.G., Jr. Thermally annealed sub-monolayers of AlF₃ on Cu(100): An STM and XPS study. *Phys. Status Solidi B* **2018**, *255*, 1800389. [\[CrossRef\]](#)
83. Greczynski, G.; Hultman, L. C1s peak of adventitious carbon aligns to the vacuum level: Dire consequences for material's bonding assignment by photoelectron spectroscopy. *ChemPhysChem* **2017**, *18*, 1507–1512. [\[CrossRef\]](#)
84. Hanawa, T. Metal ion release from metal implants. *Mater. Sci. Eng. C* **2004**, *24*, 745–752. [\[CrossRef\]](#)
85. Ghicov, A.; Tsuchiya, H.; Macak, J.M.; Schmuki, P. Titanium oxide nanotubes prepared in phosphate electrolytes. *Electrochem. Commun.* **2005**, *7*, 505–509. [\[CrossRef\]](#)
86. Macak, J.M.; Tsuchiya, H.; Ghicov, A.; Yasuda, K.; Hahn, R.; Bauer, S.; Schmuki, P. TiO₂ nanotubes: Self-organized electrochemical formation, properties and applications. *Curr. Opin. Solid State Mater. Sci.* **2007**, *11*, 3–18. [\[CrossRef\]](#)
87. Meng, R.; Hou, H.; Liu, X.; Duan, J.; Liu, S. Binder-free combination of graphene nanosheets with TiO₂ nanotube arrays for lithium ion battery anode. *J. Porous Mater.* **2016**, *23*, 569–575. [\[CrossRef\]](#)
88. Manimekalai, P.; Dhanalakshmi, R.; Manavalan, R. Preparation and characterization of ceftriaxone sodium encapsulated chitosan nanoparticles. *Int. J. Appl. Pharm.* **2017**, *9*, 10–15. [\[CrossRef\]](#)
89. Liang, Y.Q.; Cui, Z.D.; Zhu, S.L.; Yang, X.J. Characterization of self-organized TiO₂ nanotubes on Ti–4Zr–22Nb–2Sn alloys and the application in drug delivery system. *J. Mater. Sci. Mater. Med.* **2011**, *22*, 461–467. [\[CrossRef\]](#)
90. Ethiraj, R.; Thiruvengadam, E.; Sampath, V.S.; Vahid, A.; Raj, J. Development and validation of stability indicating spectroscopic method for content analysis of ceftriaxone sodium in pharmaceuticals. *Int. Sch. Res. Not.* **2014**, *2014*, 278173. [\[CrossRef\]](#) [\[PubMed\]](#)
91. Wang, Q.; Huang, J.Y.; Li, H.Q.; Chen, Z.; Zhao, A.Z.J.; Wang, Y.; Zhang, K.Q.; Sun, H.T.; Al-Deyab, S.S.; Lai, Y.K. TiO₂ nanotube platforms for smart drug delivery: A review. *Int. J. Nanomed.* **2016**, *11*, 4819–4834. [\[CrossRef\]](#)
92. Yang, W.; Xi, X.; Shen, X.; Liu, P.; Hu, Y.; Cai, K. Titania nanotubes dimensions-dependent protein adsorption and its effect on the growth of osteoblasts. *J. Biomed. Mater. Res. A* **2014**, *102*, 3598–3608. [\[CrossRef\]](#)
93. Poole, K. *Pseudomonas aeruginosa*: Resistance to the max. *Front. Microbiol.* **2011**, *2*, 65. [\[CrossRef\]](#)

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