Materials Today Communications 32 (2022) 103843

Contents lists available at ScienceDirect

Materials Today Communications

journal homepage: www.elsevier.com/locate/mtcomm

# Controlled and localized drug delivery using Titania nanotubes

Amogh Kumar Baranwal [a,1], G. Keerthiga [a,b,1], L. Mohan [a], Sayan Deb Dutta [c], Pallavi Gupta [a], Ki-Taek Lim [c], Tuhin Subhra Santra [a,*]

[a] Department of Engineering Design, Indian Institute of Technology-Madras, Chennai, Tamil Nadu 600 036, India
[b] Centre for Biotechnology, ACT Campus, Anna University, Chennai, Tamil Nadu 600032, India
[c] Department of Biosystems Engineering, Institute of Forest Science, Kangwon National University, Chuncheon 24341, Republic of Korea

ARTICLE INFO

*Keywords:*
Titania nanotubes
Ciprofloxacin
PLGA
Nanotubes drug release
Osseointegration
Controlled drug release

ABSTRACT

Titanium-dioxide nanotubes (TNTs) were fabricated by anodic oxidation of titanium (Ti) substrate and loaded with ciprofloxacin. A thin layer of Poly Lactic-co-Glycolic Acid (PLGA) was coated on top of the drug-loaded TNTs at various thicknesses. Field Emission Scanning Electron Microscopy (FESEM) was used to characterize the developed substrate. The FESEM images of anodized samples showed that, the native oxide layer on the surface of the Ti substrate was replaced with an array of ordered nanotubes up to ~700 nm in length. Drug release studies of ciprofloxacin were conducted in Phosphate Buffered Saline (PBS) solution for 24 h. The studies showed the ability of PLGA to slow down the drug release rate, depending on the thickness of the deposited layer. MG-63 cells (human osteosarcoma cell lines) were cultured on the TNT substrates after loading ciprofloxacin and coating with various concentrations of PLGA to show the potentiality for better osseointegration. An MTT assay was carried out to study cell viability and proliferation on these substrates. Anti-microbial studies were carried out to demonstrate the release of ciprofloxacin in treating infections against *Staphylococcus epidermidis*. These in-depth studies showed that local concentration of the drug released could be controlled by varying the thickness of the PLGA layer to allow for better implant osseointegration and treatment of bone infections.

## 1. Introduction

Titanium (Ti) and its alloys, known for their biocompatibility, corrosion resistance, chemical inertness, have been an excellent candidate for hard tissue replacement such as dental and orthopedic implants [1–7]. Surface modifications on Ti have been introduced for various reasons mainly to aid in better osseointegration and faster healing rate. One such surface modification technique is the formation of Titania nanotubes (TNTs) on the surface of Ti substrates. Conventional methods for TNTs fabrication, including sol-gel, seeded growth, electrospinning, nanoporous alumina templates, hydrothermal, and vaporization techniques, are very tedious, having critical steps for template removal & requiring specific precursors [8]. They also lead to formation of nanotubes dispersed in a solution in bundles, with very limited control over the distribution of their lengths. On the other hand, the anodization technique helps self-organized nanotube array formation, in a simple and cost-effective manner [1,9]. Anodization also allows for precise control over length of the fabricated nanotubes. These nanotubes (TNTs) formed on surfaces of Ti substrates can serve as agents for drug loading and different surface modifications can be made to achieve subsequent localized drug delivery. However, for these applications, fabricated TNTs must have a good volume to area ratio to allow for sufficient drug loading, and their dimensions must be controllable during fabrication [9,10]. Once loaded, the drug release profile from these TNTs can be mapped and tuned for achieving better osseointegration and faster healing post-surgery [11].

Poly Lactic-Co-Glycolic Acid (PLGA) is a copolymer of polylactic acid and polyglycolic acid, and it is one of the most commonly used biodegradable polymers for implant functionalization to improve biocompatibility. PLGA is also highly investigated for its ability to act as a controller for sustained drug delivery into the body [12], as natural degradation of PLGA in the human body promotes its ability to act as a slowly-eroding barrier for enabling sustained release of drugs. PLGA undergoes degradation via cleavage of its backbone ester linkages into oligomers and monomers. Thus, coating of PLGA on top of an implant's surface would serve as a promoter for cell adhesion and proliferation as

* Corresponding author.
*E-mail address:* tuhin@iitm.ac.in (T.S. Santra).
[1] Equally contributed

https://doi.org/10.1016/j.mtcomm.2022.103843
Received 15 September 2021; Received in revised form 19 April 2022; Accepted 12 June 2022
Available online 16 June 2022

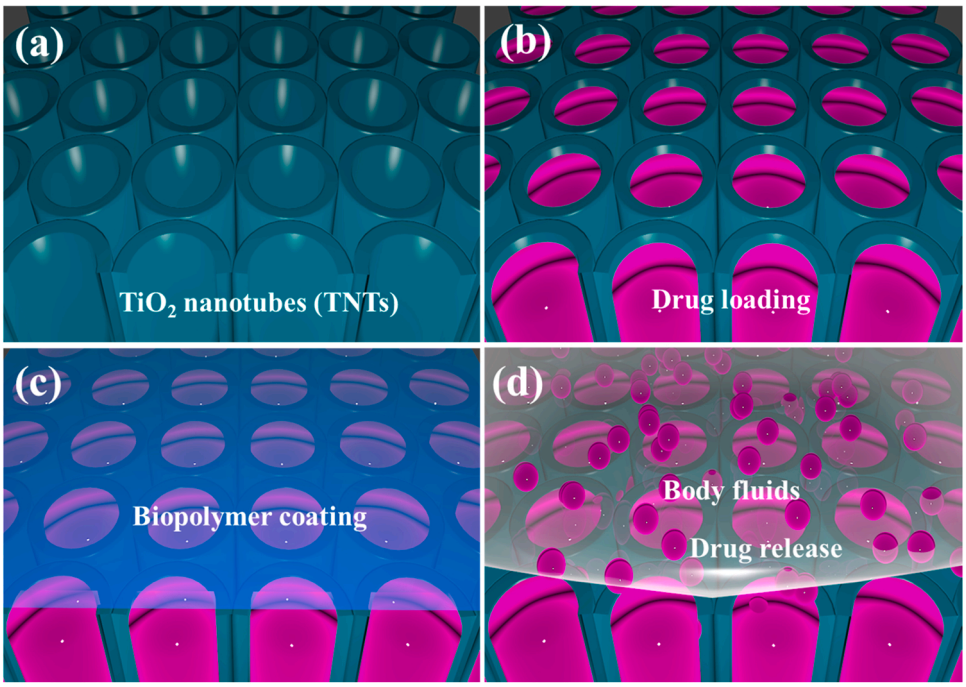


**Fig. 1.** Schematic of the drug loading and polymer coating and release process on the TNTs substrate.

well as for control drug release.

Conventional drug administration through oral, parenteral, and inhalation routes are highly followed in favor of drug delivery throughout the whole system and not at the targeted site of action. Interdisciplinary research strategies involving fields of material science, nanotechnology, clinical science, biology have demonstrated promising results on localized drug delivery (LDD) [13,14]. Drug releasing implants (DRI) for LDD have been designed for sustained and localized persistent drug release [15].

Ciprofloxacin, a fluoroquinolone class of antibiotics is used to treat a variety of bacterial infections, with specialized usage in treating bone infections, joint infections and skin infections [16]. In recent years, per capita cases of osteomyelitis, a bone infection caused by pyrogenic bacteria, have gone up due to implant surgeries becoming more common than ever before [17]. Thus, ciprofloxacin release from implants having TNTs can provide effective targeted treatment or prevention of post-operative infections. Recent studies have shown increased proliferation of dermal fibroblasts and mouse preosteoblasts in presence of PLGA and bone growth factors [12,18].

Thus, the aim of this research is to fabricate TNTs on Ti alloy to develop a functionalized surface to provide an avenue for sustained/controlled ciprofloxacin release to tackle post-operative infection and study proliferation of human fibroblasts for varying PLGA coating thickness. The designed LDD is studied for its, drug release characteristics, cytocompatibility, toxicity, osseointegration and anti-bacterial efficiency.

## 2. Materials and methods

### 2.1. Preparation of titanium samples

99.95 % pure Titanium, (Sigma Aldrich) sheets of 1 mm thickness were cut into many pieces with dimensions of 2 cm × 2 cm. The samples were etched with Kroll's reagent (91 ml distilled water ($H_2O$) + 6.5 ml nitric acid ($HNO_3$) + 2.5 ml hydrofluoric acid (HF)) to obtain a surface free from impurities.

### 2.2. Formation of Titania nanotubes

The anodization was performed using a 30 mm × 10 mm x 1 mm Platinum sample as the cathode and a prepared titanium sample as the anode. The distance between the electrodes was 2 cm. A combination of 50 ml Ethylene Glycol and 50 ml of 0.08 M HF was used as the electrolyte. The electrolyte was continuously stirred using a magnetic stirrer at 500 RPM. Anodization was carried out at 20 V for 8 h.

### 2.3. Drug loading into nanotubes

#### 2.3.1. Loading of the drug

A 0.5 mg/ml solution of ciprofloxacin in deionized (DI) water was prepared. Then 100 µl of this solution was loaded onto 2 cm × 2 cm area of TNTs samples fabricated on Ti. Thus, 50 µg of the drug was loaded onto a 2 cm × 2 cm area of the TNTs substrate. Samples were dried at room temperature for 20 min before further use.

#### 2.3.2. Polymer coating

For polymer coating, the PLGA was dissolved in 1.5 %, 0.75 %, and 0.375 % weight/volume (w/v) solutions of chloroform. Chloroform, being a volatile solvent, led to quick coating of a PLGA layer on top of the TNTs substrates. A 50 µl prepared solution was used to deposit PLGA on top of the respective drug-loaded samples. Samples were once again dried for 20 min at room temperature before further use. Fig. 1 shows the structural representation of the drug loading and polymer coating on fabricated TNTs samples.

#### 2.3.3. Drug release studies

The drug release was studied by immersing the loaded TNTs samples in 50 ml phosphate-buffered saline (PBS) solutions. The amount of drug released was estimated by measuring the absorbance of the sample at 270 nm by UV-Vis Spectrophotometer, which is the peak absorbance wavelength of ciprofloxacin (in the current condition). The measurements were taken at many intervals starting from the first minute. Five readings were taken on the first day to observe the initial burst release of the drug. A standard graph for the absorbance vs concentration of ciprofloxacin in PBS solution was prepared, and it was used to measure the

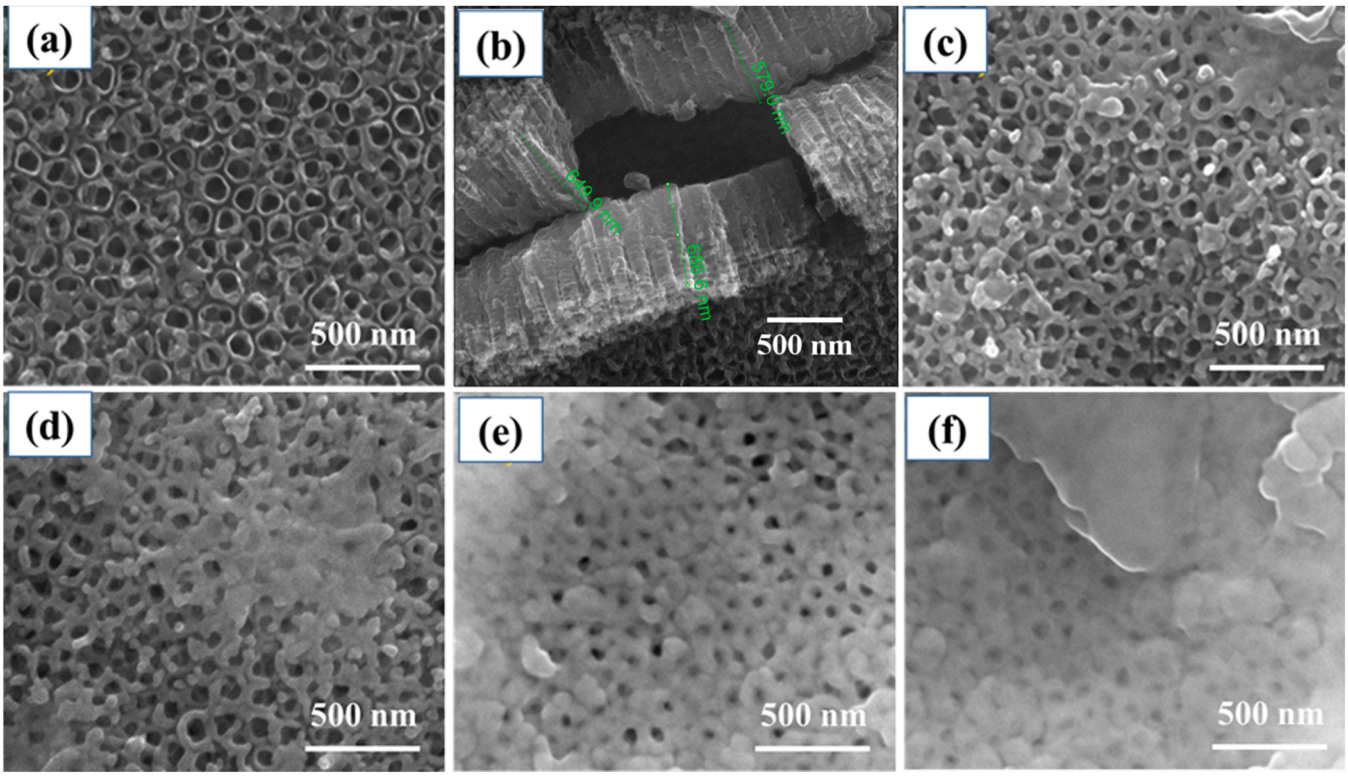


**Fig. 2.** FESEM images of (a) top view of the fabricated TNTs. (b) cross-section of the TNTs (~700 nm). TNTs loaded with ciprofloxacin and PLGA (c) 0 w/v% PLGA, (d) 0.375 w/v% PLGA, (e) 0.75 w/v% PLGA and (f) 1.5 w/v% PLGA.

drug concentration in the test samples after obtaining the absorbance. Finally, the drug release data was represented in a graph portraying the duration vs drug release percentage. The drug release percentage was calculated by dividing the amount of drug released from a sample reading by the total amount of drug-loaded on the 2 cm × 2 cm sample, multiplied by 100 [19].

### 2.4. Cell growth studies

#### 2.4.1. Viability studies of MG63 cells on TNTs

The MG-63 osteosarcoma cells were cultured on the fabricated TNTs samples. The cell culture and TNTs sample preparation details are provided in the Supplementary material. For qualitative analysis, fluorescence imaging was used to detect live and dead cells after incubation. Calcein AM and Hoechst dye were used to stain live cells and the nucleus. Calcein-AM is a cell-permeable dye, and it can cause hydrolysis inside the cell, producing green fluorescence color for live cells. The Hoechst dye is cell-permeable, and it stains the cell nucleus. The Propidium iodide (PI) dye is a cell impermeable dye, and it can stain the nucleus of dead cells [20].

#### 2.4.2. MTT assay

The MTT assay (3- [4,5-dimethylthiozol-2-yl]−2,5 diphenyl tetrazolium bromide (Analytical grade, HiMedia, Mumbai, India), test was performed to assess the viability of the MG63 cells on all the prepared samples. The MTT assay was performed as per the procedure described in earlier studies [21]. Briefly, each sample was placed in a 30 mm cell culture plate, and MG63 cells were seeded at a density of $0.5 \times 10^5$ cells/ml. Prior to cell seeding, the samples were washed with isopropyl alcohol and sterilized in UV overnight. The viability was assessed after 3 days of cell culture. A 50 µl of 5 mg/ml MTT (stock) was added to each plate, followed by incubation at 37 ºC with 5 % $CO_2$ incubator for 4 h. After that, the cell culture medium containing MTT was aspirated from the culture plate, and 500 µl dimethyl sulfoxide (DMSO, Merck) was added for dissolving the formation of formazone crystals. Then, 200 µl DMSO dissolved formazone crystal were aliquoted in a 96-well plate for the optical density measurement at 570 nm wavelength through an enzyme-linked immunosorbent assay (ELISA) plate reader (Bio-Rad, xMark, Microplate Spectrophotometer). All the assay experiments were repeated three times and the average data was collected.

### 2.5. Characterization techniques

The water contact angle measurements for the TNTs samples were performed using the sessile drop method. The surface wettability was recorded three times, where 20 µl of distilled water is allowed to make contact with the surface of the samples and then the average data is taken for generating the graph. The Quanta 200 FEG scanning electron microscope equipped with EDS from the Sophisticated Analytical Instrument Facility, IIT Madras, was used to analyze the fabricated TNTs samples and obtain the surface morphology and chemical compositions.

### 2.6. Antibacterial studies

*Staphylococcus epidermidis* (ATCC CRM-12228), a gram-positive bacterium is revived from the frozen state and transferred on Tryptine Soya Agar (TSA) (15 g/L tryptone, 5 g/L soya peptone, 5 g/L NaCl, 15 g/L neutralized agar). Bacterial pre-inoculum cultures were grown at 37 °C, overnight in 20 ml nutrient broth (with phosphate buffer, pH 7.2). This suspension was kept in a shaker at 100 rpm to achieve an inoculation concentration of $10^{-4}$ to $10^{-5}$ CFU/ml). The drug-loaded TNTs were sterilized to avoid tampering with the studies. While the control flask is devoid of drug-loaded TNTs, the other flasks containing the drug-loaded TNTs are kept in contact with the bacterial cell culture and subjected to vigorous shaking to assure contact between the sample and bacteria at 37 ± 1°C for 24 h. For a timeframe of 0–24 h, cultures are collected at regular interval, and concentration of the bacterial cells are calculated by CFU (Colony Forming Units) and by serial dilution on

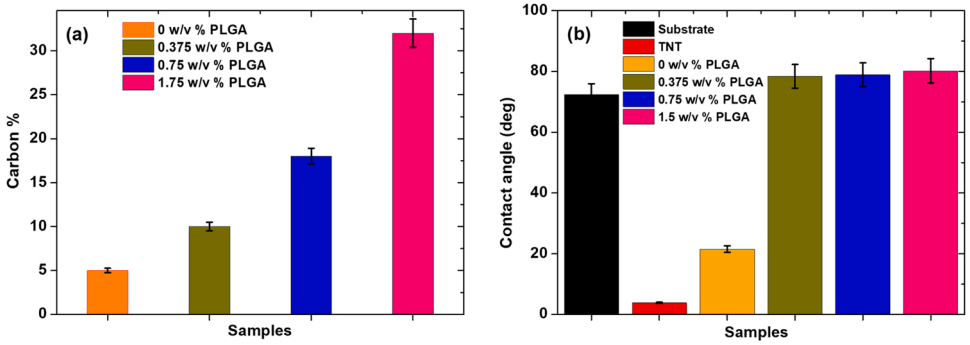


**Fig. 3.** (a) EDS Analysis showing the percentage of carbon presence on the surface of prepared drug loaded TNTs with different PLGA concentrations (b) Contact angle measurements of control & all treated samples with different polymer concentrations. (Error bars=standard deviation; N = 3).

plate count agar. Log reduction of samples at different time intervals are calculated using the formula below,

Log reduction = log (CFU $T_{24}$ control sample) - log (CFU $T_{24}$ Nanocomposite).

$T_{24}$ refers to the bacterial culture at 24 h. A log reduction of 1 is at least to be recorded for claiming antimicrobial activity.

The agar well diffusion method is used for strains that can be cultured on solidified media. The nutrient agar (NA) medium (50–70 °C) prepared is poured into the Petri plates and treated with UV for 15 min to favor solidification under maintenance of aseptic conditions. After that, the pre-incubated bacterial solution ($10^{-5}$) was spread over the solidified media and incubated at 37 °C for another 24 h, and the colony formation was photographed subsequently [22,23]. A one-way ANOVA test was performed to validate results with * $p < 0.05$.

## 3. Results and discussion

### 3.1. Fabrication and characterization of the Titania nanotubes

The field emission scanning electron microscopy (FESEM) image of the fabricated TNTs sample is shown in Fig. 2(a) for the top view and 2 (b) for the cross-sectional view. The presence of self-arrayed perpendicular, vertically aligned nanotubes is clearly visible from the image. The nanotube had an average length of ~700 nm and a diameter of ~125, which provided enough volume to load drugs.

### 3.2. Ciprofloxacin loaded Titania nanotubes coated with PLGA

Fig. 2(c)–(f) show the top view of FESEM images treated with the ciprofloxacin-loaded TNTs with different w/v% solutions of PLGA in chloroform. From these results, it is also visible that 0.375 % concentration of PLGA polymer has occupied the inter-tubular spaces as well as some space within the TNTs. The thickness of the PLGA layer appears to increase with increasing PLGA concentration substantiated using carbon content analysis through EDS in Fig. 3(a) below. The TNTs appear to be completely covered for 1.5 % concentration of PLGA. In Fig. 2(d)–(f), the TNTs appear to be filled with PLGA increase in the concentration of PLGA in chloroform used for coating. This provides further support for the claim of higher PLGA concentration in chloroform leading to deposition of thicker layers of PLGA, not only on the top of the TNTs but also on the inner tubular structures.

Energy Dispersive X-Ray Analyser (EDS) was used to study the presence of different materials on the top surface of the TNTs. Fig. 3(a) shows a trend of increasing carbon content on the top surface of TNTs with the increase in the concentration of PLGA used for coating. This further proves that a higher amount of PLGA was deposited on samples loaded with a higher concentration of PLGA in chloroform.

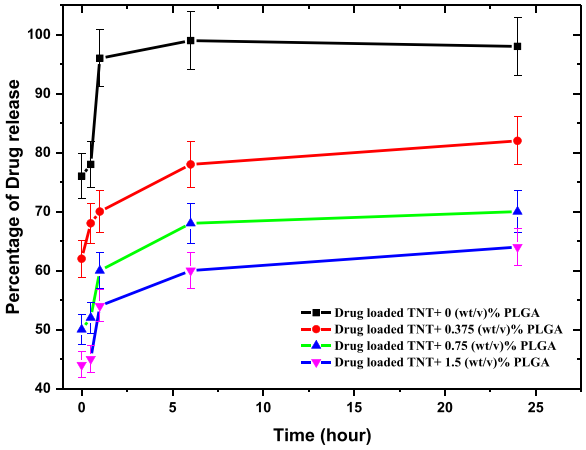


**Fig. 4.** Drug release graph of ciprofloxacin over 24 h from TNTs in PBS solution. (Error bars=standard deviation; N = 3).

### 3.3. Water contact angle measurement

The water contact angle measurement is carried out to investigate surface wettability of the substrate and the anodised samples of Titanium (Fig. 3 (b)). A sharp decrease of the contact angle measurement is observed from the Ti substrate to the anodised Ti containing TNTs. The drug-loaded surface on the anodised Ti substrate retains its hydrophilic nature, favouring the contact of the drug-loaded TNTs immersed in the simulated body fluids. The low contact angle in the sample with bare TNTs is indicative of its strong hydrophilic nature. However, the samples show faster drug release rates (burst release profile is observed) in non-coated drug-loaded Ti substrates containing TNTs. This drug diffusion phenomenon is tuned by the coating of different weight percentages of PLGA solutions, which is marked by the increase in the contact angle measurement. The polymer-coated samples remain barely hydrophilic with contact angles between 80 and 90 degrees, which is much higher than the contact angle of non-coated TNTs. The contact angle increases with coating PLGA on the substrate, which is expected owing to the hydrophobic nature of PLGA [35]. The initial interaction with the body fluids and the implant material is essential for improving osseointegration. Therefore, it is necessary to preserve a slight hydrophilic nature on the surface of the implant material. Another important factor to consider is the initial protein adsorption onto surfaces, as it is a crucial step in the process of cell adhesion, proliferation, and generation of an extracellular matrix, which finally leads to the implant being “accepted” in the body. PLGA has been shown to promote the initial adhesion of proteins such as vitronectin, which in turn promote cell adhesion through different membrane protein binding sites. Thus, the PLGA layer would overall promote cell adhesion and proliferation, despite decreasing the

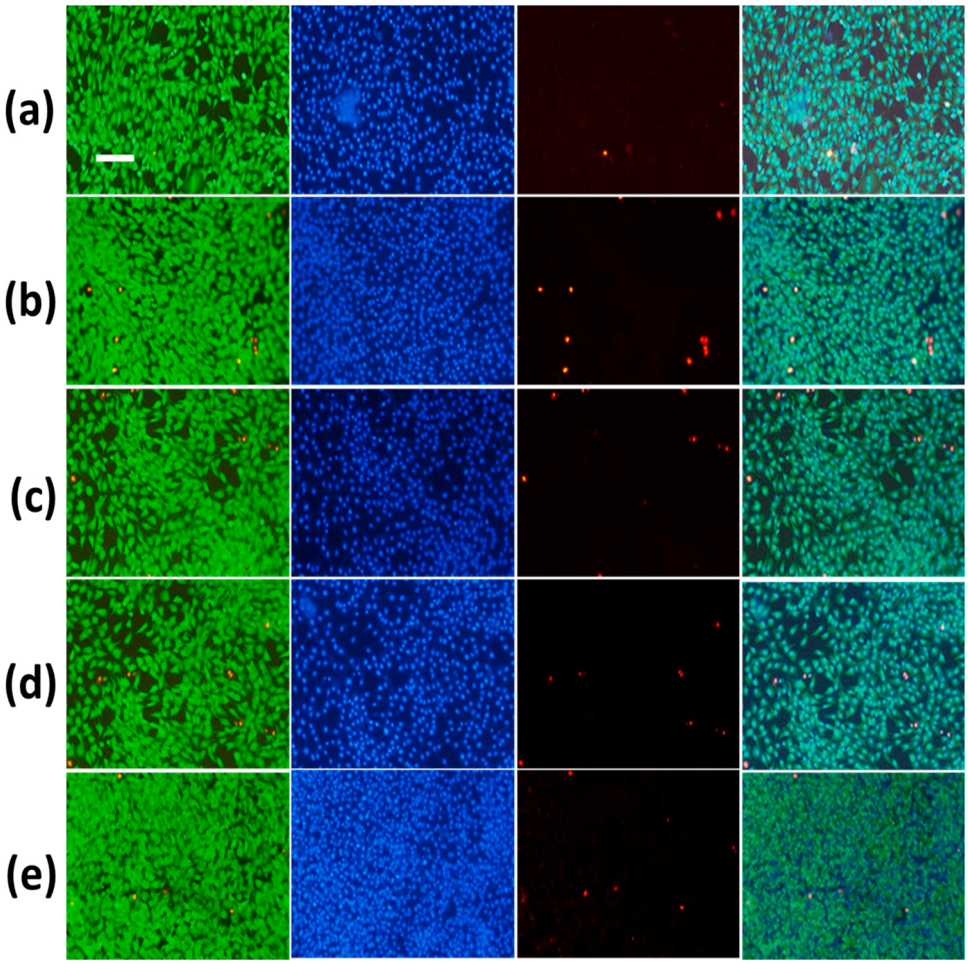


**Fig. 5.** Fluorescence imaging of cells cultured on various prepared TNTs samples (a) Bare TNTs, (b) TNTs loaded with ciprofloxacin, and TNTs loaded with ciprofloxacin and different PLGA concentration (c) 0.375 w/v% PLGA, (d) 0.75 w/v% PLGA (e) 1.5 w/v% PLGA. 1st column - calcein AM, 2nd column-Hoechst, 3rd column – PI, 4th column merge image of (1−3), (scale bar = 200 μm).

hydrophilicity of the surface by a great amount [24–27].

### 3.4. In-vitro drug release studies of ciprofloxacin

Fig. 4 shows the drug release studies of ciprofloxacin from TNTs in PBS solution. This graph shows a significant reduction in the percentage of the drug (ciprofloxacin) released when PLGA is used to control the release rate. There is approximately a 30 % reduction in the percentage of drugs released after 24 h from the TNTs not coated with PLGA and the TNTs coated with 0.375 w/v% PLGA. An increase in PLGA w/v% showed a continuous decrease in burst release of the drug. The drug diffusion rate is very high due to the high concentration gradient of ciprofloxacin between the TNTs surface and the PBS solution. The inset in the figure shows the drug release characteristics up to 1 h of studies, where it is clear that having a higher amount of PLGA deposited on the TNTs leads to a slower release rate of the loaded ciprofloxacin. Thus, following the burst release phase, the drug release followed a release profile close to zero-order release, when PLGA was coated on top of the TNTs [28].

### 3.5. Fluorescence imaging of cells

It is important for cells to be able to adhere to the substrate for enabling bone growth and integration with implants. The first row of Fig. 5 (a) shows the control experiment and the fluorescence imaging of MG63 cells cultured on the bare TNTs sample with no drug or polymer loaded on the surface. The figure shows live-cell imaging using Calcein AM (green color), which also confirmed on TNTs that most of the cells are alive after 24 h incubation in 5 % $CO_2$ incubator. The cell nucleus imaging (Hoechst (blue color)) is performed using Hoechst dye, the dead cell imaging (PI (red color)) is performed by using cell impermeable Propidium iodide dye, which also confirmed that there are very few cells are dead after 24 h cell culture on TNTs. Finally, the merged image of live cells, nucleus, and dead cells also confirmed that most of the cells are alive, indicated by blue yellowish to green-yellowish color. Finally, image analysis was done with the help of ImageJ to estimate the percentage of live and dead cells in the observed area. Around 98.5 % of the cells were alive and healthy in this analysis. From these observations, it is clear that most of the cells can adhere and proliferate on the TNTs, proving once again the excellent biocompatibility of Titania as reported elsewhere [29,30].

Fig. 5 (b) shows the fluorescence imaging of MG63 cells cultured on the TNTs sample loaded with ciprofloxacin and no PLGA coating. This figure also confirms that after 24 h of cell culture of TNTs, most of the cells are alive and proliferating (~95 % live cells on observed area), and very few cells are dead (~5 % dead cells). The merged image also confirms that most of the cells are alive with ciprofloxacin and no PLGA coating. Fig. 5 (c-e) also shows the fluorescence imaging of cells cultured on the TNTs sample loaded with ciprofloxacin with different concentrations of PLGA. The images were captured after 24 h incubation on TNTs. Rows 3–5 of Fig. 5 shows live imaging (green color) of cells on exposure to TNTs covered with different percentages of PLGA after

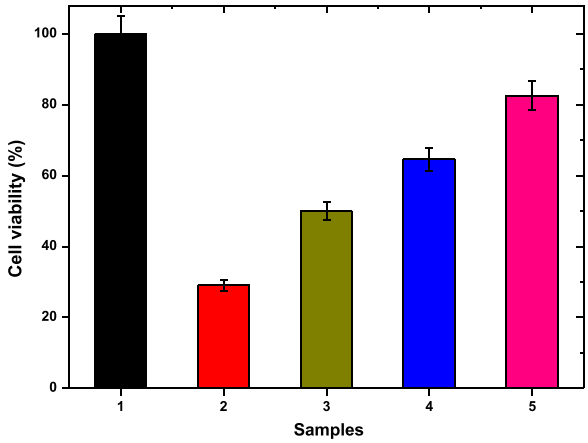


**Fig. 6.** MTT assay results showing the percentage of cell viability. Cell viability on TNTs (sample1), TNTs with ciprofloxacin (sample 2), TNTs with ciprofloxacin and different concentration (3. 0.375 w/v% 4. 0.75 w/v% 5. 1.5 w/v%) of PLGA (sample 3, 4 and 5). (Error bars=standard deviation; N = 3).

loading with ciprofloxacin. For these cases, most of the cells were alive and cells appear fully grown, healthy, flattened with their natural triangular shape with well-defined edges. Analysis with ImageJ showed greater than 97 % of cells in the observed area were live, as they were stained with Calcein-AM. The cell proliferation also increases with an increase in PLGA loaded onto the nanotubes. Rows 3–5 also show cell nucleus staining images with Hoechst dye, along with dead cells staining (red color) images with ciprofloxacin and different PLGA concentrations. Very few cells are dead after 24 h of incubation with different concentrations of PLGA coated on the substrate. There is also a lower percentage of dead cells with an increase in PLGA concentration (~3 % dead cells for 0.375 % PLGA, ~2 % dead cells for 0.75 w/v% PLGA, and ~1.3 % dead cells for 1.5 w/v% of PLGA) The last column of Fig. 5 shows the merged images of live cells, nucleus, and dead cells. These merged images also confirmed that most of the cells live, indicating blue yellowish to green-yellowish color.

### 3.6. MTT Assay for determining cell viability of samples

The cell viability results for all implants are summarized in Fig. 6 using the MTT assay study. The results show that the interactions of MG63 cells were most favored on bare TNTs surface (sample 1, considered as a control in this study). The cell viability decreased by approximately 71 % after the incorporation of the drug into the TNT surface (sample 2). The decrease in cell viability from bare TNTs to drug-loaded TNTs can be explained by the burst release of ciprofloxacin, which is known to be cytotoxic and hinders cell growth [31–33]. On the contrary, the drug improves the antibacterial activity of the implant surface. Therefore, to reduce this toxicity by controlling the drug release rate, various concentrations of polymer (0.375 %, 0.75 %, and 1.5 % for samples 3, 4, and 5) were reinforced along with the drug to the TNT surface. The MTT results show the introduction of polymer to the TNTs, including drug-treated surface, increased the MG63 cell viability. Approximately 21 %, 35.6 %, and 53.6 % higher cell viability was observed for the addition of 0.375 %, 0.75 %, and 1.5 % polymer, respectively, in comparison to the non-polymer coated, drug-loaded substrate. Thus, the increase of the concentration of PLGA with ciprofloxacin can increase the cell viability and reduce the toxicity of the sample.

### 3.7. Antibacterial studies

Fig. 7(a) shows the optical density recorded at 600 nm wavelength as a direct measure of antibacterial activity against *Staphylococcus epidermidis*. Decreasing trends of measured optical density in the liquid culture method along the antibiotics-loaded TNTs in Fig. 7(a) indicate an explicit decrease in bacterial cell concentration over time in samples loaded with ciprofloxacin. Also, it is evident that as the time of incubation is increased, sample with 0.015 wt/v % polymer coating shows very less bacterial activity in comparison to 0 wt/v % & 0.00375 wt/v % samples, as there is a controlled release of ciprofloxacin. When compared to the positive control, bare TNTs sample also exhibit antibacterial activity as reported elsewhere [34]. The plate dilution method showed that the bacterial concentration along the increasing polymer concentration was minimal along the streak direction as compared to the positive control (TNT). This corroborates with the drug release profiling, that aids in arriving as the controlled release of ciprofloxacin (antibiotics), which can help in achieving better antibiotic activity by tuning the polymer concentration. Titania nanotubes serve as a load carrier for the antibiotic with PLGA coating at various weight percentages help in tuning the antibiotic release.

## 4. Conclusions

The fabricated TNTs samples showed, increase cell viability with the increase of concentration of PLGA loaded on top of the nanotubes. Cell proliferation and adhesion were significantly improved by the presence

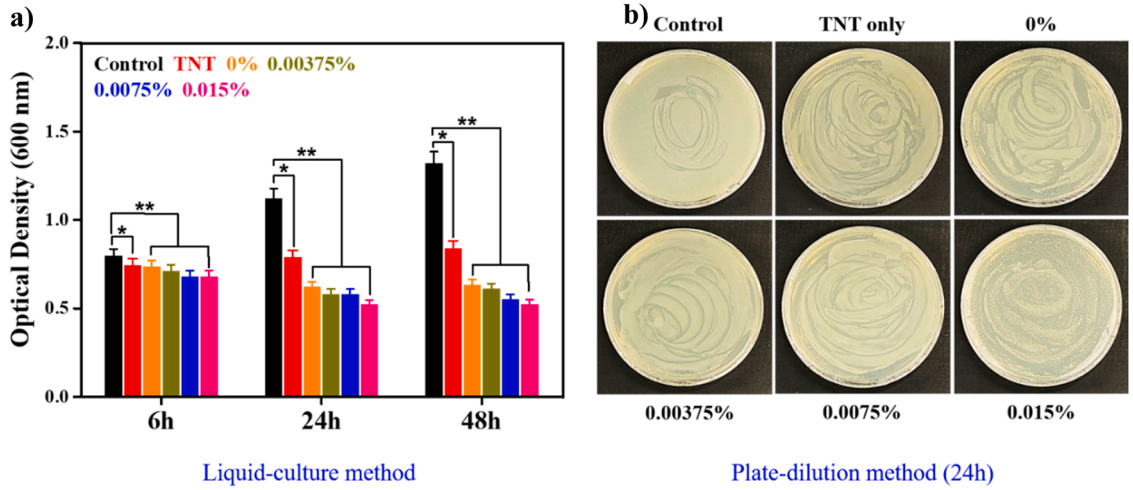


**Fig. 7.** Antibacterial performance against *Staphylococcus epidermidis* by (a) Liquid culture method * $p < 0.05$ and ** $p < 0.01$ (One-way Anova Test); (b) Plate dilution method.

of nanotubes and PLGA on the surface. With increasing concentration of polymer in the solution used for coating, the burst release of the drug was better controlled. Having shown success with controlling the drug release rate, this technique of localized/controlled drug release could be further tested in-vivo systems with small animals such as rodents before moving to clinical trials. Loading of ciprofloxacin directly onto TNT -based implants leads to localized drug delivery for treatment of osteomyelitis or other bone infections. The concentration of the PLGA used for coating could be varied to obtain a drug release profile, which would be suitable for post-operative care such as controlling inflammation and sustained treatment or prevention of infections.

## Declaration of Competing Interest

The authors declare no conflict-of-interests.

## Data Availability

The raw/processed data required to reproduce these findings cannot be shared at this time as the data also forms part of an ongoing study.

## Acknowledgments

This work was supported by DBT/Wellcome Trust India Alliance Fellowship grant number IA/E/16/1/503062 awarded to Dr. Tuhin Subhra Santra and the support from IIT Madras, Exploratory Research grant number ED/19-20/904RFER/008754. The author would like to thank SAIF, IITM for FESEM and EDS studies.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mtcomm.2022.103843.

## References

[1] D. Losic, M.S. Aw, A. Santos, K. Gulati, M. Bariana, Titania nanotube arrays for local drug delivery: recent advances and perspectives, Expert Opin. Drug Deliv. (2015) 103–127, https://doi.org/10.1517/17425247.2014.945418.

[2] A. Tian, X.F. Qin, A. Wu, H. Zhang, Q. Xu, D. Xing, H. Yang, B. Qiu, X. Xue, D. Zhang, C. Dong, Nanoscale TiO2 nanotubes govern the biological behavior of human glioma and osteosarcoma cells, Int. J. Nanomed. (2015), https://doi.org/10.2147/IJN.S71622.

[3] X. Ding, L. Zhou, J. Wang, Q. Zhao, X. Lin, Y. Gao, S. Li, J. Wu, M. Rong, Z. Guo, C. Lai, H. Lu, F. Jia, The effects of hierarchical micro/nanosurfaces decorated with TiO2 nanotubes on the bioactivity of titanium implants in vitro and in vivo, Int. J. Nanomed. (2015), https://doi.org/10.2147/IJN.S87347.

[4] Y. Lai, Y. Huang, H. Wang, J. Huang, Z. Chen, C. Lin, Selective formation of ordered arrays of octacalcium phosphate ribbons on TiO2 nanotube surface by template-assisted electrodeposition, Colloids Surf. B Biointerfaces (2010), https://doi.org/10.1016/j.colsurfb.2009.10.023.

[5] Y. Yang, Y. Lai, Q. Zhang, K. Wu, L. Zhang, C. Lin, P. Tang, A novel electrochemical strategy for improving blood compatibility of titanium-based biomaterials, Colloids Surf. B Biointerfaces (2010), https://doi.org/10.1016/j.colsurfb.2010.04.013.

[6] A.R. Rafieerad, A.R. Bushroa, E. Zalnezhad, M. Sarraf, W.J. Basirun, S. Baradaran, B. Nasiri-Tabrizi, Microstructural development and corrosion behavior of self-organized TiO2 nanotubes coated on Ti–6Al–7Nb, Ceram. Int. (2015), https://doi.org/10.1016/j.ceramint.2015.05.025.

[7] M. Chembath, J.N. Balaraju, M. Sujata, Surface characteristics, corrosion and bioactivity of chemically treated biomedical grade NiTi alloy, Mater. Sci. Eng. C (2015), https://doi.org/10.1016/j.msec.2015.06.051.

[8] D. Regonini, C.R. Bowen, A. Jaroenworaluck, R. Stevens, A review of growth mechanism, structure and crystallinity of anodized TiO2 nanotubes, Mater. Sci. Eng. R. Rep. (2013), https://doi.org/10.1016/j.mser.2013.10.001.

[9] P. Roy, S. Berger, P. Schmuki, TiO2 nanotubes: synthesis and applications, Angew. Chem. Int. Ed. 50 (2011) 2904–2939.

[10] K. Gulati, S. Ramakrishnan, M.S. Aw, G.J. Atkins, D.M. Findlay, D. Losic, Biocompatible polymer coating of Titania nanotube arrays for improved drug elution and osteoblast adhesion, Acta Biomater. 8 (2012) 449–456, https://doi.org/10.1016/J.ACTBIO.2011.09.004.

[11] L. Mohan, C. Anandan, N. Rajendran, Electrochemical behaviour and bioactivity of self-organized TiO2 nanotube arrays on Ti-6Al-4V in Hanks' solution for biomedical applications, Electrochim. Acta 155 (2015) 411–420, https://doi.org/10.1016/j.electacta.2014.12.032.

[12] S. Sun, Y. Zhang, D. Zeng, S. Zhang, F. Zhang, W. Yu, PLGA film/Titanium nanotubues as a sustained growth factor releasing system for dental implants, 2018, J. Mater. Sci. Mater. Med 299 (29) (2018) 1–8, https://doi.org/10.1007/S10856-018-6138-1.

[13] A. Santos, M. Sinn Aw, M. Bariana, T. Kumeria, Y. Wang, D. Losic, Drug-releasing implants: current progress, challenges and perspectives, J. Mater. Chem. B. (2014), https://doi.org/10.1039/c4tb00548a.

[14] T.S. Santra, L. Mohan, Nanomaterials and their Biomedical Applications. Springer Nature, Singapore, First ed.,, 2021, https://doi.org/10.1007/978-981-33-6252-9.

[15] A.S. Hoffman, The origins and evolution of "controlled" drug delivery systems, J. Control. Release (2008), https://doi.org/10.1016/j.jconrel.2008.08.012.

[16] L.M. Fisher, J.M. Lawrence, I.C. Josty, R. Hopewell, E.E.C. Margerrison, M. E. Cullen, Ciprofloxacin and the fluoroquinolones, Am. J. Med 87 (1989) S2–S8, https://doi.org/10.1016/0002-9343(89)90010-7.

[17] A.Y. Pawar, D.V. Derle, Development of cost effective biodegradable implants of ciprofloxacin hydrochloride in treatment of osteomyelitis, Asian J. Pharm. 7 (2014), https://doi.org/10.22377/AJP.V7I2.32.

[18] Q. Miao, J.L. Sun, F. Huang, J. Wang, P. Wang, Y.F. Zheng, F. Wang, C.F. Ma, Antibacterial peptide HHC-36 sustained-release coating promotes antibacterial property of percutaneous implant, Front. Bioeng. Biotechnol. 9 (2021), https://doi.org/10.3389/FBIOE.2021.735889.

[19] N. Nagiah, G. Ramanathan, L. Shobana, S. Singaravelu, T.S. Uma, T.S. Natarajan, Preparation and characterization of electrospun poly (3-hydroxybutyric acid)–poly (N-vinylpyrrolidone) and poly (caprolactone)–poly (N-vinylpyrrolidone) fibers as potential scaffolds for skin regeneration, J. Biomater. Tissue Eng. 3 (2013) 624–629.

[20] T.S. Santra, S. Kar, T.C. Chen, C.W. Chen, J. Borana, M.C. Lee, F.G. Tseng, Near-infrared nanosecond-pulsed laser-activated highly efficient intracellular delivery mediated by nano-corrugated mushroom-shaped gold-coated polystyrene nanoparticles, Nanoscale 12 (2020) 12057–12067, https://doi.org/10.1039/d0nr01792b.

[21] J. Van Meerloo, G.J.L. Kaspers, J. Cloos, Cell sensitivity assays: the MTT assay, in: Cancer Cell Cult, Springer, 2011, pp. 237–245.

[22] R.J.B. Pinto, S. Daina, P. Sadocco, C.P. Neto, T. Trindade, Antibacterial activity of nanocomposites of copper and cellulose, Biomed. Res. Int. (2013), https://doi.org/10.1155/2013/280512.

[23] M. Ginovyan, M. Petrosyan, A. Trchounian, Antimicrobial activity of some plant materials used in Armenian traditional medicine, BMC Complement. Altern. Med. (2017), https://doi.org/10.1186/s12906-017-1573-y.

[24] K. Gulati, M.S. Aw, D. Losic, Drug-eluting Ti wires with Titania nanotube arrays for bone fixation and reduced bone infection, Nanoscale Res. Lett. (2011), https://doi.org/10.1186/1556-276X-6-571.

[25] K. Vasilev, Z. Poh, K. Kant, J. Chan, A. Michelmore, D. Losic, Tailoring the surface functionalities of Titania nanotube arrays, Biomaterials (2010), https://doi.org/10.1016/j.biomaterials.2009.09.074.

[26] D.C. Miller, K.M. Haberstroh, T.J. Webster, Mechanism (s) of increased vascular cell adhesion on nanostructured poly (lactic-co-glycolic acid) films, J. Biomed. mater. Res. A 73 (2005) 476–484.

[27] J. Nikolovski, D.J. Mooney, Smooth muscle cell adhesion to tissue engineering scaffolds, Biomaterials 21 (2000) 2025–2032.

[28] S. Simovic, D. Losic, K. Vasilev, Controlled drug release from porous materials by plasma polymer deposition, Chem. Commun. 46 (2010) 1317, https://doi.org/10.1039/b919840g.

[29] E.P. Su, D.F. Justin, C.R. Pratt, V.K. Sarin, V.S. Nguyen, S. Oh, S. Jin, Effects of titanium nanotubes on the osseointegration, cell differentiation, mineralisation and antibacterial properties of orthopaedic implant surfaces, Bone Jt. J. 100 (2018) 9–16.

[30] M. Jäger, H. Jennissen, F. Dittrich, A. Fischer, H. Köhling, Antimicrobial and Osseointegration Properties of Nanostructured Titanium Orthopaedic Implants, in: Materials, 10, Basel, 2017, p. 1302.

[31] M.B. Ferreira, S. Myiagi, C.G. Nogales, M.S. Campos, J.L. Lage-Marques, Time-and concentration-dependent cytotoxicity of antibiotics used in endodontic therapy, J. Appl. Oral. Sci. 18 (2010) 259–263.

[32] T. Kloskowski, N. Gurtowska, M. Nowak, R. Joachimiak, A. Bajek, J. Olkowska, T. Drewa, The influence of ciprofloxacin on viability of A549, HepG2, A375. S2, B16 and C6 cell lines in vitro, Acta Pol. Pharm. 68 (2011) 859–865.

[33] G.L. Hoelscher, H.E. Gruber, G. Coldham, J.H. Grigsby, E.N. Hanley Jr, Effects of very high antibiotic concentrations on human intervertebral disc cell proliferation, viability, and metabolism in vitro, Spine 25 (2000) 1871–1877.

[34] Z. Xu, Y. Lai, D. Wu, W. Huang, S. Huang, L. Zhou, J. Chen, Antibacterial effects and biocompatibility of Titania nanotubes with octenidine dihydrochloride/poly (lactic-co-glycolic acid), Biomed. Res. Int. (2015) (2015), https://doi.org/10.1155/2015/836939.