A Novel Methodology for Economical Scale-Up of TiO$_2$ Nanotubes Fabricated on Ti and Ti Alloys

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1. Introduction

Nanotubular films on titanium (Ti) and Ti alloys are used in a significant number of applications including biomedical devices [1–4], dye-sensitised solar cells [5–12], and photocatalysis [13–17]. Figure 1(a) shows the increasing trend in the number of publications focusing on titanium oxide (TiO$_2$) nanotubes within the last ten years; Figure 1(b) presents the applications of TiO$_2$ nanotubes and their respective percentages among the published papers in year 2017.

For the biomedical field, a wide range of target applications have been reported regarding TiO$_2$ nanotubes grown on Ti and its alloys, for instance, drug delivery [18–20], antibacterial [21–23], biosensors [24–26], and dental and bone implants [22, 27–29]. In particular, the increasing demand for dental and hip implants is becoming a prime turnover in the orthopaedic industry [30–32]. In vitro and in vivo investigations for implant applications often require a high number of specimens for assessing the biocompatibility of the biomaterials (typically a minimum of 30 specimens for fundamental biocompatibility assays). Currently, the production rate of such surfaces is slow and limited to the production of a single specimen at a time [33, 34], hence the rationale for an improved scale-up methodology was used that allows the rapid, reproducible production of specimens.

Recently, titanium and a variety of titanium alloys, including Ti-6Al-4V, Ti-6Al-4V-ELI (extra low interstitial), Ti-6Al-7Nb, and Ti-13Nb-13Zr, have received significant attention due to their exceptional material properties. In particular, they demonstrate high corrosion resistance, high biocompatibility, low stiffness, and low density [2, 35–37]. They have thus been widely used as implant materials to replace failed hard tissues, more specifically, in the area of bone plates, dental implants, fracture fixation screws, and total knee and hip replacement [35, 38–40].
The high surface-to-volume ratio of TiO$_2$ nanotubes grown on both pure titanium and titanium alloys have been demonstrated to promote excellent protein adsorption [41–43] and to offer a platform for cell adhesion, proliferation, and differentiation, leading to the enhancement of osseointegration [22, 44–46]. Research has demonstrated that a small change in the nanotube pore size can significantly affect cellular behaviour [22, 29, 47–49], while nanotube length does not have a major impact on cellular response [50]. It is suggested that pore size changes the arrangement and strength of the focal contacts made by cells. Cells grown on nanoporous or nanotubular surfaces exhibit an upregulation in the integrin receptors that mediate the focal contacts with resulting improvements in cytoadherence, mechanotransduction, and hydroxyapatite formation [45, 51, 52].

Anodisation using a fluoride-containing electrolyte has been long recognised as a promising method to synthesise nanotubular structures on titanium substrates. Compared with other nanotube fabrication methods such as sol-gel template [39, 53–58] and hydrothermal techniques [59–64], electrochemical anodisation has the advantages of being simple, rapid, low cost, and providing more controllable and reproducible results for the preparation of highly ordered and vertically oriented TiO$_2$ nanotube layer [65–67]. The surface morphology and desired nanotube dimensions can be readily controlled by tailoring the anodising conditions, including the applied potential, electrolyte composition, and pH [1, 2, 34]. If an optimised condition is achieved, anodisation can effectively and consistently enable the formation of self-assembled and uniformly aligned nanotube arrays [68–70].

Electrochemical anodisation is an electrolytic method used to increase the thickness of the natural oxide layer on metal surfaces [71] and to create biocompatible micro- or nanoporous TiO$_2$ coatings, with increased surface energy and roughness [72, 73]. During anodisation, aqueous or organic electrolytes with fluoride ions are generally employed to produce the nanotubes. Some examples of the electrolytes include aqueous electrolyte solutions with acids,
Anodisation experiments are usually carried out in a two-electrode or three-electrode electrochemical system, with Ti or a Ti alloy as the anode, inert platinum foil as the cathode, and in the case of a three-electrode system, an Ag or AgCl electrode as the reference electrode [2]. TiO2 nanotubes can be obtained either under a constant potential (potentiostatic) or constant current (galvanostatic) mode. Anodisation can be performed using specimens of a variety of shapes and sizes, depending on the maximum allowable load of the power source [72]. The thickness and morphology of the oxide layers formed are usually determined by the applied potential, the duration of the anodisation process, and the chemical composition of the electrolyte used [71]. Previous works have concluded that, at a given point in time using the same electrolyte, the nanotube pore diameter, interpore distance, and nanotube length are directly proportional to the applied potential [67, 78–81].

A detailed growth mechanism of TiO2 nanotube using anodisation techniques is described in the literature [33, 74, 82, 83]. Briefly, the stages of nanotube formation in an aqueous electrolyte under constant potential can be monitored by the changes of current over the anodising time. As the anodising potential is initially applied, the current rapidly decays to a minimum due to the formation of a high-resistance oxide layer. Subsequently, the current rises to a maximum with the development of pore nucleation and the formation of a porous structure. The current will eventually attain an approximately constant value when an equilibrium state is achieved, i.e., the rate of oxide formation is equaled by the rate of dissolution.

Conventional anodisation systems are usually limited for production of \textit{in vitro} specimens by the following factors: (i) anodising is limited to a specific specimen rather than the entire surface, leading to material wastage; (ii) trimming of specimens for \textit{in vitro} studies causes specimen damage; (iii) usually only one specimen can be accommodated in the system, and high volume production is typically expensive in cost and time; and (iv) consistency and reproducibility cannot be guaranteed from specimens to specimens due to small variations in the anodising conditions. Figure 2 shows the schematic diagram of typical conventional anodisation systems, in which the desired anodised area is defined by an O-ring on the specimen holder.

The limitations above highlight the need for a tailored anodisation device design to produce fully anodised surfaces and to increase the productivity and repeatability of the anodisation process. In the present work, we designed an optimised anodisation setup to reduce fabrication costs and to maximise the efficiency rate of anodising titanium discs, for the first time, with the following objectives: (i) to anodise the entirety of specimen surfaces so as to reduce excessive material waste; (ii) to increase the number of specimens per anodised batch; (iii) to anodise batches of specimens with consistent results; (iv) to anodise specimens of desired TiO2 nanotube pore diameters; (v) to customise the specimen shape for actual practical application, in our case, for \textit{in vitro} biocompatibility tests; (vi) to evaluate the current cost per complete anodised specimen associated with the optimised device.

2. Materials and Methods

Pure Ti foils (thickness 0.25 mm; purity 99.5%; Alfa Aesar) and Ti-6Al-4V foils (thickness 0.4064 mm; titanium grade 5 ASTM B265; William Gregor Ltd.) were cut to the desired shape using an iPG laser ytterbium fiber laser 1 kW cutting tool, or AgieCharmilles CUT 200 Sp EDM wire-cut machine.

Before electrochemical treatment, the titanium foils were ultrasonically degreased in equal volumes of acetone, ethanol, and deionised (DI) water for 5 minutes, followed by drying under a cool air stream. An additional pickling process was introduced to Ti-6Al-4V foils prior to anodisation using a pickling mixture (Sigma-Aldrich) containing hydrofluoric acid, nitric acid, and DI water for five minutes to remove the naturally formed oxide layer.

For the electrochemical experiments, an anodising device with a three-electrode configuration was used. Specimens were mounted onto a copper rod wrapped with heat-shrink polytetrafluoroethylene (PTFE) tubing; areas other than the circular working area of the specimens were masked with lacquer (Stopper 45 MacDermid) to prevent exposure to the electrolyte. A platinum foil (thickness 0.1 mm; purity 99.99%; Alfa Aesar) served as the counter electrode, placed at 40 mm distance from the working electrode. A saturated calomel Hg/HgCl2 (1M KCl) electrode was used as a reference electrode, connected to the setup by a salt bridge placed close to the working electrode.

Constant potentials were applied using an EG&G Instruments Scanning Potentiostat (Model 362) connected to a Ministat MKIV Sycopel Scientific signal amplifier; the \textit{in situ} current-time responses (per anodisation process) were recorded using Labview software. The current density was then plotted using the current-time data per total surface area of the working specimens.

For pure Ti specimens, the electrolyte consisted of a mixture of 1M H3PO4 and 0.25 wt% HF. For Ti-6Al-4V titanium alloy specimens, 1M H2SO4 containing 0.1 wt% HF was used. All anodisation experiments were carried out at room temperature for 60 minutes. After the electrochemical treatment, the specimens were rinsed with DI water and then ultrasonically cleaned for 10 minutes and further dried under a cool air stream.

In the following, the specimens are designated according to the material type and anodising potential; for example, TiNT-2.5V refers to Ti specimens anodised at 2.5V to create nanotubes (NT), while Ti64NT-20V refers to Ti-6Al-4V specimens anodised at 20V. As shown in Figure 3, Version A of the anodisation setup used only one specimen, while Version B accommodated 10 specimens and a larger platinum foil as the counter electrode. The specimen was designed to fit into \textit{in vitro} tissue culture plates and with a tag to assist handling of specimens using tweezers. To verify that the anodisation setup was applicable to alloy, the present work was being reproduced using Ti-6Al-4V foils. The preparation time and costs for both versions were recorded and analysed.
The anodised specimens were characterised using a ZEISS Ultra 55 field emission scanning electron microscope (FE-SEM) equipped with an energy dispersive X-ray spectrometer (EDS), as reported previously [85]. The FE-SEM was operated under the InLens detector mode with 5 kV EHT in a high vacuum environment. ImageJ image analysis software was used to measure the nanotube pore diameters, interpore distances, and nanotube lengths of the anodised specimens. Statistical significance was analysed using analysis of variance (ANOVA) and counter-confirmed using the two-tailed paired Student’s t-test in Microsoft Excel. Data are presented as mean ± standard deviation (S.D.). Probability (p value) less than 0.05 was considered to be significant.

3. Results and Discussion

3.1. Design Rationales. While designing the new anodisation device, the design rationales of the device setup were initially evaluated, followed by proof of concept data, leading to further upscale and optimisation. The design rationales included the following:

(i) To create a scalable device design that was suitable for multiple specimens per batch of the anodisation process
(ii) Maintain constant electrode distances for process control
(iii) Ensure device setup that was compatible with the use of hydrofluoric acid (HF)
(iv) Allow an even distribution of potential and current to all specimens
(v) Allow monitoring of the potential of the working electrode during the anodisation process
(vi) Include a counter electrode that was at least similar or larger area to that of the working electrode
(vii) Allow no contamination from copper parts, which deleteriously affects the anodising process and in vitro cell and tissue culture tests
(viii) Allow manufacture of a specimen shape that maximises the surface area for in vitro tests
(ix) Allow ease of use when transferring the specimens during in vitro assays post manufacture

A PTFE beaker was employed to contain the electrolyte as PTFE is resistant to HF, as compared with glass or other plastic materials like low-density polyethylene (LDPE), high-density polyethylene (HDPE), polypropylene (PP), polymethylpentene (PMP), and styrene acrylonitrile (SAN). The PTFE beaker can also be autoclaved under high temperature if needed. The holder for the working electrode was a copper rod (shielded by PTFE and lacquer masking to prevent copper from exposing to the electrolyte) to which specimens were attached. Anodisation was able to take place over the entirety of the specimen, and the specimen shape could be varied according to the application.

The platinum counter-electrode was also attached to a shielded copper rod holder. The counter-electrode was positioned 40 mm from the working electrode holder. Figure 3 shows a detailed schematic of the device setup. In Version B, as the surface area of the working electrode was increased, the area of the counter-electrode was increased to be at least similar to that of the working electrode. Two square platinum foils (50 mm × 50 mm) were welded to provide an electrode of area approximately 94 mm × 50 mm.

The reference electrode was placed close to the working electrode to monitor the changes of potentials across the systems. Lacquer was used to seal connections of the platinum and specimens to the copper rods. The top cover jig of the device permitted the electrode arrangements to be preserved between batches. An opening in the cover jig allowed access of air.

As the bottom well diameter of a commonly used 24 circular well plates is 15.54 mm, as shown in Figure 3(c), a disc-shaped specimen was adopted with a diameter of 14 mm and area 3.08 cm². In Version A of the device design, a single specimen was anodised. In Version B, 10 wire-cut electrical discharge machining (EDM) connected specimens
were anodised. The specimens could be later readily separated by cutting the connecting strips between the discs provided by the metal sheet cutting procedure. The resulting tags on each disc could be folded into L shape to ease the transference of specimens without damaging the specimen surface and living cell culture during in vitro tests. The

Figure 3: (a) The design optimisation of Version A to Version B with 10x increase of specimens per anodisation; (b) spot weld of two pieces of platinum foil as the size of counter-electrode should be at least, if not larger than the overall area of the working electrode; (c) the specimen shape after cut using a precision cutting device; the tag can be folded into L shape for cell and tissue culture activity; (d) full configuration of the optimised anodisation device (Version B); (e) the schematic comparison of the two versions; note that the anodising surface on Version B was dramatically increased as compared with Version A.
number of specimens was limited to 10 to be compatible with the output of the power supply.

3.2. Proof of Concept. Version A, as illustrated in Figure 3, was used for initial proof of concept. The schematic of the full anodisation setup is displayed in Figure 3(e). The current density response recorded during anodising of a titanium specimen at 20V is shown in Figure 4. After the first few seconds of anodisation, the current fell and decayed to approximately 1.0 mA cm$^{-2}$ due to the initial formation of a barrier layer and subsequent development of the nanotube layer. A uniform array of nanotubes was observed on the anodised specimens; the top view FE-SEM micrograph is displayed in Figure 5(e). The pore diameters ($\sim$100 nm), as shown in Figure 6(a) TiNT-20V, are comparable to that reported by Bauer et al. [80].

3.3. Upscaling and Optimisation. The full 3D render of the optimised device (Version B) can be seen in Figure 3(d), and the schematic of the setup is displayed in Figure 3(e). The schematic compares the upscaling of the anodising surface in Version B as compared with Version A. To a greater extent, the actual anodising surface of Version B was two times as shown in the schematic, as there were two connected strips of five specimens (total $n = 10$) per anodisation batch. As the geometry of the working electrode and the anodisation system is symmetrical, the current passing through the system was considered evenly distributed.

When determining the maximum number of specimens allowed in the system, it is necessary to consider the maximum allowable load and current for the anodisation power supply with respect to the applied potential and total anodising surface area. In theory, considering that the current flows homogeneously across the system, the potentiostat used in this work would allow more than 20 specimens per batch if the anodising potential was under or about 20V. However, due to practical safety and constraints, the number of specimens was set to $n = 10$ per batch so to ensure that the power supply was not overloaded.

The current density responses for each experiment using the 10-specimen setup (Version B) are presented in Figure 4. These results are consistent with those of other studies [86–88] and suggest that current density increased with an increase in anodising potential.

As presented Figures 5 and 6, nanotube dimensions measured from the FE-SEM top view of the pure titanium specimens indicated that increasing potential led to an increase in the mean nanotube pore diameter, interpore distance, and nanotube length. In addition to the pore diameter and nanotube length, the interpore distance is also a crucial indicator of the consistency of the pore nucleation and dissolution rate in an anodisation process [33, 83]. The EDS analysis showed that elements of titanium and oxygen were found on the anodic layer, with traces of carbon and fluorine. The data presented above were proportional to the previously reported studies [48, 69, 80].

The nanotube dimensions, morphology, and EDS analysis of the anodised Ti-6Al-4V titanium alloy specimens showed a similar trend to those reported in previous publications [79, 89–92]. The EDS analysis revealed that the anodic layer was composed of titanium, aluminium, vanadium, oxygen, and traces of carbon and fluorine. As the applied potential (20V) was the same as that applied to pure titanium specimens (TiNT-20V), the nanotube pore diameter of the anodised Ti-6Al-4V specimens (Ti64NT-20V) was almost similar. However, the nanotube length of the anodised Ti-6Al-4V specimens appeared to be shorter than that of the anodised pure titanium specimens. It could be due to the different electrolyte and base material composition used, as well as the effect of the additional pickling process before the anodisation is carried out to remove the naturally occurred oxide layer. These phenomena have been previously demonstrated in other comparable studies [90, 93, 94]. In addition to that, the literature has also shown that changes in nanotube length are the least significant for the modulation of cellular behaviour [50], unlike the pore size and interpore distance, which are important. The overall results substantiated that the imperative dimensions of the nanotube were exquisitely controlled.

3.4. Consistency and Reproducibility Check. Current density curves were monitored during every anodisation batch to ensure the consistency of the electrochemical treatment. After anodisation, the morphology and elemental composition of the anodised specimens were characterised using FE-SEM and EDS to confirm the absence of contaminating elements, irregular distribution, or any inconsistency of the nanotube array.

As the anodisation outcomes using both Version A and Version B were expected to be similar, the nanotube dimensions, including pore diameter, interpore distance, and nanotube length, of the anodised specimens using both versions were measured and compared. The morphologies of subsequent batches of anodised specimens were also examined to evaluate the reliability and reproducibility using the optimised device (Version B). Data analysis presented in Figure 6 concluded that the multiple measurements on different batches were consistent as there was no statistically significant difference, i.e., a $p$ value greater than 0.05, between Version A and each of the four batches of Version B.
anodised at their respective anodising potentials, regardless of the position of the specimen in the specimen holder.

3.5. Cost Analysis. As the specimens were of customised shape, the use of cutting devices was included in the cost analysis. Two commonly used precision automated disc cutting techniques were compared: fiber laser cutting (Method 1) and wire-cut EDM (Method 2). The corresponding costs are displayed in Table 1.

Both cutting methods offer an accurate and precise cutting result, although some burr or burnt edges can be found following Method 1. Method 1 was favoured in terms of staff costs and turnaround time. However, the consumable cost of Method 2 was slightly lower because the sheet metal can be stacked into a pile and cut using EDM, whereas
Figure 6: Statistical plots of the nanotube dimensions correspond to their respective anodising potentials; (a) the pore diameter (lower bar, labelled PD) and the interpore distance (upper bar, labelled ITD) of the anodised specimen using Version A (first bar with solid black fill) and one specimen from each of the four batches using Version B (bars with patterned fill), chosen from different positions in the 10-specimen holder, i.e., top corner, middle, bottom corner, and random position, as indicated in Figure 3. (a) $n = 100$, error bars represent standard deviation; (b) the nanotube length of the anodised specimens, measured using the same specimen as (a) ($n = 15$, error bars represent standard deviation).
Table 1: Cost analysis of the disc cutting methods. Titanium foils (thickness 0.25 mm; purity 99.5%; 100 × 2000 mm; Alfa Aesar) were used. Staff rate of £20 per hour was considered as a typical wage for technical staff members trained to graduate level in the UK (prices current June 2018).

| Disc cutting methods | Method 1 (laser cut) | Method 2 (wire-cut EDM) |
|----------------------|----------------------|------------------------|
| Disc cutting device name | iPG laser ytterbium fiber laser 1 kW cutting tool | AgieCharmilles CUT 200 sp EDM wire-cut machine |
| Associated consumables | 2 protecting optics-£142; 2 pressurised argon gas tank-£30 | 2 EDM brass cutting wires 0.25 mm-£100 |
| Total staff hours (£20/hr) | 4 hours-£80 | 6 hours-£120 |
| Total materials costs | Ti foil of 0.25 × 100 × 2000-£364 | Ti foil of 0.25 × 100 × 2000-£364 |
| Gross cost (a) | £616 | £584 |
| Total cut specimens (b) | 313 | 450 |
| Actual usable specimens (c) | 281 | 419 |
| Rejection rate ((b - c) × 100/b) | 10.2% | 6.9% |
| Estimated cost per usable specimen (d = a/c) | £2.19 | £1.39 |

Table 2: Time and cost analysis comparing the two anodisation device designs. Wire-cut EDM (cutting Method 2) was adopted for calculating the costs due to its overall advantages. Staff rate of £20 per hour was incorporated as a typical wage for technical staff members trained to graduate level in the UK (prices current June 2018).

| Descriptions | Version A | Version B |
|--------------|-----------|-----------|
| Number of specimens at one anodisation process | 1 specimen | 10 specimens |
| Preparation time per anodisation batch* | a1 = 1.5 hr | b1 = 2 hr |
| Anodisation time per anodisation batch | a2 = 1 hr | b2 = 1 hr |
| Preparation time for 30 specimens | a3 = a1 × 30 = 45 hr | b3 = b1 × 3 = 6 hr |
| Anodisation time for 30 specimens | a4 = a2 × 30 = 30 hr | b4 = b2 × 3 = 9 hr |
| Total time for anodising 30 specimens | a5 = a3 + a4 = 75 hr | b5 = b3 + b4 = 9 hr |
| Total labour time per anodised specimen | a6 = a5/30 = 150 min | b6 = b5/30 = 18 min |
| % increase in time efficiency compared to version A (A/B × 100) | 833 | — |
| Total labour cost per specimen (staff rate £20/hour) | £5.00 | £6.00 |
| Estimated cost per usable specimen, d_{T2} (carried from Table 1) | £1.39 | £1.39 |
| Estimated price of one complete anodised specimen | £51.39 | £7.39 |
| % increase in cost efficiency compared to version A (A/B × 100) | 695% | — |

*Specimen preparation time includes deburring, degreasing, assembling, lacquering, cleaning after anodisation, disassembling, sonicating, and packing for sterilisation after anodisation. †“30 specimens” were used as a standard quantity needed for a fundamental in vitro statistical assessment, i.e., biocompatibility assays on anodised specimens of different nanotube diameters.

Method 1 can only be used on one sheet at a time, leading to increased use of consumables. Method 2 also has a significantly lower rejection rate. As no dangerous chemicals, gases, or heat dissipation were involved in Method 2, it was generally less hazardous being conducted in an enclosed chamber, and less supervision was required during the operation. Method 2 (wire-cut EDM) was chosen over Method 1 due to its overall advantages, machining quality, and lower price.

In calculating the cost, we assumed that the cutting and anodising operations were conducted by trained personnel costed at a rate of £20 per hour if in the UK. Rejection of pieces could arise due, for example, to scratches, imprecise cutting, alteration of shape, or severe burning. The costs of electricity, apparatus, and chemicals (including cutting machine energy consumption, potentiostat power supply, electrolyte, anodising device construction, etc.) have not been considered.

The time and cost for producing anodised specimens were evaluated, as shown in Table 2. As mentioned, the wire-cut EDM technique was used for the calculations. For Version B, a one-off cost for an extra piece of platinum plus spot welding process was not included. The preparation time prior to biocompatibility test was also included to provide an actual estimation of cost when applied in real applications. As no previous literature has estimated the cost and efficiency of an anodisation setup, Version A was assumed as an approximate replica of the conventional anodisation system because it was capable of producing single anodised specimen at a given time.

Based on the analysis, the total labour time per anodised specimen for Version A was approximately 8.5 times more than that of Version B, owing to anodising of only one specimen in the former. Combining the specimen cutting and labour cost for anodising the specimens, Version B has substantially reduced the cost per anodised specimen by a factor of about 7. Overall, the significant increase in time and cost efficiency as displayed in Table 2 has made the optimised device (Version B) a promising and economical option for anodisation.

4. Conclusions

Our results and cost analysis displayed a promising optimisation of an anodisation device that has a potential for use in various applications and industries, for example, laboratory researches, small-medium enterprises (SMEs), and
cross-disciplinary experiments. The design and methodology used in this study concluded that the new anodisation device is capable of producing a highly ordered uniform TiO₂ nanotube layer with tuneable pore diameters for use in, but not limited to, biocompatibility in vitro tests. Not only significantly reducing the overall time and costs associated with high volume production of anodised specimens, but also the optimised device successfully provided consistent anodisation results with increased specimen throughput, customisation of the specimen shape, and high reproducibility.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

References

[1] X. Liu, P. Chu, and C. Ding, "Surface modification of titanium, titanium alloys, and related materials for biomedical applications," Materials Science and Engineering: R: Reports, vol. 47, no. 3-4, pp. 49–121, 2004.
[2] M. Kulkarni, A. Mazare, E. Gongadze et al., "Titanium nanostructures for biomedical applications," Nanotechnology, vol. 26, no. 6, article 062002, 2015.
[3] M. Geetha, A. K. Singh, R. Asokamani, and A. K. Gogia, "Ti based biomaterials, the ultimate choice for orthopaedic implants—a review," Progress in Materials Science, vol. 54, no. 3, pp. 397–425, 2009.
[4] P. Roy, S. Berger, and P. Schmuki, "TiO₂ nanotubes: synthesis and applications," Angewandte Chemie International Edition, vol. 50, no. 13, pp. 2904–2939, 2011.
[5] G. K. Mor, K. Shankar, M. Paulose, O. K. Varghese, and C. A. Grimes, "Use of highly-ordered TiO₂ nanotube arrays in dye-sensitized solar cells," Nano Letters, vol. 6, no. 2, pp. 215–218, 2005.
[6] D. Kuang, J. Brillet, P. Chen et al., "Application of highly ordered TiO₂ nanotube arrays in flexible dye-sensitized solar cells," ACS Nano, vol. 2, no. 6, pp. 1113–1116, 2008.
[7] J. R. Jennings, A. Ghicov, L. M. Peter, P. Schmuki, and A. B. Walker, "Dye-sensitized solar cells based on oriented TiO₂ nanotube arrays: transport, trapping, and transfer of electrons," Journal of American Chemical Society, vol. 130, no. 40, pp. 13364–13372, 2008.
[8] B. Liu and E. S. Aydil, "Growth of oriented single-crystalline rutile TiO₂ nanorods on transparent conducting substrates for dye-sensitized solar cells," Journal of American Chemical Society, vol. 131, no. 11, pp. 3985–3990, 2009.
[9] K. Zhu, T. B. Vinzant, N. R. Neale, and A. J. Frank, "Removing structural disorder from oriented TiO₂ nanotube arrays: reducing the dimensionality of transport and recombination in dye-sensitized solar cells," Nano Letters, vol. 7, no. 12, pp. 3739–3746, 2007.
[10] M. Paulose, K. Shankar, O. K. Varghese, G. K. Mor, and C. A. Grimes, "Application of highly-ordered TiO₂ nanotube-arrays in heterojunction dye-sensitized solar cells," Journal of Physics D: Applied Physics, vol. 39, no. 12, pp. 2498–2503, 2006.
[11] T.-S. Kang, A. P. Smith, B. E. Taylor, and M. F. Durstock, "Fabrication of highly-ordered TiO₂ nanotube arrays and their use in dye-sensitized solar cells," Nano Letters, vol. 9, no. 2, pp. 601–606, 2009.
[12] P. Roy, D. Kim, K. Lee, E. Spiecker, and P. Schmuki, "TiO₂ nanotubes and their application in dye-sensitized solar cells," Nanoscale, vol. 2, no. 1, pp. 45–59, 2010.
[13] S. P. Albu, A. Ghicov, J. M. Macak, R. Hahn, and P. Schmuki, "Self-Organized, free-standing TiO₂ nanotube membrane for flow-through photocatalytic applications," Nano Letters, vol. 7, no. 5, pp. 1286–1289, 2007.
[14] Z. Wu, F. Dong, W. Zhao, H. Wang, Y. Liu, and B. Guan, "The fabrication and characterization of novel carbon doped TiO₂ nanotubes, nanowires and nanorods with high visible light photocatalytic activity," Nanotechnology, vol. 20, no. 23, article 235701, 2009.
[15] C. Li, J. Yuan, B. Han, L. Jiang, and W. Shangguan, "TiO₂ nanotubes incorporated with CdS for photocatalytic hydrogen production from splitting water under visible light irradiation," International Journal of Hydrogen Energy, vol. 35, no. 13, pp. 7073–7079, 2010.
[16] M. Zlamal, J. Macak, P. Schmuki, and J. Krysa, "Electrochemically assisted photocatalysis on self-organized TiO₂ nanotubes," Electrochemistry Communications, vol. 9, no. 12, pp. 2822–2826, 2007.
[17] J. M. Macak, M. Zlamal, J. Krysa, and P. Schmuki, "Self-organized TiO₂ nanotube layers as highly efficient photocatalysts," Small, vol. 3, no. 2, pp. 300–304, 2007.
[18] M. Jarosz, A. Pawlik, M. Szuwarzyński, M. Jaskula, and G. D. Sulkà, "Nanoporous anodic titanium dioxide layers as potential drug delivery systems: drug release kinetics and mechanism," Colloids and Surfaces B: Biointerfaces, vol. 143, pp. 447–454, 2016.
[19] N. Çalışkan, C. Bayram, E. Erdal, Z. Karahalioğlu, and E. B. Denkbaş, "Titania nanotubes with adjustable dimensions for drug reservoir sites and enhanced cell adhesion," Materials Science and Engineering C: Materials for Biological Applications, vol. 35, pp. 100–105, 2014.
[20] M. Lai, Z. Jin, X. Yang, H. Wang, and K. Xu, "The controlled release of simvastatin from TiO₂ nanotubes to promote osteoblast differentiation and inhibit osteoclast resorption," Applied Surface Science, vol. 396, pp. 1741–1751, 2017.
[21] X. Chen, K. Cai, J. Fang et al., "Dual action antibacterial TiO₂ nanotubes incorporated with silver nanoparticles and coated with a quaternary ammonium salt (QAS)," Surface and Coatings Technology, vol. 216, pp. 158–165, 2013.
[22] E. P. Su, D. F. Justin, C. R. Pratt et al., "Effects of titanium nanotubes on the osseointegration, cell differentiation, mineralisation and antibacterial properties of orthopaedic implant surfaces," Bone and Joint Journal, vol. 100, no. 1, pp. 9–16, 2018.
[23] A. Roguska, M. Pisarek, A. Belcarz et al., "Improvement of the bio-functional properties of TiO₂ nanotubes," Applied Surface Science, vol. 388, pp. 775–785, 2016.
[24] J. Tian, Y. Li, J. Dong, M. Huang, and J. Lu, "Photoelectrochemical TiO₂ nanotube arrays biosensor for asulam determination based on in-situ generation of quantum dots," Biosensors and Bioelectronics, vol. 110, pp. 1–7, 2018.
[25] K.-S. Mun, S. D. Alvarez, W.-Y. Choi, and M. J. Sailor, "A stable, label-free optical interferometric biosensor based on TiO₂ nanotube arrays," ACS Nano, vol. 4, no. 4, pp. 2070–2076, 2010.
[26] M. Terracciano, V. Galstyan, I. Rea, M. Casalino, L. De Stefano, and G. Sberveglieri, "Chemical modification of TiO₂ nanotube arrays for label-free optical biosensing applications," Applied Surface Science, vol. 419, pp. 235–240, 2017.

[27] J. Ni, C. J. Frandsen, K. Noh et al., "Fabrication of thin film TiO₂ nanotube arrays on Co-28Cr-6Mo alloy by anodization," Materials Science and Engineering: C, vol. 33, no. 3, pp. 1460–1466, 2013.

[28] P. Sharma, S. Cartmell, and A. J. El Haj, "Bone tissue engineering," Applications of Cell Imobilisation Biotechnology, vol. 8, pp. 153–166, 2005.

[29] K. S. Brammer, S. Oh, C. J. Cobb, L. M. Bjursten, H. v. d. Heyde, and S. Jin, “Improved bone-forming functionality on diameter-controlled TiO₂ nanotube surface,” Acta Biomaterialia, vol. 5, no. 8, pp. 3215–3223, 2009.

[30] National Joint Registry, The National Joint Registry for England, Wales, Northern Ireland and the Isle of Man 2015 Public and Patient Guide to the NJR’s 12th Annual Report 2015–Hip Replacement Edition, National Joint Registry, Hemel Hempstead, UK, 2015.

[31] A. Svedbom, E. Hernlund, M. Ivergård et al., “Osteoporosis in the European Union: a compendium of country-specific reports,” Archives of Osteoporosis, vol. 8, no. 1–2, p. 137, 2013.

[32] Accuracy Research LLP, Global Orthopedic Implants Market Worth USD 91.42 Billion by 2025 - Analysis, Technologies and Forecasts Report 2016-2025 Vendors: 3M Health Care, Abbott Labs, Baxter International Research and Markets, Business Wire, San Francisco, CA, USA, 2016.

[33] D. Regonini, Anodised TiO₂ Nanotubes: Synthesis, Growth Mechanism and Thermal Stability, University of Bath, Bath, UK, 2008.

[34] T. Molchan, Generation of Porous and Nanotubular Anodic Films on Titanium and Titanium-Aluminium alloy, The University of Manchester, Manchester, UK, 2014.

[35] C. N. Elias, J. H. C. Lima, R. Valiev, and M. A. Meyers, "Biomedical applications of titanium and its alloys," JOM, vol. 60, no. 3, pp. 46–49, 2008.

[36] N. Ren, S. Zhang, Y. Li et al., "Bone mesenchymal stem cell functions on the hierarchical micro/nanotopographies of the Ti-6Al-7Nb alloy," British Journal of Oral and Maxillofacial Surgery, vol. 52, no. 10, pp. 907–912, 2014.

[37] J. M. Hernández-López, A. Conde, J. de Damborenea, and M. A. Arenas, "Correlation of the nanostructure of the anodic layers fabricated on Ti13Nb13Zr with the electrochemical impedance response," Corrosion Science, vol. 94, pp. 61–69, 2015.

[38] S. Minagar, C. C. Berndt, J. Wang, E. Ivanova, and C. Wen, "A review of the application of anodization for the fabrication of nanotubes on metal implant surfaces," Acta Biomaterialia, vol. 8, no. 8, pp. 2875–2888, 2012.

[39] C. Covarrubias, M. Mattmann, A. Von Marttens et al., "Osteointegration properties of titanium dental implants modified with a nanostructured coating based on ordered porous silica and bioactive glass nanoparticles," Applied Surface Science, vol. 363, pp. 286–295, 2016.

[40] L. Lin, H. Wang, M. Ni et al., “Enhanced osteointegration of medical titanium implant with surface modifications in micro/nanoscale structures," Journal of Orthopaedic Translation, vol. 2, no. 1, pp. 35–42, 2014.

[41] L. S. Jung, J. S. Shumaker-Parry, C. T. Campbell, S. S. Yee, and M. H. Gelb, "Quantification of tight binding to surface-immobilized phospholipid vesicles using surface plasmon resonance: binding constant of phospholipase A₂," Journal of American Chemical Society, vol. 122, no. 17, pp. 4177–4184, 2000.

[42] M. Kulkarni, A. Mazare, J. Park et al., "Protein interactions with layers of TiO₂ nanotube and nanopore arrays: morphology and surface charge influence," Acta Biomaterialia, vol. 45, pp. 357–366, 2016.

[43] W. Yang, X. Xi, X. Shen, P. Liu, Y. Hu, and K. Cai, "Titania nanotubes dimensions-dependent protein adsorption and its effect on the growth of osteoblasts," Journal of Biomedical Materials Research Part A, vol. 102, no. 10, pp. 3598–3608, 2013.

[44] S. Minagar, J. Wang, C. C. Berndt, E. P. Ivanova, and C. Wen, "Cell response of anodized nanotubes on titanium and titanium alloys," Journal of Biomedical Materials Research Part A, vol. 101, no. 9, pp. 2726–2739, 2013.

[45] L. Zhao, L. Liu, Z. Wu, Y. Zhang, and P. K. Chu, "Effects of micropitted/nanotubular titania topographies on bone mesenchymal stem cell osteogenic differentiation," Biomaterials, vol. 33, no. 9, pp. 2629–2641, 2012.

[46] L. M. Bjursten, L. Rasmusson, S. Oh, C. G. Smith, K. S. Brammer, and S. Jin, “Titanium dioxide nanotubes enhance bone bonding in-vivo," Journal of Biomedical Materials Research Part A, vol. 9999, pp. 1218–1224, 2010.

[47] N. Wang, H. Li, W. Liu et al., "Effects of TiO₂ nanotubes with different diameters on gene expression and osseointegration of implants in minipigs," Biomaterials, vol. 32, no. 29, pp. 6900–6911, 2011.

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

[49] J. Park, S. Bauer, K. A. Schlegel, F. W. Neukam, K. von der Mark, and P. Schmuki, "TiO₂ nanotube surfaces: 15 nm-an optimal length scale of surface topography for cell adhesion and differentiation," Small, vol. 5, no. 6, pp. 666–671, 2009.

[50] S. Bauer, J. Park, J. Faltenbacher, S. Berger, K. von der Mark, and P. Schmuki, “Size selective behavior of mesenchymal stem cells on ZrO₂ and TiO₂ nanotube arrays," Integrative Biology, vol. 1, no. 8-9, pp. 525–532, 2009.

[51] J. Park, S. Bauer, K. von der Mark, and P. Schmuki, "Nanosize and vitality: TiO₂ nanotube diameter directly cell fate," Nano Letters, vol. 7, no. 6, pp. 1686–1691, 2007.

[52] M. J. Dalby, N. Gadegaard, and R. O. C. Orefio, "Harnessing nanotopography and integrin-matrix interactions to influence stem cell fate," Nature Materials, vol. 13, no. 6, pp. 558–569, 2014.

[53] C. Huang, X. Liu, Y. Liu, and Y. Wang, "Room temperature ferromagnetism of Co-doped TiO₂ nanotube arrays prepared by sol-gel template synthesis," Chemical Physics Letters, vol. 432, no. 4–6, pp. 468–472, 2006.

[54] J. Qiu, Z. Jin, Z. Liu et al., “Fabrication of TiO₂ nanotube film by well-aligned ZnO nanorod array film and sol-gel process,” Thin Solid Films, vol. 515, no. 5, pp. 2897–2902, 2007.

[55] T. Maiyalagan, B. Viswanathan, and U. V. Varadaraju, "Fabrication and characterization of uniform TiO₂ nanotube arrays by sol-gel template method," Bulletin of Materials Science, vol. 29, pp. 705–708, 2006.

[56] S. M. Liu, L. M. Gan, L. H. Liu, W. D. Zhang, and H. C. Zeng, "Synthesis of single-crystalline TiO₂ nanotubes," Chemistry of Materials, vol. 14, no. 3, pp. 1391–1397, 2002.

[57] C.-C. Wang and J. Y. Ying, "Sol–Gel synthesis and hydrothermal processing of anatase and rutile titania nanocrystals," Chemistry of Materials, vol. 11, no. 11, pp. 3113–3120, 1999.
[58] Z. Miao, D. Xu, J. Ouyang, G. Guo, X. Zhao, and Y. Tang, "Electrochemically induced Sol–Gel preparation of single-crystalline TiO2 nanowires," Nano Letters, vol. 2, no. 7, pp. 717–720, 2002.

[59] S. D. Perera, R. G. Mariano, K. Vu et al., "Hydrothermal synthesis of graphene-TiO2 nanotube composites with enhanced photocatalytic activity," ACS Catalysis, vol. 2, no. 6, pp. 949–956, 2012.

[60] M. G. Choi, Y.-G. Lee, S.-W. Song, and K. M. Kim, "Lithium-ion battery anode properties of TiO2 nanotubes prepared by the hydrothermal synthesis of mixed (anatase and rutile) particles," Electrochimica Acta, vol. 55, no. 20, pp. 5975–5983, 2010.

[61] Y.-K. Zhou, L. Cao, F.-B. Zhang, B.-L. He, and H.-L. Li, "Lithium insertion into TiO2[sub 2] nanotube prepared by the hydrothermal process," Journal of Electrochemical Society, vol. 150, no. 9, article A1246, 2003.

[62] D. V. Bavykin, V. N. Parmon, A. A. Lapkin, and F. C. Walsh, "The effect of hydrothermal conditions on the mesoporous structure of TiO2 nanotubes," Journal of Materials Chemistry, vol. 14, no. 22, p. 3370, 2004.

[63] Q. Zhang, L. Gao, J. Sun, and S. Zheng, "Preparation of long TiO2 nanotubes from ultrafine rutile nanocrystals," Chemistry Letters, vol. 31, no. 2, pp. 226–227, 2002.

[64] H. Ou and S. Lo, "Review of titania nanotubes synthesized via the hydrothermal treatment: fabrication, modification, and application," Separation and Purification Technology, vol. 58, no. 1, pp. 179–191, 2007.

[65] J.-Y. Huang, K.-Q. Zhang, and Y.-K. Lai, "Fabrication, modification, and emerging applications of TiO2 nanotube arrays by electrochemical synthesis: a review," International Journal of Photoenergy, vol. 2013, Article ID 761971, 19 pages, 2013.

[66] E. Matykin, A. Conde, J. de Damborenea, D. M. Y. Marero, and M. A. Arenas, "Growth of TiO2-based nanotubes on Ti-6Al-4V alloy," Electrochimica Acta, vol. 56, no. 25, pp. 9209–9218, 2011.

[67] D. Loic and S. Simovic, "Self-ordered nanopore and nanotube platforms for drug delivery applications," Expert Opinion on Drug Delivery, vol. 6, no. 12, pp. 1363–1381, 2009.

[68] Y. R. Smith, R. S. Ray, K. Carlson, B. Sarma, and M. Misra, "Self-ordered titanium dioxide nanotube arrays: synthesis and their photo/electro-catalytic applications," Materials, vol. 6, no. 7, pp. 2892–2957, 2013.

[69] V. S. Saji, H. C. Choe, and W. A. Brantley, "An electrochemical study on self-ordered nanoporous and nanotubular oxide on Ti-35Nb-5Ta-7Zr alloy for biomedical applications," Acta Biomaterialia, vol. 5, no. 6, pp. 2303–2310, 2009.

[70] M. F. Hossain, R. S. Mandal, M. R. Haque, and M. S. Islam, "Fabrication of TiO2 nanotube arrays in room temperature by anode oxidation method," in Proceedings of 2012 7th International Conference on Electrical and Computer Engineering, pp. 94–97, Dhaka, India, December 2012.

[71] K. Subramani, "Titanium surface modification techniques for implant fabrication - from microscale to the nanoscale," Journal of Biomimetics, Biomaterials and Tissue Engineering, vol. 5, pp. 39–56, 2010.

[72] N. Swami, Z. Cui, and L. S. Nair, "Titania nanotubes: novel nanostructures for improved osseointegration," Journal of Heat Transfer, vol. 133, no. 3, article 034002, 2011.

[73] X. Zhu, J. Chen, L. Scheideler, R. Reichl, and J. G. Gerstorfer, "Effects of topography and composition of titanium surface oxides on osteoblast responses," Biomaterials, vol. 25, no. 18, pp. 4087–4103, 2004.

[74] A. Mazzarolo, M. Curioni, A. Vicenzo, P. Skeldon, and G. E. Thompson, "Anodic growth of titanium oxide: electrochemical behaviour and morphological evolution," Electrochimica Acta, vol. 75, pp. 288–295, 2012.

[75] A. Ghicov, H. Tsuchiya, J. M. Macak, and P. Schmuki, "Titanium oxide nanotubes prepared in phosphate electrolytes," Electrochemistry Communications, vol. 7, no. 5, pp. 505–509, 2005.

[76] J. M. Macak and P. Schmuki, "Anodic growth of self-organized anodic TiO2 nanotubes in viscous electrolytes," Electrochimica Acta, vol. 52, no. 3, pp. 1258–1264, 2006.

[77] A. Valota, D. J. LeClere, P. Skeldon et al., "Influence of water content on nanotubular anodic titania formed in fluoride/glycerol electrolytes," Electrochimica Acta, vol. 54, no. 18, pp. 4321–4327, 2009.

[78] G. D. Sulka, J. Kapusta-Kolodziej, A. Brzózka, and M. Jaskula, "Fabrication of nanoporous TiO2 by electrochemical anodization," Electrochimica Acta, vol. 55, no. 14, pp. 4359–4367, 2010.

[79] Z. Bolukoglu, I. Karakaya, and M. Erdogan, "Formation of titanium dioxide nanotube arrays by anodizing Ti-6Al-4V alloy," ECS Transactions, vol. 58, pp. 45–52, 2014.

[80] S. Bauer, S. Kleber, and P. Schmuki, "TiO2 nanotubes: tailoring the geometry in H2PO4/HF electrolytes," Electrochemistry Communications, vol. 8, no. 8, pp. 1321–1325, 2006.

[81] K. Indira, U. K. Mudali, T. Nishimura, and N. Rajendran, "A review on TiO2 nanotubes: influence of anodization parameters, formation mechanism, properties, corrosion behavior, and biomedical applications," Journal of Bio- and Tribocorrosion, vol. 1, no. 4, 2015.

[82] S. Goodarzi, F. Mozfarzadeh, N. Nezafati, and H. Omidvar, "Titanium dioxide nanotube arrays: a novel approach into periodontal tissue regeneration on the surface of titanium implants," Advanced Materials Letters, vol. 7, no. 3, pp. 209–215, 2016.

[83] B. Chen, "Growth of anodic alumina nanopores and titania nanotubes and their applications," Doctoral Dissertation, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA, 2013.

[84] A. Jaroenworaluck, D. Regonini, C. R. Bowen, R. Stevens, and D. Allsopp, "Macro, micro and nanostructure of TiO2 anodised films prepared in a fluorine-containing electrolyte," Journal of Materials Science, vol. 42, no. 16, pp. 6729–6734, 2007.

[85] U. Donatus, G. E. Thompson, D. Elabar, T. Hashimoto, and S. Morsch, "Features in aluminum alloy grains and their effects on anodizing and corrosion," Surface and Coatings Technology, vol. 277, pp. 91–98, 2015.

[86] A. Atyaoui, H. Cachet, E. M. M. Sutter, and L. Bousselmi, "Effect of the anodization voltage on the dimensions and photocactivity of titania nanotube arrays," Surface and Interface Analysis, vol. 45, no. 11–12, pp. 1751–1759, 2013.

[87] S. A. Ali Yahia, L. Hamadou, A. Kadri, N. Benbrahim, and E. M. M. Sutter, "Effect of anodizing potential on the formation and EIS characteristics of TiO2 nanotube arrays," Journal of Electrochemical Society, vol. 159, no. 4, pp. 83–92, 2012.

[88] Y. Sun and K.-P. Yan, "Effect of anodization voltage on performance of TiO2 nanotube arrays for hydrogen generation in a two-compartment photoelectrochemical cell," International Journal of Hydrogen Energy, vol. 39, no. 22, pp. 11368–11375, 2014.

[89] P. W. Doll, M. Wolf, M. Weichert, R. Ahrens, A. E. Guber, and B. Spindler, "Nanostructuring of titanium by anodic oxidation
with sulfuric and hydrofluoric acid,” *Current Directions in Biomedical Engineering*, vol. 4, no. 1, pp. 641–644, 2018.

[90] T. D. Dikova, M. G. Hahm, D. P. Hashim, N. T. Narayanan, R. Vajtai, and P. M. Ajayan, “Mechanism of TiO_2 nanotubes formation on the surface of pure Ti and Ti-6Al-4V alloy,” *Advanced Materials Research*, vol. 939, pp. 655–662, 2014.

[91] T. D. Dikova, M. G. Hahm, D. P. Hashim, T. N. Narayanan, R. Vajtai, and P. M. Ajayan, “Growth mechanism of TiO_2 nanotubes on the Ti-6Al-4V surface,” in *Proceedings of IX International Congress "Machines, Technologies, Materials"*, Varna, Bulgaria, September 2012.

[92] A. Kaczmarek-Pawelska and E. Krasicka-Cydzik, “Morphological and chemical relationships in nanotubes formed by anodizing of Ti6Al4V alloy,” *De Gruyter*, vol. 14, no. 4, pp. 12–20, 2014.

[93] P. Xiao, B. B. Garcia, Q. Guo, D. Liu, and G. Cao, “TiO_2 nanotube arrays fabricated by anodization in different electrolytes for biosensing,” *Electrochem. commun.* vol. 9, no. 9, pp. 2441–2447, 2007.

[94] G. K. Mor, O. K. Varghese, M. Paulose, K. Shankar, and C. A. Grimes, “A review on highly ordered, vertically oriented TiO_2 nanotube arrays: fabrication, material properties, and solar energy applications,” *Solar Energy Materials and Solar Cells*, vol. 90, no. 14, pp. 2011–2075, 2006.
