Research Article

A Novel Methodology for Economical Scale-Up of TiO₂ Nanotubes Fabricated on Ti and Ti Alloys

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The prospective use of nanotechnology for medical devices is increasing. While the impact of material surface nanopatterning on the biological response is convincing, creating a large surface area with such nanotechnology remains an unmet challenge. In this paper, we describe, for the first time, a reproducible scale-up manufacturing technique for creating controlled nanotubes on the surfaces of Ti and Ti alloys. We describe an average of approximately 7.5-fold increase in cost and time efficiency with regards to the generation of 20, 50, and 100 nm diameter nanotubes using an anodisation technique. These novel materials have great potential in the medical field through their influence on cellular activity, in particular, protein absorption, focal adhesion, and osteoinduction. In this paper, we provide a step-by-step guide to optimise an anodisation system, starting with design rationale, proof of concept, device upscaling, consistency, and reproducibility check, followed by cost and efficiency analysis. We show that the optimised device can produce a high number of anodised specimens with customisable specimen shape at reduced cost and time, without compromising the repeatability and consistency. The device can fabricate highly uniform and vertically oriented TiO₂ nanotube layer with desired pore diameters.

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 (TiO₂) nanotubes within the last ten years; Figure 1(b) presents the applications of TiO₂ 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 TiO₂ 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].

FIGURE 1: (a) The increasing trend outlining the number of papers published on TiO₂ nanotubes from 2007 to 2017 (data were obtained from the Science Direct database on Oct 1, 2018, using keywords of “TiO₂ nanotube” with result filters of review articles and research articles under the article type); (b) range of applications of TiO₂ nanotubes and their respective percentage with regard to the number of published papers within the area of TiO₂ nanotubes in the year 2017 (data were obtained from the Science Direct database on Oct 1, 2018, using keywords “TiO₂” and “nanotube” and “corresponding keywords shown in the pie chart,” with result filters of review articles and research articles under the article type and year 2017).

The high surface-to-volume ratio of TiO₂ 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 TiO₂ 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 TiO₂ 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,

e.g., H_2SO_4 or H_3PO_4 [51, 74]; salts, e.g., $(\text{NH}_4)_2\text{SO}_4$ or Na_2SO_4 [75]; and organic electrolytes with glycerol or ethylene glycol [76, 77].

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]. TiO_2 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 time point 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 TiO_2 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 *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 *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 diagrams 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 TiO_2 nanotube pore diameters; (v) to customise the specimen shape for actual practical application, in our case, for *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 $\text{Hg}/\text{Hg}_2\text{Cl}_2$ (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 *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 H_3PO_4 and 0.25 wt% HF. For Ti-6Al-4V titanium alloy specimens, 1M H_2SO_4 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 *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.

FIGURE 2: Typical conventional anodisation systems used in material fabrication laboratories. The number and position of electrodes involved, the configuration, and the area of anodising surface; (a) top-bottom with three-electrode configuration, reported by Molchan [34]; (b) left-right with two-electrode configuration, reported by Regonini [33, 84]. The specimen (working electrode) used in both systems is bounded by an O-ring or a rubber gripper, resulting in an anodising surface of approximately 20 mm in diameters.

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 $\pm$ 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 $\times$ 50 mm) were welded to provide an electrode of area approximately 94 mm $\times$ 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

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.

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

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 ($\sim 100 \text{ nm}$), 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 in 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

FIGURE 4: Current density recorded over time corresponding to the number of specimens per anodisation and their respective anodising potentials.

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

FIGURE 5: Top view FE-SEM micrographs (A); respective EDS analysis (B); cross sections of the nanotube layer (C); base material: pure titanium (a) and Ti-6Al-4V (b); anodised specimens (c)–(f). Scale bar = 100 nm. (a) Ti. (b) Ti64. (c) TiNT-2.5V. (d) TiNT-10V. (e) TiNT-20V. (f) Ti64NT-20V.

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

(a)

(b)

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*	$a_1 = 1.5$ hr	$b_1 = 2$ hr
Anodisation time per anodisation batch	$a_2 = 1$ hr	$b_2 = 1$ hr
Preparation time for 30 specimens [†]	$a_3 = a_1 \times 30 = 45$ hr	$b_3 = b_1 \times 3 = 6$ hr
Anodisation time for 30 specimens	$a_4 = a_2 \times 30 = 30$ hr	$b_4 = b_2 \times 3 = 3$ hr
Total time for anodising 30 specimens	$a_5 = a_3 + a_4 = 75$ hr	$b_5 = b_3 + b_4 = 9$ hr
Total labour time per anodised specimen	$a_6 = a_5/30 = 150$ min	$b_6 = b_5/30 = 18$ min
% increase in time efficiency compared to version A ($A/B \times 100$)	—	833
Total labour cost per specimen (staff rate £20/hour)	$a_7 = a_6 \times 20 = £ 50.00$	$b_7 = b_6 \times 20 = £ 6.00$
Estimated cost per usable specimen, d_{T2} (carried from Table 1)	£ 1.39	£ 1.39
Estimated price of one complete anodised specimen	$a_7 + d_{T2} = £ 51.39$	$b_7 + d_{T2} = £ 7.39$
% increase in cost efficiency compared to version A [$A/B \times 100$]	—	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.

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