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Conversion of titania (TiO_2) into conductive titanium (Ti) nanotube arrays for combined drug-delivery and electrical stimulation therapy

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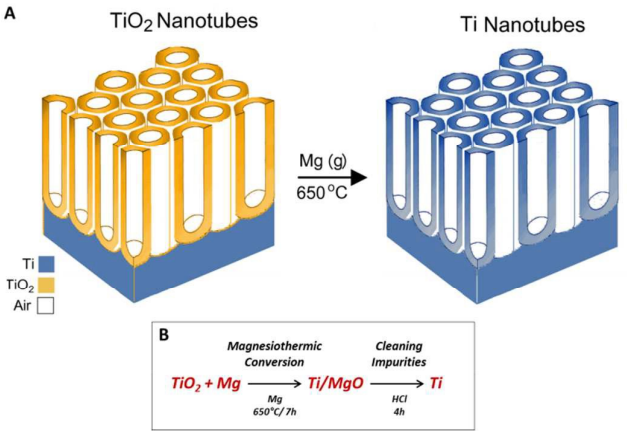
The conversion of titania (TiO_2) nanotubes into titanium (Ti), while preserving their nanotubular structures, is demonstrated. Their application as bone implants and electrodes for combined local drug delivery and electrical stimulation therapy is proposed.

Electrical stimulation therapy (EST) to treat bone fractures (nonunions and delayed unions) is approved for clinical use, and various *in-vitro* and *in-vivo* studies have established its role in accelerating bone healing.[1] Several EST strategies have been used to administer low level of current (DC), directly to the trauma site using surgical placement of electrodes (cathode at fracture site, and anode at adjacent tissue).[2] It has been reported that this process can induce cellular functions including stimulation of fibroblast activities, Ca influx and increased number of growth factor receptor sites.[1-2] In addition, studies have shown that EST reduce edema formation and improve tissue perfusion, with a significant increase in the transcutaneous oxygen pressure.[1-2] Although EST has shown considerable success in clinical applications, there is scope for improvement, for example to combine it with localized drug delivery (LDD) to simultaneously target unavoidable complications such as bacterial infection, which is often encountered in case of invasive surgeries. This can be achieved using conductive drug-releasing implants that have the ability to release therapeutics (antibiotics/proteins) inside the traumatized bone, while simultaneously providing electrical stimulation.

Drug-releasing implants based on electrochemically engineered titania (TiO_2) nanotubes (NTs), which can easily be fabricated on Ti implants of varied geometries, have been extensively explored for addressing various bone therapeutic challenges.[3] Owing to its cost-effective synthesis, favourable bioactivity, ability to load therapeutics, and demonstrated ability to achieve delayed/triggered release, TiO_2 NTs fabrication have emerged as a promising bone implant modification strategy.[4] More recently, TiO_2 NTs technology has been extended to Ti wires, pins and needles, and has been proposed as minimally-invasive, 3-dimensionally therapeutic releasing bone implants.[5-6] These implantable devices have also opened opportunities to address multiple challenges simultaneously, for example: fracture fixation and therapeutic release to combat infection/inflammation and compromised bone healing. However, the layer composed of TiO_2 NTs on Ti implants is poorly conductive (semiconductor), which limits another very attractive option, to combine their drug releasing function with electrical stimulation therapy.

To address this limitation and enable EST, while simultaneously delivering active therapeutics locally into the micro-environment of the traumatized bone, we present a simple fabrication approach to convert the layer of TiO_2 NTs into conducting Ti NTs using Ti wire as a model implant and EST electrode. Our approach is presented in



Scheme 1.

Scheme 1 Representation of conversion of TiO_2 nanotubes fabricated on Ti wires, into Ti nanotubes using magnesiothermic reduction process: (A) scheme, and (B) chemical reaction.

The process involves electrochemical anodization of Ti wire to fabricate an oxide layer with arrays of TiO_2 nanotubes on the surface. These TiO_2 nanotubes were reduced to Ti using a

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magnesiothermic reduction process.[7-8] The idea is to reduce TiO_2 into Ti while preserving the nanotubular architecture, in order to make conductive Ti wire implants with Ti nanotube arrays on the surface. Structural characterization and elemental analysis was performed to confirm conversion of TiO_2 into Ti, with preserved structures. Conductivity measurements were also performed to prove successful oxide to metal conversion. Finally *in-vitro* drug release studies were carried out to establish the suitability of the modified implant for proposed bone implant applications in combination with EST.

Commercial Ti wire (0.8 mm diameter, 5 mm length) was used as model bone implant and EST electrode. Electrochemical anodization, which represents a cost-effective and scalable technology, was used to fabricate TiO_2 NTs on the surface of electro-polished Ti wire.[4] Using ethylene glycol electrolyte (with 1% v/v water, and 0.3% w/v NH_4F) in a specially designed electrochemical cell, successful fabrication of high-quality TiO_2 nanotubes on the curved surface of the Ti wire was achieved.[5] This was confirmed by scanning electron microscopy (SEM) characterization showing images with typical structures of nanotube arrays before and after conversion. To achieve reduction of the TiO_2 layer with nanotube structures to Ti, a modified magnesiothermic reduction process was employed, as described previously.[8] SEM images of the TiO_2 NTs and converted Ti NTs are presented in Fig. 1A-D, which confirm successful transformation of Ti oxide into a Ti metallic layer, while preserving the nanotube structures. Anodization conditions using 75 V and 20 min, yielded nanotubes with ~ 12 µm length and ~ 65 nm diameter, however their dimensions (diameters, length) could easily be tailored using various anodization parameters, as reported previously.[5]

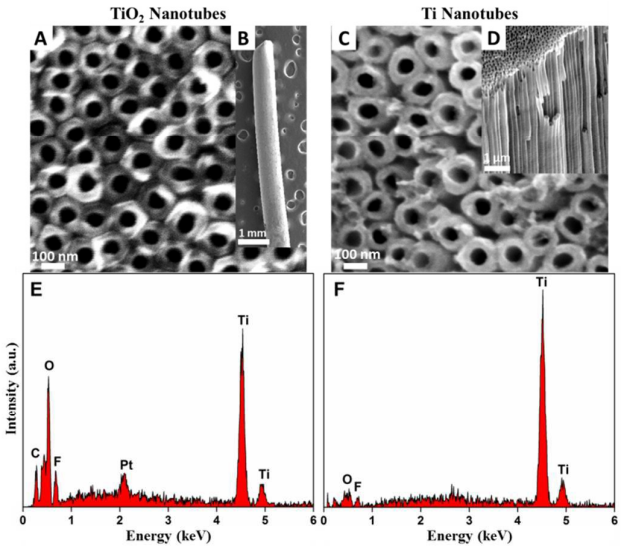

Fig. 1 Conversion of TiO_2 into Ti nanotubes by magnesiothermic reduction process: (A) SEM image showing the top view of TiO_2 NTs structures [inset (B) TiO_2 NTs wire implant], and (C) top view of Ti NTs structures after the conversion process [inset (D) cross-sectional SEM image showing that converted nanotubes retain their structural features]. Comparative EDXS analysis for (E) TiO_2 NTs and (F) Ti NTs confirms transformation of TiO_2 into Ti metallic nanotubular layer.

Energy dispersive X-ray spectroscopy (EDXS) graphs of the TiO_2 nanotubular layer before and after conversion are presented in Fig. 1E-F. The EDXS graphs for TiO_2 NTs (Fig. 1E) shows significant peaks for Ti and O, confirming an oxide layer with the presence of F and C from anodization electrolyte, and Pt from sample preparation for SEM. It is noteworthy to mention here that F ions, which are present in the anodization electrolyte, often become incorporated into the nanotube structures. An EDXS graph from Ti NTs (Fig. 1F) shows significant Ti and minor O peaks. These observations confirmed successful reduction of TiO_2 into Ti. The presence of a small oxygen peak can be explained by re-oxidation of Ti in air after sample preparation. Also reduced peaks for F and negligible presence of C infers successful removal of these impurities during the conversion and cleaning procedure.

The comparative X-ray diffraction (XRD) graphs taken from the TiO_2 and Ti NTs are presented in Fig. 2A-B, and show clear evidence of successful conversion of TiO_2 into Ti, with the presence of Mg in the nanotube structures. Weak signals for Ti are seen for TiO_2 nanotubes (Fig. 2A), whereas for Ti NTs (Fig. 2B) prominent peaks can be seen for Ti as a result of the magnesiothermic process. The incorporation of Mg from the reduction process is also observed but with a minor quantity showing the largely successful removal after the reduction process.[8] For the unavoidable presence of Mg species into the Ti NTs, the suitability for bone implant applications is not affected, as studies have shown a role for Mg ions in upregulation of bone cell functions.[9] Please note that for XRD, conductivity and water contact angle measurements, TiO_2 NTs fabricated on Ti flat foil (fabricated using same anodization conditions as Ti wire) were used.

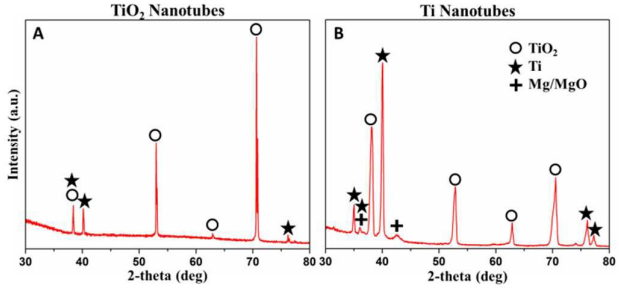

Fig. 2 XRD plots comparing the structural composition between (A) TiO_2 NTs and (B) Ti NTs. Analysis performed based on PDF card numbers 9008517/9002991/9006470 and published work.[10]

To confirm if the converted Ti NTs represents a conducting surface suitable for the proposed EST for promoting fracture healing rates, a 4-probe conductivity meter was used to measure sheet resistance of TiO_2 NTs and Ti NTs, compared with bare Ti flat foil. The results presented in Table 1 reveal very low values of sheet resistance for Ti NTs, which closely matches to conducting bare Ti. However, the resistance of TiO_2 NTs is several orders of magnitude higher. Since the sheet resistance is inversely related to conductance, it can be confirmed that the poorly-conducting oxide layer with TiO_2 NTs was successfully converted to conducting Ti NTs. The results show that Ti NTs implants can be used as electrodes for EST to stimulate bone cells to upregulate healing mechanisms. More investigations in this regard will be reported in future, using

ex-vivo and *in-vivo* models. Alternatively, studies with electrical stimulation of bare Ti and nanotube modified Ti implants have confirmed enhanced osteoblast and antibacterial functions; however, conversion of poorly-conducting TiO_2 nanotubes into metallic conducting Ti nanotubes can further improve these features.[11]

	Sheet Resistance (R) (ohms/square)	Water Contact Angle (degrees)
Titanium	198 ±6.7 R	83.70 ±4.5
TiO_2 NTs	6.44 ±7.5 MR	86.91 ±4.5
Ti NTs	206 ±11.3 R	60.73 ±8.8

Table 1 Comparing sheet resistance and water contact angle (WCA) between bare Ti, TiO_2 NTs and Ti NTs. The results confirm successful conversion of poorly-conducting TiO_2 NTs into conducting and more hydrophilic Ti NTs.

Water contact angle (WCA) data were also recorded for the bare Ti, TiO_2 NTs and Ti NTs surfaces (Table 1). Based on previous reports, which signify that the WCA on TiO_2 NTs depends on surface roughness, dimensions of the nanotubes, and the presence of impurities incorporated from electrolyte, we can conclude that after the conversion process the Ti nanotube surface became more hydrophilic. These results indicate that the magnesiothermic reduction conditions annealed the nanotubes and also contributed to removing organic impurities from the anodization electrolyte. It is noteworthy to mention that hydrophilic bone implant surfaces, especially anodized and heat treated (or annealed) ensure early onset of osseointegration (quicker bone healing rates) and also reduce the number of live and dead bacteria.[12]

Finally drug loading and releasing ability of the Ti NTs/Ti wire implants was demonstrated using the hydrophilic dye Rhodamine B (Rh B) used as a drug model. However, the 'vacant' nanotubes could be loaded with any drug, protein or growth factor, catering for specific bone conditions, as we have demonstrated with TiO_2 NTs in our previous studies.[4] Thermo-gravimetric analysis (TGA) confirmed a loading amount of ~80 µg, which is comparable with loading in TiO_2 NTs, and shows no change in drug loading performance after the conversion process. It is worth noting that drug loading could be further controlled by varying the immersion times, drug concentration or nanotube dimensions.[4] The release plot for RhB loaded Ti NTs/Ti wire implants (Fig. 3) shows the release of around ~65 µg of model drug (~80% of the total amount loaded) in the first 6 hrs, which is comparable with TiO_2 NTs. Please note that due to similar nanotube dimensions and implant lengths, the drug loading amounts and release kinetics were similar (with no significant difference) for both the systems: TiO_2 NTs and Ti NTs. This release profile shows considerable burst release due to drug present on/near the top of the nanotubes, and inside the cracks of the anodic film. The purpose of this experiment was to show that prepared Ti NTs are functional as drug-releasing applications. However, with further optimization, advanced features including maximised drug loading, controlled release, and triggered release, which have been demonstrated previously for TiO_2 NTs, can also be integrated into Ti NTs systems.[4,13]

To confirm that the EST process can be achieved from the prepared conducting Ti NTs implants, without affecting the drug releasing abilities, a special *in-vitro* release experiment was performed by applying a small DC voltage during the release process. A voltage of 10 V for 1 min in cycles with a gap of 10 min at 120 and 300 min time intervals was selected to monitor any changes in the release behaviour. The results for electrically-stimulated drug-release showed no difference in the release kinetics of the loaded dye, as compared with the release without any stimulation. It is noteworthy to mention here that any sudden change in release kinetics can adversely affect bone healing, for instance: very high concentrations can cause local tissue toxicity, and below optimum dosages could retrigger conditions such as bacterial invasion. This shows that the drug-releasing and electrically conducting functions can be combined into a single nano-engineered Ti wire implant, and can be applied for EST with localized drug delivery. This solution can address the complex therapeutic needs for instance combating infection while also simultaneously enabling quicker bone healing via electrical stimulation. It is worth noting that by changing electrical conditions using higher voltages and pulsing regimes it is possible to release drug on demand which opens another potential application for electrically-stimulated drug release.

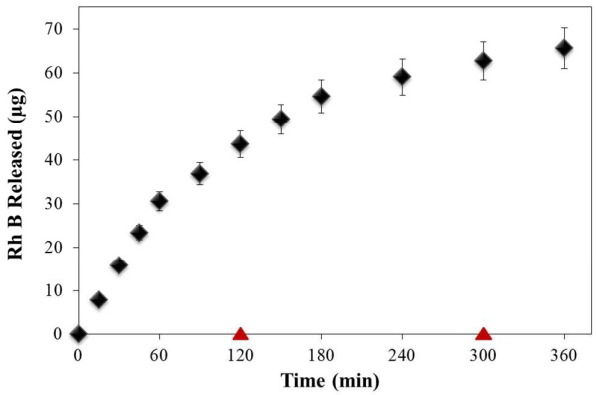

Fig. 3 *In-vitro* release profile of Rhodamine B from Ti NTs/Ti wire implants. Electrical stimulation (10 V for 1 min) on Ti NTs/Ti wire was applied at certain times (120 and 300 min, represented by red triangles on x-axis), and shows no influence on drug release.

In conclusion, this study presents new advances for the TiO_2 nanotubes implant technology, by successfully converting TiO_2 into Ti, while retaining the nanotubular morphology and providing an enhanced electrical conducting property. The advantage of these new Ti NTs/Ti wire implants is their minimally invasive implantation into the bone, and their potential use as electrodes for electrical stimulation therapy with simultaneous local release of therapeutics. Furthermore, the conducting therapeutic wire implants also qualify as drug releasing neural prostheses, which can be used to electrically stimulate specific deep lying brain regions to address complications such as Parkinson's disease.[14]

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Notes and references

***Experimental*:** High purity Ti wires (diameter 0.80 mm) and Ti flat foil (thickness 0.25 mm) were purchased from Nilaco (Japan). Rhodamine B, ethylene glycol, NH_4F, perchloric acid, butanol, ethanol, and acetone were obtained from Sigma-Aldrich (Sydney, Australia). Ti substrates were annealed at 500°C for 2 h, followed by sonication in acetone and drying in N_2. Electropolishing was performed in perchloric acid electrolyte (with butanol and ethanol, P:B:E = 1:6:9) at 40 V/1 min maintained at 4°C. The substrates were cleaned with deionized (DI) water and sonicated in acetone/ethanol. Electrochemical anodization of the Ti wires was carried out by exposing specific length (5 mm) of the Ti wire (via masking) to the ethylene glycol electrolyte [with 1 % (v/v) water and 0.3 % (w/v) NH_4F] at 75 V/ 20 min maintained at 25°C.[5] A similar anodization procedure was utilized for anodizing Ti flat foil.

For magnesiothermic conversion of TiO_2 nanotubes into Ti, Mg turnings and TiO_2 NTs/Ti implants were mixed (in the weight ratio of 0.5:1) in a ceramic boat and placed inside furnace, which was heated to 650°C at the rate of 10°C/min for 7 h, under argon gas (99.995 %) flow.[8] Afterwards the implants were soaked in HCl (1M) for 4 h to remove impurities (MgO and TiO_2). Finally the samples (now Ti NTs) were cleaned with ultra-pure water and stored in a desiccator under vacuum.

Surface characterization of the TiO_2 and Ti NTs implants was performed using a field emission scanning electron microscope/SEM (FEI Quanta 450) equipped with EDXS. Only TiO_2 NTs samples were coated with Pt (5 nm) prior to imaging with SEM, while Ti NTs samples were imaged directly without any metal coating. XRD spectra of the samples were recorded on a Rigaku Miniflex 600 instrument. Sheet resistance of bare Ti, TiO_2 NTs and Ti NTs at 50 nA current was acquired using 4-point probe conductivity meter (Jandel RM3000). Water contact angle (WCA) was also recorded for Ti, TiO_2 NTs and Ti NTs samples using Attension theta optical tensiometer (KSV instruments, Finland). Please note that for XRD, conductivity and WCA measurements, nanotubes fabricated on Ti flat foil were used.

For loading of model drug, Rhodamine B (RhB), clean nanotube samples (Ti NTs/Ti wire) were immersed in RhB solution (50 mg/ml in water) for 2 h, followed by wiping off the excess dye using soft tissue and drying. To quantify the amount of dye loaded inside the nanotubes, the loaded samples were heated to 500°C (at the rate of 10°C/min) in a TGA instrument (TA Instruments Q500), followed by analysis of the weight change. Later, the Ti NTs samples loaded with RhB were immersed in 5 ml PBS (pH 7.4, 37°C), and at predetermined time intervals 3 ml aliquots were drawn (and replaced with fresh PBS) and the absorbance was measured at 555 nm using Cary 60 spectrophotometer. The drug concentration/weight for the corresponding absorbance values was calculated based on the calibration curve and plotted against time. For electrical stimulation at specific time intervals during *in-vitro* release, Ti NTs (as cathode) and bare Ti wire (immersed in PBS, as anode) were connected to power supply and 10 V was applied for 1 min (3 cycles, separated by 10 min).

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Graphical Abstract

The conversion of titania (TiO_2) nanotubes into titanium (Ti), while preserving their nanotubular structures is demostarted for proposed application as bone implants and electrodes for combined local drug delivery and electrical stimulation therapy