Nano-engineered titanium for enhanced bone therapy

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ABSTRACT

Current treatment of a number of orthopaedic conditions, for example fractures, bone infection, joint replacement and bone cancers, could be improved if mechanical support could be combined with drug delivery. A very challenging example is that of infection following joint replacement, which is very difficult to treat, can require multiple surgeries and compromises both the implant and the patient's wellbeing. An implant capable of providing appropriate bio-mechanics and releasing drugs/proteins locally might ensure improved healing of the traumatized bone. We propose fabrication of nanoengineered titanium bone implants using bioinert titanium wires in order to achieve this goal. Titanium in the form of flat foils and wires were modified by fabrication of titania nanotubes (TNTs), which are hollow self-ordered cylindrical tubes capable of accommodating substantial drug amounts and releasing them locally. To further control the release of drug to over a period of months, a thin layer of biodegradable polymer PLGA poly(lactic-co-glycolic acid) was coated onto the drug loaded TNTs. This delayed release of drug and additionally the polymer enhanced bone cell adhesion and proliferation.

Keywords: Bone implants, titania nanotubes, local drug delivery, bone therapeutics, bone healing, bone infection

INTRODUCTION

Healing of bone fracture requires mechanical support such as that provided by titanium plates and screws. There are some circumstances, such as infection of the bone, where pharmacological therapy is also required. However, currently treatment of bone with drugs, such as antibiotics or anti-cancer drugs, is performed using systemic drug delivery, which both limits the amount of drug available at the bone site and exposes all tissues to the drug [1]. A potential solution to this dilemma lies in producing implants that combine mechanical support with enhanced therapeutic action [2].

Because of their biological inertness, titanium metal and its alloys are good candidates for bone implants and hence have been well researched and utilized extensively for both orthopedic and dental applications [3]. Titanium also has established biocompatibility and osseointegration characteristics. Various therapeutic coatings have been tested on titanium implants, including antibiotics, biopolymers, to combine bone fixation with therapeutics [4,5]. However, rapid drug diffusion and unfavorable release patterns have compromised these approaches [6]. In order to achieve prolonged drug release, drugs have been mixed with various polymers, such as PMMA or poly(methyl methacrylate), but these do not offer sufficient mechanical support and release toxic degradation byproducts [7]. Nanostructuring on titanium has been suggested to incorporate greater drug amounts and enhance bone cell functions [8]. We have suggested an alternative approach to local delivery of drug to bone sites, that has the additional advantage of the ability to load substantial amounts and delayed release kinetics of drug. This approach involves the fabrication of titania nanotubes (TNTs) on titanium surfaces, such as orthopaedic implants. TNTs, which are nano-scale vacant cylinders composed of titanium dioxide (TiO₂), can be self-ordered onto titanium substrates by electrochemical anodisation [3]. TNTs can easily be integrated into current titanium implant technology and offer improved bioactivity, mechanics and surface modification, as compared to conventional implants. The biomedical applications of TNTs include delayed drug release, enhanced bone cell adhesion/ proliferation, better integration into bone and improved bone healing [9,10,11].

In addition to conventional orthopaedic implants, nano-scale implant devices that can be surgically inserted into bone, and which are capable of releasing antibiotics, hormones, chemotherapeutics and growth factors in an effective concentration directly where they are required provides a potential solution to local drug delivery in bone. We hereby describe the production of titanium wires (diameter 0.75 mm and length 25 mm) with titania nanotubes fabricated onto the surface, which can be loaded with various drugs, each catering to a particular bone condition. TNTs have been loaded with anti-inflammatory drugs, antibiotics, and anti-cancer drugs in order to develop this novel technology aiming at

effective treatment of, for example, osteoporosis, osteomyelitis (bone infection) and primary (osteosarcoma) and metastatic (e.g. breast or prostate) bone cancer.

Figure 1. Scheme depicting (a) fabrication of titania nanotubes (TNTs) on Ti wires and drug loading into the nanotubes, and (b) adaptation of the technology to produce a bone implant that combines biomechanical support with nanotube-based drug release

MATERIAL AND METHODS

1.1 Materials

Titanium wire (99.7%) (diameter 0.75 mm) and flat foil (thickness 0.25 mm) was supplied by Alfa Aesar (MA, USA). Ethylene glycol, ammonium fluoride (NH₄F), PLGA, indomethacin, gentamicin sulfate and doxorubicin hydrochloride were purchased from Sigma-Aldrich (New South Wales, Australia). High purity Milli-Q water (Millipore Co., Billerica, MA, USA), ultra-pure grade (18.2 MΩ) and sieved through a 0.22- μm filter was used.

1.2 Fabrication of Titania Nanotubes

Titanium wires were mechanically polished using coarse/fine sand papers and porous alumina powder, followed by extended sonication in ethanol and acetone. Anodisation of the cleaned Ti wires was performed using a specially designed electrochemical cell and computer-controlled power supply (Agilent Technologies Inc.) as described previously [12]. 25 mm of the Ti wire was exposed to the electrolyte (3% water, 0.3 % NH₄F in ethylene glycol) and anodisation was carried out at 100 V for 1 hr at 20°C. For anodisation of flat Ti foil, a similar method was adopted with only a circular area (of diameter 12 mm) exposed for anodisation. The voltage and current signals were adjusted and continuously recorded during the fabrication process by specialised software (Labview, National Instruments, Austin, TX, USA). The structural characterisation of the prepared TNT samples was performed using a field emission scanning electron microscope or SEM (Philips XL 30, SEMTech Solutions, Inc., North Billerica, MA, USA).

1.3 Drug loading and analysis

Drug solutions (1% w/v) of gentamicin (Genta) and doxorubicin (Doxo) were prepared in water and for indomethacin (Indo) in pure ethanol. TNT/Ti wires were cleaned with deionised water and dried in N₂. Loading for each of the model drug was achieved by immersion of TNTs in a fixed volume of respective drug solution for 24 h. Following this, the TNTs were dried and quickly flushed with a fixed volume of sterile phosphate buffer saline (PBS) in order to remove

any surface adhered drug. To determine the amount of drug loaded, thermo-gravimetric analysis (TGA) was performed with analysis of the characteristic drug decomposition peak for each model drug. TNT/Ti Flat preloaded with indomethacin was dip-coated with a thin layer of PLGA solution (1% (w/v) in chloroform) in an attempt to delay the release of the drug.

1.4 *In-vitro* drug release

Drug loaded TNTs were immersed in 5ml PBS in a vial and the absorbance of the solution was measured at predetermined time intervals using UV-Vis spectroscopy. Each measurement involved removing a 3ml aliquot of PBS, measuring absorbance at a particular wavelength (characteristic for each model drug) and later replenishing the vial with fresh PBS (3 ml). The absorbance readings were converted into concentration using a calibration curve for the corresponding drug. Ultimately, the release profiles of each experimental set were expressed as burst (initial 6 h) and delayed release (every 24 h) in a plot with released weight percentage (wt. %) vs. time.

RESULTS

1.5 Structural Characterization of TNTs

The morphology of TNTs fabricated on Ti wires as characterised by SEM is depicted in figure 2. Figure 2a shows a top view of the TNTs with clearly visible open pores. TNTs were observed to have an average pore diameter of 180 ± 10 nm and length of 70 ± 5 μ m. The dimensions of these nanostructures could be further tailored as per the requirements; for instance, accommodating more drug or controlling its release [13]. Figure 2b shows the closed bottom of the TNTs as observed after scratching the TNT layer from the underlying substrate. The TNT layer also had few surface cracks, which is the result of radial outgrowth and mechanical stress during the fabrication of TNTs on the circular surface of the Ti wire. However, the overall structure of TNT on Ti wire was very stable, and the presence of few cracks aided in loading increased drug amounts. SEM imaging of TNTs revealed well-aligned nanotubes using this method (Fig. 2c). PLGA polymer coating resulted in effective covering of open pores, as shown in the case of indomethacin loaded TNTs fabricated on flat Ti (Fig. 2d).

Figure 2. Scanning electron microscopy (SEM) images showing (a) top view of TNTs with open pores, (b) bottom of TNTs (closed pore) obtained by removal of TNT layer, (c) cross-section with well-aligned nanotubes and, (d) PLGA

coated onto drug loaded TNTs fabricated on a flat Ti substrate to delay drug release (scratched to show underlying nanotubes). All scale bars represent 1 μm .

1.6 Drug loading

Three model drugs including Indo, Genta and Doxo were loaded into nanotubes, with each drug representing a varied chemistry, water solubility and structural weight, catering to specific bone conditions. TGA studies confirmed the loaded amounts of drugs in the TNTs on Ti wire as 0.16 mg, 0.2 mg and 1.2 mg for Indo, Genta and Doxo, respectively. TNTs with large pore diameters and lengths ensured substantial drug loading amounts, which can be further tailored by adjusting TNT dimensions, immersion times and drug solution concentrations. Variations in the amount of drug loaded were as a result of the relative attributes of the drugs with respect to their different chemistries and molecular weights. While more drug was retained inside of TNTs (post-washing the surfaces with PBS) in cases of the hydrophilic Genta and Doxo, less of the hydrophobic Indo could be loaded due the hydrophilic TNT interior walls. Due to its hydrophobic nature, more amount of Indo is expected to be loaded near the open pore of the TNTs as compared to deeper loading expected for hydrophilic drugs.

Drug Loaded	Loaded Amount (mg)	Initial Burst Release (Wt. %)	Total Release Time (Days)
Indomethacin	0.16	74	10
Gentamicin	0.20	36	11
Doxorubicin	1.20	25	8

Table 1. Release characteristics of model drugs from nanotubes.

1.7 Drug release profile

A biphasic release pattern with high initial burst release (IBR for 1st 6 hrs) followed by slow sustained release was observed for each of the model drugs, as represented in figure 3a. The drug loaded near the open pores would be eluted initially at a faster rate due to the high concentration gradient at the start of the release experiment. The slow release in the later phase is due to the diminishing concentration of drug remaining in the nanotubes. This kind of release pattern would be beneficial to treat severe conditions, such as bacterial infection, by delivering a high initial dose to reduce pathogen number, followed by a sustained release period to inhibit residual pathogen growth and thereby reduce the recurrence of bacterial adhesion [14]. For the IBR, around 74, 36 and 25 Wt. % of the Indo, Genta and Doxo were released from the TNTs, respectively. As most of the Indo is expected to be loaded near the open ends of the tubes (due to its hydrophobic nature), and hence in the initial phase of high concentration gradient, quick release is observed. The amount released of the hydrophobic Indo is very high but could be further reduced by applying a thin layer of biopolymer in order to cover the open pores, as described previously [13]. The total release of the drugs from the TNTs (~ 100% wt.) occurred over 10, 11 and 8 days for Indo, Genta and Doxo, respectively, from the surfaces of the TNT/Ti wire implants. This phenomenon again correlates with the varied water solubility, and the molecular weights of the model drugs which may have influenced not only the corresponding amounts that could be loaded but also the release behaviour. Table 1 summarises the drug loading amounts and release behaviours of the three model drugs from the TNT wire. For Indo loaded TNT/Ti flat foil, a thin layer of PLGA (2.5 μm), as confirmed by ellipsometry, reduced the IBR (from 77% to approximately 12%) and the total release was also delayed from 4 to 31 days, as compared with the uncoated samples (Fig. 3b). This delayed release technology could potentially be extended to TNT/Ti wires but this will require extensive further optimisation.

Figure 3. Plots showing release of loaded drug from TNTs into the surrounding PBS: (a) release of various drugs from TNT/Ti wires and, (b) controlled release of indomethacin from TNTs fabricated on Ti flat foil and coated with PLGA.

CONCLUSION

Titania nanotubes (TNTs) were successfully fabricated on the entire cylindrical surface area of the Ti wire. The TNTs were later loaded with a variety of model drugs, each catering to a particular bone condition, such as osteoporosis, bone infection and bone cancer. The release from the TNTs was sustained for over 10 days, which could be further extended using biopolymer coatings. These proposed wire bone implants of just 25 mm length and 0.75 mm diameter could easily be inserted inside traumatised bone and hence local drug elution could be achieved directly at the required site, potentially leading to enhanced therapeutic effects and faster bone healing.

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