Polylysine-modified titania nanotube arrays for local drug delivery

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A drug delivery system based on ε-polylysine-modified titania nanotube (ε-PL-TNTs) arrays was prepared. Polylysine on the nanotubes' surface can effectively bind with alendronate, a drug for the treatment of osteoporosis, through chemical bond. The bonds are fairly stable in an acid environment and cannot easily break up in a physiological environment. The ε-PL-TNTs increased the amount of drug loading by 9% in weight. The in vitro release profile of alendronate from ε-PL-TNTs showed a significant reduced burst release and an extended overall release of more than 15 days.

1. Introduction: In bone therapy and orthopedic implants, localised delivery of drugs in the form of drug-eluting implants is emphasised to reduce infection, prevent inflammation, and improve bone healing and tissue integration. Advances in nanoscience and nanoengineering, in particular, the application of nanotechnology to medicine, have promoted the application of a series of nanomaterials and drug carriers in drug delivery [1]. Nanoporous and nanotube structures such as nanoporous alumina and titania nanotubes (TNTs) arrays produced by electrochemical anodisation are intensively studied due to their unique features [2, 3]. Electrochemically engineered TNT arrays generated on Ti surface, due to their good biocompatibility [4], high surface area, excellent chemical inertness, and easily modifiable surfaces have been demonstrated to be good candidates for various biochemical applications [5, 6]. Moreover, they meet the requirements for prolonged and better control of drug administration.

Over the past few years, the encapsulation of various therapies into TNTs has been reported, thus proves the excellent performance of the materials used for drug delivery. On this point, the main challenges in the design of drug delivery system based on TNTs are to obtain materials with different chemical properties and functions, with high loading capacities and slow release rates. TiO_2 nanotube arrays have the advantage of a simple preparation process, and the nanotube's diameter and length can be controlled by changing the anode oxidation voltage, the composition of the electrolyte, and oxidation time [7]. Furthermore, the duration and kinetics of drug release from TNTs structures can be controlled ad-lib by engineering the nanotubes' dimensions [8], modifying their surface chemistry [9], or applying a polymeric coating on the TNT implant's surface through plasma polymerisation or dip-coating [10–13]. Losic and co-workers [14] functionalised amino-rich 3-aminopropyltriethoxysilane (APTES) onto TNTs. Due to their hydrophilic properties, the drug molecules can be better adsorbed, and the drug loading capacity is increased by 30–36 wt% compared with the unmodified TNTs, and the drug release time is prolonged (5–10 days).

Herein, we propose a drug delivery system through modifies the amino groups on the surface of the nanotubes in order to increase drug loading and to extend the duration of drug release from TNTs for water-soluble and degradable drugs. ε-Polylysine (ε-PL) is an antibacterial polypeptide with carboxyl and amino groups, which can form a strong bond with TiO_2 nanotubes through dehydration. The combination of ε-PL and drug forms an electrostatic attraction, which is much stronger than the van der Waals forces commonly used in the adsorption of drug molecules, as presented in Scheme 1 (see Fig. 1). Results from tests showed that the modified TNTs reduced burst release ~26% and extended overall release of more than 15 days.

2. Materials and methods: TiO_2 nanotube arrays were fabricated directly on Ti foils via electrochemical anodic oxidation. The titanium foils were polished with sandpaper then cleaned in acetone and deionised water for 15 min. The electrolyte contained ethylene glycol with 10 vol% DI water and 0.5 wt% NH_4F. Ti foils were placed in the electrolyte with Pt foil as the counter electrode under 60 V for 24 h.

ε-PL modified TNTs (ε-PL-TNTs) samples were prepared using a hydrothermal method. First, the previous preparation: TNTs were alkali-heat-treated with 0.5 M NaOH for 30 min at 50°C in order to increase the amount of hydroxyl groups on the surface of the TiO_2 nanotubes. The hydroxyl groups react with –COOH in ε-PL to form a stable structure after dehydration, thus grafting ε-PL to TNTs. N-2-hydroxyethylpiperazine-N-ethane-sulphonicacid (HEPES) buffer was prepared using NaOH to adjust the solutions to a final pH value of 7.4. Then, the TNTs were immersed into the Teflon-lined stainless steel autoclave containing different concentrations of ε-PL/HEPES solution, followed by hydrothermal treatment at 70°C for 6 h. Subsequently, the products were rinsed with the HEPES buffer and dried at room temperature.

A certain amount of alendronate (4 mg/ml) with a different pH was transferred to the reaction vessel. The samples were immersed in the drug solution and subsequently treated at 70°C for 6 h. Finally, the samples were allowed to dry at room temperature. In vitro drug release from all prepared the samples (modified and unmodified) was investigated by immersing the samples in 10 ml phosphate buffer solution (PBS) at pH = 7.4, placed in a water bath oscillator at 37°C for a certain number of days, and the amount NaAL loading and release was determined using an ultraviolet spectrophotometer with the indirect method. The measurement was carried out at 290 nm wavelength following the addition of an iron(III) reagent (5 mM ferric chloride in 1 M $HClO_4$) that formed a complex between the alendronate and the ion.

Scanning electron microscopy (SEM) was used to demonstrate the samples. Fourier transform infrared spectroscopy (FTIR) studies were carried out to investigate the functional group on the TiO_2 nanotube arrays. Thermogravimetric (TG) was used to analyse the drug content in the samples.

3. Results and discussion: The structure and morphology of the prepared TNTs was characterised by SEM and are summarised in

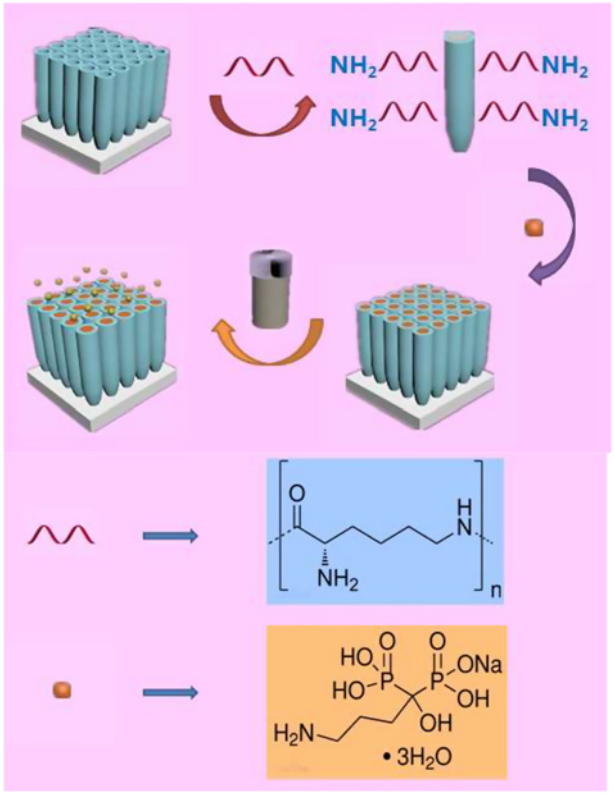

Fig. 1 *Scheme 1. Schematic diagram summarise the process of modified nanotubes combining amination and biopolymer coatings for drug release*

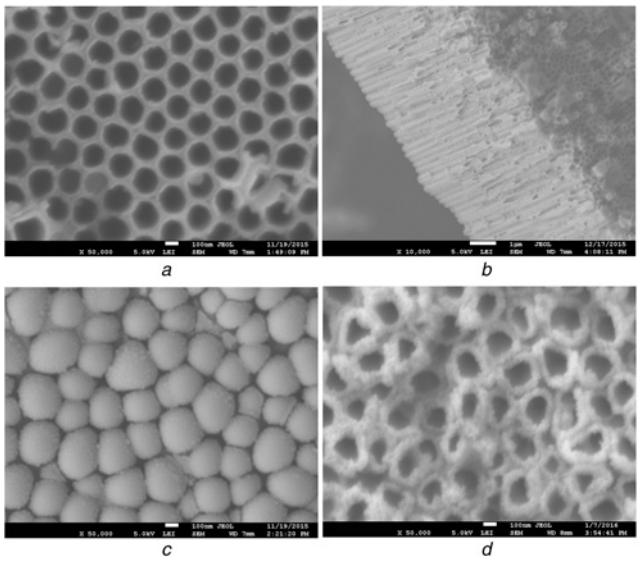

Fig. 2 *SEM images of TiO_2 nanotube array generated in an ethylene glycol electrolyte containing 0.5 wt% NH_4F and 10 vol% H_2O at 60 V for 24 h*
a Top image
b Cross-sectional image
c Bottom image
d Top surface of ε-PL-TNT

Fig. 2. SEM images of the top surface of TNT samples (Fig. 2*a*) show nanotubes with open pores featuring an average diameter of 200 ± 10 nm and a length of 5 µm. A high-resolution cross-sectional SEM image of the TNTs displaying vertically aligned, highly ordered, and densely packed arrays of nanotubes is presented in Fig. 2*b*. The bottom surface of the TNTs after

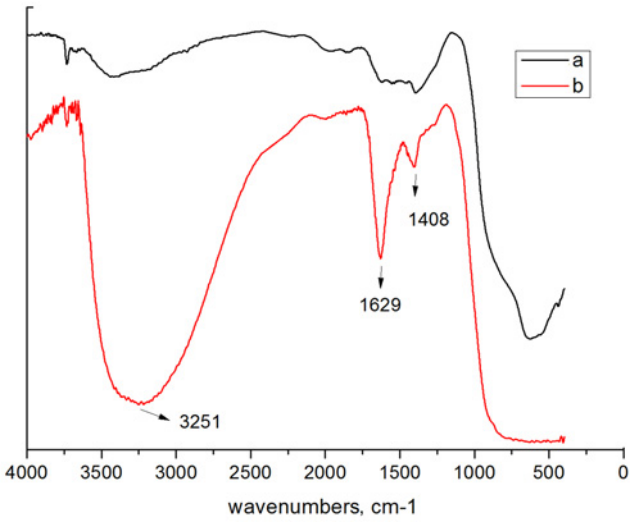

Fig. 3 *FTIR spectra of samples*
a TNTs and
b ε-PL-TNTs

detachment from the Ti substrate (Fig. 2*c*) shows that the nanotubes were closed with a spherical oxide barrier layer. Fig. 2*d* presents the SEM image of ε-PL-TNT arrays. It can be seen that the diameter of the nanotube decreases, indicating that PL has been grafted onto the nanotubes.

To further investigate the composition of the functional group and the bonding situation of the surface material, infrared (IR) spectrum technology was used to characterise the surface of the samples. Figs. 3*a* and *b* show the FTIR spectra of TNTs and ε-PL-TNTs. With respect to TiO_2, the FTIR spectra of ε-PL-TNTs exhibit three peaks at 1408, 1629 and 3251 cm^{-1}. The sharp band at 1629 cm^{-1} is the characteristic peak of ε-PL, corresponding to $–NH_2$ stretching vibration [15]. Moreover, the peak at 1408 cm^{-1} is due to the vibration of C–N bond. The broad bond at 3251 cm^{-1} is attributed to the stretching vibrations of O–H groups in H_2O or hydroxyl groups on the surface of TNTs with a wide range of hydrogen bond strengths [16].

The load capacity of the samples was measured by TG, depending on the percentage of weight loss of the NaAL relative to the TNTs. The drug loading ratio of modified TNTs was 2.3 times of pure TNTs, which proved again the good loading capacity of the modified TNTs. Figs. 4*a–d* show that the drug is completely broken down at 400°. The weight loss of the drug-loaded ε-PL-TNTs was about 18.8 wt%, remove the weight loss of ε-PL, the load of alendronate on the ε-PL-TNTs was 9, 5 wt% higher compared to unmodified nanotubes, and the weight loss of pure nanotubes was about 5 wt%.

Alendronate is soluble in water with a weak acid pH = 4.6, a portion was then removed and adjusted to pH = 7.4 with 1 M NaOH to investigate the interaction between NaAL and ε-PL. In order to assess the potential of using ε-PL-modified TiO_2 nanotubes as carriers for the controlled release of alendronate, we compared the drug release profiles of three different samples: different pH alendronate-loaded with and without modified TiO_2 nanotubes. After 15 days of release, the drug release of the unmodified nanotubes was 427 µg, while the ε-PL modified nanotubes loaded with drugs (pH = 4.6), with a total release of 732 µg. Indicating there was a reaction between ε-PL and the NaAL, which improved the drug loading. As it can be seen in Fig. 5, all release curves display a biphasic behaviour (except for the free drug), showing an initial burst in the first 24 h that released 59–85% of the drug, followed by a gradual, slow release that lasted for 15 days. As expected, drug release directly from the pure nanotubes was very

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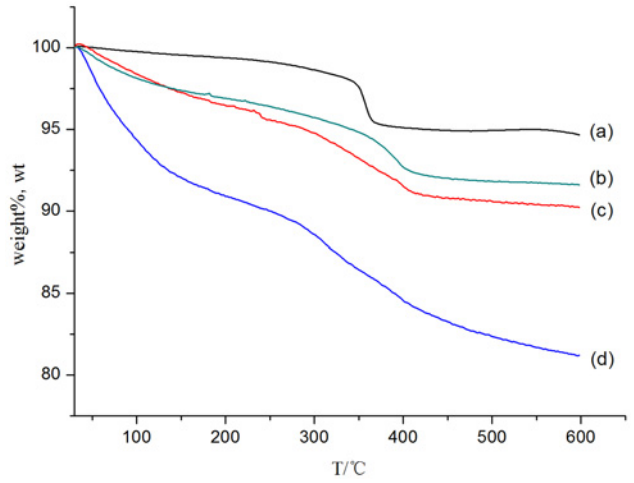

Fig. 4 *TG curves of samples*
a TNTs
b TNTs loaded with NaAL
c ε-PL-TNTs
d ε-PL-TNTs loaded with NaAL

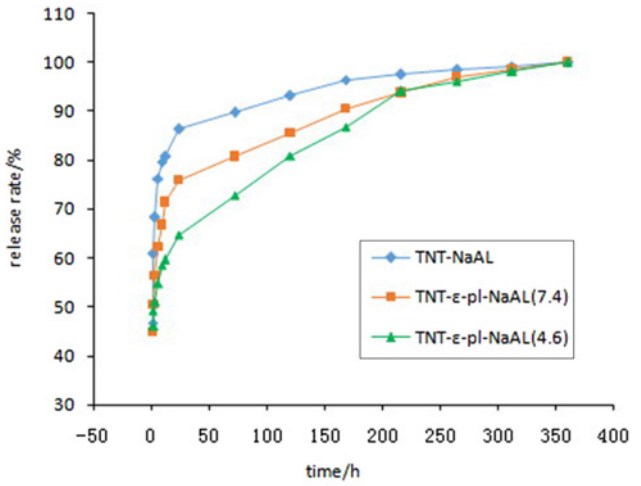

Fig. 5 *Comparative drug release graphs of anti-inflammatory drug (alendronate) from ε-PL-TNTs as drug carrier*

fast, and almost the entire amount of drug embedded in the TiO_2 nanotubes was released in the first 24 h. The drugs released at this stage are physically adsorbed on the surface of the samples, and drugs are easy to fall off. The drug release rate slows down after modifying ε-PL. This was attributed to the combination of ε-PL with alendronate. In acidic conditions, the $–NH_2$ group on the nanotubes' surface interacts with H^+ to form the positively charged NH_3^+. It is due to the electrostatic interaction between NH_3^+ and the negatively charged O, which is the P–OH group of alendronate, that drugs can be effectively loaded onto TiO_2 nanotubes [17]. In PBS (pH = 7.4), the electrostatic interaction weakens due to changes in surface charge and polarity. Thus, the drug can be released slowly in situ on the boundary between the implant and the bone tissue. When the drug pH = 7.4, the electrostatic attraction disappears, and the drug loading is relatively reduced.

4. Conclusion: In summary, a well-designed controllable drug delivery system based on ε-PL-TNTs has been developed. The electrostatic interaction between ε-PL and drugs result in a high loading of anti-osteoporosis drug molecules (NaAL). Through electrostatic interaction responsive to pH, this approach showed a significant improvement in the drug-release characteristics of TNTs, with a reduced burst release (from 85 to >59%) and an extended overall release of more than 15 days. Our results suggest that the ability of polymer-modified drug-releasing TNT-Ti samples to simultaneously deliver therapeutics, reduce implant-related infections, and promote osteointegration makes them potential candidates for future biomedical applications.

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