pH-responsive release of TiO_2 nanotube arrays/mesoporous silica composite based on tannic acid-Fe(III) complex coating

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In this work, TiO_2 nanotube arrays/mesoporous silica composite coated with tannic acid-Fe(III) complex was prepared with a simple drip coating method. The coating significantly inhibited the burst release in the early stage of drug release. The coordination conditions of the material were different under different pH values. This resulted in different stabilities of the coating, thus controlling the release of Loxoprofen Sodium (LS) at different pH values to construct a pH-responsive drug delivery system. To demonstrate this principle, the authors loaded LS into the samples and triggered pH-induced release. The excellent biological activity of the samples was confirmed in vitro by the significant growth and differentiation of MC3T3-E1 osteoblastic cells. The data has shown that the samples had achieved intelligent control of drug release and would have a wide range of applications.

1. Introduction: TiO_2 nanotubes (TNTs) are recognised as promising local drug delivery implant materials. However, to better apply TNTs in clinical practice, it is necessary to solve the problem of high dosage of TNTs in the early stage of treatment and to prolong the time of action of drugs in a local position. Nowadays, the most effective method to reduce the burst release of TNTs in the early stage of treatment is to cover the nanotubes with a polymer coating and to induce the release of drugs in a certain manner if necessary, thus achieving intelligent controlled release [1–4].

Although the modification method reported in the literature also uses nanoparticles to increase the drug loading, the principle is generally to increase the roughness of the TNTs [5]. Studies have shown that mesoporous silica can significantly prolong the drug release time and improve the drug release efficiency due to their unique properties such as high pore volume and specific surface area [6–8].

Tannic acid (TA) is a polyphenol with antibacterial and anti-oxidation [9]. TA can act on many enzymes and coagulate the protoplasm of microorganisms; thus, it can inhibit many types of bacteria such as cholera bacteria, *Escherichia coli*, and *Staphylococcus aureus*. In medicine, TA is used to stop bleeding and callus, inhibit bacteria, and resist allergy; TA also acts as an antioxidant and anticancer agent and prevents cardiovascular and cerebrovascular diseases [10]. This has made TA a hot research topic of phenolic substances in recent years. The complex of TA and Fe(III) ions can form coatings (or capsules) on diverse materials with different morphologies and properties, including planar and granular materials such as gold, polysiloxane, silica, calcium carbonate, *E. coli*, and *Staphylococcus aureus* [11, 12]. Moreover, the coordination between Fe (III) ions and TA can occur under different coordination conditions at different pH values, thus indirectly controlling the disassembly of capsules by controlling the pH [13–15].

In this Letter, the mesoporous silica was loaded onto TNTs by template method to prepare a composite. The method is novel. Meanwhile, a simple coating method was used to cover the complex of TA and Fe (III) ions on the surface of composite after the drug loading to prepare a pH-responsive drug release system, i.e. in normal bone tissue (pH = 7.4) inhibiting the release of drugs, whereas the inflammatory tissue (weak acidity) triggers the release of drugs, thus achieving intelligent control of drug release.

In general, the coatings are made of high molecular polymers [16, 17]. The complexes of TA and Fe(III) are used as coatings in this study, and their coordination conditions are different under different pH conditions to achieve simple and intelligent controlled release, and greatly inhibited the sudden release in the early stage, providing a more flexible and novel modification ideas.

2. Materials and methods: TNT arrays were fabricated directly on Ti (pure titanium with 0.8 mm thickness and 99.7 purity) foils via electrochemical anodic oxidation. The titanium foils were polished with sandpaper (continual grade 400–1500 grit) 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.

About 0.3 g dodecyltrimethylammonium bromide was added to 50 ml deionised water, 72 ml ethanol, and 20.47 ml ammonia water, stirred at 30°C until completely dissolved. Then, 2.35 ml TEOS was added to the mixture slowly. After 5 min, the TNTs were added to the solution above and stirred for 6 h. After drying, the samples were annealed at 450°C for 2 h in a muffle furnace. The sample was labelled as TNTs-SiO_2.

About 10 ml of Loxoprofen Sodium (LS) aqueous solution with a concentration of 20 mg ml^{-1} was added dropwise into TNTs-SiO_2, dried at room temperature in a vacuum drying oven (DZF-6020), and these steps were repeated ten times. The sample was labelled as TNTs-SiO_2-LS.

TA solution and $FeCl_3$ solution of 0.24 mM was prepared. Then, the $FeCl_3$ solution and TA solution were added to a test tube, oscillated for 30 s to achieve a final concentration of Fe(III): 0.1 M, TA: 0.4 M. The pH of mixture was adjusted to alkaline pH with 1 M NaOH solution. A certain amount of mixture was dripped onto the surface of samples, and dried naturally in air. This procedure was repeated five times, and the samples were labelled as TNTs-SiO_2-TA and TNTs-SiO_2-LS-TA, respectively.

Release experiments were carried out for TNTs-SiO_2-LS-TA. The samples were oscillated at 37°C in PBS of pH = 1.6, 4.5, and 7.4. The slow-release ability of coating for the drug was studied under different pH conditions. A certain amount of release solution was taken out at certain intervals for testing.

The MC3T3-E1 mouse preosteoblasts (Cell Bank of Chinese Academy of Sciences, Shanghai, China) were incubated at 37°C in a 5% CO_2 environment in α-minimum essential medium with 10% foetal bovine serum and 1% streptomycin/penicillin. We conducted

a cell-counting kit-8 (CCK-8) assay with MC3T3-E1 cells to identify potential cellular toxicity of the samples after 3 days were passed since the seeding of the cells onto the sample [18, 19].

The crystalline phase of the samples was investigated by a X-ray diffractometer (XRD, INEL Equinox 3000 France) using Cu Kα radiation ($\lambda = 0.154187$ nm) and 2θ values were between 10° and 80°. The surface morphology of samples was observed by field-emission scanning electron microscopy (FE-SEM, JSM-7500F). The vibrational spectra of samples was measured by Fourier transform infrared (FTIR, Nicole Avatar 360) spectroscopy, and the chemical composition of samples was analysed. The existence of TA-Fe(III) complex coating was confirmed by X-ray photoelectron spectroscopy (XPS, ESCALAB 250). The amount of released drug was measured by UV-vis spectroscopy (TU 1900).

3. Results and discussion

3.1. Structure and surface morphology of samples: Fig. 1 shows the XRD patterns of bare TNTs (Fig. 1(a)) and TNTs-SiO_2 (Fig. 1(b)). Both of the samples exhibited the characteristic diffraction peaks of Ti at $2\theta° = 35.2°$, 38.6°, 40.3°, 53.1°, 63.0° (JCPDS Card No. 00-044-1294). Notably, TNTs-SiO_2 exhibited the characteristic diffraction peaks of anatase TiO_2 at $2\theta° = 25.3°$, 47.8°, 53.9°, 54.9° (JCPDS Card No. 21-1272) [20]. This could be attributed to the fact that the TNTs were annealed at 450°C and the phase of TNTs was transformed from amorphous to anatase.

Figs. 2*a–d* show the SEM images of TNTs-SiO_2 coated with TA-Fe(III) for different times. Compared with the uncoated TNTs-SiO_2, with the increase in coating time, the amount of TA-Fe(III) complex on the surface of sample gradually increased. A film started to form on the surface of sample when coated three times. After coating five times, the surface of sample was completely covered with the coating. In Fig. 3, after the coating of TA-Fe(III) for five times, the surface of sample was covered with a relatively smooth film, and some protruding spots were observed on the film, presumably due to the unevenness of surface of sample after the drug loading.

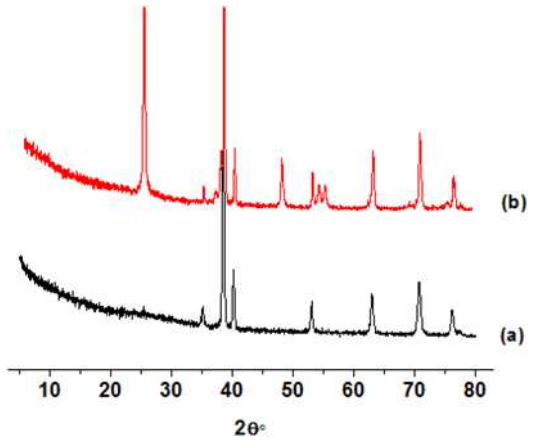

Fig. 1 *XRD patterns of the samples*
a Bare TNTs
b TNTs-SiO_2

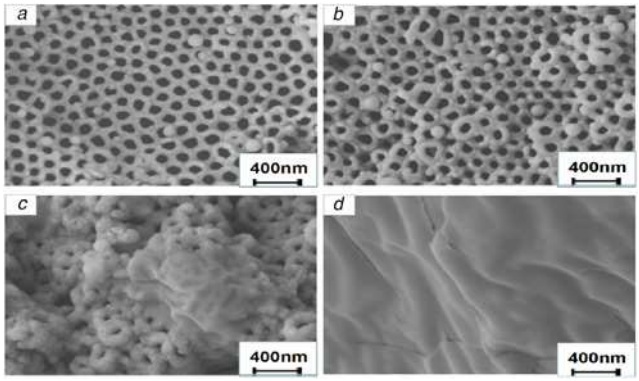

Fig. 2 *SEM images of*
a 0 time coating with TA-Fe(III) for TNTs-SiO_2
b 1 time coating with TA-Fe(III) for TNTs-SiO_2
c 3 times coating with TA-Fe(III) for TNTs-SiO_2
d 5 times coating with TA-Fe(III) for TNTs-SiO_2

Fig. 4 shows the FTIR spectra of samples. After the sample was coated with TA-Fe(III) complex, the peaks at 1574 and 1455 cm^{-1} represented the C=C bonds of aromatic ring. The peak at 1733 cm^{-1} corresponded to C=O group, and the peaks derived from C–H stretching appeared at 2970, 2925, and 2871 cm^{-1}. This shows that the entire coating process did not affect LS. Moreover, after the TA-Fe(III) coating, the intensity of aromatic C–OH stretching peaks of TA (1350–1020 cm^{-1}) was weakened, especially the peak at 1320 cm^{-1}. The decrease in intensity was dramatic, which could be interpreted as the formation of TA-Fe(III) complex [11].

3.2. XPS analysis of samples: To confirm the successful coating of TNTs-SiO_2-LS associated with the TA-Fe(III) complex, rather than the assembly or aggregation of TA alone, XPS measurements were carried out to analyse the elemental composition on the surface of TNTs-SiO_2-LS and TNTs-SiO_2-LS-TA. In the full-scan spectrum (Fig. 5*A*), the Ti 2p peak almost disappeared completely after the coating of TA-Fe(III) complex, and the intensity of Na 1 s peak clearly weakened. However, a new characteristic peak of Fe 2p appeared at 711.05 eV, even though the content of Fe was only 0.47%. In the high-resolution spectrum of O 1 s (Fig. 5*C*), only one characteristic peak appeared before the coating of TA-Fe(III) complex. This peak is located at 532.72 eV, corresponding to C–O–H bond. After the coating of TA-Fe(III) complex, a new characteristic peak appeared at 535.09 eV, corresponding to

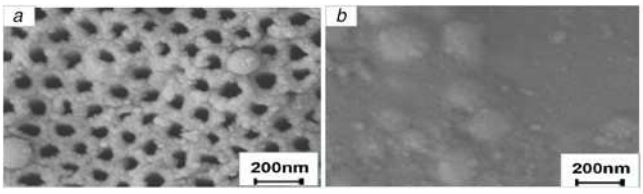

Fig. 3 *SEM images of*
a TNTs-SiO_2-LS
b TNTs-SiO_2-LS-TA

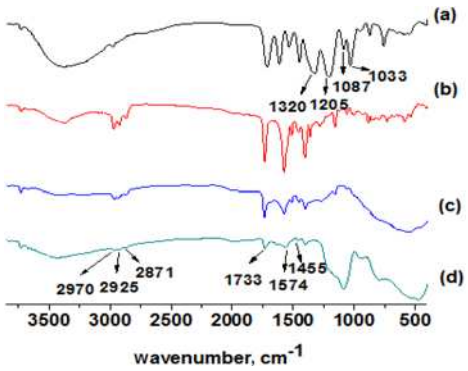

Fig. 4 *FTIR spectra of samples*
a TA
b LS
c TNTs-SiO_2-LS
d TNTs-SiO_2-LS-TA

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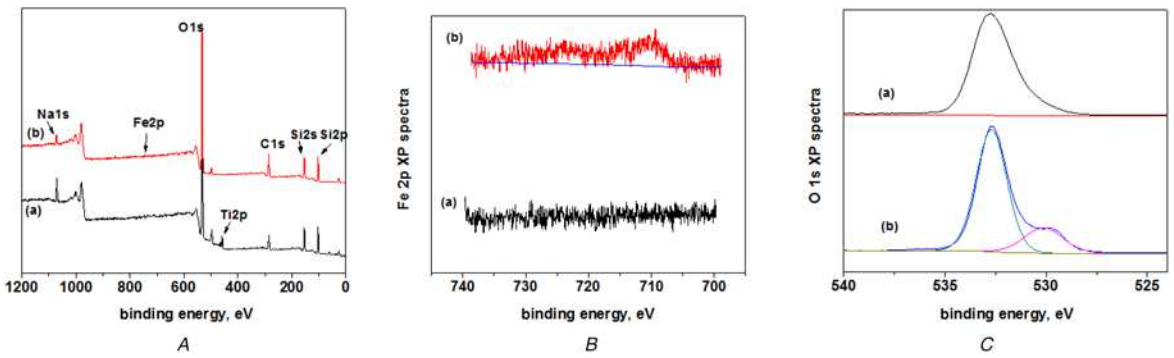

Fig. 5 *5 XPS spectra of samples*
A Full scan: (a) TNTs-SiO_2-LS and (b) TNTs-SiO_2-LS-TA
B Fe 2p spectra: (a) TNTs-SiO_2-LS and (b) TNTs-SiO2-LS-TA
C O1s spectra: (a) TNTs-SiO_2-LS and (b) TNTs-SiO_2-LS-TA

C–O–Fe. This shows that TA-Fe(III) complex was formed on the surface of sample in a complex form rather than individual forms [12].

3.3. In vitro release: As shown in Fig. 6, we found that the composite carrier modified with mesoporous silica can improve the sustained release property of the drug, but there was still a large sudden release in the early stage of release, and the entire release process cannot be intelligently controlled. After covering with the coating, the release of LS was shown in Fig. 7. After 1 h, the drug release rates of *a*, *b*, and *c* were 64.1, 28.5, and 16.8%, respectively, and after 24 h, the drug release rates of *a*, *b*, and *c* were 80.9, 54.7, and 25.8%, respectively. Obviously, the release trend of drug was significantly affected by pH. This was because that the coordination state of TA-Fe(III) complex was controlled by pH (Fig. 8*A*) [13]. TA and Fe(III) only formed a single ligand complex when pH<2. Therefore, the coating had a very large opening, and it was difficult to block the release of drugs. TA and Fe(III) formed a double ligand complex when 2<pH<7, and the opening of coating was relatively small, hindering the release of drug to some extent. TA and Fe(III) formed a triligand complex when pH>7. This complex was stable, and the coating was complete relatively. The coating can effectively block the release of drugs, but the release was not inhibited completely, probably because of the permeability of TA-Fe(III) complex to small molecules such as LS [13].

To further verify that the coating can control the release of drugs under different pH conditions, the samples released in the PBS solution at pH = 7.4 were taken out and released immediately in the PBS at pH = 4.5 and 1.6. The result was shown in Fig. 8*B*. The trend of drug release significantly varies under different pH conditions. The ratio of release amount for the three pH values was 1:8.7:40 after the release for 1 h. The results were consistent with the above explanation.

To study the application range of coating, the release behaviour of a hydrophobic drug ibuprofen was also studied. The pretreatment conditions and release environment were consistent with those of LS, and the results were shown in Fig. 8*C*. The release behaviours of hydrophobic Ibuprofen and hydrophilic LS were similar. Thus, the complex had diverse applications as coatings. The stability of coating of TA-Fe(III) complex was studied (Fig. 9). The results showed that the solubility of coating was different significantly at different pH environments. After two days of release at pH = 7.4, the coating still covered the surface of sample completely. At pH = 4.5, the surface of coating was slightly soluble, but the surface of sample remained coated. At pH = 1.6, the coating almost completely dissolved, and the mouth of nanotube was exposed completely. This showed that the stability of

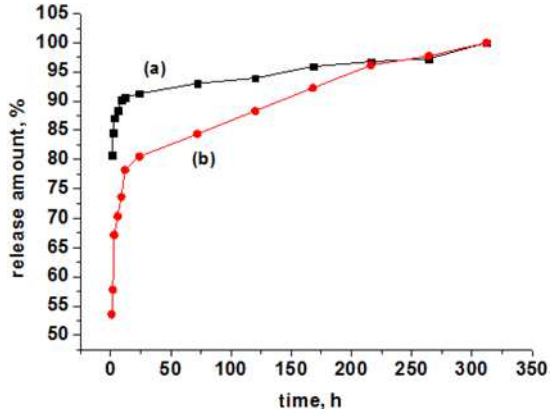

Fig. 6 *Drug release profile*
a TNTs-LS
b TNTs-SiO_2-LS

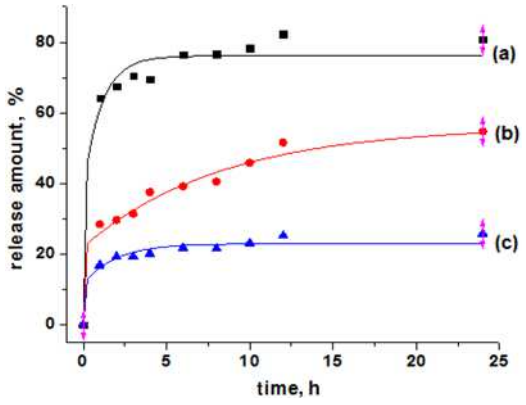

Fig. 7 *LS release profile under different pH conditions*
a pH = 1.6
b pH = 4.5
c pH = 7.4

TA-Fe(III) complex coating was different under different pH conditions.

3.4. Cell viabilities experiments: We performed cell viabilities experiments on the composite, and the results are shown in Fig. 10. After seeding the cells into different substrates, compared with 1 day of cell culture, the number of cells cultured for 3 days increased significantly, indicating that the composite was safe and non-toxic and would not have a bad effect on cell proliferation.

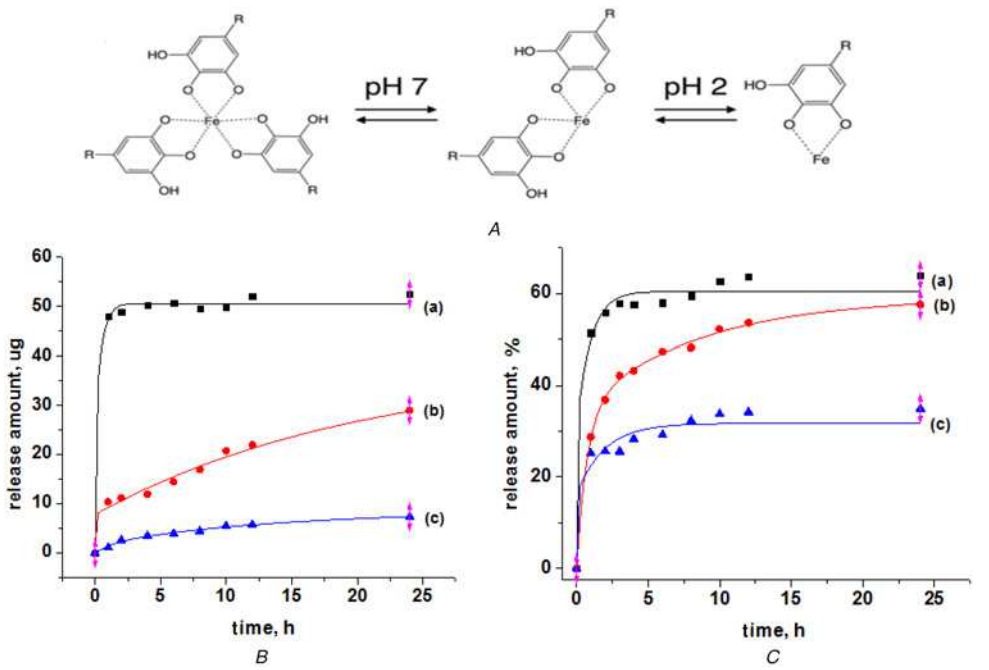

Fig. 8 *Analysis of Drug release profile*
A TA-Fe(III) coordination state under different pH conditions
B LS release profile after change pH: (a) pH = 1.6, (b) pH = 4.5 and (c) pH = 7.4
C Ibuprofen release profile under different pH conditions: (a) pH = 1.6, (b) pH = 4.5 and (c) pH = 7.4

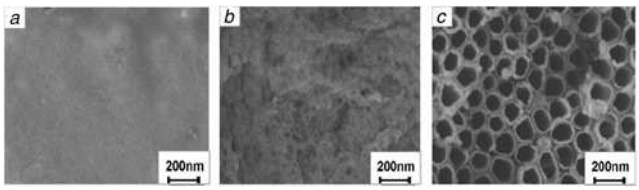

Fig. 9 *SEM images of samples*
a pH = 7.4
b pH = 4.5
c pH = 1.6

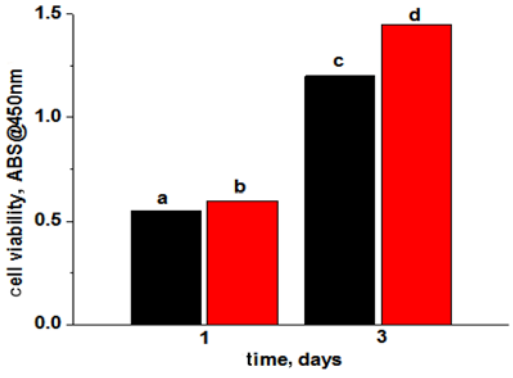

Fig. 10 *Cell grown on different substrates after one and three days of culture*
a, c TNTs
b, d TNTs-SiO_2

4. Conclusion: In summary, we use a template method to load mesoporous silica onto a nanotube to prepare a composite and a TA-Fe(III) complex was coated on the surface of a composite after drug loading by a simple drip coating method. Thus, we established a pH-controlled platform for drug delivery based on TNTs. The key trigger mechanism is the coordination conditions of TA-Fe(III) complex are different under different pH conditions and resulted in the difference of the stability of the coating and achieved intelligent control of drug release. The coating can significantly inhibit the burst release of drugs in the early stage, leading to a good application prospect of the coating. The cell viability of MC3T3 was enhanced by mesoporous silica functionalised TNTs. The results show that it would be a prospective strategy in the medical fields.

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