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# A study on PLGA sustained release icariin/ titanium dioxide nanotube composite coating

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**Abstract. – OBJECTIVE: The Chinese medicine icariin with osteoinduction and osteogenesis abilities is loaded onto the surface of $TiO_2$ nanotubes to form icariin/$TiO_2$ nanotube composite coating, which can synergistically enhance the osseointegration around the implant.**

**MATERIALS AND METHODS: However, the half-life of icariin is short, and the sustained release effect of $TiO_2$ nanotubes cannot meet the long-term stable drug release requirement. Therefore, in order to enhance the sustained release effect of icariin/$TiO_2$ nanotube composite coating, in this study, polymer PLGA was applied to the composite coating by overlay method and mixing method. Scanning electron microscopy and release detection were performed to study the effects of two PLGA loading methods on the loading and early release of composite coatings.**

**RESULTS: Results showed that the PLGA loading and sustained release effect was stronger than that of $TiO_2$ nanotubes. The sustained release effect was mainly manifested in the rapid release phase. The release time of the $TiO_2$ nanotube drug-loaded composite coating was 9 days. Overlay method and mixing method extended the drug release time to 12 d and 10 d, respectively.**

**CONCLUSIONS: Therefore, PLGA can enhance the loading and sustained release properties of icariin/$TiO_2$ nanotube composite coating. Overlay method has better sustained release effect, and the mixing method has better loading performance.**

*Key Words:*
$TiO_2$ nanotubes, Icariin, Polylactic acid-glycolic acid copolymer (PLGA), Loading, Sustained release.

## Introduction

Titanium materials have good biocompatibility and mechanical properties and have been widely used in dental implants[1-4]. However, titanium is a biological inertia material, which can cause the low speed of osseointegration, the poor initial stability and the long implantation period[5]. Therefore, surface modification of titanium has become the research focus, which can improve osseointegration around implants[4].

$TiO_2$ nanotube structure is an excellent method for surface modification of implants, which has good biocompatibility, mechanical properties, corrosion resistance and osteogenic properties[6-7]. In addition, $TiO_2$ nanotube structure is an excellent topical drug carrier. It is an important research direction to apply osteogenic drugs to its surface to form a composite coating to synergistically and effectively promote osteogenesis[8]. Among many osteogenic drugs, the traditional Chinese medicine icariin is inexpensive and stable in nature, and has significant osteoinduction effect and differentiation promotion, as well as inhibits osteoclast differentiation[9-13]. Therefore, icariin/$TiO_2$ nanotube composite coating formed by loading icariin on the surface of $TiO_2$ nanotubes may be used to enhance the speed and strength of osseointegration synergistically.

Icariin has low bioavailability and short half-life (1-2 h)[14]. However, the osseointegration is a long period (3-6 months), requiring a long-term and stable drug release to surrounding tissue. Previous studies have shown that the size of $TiO_2$ nanotubes can regulate the release process of the coating, and reducing the diameter or increasing the length of the $TiO_2$ nanotubes is beneficial to enhance the sustained release effect of the composite coating[15,16]. However, this treatment tends to reduce the drug loading of the composite coating and does not simultaneously satisfy the loading and release requirements. Studies have shown that the sustained release effect of $TiO_2$ nanotubes is poor, and the release time is difficult to meet the long-term osseointegration requirements[17,18]. The-

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refore, enhancing the sustained release properties of icariin/$TiO_2$ nanotube composite coating is a focus of current research.

At present, some polymers with better biocompatibility can be used to wrap or cover some drugs with a short half-life, easy degradation, poor stability, and high side effects. This method cannot only increase the drug loading, but also improve the sustained release effect[16,19,20]. Among them, polylactic acid-glycolic acid copolymer (PLGA) is a copolymer of polylactic acid (PLA) and polyglycolic acid (PGA), which is an FDA-approved synthetic degradable polymer material[21-23]. Compared with other natural degradable polymers (chitosan, gelatin) or inorganic calcium phosphate, PLGA is easy to prepare, stable and controllable. The hydrophobic group of PLGA slows down the degradation rate and has good sustained release properties[24,25]. Also, the sustained release effect can be regulated by adjusting the molecular mass of PLGA or the ratio of PLA and PGA[26].

Therefore, in this study, PLGA was loaded on icariin/$TiO_2$ nanotube composite coating, and the effect of PLGA on drug loading and early release of composite coating was studied by scanning electron microscopy and release detection to find a way to enhance the sustained release effect of the coating, which in turn meets the long-term bone bonding requirements.

## Sample Preparation

### Main Materials and Instruments

Pure Titanium (Northwest Nonferrous Metal Research Institute, China), Platinum Electrode (Tianjin Aida Hengsheng Technology Development Co., Ltd., China), Icariin (Sigma, USA), PLGA (Sigma, USA), high voltage DC power supply (Tianjin Dongwen High Voltage Power Supply Factory, China), Electromagnetic Stirrer (Jiangsu Jintancheng Xixiaoyang Electronic Equipment Factory, China), Scanning Electron Microscope (Hitachi Corporation, Japan).

### Preparation of $TiO_2$ Nanotube Samples

30 pieces of 1.4 cm diameter round titanium sheets were sequentially polished to 600 mesh with silicon carbide sandpaper. After polishing, titanium sheets were ultrasonically washed with acetone, absolute ethanol and deionized water for 10 min, and dried naturally. The treated pure titanium was connected to the anode, and platinum was connected to the cathode. The materials were placed in 0.34% HF electrolyte on an electromagnetic stirrer for continuous stirring. Anodic oxidation treatment was carried out for 40 minutes under the condition of a high voltage direct current power source of 18 V, and $TiO_2$ nanotubes were formed on the surface of the sample. After preparation was completed, ultrasonic cleaning with deionized water was performed for 20 seconds, followed by natural room temperature drying.

### Preparation of PLGA-Loaded Icariin/$TiO_2$ Nanotube Composite Coating

Molecular mass of PLGA in this study was 38000, PLA: PGA=85:15. Acetone was used as a solvent to dissolve PLGA and DMSO was used to dissolve icariin. $TiO_2$ nanotube samples were randomly divided into 3 groups: icariin/$TiO_2$ nanotubes (ICA-NT) group, PLGA overlay icariin/$TiO_2$ nanotubes (capped PLGA/ICA-NT) group, PLGA icariin Glycoside mixing microparticles/$TiO_2$ nanotubes (mixed PLGA/ICA-NT) group. $TiO_2$ nanotubes were immersed in $2\times10^{-3}$ mol/ml icariin solution for 3 days. Then, they were quickly rinsed with 500 μl of PBS to remove the excess icariin which was not firmly bound, and were dried in an oven to form ICA-NT group samples (20 pieces). Ten samples were randomly taken from the ICA-NT group and quickly immersed in 1% (w/v) PLGA solution for 5 s, followed by drying in an oven. This process was repeated for 3 times to form capped PLGA/ICA-NT (10 pieces). A mixed solution of PLGA and icariin with a mass ratio of 3:1 was prepared, and $TiO_2$ nanotubes were incubated with this solution for 3 days. After loading, 500 μl of PBS solution was taken to quickly rinse the surface, followed by drying in an oven to form the samples of mixed PLGA/ICA-NT group (10 pieces).

### Observation of Composite Coating by Scanning Electron Microscopy

5 pieces were randomly selected in the ICA-NT, mixed PLGA/ICA-NT, and capped PLGA/ICA-NT groups. A thin layer of gold-palladium film was formed on the surface of the sample by sputter coating, and surface morphology of the sample at a magnification of 50 k was recorded by a scanning electron microscope at an accelerating voltage of 5 kV.

### Release Rule Detection

5 samples were taken from ICA-NT, mixed PLGA/ICA-NT, and capped PLGA/ICA-NT groups, and were placed in 24-well plates, respectively soaked in 500 μl PBS, and placed in a

37°C water bath on a shaker (50 rpm/min). During the first 4 h, the infusion was aspirated every 1 h and its concentration was measured using high performance liquid chromatography, followed by the addition of 500 µl of fresh PBS to a 24-well plate. After that, measurement was performed once per day until no icariin was released into PBS solution. Average value of the doses released at different time points of each group of samples was calculated, and the cumulative release curve and cumulative release percentage curve of icariin were plotted.

## Results

### Scanning Electron Microscope Observation Results

Figure 1 shows the surface microstructure of ICA-NT, mixed PLGA/ICA-NT, and capped PLGA/ICA-NT specimens under scanning electron microscopy. It can be seen that after three drug-loading treatments, the drug has been loaded on the surface of the $TiO_2$ nanotubes. Figure 1A is a scanning electron micrograph of ICA-NT, showing that icariin particles are small and distributed in a granular or agglomerate manner on the surface of the nanotubes. Figure 1B is a scanning electron micrograph of the capped PLGA/ICA-NT group. It can be seen that the ICA-NT substrate is covered with more clustered PLGA, and $TiO_2$ nanotubes are blocked more frequently. Figure 1C shows the scanning electron micrograph of mixed PLGA/ICA-NT. It can be seen that the surface of $TiO_2$ nanotubes is coated with a large number of dense and extensive cluster-like drugs, which cannot distinguish the distribution of icariin and PLGA on the surface of $TiO_2$ nanotubes.

### Release Test Results

To study the effect of PLGA on the loading and release of icariin/$TiO_2$ nanotube composite coating, this study plots cumulative release profiles (Figure 2) and cumulative release percentage curves (Figure 2) for the first 14 days (A) and 4 h (B) of the ICA-NT, capped PLGA/ICA-NT, and mixed PLGA/ICA-NT groups.

Figure 2 A shows that the cumulative release of icariin in the first 14 days of mixed PLGA/ICA-NT group is higher, about twice as the other two groups, suggesting that PLGA is more loaded as a carrier than $TiO_2$ nanotubes. In the first 4 h (rapid release phase), capped PLGA/ICA-NT group and mixed PLGA/ICA-NT group have less cumulative drug release than the ICA-NT group (Figure 2B), suggesting that PLGA inhibits drug release during rapid release phase. In the slow release phase, the curves of each group are close to linear. At this stage, cumulative release profiles of capped PLGA/ICA-NT group and mixed PLGA/ICA-NT group show a significant increase, so the release amount of the PLGA composite coating group is higher at this stage (Figure 2A).

From Figure 3A, it can be concluded that the cumulative release percentage curve of the ICA-NT group is higher, and release time is 9 d, and curve of the capped PLGA/ICA-NT group and mixed PLGA/ICA-NT group is lower, and the release time is extended to 12 d and 10 d, respectively. Therefore, PLGA has better sustained release effect than $TiO_2$ nanotubes. As can be seen from Figure 3B, in the rapid release phase, the cumulative release percentage of the ICA-NT group is approximately 80%, while capped PLGA/ICA-NT group and mixed PLGA/ICA-NT group are reduced to 35% and 48%, respectively. Therefore, PLGA sustained release is mainly played during the rapid release phase.

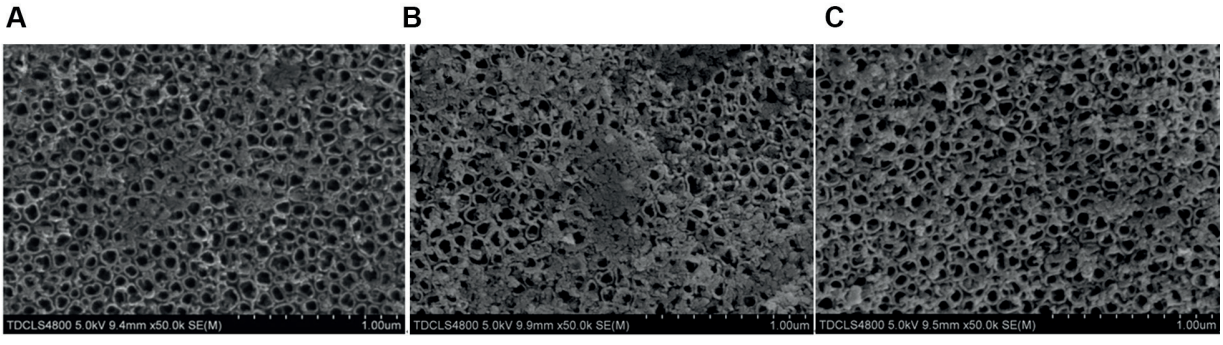


**Figure 1**. SEM observation of ICA-NT (A), capped PLGA/ICA-NT (B), and mixed PLGA/ICA-NT (C) surface morphology (50 k).

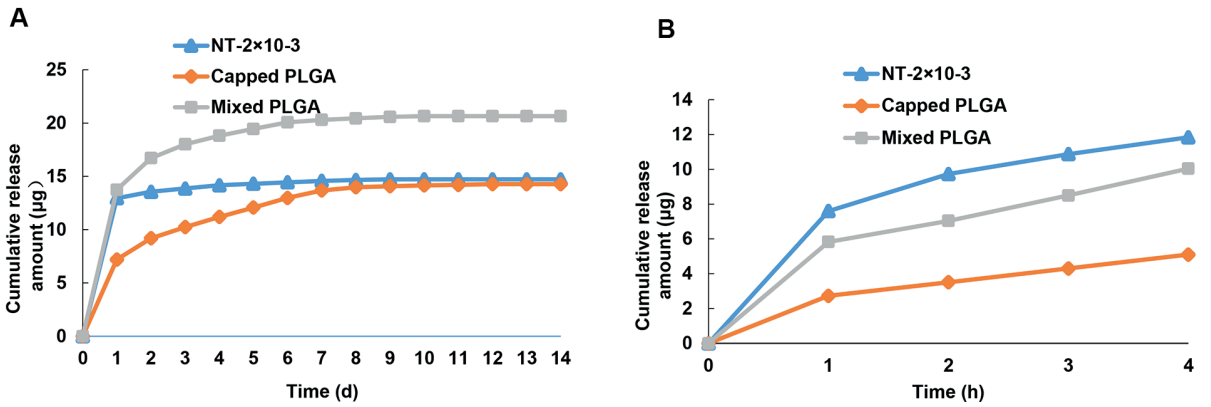


**Figure 2.** Cumulative release curve of the three groups of samples. A, NT-ICA, capped PLGA/ICA-NT and mixed PLGA/ICA-NT cumulative release curves during the first 14 d. B, NT-ICA, capped PLGA/ICA-NT and mixed PLGA/ICA-NT cumulative release curves during the first 4 h.

## Discussion

It is the goal of dental implant surgery to form osseointegration between bone tissue and implant, which can ensure the stability of implantation. However, the bioactivity of titanium implants is poor, which cause the low speed of osseointegration and the poor stability of early implantation. It will take a long time for patients. In particular, osteoporosis and diabetes can further reduce the speed and strength of implant osseointegration. Therefore, it is a hot issue to improve the osteogenic ability of titanium implants through surface modification.

In this study, icariin was loaded onto titanium nanotubes to form a composite coating of icariin/titanium nanotubes. The pure titanium was modified with the osteogenic effects of icariin and titanium nanotubes, which can coordinate to enhance the speed and strength of osseointegration, thereby shortening the time of osseointegration and enhancing the stability of implants.

The half-life of icariin is short. To ensure long-term and stable osseointegration, the good drug loading and sustained release properties of icariin/titanium dioxide nanotube composite coatings are required.

Previous studies have shown that the release process of $TiO_2$ nanotube-loaded composite coating drug can be divided into two stages, namely, the rapid release of the early surface of the nanotube and the late slow micro-release process inside the nanotube. Bone bonding is a long-term process that requires a long-term, stable release of the drug from the composite coating to the surrounding of the implant to promote osteogenesis.

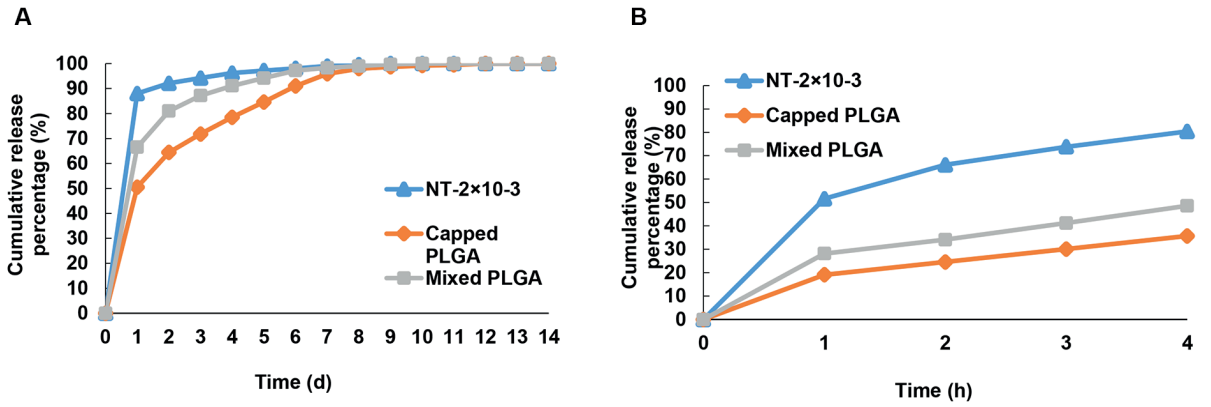


**Figure 3.** Cumulative release percentage curve of the three groups of samples. A, NT-ICA, capped PLGA/ICA-NT and mixed PLGA/ICA-NT cumulative release percentage curves during the first 14 d. B, NT-ICA, capped PLGA/ICA-NT and mixed PLGA/ICA-NT cumulative release percentage curves during the first 4 h.

In addition, the early release of the composite coating has a risk of osteocytotoxicity[27]. However, it is difficult to simultaneously meet the loading and release requirements by regulating the size of $TiO_2$ nanotubes. Therefore, further control of the burst release of icariin/$TiO_2$ nanotube composite coating and prolonging the release time are important directions of this study.

Polylactic acid-glycolic acid complex (PLGA) is an ideal drug sustained-release carrier with good capsular and film-forming properties, biocompatibility, mechanical properties and controllability[28-30]. In addition, PLGA can avoid the adverse effects of polymer on the $TiO_2$ nanotube-loaded composite coating. It is shown that the $TiO_2$ nanotube-loaded coating loaded with PLGA improves the mechanical strength and osseointegration ability of $TiO_2$ nanotubes, which in turn promotes the adhesion and proliferation of bone cells, promotes osseointegration and reduces the growth of fibrous tissue, and the hydrolysis of PLGA has minimal effect on osseointegration[31,32].

PLGA can achieve sustained drug release in two ways: First, the $TiO_2$ nanotube drug-loaded coating is prepared, and the PLGA is coated on the surface of the $TiO_2$ nanotube drug-loading system to achieve sustained release. Second, the PLGA-encapsulated drug is first formed into a mixed particle, which is then loaded onto the surface of the $TiO_2$ nanotube to form a composite coating, thereby exerting a sustained release effect[33,34]. Therefore, in this study, PLGA-covered icariin/$TiO_2$ nanotubes (capped PLGA/ICA-NT) group and PLGA icariin mixed particles/$TiO_2$ nanotubes (mixed PLGA/ICA-NT) were prepared to study the effects of two PLGA loading modes on the sustained release properties of the composite coating.

Scanning electron microscopy showed that the icariin particles were small and were mostly granular or agglomerate in the nanotubes, and PLGA was clustered. Surface of icariin/$TiO_2$ nanotubes in the capped PLGA/ICA-NT group was covered with more clustered PLGA, while in mixed PLGA/ICA-NT the distribution of icariin and PLGA cannot be distinguished, and the $TiO_2$ nanotubes were covered with a lot of dense and extensive cluster of drugs, which was the mixture of PLGA and icariin.

Results of cumulative release curve showed that the drug loading was higher in the mixed PLGA/ICA-NT group than in the other two groups, indicating that PLGA can play a carrier role to adsorb more icariin to its surroundings, and the loading performance was better than $TiO_2$ nanometer tube. Cumulative release percentage curve showed that PLGA had better sustained release effect than $TiO_2$ nanotubes, and release time of ICA-NT group was 9 days. Overlay method and mixing method extended the drug release time to 12 d and 10 d, respectively. This is because, in mixed PLGA/ICA-NT group, PLGA can exert expansion filling effect to reduce the diameter of $TiO_2$ nanotubes, thereby slowing the drug release movement. In capped PLGA/ICA-NT group, PLGA restricted the release of the drug from the surface and inside of the $TiO_2$ nanotube by covering the surface of the nanotube. Since the results show that the release time of the PLGA overlay method is longer, the effect of sustained release of this method is better. Results also show that PLGA has a strong sustained-release effect, and it can be inferred that due to a large amount of drug loading on the surface of the $TiO_2$ nanotube-loaded coating, and less in the tube, PLGA has a more pronounced effect on the sustained release of the rapid release period.

Therefore, capped PLGA/ICA-NT group has a better sustained release effect and the mixed PLGA/ICA-NT group has a better loading performance. So that, the advantages of both can be combined in the future research. The strategy is to prepare a mixed PLGA/ICA-NT composite coating and then cover the surface with PLGA to obtain an icariin/$TiO_2$ nanotube composite coating with excellent drug loading and release properties. In addition, future studies may further enhance the sustained release of PLGA by increasing the thickness of the PLGA cover, or by increasing the molecular mass of the PLGA or adjusting the PLA and PGA ratio[26].

## Conclusions

To enhance the sustained release effect of icariin/$TiO_2$ nanotube composite coating, PLGA was applied to icariin/$TiO_2$ nanotube composite coating by overlay method and mixing method. Results showed that the PLGA loading and sustained release effect was stronger than that of $TiO_2$ nanotubes. The sustained release effect was mainly manifested in the rapid release phase. The release time of the $TiO_2$ nanotube-loaded composite coating was 9 d, while overlay method and mixing method extended the drug release time to 12 d and 10 d, respectively. Overlay method has better sustained release effect and the mixing method has

better loading performance. Therefore, PLGA can enhance the loading and sustained release properties of the icariin/$TiO_2$ nanotube composite coating, and promote the long-term stable release of the drug to the surrounding tissue.

### Conflict of Interest

The Authors declare that they have no conflict of interest.

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