

Research Article Facile Fabrication of Composite Scaffolds for Long-Term Controlled Dual Drug Release

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Bone tuberculosis (TB) caused by mycobacterium tuberculosis continues to present a formidable challenge to humans. To effectively cure serious bone TB, a novel kind of composite scaffolds with long-term dual drug release behaviours were prepared to satisfy the needs of both bone regeneration and antituberculosis drug therapy. In virtue of an improved O/W emulsion technique, water-soluble isoniazid (INH)-loaded gelatin microparticles were obtained by tailoring the content of β -tricalcium phosphate (β -TCP), which played significant roles in INH entrapment efficiency and drug release behaviours. By mixing with the poly(ε -caprolactone)-*block*-poly (lactic-*co*-glycolic acid) (*b*-PLGC) solution containing oil-soluble rifampicin (RFP) via the particle leaching combined with phase separation technique, the dual drugs-loaded composite scaffolds were fabricated, which possessed interconnected porous structures and achieved the steady release of INH and RFP drugs for three months. Moreover, this dual drugs-loaded system could basically achieve their expectant roles of respective drugs without obvious influences with each other. This strategy on preparation of intelligent composite scaffolds with the multi-drugs loading capacity and controlled long-term release behaviour will be potential and promising substrates in clinical treatment of bone tuberculosis.

1. Introduction

Bacterial infections are one of the most common diseases threatening the human health, which have resulted in the severe illness to destroy the infected tissues, such as bone tuberculosis (TB) and osteomyelitis [1-4]. Traditional therapy of the infections mainly includes the disinfection/bacteriostasis and reparation of the destroyed tissues. Take serious bone TB for instance, the standard course involves a more-than-6months medication treatment to inhibit bacteria and a bone implantation after the lesion removed operation to fill and repair the cavities. A local drug delivery system (DDS) offers an effective approach for the drug therapy of bone TB. It possesses advantages of specific site delivery, drug dosage optimization and release duration. Therefore, DDS technology is recognized as the best candidate for achieving the drug therapy while tissue engineering is the most prospective method for the defect reparation [5–9]. It follows that the combination of DDS and tissue engineering should be a natural way to simultaneously satisfy the two aspects of bone TB therapy.

Recently, many researchers have been focusing on this feasible strategy. For example, Huang's groups and Kang's groups had utilized a chemical bonding technique to conjugate the drugs to biodegradable polymers to gain the tissue engineering scaffolds respectively, which could implement the prolonged drug release and satisfactory tissue regeneration. However, the chemical bonding technique always carried out a long-term drug release (about 6 months) with a slightly initial burst release. In addition, it had inevitable defectiveness of the possible drug degeneration and incomplete drug release, in particular the possible change in pharmacological effects after chemical bonding and during degradation of the polymer. When its molecular weight is lower than 5000, the degraded fragment of polylactone linked with a drug molecule can be dissolved in water and is considered as a drug molecule, but whether it can play the same role as a drug molecule is

doubtful. As a result, the physical blending technique was more preferable for achieving the controlled drug release from scaffolds [10-14]. Then, a newly designed polylactone of b-PLGC was adopted to produce a bone tissue-engineered scaffold with capability of elongating the drug release time to 98 days and reducing the initial burst release to lower than 40%. Nevertheless, it was mostly reported that with similar physical blending technique the release time could only last less than 50 days and the initial burst release reached up to higher than 60% [15]. In spite of technical differences, the above-mentioned works can meet the demands of both reparative and bacteriostatic functions. Nonetheless, the constant release of only one drug in the focus of infection may lead to the drug-resistance of bacteria; in this case, it is urgent to adopt the multiple drugs for local therapy in clinical practice [16, 17]. Therefore, establishment of long-term multiple drug release system is a correct direction to move forward not only in this research but in clinical area.

In addition to the first-line antituberculosis drugs such as isoniazid (INH), rifampicin (RFP), pyrazinamide and ethambutol, others like p-aminosalicylic acid, ethionamide, protionamide and cycloserine are also included for the multiple-drugs therapy of bone TB [18-23]. These drugs can be classified by solubility into two categories of water-soluble and oil-soluble. For the purpose of simplifying the laboratory model, one typical drug from each category was picked out to be loaded in tissue engineering scaffolds at the same time. Considering the clinically wide usage, INH was the suitable representative of water-soluble drugs and RFP was that of oil-soluble drugs. It was easy to load RFP into the tissue engineering scaffolds by the physical blending technique because the oil-soluble RFP could dissolve well in organic solvents and disperse evenly in the polylactone scaffold. Oppositely, the loading of INH was difficult. The phase separation of INH and polylactone may result in an uneven INH distribution. In addition, the water-solubility of INH demands a non-aqueous washing process to avoid uncontrolled loss, but most commonly, tissue-engineering scaffolds fabricating technique was solution cast/ porogen leaching, in which the inorganic salts (mostly NaCl particles) were adopted as porogen while the water was a necessity during leaching process [24-26]. To circumvent this problem, gelatin coating and crosslinking techniques were taken to load INH into TCP nanoparticles. Under this circumstance, the particles could be carried into polylactone/TCP composite scaffolds with little INH loss after porogen leaching process.

In this work, we incorporated the TCP nanoparticles into the RFP-containing PLGC polymer matrix to form the composite scaffolds. Since PCL has high permeability for drug molecules and PLGA has flexibility in degradation adjustment, the RFP-releasing behavior and degradation rate of scaffolds were mainly controlled by the degradation and drug permeability of *b*-PLGC polymer, which could effectively improve the uniformity, extend the release duration and suppress the initial burst release. Furthermore, by means of the complicated physical blending technique, the water-soluble INH drug was also encapsulated into the composite scaffolds to yield a novel kind of dual anti-tuberculosis drug-loaded composite scaffold and realize a controllably prolonged local drug delivery. Using this principle, many sophisticated scaffold materials with the multiple loading and tailored releases of drugs may have great applications in bone TB therapy.

2. Materials and Methods

2.1. Materials. Lactide and glycolide were purchased from Purac (Netherlands) and purified by recrystallization in ethyl acetate twice. ε-caprolactone was purchased from Acros Chemica (Belgium), dried with calcium hydride for 24h, and distilled under vacuum (82°C /133 Pa). Stannous octoate (analytical grade) and isoniazid (INH, 99%) were purchased from Sigma-Aldrich. RFP was purchased from Energy Chemical, China. β -tricalcium phosphate (β -TCP) powder with diameter of 300-500 nm was purchased from the Forth Reagent Factory of Shanghai, China. Ethyl acetate was dried by P₂O₅ overnight and then distilled. NaCl of analytical quality was purchased from Beijing Chemical Works, China. It was sieved and particles with diameter of $150-300 \,\mu\text{m}$ were selected. Gelatin (analytical grade), solvents and other compounds were obtained from Beijing Chemical Reagents Company, China. All reagents were used as received unless otherwise noted.

2.2. Characterizations. ¹H NMR spectra was obtained on a Bruker DRX-400 spectrometer in chloroform-d using tetramethylsilane (TMS) as an internal reference. Gel permeation chromatography (GPC) measurements were carried out on VE-2001 (Viscotek, UK) maintained at 35°C using chloroform as eluent at a flow rate of 1.0 mL/min. Scanning electron microscopy (SEM) images were obtained at acceleration voltage of 5kV on a JSM-6700F microscope (JEOL, Japan). The samples were sputter-coated with a thin layer of Pt for 120s to make the samples conductive before testing. Thermogravimetric analysis (TGA) measurements were performed on a TA Instruments, Inc., MDSC-2910. The temperature program was from 30 to 700°C with an increasing rate of 10°C min⁻¹ in a flow of air. UV-vis spectra were measured on a Hitachi U-3010 spectrometer and fluorescence measurements were carried out on a Hitachi F4600 photoluminescence spectrometer with a xenon lamp as a light source. Confocal laser scanning microscopy (CLSM, Zeiss LSM 510, Germany) was under excitation at 314 nm.

2.3. Preparation and Characterization of b-PLGC. According to our previous report, poly(ε -caprolactone)-*block*-poly(lactic*co*-glycolic acid) (*b*-PLGC) copolymer was synthesized via two steps [15]. Firstly, the PCL pre-polymer was prepared by ringopening polymerization of ε -caprolactone (CL) under 65 Pa in sealed glass ampoules at 140°C for 20 h in the presence of hexadecanol as initiator and stannous octoate as catalyst (0.05 wt%). Subsequently, purified PCL pre-polymer, glycolide, lactide, and stannous octoate were sealed into a deoxygenating glass tube under vacuum at 160°C for 20 h. After that, the raw product was dissolved in chloroform, precipitated into ethanol and then dried thoroughly under vacuum for 48 h.

2.4. Preparation and Characterization of Gelatin INH-Loaded Particles. INH-loaded Gelatin particles were prepared via a

modified O/W emulsion technique [27]. Firstly, INH solution with concentration of 10 wt% was made by dissolving INH in deionized water. Then a certain amount of TCP nanoparticles were added into. After the TCP nanoparticles were evenly dispersed, 1.0g of gelatin was put into the 10mL of INH solution containing TCP nanoparticles. The solution was heated to 45°C as the water phase, which was slowly added to the 350 mL of soybean oil containing the 0.675 g of span 80 as oil phase with stirring speed of 200 rpm. 10 minutes later, the stirring speed was raised up to 400 rpm and further kept for 30 min while the system was naturally cooling down to room temperature. After that, the product was washed by acetone for 4 times and isopropanol for 3 times. Next, the obtained microparticles were freeze-dried for 48h and then crosslinked by the glutaraldehyde dissolved in 100 mL of acetone. Afterwards, the particles were added into 300 mL of solution with 10 mmol glycine and 0.1 wt% span 80 to remove the residual glutaraldehyde. At last, the microparticles were washed by water for 3 times. After the freeze-dried process for 48h, the final samples were sealed and kept in a 4°C refrigerator. The microparticles with theoretical TCP content of 0 wt%, 33 wt% and 50 wt% were abbreviated as INH@GMs, INH-TCP@GMs3367 and INH-TCP@GMs5050, respectively.

After the microparticles were coated with platinum using a sputter coater (E-1010, Hitachi Ltd, Japan), their morphology was observed by SEM (JSM-6700F, JEOL Ltd, Japan). The average diameter was calculated by measuring 5 SEM pictures of each sample with more than 100 particles on every picture measured. TCP content was measured by TGA (Pyris 1, PerkinElmer, USA) with a temperature range of 30–700°C and a temperature rise speed of 10°C/min.

2.5. Preparation and Characterization of Composite Scaffolds. Based on our previous study, the composite scaffolds were fabricated by particle leaching combined with phase separation techniques [15, 24, 25]. The b-PLGC scaffolds loaded with INH-TCP@GMs and RFP were prepared as follows: Firstly, b-PLGC, RFP and 1,4-dioxane in the proportion of 5:1:90 (wt. ratio) were stirred for 24h to form a solution. Then a certain quality of INH-TCP@GMs5050 and pre-sieved NaCl particles with a weight ratio of 40/1 to *b*-PLGC were added into the solution and stirred vigorously to form a slurry mixture. Next, the slurry was fitted into a mold, frozen under -20°C and freeze-dried for 48 h. After a complete rinse with distilled water and a subsequent 24 h freeze-drying, the PLGC scaffolds loaded with INH and RFP was obtained. The content of INH-TCP@GMs5050 in the obtained scaffolds were 50 wt% so these scaffolds were abbreviated as I-M/RP50.

The *b*-PLGC scaffolds loaded with INH-TCP@GMs was prepared similarly, and the only difference was that no RFP was introduced in the *b*-PLGC solution. The contents of INH-TCP@GMs5050 in the scaffolds were 50 wt% and 70 wt%, so these scaffolds were marked as I-M/P50 and I-M/P70, respectively.

The b-PLGC scaffolds loaded with INH and without INH-TCP@GMs were prepared likewise, and the only difference was that TCP nanoparticles and INH were respectively added into the *b*-PLGC solution directly to take the place of INH-TCP@GMs5050. The INH-loaded *b*-PLGC scaffold without INH-TCP@GMs was named as INH/TCP/*b*-PLGC for short. After the scaffolds were coated with platinum using a sputter coater, their morphology was characterized by SEM. The scaffolds carrying gelatin particles loaded with Evans blue and TCP were cut into slices and observed under a confocal laser scanning microscopy (CLSM, Zeiss LSM 510, Germany) at 540 nm. Pore size was measured using the ImageJ software (National Institutes of Health, USA) according to the SEM micrographs. For each scaffold, the average diameter of the pores was calculated based on 100 measurements. The porosity of the scaffolds was determined as described previously [28]. TCP content of scaffolds was measured by TGA (Pyris 1, PerkinElmer, USA) with a temperature range of 30–700°C and a temperature rise speed of 10°C/min.

2.6. INH Loading Content and Entrapment Efficiency of Gelatin Particles. INH Loading content and entrapment efficiency of gelatin particles were confirmed by extraction method. Firstly, a standard curve was gained by a series of INH concentrations in PBS using a UV spectrophotometer (TU-1901, PERSEE, China) at λ_{max} = 263 nm. Then 10 mg of INH-loaded particles was added into NaOH solution and the solution was heated to 60°C for 2h to dissolve particles completely. Next, hydrochloric acid was put in to neutralize the alkalinity and dilute PBS to 5 mL. After that, the solution was centrifuged at 8000 rpm for 10 min to remove the TCP particles and acquire clear solution, which was analyzed using a UV spectrophotometer at $\lambda_{max} = 263 \text{ nm}$. Meanwhile, the same quality of pure particles without INH was treated in the same way as the INH-loaded particles and the clear solution obtained were served as a control. The INH concentration was calculated through a calibration curve gained from INH standard solutions at different concentrations. The percentages of INH loading and encapsulation efficiency were calculated as follows:

$$INH \text{ loading content (\%)} = \frac{(Weight of INH in particle)}{(Weight of INH-loaded particle) \times 100\%},$$
(1)
$$INH \text{ entrapment efficiency (\%)} = \frac{(Weight of INH in particle)}{(Weight of INH feeding) \times 100\%}.$$
(2)

2.7. INH Release Behaviours of Particles. In vitro release behaviours of INH@GMs, INH-TCP@GMs3367 and INH-TCP@GMs5050 were carried out at 37°C in 10 mL of 0.1 M PBS (pH = 7.4), respectively. The release medium was withdrawn at pre-determined time intervals and replaced with fresh PBS each time. Then the INH was determined by the UV spectrophotometer at λ_{max} = 263 nm. The concentration of INH was calculated with a calibration curve from INH standard solutions at different concentrations.

2.8. Drug Release Behaviours of Various Scaffolds. In vitro release behaviours of various scaffolds were performed at 37°C in 10 mL of 0.1 M PBS (pH=7.4), respectively. The release medium was taken away at pre-set time intervals and displaced with fresh PBS each time. Then the INH and RFP were determined by UV spectrophotometer at $\lambda_{max} = 263$ nm



FIGURE 1: SEM images showing the TCP and INH-loaded gelation microparticles of (a) TCP, (b) INH@GMs, (c) INH-TCP@GMs3367 and (d) INH-TCP@GMs5050.



FIGURE 2: TGA curves of various INH-loaded microparticles.

and 474 nm, respectively. The contents of INH and RFP were calculated with the calibration curve from INH and RFP standard solutions at different concentrations.

2.9. Statistical Analysis. All quantitative data were expressed as mean \pm standard deviation (n = 3). Statistical analysis was made based on *t*-test and the differences.

 TABLE 1: INH loading content and entrapment efficiency of GMs and TCP@GMs microparticles.

Parameters		Microparticles		
	CMa	TCP@	TCP@	
	GMS	GMs3367	GMs5050	
INH loading content (wt%) ^ª	3.7 ± 0.2	4.5 ± 0.3	5.0 ± 0.5	
Entrapment efficiency (%)	37.0 ± 2.0	45 ± 3.0	50 ± 5.0	

^aFeeding dose of INH to (TCP+GM) in GMs and TCP@GMs microparticles was 10:90.

3. Results and Discussion

3.1. Fabrication of b-PLGC Scaffolds Loaded with INH and RFP

3.1.1. Preparation and Characterization of the INH-Loaded Gelatin Microparticles. The morphology of INH-loaded gelatin microspheres was prepared by the modified O/W emulsion technique. As shown in Figure 1, SEM images showed the particle size of TCP with a range of 300–500 nm while the size of INH-loaded gelatin particles was $10-30 \,\mu$ m. As the increase of TCP content, the particle sizes of INH@ GMs, INH-TCP@GMs3367 and INH-TCP@GMs5050 were



FIGURE 3: SEM images showing the INH-loaded *b-PLGC* scaffolds with different contents of microparticles. (a) INH/TCP/*b*-PLGC scaffolds; (b) I-M/P50 scaffolds and (c) I-M/P70 scaffolds.

 TABLE 2: Pore parameters of INH-loaded *b*-PLGC scaffolds with different contents of microparticles.

	Scaffolds			
Parameters	INH/TCP/b- PLGC	I-M/P50	I-M/P70	
Average pore diameter (μ m)	253.0 ± 34.0	234.0±39.0	231.0 ± 27.0	
Porosity (%)	87.6 ± 1.5	84.5 ± 2.1	83.8 ± 1.4	

 $17.2 \pm 2.1 \,\mu$ m, $21.8 \pm 1.9 \,\mu$ m and $23.1 \pm 2.4 \,\mu$ m obtained from ImageJ software, respectively.

The TCP content in the microparticles was measured by thermogravimetric analysis (TGA) as showed in Figure 2. The quantities of INH@GMs, INH-TCP@GMs3367 and INH-TCP@GMs5050 were 0 wt%, 29.3 wt% and 50.0 wt% respectively, which were conformed to their theoretical contents, indicating the minimal loss of TCP in the preparation of microparticles.

As shown in Table 1, the INH loading content and entrapment efficiency of various microparticles were ranged from 3.7% to 5.0% and 37% to 50%, respectively. When the TCP content was increased, the INH loading content and entrapment efficiency grew correspondingly, which was attributed to the relatively large surface area of TCP that possessed a certain drug absorption effect. However, there was still a part of the drug loss during the microparticles preparing process due to mechanical agitation and slight solubility of INH in acetone. The above results manifested the successful preparation of the INH-loaded gelatin microparticles. 3.1. 2. Preparation and Characterization of b-PLGC Scaffolds Loaded with INH-TCP@GMs and RFP. The b-PLGC scaffolds loaded with INH-TCP@GMs were fabricated by particle leaching combined with phase separation technique. The morphology of scaffolds was determined by SEM observation and compared with the b-PLGC scaffolds loaded INH without microparticles, as shown in Figure 3. The pore size of the scaffolds loaded with INH-TCP@GMs was in a range of 200–300 μ m. Similar with the INH/TCP/b-PLGC scaffolds, numerous micro-pores (10–50 μ m) could be seen on the wall of macro-pores. The results manifested that the microparticles did not affect the characterization of scaffolds, which could provide a high surface area for the cell seeding, attachment and proliferation, deliver nutrient and elimination of metabolized products [29–32].

The pore size and porosity of scaffolds were also acquired respectively by dint of ImageJ software and volume exclusive method as shown in Table 2. The pore sizes of I-M/P50 and I-M/P70 scaffolds were in the range of $200-300 \,\mu\text{m}$ while the porosity was above 80%. These results pointed out that the pore size and porosity of scaffolds were slightly decreased after incorporating the INH-TCP@GMs.

To further analyze the distribution of microparticles, a water-soluble fluorescent polymer, Evans blue, was loaded on the microparticles instead of INH. The formed composite scaffolds, described as E-M/P50 and E-M/P70, were analyzed by the confocal laser scanning in Figure 4. The results showed that these microparticles were distributed throughout these scaffolds with a relative uniformity, e.g. some particles spread at the junction of different pores and others were embedded in the inner of scaffolds.



FIGURE 4: CLSM images of Evans blue loaded TCP@GMs/b-PLGC scaffolds of (a) E-M/P50 and (b) E-M/P70.



FIGURE 5: TGA curves of INH-loaded *b*-PLGC scaffolds with (a) different content of microparticles and (b) different parts of I-M/P50 scaffolds.



FIGURE 6: Morphologies of I-M/RP50 scaffolds.



FIGURE 7: Release behaviours of INH@GMs and INH-TCP@GMs *in vitro*.

The TCP contents of the scaffolds were also in keeping with the theoretical values with the verification by the TGA measurement (Figure 5(a)), indicating the minimal TCP loss during preparation of scaffolds. Since the TCP distribution in scaffolds exerted an influence over bone regeneration, the TCP content of I-M/P50 scaffolds in different parts was also measured by TGA (Figure 5(b)). At the top, middle and bottom parts the content was 21.2 wt%, 24.7 wt% and 27.8 wt%, respectively. The trivial distribution difference of TCP content in the whole scaffolds may be ascribed to a bit of sedimentation of microparticles during the preparing process of scaffolds, but it would not impact the usage of scaffolds.

The *b*-PLGC scaffolds were loaded with INH-TCP@GMs and RFP according to the previous methods [15]. Compared to the I-M/P50, I-M/RP50 was also in possession of random interconnected porous structures in Figure 6. Its pore size was in a range of $200-300 \,\mu\text{m}$ along with numerous micro-pores ($10-50 \,\mu\text{m}$) on the wall of macro-pores. Besides, the pore porosity and TCP content of the I-M/RP50 scaffolds were also similar to the I-M/P50 scaffolds (the corresponding data and

curves were not shown to avoid repetition). These results manifested that the RFP had no obvious influence on the morphology of scaffolds.

3.2. Drug Release Behaviours of b-PLGC Scaffolds Loaded with INH and RFP In Vitro

3.2.1. INH Release Behaviours of Microparticles In Vitro. The initial burst releases of INH@GMs, INH-TCP@GMs3367 and INH-TCP@GMs5050 were 24.4 wt%, 19.8 wt% and 17.9 wt% respectively in Figure 7. As the TCP content was increased, the burst release fell down, indicating that TCP could inhibit the initial burst release to some extent. After the initial burst release, INH release behaviours of these three microparticles were essentially uniform with linear trends. The release rate diminished as the TCP content augmented. But the drug release speed of INH-TCP@GMs accelerated after three weeks of inhibition effect, especially for the INH-TCP@GMs5050 whose cumulative release reached 98.2% on the fourth week, higher than 92.7% in INH-TCP@GMs3367 and 85.7% in INH@GMs.

After 28 days, the morphologies of microparticles were observed Figure 8. Obviously, gelatin microparticles without TCP were still able to maintain their spherical shape while the majority of those particles containing TCP were degraded as well as the exposure of TCP on the surfaces. The higher contents of TCP could cause the quicker degradation for these microparticles, which was consistent with the drug release behaviours after 3 weeks. Based on the above results of high INH entrapment efficiency, slow initial drug release rate and thorough ultimate drug release content, INH-TCP@GMs5050 was an ideal material for fabricating the INH loaded *b*-PLGC scaffolds.

3.2.2. Drug Release Behaviours of INH Loaded b-PLGC Scaffolds

In Vitro. Drug release behavior of INH loaded *b*-PLGC scaffolds is shown in Figure 9(a). The initial INH burst release of INH/TCP/*b*-PLGC was higher and the cumulative INH release in the first week exceeded 60%, but that of I-M/P50 and I-M/P70 scaffolds was less than 20%. The sustained INH release of I-M/P50 and I-M/P70 scaffolds could keep for 84 days, and the cumulative release increased with the climbing content of INH-TCP@GMs5050 in scaffolds. The rapid INH

 $\begin{array}{c} A_{1} \\ \hline \\ A_{2} \\ \hline \\ A_{2} \\ \hline \\ A_{3} \\ \hline \\ A_{4} \\ \hline \\ A_{2} \\ \hline \\ A_{3} \\ \hline \\ A_{4} \\ \hline \\ A_{2} \\ \hline \\ A_{2} \\ \hline \\ A_{3} \\ \hline \\ A_{4} \\ \hline \\ A_{2} \\ \hline \\ A_{2} \\ \hline \\ A_{3} \\ \hline \\ A_{4} \\ \hline \\ A_{2} \\ \hline \\ A_{2} \\ \hline \\ A_{3} \\ \hline \\ A_{4} \\ \hline \\ A_{2} \\ \hline \\ A_{3} \\ \hline \\ A_{4} \\ \hline \\ A_{4} \\ \hline \\ A_{2} \\ \hline \\ A_{4} \\ \hline$

FIGURE 8: SEM images showing the GMs and TCP@GMs after release for 28 days. (a) GMs; (b) TCP@GMs3367, and (c) TCP@GMs5050.



FIGURE 9: (a) Release behaviours of INH-loaded scaffolds in vitro. (b) INH and RFP Release behaviors of I-M/RP50 scaffolds in vitro.

release of INH/TCP/*b*-PLGC should be attributed to that of the water-soluble INH was directly blended in the scaffolds that were easy to contact with water. The sustained INH release of *b*-PLGC scaffolds loaded with INH-TCP@GMs5050 was mainly caused by the sustained INH release of INH-TCP@ GMs5050. In addition, since microparticles were coated with polymers during scaffold preparation process, some particles were embedded in the inner of scaffolds (Figure 5) and the INH was prevented to be directly in touch with water, resulting in the decreasing initial burst release and prolonging the release time. It is mentioned that this dual drugs-loaded system could achieve the expectant roles of respective drugs without obvious influences with each other.

The INH and RFP release profiles of I-M/RP50 scaffolds were shown in Figure 9(b). At the initial stage, a slight INH and RFP initial burst release occurred with about 15% and 20%, respectively. After that, a long-term sustained INH and RFP release profiles were obtained along with the cumulative release of ca. 75% and 82% on day 84, indicating that I-M/RP50 scaffolds could carry out a sustained INH and RFP

release for 3 months, which resembled the INH and RFP release behavior of individual INH-loaded (Figure 9(b)) and RFP-loaded scaffolds. The initial burst release of RFP should ascribe to inevitably existing RFP molecules on the surface of the substrate during the scaffold preparation. While the RFP molecules embedded inside polymeric matrixes would be slowly released via water molecule interpenetration and RFP diffusion effect. The INH and RFP release *in vivo* and its effect on repair and reconstruction of bone tissue would be further studied in our future work. In addition, there were still inevitably initial outbreak release problems that need to be improved. For example, we will design and fabricate another smart system to achieve the controllable varied-drugs delivery by further combining the supramolecular interactions and covalent bond methods.

4. Conclusions

In summary, we prepared a kind of INH-TCP@GMs microparticles with less initial burst release, higher cumulative release and longer sustained INH release, which was closely related to the TCP contents. After particle leaching combined with phase separation technique for the mixture of INH-TCP@GMs5050 and *b*-PLGC solution containing RFP, INH- and RFP-loaded b-PLGC scaffolds was obtained with the quicker INH release rate than INH-loaded b-PLGC scaffolds on account of the evenly distributed microparticles in these scaffolds. This drug release system could achieve their respective roles of two drugs, and the loading process was not affected with each other. After a slight initial burst release occurred on the first day, the INH and RFP were slowly released from the scaffolds for 84 days and the cumulative release achieved about 75% and 82% on day 84, respectively. Thus, we believe that these dual anti-tuberculosis drug-loaded composite scaffolds will have potential clinical applications in bone tuberculosis therapy.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Contributions

Dawei Li and Chao Li contributed equally to this work.

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