A mesoporous silica nanoparticulate/β-TCP/BG composite drug delivery system for osteoarticular tuberculosis therapy

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ABSTRACT

A composite scaffold drug delivery system (CS-DDS) for osteoarticular tuberculosis therapy has been prepared by loading bi-component drugs into a mesoporous silica nanoparticles (MSNs)-coated porous β-TCP scaffold, which was followed by an additional bioactive glass coating. Such a CS-DDS showed high performances in the local and extremely sustained delivery of the bi-component antitubercular drugs and excellent biocompatibility. N₂ sorption isotherms indicated greatly increased surface area of the composites compared to pure β-TCP scaffold, and the mesopores were around 2.6 nm which were large enough to encapsulate drugs such as isoniazide and rifampicin. The in vitro and in vivo release tests demonstrated extra sustained co-release profiles of rifampicin and isoniazide from such a CS-DDS, and both drug concentrations kept higher than their effective values to kill mycobacterium tuberculosis for as long as 42 days. The hepatic and renal function tests indicated that the CS-DDS had neglectable long-term lesions to liver and kidney.

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1. Introduction

With the rapid growth of floating population, the wide-spread of AIDS all over the world and the emergence of multi-drug resistant mycobacteria, tuberculosis (TB) as a global health problem continues to present a formidable challenge. According to the World Health Organization, about one third of the overall population in the world was infected with the Mycobacterium tuberculosis, and there were millions of newly infected cases annually [1–3]. Recently, there has been a spurt in extrapulmonary TB (EPTB) [4,5]. Thereinto, osteoarticular TB cases account for approximately 10–11% of EPTB, which are about 19–38 million in the world [6–10].

Strategies for treating TB, including osteoarticular TB, mainly consisted of multi-drug chemotherapy for an appropriate period, which prevented the apparent resistance of TB bacilli to single drugs. Though the optimal duration for such a chemotherapy has not been thoroughly known [11,12], it was generally recommended that a total of 9 month treatment was acceptable for adults, while no less than 12 months for children, which were undoubtedly a heavy burden to patients [13–15]. Moreover, because of toxic side effects of the drugs [16–19], degradation of drugs before reaching their target site and/or low permeability of the drugs and poor patient compliance, the effect of systematic multi-drug chemotherapy, including oral administration and intravenous injection, was unfortunately rather limited. As for osteoarticular TB, the osseous focus developed by tubercle bacillus is usually poor in blood supply, and it is difficult for antitubercular drugs carried in blood to arrive at the focus. Therefore the tubercle bacillus living in the osseous focus or survived through debridement operations were difficult to be killed completely by traditional systematic chemotherapy and easy to become latent bacillus which would reproduce rapidly once in co-morbid conditions.

Another general rule for osteoarticular TB treatment was surgical intervention [6,20]. With the development of internal fixation materials and technology, debridement of the bone infection foci was inevitably suggested to be chosen by doctors [21]. However, apart from the surgery, antitubercular multi-drug therapy was unfortunately still indispensable. The current common
regimen for treating osteoarticular TB was 1–4 weeks of pre-operative and 6–9 months of post-operative multi-drug chemotherapy in order to consolidate the curative effect. To shorten or even avoid the drug therapy following debridement and reduce lesions to hepatic and renal functions, orthopedic surgeons and researchers have been showing more and more interest in controlled drug release systems [22,23], which offer more effective and favorable methods to optimize drug dosage, deliver to specific sites or prolong delivery duration [24]. Nanoparticles [25], mesoporous materials [26], and lipids [27] were among the most investigated carriers for drugs [28–30]. Much attention has been paid to poly (lactide-co-glycolide) (PLGA) as a base compound of micro-particles for pulmonary delivery of anti-tuberculosis drugs. However, PLGA microphases embedded into the bone defect cavities would induce the significant decrease of pH values by the acidic degradation products of PLGA [31–33]. The decreased pH values could result in drug resistance of tubercle bacillus.

Alternatively, porous inorganic materials, such as mesoporous silica materials, may provide a more advantageous choice for controlled and localized antitubercular drug delivery without causing significant pH value decreases, which have been investigated as a drug delivery carrier for more than a decade, thanks to its extensive nanopore structure in mesoporous silica [26,34–36]. The mesopore structure of 2–50 nm in diameter may render good bioactivity and biocompatibility. Compared with solid nanoparticles, the mesoporous silica nanoparticles (MSNs) are apparently a more suitable drug delivery carrier due to its extensive mesoporous structure.

As far as antitubercular drugs are concerned, isoniazid (INH) and rifampicin (RFP) are two of the efficacious drugs against TB with the traditional duration of treatment [4,37,38]. With positive therapy effects to tuberculosis caused by susceptible strains, the co-encapsulation of INH and RFP had no effect on their respective virtues [39,40]. After debridement of osteoarticular TB, the residual cavity, which could give rise to common bacterial infection and rapid propagation of the residual tuberculosis germs when it was filled by blood clot, should be filled. Porous beta-tricalcium phosphate (β-TCP) bioceramics have been regarded as one of the satisfactory cations for the prolonged post-operative multi-drug chemotherapy to partly or even completely eradicate the residual tubercle bacillus. When C6STAB was dissolved completely, 5 ml tetraethoxysilane (TEOS) was added dropwise with the whole time period of about 10 min to the solution under vigorous stirring. The reaction was continued for 2 h to give rise to a white precipitate. The solid product was recovered by filtration, extensively washed with deionized water and ethanol and dried. The as-synthesized product was then calcined in air at 600 °C at a heating rate of 1°/min for 6 h to remove the templates. The morphology of MSNs was analyzed using TEM (JEOL, 2010, Japan) and N2 adsorption−desorption isotherms were obtained using a Micromeritics porosimeter (Micrometics Tristar 3800, 77 K).

2.2. Fabrication and characterization of MSN−β-TCP composite scaffolds

Synthesized MSNs in the above step were dispersed in ultrapure water at the concentration of 0.05 mg/ml through sonication. Porous β-TCP bioceramics (kindly provided by Shanghai Bio-Lu Biomaterials Co.) were soaked into the suspension and then the mixture was subjected to vacuum under ultrasonic treatment. The vacuum was turned off in 15 min and the obtained scaffolds with MSNs loaded (named as MSN−β-TCP) were separated from the solution batch. The composites were then dried and calcined at 400 °C for 4 h.

The morphology of the MSN−β-TCP was observed by FE-SEM (JSM-6700F, JEOL). The surface area and pore parameters were obtained through calculations from N2 adsorption−desorption isotherms [Micromeritics Tristar 3000, 77 K].

2.3. Encapsulation of antitubercular drugs and preparation of CS-DDS

Isoniazid (INH) and rifampicin (RFP) drug powders were dissolved in ultrapure water to obtain a mixed solution of 10 mg/ml INH and 200 μg/ml RFP. 0.1 g β-TCP or MSN−β-TCP scaffolds were immersed into 10 ml of the solution for 3 days at 37 °C. After that, the scaffolds were withdrawn and vacuum-dried at room temperature.

The drug-loaded MSN−β-TCP was further coated with a layer of bioactive glass (BG) on the MSN layer for sustained drug release. Bioactive glass sol was prepared according to the following molar ratio: TEOS: Ca(NO3)2: TEP: EOH: HCl = 1: 0.2: 0.05: 34: 0.017. The mixture was stirred for 24 h at room temperature. Put the scaffolds down into the sol for just 10 s and then promptly draw it out. Extra sol on the surface was wiped. The scaffolds were finally put under a 37 °C and 40RH% condition aging for 24 h. Such a drug-loaded, BG and MSNs-coated scaffold was named as composite scaffold drug delivery system (CS-DDS). In addition, the drug-loaded composite scaffolds without BG layer coating (named as MSN−β-TCP), and both BG and MSNs coated scaffold without drug loading (named as BG−MSN−β-TCP) were also prepared for characterization and comparison.

2.4. In vitro release

In vitro releases of RFP and INH from the β-TCP, MSN−β-TCP and CS-DDS samples (0.1 g) were carried out at 37 °C in 1 ml of SBF solution respectively. The release medium was withdrawn at pre-determined time intervals, and replaced with a fresh soaking medium each time. Then the concentrations were determined by UV−vis spectrophotometer by measuring the maximum absorbance at the wavelengths of 262 nm for INH and 480 nm for RFP. The calibration curves were obtained using solutions of INH and RFP respectively in the same concentration ranges before determination.

2.5. In vitro cell toxicity evaluation

In vitro cell toxicities of β-TCP, composite MSN−β-TCP and BG−MSN−β-TCP were evaluated using MTT assay. L-929 cells were seeded in 24-well cultured plates at a density of 5 × 103 per well with 1 ml culture medium (DMEM; 10% fatal calf serum). After 24 h of adhesion, 0.05 g samples were added in and cells were cultured with them, and the tissue culture plates without samples were used as control. On specific days, the medium and materials were removed, then 1 ml 0.5 mg/ml MTT solution was added and cells were incubated for another 4 h. Upon removal of the MTT solution, the purple formazan crystals were dissolved in 1 ml of dimethyl sulphoxide (DMSO) by shaking the plate for 10 min. Then the solutions were centrifugalized and the supernatants were transferred into another plate to record absorbance at 490 nm on a micro-plate reader (Bio-Tek ELx800).

2.6. Surgical procedures

Eighty-four young New Zealand rabbits (provided by Shanghai Second Military Medical University, Laboratory Animal Center, male or female, 2–2.5 kg body weight) were randomly divided into 14 groups (n = 6). After the rabbits were anesthetized by injecting 33 Nembutal (30 mg/kg) via ear vein, the lateral femoral cortex and the center bottom part of femur were exposed. A groove 0.5 cm away from the articular surface of lateral femoral cortex was created by an osteotome (1.5 cm in length and 0.8 cm in width). 1 g samples including drug-laden β-TCP scaffolds served as the control group and CS-DDS as the test group were respectively weighed, and then embedded into the groove. A picture was taken to explain the
surgery procedure in Fig. 25 (see SI). Finally the muscle and skin were closed. All the rabbits were monitored after surgery and fed standardly.

2.7. In vivo drug concentrations in blood and tissues

2.7.1. Specimen collecting

At 1, 3, 5, 7, 14, 28 and 42 days post-surgery, 2 ml blood samples were immediately collected through heart puncture after all the rabbits were sacrificed. The blood samples were also collected from ear veins of six randomly selected normal rabbits. In addition, samples of bones (0.5 cm away from the closer end of the groove), musculature and fibrous tissue cling to the groove and tissues from kidney, liver and spleen were collected as well.

2.7.2. In vivo drug concentrations in blood and tissues

The blood specimens were all centrifugated and the supernatants were extracted for the following tests. The tissues were ground and added into 2 ml saline before being homogenated. 0.7 ml of the tissue suspension was mixed with 2.0 ml methanol. The homogeneous mixture was centrifugated and the supernatants were reserved. The concentration of drugs distributed in blood and tissues were investigated by HPLC techniques. The HPLC conditions were described in Supporting Information.

2.8. Hepatic and renal function tests

The supernatants of blood samples including the 6 control specimens obtained in Section 2.7.2 were directly placed in an Automatic bio-chemistry analyzer (Olympus AU2700). Contents of serum Alanine Aminotransferase (AST), Aspartate Aminotransferase (AST), Blood Urea Nitrogen (BUN) and Creatinine (Cr) were examined. Independent-samples T test was used for statistical analysis by SPSS Version 13.0.

3. Results

3.1. Characterization of MSNs

The TEM images (Fig. 1) showed that the particle size of MSNs was mainly distributed at around 400 nm and the mesopores were in ordered 1-D cylindrical arrangement.

N2 sorption isotherms of the MSNs were shown in Fig. 2. The BET surface area, pore volume and pore size were calculated to be 958 m²/g, 0.91 cm³/g and 2.65 nm, respectively. The capillary
condensation jump around 0.2 of $P/P_0$ in the isotherms further confirmed the existence of mesoporous structure, which correspondingly gave a pore size distribution curve calculated from the desorption branch by the BJH model as the inset in Fig. 2. The pore size distribution of MSNs was sharp, indicating a uniform mesoporous structure, in consistence with the TEM imaging of MSNs.

3.2. Characterization of the composite scaffolds

The surface morphologies and microstructures of $\beta$-TCP scaffolds, MSN-$\beta$-TCP composites and the prepared CS-DDS are shown in Fig. 3. The interconnected open macroporous structure of $\beta$-TCP scaffolds was maintained after the introduction of MSNs and BG. The high magnification SEM (Fig. 3b2, b3) micrographs show that the surface of the composite scaffolds is covered by a heavy layer of mesoporous silica nanospheres. Due to the post-calcination of the composite systems at a high temperature, a large amount of nanospheres was joined together forming a MSNs layer sticking to the strut walls. It can also be observed that the morphology and average size of the MSNs adhibiting on the scaffold walls experienced no changes from the original nanoparticles. Fig. 3c1, 3c2 show the great change from the particulate surface morphology of MSN-$\beta$-TCP scaffold into much smoother one of CS-DDS due to the final BG coating. Spherical MSNs were no longer distinguishable, instead a layer of bioactive glass with a ternary $\text{SiO}_2-\text{CaO}-\text{P}_2\text{O}_5$ chemical constitution was extended onto the most area of the surface.

Fig. 4 reports the nitrogen adsorption/desorption isotherms for both $\beta$-TCP and MSN-$\beta$-TCP composite scaffolds. Isotherms of the
composite scaffolds exhibit a pore filling jump at \( P/P_0 \) around 0.2 and its BJH pore diameter calculated from the desorption branch is 2.57 nm, which looks very similar to those observed from MSNs powders. Comparatively, no pore filling associated with mesopores is observed of pure \( \beta-TCP \) scaffolds, as expected. The BET surface area of the composites was calculated to be 81 m\(^2\)/g, i.e. some 10 times lower than that of MSNs powders due to the presence of ceramic scaffolds.

The X-ray diffraction patterns (Fig. 1S, see SI) show ordered pore structure of both MSNs powders and the composite scaffolds, and the crystalline lattice structure of the composite scaffolds, which also demonstrates that MSNs had been successfully loaded into the scaffold.

### 3.3. In vitro drug release

Fig. 5 shows the macropore surface morphology of CS-DDS after in vitro drug release for 3 days in SBF. A layer of apatite deposits can be clearly observed in Fig. 5a, which has totally changed the surface morphology of CS-DDS as shown in Fig. 3c. A higher magnification image in Fig. 5b shows that the sizes of the elongated deposit grains were about 200–400 nm in length. As demonstrated in EDS spectra (Fig. 5c), both Ca and P peaks were detected. The Ca/P ratio was 1.52 representing the carbonated calcium-deficient hydroxyapatite, which was not far away from 1.67 of the Ca/P ratio in stoichiometric hydroxyapatite.

Similarly, after 3-day release in SBF, XRD and SEM observations were used to test the capability of apatite formation on \( \beta-TCP \) and MSN–\( \beta-TCP \) samples without BG layer. The results in Figs. 3S and 4S show less and much smaller apatite deposits formed as compared to CS-DDS.

Drug loading amounts were calculated according to the results of UV–Vis spectra. Compared to \( \beta-TCP \) scaffolds, which had low capacities of INH (8.53 mg/g) and RFP (0.91 mg/g), the CS-DDS exhibited much higher amounts of drugs incorporated: 37.89 and 3.77 mg/g for INH and RFP, respectively. The presence of mesoporous coating in the composites lead to the greatly increased surface area of the DDS, and the mesopores (~2.57 nm) were large enough for encapsulating the drug molecules.

The release profiles and release rate of INH, RFP from \( \beta-TCP \) scaffolds as references, MSN–\( \beta-TCP \) composite and the CS-DDS are shown in Fig. 6. Both INH and RFP drugs were almost completely released from the macropores of pure \( \beta-TCP \) scaffold only in the first day. In contrast, CS-DDS, in which both MSNs and BG were coated, presented an extraordinarily sustained release pattern of both drugs. Taking INH for an example, a slight initial burst release of INH is observed during the first day which accounts for around 30% of the total loaded from the CS-DDS. It is noticed that such a burst release percentage is significantly smaller than that of other composite scaffolds reported before [34] or from the MSN–\( \beta-TCP \) as illustrated in Fig. 6a, which can be most probably attributed to the loss of drugs attached on the external surface during the dipping process. Subsequently, a low dose of INH was released in the early stage and the release process lasted for more than 30 days. For comparison, it can be found that drugs released also significantly slower from the MSN–\( \beta-TCP \) composite without BG coating than pure \( \beta-TCP \), but still much faster than from CS-DDS, especially in the sustained release stage. This means that both the mesoporous structure in the MSNs coating and the additional BG coating covering the MSNs layer played an important role in achieving the extra sustained drug release properties of CS-DDS. The release rates from every sample in the form of released drug percentage per day were plotted in Fig. 6. It demonstrates more clearly the extraordinarily sustained drug releases from CS-DDS lasting for more than 30 days, as compared with the much shorter time periods for the complete release, when the release rate is zero, from \( \beta-TCP \) and MSN–\( \beta-TCP \).

Besides the much higher initial drug concentration of INH solution than that of RFP, which resulted higher physical adsorption, INH molecules can bond onto the pore surface of MSNs through hydrogen bonding with Si–OH groups in the pore surface of MSNs, therefore relatively higher amount of INH can be loaded into the CS-DDS and meanwhile release into the SBF medium in a very sustained way over a long time period. Comparatively, much less RFP can be loaded into the pore structure but it can release relatively faster due to its hydrophilic nature, which resulted in weak interaction with the hydrophilic pore surface of MSNs. Nevertheless, both drugs showed extraordinarily sustained release profiles.

### 3.4. In vitro cell toxicity evaluation

Cell toxicity of scaffolds was analyzed using MTT assay after 1, 4 and 7 days of culture. As shown in Fig. 7, an increase in absorbance from Day 1 to Day 7 was recorded, which indicated that the cells were viable within and/or on all the scaffolds. Moreover, the proliferation behavior of cells within all scaffolds was very similar to each other on every measurement day. Having been clinically used for bone tissue engineering, the low cell toxicity and excellent biocompatibility of \( \beta-TCP \) bioceramics were already widely accepted. Therefore, the results shown in Fig. 7 approved that the introduction of MSNs layer, or both of MSNs and BG layer, into \( \beta-TCP \) scaffolds (corresponding to MSNs–\( \beta-TCP \) and BG–MSNs–\( \beta-TCP \) in the figure) would add no additional cell toxicity to them.

### 3.5. In vivo release

The released concentrations of the antitubercular drugs with respect to time in tissues or blood after implantation were monitored. The data were displayed in Figs. 8 and 9. Both INH and RFP concentrations reached maxima on the fifth day from the composites, whereas they reached the maxima only in the third day.

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**Fig. 5.** SEM images of the CS-DDS after in vitro drug release for 3 days in SBF at different magnifications (a,b), and EDS spectra of the deposits.
from pure β-TCP scaffolds either in blood or in tissues. More importantly, the drug concentrations released from the β-TCP scaffold quickly drop down to nearly zero in 14 days, while those from CS-DDSs still remained above the effective concentrations (INH: 10 μg/ml; RFP: 5 μg/ml [49,50]) for over 42 days. These results were consistent with the in vitro release profiles of the composites.

Correspondingly, anti-tubercle bacillus tests with the implanted CS-DDS or β-TCP also illustrated the differences in drug release (see SI). The digital photos of the agar plates, on which different extraction liquids containing antitubercular drugs released from CS-DDS implanted after 28 or 42 days and β-TCP implanted after 7 or 14 days (named as M-28, M-42, T-7 and T-14, respectively) were smeared, are shown in Fig. 5S. No Mycobacterium tuberculosis in the plates seeded with M-28, M-42 could be observed, indicating the long-lasting maintaining of the drug concentrations for longer than 42 days. Comparatively, though T-7 seeded plates also showed absence of mycobacterium tuberculosis, however, luxuriant growth of the mycobacterium tuberculosis can be clearly noticed on T-14 seeded agar plate, which implied over-low concentrations of drug molecules in the solution of T-14 due to the quick release of the drugs from the β-TCP scaffolds within 14 days as indicated in Fig. 6, Figs. 8 and 9.

3.6. Hepatic and renal function tests

The hepatic and renal functions were tested by examining the values of some serum enzymes at different time points. The blood specimens collected from the ear vein of the rabbits without any post treatment were employed as control. As Table 1 demonstrated, ALT, AST and Cr values of the β-TCP group recovered to normal levels of those of the control group in 7 day post-surgery (p > 0.05). The test of BUN suggested its statistic rising of controlled release group only on day 3 (p < 0.05) and quickly falling back to the normal levels on the seventh day. Comparatively, the ALT, AST and Cr values of the CS-DDS group exhibited statistical differences from the control group (p < 0.05) within one week after surgery, even then, they descended to the normal levels (p > 0.05) in 14 days. The variations in these serum enzyme values were understandable and consistent with the drug in vivo release features shown in Fig. 8. As a whole, this composite drug delivery system had no significant long-term harms to either liver or kidney according to medical principles.

4. Discussion

Debridevement and antitubercular multi-drug therapy have encountered several problems such as significant side effect and unsatisfactory therapeutic effect due to low drug concentration at TB foci by systematic drug administration, long-term patient’s suffering, etc., in the treatments for patients with osteoarticular TB [6,20]. In this report, we fabricated an implantable composite scaffold drug delivery system (CS-DDS) to treat osteoarticular TB locally and regenerate the defect in the mean time. And this system has reached the requirements in three aspects to a large extent. Firstly, proper dosages of antitubercular drugs should be encapsulated in the system; Secondly, it is better to make the release duration of molecules as long as possible; Last but not least, local TB drug level must be above their minimal inhibitory concentrations (MIC).

In fact, previous reports about composite scaffolds involving mesoporous silica for bone repairing and drug delivery can be approximately divided into two categories: mesoporous particles were mixed with matrix components in advance and then fabricated directly into a scaffold [34,44,45], or a scaffold was dipped in the mesoporous silica preparing solution to introduce mesophase, in which a truly continuous mesoporous silica layer was coated by a so-called EISA process on the macropore surface [46,47]. Herein, we directly coated MSNs, instead of the preparing solution of mesoporous silica, into the macropores of β-TCP bioceramics under
Fig. 8. Concentrations of the antitubercular drug INH \((a_1, a_2)\) and RFP \((b_1, b_2)\) with respect to time in blood (1) or bone and the surrounded tissues (2).

Fig. 9. Concentrations of the antitubercular drugs INH \((a_1, a_2)\) and RFP \((b_1, b_2)\) with respect to time in liver, kidney and spleen.
vacuum condition, resulting in a MSNs coating composed of numerous mesoporous silica nanoparticles. After calcining to bond nanospheres onto the maropore surface of the scaffold, drugs were loaded through a simple immersion procedure. Finally a layer of bioactive glass sol was introduced to cover the MSNs layer. The composite scaffold a very high surface area of over 80 m²/g and coated into the scaffold macropore structure, which endows the porous structure and adsorption property of the spongy bone.

In addition, the composite scaffolds show no cytotoxicities, and the invasion of surrounding tissues. Herein, the macroporous structure of β-TCP scaffolds can fully satisfy this need, which has been demonstrated clinically. MSNs layer coated on the macropore surface has limited effect on the macropore structure of the scaffold.

In conclusion, both the mesopores and the frequent fresh release medium supplements for that extracted for concentration tests in vitro. Higher levels of TB drugs in bone tissue than in blood samples were detected due to the porous structure and adsorption property of the spongy bone.

As an implant to induce bone regeneration, the interconnected pore structure is required to offer sufficient space for tissue growth and the invasion of surrounding tissues. Herein, the macroporous structure of β-TCP scaffolds can fully satisfy this need, which has been demonstrated clinically. MSNs layer coated on the macropore surface has limited effect on the macropore structure of the scaffold.

In conclusion, both the mesoporous channels of MSNs, and a large part of which are simply physically hosted in the mesopores without chemical bonding with the pore surface, the release of this part of drugs would follow a Fickian diffusion-through-channel mechanism [48]. In addition to those physically loaded drugs, some of the drug molecules in the pore channels would interact with Si–OH groups on the mesopore surface through hydrogen bonding (state 3), as both amino and hydroxyl groups respectively in INH and RFP molecules could form hydrogen bonds with the Si–OH groups. These chemical bonds would effectively retain the drugs and slow down their release rate significantly in long terms. In addition, the BG layer coated on the MSNs, together with the precipitates of bone apatite via BG mineralization occurred when CS-DDS were put into SBF, would also play an important role in sustaining the drug release, as can be known from Figs. 3c, 5 and 6. The BG and/or apatite layer covered most of MSNs and prevented the drugs in the pore channels from fast release outwards. In conclusion, both the mesoporous structure of MSNs and the BG coating contribute to the extraordinarily sustained TB drug release behavior up to more than 30 days in vitro and in vivo.

It has been well documented that MICs of INH and RFP are 10 and 5 µg/ml, respectively [49,50], which were effective enough because they are considered to be toxic for the majority of the resident microorganisms. Correspondingly, keeping INH concentration in the range of 0.025–0.05 µg/ml and RFP in between 0.005 and 0.5 µg/ml could inhibit the growth of tuberculosis germs [49,50]. The concentrations of TB drugs detected in tissues or blood released from the CS-DDS still remained above the effective concentrations for longer than 42 days. Therefore, it is supposed that the in vivo release rate was lower than in the simulated process in vitro, which may result from free flowing of the medium liquid and the frequent fresh release medium supplements for that extracted for concentration tests in vitro. Higher levels of TB drugs in bone tissue than in blood samples were detected due to the porous structure and adsorption property of the spongy bone.

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Table 1

<table>
<thead>
<tr>
<th>Samples</th>
<th>ALT (IU/L)</th>
<th>AST (IU/L)</th>
<th>BUN (mmol/L)</th>
<th>Cr (µmol/L)</th>
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<tr>
<td>Control group*</td>
<td>59.67 ± 9.45</td>
<td>65.51 ± 13.70</td>
<td>8.51 ± 2.21</td>
<td>121.06 ± 23.82</td>
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<tr>
<td>Day 1</td>
<td>91.037 ± 4.34*</td>
<td>108.58 ± 8.64*</td>
<td>6.54 ± 1.42**</td>
<td>205.32 ± 40.93*</td>
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<tr>
<td>Day 3</td>
<td>107.48 ± 11.61*</td>
<td>154.87 ± 55.88*</td>
<td>12.28 ± 1.54**</td>
<td>306.86 ± 56.43*</td>
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<tr>
<td>Day 7</td>
<td>192.74 ± 22.77*</td>
<td>101.95 ± 5.04*</td>
<td>10.42 ± 0.55**</td>
<td>238.47 ± 30.50*</td>
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<tr>
<td>Day 14</td>
<td>84.96 ± 4.32*</td>
<td>148.19 ± 4.02*</td>
<td>8.51 ± 1.51**</td>
<td>219.80 ± 41.67*</td>
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<td>β-TCP scaffold</td>
<td>67.97 ± 9.50**</td>
<td>71.37 ± 20.74**</td>
<td>9.02 ± 1.24**</td>
<td>133.86 ± 52.15**</td>
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*<p>**P < 0.05; ***P > 0.05. Controlled correspondingly with ‘a’.

Fig. 10. Three states of TB molecules hosted in the CS-DDS: (1): TB drugs adsorbed on the external surface of MSNs; (2): TB drugs entrapped in the mesoporous channels without hydrogen bonding with Si–OH groups; (3): TB drugs entrapped in the mesopores and interacted with Si–OH groups through hydrogen bonding.
term, which is considered to be a serious side effect in oral drug administration. Therefore, the present CS-DDS can serve as promising local multi-drug delivery systems, and in the mean time, as bioactive implants for bone tissue repair.

5. Conclusion
An implantable antitubercular composite scaffold drug delivery system (CS-DDS) with mesoporous silica nanoparticles (MSNs) and bioactive glass (BG) coated in β-TCP bioceramic scaffold was fabricated. This composite system showed much higher rifampicin (INH) and isoniazid (RFP) loading capacities than pure β-TCP scaffold. Compared to the complete in vitro release of all drugs in three days from pure β-TCP scaffold, the CS-DDS displayed an extraordinarily sustained co-release pattern of INH and RFP for over 30 days. The mesostructure of MSNs and the bioactive glass coating and/or the hydroxyapatite deposits formed during release period played important roles in remarkably delaying the drug releases both in vitro and in vivo. Effective drug concentrations of both INH and RFP for treating TB in vivo can be maintained for an extra long duration over 42 days without significant long-term lesions to liver and kidney.

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Appendix
Figures with essential colour discrimination Certain figures in this article, particularly Figs. 4, 6–10 are difficult to interpret in black and white. The full colour images can be found in the on-line version, at doi:10.1016/j.biomaterials.2010.11.025.

Appendix. Supplementary material
Supplementary data related to this article can be found online, at doi:10.1016/j.biomaterials.2010.11.025.

References


