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Research Article

Synthesis, Characterization, and In Vitro Drug Delivery of Chitosan-Silica Hybrid Microspheres for Bone Tissue Engineering

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Chitosan-silica (CS-SiO_2) hybrid microspheres were prepared through the combined process of sol-gel and emulsification-crosslinking. Their composition, morphology, *in vitro* bioactivity, and drug release behavior were investigated. The results showed that, when 20 wt% SiO_2 was incorporated, the as-prepared CS-SiO_2 hybrid microspheres exhibited a regular spherical shape, a high dispersity, and a uniform microstructure. Their average particle diameter was determined to be about 24.0 μm. The in situ deposited inorganic phase of the hybrid microspheres was identified as amorphous SiO_2, and its actual content was determined by the TG analysis. As compared with the pure chitosan microspheres, the CS-SiO_2 hybrid microspheres displayed a greatly improved *in vitro* bioactivity. Vancomycin hydrochloride (VH) was selected as a model drug. It was demonstrated that the CS-SiO_2 hybrid microspheres presented a good capacity for both loading and sustained release of VH. Moreover, the increase of the SiO_2 content efficiently slowed down the drug release rate of the CS-SiO_2 hybrid microspheres.

1. Introduction

In the past few decades, microspheres have been widely used in the fields of catalysis, adsorption, drug delivery, etc. [1–5]. In particular, when serving as drug carriers, microspheres exhibit good targeting ability to specific organs/tissues and sustained and controlled release behaviors as well as various administration methods (oral, injection, filler, nasal drops, etc.) [6], thus presenting more development potentials in some specific application areas than the other types of drug carriers.

Much effort has been made to investigate the preparation processes, in vitro/in vivo evolution, or clinical performance of different kinds of the microsphere-based drug carriers [7–9]. These carrier materials mainly involve natural polymers (starch, gelatin, chitosan, cellulose, etc.) [7, 10, 11], synthetic polymers (polyvinyl alcohol, polylactic acid, poly(lactic-co-glycolic acid), etc.) [12, 13], and inorganic materials (silica, hydroxyapatite, ferroferric oxide, etc.) [14, 15]. Chitosan is one of the commonly used natural biopolymers with good sphere-forming capability, chemical stability, biocompatibility, and biodegradability [16, 17]. However, when used as a drug carrier for bone tissue engineering, the poor mechanical strength and bioactivity of the chitosan microspheres have greatly limited their clinical applications.

In this paper, silica (SiO_2) xerogel, an important inorganic biomaterial possessing good mechanical properties and bioactivity, was incorporated into the chitosan (CS) microspheres to form the chitosan-silica (CS-SiO_2) hybrid microspheres. The influence of the SiO_2 contents on the composition and morphology of the chitosan microspheres was investigated. Moreover, the feasibility of the CS-SiO_2 hybrid microspheres as a drug carrier for bone tissue engineering was preliminarily evaluated by *in vitro* bioactivity and drug delivery behavior.

2. Experimental Procedure

2.1. Preparation of the CS-SiO_2 Hybrid Microspheres. Chitosan with a medium molecular weight and a degree of deacetylation of 75-85% was purchased from Sigma-Aldrich

(Shanghai, China) and used as received. Vancomycin hydrochloride (VH) was obtained from Shanghai Macklin Biochemical Co. Ltd. (Shanghai, China). All the other chemical reagents used in this study were of analytical pure grade and supplied by Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

The CS-SiO_2 hybrid microspheres were prepared in a water-in-oil (W/O) emulsion system, and the water/oil ratio was kept at 10 : 1. Firstly, the CS powder was dissolved in acetic acid to obtain a 2% (*w*/*v*) CS solution. Then, a certain amount of the SiO_2 sol prepared by the hydrolysis of tetramethoxysilane (TMOS) in the presence of HCl was added to the CS solution. Subsequently, the resultant CS-SiO_2 hybrid sol was dropped into soybean oil containing 1% (*w*/*v*) sorbitan monooleate (Span 80) as the surfactant and stirred at 37°C for 0.5 h to generate a stable W/O emulsion. Thereafter, 0.5 mL of glutaraldehyde (25% aqueous solution) was then added into the system to solidify the CS-SiO_2 droplets, followed by adding the NaOH solution to allow the precipitation of the CS matrix. Finally, the hybrid microspheres were obtained by the successive process of centrifugation and repeated washing and air-drying. The CS-SiO_2 hybrid microspheres with 20 wt% and 40 wt% of SiO_2 (theoretical weight percentages) were prepared just by changing the amount of the added SiO_2 sol, and the samples were designated as CS-20%SiO_2 and CS-40%SiO_2, respectively. For the control group, the pure CS microspheres were also prepared using a similar procedure.

2.2. Characterization of the CS-SiO_2 Hybrid Microspheres. The morphology of the hybrid microspheres was observed with scanning electron microscopy (SEM, SU8220). A portion of the as-prepared CS-20%SiO_2 hybrid microspheres were calcined at 600°C for 4 h in a muffle furnace, and the residual powders after calcination were analyzed by SEM and transmission electron microscopy (TEM, JEOL-2010). Fourier transform infrared spectroscopy (FT-IR, PerkinElmer 983G) was applied to identify the chemical groups of the hybrid microspheres using the KBr pellet method. The crystallization behavior of the microspheres was investigated by X-ray diffraction (XRD) analysis (D8 Advance). In addition, the thermal analysis of the microspheres was carried out by thermogravimetry/differential scanning calorimetry (TG/DSC, STA 449 F3 Jupiter).

2.3. In Vitro Bioactivity of the CS-SiO_2 Hybrid Microspheres. The *in vitro* bioactivity test was performed by soaking 0.1 g of the microspheres in 5 mL simulated body fluid (SBF, pH 7.4) at 37°C. The SBF solution was refreshed every other day. After 3 days of culture, the microspheres were collected by centrifugation, washed 3 times with deionized water, and lyophilized. The dried microspheres were subjected to the SEM analysis.

2.4. Drug Loading and In Vitro Release of the CS-SiO_2 Hybrid Microspheres. The procedures of drug loading and release were performed according to the literature [18]. Briefly, the drug-loading experiment was carried out by dispersing 0.1 g of the microspheres into 20 mL of the PBS solution containing 5 mg/mL of VH. After being incubated at 37°C for 24 h, the mixture was centrifuged and the clear supernatant was collected for analysis by UV (6100S, METASH) at 281 nm. For the drug release test, the VH-loaded microspheres (0.1 g) were immersed in 10 mL of PBS at 37°C. At selected intervals, 3 mL aliquots were withdrawn and analyzed with the UV spectrophotometer. All the tests were performed in duplicate, and the data were reported as mean ± standard deviation (SD).

3. Results and Discussion

Figure 1 displayed the SEM images of the CS and CS-SiO_2 hybrid microspheres, which revealed that the SiO_2 content exerted a great influence on the dispersity and morphology of the hybrid microspheres. Among these specimens, the CS-20%SiO_2 hybrid microspheres exhibited the most desirable morphology with good spherical shape and high dispersity (Figure 1(b)). Their average particle diameter was determined to be about 24.0 μm. It was also found that there were a few fragments existing in the CS-20%SiO_2 samples, probably due to the increase in the brittleness of the microspheres with the introduction of the SiO_2 phase. Even though most of the pure CS microspheres also presented an approximately spherical form, they are more or less agglomerated together. This was inferred that the uniform hybrid of CS with SiO_2 effectively strengthened the CS microspheres, thus producing a relatively stiff network. Moreover, it was indicated by comparing the insets of Figures 1(a) and 1(b) that, after the addition of SiO_2, the microspheres exhibited a relatively rough surface. However, as the content of silica increased up to 40 wt%, the viscosity of the CS-SiO_2 hybrid sol will be enhanced correspondingly, eventually resulting in an increased average particle size to 28.0 μm and a slight adhesion between particles (Figure 1(c)).

The FT-IR spectra of pure CS and CS-SiO_2 hybrid microspheres were illustrated in Figure 2(a). The pure CS microsphere showed a wide band in the region of 3300-3500 cm^{-1}, assigning to the stretching vibrations of the N-H groups and/or the O-H groups. It was also observed that characteristic signals at 1662 and 1569 cm^{-1} may be attributed to C-O stretching and N-H stretching, respectively [19]. Moreover, the characteristic absorption peaks of the Si-O-Si groups at 448 and 793 cm^{-1} appeared in the spectra of the CS-SiO_2 hybrid microspheres [20], and the intensity of those peaks increased gradually with the increase of the SiO_2 content. A broad adsorption band of the CS-SiO_2 hybrid microspheres was centered at 1041 cm^{-1}, which was associated with the stretching vibrations of Si-O-C groups overlapping with those of the Si-O-Si groups [21]. The presence of this band confirmed the hybridization of silica with CS [22]. Figure 2(b) shows the XRD patterns of the microspheres. Pure CS microspheres exhibited a diffraction peak centered at about 19°. As observed in the XRD pattern of the CS-SiO_2 hybrid microspheres, no obvious diffraction peak was assigned to the SiO_2 phase, indicating its amorphous structure. However, with the increase of the SiO_2 content, the diffraction peak of

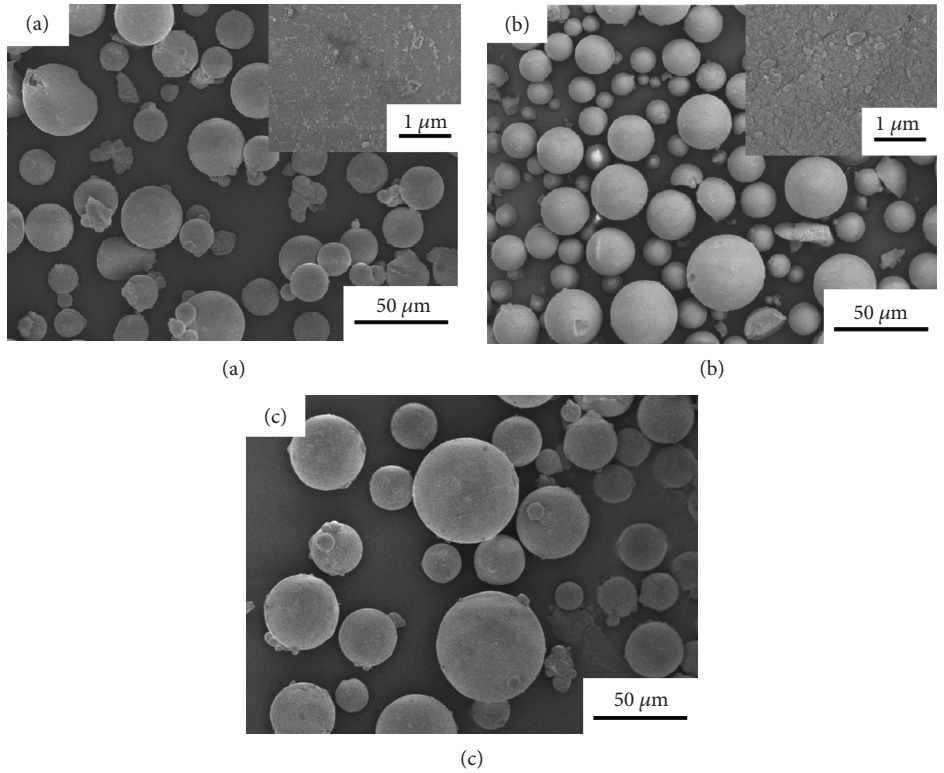


FIGURE 1: SEM images of (a) pure CS, (b) CS-20%SiO_2, and (c) CS-40%SiO_2 hybrid microspheres. The insets of (a) and (b) showed the high-magnification SEM images of the pure CS and CS-20%SiO_2 microspheres, respectively.

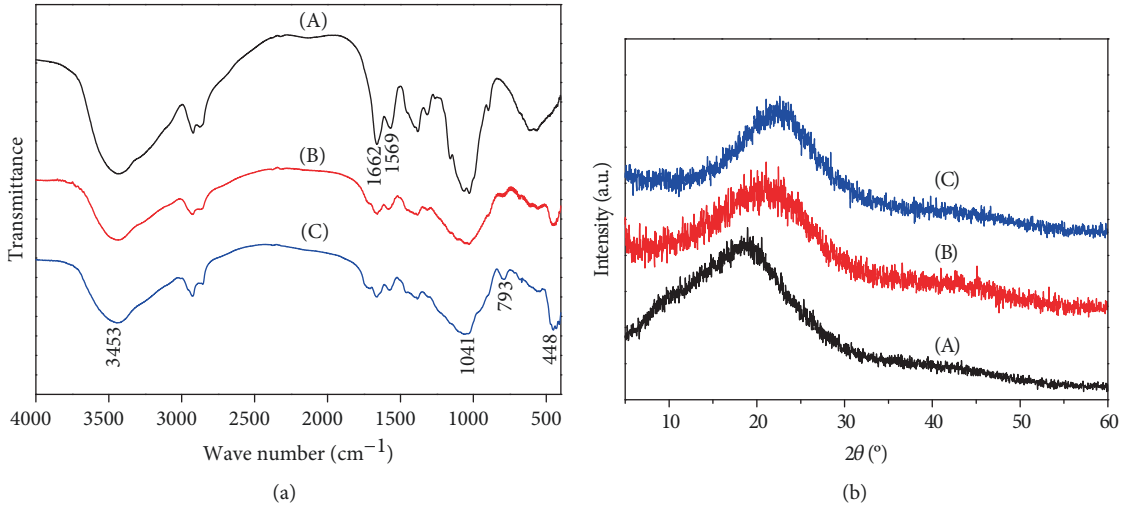


FIGURE 2: (a) FT-IR spectra and (b) XRD patterns of (A) pure CS, (B) CS-20%SiO_2, and (C) CS-40%SiO_2 hybrid microspheres.

CS was found to shift to a higher 2θ value and became less sharp, indicating the possible interaction between the SiO_2 and CS phases. In combination with the FI-IR and XRD results, it was confirmed that the inorganic phase in the CS-SiO_2 hybrid microspheres prepared herein was amorphous silica.

The thermal behavior of the CS-SiO_2 hybrid microspheres was investigated by TG/DSC. As shown in Figure 3(a), both the CS and CS-SiO_2 hybrid microspheres had almost exactly the same weight loss steps. The large weight loss occurring in the region of 200-600°C was probably associated with the decomposition of CS as well as the progressive polycondensation and dehydration of silica xerogel [23], corresponding to the strong exothermic peak in the DSC curves (Figure 3(b)). After deducting the residual amount of the pure CS microspheres (~3 wt%), the SiO_2 contents of the CS-20%SiO_2 and CS-40%SiO_2 hybrid microspheres were determined from the TG curves

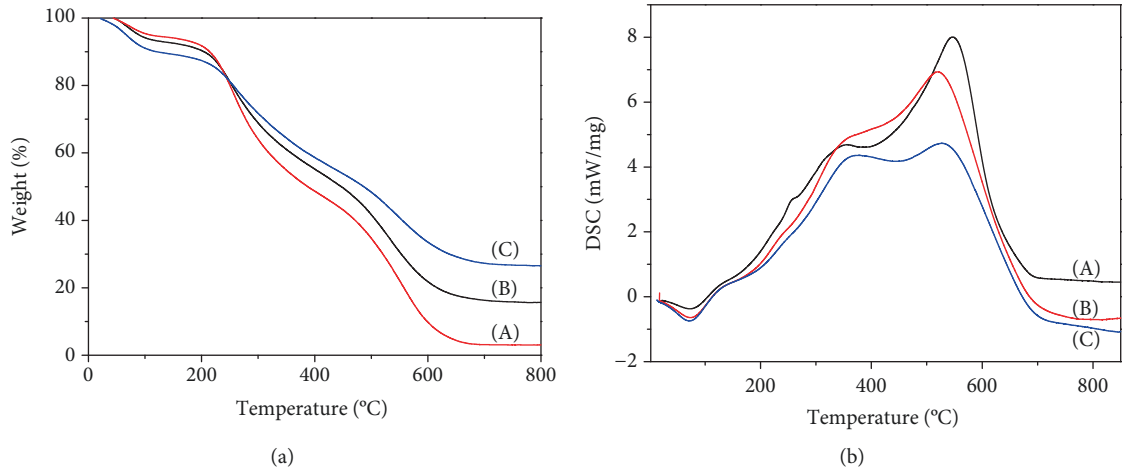


Figure 3: (a) TG and (b) DSC races of (A) pure CS, (B) CS-20%SiO_2, and (C) CS-40%SiO_2 hybrid microspheres.

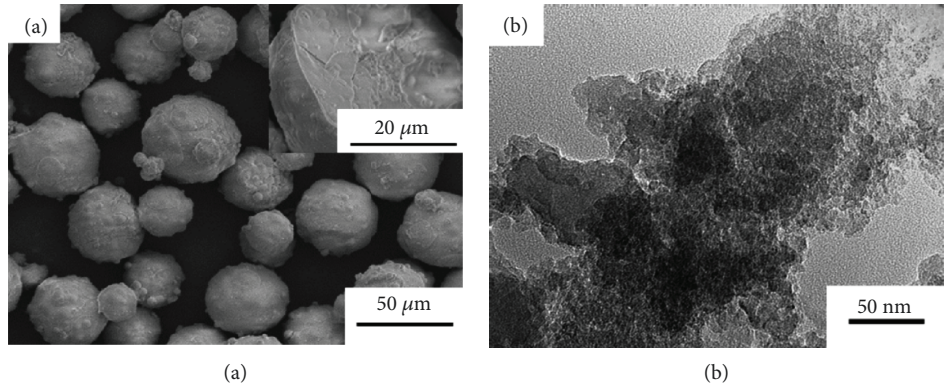


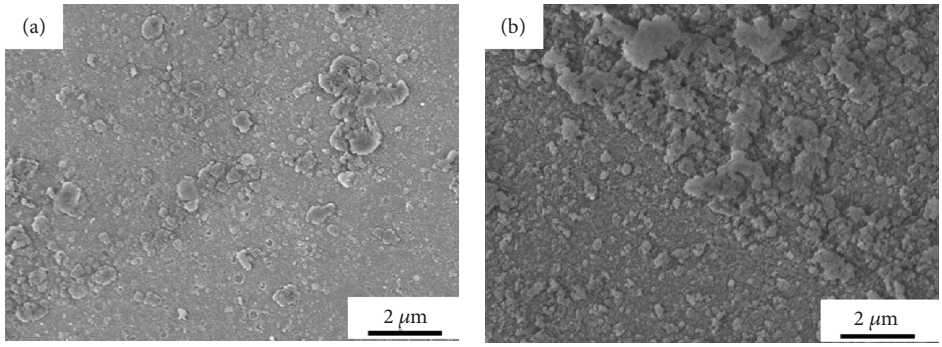
Figure 4: (a) SEM and (b) TEM images of the CS-20%SiO_2 hybrid microspheres after calcination at 600°C for 4 h.

to be about 13 wt% and 23 wt%, respectively, lower than their theoretical values. This was probably attributable to the partial loss of SiO_2 with the squeezed water during the crosslinking process. The addition of NaOH had a negligible effect on the final content of SiO_2, which was confirmed by our experiments.

To further verify the uniform hybrid of silica xerogel within the CS matrix, the CS-20%SiO_2 hybrid microspheres were calcined at 600°C for 4 h. As shown in Figure 4(a), after removal of CS by calcination, the microspheres maintained the spherical shape well and the particle size had a little change before and after calcinations although the surface turned out to be rougher. In addition, it was observed from the cross-sectional SEM image of the calcined microspheres shown in the inset of Figure 4(a) that their internal structure was very similar with the surface one, and no obvious collapse occurred during calcination. The TEM image of the crashed microspheres after calcination presented a porous structure composed of many closely packed nanopores (Figure 4(b)), which was consistent with the morphology of porous SiO_2 reported by other authors [24]. From the above analysis, it was confirmed that the SiO_2 phase was homogeneously hybridized with CS.

In vitro bioactivity is considered as one of the most important characteristics of the biomaterials for bone tissue regeneration. It was usually evaluated *in vitro* by the formation ability of bone-like apatite on the surface of the materials after immersion in the SBF solution for a period of time [25]. It was observed in Figure 5(a) that only a small amount of the mineral phase was deposited on the pure CS microspheres after 3 days of immersion. In contrast, the CS-20%SiO_2 hybrid microspheres showed a vigorous precipitation of bone-like apatite nanoparticles on the surface (Figure 5(b)), and the morphology of the particles was very similar with those reported in the SiO_2-related literatures [26, 27]. Such a result indicated the greatly improved biomineralization capacity of the CS microspheres by the uniform hybrid with silica xerogel.

Vancomycin hydrochloride (VH) was selected as a model drug and loaded into the microspheres. It was revealed in Table 1 that the CS microspheres exhibited good drug entrapment efficiency and drug-loading capacity mainly due to their strong interaction with the drug molecules *via* hydrogen bonding or ionic interaction. However, both drug entrapment efficiency and drug-loading capacity of the microspheres decreased gradually with the increase of the SiO_2 content. Even though the SiO_2 xerogel had been reported to also have strong adsorption ability of drugs by virtue of the abundant –OH groups on their surface [28], the mechanical strengthening effect of silica as an inorganic



(a) (b)

Figure 5: SEM images of (a) pure CS and (b) CS-20%SiO_2 hybrid microspheres after being soaked in the SBF solution for 3 d.

Table 1: Drug encapsulation efficiency and drug-loading capacity of the microspheres.

Samples	Drug encapsulation efficiency (%)	Drug-loading capacity (%)
CS	19.2 ± 1.5	7.7 ± 0.3
CS-20%SiO_2	17.6 ± 1.1	7.1 ± 0.2
CS-40% SiO_2	16.8 ± 0.9	6.7 ± 0.2

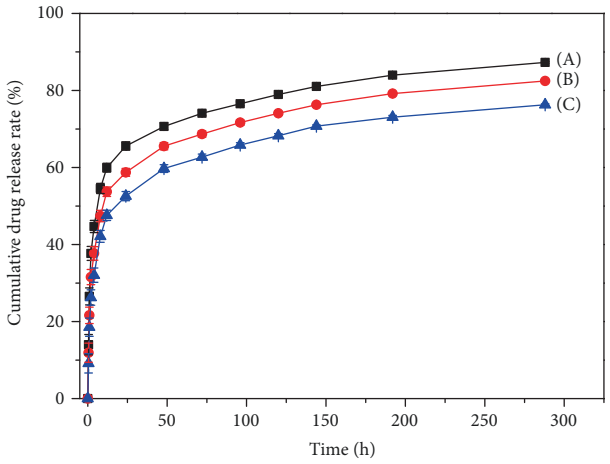


Figure 6: Drug release profiles of (A) pure CS, (B) CS-20%SiO_2, and (C) CS-40%SiO_2 hybrid microspheres.

phase for the CS microspheres would restrain their swelling behavior. Namely, the CS-SiO_2 hybrid microspheres with a higher SiO_2 content will have a higher adsorption ability but only a limited diffusion capability of drugs into the weakly swollen microspheres.

The cumulative release profiles of VH from the CS and CS-SiO_2 hybrid microspheres were depicted in Figure 6. It was implied that the release behaviors of all the microspheres typically consisted of two stages. The first stage was the burst release of VH within 12 h, which was attributed to the rapid dissolution of VH adsorbed on the surface of the microsphere or embedded in the surface layer. After 12 h of test, the cumulative amounts of VH released from the pure CS, CS-20%SiO_2, and CS-40%SiO_2 microspheres were determined as 60.0%, 53.7%, and 49.1%, respectively. In contrast, at the second stage, the VH release from 12 up to 288 h was slowed down greatly *via* gradual diffusion of the entrapped drug through the microsphere network. Moreover, the release rate during this period was decreased with the increase of the SiO_2 content, indicating that the CS-SiO_2 hybrid microspheres were more effective in releasing the drugs in a sustained manner than the pure CS microspheres. The improved drug release behavior of the CS-SiO_2 hybrid microspheres can be ascribed to their good morphologies as well as the presence of SiO_2. On the one hand, the regular shape and high dispersity of the hybrid microspheres allowed the drug to diffuse out of the microspheres more controllably and constantly. On the other hand, the abundant –OH groups as well as the strengthening effect of the SiO_2 xerogel in the hybrid microspheres would be beneficial to the sustained release of drugs. In addition, even after 288 h of test, the release of VH from all the three samples was still maintained at a comparable rate, and the cumulative amount of VH released from the pure CS, CS-20%SiO_2, and CS-40%SiO_2 microspheres reached to be about 87.2%, 82.4%, and 76.3%, respectively.

4. Conclusions

A combined process of sol-gel and emulsification-crosslinking was applied to fabricate the CS-SiO_2 hybrid microspheres in a water-in-oil emulsion. The SEM observation presented that the CS-20%SiO_2 hybrid microspheres with an average particle diameter of about 24.0 μm had the most desirable morphology. The phase composition of the microspheres was confirmed by the FT-IR, XRD, and TG/DSC measurements. After being soaked in the SBF solution for 3 days, the CS-SiO_2 hybrid microspheres were covered with bone-like apatite particles, indicating their good *in vitro* bioactivity Moreover, the CS-SiO_2 hybrid microspheres exhibited a slightly lower drug-loading capacity but a more sustained release behavior than their CS equivalents, thus potentially severing as a suitable drug carrier for bone tissue engineering.

Data Availability

The data used to support the findings of this study are included within the article. Any more specific details in the data will be delivered by the corresponding authors upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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