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Ultrasonic Controlled Morphology Transformation of Hollow Calcium Phosphate Nanospheres: A Smart and Biocompatible Drug Release System

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The studies of delivery carriers and controlled release of drugs have attracted considerable attention.[1] The synthesis of nanoparticles for applications as drug delivery in vivo is a vigorous area because ideal carriers should be small enough to travel unimpeded through the vasculature.[2] Hollow spheres become extremely attractive because they can enhance the load quantity greatly. The suggested candidates are lipids, polymer materials,[3] and porous inorganic materials such as silica-based materials.[4] Though these novel materials can improve total intake of drugs, they also bring new problems, for example, uncontrolled release kinetics and unreasonable metabolism pathway of the carriers.[5] To solve these problems, we select a biocompatible mineral phase, calcium phosphate, as the carrier material. Calcium phosphate is native to the body because it is a principle component of hard tissues such as bone and tooth enamel.[6] Therefore, the problem of the metabolism pathway should be greatly reduced, and this compound has a unique potential for the delivery of bone and dentin therapeutics. The most important feature is that the hollow-structured calcium phosphate nanospheres can be collapsed and transferred into the pin-shaped crystallites under ultrasonic treatment. During this transformation, the encapsulated compounds can be released. Ultrasound is noninvasive in the biological application, and it can penetrate deep into the interior of the body. The advantage of ultrasound is that it can be carefully controlled through a number of parameters including power density, duty cycles, and time of application.[7] Thus, ultrasonic treatment can be used to precisely regulate the morphology transformation to achieve the smart on/off and kinetic controls of drug release. Besides, the formed pin-like calcium phosphate crystallites have behaviors similar to those of the biological hydroxyapatite (HAP).[8] Here, we suggest that a combination of these hollow calcium phosphate nanospheres and the ultrasonic treatment can be developed to an ideal system for drug delivery and release.

The hollow calcium phosphate nanospheres were synthesized by an ultrasonic-assisted wet chemical reaction, and cetyltrimethylammonium bromide (CTAB) was used as a

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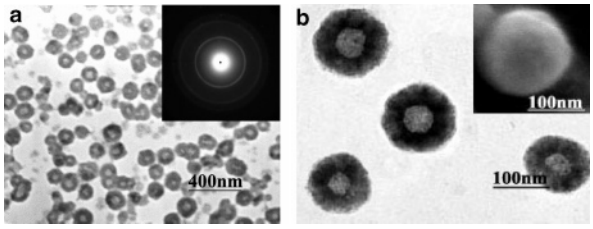

Figure 1. (a) TEM and SEAD pattern of the hollow calcium phosphate spheres. (b) TEM (higher magnification) and SEM of the nanospheres. The particles had a size distribution of 145 ± 20 nm (Figure S6, Supporting Information).

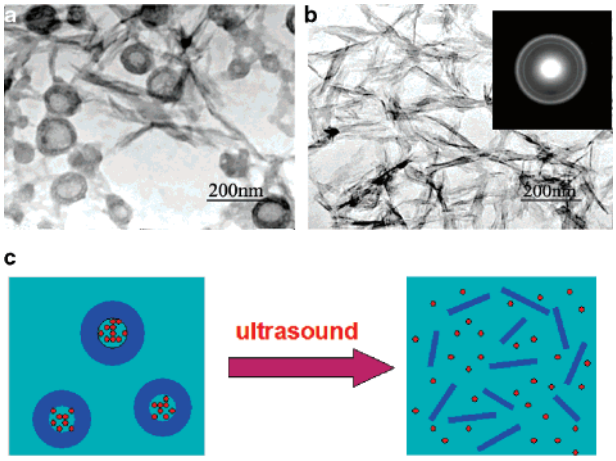

Figure 2. (a) TEM of an interim stage, the coexistence of original hollow spheres, and transferred pin-like crystallites (1 min of ultrasonic application); (b) TEM and SAED of the final stage (5 min ultrasonic application) of the transformation. (c) Scheme of a suggested drug release system.

modifier (detailed information was provided in Supporting Information). Transmission electron microscopy (TEM) showed that the uniform calcium phosphate spheres were formed and they were well mono-dispersed in the solutions (Figure 1a). The edge thickness contrast with the core of the spherical particles because of the higher density of the calcium outer shell thus confirmed that the interior of the spheres was indeed hollow. Their sizes ranged from 110 to 180 nm, and the average diameter was around 145 nm, which was confirmed by TEM, scanning electron microscopy (SEM), and dynamic light scatter (DLS, Figure S6, Supporting Information). The thickness of the shells was about 45 nm; thus, these nanospheres always had ~60 nm-sized cavities, which could be used to load drug. HAP-like phase (Figures 1, S3, and S4, Supporting Information) was detected in the nanospheres by using X-ray diffraction (XRD) and selected area electron diffraction (SAED). The results also indicated that the crystallinity of the nanoparticles was poor and the amorphous phase of calcium phosphate was present in the spheres.

These hollow nanospheres were stable in air or in the aqueous solutions without ultrasonic application. Only a slight aggregation was observed in the solutions, but their hollowed structures were not altered. However, if an ultrasonic treatment (40 kHz, 150 W) was applied, the hollow structures were deconstructed and pin-like nanocrystallites of calcium phosphate resulted (Figure 2b). This transformation kinetic was fast, and only after 4 min did the hollow spheres disappear under TEM and all calcium phosphate particles turn pin-shaped. In situ SAED indicated that these

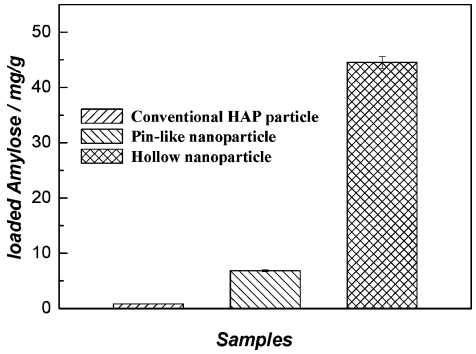

Figure 3. Maximum loads of amylose in hollow nanosphere, transferred pin-like nano crystallites and conventional rod-like HAP particles (400 nm in length and 30 nm in width).

pin-like calcium phosphate crystallites were also HAP-like, and the crystallinity was improved after the transformations (Figures S3 and S4, Supporting Information). Figure 2a illustrated an interim status of the transformation after 1 min ultrasonic treatment: the coexistence of the hollow spheres and pin-like crystallites was observed. If drug molecules were pre-loaded in the cavities of the calcium phosphate nanospheres, they could be released to the bulk solution via the ultrasonic induced morphology transformation (Figure 2c). This release pathway was different from the free penetrations and diffusions of loaded drugs in the other drug carrier systems. Besides, the resulted calcium phosphate nanocrystallites were similar to the HAP in tooth enamel,[9] implying that they were also highly biocompatible.

Ultrasound is one of the most promising external triggers for drug delivery in which the release rate of the incorporated drug can be altered by applying ultrasonic irradiation from the outside surroundings.[10] To study the dynamics of drug release from the hollow calcium phosphate nanospheres and the regulation effect of ultrasound, amylose was pre-loaded (Supporting Information). A color reaction between the released amylose molecules from the cavities and exterior iodine in bulk solution was used to trace the dynamical process accurately. The individual amylose and iodine solutions were colorless and brown, respectively. When they met together, the blue complex was formed and its maximum adsorption peak was at 614 nm (Figure S2, Supporting Information), which could be used to determine the amylose concentration quantitatively. The adsorptions of amylose onto the conventional HAP[11] (Figure S1) and onto the resulting pin-like nanocrystallites (collected at the end of morphology transformation) were also studied. As was expected, the hollow nanospheres had the highest load quantity, which was ~6.5 times and ~50 times those of the resulted pin-like and conventional HAP particles, respectively (Figure 3).

The amylose molecules were stably entrapped under "a storage condition"; the amylose-encapsulated calcium phosphate nanospheres could also remain in a well-dispersed status within at least 1 day in their 0.3% slurry, and the loaded amylose hardly escaped from the cavities (curve 1, Figure 4). Without the ultrasound, the amylose concentration

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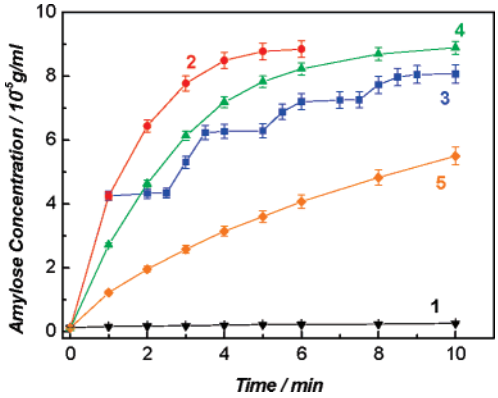

Figure 4. Curves of release kinetics of amylose from the hollow nanospheres under different experimental conditions: (1) no ultrasonic application; (2) continuous ultrasonic treatment (150 W); (3) 1 min treatment of ultrasound (150 W), the interval of duties was 1.5 min; (4) continuous ultrasonic treatment (100 W); and (5) continuous ultrasonic treatment (50 W).

in the bulk solution was kept at $\sim 1.6 \times 10^{-6}$ g/mL; this value contributed to the reaction of iodine and the adsorbed amylose molecules on the out-layers of the nanoparticles, which could not completely be eliminated during the washing. However, it was important that the amylose concentration was almost unchanged with time, indicating that no extra amylose was released from insides of the devices. When the continuous ultrasonic (40 kHz, 150 W) treatment was applied, the amylose concentrations in the bulk solutions were significantly increased, indicating a quick release of the encapsulated amylose from the nanospheres (curve 2, Figure 4). Only after 4−5 min did the concentrations of the amylose approach a steady value of $\sim 8.9 \times 10^{-5}$ g/mL, which was the maximal load amount of the total sample materials. Thus, all the amylose molecules had "escaped" from the broken cavities. It was also noted that this time period, 4−5 min, was in good agreement with the kinetics of ultrasound-induced morphology transformation.

Different from the free and slow diffusion of the encapsulated drugs from the cavity through the shells,[12] the fast release of amylose in this system was triggered by ultrasound in this case. However, the release process could be further controlled by the ultrasonic application. To evaluate the pulsatile release of the drug from the hollow nanospheres, the samples were exposed to ultrasound for 1 min and the interval of the treatments was 1.5 min. The release of amylose started instantly and continued until the irradiation was stopped. Pulsatile release of amylose was repeated, and a stepwise curve of the amylose concentration increasing in the bulk solution was displayed in Figure 4 (curve 3). The platforms of the curve also indicated the good stability of the device in the absence of ultrasonic application. Thus, the on/off control release was achieved, which was especially important clinically. It was also noted that when the same ultrasonic treatment time periods were applied the released amounts of amylose were almost not altered in curves 2 and 3. Furthermore, the dynamics of release could be also conveniently regulated by the power density of ultrasound. When the powers were decreased to 100 W and 50 W, the initial slopes of curves 4 and 5 were dropped to 2.7×10^{-5} and 1.2×10^{-5} g/mL per min, respectively. However, the normalized slope of the curve 2 (150 W) was around 4.3×10^{-5} g/mL per min. It was suggested that the release kinetics in this model system was related to ultrasonic power as well as the treatment time. This feature could be developed to a useful strategy for the smart control of drug release in vivo.

At present, the control of the release kinetics of the most reported drug-delivery nanodevices is very difficult because their pathway is the free detachment and diffusion. However, there are a number of clinical situations where such an approach is not sufficient; for example, diabetic patients require high doses of insulin after meals. Thus, a delivery system that allows real-time control of drug dosage according to alterations in chemical and physiological status is extremely important. Our current study shows a good example of such a smart control. A collapse of hollow structures of the biocompatible and biodegradable calcium phosphate nanospheres offers an effective pathway to release of the pre-loaded drug. This process is precisely controlled by ultrasonic treatments. The release of drug, which is taken into the body, can be on/off triggered, and its kinetics can be regulated by the power density, duty cycle, and time of application of ultrasound.

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Supporting Information Available: Detailed experimental procedures for material synthesis, drug load, release measurement and analysis, and FTIR, XRD, and DLS characterization results data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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