An Efficient Ultrasound-enhanced Controllable Release of Biologically Active Trace Elements on Bioactive Silica-gel-based Material

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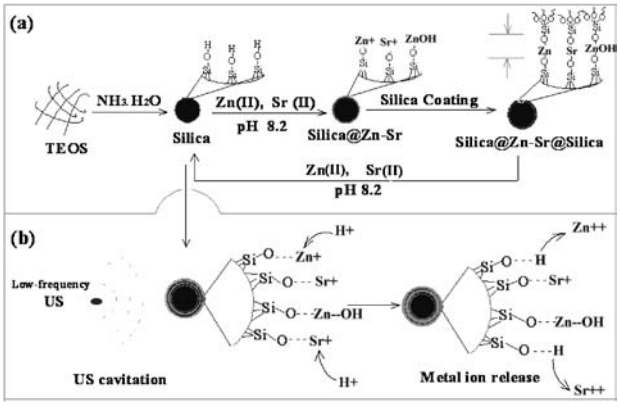
The multilayered Silica@Zn–Sr@Silica nanospheres were fabricated by a layer-by-layer (LbL) assembly route, and controlled release of active trace ions can be enhanced by low-frequency ultrasound, which is potentially advantageous not only to multicomponent administrating but also to elicit synergistic effect for promoting tissue regeneration combined the trace ion activity with ultrasound physical stimulus.

The way in which trace elemental ions are administered has gained increasing attention in nutrition and biomedicine.[1] Normally, a trace element is administered in an appropriate dose only to sustain for several hours or days. Thus, increased attention has been focused on methods of giving trace ions continually for a prolonged time and in a controlled fashion. The primary method of accomplishing this controlled release has been through incorporating trace ions into bioceramics.[2] This method is, however, inadequate to meet needs for a better control of component administration and sustained release of multiple trace elements and sometimes also results in damaging side effects for a long treatment.[3]

In recent years, utilization of external stimulus, such as ultrasound (US), has successfully provided alternative treatment in the case of polymer degradation and volume phase transition.[4] Actually, low-frequency US is now widely used form of physical stimulation to promote drug delivery and fracture healing by its cavitational effect.[5] Therefore, this technology spanning to the administration and controlled release of active trace ions with desired dose levels from the biomedical materials is possibly significantly beneficial for enhancement of therapy efficacy.

The present study evaluated the controlled release of the silica-based nanospheres with trace elements by using zinc and strontium ions as in vitro model with emphasis on possible effects of US on the trace ion release (Scheme 1). Silica gel is chosen as a reservoir because silica gel posses not only the essential trace element of silicon for bone tissue regeneration (at a dose of 30–80 ppm Si) but also negatively charged surface terminated with high-density silanol groups which aid the localization.[6] Zinc provides the structural framework for zinc fingers that regulate the function of genes in the nuclei of cells. Zinc can also increase bone protein contents and promote cell proliferation (≈3 ppm).[7] Similarly, the therapeutic potential of strontium has been primarily used as markers of calcium metabolism and enhanced the skeletal strength (≈20 ppm).[8]

A typical experimental strategy was as follows: the monodisperse silica-gel nanospheres (SGNs, ≈70 nm) were prepared by controlled hydrolysis of silicon alkoxide firstly. The electrostatic adsorption of zinc and strontium ions with a concentration (x, y) of 0.2, 1.0, or 5.0 mM on the SGNs (240 mg) was performed in a 200 mL $Zn(NO_3)_2$–$Sr(NO_3)_2$ solution (pH 8.0, 25 °C) with a 0.2 mM NaCl electrolyte under stirring for 8 h (denoted as Silica@Zn(x)–Sr(y)). The nanospheres were then coated with a thin wall of silica (≈2 nm) by hydrolysis of silicon alkoxide (denoted as Silica@Zn(x)–Sr(y)@Silica). Thereafter nanospheres were LbL assembled with metal ions and silica gel alternately until the desired multilayers were achieved. The nanospheres were washed with distilled water with a pH condition of 8.0 before each procedure. To determine the effect of US on ion-released concentration, the multilayered nanospheres were dispersed into a 50 mL of HCl–Tris buffer solution (pH 7.4, 37 °C) under mechanical stirring (120 rpm) in a 1000-cm^3 water tank with a US generator (40 kHz, 80 and 150 W). When the suspensions were stirred for 1 h, the US was also applied to initiate desorption of metal ions, and the temperature was maintained at 37 °C by a constant-temperature circulator in water tank. The release medium (2.0 mL) was removed at the scheduled time points (US working for 0, 5, 10, 15, 20, and 40 min, respectively) and centrifuged for Zn and Sr ion concentration measurement using an inductively coupled plasma atomic emission spectroscope (Vista AX). The nanospheres without US exposure were also measured as controls.



Scheme 1. Schematic procedure to generate Silica@Zn–Sr@Silica nanospheres and metal ion controlled release mediated by US.

Figure 1 shows representative TEM (JEOL JEM 2010) and EDX (INCA EDAX) images of as-prepared Silica@Zn5–Sr5 assembled LbL for 4 cycles. It was observed that the nanospheres are uniform both in size and shape (Figure 1a), and the element

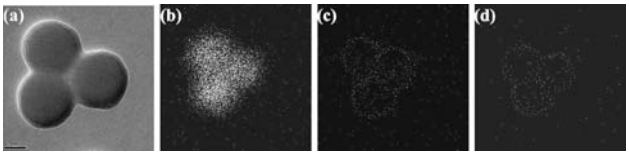


Figure 1. TEM (a) and EDX images of the elemental distribution obtained for Si (b), Zn (c), and Sr (d) of Silica@Zn5–Sr5@Silica nanospheres LbL assembled for 4 cycles.

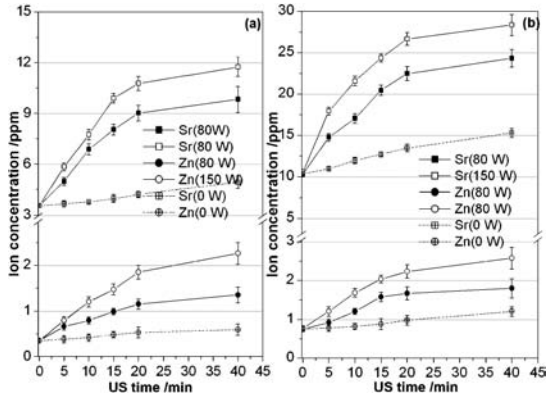


Figure 2. Effect of US on in vitro release of trace elemental ions of Silica@Zn5–Sr0.2 (a) and Silica@Zn5–Sr1 (b), the error bars represent the standard deviation ($n = 3$).

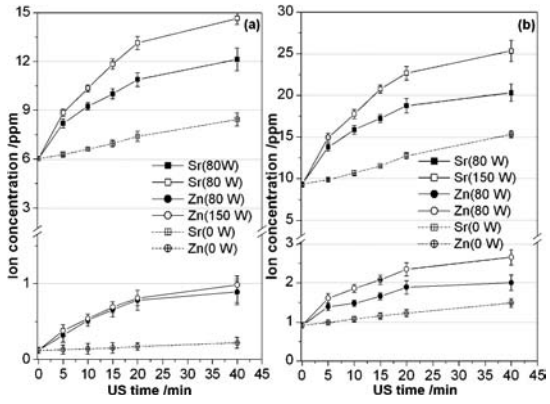


Figure 3. Effect of US on in vitro release of trace elemental ions of Silica@Zn0.2–Sr1 (a) and Silica@Zn1–Sr1 (b), the error bars represent the standard deviation ($n = 3$).

distribution images clearly reveal that Zn and Sr are incorporated into the SGNs (Figures 1b–1d).

As the adsorption ratio is concerned, the adsorption of Zn onto silica gel reached a constant level of 99.0% with an initial concentration ranges of 1–5 mM, whereas only 65.0–70.0% of the Sr was successfully anchored on the silanol groups in the same concentration ranges. Meanwhile, minor amount of metal ions release into coating solution, and the effective adsorption ratio decreased by 3–5% after silica coating, implying that the Sr and Zn ions exhibit apparently different adsorption capacity under the same pH condition, and the competitive adsorption is significant. Moreover, it can be found that the adsorption ratio on the surface of silica-gel coating (2nd cycle) by utilizing reproducible LbL assembly is effectively sustained.

The low-frequency US influence on controlled release behavior of metal ions (destroying the ionic charge) focused on two powers was determined by measuring the change in ion concentration as a function of time. Figures 2 and 3 show ion concentration in solutions under the physiological pH condition. The Zn and Sr concentrations without US utilization ($t = 0$–40 min) were only increased up to 0.6–1.2 and 4.9–15.0 ppm, respectively, which were both far lower from the expected dose levels for enhancing efficacy on bone regeneration. However, Zn and Sr ions elevated to higher concentrations significantly within 20 min by US stimuli (2–3-fold) and thereafter retaining steady concentrations. Moreover, increase of US intensity elicits favorable up-regulation of both Zn and Sr releases from the nanospheres.

As particularly noted, the storage capacity is an important factor at various ratios for both ion releases. It was observed that the Sr dose level was sustained at 15 ppm from Silica@Zn5–Sr0.2 system after 20 min of US stimulation (Figure 2a), the Sr concentration increased up to 30 ppm in Silica@Zn5–Sr1 with fivefold of Sr capacity compared with Silica@Zn5–Sr0.2 (Figure 2b). Similarly, more Zn ions released from Silica@Zn1–Sr1 with fivefold of Zn capacity compared with Silica@Zn0.2–Sr1, and furthermore US power had no positive effect on Zn release from the system with low Zn capacity (Figure 3). These findings confirm that Zn ions are essentially more strongly anchored on the silanol groups by ionic charge than Sr ions, and the appropriate high Zn storage capacity must be considered with Sr for a better dose range on demand within short time by US stimulus.

Additionally, we found that the Si concentrations (as the soluble silicate anion) were also increased up to a range between 40 and 43 ppm from the Silica@Zn5–Sr0.2 and Silica@Zn5–Sr1 systems, about 1.5-fold higher than the Si dose level without US physical stimulus, suggesting that Si release can be also strengthened by the environmental ultrasound activation.

In summary, we firstly explored the feasibility of cooperative assembly of binary Zn and Sr ions in a silica-gel nanospheres and ultrasound-responsive controlled release for a short time stage. Our results suggest that the trace metal ion release can be enhanced by ultrasound stimulus. Such new bioactive material offers a potential alternative treatment in the case of bone defect combined with the noninvasive US therapy daily that may accelerate the rate of bone regeneration and implant bioabsorption.

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