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A Bio-inspired Route to Fabricate Submicrometer-Sized Particles with Unusual Shapes − Mineralization of Calcium Carbonate within Hydrogel Spheres

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Submicrometer-sized inorganic−organic composite particles with disklike, cell-like, and hemispheroidal shapes have been fabricated by in-situ mineralization of $CaCO_3$ within hydrogel spheres of poly(*N*-isopropylacrylamide-*co*-(4-vinylpyridine)) (PNIPVP). The shape and functionality of the composite particles obtained may be manipulated by incorporating nanocrystals into hydrogel sphere templates. Our methodology paves a novel way to create sub- or micrometer-sized particles with complex shapes, inspired by biomineralization embodied in single-cellulous organisms. Also, the composite particles obtained present a transition phase between soft and hard spheres, which may self-organize into non-close-hexagonal packing structures.

Introduction

Currently, the study of sub- or micrometer-sized colloidal particles is one of the most dynamic interdisciplinary fields, spanning chemistry, materials sciences, biology, and electronic engineering.[1] Colloidal particles may be used as models for condensed matter physics, as scaffolding templates, or as building blocks for creating hierarchical nanometer- or micrometer-scale architectures. Monodispersed colloidal particles, particularly of polymer and silica, may be produced in routine ways. Most of these particles have a spherical shape, due to the isotropic nature of amorphous or disordered structures. The formation of other than spherical shapes, such as ellipsoids and cubes, is materials-specific, demanding deliberate studies of particle formation and growth mechanism.[2] The use of preformed objects as templates enables one not only to alter the chemical composition of the particles, some of which are hard to use for creating colloidal particles, but also to define the shapes of particles, replicating the structures of the templates used.[3]

Biological organisms have currently been recruited as templates to create inorganic−organic composite particles, mimicking the diverse structural complexity embodied in nature.[4] They, however, are difficult to access, especially in large scale. Adopting principles and ideas behind biomineralization in nature, one has employed a growing number of synthetic analogues, such as vesicles and micelles, to template the crystallization of minerals, which opens up a bio-inspired route to fabricate inorganic−organic composite particles.[4]

Hydrogels provide appealing replacements of biological organisms thanks to their biocompatibility, aqueous inner environment, porous structure, and facility of conjugation with various biological macromolecules such as proteins.[5] The huge demand for tissue engineering scaffolds has recently promoted substantial efforts toward biomimetic mineralization in hydrogel matrixes, either natural or synthetic ones.[6] Although hydrogel spheres found their way in pharmaceutics, serving as delivery vehicles of drugs, proteins, and genes,[5] the biomimetic mineralization within them has been little touched. Nevertheless, in nature, there exists a great deal of examples of biomineralization in biological hydrogel spheres to sculpt biominerals into diverse forms, associated with specific biological functions.[4] One example is the compound eyes of insects, each of which has a $CaCO_3$ crystal embedded in its organic gel.[7]

In the current work, we conducted in-situ mineralization within hydrogel spheres to create inorganic−organic composite particles with unusual shapes, including disks, cells, and hemispheroids. As compared to the existing templating

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methods of preparing inorganic/organic composite particles, our approach offers a number of advantages. First, hydrogel spheres, conjugated with biological macrolecules such as proteins and genes especially, present synthetic analogues of single-cell compartments. Using them as templates, our approach opens up a way to emulate biomineralization in single-cell organisms, enabling one to simply create inorganic−organic composite particles with defined dimension and complex forms. Second, the shape and functionality of the composite particles obtained may be manipulated by loading nanoparticles into gel spheres. Third, mineralization within hydrogel spheres creates transition particles between soft and hard particles, which may self-organize into unusual ordered arrays. Using the composite particles obtained, herein we demonstrate the potential of formation of non-close-ordered packing. The present work represents the first report on biomimetic mineralization within hydrogel spheres.

Experimental Section

Materials. *N*-Isopropyl acrylamide (NIPAM), *N*,*N*′-methylenebisacrylamide (MBA), 4-vinylpyridine (4-VP), potassium persulfate ($K_2S_2O_8$), calcium chloride ($CaCl_2$), ammonium carbonate ($(NH_4)_2CO_3$), cadmium chloride, tellurium, thioglycolic acid, chloroauric acid ($HAuCl_4$), sodium citrate, and tannic acid were purchased from Sigma-Aldrich. NIPAM was purified by recrystallization from a toluene/hexane mixture (1:3); 4-VP was purified by distillation under vacuum. Other commercial materials were used without further purification.

Synthesis of Hydrogel Spheres. PNIPVP microgel particles were prepared by a surfactant-free emulsion polymerization. Typically, 250 mg of NIPAM, 10 μL of 4-VP, 20 mg of $K_2S_2O_8$, and 25 mg of MBA were dissolved in 25 mL of deionized water in a 50 mL vial. The polymerization was carried out for 4 h at 70 °C under nitrogen atmosphere. The hydrogels were then purified by four successive centrifugations at 10 000*g* for 10 min (Bechman preparative ultracentrifuge), each followed by decantation and redispersion in deionized water. The hydrodynamic diameter of PNIPVP spheres is 675 nm at pH 7 at room temperature, determined by dynamic light scattering (DLS). It increases with the decrease of the environmental pH and reaches the maximum, 750 nm, at pH 3.

Synthesis of Nanocrystals. The 5 nm CdTe nanocrystals capped with thioglycolic acid were produced on the basis of our previous reports.[8] Typically, 22 mL of freshly prepared oxygen-free 5 mM NaHTe solution was added to 125 mL of 13 mM nitrogen-saturated $Cd(ClO4)_2$ aqueous solution at pH 11.2 in the presence of 0.5 mL of 1-thioglycerol as a stabilizing agent. After reflux for 48 h, the aqueous dispersion of 5 nm CdTe nanocrystals was obtained. The concentration of CdTe nanocrystals is 15 mM with reference to Cd. The 5 nm Au nanocrystals were prepared by citrate reduction of $HAuCl_4$ in the presence of tannic acid.[9] Briefly, the reducing solution, containing 2 mL aqueous solution of 1% (w/v) dehydrated sodium citrate and 0.45 mL of 1% (w/v) freshly prepared tannic acid, was rapidly poured into 100 mL boiling aqueous solution of 0.01% (w/v) $HAuCl_4$ under vigorous stirring. Within 10−15 s, the color of the reaction mixture solution became reddish. After boiling for another 5 min under vigorous stirring, followed by cooling to room temperature, monodispersed 5 nm Au nanocrystals were produced. The sizes of the nanocrystals obtained were determined by transmission electron microscopy (TEM).

Loading Nanoparticles within Hydrogel Spheres. On the basis of the pH-sensitive swelling behavior of PNIPVP spheres, we have successfully incorporated differently sized CdTe nanocrystals into their collapsed gel network.[10] In brief, after incubating PNIPVP spheres with 5 nm CdTe nanocrystals at pH 3 for 10 min, the pH of the mixtures was adjusted to pH 7. After removing excess nanocrystals by centrifugation at 2000*g* for 10 min and washing with water, nanocrystals were incorporated within PNIPVP spheres, denoted as CdTe−PNIPVP. The 5 nm Au nanocrystals were mixed with the PNIPVP sphere dispersion at 25 °C for 30 min. After excess nanocrystals were removed by centrifugation at 2000*g* for 10 min and by washing with water, nanocrystals were incorporated within the PNIPVP spheres, marked as Au−PNIPVP.

Mineralization of $CaCO_3$ within Hydrogel Spheres. In-situ mineralization of $CaCO_3$ within gel spheres was conducted on the basis of the method reported by Cölfen and co-worker.[11] In glass vials, the hydrogel spheres with or without loading of nanocrystals were well dispersed in 10 mM $CaCl_2$ aqueous solution. The concentration of PNIPVP is 1 wt %. These vials were put in a closed desiccator where solid $(NH_4)_2CO_3$ was placed as the CO_2 vapor source. All mineralization experiments were carried out at room temperature. Glass slides were placed at the bottom of the reaction vials to collect the inorganic−organic composite particles.

Characterization. DLS measurements were performed by using a commercial laser light scattering spectrometer (Malvern Autosizer 3000) with a 5 mW He−Ne laser. TEM observations were implemented on a Philips CM 120 electron microscope at an acceleration voltage of 80 kV. For all TEM observations, the copper grids used were covered with a thin carbon film. Scanning electron microscopy (SEM) images were recorded by means of a Philips XL30 electron microscope at an accelerating voltage of 3 kV. The specimens were sputtering-coated with gold before SEM imaging. Atomic force microscopy (AFM) imaging was performed by using the Nanoscope III-M system operating in tapping mode. Photoluminescent properties of CdTe nanocrystals and their loaded gel spheres were analyzed with a Spex Fluorolog 1680 spectrophotometer (the excitation wavelength is 400 nm).

Results and Discussion

In-Situ Mineralization of $CaCO_3$ within PNIPVP Gel Spheres. In our work, the mineral chosen here is $CaCO_3$, an abundant mineral in nature. Scheme 1 schematically illustrates the process of in-situ mineralization of $CaCO_3$ within PNIPVP spheres as performed by slow diffusion of CO_2 vapor at room temperature.[11] First, the gel spheres were well dispersed in $CaCl_2$ aqueous solution in a glass vial, and placed in a close desiccator where solid $(NH_4)_2CO_3$ was placed as the source of CO_2 vapor. Hydrogel spheres are porous matrices that may be swollen by aqueous solutions. In our work, the pores within PNIPVP spheres were employed as nanosized containers for mineralization of $CaCO_3$. Once CO_2 vapor slowly diffused into the porous spheres, swollen by the $CaCl_2$ aqueous solution, $CaCO_3$ nanoparticles immediately formed in the pores and agglomerated inside. Due to the accumulation of $CaCO_3$ nanoparticles within the gel matrixes, the gel spheres became

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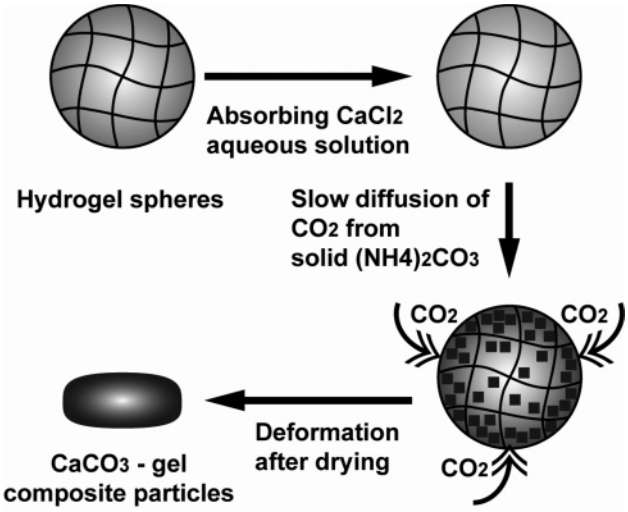
Scheme 1. Schematic Illustration of in-Situ Mineralization of $CaCO_3$ in Hydrogel Spheres



heavier and heavier and precipitated at the bottom of the reaction vial. A glass slide was placed there to collect the precipitates. After the glass slide was removed out of the dispersion and washed with water, the $CaCO_3$-loaded PNIPVP composite particles, marked as $CaCO_3$−PNIPVP, were obtained on the slide.

The formation of the $CaCO_3$−PNIPVP composite particles and their morphology were directly visualized by SEM. Figure 1a shows a SEM picture of disklike $CaCO_3$−PNIPVP composite particles of about 570 nm in diameter obtained by 5 h of calcification. AFM analysis indicated that these disks are 160 nm in thickness. In contrast, as shown in Figure 1b, after water evaporation, the original PNIPVP spheres were deformed into irregular thin sheets of around 30 nm thickness, determined by AFM, which are packed so densely that there are no obvious interstices. This morphology difference should arise from the accumulation of $CaCO_3$ nanoparticles, which to a large extent reduce the volume fraction of water in the gel matrices, leading to a smaller deformation of the composite spheres during water evaporation. In terms of swelling behavior, the $CaCO_3$−PNIPVP composite particles are the transition phase between soft and hard spheres. They may be spherical after swelling by water. Nevertheless, neither can they keep their spherical shape after drying as hard spheres do, nor can they collapse into thin sheets as hydrogel spheres do. Instead, they are deformed into a disklike shape, forming dense inorganic−organic hybrid particles. Intriguingly, as revealed in Figure 1a, the disklike composite particles are organized in a nonclose hexagonal packing array, which is rather different from the close packing structures derived from either hard spheres, such as latex and silica, or soft ones such as pure hydrogel. However, the formation of highly ordered non-close-hexagonal packing remains an open question. To in-situ monitor the swelling behavior of the $CaCO_3$−PNIPVP composite particles is an ongoing research project in our lab.

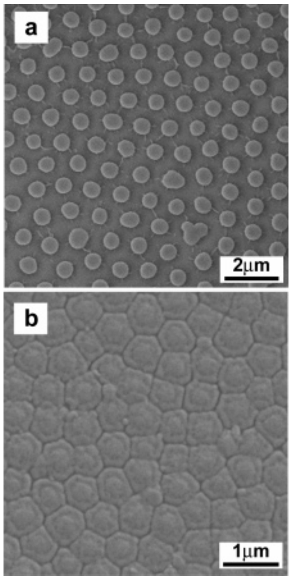


Figure 1. SEM images of the composite particles deposited on the bottom of the reaction vial after 5 h of in-situ calcification within PNIPVP hydrogel spheres (a) and the original PNIPVP hydrogel spheres collected on a glass slide after complete drying (b).

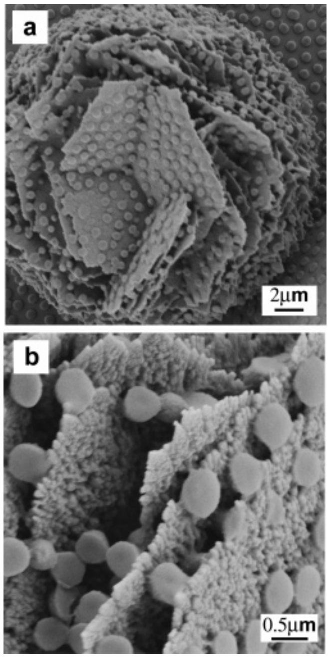


Figure 2. Low (a)- and high (b)-magnification SEM pictures of $CaCO_3$−PNIPVP composite giant aggregates, coexisting with disklike composite particles. These giant aggregates are composed of a number of micrometer-sized flakes, derived from aggregation of $CaCO_3$ nanoparticles formed without hydrogel sphere templating, which stack together, glued by the disklike composite particles.

Furthermore, we found that the disklike $CaCO_3$−PNIPVP composite particles usually coexist with pumpkin-shaped giant spheres (Figure 2). As indicated by the high-magnification SEM pictures (Figure 2b), these giant particles are composed of a number of micrometer-size flakes, derived from aggregation of $CaCO_3$ nanoparticles formed without hydrogel sphere templating, which stack together, glued by the disklike composite particles. The study of the mechanism behind the formation of these giant aggregates is underway.

X-ray diffraction patterns of the $CaCO_3$−PNIPVP composite particles and pumpkin-shaped giant aggregates identify the amorphous nature of the $CaCO_3$ formed. In our work, calcite crystals of tens micrometers in size were usually obtained on the glass slides. Nevertheless, we found that if the glass slides were covered with PNIPVP sphere mono-

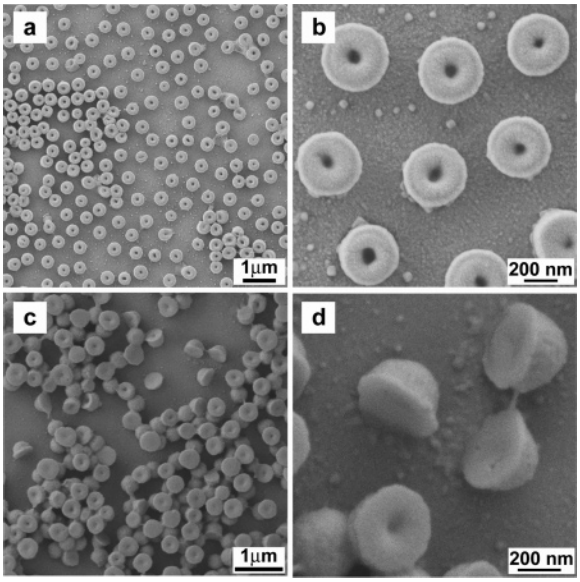


Figure 3. Low-magnification SEM image of the composite particles deposited at the bottom of the reaction vial after 5 h of in-situ calcification within PNIPVP hydrogel spheres loaded with 3 nm CdTe nanocrystals (a) and its high-magnification SEM image (b). Low-magnification SEM image of the composite particles deposited on the bottom of the reaction vial after 24 h of in-situ calcification within PNIPVP hydrogel spheres loaded with 3 nm CdTe nanocrystals (c) and its high-magnification SEM image (d).

layers, no calcite crystals were formed. This suggests that the PNIPVP spheres might inhibit the transformation from amorphous to crystal phase. To investigate the influence of hydrogel spheres on mineralization is an ongoing project in our lab.

In-Situ Mineralization of $CaCO_3$ within CdTe−PNIPVP Gel Spheres. In the current work, as the interaction between $CaCO_3$ nanoparticles and amide and pyridine groups of the gel networks of PNIPVP spheres is rather weak, the gel network has a small influence on calcification and the physical entrapment of the gel network plays the pivotal role in the accumulation of $CaCO_3$ nanoparticles or their aggregates. Biomineralization in nature is usually associated with specific functional groups of organisms that have strong interaction with biominerals, exerting a great influence on crystallization of biominerals. Recently, Au nanocrystals capped with carboxylic acid groups have been employed as nuclei for crystallization of $CaCO_3$.[12] Herein, we loaded aqueous CdTe nanocrystals of 5 nm in size, stabilized with a layer of thioglycolic acid, in PNIPVP spheres, denoted as CdTe−PNIPVP. The loaded CdTe nanocrystals not only add photoluminescence to the resulting composite particles, but their carboxylate groups also create nuclei for mineralization of $CaCO_3$. Figure 3a and b shows typical SEM images of the composite particles obtained by in-situ 5 h mineralization of $CaCO_3$ in CdTe−PNIPVP spheres, marked as $CaCO_3$−CdTe−PNIPVP. The resulting particles are about 400 nm in diameter and have a hole at the center, resembling a red blood cell. The thickness of these cell-like particles is 160 nm as determined by AFM. The formation of holes might be conjectured due to heterogeneous shrinkages or deformation of the $CaCO_3$−CdTe−PNIPVP composite particles obtained. During CO_2 vapor diffusion into the gel sphere, the carboxylate groups on CdTe nanocrystals served as nuclei, causing fast calcification on their surface. The resulting $CaCO_3$-coated CdTe particles agglomerated into a thicker and denser shell in the gel spheres, which could slow or even block diffusion of CO_2 vapor into the center of the gel spheres. This should suppress the calcification in the central part of the gel spheres; in other words, most $CaCO_3$ crystals were localized in the outer shell of the gel spheres, resembling a hollow structure with respect to the population of $CaCO_3$. Obviously, during water evaporation, the center parts of the resulting composite spheres were deformed much larger than the outer shells, driving the formation of a concave structure. The $CaCO_3$−CdTe−PNIPVP composite particles still remain photoluminescent. The influence of calcification on the photoluminescence behavior of CdTe nanocrystals is currently under exploitation.

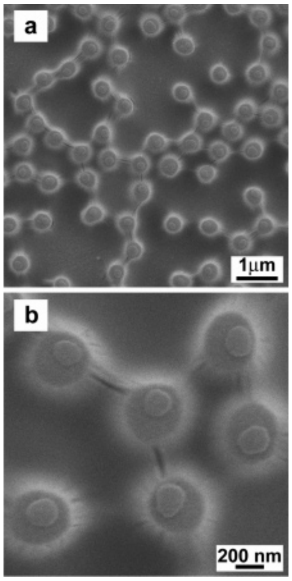


Figure 4. Low-magnification SEM image of the composite particles deposited at the bottom of the reaction vial after 5 h of in-situ calcification within PNIPVP hydrogel spheres loaded with 5 nm Au nanocrystals (a) and its high-magnification SEM image (b).

After the mineralization period was increased from 5 to 24 h, as shown in Figure 3c and d, the cell-like composite particles were converted into hemispheroids of 400 nm in diameter and 210 nm in height. The bottom of these hemispheroids is concave or has a hole at the center. The formation of this hemispheroidal shape results because the prolonged mineralization time allowed CO_2 vapor to reach the center of the composite particles deposited on the bottom of the reaction vial, augmenting their upper parts exposed to the aqueous media. On the other hand, the concave bottom of the resulting hemispheroids suggests the double concave character of the cell-like particles shown in Figure 3a and b.

In-Situ Mineralization of $CaCO_3$ within Au−PNIPVP Gel Spheres. Figure 4a and b shows typical SEM pictures of the composite particles produced by 5 h of in-situ calcification of PNIPVP spheres loaded with 5 nm Au nanocrystals. Disklike particles of 370 nm diameter are observed, each covered with a $CaCO_3$ cap. Similar to the

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previous reports,[12] the citrate groups capped on the surface of Au nanocrystals may serve as nuclei for crystallization of $CaCO_3$. The formation of the $CaCO_3$ cap is difficult to explain at present. It is likely due to the fact that $CaCO_3$ nanoparticles formed without gel templating accumulated on the top of $CaCO_3$−Au−PNIPVP spheres settled on the glass slide.

It is worthy to note that by using PNIPVP spheres loaded with either CdTe or Au nanocrystals as template for mineralization of $CaCO_3$, we usually observed the pumpkin-shaped giant aggregates, coexisting with the cell-like or hemispheroidal composite particles, similar to those shown in Figure 2.

Conclusion

In summary, we implemented in-situ mineralization of $CaCO_3$ within hydrogel spheres or those loaded with inorganic nanocrystals to construct inorganic−organic composite particles with shapes of disk, cell and hemispheroid, which are difficult to achieve by conventional routes. Incorporation of nanoparticles into gel spheres can not only provide a measure to influence mineralization of $CaCO_3$ but also enable one to add more functionality to the composite particles, for example, photoluminescence for loading of CdTe nanocrystals. Our approach evokes a bio-inspired way to create inorganic−organic composite particles with defined size and complex geometry. It can be expected that by using hydrogel spheres, incorporated with various biological nanosized objects such as proteins and genes, as templates, this bio-inspired way should enable one to imitate biomineralization embodied in single-cellulous organisms, creating diverse and complex structures encountered in nature. In the case of mineralization in pure hydrogel spheres, the resulting composite disklike particles are organized into a highly ordered array of hexagonal non-close-packing, reminiscent of compound eyes of moth whose structure provides unique antireflection properties.[13] Our ongoing research project concerns the exploitation of constructing non-close-packing arrays derived from $CaCO_3$ hydrogel composite particles and the investigation of their optical properties.

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