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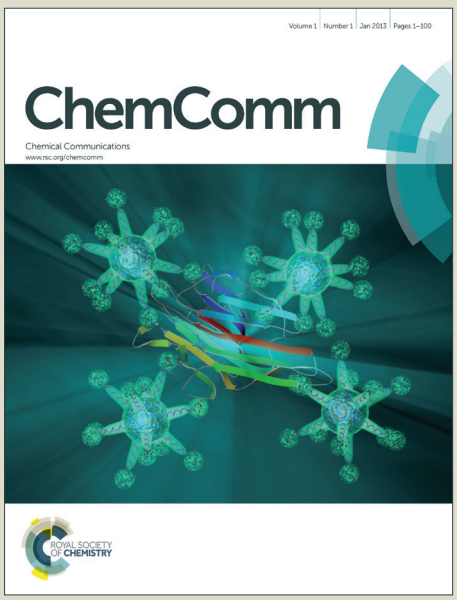

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pH responsive ATP carriers to drive kinesin movement

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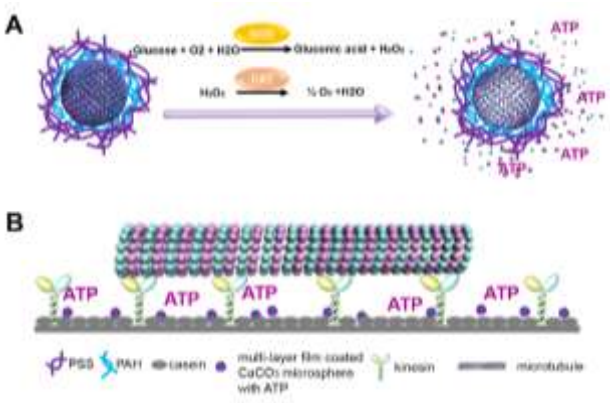
Multilayer film coated $CaCO_3$ microspheres were employed as pH responsive ATP carriers to drive kinesin run. The production of oxygen scavenger in kinesin-microtubule system induces the decomposition of ATP-loaded $CaCO_3$ microspheres and then leads to the release of ATP.

Biomotors like kinesin, myosins and dyneins play an important role in many cellular processes in eukaryotic cells. They have high energy conversion efficiency and force-generating capabilities [1-4]. Recently, several motor-protein driven devices have been successfully built to realize the cargo loading and transportation [5, 6], molecule sorting[7, 8], surface image[9], bio-sensors[10, 11] and so on. However, the great challenges on those systems are smart manipulation of motor-protein movement[1, 12-14]. As energy source, ATP is quite important in the motion generation and the motor driving. Thus to control and create ATP supply will highly affect the motor motion efficiency. We have recently reported the continuous generating of ATP by entrapping the creatine phosphate kinase (CPK) to power kinesin-based system for movement[15]. Several other methods for controlling ATP supply have also been reported such as employing ultraviolet light[16, 17], electric field[18] or microfluidics[19] to control the release of ATP or ATP analogue[16, 20].

Herein, we report a pH-responsive ATP supply system to drive kinesin movement. The inexpensive and easily synthesized porous $CaCO_3$ microspheres have been proven to have great adsorption capacity for numerous molecules and their biocompatibility has also been well exploited[21, 22]. The property that $CaCO_3$ microspheres could be decomposed in acid environment makes them perfect carriers for pH triggered cargo release. In the kinesin-microtubule system, catalase-glucose oxidase-glucose (CAT-GOD-G) mixture is usually added to the motility solution known as oxygen scavenger. However, the continuous generation of gluconic acid resulting in relatively acid surroundings may be harmful for kinesin proteins and microtubules. In our system, to avoid this, the gluconic acids were internal consumed by ATP-loaded $CaCO_3$ microspheres and ingeniously employed as triggers for smart ATP release. Thus $CaCO_3$ microspheres can not only protect the proteins from acid damage, but also act as pH-responsive ATP carriers (Scheme. 1). This system would have a potential in the building of biomotor aided nanodevices.



Scheme. 1 Illustration of the multi-layer film coated $CaCO_3$ microspheres as pH responsive ATP carriers to power the kinesin-microtubule system: A Illustration of CAT-GOD-G mixture triggered ATP release from multilayer film coated microspheres; B kinesin-microtubule system powered by ATP loaded $CaCO_3$ microspheres (All the images in Scheme 1 are not to scale).

The $CaCO_3$ microspheres were synthesized by mixing the $CaCl_2$ solution and Na_2CO_3 solution quickly with vigorous stirring. Since $CaCO_3$ microspheres could be decomposed in acid environment, to protect the microspheres from total collapse, we first coated the microspheres with multilayer film through the Layer-by-Layer assembly. For CLSM observation, a small amount of rhodamine B was also loaded into the microsphere. Fig. 1 (A、B) shows that the microspheres have an average diameter of 3 μm, and the rough surfaces make the microspheres perfect carriers for ATP and other cargoes. After incubating with rhodamine-B, the

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microspheres exhibited great fluorescence property (Fig.1 (C)), which also indicates the microspheres' strong adsorption capacity for small molecules. Fig. 1 (D) gives the UV-VIS spectra of the microspheres before (curve c) and after (curve b) incubating with ATP aqueous solution. The peak at 260 nm of curve b demonstrates the successful loading of ATP into the assembled $CaCO_3$ microspheres and the loading efficiency was calculated to be nearly 60 mg per mole of $CaCO_3$ microspheres.

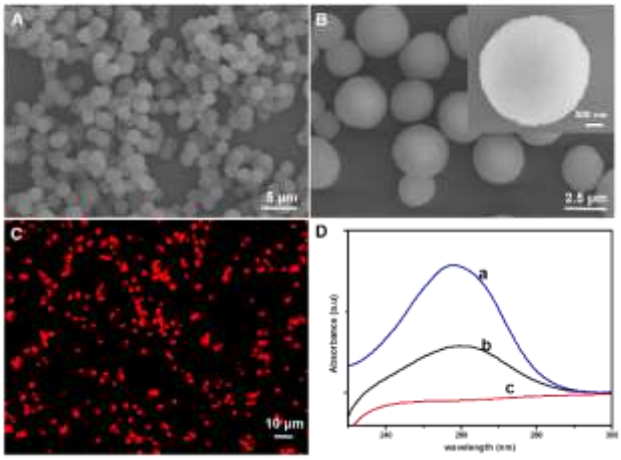

Fig. 1 A) and B) SEM images of the multi-layer film coated $CaCO_3$ microspheres; C) CLSM image of the multi-layer film coated microspheres; D) UV-VIS spectra of: a pure ATP; b multi-layer film coated $CaCO_3$ microspheres with ATP; c multi-layer film coated $CaCO_3$ microspheres without ATP loading.

To confirm that the entrapped ATP could be stimulated release by the production of CTA-GOD-G mixture, UV-VIS spectra of free ATP in supernatant of microsphere dispersion with and without the mixture were recorded. The higher the absorbance intensity, the higher ATP concentration in supernatant, which suggests the more free ATP released from the $CaCO_3$ microspheres. At first, both the absorbance intensity increased (Fig. 2 (A) and (B)), this is probably due to free diffusion of ATP from $CaCO_3$ microspheres to the surroundings. However, with the reaction time increase, the absorbance intensity of ATP in dispersion with CAT-GOD-G mixture increased remarkably (Fig. 2 (B)), while no increase trend was showed in that of the pure buffer system (Fig. 2 (A)). This significant difference indicates the continually release of free ATP from the microspheres were triggered by the production of CAT-GOD-G mixture. In the presence of glucose oxidase, glucose could be oxidized into gluconic acid and hydrogen peroxide. Under the catalysis of catalase, hydrogen peroxide could be disproportionated, which in turn promotes the oxidation of glucose into gluconic acid (Scheme. 1A). It's known that $CaCO_3$ microspheres can be decomposed under acid condition, so the continuous generation of gluconic acid could lead to the gradually decomposition of $CaCO_3$ microspheres and then result in the escape of ATP from microspheres to the surroundings.

By using the standard curve, we quantitatively estimate the release of ATP. It shows that ATP has a remarkable UV absorbance peak at 260 nm, we took the absorbance intensity at 260 nm as a standard to calculate the concentration of ATP in the supernatant according to the standard curve previously made (Fig. S3). Curve (a) in Fig. 2 (C) shows the concentration change of the free ATP in the supernatant with CAT-GOD-G mixture. As can be seen, ATP concentration increased linearly with time, it means that more and more ATP are released from the $CaCO_3$ microspheres to the supernatant. While in the controlled experiment without CAT-GOD-G mixture, the concentration of free ATP shows a slight increase due to free diffusion but quickly reached to equilibrium without further increase (curve b in Fig. 2 (C)).

A bioluminescence luciferin–luciferase assay kit was used to prove the pH triggered release of ATP from $CaCO_3$ microspheres as well. In the existence of ATP, luciferin can be rapidly oxidized by luciferase, producing light. As the luminescence intensity is proportional to the amount of ATP, the increasing luminescence intensity can also demonstrate the increasing release of free ATP. Curve a in Fig. 2 (D) shows that the luminescence intensity increases greatly when incubating ATP-loaded $CaCO_3$ microspheres with CAT-GOD-G mixture. However, no obvious increase was observed without the mixture. This difference further proves the pH responsive release of ATP from $CaCO_3$ microspheres by the production of CAT-GOD-G mixture.

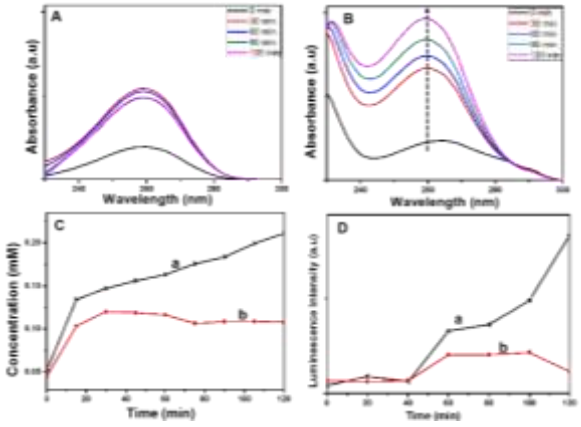

Fig. 2 A) and B) UV-VIS spectra of ATP-loaded microspheres with time in BRB 80 buffer without and with CAT-GOD-G mixture, respectively; C) ATP release as a function of time measured through UV-VIS method: Curve a with CAT-GOD-G mixture; Curve b without CAT-GOD-G mixture; D) ATP release as a function of time measured by bioluminescence luciferin–luciferase assay kit: Curve a with CAT-GOD-G mixture; Curve b without CAT-GOD-G mixture.

All the above results demonstrate that the entrapped ATP can be stimulated released from the $CaCO_3$ microspheres by the production of CAT-GOD-G mixture.

To further confirm that the ATP-loaded $CaCO_3$ microspheres can act as pH triggered "fuel" container, a motility assay with kinesin-microtubule system was conducted. Microtubule suspension with ATP-loaded $CaCO_3$ microspheres and CAT-GOD-G mixture was injected into the kinesin loaded chamber and observed with CLSM. Fig. 3 (A-E) give the time-lapse images of microtubule

motion after observed for 120 min. From these images, we can see that the microtubule slowly moved forward and moving speed was roughly estimate to be 120 nm s^{-1}. Fig. 3 (F) shows the velocity increase of microtubule with time. As can be seen, the average velocity of microtubule increased from 0 nm s^{-1} to almost 120 nm s^{-1} within 120 min (also see supporting movies S1-S4). However, no remarkable velocity of microtubule had been ever observed when there was no CAT-GOD-G mixture (supporting movies S5). This phenomenon indicates that the CAT-GOD-G mixture could stimulate the continuous release of free ATP from $CaCO_3$ microspheres to power the kinesin-microtubule system. Although free diffusion of ATP could happen between microspheres and surroundings, the amount of free diffusion ATP in solution is too low to support the move of microtubule.

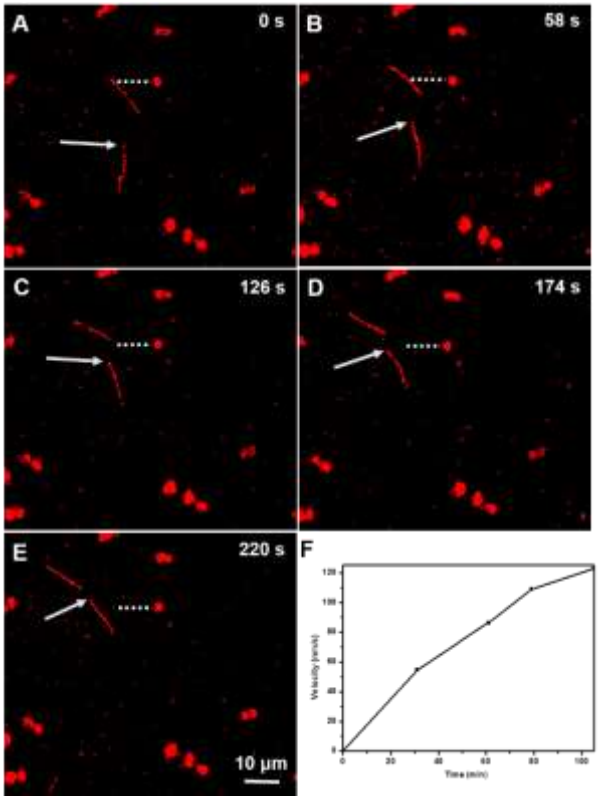

Fig. 3 (A-E) Time-lapse images of microtubule in buffer with CAT-GOD-G mixture after observed for nearly 120 min (white arrows point to the leading head of microtubule and the white dash lines serve as reference line); (F) The velocity of microtubule as a function of reaction time.

In conclusion, we have successfully employed the assembled $CaCO_3$ microspheres as pH responsive ATP carriers. These microspheres were first coated with multilayer film through Layer-by-Layer assembly. After incubating with ATP, these microspheres were used as energy source to power the kinesin-microtubule system. Besides, through consumption of gluconic acid, these microspheres can also provide the kinesin-microtubule system a mild environment to avoid the acid damage. In principle, these microspheres can be further used to load other cargos besides ATP to work as self-powered cargo delivery nano-machine, or they can be mounted as channel walls in microfluidic structures for energy supply. Such $CaCO_3$ microspheres based pH responsive ATP supplier for biomotor system may have a good potential in the building of biomotor aided nano-devices.

Acknowledgements

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