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Assembled capsules transportation driven by motor proteins

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ABSTRACT

An active biomimetic system by integrating kinesin motor, microtubule, and man-made biomimetic microcapsule has been constructed. Biomimetic microcapsules were fabricated by using the layer-by-layer technique and could serve as cargos in this active biomimetic system. Both of the hollow and filled capsules as cargos can be transported by kinesin motors along microtubules. It may help to create kinesin-powered complex hybrid micro- and nanodevices.

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The design and buildup of functional nanodevices require the introduction of various molecular motors in many cases to power the nanodevices at high efficiency. However, the availability and functionality of man-made nanomotors is currently limited, incapable of satisfying this demand. Recently, some motor proteins have been proved to be a group of suitable alternatives with the development of modern biology. A major challenge is currently how to integrate active motor proteins into the engineering of biomimetic system [1–4]. Among these motor proteins, kinesin, consisted of two heavy chains and two light chains, is responsible for intracellular transport and mitosis in cells. The amino-terminal domain of kinesin heavy chains contains the sites for ATP and microtubule binding, while the carboxyl-terminal domain is important for cargo binding. Kinesin can drive cargos such as vesicles, proteins, and organelles along microtubules in biological cells [5–7]. Kinesin works in a smart, efficient and robust manner as a nanomachine. Microtubules are polar cytoskeletal protein filaments. They provide tracks for kinesin and guide the motor from the minus to plus end of microtubules.

In some recent reports, several biomimetic systems containing kinesins, microtubules and cargos such as latex beads, DNA, and quantum dots have successfully been constructed [8–10]. However, the above mentioned cargos are individual components and mainly used to study the physical and mechanical properties of kinesin motors. There is an increasing demand to integrate multifunctional materials into devices for the nanotechnology development. The layer-by-layer (LbL) assembled capsule should be an ideal candidate because it has a hollow structure and can encapsulate some targeted materials such as polymer, DNA, protein, and drug. Additionally, it behaves like the transport of vesicles in living organism [11]. The size, shape, wall thickness, and permeability of the assembled capsules can be properly controlled. So it is expected as a novel tool for developing the technology of drug delivery and manipulating multicomponent materials [12].

In the present work, we firstly prepare poly (styrene sulfonate) (PSS)/poly (allylamine hydrochloride) (PAH) microcapsules and subsequently coat kinesin motors on the surface of as-assembled microcapsules. Finally the capsules can be transported by kinesin motors along microtubules. Moreover, after these microcapsules entrap fluorescein isothiocyanate-dextran (FITC-dextran) as a model material, they can also be delivered along microtubules (Scheme 1). This work mimics the transport of vesicles in cells. Moreover, it realizes the delivery of multicomponent materials along microtubules. The assembled capsules are desired to be applied in integrating complex materials and natural molecular machines into engineering hybrid devices.

Materials and methods

Materials. Monodisperse melamine formaldehyde particles (MF particles, 5.4 μm) were purchased from Microparticles GmbH (Germany). Full length kinesin-1 was expressed and purified as described elswhere [2]. Rhodamine-labeled microtubules were copolymerized from 1 labeled and 4 unlabeled tubulins with a final concentration of 4 mg/mL (Cytoskeleton, Denver, CO) in BRB80

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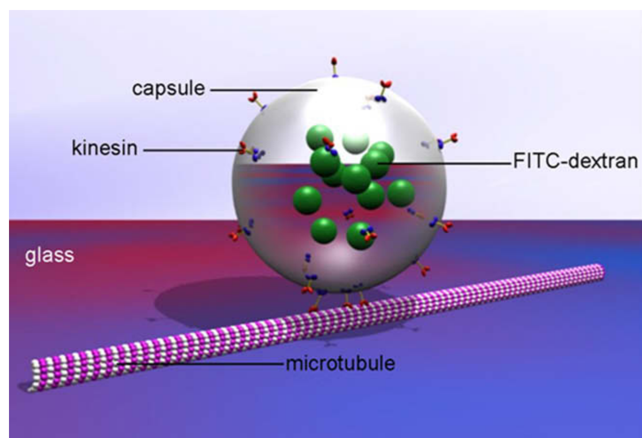

Scheme 1. Capsule as a cargo is driven by kinesin motors along a microtubule.

buffer (80 mM Pipes, pH 6.9/1 mM EGTA/1 mM $MgCl_2$) with 4 mM $MgCl_2$, 1 mM MgGTP, and 5% DMSO at 37 °C for 30 min. Microtubules were then diluted in BRB 80 buffer supplemented with 10 μM taxol.

Capsule preparation. PSS solution (2 mg/mL in 0.5 M NaCl) and FITC-PAH solution (2 mg/mL in 0.5 M NaCl) were alternately added to a MF particles suspension. During two absorption steps, three washing steps were performed to remove excess polyelectrolyte molecules. After the particles were coated by four bilayers of PSS/FITC-PAH, they were dissolved in 0.1 M hydrochloric acid and then rinsed with deionized water until neutral pH. Then the $(PSS/FITC\text{-}PAH)_4$ capsules were incubated with another PSS solution for 15 min. Subsequently, the capsule suspension was autoclaved at 121 °C for 20 min (Systec GmbH, Germany).

The filled capsules were prepared by incubating PSS/PAH capsules with a FITC-dextran solution. Firstly, $(PSS/PAH)_4$ capsules were assembled in the same way. Then FITC-dextran solution (2 mg/mL) was incubated with capsule solution in equal volume for 20 min. Subsequently the capsules were incubated with PSS solution for 15 min. The $(PSS/PAH)_4PSS$ capsules loaded by FITC-dextran were also autoclaved at 121 °C for 20 min.

A Leica TCS SP5 confocal laser scanning microscope (CLSM) is applied to observe the capsules. The sizes of capsules were measured by a Philips CM 200-FEG high resolution transmission electron microscope (TEM).

Preparation of kinesin-coated capsules. The hollow and filled capsules with the same concentration, were respectively, coated with casein by incubation with an equal volume of casein solution (2 mg/mL casein in 10 mM Tris, pH 8.0) for 30 min on ice. An equal volume of 40 nM kinesin solution was mixed with above capsule-casein mixtures and incubated on ice for another 30 min. To measure the ratio of kinesin to capsules a Casy cell counter and analyzer system (Innovates AG, Germany) was applied to counter the number of capsules.

Transport of a kinesin-coated capsule along a microtubule and observation. Motility experiments were performed in the 25 μl (24 × 10 × 0.1 mm) flow chambers. The chamber was constructed from the aminosilanized coverslip, a glass slide, and two strips of double-sided tape (Scotch 3 M, thickness 0.1 mm). Glass slides and coverslips were fornicated in KOH saturated, rinsed with ethanol and deionized water and dried by pressurized nitrogen. To prepare aminosilanized coverslips, 20 μl of 3-aminopropyl triethoxysilane (5% in acetone) solution was dropped on the surface of the coverslip, and covered with another clean coverslip. After 5 min, coverslips were washed and dried. Two aminosilanized coverslips were obtained.

Rhodamine-labeled microtubules (10 μg/mL) in BRB 80 containing 10 μM Taxol were injected into the chambers and incubated at room temperature for 15 min. An ice-cold casein solution was perfused into the microtubule-immobilized chamber to block excess amino groups. After 5 min incubation, both of the kinesin-coated hollow capsules and filled capsules with FITC-dextran in motility solutions (BRB80 buffer with 1 mM adenosine 5′-triphosphate (ATP), 20 mM glucose, 0.02 mg/mL glucose oxidase, 0.008 mg/mL catalase, 0.5% β-mercaptoethanol) were separately introduced into the chambers (chamber 1 with hollow capsules; chamber 2 with filled capsules). Finally, the chambers were sealed with silicone and observed under the Leica TCS SP5 CLSM equipped with a 63× water immersion objective.

Results and discussion

The hollow PSS/PAH microcapsules were obtained through LbL assembly on MF particles, followed by dissolving the melamine cores. Then the capsule solution was incubated at 121 °C for 20 min in order to obtain stable monodisperse microcapsules with low surface roughness. The CLSM images of fluorescent capsules before (Fig. 1A) and after (Fig. 1B) heat treatment clearly show the shrinkage of hollow $(PSS/FITC\text{-}PAH)_4PSS$ capsules. The TEM measurement was applied to precisely measure the size of the capsules before and after annealing. It can be seen that capsules with average diameter 5.4 ± 0.2 μm shrank by 70% to 1.7 ± 0.1 μm and correspondingly the wall of the capsules after annealing became thicker observed in the TEM images (the insert parts in Fig. 1). The wall of the capsules after annealing became smoother due to the rearrangement of polyelectrolyte molecules and the expulsion of water in the polyelectrolyte multilayer during heating. However, the capsules after heat treatment still remain hollow structure as shown in TEM images. The CLSM images of capsules before (Fig. 2A) and after (Fig. 2B) heating clearly show the shrinkage of FITC-dextran filled $(PSS/PAH)_4PSS$ capsules. The higher contrast of capsules in inset TEM images and the cross section profiles before and after annealing (Fig. 2C) demonstrate that FITC-dextran still remains inside the capsules after heating, and indicates that the concentration of FITC-dextran in the capsules increases. The diameters of the shrunk capsules were 2.9 ± 0.2 μm, a little more than half original size of capsules. Due to the increasing osmotic pressure of FITC-dextran inside the capsules, the capsules shrank less than the hollow capsules during autoclaving.

In order to coat kinesin motors on the surface of as-assembled capsules, PSS is used as the outmost layer due to its negative charge at neutral condition while the carboxyl-terminal domain (amino acid residues 858–1031) of kinesin heavy chains is positively charged, for it is of the highly basic nature. Ionic attraction, nonspecific binding are involved between kinesin and PSS [6]. Casein serving as blocking protein was first adsorbed so as to effectively reduce the denaturation of kinesin motors [13].

Microtubules, composed of 13 protofilaments, were formed by polymerizing global protein α, β-tubulin heterodimers. The length of rhodamine-labeled microtubules is ranging from 10 to 50 μm (Supplementary Fig. S1) [14]. The isoelectric point of tubulins is around 5.5. So the microtubules are negatively charged at neutral pH. They can thus be immobilized on the bottom of a flow chamber pretreated by an aminosilane solution via electrostatic attraction. It has been proved that aminosilanization does not affect the function of microtubules [15]. While the kinesin-coated capsules were injected into the flow chamber, they had the chance to touch with the immobilized microtubules.

After ATP was added in the flow chamber 1, the kinesin-coated hollow capsules can walk from the minus to plus end of the microtubules as shown in Fig. 3A (see also Supplementary movie S1). The

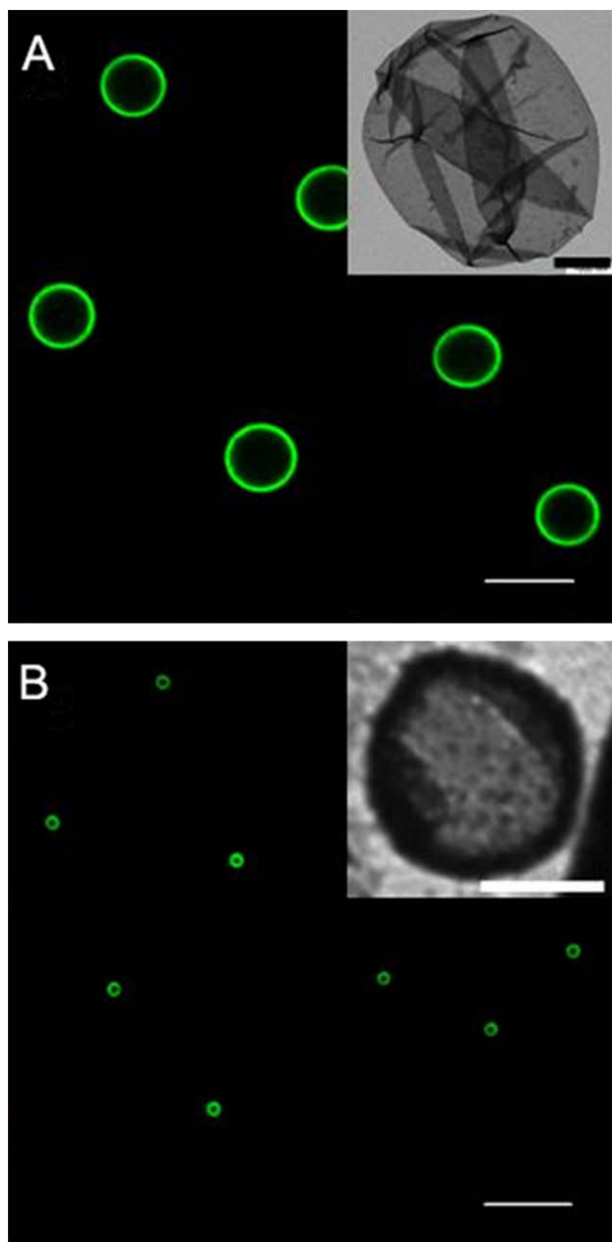

Fig. 1. CLSM and TEM images (the insert parts) of the hollow LbL-assembled (PSS/PAH)$_4$PSS capsules before (A) and after (B) sterilization at 121 °C for 20 min. Scale bar (CLSM) = 8 μm, Scale bar (TEM) = 1 μm.

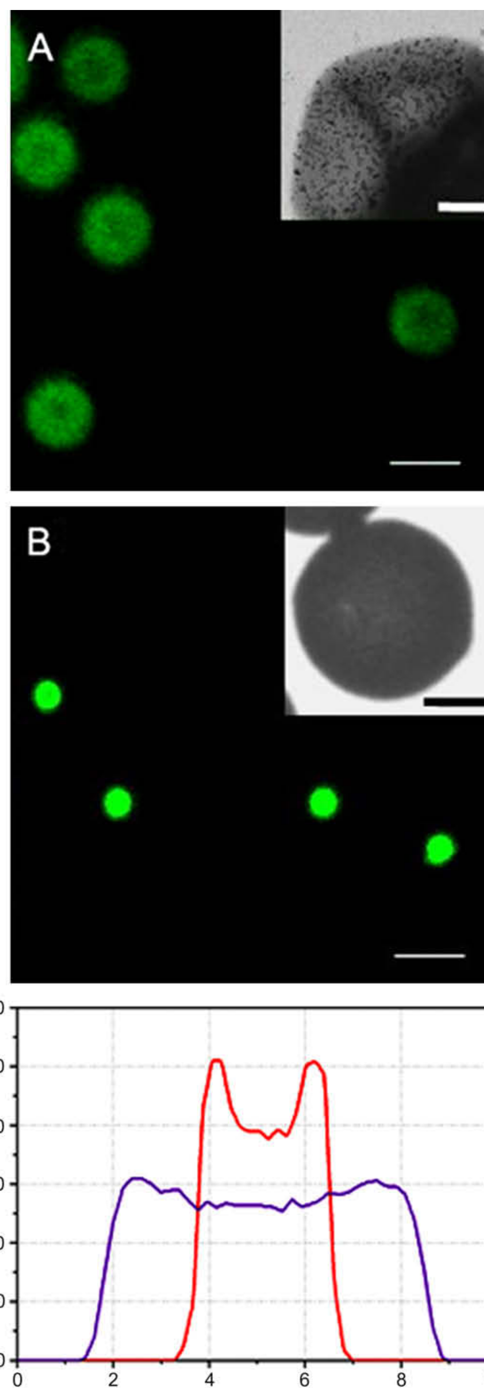

Fig. 2. CLSM and TEM images (the inset parts) of the filled LbL-assembled (PSS/PAH)$_4$PSS capsules with FITC-dextran before (A) and after (B) sterilization at 121 °C for 20 min. Scale bar (CLSM) = 8 μm, Scale bar (TEM) = 1 μm. (C) Fluorescence profiles along the lines indicated in the confocal images.

red linear structure is originated from the microtubule labeled with rhodamine. The yellow capsules are ascribed to overlapping both FITC-channel and rhodamine-channel images. ATP provides kinesin with energy to drive capsules. It demonstrates that kinesin motors successfully adsorb on the surface of capsules and they can still interact with microtubules. The "hand-over-hand" stepping mechanism of kinesin motors has been proved and widely accepted [16,17]. The amino-terminal domain of kinesin heavy chains includes two heads. In this mechanism, one head of kinesin firmly binds to the track while the other head moves forward; the two heads alternate in taking steps from the minus to plus end of microtubules. That is why kinesin motors in most cells can drive cargos from the centre of the cell towards the periphery. At the same time, one ATP molecule is hydrolyzed at each step [18]. Meanwhile, kinesin produces a force of about 7 pN with a high efficiency of conversion up to 50% [19]. The velocity of the hollow capsule could be roughly estimated by dividing run distance of the complex capsules by running time. The calculated speed is approximately 700 nm/s, which is consistent with that reported previously [10]. Apparently, these LbL-assembled microcapsules can act as cargos to move along microtubule driven by kinesin motors.

After being filled with dextran, the capsules are still able to be coated by kinesin motors and subsequently bind on microtubules. After ATP was added in the flow chamber 2, the kinesin-coated dextran filled capsules can walk along microtubules as shown in Fig. 3B (see also Supplementary movie S2). The average velocity of the running capsules containing dextran is roughly calculated

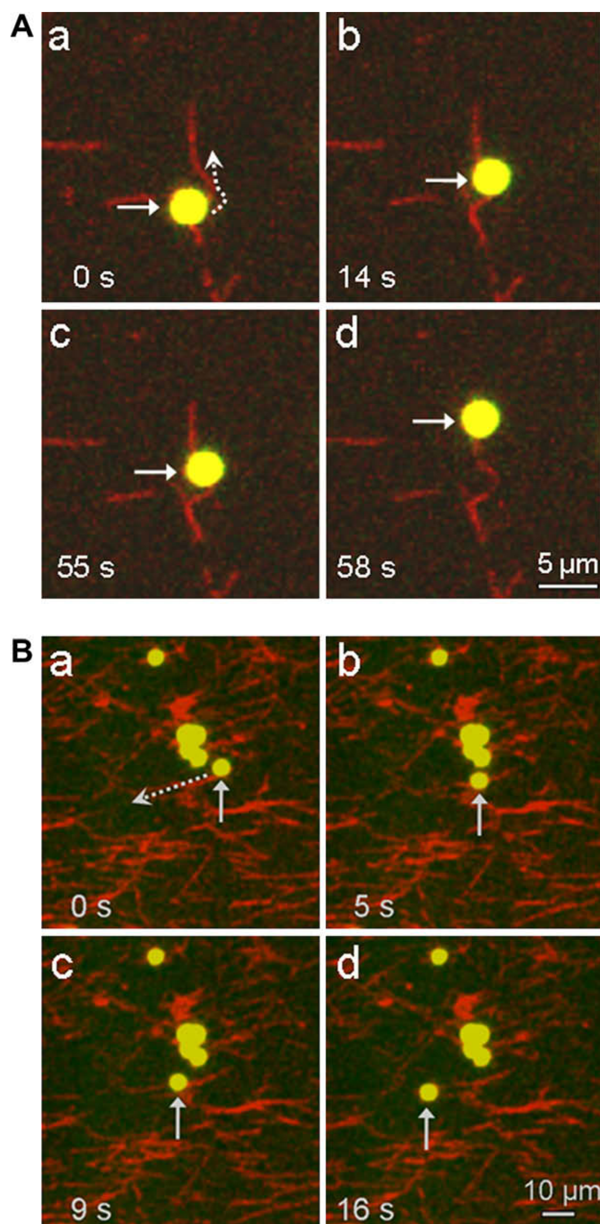

Fig. 3. (A) Time-lapse images of a hollow capsule coated by kinesin motors moving along a microtubule (see also Supplementary movie S1); (B) time-lapse images of a FITC-dextran filled capsule coated by kinesin motors moving along a microtubule (see also Supplementary movie S2). The white arrows point to the moving capsules; the dotted white line indicates the direction of transport.

to be 760 ± 100 nm/s. This demonstrates that the encapsulated materials do not have a large effect on the velocity of as-assembled system. By using artificial capsules in the kinesin-microtubule system, we confirm that the biological motor system can be applied in artificial environments. This work gives an initial example that with entrapped materials, these multicomponent cargos can be manipulated by kinesin motors. This in some sense mimics the intracellular vesicular transport. And one can envision integrating these biological motors with complex materials in artificial environments [20].

In summary, we have designed and integrated the assembled capsules with biological nanomachines in artificial environments. Both of the hollow and filled capsules with dextran can move along the microtubules driven by the kinesin motors. This approach offers the potential for simultaneously transporting multiple components, and also mimics efficient intracellular transport, which provides us with the promise to design complex hybrid nanodevices by using biological motors. Future designs will be emphasized on more precise manipulation of the multiple component materials in both position and direction.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2008.11.136.

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