Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Self-powered Kinesin-Microtubule System for Smart Cargo Delivery

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Experimental section

Materials

Creatine phosphate kinase (CPK, >30 U m^{-1}) was purchased from Sangon Biotech. Creatine phosphate (CP) was obtained from Amresco. Unlabeled tubulin, biotinylated tubulin and rhodamine-labeled tubulin were purchased from Cytoskeleton Company. Sodium carbonate (Na_2CO_3), calcium chloride ($CaCl_2$), fluorescent isothiocyanate-dextran (FITC-dextran, Mw~20 KDa), doxorubicin hydrochloride (DOX), glucose oxidase (GOD), streptavidin (SA) and poly(allylamine hydrochloride) (PAH) were obtained from Sigma-Aldrich. All chemicals were used without further purification. The water used in all experiments was prepared in a three-stage Millipore Milli-Q plus 185-purification system and had a resistivity higher than 18.2 MΩ.

Kinesin and microtubules (MTs)

Full length kinesin-1 was expressed and purified as described previously.[1] The rhodamine-labeled and/or biotinylated MTs were obtained by copolymerization of unlabeled tubulin, rhodamine-labeled tubulin and/or biotinylated tubulin with a final concentration of 2.5 mg mL^{-1} (Cytoskeleton, Denver, CO) in General Tubulin Buffer supplemented with 1 mM GTP and 5% glycerol at 37 °C for 45 min. Microtubules (MTs) were then diluted in BRB80 buffer (907 μL General Tubulin Buffer, 83 μL Glycerol Tubulin Buffer, 0.5 μL 2 mM taxol in dimethyl sulfoxide).

Preparation of CPK microspheres

Preparation of CPK microspheres with a tunable size was based on protein−calcium carbonate ($CaCO_3$) co-precipitation method.[2, 3] A mixture of Na_2CO_3 and CPK solution was first vigorously stirred in a round flask. Then same volume of $CaCl_2$ solution was added quickly to the above mixture with intense agitation on a magnetic stirrer (1200 rm min^{-1}). After 20 s or 40 s, this system was allowed to stand for 2 min. The products were washed with deionized water thoroughly for three times. To prevent the dissociation of CPK, the CPK microspheres were incubated in 0.025 wt% glutaraldehyde (GA) in phosphate buffered saline (PBS) for 2 h, followed by centrifugation and washing. Then CPK-loaded

$CaCO_3$ microspheres were collected.

Preparation of cargo-loaded CPK microspheres

The procedure for preparation of cargo-loaded CPK microspheres is the same as preparation of CPK microspheres. A mixture of Na_2CO_3, FITC-dextran solution (or DOX solution, GOD solution) and CPK solution was first mixed and vigorously stirred in a round flask. Then same volume of $CaCl_2$ solution was added quickly to the above mixture with intense agitation on a magnetic stirrer (1200 rm min^{-1}). After 20 s or 40 s, this system was allowed to stand for 2 min. The products were washed with deionized water thoroughly for three times. To prevent the dissociation, the obtained microspheres were incubated in 0.025 wt% glutaraldehyde (GA) in PBS for 2 h, followed by centrifugation and washing. For controllable triggered release of cargo, CPK and GA were alternately adsorbed onto the surface of microspheres until a desired number of layers, followed by centrifugation and washing. Then cargo-loaded CPK microspheres were collected.

Preparation of CPK/PAH/SA complex microspheres

The obtained CPK microspheres were first dispersed into 2 mg mL^{-1} PAH solution for 2 h to produce positive charge on the surface of microsphere, followed by three times centrifugation and washing with water. Then microspheres dispersion was incubated with 10 μL 5 mg mL^{-1} streptavidin (SA) solution in BRB80 buffer for 30 min. Finally, several washing steps with BRB80 buffer were performed to remove excess SA.

Binding of CPK/PAH/SA complex microsphere with microtubule

For combining the CPK/PAH/SA complex microsphere with MT, the rhodamine-labeled biotinylated MT solution (2 μL) was first diluted by BRB80 buffer and then mixed with CPK/PAH/SA complex microsphere at room temperature for 30 min. The CPK/PAH/SA complex microsphere was then attached to MT through specific recognition of streptavidin-biotin, denoted as CPK-MT complex.

Flow chamber fabrication

Glass slides and coverslips were sonicated in Paranha solution for 30 min, washed with deionized

water and ethanol, and then dried by nitrogen gas. The flow chambers were constructed as a 20 μL volume from the glass slide, the coverslip, and two strips of double-sided tape.

Measurement of ATP production

ATP generation was measured via an ENLITEN®ATP Assay System (Promega, FF2000) in a BPCL ultraweak chemiluminescence analyzer connected to a chart recorder (BPCL-1-TIC, Institute of Biophysics, Chinese Academy of Sciences, Beijing). As the luminescence intensity of luciferin is proportional to the amount of ATP, ATP production was quantified by measuring the amount of light produced by the samples and fitting the results to a standard curve of light intensity versus ATP amount. The determinations were performed at room temperature in dark.

To analyze the ATP concentration, the CPK microspheres were first dispersed into BRB80 buffer supplement with 2 μM ADP and 2 μM CP. Then 5 μL of the CPK microspheres dispersion (2.85 mg ml^{-1}) was added into a luminometer cuvette and mixed with 50 μL of luciferin-luciferase reagent (Promega, FF2000), immediately followed by registering the luminescence intensity on a recorder of BPCL ultraweak chemiluminescence analyzer.

Distance-dependent motility measurement

A casein solution (0.5 mg ml^{-1} in BRB80 buffer) was flowed into the chamber by filter paper and incubated for 5 min. Then a kinesin solution (20 nM in BRB80 buffer) was introduced and incubated for another 5 min. The MT in BRB80 buffer supplemented with 2 mM ADP, 2 mM CP and oxygen scavenger (20 mM Glucose, 0.02 mg ml^{-1} glucose oxidase, 0.008 mg ml^{-1} catalase, 0.5% b-mercaptoethanol) was flowed into the above kinesin coated chamber. Then 1 μL 2.85 mg ml^{-1} CPK microspheres dispersion was gently added to the inlet on one side of the chamber. The chamber was sealed and observed under confocal laser scanning microscopy (CLSM).

Transport of cargo-loaded CPK-MT complex on a kinesin-modified surface and motility measurement

A casein solution (0.5 mg ml^{-1} in BRB80 buffer) was flowed into the chamber by filter paper and

incubated for 5 min. Then a kinesin solution (20 nM in BRB80 buffer) was introduced and incubated for another 5 min. The suspension containing GOD-loaded CPK-MT complex was supplemented with 2 mM ADP, 2 mM CP and oxygen scavenger (20 mM Glucose, 0.02 mg ml^{-1} glucose oxidase, 0.008 mg ml^{-1} catalase, 0.5% b-mercaptoethanol) was flowed into the above kinesin coated chamber. The chamber was sealed and observed under CLSM.

Characterization

The size and surface structure of samples were characterized by scanning electron microscope (SEM, Hitachi S-4800). CLSM micrographs were taken with an Olympus FV1000MPE confocal system equipped with 60× oil-immersion objective and a numerical aperture of 1.4.

Fig. S1

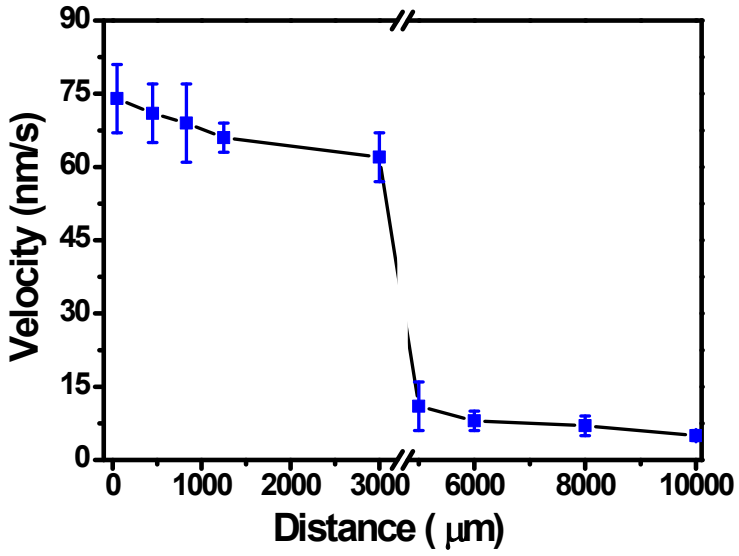

Fig. S1 The velocity of MT gliding as a function of the distances between MT and CPK microspheres.

Fig. S2

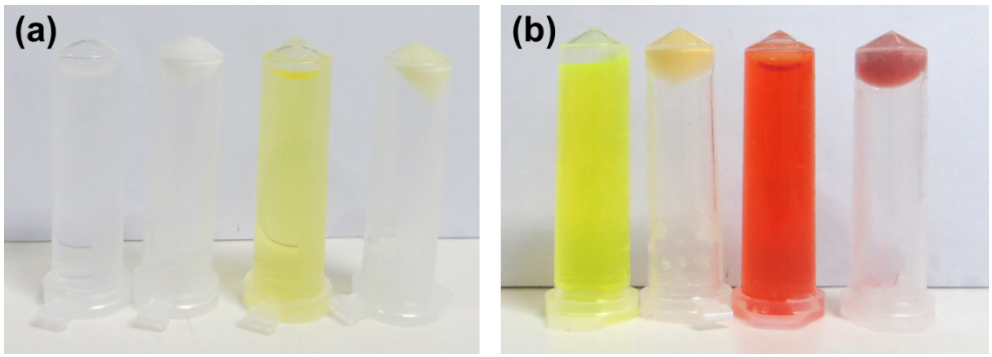

Fig. S2 (a) Photographs of CPK solution, CPK microspheres, GOD solution and GOD loaded CPK microspheres; (b) Photographs of FITC-dextran solution, FITC-dextran loaded CPK microspheres, DOX solution and DOX loaded CPK microspheres (from left to right).

Fig. S3

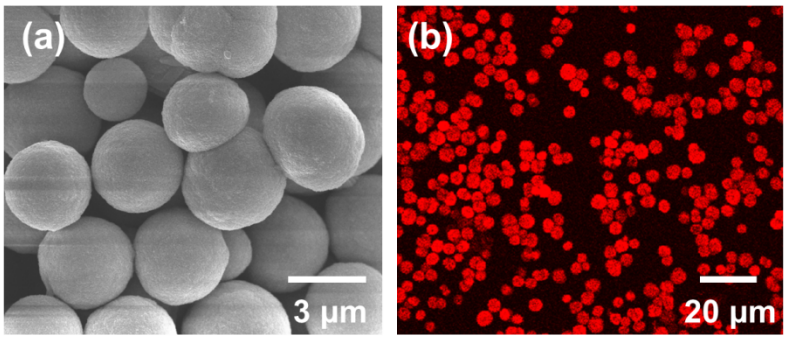

Fig. S3 SEM image (a) and CLSM image (b) of GOD loaded CPK microspheres.

Fig. S4

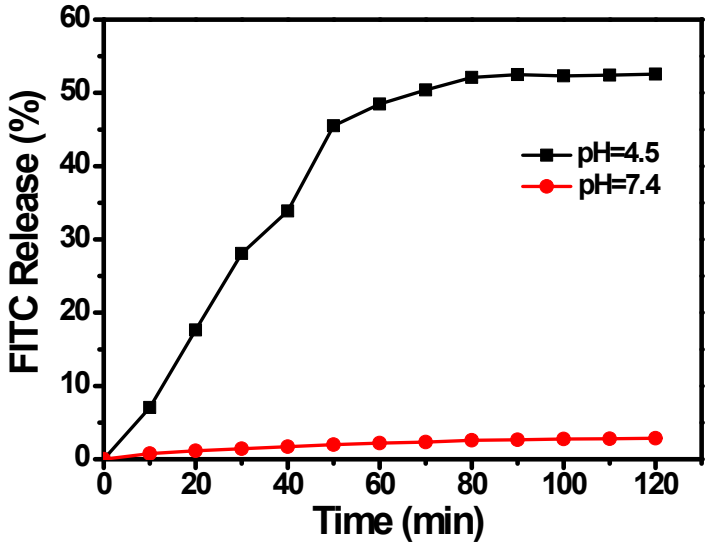

Fig. S4 Release profiles of FITC-dextran from (FITC-dextran−CPK)/$(GA/CPK)_4$ microspheres in pH 4.5 acetate-sodium acetate buffer and pH 7.4 PBS solution, respectively.

Mov. S1 The average velocity of MT gliding after adding the CPK microsphere into the chamber. (7 min, 3±1 nm s^{-1})

Mov. S2 The average velocity of MT gliding after adding the CPK microsphere into the chamber. (23 min, 6±1 nm s^{-1})

Mov. S3 The average velocity of MT gliding after adding the CPK microsphere into the chamber. (51 min, 11±2 nm s^{-1})

Mov. S4 The average velocity of MT gliding after adding the CPK microsphere into the chamber. (73 min, 34±8 nm s^{-1})

Mov. S5 The average velocity of MT gliding after adding the CPK microsphere into the chamber. (101 min, 90±10 nm s^{-1})

Mov. S6 GOD-loaded CPK-MT complex glided on kinesin-modified surface using self-supplying ATP.

Reference

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