

ADVANCED FUNCTIONAL MATERIALS

Supporting Information

for *Adv. Funct. Mater.*, DOI: 10.1002/adfm.201501050

Cell-Membrane-Coated Synthetic Nanomotors for Effective Biodetoxification

*Zhiguang Wu, Tianlong Li, Wei Gao, Tailin Xu, Beatriz
Jurado-Sánchez, Jinxing Li, Weiwei Gao, Qiang He,
Liangfang Zhang,* and Joseph Wang**

Supporting Information

Cell-Membrane Coated Synthetic Nanomotors for Effective Biodetoxification

Zhiguang Wu, Tianlong Li, Wei Gao, Tailin Xu, Beatriz Jurado-Sánchez, Jinxing Li, Weiwei Gao, Qiang He, Liangfang Zhang, and Joseph Wang**

SI Video 1 The random motion of three motor sponges in deionized water under ultrasound field (transducer voltage, 5 V; frequency, 2.83 MHz).

SI Video 2 Efficient population of moving motor sponges under ultrasound field (transducer voltage, 3V; frequency, 2.83 MHz).

SI Video 3 Ultrasound-propelled motor sponges in response to a 6 s 1-3-1 V transducer voltage steps at frequency of 2.83 MHz.

SI Video 4 The movement of motor sponges in whole blood over a period of 40 min.

SI Video 5 The movement of motor sponge in whole blood after 48 h incubation.

SI Video 6 The movement of multiple motor sponges and bare motors in whole blood.

Reagents and Solutions

Fresh RBCs were collected from male ICR mice (6–8 week, purchased from Charles River Laboratories, USA), from which the RBC membrane-derived vesicles were derived following a published protocol.³⁰ Cysteamine 4-methoxytrityl resin was obtained from Sigma-Aldrich Chemical Inc. (St Louis, MO). NHS-fluorescein (NHS-FITC), N,N-diisopropylethylamine and Glycoprotein Detection Reagent were purchased from Thermo Scientific Inc. Trypsin was obtained from G-Biosciences Inc. (St. Louis, MO). All other chemicals were of analytical grade and supplied from Sigma-Aldrich Chemical Inc. (St Louis, MO).

Equipment

Template electrochemical deposition of nanomotors was carried out with a CHI 661D potentiostat (CH Instruments, Austin, TX). Scanning electron microscopy (SEM) images were obtained with a Phillips XL30 ESEM instrument, using an acceleration potential of 20 kV. The fluorescence intensity and glycoprotein qualification was measured by a Tecan Infinite M200 microplate reader. Videos and fluorescence images were captured by an inverted optical microscope (Nikon Instrument Inc. Ti-S/L100), coupled with a 20x objective, a Hamamatsu digital camera C11440 using the NIS-Elements AR 3.2 software.

Synthesis of the motor sponges

The bare gold nanowire motors were prepared by a common template-assisted electrodeposition protocol. A silver film was first sputtered on one side of the porous polycarbonate membrane template with pore size of 400 nm (catalogue no. 6809-6022; Whatman, Maidstone, UK) to serve as a working electrode. The membrane was then assembled in a plating cell with an aluminum foil serving as a contact for the sputtered silver. Silver was initially electrodeposited in the membrane from a AgNO_3 solution, using a charge of 3 C and a potential of 0.9 V (vs Ag/AgCl reference electrode, along with a Pt-wire counter electrode). The removal of this sacrificial layer facilitates the creation of one concave-shaped

end of the wire motor. Subsequently, gold was plated from a commercial gold plating solution (Orotemp 24 RTU RACK; Technic Inc.) at 0.95 V (vs Ag/AgCl), using a charge of 3.0 C. The sputtered silver layer and silver sacrificial layer were mechanically removed from the membrane by polishing with 3–4 μm alumina slurry, followed by dissolution of any remaining silver with 8 M HNO_3 . The membrane was then dissolved in a 3 M NaOH solution for 30 min to completely release the nanowires. The nanowires were collected by centrifugation at 8000 rpm for 5 min and were washed repeatedly distilled water until a neutral pH was achieved. The resulting bare nanomotors (1 mL) were then incubated with 0.1% w/v citrate acid at room temperature overnight. After washing with Milli-Q water, the citrate-stabilized nanomotors were fused with RBC vesicles to obtain the RBC membrane-coated nanomotors using ultrasonic treatment for 20 min.

Identification of membrane coverage of the motor sponges

The fluorescein isothiocyanate (FITC)-thiol conjugate was prepared following a published protocol.³⁰ Briefly, cysteamine 4-methoxytrityl resin (25 mg, EMD Millipore) was first incubated with dimethylformamide for 4 h at room temperature. NHS-fluorescein (12 mg, 0.025 mmol, dissolved in DMF) was added to the resin together with N,N-diisopropylethylamine (20 μL , 0.11 mmol). The reaction was conducted in darkness. The resins were washed with DMF to terminate the reaction after 16 h. Then, the resin was incubated with dichloromethane followed by vacuum drying. The functionalized resin was treated with a mixture of TFA (0.1 mL) and DCM (0.4 mL). The solvent was transferred to a glass vial and evaporated at room temperature under vacuum. The fluorescence spectra of FITC-thiol (0.25 nM), FITC-thiol with motor sponges (0.25 nM and 20 μM) and FITC-thiol with bare motors (0.25 nM and 20 μM) were measured over the 500–680 nm range using a Tecani Infinite M200 microplate reader.

Identification of membrane orientation of the motor sponges

The motor sponges were first prepared through the ultrasound enhanced fusion of excess bare motors (1ml, 1 fM) and RBC vesicles. To quantify the glycoprotein, the glycoprotein on the outer surface of motor sponges was undergone a trypsinization process by incubating 1 mL of solution of the motor sponges (1 fM) with 5 μg of trypsin at room temperature for 2h. The samples were then centrifuged at 8000 rpm for 5 min and the supernatant was collected and used to quantify glycoprotein using a Glycoprotein Detection Reagent following the manufacturer's instructions.

In vitro toxin neutralization and retention study

Melittin (1 μL , 1mg mL⁻¹) was statically incubated with 10 μL solutions of PBS (1X, pH = 7.2) solution, bare motors (~1 fM) or motor sponges (~1 fM) for 2 min. Melittin (1 μL , 1mg mL⁻¹) was also mixed with motor sponges (~1 fM) and sonicated (frequency: 2.83 MHz, transducer power: 5V) for 2 min. The four formulations were added into 1.6 mL of 1% purified mouse RBCs in PBS. The absorbance of hemoglobin in the supernatant was measured at 540 nm using a Hitachi UV-4100 spectrum to determine the degree of RBC lysis. All experiments were performed in triplicates.

Supporting Figure

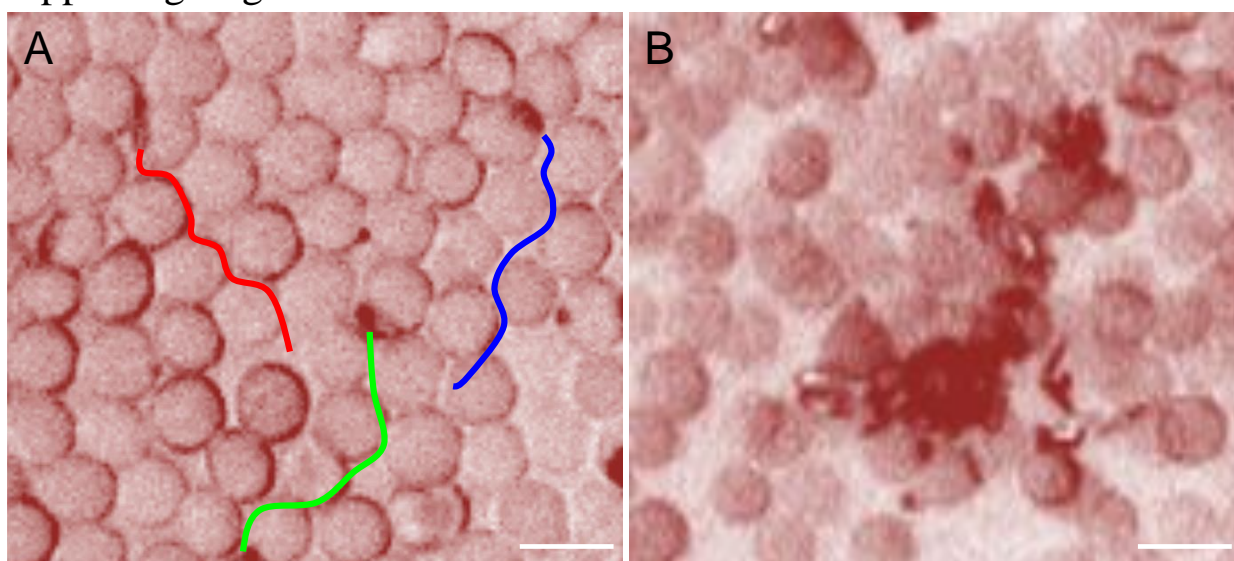


Figure S1. Ultrasound-powered movements of (A) multiple RBC sponge nanomotors and (B) bare Au nanowires in whole blood (corresponding to SI Video 6). Scale bar: 10 μm .

