Bacterial Isolation by Lectin-Modified Microengines

Susana Campuzano[1,2,†], *Jahir Orozco*[1,†], *Daniel Kagan*[1], *Maria Guix*[1,3], *Wei Gao*[1], *Sirilak Sattayasamitsathit*[1], *Jonathan Claussen*[1], *Arben Merkoçi*[3], *Joseph Wang*[1]*

[1]Department of Nanoengineering, University of California-San Diego, La Jolla, CA 92093, USA
[2]Department of Analytical Chemistry, Complutense University of Madrid, E-28040 Madrid, Spain
[3]ICREA & Nanobioelectronics & Biosensors Group, Catalan Institute of Nanotechnology, CIN2 (ICN-CSIC), Bellaterra, E-08193 Barcelona, Spain

*E-mail: josephwang@ucsd.edu

SUPPORTING INFORMATION

† These authors contributed equally to this work.

1. Supporting Videos description

Supporting Video S1. Videos showing the interaction between the ConA-modified microengines and the target bacteria in fuel-enhanced human urine samples inoculated with mixtures *E. coli* and a 5-fold excess of A) *S. cerevisiae* and B) *S. aureus*. Conditions, as in Fig. 2. Target and non-target cells are accented by dashed green and red circles, respectively.

Supporting Video S2. Selective interaction between ConA-modified and unmodified microengines and the target bacteria in peroxide fuel-containing BB solution. Conditions, as in Fig. 2.

Supporting Video S3. Pick-up and transport of *E. coli* cells by ConA-modified microengines in fuel-enhanced drinking water, apple juice and seawater samples inoculated with *E. coli*. Conditions, as in Fig. 3.

Supporting Video S4. Video showing the strong Con A-modified microengine towing force and ConA/bacteria interaction by removing an *E. coli* cell that is firmly adhered to a glass slide surface in a seawater sample. Conditions, as in Fig. 3.

Supporting Video S5. Capture and transport of multiple *E. coli* cells by the ConA-modified microengine in the peroxide fuel-containing BB solution. Conditions, as in Fig. 2.

Supporting Video S6. A) *E. coli*-ConA-modified microengine before and after 20 min navigation in a 10 mM glycine (pH 2.5) dissociation solution. B) Inability of ConA-modified microengine to capture target bacteria in the dissociation solution. Conditions, as in Fig. 4.

Supporting Video S7. Pick-up and transport of *E. coli* and a PLGA magnetic particle by a ConA-modified microengine. Conditions, as in Fig. 2.

2. Methods

Reagents and solutions

6-Mercaptohexanol (MCH), 11-mercaptoundecanoic acid (MUA), N-hydroxysuccinimide (NHS), 1-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), polyaniline (PANI), lectin from *Canavalia ensiformes* (Concanavalin A, ConA), Ulex europaeus (UEA), acetic acid sodium salt, ethanolamine, 2-(*N*-morpholino) ethanesulfonic acid (MES), $CaCl_2$ and $MnCl_2$ were purchased from Sigma-Aldrich. Hydrogen peroxide (30% w/w) and Triton X-100 were purchased from Fisher Scientific.

The BB solution consisted of a 0.1 M acetate buffer, pH 5.0, containing 1 mM Mn^{2+} and 1 mM Ca^{2+}. These two divalent metals are necessary in order to get an active ConA conformation for its binding to carbohydrates.[1,2] A 0.1 M MES buffer solution pH 5.0 was used for the modification of the microengines. A 5% (w/v) Triton X-100 solution was prepared daily in this BB solution. 1 M ethanolamine solution pH 8.5 was used as a blocking agent for amine reactive-esters. Glycine, used to promote bacteria unloading was purchased from EM Science. All chemicals were analytical-grade reagents used as received without any further purification and prepared by dilution in 18.2 MΩ cm Milli-Q deionized water when not otherwise specified. Experiments were carried out at room temperature.

For the preparation of the magnetic PLGA microparticles several chemicals were purchased: triethylamine (Sigma-Aldrich), dichloromethane (Sigma-Aldrich), PLGA-ester (50:50 lactic acid: glycolic acid, IV 0.82 dL/g from Lactel, Pelham, AL), oleic-acid coated 10 nm-Fe_3O_4 nanoparticles (OceanNanoTech; Fayetteville, AR), polyvinyl alcohol (PVA, MW 85 kDa, 80% hydrolyzed, Sigma-Aldrich) and centrifuge filters (Amicon, 100 kDa MW cut-off (MWCO). Standard White Polycarbonate "Track Etch" Membrane Filters from SPI-Pore™ were used to obtain different size of microparticles.

Bacterial strains of *E. coli* NEB 5-α (New England Biolabs) were obtained from the Clinical Microbiology Laboratory, University of California Los Angeles (UCLA), with approval from the UCLA and Veterans Affairs institutional review boards and appropriate Health Insurance Portability and Accountability Act exemptions. The pellets were received in centrifuge tubes and were stored at -80 °C until use. Overnight bacterial cultures were freshly inoculated into Luria broth (LB) and grown to logarithmic phase as measured by the optical density at 600 nm. Concentrations in the logarithmic-phase specimens were determined by serial plating. *S. aureus* cells (10% wet w/v of essentially non-viable *S. aureus* Cowan strain cells in 0.04 M sodium phosphate buffer, pH 7.2, 0.15 M NaCl containing 0.05% NaN_3) were supplied by Sigma and *S. cerevisiae* were supplied by Science Stuff. Human urine samples were collected daily, drinking water and apple juice was purchased in a local supermarket and sea water samples (pH ~8) collected from the shores of La Jolla, CA. All these real samples were inoculated with the appropriate concentration of bacteria at the moment of the experiment.

Microengine fabrication:

Microtube engines were prepared by electrodepositing sequential layers into a cyclopore polycarbonate membrane, which contains numerous double-cone-shaped micropores with a maximum diameter of 2 μm (Catalog No 7060-2511; Whatman, Maidstone, U. K.). Standard electrodepostion of an outer PANI layer and inner Pt layer was conducted via a 3-electrode set-up, as was recently described by Gao et al.[3] In brief, A 75 nm gold film was first sputtered on one side of the porous membrane to serve as working electrode. PANI was electropolymerized for 5 sec at +0.80 V (*vs* Ag/AgCl) from a plating solution

containing 0.1 M H_2SO_4, 0.5 M Na_2SO_4 and 0.1 M PANI and Pt was galvanostatically deposited for 3600 sec at a constant current of -2 mA using a commercial platinum plating solution (Platinum RTP; Technic Inc, Anaheim, CA). After electrodeposition, the gold film was removed by hand polishing with alumina slurry. In this study a shorter polishing time was used in the preparation process, to increase the area of the modified gold surface and minimize nonspecific capturing of the bacteria (by the mouth suck-in). This polishing procedure led to a larger inner opening on the smaller-diameter side of the microengine, and hence to oxygen bubbles emerging from that side, while maintaining the characteristic efficient propulsion and high towing force.[3] The membrane was then dissolved and repeatedly washed in methylene chloride, ethanol and ultrapure water. The wires solution was then evaporated onto glass slides before the sequential deposition of 10 nm Ti (adhesion layer), 26 nm Ni (magnetic layer), and 12 nm of Au (functionalization layer) over the microtubes using electron beam deposition. This additional steps provide the necessary magnetic directional control and surface modification capabilities for the appropriate guidance and pick-up of target bacterial cells.

Microengines modification:

The external gold surface of the microengines was modified by an overnight immersion in a binary mixture of 0.25 mM of MUA and 0.75 mM of MCH in absolute ethanol. After washing with Milli-Q water, the resulting mixed monolayer-modified microengines were treated with a 20 mM NHS and 10 mM EDC in 0.1 M MES buffer solution pH 5.0 for 30 min, washed 1 min with BB solution and immersed 2 h in a BB solution containing 9 mg/ml of ConA (or UEA) receptor. The remaining amine reactive-esters from the activated monolayer were blocked with 1 M ethanolamine solution, pH 8.5, for 30 min and later resuspended in BB solution. Between each incubation and washing steps the microengines were isolated by centrifugation at 6,000 rpm during 4 min; all experiements were carried out at room temperature.

'Control' microengines (without the lectin receptor) were prepared using the same protocol (with the SAM assembly, activation and blocking steps) but omitting the addition of the Con A and carrying out the corresponding incubation in BB.

Preparation of bacterial suspension:

E. coli stock suspension were prepared by resuspending the appropriate pellet containing ~ 10^7 colony forming units (cfu) bacteria in 100 μL of BB solution or in the undiluted sample matrix under study. This suspension was serially diluted in the same buffer (or sample matrix) to provide different concentrations of bacterial cells.

S. cerevisiae and *S. aureus* stock solutions were prepared daily by resuspending the appropriate weight of the yeast or by diluting the required volume of the commercially-attenuated *S. aureus* cells suspension in the BB solution or in the sample under study.

Identification and isolation of target bacteria:

For the detection and isolation of the target bacteria, 2 µl of modified-microengines suspension, 2 µl of 5% (w/v) Triton X-100 (prepared also in BB solution) and 2 µl of 30% (w/v) H_2O_2 solutions were mixed onto a freshly cleaned glass slide. Once the microrockets were deemed to possess the proper movement and magnetic guidance, 2 µl of the diluted bacterial cell suspension (prepared in the sample matrix or in the BB solution) were added to the mixture. The microengines were then magnetically guided towards the target cells and monitored using a Nikon Eclipse Ti-S/L100 optical microscope. Videos were captured using Hamamatsu digital camera C11440, 40×objective (unless mentioned otherwise) and acquired at the frame rate of 20 fps using the NIS-Elements AR 3.2 software. To test the performance of the lectin-based microengines in complex matrixes the undiluted sample under study was inoculated with the appropriate concentration of bacteria.

It is worth to mention that according to the protocol described all the inoculated matrixes (originally undiluted) were finally diluted 4 times after mixing with equal volumes of the other 3 solutions required by the bioassay (microengines, H_2O_2 and Triton X-100, to yield a final fuel and surfactant concentrations of 7.5% (w/v) and 1.25% (w/v), respectively. Arana et al.[4] demonstrated that the number of bacteria with intact cytoplasmic membranes (viable) is still high even working with more than 1 % of H_2O_2, despite a sharp loss in culturability.

Unloading experiments:

After capturing target bacteria following exactly the same protocol described in the previous section, 12 µL of the dissociation solution (10 mM glycine pH 2.5) were added to the glass slide. We monitored the navigation of the Con A-modified microengines in this solution during the required time to release the captured bacteria.

Preparation of the magnetic PLGA microparticles:

The magnetic PLGA microparticles were freshly prepared by oil-in-water emulsion method.[5] The PLGA-ester (50:50 lactic acid: glycolic acid, IV 0.82 dl/g from Lactel, Pelham, AL) was dissolved in chloroform and mixed with oleic-acid coated 10 nm-Fe_3O_4 nanoparticles (400 µL, OceanNanoTech; Fayetteville, AR). This oil phase solution was added to water phase PVA (1.5 mL, 2% (w/v)) to stabilize the formed magnetic PLGA particles. The mixture was vortexed for 3 minutes and sonicated for 6 min. Chloroform was evaporated overnight by gentle stirring. Particles with 1-2 µm were separated from the

bulk solution by using 1 and 2 μm pore diameter polycarbonate filter membranes (Whatman Inc.). This PLGA fraction was diluted and 2 μl of the solution were added to the above microengine/bacteria mixture when performing experiments.

3. References

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