Supporting Information

Fluid Actuation and Buoyancy Driven Oscillation by Enzyme Immobilized Microfluidic Microcapsules

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Contents:

Experimental Section/Methods

Materials. Gold(III) chloride trihydrate (≥99.9% trace metal basis), catalase from bovine liver (lyophilized powder, 2000−5000 units/mg protein), Grubbs catalyst, Dicyclopentadiene (DCPD), and 1,2,4-trichlorobenzene (TCB) were purchased from Sigma-Aldrich. Hydrogen peroxide (H_2O_2, 30%) was purchased from MERCK. Millipore water (18.2 MΩ·cm at 25 °C) was used in all experiments. SU-8 2035 photoresist and developer solution were purchased from MicroChem Corp., USA for fabricating the silica master with designed microchannels. Silicon tubings were purchased from Polymax India. The polydimethylsiloxane (PDMS; SYLGARD 184) package was purchased from Dow corning corporation and used for fabricating the microfluidic chip.

Synthesis of trimethylammonium tetraethylene glycol-functionalized Au nanoparticles. Cationic Au nanoparticles were prepared through a two-step process reported earlier.[1] In brief, 100 mg of thiol ligand, that is, HS-C_{11}-tetra(ethylene glycol)lyated trimethylammonium bromide, in 3 mL of dichloromethane was added to 20 mg of hydrophobic Au nanoparticles dispersed in 15 mL of dichloromethane. The resulting mixture was stirred in an oil bath at 35

°C for 2 days forming black precipitation. The black precipitate was then washed five times with dichloromethane and finally dissolved in Millipore water for further studies.

Preparation of microfluidic device. To fabricate the microfluidic device, commercially available SU-8 2035 negative photoresist and SU-8 developer were purchased from MicroChem Corp., USA. Before the start of the lithography process, substrates were thoroughly cleaned with a piranha wet etch (using H_2SO_4 & H_2O_2) followed by a de-ionized water rinse. Stream of dry nitrogen gas was used to remove the excess water droplets. The substrate may also be cleaned by using reactive oxygen plasma to remove contaminants from the surface of Si wafer. Then, a negative photoresist SU-8 2035 was spin-coated onto a 4 × 4 silicon wafer as a substrate material, and then design was transferred on the silicon wafers using maskless photolithography (X-pert SF100, Intelligent Instruments). The next step involves dipping the surface in the SU-8 developer and followed by rinsing with 2-propanol. Finally, the patterned surface was baked for 2 min to stabilize the structure. Next, PDMS 184 SYLGARD with a curing agent in a ratio of 10:1(w/w) was poured onto the wafer and degassed in a vacuum desiccator until the trapped air bubbles disappeared. The unit was placed in an oven at 70 °C for 1 h. Once cured, the microchannels imprinted on the PDMS mold were peeled from the wafer and punched with a biopsy punch with a diameter of 2.5 mm. PDMS was then plasma-bonded to a glass slide with oxygen plasma treatment in a radio frequency plasma cleaner system for 60 s. Finally, the device was placed in a 70 °C oven for 5 h and cooled to room temperature for further use.

Micropump Fabrication. To monitor the pumping ability of catalase immobilized MCs, a customized chamber [l= 1cm; w= 1cm, h= 1.8 mm] was made using FT-1201 mounting foam tape. Afterwards, MCs were carefully placed inside chamber by using micropipette and the chamber was sealed by a PET sheet. It is important to create a leak proof chamber to avoid unwanted fluid flow and turbulence during measurement. To initiate the fluid pumping, an

aqueous solution of substrate with suspended tracer particles (5 μm in diameter) was injected into the chamber and the fluid flow was observed by optical microscope.

Particle Tracking. The real time videos of particles motion was captured for further analysis. For measuring fluid velocity, 20 particles were tracked for 30s using Tracker, a motion analysis software.

Oxygen Bubble Nucleation and Growth. Bubble nucleation: Approximately 2-5 catalase immobilized microcapsules having a size around 480 μm were placed through fine needle in customized well fabricated on glass slide. 50μl of aqueous solution of hydrogen peroxide (>10mM) were then added to well. The number of oxygen bubble nucleation events occurring per microcapsules was monitored using optical microscopy. Growth: Using the above procedure, growth of oxygen bubbles on catalase immobilized microcapsules was monitored by optical microscopy recordings of time-dependent changes in the volume of the bubble. Image analysis was carried out using Image J, assuming that bubbles were spherical.

Enzyme-Powered vertical oscillatory movement of catalase immobilized Microcapsules. To study the oscillatory motion of the buoyant catalase immobilized MCs, real time videos were captured using a camera. An aqueous dispersion of catalase immobilized MCs were pipetted into a vial and microcapsules allowed to sink to the bottom of the vial. Then vertical displacement of MCs having various nucleated bubble in the presence of hydrogen peroxide were recorded.

Measurements. Cationic gold nanoparticles and catalase immobilized microcapsules (MCs) of varied diameter were synthesized according to the reported literature. The dried MCs and their morphology were observed with SEM (JEOL JSM-IT300 microscope); the samples were coated with gold to render them conductive. Microcapsules (MCs) and its trajectories were observed with an optical microscope (Olympus IX73 inverted microscope). Microparticles

(polystyrene particles) tracking analysis of tracer particles was performed by Tracker software.

S1. Scanning Electron Micrographs:

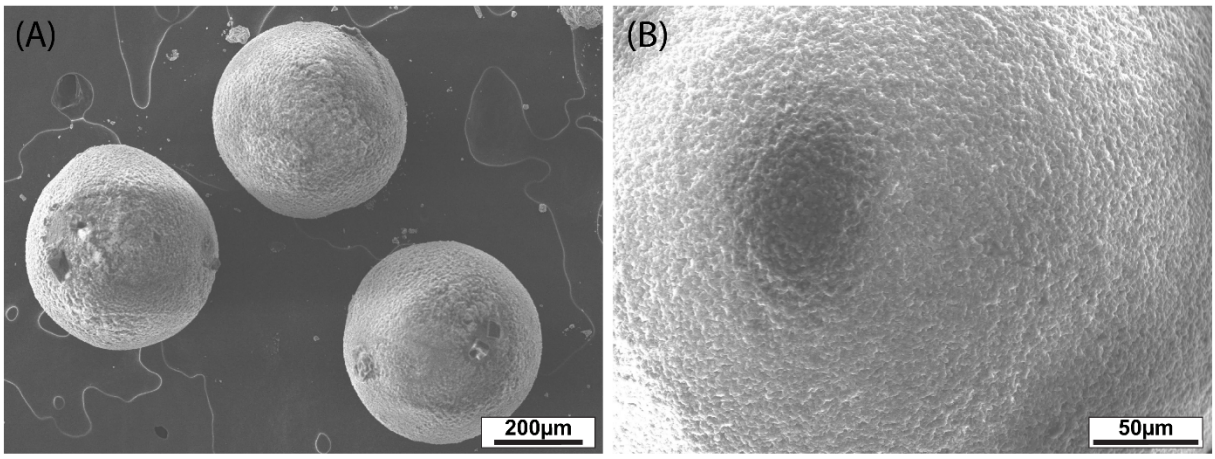

Figure S1: (a) SEM images of dried Catalase immobilized Microfluidic MCs (low resolution) (b) High resolution image of corresponding MC.

S2. Illustration of reaction rate calculation using catalase:

The radius of the enzyme immobilized beads is 240 µm. Therefore, its area is given by $4\pi r^2 = 4\pi (0.24\times 10^{-3})^2\ m^2 = 0.723 \mu m^2$

Diameter of a single catalase molecule is 10.2 nm.

Cross sectional a single enzyme molecule:-

$$= \frac{\pi(10.2 \times 10^{-9})^2}{4} = 8.2 \times 10^{-17} m^2$$

The approximate number of enzyme molecule on the beads:-

$$= \frac{0.723 \times 10^{-6} m^2}{8.2 \times 10^{-17} m^2} = 8.82 \times 10^{9}$$

Using Avogadro's number (6.02×10^{23} molecules/mole), the moles of enzyme molecules were determined (1.46×10^{-14} moles), and the concentration was then calculated using the volume of solution inside the spacer ($1.5\times 10^{-7}\ m^3$).

Therefore,

$$[E] = \frac{1.46 \times 10^{-14} moles}{0.00015\ L} = 9.73 \times 10^{-11} M$$

Following Michaelis-Menten kinetics, for an enzyme concentration and substrate concentration, the reaction rate can be expressed as

$$v = \frac{4K_{cat}[E][S]}{K_M + [S]}$$

Here, we have considered four active sites per molecule of catalase. Now, for bovine liver catalase, K_M = 93 mM. Therefore, we have chosen different substrate concentration, [S] = 2.5mM to 10mM, the rate can be expressed as

$$v = \frac{4K_{cat}[E][S]}{K_M + [S]}$$

K_{cat} (catalase, per active site) = 2.12×10^5 s^{-1}

$$v = \frac{4\left(2.12 \times 10^5\, s^{-1}\right)\left(9.73 \times 10^{-11} M\right)(0.0025 M)}{0.093 + (0.0025 M)}$$

$$v = 2.16\ \mu M.\, s^{-1}$$

Supplementary Table S1 : Substrate concentrations used with catalase immobilized microparticle micropumps, corresponding reaction rates and pumping speeds obtained ([E] = 9.73 x 10^{-11} M; kcat = 2.12 x 10^5 s^{-1}; K_M = 0.093 M, active sites = 4)

Substrate Concentration (mM)	Reaction Rate (µM/s)	Pumping Speed (µm/s)
2.5mM	2.16	0.902±0.21
5mM	4.21	2.96±0.27
7.5mM	6.15	5.19±0.56
10mM	8.01	7.08±0.72

Supplementary Table S2 : Substrate concentration (5mM) was kept constant with varied number of catalase immobilized microparticle, corresponding reaction rates and pumping speeds obtained ([E] = 9.73 x 10^{-11} M; kcat = 2.12 x 10^5 s^{-1}; K_M = 0.093 M, active sites = 4).

No. of Particles (same size =480µm)	Reaction Rate (µM/s)	Pumping Speed (µm/s)
1 particle	4.21	2.96±0.27
2 particle	8.42	5.22±0.84
3 particle	12.63	7.13±0.72

Supplementary Table S3 : Substrate concentration (5mM) was kept constant with different size of catalase immobilized microparticle, corresponding reaction rates and pumping speeds obtained ([E] = 8.11 x 10^{-11} M; kcat = 2.12 x 10^5 s^{-1}; K_M = 0.093 M, active sites = 4).

Diameter of Particles	Reaction Rate (µM/s)	Pumping Speed (µm/s)
480µm	4.21	2.99±0.24
715µm	9.2	5.25±0.67

S3. Recyclability Plot :

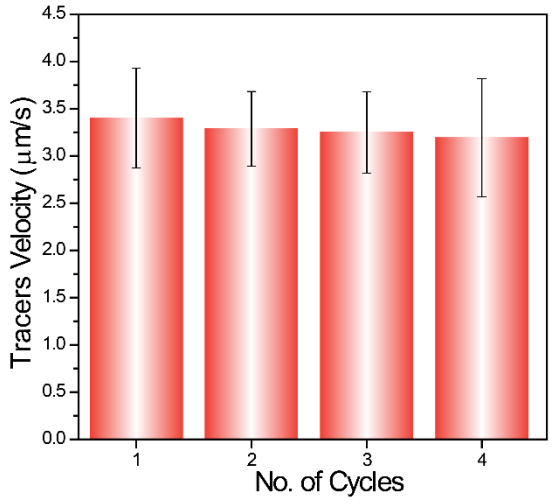

Figure S3: Recyclability plot for reused enzyme immobilized MCs in presence of 5mM substrate.

S4. Oscillatory vertical motion of catalase immobilized MCs:

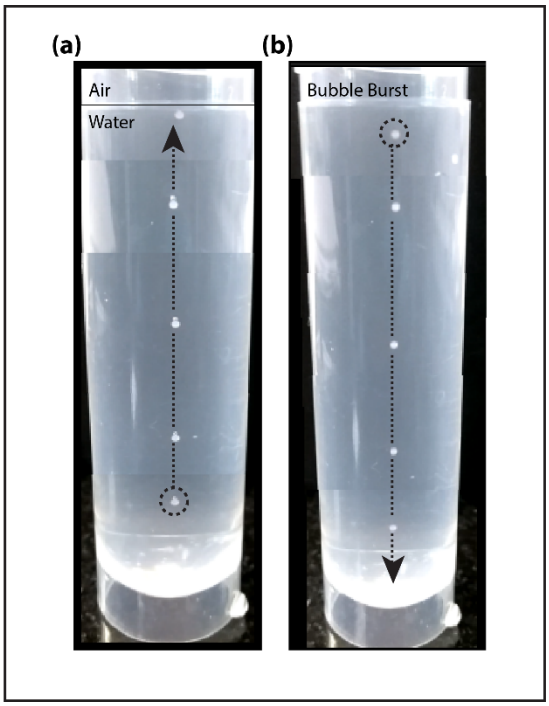

Figure S4. A time lapse sequence of photographic images (a,b) that shows oscillatory vertical movement of single catalase immobilized MCs that results from bubble evolution on consumption of substrate and bubble burst at the interface .

Reference

1) B. Samanta, X. C. Yang, Y. Ofir, M. H. Park, D. Patra, S. S. Agasti, O. R. Miranda, Z. H. Mo and V. M. Rotello, *Angew. Chem. Int. Ed.*, 2009, **48**, 5341-5344.