Supporting Information

Cell Isolation and Recovery Using Hollow Glass Microspheres Coated with Nanolayered Films for Applications in Resource-limited Settings

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Biotin modification of ALG

Alginate was conjugated with biotin hydrazide (Sigma B7639) using a standard carbodiimide reaction, as described in previous literatures.[1,2] Briefly, ALG was dissolved in pH 6.1 MES buffer to form a 1.0 wt.% solution. Per 50 mL of ALG solution, 80 mg of biotin hydrazide, 360 mg of 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC, Pierce 22980), and 204 mg of hydroxysulfosuccinimide (Sulfo-NHS, Pierce 24510) were added and reacted for 3 h, after which time the solution was dialyzed against deionized water for 48 h and lyophilized.

Modification of PAH with fluorescein

PAH (214 mg) was dissolved in pH 7.4 Phosphate-buffered saline (PBS) solution (4.3 mL) and placed in ice bath with stirring. Fluorescein NHS ester (10 mg) was dissolved in Dimethylformamide (DMF) (500 μL) and added dropwise into the stirring PAH solution. After 3 h, the reaction solution was dialyzed against deionized water for 48 h and lyophilized.

Buoyancy force calculation

For the hollow glass microspheres to be able to lift the cancer cell upwards, the following condition should be fulfilled:

$$F - G > 0 \qquad (1)$$

$$F = \frac{4}{3}\pi \times (R_{cell}^3 + R_{HGMS}^3) \times \rho_{water} \qquad (2)$$

$$G = \frac{4}{3}\pi \times R_{cell}^3 \times \rho_{cell} + \frac{4}{3}\pi \times R_{HGMS}^3 \times \rho_{HGMS} \qquad (3)$$

Where F is the total buoyancy and G is the total gravity of cell and HGMS. The density of cancer cell and HGMS are 1.08 g/ml and 0.47 g/ml, respectively.[3]

Shear stress calculation

For a solid sphere flow through a stationary liquid with uniform velocity U, the shear stress at the sphere surface can be calculated as following:[4]

$$\tau_\theta = -\frac{3}{2}\frac{\mu U}{R} sin\theta \qquad (4)$$

Where τ_θ is shear stress at angle θ; μ is viscosity of the liquid; R is radius of the sphere; θ is azimuth angle in Spherical coordinate.

The μ of water at 20 ºC is 0.001 Pa•S and R of PC-3 cell is approximate 7 μm. A velocity (U) of 0.001 m/s was measured in experiment. The above values were substituted into the equation (4) and the maxima shear stress of 0.21 Pa was obtained at $\theta = \pi/2$. This value is comparable with shear stress (0.1-1 Pa) in microfluidic devices. [5, 6]

Film stability test

Film stability test was performed by incubating PARG/ALG-PEG-anti-EpCAM film with blood plasma for 3 h. Similar to our previous work,[6] 5 bilayers of PARG/ALG films were deposited along the walls and floor of microfluidic channels formed by bonding a polydimethylsiloxane (PDMS) mold to an oxygen plasma treated glass slide. NH_2-PEG/ NH_2-PEG-biotin was then modified on the film by EDC chemistry. Texas Red-labeled Neutravidin was used to conjugate biotin-anti-EpCAM onto PEG-biotin on the film. The prepared film was ready for stability test as PARG/ALG-PEG-anti-EpCAM film. Human blood was centrifuged at 300 g for 5 min to sink down all the blood cells and supernatant was kept as blood plasma. Right after the blood plasma was introduced to the PARG/ALG-PEG-anti-EpCAM film, optical fluorescent images were taken by an Olympus BX53 fluorescence microscope with 200 ms exposure time for 3 h. The result images were analyzed by ImageJ.

Supplementary Figures

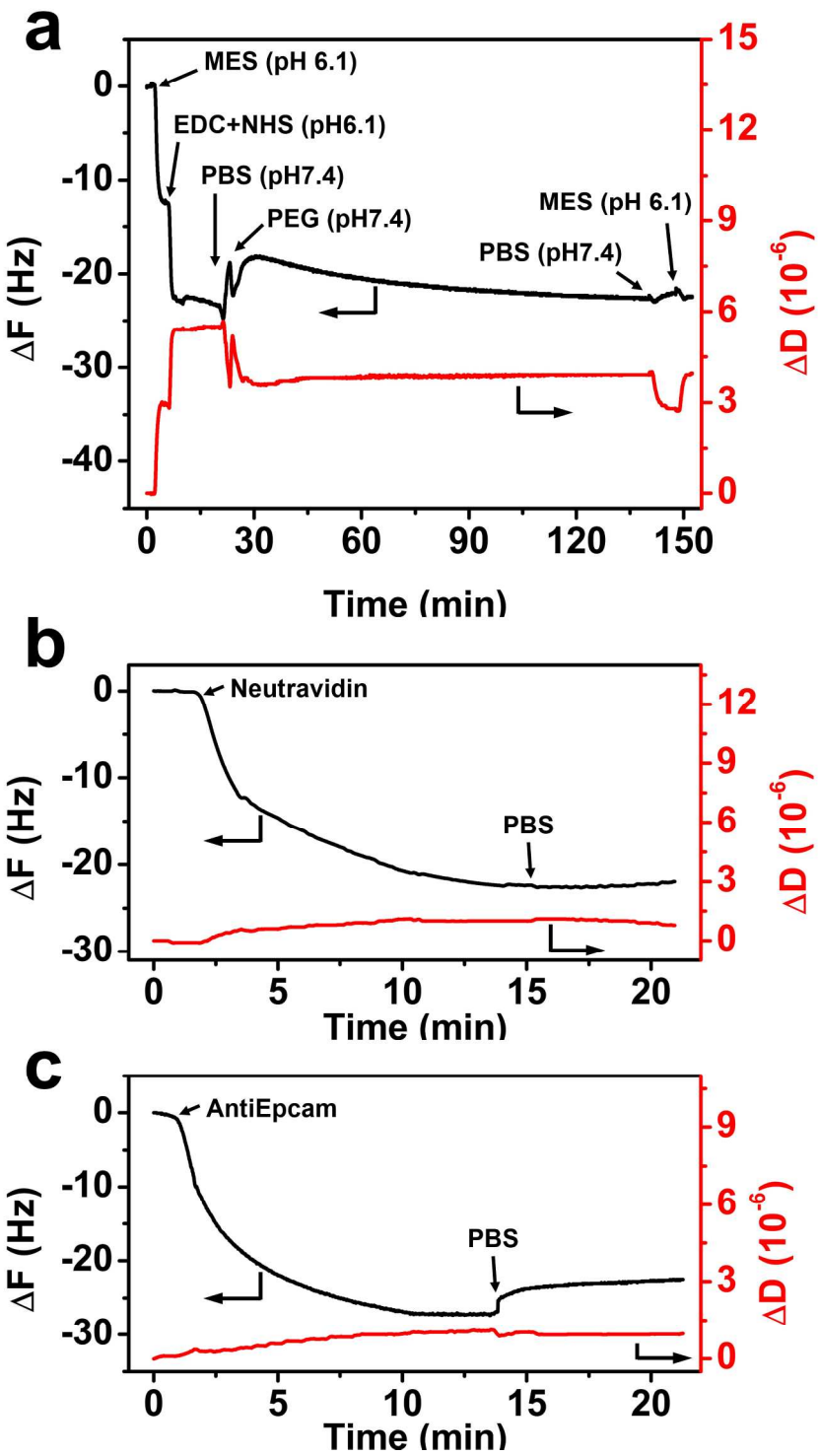

Fig. S1. QCM-D monitoring of surface modifications: a. NH_2-PEG/NH_2-PEG-Biotin conjugation via EDC chemistry; b. Neutravidin modification; c. antiEpCAM modification.

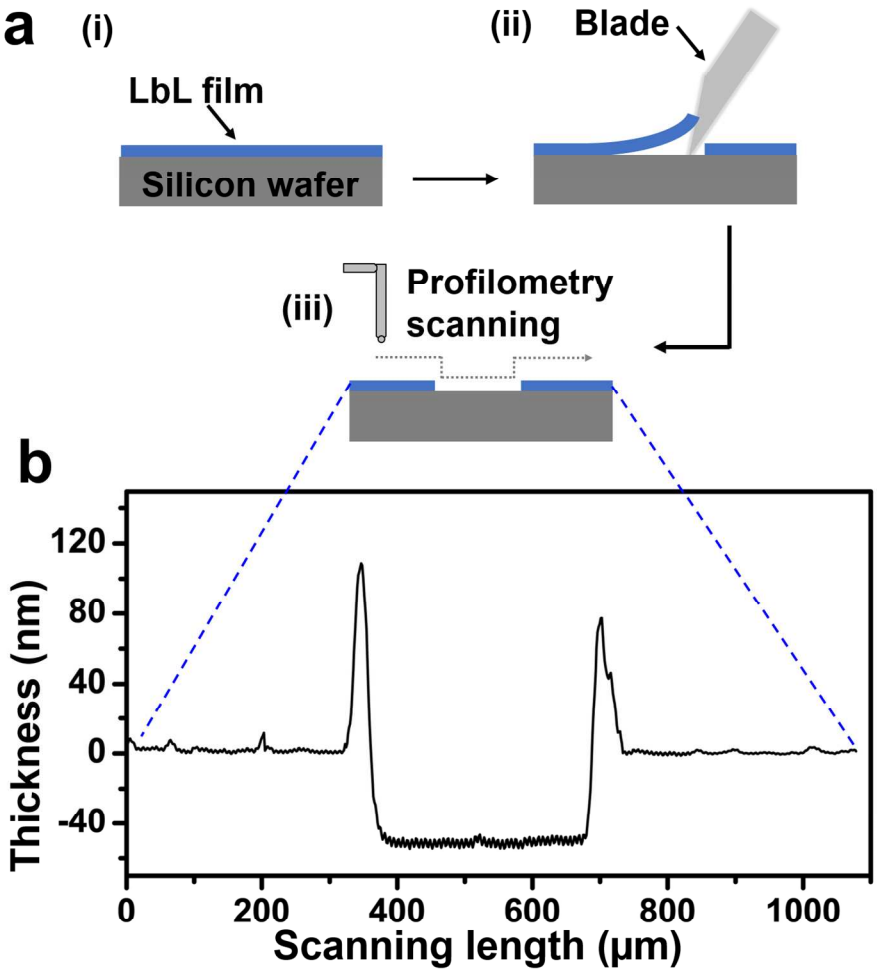

Fig. S2. Film thickness measurement by profilometry: a. (i) prepare LbL film on silicon wafers; (ii) scratch LbL film all the way to the silicon to form a gap; (iii) scan the gap by profilometry to get film thickness; b. profilometry result of 5 bilayer PARG/ALG on silicon wafer.

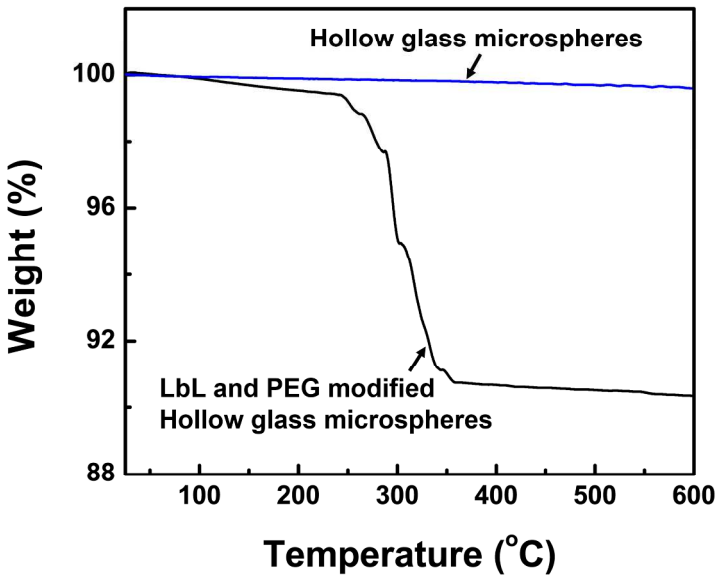

Fig. S3. TGA characterization of nanolayer modified and unmodified hollow glass microspheres.

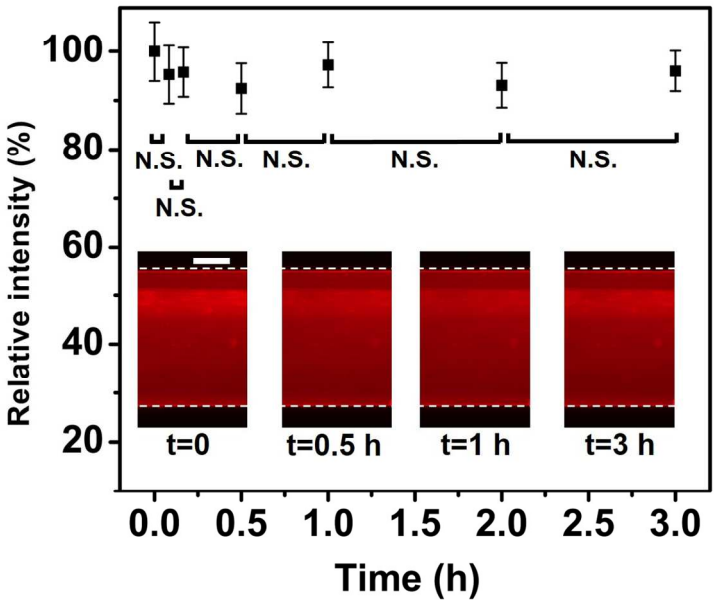

Fig. S4. Stability of antibody modified LbL film: Optical fluorescent images of Texas Red labeled PARG/ALG-PEG-anti-EpCAM films after being incubated for various times in blood plasma. Film edges are marked by a white dashed line. Scale is 100 μm.

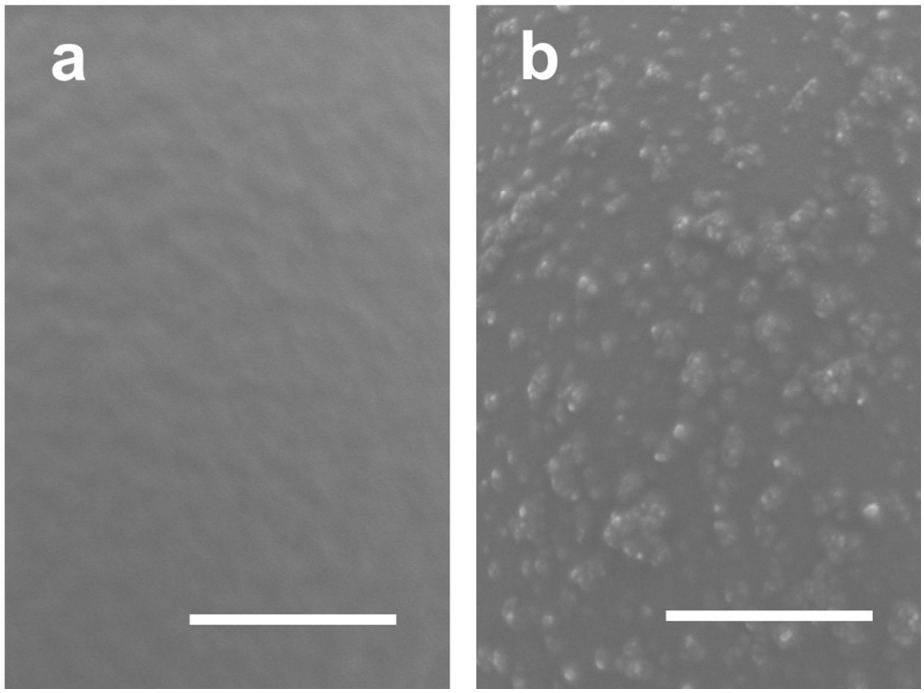

Fig. S5. SEM images of surface of the HGMS before (a) and after (b) cell capture and release process. Scale is 2 μm.

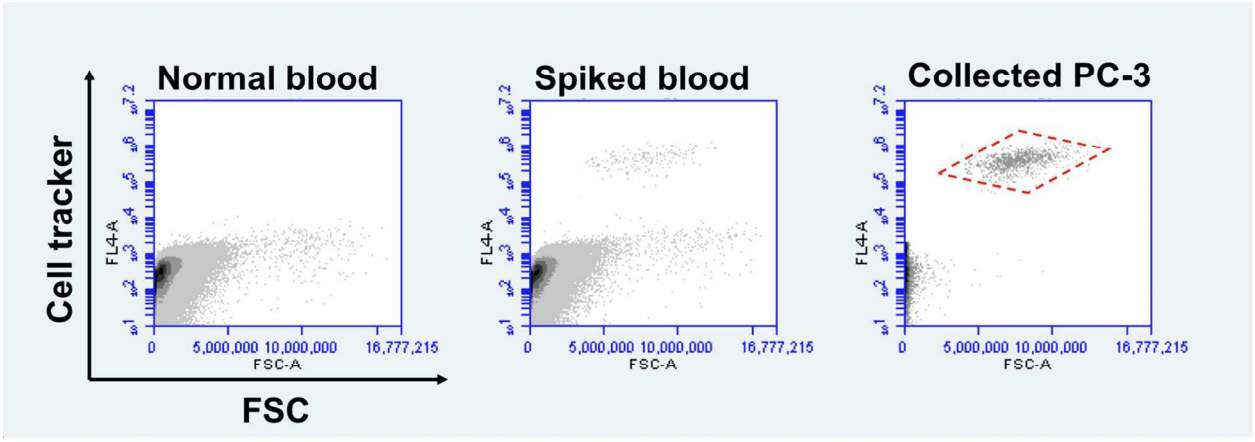

Fig. S6. Typical flow cytometry results normal blood, spiked blood and PC-3 cell recovered from blood.

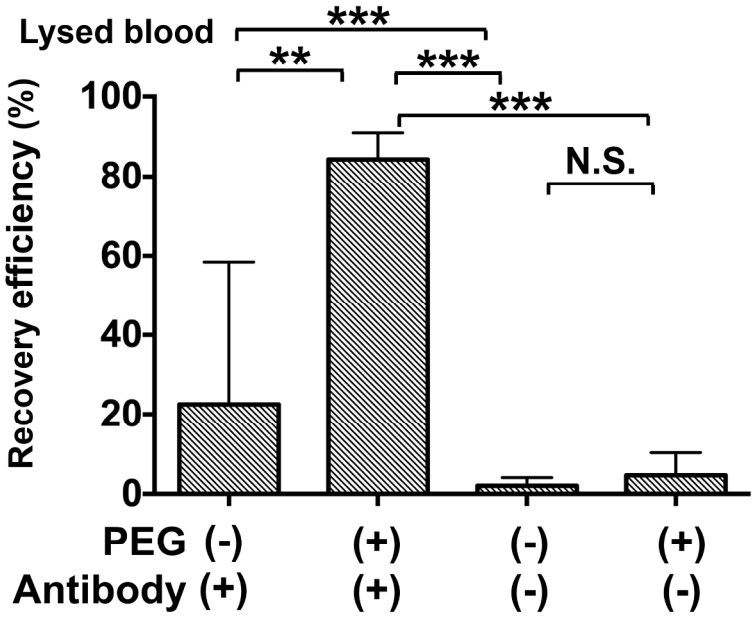

Fig. S7. Capture and release of PC-3 from lysed blood.

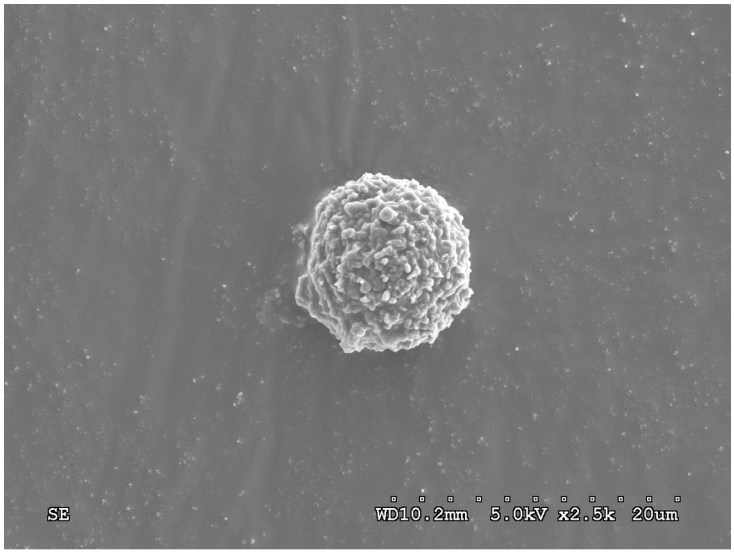

Fig. S8. SEM image of a PC-3 cell.

References:

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