Supporting Information for

Antibody modified porous silicon microparticles for the selective capture of cells

Bin Guan [1,2], Astrid Magenau [2,4], Simone Ciampi [1], Katharina Gaus [2,4], Peter J. Reece [5], J. Justin Gooding [1,2,3*]

1. School of Chemistry; 2. The Australian Centre for NanoMedicine; 3. ARC Centre of Excellence in Convergent Bio-Nano Science and Technology, 4. Centre for Vascular Research; 5. School of Physics

The University of New South Wales, Sydney, Australia

Email: Justin.gooding@unsw.edu.au

Scanning electron micrographs of functionalized PSi particles

The tetra (ethylene glycol) (EO_4) functionalized PSi particles were fabricated *via* surface modification and ultrasonication processes. Scanning electron microscopy (SEM) was employed to evaluate the resulting particle size and the pore morphology of PSi structure. Figure S1a shows the SEM images of randomly shaped particles immobilized on carbon tape. By the analysis of dozens of particles, the Feret diameter[1] for PSi particles lies mostly in the range of 20 to 80 μm. The distribution histogram is displayed in Figure S1c. The top view of one of the EO_4 modified particles (Figure S1b) indicates PSi particles retain a good porosity with no destruction of porous structure during the sonication. The average pore size for EO_4 particles is 16 nm, suited to further antibody immobilization or drug loading.

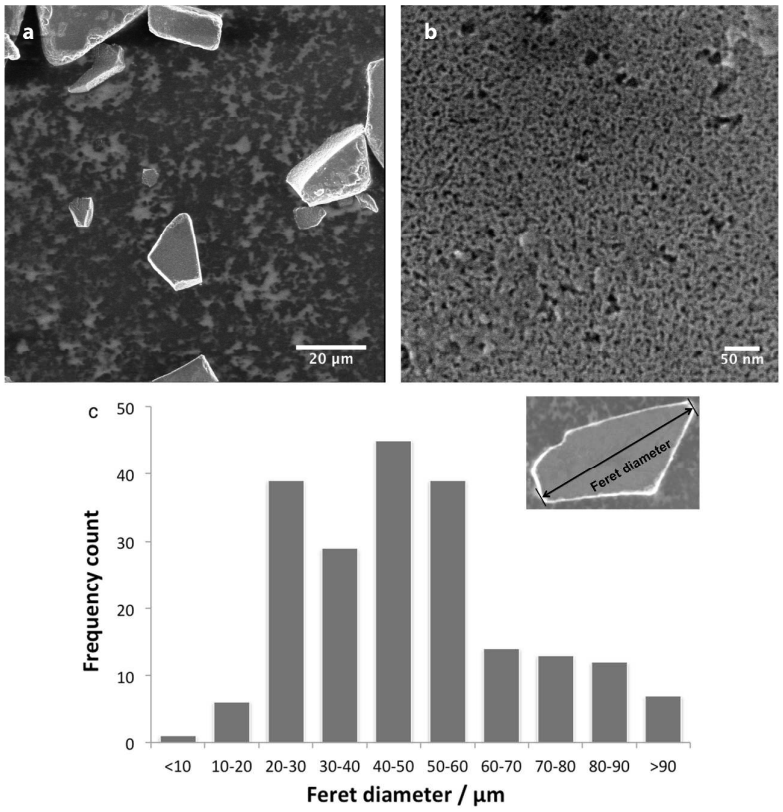

Figure S1. Scanning electron micrographs of a) EO_4-functionalized PSi microparticles with a size range between 20 and 80 µm, and b) top view of the porous surface showing an average pore size of 16 nm. c) Feret diameter distribution histogram for PSi microparticles. The inset indicates how the Feret diameter of a PSi particle is defined for this work.

Surface-bound antibodies functionality

To further confirm the immobilisation of antibodies on surfaces and to illustrate that antibodies bound to the particles remain capable of binding to specific epitopes of cells, an antibody functioniality test was conducted. Anti-BSA antibody was coupled onto the surface of PSi particles for specifically binding to free FITC-labeled BSA in solution. The particles with distal EO_4 moieties, *i.e.* antifouling particles, acted as control.

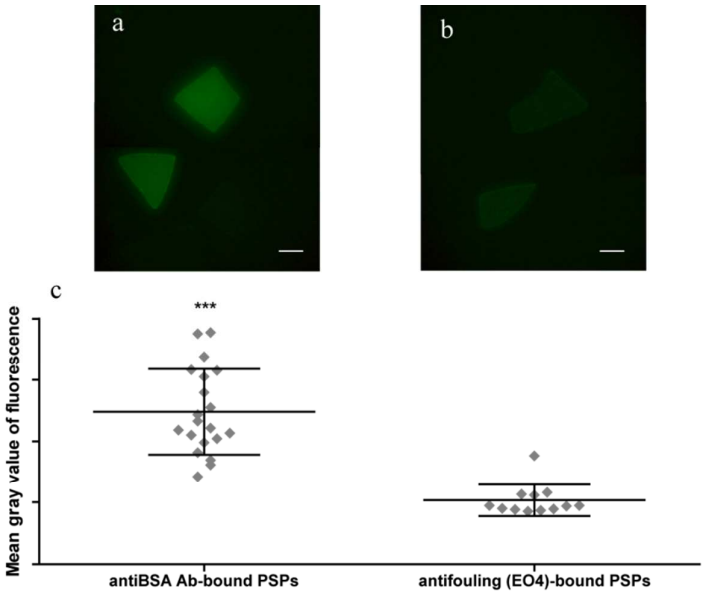

Figure S2. Comparison of the fluorescence intensities obtained from anti-BSA modified PSi particles and antifouling particles after interacting with free FITC-BSA in solution. Green fluorescence images of (a) anti-BSA modified PSi particles and (b) antifouling particles. c) Statistic analysis of fluorescence emissions from FITC-BSA incubated PSi particles. Asterisks represent significant difference based on one-way ANOVA analysis, while *** stands for significance at 99.9% confidence interval. Scale bar equals 50 μm.

Figure S2 shows the fluorescence micrographs of anti-BSA modified particles and antifouling particles after incubation with FITC-BSA solution. The anti-BSA modified particles clearly display a bright green fluorescence (Figure S2a), demonstrating the strong binding between free FITC-BSA and anti-BSA antibody on the surface. Some extent of non-specific binding is observed on the antifouling particles as weak green fluorescence can be viewed in Figure S2b. In order to quantify the intensity difference between the green fluorescence observed in Figure S2a and S2b, the mean grey value from each particles was measured and recorded by ImageJ. One-way ANOVA analysis performed on the mean grey values from more than 30 particles with different functionalities (Figure 1c) illustrates significantly higher binding affinity of FITC-BSA to anti-BSA particles than to antifouling particles. This result provided evidence that 1) antibodies were successfully coupled onto PSi particles, and 2) surface-bound antibodies were capable of binding to their corresponding affinity partners.

Optical properties of cell-bound PSi microparticles

As indicated in the experimental section, the PSi microparticles were fabricated to retain a multilayer structure by a periodical switching of the current density in the electrochemical etching process. The PSi multilayer structure is called a rugate filter, usually featuring a high reflectivity band gap on a tunable wavelength. The position of the high reflectivity band gap in the spectrum can be easily tailored by the current density and the etching time in the electrochemical process, which are related to the refractive index and thickness of multilayers. The reflectivity properties of the PSi particles are critical to cell-based sensing or drug delivery applications, as the reflectivity spectra of particles can indicate any changes occurred inside the pores of PSi, such as drug loading or releasing.

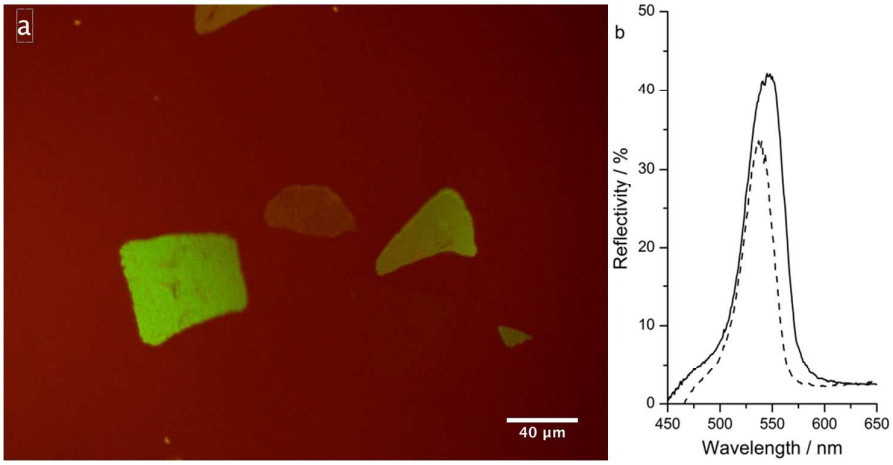

Figure S3. a) Epi-illumination image of anti-GFP PSi particles adhering on HeLa cells and b) the reflectivity spectra of anti-GFP particles.

Here, the particles being attached with cells were examined for their optical properties. Figure S3 presents the epi-illumination image of anti-GFP particles associated with GFP-transfected HeLa cells and their reflectivity spectra. As shown in the figure, anti-GFP particles show green color in the reflectance imaging mode, as distinct from the anti-mCherry particles in red under reflectance mode. The optical properties of the PSi particles displayed here enhance the possibility of using these PSi particles for further specific cell-targeting biosensing or drug delivery.

Cell targeting with antibody modified PSi microparticles

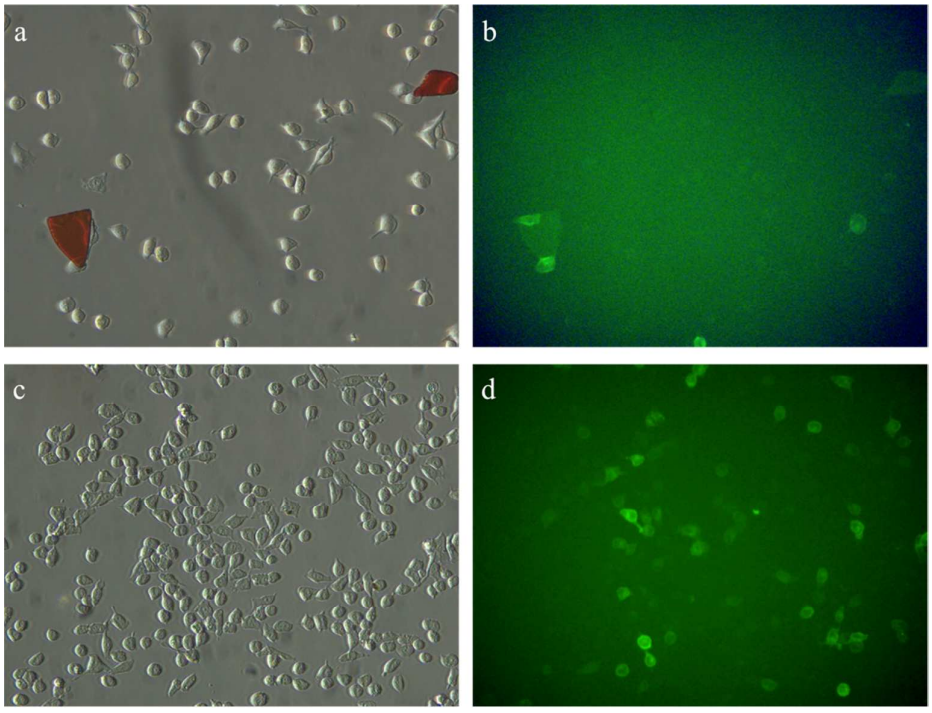

Figure S4. a-b) Bright-field microscope image (a) and fluorescence image (b) of antifouling particles and GPI-GFP transfected HeLa cells. Very minor amounts of non-specific adsorption was observed, as few antifouling particles were bound to transfected HeLa cells. c-d) Bright-field micrograph (c) and fluorescence image (d) of anti-BSA particles and GPI-GFP-transfected HeLa cells interaction experiment. Negligible amount of antifouling and antiBSA particles were associated with transfected cells, suggesting specific targeting by antibody modified particles.

References

1. Merkus, H. G., Particle size, size distributions and shape. In *Particle Size Measurements: Fundamentals, Practice, Quality*, Springer: 2009; p 15.