Supporting Information

Elastomeric Negative Acoustic Contrast Particles for Affinity Capture Assays

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Silicon Acoustic Sample Preparation Chip

An acoustic sample preparation chip was fabricated in silicon via photolithography using a photomask designed from AutoCAD software (Autodesk Inc., San Rafael, CA) and a deep reactive ion etching (DRIE) using a protocol described by Suthanthiraraj et al.[1] DRIE allowed specific micro-channel patterns to be etched onto the surface of the silicon wafer (WaferNet Inc., San Jose, CA). A borosilicate glass slide (Borofloat 33, Schott North America, Inc., Elms Ford, NY) with 1 mm holes drilled through it to accommodate inlet and outlet ports (tube connection sites) was then sealed on top of the etched silicon wafer using anodic bonding.[1] To allow easy insertion of silicone tubing (Cole-Parmer Instrument Company, Vernon Hills IL) into inlet and outlet ports, square PDMS blocks, with punctured holes, were aligned and plasma sealed on top of the inlet and outlet ports.[2] Silicone tubing was then inserted and epoxy glued into the PDMS inlet and outlet ports. To complete the fabrication, a lead zirconate titanate piezoelectric transducer (PZT: 5 mm width x 30 mm length x ~1 mm thickness) (Boston Piezo-Optics Inc., Bellingham, MA) was attached to the bottom side of the chip using cyanoacrylate glue.

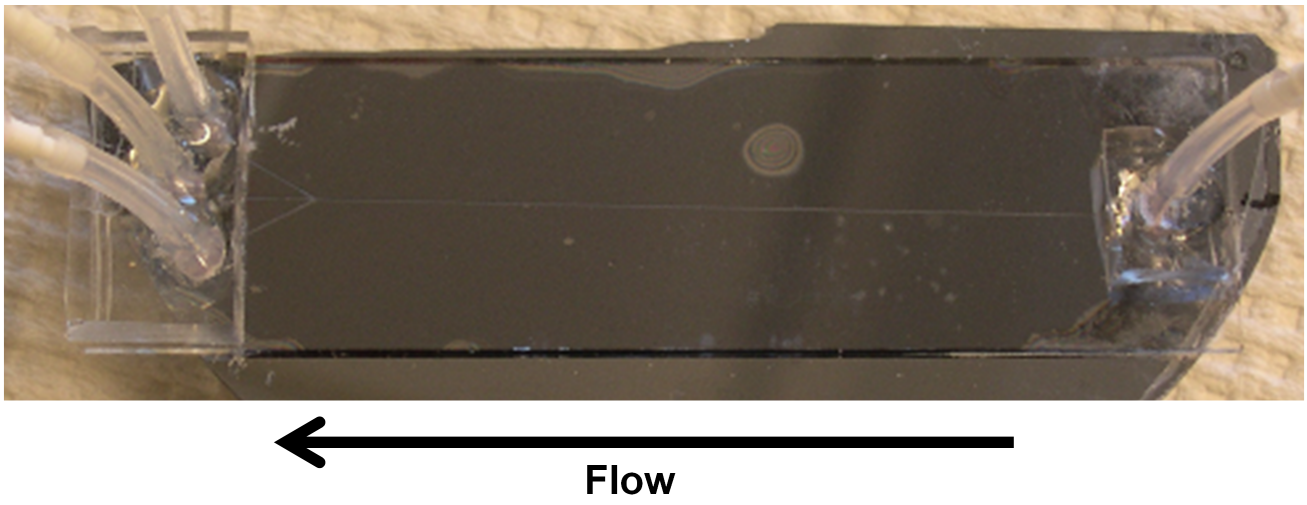

Figure S1. Acoustic sample preparation chip.

1. Austin Suthanthiraraj, P. P.; Piyasena, M. E.; Woods, T. A.; Naivar, M. A.; Lopez, G. P.; Graves, S. W., One-dimensional acoustic standing waves in rectangular channels for flow cytometry. *Methods* **2012**.

2. Saarela, V.; Franssila, S.; Tuomikoski, S.; Marttila, S.; Östman, P.; Sikanen, T.; Kotiaho, T.; Kostiainen, R., Re-usable multi-inlet PDMS fluidic connector. *Sensors and Actuators B: Chemical* **2006,** *114* (1), 552-557.

Avidin Adsorption and Biotin-Binding

Elastomeric particles (2.5 x 10^7) where incubated in 1 µM avidin (Molecular Probes, Eugene, Oregon) in 1 mL of phosphate buffered saline (1X PBS with 7.4 pH: 10 mM Na_2HPO_4; 1 mM KH_2PO_4; 138 mM NaCl; 3 mM KCl) for 30 minutes with continuous rocking at room temperature. They were then centrifugally (2900 g for 5 min) washed and resuspended in 1 mL of washing/blocking buffer (0.1 % BSA in PBS) and incubated with continuous rocking at room temperature for 30 minutes. 5.0 x 10^5 avidinylated-elastomeric particles were then incubated in 200 µL in PBS with different concentrations of biotin-4-fluorescein (0, 0.1, 1, 10, 100 nM; Invitrogen) for 30 minutes with continuous rocking at room temperature. The particles were then analyzed with an Accuri C6 flow cytometer without prior washing. The biotin-block controls were performed in a similar manner but were incubated with 400 µM of free-biotin (Sigma-Aldrich) prior to incubation with biotin-4-fluorescein.

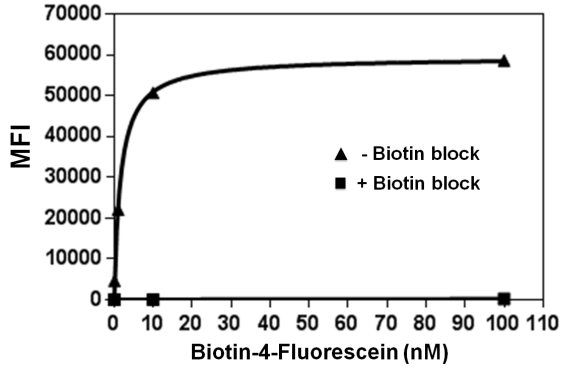

Figure S2. Binding curve for avidinylated-elastomeric particles that were incubated with different concentrations of biotin-4-fluorescein and then analyzed using flow cytometry (Accuri C6). The data were fit to a 1-site binding model (R^2= 0.99) using GraphPad PRISM 6.

Attachment of Biotinylated Capture Antibody

Avidinylated-elastomeric particles (2.5 x 10^7) were prepared as described above. A biotinylated mouse anti-human PSA monoclonal antibody (capture antibody) (HyTest Ltd., Turku, Finland) was added to make a 6 nM antibody solution in 1 mL of PBS, which was then incubated for 30 minutes with rocking at room temperature. The resulting ECµPs were centrifugally washed and resuspended in washing/blocking buffer. 5.0 x 10^5 ECµPs were then incubated in 200 µL of 1 nM of goat anti-mouse IgG-phycoerythrin (PE) (detection antibody) (Abcam, Cambridge MA) for 30 minutes with rocking at room temperature. Ligand-bound ECµPs were then analyzed by flow cytometry with a 585 ± 20 nm bandpass filter without prior washing. To determine the level of non-specific binding of the detection antibody, a negative

control was performed in the same way, but without the inclusion of the biotinylated capture antibody.

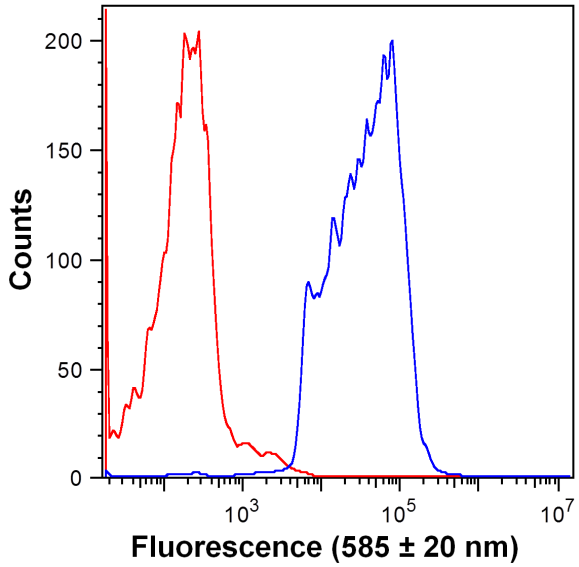

Figure S3. Fluorescence histograms showing the attachment of a biotinylated mouse anti human PSA monoclonal antibody (capture antibody) to avidinylated-elastomeric particles as measured by flow cytometry. Attachment of the biotinylated capture antibody was detected using a goat anti mouse polyclonal antibody-PE (detection antibody) (blue trace). To determine the non-specific binding of the detection antibody, a negative control (red trace) was performed in the same way, but without the inclusion of the biotinylated capture antibody. The median fluorescence intensity (MFI) of the negative control was 148, whereas the MFI of the sample treated with the biotinylated capture antibody was 39,270. These results indicate that ECμPs can be easily formed through the specific adsorption of high molecular weight biotinylated biomolecules (e.g., ~150,000 Da IgGs) to avidinylated PDMS microparticles.

Gating of Flow Cytometry Data for Quantifying Separation Efficiency

To identify the blood cell population in subsequent measurements of separation efficiency, 0.1 % whole porcine blood (diluted in PBS with 0.1% BSA) in 100 µL was analyzed using flow cytometry (Accuri C6). The data collected from this analysis was graphed on a scatter plot (fluorescence (585 ± 20 nm) vs. forward scatter, see Fig. S4a). The above-mentioned blood sample was then diluted into 900 µL of a common lysing buffer (recipe from Cold Spring Harbor Laboratory: 155 mM NH_4Cl; 12 mM $NaHCO_3$; and 0.1 mM EDTA in DI water) and incubated for 10 minutes at room temperature. The blood sample was then also analyzed using flow cytometry under identical conditions as mentioned-above. Based on the two analyses a gate was set to count particles with the fluorescence and scatter characteristics of blood cells (see Fig. S4a,b).

5.0 x 10^5 ligand-bound ECµPs (fluorescently labeled ligand = goat anti mouse IgG antibody conjugated to phycoerythrin, 1 nM) in 200 µL of PBS (0.1% BSA) were analyzed using flow cytometry. The fluorescence (585 ± 20 nm) vs. forward scatter for the ECµPs is given in Fig. S4c. The ligand-bound ECµP population was clearly distinct using these parameters from the blood cell population and thus the gating shown in Fig. S4 for each population can be used to distinguish each type of particle and to quantify separation efficiency.

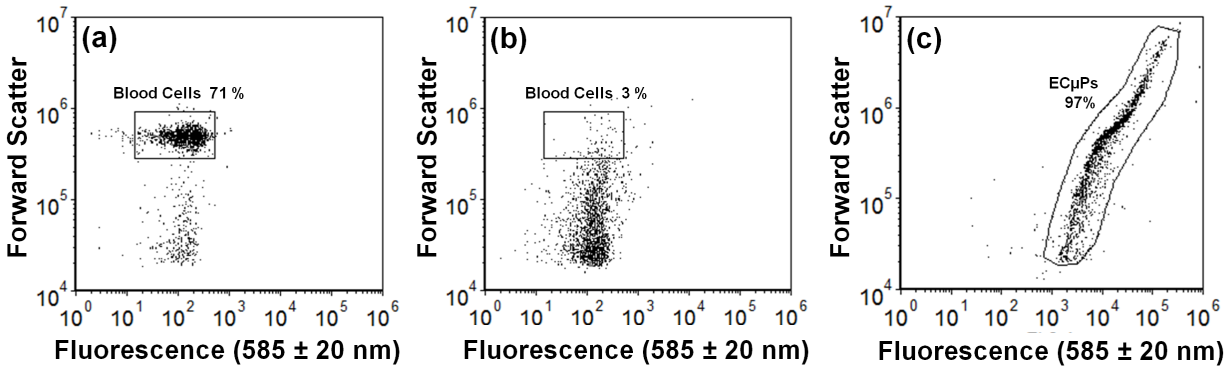

Figure S4. Porcine blood cells and ligand-bound ECµPs were identified using flow cytometry scatter plots (fluorescence (585±20 nm) vs. forward scatter). To quantify blood cells and ligand-bound ECµPs (fluorescently labeled ligand = goat anti mouse IgG antibody conjugated to phycoerythrin) it was necessary to determine the location of the blood cells and ligand-bound ECµPs on the scatter plots. 0.1% whole porcine blood was (a) analyzed using flow cytometry followed by lysis (b) and then re-analysis. (c) Ligand-bound ECµPs were analyzed using flow cytometry to determine the location of their appropriate gate. The numbers reported in the plots indicate the fraction of particles within the gating region in each case.

Synthesis of Monodisperse Elastomeric Particles

Sylgard 184 (20% crosslinking agent) was used to form microdroplets in a T-junction microfluidic chip (polydimethylsiloxane-based).[1] The continuous phase contained ultrapure water with 5% polyvinyl alcohol (PVA).[2] To prevent Sylgard 184 (with crosslinking agent) from wetting the micro-channel walls, the continuous phase was first injected using a syringe pump (Nexus 3000, Chemyx Inc. Stafford, TX) at a flow rate of 5 µL/min. The dispersed phase (Sylgard 184 with 20 wt.% crosslinking agent) was then injected into the microfluidic chip at a flow rate of 0.08 µL/min. Monodisperse elastomeric droplets were collected and cured at 70 °C overnight, with stirring, to form crosslinked monodisperse elastomeric particles. A Coulter counter (Z2 Coulter Particle Count and Size Analyzer, Becton Dickinson, Franklin Lakes, NJ) was used to determine the size distribution of the near-monodisperse elastomeric particles.

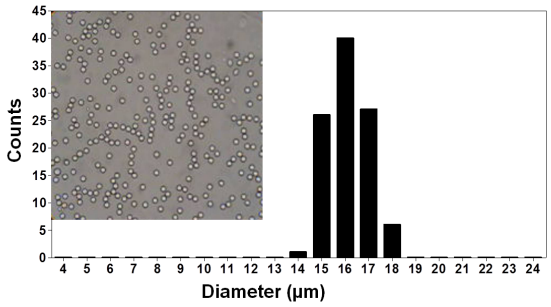

Figure S5. Uniformly sized elastomeric particles were synthesized using a T-Junction PDMS microfluidic chip. The mean diameter was measured to be 16.1 µm with a standard deviation (S.D.) of ± 0.9 µm and a coefficient of variation (C.V.) of 5.6 %.

1. Xu, J.; Li, S.; Tan, J.; Wang, Y.; Luo, G., Preparation of highly monodisperse droplet in a T-junction microfluidic device. *AIChE journal* **2006,** *52* (9), 3005-3010.

2. Elbert, D. L.; Hubbell, J. A., Surface treatments of polymers for biocompatibility. *Annual Review of Materials Science* **1996,** *26* (1).