Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information

Self-concentrating buoyant glass microbubbles for high sensitivity immunoassays

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Materials and Methods

Reagents

Glass microbubbles (iM30K) were a gift from 3M Inc. 3-Aminopropyltriethoxysilane (APTES), and Glutaraldehyde (50%) were purchased from Sigma-Aldrich. Capture antibodies (anti-human TNF-α, anti-human IL-6), recombinant human cytokines (TNF-α, IL-6), and biotinylated detection antibodies (anti-human TNF-α, anti-human IL-6) were purchased from R&D Systems. Streptavidin-phycoerythrin (SAPE) was purchased from BDbiosciences. Negative photoresist (SU-8) was purchased from MicroChem. Polydimethylsiloxane (PDMS) was purchased from Dow Corning.

Microfluidic liquid hemisphere array device fabrication.

The microfluidic device was fabricated in polydimethylsiloxane (PDMS) using an open-air channel soft lithography technique[1]. In brief, the device design was drawn with AutoCAD software, then printed onto a photoresist mask. In a cleanroom, we spincoated photoresist (SU-8) onto a silicon wafer with a thickness of 100 μm and 130 μm corresponding to the required channel height. Then, the patterned mask was placed over the SU-8 coated silicon wafer for UV exposure, which crosslinks the photoresist in the exposed regions, and the unexposed regions were washed away with developer solvent to generate a master mold. PDMS was poured onto the master mold, then a PE sheet and glass plates were placed atop the master and compressed to squeeze out the excess PDMS from the top of the master mold structures. The PDMS prepolymer on the master was then baked in an oven at 65˚C for 24 hours to solidify. Subsequently, the PE sheet together with the PDMS structure was removed from the master. The PDMS structure was then bonded to a glass slide via oxygen plasma treatment, and the PE sheet was peeled off the PDMS to leave an open channel structure.

Glass microbubble antibody conjugation.

Although multiple glass surface modification and antibody conjugation methods have been reported previously, we adapted a protocol used for microcontact printing[2] with a few adjustments to suit our application. Glass microbubbles were first treated with 1:1 (v/v) methanol in HCl for 30 minutes on a rocker to hydroxylate the glass surface, then washed in 99.8% ethanol for 3 times to remove the acid. The microbubbles were then treated with 10%

(v/v) solution of 3-Aminopropyltriethoxysilane (APTES) in ethanol for 60 min on a rocker to silanize the glass surface, washed once with ethanol, once with PBS, then treated with 2.5% (v/v) Glutaraldehyde in PBS for 2 hours on a rocker. After 2 washes with PBS, the glass microbubbles were incubated with capture antibody solution (96 μg/mL anti-human TNF-α, 48 μg/mL anti-human IL-6), or 1% BSA (negative control) in PBS for 1 hour to enable covalent conjugation of the antibody to the glass surface. All steps were performed at room temperature.

We noted that the packaged glass microbubbles actually contained some broken bubble fragments, which sink to the bottom of the liquid, whereas intact bubbles remain buoyant. Thus we removed the broken bubbles via simple centrifugation (1500 rpm, 1 min). For changing the reaction and wash solutions for the microbubbles, the bubble-solution mixture was centrifuged at 1500 rpm for 1 min, which "spins up" the bubbles to the top of the centrifuge tube, whereas the waste solution at the bottom was withdrawn by a syringe attached with a long Teflon tubing.

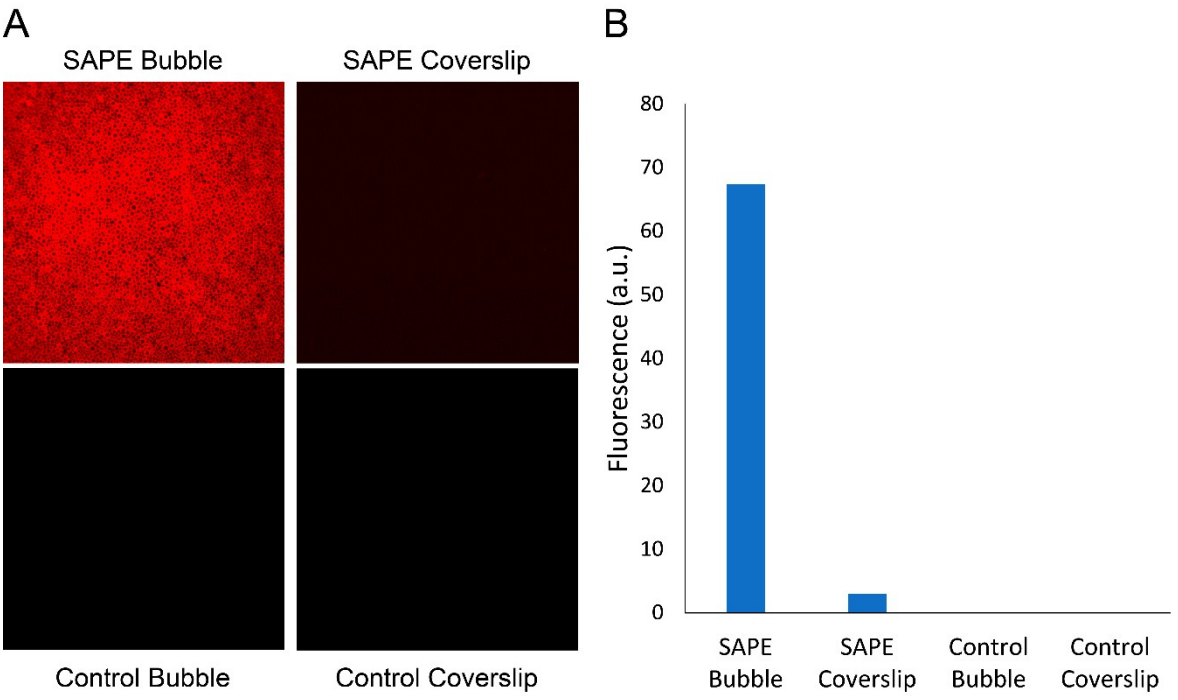

Fig. S1 Signal amplification of glass microbubbles. (A) Fluorescent images of glass coverslips and microbubbles with or without fluorescent streptavidin-phycoerythrin (SAPE) conjugation. (B) Quantification of fluorescent image intensity. Microbubbles displayed a signal amplifying effect of up to 22.7 times (arbitrary unit) compared to that of a glass coverslip. Both control (non-coated) microbubbles and control coverslips display very low background noise.

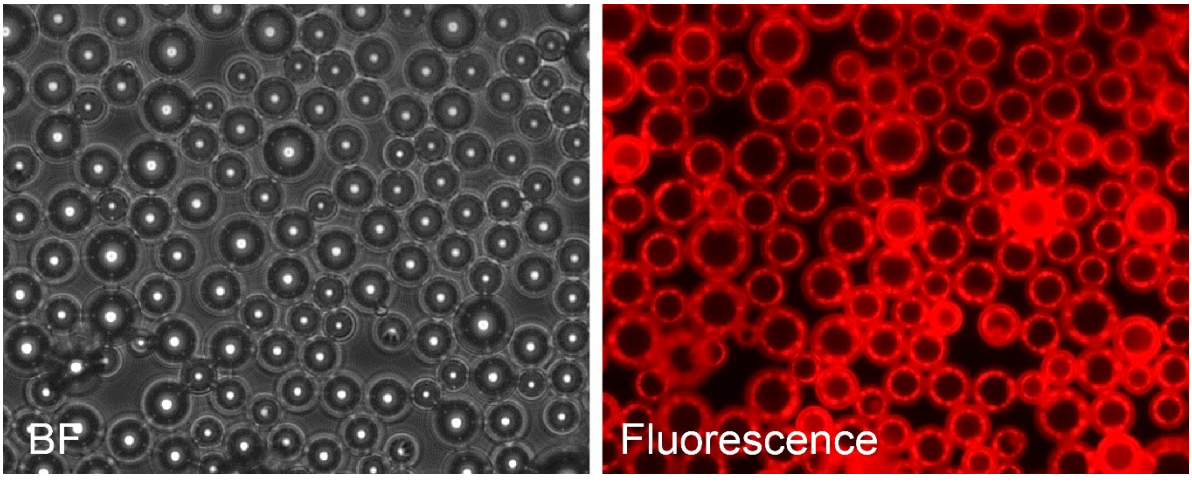

Fig. S2 Lensing effect of the glass microbubbles. Left, under bright field microscopy, glass microbubbles can focus light to a single spot on the center of the bubble, whereas in fluorescence (right), the glass bubbles concentrate the fluorescent signal to a circular ring (halo) around the bubbles when focused on the focal plane of the microscope.

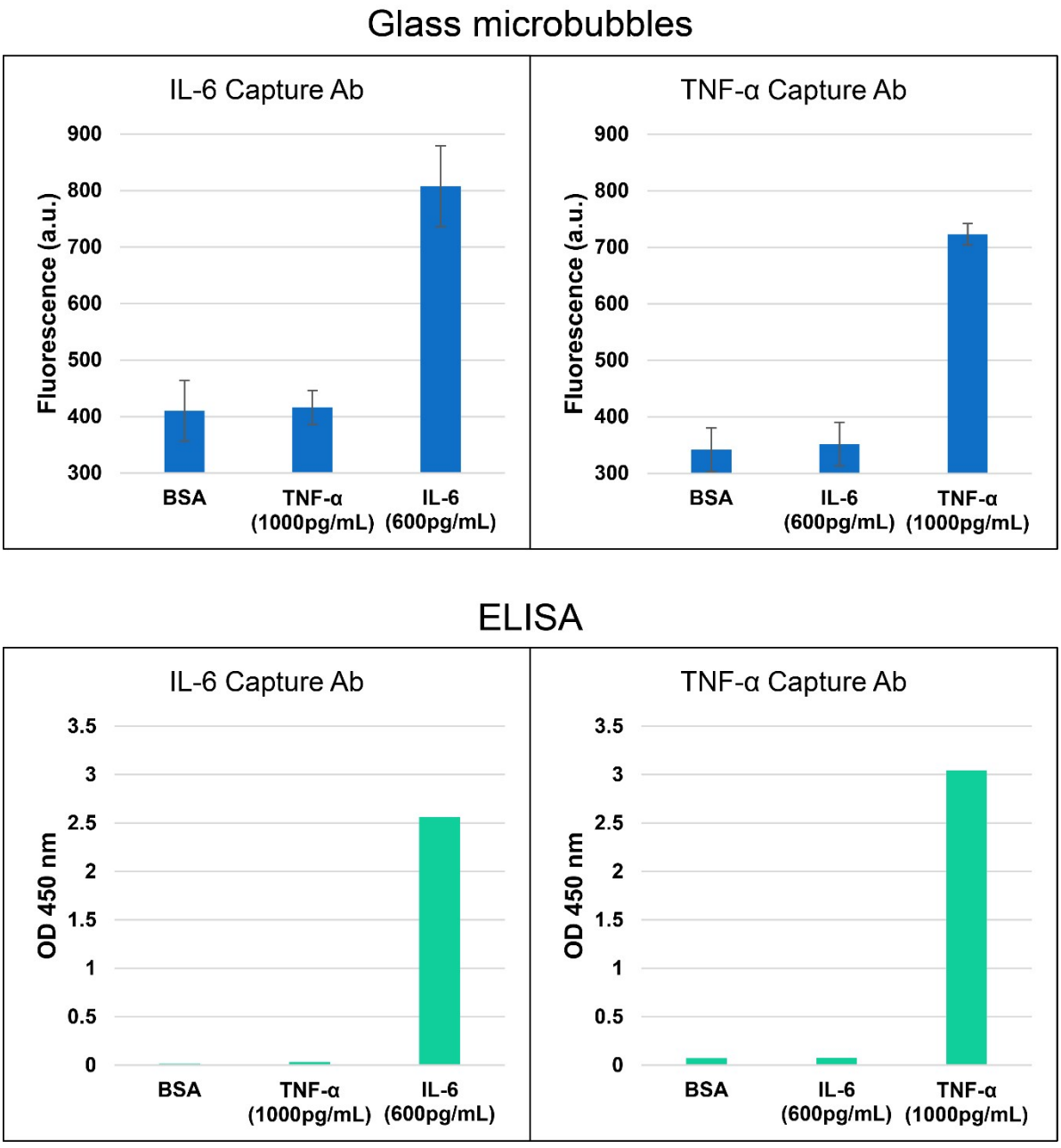

Fig. S3 Validation of specificity of antibodies used in the experiment. Both antibody functionalized glass microbubbles (top) and standard ELISA (bottom) displayed high specificity towards the target antigen with no detectable cross reactivity towards the non-target antigen, even at high concentrations.

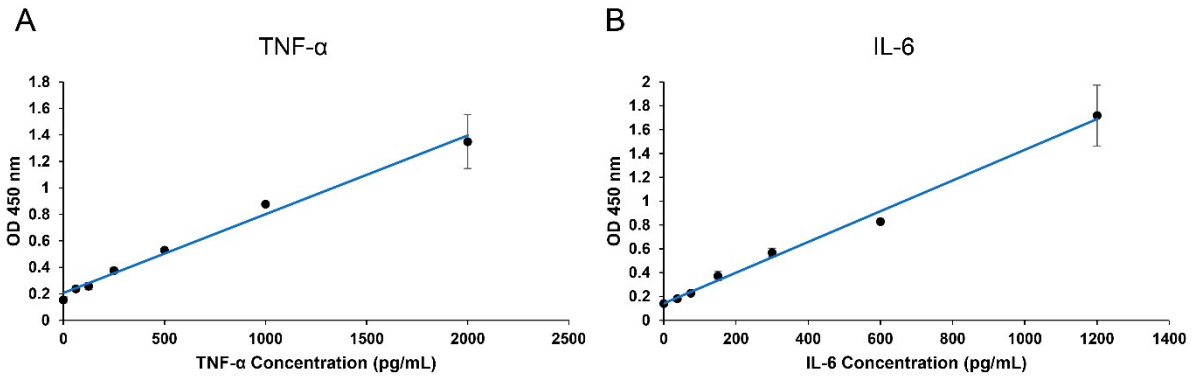

Fig. S4 Sensitivity of standard ELISA using the same antibodies under same conditions as the microbubble array platform. The limit of detection for TNF-α and IL-6 on ELISA are 24.04 pg/mL and 32.51 pg/mL, respectively.

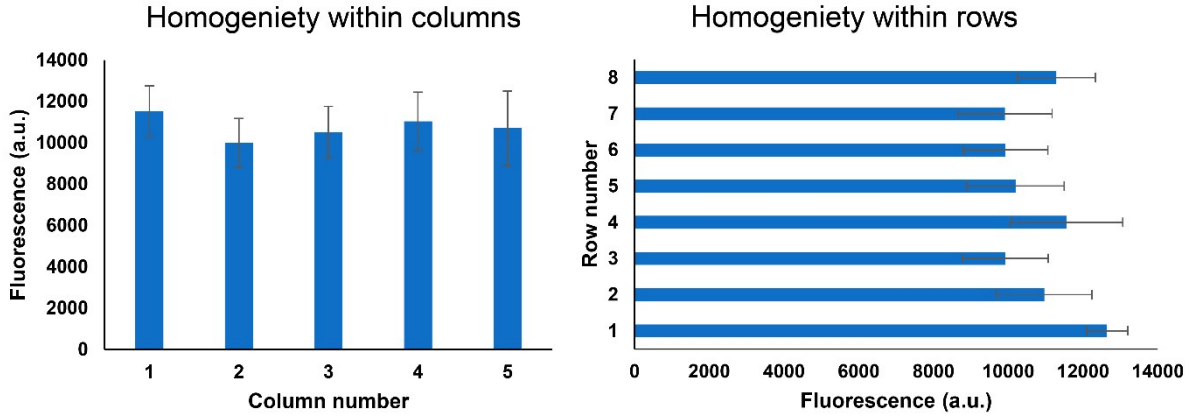

Fig. S5 Signal homogeneity within the columns and rows of the microbubble array platform. Coefficient of variation (CV) = 12.95%

References

1. C. H. Hsu, C. Chen and A. Folch, *Lab on a chip*, 2004, **4**, 420-424.
2. E. Blinka, K. Loeffler, Y. Hu, A. Gopal, K. Hoshino, K. Lin, X. Liu, M. Ferrari and J. X. Zhang, *Nanotechnology*, 2010, **21**, 415302.