Supporting Information

Microfluidic Particle Dam for Visual and Quantitative Detection of Lead Ions

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Experimental Section

Design and fabrication of microchip

The layout of the microfluidic chip is based on our previous design for visual quantification of DNA oligonucleotide (Figure S1).[1] The device fabrication is based on conventional soft-lithography (Figure S2). Here, instead of the conventional polydimethylsiloxane (PDMS), Norland optical adhesive 63 (NOA63, Norland Products, USA) is used as the channel material to bond on glass substrate because it can maintain its hydrophilic property in long term.[2] After plasma treatment (Harrick Plasma, 400 mTorr, 2 min), the contact angle of NOA can be reduced from 79.8° to 60.9° for many days, which is similar to the plasma-treated glass and much stable than plasma-treated PDMS (Figure S3). To fabricate it, SU8 2015 photoresist (Gersteltec Sarl, Switzerland) was first spin-coated onto the silicon wafer (Suzhou Crystal Silicon Electronic & Technology Co., Ltd.) at 1200 rpm so that SU8 master with a depth of 25 ± 0.3 μm can be obtained. After UV exposure and development, PDMS precursor (elastomer base: curing agent = 10:1, Sylgard^{TM} 184, Dow Corning, USA) was poured onto the SU8 master mold, cured at 70 ℃ for 2 hours, and peeled off. Next, the patterned surface of the PDMS was sequentially treated with plasma (Harrick Plasma, 400 mTorr, 2 min) and coated with (3-Aminopropyl) trimethoxysilane (Sigma Aldrich, USA) by gas-phase deposition at room temperature for 6 hours, followed by a secondary PDMS casting on top of it. After curing and demolding, NOA was smeared onto the patterned surface of the secondary PDMS. A commercially available polypropylene film with 100 μm thickness (KOKUYO, Japan) was utilized to put on top of the uncured NOA63 glue before UV-curing for 50 seconds, which facilitates the peeling process of the NOA63. In particular, the outer region of the inlet was made to be hydrophobic to ensure that all

particles can be introduced into the hydrophilic microchannel (Figure S1). To fabricate it, a glass slide (Sail Brand, 1" × 3", Boshida Ltd.) was coated with trichloro(1H,1H,2H,2H-perfluorooctyl)silane 97% (J&K Scientific Ltd.) diluted in toluene (10 wt%) inside a vacuum chamber for 40 min. After coating, the outer region of the inlet was protected by a tape to retain its hydrophobicity while leaving the other region exposed. After plasma treatment (800 mTorr, 2 min) on the glass slide and the patterned surface of NOA63, the NOA63 chip was bonded onto the glass slide. In this way, the device can maintain the outer region (silanized glass) hydrophobic (contact angle: 123.3º ± 7.21) and inner wall of NOA63 microchannel hydrophilic (Figure S2). A neodymium magnet in the size of 2.6 mm × 1.8 mm × 1.5 mm was then glued at 1 mm next to the magnetic separator. All chemicals were of analytical grade.

Modification of MMPs and PMPs

Briefly, 1 μl of MMPs (0.36 μm in diameter, 1.07 μg biotin-FITC per mg MMPs, 10 mg/ml, Bangs Laboratories, Inc., USA) or PMPs (15.34 μm in diameter, 0.038 μg biotin-FITC per mg PMPs,10 mg/ml, Bangs Laboratories, Inc., USA) solution was added with 1 μl of oligonucleotide probes (Probe 1 for MMPs and Probe 2 for PMPs, 100 μM in diethyl pyrocarbonate (DEPC)-treated water, BGI-Tech Solutions Co., Ltd., sequence in Table S1). Note that both MMPs and PMPs were fully loaded as the added amount of oligonucleotides was 3×10^{-10} moles per particle, which is beyond the maximum loading capacity of MMPs and PMPs (3.86×10^{-11} mole and 1.37×10^{-12} mole per particle, respectively). The mixture of MMPs + Probe 1 and PMPs + Probe 2 were incubated with gentle shaking for 30 min at room temperature. The excess amount of oligonucleotides were removed by rinsing three

times with 200 μl TA buffer (50 mM Tris Acetate consisting of Tris(hydroxymethyl) aminomethane (J&K Scientific) and acetic acid (J&K Scientific), 0.2 M NaCl, 0.2% Tween 20). In each washing step, MMPs were isolated using a magnetic separation rack, while the PMPs were isolated by a centrifuge (13.8 × g for 3 min). At last, the Probe 1-modified MMPs and Probe 2-modified PMPs were mixed together and re-suspended in 2 μl of TA buffer.

Preparation of GR-5 DNAzyme

The GRDS and GRE were designed based on the original form[3] with extension on its two termini, allowing hybridization with Probe 1 at the 3' end and Probe 2 at the 5' end (Table S1). Once receiving, the oligonucleotides were dissolved in DEPC-treated water (Thermo Fisher Scientific). To prepare the GR-5 DNAzyme, the GRDS (Integrated DNA Technologies Pte. Ltd., sequence in Table S1) and GRE (BGI-Tech Solutions Co., Ltd., sequence in Table S1), diluted in TA buffer, were first mixed with 1:1 ratio at the concentration of 1 μM each for 1-hour incubation. Dissolving in TA buffer or DEPC-treated water allows the DNA solution stable in the refrigerator (4 °C) for several months without losing its normal function.

Detection of lead ions

The Lead (Pb^{2+}), Nickel (Ni^{2+}), Copper (Cu^{2+}), Cadmium (Cd^{2+}), Calcium (Ca^{2+}), Barium (Ba^{2+}) or Mercury (Hg^{2+}) solution (J&K Scientific (Hong Kong) Ltd, Hong Kong) was diluted in Deionized (DI) water (Milli-Q Plus system, with a resistivity of 18.2 MΩ·cm) with different concentration. For reaction in different pH values, DEPC-treated water with

50 nM of lead ions was first adjusted by addition of NaOH or HCl until the pH reached 6, 6.5, 7, 7.5, 8, or 8.5. For detection with different water hardness, 50 nM of lead was diluted in four kinds of water samples with different hardness levels (Soft (55 mg/L), Moderately Hard (107.5 mg/L), Hard (157.7 mg/L), and Very Hard (318.3 mg/L). For tests in tap water, lead ions with different concentration were spiked in tap water. Next, the metal ion solution of 0.5 μl was mixed with the 0.5 μl of DNAzyme solution (150 nM) for 1 hour. Afterward, the solution of DNAzyme and metal ions of 1 μl was mixed with the solution containing MMPs and PMPs in 2 μl in microcentrifuge tube coated with BSA (1%) for 30 min. At last, the total of 3 μl of the final solution was loaded into our device. Thus, the total sample-to-answer time is around 1.5 hours.

Agarose gel electrophoresis

To obtain the 5% agarose gel, five grams of agarose powder (Thermo Fisher Scientific) was dissolved in 100 ml of 1X TAE buffer (diluted from 10X TAE buffer, Thermo Fisher Scientific). All the agarose particles were dissolved after microwaved until boiling. After mixing with 3 μl of 10,000X gel red (BIOTIUM), the solution was poured into the casting tray until cooled down and gelation. All oligonucleotides, including GRDS, GRE, and GR-5, were prepared at 200 nM diluted by TA buffer. For GR-5 + Pb^{2+}, 200 nM lead solution was applied. Next, the electrophoresis was conducted inside a gel tank (electrophoresis unit) prefilled with the 1X TAE buffer under 130 V for 45 mins after loading. To visualize the gel, BIO-RAD Gel Doc EZ Imager was used with reference to GeneRuler Ultra Low Range DNA Ladder (SM1211, Thermo Fisher Scientific).

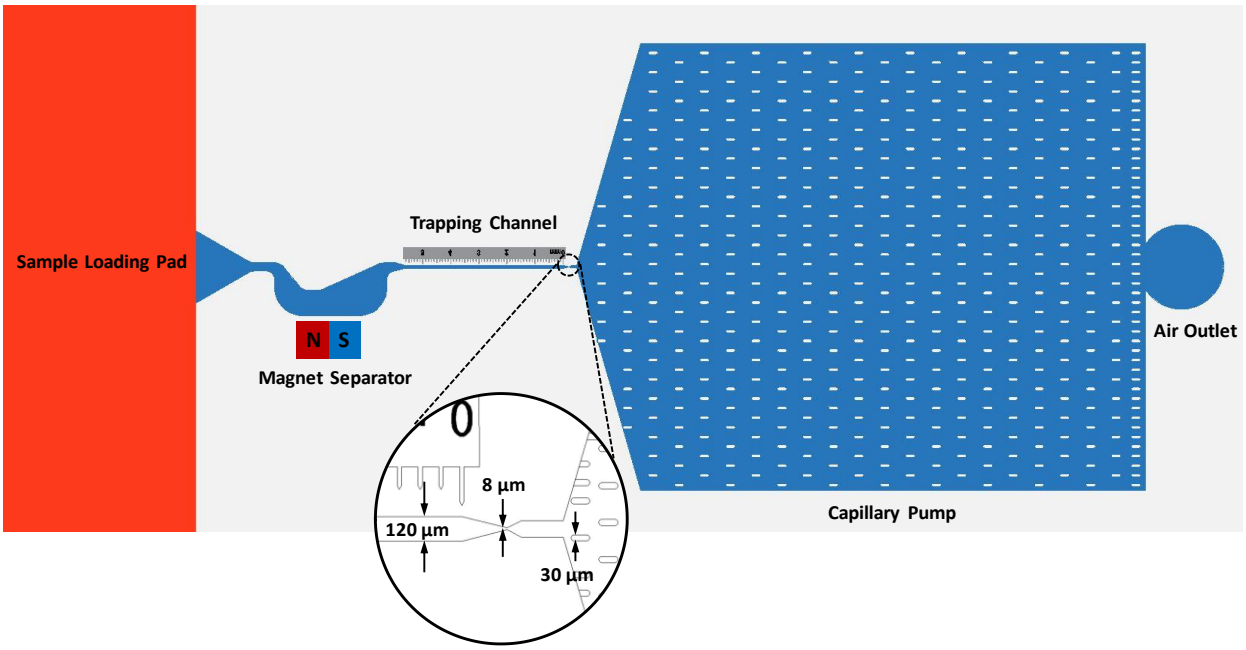

Figure S1. Layout of the microfluidic chip. A top view of the microfluidic system consists of a hydrophobic sample loading pad, a magnetic separator, a trapping channel, a particle dam with a nozzle, a capillary pump, and an air outlet. The width of the nozzle, 8 µm, was designed to trap PMPs with diameter of 15.34 µm.

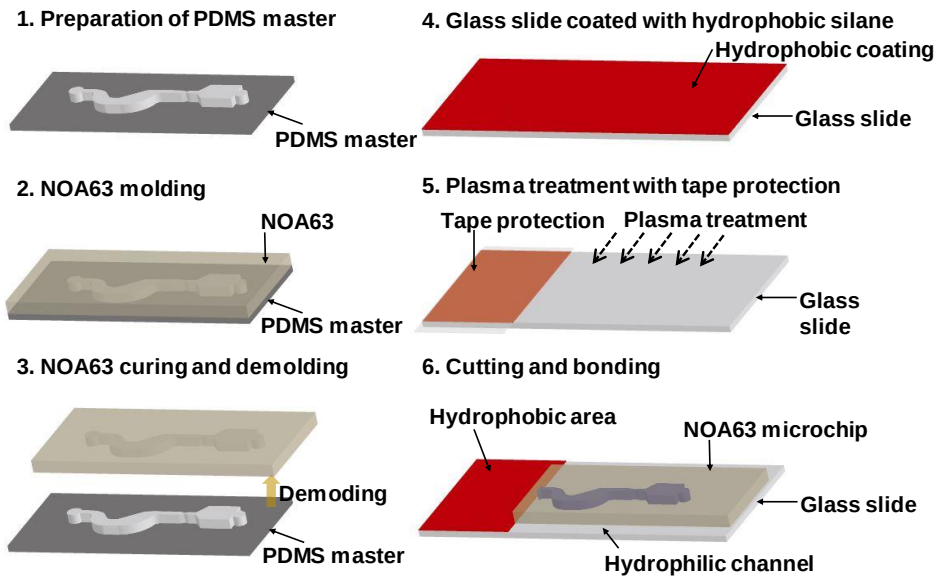

Figure S2. Fabrication process. (1) PDMS master prepared by casting. (2) Norland Optical Adhesive 63 molding (NOA63). (3) UV curing and demolding. (4) Glass slide surface treatment with Trichloro (1H,1H,2H,2H-perfluorooctyl) silane 97% in vacuum chamber for 40 min. (5) Selectively plasma treatment with tape protection. (6) Cutting and bonding.

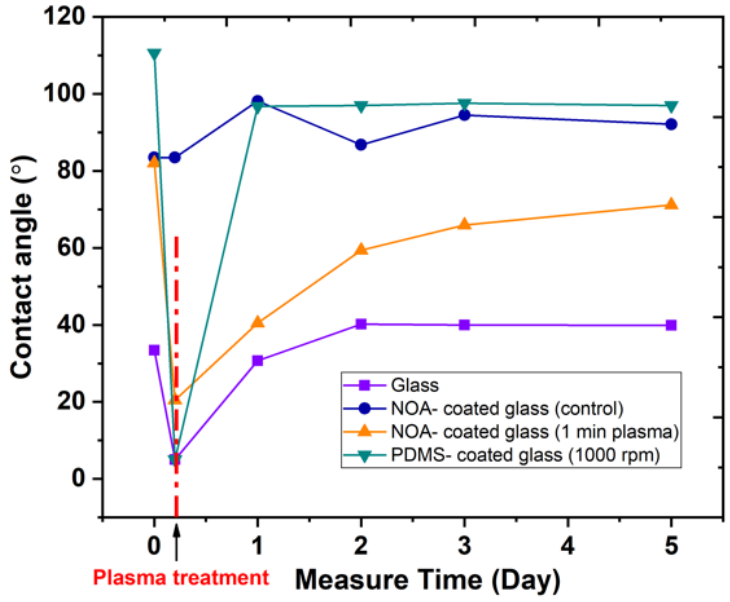

Figure S3. Contact angle measurement of different surface-treatment on NOA and glass.

Table S1. The sequence of oligonucleotides

Strand name	Sequence
MB155	5’ -TTAATGCTAATCGTGATAGGGG-3’
Probe1	5’ -/biotin/-CCCCTATCACG-3’
Probe2	5’ -ATTAGCATTAA-/biotin/-3’
GRDS	5’ -TTAATGCTA ATACTCACTAT rA GGAAGAGATGATGTCTGTCGTGATAGGGG-3’
GRE	5’ -ACAGACATCATCTCTGAAGTAGCGCCGCCGTATAGTGAG -3’

Supporting video caption

Video S1. Magnetic separation without lead ions (4X magnification)

Video S2. Magnetic separation with 200 nM of lead ions (4X magnification)

Video S3. PMP accumulation at the particle dam (4X magnification)

References

(1) Zhao, Z.; Bao, Y.; Chu, L. T.; Ho, J. K. L.; Chieng, C. C.; Chen, T. H. Microfluidic bead trap as a visual bar for quantitative detection of oligonucleotides. *Lab Chip* **2017**, *17*, 3240-3245.

(2) Dupont, E. P.; Luisier, R.; Gijs, M. A. M. NOA 63 as a UV-curable material for fabrication of microfluidic channels with native hydrophilicity. *Microelectron. Eng.* **2010**, *87*, 1253-1255.

(3) Lan, T.; Furuya, K.; Lu, Y. A highly selective lead sensor based on a classic lead DNAzyme. *Chem. Commun.* **2010**, *46*, 3896-3898.