Fabrication of the microdevice

The microdevice shown in Fig. 1 comprises of a glass substrate patterned with electrodes and a PDMS microchannel. The electrodes were fabricated using the conventional photolithography techniques including sputter-deposition of metals, photoresist patterning, chemical etching, and lift-off. A gold layer (230 nm) was first deposited on a glass wafer with a chromium intermediate layer (60 nm) and patterned for the working and auxiliary electrodes. The active area of the working electrode was 500 μm × 500 μm. The reference electrode was formed with a silver layer (650 nm). A polyimide layer (3.0 μm, Toray Industries, Japan) was formed to delineate the active areas for the electrodes and the pad areas. The silver layer was also covered with the polyimide layer, except for two pinholes of 70 μm diameter, which were used to make the reference electrode durable in a concentrated KCl solution. Subsequently, an array of micropillars (30 μm in diameter) was fabricated on the active area of the gold working electrode with a thick film photoresist SU-8 (Microchem, US). A gold layer (230 nm) was again sputter-deposited on the working electrode with the micropillars through a stainless steel mask with a square hole of 500 μm × 500 μm. The wafer was then cut into small pieces (20 mm × 15 mm). The surface of the micropillars was modified with gold black at a current density of –60 $\mu A/mm^2$ for 5 min in a vigorously

stirred solution containing 83 mM hydrogen tetrachloroaurate(III) tetrahydrate and 1.58 mM lead(II) acetate (Wako Pure Chemicals Industries, Japan). Prior to experiments, a silver chloride layer was grown from the pinholes by applying 50 nA for 5 min in a 0.1 M KCl solution with respect to a platinum plate electrode.

The PDMS microstructure was fabricated using micromolding. The master for preparing the PDMS microstructure was fabricated with SU-8 photoresist using a photomask having the desired shape. The liquid PDMS prepolymers composed of a mixture of 10:1 silicon elastomer and the curing agent (Shin-Etsu Chemical, Japan) were casted onto the SU-8 master. The mixture was cured at 80ºC for 1 h, and the PDMS replica was then peeled off from the mold. The serpentine channel was 55 μm in height, 500 μm in width, and 50 mm in length. The PDMS micropillars were 55 μm in height and 50 μm in diameter. The inter-pillar distance (edge to edge) was 50 μm, and the number of PDMS micropillars was approximately 6000. Finally, the PDMS substrate was aligned and placed on the substrate with the electrodes under a microscope, and was reversibly sealed by applying slight pressure between the two substrates.

Measurement procedures

Fig. 2: The 3D micropillar electrode modified with gold black and having no microchannel was used to perform ELISA of bone alkaline phosphatase (BAP). A drop of 20 μg/ml primary monoclonal antibody against human BAP (Abcam, UK) was placed on four different working electrodes: two 3D electrodes with and without gold black, and two 2D electrodes with and without gold black. The antibody was then physically adsorbed for 10 min. After blocking with lactoprotein, a drop of 10 U/L BAP (Abcam, UK) was placed on the electrodes and incubated for 10 min. After washing with PBS, 20 μg/ml secondary biotinylated antibody against human BAP (Abcam, UK) and 0.5 U/ml β-galactosidase-streptavidin complex (Vector Laboratories, Switzerland) were subsequently placed on the electrodes and incubated for 10 and 15 min, respectively. Finally, a drop of 4.5 μM *p*-aminophenyl-β-D-galactopyranoside (PAPG, Sigma-Aldrich, USA) was placed on the electrodes. β-galactosidase converts PAPG to *p*-aminophenol (PAP). After 10-min incubation, cyclic voltammograms were recorded at a scanning rate of 20 mV/s from +0.8 to –0.4 V vs. the Ag/AgCl reference electrode by using an Autolab PGSTAT12 potentiostat (Eco Chemie, Netherlands).

Fig. 3(a): To directly immobilize β-galactosidase onto the PDMS micropillars, the PDMS substrate was first immersed in acetone for 5 min, washed with distilled water, and immersed in 30%(v/v) hydrogen peroxide for 1 h. The surface was then reacted

with 2.0%(v/v) 3-aminopropyl-triethoxysilane for 2 h at room temperature and subsequently for 5 min at 50ºC. After washing with acetone, the PDMS substrate was placed on a plain glass substrate to form a microchannel. The amino group on the PDMS surface was activated by passing 5.0%(v/v) glutaraldehyde at a flow rate of 1 μl/min for 1 h. During this step, the color of PDMS changed to light orange. After washing with distilled water, 0.5 U/ml β-galactosidase solution was introduced into the microchannel at a flow rate of 1 μl/min for 15 min. Following this, PAPG with six different concentrations was introduced into the microchannel at 1 μl/min and enzymatically converted PAP was oxidized downstream at the planar working electrode. The potential of the working electrode was set to +0.3 V (vs. Ag/AgCl), and the steady oxidation current was recorded using the potentiostat.

Fig. 3(b): The reactions required for ELISA were performed upstream on the surface of PDMS micropillars in a serpentine channel and the enzymatically converted analytes were detected downstream with the 3D working electrode. First, antibody against tartrate-resistant acid phosphatase-5b (TRACP-5b) was immobilized on the PDMS micropillars by using a covalent binding method. Briefly, the PDMS substrate with the micropillars in the serpentine channel was placed on a bare glass slide. After activating the surface of the PDMS micropillars as described above, 20 μg/ml primary

antibody against human TRACP-5b (Santa Cruz Biotechnology, US) was introduced into the microchannel at a flow rate of 1 μl/min for 10 min. The entire surface of the microchannel was then blocked with lactoprotein, and TRACP-5b with five different concentrations was introduced into the microchannel at 1 μl/min and allowed to react with the immobilized TRACP for 10 min. After washing with PBS, 20 μg/ml secondary biotinylated antibody against human TRACP-5b (Santa Cruz Biotechnology, US) was introduced into the microchannel for 10 min. Then, 0.5 U/ml β-galactosidase-streptavidin complex was introduced at a flow rate of 1 μl/min and allowed to react with the secondary antibody labeled with biotin for 15 min. The PDMS substrate was subsequently peeled off from the glass slide and placed on the substrate with the 3D working electrode. Finally, 4.5 μM PAPG was introduced into the microchannel at 1 μl/min and the detection current was measured. The potential was kept at +0.3 V with respect to the Ag/AgCl reference electrode, and the output current caused by oxidation of PAP to *p*-quinoneimine on the electrode was measured with a potentiostat.