Electronic Supplementary Material (ESI) for Analyst.

Supplementary Material (ESI)

Nanoporous anodic aluminum oxide internalized with gold nanoparticles for on-chip PCR and direct detection by surface-enhanced Raman scattering

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Experimental Procedures

Material. Aluminum foils (Alfa Aesar, 99.99%), perchloric acid (DC Chemical, 70%), oxalic acid (OCI company, 99.5%), phosphoric acid (DC chemical, 85%), tetraethyl orthosilicate (TEOS, Aldrich, 98%), ammonium hydroxide (Daejung, 25-28%) oleylamine (ACROS, 80–90%), gold (III) chloride hydrate ($HAuCl_4$, Aldrich, 99.999%) and Polystyrene (Aldrich, molecular weight 35 000 g/mol M_w) were used as supplied without further purification. The PDMS prepolymer (Sylgard 184) and a curing agent were purchased from Dow Corning. Poly(methyl methacrylate) (PMMA), and poly(ethylene terephthalate) (PET) sheets were purchased from Goodfellow. Norland Optical Adhesive 63 (NOA 63) was purchased from Norland Products, Inc. The PCR kit including *Taq* polymerase was purchased from BioFact. Genomic DNA of *Escherichia coli* O157:H7 was extracted from overnight cultures using a Wizard genomic DNA extraction kit (Promega). SYBR Green I was purchased from Lonza.

Preparation of porous anodic aluminum oxide (AAO). The AAO templates were prepared by a two-step anodization process described in detail elsewhere.[6] In brief, as-annealed aluminum foils (0.25 mm thick) were electrochemically polished in a mixed solution of perchloric acid and ethanol (1:5, v/v) at 15 V and 5 °C. The first anodization was carried out in a 0.3 M oxalic acid solution for 7 h at 10 °C and 40 V. The irregular oxide layer that resulted from the first anodization was etched away by the solution of phosphoric acid (6 wt.%) and chromic acid (1.5 wt.%) at 60 °C for 4 h 15 min. The oxide layer of highly ordered pores (100 nm x 50 nm) was obtained by the second anodization for 90 s followed by pore widening in phosphoric acid (5 wt.%) for 18 min.

Preparation of gold nanoparticles (AuNPs). First, 70 mg of $HAuCl_4$ was dissolved in a mixture of 1 mL oleylamine and 1 mL of toluene. This solution then was quickly injected into a pre-heated (80 °C) mixture (1 mL oleylamine:15 mL toluene). The reaction solution was then refluxed for 2 h at 120 °C. After precipitation by adding 50 mL of methanol, the particles were collected and purified by centrifugation. Reaction cycles were repeated five times to obtain approximately 40 nm AuNPs.

Internalization of AuNPs into AAO nanopores (AuNPs@AAO). The AAO template (1.5 cm x 3 cm) containing three AuNPs@AAO chambers was fabricated as described in Scheme 1a. Briefly, the AAO was temporarily blocked by the plastic mask except for the area in contact with the PCR chamber. Then, the insertion of AuNPs processes were carried out as described in a previous report. [6] The AAO was placed inside a vacuum chamber on a rocking platform. Each PCR chamber hole was then covered with 10 µL of AuNPs solution dissolved in toluene (3 mg mL^{-1}). After complete evaporation, 10 µL of toluene was added. The evaporation of toluene stimulated the AuNPs to move deeper into the AAO pores. A sonication was then performed to remove the AuNPs bound to the AAO surface. These procedures were repeated 2-3 times to obtain full AuNPs internalized in the pores of AAO.

Preparation of polystyrene replica of AuNPs@AAO. The AuNPs@AAO template (0.5 cm x 0.5 cm) was dropped with 20 µL polystyrene solution dissolved in toluene (5 wt.%). The template was heated at 160 °C on a hot plate for 1 h. The infiltration of melt-cast polystyrene on AAO was then cured at room temperature. Finally, the AAO was removed from the polymer replica by dissolving in $HgCl_2$ (saturation) and phosphoric acid (25 wt.%), respectively. The final structure of polystyrene replica of AuNPs@AAO was used for UV-vis absorption measurement.

Preparation of a PDMS-AAO hybrid chip. The top PDMS layer contained three PCR chambers. To fabricate PCR chambers on the PDMS layer, first, the designated chambers were engraved on a PMMA substrate using a computer numeric control (CNC) milling machine. Next, a UV-curable NOA 63 prepolymer was poured onto the PMMA mold and then sandwich stacked with plasma treated PET and cured to fabricate a negative replica structure from the PMMA mold. Specifically, after an exposure under UV (ultraviolet) light for 6 s (225 mW cm^{-2}) (Dymax 5000-EC Series UV Curing Systems), NOA 63 was hardened and formed on the PET substrate. Then, the NOA mold was left fully cured upon UV photopolymerization (135 mW cm^{-2}) overnight. After 12 h, the NOA 63 mold was placed on a square dish and a mixture of PDMS prepolymer and a curing agent in a 10:1 (w/w) ratio was degassed and poured onto the mold. After thermal curing at 80 °C for 30 min, the PDMS was peeled off the mold. After hole punching (300 µm) at the inlet and outlet, the patterned side of the PDMS was treated with oxygen plasma (60 W) (Femto Science, Korea) for 1 min to facilitate bonding with the AAO substrate to form a closed fluidic system. At each chamber, AuNPs were internalized into the pores by the above-mentioned method. In this way, PDMS-AAO hybrid chips with and without AuNPs were fabricated. Except for the area in contact with the PCR chamber on PDMS, the surface of the rest of the AAO bottom layer was fully coated with a thin silica layer via a modified Stöber method,[30] (TEOS 1.3% in EtOH 95% catalyzed by NH_4OH 28-30%). Then, the AAO bottom layer was bonded with the top PDMS layer via plasma oxidization.

Temperature measurement. Prior to conducting on-chip PCR, the heat transfer through the hybrid chip was evaluated. The AAO substrate, with a thickness of 165 µm, which served as the bottom layer of the PDMS–AAO hybrid chip, was placed on a tailor-made flat heater inside a commercial thermal cycler (GeneTouch TC-E-96GA, Bioer), and its surface temperature was measured

using an infrared (IR) camera (FLIR Thermovision A320). Five spots were randomly selected for temperature measurement, and the average temperature was evaluated using an image analyzer (ThermaCAM researcher 2.8). The temperature of the flat heat block was also directly measured along with the temperature of the AAO substrate surface. Black insulating tape was adhered to the top surface of the AAO substrate and the heat block for a precise measurement of the temperature.

On-chip PCR using the PDMS-AAO hybrid chip. The PCR mixture (12.5 μL) contained 10× reaction buffer, 10 mM deoxynucleotides (dNTPs), 5 U μL^{-1} *Taq* polymerase, 0.1 mM forward and reverse primers and a 5x band helper solution. The band helper solution was used to facilitate PCR when the target contains high GC content. Genomic DNA of *E. coli* O157:H7 was extracted following the protocol of the manufacturer. Purified genomic DNA (20 ng μL^{-1}) was added to the PCR mixture with a volume of 0.3 μL. The primer sequences used to amplify a 183-bp *eaeA* gene fragment from *E. coli* O157:H7 were as follows: 5' AGA AGT CGT TGT TAA GTC A 3' (forward) and 5' CCA CCA CCA TGA GTT AGA 3' (reverse). PCR on the hybrid chip was performed using a commercial thermal cycler (GeneTouch TC-E-96GA, Bioer) whose typical heat block, which is equipped with 48 or 96 wells, was replaced with a flat block on which the PDMS-AAO hybrid chip was placed for programmed thermal cycling. Amplification was performed at 95 °C for 15 s, 48 °C for 15 s, and 72 °C for 20 s, for a total of 30 thermal cycles. On-chip PCRs were conducted on hybrid chips with and without internalized AuNPs. For comparison, conventional PCR was also conducted using a thermal cycler (Bio-Rad C1000), and amplification was performed at the same temperatures as those used for performing the on-chip PCR for a total of 30 thermal cycles. The PCR products were analysed by agarose gel electrophoresis with ethidium bromide (EtBr) staining and detected using a Gel Doc EZ system (Bio-Rad).

Raman measurement. The Raman spectra were recorded by a micro-Raman spectrometer (ANDOR Monora 500i) using a He-Ne laser beam wavelength of 632.8 nm. The suspension mixture of PCR product and SYBR Green I (10:1, v/v) was immediately measured after preparation on AuNPs@AAO substrate (Fig. 1b). The exposure time and minimum trigger period were 1 s and 0.5 s, respectively. The number of accumulations was 10 and the detecting spectra grafting was 600/500 nm.

Table S1 The statistics summary of diameter of AuNPs and AAO.

	N total	Mean	SD	Minimum	Maximum
AuNPs diameter (nm)	100	39.90	3.18	33.3	48.5
AAO diameter (nm)	100	50.15	5.48	40.6	60.7

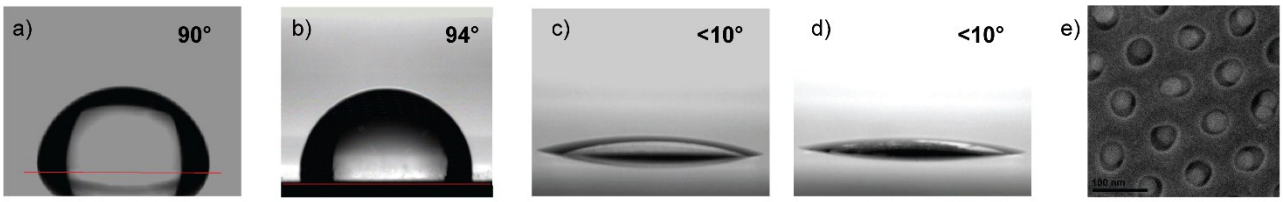

Figure S1 Results of water contact angles measured on a) pristine AAO substrate, b) AAO substrate internalized with AuNPs (AuNPs@AAO), c) O_2 plasma-treated AuNPs@AAO substrate, d) O_2 plasma-treated AuNPs@AAO substrate after 12 h, and e) SEM image of AuNPs@AAO after O_2 plasma treatment.

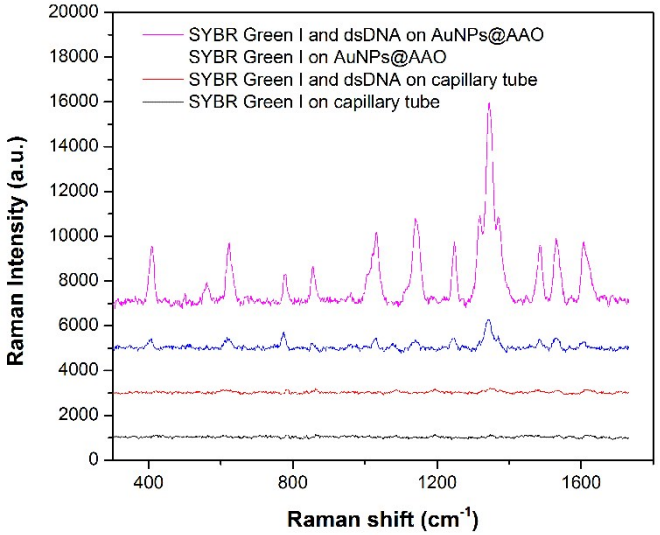

Figure S2 Raman spectra of SYBR Green I and the SYBR Green I/dsDNA mixture measured on the capillary tubes and AuNPs@AAO platform.

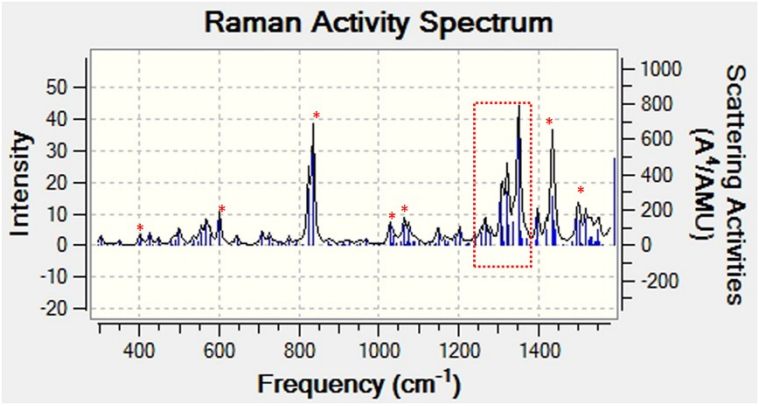

Figure S3 Density functional theory (DFT) simulation result of SYBR Green I.