Electronic Supplementary Material (ESI) for Biomaterials Science.

Dual Stimuli-Responsive Smart Beads that Allow "On–Off" Manipulation of Cancer Cells

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Supporting Information

Contents

1. Theory

Dielectrophoresis: When a particle is subjected to a non-uniform electric field, a dielectrophoresis (DEP) force is exerted on the particle. The time-averaged DEP force ($\mathbf{F}_{DEP}$) acting on a spherical particle of radius (a) can be approximated by

$$\mathbf{F}_{DEP} = 2\pi\varepsilon_e a^3 \,\mathrm{Re}[K(2\pi f)]\nabla\left|E_{\mathbf{e}}\right|^2, \tag{1}$$

where ε_e and E_e are the permittivity of the suspending medium and applied electric field, respectively. The Clausius–Mossotti (CM) factor, $K(2\pi f)$, which represents the relative permittivity between the particle and suspending medium, is

$$K(2\pi f) = \frac{\varepsilon_p^* - \varepsilon_e^*}{\varepsilon_p^* + 2\varepsilon_e^*}, \tag{2}$$

where $\varepsilon^* = \varepsilon + \frac{\sigma}{2\pi f} j$ is complex permittivity, and $j = (-1)^{1/2}$, and σ and f are the conductivity and frequency of the applied electric potential, respectively. Subscripts p and e represent particle and suspending medium, respectively. When the real part of the CM factor, Re[K(2πf)], is bigger than 0, the particle will be attracted toward the strong electric field, which is termed positive DEP (pDEP). Conversely, the particle will be pushed away by a negative DEP when the factor is smaller than 0. The CM factor can be controlled by adjusting the conductivity of the external medium and frequency of the applied electric field. In the present study, we induced pDEP to localize smart beads at the edge of the electrodes by optimizing DEP conditions and using a sucrose-based low-conductivity buffer to adjust the conductivity of the medium and osmotic pressure.

2. Experimental Section

Materials: *N*-Isopropylacrylamide (NIPAAm) was kindly provided by Kohjin Co., Ltd. (Tokyo, Japan) and purified by recrystallization from benzene and hexane before use. Initiator 2,2'-azobis(isobutyronitrile) (AIBN) was purchased from Wako Pure Chemical Industries (Osaka, Japan) and purified by recrystallization from methanol before use. Chain transfer agent 2-mercaptoethylamine (MEA) was purchased from Alfa Aesar (Ward Hill, MA, USA). Carboxylated polystyrene (COOH-PS) beads with a diameter of 6 μm were obtained from Corpuscular Inc. (Cold Spring, NY, USA). Ethyl(dimethylaminopropyl)carbodiimide (EDC) and *N*-hydroxysulfosuccinimide (NHS) were purchased from TCI (Tokyo, Japan). Anti-epithelial cell adhesion molecule (anti-EpCAM) antibody (MAB960) was obtained from R&D Systems (Minneapolis, MN, USA). RPMI 1640, trypsin (0.25%) EDTA solution and penicillin-streptomycin were purchased from Wako Pure Chemical Industries. Fetal bovine serum (FBS) was obtained from Japan Bio Serum (Fukuyama, Hiroshima, Japan). Normal human prostate cancer (PC3) cells were provided by RIKEN Bio Resource Center (Tsukuba, Ibaraki, Japan). The PC3 cells were cultured at 37 °C with 5% CO_2 in RPMI-1640 containing 10% FBS and 1% penicillin-streptomycin. All other solvents and chemicals were used as received. MilliQ water (Merck Millipore, Darmstadt, Germany) was used in all experiments.

Preparation of smart beads: The surface of COOH-PS beads was conjugated with NH_2-PNIPAAm and anti-EpCAM antibody to prepare smart beads as follows. NIPAAm (20 mmol), MEA (5 mmol) and AIBN (0.2 mmol) were dissolved in *N,N*-dimethylformamide (DMF, 20 mL). After three freeze–pump–thaw cycles, polymerization was carried out at 60 °C for 24 h. The polymerized solution was purified by dialysis against ethanol for 3 days and MilliQ water for 4 days using a dialysis membrane (Spectrum™

Spectra/Por™, MWCO = 3,500, Fisher Scientific, Waltham, MA, USA). The dialyzed solution was lyophilized for 4 days and then NH_2-PNIPAAm was obtained.

The surface of COOH-PS beads was conjugated with NH_2-PNIPAAm and anti-EpCAM antibody after activation of the carboxyl groups using NHS and EDC. Briefly, COOH-beads (20 mg) were washed with 0.1 M 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer (pH 4.7) three times by centrifugation at 16,600g. Then, EDC (0.8 mg) and NHS (2.2 mg) were added to the COOH-bead dispersed in MES buffer (1 mL). The carboxyl groups were activated for 4 h at 25 °C. The NHS-activated beads were obtained after washing with phosphate-buffered saline (PBS, pH 7.8) three times by centrifugation at 16,600g. The NHS-activated beads (4 mg) were reacted with anti-EpCAM antibody (50 μg/mL, 200 μL) and NH_2-PNIPAAm (100 mg) in PBS (2 mL) overnight at 4 °C. The smart beads were obtained after washing with PBS three times by centrifugation at 16,600g. In addition, anti-EpCAM antibody-conjugated beads were also obtained in a similar manner.

Characterizations of the polymer and smart beads: The ^{1}H NMR spectrum of NH_2-PNIPAAm was obtained by dissolving the polymer in D_2O (600 MHz Ultra-Shield Bruker NMR, AZ Core Labs: Phoenix, AZ, USA) to determine its chemical composition. ^{1}H NMR (600 MHz), δ (D_2O, ppm): 1.0 (6H, -C***H***$(CH_3)_2$), 3.8 (1H, -CH(C***H*****$_3$**$)_2$), 1.8 – 2.1 (1H, -CH_2C***H***-), 1.3 – 1.7 (2H, -C***H*****$_2$**CH-), 2.7 (2H, -S-C***H*****$_2$**CH_2-NH_2), 3.4 (2H, -S-CH_2C***H*****$_2$**-NH_2).

To determine the LCST of the polymer solution, the transmittance of a solution of NH_2-PNIPAAm in PBS (pH=7.4, 0.1 w/v %) at 500 nm was continuously recorded at a heating rate of 1.0 °C min^{-1} by a UV-Vis spectrometer (JASCO International Co., Ltd., Tokyo, Japan) equipped with a temperature controller. The LCST of the polymer solution was determined at 50% transmittance.

"On–off" reversible switching of the prepared smart beads was evaluated by measuring their zeta (ζ)-potential and hydrodynamic diameter by dynamic light scattering (DLS, Nano ZS, Malvern Instruments Ltd., Malvern, UK) at both 25 and 37 °C.

Preparation of ITO-patterned surfaces: Commercially available indium tin oxide (ITO)-coated glass substrates (Geomatec Co. Ltd., Yokohama, Japan) were used in this study. Patterns in the ITO-coated glass substrates were prepared by standard photolithography and etching processes. Briefly, S1813 positive-type photoresist (Shipley Far East Ltd., Tokyo, Japan) was spin-coated onto the ITO-coated glass surface at 4,000 rpm for 30 s. The substrate was subsequently baked at 120 °C for 2 min on a hot plate. The ITO-coated glass was then exposed to UV light for 5 s through a patterned photomask. After development using AZ developer (Microchemicals, Ulm, Germany), the ITO-coated glass substrate was etched using etching solution (mixture of 0.2 M $FeCl_3$ and 6 M HCl) for 30 min at 40 °C with shaking. Finally, the patterned ITO glass substrate was obtained after sufficient washing with acetone, isopropyl alcohol and running water.

Evaluation of temperature-responsive "on–off" capture and release for cancer cells and electrical field-responsive "on-off" localization for smart beads: To examine the capture of cancer cells by the smart beads by temperature changes, the smart beads were first incubated with a suspension of PC3 cells at 37 °C for 5 min to capture cells (The ratio of cancer cells to smart beads is almost 1 : 1). To investigate the release of the cells from the beads, the cell-captured beads were incubated at 4 °C for 5 min. Images of the cells were captured by phase-contrast microscopy

(AxioObserver D1, Carl Zeiss, Oberkochen, Germany). The percentage of attached cells was calculated from more than five randomly selected images. Data are expressed as standard errors of the means.

The "on–off" localization of PC3 cell-captured smart beads on the ITO-patterned surface was performed in low-conductivity DEP solution (pH 7.4, conductivity: 22.4 mS/m) that consisted of 1 mM calcium chloride, 236 mM sucrose, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and 59 mM glucose. In addition, to avoid non-specific cell adhesion on the beads, 2% of bovine serum albumin (BSA) was added. This DEP solution was used after filtration through a 0.2-μm filter membrane. The PC3 cells were dispersed in DEP solution with smart beads at 37 °C for 5 min. The PC3 cell-captured smart bead solution was dropped on the ITO-pattered glass surface and then an electrical field (100 Hz / 5 V_{pp}) was applied with "on–off" switching using a function generator (WF1948, NF Corp., Tokyo, Japan). Images of the solution were taken by an inverted microscope (IX 71, Olympus, Tokyo, Japan) that was equipped with a digital CCD camera (ORCA-R2, Hamamatsu Photonics, Tokyo, Japan).

The whole steps for targeted cancer cell isolation methods are described as follows:

(1) enrichment of cancer cells on the smart beads surface (temperature "on" and DEP "off")

(2) enrichment of cancer cell-captured smart beads on the ITO-patterned surface (temperature "on" and DEP "on")

(3) separation of cancer cells from cancer cell-captured smart beads (temperature "off" and DEP "off")

(4) enrichment of separated-smart beads on the ITO-patterned surface and isolation of only cancer cells (temperature "off" and DEP "on")

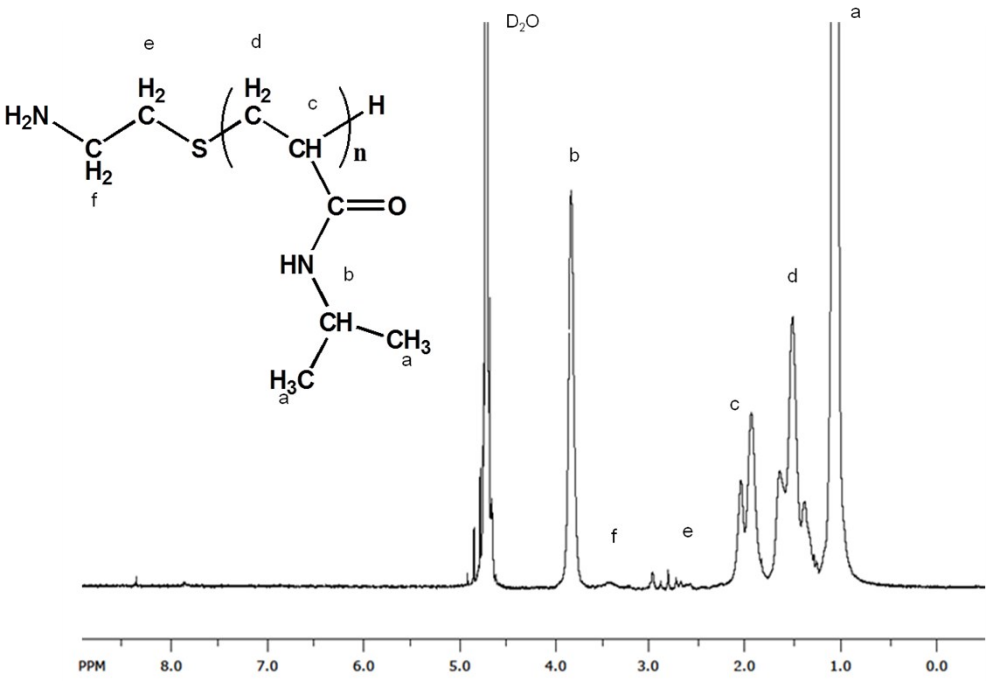

Figure S1. ^{1}H NMR spectrum of NH_2-PNIPAAm (solvent: D_2O).

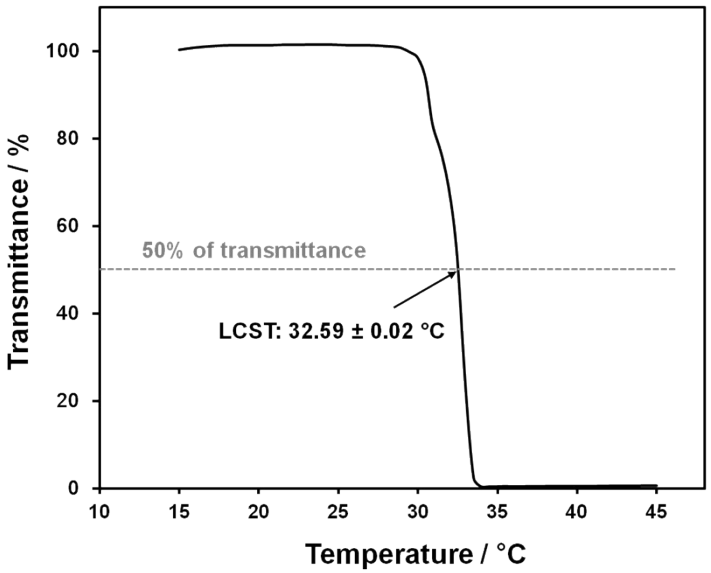

Figure S2. Temperature dependence of the optical transmittance NH_2-PNIPAAm in aqueous solution (0.1 wt/v%, pH 7.8).