Electronic Supporting Information

Inverted Pattern Formation of Cell Microarrays on PEG Gel Patterned Surface and Construction of Hepatocyte Spheroids on the Unmodified PEG Gel Microdomains

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Materials and Methdos

Materials Poly(ethylene glycol) (MW: 4,600), 3-(trimethoxysilyl)propyl methacrylate (TSPM) and 3-aminopropyltriethoxysilane (APTS) were purchased from Sigma-Aldrich (Milwaukee, WI, USA) and were used as received. Commercial tetrahydrofuran (THF; Kanto Chemical, Tokyo Japan) and methacrylic anhydride (Aldrich) were purified by conventional methods. A THF solution of potassium naphthalene was prepared by reacting naphthalene (13.4 g, 0.105 mol) with metallic potassium (4.30 g, 0.110 mol) in dry THF (300 mL). The mixture was allowed to react overnight at room temperature, and the concentration of the mixture was determined by titration (0.35 mol/L). Dulbecco's modified Eagle's medium (DMEM) was purchased from Sigma-Aldrich, and fetal bovine serum (FBS) and antibiotic-antimycotic were purchased from Gibco-Invitrogen (Grand Island, NY, USA). The water used in this study was purified using a Milli-Q system (Nihon Millipore Co., Tokyo, Japan).

Synthesis of methacrylate-ended telechelic PEG To a THF solution (60 mL) of 1 mmol PEG with hydroxyl groups at both ends (MW; 4,600) in a 300 mL flask equipped with a three-way stopcock, 2 mmol of potassium naphthalene and 6 mmol of methacrylic anhydride were added under a nitrogen atmosphere. After the mixture was allowed to react for several hours with magnetic stirring, the solution was poured into isopropyl alcohol (IPA) (1.5 L). The precipitate was centrifuged and the corrected precipitate was dissolved in THF (50 mL). The solution was poured again into IPA (1.5 L). The precipitated polymer thus obtained was finally freeze-dried with benzene (yield: 98%). The obtained polymer was analyzed by size exclusion chromatography (SEC) and ^{1}H NMR spectra, and an end-functionality of approximately 90 % was confirmed. The SEC pattern and ^{1}H NMR spectrum of it are shown in Figures S1 and S2.

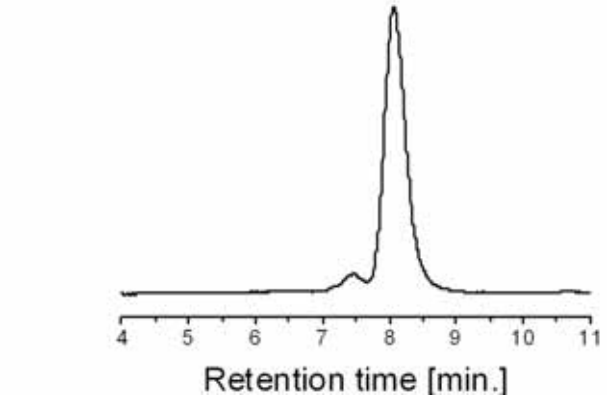

Figure S1 Size exclusion chromatogram of PEG-DMA. The molecular weight and the molecular weight distribution of the PEG-DMA were determined by Gel permeation chromatography (TOSOH HLC-8120, TOSHO Co., Tokyo, Japan) with TSK gel columns (TSKgel SuperHZ3000 + HZ2000) and an internal refractive index (RI) detector (TOSOH HLC-8020RI). THF containing 0.5 wt % triethylamine was used as the eluent at a flow rate of 0.35 mL/min at 40 °C. The weight-average molecular weight and the polydispersion of PEG-DMA were 4500 and 1.03, respectively.

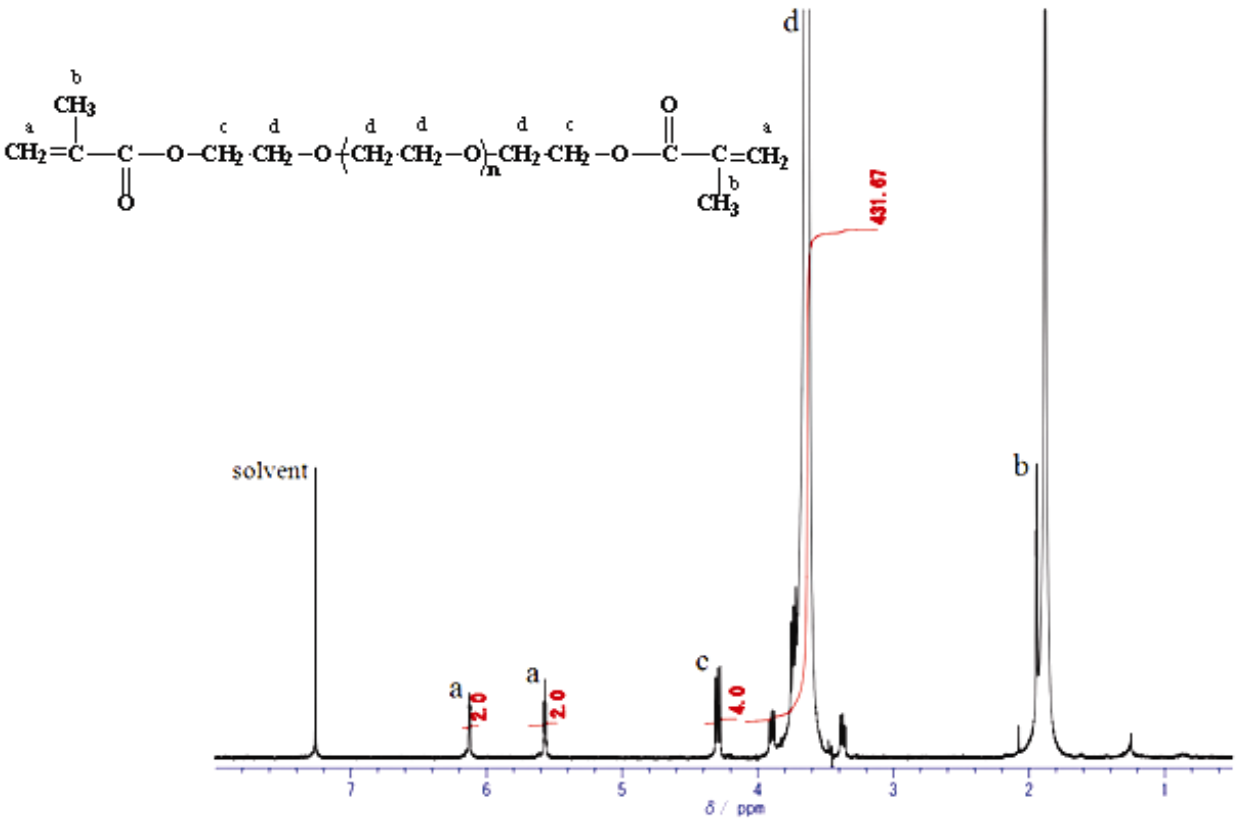

Figure S2 ^{1}H NMR spectrum of PEG-DMA. The characterization of the PEG-DMA was carried out by ^{1}H-NMR (JEOL EX-270 spectrometer, JEOL Ltd., Tokyo, Japan) at 270 MHz in $CDCl_3$ solution at room temperature. The signals based on DMA installed at the both chain ends of PEG (a, b, c) were clearly observed in the spectrum. An end-functionality of approximately 90 % was confirmed by the a/d and c/d ratio of integration values. The yield of the polymer was 98 %. On the basis of these results, the quantitative preparation of the PEG-DMA was confirmed.

Construction of a PEG gel micro-patterned surface Figure S3 shows the process flow for the fabrication of PEG hydrogel microstructures by photolithography technique. After the glass surface was washed with piranha solution (1:1 volume of concentrated H_2SO_4 and hydrogen peroxide (30 w/v%) for 1 h), the glass surface was modified with an ethanol solution of 3-(trimethoxysilyl)propyl methacrylate (1.6 vol.%) for 30 min and gently rinsed with ethanol. Then, the vinyl group-introduced glass surface thus prepared was modified with an ethanol solution of 3-aminopropyltrimethoxysilane (4 vol. %) for 1 hour in order to increase the hydrophilicity of the glass surface. If the silanization with amino silane was not demonstrated, the PEG solution was repelled from the glass surface during spin-coat due to the strong hydrophobicity of vinyl groups on the surface. On the other hand, in the case of glass surface silanized with amino silane only, the PEG solution extended uniformly on the silanized glass, though the gels flaked off from the glass substrates easily by rinsing. Then, the glass was washed with deionized water three times and allowed to stand for 12 h at 100 °C in vacuo. The mixture of PEG-DMA (MW: 4,600, 50 mg) and Irgacure 2959 (50 mg) in methanol (1 mL) (Method A) and/or in a mixture of water and methanol (50 % vol/vol, 1 mL) (Method B) were spin-coated at 3,000 rpm on the silanized glass surface. This amount of initiator was not dissolved completely in the methanol/water co-solvent that contains less than 50 % v/v methanol. After the casting solvent was completely removed by evaporation at ambient temperature for 10 min, the micro-pattern was prepared by UV exposure (254 nm, 240 mJ/cm^2). A metal mask with 100 μm aligned cavities separated by 100 μm intervals (edge-to-edge distance) was used for patterning through the metal mask under a narrow bandwidth. The surface was developed by running distilled water over it for 30 s. The obtained micro-patterned surface was soaked in PBS for 30 min prior to use.

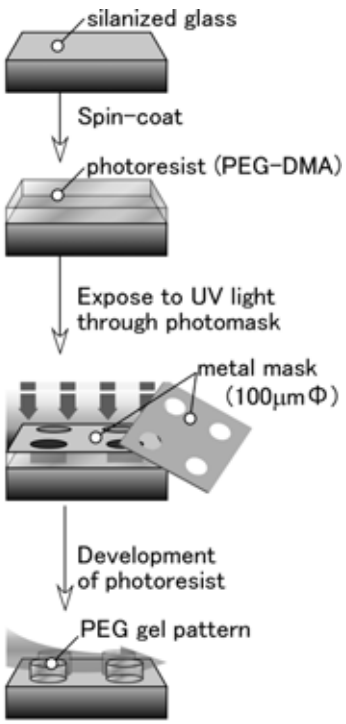

Fig. S3 Process flow for the fabrication of PEG hydrogel microstructures by photolithography technique.

DIM observation High-resolution images of the hydrogel microstructures were obtained using a the differential interference microscope (DIM) (Carl Zeiss Co. Ltd, Inc., Axiovert 200M). The PEG gel pattern sample was prepared by casting using methanol solvent and methanol/water co-solvent in photolithography using a metal mask. Figure S4a and S4b show DIM images of a PEG gel pattern on a silanized glass surface after the photolithographic process (irradiation and development) were applied to spin-coated films prepared by Method A and Method B. As shown in the images, PEG gel dots with a 100 μm diameter at 100 μm intervals on the substrate were created in exactly the same pattern as that of the photomask used in this study (Figure S4c). Thus, it was confirmed that a precisely controlled PEG gel micropattern on a hydrophobic glass surface can be obtained by using either of the spin-coated films (Methods A and B).

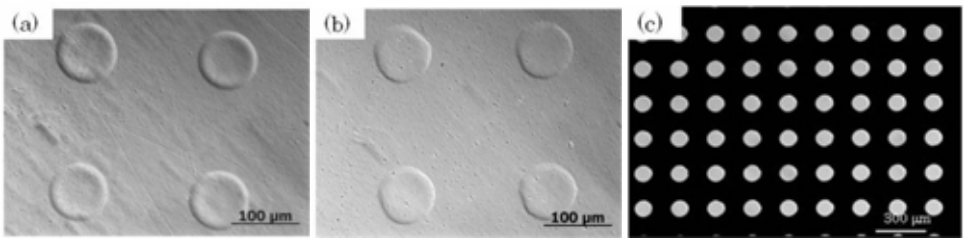

Fig. S4 DIM images of the PEG microstructures of photolithographically patterned PEG gels prepared using (a) Method A and (b) Method B. The spots were approximately 100 μm in diameter, with a 100 μm edge-to-edge distance. The PEG gel pattern, comprised of circular 100 μm dots, allows a feature density of 25 dots/mm^2. These thicknesses of each PEG gel film were measured using a surface profilometer (Alpha-Step 500 surface profiler, Tencor Instruments.). Both of thicknesses were approximately 40 nm. (c) Phase-contrast micrographs of photomask patterns with 100 μm circular holes separated by 100 μm intervals (edge-to-edge distance).

Endothelial cell and hepatic cancer cell culture on PEG patterned gel surfaces Bovine aortic endothelial

cells (BAECs) and Human hepatoma-derived cell line (FLC-4) (supplied from BIOS Inc.) were purchased from the Health Science Research Resources Bank (JCRB0099, Osaka, Japan). BAECs under 20 passages were used in all cell culture experiments. All modified glass substrates were sterilized in 70 % ethanol and then immersed in DMEM supplemented with 10 % FBS and 1 % antibiotic-antimycotic for 30 min at room temperature. These PEG gel patterned surface were seeded with each cells to be 100 % confluent to these glass substrates (approximately 2.0 x 10^5 cells/cm^2 of BAECs or 1.8 x 10^5 cells/cm^2 of FLC-4) and incubated at 37 °C with DMEM in a humidified atmosphere with 5 % CO_2. After 24 h of cultivation at 37 °C, the unattached cells were washed away with PBS. The cell morphology was monitored using a phase-contrast microscope (OLYMPUS IX71, OLYMPUS Co., Tokyo, Japan).

Protein adsorption experiments Protein adsorptions were evaluated by fluorescence-based analysis on the five testing samples (Gel A, Gel B, Glass A, Glass B, and silanized glass), which were prepared as follows: Gel A and Glass A were cast from a methanol solution of PEG-DMA and photoinitiator; Gel B and Glass B were cast from a mixture solution of methanol/water (50/50 vol.%); then Gel A and Gel B were washed with water after UV irradiation; Glass A and Glass B were washed without UV irradiation, *viz*., the surfaces of Gel A and Gel B were formed PEG gels, and Glass A and Glass B were coated and developed glass samples without UV irradiation. In addition, silanized glass, which was not treated with PEG solution, was prepared as a control. The treatments for each sample are summarized in Table 1. An FITC-BSA (Sigma Chemical Co. USA) adsorption test was carried out on the patterned PEG hydrogel surface by soaking each substrate of FITC-BSA (1 mg/mL) in PBS for 90 min at room temperature after preincubation in PBS. Subsequently, the samples were rinsed three times with PBS for 10 min. The adsorbed protein on the surface was analyzed using a fluorescence plate reader (ARVO™ MX, PerkinElmer Japan Co., Ltd., Yokohama, Japan) and a fluorescence microscope (OLYMPUS IX71, OLYMPUS Co., Tokyo, Japan). The fluorescence intensity was measured at 485 nm excitation and 535 nm emission.

Contact angle measurements The contact angles of water on the Gel A, Gel B, Glass A, Glass B, and silanized glass surfaces were measured using a contact angle analyzer (CA-X, Kyowa Interface Science Co., Ltd., Asaka, Japan) as follows: a liquid droplet was gently placed onto the surface, and the contact angles were measured 10 s after placement by the θ/2 method. The error bars are standard deviations; n = 5.

XPS analysis The chemical composition of the silanized glass, Glass A, Glass B, and Glass C surface were determined by XPS using a magnesium anode nonmonochromatic source (AXIS-Hsi, Shimadzu/KRATOS ANALYTICAL, Ltd., Kyoto, Japan). Glass C was prepared by casting from a water and then washed without UV irradiation. All samples were completely dried in vacuo before measurement. Survey scans (0-1100 eV) were performed to identify the C and Si elements. The takeoff angle of the photoelectrons was 90°. All the binding energies referenced the peak of C-C bond at 285.0 eV. Their elemental compositions were determined based on the peak areas corresponding to these elements.