Supplementary Information

Photodegradable avidin-biotinylated polymer conjugate hydrogels for cell manipulation

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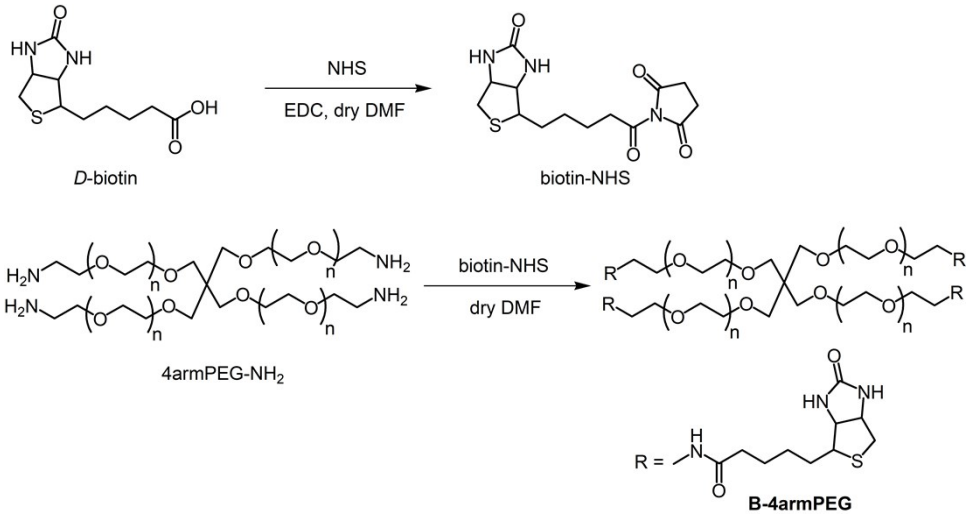
1. Synthesis of biotinylated macromonomers

1-1. General procedures

Column chromatography was performed on a silica gel (60N spherical, 40–50 μm), provided by Kanto Chemical Co. Inc. (Tokyo, Japan). NMR chemical shifts are reported in ppm downfield of tetramethylsilane using a residual solvent as an internal reference. NMR spectra were recorded using Avance 600 (600 MHz; Bruker, Germany). MALDI-TOF mass spectra were measured with Autoflex Speed (Bruker, Germany).

1-2. Synthesis of B-4armPEG



Scheme S1. Synthetic scheme of **B-4armPEG**

Synthesis of biotin-NHS. D-biotin (500 mg, 2.05 mmol) and *N*-Hydroxysuccinimide (NHS) (320 mg, 2.78 mmol) was dissolved into 30 ml of anhydrous *N,N'*-dimethyl formamide (DMF). Then, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) (471 mg, 2.46 mmol) was added to start reaction. After reacted for 23 hours, DMF was removed by evaporator. The crude product was suspended in Milli-Q water (Nihon Millipore Ltd., Tokyo, Japan), followed with filtration. The product was dried in a vacuum for 4 hours. White solid of **biotin-NHS** was obtained (398 mg, yield: 57 %).

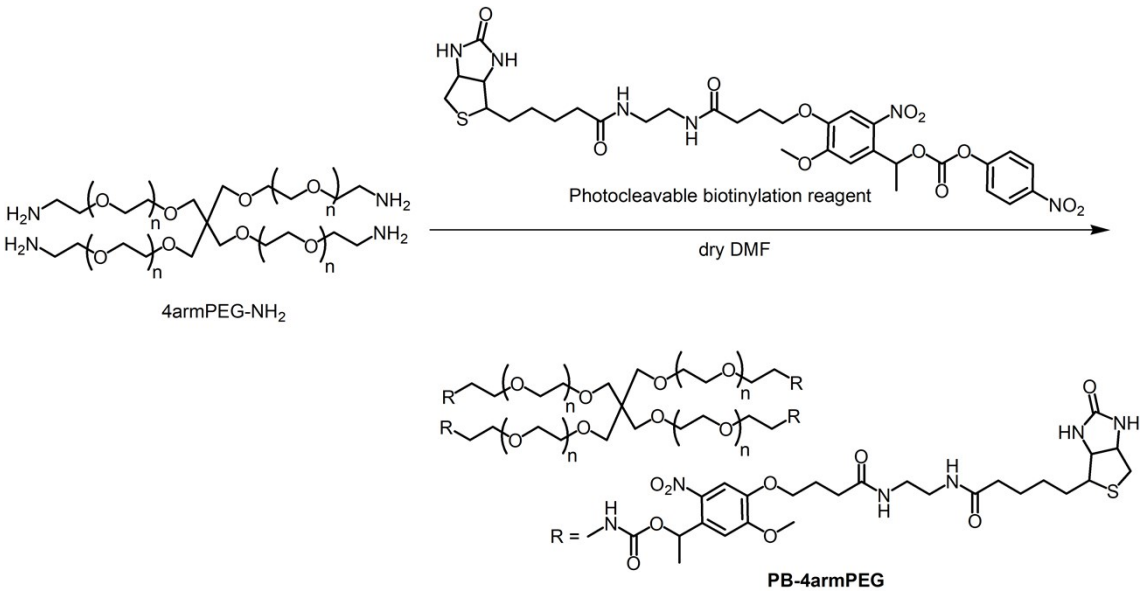
^{1}H-NMR of one (600 MHz, DMSO-d6) δ: 6.42 (s, 1H), 6.37 (s, 1H), 4.30 (m, 1H), 4.15 (m, 1H), 3.10 (m, 1H), 2.82 (t, 4H), 2.67 (t, 2H), 2.57 (d, 2H), 1.41-1.66 (m, 6H).

Synthesis of B-4armPEG. PTE-200PA (Sunbright, 4armPEG-NH_2, Mw = 20,000; 60.3 mg, 3.00 μmol) and biotin-NHS (8.12 mg, 25.0 μmol) were dried under reduced pressure, and the atmosphere in the flask was replaced with argon. Anhydrous DMF (1 mL) was

added to the flask. After stirring at room temperature for 210 min, the crude product was precipitated by dropping the reaction mixture into cold diethyl ether (40 mL) and collected by centrifugation. The crude product was dissolved in 5 mL of 50 mM Tris-HCl buffer (pH 8.0) and centrifuged. The supernatant was further purified by dialysis (MWCO 3.5 kDa) in Milli-Q water. The purified solution was filtered and lyophilized to yield a white solid (58.6 mg, yield: 93%). ^{1}H-NMR: (600 MHz, $CDCl_3$) δ: 6.49 (s, 4H), 5.33 (s, 4H), 4.72 (s, 4H), 4.50 (m, 4H), 4.32 (m, 4H), 3.41–3.80 (m, PEG), 3.14 (m, 4H), 2.89 (d, 4H), 2.71 (d, 4H), 2.19 (t, 8H), 1.57–1.76 (m, -CH_2-), 1.45 (m, 8H).

The biotinylation number of 4armPEG was evaluated by MALDI-TOF Mass spectrometry. The observed masses (the peak center of the spectrum) of 4armPEG-NH_2 (starting materials) and B-4armPEG (product) were 21042.4 and 21946.2 (m/z), respectively. From the difference in the mass attributed to biotinylation, the average modification number was calculated to be 3.98.

1-3. Synthesis of PB-4armPEG



Scheme S2. Synthetic scheme of **PB-4armPEG**

Synthesis of PB-4armPEG. Photocleavable biotinylation reagent was synthesized according to our previous report.[S1] PTE-200PA (122 mg, 6.10 μmol) and photocleavable biotinylation reagent (41.4 mg, 56.4 μmol) were dried under reduced pressure, and the atmosphere in the flask was replaced with argon. Anhydrous DMF (1.1 mL) was added to the flask. After stirring at room temperature for 28 hours, the crude product was precipitated by dropping the reaction mixture into diethyl ether (40 mL) and collected by

centrifugation. The crude product was dissolved in 5 mL of 50 mM Tris-HCl buffer (pH 8.0) and centrifuged. The supernatant was further purified by dialysis (MWCO 3.5 kDa) in Milli-Q water. The purified solution was filtered and lyophilized to yield a yellow solid (98.0 mg, yield: 77%). ^{1}H-NMR: (600 MHz, $CDCl_3$) δ: 7.59 (s, 4H), δ: 7.02 (s, 4H), 6.81 (bs, 4H), 6.72 (bs, 4H), 6.33 (bs, 4H), 5.81 (s, 4H), 5.53 (q, 4H), 5.08 (s, 4H), 4.50 (m, 4H), 4.32 (m, 4H), 4.11 (t, 8H), 3.96 (s, 12H), 3.49–3.79 (m, PEG), 3.24 (m, 16H), 3.14 (m, 4H), 2.89 (d, 4H), 2.71 (d, 4H), 2.42 (t, 8H), 2.20 (m, 8H), 2.14 (t, 8H), 1.57–1.76 (m, 28H), 1.44 (m, 8H).

The biotinylation number was evaluated by MALDI-TOF Mass spectrometry. The observed masses (the peak center of the spectrum) of 4armPEG-NH_2 (starting materials) and PB-4armPEG (product) were 21042.4 and 23428.3 (m/z), respectively. From the difference in the mass attributed to biotinylation, the average modification number was calculated to be 4.02.

2. Concentration determination of biotinylated macromonomers

Biotinylated macromonomers (**B-4armPEG** and **PB-4armPEG**) were dissolved in Dulbecco's phosphate buffered saline (PBS). The concentration of the solutions of B-4armPEG and PB-4armPEG was determined by the colorimetric biotin assay based on the 2-(4'-hydroxy- azobenzene) benzoic acid (HABA)-avidin complex. To prepare a HABA-avidin complex solution, both HABA and avidin were dissolved into PBS at the final concentrations of 0.25 mM and 1.0 mg/ml, respectively. Biotin standard solutions were similarly prepared at the concentrations from 18.8 to 500 μM. The biotinylated macromonomer solutions and the biotin standard solutions were diluted ten times with the HABA-avidin complex solution, and then, the absorbance of the mixture solutions at 500 nm was measured with a NanoDrop1000 spectrometer (Thermo Fisher Scientific K.K., Japan). The calibration line for the biotin concentration was prepared by fitting to the absorbance of the biotin standard solutions. By using this calibration line, the biotin concentration of the macromonomer solutions was determined from the absorbance, and then, the macromonomer concentrations were calculated from the biotin concentrations.

3. Evaluation of stability of hydrogels

We evaluated the stability of the hydrogel consisting of **B-4armPEG** and avidin against overlaying of the solutions. 12 mg of **B-4armPEG** was dissolved with 100 μL of PBS, and after preparation of the dilution series of the **B-4armPEG** solution (60~480 times), the biotin concentration of the solutions was determined by using the HABA-avidin complex as described above. From the sloop of the line fitted to the plot of the

biotin concentration versus the dilution ratio, the biotin concentration of the original **B-4armPEG** solution was determined, and then, the solution was diluted with PBS including yellow-green fluorescent particles with the diameter of 2.0 μm (the particle suspension of the purchased product was diluted 100 times with PBS), to set the biotin concentration of 8 mM (**B-4armPEG** conc.: 2 mM). 50 μL of the **B-4armPEG** solution (2 mM in PBS, including fluorescent microparticles) was mixed with 50 μL of the avidin solution (2 mM in PBS) in glass vials. After gelation at room temperature for 10 min, 500 μL of PBS or a culture medium (RPMI-1640, supplemented with 10% fetal bovine serum (FBS) and 0.5% penicillin/streptomycin) was layered on the hydrogel (hydrogel volume: 100 μL) and incubation at room temperature. After incubation for 4 hours, the stability of the hydrogel was checked by inverting the glass vials.

Figure S1 shows the photographs of the hydrogel overlayed with PBS and the culture medium in the inversion test, respectively. These photographs confirm that the hydrogels did not fall before and after incubation. This result indicates that the hydrogel was stable against overlays of PBS and even culture medium for 4 hours.

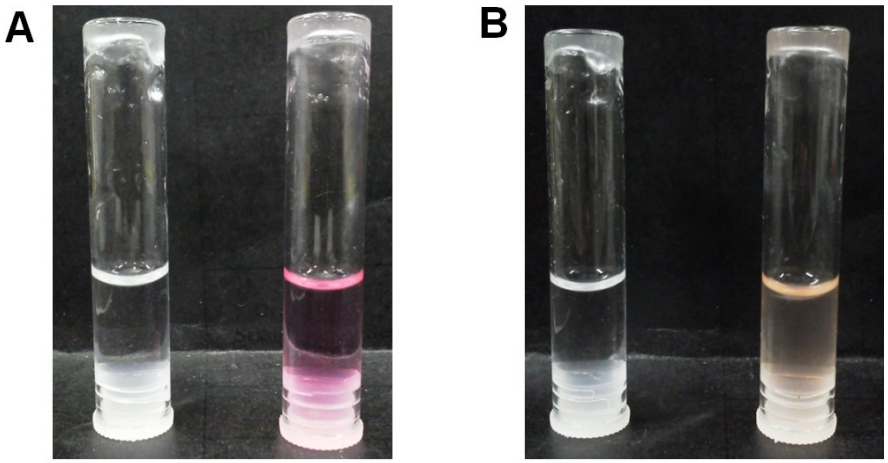


Fig. S1 Photograph of the PEG-avidin hybrid hydrogel overlayered with solutions in the inversion test. The **B-4armPEG**-avdin hydrogel was overlayed with (*left*) PBS and (*right*) the culture medium, respectively. The photographs were taken (A) before and (B) after incubation at room temperature for 4 hours.

4. Evaluation of molecular permeability of hydrogels

Release of enhanced green fluorescent protein (EGFP) from the hydrogel consisting of **B-4armPEG** and avidin was quantified to evaluate the protein permeability of the gel networks. The hydrogel including EGFP (final conc.: 4 μM) was prepared by mixing the

B-4armPEG and avidin solutions in glass vials as described above. After gelation, 100 μL of PBS was layered on the hydrogel (hydrogel volume: 100 μL) and incubation at room temperature. After incubation for various periods from 1 to 5 hours, the fluorescent spectra of the PBS layer on the hydrogel were measured by excitation at the wavelength of 489 nm with a fluorescent spectrometer (FP-6500; Jasco, Tokyo, Japan). As a control, the EGFP solution without **B-4armPEG** and avidin (4 μM EGFP, 100 μL) was mixed with 100 μL of PBS and then served to the same fluorescence measurement.

Figure S2 shows the fluorescent spectra of the PBS layer on the hydrogel. EGFP was shown to be released from the hydrogel to the PBS layer. This result indicates that EGFP is permeable in the gel networks by free diffusion.

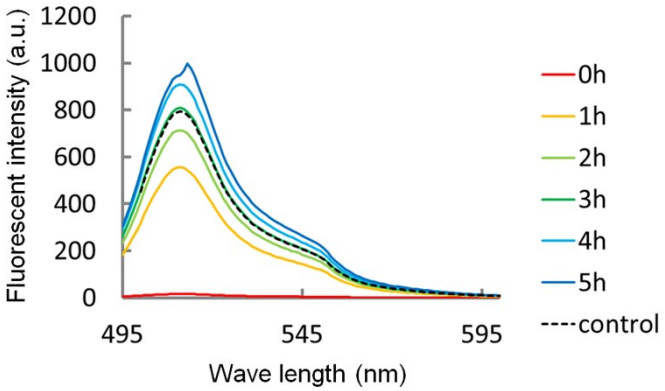


Fig. S2 Fluorescence of EGFP released from the PEG-avidin hybrid hydrogel after incubation for various periods. The fluorescent spectra of the PBS solution on the EGFP-including hydrogel were measured after incubation for 0 (red), 1 (orange), 2 (light green), 3 (green), 4 (light blue) and 5 hours (blue). Simultaneously, that of the EGFP solution without both the macromonomer and avidin was measured as a control.

5. Formation and light-induced degradation of photodegradable hydrogels

The **PB-4armPEG** solution (2 mM) was prepared by dissolving in PBS including yellow-green fluorescent particles (diameter: 2.0 μm) as described in the preparation of the **B-4armPEG** solution. 50 μL of the **PB-4armPEG** solution (2 mM in PBS, including fluorescent microparticles) was mixed with 50 μL of the avidin solution (2 mM in PBS) in glass vials. The **PB-4armPEG**-avidin solution was incubated at room temperature for 10 min. Gelation was evaluated using a simple inversion test. The glass vial containing the solution was inverted, and the solution was considered to have formed a gel when it did not fall under gravity.

To induce photo-degradation, the **PB-4armPEG**-avidin hydrogels in the glass vials were exposed to an ultraviolet (UV) irradiator (LAX-102, from Asahi Spectra Co., Ltd., Tokyo, Japan) equipped with a cylindrical lens and bandpass filter (wavelength: 365 ± 5 nm, 2.5 mW/cm^2). After light exposure from below at room temperature for 4000 sec, degradation was determined using the inversion test described above.

Furthermore, after exposure to light, a portion of the solution of the photodegraded product was collected, and the UV/vis absorbance was measured with NanoDrop One (from Thermo Fisher Scientific Japan, Yokohama, Japan). Figure S3 shows the absorbance of the photodegraded product solution and the **PB-4armPEG** solution which was prepared at the same **PB-4armPEG** concentration as the hydrogel formation. The difference between two spectra clearly shows that **PB-4armPEG** in the hydrogel photoconverted during photodegradation. Moreover, the spectral change in the photodegraded product was almost the same as previously reported to be observed in the photocleavage of the similar MeNVoc linker.[S2] This result suggests that the present photodegradation of the hydrogel occurred through photocleavage of the MeNVoc linker as designed.

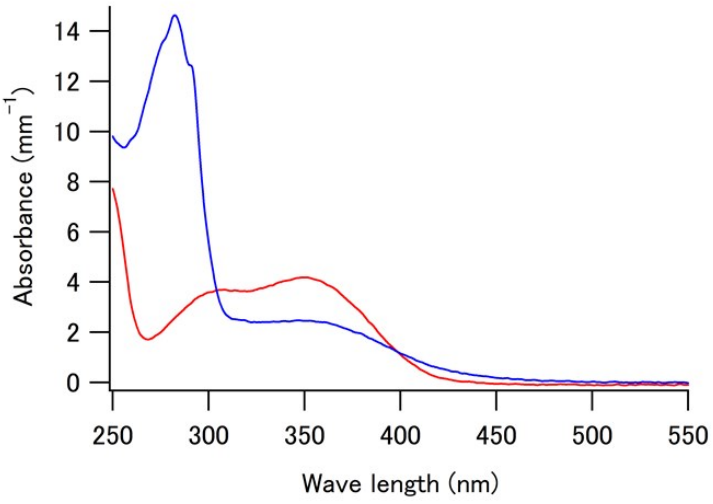


Fig. S3 UV/vis absorbance spectra of the **PB-4armPEG** solution (red line) and the photodegraded product from the **PB-4armPEG**-avidin hydrogel (blue line).

6. Single particle tracking analysis in the hydrogels

The **PB-4armPEG** solution (2 mM in PBS, including yellow-green fluorescent particles with the diameter of 2.0 μm) was prepared as described above. 50 μL of the **PB-4armPEG** solution was put onto the glass-bottom part (Φ:10 mm) on the glass-bottom

dish (Φ: 35 mm) for microscopic observation and was mixed with 50 μL of the avidin solution (2 mM in PBS) on the glass-bottom dish. After gelation at room temperature for 10 min, the fluorescent particles in the hydrogel were observed with a confocal laser scanning microscope (CLSM) (LSM 510 META, Carl Zeiss, Germany). Under the observation, a part of the observation flame was irradiated with a diode laser (405 nm, < 30 mW/cm^2) for 2 min, using the region of interest (ROI) mode of the microscope. Time-lapse images were obtained both in the light-irradiated and non-irradiated area for 1 min at 3 sec intervals. The velocity of the microparticle motion was calculated with the Particle Track and Analysis (PTA ver 1.2) plug-in of ImageJ (NIH, Bethesda, MD). In the two trials, total seven and eight particles were observed in the irradiated and non-irradiated area, respectively. The mean velocities and the standard errors were calculated from the data. As a control, the same experiment was performed by using **B-4armPEG** instead of **PB-4armPEG**.

In the **PB-4armPEG**-avidin hydrogel, the Brownian motion of the microparticles was observed at the lower-right quadrant area (the light-exposure area) (**Movie S2**), while the motion of the microparticles was observed to stop over the observation frame in the control **B-4armPEG**-avidin hydrogel (**Movie S1**). These results clearly show that the specific local area of the hydrogel can be degraded due to cleavage of the photocleavable linker of **PB-4armPEG** by site-specific light exposure.

Movie S1. The motion of the microparticles in the **PB-4armPEG**-avidin hydrogel after light exposure at the lower-right quadrant area of the observation frame. High-speed playback movie (approx. 30-time faster) of the twenty of time-lapse images observed at 3 s intervals.

Movie S2. The motion of the microparticles in the **B-4armPEG**-avidin hydrogel after light exposure at the lower-right quadrant area of the observation frame. High-speed playback movie (approx. 30-time faster) of the twenty of time-lapse images observed at 3 s intervals.

7. Oscillatory rheology measurements in the culture medium

The oscillatory shear rheological properties, that is, the storage elastic modulus (G') and the loss elastic modulus (G''), were measured by sweeping frequency from 10 Hz to 0.01Hz at 25 °C on an Anton Paar Physica MCR 301 rheometer (Anton Paar, Graz, Austria). **PB-4armPEG** and avidin were dissolved in PBS and the culture medium (RPMI-1640, supplemented with 10% fetal bovine serum (FBS) and 0.5%

penicillin/streptomycin) at the concentration of 1 mM, respectively. A cylindrical hydrogels with a thickness of 0.3 mm were prepared by placing the **PB-4armPEG**-avidin solutions in a mold at room temperature for 10 min. Parallel-plate geometry was employed using a 25 mm diameter plate with a constant deformation of 1%.

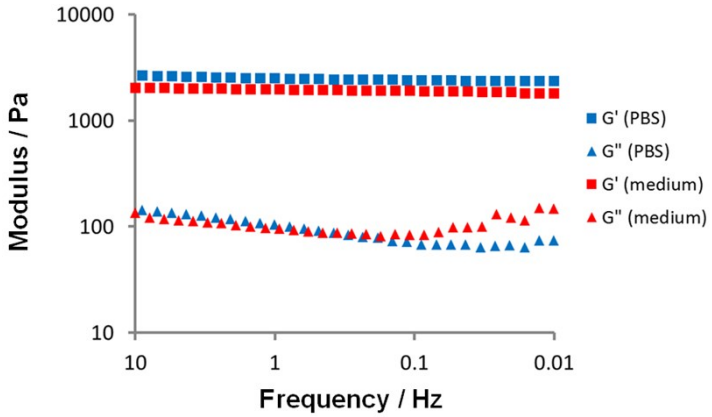


Fig. S4 Dynamic viscoelasticity measurement of photodegradable PEG-avidin hybrid hydrogels (*G'*: the storage modulus (squire), *G"*: the loss modulus (triangle)). **PB-4armPEG** and avidin were dissolved in PBS (blue) and the culture medium (red).

8. Cell culture and Preparation

BaF3 murine IL-3-dependent pro-B cell line and NIH3T3 murine fetal fibroblast were purchased from RIKEN Bioresourse Center (Saitama, Japan). BaF3 cells were cultured in RPMI-1640 medium supplemented with 1 ng/mL murine IL-3, 10% FBS and 0.5% penicillin/streptomycin at 37°C under 5% CO_2. NIH3T3 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS and 0.5% penicillin/streptomycin.

Cells in the culture medium were collected by centrifugation (100 ×g, 3 min). BaF3 cells were washed with PBS twice by resuspension and centrifugation and were finally suspended in PBS to prepare cell suspensions for cell embedding for hydrogels. NIH3T3 cells were detached from the culture dish by 0.25% trypsin/EDTA treatment and harvested by rinsing with the culture medium including FBS. Then, the harvested cells were washed with PBS and finally suspended as described above.

9. Light-induced cell release from hydrogel

The **PB-4armPEG** solution in the culture medium was put onto the glass-bottom part (*Φ*:10 mm) on the glass-bottom dish (*Φ*: 35 mm). BaF3 cell was suspended in the avidin solution of the culture medium, and then, the solution was dropped onto the **PB-4armPEG** solution on the dish, immediately followed by mixing with a pipette to prepare the **PB-4armPEG**-avidin solution (both final concentration: 1 mM, total volume: 30 μL) including BaF3 cells (final cell density: 2×10^7 cells/mL). After gelation for 10 min, the cell-embedded hydrogel was rinsed with PBS (1 mL), and then, 100 μL of the culture medium was overlaid on the hydrogel in the glass-bottom part. The hydrogel was exposed to light at 2.0 J/cm^2 with an ultraviolet (UV) irradiator (LAX-102, from Asahi Spectra Co., Ltd., Tokyo, Japan) equipped with a cylindrical lens through a bandpass filter (wavelength: 360 ± 5 nm, 4.0 mmW/cm^2). The photodegraded hydrogel including cells (calc. 6×10^4 cells) was collected with the culture medium by a pipette. The collected cells were stained with PI, and then analysed with a flow cytometer equipped with an argon laser (FACS-Calibur, from Becton-Dickinson, USA). The viability was obtained from the rate of the PI-negative cells. The same experiments were replicated three times, and the viability was expressed as the mean value of the three experiments and its standard error.

Figure S5 shows the viability of the cells collected from the photodegraded gel solution. The viability (94.2±1.0 %) was almost the same as that of the control intact cells (98.1±0.3 %).

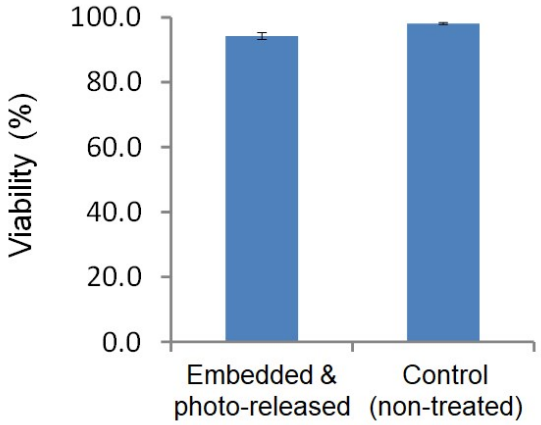


Fig. S5 Viability of the cells released from photodegradable PEG-avidin hybrid hydrogels. As a control, intact cell was employed. Each bar represents the mean ± S.E. (n = 3).

10. Adherent cell culture on functionalized hydrogels (2D culture)

The **PB-4armPEG** solution was pored into the glass-bottom dish (35 mm). Biotinylated RGD peptide (Biotin-CGGGKEKEKEKGRGDSP) was dissolved into the avidin solution. After incubation for 10 min, this RGD peptide-avidin complex solution was dropped onto the **PB-4armPEG** solution on the dish, immediately followed by mixing with a pipette to prepare the RGD peptide-decorated **PB-4armPEG**-avidin solution (**PB-4armPEG**: 1 mM, avidin: 1 mM, biotinylated RGD peptide: 0.25 mM, total volume: 30 μL). Then, a concave mold made of PDMS was pressed against a droplet of the solution. After gelation for 5 min, the mold was removed, and a thin hydrogel with a flat molded surface (thickness: 100 μm) was washed with PBS (1 mL). As negative controls, non-decorated and biotin-decorated hydrogels were prepared by using the avidin solution and the biotin-avidin complex solution (the final concentration of biotin: 1 mM) instead of the RGD peptide-avidin complex solution.

Murine embryonic fibroblast (NIH3T3 cell) was harvested from culture dishes and suspended in DMEM supplemented with 10% FBS (5×10^3 cells/mL). This cell suspension (1.5 mL) was added onto the glass-bottom dish with the molded hydrogel, followed with incubation at 37 °C under 5% CO_2 for 24 h. To fluorescently stain alive and dead cells on the hydrogel, a staining solution including 2 μM Calcein AM and 1.5 μM PI (volume: 1 mL) was added into the dish, followed with incubation at 37 °C for 1 hour. After washing with PBS, the cells on the hydrogels were observed with the CLSM. As a positive control, the cells on the glass-bottom dish were also observed. To quantitatively evaluate cell elongation on the functionalized hydrogels, the green fluorescent image of the cells was analysed with ImageJ (NIH, Bethesda, MD). The mean aspect ratio of the cell shape was determined by using the images from nine randomly selected locations.

Figure S6 shows the confocal microscopic images and the mean aspect ratio of the cells on various surfaces. The cells on RGDS peptide-decorated hydrogels were observed to elongate more (Fig. S6B), compared with those on non-decorated and biotin-decorated hydrogels (Fig. S6C and D). The aspect ratio of the cells on RGDS peptide-decorated hydrogels was almost two times larger than that on non-decorated ones. This result indicates that the cells could elongate on the hydrogel surface due to functionalization with RGDS peptide.

However, the cell morphology on the hydrogel surface were different from that on the glass-bottom dish (Fig. S6A). From this result, the concentration of RGDS peptide on the hydrogel was presumably low, and therefore, cells could not tightly adhere on the

hydrogel surface. Additionally, the cell numbers on the hydrogels were smaller than that on the glass-bottom dish. In particular, those on non-decorated and biotin-decorated hydrogels were much smaller. These results were assumed to be derived from weak adhesion of cells onto the hydrogel, leading to the low cell growth and the loss of cells during washing of the surface to remove the staining reagents.

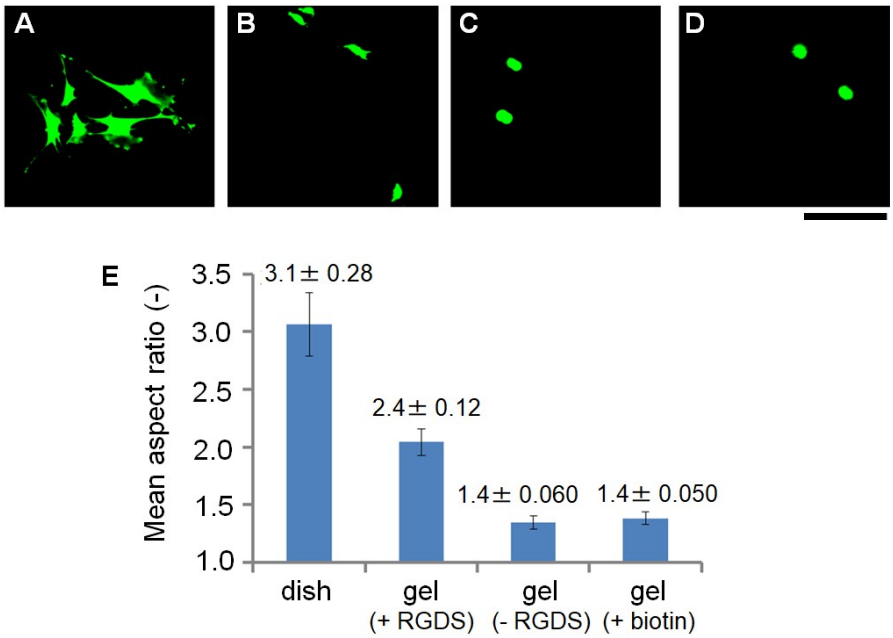


Fig. S6 Morphology of NIH3T3 cells on PEG-avidin hybrid hydrogels with and without RGD-peptides after culture for 2 days. The green fluorescent images of the cells were obtained with a confocal microscope after culture on (A) the glass-bottom dish surface, (B) RGDS-decorated hydrogel surface, (C) non-decorated hydrogel surface and (D) biotin-decorated hydrogel surface. Scale bar: 50 μm. (E) Mean aspect ratio of the cells on the hydrogels with and without RGDS-peptide. The mean aspect ratios were calculated from the microscopic images of the cells on the glass-bottom dish (**dish**), RGDS peptide-decorated hydrogel (**gel (+ RGD)**), non-decorated hydrogel (**gel (- RGD)**) and biotin-decorated hydrogel (**gel (+ biotin)**). Each bar represents the mean ± S.E. (total more than fourteen cells were observed from three images in three trials).

References

[S1] S. Takamori, S. Yamaguchi, H. Ohashi, T. Nagamune, *Chem. Commun.*, 2013, **49**, 3013–3015.

[S2] A. A. Pogodaev, A. S. Y. Wong, W. T. S. Huck, *J. Am. Chem. Soc.*, 2017, **139**,

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