Electronic Supplementary Information

Selective capture and noninvasive release of cells using a thermoresponsive polymer brush with affinity peptides

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S1. Synthesis of propargyl acrylate

Propargyl acrylate was synthesized via the reaction between propargyl alcohol and acryloyl chloride by the following procedure. Dichloromethane (200 mL), propargyl alcohol (17.5 mL, 0.30 mol), and purified triethylamine (58.5 mL, 0.42 mol) were added into a 500 mL flask, and the solution was stirred at 0 °C. Acryloyl chloride (24.8 mL, 0.3 mol) was dissolved in dichloromethane (50 mL), and the obtained solution was added drop by drop to the propargyl alcohol solution. These solutions were reacted for 4 h at 0 °C. After the reaction, the solution was rinsed using a separating funnel, thrice with 1 M HCl aq (150 mL), twice with 0.2 M NaOH aq (150 mL), and once with NaCl saturated solution (150 mL). The monomer was then dehydrated using magnesium sulfate. Dichloromethane was distilled away from the product to obtain the purified monomer.

S2 Peptide synthesis

S2-1 Synthesis of cyclic-RGD

Peptides were synthesized using were synthesized through solid-phase peptide synthesis using a peptide synthesis apparatus (Initiator+ SP Wave; Biotage, Uppsala). Lysine with azido group were added to the terminal ends of peptides as the reaction group in the click reaction to conjugate the block copolymer brushes.

H-Gly-Trt(2-Cl)-resin (0.10 mmol) was added to a 5-mL vial, and dichloromethane (DCM; 0.8 mL) was added to the resin. Then, Fmoc-Arg(Pbf)-OH (0.3 mmol), *O*-(1*H*-benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HBTU; 0.3 mmol), 1-hydroxybenzotriazole (HOBt; 0.3 mmol), and *N,N*-diisopropylethylamine (DIPEA; 0.6 mmol) were dissolved in 1.5 mL *N,N*-dimethylformamide (DMF). The solution was added to a vial and reacted to H-Gly-Trt(2-Cl)-resin at 25°C for 30 min with continuous shaking. After the reaction, the resin was rinsed with DMF. Then, 20% piperidine/DMF (1.75 mL) was added to the resin, and the solution was stirred continuously for 3 min. The reaction solution was collected, and 20% piperidine/DMF (1.75 mL) was added to the resin. The solution was then stirred for 20 min, and the reaction solution was collected. Next, the resin was rinsed with DMF three times, and DMF was collected. The Fmoc concentration of the collected solution was measured by determining the absorption at 301 nm, and the amino acid introduction rate to the resin was estimated by determining the Fmoc amount in the collected solution.

Amino acids were reacted with the amino acid resin in the order of Fmoc-Lys(N_3)-OH, Fmoc-D-Phe-OH, and Fmoc-Asp(OtBu)-OH at 75°C for 5 min to synthesize H-Asp(OtBu)-D-Phe-Lys(N_3)-Arg(Pbf)-Gly-resin.

Ten milliliters of acetic acid/2,2,2-trifluoroethanol/DCM mixed solution (20:20:60) was added to H-Asp(OtBu)-D-Phe-Lys(N_3)-Arg(Pbf)-Gly-resin, and the suspension was stirred at 25°C for 1 h. Then, the solution was filtered, and the filtrate was mixed with toluene (50 mL). Acetic acid in the solution

was removed by evaporation. The residue was purified by dropping into cooled diethyl ether (30 mL), and crude peptide was obtained.

DCM (3 μL), 4-dimethylaminopyridine (2.75 mg), propylphosphonic anhydride solution (> 50 wt. % in ethyl acetate; 196 μL), and triethylamine (226 μL) were added into a glass flask. The crude peptide then was dissolved in DCM (1.2 mL), and the peptide solution was dropped into the solution in the flask. After incubation for 4 h, the solution was stirred at 25°C for 18 h to circulate the peptide. Next, the solution was dried in vacuo, and a crude cyclic peptide was obtained. The peptide was dissolved in trifluoroacetic acid (TFA; 2.15 mL), water (0.15 mL), and DL-dithiothreitol (0.05 mg), and the solution was stirred at 25°C for 30 min. The solution was dropped into cooled diethyl ether, and the precipitate peptide was dried. The obtained peptide was purified using high-performance liquid chromatography (HPLC), and the dried peptide was obtained by lyophilization. The obtained peptide was confirmed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry.

S2-2 Synthesis of REDV and GGGREDV

Cl-Trt(2-Cl)-resin (0.10 mmol) was added to a 5-mL vial and DCM (0.8 mL) was added to the resin. Next, Fmoc-Val-OH (0.3 mmol), HBTU (0.3 mmol), HOBt (0.3 mmol), and DIPEA (0.6 mmol) were dissolved in 1.5 mL DMF. The solution was added to the vial and reacted with Cl-Trt(2-Cl)-resin at 25°C for 30 min with continuous shaking. After the reaction, the resin was rinsed with DMF. Then, 20% piperidine/DMF (1.75 mL) was added to the resin, and the solution was stirred continuously for 3 min. The reaction solution was collected, and 20% piperidine/DMF (1.75 mL) was added to the resin. The solution was then stirred for 10 min, and the reaction solution was collected. Next, the resin was rinsed with DMF three times, and DMF was collected. The Fmoc concentration of the collected solution was measured by determining the absorption at 301 nm, and the amino acid introduction rate to the resin was estimated by the amount of Fmoc in the collected solution.

Amino acids were reacted with the amino acid resin at 75°C for 5 min by repeated deprotection and coupling reactions. After the reactions, the resin peptide was reacted with 2.5 mL mixed TFA/water/triisopropylsilane (95/2.5/2.5) solution at 25°C for 90 min. The filtrate was then washed with TFA and added to diethylether (40 mL). The solution was centrifuged at 3500 × *g* for 5 min, and the supernatant was removed. This process was repeated three times. The precipitate was then dried, and a crude peptide was obtained. The peptide was purified by HPLC and obtained by lyophilization. The obtained peptide was confirmed by electrospray ionization time-of-flight mass spectrometry.

Table S1 Cell culture media

Cells	Culture media [a)]	Additives [b)]
Normal human umbilical vein endothelial cells (HUVECs)	Endothelial Cell Basal Medium 2	FCS (10 mL) hEGF (2.5 μg) Hydrocortisone (100 μg) VEGF (0.25 μg) hbFGF (5 μg) R3-IGF (1 μg) Ascorbic acid (500 μg) Heparin (11.25 mg) Penicillin-Streptomycin (5 mL)
Human aortic smooth muscle cells (AoSMCs)	Smooth muscle cell basal medium	Insulin (0.5 mL) hFGF-β (1.0 mL) GA-1000 (0.5 mL) FBS (25 mL) hEGF (0.5 mL) Gentamicin/Amphotericin-B (GA) (0.5 mL)
Neonatal normal human dermal fibroblasts (NHDFs)	FBM basal medium	hFGF-β (0.5 mL) Insulin (0.5 mL) FBS (10 mL) Gentamicin/Amphotericin-B (GA) (0.5 mL)
Cell separation experiment	DMEM	FBS (50 mL)

a) Volume of cell culture medium was 500 mL. b) Additives were added to 500 mL cell culture medium.

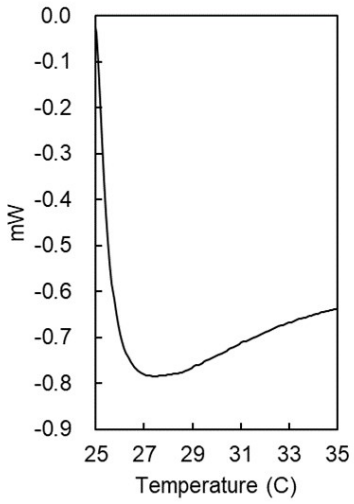

Fig. S1 Phase transition behavior of P(NIPAAm-*co*-HEMA) observed by differential scanning calorimetry.

Table S2 Elemental analyses of polymer brush-modified glass surfaces by X-ray photoelectron spectroscopy at a take-off angle of 90°.

Code	Atom (%)					N/C ratio
	C	N	O	Si	Cl	
Initiator modified glass	23.9	0.15	58.1	17.3	0.69	0.049
P1	25.4	0.97	58.0	15.4	0.29	0.038
P1T1000	32.1	1.55	52.7	13.4	0.29	0.048
P1T1000-cRGD	37.0	2.89	47.6	12.4	0.11	0.078
Calcd of P(HEMA-*co*-PgA) [a)]	67.5	0	32.5	-	-	0
Calcd of P(NIPAAm-*co*-HEMA) [a)]	74.2	0.11	0.14	-	-	0.152

a) Theoretical atomic composition of each monomer.

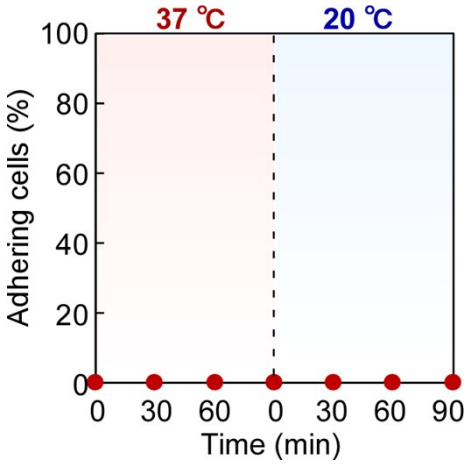

Fig. S2 HUVEC adhesion profile on P1T500-REDV.

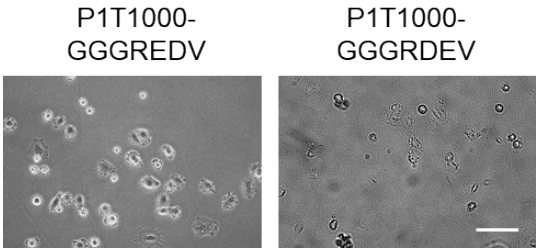

Fig. S3 Comparison of HUVEC adhesion between P1T1000-GGGREDV and P1T1000-GGGRDEV after incubation at 75°C for 90 min.

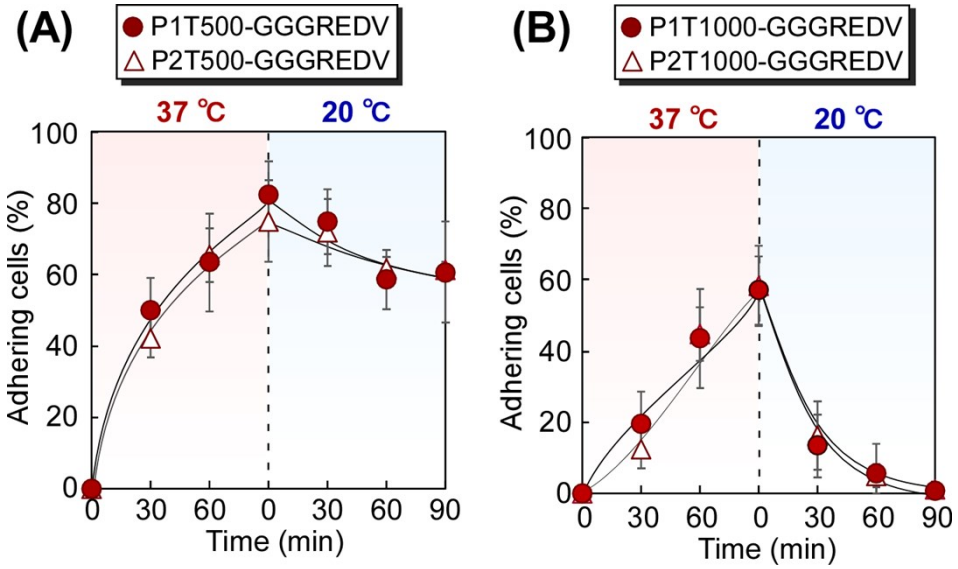

Fig. S4 Effects of the bottom peptide conjugated segment on HUVEC adhesion. (A) Comparison between P1T500-GGGREDV and P2T500-GGGREDV. (B) Comparison between P1T1000-GGGREDV and P2T1000-GGGREDV.

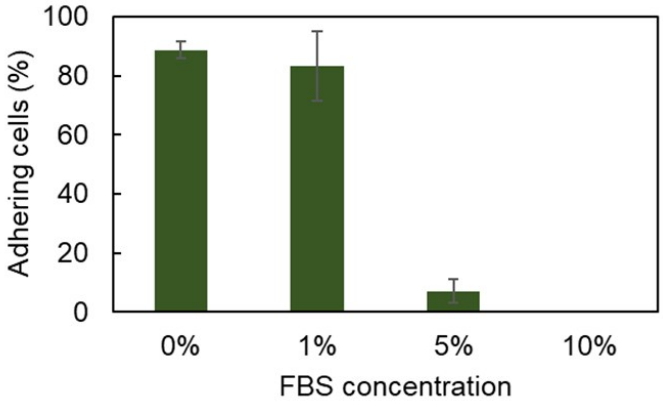

Fig. S5 Cell adhesion ratio of NHDFs on P1T1000 after incubation at 37°C for 90 min using DMEM containing various concentrations of FBS.

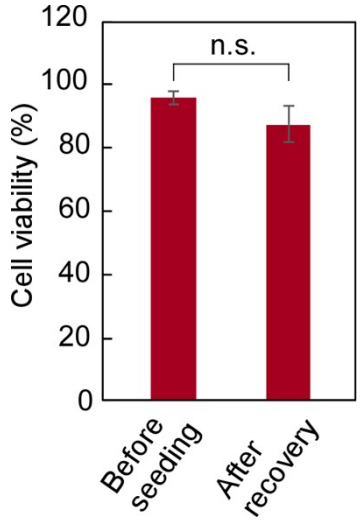

Fig. S6 Viability of HUVECs before seeding and after recovery of P1T1000-GGGREDV. Statistics analysis was performed using Student's t tests. "n.s." indicates not significant ($p > 0.05$); n = 3.