Electronic Supplementary Material (ESI) for Soft Matter

Supplementary Information

Reversible Regulation of Bioactive Ligands Presented on Immobilized Gold Nanoparticles

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Synthesis and purification of polypeptides and the B-PEG conjugate

The genes encoding cysA-RGD, Bcys, A, and cysA (the sequences are shown in Table S1) were each constructed in the Qiagen pQE9 expression vector through standard recombinant DNA manipulation. Each polypeptide was expressed in the *E.coli* strain SG13009 under control of the bacteriophage T5 promoter and purified through nickelnitrilotriacetic acid (Ni-NTA) metal-affinity chromatography (Qiagen). To synthesize the B-PEG conjugate, the solution of Bcys was prepared in 8 M urea (pH 4.5) containing 20 mM tris(2-carboxyethyl)phosphine

hydrochloride (TCEP·HCl, Pierce) and the solution was incubated at room temperature for 1 h to allow reduction of disulfide bonds. The pH of the solution was adjusted to 7.0, followed by addition of mPEG-maleimide (10kDa, Laysan Bio Inc.) at a weight ratio of 10:1 (mPEG-maleimide:Bcys). The mixture was stirred overnight in the dark. The resulting B-PEG conjugate was purified by using Ni-NTA resin and Sepharose 6B resin (Sigma) sequentially. Purified CysA-RGD, Bcys, A, cysA, and B-PEG were characterized using SDS-polyacrylamide gel electrophoresis (Figure S1) and MALDI mass spectrometry.

Functionalization of gold nanoparticles with polypeptides

The solution of cysA-RGD or cysA (100 μM) was prepared in PBS (pH7.5) containing 2 mM TCEP. Each solution was incubated at room temperature for 1 h to allow reduction of disulfide bonds. Gold nanoparticles (AuNPs) having a diameter of 250 nm (purchased from Ted Pella Inc.) were collected by centrifugation at 14,000 RCF for 15 mins and resuspended in the cysA-RGD or cysA solution, followed by incubation overnight. The AuNPs were then harvested by centrifugation and washed three times with PBS containing 0.01% Tween-20 (pH 7.5). The AuNPs were re-suspended in the same buffer with a sonicator (Sonic Dismembrator, Fisher Scientific). To confirm polypeptides were immobilized on AuNPs through this procedure, the AuNPs modified with cysA-RGD were incubated with 0.1% w/v fluorescamine (Acros Organics, prepared in PBS, pH 8.0) at room temperature for 10 mins, followed by examination of the fluorescence spectrum (Ex: 390 nm) with a Cary Eclipse fluorescence spectrophotometer (Varian Inc.). Unmodified AuNPs and the cysA-RGD solution were used as controls.

Immobilization of polypeptide-functionalized AuNPs on substrates

Substrates bearing the *N*-hydroxysuccinimide (NHS) ester group were prepared by modifying polystyrene substrates with *N*-sulfosuccinimidyl-6-(4'-azido-2'-nitrophenylamino) hexanoate (sulfo-SANPAH, Pierce) through a procedure adapted from a previously published method (reference 23 cited in the main text). Briefly, 2mM sulfo-SANPAH was placed on the surface of each well of 48-well suspension plates (Greiner), followed by irradiation (365 nm, 125W) for 10 mins. The majority of the sulfo-SANPAH solution was removed with a thin layer left on the surface, followed by irradiation for an additional 15 mins. The plates were then washed three times with PBS to remove sulfo-SANPAH completely. To examine whether the NHS group was functionalized on the resulting substrates, samples were incubated with rabbit anti-human IgG (Pierce) at room temperature for 1 h, washed with PBS containing 0.05% Tween-20, incubated with Alexa Fluor 488-conjugated goat anti-rabbit antibody (Invitrogen) for 1 h, and washed with PBS containing 0.05% Tween-20 four times. Each sample was examined with a 2.5× objective (EC Plan-Neofluar, Zeiss) on a fluorescence microscope. To modify the NHS-functionalized substrates with polypeptide-functionalized AuNPs, the substrates were coated with cysA-RGD-functionalized or cysA- functionalized AuNPs and the reaction was allowed to proceed at room temperature overnight. The substrates were washed three times with PBS to remove non-immobilized AuNPs, followed by passivation of unreacted NHS with 5mM mPEG-NH_2 (500 Da, Laysan Bio Inc.) at room temperature for 1 h. The resulting substrates were examined with a 40× objective (EC Plan-Neofluar, Zeiss) on a Zeiss Axio Observer inverted microscope.

Reversible presentation of the RGD immobilized in submicron patches to cells

Fibroblasts (NIH 3T3) were maintained in Dulbecco's modified Eagle's medium (DMEM, Hyclone) supplemented with 5% fetal bovine serum (FBS, Hyclone), 100 U/mL penicillin, and 100 μg/mL streptomycin (Invitrogen). To examine whether the RGD ligand immobilized in submicron patches was presented to cell surface receptors, cells (4×10^4 per well) were seeded on substrates modified with cysA-RGD-functionalized AuNPs and cysA-functionalized AuNPs, respectively, and incubated in DMEM containing 1% FBS for 3 h. Each sample was washed to remove non-adherent cells and stained with calcein AM and ethidium homodimer-1 (Live/Dead Assay, Invitrogen), followed by examination with a 2.5× objective (EC Plan-Neofluar, Zeiss) on a Zeiss Axio Observer inverted fluorescent microscope. To examine whether B-PEG could be co-immobilized and shield the RGD from access by cells, a substrate modified with cysA-RGD-functionalized AuNPs was incubated with B-PEG (300 μM) with gentle shaking for 3 h. Fibroblasts (4×10^4 cells per well) were seeded on the resulting substrate and incubated for 3 h, followed by washing and staining of the remaining cells with calcein AM and ethidium homodimer-1 to determine whether the substrate was non-adhesive to cells (the RGD ligand was inaccessible).

To examine whether the RGD ligand could be converted from a cell-accessible to a cell-inaccessible state, cells were allowed to adhere on a substrate modified with cysA-RGD-functionalized AuNPs in DMEM containing 1% FBS. B-PEG was added in the medium at a concentration of 300 μM, and the sample was incubated with gentle shaking (at a speed at which the liquid started to move to enhance mass transfer) at 37 °C for 3 h. A control in which B-PEG was not added was performed. After removing detached cells, the undetached cells were stained with calcein AM and ethidium homodimer-1, followed by examination with a 2.5× objective on

a Zeiss Axio Observer inverted fluorescent microscope. The detached cells were collected, re-plated in a cell culture plate, and incubated in DMEM with 5% FBS. The sample was examined with the cell Live/Dead assay at 3 h and 3 days, respectively. To examine whether the RGD ligand could be converted from a cell-inaccessible to a cell-accessible state, a non-adhesive substrate on which cysA-RGD-functionalized AuNPs were immobilized and B-PEG was co-immobilized was incubated with a solution of A (600 μM) with gentle shaking at 37°C for 3 h, followed by cell seeding (4×10^4 cells per well) and static incubation for 3 h to allow cell adhesion. The sample was washed and stained with calcein AM and ethidium homodimer-1 to determine whether the substrate was cell-adhesive (the RGD ligand was accessible).

The effects of the molecular triggers on cell viability

Fibroblasts were seeded in 48-well tissue culture plates at a density of 4×10^4/well, followed by incubation in the maintenance medium for 24 h. The cells were then incubated overnight in the presence of the molecular triggers B-PEG and nonimmobilized polypeptide A, respectively (in DMEM containing 1% FBS). Cell viability before and after addition of B-PEG or A was characterized using the Live/Dead assay (Figure S4). Images were acquired with a 2.5× objective (Zeiss EC Plan-Neofluar) on a Zeiss Axio Observer inverted fluorescent microscope.

Table S1. The sequences of the polypeptides used in this study.

Polypeptide	Sequence
cysA-RGD	MRGS HHHHHHGSDDDDKASSGSGCSGSGT SGDLENEVAQLEREVRSLED EAAELEQKVSRLKNEIEDLKAE IGDHVAPRDTSYAVTGRGDSPASSTSW
Bcys	MRGS HHHHHH GSDDDDKWASGT SGDLKNKVAQLKRKVRSLKDKAAEL KQEVSRLENEIEDLKAK IGDHVAPRDTSMGGC
A	MRGS HHHHHH GSDDDDKA SGDLENEVAQLEREVRSLEDEAAELEQKVSRL KNEIEDLKAE IGDHVAPRDTSW
cysA	MRGSHHHHHHGSDDDDKASSGSGCSGSGTSGDLENEVAQLEREVRSLEDEAAELE QKVSRLKNEIEDLKAEIGDHVAPRDTSW

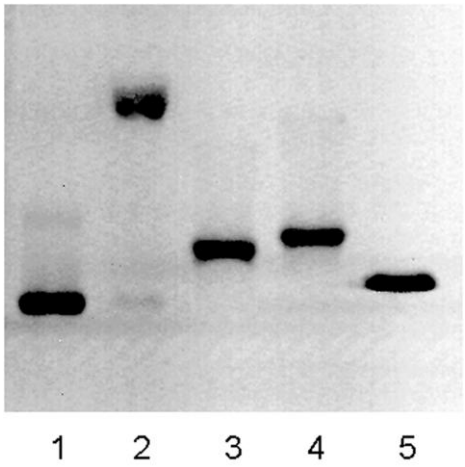

Figure S1. SDS-PAGE of purified polypeptides. From lane 1 to 5: Bcys (8.5 kDa), B-PEG (18.5 kDa), cysA (9 kDa), cysA-RGD (10.5 kDa) and A (8.2 kDa).

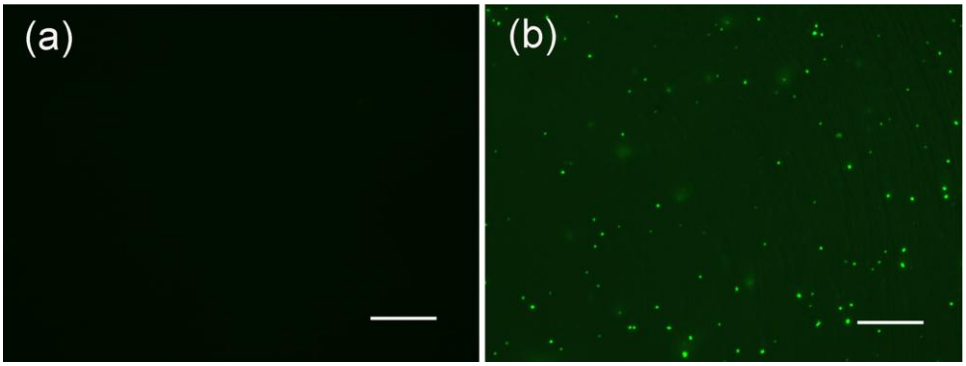

Figure S2. Fluorescent images of substrates after sequential incubation with rabbit anti-human IgG and Alex Fluor 488-conjugated goat anti-rabbit antibody: (a) unmodified polystyrene; (b) sulfo-SANPAH-modified polystyrene. The scale bars are 500 μm.

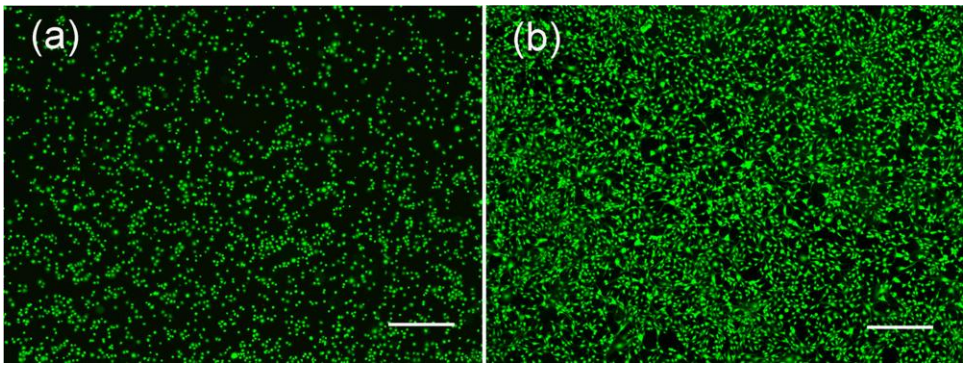

Figure S3. Cells detached from a substrate modified with cysA-RGD-functionalized AuNPs upon addition of B-PEG were viable. (a) Cell adhesion on the surface of a tissue culture plate was observed 3 h after re-plating. (b) The cells grew to confluence after 3 days. The scale bars are 500 μm.

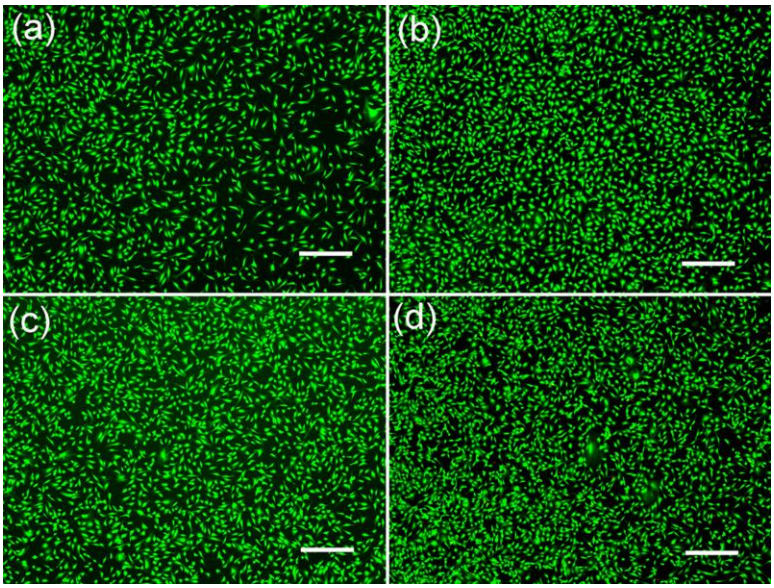

Figure S4. The effects of the molecular triggers on cell viability: (a) before addition of B-PEG; (b) after overnight incubation in the presence of 300 μM B-PEG; (c) before addition of A; and (d) after overnight incubation in the presence of 600 μM A. The scale bars are 500 μm.