Electronic supplementary information

Sticktight-inspired PEGylation for low-fouling coatings

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Contents

Experimental section

Materials

Methoxy PEG thiol (PEG-1SH, 5 kDa), PEG dithiol (PEG-2SH, 10 kDa), 4-arm PEG thiol (PEG-4SH, 20 kDa), 8-arm PEG thiol (PEG-8SH, 40 kDa), methoxy PEG succinimidyl carboxymethyl ester (PEG-1NHS, 5 kDa), and 8-arm PEG succinimidyl carboxymethyl ester (PEG-8NHS, 40 kDa) were purchased from Shanghai ToyangBio Tech. Inc. (China). 8-arm PEG hydroxyl (PEG-8OH, 40 kDa) was obtained from SINOPEG Biotech Co., Ltd. (China). 5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB) and branched polyethylenimine (PEI, 25 kDa) was obtained from Sigma-Aldrich (China). Au chips were purchased from RenLux Crystal Co., Ltd. (China). Micro total mercapto assay kit was bought from Solarbio Science & Technology Co., Ltd. (China). Dulbecco's modified Eagle's medium (DMEM, High Glucose) was bought from Beijing Neuronbc Laboratories Co., Ltd. (China). Fetal bovine serum (FBS) was obtained from Gibco (Germany). HeLa cells were purchased from Cell Bank of Chinese Academy of Sciences (Shanghai, China). *Escherichia coli* (*E. coli*) was obtained from China General Microbiological Culture Collection Center. Calcein-AM and SYTO 9 were bought from Dalian Meilun Biotechnology Co., Ltd. (China). Water was obtained by using Milli-Q ultrapure water apparatus with a resistivity of 18.2 MΩ cm.

Characterization methods

The adhesion performances of PEG and FBS were analyzed by a quartz crystal microbalance with dissipation monitoring (QCM, Biolin Scientific, Qsense Analyzer, Sweden). The water contact angle was detected by a contact angle apparatus (Kruss, DSA100S, Germany). UV–visible (UV–vis) absorption spectra were recorded on a UV–vis spectrophotometer (Shimadzu, UV-2600, Japan). Fluorescence images of cells and bacteria were observed on a fluorescence microscope (ZEISS, Axio Observer 3, Germany). The molecular weight (M_w) and polydispersity index (PDI) were determined by gel permeation chromatography (GPC) (Brookhaven, USA) equipped with a differential refractive index detector. PEG was used as the standard for calibration. The eluent of tetrahydrofuran was flowing at a rate of 1 mL/min.. Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained on a Bruker Avance 400 NMR spectrometer (Bruker, USA). The presence of PEG on the surface of Au chips was determined by an X-ray photoelectron spectroscopy (XPS, Thermo Fisher Scientific, ESCALABXI+, USA). The morphology of PEG coatings was measured by atomic force microscopy (AFM, Bruker, JPK NanoWizard 4XP, USA).

Characterization of thiolated PEG

The average number of thiol groups in thiolated PEG was determined by micro total mercapto assay kit with a series concentrations of cysteine solution as standards

according to the operation protocol. GPC tests was performed with tetrahydrofuran as the eluting agent. The chemical structures were examined through NMR with D_2O as the solvent.

Synthesis of PEG-TNB

PEG-SH (M_w = 5 kDa, 250 mg, 50 μmol) and DTNB (29.7 mg, 75 μmol) were dissolved in PBS (100 mM, pH 8, 10 mL) in a round bottom flask and stirred for 12 h. The mixture was purified by dialysis (MWCO 1000 Da) in water for 48 h. PEG-TNB was obtained after freeze drying. The chemical structure was confirmed by ^{1}H NMR with D_2O as the solvent.

Substrate preparation

Au wafers with the size of 9 mm × 9 mm were treated with Piranha solution (7:3 v/v mixture of 98% sulfuric acid and 30% hydrogen peroxide) for 15 min, followed by washing with water and drying with N_2. The wafer was immersed in thiolated PEG solution (5 mg/mL) for 3 h and subsequently washed with water to remove free PEG molecules. The component of the layer on the surface of Au wafer was analyzed by XPS.

Contact angle measurements

The contact angles of the bare and PEGylated substrates were measured and recorded by dropping a 20 μL of water onto the substrate surfaces. All tests were performed in triplicates.

Measurements of PEG and FBS adsorption by QCM

Thiolated PEG solution in PBS (5 mg/mL, pH=7.4) was pumped into the QCM chambers at a rate of 100 μL/min until the adsorption equilibrium on Au chips was obtained. The sample concentration, flowing rates and pH values did not significantly influence the adsosrption of PEG, as shown in **Fig. S7**. PBS was pumped to rinse away the unadsorbed PEG molecules. Subsequently, FBS solution was pumped into the QCM chambers until adsorption equilibrium, followed by rinsing with water to remove free FBS. The quantitative amount of PEG modification and FBS adsorption were analyzed using D-find software. To block the free thiol groups on the substrates, PEG-TNB (5 mg/mL) was introduced before the FBS adsorption. To study the PEGylation with PEG-1NHS and PEG-8NHS, PEI (2 mg/mL) was coated on the substrate to introduce amine groups. All solutions used in the tests were deoxygenized by N_2 bubbling before introduction into the chambers.

HeLa cells adhesion

HeLa cells were cultured in DMEM containing 10% (v/v) of FBS at 37°C supplied with 5% CO_2. PEGylated Au wafers were placed into a 24-well plate and cells were seeded with a density of 5×10^4 cells per well. After incubation overnight, the media and

unattached cells were removed by rinsing with PBS three times. Cells were fluorescently stained with Calcein-AM (2 μM, 100 μL per well) for 15 min. Fluorescence images were obtained by an inverted fluorescence microscope. Five random areas were chosen to determine the cell numbers.

E. coli adhesion

PEGylated Au wafers were placed into a 24-well plate. *E. coli* was suspended in PBS with a density of 10^7 bacteria per mL before being seeded into the wells of the plate (500 μL/well). After incubation for 8 h, unattached bacteria were removed by gently rinsing with PBS three times. The adhered bacteria were fluorescently stained by SYTO 9 (33 μM, 75 μL per well) for 15 min. The attached bacteria were observed by using an inverted fluorescence microscope equipped with a 63× oil immersion objective. The number of bacteria in microscopy images was analyzed by ImageJ software.

Statistical analysis

All data obtained were presented as mean ± standard deviation (SD). Student's t-test was engaged to analyze the difference between the groups. *p*-values calculated lower than 0.05 were considered statistically significant.

Supplementary figures and tables

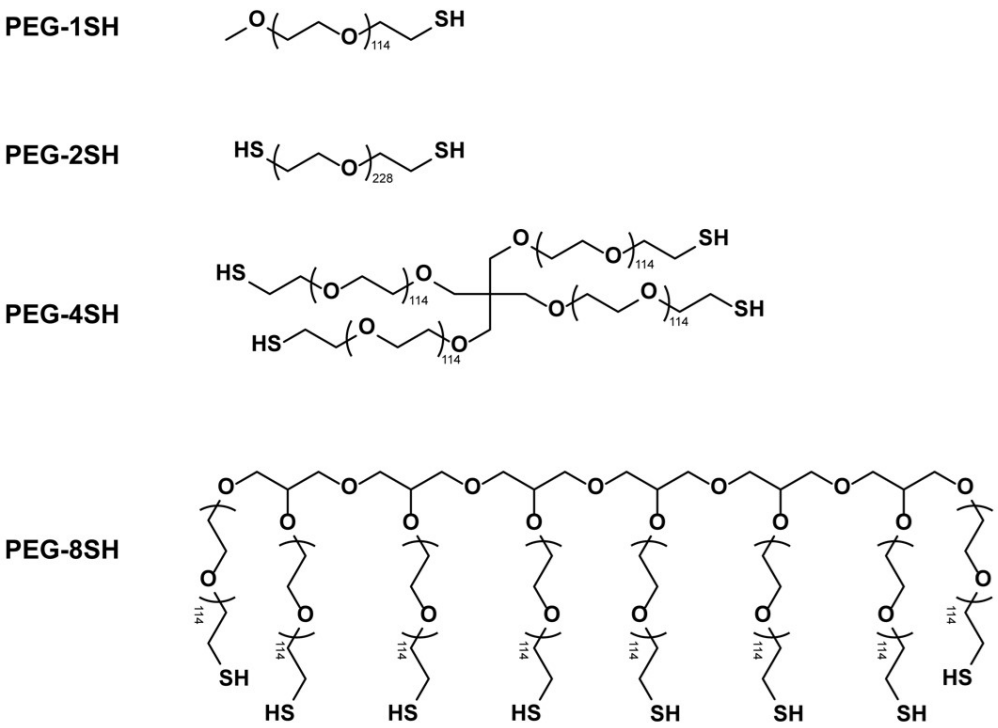

Fig. S1 Chemical structures of thiolated PEG.

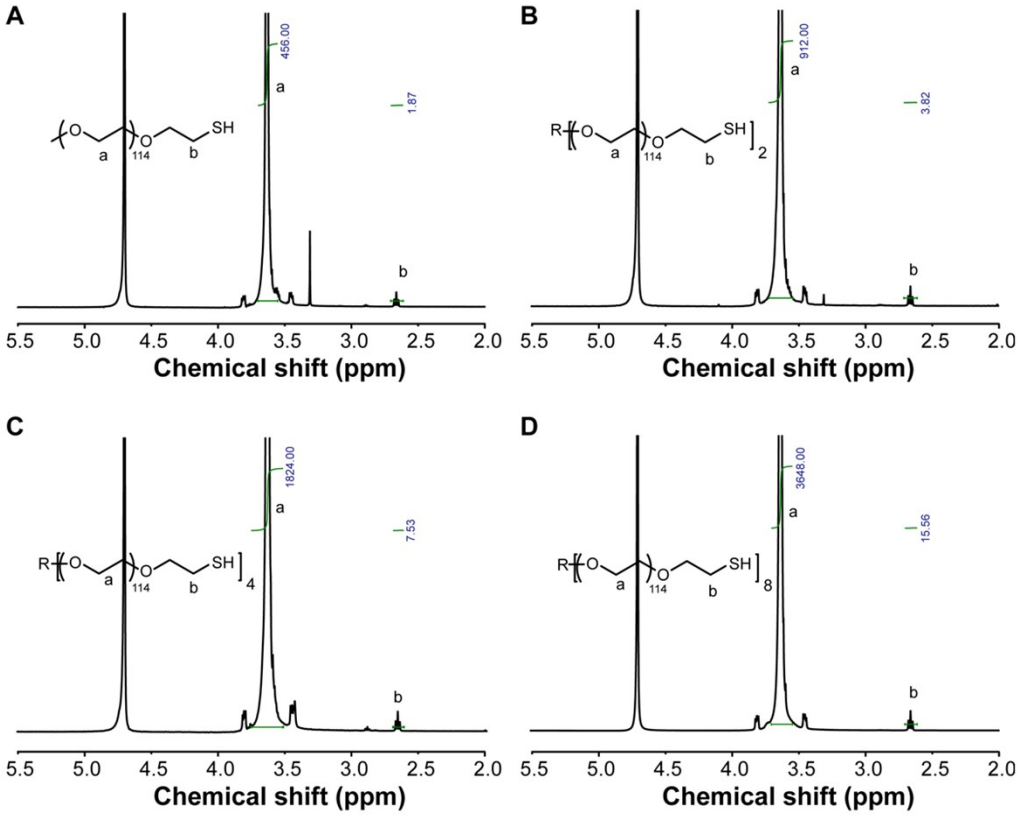

Fig. S2 ^{1}H NMR (400 MHz, D_2O) spectra of PEG-1SH (A), PEG-2SH (B), PEG-4SH (C), and PEG-8SH (D).

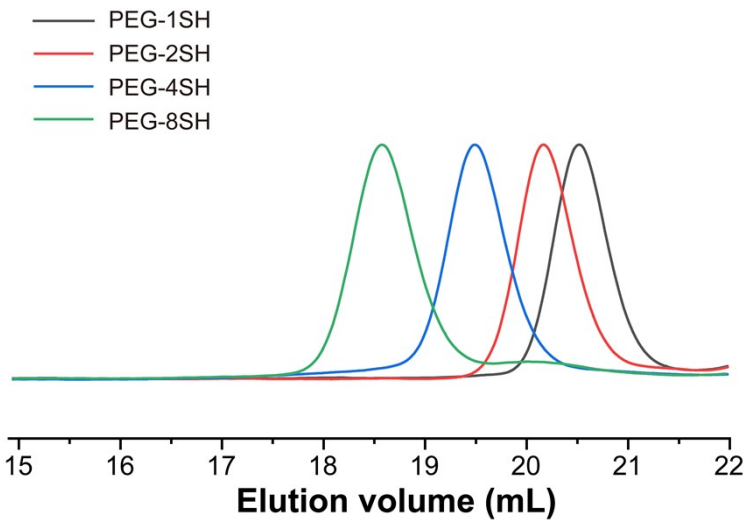

Fig. S3 GPC traces of thiolated PEG.

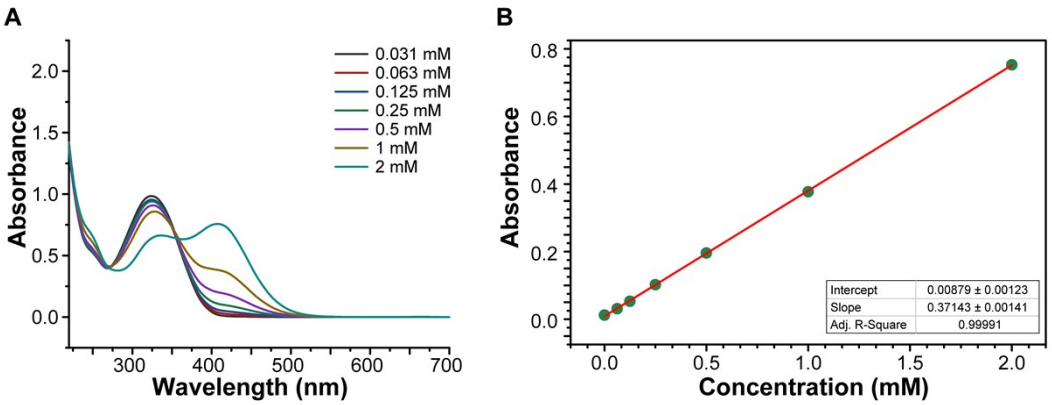

Fig. S4 (A) UV absorbance of cysteine solutions after mixing with Ellman's reagent (5 mM). (B) Standard fitting curve of the absorbance at 412 nm.

Table S1. List of thiolated PEG in this study.

Sample	$Mw_{theoretical}$ (Da)	Mw_{GPC} (Da)	PDI	N_{thiol}
PEG-1SH	5000	5387	1.13	0.98
PEG-2SH	10000	11820	1.24	1.97
PEG-4SH	20000	20640	1.11	3.71
PEG-8SH	40000	42290	1.16	7.75

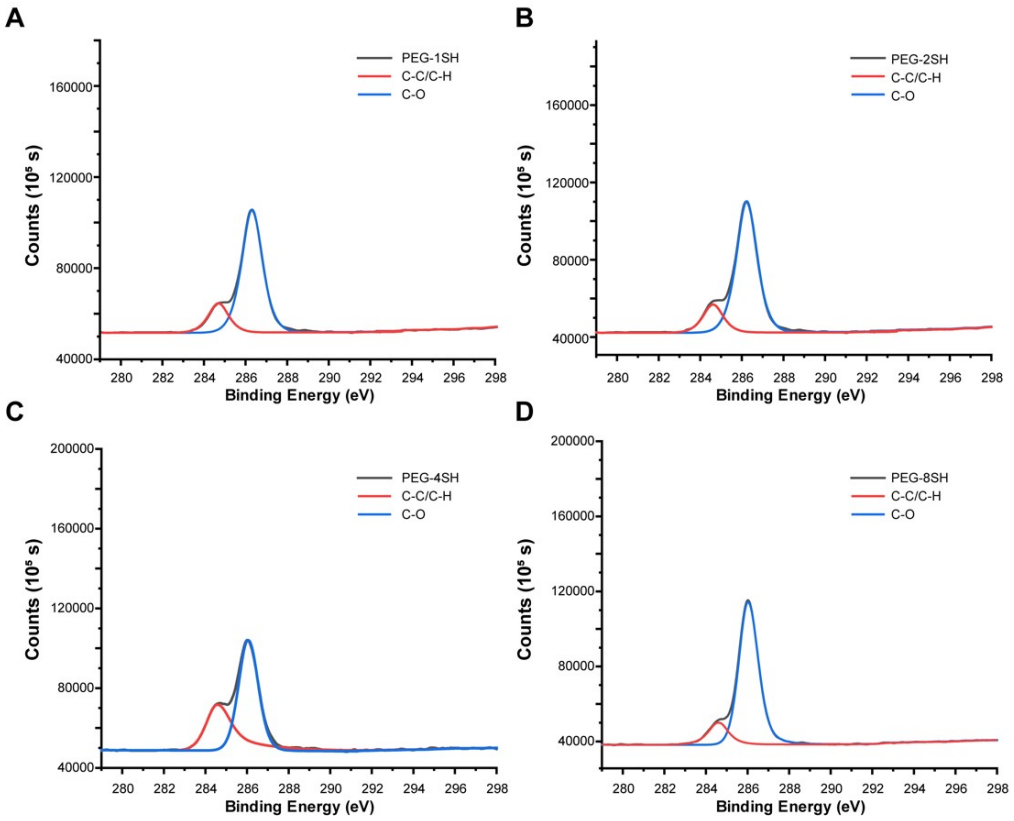

Fig. S5 XPS of C1s on Au wafers modified with PEG-1SH (A), PEG-2SH (B), PEG-4SH (C) and PEG-8SH (D).

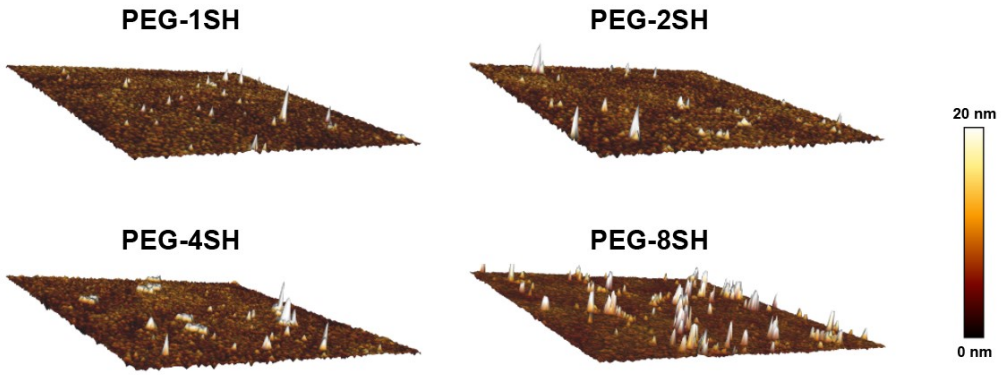

Fig. S6 AFM images of PEGylated coatings.

Table S2 Surface roughness of PEG coatings.

Sample	R_a (nm)	R_q (nm)	R_t (nm)
Au	1.03	1.29	11.10
PEG-1SH	1.22	1.83	56.17
PEG-2SH	1.21	1.90	76.03
PEG-4SH	1.25	2.44	82.71
PEG-8SH	1.63	3.24	85.01

R_a: Average Roughness, R_q: RMS Roughness, R_t: Peak to Valley Roughness

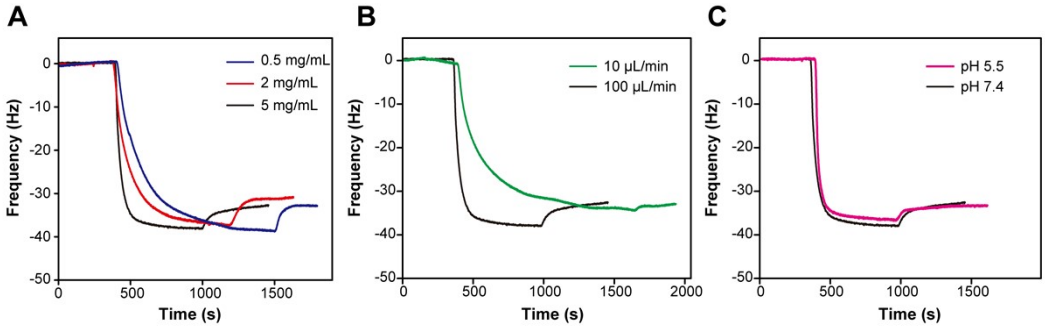

Fig. S7 QCM frequency changes for the monitoring of the PEGylation using PEG-1SH. (A) Concentration-dependent PEGylation with the flowing rate of 100 μL/min (pH 7.4). (B) Flowing rate-dependent PEGylation with the PEG concentration of 5 mg/mL (pH 7.4). (C) pH-dependent PEGylation at the PEG concentratin of 5 mg/mL and flowing rate of 100 μL/min.

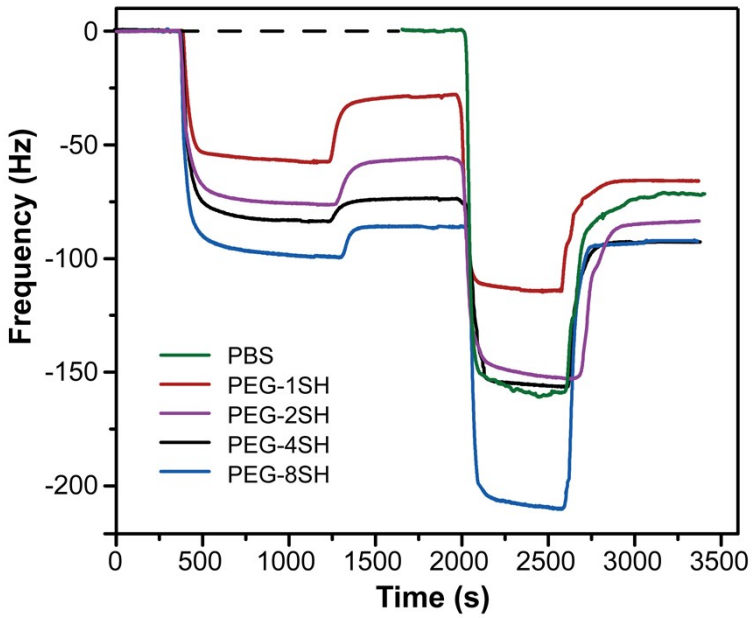

Fig. S8 QCM frequency changes to monitor PEGylation and the subsequent protein adhesion (5 mg/mL of PEG with a flowing rate of 100 μL/min).

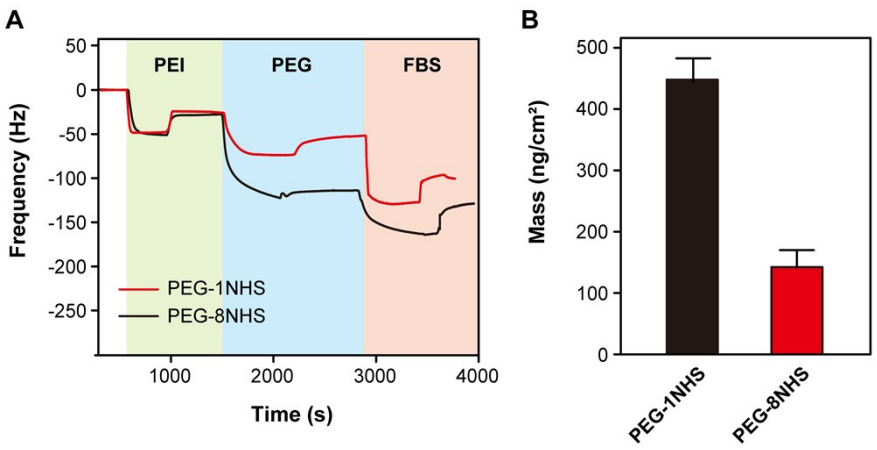

Fig. S9 (A) QCM frequency changes against time to monitor PEGylation with PEG-1NHS and PEG-8NHS. (B) Mass of adsorbed FBS on PEGylated surfaces.

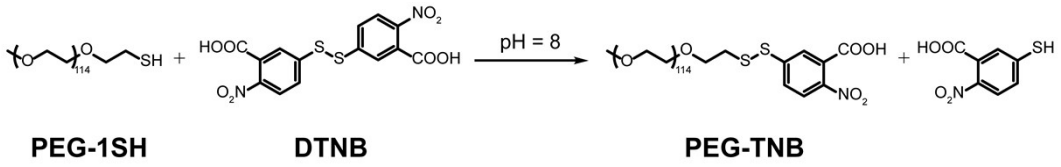

Fig. S10 Synthesis of PEG-TNB.

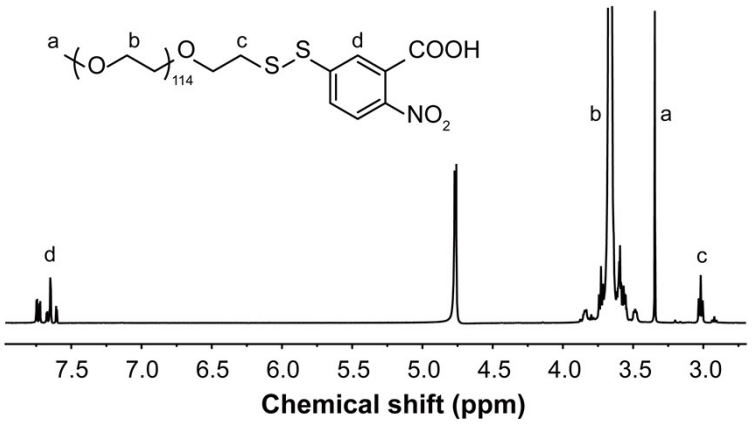

Fig. S11 ^{1}H NMR spectrum of PEG-TNB in D_2O.

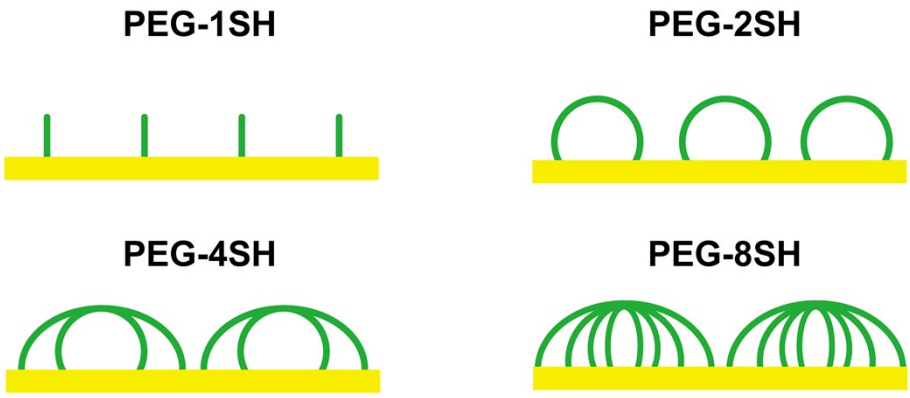

Fig. S12 Speculated illustration of multi-arm PEG coatings on Au substrates.

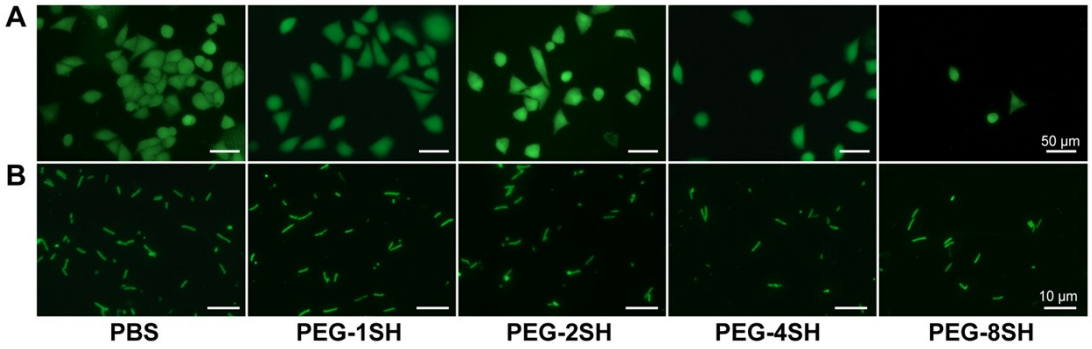

Fig. S13 Representative fluorescence microscopy images of adsorbed HeLa cells (A) and *E. coli* (B) on PEGylated substrates followed by incubation with PEG-TNB.

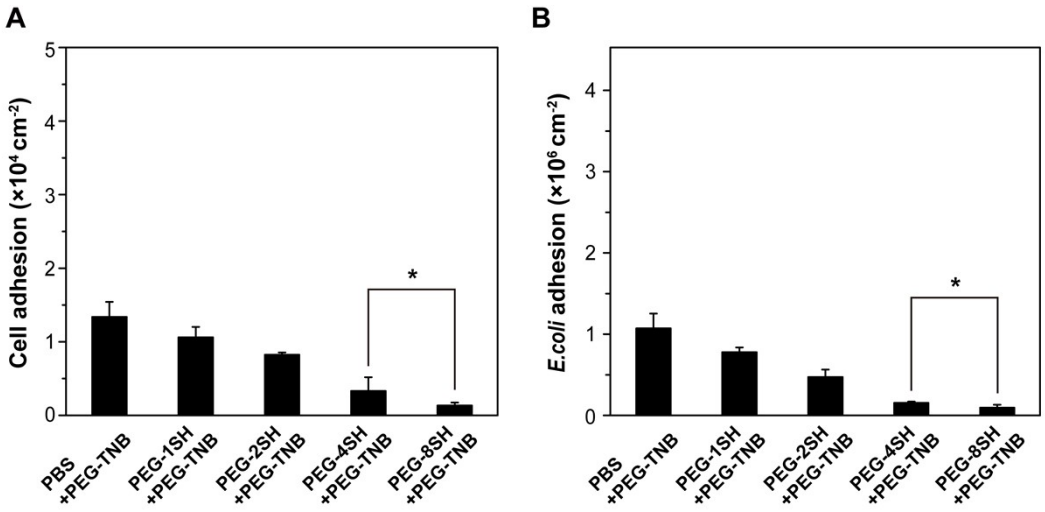

Fig. S14 Quantitative densities of adhered HeLa cells (A) and *E. coli* (B) on PEGylated substrates followed by incubation with PEG-TNB. Data represent mean ± SD (n = 5, *$p < 0.05$).