Electronic Supplementary Information

of

Photoresponsive Smart Template for Reversible Cell Micropatterning

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1. Materials and Methods

N, N-dimethylformamide (DMF), pyridine, piperidine, toluene, aether, trifluoroacetic acid (TFA), acetone, triethylamine, 98 % (v/v) sulfuric acid and 30 % (w/v) hydrogen peroxide were purchased from Shanghai Reagent Chemical Co. (PR China). DMF, pyridine, toluene and acetone were purified by distillation. 3-acryloxypropyl trichlorosilane, poly(ethylene glycol) diacrylate (PEG-DA; MW 575), 2,2'-dimethoxy-2-phenylacetophenone (DMPA), 4-aminoazobenzene, 5-aminofluorescein and succininc anhydride were obtained from Shanghai Reagent Chemical Co. (PR China). 3A-amino-3A-deoxy-(2AS,3AS)-α-cyclodextrin hydrate (NH_2-*α*-CD) was purchased from Tokyo Chemical Industries (TCI). *N*-Fluorenyl-9-methoxycarbonyl (Fmoc) protected L-amino acids (Fmoc-Arg(Pbf)-OH, Fmoc-Gly-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Ser(tBu)-OH), 2-chlorotrityl chloride resin (100–200 mesh, loading: 0.4 mmol g^{-1}, 1 % DVB) and *o*-benzotriazole-*N*,*N*,*N*',*N*'-tetramethyluroniumhexafluorophosphate (HBTU) were purchased from GL Biochem Ltd. (Shanghai, China) and used as received. Molecular probe (Hoechst 33258) was purchased from Invitrogen (CA, USA). Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), penicillin streptomycin and Dubelcco's phosphate buffered saline (PBS) were purchased from Invitrogen Corp.

2. Synthesis of azobenzene-GRGDS and azobenzene-GDSRG

Preparation of azobenzene-COOH

4-Aminoazobene (1.97 g, 10 mmol) and succinic anhydride (1.20 g, 12 mmol) were dissolved into 25 ml distilled acetone. 0.79 g (10 mmol) anhydrous pyridine was added into the solution and the mixture was stirred for 6 h at 60 ^{o}C. The obtained suspension was filtered and after been dried at 50 ^{o}C for 48 h under vacuum drying, 2.85 g azobenzene-COOH (azo-COOH) was obtained. (yield: 97 %). Fig. S1 shows the ^{1}H NMR spectrum of azo-COOH.

Preparation of azobenzene-GRGDS and azobenzene-GDSRG

GRGDS peptide was manually synthesized in 0.6 mmol scale on the 2-chlorotrityl chloride resin, employing a standard Fmoc chemistry by solid phase peptide synthesis (SPPS) method. The coupling of the first residue used 4 equiv of Fmoc-protected amino acid (Fmoc-Ser(tBu)-OH) relative to resin substitution degree with 6 equiv of DIEA in a DMF solution. Other amino acid couplings and azo-COOH were carried out with 4 equiv of Fmoc-protecting amino acid or azo-COOH, 6 equiv of DIEA and 4 equiv of HBTU for 4 h. During the synthesis, the Fmoc protecting groups were deprotected with 20 % (v/v) piperidine/ DMF twice. The cleavage of peptide was performed in a mixture of deionized water, TIS and TFA in the ratio of 2.5:2.5:95. After 2 h stirring at room temperature, the mixture was collected. The excess TFA was removed by rotary evaporation and the remaining viscous peptide solution was precipitated in the cold ether. The resulting orange product was collected and vacuum dried. The preparation of azo-GDSRG was the same as azo-GRGDS. The molecular weight of azo-GRGDS ([M^{+}]) measured by ESI-MS was 768.5 (theoretical value: 768.3). Fig. S2 shows ESI-MS spectrum of azo-GRGDS.

3. Pretreatment of quartz substrates

The quartz substrates were cleaned prior to alkanesilane assembly. The substrates were immersed for 2 h at 80 ^{o}C in “piranha” solution consisting of 3:7 ratio of aqueous solutions of 30 % (w/v) hydrogen peroxide and 98 % (v/v) sulfuric acid. (*Caution: this “piranha”*

solution has causticity and reacts violently with organic materials, so it needs to be handled with extreme care). After removal from "piranha" solution, quartz substrates were rinsed with deionized (DI) water and dried under nitrogen, then stored at room temperature.

4. Synthesis of acrylate-terminated silane SAMs (See Scheme S1)

3-acryloxypropyl trichlorosilane (0.06 mmol) was dissolved in 30 ml anhydrous toluene. The pretreated quartz substrates were immersed into the silane solution for 12 h. The reaction was conducted under a nitrogen atmosphere at room temperature. After removal from the silane solution, the substrates were gently rinsed in anhydrous toluene and were blown dry in a nitrogen stream.

5. Fabrication of PEG hydrogel micropatterned substrates (Photolithography)

PEG hydrogel patterns were fabricated from the precursor solution of PEG-DA (MW 575) with 1% (w/v) photoinitiator (DMPA). This solution was spun at 1000 rpm for 6 s onto the silane-treated substrates containing terminal acrylate group using a spin-coater. The uniform layer of the PEG-DA precursor solution on a substrate was then exposed through a photomask (the desired patterns were prepared using Photoshop and printed onto transparencies using a standard laser jet printer (LaserWriter16/600PS). The exposure times ranged from1 to 2 s. The regions of PEG-DA exposed to UV light underwent free-radical polymerization and became cross-linked, while unexposed regions were dissolved in DI water after 5min of development. The micropatterns were measured with a scanning electron microscope (SEM).

6. Fabrication of fluorescein terminated SAMs and micopattered fluorescein substrates (Compound 2)

5-aminofluorescein (10 mg) was dissolved in 10 ml anhydrous DMF. The silane treated substrates and micropatterned PEG hydrogel substrates were immersed into this solution for 2 h at room temperature and 4 μl triethylamine was added as the catalyst. This Michael Addition Reaction was conducted under a nitrogen atmosphere. After removal from the solution, the substrates were gently rinsed in anhydrous DMF and were blown dry in a nitrogen stream respectively. These two different substrates were visualized by a confocal laser scanning microscope (C1-Si, Nikon, Japan).

7. Fabrication of micropatterned α-CD/azo-peptide SAMs

Preparation of micropatterned α-CD SAMs

30 mg NH_2-α-CD was dissolved in 15 ml anhydrous DMF. The micropatterned PEG hydrogel substrates were immersed into this 2 mg/ml solution with 6 μl triethylamine for 2 h at room temperature. The Michael Addition Reaction was conducted under a nitrogen atmosphere. After that, the substrates were gently rinsed in anhydrous DMF and were blown dry in a nitrogen stream. Then the micropatterned α-CD SAMs were obtained.

Preparation of micropattered α-CD/azo-peptide SAMs (Compound 1)

15 mg azo-GRGDS (azo-GDSRG) was dissolved in 1.5 ml CH_3OH and this solution was dropped into 13.5 ml DI water. The micropatterned α-CD SAMs were put into this mixture and shaked gently for 2 h at room temperature. After assembly, the substrates were rinsed with DI water scrupulously and dried with a nitrogen stream. The micropatterned α-CD/azo-peptide SAMs were obtained.

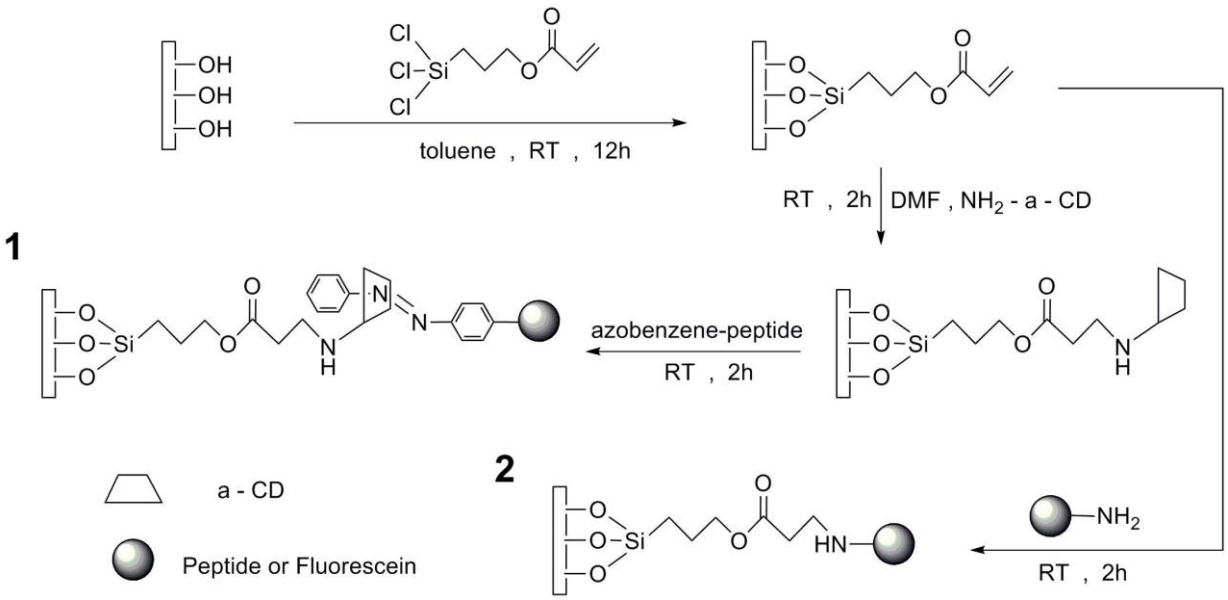
8. Evaluation of cell adhesion and proliferation on different surfaces

Human cervix carcinoma (HeLa) cells were cultured in DMEM medium with 10 % FBS and 1 % antibiotics (penicillin-streptomycin, 10000 U/mL) at 37 °C in a humidified atmosphere containing 5 % CO2. Before seeding, all of substrates (α-CD/azo-GRGDS, fixed-

GRGDS and clean quartz substrate) were sterilized with high temperature. 1 ml DMEM culture medium and 150 ul DMEM medium containing 2×10^5 cells/ml was added to each substrate and this culture was maintained for 24 h. Then clean quartz substrates, α-CD/azo-GRGDS SAMs and fixed-GRGDS SAMs were irradiated with UV light (365 nm, 15 W) for 10 min, the old medium was removed and new DMEM medium was added. These samples were incubated at 37 oC, 5 % CO2 for 1 h. The UV irradiation process was repeated three times and then the cells were counted and recorded.

9. Cell micropatterning

Human cervix carcinoma (HeLa) cells were cultured in DMEM medium with 10 % FBS and 1 % antibiotics (penicillin-streptomycin, 10000 U/mL) at 37 °C in a humidified atmosphere containing 5 % CO_2. 1 ml DMEM culture medium and 150 ul DMEM medium containing 2×10^5 cells/ml was added to each substrate and this culture was maintained for 12 h. Then clean quartz substrates and α-CD/azo-GRGDS SAMs were irradiated with UV light (365 nm, 15 W) for 10 min, the old medium was removed and new DMEM medium was added. These samples were incubated at 37 ^oC, 5 % CO_2 for 1 h. The UV irradiation process was repeated three times. The nucleus of HeLa cells was stained with 20 ul (2 ug/ul) of Hoechst 33258 for 15 min at 37 ^oC, after which the cells were further washed with PBS three times and incubated with 200 ul DMEM. The cells were observed with a scanning electron microscope (SEM), phase contrast microscope and a confocal laser scanning microscope (CLSM, C1-Si, Nikon, Japan) equipped with a 405 nm diode for Hoechst 33258.



Scheme S1 Preparation of SAMs terminated with (1) α-CD/azo-peptide and (2) peptide (or fluorescein).

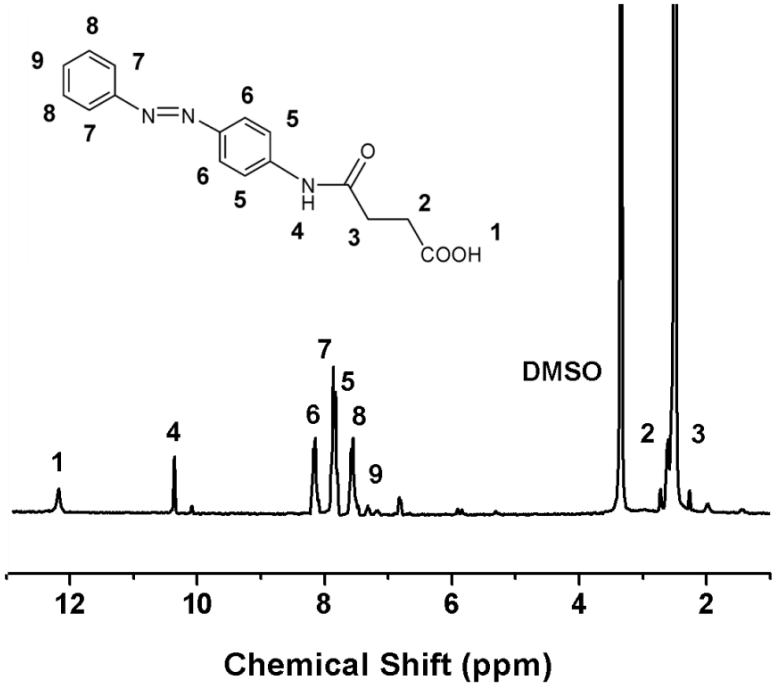


Fig. S1 ^{1}H NMR spectrum of azo-COOH.

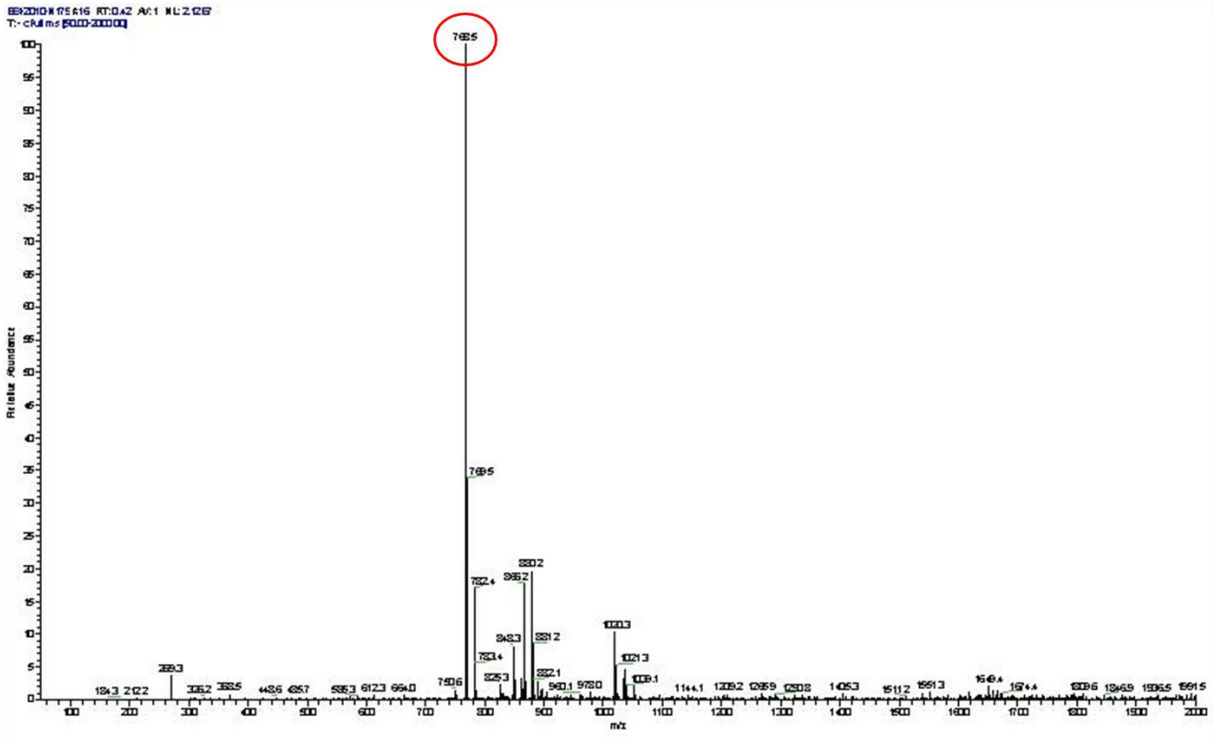


Fig. S2 ESI-MS spectrum of azo-GRGDS.

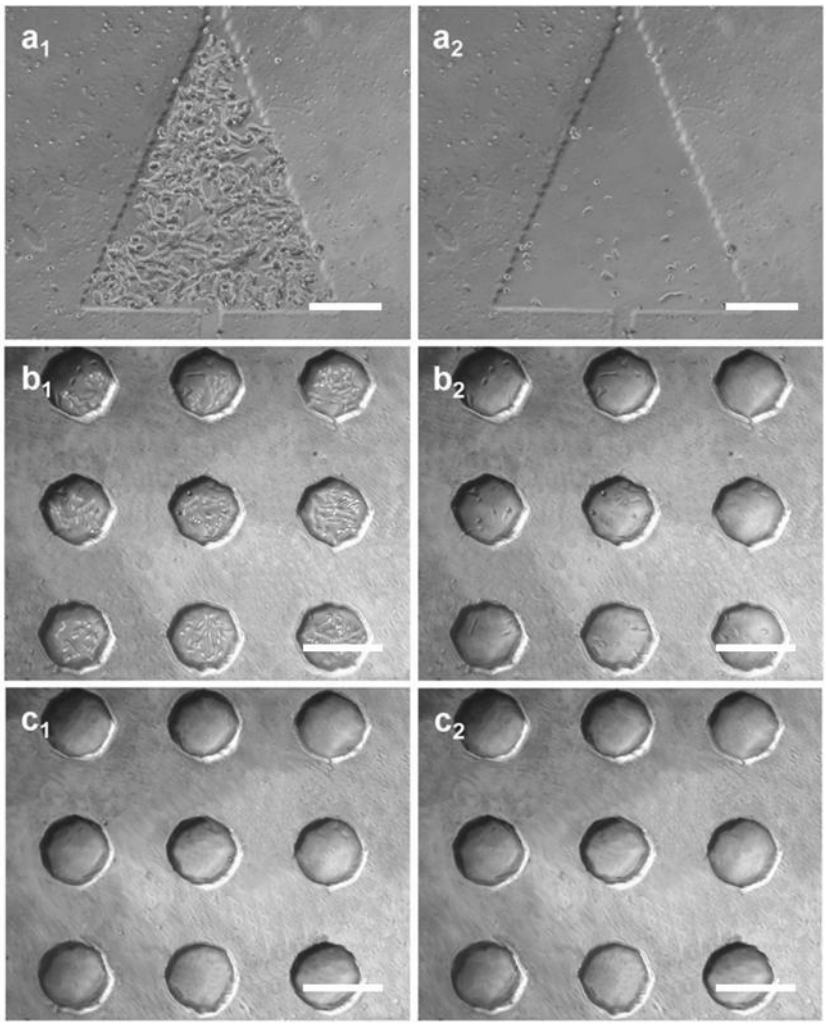


Fig. S3 Phase contrast micrographs of Hela cells on the different substrates: (a_1) α-CD/azo-GRGDS (first pattern), (a_2) α-CD/azo-GRGDS (first pattern, UV), (b_1) α-CD/azo-GRGDS (second pattern), (b_2) α-CD/azo-GRGDS (second pattern, UV), (c_1) α-CD/azo-GDSRG (second pattern), (c_2) α-CD/azo-GDSRG (second pattern, UV). Scale bar: 200um.