Supporting Information

A facile method to *in situ* fabricate three dimensional gold nanoparticles micropatterns in a cell-resistant hydrogel

Ming-Hao Yao, Jie Yang, Dong-Hui Zhao, Rui-Xue Xia, Rui-Mei Jin, Yuan-Di Zhao, and Bo Liu**

Britton Chance Center for Biomedical Photonics at Wuhan National Laboratory for Optoelectronics - Hubei Bioinformatics & Molecular Imaging Key Laboratory, Department of Biomedical Engineering, College of Life Science and Technology, Huazhong University of Science and Technology, Hubei, Wuhan 430074, P. R. China

Experiments section

Materials

Restriction endonuclease SpeI, NheI, and T4 DNA ligase were purchased from New England Biolabs Inc. (Beijing, China). Tris(2-carboxyethyl)phosphine (TCEP), β–mercaptoethanol, isopropyl-b-D-thiogalactoside (IPTG), ampicillin and kanamycin were purchased from Sigma-Aldrich, Inc. (St. Louis, MO). Ni-NTA separation column was obtained from Qiagen China (Shanghai) Co., Ltd. UV radiometer (peak λ = 365 nm, 500 W, 300 W) was purchased from Beijing Changtuo Technology Co. Ltd. All other reagents were purchased from Sigma-Aldrich, Inc. (St. Louis, MO) unless otherwise specified.

Synthesis of polypeptide

The segment encoding NcysBRGD and containing NheI and SpeI restriction sites was synthesized by the method of polymerase chain reaction (PCR). The NcysBRGD segment and the PQE9 plasmid (containing NheI and SpeI restriction sites) were digested by NheI and SpeI to yield cohesive ends. Digested NcysBRGD and PQE9 were ligated with T4 DNA ligase to construct PQE9 NcysBRGD plasmid. The DNA sequences of PQE9NcysBRGD were verified at the DNA sequencing core facility of Sunny Institute at Shanghai. PQE9NcysBRGD plasmid was transformed into *E. coli* strain M15. Bacterial culture was grown at 37 ^{o}C in 1 L of 2xYT media supplemented with 25 mg L^{-1} of kanamycin and 50 mg L^{-1} of ampicillin. The culture was induced with 1 mM isopropyl-*β*-D-thiogalactoside (IPTG) when the optical density at 600 nm reached 0.6-1.0. The culture was continued for an additional 4.5 h. Cells were harvested by centrifugation (6,000 g, 30 min) and lysed in 8 M urea (pH = 8.0). The cell lysate was centrifuged at 12,000 g for 1 h, and the supernatant was collected for purification. A 6× histidine tag encoded in pQE9 vector allows the polypeptides to be purified by affinity chromatography on a Ni-NTA resin following the denaturing protocol provided by Qiagen. The eluted fractions were dialyzed against sterile water for 3 days at room temperature, frozen at -80 ^{o}C overnight, and lyophilized for 4 days. The purified polypeptide was characterized using 12% SDS-PAGE and MALDI mass spectrometry. For mass spectral characterization, the sample was prepared by adding 4 μL of MALDI matrix 3,5-dimethoxy-4-hydroxycinnamic acid (10 mg mL^{-1} in 50% acetonitrile and 0.1% trifluoroacetic acid) to 1 μL of polypeptide solution and analyzed on a Bruker Reflex III reflectron MALDI-TOF mass spectrometer.

Preparation of cell-resistant PEG hydrogel substrate and other samples

The cell-resistant PEG hydrogel substrate was fabricated by photopolymerization of 20% $PEGDA_{2k}$ under UV light

(365 nm, 500 W) condition for 1 minute at a distance of 5 cm. To prepare the unpatterned GNPs hydrogel, the PEG hydrogel was immersed into 1% 2959 solution containing 2.4 mM $HAuCl_4.4H_2O$ for several minutes to allow Au^{3+} seeping into hydrogel and exposed to UV light for 1 minute and 20 seconds at a distance of 5 cm. During the second exposure, photomask with different micropattern was used for synthesis GNPs micropatterned hydrogel.

To promote cell behaviors, GNPs micropatterns were further modified with a polypeptide NcysBRGD containing a cell-binding tripeptide RGD. In brief, the GNPs micropatterns hydrogel was processed with 24 mM NcysBRGD solution (TCEP used to break the disulfide bond before using) at room temperature for 1 hour, and the unreacted NcysBRGD was removed by PBS (0.01 M pH = 7.2) for another one hour (refreshing the PBS every 10 min). The control test PEG hydrogel modified with NcysBRGD solution was prepared likewise.

UV-vis absorption spectrum, TEM and SEM characterization of samples

The UV-vis absorption spectrum of the samples was measured by an UV-vis spectrophotometer (UV-2550, Shimadzu, Japan). The electron images of samples were obtained by TEM microscopy (FEI Tecnai G2 20, USA). The morphologys of the samples were observed by a SEM (Nova NanoSEM 450). For SEM analysis, carbon element was used to spray on samples for increasing the electrical conductivity of samples.

2D cell experiments of samples

Before cell experiments, the samples, including PEG hydrogel, unpatterned GNPs hydrogel, PEG hydrogel (modified with NcysBRGD), unpatterned GNPs hydrogel (modified with NcysBRGD), and micropatterned GNPs hydrogel (modified with NcysBRGD) were sterilized by exposed to UV light for 20 minutes. The HeLa cells were seeded onto the surface of hydrogel at a density of 2.5×10^5 cells cm^{-2} and allowed to adhere for 2 h at 37 ^oC, 5% CO_2 in cell incubator. The culture media DMEM was refreshed, and the sample was stained with the calcein AM/ethidium homodimer for 20 min and examined with a 10× objective on an inverted fluorescent microscope (Olympus IX71, Japan) equipped with a cool color charge-coupled device (CCD) (Pixera Penguin 150CL, USA).

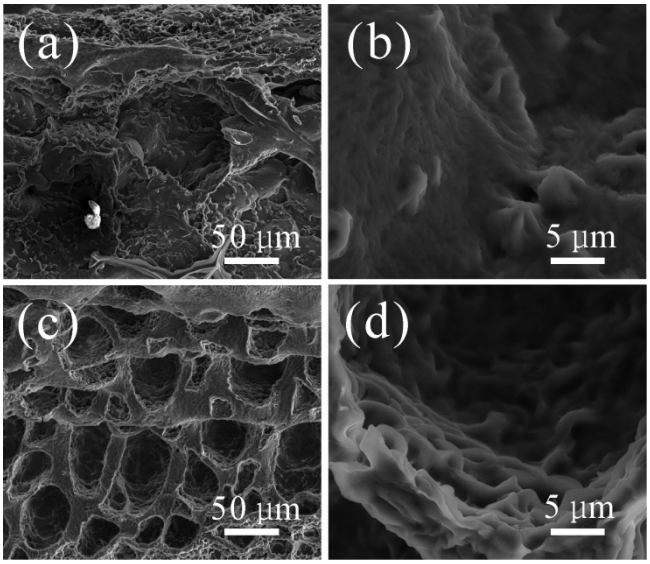

Fig. S1 SEM image of GNPs hydrogels: (a) 10% (w/v) PEGDA and 1.2 mM Au^{3+}, (b) amplification of a, (c) 20% (w/v) PEGDA and 2.4 mM Au^{3+}, (d) amplification of c.