

Supporting information

A Light-Induced Hydrogel Responsive Platform to Capture and Selective Isolate Single Circulating Tumor Cell

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Experiment section

Materials

Streptavidin (SA) was obtained from Invitrogen. Glutaraldehyde (GA), acetone, 3-aminopropyltriethoxysilane (APTES), carboxyl ethyl silanetriol sodium salt, 2-(4-morpholino)-ethane sulfonic acid (MES), 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS) were purchased from Aladdin. Phycoerythrin (PE)-labeled anti-cytokeratin (PE-CK) and fluorescein isothiocyanate (FITC)-labeled anti-CD45 (FITC-CD45) were purchased from BD Biosciences. matrix metalloproteinase-9 (MMP-9), paraformaldehyde (PFA, 36% in water), Triton X-100, 4,6-diamidino-2-phenylindole dihydrochloride (DAPI), fluorescein diacetate (FDA), and lymphocyte separation medium were purchased from Sigma-Aldrich. CCK8 was purchased from Solarbio, gelatin methacryloyl (GelMA), lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP), and chondroitin Sulfate methacryloyl (CSMA) were purchased from StemEasy. The biotinylated anti-human EpCAM antibody was obtained from R&D systems. Phosphate buffer saline (PBS) was obtained from Biosharp.

Fabrication and modification of Gnp substrate

The preparation method of Gnps was as reported previously.^{1, 2} Firstly, we used 200 μL MES solution (4 mg/mL EDC and 6 mg/ mL NHS in 0.1 M MES solution) to cover the Gnps for 1 h. A glass (1×1 cm) was first immersed in 5% APTES in anhydrous ethanol for 1 h, and we used ethanol and PBS to wash three times. Then glass was reacted with functionalized Gnps for 1 h to get Gnp substrate. We used 100 μL streptavidin (50 $\mu\text{g}/\text{mL}$) to immerse Gnp substrate at 4 °C for 10 h. The Gnp substrate was washed three times with PBS and incubated with 20 $\mu\text{g}/\text{mL}$ anti-EpCAM antibody for 2 h. The Gnp substrate has washed three times again before cell capture experiments.

Biocompatibility tests of Gnp substrate

Hemolysis test with Gnp substrate. The red blood cells were collected, centrifuged at 1500 rpm for 15 min, and washed with PBS for 3 times. We mixed the centrifuged red

blood cells into PBS, and the red blood cells ended up with a red blood cell concentration level of about 2%. 1 mL red blood cell blood was immersed in flat glass, Gnp substrate, and incubated at 37°C for 2 h. 100 µL of the red blood cell solution was taken to a 96-well plate, centrifuged at 1500 rpm for 15 min, and the hemolysis percentage of the supernatant was determined by enzyme standard instrument analysis under the absorbance of 540 nm. The negative control was PBS and the positive control was deionized water (DI water).

To test Gnp substrate would be have toxic to the captured cells. First, Gnp substrate was placed in 12-well plates, and then 5×10^4 fresh MCF-7 cells were inoculated on Gnp substrate. Then, 2 mL of DMEM medium was added and beaten evenly. The well plates containing Gnp substrate were incubated at 37°C and 5% CO₂ for 3 days, and the Gnp substrate was stained with 50 µg/mL FDA/PI solution, and the activity of cells was observed under a fluorescence microscope.

Preparation cell samples

The human breast cancer cell line MCF-7, HepG2, Hela, healthy blood samples, and the cancer patient's blood were obtained from Zhongnan Hospital, Wuhan University. MCF-7, Hepg2, and Hela cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with fetal bovine serum (FBS) (10% v/v) and penicillin–streptomycin (1% v/v) in the conditions of 5% CO₂ and 37 °C.

Capture performance of Gnp substrate

Characterization of captured cell on flat Glass and Gnp substrate. The antibodies were modified on flat glass and Gnp substrate, and MCF-7 cell suspension droplets were added to the substrate. After incubation at room temperature for 1 h, the substrate was gently rinsed with PBS. Then the sample of the cell-captured substrate was observed by SEM. Before observation, the captured cells on the substrates were fixed with 2.5% glutaraldehyde. Cells were then postfixed in 1% osmium tetroxide for 1 h, and 1% tannic acid was used as a mordant. Then the samples were dehydrated through a series of alcohol concentrations (15%, 30%, 50%, 70%, 80%, 90%, 100%) followed by drying with supercritical carbon dioxide.

Efficiency evaluation with the different time. To optimize capture time, we used 1×10^4 FDA stained MCF-7 cells, which positively expressed EpCAM, and loaded on the Gnp substrate for different times (15, 30, 60, 90, and 120 min). And then, the chip was gently washed with PBS to remove no-captured cells. After the capture process, the cell-attached Gnp substrate was observed and counted under a fluorescence microscope. Finally, capture efficiency = number of cells captured / number of cells put in $\times 100\%$

Efficiency evaluation in different substrates. To verify the specific capture of substrates, we placed three different cell lines (MCF-7, HepG2, and HeLa cells) on various substrates (flat glass, Gnp substrate, anti-EpCAM modified Gnp substrate, and anti-EpCAM modified flat glass). The 10^5 cell were stained by FDA, and the stained cells were co-incubated in different substrates for 1 h. Then the number of cells was calculated under a fluorescence microscope, and the capture efficiency of different substrates for different cell lines was obtained by using the above formula. And then, the MCF-7 cell-attached different substrates were observed under a fluorescence microscope.

Efficiency evaluation in PBMC and whole blood. MCF-7 cells were first stained with FDA to facilitate the following counting process, and a certain number of MCF-7 (25, 50, 100, 150, and 200) spiked into the prepared PBMCs, or whole blood samples to make 100 μL cell suspensions. The as-prepared artificial CTC samples were loaded onto the Gnp substrate for 1 h of incubation time, after which the chip was gently washed with PBS. After the capture process, the cell-attached Gnp substrate was observed and counted under a fluorescence microscope.

Drug sensitivity test

The 5×10^4 cells captured by the Gnp substrate were immersed in a cisplatin solution for drug susceptibility tests. Cisplatin was mixed with DMEM medium to the desired concentration to prepare different drugs concentrations (0 μM , 5 μM , 50 μM and 500 μM) for co-culture with cells. At least 1 day in a wet incubator at 37 $^\circ\text{C}$ and 5% CO_2 . After incubation, the medium was removed and use the PBS to wash, the cells were

stained with FDA/PI, and the treated cells were observed under a fluorescence microscope.

CCK8 test of CSMA and LAP

Using CCK8 to determinate the cytotoxicity of CSMA and LAP to cancer cell. Firstly, 100 μ L cell suspension was inoculated with 96-well plates and placed in 5% CO₂ at 37°C for 12 h. Next, adding 10 μ L of CSMA and LAP of different concentrations to the well plate, and culture for 24 h. And then adding 10 μ L CCK8 reagents to each well plate for 4 h. Finally, the optical density (OD) of the experimental group (As) with CSMA and LAP solution and control group (Ac) without CSMA and LAP were measured at 450 nm using an enzyme standard instrument analyzer. In addition, the optical density of blank groups contained DMEM and CCK8 also measured.

$$\text{Cell Activity (\%)} = (\text{As} - \text{Ab}) / (\text{Ac} - \text{Ab}) \times 100\%$$

Cell activity test of 405 nm Laser

The power of the 405 nm laser was 50 mW, captured MCF-7 cells on the Gnp substrate were irradiated for different times (5 s, 10 s, 20 s, 40 s, 60 s), then the FDA/PI reagent was added to stain the cells. When the FDA stained the living cells, they would appear green under the fluorescence microscope, while the PI stained the dead cells, which would appear red under the fluorescence microscope. After staining for 10 min, the number of living and dead cells was observed under a fluorescence microscope, and the cell activity was calculated

The irradiation time of 405 nm laser was 30 s, captured MCF-7 cells on the Gnp substrate were irradiated with different laser power (12.5 mW, 25 mW, 50 mW, 75 mW, and 100 mW), and then the cells were stained with FDA/PI reagent. After staining for 10 min, the number of living and dead cells was observed under a fluorescence microscope, and the cell activity was calculated. To further prove that the 405 nm laser had no effect on the cells' activity, the cells captured on the Gnp substrate were exposed to 405 nm laser radiation. We then re-cultured and stained the cells with FDA/PI, and observed the proliferation of the cells for three days.

Construction of the light-irradiation system

A schematic diagram of the light-irradiation system developed in this study is shown in Figure S2. The developed system contained the following basic components: 405 nm laser (Fig. 7a-A), plano-convex lens with a focal length of 50 mm (Fig. 7a-B), Small aperture aperture (Fig. 7a-C), plano-convex lens with focal length of 25.4 mm (Fig. 7a-D), focused plane-convex lens with a focal length of 500 mm (Fig. 7a-E), reflecting plane mirror (Fig. 7a-F,G), the eyepiece of the microscope (Fig. 7a-H), microscope stage (Fig. 7a-I).

The red line represents the laser transmission process (Fig. 7b). The incident light from the 405 nm laser (A) passes through the plane convex lens (B) with a focal length of 50 mm to the small aperture (C). Then the light passed through the planar convex lens (D) with a focal length of 25.4 mm to achieve the contraction of the 4 mm incident light, and then through the focusing lens (E) with a focal length of 500 mm to focus, and then the focused beam is incident to the sample surface (I) through the guidance of the mirror (F-G). All lenses used in this proposed system were selected using Optic Studio optical simulation software (Zemax, Kirkland, WA).

The system of photocurable hydrogels selectively encapsulate cell

The cells stained with FDA were captured on the Gnp substrate. Next, 0.1 mg/mL of MMP-9 was immersion Gnp substrate at room temperature for 30 min. Then, gently rinse the Gnp substrate with PBS. The Gnp substrate before and after MMP-9 enzyme treatment were observed under a fluorescence microscope.

The cells stained with FDA were captured on the Gnp substrate, and then the Gnp substrate was covered with a layer of CSMA and LAP mixed solution, the concertation of CSMA and LAP was 5% and 0.5%, and then curing the selected cell by using the 405 nm laser (25 mW, 15 s) under a microscope. Next, 0.1 mg/mL of MMP - 9 was used to dissolve in suit the Gnp substrate to remove uncured cells. The Gnp substrate before and after MMP-9 enzyme treatment were observed under a fluorescence microscope.

Single CTC isolation using clinical samples

First, the patients' blood samples were treated with lymphocyte separation solution, and PBMC cells were extracted and cleaned for later use. Lymphocyte separation

fluid was used to separate human blood. First, mix 1 mL of fresh blood with 1 mL of PBS. Then, remove 2 mL of lymphocyte separation solution in 15 mL centrifuge tube, and slowly add the diluted 2 mL of blood sample into 15 mL centrifuge tube containing lymphocyte separation solution. Place the centrifuge tube in a horizontal centrifuge at 1500 rpm and centrifuge for 30 min. After the centrifugation, the supernatant on the peripheral blood mononuclear cells (PBMCs) were discarded, and the peripheral blood mononuclear cells (PBMCs) were removed for centrifugation and washing for subsequent use. Next, PBMC cells were dropped onto the Gnp substrate modified antibody for static culture for 1h. And then we used PBS to remove uncaptured cells by gently rinsing. Then the captured cells were identified by trichromatic fluorescence. Next, cells were fixed with 2% paraformaldehyde (10 min), permeabilized with 0.3% Triton-X 100 (10 min), and stained with 10 µg/mL DAPI (10 min). FITC-CD45 and PE-CK were used to stain CTCs and leukocyte for 2 h at 4 °C. After washing, the captured cells were observed by fluorescent microscopy, and choose CTCs expressed DAPI+, CK+, CD45- to cure. Next, the Gnp substrate was covered with a layer of CSMA and LAP mixed solution, and then we used a 405 nm laser (25 mW, 15 s) through light-irradiation system to the location of selected CTCs. Next, 0.1 mg/mL of MMP-9 was used to dissolve the Gnp substrate for 30 min to remove uncured cell. Finally, we skillfully operate microcapillary to gently extract hydrogel-encapsulated cells for gene analysis.

CTC-Based gene analysis

The released CTC was collected, DNAs were extracted and the genome was amplified. Next, the TP53 gene was amplified by polymerase chain reaction (PCR) using the following primers. Finally, we sent the gene samples to qingke biotech for sequencing. The following primers were used for TP53 gene PCR amplification to detect TP53 mutations

TP53-2-3-4-Forward: 5'-GGAAGCCGAGCTGTCTCAGACA-3'

TP53-2-3-4-Reverse: 5'-GGGGATACGGCCAGGCAT-3'

TP53-7-Forward: 5'-GAGGCTGAGGAAGGAGAATGG-3'

TP53-7-Reverse: 5'-GCCCAGGGGTCAGAGGCA-3'

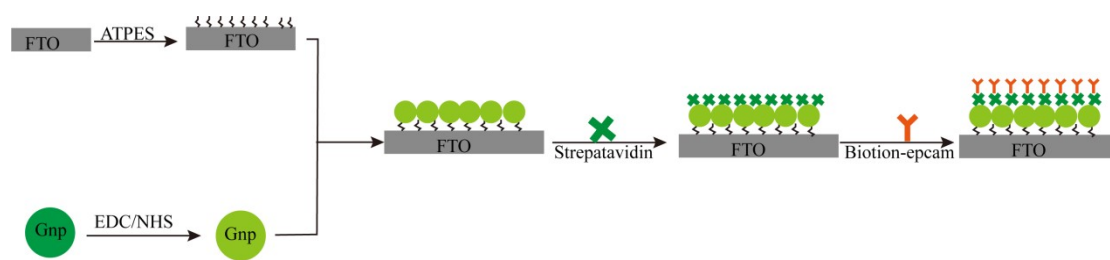


Fig. S1. Schematic diagram of surface modification of the Gnp substrate.

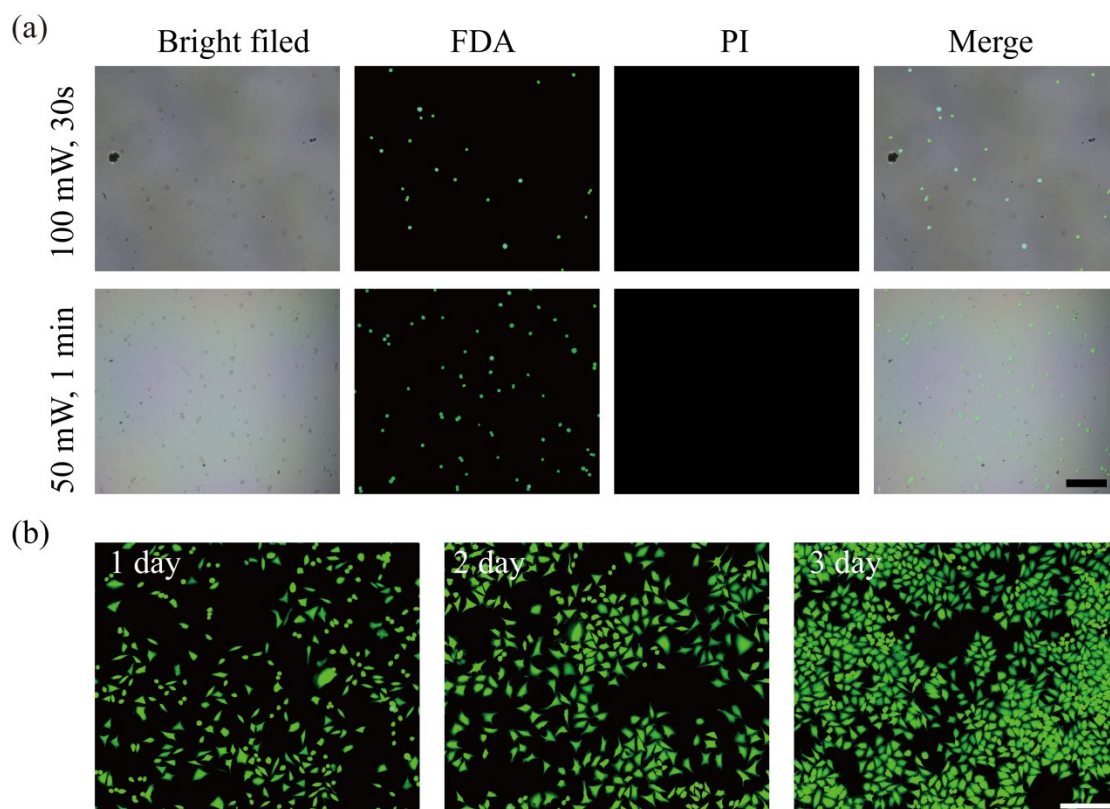


Fig. S2. Effect of 405 nm laser on cell activity. (c) FDA/PI staining images of captured cells after 405 nm laser irradiation. Scale bar=100 μ m. (d) Fluorescent image of 405 nm laser-irradiated MCF-7 cells after cultured for 3 days. Scale bar=100 μ m.

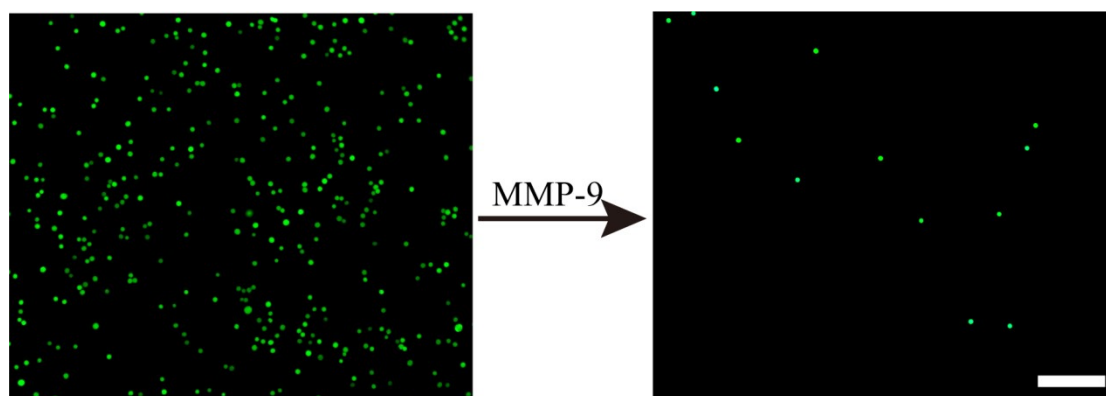


Fig. S3. Fluorescent images of capture and released cell treated with MMP-9. Scale bar=100 μm

Table S1. The information of patient.

Cancer Type	Age	Gender	Stage	Volume Sample/mL	CTCs
Liver	58	Male	T2 N2 M0 IIIA	2	13

Reference

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2. B. Cai, L. Rao, X. Ji, L. L. Bu, Z. He, D. Wan, Y. Yang, W. Liu, S. Guo and X. Z. Zhao, *J Biomed Mater Res A*, 2016, **104**, 2854-2860.