Supporting Information

Plasmonic Surfaces for Cell Growth and Retrieval Triggered by Near-Infrared Light

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Experimental section.

Materials. Hexadecyltrimethylammonium bromide (CTAB, >96%), hydrogen tetrachloroaurate trihydrate (HAuCl₄·3H₂O, >99.9%), L-ascorbic acid, (>99%), Triton X-100 (laboratory grade), Tween 20, sodium chloride (>99%), sodium phosphate (>99%), Dimethylsulfoxide (>99.9%) and ammonium hydroxide solution (30% w/w) were purchased from Aldrich. Hydrogen peroxide solution (35% w/w) and Toluene were purchased from Scharlau. Poly(styrene-b-2-vinyl pyridine) (PS₁₀₅₀-P2VP₄₉₅) was purchased from Polymer Source, Inc. DMEM, FBS, PS, trypsin-EDTA, Dapi, and WGA-AF647 were purchased from Invitrogen. All chemicals were used as received. Milli-Q water (resistivity 18.2MΩ cm at 25 ºC) was used in all experiments. Picodent twissil® (Picodent, Wipperfürth, Germany) was used to glue the eppendorf tubes to the plasmonic substrates. HeLa cells and 3T3 NIH fibroblasts were kindly given by Charles Lawrie and Ander Izeta, respectively (Biodonostia, Donostia-San Sebastián, Spain). A549, HUVEC, and J774 cells were kindly given by Sergio Moya, Marco Marradi, and Juan Mareque, respectively (CIC biomaGUNE).

Methods. All glassware was washed with aqua regia, rinsed with water, sonicated three times for 3 min with Milli-Q water, and dried before use. Oxygen plasma treatment was performed in a Diener plasma chamber (Diener, Ebhausen, Germany). Optical extinction spectra were recorded using an Agilent 8453 UV-vis diode-array spectrophotometer and a Cary 5000 UV-vis-NIR spectrophotometer (Varian, Inc). SEM images were obtained using an ESEM Quanta250 FEG (FEI, The Netherlands). Sergey Novikov is acknowledged for SEM imaging. XPS experiments were performed in a SPECS Sage HR 100 spectrometer with a non monochromatic X ray source (Magnesium Kα line of 1253.6 eV energy and a power applied of 250 W and calibrated using the 3d5/2 line of Ag with a full width at half maximum (FWHM) of 1.1 eV. An electron flood gun was used to compensate for charging during XPS data acquisition. The selected resolution for the spectra was 15 eV of Pass Energy and 0.15 eV/step. All measurements were made in an ultra high vacuum (UHV) chamber at a pressure around 5x10⁻⁸ mbar. Static contact angle measurements were performed with a DSA100, Krüss GmbH, (Hamburg, Germany). A Cell Observer Zeiss microscope was used to record bright field and fluorescence pictures of the cells on the substrates.

Fabrication of plasmonic substrates. Glass substrates were cleaned prior to the deposition and growth of the monolayer of plasmonic nanoparticles with basic piranha solution (1:1:5 volume fraction of hydrogen peroxide solution, ammonium hydroxide solution, and water, respectively) for 2 hours at 60 ºC. (Caution: piranha solution is extremely corrosive and oxidizing, therefore great care should be taken when preparing and handling this solution). The fabrication of the plasmonic substrates is divided into two steps: 1) deposition of Au seeds onto the surface, and 2) growth of Au seeds into anisotropic nanoparticles.

Deposition of the Au seeds was based on the block copolymer micellar nanolithography (BCML) technique. Basically, a monolayer of inverse micelles of a copolymer with Au
atoms contained in the core was deposited by dip-coating. The dispersion of the inverse micelles was prepared as follows; i) a 5 mg/mL dispersion of the block copolymer was prepared in toluene and stirred for 24 hours, ii) 0.5 mol of Au atoms (as hydrogen tetrachloroaurate trihydrate) per each P2VP unit was added, and the dispersion was stirred for 24 hours, iii) the dispersion was let without stirring for 24 hours, iv) the dispersion was transferred without using a few mL from the bottom, usually appearing turbid. Note that all fabrication steps of the dispersion, as well as the storage, were performed under dark conditions. The glass substrates were dip coated (using a mechanic, home-made dip coater), with a dip speed of 26 mm/min in the BCML dispersion. Immediately after dip-coating, the substrates were treated with oxygen plasma at the following conditions: 1.47 mbar, 200W, 4 min. At this point, the substrates are covered with a monolayer of Au nanoparticles seeds with certain coverage of copolymer. Longer times of oxygen plasma treatment at this stage render Au nanoparticle seeds which cannot be further grown.

**Growth of Au seeds into anisotropic nanoparticles.** The substrates were subjected to two growth steps by immersing the substrates into an aqueous growth solution. This growth solution is composed as follows: HAuCl₄:AA:CTAB 1:12.5:50 in molar ratio. The concentration of ascorbic acid (AA) was 1 mM. The ascorbic acid was freshly prepared and immediately used. No waiting time was allocated between the two growth steps. The substrates were rinsed with Milli-Q water and gently blow-dried with a stream of nitrogen gas. A final treatment with oxygen plasma is required with the following conditions: 1.47 mbar, 200W, 50 min. The plasmonic substrates were stored in Petri dishes.

**Biofunctionalization of the plasmonic substrates with c-RGD.** The cyclic-RGD (cyclo[Arg-Gly-Asp-D-Phe-Lys(Ac-SCH₂CO)] 1) was synthesized at a purity of 95% by Peptides International. The peptide was equipped with a thioacetyl group at the lysine residue for linking to Au surfaces. Acetyl protected RGD, typically (7.12 mg) was deacetylated in an aqueous solution of 0.05 M hydroxylamine-HCl/0.03 M of ethylenediaminetetraacetic acid of pH 7.0 for two days to yield deacetylated RGD peptide 2 (94%). The reaction was monitored by 1H-NMR and proceed until the singlet signal at 2.3 ppm, assigned to CH₃CO-S (S-thioacetyl group) disappeared, see Figure S3, below. The plasmonic substrates were soaked in a solution of the c-RGD peptide with a concentration of 50 μM during 12 hours.

**Cell culture.** HeLa, A549, J774 and 3T3 cells were grown in DMEM media supplemented with 10% FBS and 1% Pen-Strep. HUVEC cells were grown in F12-K media supplemented with 10% FBS, 1% Pen-Strep, heparin (0.1 mg/ml) and endothelial cell growth factor (ECGF; 30 ug/ml). Cells were passaged using Trypsin-EDTA, except in the case of J774 cells which were passaged using pipetting. For all experiments cells were plated in a specialised holder made of a cut-off plastic eppendorf tube (0.5 or 1.5 ml volume) glued onto a glass coverslip (#1.5 thickness), with or with gold/gold-RGD covering. The cells were allowed to grow and adhere to the substrates overnight.
**Flow cytometry.** A549, HUVEC and HeLa cell monolayers were washed with PBS and Accutase (Sigma), heated to room temperature, was added to bring the cells into suspension. Accutase rather than trypsin was used to prevent loss of surface expressed \(\alpha V\beta 3\) integrin. Cells were counted, washed with 1% BSA/PBS and placed in cytometry tubes at 1x10^5 cells/tube. Cells were stained with anti-\(\alpha V\beta 3\) mouse anti-human monoclonal antibody (LM609; Milipore), diluted 1/400 in 0.1% BSA/5% donkey serum/PBS and left on ice for 30 minutes. Cells were washed twice with BSA/PBS and resuspended in donkey anti-mouse polyclonal IgG-AF647 (Abcam), diluted 1/2000 in BSA/donkey serum/PBS. Cells were left in the dark on ice for 30 minutes followed by two more BSA/PBS washes and finally resuspended in 200 ul BSA/PBS. Cells were run on a Canto II BD flow cytometer and \(\alpha V\beta 3\) expression measured in the APC channel, collecting 5000 cell events.

**Fluorescence staining.** Cells, grown on glass (with or without gold) were left in their wells and first treated with formaldehyde (2% in PBS) for 20 minutes at room temperature (RT). After washing with ice-cold PBS, cells were stained with Wheat-germ agglutinin (WGA)-Alexa Fluor 647 (1/200 in PBS) for 20 minutes at RT. Following washing with ice-cold PBS, cells were permeabilized using 0.25 % Triton X-100 (10 min, RT) and then blocked using 1 % BSA in PBST (PBS 10 mM with 0.1 % Tween-20) for 30 mins at 37C. Block was removed and without washing, anti- \(\alpha V\beta 3\) (LM609; Milipore) or anti-vinculin (SMP227; Abcam) were added, diluted in PBST. After 1 hr incubation at 37C, samples were washed with ice-cold PBS and anti-mouse IgG-AF488 added (1/500 dilution in 1% BSA in PBS). Samples were left in the dark for 1 hr at RT, followed by washing and then addition of actin stain (1/50 in PBS). After 20 mins at RT, samples were washed again and then stained with DAPI (1/600 in media) followed by extensive washing. Brightfield and fluorescence microscopy photos were taken on a Cell Observer Zeiss microscope using either a x20 (NA 0.8), x40 (NA 1.3) or x63 (NA 1.3) objective. Dapi, GFP and RFP filters were used to detect the cell nucleus, integrin/vinculin and plasma membrane staining, respectively. At least 4 images/format were taken.

**Live cellular imaging.** For experiments in which cells were subjected to laser treatment and then followed over time, no fluorescence staining was conducted but cells were simply imaged using brightfield microscopy. Cells, grown on glass coverslips were left overnight to adhere and were then imaged to obtain control pre-laser morphology and information relating to cell numbers. After applying laser treatment to cells, samples were returned to a cell culture incubator (37C, 5% CO2, humidity) and at various time points thereafter removed and photos taken using brightfield microscopy. In certain cases cells, which had become dislodged post laser treatment, were removed and replated in either a 96-well plate or a 384-well plate (the later when the number of dislodged cells was very low). Between 4-8 hours post laser treatment, cell media was replaced with a solution of live and dead stains, diluted 1/1000 in staining buffer (Live/Dead cell staining kit, Abcam). Brightfield and fluorescence microscopy photos were taken 15 mins after using filters for GFP and RFP for live and dead stains.
respectively. A Cell Observer Zeiss microscope with a x10 or x20 objectives was used to take all images. Three to five images per format were collected to gain an average.

*Cell viability MTT assay.* In order to determine whether cells were still metabolically active after laser treatment we used the MTT reagent. Cells were grown as described above; approximately 4 hrs post laser treatment dislodged cells were removed and plated in a 96-well plate. Cells which did not lift up were dislodged using trypsin-EDTA and placed in separate wells of the same 96-well plate. Control wells were also plated which comprised cells A) grown directly in the 96-well plate, and B) cells grown under the same conditions of glass/gold/gold-RGD but without any laser treatment. In all cases the initial number of cells was the same for each cell line. The following day the media of all wells was removed and 100ul MTT reagent (1/20 dilution in media) added. Cells were incubated for 2hrs at 37°C followed by removal of the media and addition of 100 ul/well DMSO. Absorbance at 550nm was read using a plate reader.

*Fibroblast culture.* 3T3 NIH fibroblasts were seeded in the same specialized holders described above. A 1.5ml eppendorf was used with a glass slide (not coverslip). 1 x 10^5 cells/well were added to each eppendorf and capped with loose Parafilm. The cells were incubated at 37 °C for 2 weeks with changing of the media every 3-4 days. After laser treatment, cells were left for 4 hrs and then, using light pipetting, a cell sheet was released from the gold surface. In order to take an image, the sheet was carefully transferred to a clean glass slide and a mosaic image (software available from Zeiss) taken using a x5 objective.
Figure S1 SEM pictures of the Au seeds deposited on the surface of glass after the short treatment with oxygen plasma. A) Scale bar is 300 nm B, C, D) Scale bar is 1 micron.

Figure S2 SEM picture of the plasmonic surface after the chemical growth step and cleaning with a long treatment of oxygen plasma. A) Scale bar is 500 nm B) Scale bar is 1 micron C) Scale bar is 3 microns D) Scale bar is 10 microns

Figure S3 XPS N 1s spectra of the plasmonic substrate before (green line) and after (red line) growth.
Figure S4 A) Chemical formula depicting the activation of the thiolated c-RGD peptide. B) NMR spectra of the acetylated c-RGD peptide. C) NMR spectra of the activated c-RGD peptide previous to functionalization of the plasmonic substrate.
**Figure S5** Fluorescence microscopy images of cells as grown on glass. A) HeLa B) A549 C) HUVEC D) J774. Staining was as follows: nuclei (blue), vinculin (green), actin (red). Scale bar is 50 μm.

**Figure S6** Cell area of A) HeLa B) A549 C) HUVEC D) J774 as grown on glass, bare plasmonic substrates and c-RGD coated plasmonic substrates. Error bars represent SD of at least 30 counted cells.
**Figure S7** Flow cytometry results for the expression of αVβ3 integrin (colored line) and background staining (filled curve). From left to right: HeLa (red), A549 (green) and HUVEC (blue).

**Figure S8** Fluorescence microscopy pictures of A) HeLa B) A549 C) HUVEC D) J774 grown on a 20 nm smooth Au film.
Figure S9  Aspect ratio of HeLa, A549, HUVEC, and J774 of cells cultured on plasmonic substrates (nanostructured) and on a 20 nm smooth Au film (smooth). Error bars represent SD of at least 30 counted cells.

Figure S10  Area per cell of HeLa, A549, HUVEC, and J774 of cells cultured on plasmonic substrates (nanostructured) and on a 20 nm smooth Au film (smooth). Error bars represent SD of at least 30 counted cells.
Figure S11 Microscopy images of HeLa cells grown on a plasmonic substrate functionalized with thiolated c-RGD before and after irradiation with NIR light in left and right, respectively. Scale bar is 200 microns.

Figure S12 Microscopy images of A549 cells grown on glass (Glass), bare plasmonic substrates (Gold) and c-RGD coated plasmonic substrates (RGD). Microscopy images were taken before and after irradiation with NIR light in left and right, respectively. Scale bar is 200 microns.
**Figure S13** Microscopy images of HUVEC cells grown on glass (Glass), bare plasmonic substrates (Gold) and c-RGD coated plasmonic substrates (RGD). Microscopy images were taken before and after irradiation with NIR light in left and right, respectively. Scale bar is 200 microns.
Figure S14 Microscopy images of J774 cells grown on glass (Glass), bare plasmonic substrates (Gold) and c-RGD coated plasmonic substrates (RGD). Microscopy images were taken before and after irradiation with NIR light in left and right, respectively. Scale bar is 200 microns.
**Figure S15** Effect of NIR laser on the temperature of culture media on a glass substrate (blue circles) and on a plasmonic substrate (red squares) as a function of continuous irradiation time. Initial temperature is 21ºC.

**Figure S16** Area per cell (left) and aspect ratio (right) of HeLa (red), A549 (green), HUVEC (blue), and J774 (dark yellow) of cells detached and re-cultured on plasmonic substrates. Control values are the morphological data of cells cultured on plasmonic substrates prior to detachment. Error bars represent SD of at least 30 counted cells.
**Figure S17** Live/dead assay conducted on A549 (A), J774 (B), Hela (C) and HUVEC (D) cells which are removed from glass (top) of gold (bottom) surfaces after laser excitation (980nm). Cells were transferred to separate optical wells and once sufficiently adhered, live (green) and dead (red) stains added.

**Figure S18** Cell detachment rates of re-planted cells upon NIR irradiation of HeLa (red), A549 (green), HUVEC (blue), and J774 (dark yellow). The cells were grown on bare plasmonic substrates. Error bars represent SD of at least 200 counted cells.
Figure S19 Cell survival rate measured with the MTT assay, after NIR-mediated detachment from plasmonic substrates. The detachment was performed on a reusability assay, in which the plasmonic substrates were subjected to two subsequent cycles of cell growth and detachment with NIR laser.