Supplementary Information

Smart surfaces for pH-controlled cell staining

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Materials.

All reagents were purchased from Sigma-Aldrich unless otherwise specified. The 4-nitrophenyl methacrylate monomer (NPMA) was purchased from Monomer and Polymer, whereas the O,O'-bis(2-aminoethyl)polyethylene Glycol (diamino-PEG), having a molecular weight (MW) of 2000 Dalton, was purchased from Rapp Polymere. All the reagents were used as received. Oligothiophene-conjugated anti-human CD4 (TF-antiCD4) antibody was kindly provided by Mediteknology srl. The chemical structure and the optical characteristics of the oligothiophene fluorophore TF were reported elsewhere.[1] Deionized water with a resistivity of ≥ 18.0 MΩ/cm was employed in the experiments. Glass supports with a roughness of few nanometers was supplied by VisionTek, UK. The Jurkat T-lymphocytic cell line was purchased from the American Type Culture Collection (ATTC TIB 152) and grown as instructed by the vendor.

Microgel Synthesis.

The PMAA microgels were synthesized according to the procedure reported in literature.[2] Briefly, the monomers methacrylic acid (MAA) and NPMA, and the N,N′-methylene bis-acrylamide crosslinker (MBA) at a molar ratio of 2:2:1 were dispersed in the reaction solution consisting of methanol, acetone and ethanol in a volume ratio of 3:2:3,3. The reaction was degassed for 20 min by bubbling nitrogen, immersed in a mineral oil bath, and warmed up to 60°C. Within 10 min after the addition of the initiator AIBN the polymerization started, as the solution became turbid. The reaction ran for 1 h and then cooled down at room temperature (RT).

The reaction mixture was centrifuged at 2000 rcf at RT and the obtained white pellet was resuspended in fresh ethanol. This step was repeated five times. The dried reaction product was suspended in a sodium hydroxide solution (1.0 M) to hydrolyze the nitrophenol groups of the microgels and thus forming a copolymer of MAA and MBA. After 5 h the resulting hydrolysis product was centrifuged at 2000 rcf for 15 min and resuspended in bidistilled water several times to remove the nitrophenol from the mixture.

The PMAA microgels were characterized by optical microscopy (BX-61, Olympus) and the average size was found to be 3-6 μm.

Glass substrate treatment.

Precleaning. Prior to silanization, the glass substrates were precleaned by sonication in acetone and then isopropylic alcohol. The glass supports were treated in oxygen plasmas generated in a RIE IONVAC inductively coupled (ICP) plasma reactor (PGF 600 RF HUTTER), to increase the hydroxyl groups content on their surfaces. The oxygen flow rate was set to 20 sccm, at power of 30 W and pressure of 30 mTorr for 3 s.

Silanization. After that, the glass surfaces were silylated with 1% (v/v) 3-triethoxysilylpropyl isocyanate (TESPI) in toluene at RT overnight, with gentle agitation of the solution on a rotator. TESPI reacted with hydroxyl groups of the glass surfaces, leaving free isocyanate terminals for further reactions with a nucleophilic group. The glass samples were removed, thoroughly rinsed with toluene and dried by nitrogen flow.

Diamino-PEG linking. Immediately after the silanization step, the diamino-PEG spacers were linked by nucleophilic attack of the amino groups on the isocyanate terminals. A solution of diamino-PEG (0.8% w/v) in THF was added to the isocyanate-functionalized glass substrates and kept with gentle agitation for 1 h to induce the formation of the ureidic bond. After this time the solution was removed, whereas the glass substrates were rinsed repeatedly with THF and dried under nitrogen.

Amide formation. Carboxylic groups of the PMAA microgels were activated by suspending the microgels in a 0.6 mM 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) aqueous solution (MES buffer 1.0 mM) at pH 4.0, and the reaction was allowed to proceed at RT for 1 h. After the incubation time, the samples were removed, rinsed carefully with distilled water and sonicated in distilled water for 10 min, in order to remove the non-covalently bonded microgels. Finally they were dried under nitrogen. The functionalized glass surfaces were stored in dry form at room temperature.

Oligothiophene-labeled antibody uptake.

TF-labeled anti-CD4 antibodies were prepared according to the modalities described in reference 3. The TF-antiCD4 antibody solutions were diluted with MES buffer (1.0 mM, pH 5.0) to a final concentration of 5.0 μg/mL and added in a beaker containing the previously treated glass substrates. The system consisting of the covalently immobilized microgel and the labeled antibodies was incubated on a rotary mixer at RT overnight. The pH of the incubating solution was set to the value of 5.0 by adding submicroliter amounts of 0.5 M NaOH and 0.5 M HCl. After the incubation time, the glass substrates, bearing the antibody-loaded microgel, were rinsed thoroughly with bidistilled water, to remove the free labeled antibodies, and finally dried under a nitrogen flow.

Oligothiophene-labeled antibody release and cellular staining experiments.

Jurkat cells, a human T lymphoblastoid cell line, were suspended in TBS-Tween (Tris buffered saline 0.1% Tween) and centrifuged at 1080 rcf for 5 min. Then the supernatant was removed from the cell pellet. After this step the pellet was resuspended completely and incubated with the blocking solution (1% BSA in TBS-Tween) for 1 h at RT. After the centrifuging step, the cell pellet was fixed in 4% formaldehyde in PEM buffer [1.0 M PIPES buffer containing MgCl2 (2.0 mM) and EGTA (5.0 mM)] for 15 min at RT. At this point, to quench autofluorescence, the pellet was resuspended in 100 mM ammonium chloride in PEM buffer, then the cells were washed two times in TBS-tween, centrifuged for 5 min at 1080 rcf, and the supernatant was removed. After this few drops of cell pellet were incubated overnight at pH 7.8 with the glass substrates bearing the antibody-loaded microgel. This basic pH allowed the release of labeled antibodies through the enlarged meshes of the microgel network and therefore the *in situ* cellular staining.

As a control experiment, a Jurkat cell suspension was incubated with the immobilized, antibody-loaded microgels at pH 5.0. After the incubation time, confocal microscopy analysis was performed for determining the localization of the labeled antibody within the sample. As observed in Figure S1, the microgels are visible as bright fluorescent spots, whereas the cells are not stained.

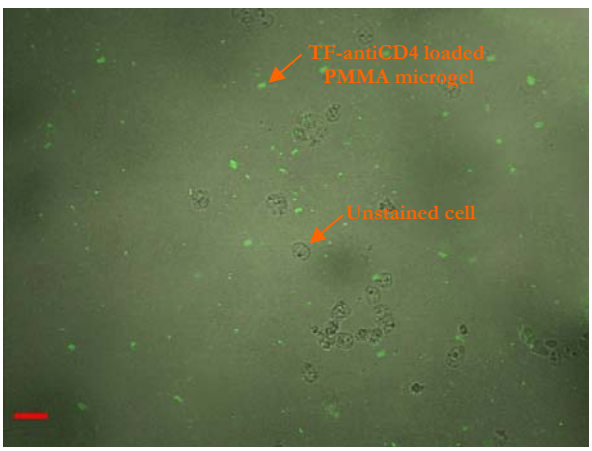

Figure S1. Confocal image of Jurkat cells after the incubation with antibody-loaded microgels at pH 5.0. The image was obtained by merging the phase contrast image and the fluorescent image.
The samples were excited with a diode laser (λ 488 nm) passed through an objective lens 40x, numerical aperture 0.85. The scale bar was 25 μm.

This is a clear evidence that the labeled antibodies were not released from the PMAA microgels, and therefore the cellular staining didn't occur at pH 5.0.

AFM characterization.

Atomic force microscopy (AFM) have been used to probe the properties of the covalently immobilized PMAA microgel as a function of environmental pH. Air and fluid Non-Contact mode AFM (NC-AFM) images were obtained at RT using a XE-100 microscope (PSIA, Korea). NSC36 silicon probes (PSIA, Korea) were employed. Imaging and analysis was performed with XEI 1.6 software.

Confocal laser scanning microscopy (CLSM) characterization.

After cellular staining with TF-antiCD4 antibody, the glass substrates were then analysed by using a confocal laser scanning microscope (FV-1000 Olympus) in epilayer configuration. The samples were excited with a diode laser (λ 488 nm) passed through an objective lens 60x, oil immersion, numerical aperture 1.4. Jurkat cells were analysed cell-by-cell and autofluorescence was excluded by performing spectral analysis of individual cells.

Notes and references

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