Material and methods

Materials and reagents

Prisms bearing a 48 nm thick gold layer above a 2 nm thick chromium layer were provided by Genoptics (Orsay, France). Chemicals were obtained from the following sources: bovine serum albumin (BSA), phosphate buffer saline (PBS) tablets, and human serum-purified IgG were from Sigma–Aldrich (St Quentin-Fallavier, France); Tween-20 is from CIS bio international (Bagnols-sur-Cèze, France); purified rat anti-mouse CD19 monoclonal antibody and purified hamster anti-mouse CD3-ε monoclonal antibody were supplied by BD Pharmingen (Le Pont-de-Claix, France); DMEM and foetal calf serum (FCS) are from Gibco (Invitrogen, Cergy Pontoise, France). LS102.9 B cell hybridoma and 13G7 T cell hybridoma were kindly provided by L. Vidard (Institut Curie, France).[1]

Cell culture and sample preparation

Cell culture. Cells were maintained in culture at 0.5 – 2.5x10^5 cells/mL in DMEM supplemented with 10% heat inactivated FCS, non essential amino acids (0.1mM/mL), sodium pyruvate (1mM/mL), 50µM 2-mercaptoethanol, 50U/mL penicillin and 50mg/mL streptomycin. Culture were routinely diluted every 2-3days.

Sample preparation. Cells were centrifuged for 5 minutes at 400 g and resuspended in DMEM supplemented with 5% heat inactivated FCS. The cell concentration was then determined and adjusted to 1x10^6 cells.mL^{-1}. Cell suspensions were stored over day at 4°C and diluted to the appropriate concentration by adding non-supplemented DMEM just before injection.

Preparation of pyrrole–protein conjugates

N-hydroxysuccinimidyl 6-(pyrrol-yl)-caproate (NHS–pyrrole) was prepared as previously described.[2] Antibodies (1.5 µM) were conjugated to NHS–pyrrole (15 µM) in phosphate-buffered saline (PBS) pH 7 overnight at room temperature. After coupling, unreacted NHS–pyrrole was removed by filtration through Vivaspin 500 µL concentrators (100-kDa cut off, Vivascience, Hannover, Germany) for 15 minutes at 15 000 g. The conjugated proteins were resuspended using the spotting buffer (50 mM phosphate buffer, pH 6.8, containing 50 mM NaCl and 10% glycerol) and the protein concentration was measured by UV absorbance (280 nm).

Biochip functionalization

Biochips are glass prisms covered with a gold layer used as a working electrode. Before sample electropolymerization, an hydrophobic mask was transferred on the metallic surface following Do Lago's procedure.[3] The mask was designed to fit the biochip surface with 12-16 unmasked regions of 700-800 µm in diameter. Masks

were first printed with a laser printer on a sticker-compatible A4 piece of paper and then heated (175°C) for 150 seconds on the prism surface for toner transfer. After cooling, the paper was gently peeled off and prisms were used for electropolymerization. Pyrrole conjugated proteins were arrayed on the biochip as described elsewhere.[2] Briefly, solutions of pyrrole-modified proteins (3 µM) were diluted with free pyrrole (20 mM) in the spotting buffer. The electro-copolymerization of free pyrrole and pyrrole-modified proteins was carried out in a pipette tip containing both the solution to be polymerized and a platinum wire used as a counter electrode, according to the process described by Guedon *et al*[4]. This tip was driven in the vicinity of the gold layer, above an unmasked region, till an electrical contact was enabled between the working (gold surface) and counter (platinum wire) electrodes. The polymerization on the prism gold layer was performed with a 100 ms electric pulse (2.0 V). Each sample was spotted at least in duplicate (pyrrole and IgG control spots) or four times on the same chip (monoclonal antibodies). The addressing of each sample on the unmasked regions was carried out with a x/y/z robot from Microcontrole (Newport Instruments). Following spotting, the surface was rinsed with PBS and prisms were stored at 4 °C in PBS.

Cell binding on the chip

SPRi detection. The reactions were carried out in a 200 µL PEEK reactor connected to a peristaltic or a syringe pump (non-supplemented DMEM, 100 µL/min) and a waste vial. After saturation of the biochip with BSA 2% (w/v), Tween-20 0.01% (w/v) in PBS for 20 minutes at room temperature, the prism was carefully washed with PBS and placed in the SPR imager (Genoptics SA, Orsay, France). Then, the reactor was filled with DMEM and plasmon curves were plotted. Cellular samples were injected through a syringe into the circuit and moved with a pump. Once the reactor is filled with the cellular sample, the flow is stopped and cells are allowed to gravitationally settle on the chip surface for several minutes. Cell–antibody interactions induced a modification in the refractive index near the gold surface, resulting in a change of the reflectivity which was monitored using a 12-bit CDD camera as a grey level contrast.[4] Images were recorded every 0.02 seconds and analyzed with a dedicated software (Genovision, Genoptics).

Optical microscopy detection. After cell binding, the prism was gently washed with DMEM and observed under an microscope (BX 60, Olympus) equipped with a Peltier cooled CCD camera (Hamamatsu). Images were recorded and analyzed with dedicated software (Imagepro Plus, Media Cybernetics, MD, USA).

References

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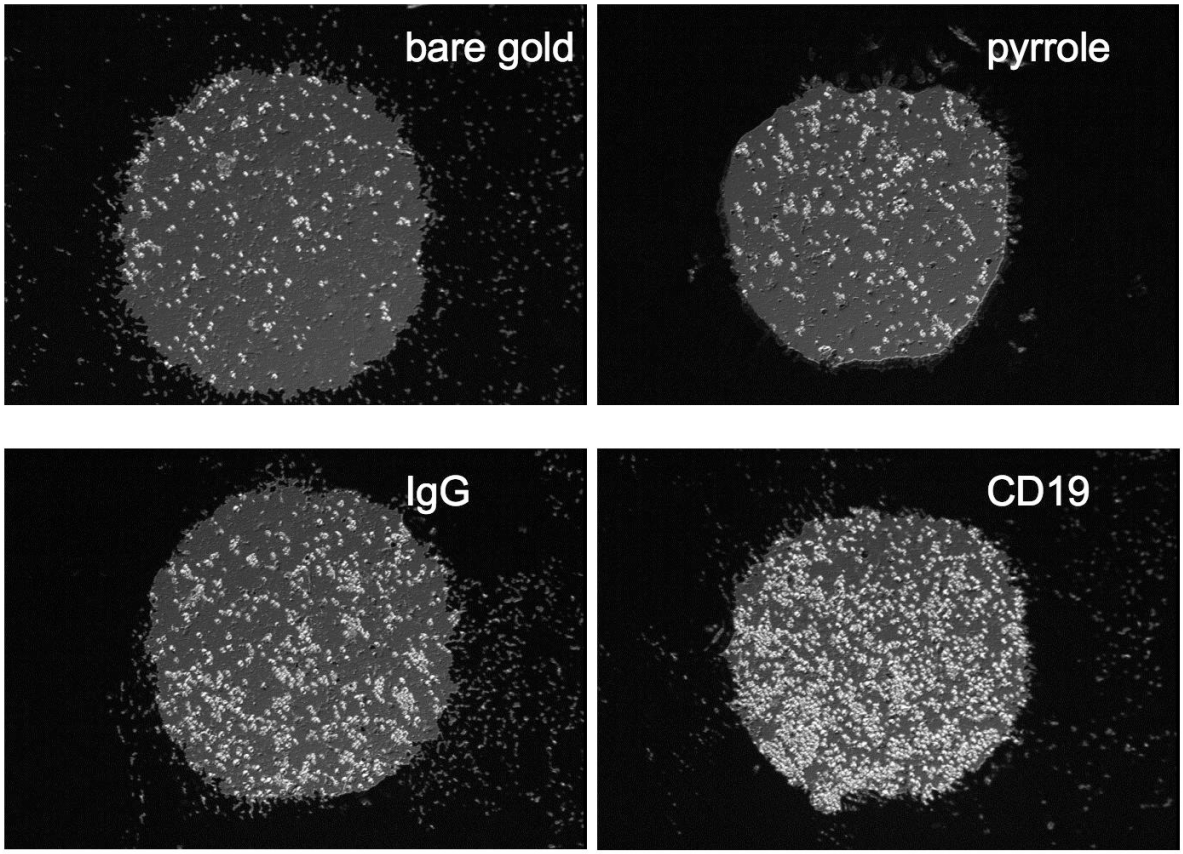
Differential interference contrast (DIC) microscopy of the spotted regions after cell binding



End-point DIC images recorded after a SPR imaging experiment: 3 injections of LS102.9 cells (300 μL, 500.000 cells/mL) were loaded on the biochip and incubated for *c.a.* 30 minutes each time. After cell binding, the prism was removed from the imager, the surface was gently rinsed with DMEM and the functionalized surface was observed by DIC microscopy.

Comment: Because cell binding involves non-covalent interactions between antibodies and antigens, bound cell layers were quite delicate to handle and impeded any comparison of results obtained with the same spot by both SPR imaging and DIC microscopy. But control experiments analyzed by DIC microscopy yielded results strengthening the data obtained by SPR images. In both cases, a specific response was detected on cell-type specific spots and only weak non-specific cell binding was observed on control spots though more cells were seen on control spots by DIC microscopy. It must be stressed that, using SPR imaging as a near field approach detecting cells only in the first 100nm layer, cells tightly bound to the surface are easily detected but not the cells suspended in the carrying medium nor cells loosely linked to the surface. This may account for the weak non-specific cell binding detected by SPR imaging compared to DIC pictures. Our experimental setup is thus a convenient approach for studying cells physically interacting on a surface (*i.e.* interacting within a layer of 100 nm above the surface).