

Adhesive microarrays for multipurpose diagnostic tools

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Materials and methods

Materials

Synthetic oligonucleotide probes were supplied by Eurogentec (Belgium): 5'-NH₂-C₆-TTGAGGTGCATGTTTGTGCC-3', 5'-NH₂-C₆-ATCTCGGGAATCTCAATGTTAGT-3' and 5'-Biotin-GGCACAAACATGCACCTCAA-3'. Alkaline phosphatase-conjugated streptavidin, BCIP/NBT (4-bromo-5-chloroindolyl phosphate/ nitro-blue tetrazolium) ready-to-use solution, bovine serum albumin (BSA), polyoxyethylene-sorbitan monolaureate (Tween20) and peroxidase-conjugated streptavidin were purchased from Sigma-Aldrich Chimie S.a.r.l (Lyon, France). Sodium nitrite and Veronal (diethylmalonylurea sodium) were obtained from Prolabo (Fontenay Sous Bois, France). C-reactive protein (CRP) from human fluid was obtained from Biodesign International (Meridian Life Science Inc., Saco, USA). All buffers and aqueous solutions were prepared with distilled demineralised (d.d.) water.

The bottomless 96-well plates were purchased from Greiner bio-one SAS (Courtaboeuf, France). White double sided adhesive 7966WDL, medical grade was obtained from 3M (France).

Spotting procedure

For oligonucleotide microarrays preparation, the 5'-amino modified 20^{mer} sequences (complementary 5'-NH₂-C₆-TTGAGGTGCATGTTTGTGCC-3' and non-complementary 5'-NH₂-C₆-ATCTCGGGAATCTCAATGTTAGT-3') were prepared in acetate buffer 0.1 mol.L⁻¹, KCl 0.1 mol.L⁻¹, bromophenol blue 0.25 mg.mL⁻¹, pH 5.5 to reach a final concentration of 50 µmol.L⁻¹.

For CRP specific microarrays, anti-CRP monoclonal antibodies (C5 clone from Biodesign International [Meridian Life Science Inc., Saco, USA]) and BSA (as negative control) were prepared at a concentration of 500 µg.mL⁻¹ in acetate buffer 0.1 mol.L⁻¹, KCl 0.1 mol.L⁻¹, bromophenol blue 0.25 mg.mL⁻¹, pH 5.5.

These solutions were spotted as 3.2 nL drops (pitch = 600 µm) on the flat adhesive polymer 3M 7966WDL using a piezoelectric spotter (sciFLEXARRAYER S3, SCIENION, Germany). The substrate was air-dried at room temperature and was then ready to be used.

Oligonucleotide assay on adhesive bottom 96-well plate

Oligonucleotide assays were carried out on an EVO75 robot (TECAN, Switzerland) equipped with a heater. For all experiments, the protocol involved the following steps. i) Wells were washed with phosphate buffer saline (PBS, 0.1 mol.L⁻¹, NaCl, 0.15 mol.L⁻¹, pH 7.4) and saturated with PBS mixed with

LowCross Buffer 1:5(v:v) (Candor Bioscience, Wangen, Germany). ii) Samples (biotinylated probes) were transferred to the wells and incubated for 30 min at 37°C. iii) The wells were then loaded with the alkaline phosphatase-conjugated streptavidin solution (2 µg.mL⁻¹) and incubated for 30 min at 37°C. iv) Finally, 200 µL of BCIP/NBT substrate solution were added in each well for signal generation. Wells were washed with PBS after hybridization, labelling and signal generation steps. The microtiter plate bottom was imaged using a flatbed scanner (HP Scanjet 3770, Hewlett-Packard) in greyscale (from 0 to 65535 arbitrary units (a.u.)) with a 2400 dpi resolution.

For blood group genotyping, the protocol was similar except for the hybridization temperature which was raised to 55°C to achieve selectivity. Blood samples were prepared according to reference ¹.

CRP assay on adhesive bottom 96-well plate

CRP assays were carried out on an EVO75 robot (TECAN, Switzerland) equipped with a heater. For all experiments, the protocol involved the following steps; i) Wells were washed with, **PBS** and saturated with PBS mixed with LowCross Buffer 1:5(v:v) (Candor Bioscience, Wangen, Germany); ii) Samples (CRP protein) were mixed with biotinylated anti-CRP (C7 clone from EXBIO, Czech Republic) at a concentration of 0.1 µg.mL⁻¹ and alkaline phosphatase-conjugated streptavidin at a concentration of 1 µg.mL⁻¹, transferred to the wells and incubated for 1 hour at 37°C; iii) Finally, 200 µL of BCIP/NBT substrate solution were added in each well and incubated 15 minutes for signal generation. iv) Wells were washed with PBS after incubation, labelling and signal generation steps. The microtiter plate bottom was imaged using a flatbed scanner (HP Scanjet 3770, Hewlett-Packard) in greyscale (from 0 to 65535 arbitrary units (a.u.)) with a 2400 dpi resolution.

CRP assay on integrated adhesive microarray

CRP assays were carried out within a laminated microfluidic system composed of one adhesive layer supporting anti-CRP microarray, one double adhesive layer in which a microchannel and an incubation chamber were cut out using a desktop digital craft cutter (Silhouette SD Quickutz[®], US)² and one cover layer (glass or plastic) equipped with two drilled apertures for fluid inlet and outlet.

For the assay, the different solutions were injected using a micropipette directly in microsystem inlet. The protocol involved the following steps; i) Injection of 200 µL of **PBS**; ii) Injection of 200 µL of PBS added of LowCross Buffer 1:5(v:v) (Candor Bioscience, Wangen Germany); iii) Injection of 200 µL of samples (CRP protein) mixed with biotinylated anti-CRP (C7

clone) at a concentration of $0.1 \mu\text{g.mL}^{-1}$ and alkaline phosphatase-conjugated streptavidin at a concentration of $1 \mu\text{g.mL}^{-1}$, and incubation for 30 minutes at 37°C ; iv) Finally, $200 \mu\text{L}$ of BCIP/NBT substrate solution were injected for signal generation and incubated for 15 minutes. The chambers were washed with PBS after incubation, labelling and signal generation steps.

The entire system was then imaged using a flatbed scanner (HP Scanjet 3770, Hewlett-Packard) in greyscale (from 0 to 65535 arbitrary units [a.u.]) with a 2400 dpi resolution.

Data analysis

Images analysis (signal quantification) was performed using GenePix Pro 6.0 software (Molecular Devices France, St Grégoire, France). The signal intensity per spot was calculated as the median intensity for all pixels included in a circular feature defining the spot and corrected using a local background evaluation. The resulting net specific intensity on a given probe was calculated as the mean intensity for all replicate spots.

Complementary results

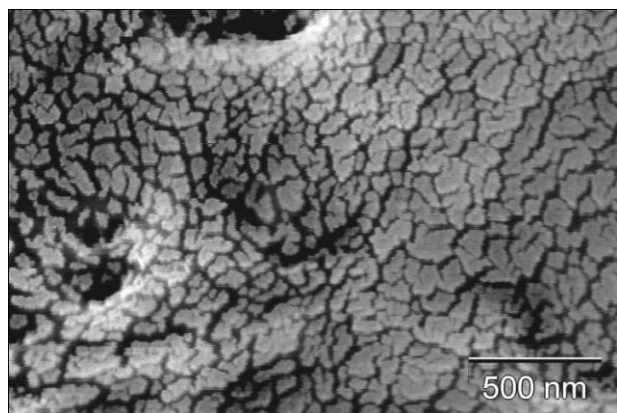


Fig. 1 a) SEM picture of a bare acrylic based polymeric adhesive surface. (15kV under vacuum)

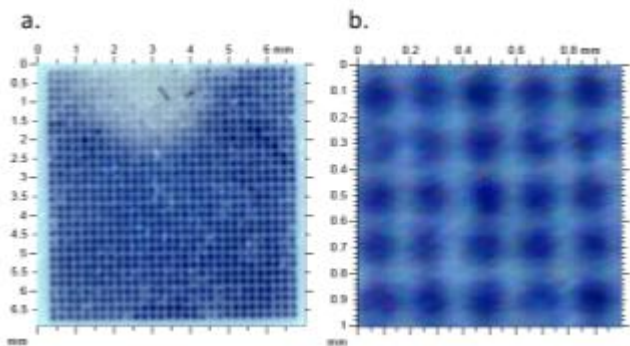


Fig. 2 a) 30x30 spots microarray (900 spots) with a $200 \mu\text{m}$ pitch.
b) Further magnification of the array

2. P. K. Yuen and V. N. Goral, *Lab Chip*, 2010, **10**, 384-387.

1. G. C. Le Goff, J.-C. Brès, D. Rigal, L. J. Blum and C. A. Marquette, *Anal Chem*, 2010, **82**, 6185-6192.