Real-time detection of lymphocytes binding on an antibody chip using SPR imaging

Emmanuel SURANITI[1], Elodie SOLLIER[2], Roberto CALEMCZUK[1], Thierry LIVACHE[1], Patrice MARCHE[2], Marie-Bernadette VILLIERS[2], Yoann ROUPIOZ[1]

1 CREAB, UMR 5819 (CEA-CNRS-UJF), DRFMC. CEA Grenoble France.

2 INSERM U823, equipe 8, Institut Albert Bonniot La Tronche France

Whilst biochips dedicated to cell analysis have generated interest in developing new tools for diagnostic and research purposes, microarray technology is playing a crucial role in detecting interactions. Further, optical detection by Surface Plasmon Resonance imaging (SPRi) combines both techniques and offers great interest for the real-time direct measurement of label-free cell-surface binding. In particular

SPRi technology allows the detection of site-specific binding of either B or T lymphocyte populations on an antibody array.

Principle

Antibodies were pyrrole-modified and electropolymerized on a SPRi Biochip™. Chosen murine cell lines were injected on the SPR imager system and antibodies-cells interactions were monitored with direct label-free detection using Surface Plasmon Resonance imaging (SPRi) technique.

Materials and methods

1) Preparation and immobilization of pyrrole-antibody conjugate on the biochip.

A hydrophobic mask was transferred onto the gold surface of a biochip leaving unmasked regions for biomolecules electrodeposition. Then three pyrrole-modified antibodies were electropolymerized on the free gold layer of the biochip following the pyrrole chemistry previously described in other notes. CD19 and CD3 monoclonal antibodies directed against lymphocyte membrane proteins, and a human IgG as a negative control antibody, were chosen for the assay. Each sample was spotted more than once on the chip.

2) SPRi experiment

After spotting, the SPRi Biochip™ was introduced into a SPR imager platform with DMEM being used as running buffer.

Injected solutions

Cell pellets LS102.9 B-type lymphocytes and 13G7 T-type lymphocytes from murine cell lines resuspended in DMEM supplemented with 5% FCS. Prior to injection cellular samples were diluted in non-supplemented DMEM to the desired injection concentration and from 0.3 to 1mL were injected in the flow cell system.

For cell binding specificity LS102.9 cells had to respond with anti-CD19 spots and 13G7 cells had to respond with anti-CD3 spots.

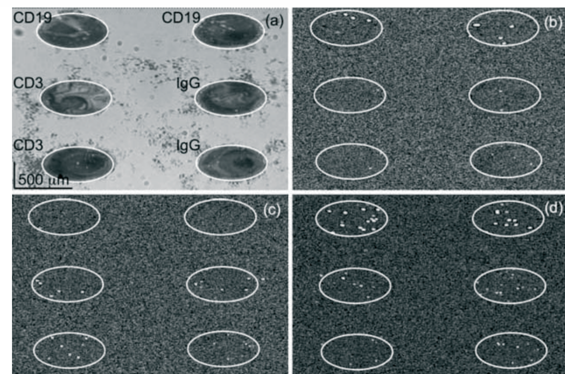

Figure 1:
Cell-specific binding of pure lymphocyte populations on a biochip.

(a) SPR image of the surface functionalized with three different antibodies (each spotted in duplicate): anti-CD19, anti-CD3 and a control human IgG with no specific affinity for lymphocytes.

(b) Differential SPR image recorded 50 min after a first cell injection (LS102.9 lymphocytes, 300 mL at 10^4 cells.mL^{-1}).

(c) Differential SPRimage recorded 50 min after a second cell injection (13G7 lymphocytes, 300 mL at 10^4 cells.mL^{-1}).

(d) Differential SPR image recorded 50 min after a third cell injection (LS102.9 lymphocytes, 300 mL at 3.104 cells.mL^{-1}).

Results and discussion

Detection of cell binding to antibodies chips by SPRi

Firstly, LS102.9 cells at 10^4 cells.mL^{-1} were injected. Four and five cells could be counted on the anti-CD19 spots. Secondly 13G7 cell sample at 10^4 cells.mL^{-1} were injected. Five and seven cells could be seen on the SPR image on the anti-CD3 spots. No cell was visible on control anti-CD19 spots. However, cells binding to the control IgG spots were observed. Subsequent injection of LS102.9 with higher cell concentration showed cells binding not only on the associate anti-CD19 but also on IgG and anti-CD3 spots. The explanation given for this is the close proximity to the first cell sample injected occurring with the following cells injection, so increasing the cell binding response from the previous injection.
However higher cell concentrations injections of mixed lymphocytes populations increase the reflectivity variation on the anti-CD3 and anti-CD9 areas but no significant reflectivity increase was measured on the control IgG spots.

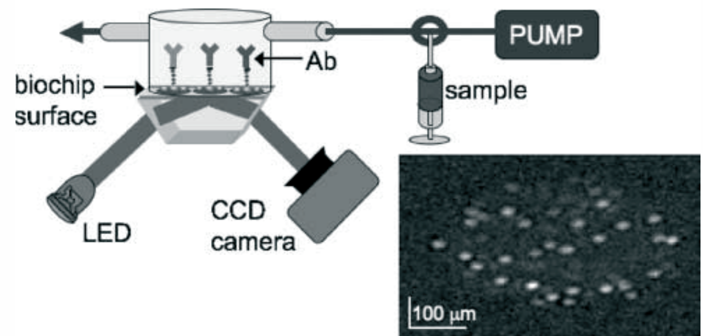

Figure 2: Schematic view of the SPR imaging setup

Conclusion

SPR imaging system can be used for cell-surface binding detection. The technique is convenient for studying in real time, label-free cells physically interacting on a surface.

Applications for diagnostic analysis can be validate using blood samples.

The results of the experiment are published in Lab. Chip., 2007, 7, 1206-8.

HORIBA Scientific

GENOPTICS BIO INTERACTIONS

www.horiba.com/scientific

USA: +1-732-494-8660
UK: +44 (0)20 8204 8142
China: +86 (0)10 8567 9966
France: +33 (0)1 64 54 13 00
Italy: +39 0 2 57 60 30 50
Other Countries: +33 (0)1 64 54 13 00
Germany: +49 (0)89 46 23 17-0
Japan: +81 (0)3 3861 8231