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Title:

Label Free Emerging Technology Based on SPR Biochips Combined with Mass Spectrometry for Deep Characterizations of Protein Markers in Complex Media.

Authors & affiliations:

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Abstract: (Your abstract must use **Normal style** and must fit in this box. Your abstract should be no longer than 300 words. The box will 'expand' over 2 pages as you add text/diagrams into it.)

Introduction:

Surface Plasmon Resonance in Array coupled to Mass Spectrometry (SUPRA-MS) is a promising approach in clinical research to go beyond classical bioassays in allowing the validation of biomarkers (1), elucidation of proteins and fine analysis of protein variants. We have developed a versatile platform (2) in macro and microarray formats for cancer and sepsis biomarkers characterizations in human plasma at the femtomol level.

Results:

Homemade SPR chips were chemically functionalized by SAMs compatible with investigations in biological fluids. Immobilization of ligands in various array formats (16 to 96 spots per chip) has been validated with a manual spotter or using the Continuous Flow Microspotter.

The capture of analytes in human plasma has been monitored in real time without any labeling by SPRi. Biosensor performances have been established and efficient coupling with mass spectrometry on arrays has been validated in MS and MS/MS modes.

In particular, we have performed the capture of LAG3 protein, a potential marker of breast cancer, in human plasma (2.5%).The spots of α-LAG3 and controls were obtained after optimization of grafting conditions leading to the high surface coverage of 15 femtomol/mm^2 (Fig1). The capture of the biomarker (1.6µg/mL) was achieved at 4 to 7 femtomol/mm^2 on α-LAG3 spots with high S/N ratio (>10). An original treatment of arrays by spray before MS allows the identification of LAG-3 protein with a high significant score (>58) and its structural elucidation (Fig2).

Regarding sepsis grades, we have investigated potential interacting partners of protein TREM1 in human plasma through SUPRA-MS. First results of this clinical study will be presented.

Conclusion:

We demonstrate the capacity of this "SUPRA-MS" platform analysis to detect and identify biomarker in human plasma. One possible application of this platform is the quantitative and qualitative characterizations in multiplex format of human protein variants described as potential biomarkers.

(1) Sparbier K, Wenzel T, Dihazi H, Blaschke S, Müller G-A, Deelder A, Flad T, Kostrzewa M (2009) Proteomics 9:1442-1450

(2) Remy-Martin F., El Osta M., Lucchi G., Zeggari R., Leblois T., Bellon S., Ducoroy P., Boireau W. (2012) Procedia Chemistry, to be published

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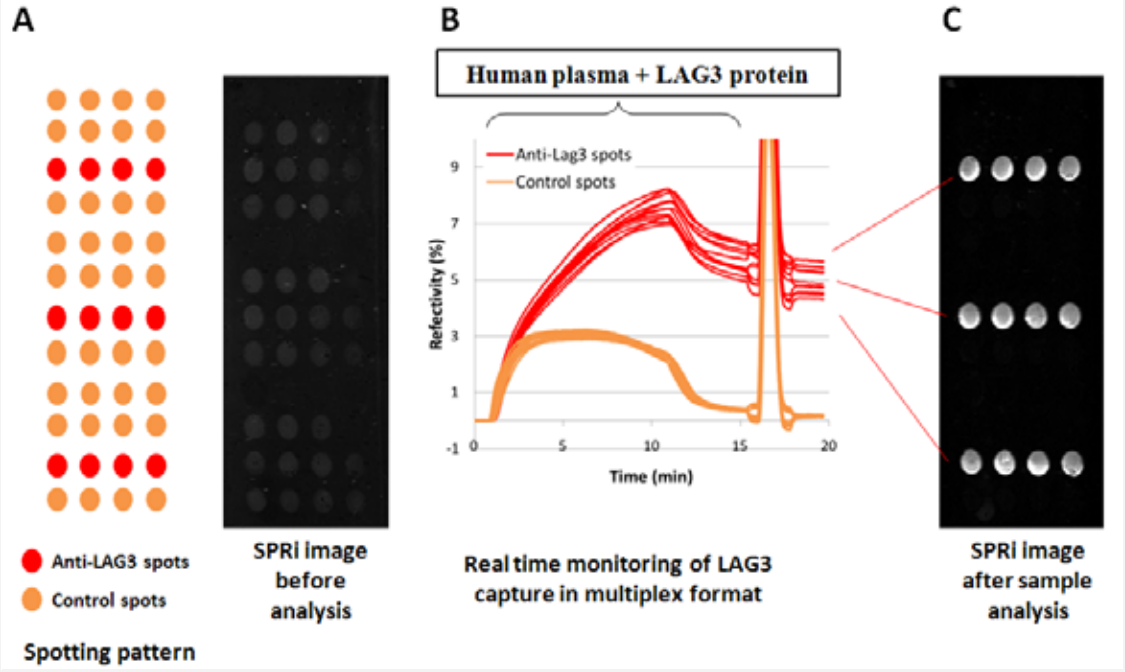
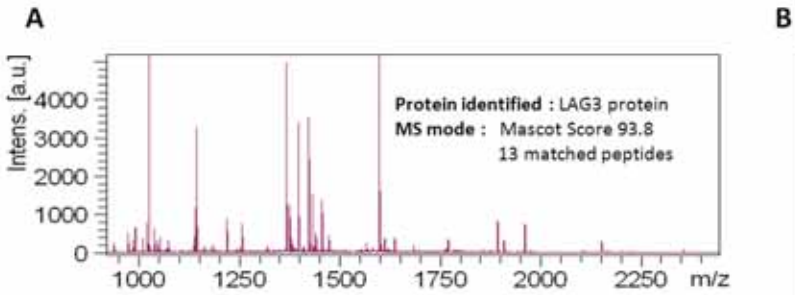


Figure 1. (A) Spotting pattern compared to the SPR image of the chip before analysis (B) SPR monitoring of spiked LAG-3 injection at 20 µL/min during 10 min followed by a detergent injection (C) SPR image of the chip after analysis.



B

Anti-LAG3 spot	MS score	Number of peptides
1	/	/
2	61.9	10
3	36.3	6
4	23.2	4
5	93.8	13
6	72.6	8
7	44.2	7
8	72	9
9	48.4	7
10	/	/
11	60.8	12
12	87.4	11

Figure 2. (A) MS spectrum of one anti-LAG3 spot (B) MS results of the anti-LAG3 spots showing 83% of LAG3 protein identification in microarray format.