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Application Note

Environment

SPRi

SPR30

Detection of birch pollen allergen in the air: First feasibility study

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Over 20% of the European population suffers from respiratory allergies. Many factors can cause these immune dysfunctions. They can be classified into three categories:

- **Internal environmental factors, which refer to all potential allergens inhaled indoors (dust mites, molds, cat or dog fur, etc.).**
- **External environmental factors, which refer to all potential allergens inhaled outdoors (pollens, molds).**
- **Factors of air pollution: there is a triangular relationship between pollution, pollen and allergy. Pollution can not only affect pollen by changing its external biochemical structure and thus its allergenicity, but also the human respiratory mucosa by changing its immunological sensitivity to pollen particles.**

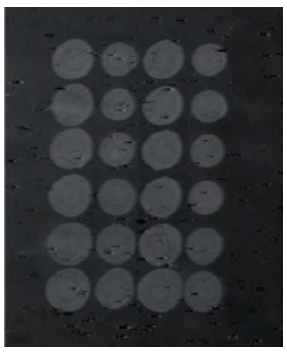
The purpose of this proof of concept is to show that Surface Plasmon Resonance imaging (SPRi) is a suitable technique to detect allergens. Compared with other techniques, such as ELISA or Luminex, which are based on colorimetric or fluorescence detection, this technology does not require the labeling of antibodies. We focus here on the real-time detection, in a multiplex format, of a recombinant birch pollen allergen (rBet v 1) and a natural birch pollen allergen (Bet v 1) contained in pollen grains by monitoring the kinetics of the interaction between an anti-Bet v 1 antibody and the birch pollen allergens. This unique technique allows, for the first time, the combination of both real-time and multiplex detection of pollen allergens.

The ultimate goal is to design a "pollen" biochip, which would permit the detection and quantification of different pollen allergens in the air.

Materials and Methods

Immobilization of antibodies directed against allergens on the SPRi-Biochip™

Two ligands were immobilized using the SPRi-Arrayer™ on a CS SPRi-Biochip™. The first one is a monoclonal antibody 6H4 directed against Bet v 1 allergen 5 (Stallergenes, Antony, France) and the second one is a negative control monoclonal antibody Kori75 (Stallergenes, Antony, France) which does not react with Bet v 1. Figure 1 shows the image of the antibodies spotted at 6 µM and the spotting map.



Kori75	6H4	Kori75	6H4
Kori75	6H4	Kori75	6H4
Kori75	6H4	Kori75	6H4
Kori75	6H4	Kori75	6H4
Kori75	6H4	Kori75	6H4
Kori75	6H4	Kori75	6H4

Figure 1: Spotting map and image of the spotted antibodies matrix on the SPRi-Biochip™.

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Experimental device

The spotted biochip was inserted into the SPRi-Plex II™ instrument from HORIBA Scientific. The flow rate was set to 50 µL/min and the working temperate was fixed at 25°C. First, a solution of rBet v 1 allergen was injected at 8 concentrations (41 ng/mL, 72 ng/mL, 102 ng/mL, 256 ng/mL, 640 ng/mL, 1.6 µg/mL, 4 µg/mL and 10 µg/mL) to determine the affinity constant between 6H4 and rBet v 1 and to plot a calibration curve. Second, birch pollen allergens were extracted from the birch pollen grains. 1 mg/mL birch pollen was incubated in PBS 10mM over the night to extract allergens.Then, the solution was centrifuged and filtrated. The supernatant was recovered and injected at different dilutions.

Between each sample injection, the biochip was rinsed with 10 mM PBS buffer and the interaction was regenerated with a 0.1 M glycine/HCl pH=2 solution. The interaction between the antibody and the allergen was monitored in real time by SPR imaging, and for each injected concentration, the variations of reflectivity representing the quantity of rBet v 1 interacting with antibodies were determined.

Results and Discussion

1. Affinity between 6H4 and purified birch allergen

Figure 2 shows the variation of reflectivity (%) versus time for the 8 injected rBet v 1 concentrations. Each kinetic curve corresponds to an average on the twelve 6H4 spots. The signal obtained on the Kori 75 spots was subtracted from the response from 6H4 spots. Figure 3 displays the "difference image" after the injection of the rBet v 1 solution at 256 ng/mL.

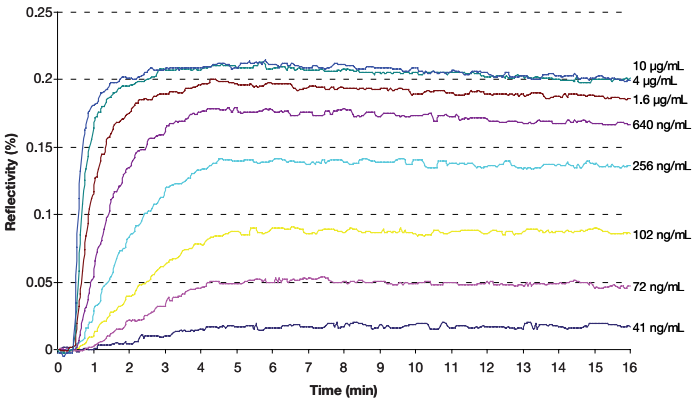

Figure 2: Kinetic curves obtained after the injection of 8 concentrations of rBet v 1 on 6H4 spots.

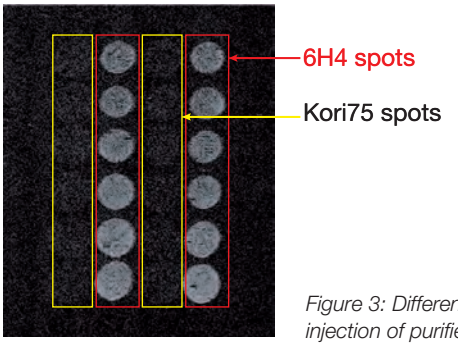

Figure 3: Difference image after the injection of purified rBet v 1 at 256 ng/mL.

As observed in Figures 2 and 3, there is a specific response on the 6H4 spots and no variation of reflectivity on Kori75 spots after the injection of the allergen solution. The signal increases with the concentration injected and reaches a plateau at 4 µg/mL. The limit of detection is about 40 ng/mL.

Figure 4 shows the fitted kinetic curves after the injection of rBet v 1 at 102, 256 and 640 ng/mL, i.e. 5.8, 16.6 and 36.6 nM. A 1:1 interaction model was used to fit the curves globally. Based on these fits, kinetic constants (k_a and k_d) and the affinity (K_D) were determined for the interaction 6H4/rBet v 1 with the ScrubberGen Software.

The affinity between the 6H4 antibody and the rBet v 1 protein was measured and corresponded to 192 pM.

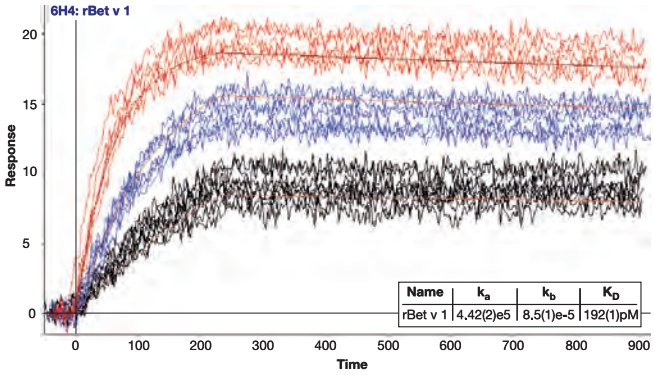

Figure 4: Fitted kinetic curves allowing to determine kinetic constants and the affinity for the interaction 6H4/rBet v 1.

2. Calibration curve

There is a link between the variation of reflectivity and the quantity of allergen that reacts with the antibodies spotted on the biochip. From the kinetic curves above, a calibration curve was plotted. It represents the quantity of rBet v 1 retained on the 6H4 spots versus the concentration of injected allergen (Figure 5).

The equation of the linear trendline (between 2.3 and 16.6 nM) is :

$$y\ (pg/mm^2) = 5.8731x\ (nM)$$

with a R^2 coefficient of 0.9923.

Based on this calibration curve, the concentration of birch allergen in a pollen extract can be determined, and this value can be correlated to the quantity of collected pollen.

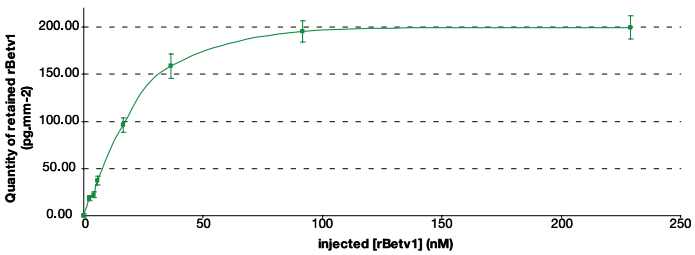

Figure 5: Quantity of retained rBet v 1 on 6H4 spots in pg/mm² versus the concentration of rBet v 1 injected.

Figure 6 shows an example of kinetic curves obtained after the injection of birch pollen extract at 72 µg/mL and 102 µg/mL in pollen. Between each injection, the biochip surface was regenerated with the injection of a 0.1 M glycine/HCl pH=2 solution. Each kinetic curve in figure 6 corresponds to an average curve on 12 spots for each family (6H4 and Kori75).

Figure 7 represents the difference image after the injection of birch pollen extract at 102 µg/mL in pollen.

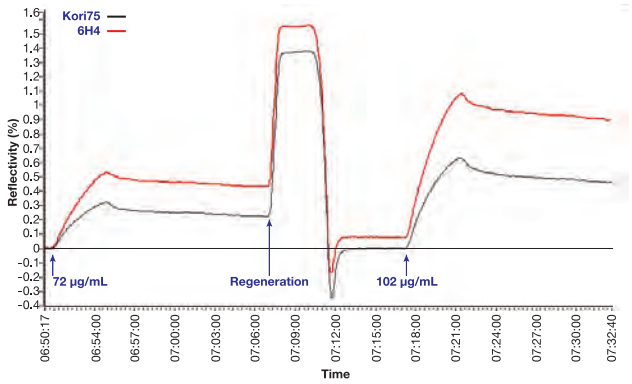

Figure 6: Kinetic curves after the injection of birch pollen supernatant at 72 µg/mL and 102 µg/mL in pollen on anti-Bet v 1 antibodies 6H4 spots and on Kori75 antibodies spots (negative control).

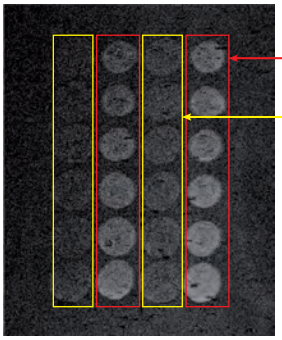

Figure 7: Difference image of the biochip after injection of the birch pollen supernatant (102 µg/mL in pollen).

We can observe on the kinetic curves and on the difference image that there is some non-specific adsorption on Kori75 spots after injecting the supernatant solution. This non-specific adsorption is due to the fact that a complex solution is injected. This solution contains other proteins and particles that can disrupt the signal; but by subtracting the signal obtained on negative controls spots, the specific signal representing the interaction between the antibody 6H4 and the protein Bet v 1 can be obtained.

Thanks to the calibration curve, the concentration of Bet v 1 in the pollen extract can be determined. An equivalent of 1 µM of Bet v 1 was contained in the extract, which corresponds to 17.8 µg/mL. Hence, the quantity of Bet v1 in 10 mg of pollen is about 17.8 µg. This value is consistent with the results obtained by J. Buters et al [1, 2].

Conclusion

We demonstrate here, the interest of the SPRi technology for the detection of allergens from freshly collected pollen grains. Indeed, there is a real need to correlate the exposure to pollens and the health impact. In addition, there is a strong demand to measure the presence of allergens in different locations, such as offices, nurseries, hotels, pet stores, etc. For now, no device is able to estimate, in real time and without labeling, the amount of allergens collected in a particular environment.

Here we show that the multiplex approach allows the simultaneous detection of different allergens in order to determine the quantity of pollen in the air. This can help notify allergic patients quickly about the presence of specific pollen in a particular area.

References

[1] J. T. M. Buters et al., Allergy (2010), Jul;65(7):850-8.
[2] J. T.M. Buters et al., Int. Arch Allergy Immunol (2008), 145:122–130.

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