A new ex vivo skin model to mimic pollen allergens exposition and evaluate preventive or cleansing effects of skin care products

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Abstract

Background:
Airborne pollen exposure leads to an accumulation of allergens in infundibulum and is responsible of irritation, discomfort and allergic sensitization. In order to evaluate the preventive or cleansing efficacy of care or hygiene skin products, a new study model using hairy skin explants on Perfex *vivo* supports was developed to mimic the deposit of pollen allergen in the hair follicle infundibula.

Methods:
A first step aimed at mimicking pollen accumulation and visualizing it in infundibula on large *ex vivo* hairy skin explants maintained in survival on Perfex *vivo* supports. After 6 hours of contact, pollen accumulation was revealed by immunostaining. In a second step, film forming product was applied prior to pollen exposure and cleansing product was applied after pollen exposure.

Results:
Firstly, the optimal immunostaining method to identify and reveal the accumulation of pollen allergen in the infundibula was determined.
Secondly, the activity of different cosmetic products was characterized. A film forming product applied prior to pollen exposure was able to significantly reduces pollen deposit in infundibula, offering a preventive benefit effect. Cleansing with cosmetic product after pollen exposure managed to significantly remove accumulated pollen.

Conclusion:
The model based on hairy skin explants on Perfex *vivo* support allows to mimic skin exposure to pollinic allergens and their deposit in infundibula.
This model can highlight protective effects of film forming products by reducing the accumulation of pollen on the skin. In addition, this model allows to evaluate cleansing effects of products used to remove pollen from the skin.

Keywords: Pollen; Ex *vivo*; skin model; allergen; care product.

Introduction.

By their morphology and function, infundibula are natural site of accumulation of many exogenous elements, such as microorganisms, chemicals, pollutant matters or allergens such as pollen allergens.

In most cases this accumulation is a source of discomfort but it may lead to severe allergic reactions in sensitive subjects. The development of care products protecting against the accumulation of pollen in the infundibula or of products facilitating the elimination of pollen deposits is of great importance for people with allergies and can limit the sensitization of new subjects. In order to evaluate the preventive or cleansing efficacy of care or hygiene skin products, a new study model using hairy skin explants on Perfex *vivo* supports was developed to mimic the deposit of pollen allergen in the infundibula of hair follicle.

Materials and Methods.

Hairy abdominal skin was obtained from surgical residues after written informed consent from the donor and in full accordance with the Declaration of Helsinki and article L.1245–2 of the French Public Health Code. The latter does not require any prior authorization by an ethics committee for use of surgical waste.

Large explants containing several infundibula were prepared from three donors (27-, 30- and 33-year-old Caucasian males) and maintained on Perfex *vivo* culture supports (BIO-EC culture device, fig. 1). After few hours of stabilization in open air under controlled temperature and with a continuous flux of nutrition culture medium thanks to fluidic system, stratum corneum reaches its normal physiology and environment. The Perfex *vivo* skin explants present optimal conditions of tension, hydration and temperature [1].

Figure 1: Perfex *vivo* explants

Then, recombinant pollen allergen Phl p 5b protein (Abcam ref. ab225974) was applied on the whole surface of the explant at a rate of 15,75 µg/cm^2 to mimic a real pollen exposure.
After 6 hours of contact, skin explants comprising several infundibula were collected and cut in two parts. Half was fixed with formalin solution, then impregnated and embedded in paraffin for immunohistochemistry analysis and half was frozen and kept à -80°C.
5-µm-thick serial sections were made using a microtome, and the sections were mounted on histological glass slides. The frozen samples were cut into 7-µm-thick serial sections using a cryostat. Sections were then mounted on glass slides.

The presence of pollen allergen was revealed by immunostaining on formol-fixed paraffin embedded (FFPE) or frozen skin sections with a polyclonal anti-Pollen allergen Phl p5b antibody (Biorbyt, ref. orb51666) or anti-Pollen allergen Phl p5b-FITC antibody (Biorbyt, ref. orb51668) in PBS-BSA 0.3% and incubated overnight at room temperature. FFPE skin sections were then incubated with a Vectastain Kit Vector amplifier system avidin/biotin, and revealed by VIP (violet substrate of peroxidase from Vector laboratories, Ref. SK-4600). Sodium citrate antigen retrieval at pH6 was performed on half of the FFPE slides. Frozen skin sections stained with orb51668 FITC-coupled antibody were directly observed whereas those stained with orb51666 antibody were revealed by AlexaFluor 488 (Lifetechnologies, ref. A11008). The nuclei were counterstained by propidium iodide.

Observations were realized using a Leica DMLB, an Olympus BX43 or BX63 microscope. Images were acquired with a numeric DP72 or DP74 Olympus camera with Olympus cellSens storing software. For each batch of explants, the percentage of the region of interest (infundibulum) covered by the staining (stained surface percentage) was determined by image analysis, using Olympus cellSens software. The stained surface percentage (Surf%) for the allergen exposed explant was compared to the unexposed condition.

In a second step, two types of product application (preventive or curative) were carried out to define if pollen allergen accumulation in infundibulum can be reduced or removed. In order to evaluate if a product was able to reduce the accumulation of pollen protein in the infundibula, it was applied topically at the rate of 2µL/cm² 10 minutes before the application of the pollen allergen (batch PPA Tab.1).

To investigate the performance of a cleansing product, 6 hours after pollen allergen application, curative product was used to remove allergen by rubbing the skin surface with two soaked cotton discs soaked with the product (batch ACP). The control batches C and AC did not receive any product.

Batches	*Conditions*
C	Unexposed control
AC	Pollen allergen
PPA	Preventive product + Pollen allergen
ACP	Pollen allergen + Curative Product

Table 1: Conditions of product treatment and Pollen allergen exposure

Statistical analysis was performed using excel software. Two-tailed, unpaired t tests were performed to compare experimental groups. The results are reported in the figures and figure legends

Results.

In the first step, several trials with different skin types, mode of application, tissue processing and antibodies to visualize the allergen accumulation in the skin were conducted. Pre-treatment as Heat-Induced Epitope Retrieval *(*HIER*)* was also carried out, to determine optimum conditions to detect pollen protein in the infundibulum.

Immunostainings showed that pollen accumulation was localized mainly in the infundibulum but deposit on the stratum corneum of the interfollicular epidermis was also observed. Consequently, pollen accumulation was evaluated in the infundibulum and on the stratum corneum (fig. 2).

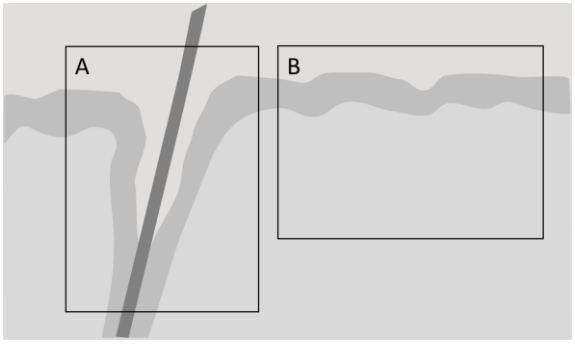

Figure 2: Pollen accumulation was evaluated on infundibulum (A) and on stratum corneum of interfollicular epidermis (B).

The pollen allergen immunostainings on frozen sections, showed a non-specific staining observed at the base of the stratum corneum (fig. 3). Further investigations (data not shown) have exhibited a possible cross reaction with filaggrin.
The pollen allergen immunostainings on the FFPE sections showed a more specific staining than the one obtained on frozen sections (fig.4).

In conclusion, the best method to identify and reveal the accumulation of pollen in the infundibula was immunostaining of Phl p 5b pollen protein on FFPE skin sections revealed by immunohistochemistry (peroxidase coupled secondary antibody) with Orb51666 antibody without HIER.

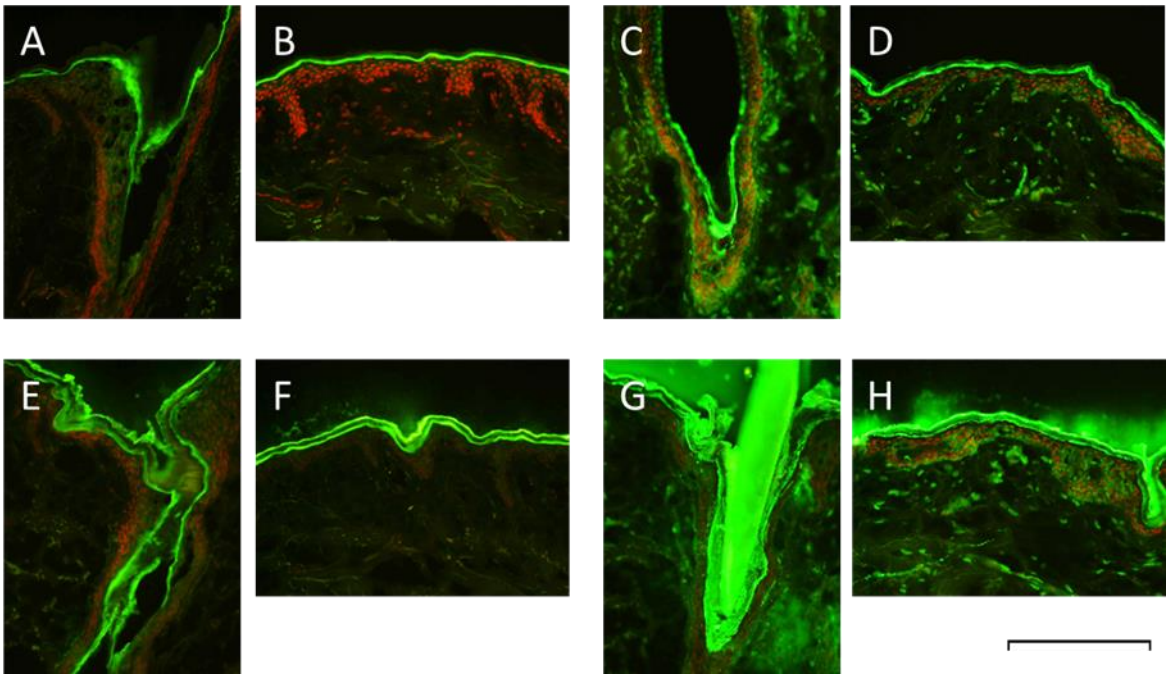

Figure 3: Immunostaining of pollen allergen Phl p 5b on frozen skin sections using FITC-coupled Orb51668 antibody (A, B, E, F) or AlexaFluor® 488-revealed Orb51666 antibody (C, D, G, H). Nonspecific fluorescence was observed on control conditions (A, B, C, D) in infundibulum (A, C) and on the stratum corneum (B, D). Additional fluorescence was observed under pollen allergen-exposed conditions (E, F, G, H) in infundibula (E, F) and on the surface of the stratum corneum (F, H).

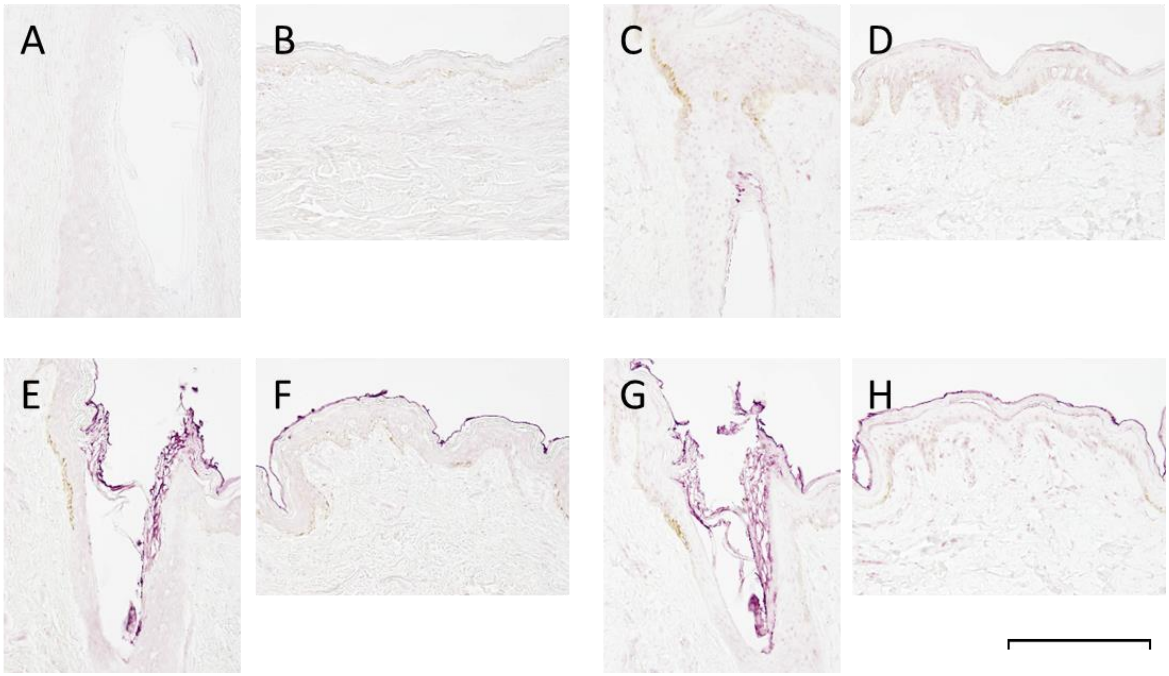

Figure 4: Immunostaining of Pollen allergen Phl p 5b protein on FFPE skin sections using peroxidase-revealed Orb51666 antibody (A, B, E, F). HIER pre-treatment was also tested (C, D, G, H). Slight non-specific staining was observed on control conditions (A, B, C, D). Clear staining was observed under pollen allergen-exposed conditions (E, F, G, H) in infundibula (E, F) and on the surface of the stratum corneum (F, H). HIER pre-treatment doesn't offer better results.

In a second step, the model has been implemented by testing different types of skin care products. Film forming product was applied prior to pollen allergen exposure to prevent accumulation and cleansing product was applied after exposure to remove pollen allergen. A moderate staining was observed on keratinized areas of the stratum corneum in the infundibulum. This non-specific staining was stronger than the one observed in the first step, but did not mask specific staining observed when pollen was applied.

Effect of products was assessed by scoring the pollen allergen accumulation evaluated by trained histologist (fig. 5) and completed by image analysis (Fig. 6).

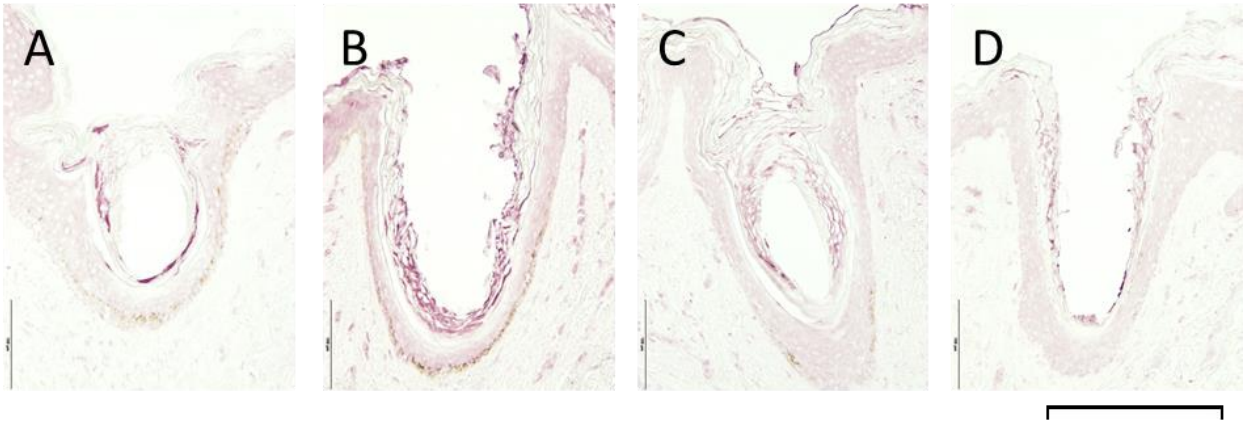

Figure 5: Immunostaining of pollen allergen in infundibulum. Non exposed control (A) showed a slight to moderate non-specific staining. After application of the pollen allergen a clear staining was observed in infundibulum of pollen allergen exposed control (B). Preventive treatment with film-forming product dramatically inhibited pollen allergen accumulation in infundibulum (C). Cleansing product CP removed a high amount of pollen allergen (D).

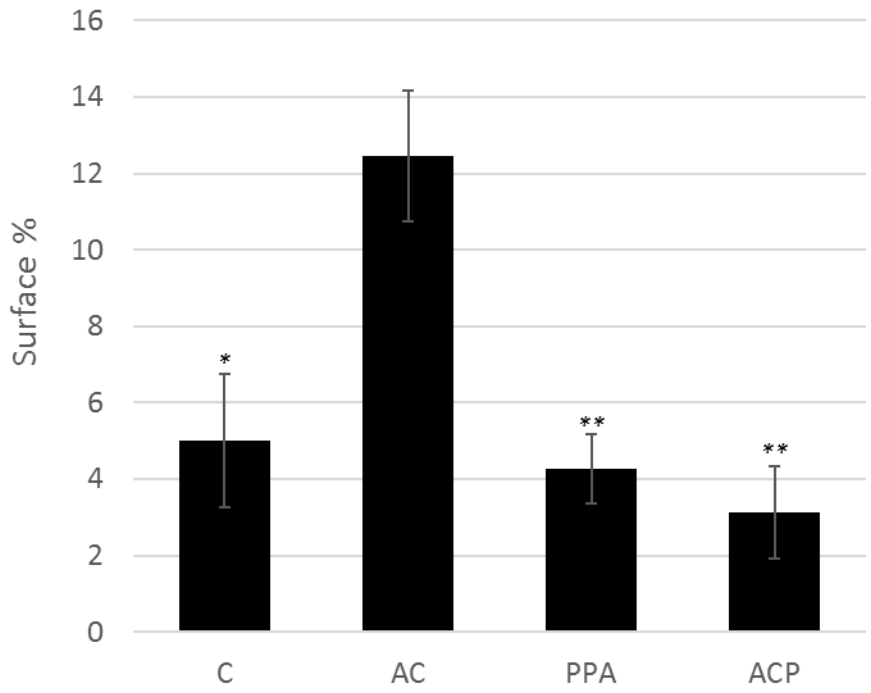

Figure 6: Image analysis of pictures as in fig 5 to measure the surface percentage of the infundibula positive to pollen staining. Non-exposed control (C), pollen allergen control (AC), preventive treatment before pollen allergen exposure (PPA) and curative treatment after pollen allergen exposure (ACP). Error bars, SD or SEM, * $p<0.05$ and ** $p<0.01$ vs AC.

Exposure to pollen allergen induced a significant increase in pollen allergen accumulation in infundibula by 149% ($p < 0.05$).

The preventive application of the film forming product (PP) completely prevented pollen allergen accumulation in the infundibulum of the hair follicles, offering a preventive benefit effect.

Cleansing with a cosmetic product (CP) following pollen exposure significantly remove accumulated pollen from infundibula.

Discussion.

Due to the permanent exposure of the skin to various pollen allergens, contact allergies multiply [2] [3] and constitute a source of discomfort that it is important to reduce. Until now, studies aimed to asses skin exposure to pollens were mainly conducted *in vitro* or *in vivo* [4] [5]. *In vitro* studies are very useful, but essentially limited to the evaluation of compounds or active ingredients. While *in vivo* studies are suitable for evaluating finished products, they are ethically difficult to conduct because of the risks they pose to volunteers. The *ex vivo* model we developed is closer to reality by allowing pollen allergens to be applied to healthy skin containing hair follicles. The skin and in particular its stratum corneum are maintained in conditions very close to reality. The application of pollen allergens makes it possible to reproduce a realistic exposure with accumulation of allergens in the infundibula. Application of cosmetic products can also mimic utilization similar to the *in vivo* conditions. Thus, this model allows to evaluate the capacity of cosmetic products to reduce the accumulation of pollen or to facilitate its elimination. Although it presents the classic disadvantages of *ex vivo* survival, this model is nevertheless a model of choice for the evaluation of products with regard to the accumulation of pollen allergen in the infundibula.

Conclusion.

The model based on hairy skin explants on Perfex *vivo* support allows to mimic skin exposure to pollen allergens and their deposit in infundibula. This model can highlight protective effects of film forming products by reducing the accumulation of pollen on the skin.

In addition, this model permits to evaluate cleansing effects of products applied to remove pollen from the skin.

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Conflict of Interest Statement.

Nicolas Lecland, Julie Scalia and Sandra Trompeszinski are employees of NAOS group (Aix-en-Provence).

References.

1. Peno-mazzarino L, Besse R, Percoco G, et al (2018) The missing bridge between ex vivo and in vivo models: the perfex vivo system. Conference IFSCC 2018 Podium presentations: Skin biology, Session 3, Paper n°: O-S4-06.
2. D'Amato G, Spieksma FThM., et al (1998) Pollen-related allergy in Europe. Allergy: 53:567-578.
3. Ellwood P., Asher M.I., et al (2001) Diet and asthma, allergic rhinoconjunctivitis and atopic eczema symptom prevalence: an ecological analysis of the International Study of Asthma and Allergies in Childhood (ISAAC) data. Eur Respir J 2001; 17: 436–443.
4. Jacobi U, Engel K, et al (2007) Penetration of Pollen Proteins into the Skin. Skin Pharmacol Physiol 2007;20:297–304.
5. Meinkea M, Patzelta A, et al (2007) In-vitro and in-vivo investigations of the penetration of pollen allergens in the skin and a prevention strategy. Congress Abstracts / Medical Laser Application 24 (2009) 120–142.