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ORIGINAL ARTICLE

Reduction of Cedar Pollen Adhesion by Lecithin Polymer Coating

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ABSTRACT

Background: Avoidance of allergens is one of the most effective treatments of allergenic diseases, including pollinosis. Although various masks and goggles for pollinosis sufferers are commercially available, little work has been done on preventing adhesion of pollen to the human body and clothes. We have examined the effect of polymer coating on adhesion of cedar pollen to skin, hair and fabrics, and found that a lecithin polymer (LP), containing both cationic and anionic sites, is highly effective in reducing adhesion levels.
Methods: We evaluated the changes in electrostatic charge and frictional coefficient of sample surfaces after coating with an anionic polymer (AP), a cationic polymer (CP), a nonionic polymer (NIP), or LP (each applied by spraying 0.10% polymer solution in ethanol/water). Pollen adhesion level was evaluated by observation of the number of cedar pollen particles on video microscope (VMS) images and by quantification of cedar pollen antigen Cry j1 with a sandwich ELISA system. Adhesion of pollen to human skin or hair was also examined by VMS observation.
Results: LP-coated samples showed a significant reduction in electrostatic charge level and frictional coefficient. We presumed that these properties are closely related to the significant reduction in the adhesion level of cedar pollen or Cry j1 compared with CP, AP or NIP-coated samples. A similar effect was seen on LP-treated skin, and LP-treated waxed hair.
Conclusions: Our results suggest that coating with LP could help in the management of pollinosis by reducing the contact level of patients with cedar pollen antigens.

KEY WORDS

adhesion, cedar pollen, electric charge, frictional coefficient, lecithin polymer

INTRODUCTION

Pollinosis is an immunological disease involving a typical type-I hypersensitivity reaction to pollen antigens, and symptoms include itching of the eyes, nasal congestion, rhinorrhea and sneezing. Current therapies for the treatment of pollinosis consist of pharmacotherapy using oral H_1 antihistamines or intranasal corticosteroids, immunotherapy using a specific form of controlled allergen,[1,2] and surgical laser treatment.[3] More recently, a DNA vaccine treatment exploiting a T cell epitope associated with pollinosis has been investigated.[4] However, the avoidance of allergens remains a primary objective of allergy treatment, and is the logical first step in managing allergic disease.[5-7] Primary avoidance studies have been reported by several researchers. Carswell *et al.*[8] suggested that mite removal procedures may modestly improve the condition of mite-sensitive asthma patients, and van der Heide *et al.*[9] reported that air-cleaners contributed to diminution of allergen exposure and improvement of airway hyperresponsiveness in asthma patients.

Various kinds of masks and goggles to prevent contact and inhalation of pollen are currently available, but little work has been done on the effective prevention of pollen adhesion to the human body and clothes, and only a few products aimed at pollen antigen avoidance are commercially available. Of those that are available, application is limited to cloths, and none of them can be applied directly to the human body and hair. Pollen adhering to the human body

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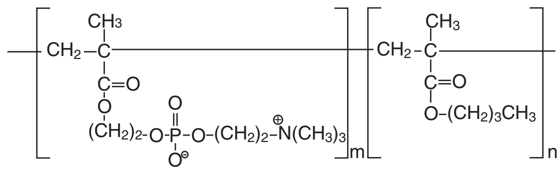
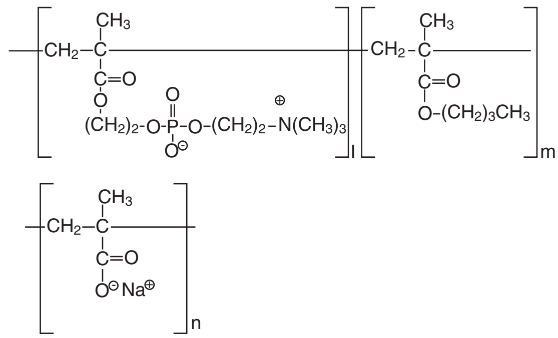
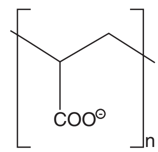
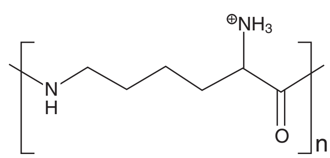
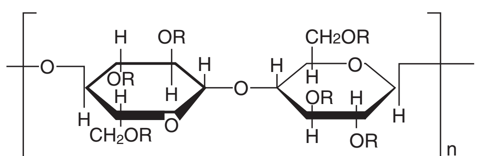
Table 1 Coating polymer agents used

Chemical name	Abbreviation	Type	MW (kDa)	Formula
2-Methacryloyloxy ethylphosphorylcholine butylmethacrylate copolymer	PQ-51	Lecithin polymer	100	
2-Methacryloyloxy ethylphosphorylcholine n-butylmethacrylate sodium methacrylate copolymer	PQ-65	Lecithin polymer	600	
Polyacrylic acid	AP	Anionic polymer	25	
Poly-D, L-lysine	CP	Cationic polymer	3	
Hydroxypropylmethyl cellulose	NIP	Nonionic polymer	30	 R:H, CH_3 or $CH_3CH(OH)CH_2$-

Chemical name, abbreviation, polymer type, molecular weight, and side chains of polymer agents are shown.

and clothes might easily be inhaled during daily life, so measures to prevent pollen adhesion might be highly effective for ameliorating pollinosis.

Electrostatic interaction and friction with rough surfaces are likely to be major factors promoting the adhesion of microparticles to surfaces. Further, microparticles of pollen may be readily adsorbed on wet surfaces. Therefore, we hypothesized that a dry, smooth surface with low electrostatic charge potential would be a promising candidate to reduce pollen adhesion.

Among raw materials for cosmetics, LP is a widely used ingredient with high safety and biocompatibility.[10,11] It is related to lecithin, a fundamental biomembrane constituent. In the present study, we focused on the effect of LP on the surface properties of human skin, hair and cloths, and on the adhesion level of cedar pollen to them.

METHODS

CEDAR POLLEN

Cedar pollen was purchased from Hayashibara Bio-

Table 2 Effect of LP (PQ-65) on electrostatic potential of PS films

	Electrostatic potential (kV)	
	PVDC	Nylon
Non-polymer coating	2.79 ± 0.13	− 2.34 ± 0.20
LP (PQ-65) coating	0.96 ± 0.17***	− 0.96 ± 0.08***

PS films were positively or negatively charged by rubbing with PVDC and nylon, respectively. $N = 5$, Student's *t*-test, ***: $p < 0.001$, *vs*. non-polymer coating.

Table 3 Effect of LP (PQ-65) coating on frictional coefficient of cotton and PE fabrics

	Frictional coefficient	
	Cotton	PE
Non-polymer coating	0.244 ± 0.011	0.201 ± 0.011
LP (PQ-65) coating	0.235 ± 0.012*	0.174 ± 0.013***

$N = 15$, data shows mean ± SD, Student's *t*-test, ***: $p < 0.001$, *: $p < 0.05$, *vs*. Non-polymer coating

chemical Laboratories (Okayama, Japan), and was desiccated *in vacuo* at 50℃ for 5 hours prior to use.

POLYMER COATING

Polymers used in this study are listed in Table 1. LP coating was performed with 2-methacryloyloxy ethylphosphorylcholine butylmethacrylate copolymer (PQ-51) or 2-methacryloyloxy ethylphosphorylcholine n-butylmethacrylate sodium methacrylate copolymer (PQ-65) (NOF Corporation, Tokyo, Japan). AP coating was performed with polyacrylic acid (Wako Pure Chemical Industries, Osaka, Japan), and CP coating was performed with poly-D, L-lysine (Chisso Corporation, Tokyo, Japan). NIP coating was performed with hydroxypropylmethylcellulose (Shin-Etsu Chemical, Tokyo, Japan) (Table 1). Sample surfaces were sprayed with 1 mL of 0.10% polymer solution in ethanol/water (9/1), resulting in a coating of 2.0 μL/cm² of each polymer.

EVALUATION OF THE ELECTROSTATIC CHARGE OF SURFACES

A polystyrene (PS) film was coated with PQ-65. A positive charge was loaded by rubbing with polyvinylidene chloride (PVDC) 10 times. A negative charge was loaded by the same procedure but using nylon instead of PVDC. Thereafter, the electrostatic potential of each sample was measured at 25℃ under 50% relative humidity using an electrostatic field meter FMX-002 (Simco Japan, Inc., Kobe, Japan). A film without polymer coating was used as the control.

EVALUATION OF THE FRICTIONAL COEFFICIENT

The frictional coefficient of surfaces was measured at 25℃ under 50% relative humidity with a Tribogear Heidon Type-14DR (Shinto Scientific Co., Ltd., Tokyo, Japan). Cotton fabric (size: 80 mm × 20 mm, fiber diameter: 20 μm, fiber density: 30 × 25 fibers/cm, thickness: 340 μm, fabric density: 160 g/m²), and polyethylene (PE) fabric (size: 80 mm × 20 mm, fiber diameter: 20 μm, fiber density: 100 × 34 fibers/cm, thickness: 180 μm, fabric density: 120 g/m²) were clipped to an appropriate size, and the frictional coefficient was recorded under conditions of 100 gf weight loading and 120 mm/min sliding speed.

CEDAR POLLEN ADHESION TO FABRIC

PE and cotton fabric circles 13 mm in diameter, coated with the above-mentioned polymers, were dried *in vacuo* at 50℃ for 5 hours, followed by equilibration at 25℃ under 50% relative humidity for over 3 hours. Then, they were fixed on the lid of a 6-well tissue culture plate with double-sided adhesive tape. Ten mg of cedar pollen was applied into each well of an antistatic-treated culture plate, and the lid was fixed firmly in place and sealed with a tape. The sealed plates were horizontally shaken at 1000 rpm for 1 minute using an MS-1 Mini shaker (Ika-Werke GmbH & Co., KG, Germany). Fabric samples without polymer coating were used as the control. The adhesion level of cedar pollen to PE or cotton fabric was counted at 0 (3 minutes), 3, 6 and 24 hours after coating with PQ-65, as described above.

A non-woven fabric from a mask for pollinosis sufferers (Unicharm Co. Ehime, Japan) was soaked in 0.1% PQ-65, 0.1% AP and 0.1% CP aqueous solution, and dried. Thirty mg/cm² of cedar pollen was applied to the fabric samples in glass test tubes, and the tubes were shaken at 1500 rpm for 1 minute. Samples were carefully removed and soaked in phosphate-buffered saline to dissolve adhering protein. The amount of Cry j1 in the extract was measured with a Cry j1 ELISA system. Fabric samples without any polymer coating were used as the control.

CEDAR POLLEN ADHESION TO HAIR

Non-bleached hair fibers (50 fibers, human female adult) were cut to approximately 10 cm in length, washed with a nonionic detergent and rinsed with deionized water. After incubation at 25℃ under 50% relative humidity for over 3 hours, the hair samples were coated with the above-mentioned polymers and fixed on the cap of a 1 L polypropylene bottle so that they hung within the bottle. Then 200 mg of cedar pollen were added to each bottle, and the bottle was shaken 20 times. The hair samples were carefully removed without allowing them to touch the wall of the sample bottle. In case of examination of wax-treated hair, 10 *g* of hair-wax was applied to the hair samples, followed by coating with PQ-65, and then the cedar pollen adhesion test was conducted in the same way as described above.

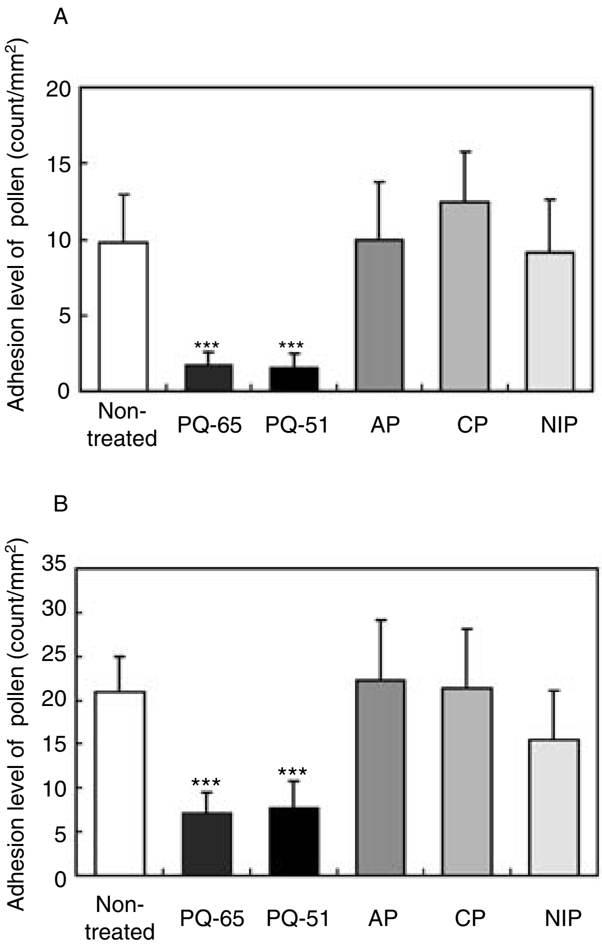


Fig. 1 Effect of polymer coating on the adhesion level of cedar pollen to polyester fabric (**A**) and cotton fabric (**B**). The data are represented as means ± SD of samples. $N = 9$, Mann-Whitney test, ***: $p < 0.001$, *vs*. non-treated.

CEDAR POLLEN ADHESION TO HUMAN SKIN

The subject of this study was a healthy male volunteer who had given informed consent to participate. The skin area used for the experiment was the inner side of the forearm. This was washed twice with soap and water, then held in an environment at 25℃ under 50% relative humidity for over 30 minutes. Next, 50 μL of PQ-65 was sprayed on the test area. Cedar pollen (5 mg) was applied to the skin, and the excess cedar pollen was removed with a rubber blower. A skin area without polymer coating was used as the control.

EVALUATION OF THE ADHESION LEVEL OF CEDAR POLLEN

High-magnification images of sample surfaces were taken with a video microscope (VMS, VH-8000, Keyence, Tokyo, Japan) in order to count adhering pollen particles.

SANDWICH ELISA FOR CRY J1

ELISA for Cry j1 was carried out essentially according to the method of Sawatani *et al.*[12] Ninety-six-well polystyrene micro plates (Costar®, Corning Inc., NY, USA) were coated by incubation overnight at 4℃ with 0.5 μg/mL of rabbit anti-Cry j1 IgG fraction (Hayashibara, Okayama, Japan) in 0.1 M sodium carbonate buffer, pH 9.7. The plates were washed three times with Tris-buffered saline-0.05% Tween 20 (TBS-T, pH 7.4), and remaining protein binding sites on the plates were blocked by incubation with Block Ace (Dainippon Seiyaku, Osaka, Japan) at 37℃ for 1 hour. Samples and standards in a volume of 100 μL were applied to the wells, in duplicate, and the plate was incubated for 2 hours at room temperature. The wells were washed, and 500 pg/mL of horseradish peroxidase-conjugated mouse anti-Cry j1 IgG (Hayashibara, Okayama, Japan) was added to each well. Incubation was continued at 37℃ for 2 hours. Bound peroxidase was determined using 100 μL of TMB One solution (Promega, Madison, WI, USA) as the substrate. The color reaction was developed for 5 minutes, and then stopped by the addition of 100 μL of 1 M HCl to each well, and the absorbance was monitored at 450 nm using a micro plate reader.

STATISTICAL ANALYSIS

The data were analyzed by means of Student's *t*-test or the Mann-Whitney test, and *p* values less than 0.05 were considered to indicate significant differences.

RESULTS

REDUCTION OF ELECTROSTATIC CHARGE AND FRICTION BY LP COATING

The electrostatic charge of PS films charged with PVDC or nylon was significantly decreased by PQ-65 coating compared with the control (Table 2). Frictional coefficients of both cotton and PE samples coated with PQ-65 were significantly lower than the control (Table 3). These data indicated that different types of materials had been successfully coated with PQ-65.

REDUCTION OF THE ADHESION LEVEL OF POLLEN TO PE AND COTTON FABRICS BY LP COATING

The adhesion level of pollen to the untreated PE sample was lower than that to the untreated cotton sample, which is consistent with the difference of their frictional coefficients. We next carried out experiments to examine whether LP coating could reduce the adhesion level of pollen to PE and cotton samples. In both cases, the adhesion level to PQ-51- or PQ-65-coated samples was significantly decreased compared with the control (Figs. 1A, 1B). On the other hand, the adhesion level to AP-, CP-, or NIP-coated samples was not significantly different from the control. Representative VMS images confirming the reduction of

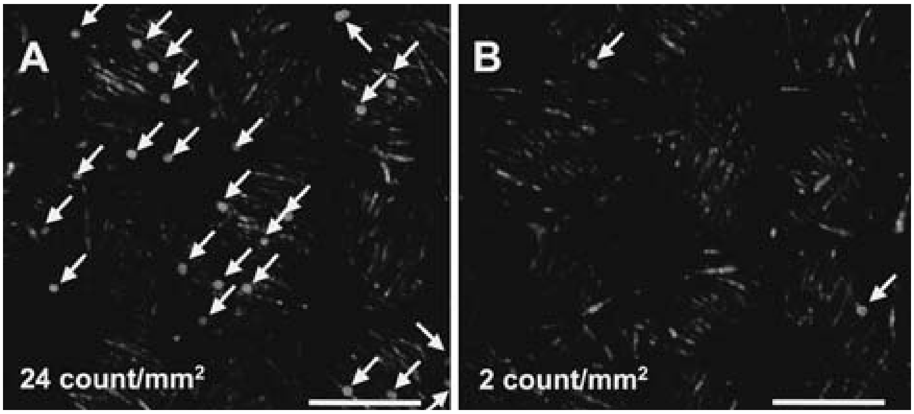


Fig. 2 Video microscope images of cedar pollens adhering to non-treated (**A**) and PQ-65-treated (**B**) cotton fabric. Scale bars represent 250 μm. Arrows show cedar pollen grains.

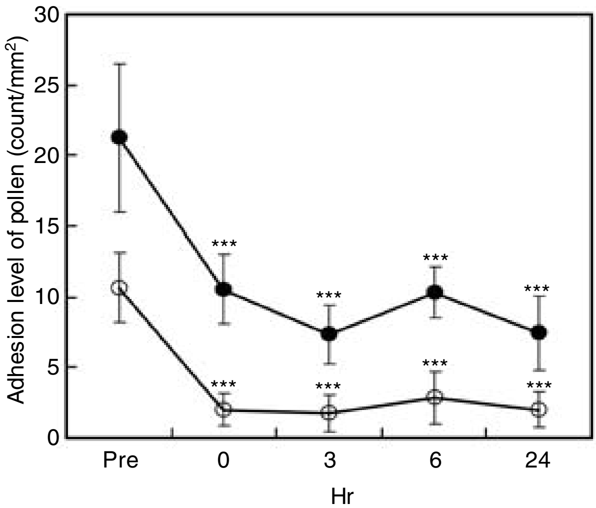


Fig. 3 Time course of adhesion level of cedar pollen to cotton (-●-) or PE (-○-) fabric coated with PQ-65. The data are represented as means ± SD. $N = 9$, Student's *t*-test, ***: $p < 0.001$, *vs*. non-treated.

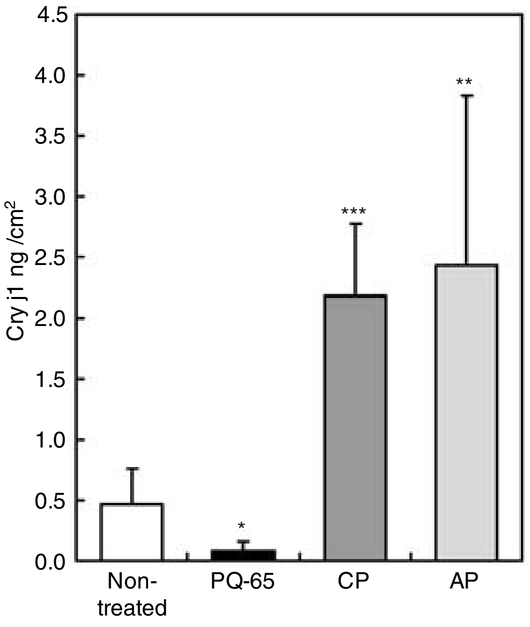


Fig. 4 The adhesion level of Cry j1 to nonwoven fabric for masks. Cry j1 was detected by sandwich ELISA. The data are represented as means ± SD of samples. $N = 5$, Student's *t*-test, ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, *vs*. non-treated.

cedar pollen adhesion to the PQ-65-coated cotton sample are shown in Figure 2. Next, we quantitatively evaluated the effect of PQ-65 coating on the adhesion level of pollen to cotton or PE fabric. In both cases, the adhesion level of the PQ-65-coated samples was significantly decreased compared with that of the untreated samples (Fig. 3). Furthermore, no remarkable change in adhesion level was observed during 24 hours after coating.

REDUCTION OF THE ADHESION LEVEL OF CRY J1 TO A POLLINOSIS MASK BY LP COATING

Next, we evaluated whether LP coating could reduce the adhesion level of pollen to the non-woven fabric of a mask for pollinosis sufferers, by determining the Cry j1 content. The level of Cry j1 on a pollen-exposed, PQ-65-coated non-woven mask fabric sample was significantly decreased compared with the control. On the other hand, the adhesion level of pollen to a CP- or AP-coated mask fabric sample was approximately 4-fold higher than the control (Fig. 4).

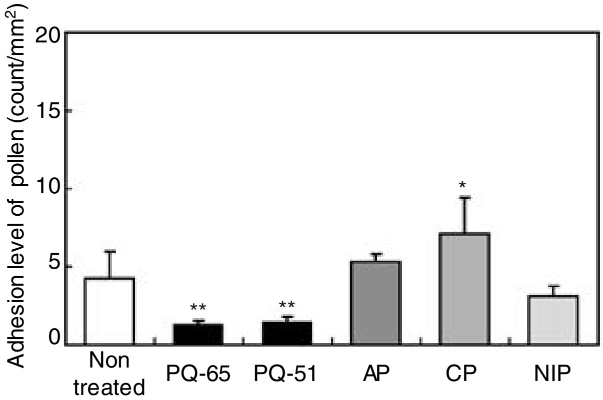


Fig. 5 Effect of polymer coating on the adhesion level of cedar pollen to human hair. The data are represented as means ± SD of samples. $N = 5$, Student's *t*-test, **: $p < 0.01$, *: $p < 0.05$, *vs*. non-treated.

REDUCTION OF THE ADHESION LEVEL OF POLLEN TO SKIN AND HAIR WITH LP COATING

The adhesion level of pollen to PQ-51- or PQ-65-coated hair was significantly reduced as compared with the control, whereas AP, CP, or NIP coating produced no remarkable changes in the adhesion level as compared with the control (Fig. 5). In the case of hair treated with a hair-wax, the inhibitory effect of PQ-65 coating on the adhesion of pollen was confirmed on VMS images (Fig. 6).

Similarly, pollen adhesion to PQ-65-coated skin was obviously reduced compared with that to non-treated skin (Fig. 7).

DISCUSSION

In the present study, we have shown that LP coating can reduce the adhesion of cedar pollen to fabrics and to the human body. This is the first report dealing with the reduction of pollen adhesion to the human body by application of a chemical barrier.

Fukuda and Flowere[13] reported that a PS film could be readily charged electrostatically, compared with other fabrics; the surface of PS was negatively charged by rubbing with PVDC, and positively charged by rubbing with nylon.[14] Therefore, we chose PS film to evaluate the effect of LP coating on the surface electrostatic charge. We found that LP (PQ-65) -coated PS film was only weakly charged as compared with a non-coated PS sample, after rubbing it with PVDC or nylon to load electric charge (Table 2). Since LP contains both cationic and anionic sites originating from choline and phosphoric acid, respectively (Table 1), it might have an electrical buffering effect, tending to maintain a neutral charge on a surface covered with LP. In addition, LP coating produces smooth surfaces (Table 3). Adhesion of pollen to a rough surface might be relatively easy, while adhesion to a smooth surface may be reduced. Thus by using LP to reduce electrostatic interaction and to increase the smoothness of the surface, we were able to reduce pollen adhesion substantially.

We consider that the reason why a larger amount of pollen adhered to the untreated cotton sample compared with the untreated PE sample is the greater surface roughness of the cotton fabric (Fig. 1). Indeed, the frictional coefficient of the cotton sample was higher than that of the PE sample, and the difference between LP-coated and non-coated PE was rather small (Table 3). The inhibitory effect of LP coating on pollen adhesion was observed on skin and hair, as well as fabric. In addition, a lot of pollen adhered to surfaces with high electrostatic charge, such as non-polymer coated films (Table 2), and the amount of pollen that adhered appeared to depend simply on the absolute value of the electrostatic potential (data not shown).

The electrostatic charge of human hair depends on the material used for combing,[15] and therefore, the ability to neutralize both a positive and a negative charge is considered to be important to prevent pollen adhesion. Cancellation of the electrostatic interaction by LP coating resulted in a marked reduction in pollen adhesion to the hair. Furthermore, Harusawa *et al.*[16] reported that application of a hair wax remarkably increased the frictional coefficient of the hair, and the waxed hair easily captured pollen. It is noteworthy that LP coating after application of a hair wax resulted in striking suppression of pollen adhesion. In addition, the effect of PQ-65 coating persisted for 24 hours after the treatment (Fig. 3), suggesting that PQ-65 coating would actually be effective applied once daily. These results suggest that we could substantially reduce pollen adhesion to hair and clothes by applying LP coating, and thereby reduce the amount of pollen carried into people's houses. This could improve the quality of life of pollinosis patients.

Maeda[17] reported that not only the internal core of pollen, but also the outermost layer of pollen, called the ubisch body, carries Cry j1, which is a major allergen of cedar pollen. Because the ubisch body is very small, there is a possibility that Cry j1 might be inhaled by persons wearing a mask, even if pollen grains could not pass through the mask. Therefore, measurement of total Cry j1 is important, as well as counting the number of pollen particles adhering to the mask. We found that that the amount of Cry j1 adhered to the mask was decreased by coating with LP, suggesting that the risk of inhalation of Cry j1 through the mask would also be reduced.

Platts-Mills[18] noted that pollinosis can be ameliorated by wearing goggles or a mask to protect the eyes, nose, and mouth from pollen during the peak season. The conventional way to avoid pollen inhalation is to capture it with an absorbent.[19] On the other hand, we propose that pollen inhalation might be reduced by coating the surfaces of skin, hair and cloth-

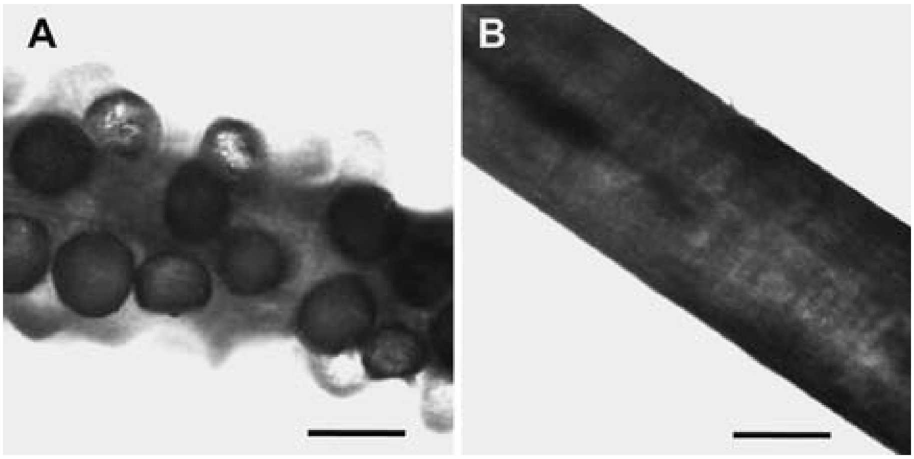


Fig. 6 Video microscope images of adhesion of cedar pollen grains to waxed hair (**A**) and waxed PQ-65-treated hair (**B**). Scale bars represent 5 μm.

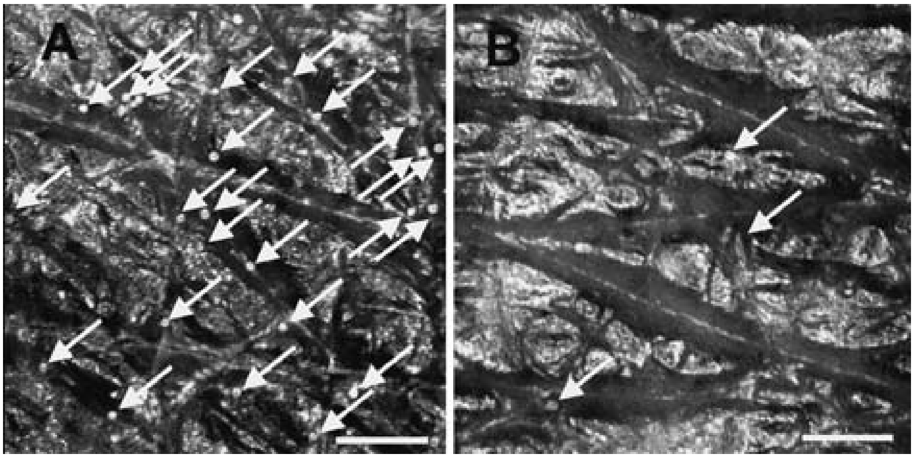


Fig. 7 Video microscope images of adhesion of cedar pollen grains to normal human skin (**A**) and PQ-65-treated human skin (**B**). Scale bars represent 200 μm. Arrows show cedar pollen grains.

ing with LP to reduce adhesion of pollen. LP incorporates lecithin, which is a component of biomembranes,[20] so LP should have good biocompatibility and safety when applied to skin and hair.

In summary, we have demonstrated that LP coating can reduce adhesion of pollen to clothing materials, as well as to the human body and hair. We believe that LP sprays may be useful in the management of pollinosis by contributing to the avoidance of allergen exposure.

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