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Rapid Allergen Inactivation Using Atmospheric Pressure Cold Plasma

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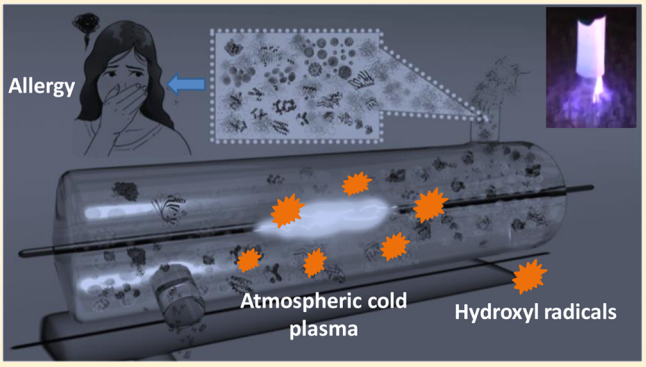
Supporting Information

ABSTRACT: Allergies have become a global problem, and effective control is greatly needed. Here, the inactivation effects of the atmospheric pressure cold plasma (APCP) on aerosolized allergens including Der p 1, Der f 1, Asp f 1, Alt a 1, and Can f 1 as well as those from indoor and outdoor environments were investigated. The effectiveness of the APCP treatment was further studied using blood sera from the allergen sensitized humans. In addition, the allergen samples were also analyzed using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Results revealed that the APCP was highly effective in reducing the allergenicity of both lab-prepared and environmental allergen aerosols. The airborne reductions were shown to range from 30% for Der p 1 to 80% for Can f 1 allergen for 0.12 s exposure. Allergnicity tests showed that the APCP treated Asp f 1 allergens caused 50% less binding with IgEs in the blood sera compared to the control. The observed allergenicity loss was due to hydroxyl radicals produced by the plasma device. The results from SDS-PAGE showed that the plasma treatment resulted in decreased size of the Asp f 1 allergen. The developed technology holds great promise in combating the allergic diseases.



INTRODUCTION

Asthma is one of the leading human diseases[1,2] and the problem continues to emerge.[3] For example, asthma prevalence among children in the United States alone was indicated to have nearly doubled over the last two decades.[4] Among other environmental pollutants, exposure to environmental allergens is shown to play an important role in the development of such an illness. Among the allergens, dog allergen Can f 1, house dust mite allergens (Der p 1 and Der f 1), and fungal allergens (Asp f 1 and Alt a 1) were shown to be associated with the development of asthma in humans, especially for young children.[1,5−9] For instance, dog allergen Can f 1 was shown to have great potential to cause asthma if inhaled by humans into the lower respiratory tract.[10] As one of the dust mite allergens, Der p 1 has been routinely detected in residential dust and indoor air due to disturbance.[6] Extensive experimental studies found that exposure to high levels of allergen Der p 1 can cause airway inflammation and asthma to sensitive people.[11] Long-term exposure to even low and naturally occurring levels of these allergens was indicated to increase the severity of asthma to sensitized asthmatic individuals.[6,11,12] On the other hand, fungal allergens such as *Alternaria alternata* allergen (Alt a 1) and *Aspergillus fumigatus* allergen (Asp f 1) are two of the most common and major fungal allergens which are recognized to cause many allergic diseases to human beings.[13,14] As described in the literature, more than 80% of patients sensitized to the fungi were diagnosed to have IgE antibody to Alt a 1 and Asp f 1.[15,16] For example, *Alternaria alternata* allergen Alt a 1 was shown to be one of major allergens associated with the development of asthma in 6-year-old children raised in a semiarid area.[17] When inhaled, these allergens could enter the blood system and further interact with IgEs present on mast cells, triggering histamine release and causing allergic diseases.[18−20] Accordingly, it is of vital importance to design engineering solutions to abate the allergen exposure.

Traditionally, both physical methods and chemical treatments are employed to reduce or control the allergen exposure/levels. For example, particulate filters such as those installed in the heating, ventilating, and air-conditioning (HVAC) system and the high efficiency particulate air (HEPA) cleaner were demonstarted to significantly reduce the levels of indoor airborne allergens.[21] As another physical approach, no-returning home for nine continuous months for some children was found to not only decrease the allergen-specific IgE levels in their sera, but also decrease late allergen-induced bronchial reactions.[22] In another study, washing bedding by eucalyptus oil has been shown to be effective in

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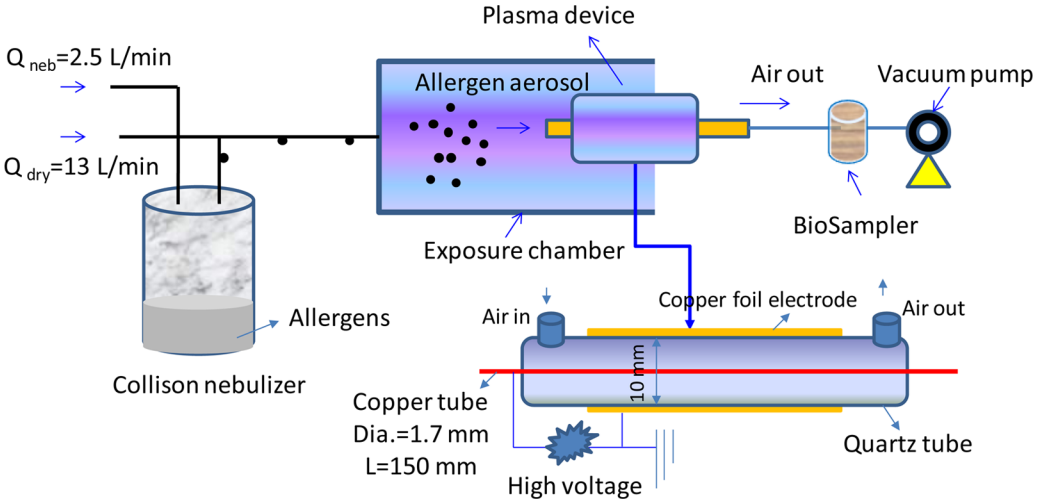

Figure 1. Experimental setup for airborne allergen inactivation by the atmospheric pressure cold plasma (APCP); The APCP was produced at a voltage of 14 kV with a frequency of 10 kHz using an energy level of 28 W.

reducing the house dust mites and their allergens.[23] Although these physical treatments can remove allergens from the air or dust, they do not inactivate them. As expected, chemical treatments were also attempted to control the exposure to indoor allergens. For example, benzyl benzoate, a popular chemical, was usually used by U.S. families and had pronounced reduction of the effects of indoor allergens.[24,25] Tannic acid, another popular chemical, was also utilized to chemically treat the carpets and found to significantly reduce the allergen levels including Der p 1, Der f 1, and Fel d 1.[26] Recently, a study found that using vinyl mattress covers and acaricide in bedrooms and living rooms for an extended time was very effective in preventing the development of asthma in individuals who are considered at high risk to allergic diseases.[27] Despite of these successful applications, possible environmental side effects from chemical treatments await to be further evaluated.

While extensive literature with respect to allergen control by physical or chemical methods exists in the indoor house dust and food industry,[19,28−33] very limited research was conducted in inactivating airborne allergens which are otherwise closely related to the development of allergic diseases through airborne route. To tackle this problem, we previously investigated the effects of microwave irradiation on allergens, which is believed to be the first study of its kind in the literature. While reductions for certain allergens using microwave irradiation were achieved, some enhancing effects took place, for example, with dog allergen Can f 1.[20] In recent years, the atmospheric pressure cold plasma (APCP) has attracted a great attention for inactivation of airborne biological materials due to its high efficiency.[34,35] The APCP is very different from microwave irradiation regarding their generation and inactivation mechanisms. Microwave irradiation inactivates biological molecules by vibrating polar molecules despite controversy about the mechanisms (thermal and nonthermal effects), and in contrast, APCP inactivates biological cells by directly attacking the membranes and damaging DNA and RNA by the radicals, charged particles, and ions. When exposed to plasma cluster ions, dust mite allergen extracts were found to lose about 95% of their allergenicity, and a remarkable decrease in allergenicity was also detected in pollen.[36,37] To our best knowledge, this is only reference in the literature related to plasma ions in treating dust mite allergens. In addition, the APCP has not been used to inactivate airborne allergens. To search for an effective allergen control solution, here we directly exposed various airborne allergens including those from dust mites, dogs, cats, and fungal species to APCP, and their inactivation efficiencies and ability in binding IgEs in human blood sera after the treatments were further explored.

■ MATERIALS AND METHODS

Atmospheric Pressure Cold Plasma Generation and Experimental Setup. In this study, a dielectric barrier discharge (DBD) system designed in our previous study[35] was used to inactivate allergens. Here, the APCP was produced using an energy level of 28 W at a voltage of 14 kV with a frequency of 10 kHz provided by a high voltage high frequency power supply (CTP-2000K, Suman, Nanjing, China) under an ambient temperature of 20 °C. Using the device shown in Figure 1, airborne allergens can be directly exposed to the APCP produced. In addition, we also analyzed the compositions of the plasma produced with room air at different energy levels (20, 24, and 28 W) using an optical emission spectroscopy (OES) via a multi channel fiber optic spectrometer (AvaSpec-2048-8-USB2, Avantes, Eerbeek, The Netherlands). The temperature inside the plasma tube was measured around 60 °C when the device was being operated. Because airborne exposure is generally very short, for example, within seconds; whereas water-borne exposure allowed us to perform extended exposure, for example, up to minutes or hours. In addition, investigation of inactivation mechanisms would require higher doses of allergens, which is often not possible with airborne exposure. Also the sampling device is currently limited to the BioSampler from SKC, which requires 20 mL of liquid for collection, which would significantly dilute the allergen concentration. For these reasons, we have included the water-borne exposure experiments. For water-borne allergen exposure, microhollow cathode discharge via a different device called microjet system as shown in Supporting Information (SI) Figure S1 was used. The detailed description about the device can be found in SS File S1.

Allergens Used. In this work, cat allergens (Fel d 1), dog allergens (Can f 1), *Alternaria alternata* allergens (Alt a 1), *Aspergillus fumigatus* allergens (Asp f 1), and dust mites allergens (Der p 1 and Der f 1) were tested against the APCP

produced. These allergens are found universally present in both natural and built environments, and they represent significant risks of allergic reactions. In addition to the documented health effects from dog and cat allergens, more than 80% of patients sensitized to the fungi were diagnosed to have IgE antibodies to Alt a 1 and Asp f 1 in their blood sera.[15,16,38,39] Accordingly, these allergen types either in airborne or water-borne state were selected to be tested against the APCP, and relevant allergen standards from an ELISA kit (Indoor Biotechnologies Inc., Charlottesville, VA) were used for the experiments. Besides, we have also investigated the inactivation efficiencies of the APCP for naturally occurring airborne Der p 1, Der f 1, Asp f 1, and Alt a 1 allergens in both indoor and outdoor environments in Beijing.

Air Sampler Used for Collecting Allergen Aerosols. In this study, a SKC BioSampler (SKC Inc., Eighty Four, PA) was employed to collect airborne allergens using 20 mL deionized (DI) water (Milli-Q, Millipore, Billerica, MA) at its standard sampling flow rate of 12.5 L/min. The flow rate for the sampler was calibrated using a mini-Buck calibrator (A.P. Buck, Inc., Orlando, FL) prior to sampling. In order to maintain high collection efficiency, 20 min sampling time was chosen in this study according to the manufacturer's recommendation.

■ EXPERIMENTAL PROCEDURES

Allergen Exposure to Cold Plasma and Reaction with IgE in Human Blood Sera. The atmospheric pressure cold plasma was applied to inactivating various allergens including those from dust mites, dogs, cats, and fungal species. For airborne allergen exposure experiments, the allergen standards (Can f 1, Der p 1, Asp f1, and Alt a 1) were aerosolized and directly exposed to the atmospheric pressure cold plasma. The schematic description of experimental setup used for this work is shown in Figure 1. A three-jet Collison nebulizer (BGI Inc., Waltham, MA), operating at a suggested flow rate of 4.2 L/min with a pressure of about 50 psi, was used to aerosolize allergens. The allergens used for the aerosolization were from the same allergen suspensions both for the control and exposed experiments. The aerosolized allergens were further dried and diluted with a pure dry N_2 air flow of 13 L/min. The allergen aerosol flow was further drawn into the exposure chamber and exposed to the cold plasma produced by the DBD system shown in Figure 1 at an output power 24 W. In this work, the control and exposed allergen aerosols were being continuously aerosolized and the control and exposed allergens (with plasma device turned on) were alternatively collected by a BioSampler (SKC Inc., Eighty Four, PA) at its standard flow rate of 12.5 L/min for 20 min from the exposure chamber as shown in Figure 1. The DBD system has a total volume of 0.025 Liter, therefore the airborne allergens exposure time was approximately 0.12 s by calculation. The collected airborne allergen samples were individually poured into 50 L corning tubes and stored at 4 °C for subsequent allergenicity analyses using ELISA assay. For each test allergen, at least three independent repeats were conducted both for control and exposed experiments. The relative humidity of bioaerosol-flow stream was measured around 38% for all experimental conditions tested.

In addition, allergen aerosols in indoor and outdoor environments were also directly exposed to the cold plasma produced by the DBD system shown in Figure 1. The control and exposed allergen aerosols were continuously collected by a BioSampler (BGI Inc., Waltham, MA) for 60 min at standard flow rate of 12.5 L/min. Similar to the lab-generated allergen aerosol inactivation, the exposure time was also 0.12 s. The collected airborne allergen samples were individually stored at 4 ∘C for subsequent allergenicity analyses using ELISA. The relative humidity of indoor and outdoor air was around 35% for all experimental conditions tested. For water-borne allergen exposure experiments, dog allergen Can f 1 and house dust allergen Der p 1 were tested against the APCP. In brief, 5 mL of allergen standard was added to a 15 mL tube (Corning Premium Quality, Acton, MA) with three replicates. Then the allergen suspensions were exposed to the APCP produced using the device shown in SI Figure S1 for varying exposure times (2 and 5 min) at an output power of 28 W. As a comparison, the effects of traditional methods such as ultraviolet (UV) exposure and water-bath heating on the water-borne dog allergen Can f 1 were studied and the procedure was described in details in SI File S2. The allergen samples were further analyzed using standard ELISA method as mentioned above.

Importantly, to further confirm the allergen inactivation the control and plasma-treated water-borne fungal allergen Asp f 1 samples were also tested with IgEs in human blood sera. In this study, blood sera samples from six individuals who were sensitized to Asp f 1 allergens were obtained without their identity information from Jiangsu Centers for Diseases Prevention and Control (JCDPC), Nanjing, China. These blood sera samples were those leftover from one of the completed projects, and reuse of the sera samples did not require an IRB-type permission as informed by the JCDPC at the time of their delivery. As described in our previous study,[20] 75 μL control or plasma-treated water-borne Asp f 1 allergens (0.2 ng/mL) at 24 W for 2 min were incubated with 25 μL human blood sera (prediluted 5 times) for 1 h. Then, a radioallergosorbent test (RAST) method was used to analyze the mixtures of human blood sera and allergen samples following the procedure described in our previous study[20] and below.

Analysis of Allergen Samples Using ELISA,RAST and Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) Methods. A sandwich enzyme-linked immunosorbent assay (ELISA) kit (Indoor Biotechnologies, Charlottesville, VA) was used to quantify both the waterborne and airborne allergens. In this work, standard series from 200 to 0.5 ng/mL were prepared using the PBS-T containing 0.05% Tween 20. As described in our previous study,[20] 100 μL volumes of standards with doubling dilutions (from the ELISA kit) in duplicate, allergen samples in triplicate, negative controls (PBS-T containing 0.05% Tween 20) were pipetted into precoated wells in a 96-well microplate (NUNC, Maxisorp Cert., Rochester, NY). All the procedures were followed per the manufacturer's instructions. In addition, we have also studied the recoveries of house dust mite allergens (Der p 1 and Der f 1), *Alternaria alternata* allergen (Alt a 1) and *Aspergillus fumigatus* allergen (Asp f 1) when spiked into the sterile DI water treated by the APCP produced by microjet device shown in the SI Figure S1. The microplate was finally placed inside a spectrophotometer, SpectraMax (Molecular Devices, Inc., Sunnyvale, CA), and the optical density was measured using an end point assay at 405 nm after 25 min color development. The inactivation efficiency of cold plasma, R, was measured using the following equation:

$$R = (1 - C_{\text{allergen_exposed}}/C_{\text{allergen_control}}) \times 100\% \quad (1)$$

where $C_{allergen_control}$ is the allergen concentration without exposure to cold plasma, and $C_{allergen_exposed}$ is the allergen concentration in the samples exposed to cold plasma.

The IgE levels of human blood sera samples (without any allergen, mixed with the control or the plasma exposed waterborne allergen) were quantified using the RAST method. Human allergen specific IgE ELISA blood assay from ALerCHEK, Inc. (Springvale, ME) was used for the analysis following the steps of the manufacturer's instructions. The precoated strip with Asp f 1 antigen was used in this study. Horseradish peroxidase (HRP) conjugated mouse monoclonal antihuman IgE and TMB/peroxide substrate (ColorburstTM Blue II) were used together to determine the IgE levels in the blood sera samples. The optical density of the final solution was read using the SpectraMax (Molecular Devices) at 450 nm, and IgE levels were normalized for the mixture of human blood sera and allergen samples (control and plasma-exposed).

Here, to investigate the allergen inactivation mechanisms, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was also employed. The gel was prepared using a kit (Real-Times (Beijing) Biotechnology Co., Ltd.). For the electrophoresis buffer, 1 × Tris-Glycine was used with a total volume of 300 mL. Control Asp f 1 concentration was 0.08 ng/mL. The Asp f 1 allergen was treated by the APCP for 3 min at an energy level of 28 W and boiling water for 3 min, respectively. The control and treated Asp f 1 allergens as well as a protein ladder (10−170 kDa) were subjected to SDS-PAGE analysis using a vertical electrophoresis equipment (Beijing Liuyi instrument factory) operated at a voltage of 80 for 1 h. After the experiments, the gel was stained using the silver stain agent (Beijing ComWin Biotech Co., Ltd.).

Statistical Analysis. The experimental data were analyzed by *t* test via SigmaPlot version 10.0 software package, and one-way ANOVA tests. *P*-values less than 0.05 generally indicated a statistically significant difference.

RESULTS AND DISCUSSION

In the effort of searching the allergy mitigation solutions, we previously investigated the use of microwave irradiation for inactivating airborne allergens, however both inhibition and enhancing effects were observed depending on the allergen types.[20] Here, we further investigated the application of the APCP in inactivating various allergen types. The results for the aerosolized allergens when exposed to the APCP for 0.12 s are shown in Figure 2. In this work, the aerosolized allergen concentrations were shown to reach 230 ng/m^3 for Alt a 1 as the highest and 9 ng/m^3 for Der p 1 as the lowest, respectively. As also seen from the figure, the highest inactivation (∼80%) was observed with dog allergen Can f 1, while the lowest (∼30%) was observed with dust mite allergen Der p 1. For two major fungal allergens (Alt a 1 and Asp f 1), the inactivations were shown to be more than 50% as observed in Figure 2. For all allergen types tested, there were statistically significant differences in the allergenicity between the APCP treated and control samples (p-values <0.05; t test). These results imply that different allergen types could have responded differently to the APCP, and such a difference might be resulting from their different forms of protein structures. Can f 1 is the primary allergens secreted from dogs, and its sequence encodes 26 amino acid signal peptide followed by a 148 amino acid protein.[40] For fungal allergens, the majority of the Asp f 1 specific T-cell clones responded to its two peptide fragments which represent amino acid residues 46−65 and 106−125.[41,42]

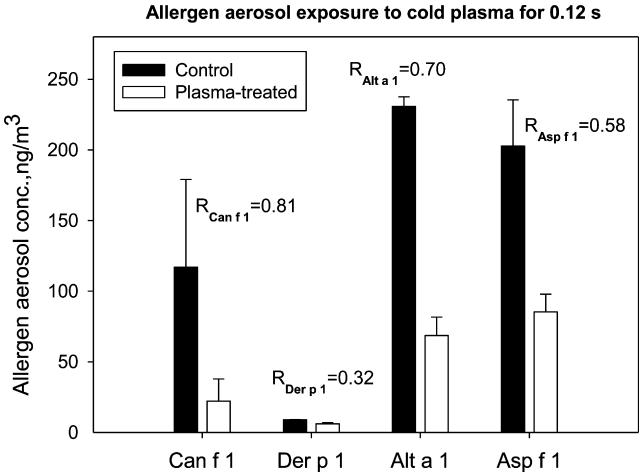

Figure 2. Exposure of lab-aerosolized pure allergens (Can f 1, Der p 1, Alt a 1, and Asp f 1) to the APCP for 0.12 s; data points and error bars represent averages of three independent repeats and their standard deviations; R stands for the allergen inactivation efficiency of the plasma; statistically significant differences were detected for all allergens tested (p-values <0.05 for allergen types; t test analysis).

While for Alt a 1, it is a heat-stable dimer of 28 kDa, which dissociates into 14.5-kDa and 16-kDa subunits under reducing conditions.[43,44] Alt a 1 protein with two subunits of 15 kDa each was shown to interact with IgE antibody only when present in unreduced form, and this suggests that the presence of conformational epitopes specific for IgE antibodies to Alt a 1.[45] For Der p 1 allergens, they are secreted by *Dermatophagoides pteronyssinus* with an N-terminal pro-region which is autocatalytically cleaved upon enzyme maturation under acidic conditions.[46] The pro-region blocks not only the catalytic activity but also conformational IgE antibody binding epitopes.[47] These different surface structures and properties could have reacted differently with the plasma contents produced. Nonetheless, our study showed that exposure of these airborne allergens to atmospheric cold plasma for a very short time could result in their significant reductions up to 80%.

In addition to the aerosolized allergens, we also conducted experiments with airborne allergens present in natural environments. Figure 3 shows the inactivations of major airborne Der p 1, Der f 1, Alt a 1, and Asp f 1 allergens by the APCP which was produced using indoor or ambient air at the energy level of 28 W. As observed from the figure, naturally occurring allergen concentrations can be up to 30 ng/m^3 for Der f 1, 17 ng/m^3 for Alt a 1, 8 ng/m^3 for Asp f 1, and 12 ng/m^3 for Der p 1 in Beijing outdoor environments. As shown in the figure, those in the indoor environment (lab office) were shown to have lower allergen levels compared to the outdoors. The observed dust mite allergen levels here were slightly lower than those previously observed in outdoor environments.[19] Compared to aerosolized experiments, comparable inactivation rates were observed with Der p 1, Alt a 1, and Asp f 1 allergens. For allergen Alt a 1, the inactivation efficiency was doubled up to about 80% in outdoor environments compared to that of the indoor environment. The difference between the outdoor and indoor environments could be a result of different air compositions by which the APCP was produced. Except the Alt a 1 allergen, the inactivation efficiencies ranging from 30% to 65% in both environments were found comparable among the allergen types investigated as shown in Figure 3. For Alt a 1 allergen, the results could be attributed to the differences in Alt

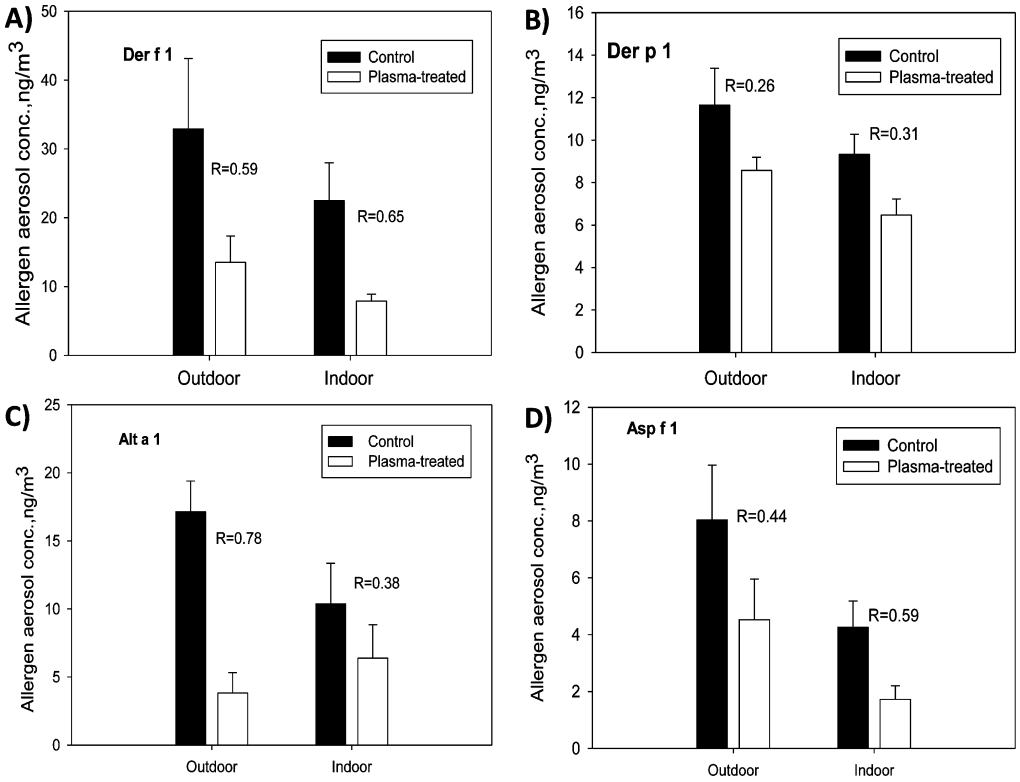

Figure 3. Exposure of indoor and outdoor airborne allergens (Der f 1, Der p 1, Alt a 1, and Asp f 1) to the APCP for 0.12 s; data points represent averages of three independent concentration measurements and their standard deviations; R stands the allergen inactivation efficiency of the plasma; statistically significant differences were detected for all allergens tested (p-values <0.02 for allergen types; t test analysis).

a 1 produced in different environments and also its sensitivity to the differences in plasma produced in different environments. Although much data are available for allergens in the settled dusts, little information is available in their airborne states. The results from this work indicated that outdoor environments might also present significant allergy risks from dust mites and fungi. In addition to pathogens, our work shows that the APCP can also offer an effective airborne allergen control solution.

In our work, we analyzed the compositions of the APCP generated by the device shown in Figure 1 using N_2 gas at different output energy levels (20, 24, and 28 W) using the optical emission spectroscopy, and the results were presented in Figure 4. As observed from the figure, among other contents the level of reactive oxygen species such as hydroxyl radicals (OH) increased with increasing output power. In our work, we used pure N_2 gas (total flow rate of 17.2 L/min (dry flow 13 L/min) and the allergen aerosol flow rate was 12.5 L/min, therefore there was little oxygen available during the plasma production. Accordingly, we did not detect the peak of atomic oxygen at 777 nm or its concentration was below the detection limit. On the other hand, if there were atomic oxygen present, it would have reacted with O_2 to form O_3. The observed OH peak in the OES spectra was due to water content accompanying the aerosolization process. Among the emission peaks we have detected in the OES spectra, only the OH is known to have oxidizing capacity, therefore we believe that OH played a role in the reduction of the allergen allergenicity. The allergen binding sites (epitopes) could have been damaged by OH, thus losing its ability in causing allergic reactions in sensitized individuals. In one study, cold plasma cluster ions were employed to inactivate dust mite allergens, and about 95%

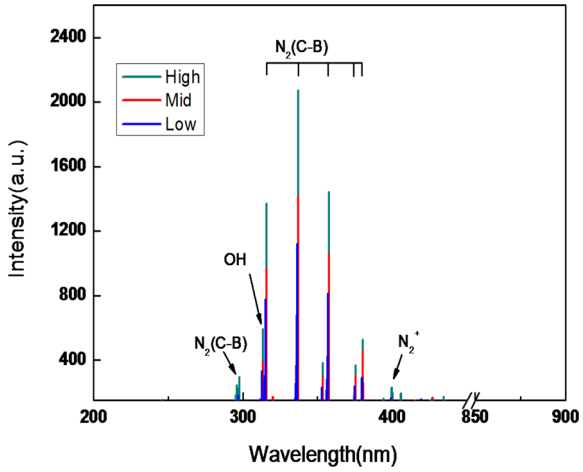

Figure 4. Optical emission spectroscopy (OES) of the APCP produced using pure nitrogen (N_2) gas with different energy levels (20[Low], 24[Mid], and 28[High] watts); higher energy levels produced higher amounts of reactive oxygen species, that is, OH, as indicated in the figure; OH was produced due to the water content accompanying the aerosolization process.

of the epitopes of dust mite allergens was shown to lose their binding ability to IgE in atopic dermatitis (AD) mice sera after exposure to the plasma ions.[36] In addition, they also conducted a mice control study in which they exposed AD mice to plasma ions in an animal cage for two weeks.[36] Compared to the control, those exposed to plasma ions exhibited improvements in AD-like skin lesion.[36] They have attributed the findings to the destruction of allergenic epitopes present on the dust mite allergens by the hydroxyl radicals being formed by the plasma

ions.[36] To extend the plasma exposure time and to study inactivation mechanisms, we investigated the water-borne allergens exposure (up to 5 min) to the APCP produced using the device shown in SI Figure S1. As observed in SI Figure S2, for Can f 1 we obtained an efficiency of 53% for 2 min exposure. Compared to short exposure (less than a second) in their airborne states, the inactivation of Can f 1 was shown to be a little less. However, as observed in SI Figure S2 extending the exposure time to 5 min did not result in further increases in their inactivations (p-value = 0.859). These differences between airborne and water-borne exposure were likely due to the differences in plasma compositions and their die-off rates inside the liquid (water). The plasma exposure time also played a role, but not a linear dose−response relationship. Overall, increased exposure time for water-borne allergens, that is, from 2 to 5 min, did not result in significant improvements of their inactivations (p-values = 0.859 for Can f 1; t test analysis).

As a comparison, we also investigated the effects of traditional inactivation methods such as UV light and water heating on the allergenicity of allergens. As an example, dog allergen Can f 1 was tested with different temperatures and also tested with different UV irradiation times as shown in SI Figures S3 and S4, respectively. As observed in both figures, none of the methods could achieve reductions of dog allergen Can f 1 even with increased temperature up to 100 °C for 30 min (p-value = 0.33) and extended UV exposure time up to 1 h (p-value = 0.38). Despite of its ability to kill spores with extended exposure, the UV has very limited penetration power,[48] which could be the reason for relevant findings in SI Figures S3 and S4. On the other hand, pulsed UV light with 220.8 J/cm^2 was shown to have significant reduction on peanut allergens while the water boiling treatment had little effect.[49] In another study, the pulsed UV was also shown to reduce IgE binding of shrimp allergens, while the water boiling was found to increase the IgE binding.[50] These differences were likely due to different UV dose and allergen types tested. The pulsed UV has greater energy doses and penetration power.[50] In this work, both methods, although generally effective for eliminating pathogens, were shown to have little effect on the dog allergen Can f 1. Nonetheless, only Can f 1 was tested with these two methods, and it is possible that the results for other allergens if treated in a similar manner could vary.

To further confirm the effects of plasma exposure on allergenicity of the studied allergens, we also investigated the binding ability (allergnicity) of the treated water-borne fungal Asp f 1 allergens with relevant IgEs present in human blood sera from six patients who are sensitized to Asp f 1 allergens. Figure 5 shows the reactions of control and plasma-treated Asp f 1 with IgEs present in human blood sera from the patients. Regardless of patients' sera, we all observed an reduced ability of the plasma treated Asp f 1 allergens in binding free IgEs present in the blood sera as shown in Figure 5 (p = 0.05; paired t test). When the human sera were mixed with Asp f 1 allergens without the plasma treatment, the free IgE levels decreased with an average of 48% in the blood sera, up to 70% for patient F as shown in Figure 5. This suggests that those allergens without the plasma treatment reacted more with free IgEs in the human blood samples, thus lowering its levels in the blood samples. However, when the blood sera were mixed with the same Asp f 1 allergens but treated with the plasma the free IgE levels decreased less as observed in Figure 5, having an average of ∼20% for the patients' blood sera. In our airborne exposure experiments, we have observed a reduction rate of about 50% for airborne Asp f 1 when exposed to the APCP for less than a second as shown in Figures 2 and 3. These inactivation efficiencies are directly reflected in our human blood sera experiments with the plasma treated samples. These results indicated that after the cold plasma treatments some Asp f 1 allergens could significantly lose their allergenicity, thus losing its ability to cause allergic reactions in individuals who are sensitized to the allergens. In another study, remarkable decreases both in in vitro and in vivo allergenicities of atomized Japanese cedar pollen extracts after exposed to the plasma ions were also observed, and an inhibition of 80% of the binding to pooled sera IgE from patients allergic to Japanese cedar pollen on ELISA inhibition was achieved.[37] In contrast, microwave-irradiated Asp f 1 did not result in its allergenicity loss against human sera IgEs.[20] The allergen inactivations were further studied using SDS-Page analysis. As observed in Figure 6, after the plasma treatment under the conditions used, the gel bands

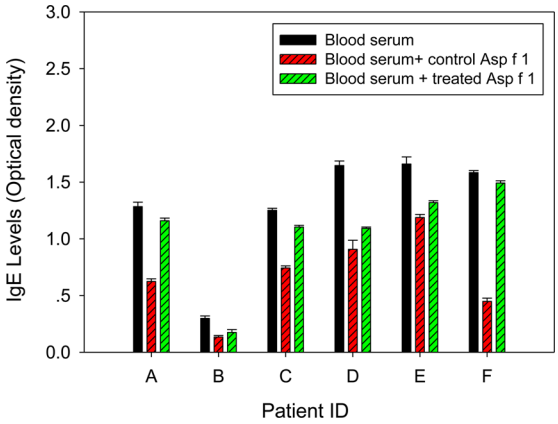

Figure 5. Allergenicity change of water-borne fungal allergen Asp f 1 when exposed to the APCP for 2 min which was produced using indoor air at the energy level of 28 W; high IgE levels (optical density) in the blood sera and mixed samples (y-axis) indicate lower allergen concentrations in the allergen samples that were mixed with blood sera; data points and error bars represent averages and standard deviations from three independent experiments; A−F refer to patient IDs.

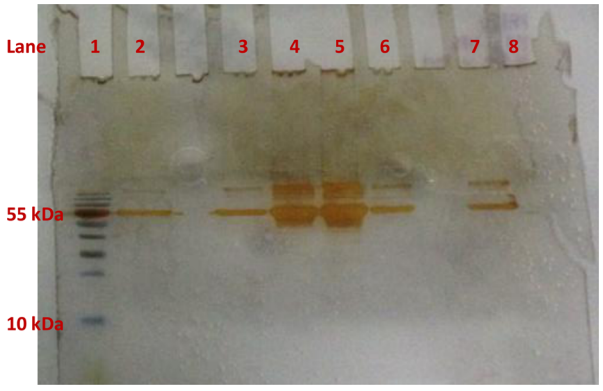

Figure 6. SDS-Page gel image of the plasma and boiling water treated and control Asp f 1 allergen; lane 1-protein ladder (from bottom to the top: 10,15, 25, 35, 40, 55, 70, 100, 130,170 kDa); lane 2-allergen control #1; lane 3-allergen control #2; lane 4-plasma treated #1; lane 5-plasma treated #2; lane 6-boiling treated #1; lane 7-boiling treated #2; lane 8-plasma treated water negative control.

became much wider than those of control and those treated by boiling water (30 min). This has presented direct evidence that after the plasma treatment the allergen protein might have been damaged, that is, the reduced allergen size. In contrast, boiling water treatment did not result in changes to the allergen compared to the control. Our data also indicated that plasma-treated water did not produce any visible gel bands as shown in Figure 6, suggesting no interference from the plasma. From the figure, we can also estimate that the majority of the ASP f 1 allergen we used from the ELISA kit has a molecular size of 55 kDa. The results from this work suggest that the APCP can offer a promising remediation solution for a variety of airborne allergens including those from dust mites, dogs, cats, fungi, and pollens.

Based on the dimension of the plasma device and the flow rate we used, the residence time for the allergen aerosol was about 0.12 s. To our best knowledge, there is no related information about the sizes of the allergens we have tested in the literature. However, in our previous work,[20] we presented SEM images of allergen Asp f 1. According to the image, we can estimate the size of the allergen to be around 2.5−5 μm. By calculation, the electrical drift terminal velocity for the allergen aerosol (given its size of 2.5 μm) is about 0.00016 m/s. Therefore, it takes about 1 min to allow the allergen aerosol particles to settle inside the plasma tube if only the electrical force is exerted. Given the overall residence time (only 0.12 s), it is less likely that the allergen aerosol particles will be deposited solely by the electrical force. Nonetheless, some of the aerosol particles might have been impacted and absorbed by the charged particles produced by the plasma or precipitated by electrical force due to low flow rate, thus possibly leading to their loss to the plasma tube as discussed previously.[51] This can be analyzed by washing the plasma device tube in our future research endeavors. In our work, we also investigated the possible interferences of plasma contents with ELISA assay by mixing plasma treated water with four different allergens (Der p 1, Der f 1, Asp f 1, and Alt a 1). As observed in SI Figure S5, for all allergens no significant differences were detected in the measurements by ELISA (p-value = 0.37; paired t test). These results eliminated the possibility of enhancement or inhibition of ELISA array by the plasma. Therefore, the differences detected in allergen concentration levels between the control and exposed were solely due to the changes of their allergenicity upon the plasma treatments.

Airborne exposure to allergens plays an important role in many human allergenic diseases, and such an impact is likely to increase in the future.[17,27,52−54] Out of other allergen control strategies, this work provides an efficient yet low-cost technology for remediating a variety of airborne allergens both from microbial and animal sources. Use of APCP technology directly in an occupied space is applicable when the space is evacuated. After the treatment, blowing fresh air into the space would immediately diminish the plasma byproducts such as ozone. However, if high energy level is not required for the plasma generation, the ozone level generated could be very low, even comparable to those commonly found in ambient air, which allows the technology immediately applicable even to an occupied setting. Further commercialization of the technology and utilization in living environments would lend a great help in the combat with allergic diseases.

■ ASSOCIATED CONTENT

Supporting Information

Additonal information as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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