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Soft-X-Ray-Enhanced Electrostatic Precipitation for Protection against Inhalable Allergens, Ultrafine Particles, and Microbial Infections

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Protection of the human lung from infectious agents, allergens, and ultrafine particles is difficult with current technologies. High-efficiency particulate air (HEPA) filters remove airborne particles of $>0.3\ \mu\text{m}$ with 99.97% efficiency, but they are expensive to maintain. Electrostatic precipitation has been used as an inexpensive approach to remove large particles from airflows, but it has a collection efficiency minimum in the submicrometer size range, allowing for a penetration window for some allergens and ultrafine particles. Incorporating soft X-ray irradiation as an *in situ* component of the electrostatic precipitation process greatly improves capture efficiency of ultrafine particles. Here we demonstrate the removal and inactivation capabilities of soft-X-ray-enhanced electrostatic precipitation technology targeting infectious agents (*Bacillus anthracis*, *Mycobacterium bovis* BCG, and poxviruses), allergens, and ultrafine particles. Incorporation of *in situ* soft X-ray irradiation at low-intensity corona conditions resulted in (i) 2-fold to 9-fold increase in capture efficiency of 200- to 600-nm particles and (ii) a considerable delay in the mean day of death as well as lower overall mortality rates in ectromelia virus (ECTV) cohorts. At the high-intensity corona conditions, nearly complete protection from viral and bacterial respiratory infection was afforded to the murine models for all biological agents tested. When optimized for combined efficient particle removal with limited ozone production, this technology could be incorporated into stand-alone indoor air cleaners or scaled for installation in aircraft cabin, office, and residential heating, ventilating, and air-conditioning (HVAC) systems.

The simple, involuntary act of breathing constantly exposes the human respiratory system to a host of biological and nonbiological particles capable of causing diseases that range from microbial respiratory infections to allergic reactions that may exacerbate underlying asthmatic conditions. Aerosol transmission has been implicated to various degrees in a number of viral and bacterial infections, including smallpox (1), influenza (2), tuberculosis (3), and anthrax (4). Asthma, a chronic respiratory disease that can be triggered by inhalation exposure to diesel soot and allergens, such as fungal spores, pollen, and pet dander, ranked as the fifth most costly health care expenditure in the United States at \$51.3 billion in 2006 (5). Considering that citizens of developed nations normally spend 87% of their time indoors (6, 7), properly maintaining indoor air quality is clearly an absolute necessity for the protection of public health.

The capture of aerosol particles by filtration is the most common method for air cleaning. High-efficiency particulate air (HEPA) filters, which remove airborne particles of $>0.3\ \mu\text{m}$ with 99.97% efficiency, are generally employed for applications requiring the highest level of particle removal. For example, HEPA filters have been incorporated into hospital air handling systems that service operating rooms where bone marrow transplant (8, 9) and medical device implant surgeries (10) are performed. Over time, however, as particle loading increases, pores can become clogged, resulting in a high-pressure drop across the filter material. This pressure drop, in turn, requires additional energy consumption in order to maintain a consistent airflow rate. Regular filter replacement can alleviate pressure drop concerns, but at a price.

Because of lower power requirements and reduced maintenance costs, electrostatic precipitation (ESP) has established itself as a feasible particulate control alternative. ESPs act by imparting charge on airborne particles which, in the presence of an electric field, are then directed to and deposited on a metallic collection

plate. Corona inception is an operational condition in which a dense cloud of free electrons and positive ions is first established around a discharge electrode (11). In the presence of this corona, noncharged or weakly charged particles that otherwise are inefficiently collected by ESPs become charged as they collide with ambient unipolar ions. An electric field then drives these particles toward an oppositely charged collection electrode, with physical capture occurring upon contact with the inner wall of the ESP. With airflow occurring tangential to the collected material, these devices are characterized by low-pressure drop and, subsequently, have lower power requirement than HEPA filtration. The usefulness of ESP technology in mitigating biological aerosols of $>1\ \mu\text{m}$ in diameter has been demonstrated using bacterial endospores and various bacterial species (12, 13). Other research involving electrostatic precipitation of bacterial cells and spores has focused on survival rates (i.e., bioavailability) after electrical charging as well as sampling efficiency for exposure assessment (14–18).

ESPs have size-dependent collection efficiencies, and while overall mass-based collection efficiencies may be high (e.g., 99%), the collection efficiencies of particles in the submicrometer and nanometer particle size range, the size range associated with viruses and some allergens, are typically low (19–22). In this ESP penetration window (0.1- to $1\text{-}\mu\text{m}$ size range), electrical mobility,

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which is influenced by both particle charge and mechanical mobility, is at a minimum, and consequently, collection efficiency is also at a minimum. At diameters below this size range, diffusion charging becomes more efficient, and particle collection efficiency increases. Similarly, at particle sizes above this size range, field charging becomes more efficient, leading to an increase in particle collection efficiency. The collection efficiency of particles in this ESP penetration window can be enhanced through incorporation of *in situ* soft X-ray irradiation, which increases bipolar ion concentration and direct particle photoionization (19, 23). Soft X-rays generated by the emitter have wavelengths of 0.12 to 0.41 nm, which slightly overlap the range of extreme UV light. At these wavelengths, the soft X-rays have energies of 3.5 to 9.5 keV, which makes them less energetic than hard X-rays (medical diagnostics) but more energetic than the majority of UV light. Hogan et al. (23) demonstrated both enhanced charging and improved collection efficiency of aerosolized bacteriophage MS2. In a follow-up study, molecular microbiological techniques were used to quantify the level of in-flight inactivation of bacteriophages T3 and MS2 passing through a soft-X-ray-enhanced ESP system (24).

This study aimed to apply soft-X-ray-enhanced electrostatic precipitation in a fashion relevant to (i) infectious disease control for a range of microorganisms and (ii) attenuation of airborne allergen concentration. With handling ease and worker safety concerns in mind, agents were chosen to represent a very broad spectrum of particle sizes and morphologies, including surrogates for potential bioterror agents as well as more typically encountered biological particles. Specifically, surrogates for smallpox, tuberculosis, anthrax, and airborne allergens were aerosolized, challenged by the ESP system, and then delivered to murine models in a nose-only exposure chamber. While filter testing or biological air samplers could quantify particle penetration, the use of animal exposures allows for a truer assessment of system protectiveness. Biological indicators of infection, i.e., seroconversion, lung infectivity burden, and mortality, were monitored postexposure to evaluate system effectiveness for prevention of respiratory infections. Airway infiltrations of inflammatory cells such as granulocytes (eosinophils and neutrophils) as well as the presence of cytokines in pulmonary secretions, which mimic outcomes in human asthma (25), were used to assess allergic responses.

MATERIALS AND METHODS

Mice. Specific-pathogen-free mice were used at 6 to 8 weeks of age. A/J and C57BL/6 mice were obtained from the National Cancer Institute-Frederick Animal Production Program (Frederick, MD). All mice received food and water *ad libitum*. All protocols and experimental procedures were approved by the Saint Louis University Institutional Biosafety and Institutional Animal Care and Use Committees.

Cells and virus. BSC-1 cells were obtained from the American Type Culture Collection (ATCC) (Rockville, MD). BSC-1 cells were maintained in Dulbecco modified Eagle medium (DMEM) (BioWhittaker) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 120 $\mu\text{g ml}^{-1}$ penicillin, and 200 $\mu\text{g ml}^{-1}$ streptomycin and neomycin sulfate and grown at 37°C with 5% CO₂. Ectromelia virus, strain Moscow (ECTV-MOS), was derived from a plaque-purified isolate (ATCC VR-1374). ECTV, a member of the *Orthopoxvirus* genus, was used as a surrogate for variola virus, the causative agent of smallpox, and a potential bioterror agent. ECTV was propagated in BSC-1 cells, and purified through a sucrose cushion gradient, and the titers of the virus were determined by viral plaque assay (26), generating a stock concentration of 3.0×10^8 PFU ml⁻¹.

Bacteria. *Mycobacterium bovis* bacillus Calmette-Guérin (BCG), a live attenuated vaccine strain against tuberculosis, was kindly provided by Daniel F. Hoft (Saint Louis University) and used as a surrogate for *Mycobacterium tuberculosis*. BCG was enumerated by growing serial dilutions on sealed oleate-albumin-dextrose-catalase-enriched Middlebrook 7H10 agar plates for 2 to 3 weeks at 37°C with 5% CO₂.

Bacterial endospores. *Bacillus anthracis* Sterne strain 7702 (pXO1⁺ pXO2⁻) was kindly provided by Adam Driks (Loyola University Medical Center). Spores were prepared by the method described by Harwood and Cutting (27). Briefly, the Sterne strain was grown vegetatively in LB medium (overnight at 37°C). Schaeffer's sporulation agar (SSA) plates were then inoculated with these vegetative cells and incubated overnight at 30°C. Isolated colonies from the SSA plates were picked and suspended in Schaeffer's sporulation medium (SSM). Aliquots (200 μl) of this bacterial suspension were spread on SSA plates, which were then incubated for 3 h at 37°C. Following incubation, the SSA plates were flooded with 5 ml of SSM to suspend the cells. This bacterial suspension was used to inoculate 30-ml volumes of SSM at an optical density at 600 nm (OD₆₀₀) of 0.01. These cell cultures were then incubated at 37°C with vigorous aeration (250 rpm). Spores were harvested via centrifugation at $5,250 \times g$ for 10 min in a swinging bucket rotor 20 h after the cell culture reached stationary growth phase. The spore pellets were then washed 5 times with cold deionized (DI) water and pooled to form a spore stock with a final concentration of $\sim 1.7 \times 10^9$ spores ml⁻¹. Finally, the stock was heated at 65°C for 30 min to kill any remaining viable vegetative cells.

ELISA for detection of ECTV-specific antibody. ECTV-specific immunoglobulin G (IgG) levels in sera were determined by enzyme-linked immunosorbent assay (ELISA) using purified ECTV as previously described (28). Briefly, flat-bottom 96-well Immulon 2B ELISA plates (Thermo Scientific) were coated with purified ECTV. Sera were assayed at a dilution of 1:50, and ECTV-specific antibody (Ab) was detected by using horseradish peroxidase-conjugated goat anti-mouse IgG.

Soft-X-ray-enhanced ESP. The soft-X-ray-enhanced ESP used in this study was designed and built in-house and has been described in detail elsewhere (19, 23). Briefly, the ESP consisted of a cylindrical, stainless steel collection electrode (25.4 cm in length and 5 cm in diameter) surrounding a discharge electrode (0.323-mm-diameter stainless steel wire). The collecting electrode was enclosed on the outside by a polyvinyl chloride (PVC) tube to shield against leaking X-rays. A circular hole (2 cm in diameter) was drilled in the middle of the PVC tube and collection electrode through which a fitted soft-X-ray emitter (model L6941-1; Hamamatsu Photonics Ltd., Japan) could irradiate the region inside the ESP. A high-voltage power supply (EL series; Glassman High Voltage Inc., High Bridge, NJ) was used to apply a potential difference between the centrally located charging electrode and the cylindrical collection electrode.

Physical characterization of soft-X-ray-enhanced ESP. (i) Corona inception voltage. A buffer solution (Dulbecco's modified Eagle's medium) was aerosolized in a bioaerosol nebulizing generator (BANG) using compressed air and delivered at 1.5 liters per min (lpm) directly to the ESP system through 3/8-in.-inner diameter (i.d.) flexible Tygon tubing. Physical measurements of total particle concentration were taken at the outlet of the ESP using an ultrafine condensation particle counter (CPC) (model 3022a; TSI Inc., Shoreview, MN) while various potential differences (i.e., applied voltages ranging from 0 to -8 kV) were formed across the discharge and collecting electrodes with and without soft X-ray irradiation.

(ii) Ozone generation. Particle-free compressed air was delivered at 1.5 lpm to the ESP system through 3/8-in.-i.d. flexible Tygon tubing. Ozone concentration was measured at the outlet of the ESP using an ozone analyzer (model 49i; Thermo Scientific) while various potential differences (i.e., applied voltages ranging from -10 kV to +10 kV) were formed across the discharge and collecting electrodes with and without soft X-ray irradiation.

(iii) Particle collection efficiencies. A suspension containing 100 μl each of 50-nm, 100-nm, and 200-nm polystyrene latex (PSL) particles

(Ladd Research, Williston, VT) in 9.7 ml DI water (giving concentrations of $\sim 2.9 \times 10^{13}$, $\sim 3.7 \times 10^{12}$, and $\sim 4.6 \times 10^{11}$ particles/ml, respectively) was aerosolized in a bioaerosol nebulizing generator and delivered at 1.5 lpm to the ESP system which was operated under negative applied potentials (-4 , -5 , -6 , and -7 kV) and positive applied potentials ($+4$, $+5$, $+6$, and $+7$ kV) with and without soft X-ray irradiation. Size-specific particle collection efficiencies of the ESP system were determined using a scanning mobility particle sizer (SMPS) to sample the outlet of the ESP. The particle collection efficiency E_{dp} (percentage) was defined as follows:

$$E_{dp} = 100 \left(1 - \frac{N_{dp,AV,X}}{N_{dp,baseline}} \right)$$

where $N_{dp,AV,X}$ is the number concentration (number cm^{-3}) of particles with diameter dp at the outlet of the ESP for a given applied voltage (AV) and X-ray setting (on or off) and $N_{dp,baseline}$ is the number concentration (number cm^{-3}) of particles with diameter dp at the outlet of the ESP at 0 kV applied voltage and X-ray turned off.

Microbial aerosol exposures. The same general exposure procedure was performed in each of the microbial challenge experiments. A biological suspension was aerosolized using the single-jet BANG with an air flow rate of 1.5 lpm and a liquid flow rate of 0.5 ml/min. The liquid flow and airflow were controlled using a recirculating pump (model QG50/QG50MB; Fluiding Metering, Inc., Syosset, NY) and an automated bio-aerosol exposure system (29), respectively. The biological aerosol generated by the BANG unit was delivered at 1.5 lpm to the inlet of the soft-X-ray-enhanced ESP. Following electrostatic treatment in the ESP, the air stream was then sent to a nose-only inhalation exposure system (NOIES) [CH Technologies (USA), Inc., Westwood, NJ], which was comprised of a series of 12-port stackable manifolds, allowing simultaneous aerosol exposure to multiple mice. The BANG, ESP, and NOIES were connected in series using 3/8-in.-i.d. flexible Tygon tubing. The exhaust of the NOIES was sampled in its entirety using an AGI-4 impinger (Ace Glass Inc., Vineland, NJ) containing 20 ml DMEM (for ECTV) or 20 ml phosphate-buffered saline (PBS) (for BCG and *B. anthracis*) at the manufacturer's recommended flow rate of 5 lpm. ECTV, BCG, and *B. anthracis* exposures were 15 min in duration. All aspects of the microbial challenge experiments, including aerosolization, electrostatic treatment in the ESP, nose-only exposure, and effluent sampling were conducted inside a biosafety level 2 (BSL2) safety cabinet. The collection electrode was wiped with Spor-Klenz (Steris Life Sciences, Menton, OH) after each trial, and the system was flushed with clean, dry air for 5 to 10 min preceding the next trial. Air samples (blanks) were collected following the decontamination process on random occasions to assess carryover contamination, accounting for $\sim 5\%$ of all samples.

ECTV. The ECTV aerosol was generated from a suspension of ECTV in DMEM medium at an initial titer of 5.5×10^6 PFU/ml. The applied voltage regime of -5.25 to -5.75 kV was investigated because of the very low-intensity corona determined to result during soft X-ray irradiation at this setting. Two high-intensity corona conditions (-8 kV; X-ray off and on), in addition to positive and negative controls, were included for comparison.

BCG. The BCG aerosol was generated from a suspension of BCG diluted in PBS containing 0.04% Tween 80 to an initial titer of 2.4×10^7 CFU/ml. C57BL/6 mice were divided into six groups, including positive and negative controls, to investigate a low-intensity corona (-6 kV) and a high-intensity corona (-10 kV) condition with and without soft X-ray irradiation. At 1 h postexposure, whole lungs were extricated, homogenized, and plated in serial dilutions on sealed oleate-albumin-dextrose-catalase-enriched Middlebrook 7H10 agar plates from 5 animals to assess the initial lung burden in the mice.

B. anthracis. The *B. anthracis* endospore aerosol was generated from *B. anthracis* endospores suspended in sterile DI water at an initial titer of 1.7×10^9 CFU/ml. A/J mice were divided into six groups, including positive and negative controls, to investigate a low-intensity corona (-6 kV) and a high-intensity corona (-10 kV) condition with and without soft

X-ray irradiation. At 1 h postexposure, whole lungs were extricated, homogenized, and plated in serial dilutions on LB agar plates from 10 animals to assess the initial lung burden in the mice.

OVA sensitization and aerosol exposure protocol. An acute, ovalbumin (OVA)-induced allergic airway disease model similar to one described by Secor, Jr. et al. (30) was used. Briefly, male C57BL/6 mice were immunized with three weekly intraperitoneal injections of a suspension containing 25 μg of OVA (grade V; Sigma, St. Louis, MO) and 2 mg of aluminum hydroxide in 0.5 ml of 0.9% sodium chloride (Hospira, Inc., Lake Forest, IL). One week after the last injection, the mice were placed in the NOIES and exposed to an electrostatically treated OVA-laden air-stream for 20 min per day for 3 days. A 1% (wt/vol) OVA/DI water suspension was aerosolized using a single-jet BANG as previously described. Mice were divided into 11 groups, including positive and negative controls, to investigate a range of potential differences across the electrodes ($+4$ or -4 [± 4], ± 6 , ± 8 , and ± 10 kV) with and without soft X-ray irradiation. The exhaust of the NOIES was sampled in its entirety using an AGI-4 impinger containing 20 ml DI water at the manufacturer's recommended flow rate of 5 lpm. All aspects of the OVA aerosol challenge experiments were conducted inside a BSL2 safety cabinet with the same decontamination procedures used as described above. The mice were monitored for weight change during the 3-week sensitization period and the 3-day exposure period.

BAL. Twenty-four hours after the final OVA aerosol exposure, the mice were sacrificed by overdose of a ketamine/xylazine cocktail. At the time of sacrifice, the lungs were lavaged *in situ* with three 1-ml aliquots of PBS. The BAL fluid was centrifuged at $200 \times g$ for 10 min. The bronchoalveolar lavage (BAL) fluid supernatant was decanted and stored in cryovials at -70°C for subsequent cytokine analysis. The remaining cells were analyzed via flow cytometry for various leukocyte populations.

Flow cytometry. BAL fluid samples were incubated with 20 μl of appropriately diluted antibodies for 20 min at 4°C . After the cells were stained, they were washed twice with PBS containing 1% fetal calf serum (PBS-1) and fixed with 1% methanol-free formaldehyde in PBS-1, and relative fluorescence intensities were determined via flow cytometry using a fluorescence-activated cell sorter (FACS) (LSR II; BD Biosciences) and analyzed with FlowJo v7.2.2 software (Tree Star, Inc., Ashland, OR). The following fluorescence-labeled monoclonal antibodies were used: CD8a labeled with Alexa Fluor 700 (CD8a-Alexa Fluor 700) (Caltag Laboratories, Burlingame, CA), CD4 labeled with Pacific Blue (CD4-PAC Blue) (RM4-5), CD11b labeled with peridinin chlorophyll protein (CD11b-PerCP) (M1/70), CD19 labeled with phycoerythrin and Cy7 (CD19-PE Cy7) (1D3), CD45 labeled with allophycocyanin (CD45-APC) (30-F11), and GR1 labeled with fluorescein isothiocyanate (GR1-FITC) (RB6-8C5) (BD Pharmingen, San Jose, CA). CD45 profiles, in combination with side-scatter intensities, were first used to differentiate CD45⁺ cells from erythrocytes, epithelial cells, and other debris in the sample. CD45⁺ cells (e.g., macrophages/monocytes, T cells, B cells, and granulocytes) were then separated into distinct populations based on the amounts of CD11b and GR-1 proteins detected on their cell surface (31).

BAL fluid cytokine analysis. The BAL fluid supernatant was allowed to thaw and then concentrated using an Amicon Centriplus YM-10 filtration device (Millipore Corporation, Bedford, MA). Concentrated samples were then analyzed for interleukin 5 (IL-5) using cytometric bead array kits (BD Biosciences, San Jose, CA) according to the manufacturer's directions. The limit of detection was 10 pg ml^{-1} .

Statistical analysis. Where applicable, data were analyzed by one-way analysis of variance (ANOVA) followed by the Tukey multiple comparison test using GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA).

RESULTS

Determination of corona inception voltage and other system characteristics of the soft-X-ray-enhanced electrostatic precipitator. At the baseline condition of 0 kV applied voltage (X-ray off), a broad distribution of particle sizes was obtained which

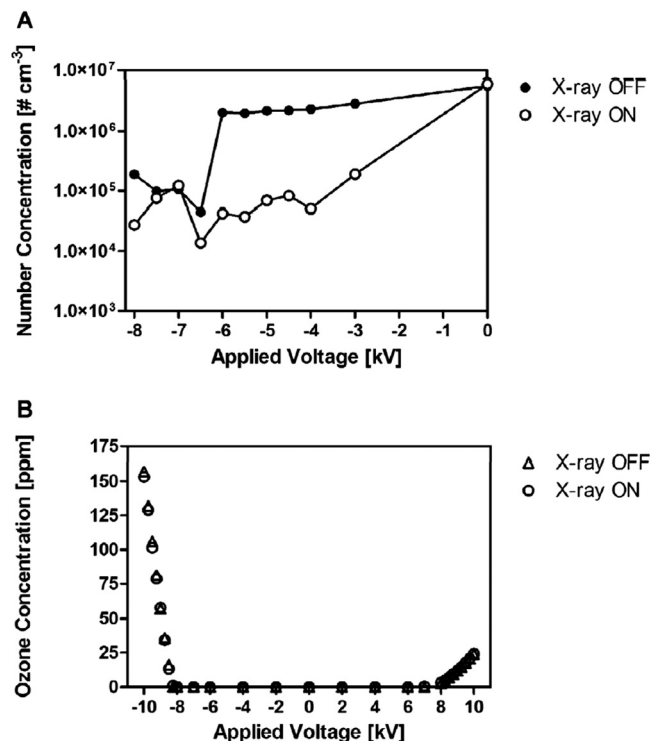


FIG 1 System characteristics of the soft-X-ray-enhanced electrostatic precipitator. (A) Particle number or concentration as a function of applied voltage at the outlet of the ESP. Values are means of triplicate measurements. Error is less than 10% of the mean. (B) Ozone concentration at the outlet of the ESP as a function of applied voltage with and without soft X-ray irradiation. Values are means of triplicate measurements. Error is less than 5% of the mean.

peaked at approximately 3.0×10^5 particles cm^{-3} at a diameter of 75 nm. The increase in corona current and precipitous drop in outlet particle number concentration was evidence of corona inception at approximately -6 kV applied voltage (X-ray off) (Fig. 1A). The corona inception voltage is less clear under conditions when the X-ray was turned on (X-ray-on condition). However, based on the magnitude of reduction in applied voltage shown in previous studies incorporating *in situ* soft X-ray irradiation (19, 23, 24), a corona inception voltage of approximately -5.25 kV would be expected in the X-ray-on condition (Fig. 1A).

Ozone production was evaluated as a function of applied voltage in the presence and absence of soft X-ray irradiation. In general, ozone concentration increased as corona intensity increased at the outlet of the ESP (Fig. 1B). Trace levels of ozone were first detected at applied voltages corresponding to corona initiation (Fig. 1A) and increased as the magnitude of applied voltage increased. Peak ozone concentrations occurred at the highest-intensity corona conditions, with ozone levels reaching 156 ppm at -10 kV, which was more than six times higher than at the corresponding positive applied potential ($+10$ kV). No appreciable difference in ozone concentration was observed with and without soft X-ray irradiation.

To quantify size-specific particle collection efficiencies, a suspension of PSL particles was aerosolized and challenged by the ESP system. We observed a collection efficiency minimum in the 200- to 600-nm size range without soft X-ray irradiation (Fig. 2A and B). Incorporation of *in situ* soft X-ray irradiation enhanced

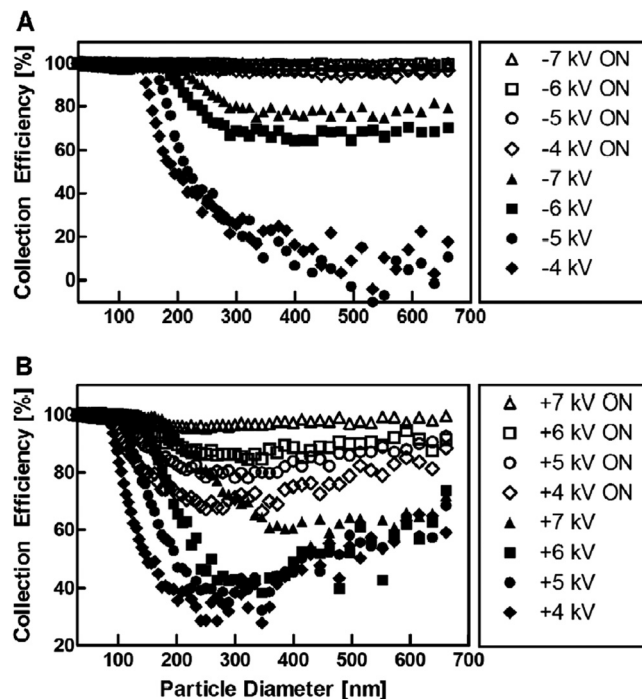


FIG 2 Particle collection efficiency of the soft-X-ray-enhanced electrostatic precipitator when operating at an air flow rate of 1.5 lpm. (A) Negative applied voltages with and without soft X-ray irradiation. (B) Positive applied voltages with and without soft X-ray irradiation. Particle collection efficiency is shown as the mean of triplicate measurements. Error is less than 5% of the mean.

the capture efficiency of PSL particles in this size regime for both negative and positive applied potential conditions (Fig. 2A and B). Capture efficiency was increased 2-fold to 9-fold depending on the magnitude of the applied voltage. Below 200 nm, particles are collected with near 100% efficiency without the aid of *in situ* soft X-ray irradiation due to highly efficient diffusion charging of these particles. Overall, particle collection efficiency was generally higher for negative applied voltages compared to the same positive magnitude.

Soft-X-ray-enhanced electrostatic precipitation protects against viral aerosol infection. To investigate conditions where soft X-ray irradiation was most likely to have the greatest contribution to particle removal, we focused on two applied voltages (-5.25 kV and -5.75 kV) where soft X-ray irradiation actually initiated a very low-intensity corona (Fig. 1A). Because ECTV infection is lethal for A/J mice, mortality was used as a biological indicator of ESP system performance. Mortality as a function of day postexposure is plotted in Fig. 3A. The two X-ray-off cohorts (-5.25 kV and -5.75 kV applied voltage) exhibited 100% mortality by 11 and 15 days postexposure, respectively. The mortality rates in these two groups essentially followed that of the positive-control group, which was exposed to the aerosolized ECTV agent with no electrostatic treatment. Not surprisingly, similar weight loss trends were also observed for these three groups, with nearly 20% loss in body weight precipitating death (data not shown). Incorporation of *in situ* soft X-ray irradiation at the -5.25 and -5.75 kV applied voltage conditions resulted in a considerable delay in the mean day of death of both cohorts, as well as lowered overall mortality rates (cohort mortality was reduced from 100%

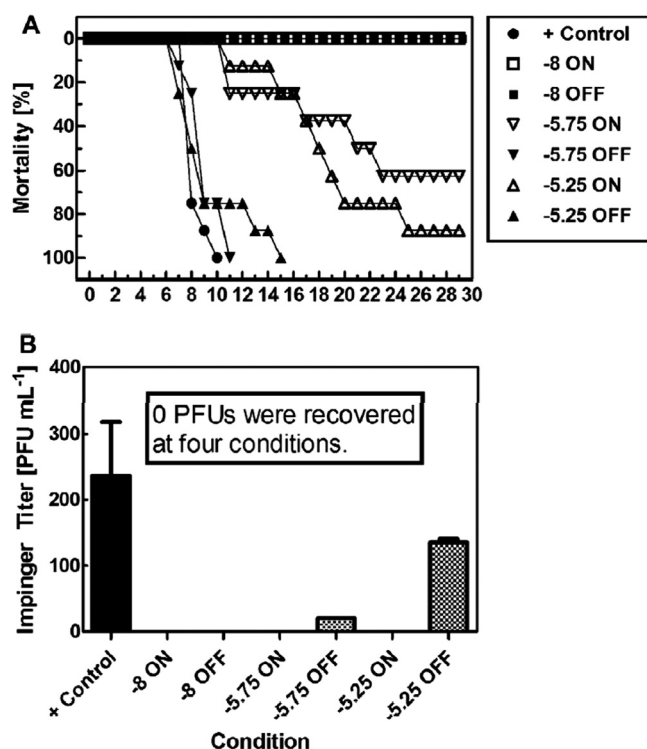


FIG 3 Effects of soft-X-ray-enhanced electrostatic precipitation on an ECTV-laden aerosol. (A) Percent mortality in female A/J mice during the 29-day postexposure observation period. The x axis shows time after exposure (in days). The different conditions are shown (X rays on or off and the voltage). Values are means of 8 animals per group except for the positive-control (+ Control) group ($n = 16$). (B) ECTV concentration in the AGI-4 impinger downstream of the NOIES. Error bars represent standard deviations of duplicate measurements.

to 88% at -5.25 kV and from 100% to 63% at -5.75 kV). No mortality was observed in either of the -8 kV cohorts.

Similar trends were also observed in the number of viable ECTV PFU sampled from the effluent of the NOIES, where viable PFU recovered in the impinger decreased as the corona intensity increased (Fig. 3B). For example, at both the -5.25 and -5.75 kV applied potentials, impinger PFU decreased when soft X-ray irradiation was included in the electrostatic treatment process (which coincided with initiation of very low-intensity coronas). No viable ECTV was recovered from the impinger after ramping up the applied voltage to -8 kV, an act that also increases corona intensity.

Blood serum samples were analyzed from all surviving mice at 29 days postexposure for the presence of ECTV-specific IgG. The high-intensity-corona cohorts (-8 kV, X-ray on and off) exhibited 0% seroconversion (Table 1), suggesting that the ESP system afforded the mice complete protection from the ECTV aerosol under these conditions.

Mycobacterial respiratory infection is preventable using soft-X-ray-enhanced electrostatic precipitation. To demonstrate that the soft-X-ray-enhanced ESP system could effectively remove larger microorganisms, such as bacteria, from an airstream, we challenged the ESP with BCG, which causes a nonlethal infection in C57BL/6 mice. No appreciable difference in weight change was observed between the negative-control and positive-control groups and both of the low-intensity-corona (-6 kV)

groups (data not shown). The mice in both high-intensity-corona (-10 kV) groups did, however, experience a large transient weight loss in the first few days postexposure ($\sim 15\%$ of body weight at -10 kV with X-ray off, $\sim 20\%$ at -10 kV with X-ray on), possibly due to ozone generation; however, this was quickly reversed by 6 days after exposure to parallel weight change in the other exposure groups. Lung infectivity burdens, the primary biological indicators of infection measured in this aerosol challenge, were obtained at 1 h postexposure. Results indicate that only mice in the positive-control group (BCG agent with no electrostatic treatment of the aerosol) had any measurable bacterial entrainment in their lungs ($\sim 1 \times 10^3$ CFU lung⁻¹) at 1 h postexposure. All other plated lung samples were negative for BCG (data not shown). Aerosol measurements of the NOIES effluent also indicate efficient removal of airborne BCG, with impinger samples for the four various corona groups all negative for BCG compared to $\sim 1 \times 10^3$ CFU ml⁻¹ for the positive control (data not shown).

Anthrax spores are effectively removed from an airstream using soft-X-ray-enhanced electrostatic precipitation. To study whether the ESP system could efficiently remove bacterial endospores, we challenged the ESP with *B. anthracis* (Sterne strain) spores, which can cause a lethal infection in A/J mice. Similar to the BCG aerosol challenge, no appreciable difference in weight change was observed between the negative-control and positive-control groups and both of the low-intensity-corona (-6 kV) groups (Fig. 4A). Again, the mice in both high-intensity-corona (-10 kV) groups did, however, experience a large weight loss in the first few days postexposure and then subsequently gained weight in parallel with all other exposure groups. Mortalities were observed only in the positive-control mice (*B. anthracis* spores with no electrostatic treatment of the aerosol), with a 40% mortality rate obtained at 7 day postexposure. No other group exhibited mortality during the 14-day postexposure observation period (data not shown). Lung infectivity burdens obtained at 1 h postexposure (Fig. 4B) indicated that the level of spores deposited in the lungs decreased as the ESP corona intensity increased, with fewer than 16 CFU lung⁻¹ obtained for either high-intensity-corona group. No appreciable difference in lung infectivity burdens was observed with and without soft X-ray irradiation. Aerosol measurements of the NOIES effluent mirrored the lung infectivity burden patterns (Fig. 4C). Fewer CFU were obtained from the impinger samples as the ESP corona intensity increased, with fewer than 5 CFU ml⁻¹ obtained for the high-intensity-corona

TABLE 1 ECTV-specific IgG detection in the sera of surviving mice measured at 29 days postexposure

Applied voltage (kV)	Soft X-ray	Mortality (no. of mice/total no. of mice) (%)	No. of seropositive mice/total no. of mice (%)
-8	On	0/8 (0)	0/8 (0)
	Off	0/8 (0)	0/8 (0)
-5.75	On	5/8 (62.5)	3/3 (100)
	Off	8/8 (100)	
-5.25	On	7/8 (87.5)	1/1 (100)
	Off	8/8 (100)	
0	Off	8/8 (100)	

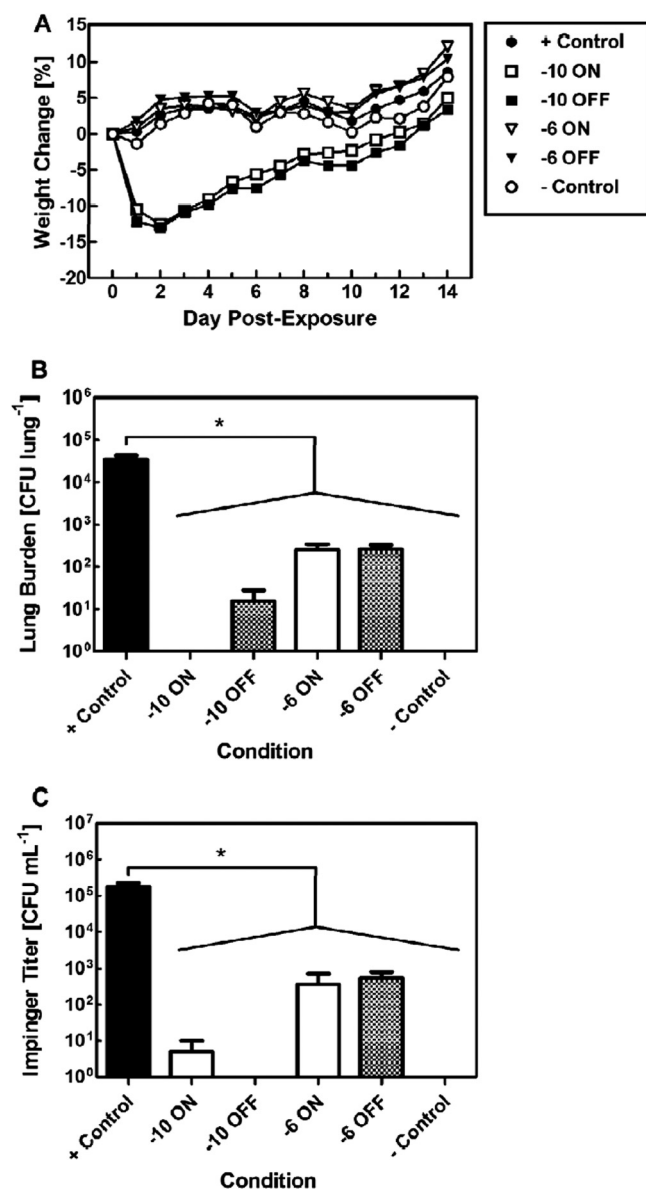


FIG 4 Effects of soft-X-ray-enhanced electrostatic precipitation of a *B. anthracis*-laden aerosol. (A and B) Percent weight change (A) and lung infectivity burden (B) in female A/J mice. Values are means of 10 animals per group. (C) *B. anthracis* concentration in the AGI-4 impinger downstream of the NOIES measured in duplicate. Error bars represent standard errors of the means. Data were analyzed using 1-way ANOVA followed by the Tukey multiple comparison test. Values that are significantly different ($P \leq 0.05$) are indicated by the bar and asterisk. Values for positive-control (+ Control) and negative-control (– Control) groups are shown.

groups. Again, no appreciable difference was observed with and without soft X-ray irradiation.

Airborne allergen concentration is attenuated using soft-X-ray-enhanced electrostatic precipitation. To test the ability of the ESP system to reduce the concentration of airborne allergens, we employed an ovalbumin-induced allergic airway disease (AAD) murine model. Ovalbumin-induced AAD, as defined in this study by the positive-control group, had eosinophil and neutrophil frequencies of 79.3% and 3.4%, respectively, in the bronchoalveolar lavage fluid specimens (Fig. 5A and D). The negative-control BAL

fluid specimens, by comparison, consisted of 0.99% eosinophils and 1.14% neutrophils. Thus, an influx of eosinophils in the bronchoalveolar fluid after inhalation of the OVA allergen was the key parameter detectable via flow cytometric analysis. When the potential applied to the system was set to the mid- and low-intensity corona conditions (–8 kV and –6 kV), the resulting eosinophil frequencies in the BAL fluid specimens reflected that of the negative control where no OVA was inhaled (Fig. 5A). A further decrease in the applied potential to –4 kV (i.e., no corona) resulted in a measurable increase in the eosinophil population in the BAL fluid, indicating OVA penetration through the ESP system with subsequent downstream inhalation in the exposure chamber. This increase in eosinophil frequency at –4 kV was diminished under soft X-ray irradiation (although not statistically significantly different), indicating that some degree of enhanced charging and particle removal was taking place. No other condition exhibited any effect of soft X-ray irradiation. No positive applied potential conditions exhibited elevated eosinophil frequencies (Fig. 5B). Overall, both eosinophil and neutrophil frequencies were lower under positive applied potentials compared to their respective negative applied potentials according to flow cytometric analysis.

Two items of note were observed at applied voltage conditions where ozone exposure was expected to occur (i.e., ± 8 kV and ± 10 kV). (i) A statistically significant ($P \leq 0.05$) influx of eosinophils was observed at the high corona intensity –10 kV condition (Fig. 5A). (ii) The primary cell population in the BAL fluid specimens was neutrophils, not eosinophils (Fig. 5D and E compared to Fig. 5A and B). In an effort to control for these confounding effects, additional exposure experiments were conducted at ± 10 kV applied voltage (X-ray off and on) using an aerosol generated from DI water devoid of OVA particles. Small statistical differences in eosinophil frequencies were found at –10 kV off and +10 kV off conditions compared to their respective “NO OVA” condition (Fig. 5C), as well as in neutrophil frequencies at –10 kV off compared to the “NO OVA” condition (Fig. 5F). Presumably, this is an artifact of low n values ($n = 2$ to 5) and the inherent variability in animal models.

Cytokine IL-5 levels in the BAL fluid were used as a complementary measure to flow cytometric analysis to quantify the physiological response to ovalbumin-induced AAD. The trends closely resembled those in the eosinophil frequency data presented in Fig. 5A to C. AAD-positive mice expressed IL-5 levels of 9.2 pg mL^{-1} , a value 26 times higher than the negative control (see Fig. S1A and S1B in the supplemental material). Elevated levels of IL-5 were detected only in the high-intensity (10 kV) and midintensity (8 kV) corona conditions when operating under negative applied potentials (Fig. S1A). When we ramped down the potential applied to the soft X-ray corona (SXC) system to the low-intensity corona condition (–6 kV), the resulting IL-5 concentration in the BAL fluid reflected that of the negative control where no OVA was inhaled. A further decrease in the applied potential to –4 kV (i.e., no corona) resulted in a small increase in the BAL fluid, indicating potential OVA penetration through the ESP system with subsequent downstream inhalation in the exposure chamber. When a positive applied potential was used, elevated levels of IL-5 were measured only at +0 kV (Fig. S1B). IL-5 concentrations increased by 17.4 and 7.9 times with and without soft X-ray irradiation, respectively, at this high-intensity corona condition compared to the negative control. No significance difference was observed in the IL-5 concentration in the BAL fluid specimens when control-

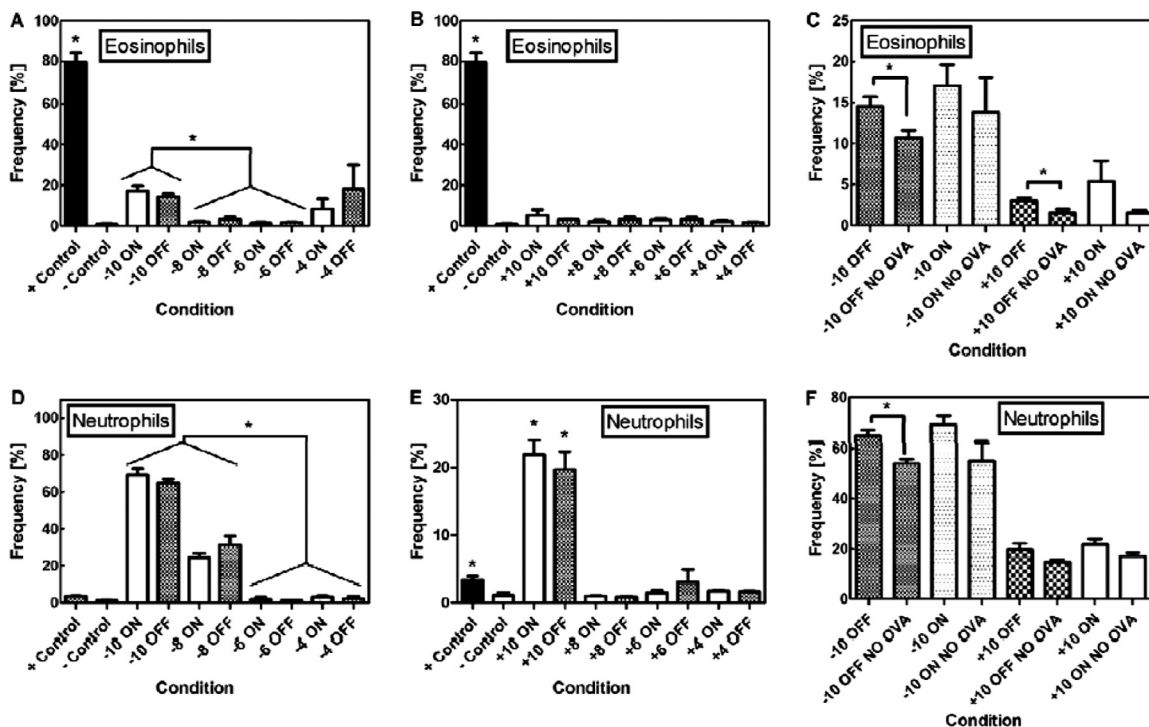


FIG 5 Assessment of allergic response in mice after exposure to an electrostatically treated OVA-laden aerosol. (A to C) Eosinophil frequency in BAL fluid at negative (A) and positive (B) applied potential conditions and while controlling for OVA in the aerosol stream at high-intensity corona conditions (± 10 kV) (C). (D to F) Neutrophil frequency in BAL fluid at negative (D) and positive (E) applied potential conditions and while controlling for OVA in the aerosol stream at high-intensity corona conditions (± 10 kV) (F). Values are means of 2 to 5 samples per group. Error bars represent standard errors of the means. Data were analyzed using 1-way ANOVA followed by the Tukey multiple comparison test. Values that are significantly different ($P \leq 0.05$) are indicated by the bar and asterisk.

ling for the presence of OVA in the aerosol at the ± 10 kV conditions (Fig. S1C).

The increase in neutrophil frequency and IL-5 concentration in BAL fluid specimens at high-intensity (± 10 kV) corona conditions correlated with observed weight loss trends. Similar to the BCG and *B. anthracis* aerosol challenges, no appreciable difference in weight change was observed between the negative-control, positive-control, low-intensity (± 6 kV), and no-corona (± 4 kV) groups (data not shown). Mice in the high-intensity-corona groups (-10 kV off/on and $+10$ kV off/on), however, experienced weight losses of $\sim 26\%$ and $\sim 6\%$, respectively, during the 3-day OVA exposure period beginning at time zero. We observed similar weight loss trends when controlling for the presence of OVA in the aerosol at the ± 10 kV conditions (data not shown).

DISCUSSION

Our study demonstrates the ability of a soft-X-ray-enhanced electrostatic precipitator to efficiently remove airborne biological agents of various sizes and characteristics, thereby preventing respiratory infections and attenuating inhalation-induced allergic reactions. Surrogates for ultrafine particles, smallpox, tuberculosis, anthrax, and airborne allergens were all effectively removed from an airstream with the aid of a corona charger working in conjunction with soft X-ray irradiation.

Soft X-ray effect on particle removal. Previously published data suggest that soft X-ray irradiation enhances the capture efficiency of ultrafine particles in an electrostatic precipitator (19). However, that study investigated only inorganic particles. Therefore, we wanted to verify this soft X-ray effect on surro-

gate organic particles before proceeding with full-scale microbial and allergen exposures. Here, polystyrene latex spheres between 200 to 600 nm in diameter were collected more efficiently using *in situ* soft X-ray irradiation (Fig. 2A and B). Soft X-rays enhance the collection efficiency of particles in this ESP penetration window through an increase in particle charging efficiency brought about by the generation of more positive and negative ions in the headspace of the ESP as well as by direct particle photoionization (19, 23).

From a biological perspective, the submicrometer size class is an important regime for virus and pollen fragment aerosols. ECTV was chosen as a test particle due to its size, which in its native state is 200 by 400 nm (32). In an effort to mimic droplet nuclei generated by a coughing individual associated with aerosol transmission, the ECTV aerosol was generated from a liquid suspension. Enhanced charging and removal of ECTV particles and, hence, lower delivered doses of ECTV to the mice were evident with *in situ* soft X-ray irradiation at low-intensity corona conditions. The pathogenesis of ECTV infection is a series of viral replication and dissemination events that eventually lead to systemic infection (32, 33). With fewer ECTV virions delivered to the lungs at the -5.25 and -5.75 kV applied voltage conditions with *in situ* soft X-ray irradiation, the kinetics of viral replication at the site of infection followed by spread to the regional lymph nodes, bloodstream, and organs such as the spleen and liver would be slowed, thereby accounting for the observed decrease in mortality and overall delay in the mean day of death (Fig. 3A).

With asthma rates on the rise and inhalation of an assortment

of allergens acting, in part, as an asthma trigger, we wanted to test the ability of the soft X-ray corona system to reduce the concentration of airborne allergens. To do so, ovalbumin protein was aerosolized from a liquid suspension (i.e., the same nebulizer as in the microbial exposures), which would most closely represent a high-relative-humidity environment. Busse and Lemanske (34), in their detailed review of the immunology of asthma, explain that the challenge of the airway with allergen increases the local concentration of IL-5, which correlates directly with the degree of airway eosinophilia. Eosinophil frequency in the BAL fluid (attributable to OVA allergen exposure) was reduced in this study prior to the onset of a corona (Fig. 5A) with the aid of *in situ* soft X-ray irradiation, another example of soft X-rays enhancing the charging and collection efficiency of submicrometer particles, which subsequently led to lower exposure doses.

The BCG, *B. anthracis* spores, and a segment of the ECTV and OVA particle distributions were larger than the targeted size regime for soft-X-ray-enhanced charging, and consequently, little effect was observed with the addition of soft X-ray irradiation. As demonstrated in the PSL experiments, the bioaerosol nebulizer used in this study generates an aerosol with a distribution of particle sizes meaning that the particles entering the ESP are often different (i.e., larger) than the native-state size of the biological particles. Relative humidity, drying time, buffer salt concentration, and in-flight agglomeration all play a role in dictating the overall size distribution. Particles the size of bacteria and bacterial endospores, which typically have diameters on the order of 1 to 2 μm , are very efficiently charged in the ESP without the need for X-ray enhancement. For these particles, a mechanism known as field charging, which is governed by the geometry and operational conditions of the ESP, is suitable for this task. Despite falling outside the targeted size regime of soft-X-ray-enhanced charging, BCG and *B. anthracis* were included in the exposure study to lend credence to the assertion that the soft X-ray ESP system is capable of removing biological particles across all size regimes.

Overall, the contribution of soft X-ray irradiation was also less evident as corona intensity increased due to the overwhelming effects of ions generated by the corona itself. At the high-intensity corona conditions, where ion concentrations can approach 10^6 to 10^9 cm^{-3} (11), nearly complete protection from viral and bacterial respiratory infection was afforded to the murine models for all biological agents tested. In general, as corona intensity increased, the primary indicators of infection (mortality and lung infectivity burden) decreased.

Confounding effects of ozone generation. The soft X-ray corona system through which these particles are traveling produces a localized plasma, or region of ionized gas, in the immediate vicinity surrounding the stainless steel wire (or discharge electrode). These ions then attach to or interact with gas molecules generating reactive species (35), including O_3 and various radicals. In addition to charging the biological agents and removing them in the electrical field, the ions and O_3 in the system may act as both a bactericide and viricide as the ability of O_3 to inactivate microbes is well-established. The amount of viral inactivation occurring in an ESP has been reported for bacteriophages (24). Additionally, studies investigating the effect of reactive species (excluding O_3) from a corona discharge on dust mite and cat allergens have shown that these allergens, which differ structurally for one another, can be destroyed by exposure to the corona discharge prod-

ucts (36, 37). Similar results were obtained with Japanese cedar pollen (38).

Aside from the effluent ozone concentrations reported (Fig. 1B), three pieces of evidence in this study attest to the “reactive” nature within the ESP. First, in the ECTV challenge, only two of the four low-intensity corona conditions (-5.25 kV off and -5.75 kV off) had impinger samples that tested positive for viable ECTV, whereas mice succumbed to infection and either died or seroconverted in all four of these conditions. One could argue that infectious ECTV particles were present in the NOIES but were inactivated downstream during the aerosol sampling process. The 15-min sampling period may have provided additional time for reactive species generated in the ESP to contact the ECTV virions. Alternatively, sufficient virus was present in the NOIES to infect, just not at detectable levels for the infectivity plaque assay. Second, mice in all high-intensity corona exposure groups (i.e., -10 kV) experienced considerable weight loss in the first few days postexposure, despite showing no indication of infection. Last, AAD symptoms such as influxes in neutrophils and increases in IL-5 cytokine levels were present in mice exposed to a mock aerosol devoid of any OVA particles that had been electrostatically treated at high-intensity corona conditions (Fig. 5C and F). O_3 -induced acute lung injury is well-documented in inbred mouse strains (39–41) and has been shown to elevate neutrophil levels in BAL fluid (42), exacerbate eosinophilic airway inflammation (43), and increase the levels of eosinophil survival factors (including IL-5) in BAL fluid (43).

The measured ozone concentrations taken directly from the outlet of the ESP are sufficiently high to pose a human health hazard. For comparison, the National Institute for Occupational Health and Safety-recommended ozone exposure limit for humans is 0.1 ppm (44). When operated under positive applied potentials, however, O_3 production was considerably minimized while still maintaining efficient collection of all particle types. A downstream ozone absorber, such as granular activated carbon, could be incorporated into the instrument design to meet this ozone exposure threshold.

In summary, HEPA performance specifications require removal of airborne particles of $>0.3 \mu\text{m}$ with 99.97% efficiency, which as a target, was met and exceeded by the soft X-ray corona system. As a control device, the soft X-ray corona system is well-suited for removal of ultrafine particles, infectious biological aerosols, and airborne allergens. This advanced proof of concept study has demonstrated effective control of respiratory infections from agents across a broad spectrum of particle sizes and morphologies. The antimicrobial effects of the generated O_3 , in theory, could be harnessed to allow the system to act as a hybrid mitigation technology—combining particle capture with the destruction of the biological activity of captured particles to make system cleaning and maintenance safer compared to a HEPA system. Incorporation of soft X-ray irradiation lowers the corona inception voltage and targets an important size class that traditional electrostatic precipitation inefficiently captures. The prototype investigated here could be easily modified and scaled for installation in HVAC systems in aircraft cabins and residential or commercial buildings. It is a far superior alternative to HEPA filtration, both from a performance viewpoint and operational cost. While flowthrough filtration systems can be designed to capture fine particles, they do not inactivate them and are plagued with very high pressure drops and operational constraints. Optimal system design and operating conditions would have to be explicitly studied, but they would

most likely include a positive-potential-induced low-intensity corona with downstream ozone absorber to provide efficient particle removal while limiting ozone emissions.

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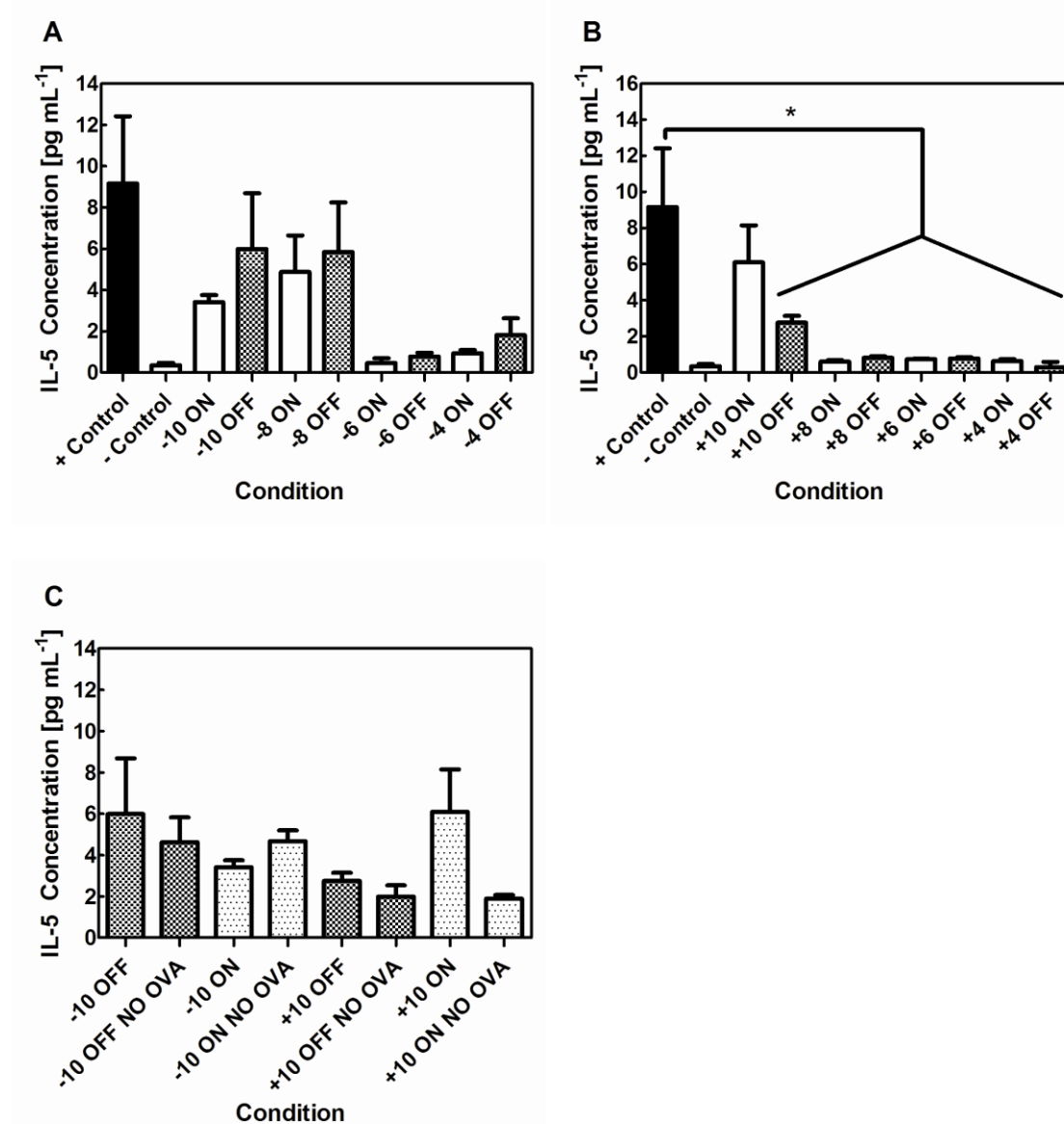


FIG S1. Cytokine IL-5 concentration in BAL. **(A)** Negative applied potential conditions. **(B)** Positive applied potential conditions. **(C)** Controlling for OVA in the aerosol stream at high-intensity corona conditions (± 10 kV). Values are means of $n = 2-5$ samples per group. Error bars represent standard error of the mean. Data were analyzed using 1-way ANOVA followed by the Tukey multiple comparison test, $*p \leq 0.05$.