

# Inhaling to mitigate exhaled bioaerosols

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**Humans commonly exhale aerosols comprised of small droplets of airway-lining fluid during normal breathing. These “exhaled bioaerosols” may carry airborne pathogens and thereby magnify the spread of certain infectious diseases, such as influenza, tuberculosis, and severe acute respiratory syndrome. We hypothesize that, by altering lung airway surface properties through an inhaled nontoxic aerosol, we might substantially diminish the number of exhaled bioaerosol droplets and thereby provide a simple means to potentially mitigate the spread of airborne infectious disease independently of the identity of the airborne pathogen or the nature of any specific therapy. We find that some normal human subjects expire many more bioaerosol particles than other individuals during quiet breathing and therefore bear the burden of production of exhaled bioaerosols. Administering nebulized isotonic saline to these “high-producer” individuals diminishes the number of exhaled bioaerosol particles expired by  $72.10 \pm 8.19\%$  for up to 6 h. *In vitro* and *in vivo* experiments with saline and surfactants suggest that the mechanism of action of the nebulized saline relates to modification of the physical properties of the airway-lining fluid, notably surface tension.**

drug delivery | lung | infectious disease | influenza

It has long been understood that exhaled bioaerosol particles provide an important vector for the spread of certain infectious diseases (1, 2). Viruses known to spread from humans and/or animals through breathing, sneezing, and coughing include measles, influenza virus (3, 4), adenovirus (5), African swine fever virus (6), foot and mouth disease virus (7), varicella-zoster virus (chicken pox) (8), infectious bronchitis virus (9), and smallpox, among others (10). Airborne bacteria include anthrax, *Escherichia coli* (11), *Klebsiella pneumoniae* (12), *Francisella tularensis* (13), and tuberculosis (14). Normal mouth breathing (more than coughing, nose breathing, or talking) has been observed to produce the largest number of airborne droplets (15, 16). These droplets are primarily  $<1 \mu\text{m}$  in size, because larger droplets tend to be filtered out of the expired air by the lungs (16). Given the variable dimensions of common viral and bacterial pathogens ( $\approx 25 \text{ nm}$  to  $5 \mu\text{m}$ ), the ability of exhaled bioaerosol droplets of a given size to carry pathogen obviously varies with pathogen type. Bioaerosols seem to form by the passage of air, during inhalation and exhalation, over the mucus layer lining the lungs (17) or possibly through the reopening of closed small airways, destabilizing the mucus surface through an interplay of surface tension and viscous forces to form small airborne droplets, as has been simulated *in vitro* via “cough machine” experiments (18). In this study, we aimed to explore the ability to transiently diminish the number of exhaled bioaerosol droplets in normal human subjects by delivery of a simple, safe, liquid aerosol. We also aimed to understand the mechanism of the effect of the inhaled aerosol through *in vitro* cough machine experiments.

## Materials and Methods

**Materials.** 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol (POPG) were purchased from Genzyme. Aqueous solutions for aerosoliza-

tion were prepared either as a 0.9% isotonic saline formulation or as a 7:3 wt/wt mixture of DPPC and POPG suspended at 100 mg/ml in 0.9% saline. Mucus mimetics for bioaerosol formation in the simulated cough machine were prepared by adding a small amount of concentrated sodium tetraborate in distilled water solution ( $\text{Na}_2\text{B}_4\text{O}_7$ , 50 g/liter; J.T. Baker) to locust bean gum (LBG) in distilled water mixture (2% wt/vol; Fluka) and mixing for 1 min, pipetting onto the trough mimetic and allowing 30 min for proper crosslinking. Two percent LBG and 2 mM Borax were chosen as representative mucus simulants and were in the literature range of 0.4–2% and 1–3 mM (18). Further information related to materials and methods can be found in the *Supporting Text*, which is published as supporting information on the PNAS web site.

**Exhaled Bioaerosol Measurement System.** A mouthpiece was connected to a Fleisch pneumotachograph with thermister to prevent condensation (model no. 1, Phipps and Bird, Richmond, VA). A pressure transducer (model no. 239, Setra, Boxborough, MA) measured the pressure drop through the pneumotachograph. The signal from the pressure transducer was amplified and converted to a flow profile (LABVIEW software, National Instruments, Austin, TX) for data acquisition and real-time visualization of the patient’s flow signal on an external oscilloscope. The outlet of the pneumotachograph was connected to a sampling “T” adapter. One end of the sampling T was connected to a six-channel optical particle counter (OPC; Climet, Ultimate 100, Redlands, CA) to measure expired particle count and size. Each channel on the OPC tabulates particle counts within a size-selective range for a total of six bins: 0.085–0.1, 0.1–0.15, 0.15–0.2, 0.2–0.3, 0.3–0.5, and  $>0.5 \mu\text{m}$ . The other end of the sampling T was connected to a Delbag-Luftfilter air filter (COPULAR CKL Macropur-F Acelan, GEA, Berlin), which removed any airborne particulates from the inhaled ambient air stream. A physical representation of the system used to assess *in vivo* particle exhalation can be seen in Fig. 5, which is published as supporting information on the PNAS web site.

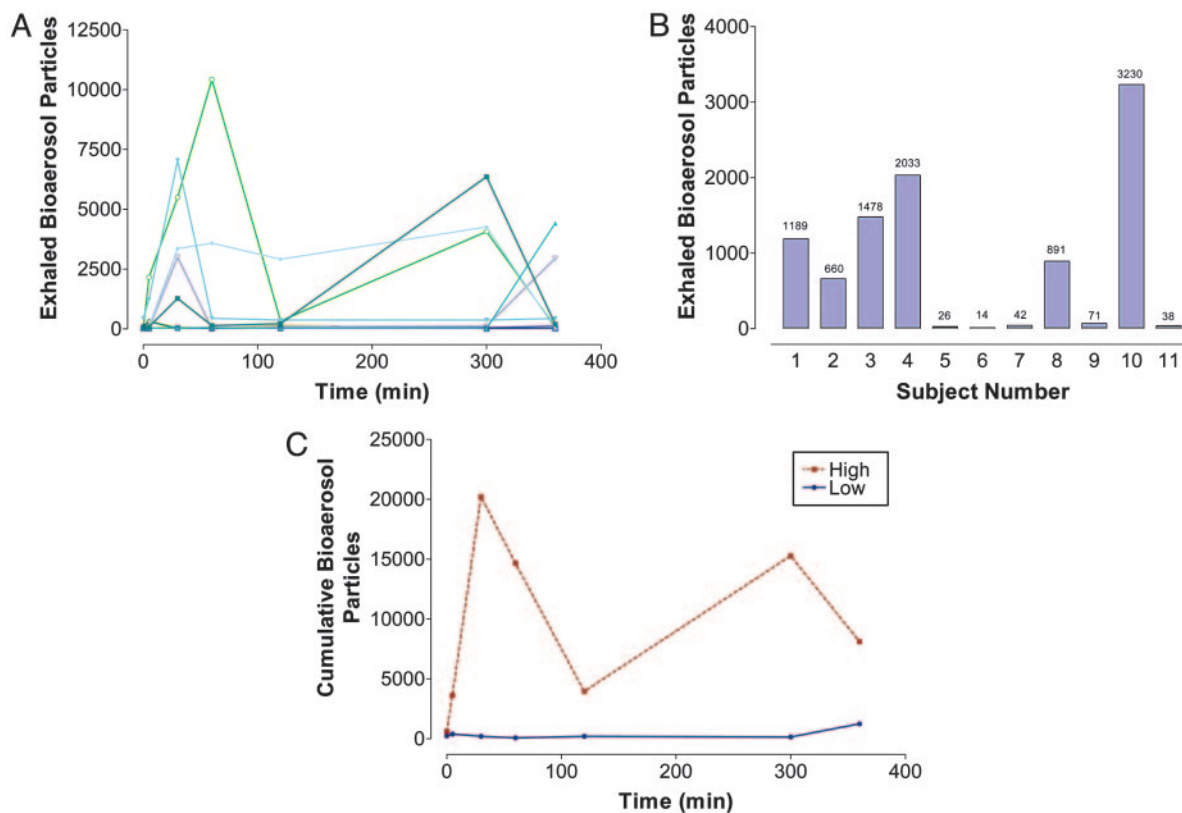
**Human Study.** To test the hypothesis that by transiently perturbing the balance between surface tension and viscous forces acting on or within lung-lining fluid, we might substantially reduce the number of exhaled bioaerosol particles expired during normal mouth breathing, we constructed a device similar to that of Papineni and Rosenthal (16) for monitoring the number and size of particles exhaled by human subjects breathing particle-free air. This device and the clinical trial are further described here. Eleven healthy adult volunteers ages 18–65 with normal lung function [% forced expiratory volume (FEV1)  $>80\%$ ] provided informed written consent and were selected for participation in this crossover placebo-controlled study. Exclusion criteria included history or evidence of significant pulmonary disease (e.g., cystic fibrosis, chronic obstructive pulmonary disease, or severe

Abbreviations: DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; POPG, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol; LBG, locust bean gum.

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**Fig. 1.** Exhaled bioaerosol particles during normal breathing of 11 healthy human subjects. (A) Exhaled bioaerosol particles per liter vs. time for 11 healthy human subjects. Particles of  $>150$  nm were counted in these measurements, after a period of 2-min expiration at each represented time point. (B) Average exhaled bioaerosol particles per liter vs. human subject over the 6-h measurement interval. The average exhaled particle per liter number was obtained by summing the measured particle numbers for every time point during the 6-h period and dividing by the number of time points. High producers are defined as those subjects who expire on average  $>500$  particles per liter. (C) Cumulative expired particles per liter vs. time for the high- ( $n = 6$  subjects) and low-producer ( $n = 5$  subjects) groups. Cumulative expired particles were determined by summing up the expired particles per liter for all individuals of a group at each time point.

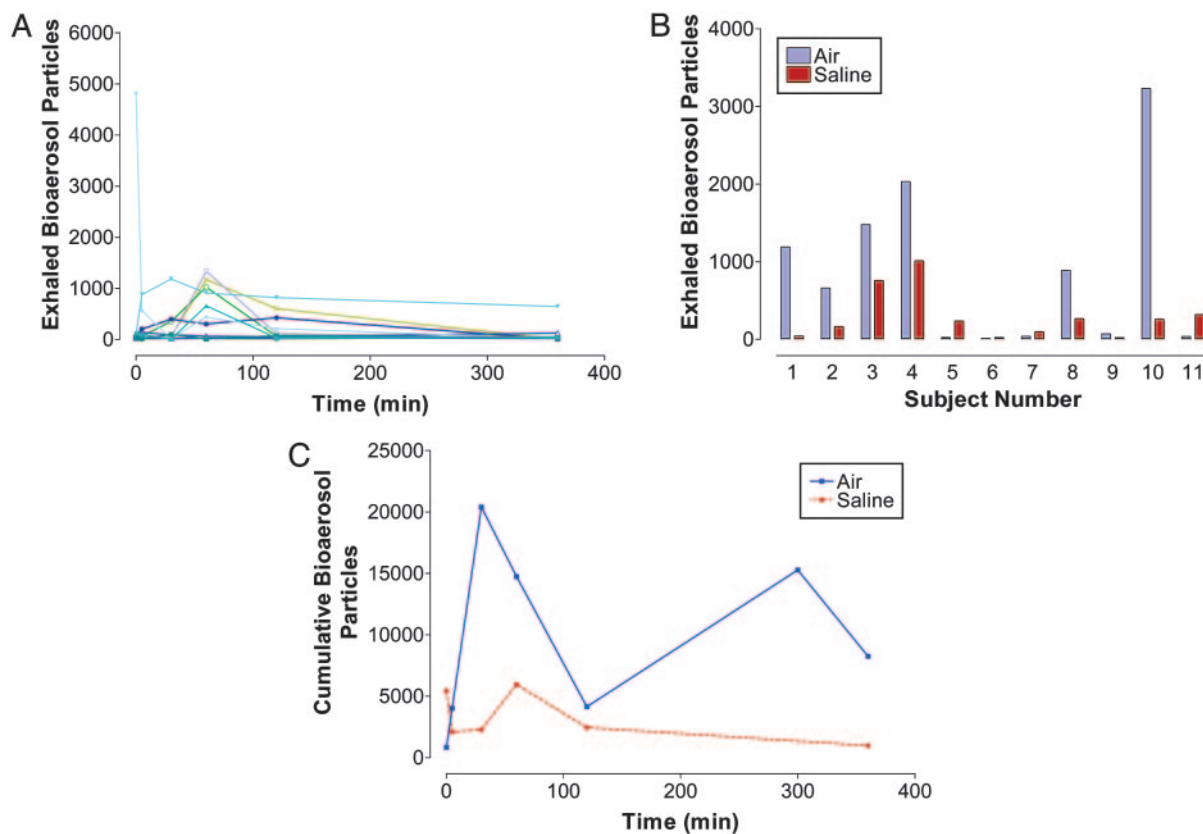
groups: high-producer human subjects, whom we define as those human subjects expiring on average  $>500$  particles per liter over the 6-h interval, and low-producer human subjects, expiring less than an average of 500 particles per liter over the 6-h period. The high-producer subjects ( $n = 6$ ) bear the burden of total bioaerosol production (98.16% of all particles counted) within the human subject group; this is shown graphically in Fig. 1C, which compares the cumulative bioaerosol particle numbers at each time point over the 6-h interval for high versus low producers.

To test our hypothesis that by altering the surface tension properties of the lung-lining fluid we might significantly diminish exhaled bioaerosol in healthy subjects, we next delivered to the same 11 human subjects isotonic saline by nebulization. As with the baseline results of Fig. 1A, we observed considerable inter-subject variability (Fig. 2A). The effect of saline delivery on the average number of expired bioaerosol particles is shown in Fig. 2B on a per-subject basis. High-producer subjects ( $n = 6$ ) responded to saline delivery with a statistically significant drop in average expired bioaerosol particle number ( $-72.10 \pm 8.19\%$ ) relative to baseline. The opposite trend was observed for low-producer subjects ( $n = 5$ ), who expired more particles on average after saline ( $340.35 \pm 181.88\%$ ) relative to baseline. Note that after saline delivery the major change in exhaled particle number was dominated by the high-producer subjects, resulting in a substantial diminishment of cumulative particle count for all 11 subjects combined (Fig. 2C).

To test our hypothesis that modification of the surface properties of the lung-lining fluid underlies the suppression of exhaled bio-

aerosol produced in high-producer subjects, we constructed a cough machine apparatus (see *Materials and Methods*). We delivered a burst of air (12 liters/s for 30–50 ms) over a model mucus layer (formed of 2% LBG crosslinked with sodium tetraborate in distilled water) to simulate a typical cough profile as previously measured by King *et al.* (18). In the absence of nebulized saline, the burst of air destabilized the mucus/air surface to form submicron droplets with volume-averaged median size of  $\approx 320$  nm (Fig. 3A), as measured by a Sympatec (Lawrenceville, NJ) HELOS/KF laser-diffraction particle sizer. After nebulization for 6 min of normal saline through the model trachea/trough of the cough machine, the size distribution of mucus droplets shifted to larger values. Fig. 3A also reveals the mucus droplet size distribution after saline delivery at  $t = 0, 30,$  and  $60$  min, respectively. Median droplet size increased from  $\approx 320$  nm in the absence of saline to  $\approx 1 \mu\text{m}$  with saline at  $t = 0$  min,  $65 \mu\text{m}$  at  $t = 30$  min, and  $30 \mu\text{m}$  at  $t = 60$  min. Given that saline and mucus simulant are relatively immiscible at room temperature, application of saline to mucus simulant produces a thin surface layer of relatively high surface tension [isotonic saline has a surface tension of 72 dyne/cm, which is significantly higher than that reported for mucus and mucus simulant materials (21, 22)]. Isotonic saline also has much lower viscosity in comparison with mucus or mucus simulant. Relatively high surface tension favors the formation of droplets that are relatively large, by equilibrium thermodynamic considerations (23). On the other hand, smaller fluid viscosity tends to favor the creation of smaller droplets (24), suggesting that viscous forces may play a secondary role in the size shift observed in Fig. 3A. Our *in vitro* results may shed important light





**Fig. 2.** Exhaled bioaerosol particles after delivery of isotonic saline during normal breathing in 11 healthy human subjects. (A) Exhaled bioaerosol particles per liter vs. time for 11 healthy human subjects after inhalation of isotonic saline at  $t = 0$ . Particles of  $>150$  nm were counted in these measurements, after a period of 2-min expiration at each represented time point. (B) Average exhaled bioaerosol particles per liter vs. human subject over the 6-h measurement interval for the cases of baseline and saline delivery. The average exhaled particle per liter number was obtained by summing the measured particle numbers for every time point during the 6-h period and dividing by the number of time points. (C) Cumulative expired particles per liter vs. time for all human subjects after inspiration of saline ( $n = 11$  subjects) and of air (i.e., baseline) ( $n = 11$  subjects). Cumulative expired particles were determined by summing up the expired particles per liter for all individuals of a group at each time point.

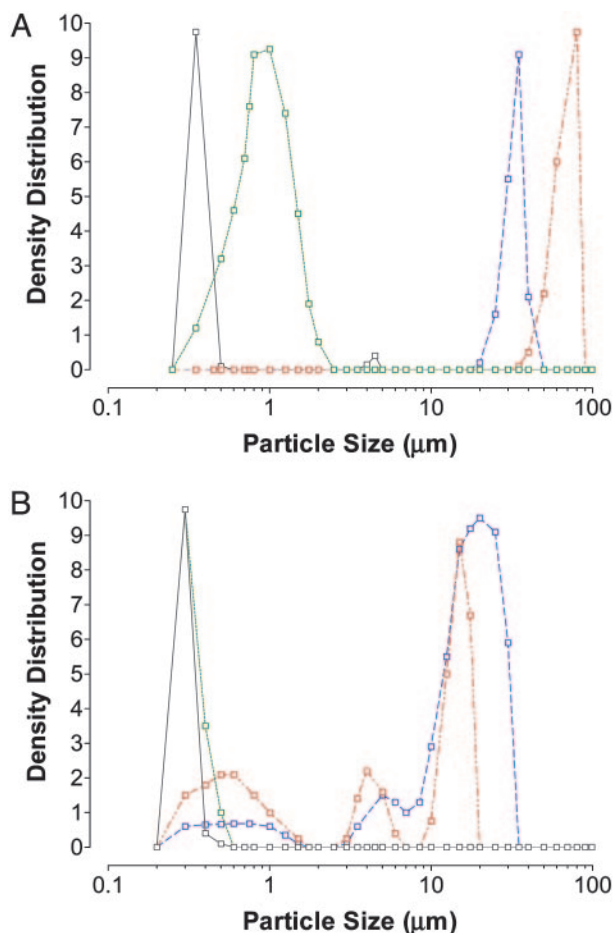
on the differences observed in Fig. 1 between high and low producers and in the response of the two groups to saline delivery, as revealed in Fig. 2. Subjects breathing out relatively large numbers of particles may tend to produce more particles in the nanometer range during respiration than subjects breathing out relatively fewer numbers of particles, possibly because of differences in surface tension of the lung-lining fluid. Delivery of saline to high-producer individuals may thus shift droplet sizes on breakup toward droplet sizes of approximately  $>10$   $\mu\text{m}$ , i.e., to droplets that are effectively filtered by gravity sedimentation and inertia in the respiratory tract (25).

To test our hypothesis that surface tension plays a key role in the diminution of expired bioaerosol via saline delivery to the lungs, we formed a suspension of isotonic saline and lung surfactant phospholipid surfactants DPPC and POPG. The mixture consisted of 7% (wt/wt) DPPC and 3% (wt/wt) POPG in isotonic saline. This mixture exhibited an equilibrium surface tension of  $42 \pm 2$  dyne/cm versus a surface tension of 72 dyne/cm for pure isotonic saline, as measured by a Wilhelmy Microbalance (see *Materials and Methods*). The apparent viscosity of the mixture, as measured by a rotating disk viscometer, was found to be somewhat greater than that of isotonic saline ( $\approx 0.03$  vs. 0.01 poise). We hypothesized that delivery of this surfactant mixture would produce in our *in vitro* and *in vivo* studies surface tensions less than those observed upon delivery of saline alone and therefore have less impact on bioaerosol diminution in high-producer individuals relative to baseline. We based this hypothesis in part on published results (22) that show

surfactant delivery to the trachea of horses produces tracheal surface tension significantly below ( $24.5 \pm 0.51$  dyne/cm) that achieved by delivery of saline alone ( $31.9 \pm 0.54$  dyne/cm). We first nebulized the surfactant mixture into the cough machine apparatus to produce the aerosol profile shown in Fig. 3B. We find that the median droplet size is consistently smaller in the case of surfactant relative to saline delivery, in line with the smaller surface tension of the mixture in comparison with pure saline. This also supported the secondary role of viscosity in the droplet creation process, in that the surfactant mixture displayed a (modestly) greater viscosity than the pure saline.

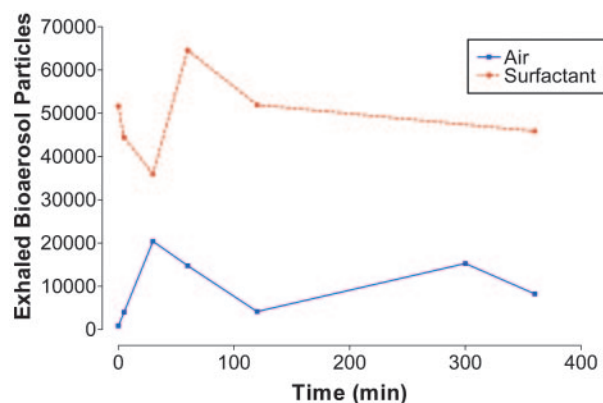
To test our hypothesis in humans, we next delivered to 11 human subjects the mixture of isotonic saline and LS phospholipid surfactants DPPC and POPG. Fig. 4 compares the total number of expired particles for the entire group with baseline particles for the 11 human subjects, revealing that surfactant delivery substantially amplifies exhaled bioaerosol. We observe this amplification in both high and low producers. Thus, surfactant delivery dramatically amplifies bioaerosol particle production among high producers by  $325.79 \pm 172.92\%$  and among low producers by  $5,954.82 \pm 5,447.44\%$ .

The saline versus surfactant results of our human studies combined with our *in vitro* data raise the question of the effect of administered aerosol on the size (and thereby mass) distribution of exhaled bioaerosol. We did not, however, observe any statistical tendency for saline or surfactant to increase the size or mass of exhaled aerosol, possibly because of the filtration capacity of the human lungs, which tend to remove by deposition



**Fig. 3.** Aerosol concentration of particles produced in the *in vitro* cough machine after delivery of isotonic saline or surfactant as a consequence of a burst of air over the simulated mucus. (A) The effect of saline delivery on density distribution of aerosol particles formed after exposure of mucus simulant surface to a burst of air in the *in vitro* cough machine. Four cases are shown: (i) mucus simulant (solid grey line), (ii) mucus simulant immediately after application of nebulized isotonic saline (dotted green line), (iii) mucus simulant 30 min after application of nebulized isotonic saline (dashed-dotted red line), and (iv) mucus simulant 60 min after application of nebulized isotonic saline (dashed blue line). (B) The effect of surfactant delivery on density distribution of aerosol particles formed after exposure of mucus simulant surface to a burst of air in the *in vitro* cough machine. Four cases are shown: (i) mucus simulant (solid grey line), (ii) mucus simulant immediately after application of nebulized isotonic saline (dotted green line), (iii) mucus simulant 30 min after application of nebulized isotonic saline (dashed-dotted red line), and (iv) mucus simulant 60 min after application of nebulized isotonic saline (dotted blue line).

particles in the inertial size range. These observations must nonetheless be considered within the constraints of our particle-sizing apparatus. As pointed out in *Materials and Methods*, the Climet particle sizer accurately measures particle size in the 150- to 500-nm range by classifying particles in the following (unequal) size ranges: 150–199, 200–299, and 300–499 nm. We observed that for all human subjects after administration of air only, saline, and surfactant, the distribution of particle sizes within these three particle size ranges showed no statistical variance with time after administration of air, saline, or surfactant. Dividing the three size bins into eight bins of equal size ranges (i.e., spanning 50 nm) and assuming homogeneous particle distribution in the ranges 200–299 and 300–499 nm, the predominant size of exhaled aerosol particles for all subjects was



**Fig. 4.** Cumulative expired particles per liter vs. time for all human subjects after inspiration of saline ( $n = 11$  subjects) and of surfactant ( $n = 11$  subjects). Cumulative expired particles were determined by summing up the expired particles per liter for all individuals of a group at each time point.

150–199 nm. This did not change after saline or surfactant administration.

In our studies, performed over the course of 3 months (February through April) in the spring of 2004, high-producer subjects tended to remain high producers, as defined here. Thus, the six subjects defined as high producers on the basis of our baseline measurements (Fig. 1) accounted for 98.16% of all expired particles for baseline, 78.15% of all particles in the case of saline delivery, and 62.43% of total expired aerosol in the case of surfactant delivery. Whether high-producer individuals are more prone, barring mitigation of bioaerosol expiration, to spread inhaled infectious diseases such as influenza, tuberculosis, and severe acute respiratory syndrome needs to be clarified by further studies.

### Conclusion

We have found that, among a group of 11 healthy human subjects, two distinct groups of individuals can be identified, the one expiring on average many more bioaerosol droplets than the other. Delivery of isotonic saline can markedly diminish the number of expired bioaerosol particles among the high-producer subjects for up to 6 h after inhalation. *In vitro* experiments suggest that the mechanism of action of the saline suppression of exhaled bioaerosol relates to alteration of the surface properties of the airway-lining fluid, notably surface tension. Thus, delivery of a surfactant solution to the lungs actually magnified exhaled bioaerosol expiration, consistent with *in vitro* experiments that show its effect on droplet formation is unlike that of isotonic saline. Many questions must be resolved by future studies, including those related to the roles of other physical properties of lung mucus, like surface elasticity and surface viscosity, the role of physiological and/or environmental conditions on expired bioaerosol number, and those touching on dose and duration of effect. Recent studies on the characteristics of atmospheric aerosols or those created by nebulizers and inhalation devices have suggested a primary role for surface tension and viscosity in the formation and size distribution of droplets (26–30). Additionally, further investigations into the role of nanoparticles in airborne transmission are warranted. Clearly, the effect of normal saline solution, as well as other inhaled agents that alter surface properties of the lung-lining fluid, on transmission of airborne pathogens needs to be explored further.

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