MOLECULAR FRONTIERS JOURNAL

Nasal Calcium-Rich Salts for Cleaning Airborne Particles from the Airways of Essential Workers, Students, and a Family in Quarantine

David Edwards[1,3], Jonathan Salzman[3], Tom Devlin[3] and Robert Langer[2]

To improve hygiene in the COVID-19 pandemic, we evaluated the intranasal delivery of calcium-enriched saline to suppress breath particles in practical settings reflecting essential worker hygiene, quarantine hygiene, and back-to-school hygiene. In studies with 92 men, women, and children, we observed that nasal salt aerosols lowered exhaled particles for the group by around 75%, and outperformed surgical face masks in the clearing of sub-micron particles.

***Keywords*:** Nasal Saline; COVID-19; Calcium; Exhaled Breath; Respiratory Droplets; Hygiene; Airborne Infectious Disease.

INTRODUCTION

The COVID-19 pandemic has placed the global human population under the threat of infection, and, if not at risk of the severest of symptoms, at risk of transmitting the infection to one or more who might be.

An estimated 2.7 billion workers and 1.6 billion students are today especially threatened by the need to work and study in socially intimate settings as a consequence of the COVID-19 pandemic[1,2]. Nearly half of these over four billion essential workers and school-age children live in low- and middle-income countries where lack of access to effective hygiene places them at high risk of infection, loss of work, and damage to their education[1].

Meanwhile, for the elderly, incarcerated, and anyone today seeking the presence of others outside of their own bubble of safety, even full access to hygienic protection may not eliminate an elevated risk of infection by SARS-CoV-2, as evidenced by elevated infection rates among frontline healthcare workers even while wearing full personal protective equipment[3].

SARS-CoV-2, like pneumonia and influenza, transmits through the air[4]. Large respiratory droplets coughed or sneezed into the environment by infected individuals can pose a risk of infection to those within near proximity of the host. Surgical face masks and social distancing are effective measures to combat this risk[5], while SARS-CoV-2 also transmits by the very small droplets we breathe out of our airways with every breath[6]: these droplets are generally too small to be effectively filtered by conventional surgical masks[7] and travel further than the 2 m social distance rule[8]. Small respiratory droplets (<1 μm diameter) are especially problematic within poorly circulated indoor environments where they can linger, accumulate, and travel through the air and into the human respiratory tract[9].

The human respiratory system is designed to filter out most small and large particles and to clear them before they

[1]Harvard John A. Paulson School of Engineering & Applied Sciences, Harvard University, Cambridge, Massachusetts. [2]Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts. [3]Sensory Cloud Inc, 50 Milk St, Boston, Massachusetts.
Published online 29 September 2020; doi:10.1142/S2529732520400040

reach the gas-exchange regions of the lungs where respiratory infections occur. Humans inhale each day approximately 10,000 L of air containing approximately 100 million and 10 billion airborne particles[10]. Most of these particles deposit in the nasopharynx, trachea, and main bronchi, notably when they are larger than a few hundred nanometers, that is, large enough to carry one or more airborne viruses, such as SARS-CoV-2, which is approximately 100 nm in diameter[11].

The nose, trachea, and main bronchi are lined with mucus and densely ciliated to capture inhaled airborne particles and move them from the respiratory system to the mouth for ingestion[12]. Mucus clearance times in the nose, trachea, and main bronchi range from minutes to hours, which is the same approximate time needed for a viral or bacterial pathogen to penetrate the mucus to the less viscous airway lining fluid and the epithelial cells lining the airway[13]. This creates a battle between clearance and infection, which is significantly controlled by the efficiency and health of the mucus layer itself. Pathogen entering the airways, and depositing in the nose and upper airways, can be cleared, and infection eliminated from the body, or escape the clearance mechanism of the body, and penetrate deeper into the lungs, where it promotes infection.

In the case of an airborne viral infection, escape from the standard clearance processes of the upper airways involves the re-entrainment of virus in the air, caused by the breakup into airborne droplets of mucus as happens in the very fast movement of air over the mucus during natural breathing[14]. Peak air flows during natural breathing can reach 2.5 m/s, near turbulent conditions[15]. These powerful air flows generate shear stress on the mucus surface, a thin layer of about 5–10 μm deep, generating tiny droplets, like sea mist. Pathogen picked back up into the air by the shedding of droplets of mucus and airway lining fluid can carry pathogen deeper into the lungs or back into the environment—potentially leading to pulmonary infection of the host and those in the vicinity of the host[16].

Divalent cations, such as calcium, when delivered onto mucus, bind mucin through electrostatic interactions[14]. These interactions increase the surface elasticity of the mucus/airway surface and strengthen the ability of the mucus surface to resist the breakup under the shear stress created by the airflow[14]. Temporal in nature, these interactions are natural, occurring whenever humans inhale sea mist, and occasionally medicinal, as with nasal saline treatments with Ringer's Solution, rich in calcium and magnesium[17].

Recently[16], we reported that a solution of calcium chloride and sodium chloride, with salinity similar to ocean salinity while with a particularly high-calcium chloride content, delivered to the airways of 10 human subjects with a mist of droplets possessing a diameter between 9 and 10 μm, suited to nasal and upper airway deposition, cleans the airways of exhaled aerosol particles, with particularly significant effect in those subjects who exhale the most particles.

We hypothesized that this calcium-rich nasal saline, called FEND might be more broadly useful to a larger cohort of healthy and COVID-infected individuals in real-world hygienic circumstances. We conducted human volunteer studies in three particular settings, with a focus on essential worker hygiene, school hygiene, and COVID-19 quarantine hygiene. We report on these studies here.

METHODS

FEND is a drug-free nasal saline hygiene formulation composed of calcium chloride and sodium chloride in distilled water. Overall salt composition ($4\times$ isotonic composition) is in the range of sea water with 0.43M $CaCl_2$, 0.05M NaCl (4.72% $CaCl_2$, 0.31% NaCl). FEND compositions were manufactured at Pharmasol (Massachusetts) in a good manufacturing practice (GMP) mixing and filling facility and contained in sealed plastic bottles (0.5 oz).

FEND bottles were opened and emptied into glass vials of the FEND Mister device (Nimbus) as shown in Figure 1a. The handheld, vibrating-mesh nebulizer (Nimbus), produced at Perfect Electronics in Shenzhen, China, has a 6-μm pore size to produce, on tipping of the device, an aerosol cloud with a particle-size distribution optimal for delivery to the nose through natural nasal inspiration. Generating a median volume particle diameter of 9–10 μm[16], optimal for nasal and upper airway deposition of aerosol following a deep natural tidal inspiration through the nose and with relatively uniform distribution of deposition from the anterior to the posterior of the nose, Nimbus produces on tipping 57 mg +/– 2 mg within a 10-second actuation, after which power ceases until tipped back upright and again overturned. The device delivers a dose of approximately 33 mg (1.56 mg $CaCl_2$) by filling an empty 6 oz glass with the cloud for the internally programmed 10 s actuation of the device and then inspiring the cloud directly from the glass into the nose (Figure 1b). Dosing can also be achieved by creating the cloud before the nose with deep nasal inspiration (Figure 1b).

Simply Saline by Arm & Hammer, a nasal spray of isotonic sodium chloride available on the market, was bought and used (one spray per nostril) as a placebo control.

In each of our human volunteer studies, exhaled particles were measured, before and after FEND administration, by a particle detector (Climet 450-t) designed to count airborne particles in the size range of 0.3–5 μm. The particle detector was connected to standard nebulizer tubing and mouthpiece that filters incoming air through a high-efficiency particulate air (HEPA) filter. Each standard nebulizer tubing and mouthpiece were removed from sealed packaging before each subject prior to the subject's first

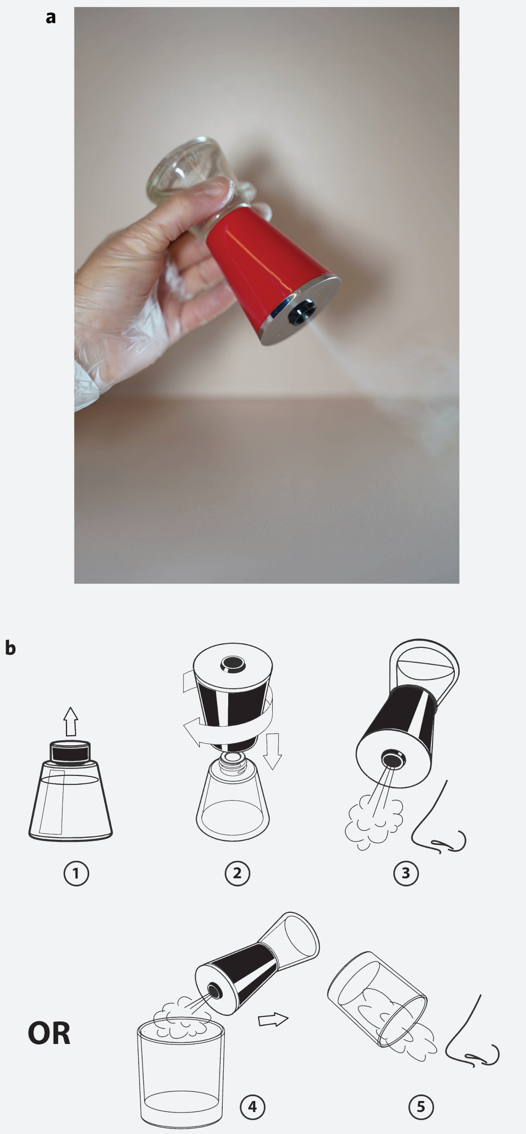

Figure 1. FEND Mister (Nimbus). (a) The handheld nebulizer activates on tipping, vibrating a mesh that generates an aerosol cloud for dosing. (b) FEND can be administered by Nimbus with a deep nasal inspiration either in an uncontrolled fashion before the nose or in a controlled fashion by containing the cloud in a glass.

exhaled particle detection. On subsequent counting maneuvers, the same mouthpiece, tubing, and HEPA filter were replaced into the particle counter system by the participant to insure effective hygiene. Subjects performed normal tidal breathing through a mouthpiece while plugging their noses over one to two minutes—beginning with two deep breaths to empty their lungs of environmental particles. Over this time frame, particle counts per liter diminished to a lower baseline number reflecting particles emitted from breakup of airway lining fluid surfaces in the subject's airways. Once the lower plateau of particle counts was reached, subjects continued to breathe normally. Three to eight particle counts (average values of particle counts assessed over six seconds) were then averaged to determine the mean exhaled particle count and standard deviation. Participants sat opposite to the study administrator with a plexiglass barrier in between.

Breathing through masks was achieved in the same manner as described earlier with fresh previously unused surgical masks (disposable Earloop Face Mask FIK0906U) and cotton masks (WD37U, 100% Jersey Cotton 3-Ply SMasks).

For our first human volunteer (non-drug, cosmetic) study, we recruited healthy subjects 15–66 years of age, all essential workers (and two children of one of the workers) at No Evil Foods (Asheville, North Carolina). The trial was conducted on the premises of No Evil Foods. We conducted a second human volunteer study over a four-day period in a Winchester home with four human volunteers, members of a family quarantined with one member of the family screened positive for SARS CoV-2 infection by polymerase chain reaction (PCR). All participants were screened for SARS CoV-2 infection by nasal swab and PCR. Three tested negative (ages 54, 14, and 16) and one tested positive (age 51). For our third study, we recruited parents and children ages 10 through 59 in the Boston Area. The trial was conducted at the offices of Sensory Cloud (Cambridge, Massachusetts) and in the home of one of the participating families (Wellesley, Massachusetts). Participants were not screened for SARS CoV-2 infection by serology or PCR before enrolment. The general protocol for the studies is pictorially represented in Figures 2a and 2b. All participants in all studies provided written informed consent prior to the enrolment.

COVID-19 testing occurred at Winchester Hospital and Newton Wellesley Hospital by nasal swab with PCR analysis.

RESULTS

We evaluated the effect of FEND nasal inspiration on exhaled aerosol in a 76 volunteer study at No Evil Foods in Asheville, North Carolina. Results are shown in Figures 3a–c.

Individual data are grouped in three categories according to the amount of exhaled aerosol. Those exhaling greater than 850 particles per liter of air are shown in Figure 3a, those exhaling 250–850 particles per liter are shown in Figure 3b, and those exhaling less than 250 particles per liter are shown in Figure 3c. We categorized subjects in this manner as the first two groups (Figures 3a, 3b) represent 20% of the participants in the study and accounting for 80% of the overall exhaled aerosol (on day 1 of the study)—meeting the classical 20:80 definition of super spreading. Among super spreaders, those exhaling above 850 particles per liter, and representing 10% of the

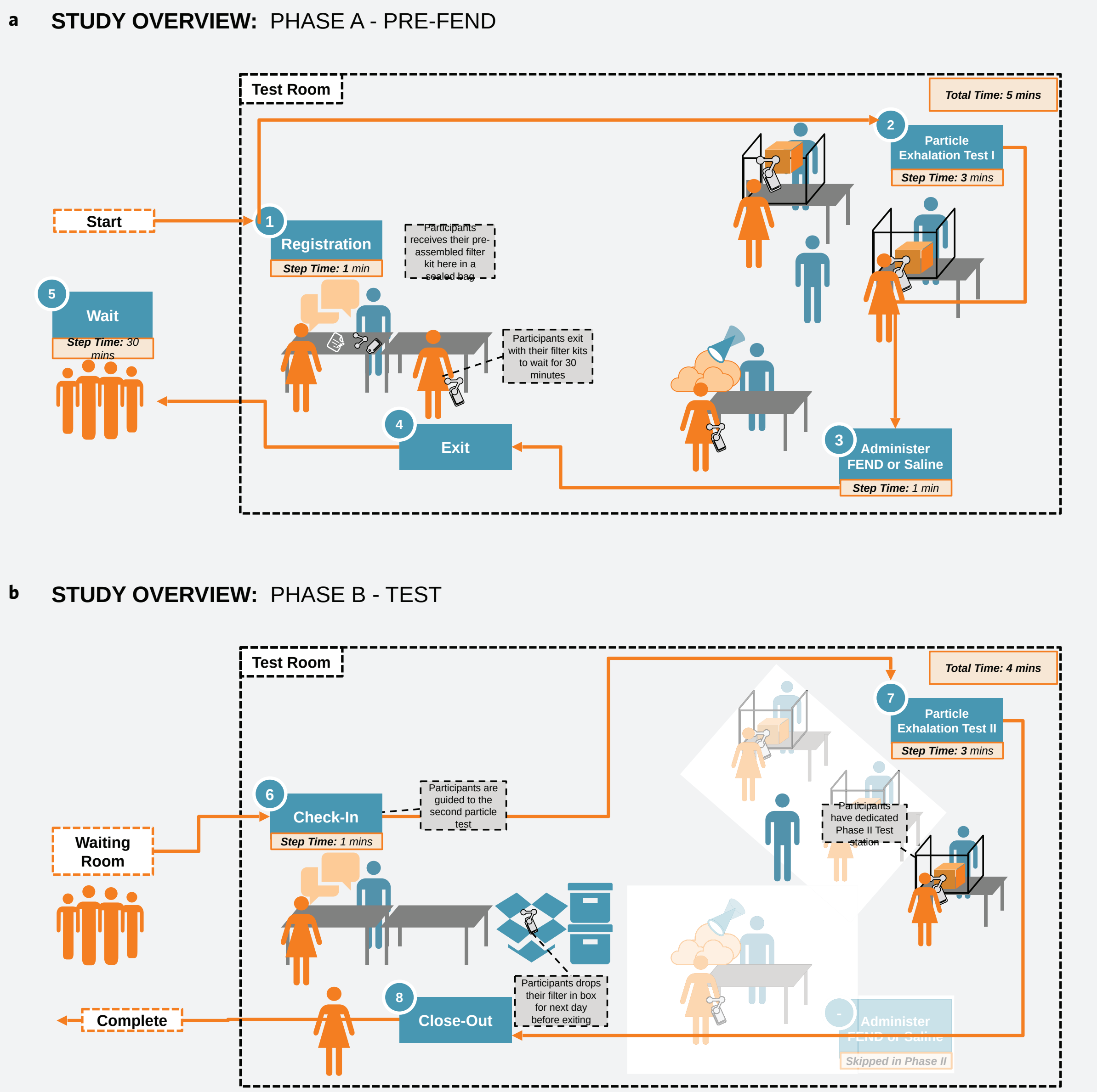

Figure 2. Protocol for measuring exhaled aerosol, dosing, and reassessing exhaled aerosol. (a) The pre-FEND or pre-control process; (b) The post-FEND or post-control process.

overall group (Figure 3a), are responsible for 80% of the super spreader production—or 64% of the total particle production.

FEND delivery suppresses exhaled aerosol 30-minutes post treatment by 84% overall for the highest producing super spreaders (Figure 3a) and 78% overall for all super spreaders, with reduction of exhaled aerosol by individual Super Spreaders ranging between 36% and 99%. Overall suppression of exhaled aerosol for the low spreaders was minor, and in several cases, natural variability of exhaled aerosol from one sampling time to the next was more significant than any respiratory droplet cleansing effect of FEND.

This natural variability in exhaled aerosol is particularly apparent in the observation of the nasal saline spray control study (Figure 4). Although exhaled aerosol before and after the Simply Saline control delivery varied greatly from subject to subject, overall variability of exhaled aerosol for the group before and after the control was insignificant (with 9,835 total

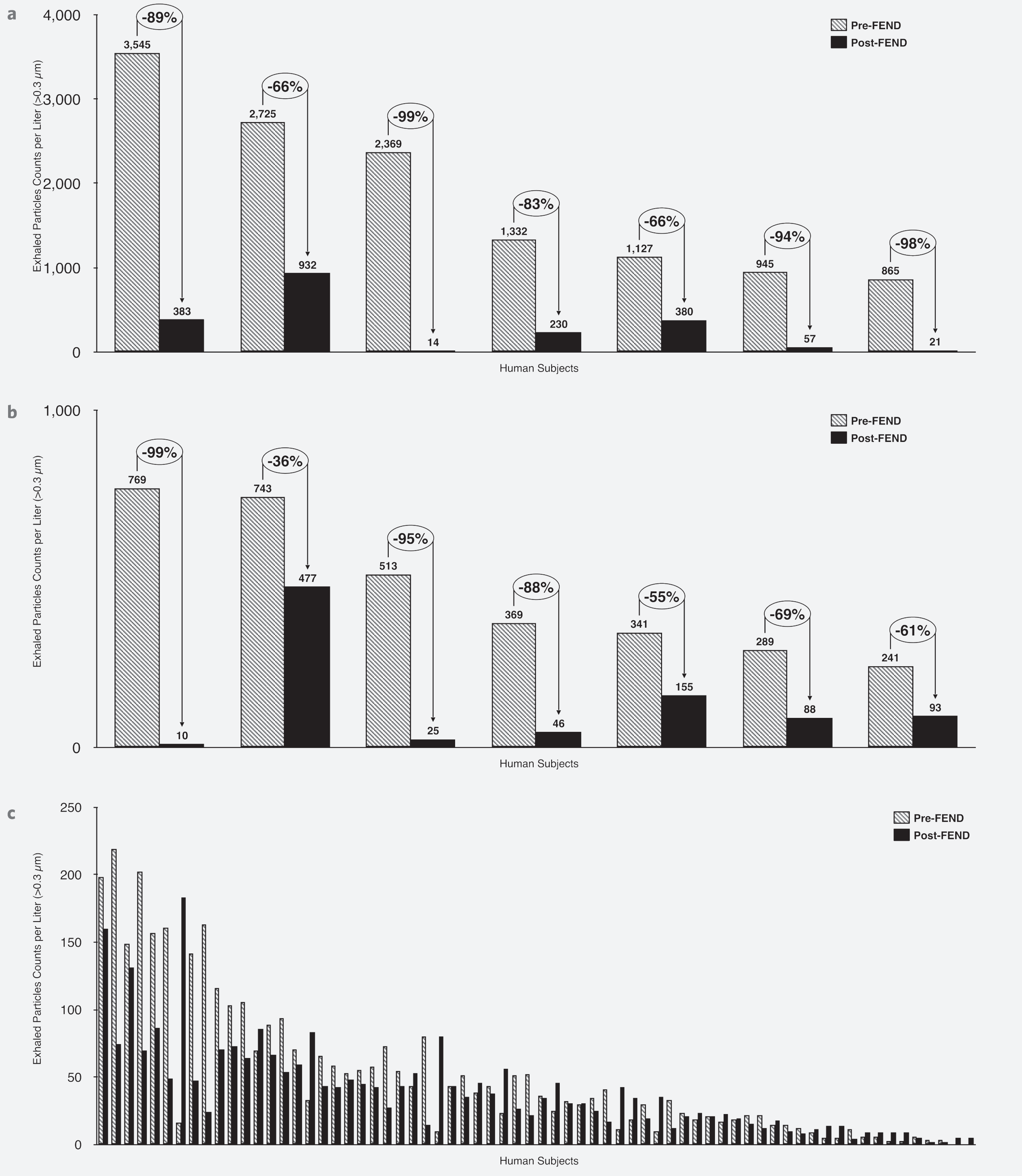

Figure 3. Effect of FEND on exhaled breath particles of 76 essential workers at No Evil Foods (Day 1): (a) "super spreader" Participants (1st Decile); (b) "super spreader" Participants (2nd Decile); (c) "Low Spreader" Participants.

exhaled particles before Nasal Saline treatment, 9,302 particles post–Nasal Saline treatment).

Two individuals in the 76 human volunteer study, both Super Spreaders, had an impairment to nasal inspiration (deviated septum and sleep apnea), and were for this reason excluded from the study. A third individual, included in the study and with non-allergenic rhinitis (chronically persistent runny nose), prior to FEND exhaled 39 +/– 47 particles per liter, while post-FEND exhaled 354 +/– 310 particles.

In a second study, we evaluated the effect of FEND on the cleansing of aerosol particles from the airways of a

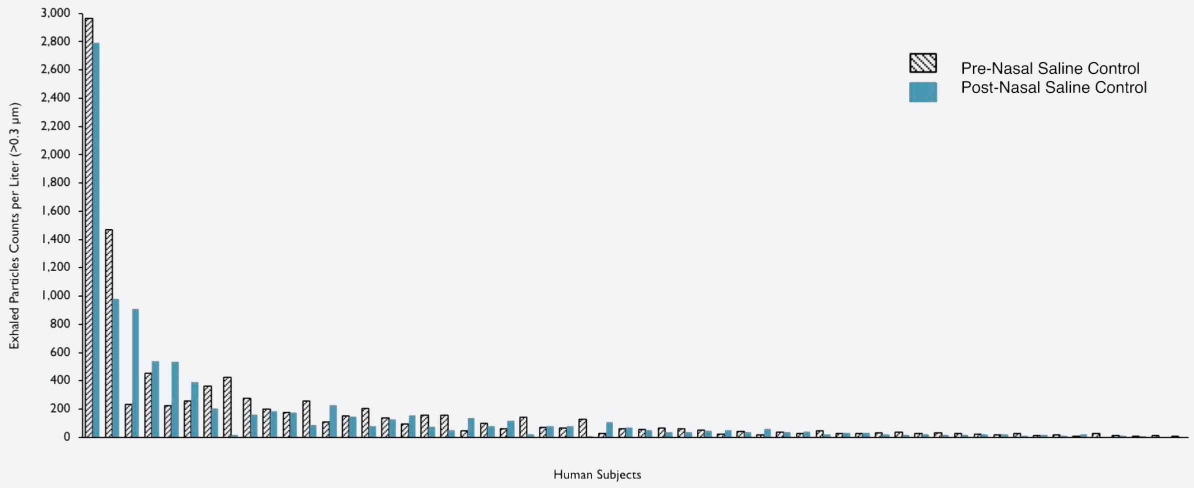

Figure 4. Exhaled breath particles before and after nasal saline control. Study performed on day 2 of the protocol.

COVID-positive member of a family of 4 (Figures 5a, b, c). On Monday August 24, a woman (Stephanie, age 51, BMI 26) began to experience symptoms including shortness of breath and cough. After five days of continued symptoms Stephanie was tested at Winchester Hospital MA by nasal swab for presence of SARS-CoV-2 virus. On day 7, August 31, following a positive PCR result, she and her husband (54 years) and two children (14 and 16 years) quarantined in their home in Winchester. On September 1 (day 8 post symptoms) we began to evaluate the exhaled aerosol of Stephanie as well as the other three members of her family.

On day 8, Stephanie exhaled 2754 +/– 1577 respiratory droplets, with 5% (138 +/– 15) of these droplets larger than 1 μm. These numbers of exhaled aerosol particles are significantly higher than what would be anticipated based on her BMI-years (1326) (Figure 2c). Day 9 Stephanie exhaled 1353 +/– 1517 particles, with 43% (52 +/– 20) larger than 1 μm. Stephanie's exhaled aerosol particles dramatically diminished in number (and variance) on days 10 and 11 to 224 and 29 with between 27% and 20% of particles larger than 1 μm, and to 12 +/– 5 particles on day 12. On these last three days of her quarantine, Stephanie's exhaled aerosol particle numbers reached levels similar to those of her other family members on days 8 through 12 (see Figure 5a).

Stephanie self-administered with FEND on day 8 following the peak of her exhaled aerosol (Figure 6a). The results of her exhaled aerosol following administration for up to 4 hours are shown in Figure 5b. Stephanie's exhaled aerosol remained low and in the range of her family members (Figure 5a). On day 9 her exhaled aerosol numbers increased. She again self-administered FEND, in the morning and the evening. Stephanie self-administered a fourth time on day 10, even as her baseline numbers had returned to a low spreader level (Figure 6a). The results of all of Stephanie's administrations are shown in Figure 5c (as before and after comparisons), as are the results following self-administration of FEND to the highest exhaled aerosol emitter of her family, on day 10.

In our third study, we assessed the airborne particle clearance effect of FEND in comparison to surgical masks with children and their parents. Figure 6a shows the variation of exhaled aerosol for the group from the highest to the lowest particle exhaling human subject. Three of the 12 participants exhaled many more particles than the other nine—and within or very close to the "super spreading" range based on the 76 person study.

Figure 6b shows the suppressive effect of FEND compared to that of the surgical mask in the case of the high-producing group. In two of the three individuals, FEND and surgical masks reduce exhaled particles by 98% and 82% (FEND) and by 93% and 76% (masks) relative to baseline. In the third individual, either the mask had no statistical filtration effect or FEND had no clearance effect—possibly owing to poor application. When properly applied, the masks were most effective at reducing large particles (reducing the large particle ratio from 1% to 0.02%, and from 44% to 4%), while FEND was more effective at reducing the presence of the smaller particles, and reduced all particles equally (the small particle ratio remained unchanged after suppression).

Figure 6c shows the other subjects as a function of age and compares the effect of FEND to the surgical masks. Omitted are the children who did not have a good

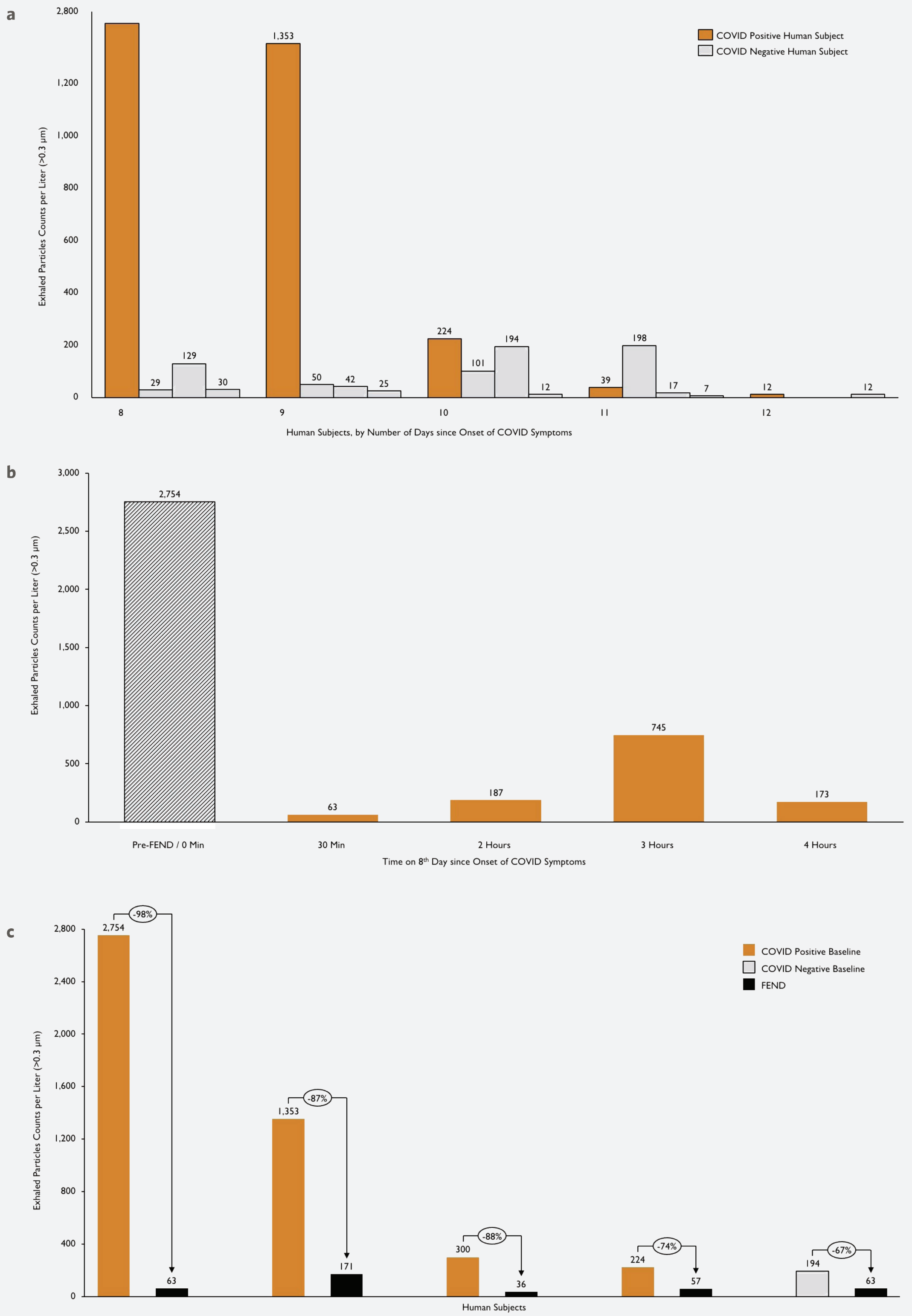

Figure 5. Exhaled breath particles in quarantine. (a) Entire four member family's results on day 8 post symptoms, including Stephanie (orange), child (16), husband (54), and son (14). (b) Stephanie day 8, pre- and post-FEND delivery for up to 4 hours post dosing, c) Stephanie before and after FEND administration on (starting from the left) day 8, day 9 (morning), day 9 (afternoon), day 10, and Stephanie's daughter (16 years) at day 10. The after FEND measurements were assessed between 30 minutes and 3 hours post dosing.

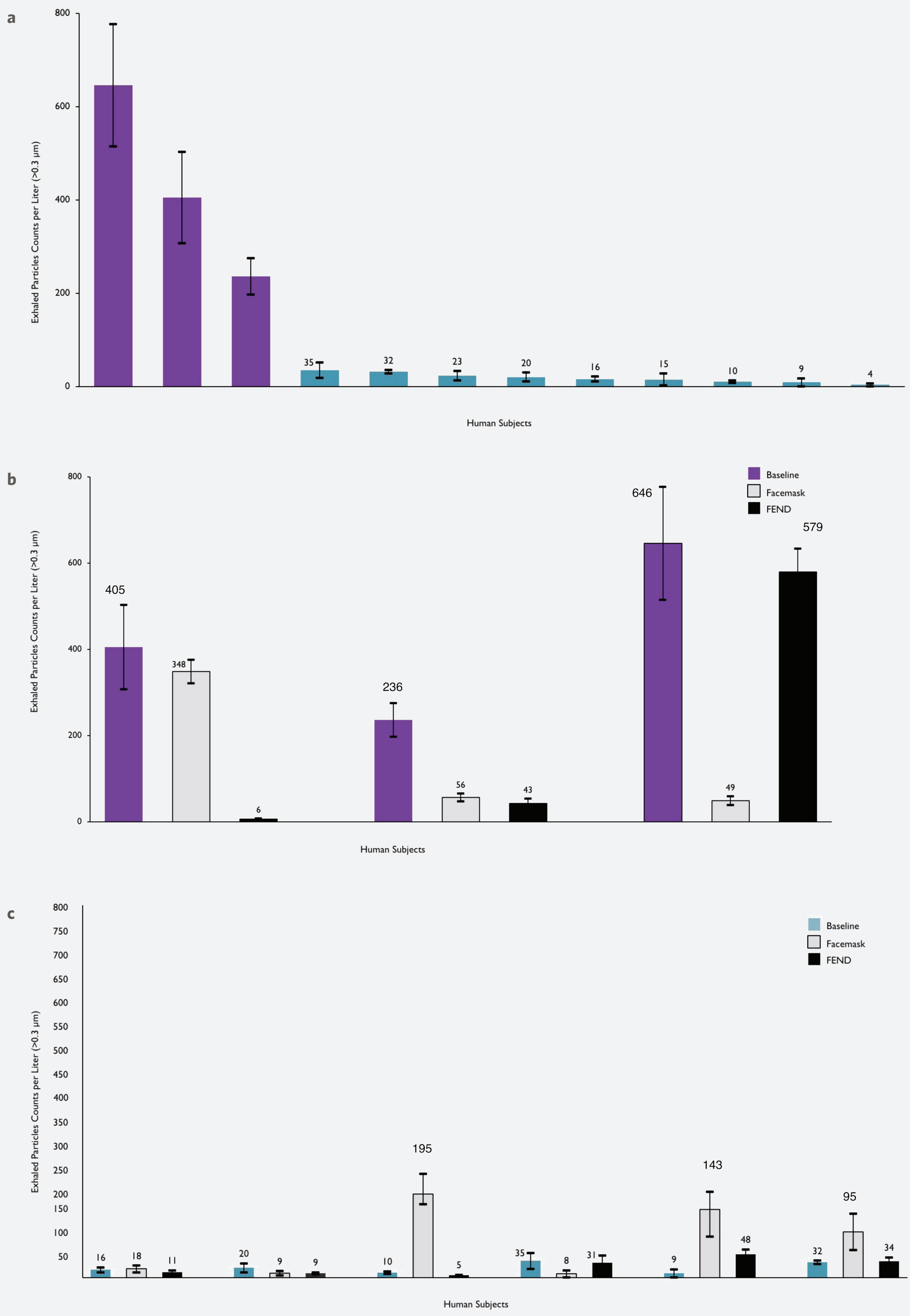

Figure 6. Exhaled breath particles of children and parents following FEND and masks. (a) 12 human volunteers at baseline. (b) High aerosol–producing subjects before and after FEND and surgical masks. (c) Low aerosol–producing subjects before and after FEND and surgical masks.

seal with the mouthpiece, reflected by an order of magnitude or more increase in large particles on use of the mask. There is a general trend for children to exhale many fewer particles than adults. As in the 76 person study for the low-spreader group (Figure 3c), there is high variability from one measurement to the next in these low-emitting children and adults. Swings in exhaled aerosol are greater with the mask than with FEND. This may reflect the fact that natural variability in exhaled aerosol leads to variability in particles emitted through the mask, whereas the nature of FEND action on mucus surfaces suppresses this variability.

The overall diminution of exhaled aerosol for the parents and children relative to baseline was for the masks 34% and for FEND 46%. These filtration numbers are weighted down by the single outlier individuals who seem to have poorly applied their mask or FEND.

DISCUSSION

Airborne pathogen transmits from person to person by the generation of small droplets of airway lining fluid generated in the processes of breathing, speaking, coughing, and sneezing[13]. During normal breathing, these particles vary significantly from person to person, and within subjects over time (Figure 2). In our study of 76 human subjects, the distribution of exhaled particles followed a classical "super spreading" distribution, with 20% of the individuals accounting for 80% of the aerosol (Figures 3a, b). In this distribution, young people were most commonly in the "low-spreader" group, an observation we made as well in our study of 12 parents and children (Figure 6c). While face masks and social distancing are useful practices to limit exhaled aerosol droplet spread, the results of these studies reveal that the majority of particles exhaled during normal breathing are smaller in size (sub-micron) than either practice is designed to limit. Administration of FEND, a hypertonic salt solution composed of calcium and sodium chloride generally reduced these sub-micron exhaled particles, and as a cleanser, or "invisible mask," the effect of FEND increased with increasing number of exhaled small particles (Figure 3).

In our evaluation of exhaled aerosol within the quarantined family, while only involving a single COVID-19 infected family member, we found an unusually high proportion of sub-micron exhaled particles on days 8 and 9 post-infection, when Stephanie had unusually high exhaled aerosol count. The ability of FEND to suppress the smallest exhaled aerosol particles may make it particularly valuable as an airway cleanser in these circumstances.

The results of our study with children and parents suggest that while clean surgical masks are more effective at filtering the largest particles, and FEND more effective at clearing the smallest particles, the combination of clean masks and FEND has not only the advantage of both, it also provides the benefit of a backup should either the mask or FEND be improperly applied.

FEND can be used as a personal hygiene practice. It can also be easily administered by a gloved individual to workers, frontline healthcare providers, students, and family members—without contacting the device in a stadium or other large gathering place, FEND can permit mass hygiene interventions. With 10 seconds per application, and 250 applications per 1 oz bottle, 100 FEND administrators treat 25,000 people in less than an hour, allowing rapid large-scale airway hygiene for those entering indoor environments or situations where social distancing may be difficult to constantly adhere to.

There is a need for practical and quantifiably effective human hygiene measures that can be adopted widely and fairly. Calcium-enriched nasal saline aerosol may be such a new measure, a complement to existing hygiene measures, and a useful hygienic option for those who cannot wear or do not have access to clean effective face masks[18].

ACKNOWLEDGMENTS

The authors wish to thank the team at No Evil Foods and all the human volunteers in our studies, and above all Stephanie and her family.

CONFLICT OF INTEREST

DE is the founder, CSO and CEO of Sensory Cloud; TD and JS are employees of Sensory Cloud. RL has no relationship with Sensory Cloud, the manufacturer of FEND.

REFERENCES

1. Editorial. The plight of essential workers during the COVID-19 pandemic. *The Lancet*. **5**(9), E475–E483 (2020).
2. https://en.unesco.org/covid19/educationresponse.
3. Nguyen, L.H., Drew, D.A. & Graham, M.S. Risk of COVID-19 among front-line health-care workers and the general community: a prospective cohort study. *The Lancet*. **5**(9), E475–E483 (2020).
4. Zhang, R., Li, Y., Zhang, A.L., Wang, Y. & Molina, M.J. Identifying airborne transmission as the dominant route for the spread of COVID-19. *Proc. Natl. Acad. Sci.* **117**(26), 14857–14863 (2020). DOI: 10.1073/pnas.2009637117
5. Bunyan, D. *et al*. Respiratory and facial protection: a critical review of recent literature. *J. Hosp. Infect*. **85**(3), 165–169 (2013). DOI: 10.1016/j.jhin.2013.07.011
6. Edwards, D.A. *et al*. Inhaling to mitigate exhaled bioaerosols. *Proc. Natl. Acad. Sci*. **101**(50), 17383–17388 (2004). DOI: 10.1073/pnas.0408159101

7. Leung, N.H.L. *et al*. Respiratory virus shedding in exhaled breath and efficacy of face masks. *Nat. Med*. (2020). DOI: 10.21203/rs.3.rs-16836/v1
8. Bourouiba, L. Turbulent gas clouds and respiratory pathogen emissions: potential Implications for reducing transmission of COVID-19. *JAMA*. **323**(18), 1837–1838 (2020). DOI: 10.1001/jama.2020.4756
9. Morawska, L. & Cao, J. Airborne transmission of SARS-CoV-2: the world should face the reality. *Environ. Int*. **139**, 105730 (2020, June). DOI: 10.1016/j.envint.2020.105730
10. Tsuda, A., Henry, F.S. & Butler, J.P. Particle transport and deposition: basic physics of particle kinetics. *Compr. Physiol*. **3**(4), 1437–1471 (2013). DOI: 10.1002/cphy.c100085
11. Bar-On, Y.M., Flamholz, A., Phillips, R. & Milo, R. SARS-CoV-2 (COVID-19) by the numbers. *eLife*. **9**, e57309 (2020). DOI: 10.7554/eLife.57309
12. Hounam, R., Black, A. & Walsh, M. Deposition of aerosol particles in the nasopharyngeal region of the human respiratory tract. *Nature*. **221**, 1254–1255 (1969). DOI: 10.1038/2211254a0
13. James, A.C. *et al*. The respiratory tract deposition model proposed by the ICRP task group. *Radiat. Prot. Dosim*. **38**(1–3), 159–165 (1991). DOI: 10.1093/oxfordjournals.rpd.a081085
14. Watanabe, W. *et al*. Why inhaling salt water changes what we exhale. *J. Colloid. Interface. Sci*. **307**(1), 71–78 (2007). DOI: 10.1016/j.jcis.2006.11.017
15. Dekker, E. Transition between laminar and turbulent flow in human trachea. *J. Appl. Physiol*. **16**(6), 1060–1064 (1961). DOI: 10.1152/jappl.1961.16.6.1060
16. Edwards, D.A. *et al*. A new natural defense against airborne pathogens. *QRB Discovery*. **1**(E5) (2020). DOI: 10.1017/qrd.2020.9
17. Bastier, P.L., Lechot, A., Bordenave, L., Durand, M. & de Gabory, L. Nasal irrigation: from empiricism to evidence-based medicine. A review. *Eur. Ann. Otorhinolaryngol. Head. Neck. Dis*. **132**(5), 281–285 (2015). DOI: 10.1016/j.anorl.2015.08.001
18. Caruso, B.A. & Freeman, B.A. Shared sanitation and the spread of COVID-19: risks and next steps. *The Lancet*. **4**(5), E173 (2020). DOI: 10.1016/S2542-5196(20)30086-3