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Fluorescent Bioaerosol Particles Resulting from Human Occupancy with and without Respirators

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ABSTRACT

Airborne transmission of pathogenic aerosols via human breath plays a major role in infectious disease outbreaks in indoor environments. Yet, their bioaerosol emission profiles are still not well quantified. Here, we first studied bioaerosol emission rates of human exhaled breath from 12 healthy subjects, and then evaluated the bioaerosol emissions when wearing two different respirators "Doctor masks" and N95 in a controlled environment (27 m^3) using a bioaerosol sensor-ultraviolet aerodynamic particle spectrometer (UV-APS). The human bioaerosol contribution was further confirmed through classroom observation. The results showed that there was a peak around 1.5 μm for the fluorescent particles emitted from humans' breath. For the controlled environment, the presence of 5 people without wearing masks increased bioaerosol concentration by 107% within 30 min at an average emission rate of 8.4×10^5 fluorescent particles $person^{-1}$ $hour^{-1}$ resulting from the occupancy. When wearing N95 masks or "Doctor masks", bioaerosol increases were observed to be 81% or 31% for the controlled environment, respectively, lower compared to those without masks. In-classroom observation also showed a fluorescent particle concentration increase of about 50%. In all experiments, we observed a decline in PM number concentration. Bioaerosol emission from exhaled breath was calculated to account for about 17% of the increase in the controlled environment. The results here suggest the need for re-evaluating microbial aerosol exposure risks for medical sites that demand high levels of hygiene even while wearing a respirator.

Keywords: Bioaerosols; Exhaled breath; Respiratory masks; Shielding efficiencies; Controlled environment.

INTRODUCTION

Human exposure to bioaerosols can cause a variety of adverse health effects including infectious disease, respiratory impairment, and other allergenic reactions. Ironically, humans themselves are also important sources of bioaerosol particles, emitting millions $person^{-1}$ $hour^{-1}$, and this topic is currently under extensive investigation (Qian *et al.*, 2012; Xu *et al.*, 2012; Hospodsky *et al.*, 2012; Bhangar *et al.*, 2014; Hospodsky *et al.*, 2014; Bhangar *et al.*, 2015). Among others, exhaled breath is an important contributor to human emission of bioaerosol particles (Noti *et al.*, 2012; Shen *et al.*, 2012; Xu *et al.*, 2012; Milton *et al.*, 2013). Exhaled bioaerosol particles can remain airborne and subsequently disperse via airborne transmission (Gralton *et al.*, 2011). For example, the SARS outbreak in 2003 was largely facilitated by the airborne spread of the corona viruses (Yassi *et al.*, 2004; Gao *et al.*, 2013). Correspondingly, knowledge of the size distribution and concentration of bioaerosols emitted from human breath is necessary for evaluating microbial aerosol exposure risks. In our previous work, we have shown using culturing method the exhaled breath contains up to 7000 CFU m^{-3} bacteria (Xu *et al.*, 2012). It is also important to quantify how much viable bioaerosol (not just culturable) released from human exhaled breath in an indoor environment for a given time duration.

In recent years, there are an increasing number of studies investigating bioaerosols from indoor and occupational environments including impacts from ambient ones (Hsu *et al.*, 2012; Fang *et al.*, 2014; Qian *et al.*, 2014; Galès *et al.*, 2015; Li *et al.*, 2015; Sidra *et al.*, 2015; Yamamoto *et al.*, 2015; Goudarzi *et al.*, 2016; Jahne *et al.*, 2016; Li *et al.*, 2016; Mirhoseini *et al.*, 2016; Smets *et al.*, 2016). Most recently, a research focus has been developed, particularly for the bioaerosol emission from human occupancy in an indoor environment. For example, a study using the UV-APS has shown that for the particle size range of 2.5–10 μm, there was an average 0.9 ± 0.3 million particles per person-h

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(Bhangar *et al.*, 2015). They attributed the main bioaerosol emissions to the release from the floor (about 60–70% or more), and walking was described to be responsible for a 5–6 times increase in the emission rate (Bhangar *et al.*, 2015). In their work, they have demonstrated a dominant bioaerosol mode of 3–5 μm diameter range (Bhangar *et al.*, 2015). The indoor bioaerosol concentration depends on a number of factors, including, but not limited to, regional climatic conditions, human activity strength, duration of human presence, floor characteristics, and environmental settings (e.g., possible mold growth). Particularly, human activity strength is shown to be a strong indicator for indoor bioaerosol levels (Chen and Hildeman, 2009; Hospodsky *et al.*, 2014). Using qPCR together with aerosol sampling, it was shown in an occupied classroom that human emission rate was about 37 million genome copies (empirically about 4 $\times$ 10^6 particles person^{-1} hour^{-1}), and ~18% of bacterial emissions were described to be from taxa that are closely associated with the human skin microbiome (Qian *et al.*, 2012). In another work, bioaerosols from human occupied space were found to be associated with nostrils and hair of humans (Hospodsky *et al.*, 2012). Overall, different studies obtained similar magnitudes of human bioaerosol emission rates (about several million per hour per person) even in different geophysical settings.

Accordingly, people often choose respiratory masks to protect either themselves or environments from breath-borne bioaerosol infection or contamination. For example, "Doctor masks" or Surgical masks are usually used to prevent release of exhaled bioaerosols into the environment, while N95 respirators are generally used to prevent people from inhaling infectious aerosol particles in the environment. Current tests with different masks available on the market show that the filtration efficiencies of different masks vary greatly and the filtration efficiencies of N95 respirators and "Doctor masks" can generally reach above 90%, but for ultrafine particles their efficiencies decrease substantially (Balazy *et al.*, 2006; Viscusi *et al.*, 2009; Maclntyre *et al.*, 2014; Zou and Yao, 2015). Particularly, N95 respirators may not achieve the expected filtration efficiency against bacteria and viruses (Lee *et al.*, 2008). Particle size, shape and density of airborne microorganisms were shown to have important impacts on the protection provided by N95 respirators (Lee *et al.*, 2005). However, the filtration efficiency of masks is not the same as actual shielding efficiency because there is a bioaerosol leak due to the problem of the fit between mask and face. For example, a randomized trial of nurses in Ontario tertiary care hospitals showed that influenza infection occurred in 23.6% of participants wearing surgical masks and 22.9% of participants wearing N95 respirators, due to face leakage (Loeb *et al.*, 2009). Face leakage led to the nearly equal shielding efficiencies between the surgical masks and N95 respirators (Loeb *et al.*, 2009). It is still unclear whether N95 respirators provide a higher protection level than surgical masks for patient care activities (Gamage *et al.*, 2005). Specifically, "Doctor masks" or surgical masks are frequently used by doctors to protect patients' wounds from infection. However, a study shows that it is unclear whether the wearing of surgical masks by doctors has any influence on infection rates of patients' surgical wounds in surgery (Lipp and Edwards, 2012). Overall, there is a large volume of past studies focusing on protection efficiencies of masks against airborne viral particles; however, little information exists for breath-borne bacterial aerosol particles released from humans when wearing these respiratory masks. Accordingly, the actual protection is not quantified.

In this work, we first quantified the size distribution and concentration level of viable bioaerosols in human breath samples using an ultraviolet aerodynamic particle spectrometer (UV-APS) in real-time. Then, the bioaerosol contributions of humans were studied in a controlled manner in a controlled environment with and without the use of different respiratory masks. Last, we monitored the viable bioaerosol concentration levels in a classroom (before, on and after class session) using the UV-APS. The results from this study will provide information about the influence of people on environmental bioaerosols, the emission rate of bioaerosols from humans, and bioaerosol shielding efficiencies of "Doctor masks" and N95 respirators. The information obtained here indicates the need for re-evaluating microbial aerosol risks, especially in settings that demand high levels of hygiene.

METHODS

The UV-APS Instrument

In this work, we utilized the UV-APS (TSI, Inc) for monitoring fluorescent bioaerosol size distributions both for human breath and natural environments. The UV-APS is an instrument that couples the UV laser-induced fluorescence technique with the aerodynamic particle sizer, and it can achieve real-time monitoring on metabolically active microorganisms. It detects viable biological aerosol particles by exciting and detecting fluorescence that is being emitted by NADH, NADPH, and riboflavin generated from metabolically active biological cells. NADH and NADPH respectively refer to the reduced forms of nicotinamide adenine dinucleotide, and they are produced and utilized during the bacterial metabolism. So, the UV-APS can distinguish viable biological aerosol particles from all particles present. A higher fluorescent intensity indicates that a particle has a higher probability of being a biological particle (Jung *et al.*, 2012). The total air sampling flow rate of the UV-APS was 5 L min^{-1}, including a sheath flow rate of 4 L min^{-1} and an aerosol sampling rate of 1 L min^{-1} (Huffman *et al.*, 2012). Particle aerodynamic diameters obtained by the UV-APS were in the range of 0.5–20 μm, and distributed into 52 size groups.

Experimental Procedures

First, we recruited 12 healthy people (male-to-female ratio = 1:1) with an age range of 20–26 years to study the bioaerosol size distribution from human exhaled breath. The human subjects (no smoking history) were healthy without pre-existing or onset of respiratory problems. The UV-APS instrument was connected with a one-time-use mouthpiece through 20-cm long inlet tubing, through which subjects were advised to actively exhale once through the

mouthpiece into the tubing about every 3–5 seconds for 6 minutes. The human subjects inhaled indoor air through their noses during the measurements. The measurements were taken generally 3 hours later after the subjects took their meals. Monitoring data were recorded by the instrument for every 1 minute, and only the last 5 minutes of data were analyzed (the first minute of data had large variations). The healthy human subjects were divided into 3 groups, and each group was tested at different time. When performing experiments with each group, we also measured the environmental bioaerosols present at the testing location.

Next, we performed experiments in an office room to further quantify emission rates of bioaerosols from humans and bioaerosol shielding efficiencies of "Doctor masks" and N95 respirators. The enclosing (its floor is covered using glazed bricks) was an empty room with a total air space of 27 m^3 whose door and window were all closed during the measurements. We selected two typical masks: N95 respirators (model 9010, 3M) and "Doctor masks" (model 101, DOCTOR MASK). Firstly, the office space was ventilated with an exhaust fan for 20 minutes at flow rate of 724.8 m^3 $hour^{-1}$ (the entire room is ventilated about 9 times) and then the UV-APS was utilized to monitor the environmental bioaerosols in the space without the presence of people for 30 minutes while the exhaust fan was turned off. Secondly, 5 subjects (male = 2, and female = 3) were asked to sit quietly in the space for 30 minutes, while the UV-APS (placed 1 m above the ground) was utilized to monitor bioaerosol size distributions with the window and door closed and the ventilation off. In addition, the same procedures were repeated but with human subjects wearing different respiratory masks such as N95 respirators and "Doctor masks". In total, we performed five independent experiments with 5 human subjects with or without wearing N95 and "Doctor masks" on the same day. Each time, we monitored the background bioaerosol levels in the room before subjects entering. For the last experiment, the monitoring time was set to be 15 minutes for studying the time dependence of human bioaerosol contribution. The bioaerosol contributions of humans and the shielding efficiencies of these respiratory masks against exhaled bioaerosols were examined. The emission rate (E_{bio}) of bioaerosols resulting from human occupancy was calculated by the following equation:

$$E_{bio} = (c_1 - c_0) \times V/n/t \quad (1)$$

where c_1 and c_0 represent the average bioaerosol concentrations in the studied environment with and without the presence of 5 human subjects, respectively; V represents the volume of the studied environment, n represents the number of people and t represents monitoring time. The shielding efficiencies of different respiratory masks were studied in relation to their relevant bioaerosol concentration increase in the environment.

Finally, we quantified human bioaerosol contributions in a classroom (its floor is covered with glazed bricks) on Peking University campus with an attendance of close to 100 students. The bioaerosol size distributions inside the classroom were monitored in real-time by the UV-APS from 18:16 to 21:06 on March 18, 2014. Both fluorescent and total aerosol particle numbers were recorded very minute during the measurement. The monitoring time periods were classified into three different groups: before class (18:06–18:40), during class (18:41–20:30) and after class (20:31–21:06). For the entire monitoring process, we recorded the number of students present in the classroom every 10 minutes. The average number of students in the classroom was 50 before class, 100 during class and 25 after class. The bioaerosol and PM number concentration levels were analyzed for each class time period. All measurements were conducted during the winter (Jan–March, 2014) under the ambient humidity level (about 40%) and room temperature around 25°C.

Statistical Analysis

The concentration differences of bioaerosols under different test conditions were analyzed by independent sample t-test analysis with SPSS 16.0, and a p-value of less than 0.05 indicates a statistically significant difference. In this work, we counted biological aerosol particles from the fluorescence channel that had significantly higher fluorescence intensity (i.e., usually at least 10 times higher than the preceding fluorescence channel). By doing so, we eliminated the counts that could be from interfering chemical compounds with weak fluorescence signals from the background.

RESULTS AND DISCUSSION

Exhaled Bioaerosols

As observed in Fig. 1, exhaled bioaerosol particles followed a unimodal distribution with a number concentration peak at 1.5 μm. On average, the concentration of exhaled viable bioaerosols (fluorescent particles) was found to be about $1.93 \pm 1.83 \times 10^5$ particles m^{-3}. In a previous study, it was shown that most exhaled bacterial aerosol particles were in the range of 0.5–1 μm using scanning electron microscope in conjunction with a new exhaled breath collection device (Xu *et al.*, 2012), and such identical size ranges were also observed in another study (Wan *et al.*, 2014). The differences could be due to different measurement techniques and also water droplet and evaporation effects on breath-borne particles. Using a VITEK 2 system, *Sphingomonas paucimobilis*, *Kocuria rosea*, *Bacillus lentus*, *Aerococcus viridians*, *Bacillus firmus*, *Kocuria kristinae* and *Staph. Xylosus* were detected in exhaled breath (Xu *et al.*, 2012). These species (individual or in aggregates) could have contributed to the 1.5 μm peak from the exhaled breath. In another study, it was shown that 99% of exhaled particles were below 1 μm with a production rate of mostly approaching 1.5×10^5 particles m^{-3} (Wurie *et al.*, 2013). However, their data were obtained using an optical particle counter which cannot distinguish between biological and non-biological aerosols, and thus the bioaerosol percentage for their detected concentration level was not clear. The smaller biological particles (less than 1 μm) detected from human exhaled breath could remain airborne for a much longer time period, thus increasing the risk of airborne

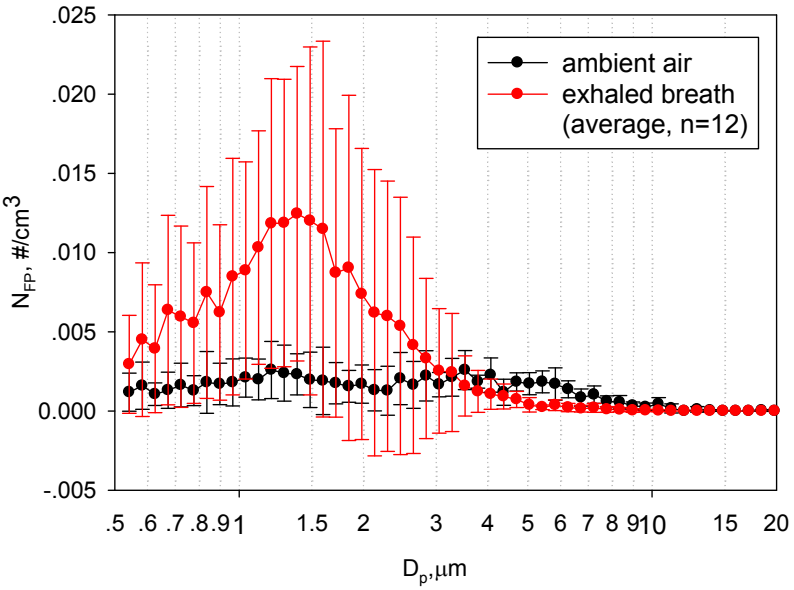

Fig. 1. The average size distributions of the fluorescent biological aerosol (viable bioaerosol) particles of ambient environment and exhaled breath from 12 healthy people; D_p represents particle aerodynamic diameter, and N_{FP} represents the concentration of fluorescent biological aerosol particles; data points represent means and standard deviations of average environmental bioaerosol levels and 12 human subjects' averages of breath-borne bioaerosol levels over a time period of 5 minutes, and data was recorded every 1 minute. Data points represent averages and standard deviation of measurements.

transmission of potential infectious diseases. Clearly, as shown in Fig. 1, there were large variations in viable exhaled bioaerosol particle levels among 12 healthy subjects. This variation was also observed in Wurie *et al.*'s study, and the production was shown to increase with age, which was shown to be influenced by airway inflammation as a result of environmental irritants (Wurie *et al.*, 2013). Our subjects had minor age differences. In contrast, outside ambient air was found to have a lower viable bioaerosol concentration level, up to $(6.6 \pm 4.2) \times 10^4$ particles m^{-3}, which was about one third of those from exhaled breath in average as observed in Fig. 1 and shown in Table S1 (Supporting Information). Different from that of exhaled breath, two peaks (1–1.5 µm and 3–4 µm) as shown in Fig. 1 were detected for viable bioaerosol size distribution for the outside ambient environment. From the gene sequence results for Beijing's air samples in a previous study (Li *et al.*, 2013), these peaks could be contributed by *Exiguobacterium*, *Microbacterium*, and *Bacillus* for the 1–1.5 µm peak; and *Aspergillus, Cladsporium, Penicillium*, and *Alternaria* for the 3–4 µm peak. However, it is also possible that the larger peak could be contributed by larger bacterial species or their aggregates from the ambient environment. Overall, the difference observed between exhaled breath and ambient air is due to different bioaerosol emission sources.

Human Contribution to Environmental Bioaerosols without Wearing Respirators

Fig. 2 shows the viable bioaerosol concentration levels when 5 or no people were present in the studied environment (27 m^3). As observed from the figure, for all experiments elevated bioaerosol concentration levels were observed when five people were present in the environment compared to that of without human presence. However, quantitative contribution depended on the duration of human stay. As indicated in Table S2 human presence for 15 minutes in the controlled environment in the 5th experiment contributed fewer bioaerosol particles compared to 30 min presence. As shown in Fig. S1, the presence of humans did not significantly modify the viable bioaerosol size distributions. On average, the viable bioaerosol concentration was detected to range from 8.82×10^4 particles m^{-3} without the presence of humans to 1.66×10^5 particles m^{-3} with 5 people present. The environment was observed to have an average bioaerosol increase of 107% within 30 minutes upon the human occupancy. By calculation using the breath-borne bioaerosol concentration described above, fluorescent bioaerosol particles via breathing at 12.5 L min^{-1} alone from 5 subjects for 30 min contributed to about 17% of the increase in the environment (27 m^3) studied here. The remaining increase can be attributed to the skin and clothing shielding bioaerosol particles and also to the resuspension from the floor. The percentage of bioaerosols of the total particles (> 0.5 µm) with and without human occupancy was observed to increase from 13.0% to 26.0%. By calculation as shown in Table S2, average human emission rate resulting from the human occupancy in the studied environment study was about 8.4×10^5 particles $person^{-1}$ $hour^{-1}$, which is slightly lower than the previously reported value (2.1×10^6 particles $person^{-1}$ $hour^{-1}$) (Bhangar *et al.*, 2014). Here, for each experiment the background bioaerosol levels could be affected by the ventilation process, e.g., enhancing the floor resuspension. Overall, according to the results of this work and the literature data, the bioaerosol emission can be approximately attributed

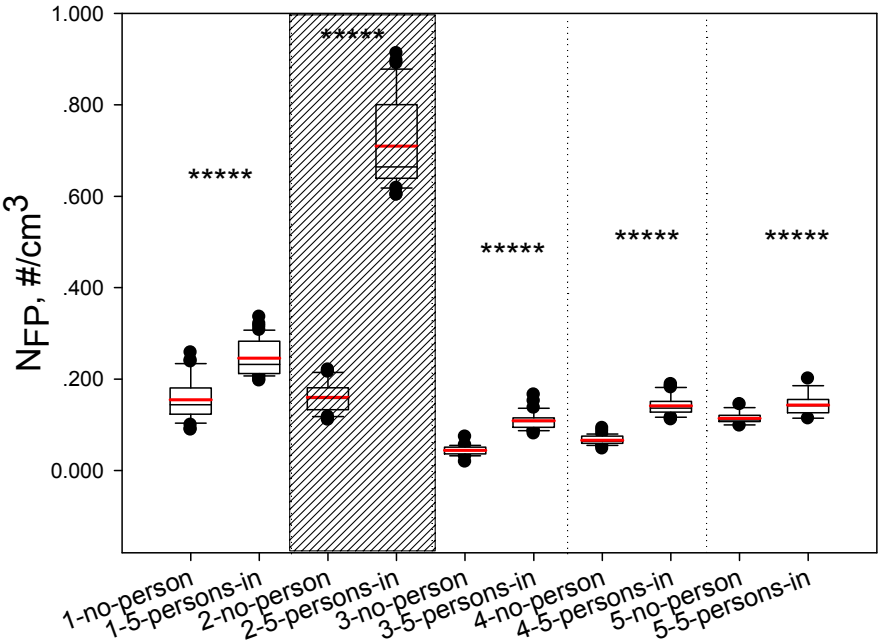

Fig. 2. The concentration levels (Boxplots) of the fluorescent biological aerosol particles detected using the UV-APS when there were no people or 5 people in the controlled environment; N_{FP} represents the concentration of fluorescent biological aerosol particles and the data points represent fluorescent biological aerosol particle concentration values of every 1 minute in the studied environment. Different from the former four experiments (e.g., 1-no-person, 2-no-person, 3-no-person, and 4-no-person) which were monitored 30 minutes, the monitoring time of the fifth experiment (5-no-person) was only 15 minutes. ***** indicates a statistically significant difference between two tests. For the second experiment (2-5-persons-in) as marked in shadow plots, the results of the fluorescent biological aerosol particles when 5 people in the controlled environment was detected to be an outlier (possibly due to malfunction of the UV-APS during that time period, Grubbs' test, 90% confidence).

to ~17% from breathing (this work), ~18% from human skin and clothing shielding(Qian *et al.*, 2012) and the rest (~65%) from the floor resuspension(Bhangar *et al.*, 2015).

Bioaerosol Shielding Efficiencies of "Doctor Masks" and N95 Respirators

Humans are important contributors to indoor bioaerosols, so the study of human emission rates of bioaerosols is necessary. However, compared with other human sources, exhaled bioaerosols are more important because some of the exhaled bioaerosol particles are possible human pathogens. Thus, in many different scenarios, including medical operations such as dental procedures and surgeries, healthcare providers wear protective masks to protect either themselves or safeguard the surgery from infection. In our previous work, we have shown that filter materials from N95, Surgical mask and "Doctor mask" had more than 95% filtration efficiency for 1.5 μm size particles, and close to 100% for 3 μm ones (Zou and Yao, 2015). Overall, N95 had a higher filtration efficiency, followed by Surgical mask and "Doctor mask" (Zou and Yao, 2015). Here, we selected two common respirators: N95 and "Doctor masks" for which results are shown in Fig. 3 and Fig. S2. As observed from Fig. 2, the bioaerosol concentration levels increased when 5 people were present in the environment regardless of use of a respirator or not. Although the bioaerosol emission via breathing is important, the increase in the studied environment was largely due to the floor resuspension and skin shielding in this work. For each different experiment, the actual contribution from the floor resuspension of the controlled environment could vary. As listed in Table S2, when 5 people without wearing respiratory masks were present in the environment, the bioaerosol level increased by 107% for 30 min; however, when wearing an N95 or "Doctor masks" the bioaerosol level increased only 81% and 31%, respectively, as listed in Table S3. Results from Fig. 3 suggested that use of a respiratory mask can help reduce human emission of bioaerosols into the environment, and the effectiveness varies with the respiratory type used. Wearing a respirator can have two benefits in terms with the bioaerosol levels in the environment: 1) use of the respiratory will help prevent humans from releasing the bioaerosols; 2) The respiratory material can also filter indoor aerosol particles during the human breathing. Nonetheless, an important benefit of wearing a respiratory mask is to protect people from pathogenic aerosols in many occupational environments. Smaller increase in bioaerosol levels via the use of "Doctor Masks" could be also due to possible increases in breathing rate (more airborne particles are filtered through the mask during 30 min time) because of its tighter fit compared to that of N95.

The N95 respirator is commonly used in protecting individuals from environmental bioaerosol infection, while a surgical mask is often used to safeguard the surgery

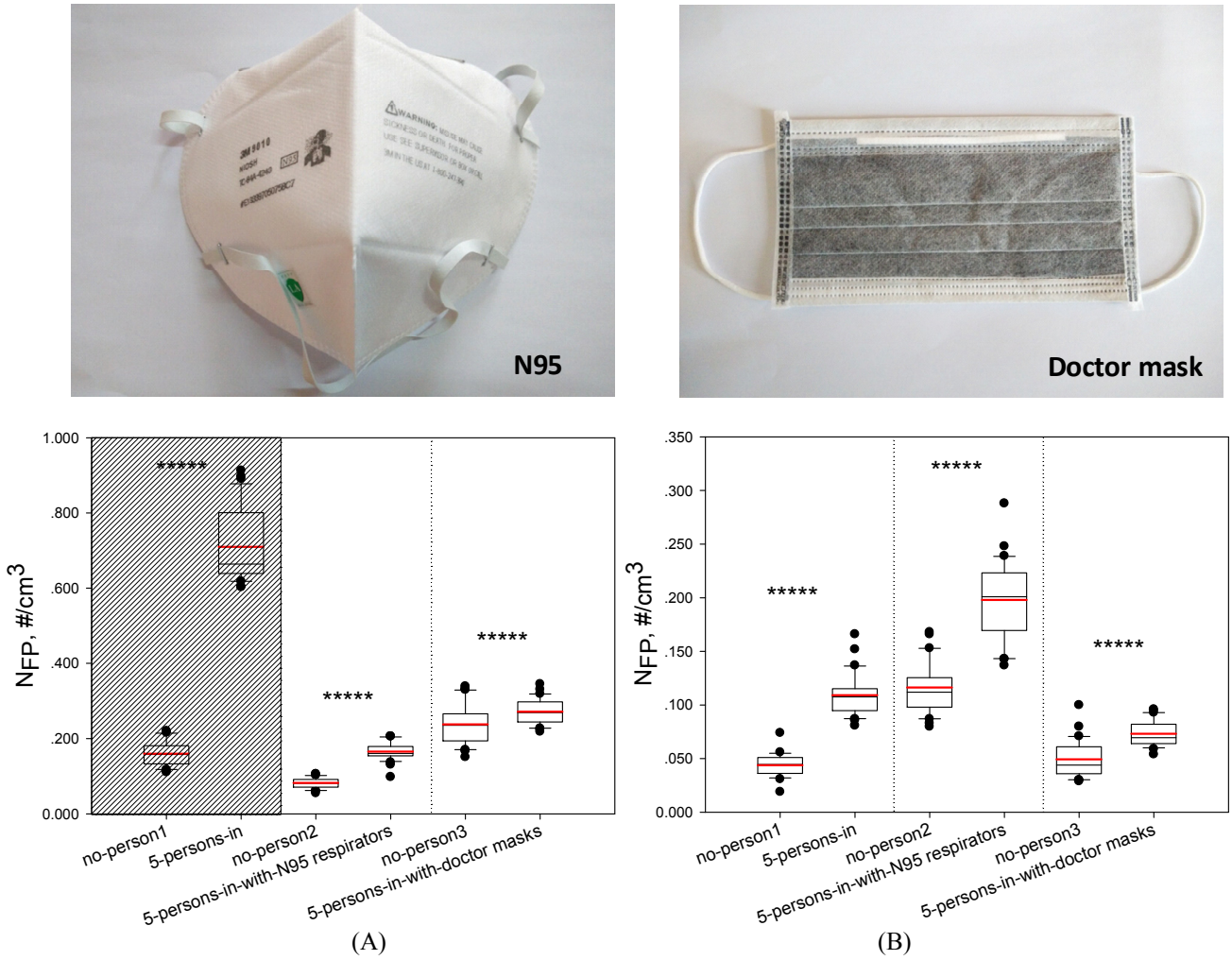

Fig. 3. The concentration levels (Boxplots) (two different replicates) of the viable bioaerosols in the controlled environment under different tests (no-person1, no-person2, no-person3) with and without N95 (A) /"Doctor masks (B)" shown in the figure; the monitoring time under each test was 30 minutes. N_{FP} represents the concentration of fluorescent biological aerosol particles in the controlled environment; data was recorded every 1 minute. Data points represent concentration values of every 1 minute data in the studied environment. ***** indicates a statistically significant difference. For the second experiment (5-persons-in) (the same in Fig. 2), the results of the fluorescent biological aerosol particles when 5 people in the controlled environment was detected to be an outlier (possibly due to malfunction of the UV-APS during that time period).

during a medical operation. However, one study indicated that influenza infection occurred in 23.6% of nurses wearing surgical masks and in 22.9% of nurses wearing N95 respirators for which infection resulted from face leakage (Loeb *et al.*, 2009). The N95 respirator usually has a fairly rigid mask surface, which is very difficult to adjust to fit an individual face frame, while the surgical mask on the other hand has a soft mask surface that can easily accommodate the individual face, accordingly having less leakage. The observed difference in infection rates between N95 respirators and surgical masks in the Loeb's study was due to their different designs and materials. Another study suggested that surgical masks can help patients reduce aerosol shedding of viruses by 3.4 fold, and more viral copies were detected in fine particles (< 5 μm) than coarse particles (> 5 μm) (Milton *et al.* 2013). However, other studies suggested that surgical masks do not provide intended protection, e.g., protecting wounds from infection (Bałazy *et al.*, 2006; Oberg and Brosseau, 2008). Most of the studies discuss the protection efficiencies of respirators against viruses, while little information is available for their breath-borne bacteria shielding. Overall, the facial fit of the respirator plays an important role in bioaerosol shielding efficiencies for the wearer.

In contrast to viable bioaerosols, as shown in Table S4 and Fig. S3, we found a significant decline in the number concentration of particle matter (PM) when 5 people with or without wearing respirators were present in the studied environment. The environmental PM aerosol concentration of the studied environment decreased about 23% when 5 people stayed (sitting quietly) inside for 30 minutes without wearing respirators; however an increase of 7% was detected

when wearing N95 respirators, and a decline of 15% was found when "Doctor masks" were worn as listed in Table S4. Due to leakage, environmental aerosol particles can still be inhaled by humans in addition to those filtered by the mask; however because of the shielding effects of the respirator, humans emitted fewer particles to the environment (most of them were filtered by the respirator material), accordingly reducing environmental particle concentration levels. These results suggest that humans without using respirators might serve as "air purifiers" for the environment in which they reside (they emit bioaerosol particles, but also inhale aerosol particles). This on the other hand indicates that particles inhaled by humans could eventually deposit in the lung. Also it needs to be pointed out that particle losses on the skin, clothes and hair are also possible and worthy to be further studied. Results from this work indirectly show that the N95 respirator was more effective than the "Doctor masks" in terms of protecting people from environmental aerosol particles. We only studied the number concentration of PM, while PM mass concentration was found to be elevated 9 times under the studied situation in another work (Hospodsky *et al.*, 2014). As discussed, the difference might be due to many different factors, including human activity strength. Another study indicates that coarser fractions of particle mass were strongly correlated with strength of human activity (Chen and Hildemann, 2009). The bioaerosol contribution to the environment arising from human presence might come from various sources, e.g., floor resuspension (floor characteristics are thus important, e.g., carpet contribution is very different from brick or tile), human exhaled breath, skin, clothes and many others. Wearing a respirator would not only help prevent breath-borne bioparticle release, but also serve as a filter to remove particles from the air in the environment (humans inhale air). The actual percentage of the bioaerosol contribution to the environment via breathing depends on the floor characteristics, e.g., type of the floor, cleanness, previous occupancy and others. In some very clean environment, e.g., medical surgery room, human emission via breathing could be a major bioaerosol contributor.

Bioaerosol Concentration Monitoring in a Classroom before, during and after Class

For the classroom study, as shown in Fig. 4(B) we detected significantly higher levels of bioaerosol particles during class when about 100 students were present compared to before and after class, although there were still 50 students and 25 students present on average, respectively. The bioaerosol concentrations before, during and after class were 1.38, 2.8 and 1.37 × 10^5 particles m^{-3} in average for each time period, respectively. During the class, the bioaerosol concentration nearly doubled compared to before and after class. However, no differences were observed for bioaerosol size distribution during the three different time periods with different numbers of students present. As observed in Fig. 4(A), there were two bioaerosol peaks detected at 1–1.5 μm and 2–3 μm, which was similar to those observed in our control study in the studied environment. Bioaerosol particles in the classroom are contributed from various sources including outdoors by penetration, indoor emission sources (humans, growing/accumulating bacteria on surfaces emitted via aerosolization or vibration), and floor resuspension. Unlike outdoors, indoor environments could provide desirable conditions that facilitate microbial growth, such as moisture, warm temperature, and shielding of atmospheric irradiation. Accordingly, pathogens released by humans could propagate rapidly and potentially impose an infection risk to indoor occupants. Bacteria emitted to the ground by previous occupants in an indoor environment could be resuspended through human activities, thus presenting a

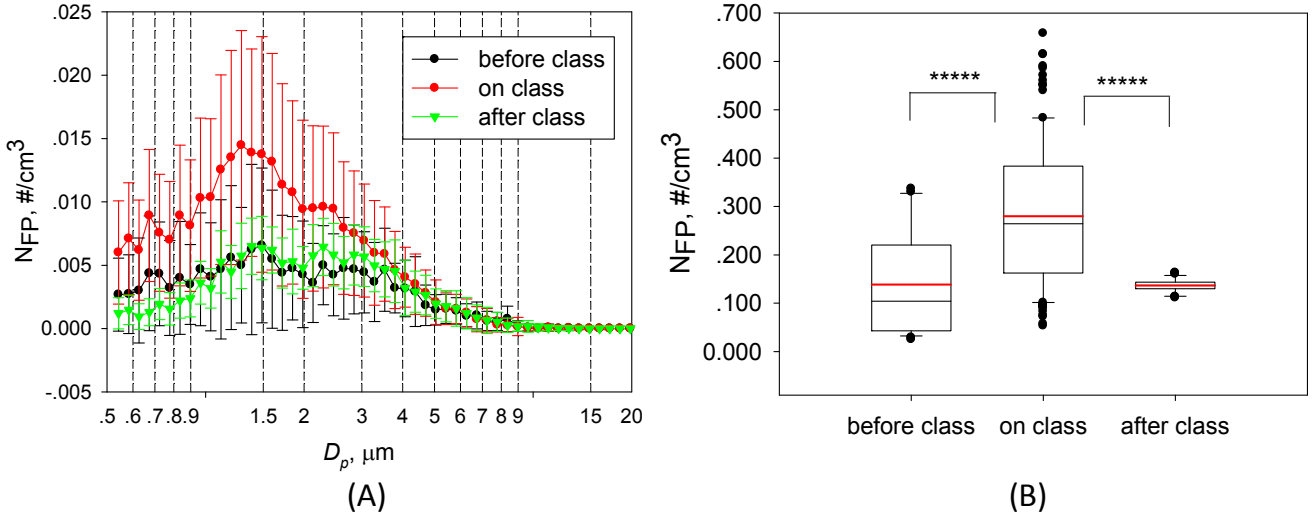

Fig. 4. The size distributions (A) and concentration levels (B) of the fluorescent biological aerosol particles in the classroom over different class time periods (before, during and after class); D_p represents particle aerodynamic diameter, and N_{FP} represents the concentration of fluorescent biological aerosol particles; data set was recorded every 1 minute. Data points represent means of monitoring data over the time period studied. ***** indicates a statistically significant difference.

microbial exposure risk. Based on this work and previous literature data, the bioaerosol increase might be also dominantly contributed by the floor resuspension.

Similar to our controlled study in the studied environment, we also observed a decline of total PM number concentration during class when 100 students were present compared to before and after class (Fig. 5(B)). The PM (0.5–20 μm) number concentrations before, during and after class were 1.99, 1.55 and 2.31 × 10^6 particles m^{-3}, respectively. On average, viable bioaerosol particles accounted for about 10% of the total aerosol particles of > 0.5 μm. Likewise, we did not detect a significant difference in their size distributions with a peak at 1.5 μm (PM) for three different class time periods (Fig. 5 (A)).

There have been an increasing number of occurrences of infectious disease outbreaks in recent decades. Humans play an important role in the transmission of infectious diseases by emitting pathogenic aerosols, e.g., SARS in 2003 (Yu *et al.*, 2004). In past studies, both viruses and bacteria were detected from exhaled breath (Fabian *et al.*, 2008; Shen *et al.*, 2012; Xu *et al.*, 2012; Milton *et al.*, 2013). Therefore, wearing a respirator, especially during an outbreak, can help protect the wearer from pathogenic aerosol exposure. However, growing evidence shows that currently available respirator masks often fail to offer the desired protection (Loeb *et al.*, 2009; Zou and Yao, 2015). This is largely due to leakage between the mask and human face, i.e., the problem of poor facial fit. For future respirator design, facial fit is a critically important parameter to consider for desired protection efficiency. Another problem lies with common materials of masks, such as active carbon and polypropylene melt-brown fabric, which might serve as hotbeds for bacterial growth and virus breeding on the mask, eventually causing secondary pollutants to people and the environment (Lin *et al.*, 2015). In an effort to minimize the problem, some studies focus on developing new materials for respiratory masks, e.g., adding antibacterial agents such as nano-silver to the material of masks (Borkow *et al.*, 2010; Natarajan *et al.*, 2016) or using carbon nanotubes as filtration material (Guan and Yao, 2010; Xu and Yao, 2011; Zou and Yao, 2015). Another major problem with current respirators is a high pressure drop, which leads to significant breathing discomfort. Materials used in future respirator designs should not only provide high filtration efficiencies, but also low airflow resistance.

The UV-APS used in this work has an upper detection limit of 6 × 10^7 particles m^{-3} (Agranovski *et al.*, 2003). Our detected bioaerosol concentration did not reach such a magnitude; therefore the results were not impacted by the detection limit. On the other hand, in exhaled breath, non-biological compounds or biological debris could be present that fluoresce at the same wavelength as bioaerosols, which thus could interfere with the detection of true microbial aerosol particles by the UV-APS. However, detailed information regarding the possible fluorescence of substances that can be present in exhaled breath is not currently available, and certainly this topic deserves thorough investigation. Nonetheless, in our work, we counted breath-borne bioaerosol particles from the fluorescence channel that had substantially higher fluorescence intensity (i.e., usually at least 10 times higher) than the preceding fluorescence channel. By doing so, we eliminated fluorescent counts that were from interfering chemical compounds with weak fluorescence signals possibly from exhaled breath.

CONCLUSIONS

The results here suggest that human occupancy in the environment can contribute significantly to its bioaerosol levels via breathing (17%; this work), and previously reported skin emission and resuspension. The bioaerosol emission rate via human breathing was found to be about 1.45 × 10^5

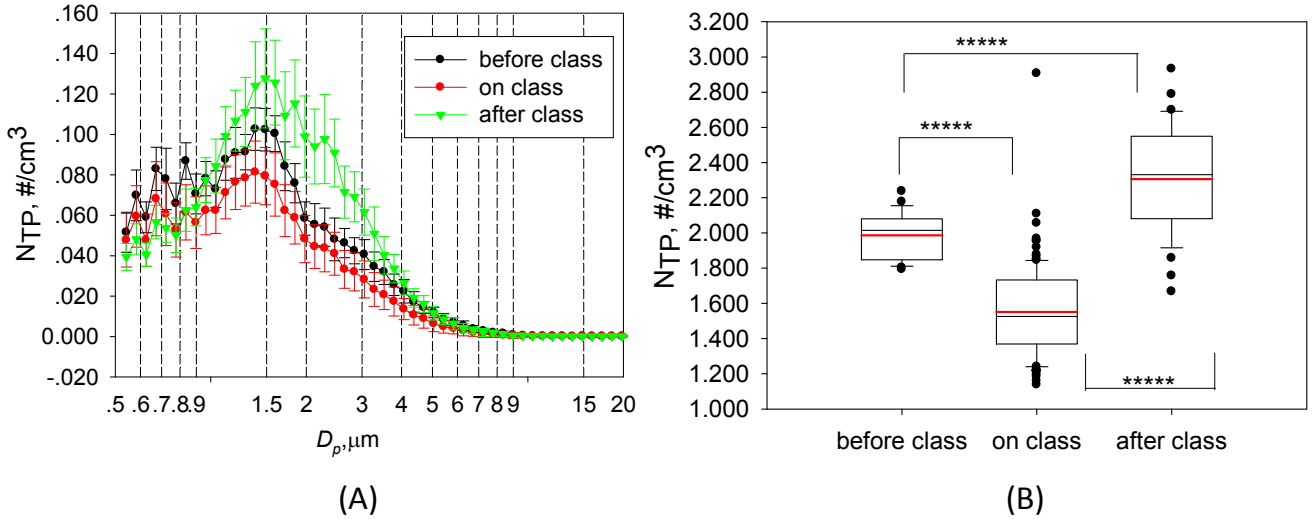

Fig. 5. The size distributions (A) and number (larger than 0.5 μm) concentration (B) levels of particulate matters in the classroom at different time periods (before, during and after class); D_p represents particle aerodynamic diameter, and N_{TP} represents the concentration of aerosol particles; data set was recorded every 1 minute. Data points represent means of monitoring data over the entire monitoring time period. ***** indicates a statistically significant difference.

particles person^{-1} hour^{-1}. There was a peak around 1.5 μm observed for the fluorescent particles in human exhaled breath. In the studied environment (27 m^3), the presence of 5 people without wearing masks increased bioaerosol concentration by 107% within 30 min with an average bioaerosol emission rate of 8.4×10^5 fluorescent particles person^{-1} hour^{-1} resulting from one's occupancy. The percentage of bioaerosols out of the total particles (> 0.5 μm) was observed to increase from 13.0% to 26.0% with human occupancy. In-classroom observation also showed a fluorescent particle concentration increase of about 50%. Bioaerosols emitted via breathing from infected individuals could be pathogenic. Use of respirator could prevent humans from releasing bioaerosols (especially those pathogenic ones) into the environment, and the efficiency depends on the respiratory type. Because of the difference in facial fit, it seems that the "Doctor masks" is better than the N95 mask in terms of preventing humans from releasing bioaerosol into the environment. Based on previous literature data and this work, skin emission and floor resuspension (those from the floor are also contributed directly by humans or carry-ons from the environment) resulting human occupancy accounted for the major part (in this work, it is more than 80% by calculation) of the bioaerosol increase in an indoor environment. Information derived herein help differentiate the bioaerosol emission pathways of human occupancy and can assist in risk assessment of microbial aerosol exposure with and without using respirators in various settings including public spaces such as subway system and occupational settings such as flight cabins and medical surgery and transplant rooms.

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SUPPLEMENTARY MATERIAL

Supplementary data associated with this article can be found in the online version at http://www.aaqr.org.

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