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Development of an automated wet-cyclone system for rapid, continuous and enriched bioaerosol sampling and its application to real-time detection

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ABSTRACT

We present a novel bioaerosol sampling system based on a wet-cyclone for real-time and continuous monitoring of airborne microorganisms. The Automated and Real-time Bioaerosol Sampler based on Wet-cyclone (ARBSW) continuously collects bioaerosols in a liquid medium and delivers the samples to a sensing device using a wireless remote control system. Based on a high air-to-liquid-flow-rate ratio (∼1.4 × 10⁵) and a stable liquid thin film within a wet-cyclone, the system achieved excellent sampling performance as indicated by the high concentration and viability of bioaerosols (>95% collection efficiency for >0.5-μm-diameter particles, >95% biological collection efficiency for Staphylococcus epidermidis and Micrococcus luteus). Furthermore, the continuous and real-time sampling performance of the ARBSW system under test-bed conditions and during a field test demonstrated that the ARBSW is capable of continuously monitoring bioaerosols in real time with high sensitivity. Therefore, the ARBSW shows promise for continuous real-time monitoring of bioaerosols and will facilitate the management of bioaerosol-related health and environmental issues.

1. Introduction

Concerns over airborne microorganisms, called bioaerosols, have increased due to their adverse effects on the human body and the environment [1–4]. Bioaerosols, such as pathogenic viruses, bacteria, and fungal spores, are associated with infectious diseases, allergies, and asthma [2,5–7]. For example, the Middle East respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS) coronaviruses, which have recently killed hundreds of people in East Asia, are transmitted by direct inhalation of contaminated bioaerosols [8]. Therefore, the World Health Organization (WHO) recommends that the bioaerosol concentration within indoor air be less than 500 CFU/m³ [9,10].

A technique enabling rapid real-time detection of airborne microorganisms would be advantageous for public health research and bioterrorism defense; however, the development of such a system is at an early stage. A major reason for the slow pace of development is the difficulty of determining the concentration and type of bioaerosols in the air stream [11–16]. Bioaerosols are typically detected by first collecting them in a liquid or on a surface and analyzing the collected particles using culture-based techniques, biochemical assays (e.g., polymerase chain reaction (PCR)) and/or enzyme-linked immunosorbent assay (ELISA) [14,17–20]. Recently, the development of real-time detection systems for microorganisms in liquid medium has made considerable progress [14,21–23]; however, the performance of bioaerosol sampling systems with regard to biological stability, particle collection efficiency, and particle enrichment is still insufficient for use as a real-time system. In addition, integration of the bioaerosol sampling system and the detection system, including rapid and stable sample transfer, would be desirable for continuous real-time monitoring of bioaerosols.

The ideal bioaerosol sampler should have the following characteristics: (i) rapid and continuous sampling, (ii) high collection efficiency and stable microbial recovery (e.g., liquid or semi-liquid collection medium), (iii) high particle enrichment, (iv) integration with the detection system (e.g., continuous and consistent sample delivery) and (v) portability and automated operation. Tan et al. developed an
automated electrostatic sampler that involves collection of bioaerosols in a liquid reservoir and their delivery to sensing devices [24]. Liu et al. and other researchers described an airborne pathogen direct analysis system based on microfluidic enrichment [11–13]; however, these systems require prolonged sampling for enrichment. A novel bioaerosol sampling system, the MicroSampler, based on two-phase fluid control in a microchip can be used with a real-time bioaerosol sensor [25]; however, due to the low throughput, adequate sampling is difficult in the presence of bioaerosol concentrations < 500 CFU/m³.

A wet-cyclone collects aerosols into a liquid film on the inner wall of the cyclone using the particle centrifugal force and the liquid surface tension. Such systems achieve high sampling performance and enable the concentration of samples due to the high flow rate ratio between the incoming air and drainage liquid. Considerable research has focused on the development of wet-cyclone bioaerosol samplers [26–30]; however, these have a large particle cut-off diameter, and a low particle collection efficiency and aerosol-to-liquid transfer rate; furthermore, the two-phase flow operation is unstable and few fully integrated bioaerosol sampling systems are capable of continuous real-time sampling.

Here, the Automated and Real-time Bioaerosol Sampler based on Wet-cyclone (ARBSW) system for continuous real-time monitoring of bioaerosols is presented. The ARBSW system continuously collects airborne microorganisms and automatically delivers them to an analytical sensor. The aerosol collection efficiency, particle air-to-liquid transfer efficiency, sample enrichment, and microbial recovery of the ARBSW are evaluated. In addition, the ARBSW was subjected to sampling sensitivity tests of bioaerosol detection under test-bed conditions, as well as real-time bioaerosol monitoring in a real atmosphere environment. The results demonstrate that the ARBSW facilitates continuous bioaerosol monitoring with high sensitivity in real-world environments.

2. Materials and methods

2.1. Test particles and microorganisms

Standard-size particles and bacteria were used as test aerosols to evaluate the performance of the ARBSW system. Monodisperse and spherical standard polystyrene-latex (PSL) particles (0.1, 0.3, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 and 2.0 μm in diameter; 1.06 g/cm³ density; Duke Scientific Corp., Palo Alto, CA, USA) and red fluorescent PSL (FPSL) particles (0.3, 0.48, 0.8, 1.0 and 2.1 μm in diameter; 1.05 g/cm³ density; Fluoro-Max™, Thermo Scientific, Waltham, MA, USA) were used to evaluate the aerosol collection efficiency and air-to-liquid particle transfer efficiency of the ARBSW. Staphylococcus epidermidis (ATCC 12228) and Micrococcus luteus (ATCC 9341) were used as the test airborne microorganisms. These Gram-positive bacteria are commonly found in indoor environments and on human skin [31,32]; and are used widely in bioaerosol research [21,33–35]. In particular, S. epidermidis is an important opportunistic pathogen and is the most common source of infections on indwelling medical devices [36]. The bacteria were incubated in nutrient broth (Becton Dickinson, Franklin Lakes, NJ, USA) at 37 °C for 24 h. Upon reaching an optical density at 600 nm of 0.6, bacterial suspensions were harvested by centrifugation (5000 × g, 10 min), washed three times with sterilized deionized water (SDW) and diluted with 20 mL of SDW. Subsequently, a 30 μL aliquot (~ 10⁹ colony forming units (CFU)/mL) was poured into the nebulizer.

2.2. Test aerosol generation

Figure S1(a) shows a schematic diagram of the test aerosol generation. Compressed clean air was passed into a six-jet Collison nebulizer (BGI Inc., Waltham, MA, USA) via a mass flow controller (MFC, FC-280S; Mykrolis Corp., Billerica, MA, USA) at a flow rate of 5 L/min. To remove moisture and electrical charge from the aerosols, the nebulized particles were sequentially passed through a diffusion dryer and ²¹⁹Po neutralizer. The test aerosol flow was diluted with an additional clean air flow (7–11 L/min) in a mixing chamber and inserted into the ARBSW system.

2.3. Real-time aerosol measurement

As shown in Figure S1(b), the size distribution and number concentration of the test aerosols before and after passing through the ARBSW system were measured in real-time using a wide-range particle spectrometer (WPS) (1000XP; MSP Corp., Shoreview, MN, USA; particle size range 10 nm to 10 μm) and an aerodynamic particle sizer (APS) (3314; TSI Inc., St. Paul, MN, USA; particle size range 0.5–20 μm), respectively.

2.4. The wet-cyclone module operation

Figure S1(c) shows the schematic diagram of the wet-cyclone module operation. There is one aerosol inlet and three sampling liquid inlets in the side of the wet-cyclone, and the outlets for the exhausted air and hydrosol liquid (including particle sample) are in the upper and bottom side. During operation, the liquid sampling medium (e.g., SDW) was injected at 5–13 mL/h through the three ports of the wet-cyclone using a syringe pump (KD200; KD Scientific Inc., Holliston, MA, USA). The liquid drainage flow rate was controlled at 2–13 mL/h using a peristaltic pump (T60-WX10; Longer Corp., Hebei, China). The liquid sampling medium can be transferred continuously and directly to the particle detection part or storage container array for later processing.

2.5. Particle characterization

Figure S1(d) shows a schematic diagram of the particle characterization after sample collection. Airborne bacterial particles were deposited onto copper transmission electron microscopy (TEM) grids (carbon film on a copper mesh; CF300-Cu; Electron Microscopy Sciences, Hatfield, PA, USA) using an electrostatic precipitating nanoparticle collector (model 4650; HCT Inc., Icheon, Republic of Korea). A field emission-scanning electron microscope (FE-SEM; Teneo VolumeScope, FEI, Hillsboro, OR, USA) was used to visualize the structure and morphology of surface-deposited airborne bacterial particles. To calculate the FPSL particle concentration in the drainage liquid medium, aliquots (~ 10 μL) from the wet-cyclone outlet were injected into a disposable hemocytometer (DHNC01; INCYTO, Cheonan, Republic of Korea). Next, the FPSL particles were counted using a fluorescence microscope (BX51; Olympus, Tokyo, Japan) with a U-MWG2 filter set (excitation, 510–550 nm; emission, > 590 nm). Images of at least nine microscopic fields were captured using a charge-coupled device (CCD) array camera. Particles were enumerated using ImageJ software (National Institutes of Health, Bethesda, MD, USA). For the colony counting assay, the collected bacterial suspensions were serially diluted in SDW. Aliquots of 100 μL of the suspensions were spread on the surface of nutrient agar (Becton Dickinson) in Petri dishes. Colonies were counted after incubating the Petri dishes for 24 h at 37 °C.

2.6. Characteristics and performance evaluation of the ARBSW system

The prototype ARBSW system automatically controls the air sampling, liquid supply, liquid drainage flow rate and sample delivery using software based on Arduino and MATLAB. The system can be remotely controlled using the wireless control panel and is 25 × 15 × 20 cm in size. Aerosols are entered through the inlet of the ARBSW by an air pump and continuously collected in the sampling liquid flow. Two peristaltic pumps enable a continuous supply of liquid to the ARBSW system and a transfer of liquid to the analytical detection part at the appropriate flow rate.

For evaluating the bioaerosol collection efficiency of the ARBSW,
the number concentrations of the test bioaerosols before and after transmission through the ARBSW system were measured using an APS. Simultaneously, the drainage liquid was sampled (∼ 100μL) and the colony number was determined using the colony counting assay. Finally, the culturable bioaerosol number concentration (CFU/mL) was determined according to the total liquid volume.

The BioSampler (SKC Inc., Eighty Four, PA, USA) was used for performance evaluation in terms of the relative microbial recovery (%) and sample enrichment ratio of bioaerosol. The BioSampler was formed of glass and consisted of three parts: an inlet, a nozzle section with three tangential sonic nozzles and a collection vessel. The collection vessel was filled with a liquid collection medium (20mL). The nozzles of the BioSampler create a swirling air flow (12.5L/min air of air supply) that maintains microorganism viability by gently moving particles onto the collection surface without re-aerosolization [25,37–39]. Under the same bioaerosol exposure condition (∼ 100 particles/cm³ air of total aerosol number concentration (TANC)), we sampled the bioaerosols using the ARBSW and BioSampler and obtained the culturable bioaerosol number concentration, respectively. Finally, we compared the relative microbial recovery (%) and the sample enrichment ratio between the ARBSW and BioSampler under various sampling times.

2.7. Experimental setup for sensitivity test and real-world field test

Figure S2 shows a schematic diagram of the sensitivity test setup to evaluate the real-time sampling responsivity of the ARBSW under the abrupt bioaerosol exposure conditions. The S. epidermidis suspension was nebulized for 40 s at 10-min intervals in a biosafety cabinet. While the ARBSW system samples surrounding bioaerosols in a real-time manner, the APS monitors the change of TANC at 1-min intervals. The real-time colony concentration of sampled particles using ARBSW was determined using the colony counting assays and compared with the real-time data of APS.

Figure S3 shows a schematic diagram of the real-world field test setup. The temperature, relative humidity and wind speed were monitored using a hot-wire anemometer with thermohygrometer (TES-1341; TES Electrical Electronic Corp., Taipei, Taiwan). The atmospheric particulate matter (PM) concentration and TANC were monitored using an optical particle counter (OPC) (model 1.109; GRIMM Aerosol Technik Airing GmbH & Co. KG, Airing, Germany). The ARBSW also sampled the atmospheric bioaerosols continuously and the liquid samples were stored every 5 min. These samples were tested using colony counting assays to obtain the bioaerosol colony concentration.

3. Results and discussion

3.1. Principle and design of the wet-cyclone module for the ARBSW

Fig. 1(a) shows the aerosol collection principle of the wet-cyclone module for the ARBSW system. The cyclone is a conical device that creates an internal helical air stream and collects aerosols on the inner wall through centrifugal force. In our wet-cyclone module, a stable liquid thin film is formed on the inner wall of the cyclone by centrifugal force and the shear force generated by the high air-flow rate. At the same time, the liquid film slowly and continuously flows down the cyclone according to the balance between the supply and drainage liquid flow for delivering samples. Due to the large difference between the air and liquid flow rates, aerosols entering the wet-cyclone are completely collected in the liquid film, rapidly concentrated to a high enrichment ratio and continuously delivered to an analytical sensor.

Our bioaerosol sampling system has a cut-off diameter of 0.3μm and collection efficiency of > 99% for aerosols > 1μm in diameter with high-throughput operation. The cut-off diameter ($d_{50}$), defined as the diameter of the aerosol having a collection efficiency of 50%, is expressed as follows [40]:

$$d_{50} = \frac{9\mu b}{2\pi \rho_p \cdot U_a \cdot C_s \cdot N_t}$$

(1)

where $\mu$ is the air dynamic viscosity, $b$ is the width of the air inlet, $\rho_p$ is the particle density, $U_a$ is the air velocity at the inlet, $C_s$ is the Cunningham slip correction factor calculated using $C_s = 1 + 0.5Kn_p$ [2.34 + 1.05 exp(-0.195Kn_p)], $Kn_p$ is the Knudsen number of the particle and $N_t$ is the number of turns of the air stream in the cyclone.

As shown in Eq. (1), the characteristics of the air flow inside the cyclone play a decisive role in the cut-off diameter. The behavior of the air stream directly depends on the geometry of the cyclone; for this
We used the numerical analysis for optimization of the air and liquid flow conditions in the wet-cyclone. The air flow rate \(Q_a\) is 16 L/min. The supplied liquid flow rate \(Q_s\) is 9 mL/h and the drainage liquid flow rate \(Q_d\) is (i) 10, (ii) 8.2 and (iii) 5 mL/h. The dark area within the wet-cyclone indicates the liquid film. (b) Optimized operating conditions for the supplied- and drainage-liquid flow rate to obtain the stable status of the two-phase flow at the wet-cyclone. The intake air flow rate is 12, 14 and 16 L/min. (c) Photographs of the status of the wet-cyclone according to the operating conditions [(i), (ii), and (iii)] indicated in Fig. 2(b). For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.

Fig. 2. Characteristics of the stabilization of two-phase flow in the wet-cyclone. (a) Numerical analysis for optimization of the air and liquid flow conditions in the wet-cyclone. The air flow rate \(Q_a\) is 16 L/min. The supplied liquid flow rate \(Q_s\) is 9 mL/h and the drainage liquid flow rate \(Q_d\) is (i) 10, (ii) 8.2 and (iii) 5 mL/h. The dark area within the wet-cyclone indicates the liquid film. (b) Optimized operating conditions for the supplied- and drainage-liquid flow rate to obtain the stable status of the two-phase flow at the wet-cyclone. The intake air flow rate is 12, 14 and 16 L/min. (c) Photographs of the status of the wet-cyclone according to the operating conditions [(i), (ii), and (iii)] indicated in Fig. 2(b). For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.

reason, we analyzed the air flow and aerosol behavior in the wet-cyclone to optimize the cyclone design parameters using commercial computational fluid dynamics software (Fluent 16.0; ANSYS Inc., Canonsburg, PA, USA). The finite volume method was employed to solve the governing equations and the discrete phase model in Fluent code was used for particle tracking. Figure S4 shows the optimal cyclone geometry and dimensions and Figure S5(a) shows the trajectories of aerosols entering the cyclone at the optimal inlet air flow rate of 16 L/min. The particle collection position gradually lowers with decreasing particle size; all particles ≥ 0.75 μm diameter were captured in the liquid film (Fig. S5(c)) indicated a cut-off diameter of ~ 0.3 μm. Fig. 1(b) shows photographs of the wet-cyclone module based on the simulation results.

3.2. Stabilization of two-phase flow in the wet-cyclone module

For real-time continuous particle sampling with a high concentration ratio, the liquid sampling medium should form a stable film on the wall of the cyclone under the high air flow rate condition. The stable liquid film means that the liquid covers the entire surface of the cyclone wall without being sprayed out of the film, to prevent sampling loss in the cyclone. The drag force of the fluid on the particle is proportional to the cube of the particle size, whereas the force between the particle and the wall is proportional to the particle size. Therefore, if fine aerosols directly adhere to the cyclone wall, there is no way to remove the particles, resulting in sampling loss [41]. The influence of air flow on a liquid film can be expressed by the modified Webber number, \(W_{\text{ef}}\). This can be conceived of as a measure of the relative importance of the inertia of a fluid compared to its surface tension [25]:

\[
W_{\text{ef}} = \frac{\rho_l \cdot U^2 \cdot t}{\sigma_l}
\]  

(2)

where \(\rho_l\) is the liquid density, \(t\) is the thickness of the liquid film and \(\sigma_l\) is the surface tension of the liquid.

When \(W_{\text{ef}}\) increases the inertia of the liquid due to the shear force generated at the interface between the liquid film, the air flow overwhelms the surface tension of the film surface and the liquid is separated from the surface and sprayed into the surrounding air stream. To maintain a stable liquid film the \(W_{\text{ef}}\) should be lowered by adjusting the air flow rate and the liquid flow rate. However, reducing the air flow rate to lower \(W_{\text{ef}}\) hampers particle concentration and decreases the collection efficiency of fine aerosols (see Eq. (1)). Therefore, it is important to optimize the liquid flow inside the cyclone by controlling the liquid supply and drainage flow rates.

A numerical analysis was conducted to identify the optimal air flow and liquid flow conditions for the stable film. The volume-of-fluid (VOF) model, a two-phase flow model in Fluent code, was developed to calculate the hydrodynamics of liquid film formation inside the cyclone under various operating conditions. For the reasons mentioned above, the air flow rate was set at > 12 L/min and the liquid drainage flow rate was limited to 12 mL/h to attain an enrichment ratio of > 10⁴. Within the cyclone the surface of the thin liquid film rapidly evaporates due to the high air flow rate; therefore, if the liquid supply flow rate was lower than or equal to the drainage flow rate, a stable liquid film would not be formed and the liquid would split into multiple streams (Fig. 2(a)(i)); supplied liquid flow rate \(Q_s\) = 9 mL/h, drainage liquid flow rate \(Q_d\) = 10 mL/h). In contrast, if the supply flow rate were larger than the drainage flow rate, the liquid would float inside the cyclone and tend to clog (Fig. 2(a)(iii)); \(Q_s\) = 9 mL/h, \(Q_d\) = 5 mL/h). The optimal supply and drainage flow rate ratio is around 1.1 (Fig. 2(a)(ii)); \(Q_s\) = 9 mL/h, \(Q_d\) = 8.2 mL/h).

The conditions for the formation of a stable liquid film in the real wet-cyclone were optimized using the numerical analysis. Fig. 2(b) shows the optimized operating domains, where stable liquid films are formed at air flow rates of 12, 14 and 16 L/min. For 12 L/min, the optimal liquid supply-to-drainage-flow rate ratio is around 1.12, similar to the numerical analysis result (Fig. 2(c)(ii)). The optimal ratio slightly increased with an increasing air flow rate due to faster evaporation (1.17 and 1.22 at air flow rates of 14 and 16 L/min, respectively). If the amount of supplied liquid was too low (Fig. 2(c)(i)) or too high (Fig. 2(c)(iii)) unstable films were formed, similar to the numerical analysis result. These cases are also shown in Movie S1.
3.3. Performance evaluation of the aerosol sampling in the wet-cyclone module

We evaluated the aerosol collection performance of the wet-cyclone module at liquid supply flow rates of 0, 5, 7, 9, 11 and 13 mL/h and sampling air flow rates of 12, 14 and 16 L/min using standard PSL particles (Fig. 3a). The total aerosol collection efficiency (\( \eta_T \)) was defined as the fraction of the total number concentration of the entering PM retained by the wet-cyclone:

\[
\eta_T = \frac{C_{\text{in}} - C_{\text{out}}}{C_{\text{in}}} \quad (3)
\]

where, \( C_{\text{in}} \) and \( C_{\text{out}} \) represent the aerosol number concentration at the air inlet and outlet, respectively.

At a 12 L/min air flow rate (Fig. 3a1), the collection efficiency of aerosols over 0.5 \( \mu \)m in diameter was >95%, similar to the simulation result. As the air flow rate was increased, the particle collection performance improved due to the increased centrifugal force; the collection efficiency of aerosols > 0.5 \( \mu \)m in diameter was \( \sim \) 98.98% at 14 L/min (Fig. 3a2)) and \( \sim \) 99.72% at 16 L/min (Fig. 3a3)). However, the liquid supply flow rate had little effect on the aerosol collection performance.

The aerosol-to-liquid transfer efficiency of the wet-cyclone system was evaluated using fluorescent PSL particles under sampling air flow rates of (b1) 12 L/min, (b2) 14 L/min and (b3) 16 L/min.

The proportion of the input aerosols delivered to the analytical sensor part was also assessed. In Eq. (3), the \( \eta_T \) is equal to the sum of the air-to-liquid particle transfer efficiency and the fraction of particles lost to the inner wall of the cyclone. The air-to-liquid particle transfer efficiency (\( \eta_{AL} \)) is defined as the transfer fraction of aerosol in the air to the liquid medium in the cyclone. We measured \( \eta_{AL} \) by comparing the total number of FPSL particles collected with that in the liquid drainage medium:

\[
\eta_{AL} = \frac{N_d}{N_{in} \cdot \eta_T} = \frac{C_{d} \cdot Q_a}{C_{in} \cdot Q_a \cdot \eta_T} \quad (4)
\]

where \( N_d \) is the total number of particles in the liquid drainage medium, \( N_{in} \) is the total number of aerosols in the inlet air, \( C_{d} \) is the number concentration of particles in the liquid drainage medium and \( Q_a \) is the air flow rate.

As shown in Fig. 3(b), \( \eta_{AL} \) increased as the liquid flow rate and air flow rate were increased; however, the particle size had little effect on the \( \eta_{AL} \) of the wet-cyclone. These results are related to the uniformity and coverage of liquid film on the inner wall of the wet-cyclone. The higher air and liquid flow rates yield a more uniform liquid film with greater coverage in the wet-cyclone, which can decrease the particle
3.4. Characteristics and performance evaluation of the ARBSW system

The ARBSW system was developed using the above numerical and experimental results and all parts are operated automatically. Fig. 4 is a photograph of the ARBSW and wireless remote-control panel. The real-time bioaerosol sampling performance of the ARBSW system was evaluated using test microorganisms (S. epidermidis and M. luteus). Fig. 5(a) shows the size distributions of the test bioaerosols as unimodal curves. The specific geometric mean diameter (GMD) of S. epidermidis and M. luteus was 0.86 ± 0.01 μm and 1.10 ± 0.009 μm, respectively. The GMD is defined as $\sum n_j l n j/N$, where $n_j$ is the number of particles in the $j$th group, $d_j$ is the diameter of an individual particle and $N$ is the total number of particles. The maximum and minimum aerodynamic diameters of S. epidermidis and M. luteus were ∼ 0.55 and ∼ 1.3 μm, and ∼ 0.55 and ∼ 2.1 μm, respectively. SEM showed that cells of both taxa are spherical (Fig. 5(b)).

The bioaerosol collection efficiency of the ARBSW system was initially assessed. The collection efficiency of S. epidermidis and M. luteus was more than 95% over their entire size range (Fig. 5(c1)), similar to the standard PSL particles. The total collection efficiency of both bioaerosols was > 99% (Fig. 5(c2)). Next, the microbial recovery of
bioaerosols sampled by the ARBSW system was assessed. A comparative test was conducted with the conventional verified bioaerosol sampler, called BioSampler, which has a collection efficiency of > 90% for bioaerosol sizes of > 0.5 μm and a microbial recovery of > 99%. According to the BioSampler’s operational manual, 12.5 L/min of air enters 20 mL of sampling liquid in a reservoir and the enrichment of the collected particles is proportional to the sampling time (typically 20 min to reduce the desiccation effect) [25,37–39]. In contrast, the ARBSW system maintains a high particle enrichment ratio regardless of the sampling time, due to the constant air-to-liquid flow rate ratio (∼ 1.4 × 10^5; use of the continuous entered air flow (16L/min) and the sampling time, due to the constant air-to-liquid flow rate ratio (∼ 20min to reduce the desiccation effect) [25,37–39].

According to the BioSampler’s operational manual, 12.5 L/min of air-enriched bioaerosol sizes of >0.5 μm and a microbial recovery of >99%. Called BioSampler, which has a collection efficiency of >90% for biological aerosols, was kept constant at ∼ 100 particles/cm3 after collection. The sampling time was 20 min and the TANC of test bacterial bioaerosols was kept constant at ∼ 100 particles/cm³. The concentration was<0.01 CFU/cm³ at<0.1 CFU/cm³. After 2 min of nebulization, the TANC was<0.01 CFU/cm³. In the second nebulization, the TANC increased from 0 to 1.6 particles/cm³, indicating removal of >90% of bioaerosols. The colony concentration increased to 0.24 CFU/cm³ during the nebulization and decreased to 0.06 CFU/cm³ after 1 min of nebulization. After 2 min of nebulization, the TANC was<0.01 particles/cm³, indicating removal of >90% of bioaerosols. The colony concentration increased to 0.24 CFU/cm³ during the nebulization and decreased to 0.06 CFU/cm³ after 1 min of nebulization. After 2 min of nebulization, the TANC was<0.01 CFU/cm³. In the second nebulization, the TANC increased from 0 to 1.2 particles/cm³ and the colony concentration increased from 0 to 0.2 CFU/cm³. Thus, the colony concentration results of the ARBSW correspond well to the TANC variation measured by APS. Additionally, the results demonstrate that the ARBSW system is capable of continuous and accurate real-time bioaerosol monitoring, even in the presence of rapid changes in concentration.

3.5. Real-time and continuous sampling performance evaluation using a test-bed condition

Real-time airborne microorganism monitoring systems should be capable of responding to abrupt changes in bioaerosol concentrations. To evaluate the responsibility of the ARBSW system, a test-bed environment that allows for sudden changes in bioaerosol concentration was used, as shown in Fig. 6(a) (details of the experimental setup are in Fig. S2). Fig. 6(b) shows the TANC and colony concentration of the bioaerosols at 1-min intervals during the test. APS showed that the TANC rapidly increased from 0 to 1.6 particles/cm³ during the first nebulization, and decreased to 0.4 particles/cm³ after 1 min of nebulization. After 2 min of nebulization, the TANC was<0.1 particles/cm³, indicating removal of >90% of bioaerosols. The colony concentration increased to 0.24 CFU/cm³ during the nebulization and decreased to 0.06 CFU/cm³ after 1 min of nebulization. After 2 min of nebulization, the concentration was<0.01 CFU/cm³. In the second nebulization, the TANC increased from 0 to 1.2 particles/cm³ and the colony concentration increased from 0 to 0.2 CFU/cm³. Thus, the colony concentration results of the ARBSW correspond well to the TANC variation measured by APS. Additionally, the results demonstrate that the ARBSW system is capable of continuous and accurate real-time bioaerosol monitoring, even in the presence of rapid changes in bioaerosol concentrations.

3.6. Real-world field test

A field test of the ARBSW system was conducted, where real-time monitoring of bioaerosols at a pond was performed at the Korea Institute of Science and Technology (KIST, Seoul, Republic of Korea) on August 23, 2017 (14:00–17:00) (Fig. 7(a)). Fig. 7(b) shows the variations in atmospheric PM and total culturable bioaerosol concentrations during the field test (details of the experimental setup are in Fig. S3). PM10 and PM2.5 are defined as the mass fractions (μg/m³) of aerosols with an aerodynamic diameter smaller than 10 and 2.5 μm, respectively [40]. The PM10 concentration decreased from 13 to 10 μg/m³, and the PM2.5 decreased from 11 to 9 μg/m³. During the field test, the TANC also decreased from ∼ 5.5 × 10^5 to ∼ 3.7 × 10^5 particles/m³ at a particle size range >1.0 μm (∼ 33% reduction) (Fig. S6). The total bioaerosol concentration monitored by the ARBSW decreased from 220 to 180 CFU/m³ at a rate similar to those of PM10 and PM2.5. These results indicate that the bioaerosol concentration was influenced predominantly by the PM concentration. More environmental monitoring data are shown in the supporting information (Fig. S6). These results demonstrate that the ARBSW system can perform continuous and real-time bioaerosol monitoring in real-world environments.

Fig. 6. Real-time bioaerosol sampling experiment in a test-bed environment. (a) Photograph of the test-bed experimental setup to evaluate the real-time sampling performance of the ARBSW system. (b) Variation of real-time bioaerosol and colony concentrations monitored by the APS and the ARBSW system during bioaerosol exposure events.
J.H.J. conceived the initial idea; Y.S.C., J.C., and S.C.H. designed and implemented the experiments; and J.H.J. and S.C.H. developed and refined the concept and wrote the paper.

Author contributions

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4. Conclusion

The ARBSW system enables real-time monitoring of bioaerosols in real environments. This system has superior particle collection and particle transfer efficiency into a liquid medium. Compared with a conventional bioaerosol sampler, the ARBSW system enables marked particle enrichment and stable microbial recovery. Furthermore, the ARBSW not only has an excellent sampling performance with rapid particle transfer efficiency into a liquid medium, but is also suitable for the real-time monitoring of bioaerosols in real-world environments. The continuous liquid-based particle sampling used by the ARBSW system, when integrated with a real-time particle analysis system (e.g., microfluidic flow cytometer), enables continuous quantitative characterization of airborne microorganisms and facilitates analysis of the physicochemical and biological properties of bioaerosols, for example using markers for specific aptamers or antibodies.

Notes

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.snb.2018.12.155.

References

[1] W.W. Nazaroff, Indoor bioaerosol dynamics, Indoor Air 26 (2016) 61–78.
[2] J. Douwes, P. Thorne, N. Pearce, D. Heederik, Bioaerosol health effects and exposure assessment: progress and prospects, Ann. Occup. Hyg. 47 (2003) 187–200.
[3] H. Burger, Bioaerosols: prevalence and health effects in the indoor environment, J. Allergy Clin. Immunol. 86 (1990) 687–701.
[4] M. Nicas, A. Hubbard, A risk analysis approach to selecting respiratory protection against airborne pathogens used for bioterrorism, AJHA J. 64 (2003) 95–101.
[5] P.M. Blachere, W.G. Lindsley, T.A. Pearce, S.E. Anderson, M. Fisher, R. Khakhoo, B.J. Meade, O. Lander, S. Davis, R.E. Thewlis, Measurement of airborne influenza virus in a hospital emergency department, Clin. Infect. Dis. 48 (2009) 438–440.
[6] R.K. Bush, J.M. Portnoy, The role and abatement of fungal allergens in allergic diseases, J. Allergy Clin. Immunol. 107 (2001) S430-S40.
[7] S.-A. Lee, C.-H. Liao, Size-selective assessment of agricultural workers' personal exposure to airborne fungi and fungal fragments, Sci. Total Environ. 460 (2014) 725–732.
[8] R. Hilgenfeld, M. Peiris, From SARS to MERS: 10 years of research on highly pathogenic human coronaviruses, Antivir Res 100 (2013) 286–295.
[9] B.U. Lee, I.G. Hong, D.H. Lee, E.-S. Chung, J.H. Jung, J.H. Lee, I.H. Kim, I.-S. Lee, Bacterial bioaerosol concentrations in public restroom environments, Aerosol Air Qual. Res. 12 (2012) 251–255.
[10] C.Y. Rao, H.A. Burge, J.C. Chang, Review of quantitative standards and guidelines for fungi in indoor air, J. Air Waste Manage. 46 (1996) 899–908.
[11] Q. Liu, Y. Zhang, W. Jing, S. Liu, D. Zhang, G. Sui, First airborne pathogen direct analysis system, Analyst 141 (2016) 1637–1640.
[12] W. Jing, W. Zhao, S. Liu, L. Li, C.-T. Tsai, X. Fan, W. Wu, J. Li, X. Yang, G. Sui, Microfluidic device for efficient airborne bacteria capture and enrichment, Anal. Chem. 85 (2013) 5255–5262.
[13] J.S. Kang, K.S. Lee, S.S. Kim, G.-N. Bae, J.H. Jung, Real-time detection of an airborne microorganism using inertial impaction and mini-fluorescent microscopy, Lab Chip 14 (2014) 244–251.
[14] J. Ho, Future of biological aerosol detection, Anal. Chim. Acta 457 (2001) 125–148.
[15] J. Choi, J.S. Kang, S.C. Hong, G.-N. Bae, J.H. Jung, A new method for the real-time quantification of airborne biological particles using a coupled inertial aerosol system with in situ fluorescence imaging, Sens. Actuators B: Chem. 244 (2017) 635–641.
[16] J. Chung, J.S. Kang, J.S. Jung, J.H. Jung, B.C. Kim, Fast and continuous microorganism detection using aptamer-conjugated fluorescent nanoparticles on an optofluidic platform, Biosens. Bioelectron. 67 (2015) 303–308.
[17] A. Alvarez, M. Buttnar, G. Tornazs, E. Dvorsky, A. Tore, T. Teikes, L. Meritakis-Pifer, L. Stezenbach, Use of solid-phase PCR for enhanced detection of airborne microorganisms, Appl. Environ. Microbiol. 60 (1994) 374–376.
[18] R.H. Williams, E. Ward, H.A. McCartney, Methods for integrated air sampling and DNA analysis for detection of airborne fungal spores, Appl. Environ. Microbiol. 67 (2001) 2453–2459.
[19] T. Rinsoz, P. Duquenne, G. Greff-Mirguet, A. Oppliger, Application of real-time PCR for total airborne bacterial assessment: comparison with epifluorescence microscopy and culture-dependent methods, Atmos. Environ. 42 (2008) 6767–6774.
[20] S. Speight, B. Hallis, A. Bennett, J. Benbough, Enzyme-linked immunosorbent assay for the detection of airborne microorganisms used in biotechnology, J. Aerosol Sci. 28 (1997) 483–492.
[21] J. Choi, M. Kang, J.H. Jung, Integrated micro-optofluidic platform for real-time detection of airborne microorganisms, Sci. Rep. 5 (2015) 15983.
[22] J.H. Jung, J.E. Lee, G.-N. Bae, Real-time fluorescence measurement of airborne bacterial particles using an aerosol fluorescence sensor with dual ultraviolet-and visible-fluorescence channels, Environ. Eng. Sci. 29 (2012) 987–993.
[23] H.-S. Moon, J.-H. Lee, K. Kwon, H.-I. Jung, Review of recent progress in microsystems for the detection and analysis of airborne microorganisms, Anal. Lett. 45 (2012) 113–129.
[24] M. Tan, F. Shen, M. Yao, T. Zhu, Development of an automated electrostatic sampler (AES) for bioaerosol detection, Aerosol Sci. Technol. 45 (2011) 1154–1160.
[25] J. Choi, S.C. Hong, W. Kim, J.H. Jung, Highly enriched, controllable, continuous aerosol sampling using inertial microfluidics and its application to real-time

Fig. 7. Real-world field test. (a) Photograph of the real-time continuous monitoring of atmospheric air quality nearby Korea Institute of Science and Technology (KIST). (b) Variations in atmospheric PM\textsubscript{10}, PM\textsubscript{2.5}, and total culturable bioaerosol concentrations during the field test.
detection of airborne bacteria, ACS Sens. 2 (2017) 513–521.

[26] M.D. King, A.R. McFarland. Bioaerosol sampling with a wetted wall cyclone: cell culturability and DNA integrity of Escherichia coli bacteria, Aerosol Sci. Technol. 46 (2012) 82–93.

[27] M.D. King, B.P. Thim, S. Tüürikkäinen, A.R. McFarland. Collection characteristics of a batch-type wetted wall bioaerosol sampling cyclone, Aerobiologia 25 (2009) 239–247.

[28] A.D. Tolchinsky, V.I. Sigaev, G.I. Sigaev, A.N. Varfolomeev, E.V. Zvyagina, T. Brasil, Y.S. Cheng. Development of a personal bioaerosol sampler based on a conical cyclone with recirculating liquid film, J. Occup. Environ. Hyg. 7 (2010) 156–162.

[29] B.A. Orsini, K. Rhoads, K. McElhoney, E. Schick, D. Koehler, O. Hogrefe. A water cyclone to preserve insoluble aerosols in liquid flow—an interface to flow cytometry to detect airborne nucleic acid, Aerosol Sci. Technol. 42 (2008) 343–356.

[30] B. Lighthart, Y. Tong. Measurements of total and culturable bacteria in the al fresco atmosphere using a wet-cyclone sampler, Aerobiologia 14 (1998) 325–332.

[31] W.E. Kloos, M.S. Musself, W. White, D. and persistence of Staphylococcus and Micrococcus species and other aerobic bacteria on human skin, Appl. Microbiol. 30 (1975) 381–395.

[32] G.B. Hwang, K.J. Heo, J.H. Yun, J.E. Lee, C.W. Nho, G.-N. Bae, J.H. Jung. Antimicrobial air filters using natural Euscaphis japonica nanoparticles, PLoS One 10 (2015) e0126481.

[33] D.Y. Choi, K.J. Heo, J. Kang, E.J. An, S.-H. Jung, B.U. Lee, H.M. Lee, J.H. Jung. Washable antimicrobial polyester/aluminum air filter with a high capture efficiency and low pressure drop, J Hazard Mat 351 (2018) 29–37.

[34] J.H. Jung, J.E. Lee. Real-time bacterial microcolony counting using on-chip microscopy, Sci. Rep. 6 (2016) 21473.

[35] M. Otto. Staphylococcus epidermidis—the accidental pathogen, Nat. Rev. Microbiol. 7 (2009) 555–567.

[36] K. Willeke, X. Lin, S.A. Grinspun. Improved aerosol collection by combined impaction and centrifugal motion, Aerosol Sci. Technol. 28 (1998) 439–456.

[37] X. Lin, T.A. Reponen, K. Willeke, S.A. Grinspun, K.K. Forder, D.S. Ensor. Long-term sampling of airborne bacteria and fungi into a non-evaporating liquid, Atmos. Environ. 33 (1999) 4291–4298.

[38] X. Lin, T. Reponen, K. Willeke, Z. Wang, S.A. Grinspun, M. Trunov. Survival of airborne microorganisms during swirling aerosol collection, Aerosol Sci. Technol. 32 (2000) 184–196.

[39] W.C. Hinds. Aerosol Technology: Properties, Behavior, and Measurement of Airborne Particles, John Wiley & Sons, 2012.

[40] Y. S. Cho et al. Sensors & Actuators: B. Chemical 284 (2019) 525–533

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