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An Exhaled Breath Sampler Based on Condensational Growth and Cyclone Centrifugation (BSCC)

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ABSTRACT

An exhaled breath sampler based on condensational growth and cyclone centrifugation (BSCC) was developed and evaluated. The BSCC increases the size of exhaled breath aerosols through condensational growth and then collects them as liquid sample via centrifugation. This enables rapid sample collection and eliminates certain pre-treatment steps for pathogenic microorganism analysis. Laboratory-generated aerosols were mixed with saturated water vapor to simulate exhaled breath, and the collection efficiency and the virus infectivity conservation efficiency of the BSCC were evaluated. The collection efficiency of the BSCC was approximately 66.7% for 100 nm aerosols and increased to nearly 100% for 3 μm aerosols. Besides, the BSCC maintained approximately 93.5% infectivity of atomized model virus aerosol (*Pseudomonas* bacteriophage Phi6). When collecting exhaled breath samples from nine volunteers, the average collection rate was 248.7 μL min^{-1}. The BSCC achieved superior overall performance (i.e., 60% high collection efficiency and 40% higher infectivity conservation efficiency) compared with RTube, a commercial used exhaled breath sampler, indicating its potential for diagnosis of respiratory infection and measurements of exhaled viral aerosols.

Keywords: Viral aerosol collection, Exhaled breath, Sampler development, Collection efficiency, Viral infectivity conservation

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1 INTRODUCTION

Coronavirus disease 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has quickly spread throughout the world and has resulted in mass casualties (Udugama *et al.*, 2020). COVID-19 patients can release viral aerosols into the air when breathing, coughing, sneezing or talking (Yan *et al.*, 2018; Jiang *et al.*, 2020; Ma *et al.*, 2020; Morawska and Milton, 2020), endangering others' lives (Jones and Brosseau, 2015; Scheuch, 2020). Previous studies have demonstrated that SARS-CoV-2 can be transmitted via airborne routes (Miller *et al.*, 2020; Morawska and Cao, 2020; Morawska and Milton, 2020) and COVID-19 patients can exhale SARS-CoV-2 virus (Ma *et al.*, 2020; Zhou *et al.*, 2020). Exhaled breath samples can be used for disease diagnosis by detecting viral aerosols or biomarkers in it. For example, exhaled breath samples of COVID-19 patients were collected using RTube and SARS-CoV-2 nucleic acid were analyzed by reverse-transcription quantitative polymerase chain reaction (RT-qPCR). It is suggested that a 93.5% positive detection rate was achieved among exhaled breath samples, indicating the capability of using exhaled breath samples for diagnosis (Ryan *et al.*, 2020). In another study, influenza viruses in the exhaled breath from infected patients were collected using G-II system and also showed positive results in virus nucleic acid testing (Milton *et al.*, 2013). Besides, bacterial pathogens such as H. influenzae were detected from exhaled breath using PKU BioScreen (Zheng *et al.*, 2018). Moreover, the concentration of biomarkers such as ethyl butanoate, butyraldehyde and isopropanol in exhaled breath may offer support in COVID-19 diagnosis (Chen *et al.*, 2021). Therefore, exhaled breath samples can be used for respiratory disease diagnosis and pathogen identifications.

Currently, exhaled breath can be collected using condensation devices such as RTube and EcoScreen (Xu *et al.*, 2012; Ahmadzai *et al.*, 2013; Kuban and Foret, 2013; Vasilescu *et al.*, 2021). When warm and humid breath is directed through a chilled device, water vapor and volatile components are condensed into liquid and can be collected for subsequent analysis (Konstantinidi *et al.*, 2015). However, such devices are only designed for exhaled gas collection, and cannot efficiently collect exhaled aerosols which comprise a major portion of particles under 1 μm (Li *et al.*, 2021b). Novel techniques have been developed which utilizes advanced condensational growth module for bioaerosol collections, for example the BioSpot-VIVAS (Aerosol Devices Inc., Fort Collins, CO) (Lednicky *et al.*, 2016; Pan *et al.*, 2016; Ward *et al.*, 2020). Such devices significantly promoted current bioaerosol sampling techniques and have successfully accomplished real world sampling of airborne SAR-CoV-2 viruses (Li *et al.*, 2021a; Li *et al.*, 2022). Some techniques, such as filtration (Kintz *et al.*, 2016) and cyclone (Lindsley *et al.*, 2010), are also used to collect exhaled breath aerosols targeted on certain size distribution. A facemask-based sampler for filtering exhaled breath viruses was developed and respiratory virus including rhinovirus and influenza virus were detected using RT-qPCR from patients with cold symptoms (Hu, 2022). However, when exhaled breath bioaerosols are collected for culture analysis, the dehydration that occurs during sampling with these "dry-type" samplers may cause loss of virus infectivity (Fabian *et al.*, 2009; McDevitt *et al.*, 2013). The elevated osmotic stress resulting from desiccation can also lead to cell rupture as well (Zhen *et al.*, 2013). To sum up, previous studies tended to use condensation method alone or filter-based samplers, which cannot simultaneously satisfy the needs of effectively capturing viral aerosols and conserving virus infectivity. Therefore, novel exhaled breath sampling techniques which can preserve high collection efficiency as well as high virus infectivity conservation efficiency is urgently required, especially under the COVID-19 pandemic.

In this study, we developed a novel exhaled breath sampler based on condensational growth and cyclone centrifugation (called BSCC). Laboratory-generated aerosols, including sodium chloride (NaCl), ammonium sulfate and poly-α-olefin (PAO), were used to evaluate the collection efficiency of the BSCC. *Pseudomonas* bacteriophage Phi6, a readily available surrogate for respiratory viruses, was employed to evaluate the infectivity conservation efficiency of the BSCC. Eventually, the sample collection rate was tested on the exhaled breath samples of recruited volunteers and potential pathogen such as influenza B virus within these samples were analyzed.

2 METHODS

2.1 Design of the Sampler

A schematic and photograph of the BSCC are displayed in Fig. 1. The main body of BSCC is manufactured of polylactic acid (PLA) via 3D printing. Considering its low cost and convenience of manufacturing, BSCC can be used as disposable items after each breath sample collection, which its beneficial for disease diagnosis. For exhaled breath collections, users exhale through their mouths into the breath inlet of the BSCC. A saliva trapper is placed after the breath inlet to remove saliva droplets from the breath, and the saliva trapper can be easily removed if needed. The exhaled breath is directed into a centrifuge tube via a lateral tube, making the centrifuge tube working as a cyclone. Given the fact that exhaled breath has a high temperature and relative humidity (RH; RH is approximately 100% at 37°C), exhaled breath aerosols can be enlarged into larger droplets via condensational growth by cooling the centrifuge tube, and then collected by cyclone centrifugation. Such collected droplets gradually form liquid samples at the bottom of the centrifuge tube. Finally, the exhaust is filtered through a high efficiency particulate air (HEPA) filter before being discharged into the air.

The outer casing of the BSCC is made of three-dimensionally polycarbonate (54-mm outer diameter [OD] × 52-mm inner diameter [ID] × 153-mm length [L]) insulated with a neoprene cover (0.5-mm thickness). The outer casing and the internal 50 mL standard medical-grade centrifuge tube (CentriStar, Corning, Tewks-bury, MA, USA) are covered by a screw-on lid. The included angle between the lateral tube (8 mm OD × 6 mm ID × 15 mm L) and vertical tube (10 mm OD ×8 mm ID × 15 mm L) is 60°, and the lateral tube is tangent to the centrifuge tube. The space between the centrifuge tube and the outer casing is filled with 120 mL of water and is frozen into ice before each sampling to serve as a condenser.

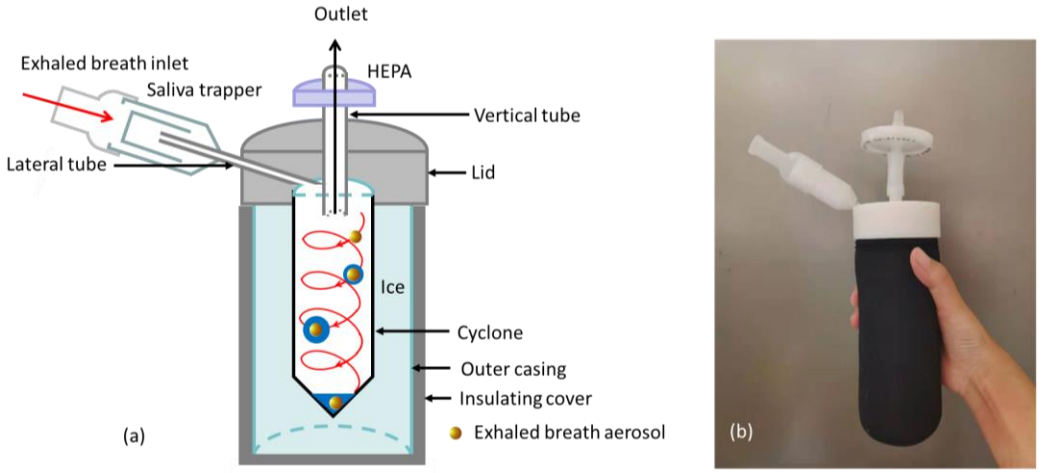

Fig. 1. (a) Schematic and (b) photograph of the BSCC.

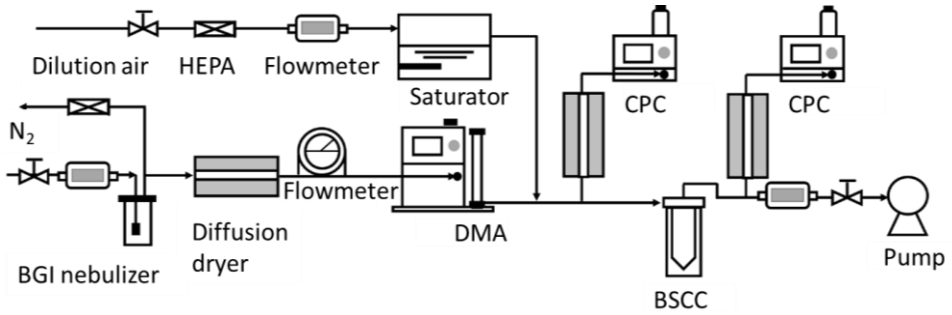

Fig. 2. Experimental setup for collection efficiency tests.

2.2 Sampler Evaluation

2.2.1 Determination of collection efficiency

Fig. 2 illustrates the experimental setup for testing the collection efficiency of the BSCC. PAO and ammonium sulfate aerosols were generated using a six-jet collision nebulizer (Model CN25, BGI, Waltham, MA, USA) with nitrogen as the carrier gas at a flow rate of 6.1 L min^{-1}. Ammonium sulfate aerosols are highly hydrophilic, whereas PAO aerosols are hydrophobic representing the unfavorable scenario for condensational growth. The generated polydisperse aerosols were directed into a diffusion dryer filled with silica gel or activated carbon to remove the water vapor or butanol used for the generation of the ammonium sulfate or PAO aerosols, respectively. The aerosols then passed through a neutralizer (Model 3088, TSI, Shoreview, MN, USA) and were directed into a differential mobility analyzer (DMA, Model 3081A, TSI, Shoreview, MN, USA). Particles with diameters of 100, 200, 300 and 400 nm were selected by the DMA. HEPA-filtered air was directed into a saturator (stainless steel, 400 mm × 150 mm × 120 mm in dimension) filled with heated deionized water for saturation. The saturated air (measured by a type K thermal couple) was maintained 42°C and then mixed with the aerosols selected by the DMA. The RH and temperature of the aerosol-vapor mixture, which were monitored by a temperature and humidity sensor (XMS616, Huayangdongzhuo, Zhengzhou, China), were maintained at 99% and 37°C before the mixture entered the BSCC to ensure exhaled breath simulation (Chen *et al.*, 2020). The overall flowrate of the aerosol-vapor mixture directed into the BSCC was maintained at 8 L min^{-1}, an average breathing rate for an adult (Winters *et al.*, 2017).

In addition to DMA-selected PAO and ammonium sulfate aerosols, we used monodisperse 0.7 μm, 1.6 μm, and 3.0 μm polystyrene latex (PSL) spheres (Thermo Fisher Scientific, Barrington, IL, USA) to test the collection efficiencies above 400 nm. A condensation particle counter (CPC; Model 3776, TSI, MN, USA) was used to measure the number concentration of the aerosols before and after the tested samplers. Collection efficiency was calculated by Eq. (1):

$$Colletion\ efficiency = \frac{C_{up} - C_{down}}{C_{up}} \times 100\% \quad (1)$$

where C_{up} (# cm^{-3}) and C_{down} (# cm^{-3}) are the upstream and downstream aerosol number concentration, respectively, of the tested samplers. RTube (Respiratory Research, Charlottesville, VA, USA) was evaluated with the same system configuration.

Additionally, the collection efficiency of the cyclone of the BSCC (i.e., the device without the condenser) was characterized using polydisperse NaCl particles. In brief, NaCl solution was injected via a syringe pump into an ultrasonic nozzle (60 KHz, Model 8700-60, Sono-Tek, NY, USA) motored by an ultrasonic generator (Model 06-05018, Sono-Tek, NY, USA) to generate micron-sized NaCl droplets. A mixing chamber was placed beneath the ultrasonic nozzle where filtered air was supplied and mixed with generated droplets for dilution and stabilization (Chen *et al.*, 2016). The cyclone of BSCC was placed inside the chamber and particle size distributions before and after the tested device were measured by an Aerodynamic Particle Sizer (APS; Model 3321, TSI, MN, USA). The collection efficiency of the cyclone of BSCC was determined by comparing the upstream and downstream APS measurements.

2.2.2 Determination of virus infectivity conservation efficiency

The experimental setup for testing the infectivity conservation efficiency of Phi6 is depicted in Fig. 3. Phi6, which has a lipid envelope and double-stranded RNA genome similar to those of other respiratory viruses (Turgeon *et al.*, 2014), was selected as model virus for evaluating the infectivity conservation efficiency of RTube, the BSCC, and its cyclone. Phi6 aerosols were generated with a 6-jet collison nebulizer (BGI, MA, USA) that was filled with 10 mL Phi6 solution (10^8 PFU mL^{-1}) diluted by 40 mL SM buffer (Sangon Biotech, Shanghai, China). The aerosol-vapor mixture was divided into three branches and directed into RTube, the BSCC and its cyclone, respectively. The mean diameter of generated Phi 6 aerosols was 104 nm. The sampling time and flowrate of each sampler were set to 10 min and 8 L min^{-1}, respectively. To prevent a decrease in infectivity of the Phi6 solution due to high temperature and sunlight during nebulization, we placed an ice plate around the nebulizer and the aerosol generator was covered with aluminum foil. The samples collected with each aerosol sampler, nebulizer liquid before and after experiment were immediately analyzed by plaque assay and RT-qPCR.

Viral virus concentration was determined using plaque assay. To propagate Phi6, *Pseudomonas syringae* were grown in tryptic soy broth medium (TSB; Solarbio Science & Technology, Beijing, China). Phi6 was mixed with the bacteria host at 1:10 ratio and then incubated overnight. Sediments were removed from the virus suspension through centrifugation at 5000 rpm, and the residual bacterial host and debris were removed by passing through a 0.22 μm polyethersulfone membrane filter (Merck, Darmstadt, Germany). The Phi6 concentration was approximately 10^{10} PFU mL^{-1}, and the Phi6 suspension was stored at 4°C until use.

A double agar layer plaque assay was used to determine the infectivity of the bacteriophages collected in the liquid sample. 100 μL diluted sample was mixed with 800 μL of bacteria host and incubated for 30 min at room temperature. Next, 3 mL TSB semisolid medium (0.75%) was added

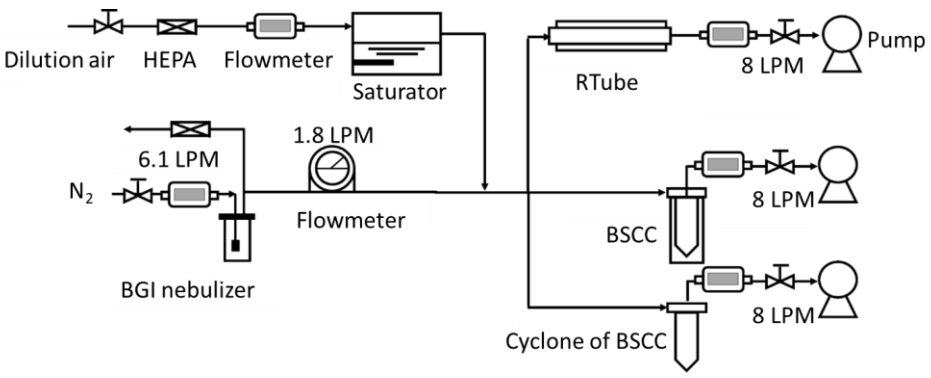

Fig. 3. Experimental setup for testing Phi6 infectivity conservation efficiency.

into the solution, which was then poured onto a culture plate coated with TSB-supporting agar (1.5%). The culture plate was rotated to distribute the medium evenly and then stood at room temperature for 15 min. Finally, culture plate was inverted and incubated overnight at 26°C. Each sample was tested in triplicate, and a bacteria host suspension without Phi6 was used as a negative control. The plaques were counted and used to calculate the viable virus concentration of the sample according to Eq. (2):

$$C_{PFU} = N \times T \times 10 \tag{2}$$

where C_{PFU} (PFU mL^{-1}) is the viable virus concentration of the sample, N is the number of plaques on the plate and T is the dilution ratio.

Total virus concentration was determined using RT-qPCR. Phi6 genomes were extracted with QIAamp viral RNA mini kits (Qiagen, Hilden, Germany). Extracted RNA was converted to cDNA by the GoScript Reverse Transcription System (Promega, WI, USA) and then stored at –20°C until analysis. Quantitative PCR (qPCR) was performed with the ABI QuantStudio 5 detection system (Thermo Fisher Scientific, MA, USA) with the following primers and probe: forward primer of TGGGATCGGAGGGATCTTC, reverse primer of GACGTCAGCGTAGGCGTGAT and probe of FAM-CCTCTATCGCAACCAC-MGB. The RNA standards used for the qPCR calibration curves were based on Phi6 genomes extracted from the purified stock and quantified in Quant Studio 3D Digital PCR (Applied Biosystems, Foster City, USA). All experiments were performed in triplicate.

Phi6 infectivity conservation efficiency, the ability of the samplers to maintain the Phi6 infectivity of the samples, was calculated by Eq. (3):

$$Phi6\ infectivity\ conservation\ efficiency = \frac{I_s/T_s}{I_N/T_N} \times 100\% \tag{3}$$

where I_S (PFU mL^{-1}) is the infectious Phi6 concentration of the sample, T_S (copies mL^{-1}) is the total Phi6 concentration of the sample, I_N (PFU mL^{-1}) is the average value of infectious Phi6 concentration of nebulizer liquid before and after experiment, and T_N (copies mL^{-1}) is the average value of total Phi6 concentration of nebulizer liquid before and after experiment. Infectious Phi6 concentration was determined using double agar layer culture and the total Phi6 concentration containing both infective and inactivated viruses, was determined through RT-qPCR.

2.2.3 Determination of sample collection in real-world tests

Sample collection rate is an important parameter which indicates the feasibility of actual exhale breath collection. In short, a higher sample collection rate corresponds to a shorter collection time, which is favorable by making it more convenient for the test subject to breath less times. To compare the sample collection rates of the BSCC and RTube (Respiratory Research, Charlottesville, VA, USA), we recruited nine healthy adult volunteers and collected their exhaled breath samples by using both devices for 2, 4, 6, 8 or 10 min, respectively. Before sampling, both the BSCC and RTube were stored overnight at −20°C (an optimal cooling temperature as revealed in the following sections).

Besides, breath samples from eight volunteers with respiratory symptoms were collected and potential pathogens within these samples (i.e., respiratory syncytial virus (RSV), influenza B virus and etc.) were analyzed by corresponding nucleic acid testing (NAT).

3 RESULTS AND DISCUSSION

3.1 Collection Efficiency

The collection performance of the BSCC was significantly higher than that of RTube. Fig. 4(a) presents the collection efficiency of the BSCC for two types of test aerosols at three condensation temperatures and a constant flowrate (8 L min^{-1}). In the absence of condenser (i.e., at room temperature 25°C), the BSCC had a collection efficiency below 5% for aerosols with diameters of 100 to 400 nm; collection efficiency was considerably improved after the addition of condenser. At –20°C, the collection efficiency was approximately 66.7% for 100-nm aerosols, and increased

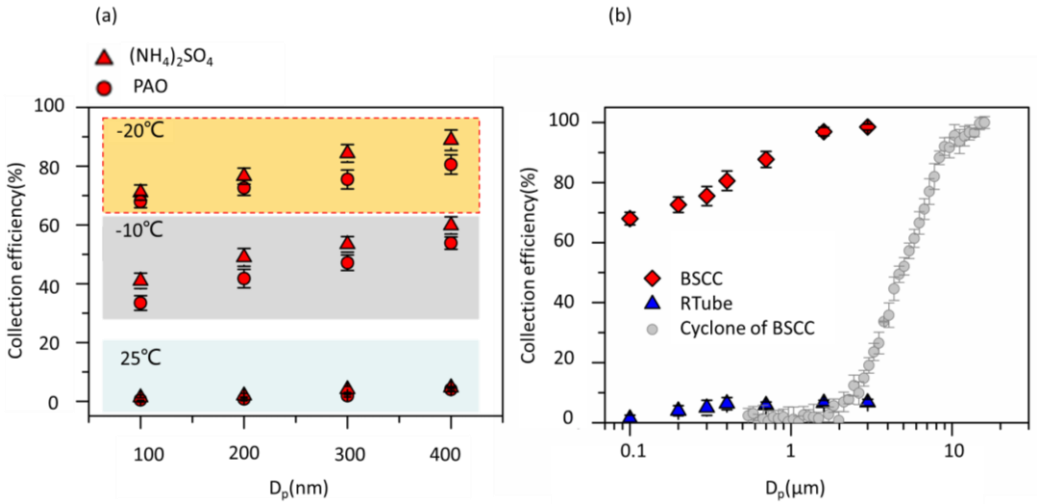

Fig. 4. Collection efficiencies as a function of aerodynamic diameter for: (a) BSCC at condensation temperatures of –20, –10 and 25°C. (b) Cyclone (using NaCl aerosols), BSCC and RTube (using PAO aerosols with diameters of 100, 200, 300 and 400 nm and PSL spheres with diameters of 0.7, 1.6 and 3 μm) at the condensation temperature of –20°C). Error bars represent the standard deviations of three measurements.

to approximately 80% for 400-nm aerosols. Therefore, –20°C was determined to be the optimal condenser temperature and employed in all subsequent experiments.

As displayed in Fig. 4(b), the collection efficiency of RTube was below 10% for aerosols with diameter of 100 nm to 3 μm. By contrast, with increasing aerosol diameter, the collection efficiency of the BSCC increased to 99.2% for 3 μm aerosols. The 50% cutoff size of the cyclone was approximately 4.6 μm, which indicates that it is difficult to effectively collect submicron aerosols with the cyclone alone. However, the relative high collection efficiencies of the BSCC for 100–400 nm aerosols demonstrate its ability to effectively grow submicron aerosols through condensation growth process. The BSCC utilized the high-humidity characteristic of exhaled breath to make the water vapor condensed on the submicron exhaled breath aerosols and grow into larger sizes. Combining condensation growth and centrifugation collection, BSCC shows a better collection performance than RTube which only utilizes condensation, especially for submicron aerosols.

3.2 Phi6 Infectivity Conservation Efficiency

In addition to collection efficiency, the ability of an exhaled breath sampler to maintain virus infectivity in the sample is a critical performance parameter. Fig. 5 presents the Phi6 infectivity conservation efficiency of the BSCC, RTube and cyclone of the BSCC. The Phi6 infectivity conservation efficiency of BSCC was 93.5% which was greater than that of RTube (54.2%). However, the temperature of simulated exhaled breath inside the BSCC (0–10°C) was lower than that of RTube (10–20°C) during the 8-min experiment; therefore, the lower temperature may have benefited Phi6 survival. Compared with the cyclone, the BSCC (cyclone plus condenser) achieved a higher infectivity conservation efficiency. Our results suggest that collecting bioaerosols into liquid samples may help to preserve their biological activity more effectively than "dry media" collections (Fabian *et al.*, 2009). In another study, a comparison of performance of three bioaerosol samplers for influenza revealed that SKC BioSampler with liquid media preserved more virus infectivity than gelatin filter (Li *et al.*, 2018). We observed that liquid collection (BSCC) outperformed dry media collection (cyclone), which was similar to the result of Li's study mentioned above.

3.3 Sample Collection in Real-world Tests

Sample collection rate is also another important parameter for evaluating the applicability of exhaled breath samplers. In this study, we recruited nine adult volunteers for testing the collection rates of the BSCC and RTube. Fig. 6 displays the volumes of the samples collected by the BSCC and RTube for different durations. In general, the sample volume increased with the prolonging

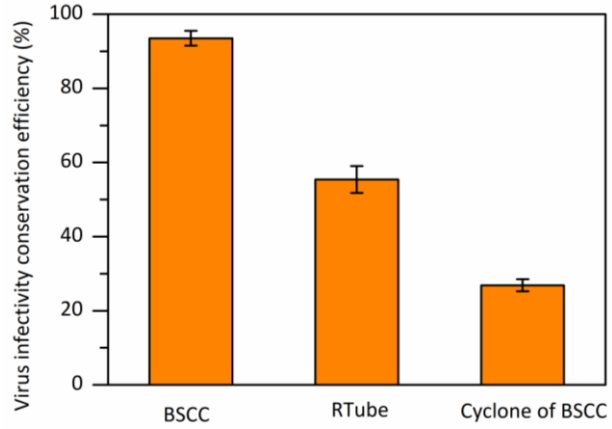

Fig. 5. Phi6 infectivity conservation efficiency of the BSCC, RTube and cyclone of BSCC. Error bars represent the standard deviations of three measurements.

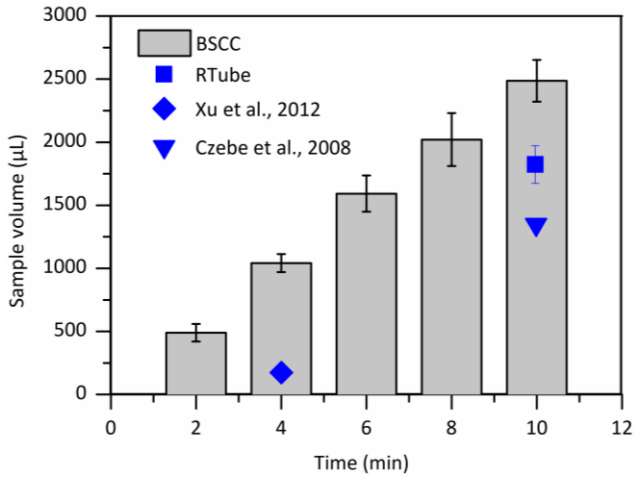

Fig. 6. Volumes of samples collected by the BSCC, RTube and some exhaled breath samplers in previous studies. Error bars represent the standard deviations of three measurements.

of collection time. The average sample volume collected by BSCC per unit of time (i.e., sample collection rate) was approximately 248.7 μL min^{-1}, approximately 1.4 times higher than that of RTube and higher than those of other exhaled breath samplers reported previously (Czebe *et al.*, 2008; Xu *et al.*, 2012).

During the COVID-19 pandemic, the most common method for diagnosing SARS-CoV-2 infection is testing nucleic acid through RT-qPCR. The usual sample volume required for RT-qPCR analysis is 200 μL. Given its sample collection rate, the BSCC can provide sufficient sample in just one minute, making it an applicable exhaled breath sampler for RT-qPCR analysis. The BSCC also uses standard centrifuge tubes for real-world sampling, and are more convenient for sample transfer and analysis.

Regarding potential pathogen analysis of real exhale samples, our preliminary results indicate that positive NAT results (with CT values between 33-38) were observed in several samples from volunteers who are infected with influenza. Although such results need further interpretations, these preliminary findings indicate that BSCC is a promising tool in real-world diseases diagnosis.

4 CONCLUSIONS

We developed a new exhaled breath sampler based on condensational growth and cyclone centrifugation for collecting bioaerosols. The collection efficiency of the BSCC for 100 nm test

aerosols is 66.7% and goes up to above 80% for 400 nm aerosols indicating that it can efficiently collect exhaled breath viral aerosols. The Phi6 infectivity conservation efficiency of the BSCC was approximately 93.5%, demonstrating that the sampler can effectively preserves the infectivity of model viruses for subsequent culture experiment. Because of its high sample collection rate, the BSCC can achieves a relative short sample collection time and thus is suitable for real exhaled breath collections. In addition to its good performance, the BSCC is beneficial in its simpler structure, lower cost and most importantly the flexibility of disposable after each sampling in respiratory diseases diagnosis. Such advantages make BSCC a valuable technique for diagnosing respiratory infections and investigating airborne transmission, especially in the context of current global COVID-19 pandemics.

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REFERENCES

Ahmadzai, H., Huang, S., Hettiarachchi, R., Lin, J.L., Thomas, P.S., Zhang, Q. (2013). Exhaled breath condensate: A comprehensive update. Clin. Chem. Lab. Med. 51, 1343–1361. https://doi.org/10.1515/cclm-2012-0593

Chen, D., Bryden, W.A., McLoughlin, M. (2020). A Novel system for the comprehensive collection of nonvolatile molecules from human exhaled breath. J. Breath Res. 15, 016001. https://doi.org/10.1088/1752-7163/abba87

Chen, H.X., Qi, X., Zhang, L., Li, X.Y., Ma, J.X., Zhang, C.Y., Feng, H.S., Yao, M.S. (2021). COVID-19 screening using breath-borne volatile organic compounds. J. Breath Res. 15, 047104. https://doi.org/10.1088/1752-7163/ac2e57

Chen, X., Jiang, J., Deng, J., Duan, L., Hao, J. (2016). Development and performance evaluation of a supermicron particle generation system for aerosol instrument calibration. Environ. Sci. 37, 789–794. https://doi.org/10.13227/j.hjkx.2016.03.001 (in Chinese).

Czebe, K., Barta, I., Antus, B., Valyon, M., Horvath, I., Kullmann, T. (2008). Influence of condensing equipment and temperature on exhaled breath condensate PH, total protein and leukotriene concentrations. Resp. Med. 102, 720–725. https://doi.org/10.1016/j.rmed.2007.12.013

Fabian, P., McDevitt, J.J., Houseman, E.A., Milton, D.K. (2009). Airborne influenza virus detection with four aerosol samplers using molecular and infectivity assays: Considerations for a new infectious virus aerosol sampler. Indoor Air 19, 433–441. https://doi.org/10.1111/j.1600-0668.2009.00609.x

Hu, B. (2022). Recent advances in facemask devices for in vivo sampling of human exhaled breath aerosols and inhalable environmental exposures. Trends Analyt. Chem. 151, 116600. https://doi.org/10.1016/j.trac.2022.116600

Jiang, J., Vincent Fu, Y., Liu, L., Kulmala, M. (2020). Transmission via aerosols: Plausible differences among emerging coronaviruses. Aerosol Sci. Technol. 54, 865–868. https://doi.org/10.1080/02786826.2020.1769020

Jones, R.M., Brosseau, L.M. (2015). Aerosol transmission of infectious disease. J. Occup. Environ. Med. 57, 501–508. https://doi.org/10.1097/JOM.0000000000000448

Kintz, P., Mathiaux, F., Villeger, P., Gaulier, J.M. (2016). Testing for drugs in exhaled breath collected with Exabreath in a drug dependence population: Comparison with data obtained in urine after liquid chromatographic-tandem mass spectrometric analyses. Ther. Drug Monit. 38, 135–139. https://doi.org/10.1097/ftd.0000000000000228

Konstantinidi, E.M., Lappas, A.S., Tzortzi, A.S., Behrakis, P.K. (2015). Exhaled breath condensate: Technical and diagnostic aspects. Sci. World J. 2015, 1–25. https://doi.org/10.1155/2015/435160

Kuban, P., Foret, F. (2013). Exhaled breath condensate: Determination of non-volatile compounds and their potential for clinical diagnosis and monitoring. A review. Anal. Chim. Acta. 805, 1–18. https://doi.org/10.1016/j.aca.2013.07.049

Lednicky, J., Pan, M., Loeb, J., Hsieh, H., Eiguren-Fernandez, A., Hering, S., Fan, Z.H., Wu, C.Y. (2016). Highly efficient collection of infectious pandemic influenza H1N1 virus (2009) through laminar-flow water based condensation. Aerosol Sci. Technol. 50, i–iv. https://doi.org/10.1080/02786826.2016.1179254

Li, H., Shankar, S.N., Witanachchi, C.T., Lednicky, J.A., Loeb, J.C., Alam, M.M., Fan, Z.H., Mohamed, K., Eiguren-Fernandez, A., Wu, C.Y. (2021a). Environmental surveillance and transmission risk assessments for SARS-CoV-2 in a fitness center. Aerosol Air Qual. Res. 21, 210106. https://doi.org/10.4209/aaqr.210106

Li, H., Shankar, S.N., Witanachchi, C.T., Lednicky, J.A., Loeb, J.C., Alam, M.M., Fan, Z.H., Mohamed, K., Boyette, J.A., Eiguren-Fernandez, A., Wu, C.Y. (2022). Environmental surveillance for SARS-CoV-2 in two restaurants from a mid-scale city that followed U.S. CDC reopening guidance. Aerosol Air Qual. Res. 22, 210304. https://doi.org/10.4209/aaqr.210304

Li, J., Leavey, A., Wang, Y., O'Neil, C., Wallace, M.A., Burnham, C.D., Boon, A.C., Babcock, H., Biswas, P. (2018). Comparing the performance of 3 bioaerosol samplers for influenza virus. J. Aerosol Sci. 115, 133–145. https://doi.org/10.4209/aaqr.210106

Li, X., Jiang, J., Wang, D., Deng, J., He, K., Hao, J. (2021b). Transmission of coronavirus via aerosols and influence of environmental conditions on its transmission. Environ. Sci. 42, 3091–3098. https://doi.org/10.13227/j.hjkx.202010033 (in Chinese).

Lindsley, W.G., Blachere, F.M., Thewlis, R.E., Vishnu, A., Davis, K.A., Cao, G., Palmer, J.E., Clark, K.E., Fisher, M.A., Khakoo, R., Beezhold, D.H. (2010). Measurements of airborne influenza virus in aerosol particles from human coughs. Plos One 5, e15100. https://doi.org/10.1371/journal.pone.0015100

Ma, J., Qi, X., Chen, H., Li, X., Zhang, Z., Wang, H., Sun, L., Zhang, L., Guo, J., Morawska, L., Grinshpun, S.A., Biswas, P., Flagan, R.C., Yao, M. (2020). COVID-19 patients in earlier stages exhaled millions of SARS-CoV-2 per hour. Clin. Infect. Dis. 72, E652–E654. https://doi.org/10.1093/cid/ciaa1283

McDevitt, J.J., Koutrakis, P., Ferguson, S.T., Wolfson, J.M., Fabian, M.P., Martins, M., Pantelic, J., Milton, D.K. (2013). Development and performance evaluation of an exhaled-breath bioaerosol collector for influenza virus. Aerosol Sci. Technol. 47, 444–451. https://doi.org/10.1093/cid/ciaa1283

Miller, S.L., Nazaroff, W.W., Jimenez, J.L., Boerstra, A., Buonanno, G., Dancer, S.J., Kurnitski, J., Marr, L.C., Morawska, L., Noakes, C. (2020). Transmission of SARS-CoV-2 by inhalation of respiratory aerosol in the Skagit Valley chorale superspreading event. Indoor Air 31, 314–323. https://doi.org/10.1101/2020.06.15.20132027

Milton, D.K., Fabian, M.P., Cowling, B.J., Grantham, M.L., McDevitt, J.J. (2013). Influenza virus aerosols in human exhaled breath: Particle size, culturability, and effect of surgical masks. PLoS Pathog. 22, S51–S51. https://doi.org/10.1371/journal.ppat.1003205

Morawska, L., Cao, J. (2020). Airborne transmission of SARS-CoV-2: The world should face the reality. Environ. Int. 139, 105730. https://doi.org/10.1016/j.envint.2020.105730

Morawska, L., Milton, D.K. (2020). It is time to address airborne transmission of coronavirus disease 2019 (COVID-19). Clin. Infect. Dis. 71, 2311–2313. https://doi.org/10.1093/cid/ciaa939

Pan, M., Eiguren-Fernandez, A., Hsieh, H., Afshar-Mohajer, N., Hering, S.V., Lednicky, J., Hugh Fan, Z., Wu, C.Y. (2016). Efficient collection of viable virus aerosol through laminar-flow, water-based condensational particle growth. J. Appl. Microbiol. 120, 805–815. https://doi.org/10.1111/jam.13051

Ryan, D.J., Toomey, S., Madden, S.F., Casey, M., Breathnach, O.S., Morris, P.G., Grogan, L., Branagan, P., Costello, R.W., De Barra, E., Hurley, K., Gunaratnam, C., McElvaney, N.G., ME, O.B., Sulaiman, I., Morgan, R.K., Hennessy, B.T. (2020). Use of exhaled breath condensate (EBC) in the diagnosis of SARS-CoV-2 (COVID-19). Thorax 76, 86–88. https://doi.org/10.1136/thoraxjnl-2020-215705

Scheuch, G. (2020). Breathing is enough: For the spread of influenza virus and SARS-CoV-2 by breathing only. J. Aerosol Med. Pulm. Drug Deliv. 33, 230–234. https://doi.org/10.1089/jamp.2020.1616

Turgeon, N., Toulouse, M.J., Martel, B., Moineau, S., Duchaine, C. (2014). Comparison of five bacteriophages as models for viral aerosol studies. Appl. Environ. Microbiol. 80, 4242–4250. https://doi.org/10.1128/AEM.00767-14

Udugama, B., Kadhiresan, P., Kozlowski, H.N., Malekjahani, A., Osborne, M., Li, V.Y.C., Chen, H., Mubareka, S., Gubbay, J.B., Chan, W.C.W. (2020). Diagnosing COVID-19: The disease and tools for detection. ACS Appl. Nano Mater. 14, 3822–3835. https://doi.org/10.1021/acsnano.0c02624

Vasilescu, A., Hrinczenko, B., Swain, G.M., Peteu, S.F. (2021). Exhaled breath biomarker sensing. Biosens. Bioelectron. 182, 113193. https://doi.org/10.1016/j.bios.2021.113193

Ward, R.X., Tilly, T.B., Mazhar, S.I., Robinson, S.E., Eiguren-Fernandez, A., Wang, J., Sabo-Attwood, T., Wu, C.Y. (2020). Mimicking the human respiratory system: Online in vitro cell exposure for toxicity assessment of welding fume aerosol. J. Hazard Mater. 395, 122687. https://doi.org/10.1016/j.jhazmat.2020.122687

Winters, B.R., Pleil, J.D., Angrish, M.M., Stiegel, M.A., Risby, T.H., Madden, M.C. (2017). Standardization of the collection of exhaled breath condensate and exhaled breath aerosol using a feedback regulated sampling device. J. Breath Res. 11, 047107. https://doi.org/10.1088/1752-7163/aa8bbc

Xu, Z., Shen, F., Li, X., Wu, Y., Chen, Q., Jie, X., Yao, M. (2012). Molecular and microscopic analysis of bacteria and viruses in exhaled breath collected using a simple impaction and condensing method. PLoS One 7, e41137. https://doi.org/10.1371/journal.pone.0041137

Yan, J., Grantham, M., Pantelic, J., Bueno de Mesquita, P.J., Albert, B., Liu, F., Ehrman, S., Milton, D.K., Consortium, E. (2018). Infectious virus in exhaled breath of symptomatic seasonal influenza cases from a college community. Proc. Natl. Acad. Sci. USA. 115, 1081–1086. https://doi.org/10.1073/pnas.1716561115

Zhen, H., Han, T., Fennell, D.E., Mainelis, G. (2013). Release of free DNA by membrane-impaired bacterial aerosols due to aerosolization and air sampling. Appl. Environ. Microbiol. 79, 7780–7789. https://doi.org/10.1128/AEM.02859-13

Zheng, Y., Chen, H., Yao, M., Li, X. (2018). Bacterial pathogens were detected from human exhaled breath using a novel protocol. J. Aerosol Sci. 117, 224–234. https://doi.org/10.1016/j.jaerosci.2017.12.009

Zhou, L., Yao, M., Zhang, X., Hu, B., Li, X., Chen, H., Zhang, L., Liu, Y., Du, M., Sun, B., Jiang, Y., Zhou, K., Hong, J., Yu, N., Ding, Z., Xu, Y., Hu, M., Morawska, L., Grinshpun, S.A., Biswas, P., Flagan, R.C., Zhu, B., Liu, W., Zhang, Y. (2020). Breath-, air- and surface-borne SARS-CoV-2 in hospitals. J. Aerosol Sci. 152, 105693. https://doi.org/10.1016/j.jaerosci.2020.105693