**Supplementary Materials**

**Filtration efficiency of face masks against aerosolized surrogate SARS-CoV-2 at different social distances**

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**10 Pages, 4 Tables, and 2 Figures**

**Materials and Methods**

***Materials***

As a proxy for SARS-CoV-2, SARS-CoV-2 spike-pseudovirus (Sino Biological Inc., Beijing, China), which has no autonomous replication ability, was used in this study. Characterization of the pseudovirus by scanning electron microscopy (ZEISS Sigma 300, Oberkochen, Germany) found no obvious difference with respect to the size (80–120 nm) and intact envelope (full-length spike protein on the surface) compared with SARS-CoV-2 (**Fig. S1**).

The study included one representative mask from each of the four major mask categories commonly used by the general public: (1) medical N95 respirator [22.0 mm × 16.0 mm, five layers: S (spunbond)-M (meltblown)-M-M-S, Zhongjian Medical Equipment Co. Ltd, Henan, China]; (2) medical grade surgical mask (18.0 mm × 9.5 mm, three layers: S-M-S, Hengxin Medical Supplies Co. Ltd, Qiqihar, China); (3) single-use disposable mask (non-medical grade, 17.5 mm × 9.0 mm, three layers of nonwoven cloth, Kangminweicai Co. Ltd, Xinxiang, China); and (4) home-made cloth mask (20.0 mm × 10.5 mm, three layers of cotton fabric) as shown in **Fig. S2(a**).

***Filtering performance of different mask types at different distances when simulating sneezing***

The sneezing experiment was carried out in a closed exposure chamber (3.0 m × 1.5 m × 2.0 m) with a quiescent environment. A high-efficiency particulate filter was used to ensure the chamber air cleanliness at the start of each experiment. The human sneeze velocity varies from 10 to 50 m/s, and the duration of a sneeze varies from 0.06 to 0.3 s [1].A sneezing aerosol source simulator was made in-house and included a compressor, an automated (on/off) electrical modulating valve, a manual electrical modulating valve, and a spray gun as shown in **Fig. S2(b)-**(**c**). The electrically modulated valve was used to control the sneezing duration at 1 s. Another manual electrically modulated valve was used to control the flow rate of sneezing at 11 ± 2 m/s and the number of droplets at 106. The generated droplets/aerosols were 0.1 to 100 μm in diameter [2] and had a total aerosol volume of 70 μL per sneeze. We produced not only droplets but also aerosols (**Table S1)**. The relative humidity (RH) and temperature of the test chamber were maintained at 45–50% and 22–24°C, respectively.

For the outward sneezing experiment shown in **Fig. S2(b**), the pseudovirus solution (108 copies/mL pseudovirus in artificial saliva) was ejected five times (simulating five sneezes) through the mouth of a standard mannequin head using a sneezing simulator [3, 4].The mannequin head with elastomeric skin was fitted with a mask or was not masked. Droplets/aerosols were sampled over 8 min at distances of 0, 0.5, 1.0, 1.5, and 2.0 m away from the mannequin head’s mouth. Concentrations measured when the mannequin was not wearing a mask were denoted as Cu. Concentrations measured when the mannequin was wearing a mask were denoted as Cd.

For the inward experiment shown in **Fig. S2(c**), the pseudovirus solution was ejected five times (simulating five sneezes) toward the mouth of the mannequin head. The solutions were placed at 0, 0.5, 1.0, 1.5 and 2.0 m away from the sneeze outlet. The flow rate of the breathing simulator was adjusted to 10 L/min. Concentrations measured when the mannequin was not wearing a mask were denoted as Cu. Concentrations measured when the mannequin was wearing a mask were denoted as Cd.

Aerosol concentrations were measured with a Sioutas Cascade Impactor 225-370 (SKC Inc., California, USA), operated at 10 L/min. To address potential variations, tests with each mask type were repeated three times (n = 3).

***Digital PCR assay***

After collection, all of the samples were immediately sent to the laboratory for analysis using cold-chain shipment. The pseudovirus on the filter membranes was eluted with 1 mL of viral preservation medium (Dakewe Biological Engineering Co. Ltd, Shenzhen, China). Viral RNA was extracted using a QIAamp Viral RNA Mini Kit (QIAGEN Inc., Hilden, Germany) according to the manufacturer’s instructions. To determine the viral RNA copy number, a droplet digital PCR (ddPCR) assay targeting the gene encoding *WPRE* was developed using a QX200 Droplet Digital PCR System (Bio-Rad, California, USA). The primer and probe sequences are provided in **Table S2**. The reaction mixture (22 μL) contained 11 μL of Supermix (ddPCRTM Supermix for Probe, Bio-Rad, California, USA), 820 nM of WPRE gene primers, 230 nM of WPRE gene probes, and 6.9 μL of five-fold diluted cDNA. PCR amplification was performed using 96-well plates as follows: initial denaturation at 95℃ for 10 min; 41 cycles of two-step amplification (denaturation at 94℃ for 30 s and extension at 60℃ for 1 min); and 98℃ for 10 min. All samples were subjected to three technical replicates, and each experimental batch was loaded with negative and positive controls. Detailed methods and quality control (QC) [e.g., negative and positive controls, limit of blank (LoB), and limit of detection (LoD)] are presented in **Table** **S3**.

To establish the limit of blank (LoB), 50 blank measurements were obtained from five blank controls of the experiments within two different days. The LoB was estimated nonparametrically as the 95th percentile of the measurements. Linear interpolation between the 47th and 48th observations yielded a LoB estimate of 0.04 copies/μL and 0.8 copies/reaction, respectively. A total of 80 measurements from four samples with low concentrations (1 to 6 copies/reaction) were used to determine the limit of detection (LoD) according to the CLSI guideline of EP17-A1 [5]. The LoD for the SARS-CoV-2 spike-pseudovirus gene was 2 copies/reaction.

Each batch of ddPCR experiments was loaded with a negative control and a positive control. Based on the measured values of the negative and positive controls, a threshold was set to distinguish between negative and positive droplets. In all of the negative controls, only one had a positive droplet. Therefore, samples with values lower than the LoB were reported as negative, and samples with values greater than the LoD were reported as the measured values. If the sample had a value greater than the LoB but less than the LoD in addition to the number of positive droplets greater than 2, the measured value was reported.

***Filtration Efficiency***

The outward or inward filtration efficiency (FE, %) of each mask type was calculated using the following equation according to a previous study [6]:

FEi = [(Cu,i – Cd,i)/C­u,i­)] × 100%

Cuand Cd are the aerosol concentrations measured without and with masks, and i is the index for distance from the source.

***Statistics***

All data analysis was performed in R version 4.0.1. Various packages (e.g., *ggplot2*, *dplyr*, *ggpubr*, *agricolae,* and *reshape2*) were used for data analyses and visualization. Analysis of variance (ANOVA) and Fisher’s least significant difference (LSD) test were employed to determine the significance of the overall difference and the difference between two groups, respectively. *P* < 0.05 was considered statistically significant.

**Table S1.** The number of droplets/aerosols of various sizes identified at different social distances using a laser particle counter (Y09-301, AC-DC, Jiangsu Sujing Group Co., Ltd., China) produced by a sneezing aerosol simulator in this study. Each data point is the average of five replicates (n = 5).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Distance Size** | **0.3 μm** | **0.5 μm** | **1 μm** | **3 μm** | **5 μm** | **10 μm** |
| **0 m** | 264234.6 | 254980 | 212568 | 112385.4 | 10884.6 | 8382.4 |
| **0.5 m** | 242930 | 229005.2 | 189722.2 | 93651 | 9020.2 | 6985.2 |
| **1 m** | 219982.4 | 212341 | 167780.2 | 87077.2 | 8606.4 | 5548.2 |
| **1.5 m** | 87920.4 | 77856.6 | 68555.4 | 8568.4 | 858 | 621.4 |
| **2 m** | 6038.6 | 4783.6 | 3611.2 | 781.4 | 385 | 114.6 |

**Table S2.** Theprimers and probe sequences used in the digital PCR assay.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Gene** | **Type** | **Name** | **Sequence** | **Modification** |
| *WPRE* | Primers | S-F | CCTTTCCGGGACTTTCGCTTT |  |
| S-R | GCAGAATCCAGGTGGCAACA |  |
| Probes | S-P | ACTCATCGCCGCCTGCCTTGCC | 5´FAM, 3´TAMRA |

**Table S3.** The outward and inward copy numbers of viral RNA from four different masks filtering against sneeze-generated droplets/aerosols of SARS-CoV-2 spike-pseudovirus at 0, 0.5, 1, 1.5 and 2 m away from the sneeze outlet. Data are represented as the mean ± standard error of the mean (SEM, n =1 to 3) after excluding the values < LoD. N.D., none detected (< LoD). The significance of the overall difference between the five distances was determined using analysis of variance (ANOVA). Different letters (within the same column) indicate significant differences from the no-mask-group values (*P* < 0.05), based on the LSD-t test.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Mask Type** | **Outward Viral RNA Copies** | | | | |  | **Inward Viral RNA Copies** | | | | |
| **0 m** | **0.5 m** | **1 m** | **1.5 m** | **2 m** | **0 m** | **0.5 m** | **1 m** | **1.5 m** | **2 m** |
| N95 Mask | 7029a | 7029.50±1814.25a | 6576.00±685.89a | N.D. | N.D. |  | 4308a | 2721±0a | 2494a | N.D. | N.D. |
| Surgical Mask | 2721a | 7710.00±907.00a | 4006.00±240.32a | N.D. | N.D. |  | 5896.00a | 10506.33±2330.71a | N.D. | N.D. | N.D. |
| Single-use Mask | 53635.33±6470.77a | 6727.00±611.45b | 6802.67±545.02b | N.D. | N.D. |  | 56296.00±6042.18a | 5215.50±793.75b | 6046.67±549.12b | N.D. | N.D. |
| Cloth Mask | 145336.33±15431.10a | 92214.67±5279.08a | 111867.00±4147.67a | N.D. | N.D. |  | 139410.33±2847.32a | 38019.67±3528.16b | 58881.33±2640.16b | N.D. | N.D. |
| No Mask | 423733.83±14326.91a | 301738.33±3957.83a | 282237.33±2394.50a | 4233.00±352.67b | N.D. |  | 423733.83±14326.91a | 301738.33±3957.83a | 282237.33±2394.50a | 4233.00±352.67b | N.D. |

**Table S4**. The outward and inward filtration efficiencies (FEs, %) of four different masks against the sneeze-generated pseudovirus as a function of distance from the source (0, 0.5, 1, 1.5 and 2 m). The mean ± SEM of the FE is shown for three experimental replicates (n = 3), after excluding unrealistic values (FE < 0). The significance of the overall difference between the four mask types was observed using ANOVA, and different letters (within the same column) indicate significant differences between the values of each mask type, using the LSD-t test (*P* < 0.05).

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Mask Type** | **Outward Filtration Efficiencies (%)**  **Mean ± SEM** | | | | |  | **Inward Filtration Efficiencies (%)**  **Mean ± SEM** | | | | |
| **0 m** | **0.5 m** | **1 m** | **1.5 m** | **2 m** | **0 m** | **0.5 m** | **1 m** | **1.5 m** | **2 m** |
| N95 Mask | 99.00±0.33a | 98.33±0.29a | 97.67±0.29a | N.D. | N.D. |  | 99.67±0.11a | 99.33±0.11a | 99.33±0.11a | N.D. | N.D. |
| Surgical Mask | 99.67±0.11a | 98.00±0.19a | 98.67±0.11a | N.D. | N.D. |  | 99.33±0.22a | 96.67±0.78a | N.D. | N.D. | N.D. |
| Single-use Mask | 80.33±3.09a | 97.33±0.40a | 97.67±0.11a | N.D. | N.D. |  | 88.00±1.45a | 98.67±0.11a | 97.67±0.29a | N.D. | N.D. |
| Cloth Mask | 55.00±2.52b | 68.33±0.48b | 62.33±1.87b | N.D. | N.D. |  | 69.33±3.08b | 87.33±1.13b | 77.33±1.06b | N.D. | N.D. |

**Fig. S1.** (a)A representative scanning electron microscope (SEM) image of SARS-CoV-2 spike-pseudovirus coated with gold. (b)A representative SEM image of SARS-CoV-2 spike-pseudovirus.

1. (b)

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**Fig. S2**. Schematic of the experimental setup in an exposure chamber. (a) Representative photos of the N95 respirator, surgical mask, single-use disposable mask, and cloth mask used in this study. For the sneezing experiment (b and c), the polydisperse SARS-CoV-2 spike-pseudovirus solution was ejected five times (simulating five sneezes) from artificial saliva [3]with 108 copies/mL pseudovirus using a sneeze generator through (outward) or toward (inward) the mouth of a mannequin head with elastomeric skin (mimicking a spreader or receiver, respectively). Viral droplets/aerosols filtered through a mask (Cd) or without a mask (Cu) were collected at 0, 0.5, 1.0, 1.5, and 2.0 m away from the sneeze outlet using a collection unit (sampling time: 8 mins in total; sampling flow:10 L/min). The edges of the masks attached on the mannequin head were completely sealed using medical tape.

图示, 示意图

描述已自动生成

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