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Detection of human coronavirus RNA in surgical smoke generated by surgical devices

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SUMMARY

Background: Gaseous by-products generated by surgical devices — collectively referred to as 'surgical smoke' — present the hazard of transmitting infective viruses from patients to surgical teams. However, insufficient evidence exists to evaluate and mitigate the risks of SARS-CoV-2 transmission via surgical smoke.

Aim: To demonstrate the existence and infectivity of human coronavirus RNA in surgical smoke using a model experiment and to evaluate the possibility of lowering transmission risk by filtration through a surgical mask.

Methods: Pelleted HeLa-ACE2-TMPRSS2 cells infected with human coronavirus were incised by electric scalpel and ultrasonic scalpel, separately. A vacuum system was used to obtain surgical smoke in the form of hydrosol. Reverse transcription–quantitative polymerase chain reaction was used to analyse samples for the presence of viral RNA, and infectivity was determined through plaque assay. Furthermore, a surgical mask was placed centrally in the vacuum line to evaluate its ability to filter viral RNA present in the surgical smoke.

Findings: In this model, $1/10^6$ to $1/10^5$ of the viral RNA contained in the incision target was detected in the collected surgical smoke. The virus present in the smoke was unable to induce plaque formation in cultured cells. In addition, filtration of surgical smoke through a surgical mask effectively reduced the amount of viral RNA by at least 99.80%.

Conclusion: This study demonstrated that surgical smoke may carry human coronavirus, though viral infectivity was considerably reduced. In clinical settings, surgical mask filtration should provide sufficient additional protection against potential coronavirus, including SARS-CoV-2, infection facilitated by surgical smoke.

Introduction

Coronavirus disease 2019 (COVID-19), caused by the coronavirus SARS-CoV-2, has become a pandemic. This respiratory virus is transmitted via aerosols generated when an infected individual sneezes or coughs [1,2]. Accumulating studies have indicated the widespread existence of asymptomatic carriers of SARS-CoV-2; these potential sources of infection may warrant reassessment of the transmission dynamics in the current outbreak [3–5]. A study performed in China demonstrated a lack of radiographic or computed tomography abnormalities in 17.90% of COVID-19 patients [6]. Another study performed

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in Iceland demonstrated that 43.00% of patients who tested positive for SARS-CoV-2 with a reverse transcription–quantitative polymerase chain reaction (RT-qPCR) assay were asymptomatic [7].

Given this information, there is notable concern that asymptomatic but infected patients undergoing operations may pose a substantial threat to operating room staff, including surgeons. For example, in the operating room, aerosols generated during tracheal intubation may cause transmission of SARS-CoV-2 from patients to operating room staff. To mitigate this risk, the American Statistical Association and the Anesthesia Patient Safety Foundation recommend preoperative screening with viral RNA (PCR) for all patients undergoing non-emergency surgery [3]. However, even if SARS-CoV-2 RNA is detected by PCR, the surgeon may have to proceed with surgery on the patient. As viral RNA has been detected in respiratory, faecal, and serum samples of patients with COVID-19, all types of surgical physicians may be exposed to the virus during surgery [3,8,9].

Many surgeons use high-frequency electrical current or ultrasonic vibration devices to cut tissue and coagulate small vessels during procedures. Surgical smoke — gaseous by-products generated from the thermal decomposition of tissues by such surgical tools — can be potentially harmful to operating room staff. Indeed, numerous studies have indicated the presence of hazardous agents, including carcinogens, bacteria, malignant cells, and viruses (e.g. hepatitis B virus, human immunodeficiency virus, and human papillomavirus) in surgical smoke [10–19]. However, insufficient information currently exists to evaluate the risk of SARS-CoV-2 transmission via surgical smoke [20].

Here, we aimed to evaluate the possibility that infective coronaviruses might exist in surgical smoke. Moreover, we sought to determine whether transmission of infection from this source could be prevented by wearing a surgical mask.

Methods

Cells and virus strain

HeLa-ACE2-TMPRSS2 cells were provided by Dr S. Matsuyama of the Department of Virology III, National Institute of Infectious Diseases, Japan [21]. The cells were cultured at 37°C in Dulbecco's modified Eagle medium with low glucose (D5546; Sigma, St Louis, MO, USA) containing 10.00% (v/v) foetal bovine serum. Human coronavirus 229E (HCoV-299E; GenBank: KU291448.1) was provided by Dr K. Mizuta of the Yamagata Prefectural Institute of Public Health, Japan [22].

Preparation of infected cells

HeLa-ACE2-TMPRSS2 cells (1×10^7) grown in 100 mm dishes were infected with HCoV-229E at a multiplicity of infection of 0.001 and incubated at 34°C for 4 days. After incubation, the infected cells were trypsinized and pelleted by centrifugation at 300 *g* for 3 min at 20°C. A portion of the pelleted cells was used for RNA extraction to determine the intact virus copy number, and the remaining portion was subjected to treatment with surgical devices. The culture supernatant was collected for virus titration.

Virus titration

A plaque assay was performed using the ISO18184 test as a reference (ISO 18184: https://www.iso.org/obp/ui/#iso:std:71292:en). Briefly, HeLa-ACE2-TMPRSS2 cells grown to 100% confluence in 12-well dishes were incubated with 200 μL of serially diluted virus stock in serum-free Eagle's minimum essential medium (EMEM, 05901; Nissui, Tokyo, Japan) at 34°C for 85 min. After washing the cells with phosphate-buffered saline solution, they were overlaid with serum-free EMEM containing 0.75% INA agar (TC-5, INA-1902-05; Nagano, Japan) and 1 mM L-glutamine, 0.15% sodium bicarbonate, and 0.01% DEAE–dextran. After incubation at 34°C for 5 days, the cells were fixed with 3.70% formalin and stained with methylene blue to count the number of plaques.

RNA isolation and RT-qPCR

Total RNA was extracted from 100 μL of the sample solution using the ZymoSpin kit (R2050; Zymos Research, Irvine, CA, USA) following the manufacturer's instructions, and eluted with 20 μL of RNase-free distilled water. Next, 10 μL of extracted RNA was reverse-transcribed to cDNA using First Strand cDNA Synthesis Kit ReverTra Ace-α (FSK-101; Toyobo, Osaka, Japan), according to the manufacturer's instructions. qPCR was then performed with the cDNA as a template, using a CFX96 real-time PCR system (1855196J1; Bio-Rad, Hercules, CA, USA) with TaqMan Fast Advanced Master Mix (4444558; Thermo Fisher, Waltham, MA, USA) under the following amplification conditions: annealing for 2 min at 50°C, denaturing at 95°C for 2 min, and extension at 60°C for 50 cycles of 30 s. For virus quantification, the following primers and FAM-labelled probe sets were used: forward primer, 5′-TCTCTTTA-TAGCCCTTTGCTTG-3′ (HCoV-229E *N* region, nt 25,752–25,773); reverse primer, 5′-ACCCGTTTGCCCTTTCTAGT-3′ (HCoV-229E *N* region, nt 25,900–25,881); probe, 5′-CAACCTTGGAAGGTGA-TACCTCGT-3′ (FAM, nt 25,785–25,808).

To prepare the internal standard DNA, the fragment of viral cDNA was amplified using the following primer sets: forward primer, 5′-CAGTGAGCTCTCCCATGAGC-3′ (HCoV-229E *N* region, nt 25,614–25,633); reverse primer, 5′-GCAAAATTCCAAC-TAAAGCCTG-3′ (HCoV-229E *N* region, nt 26,902–26,881). An expected 1289-base pair PCR product was ligated into the pMD20 plasmid (3270; Takara Bio, Kyoto, Japan) using NEBuilder HiFi DNA Assembly Cloning Kit (E5520S; New England Biolabs, Ipswich, MA, USA), and its sequence was confirmed by DNA sequencing analysis. The limit of detection using this internal standard curve was estimated to be 10 copies.

Generation of surgical smoke

HeLa-ACE2-TMPRSS2 cells (1×10^6) infected with HCoV-229E were resuspended in 300 μL of culture medium and then mixed with 60 mg of sterilized chopped paper towel (Kim-wiper; Nippon Paper Crecia, Tokyo, Japan) to soak up the excess liquid.

The infected cell pellet or supernatant mixed with chopped paper towel were placed in contact with the following surgical devices to generate surgical smoke: the blade of the Harmonic HC (Ethicon Endo Surgery Inc., Johnson & Johnson Medical SPA, Cincinnati, OH, USA) ultrasonic surgical device and a bipolar electrosurgical scalpel (MGI-202; Honest Medical Co. Ltd.,

Matsumoto, Japan) high-frequency surgical unit. The instruments were operated for 3 min at intervals of 10 s of work and 10 s of pause.

The surgical smoke was collected with a vacuum pump at a flow rate of 18 L/min and introduced into 1.0 mL of EMEM in a microtube under vacuum (Figure 1). The EMEM containing the surgical smoke was analysed for the presence of viral RNA, and its infectivity was assessed. Additionally, a surgical mask (Surgical face mask YM-1; Hogy Medical, Tokyo, Japan) was cut to size and placed in the filter holder (SX0004700; Merck Millipore, Darmstadt, Germany), set in the centre of the vacuum line between the material and the collection tube, to evaluate its ability to filter viral RNA from the surgical smoke.

Statistical analysis

Student's *t*-test was used to analyse the statistical significance of the differences between the groups with or without mask filtration. $P < 0.05$ was considered statistically significant.

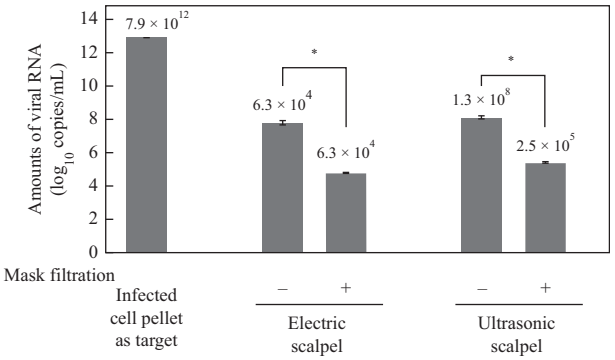

Figure 2. Amount of viral RNA in surgical smoke generated by different types of surgical devices, and the effect of filtration through a surgical mask. The presence or absence of a mask filter is indicated as (+) or (−), respectively. Student's *t*-test was used to analyse the statistical significance of differences between the groups with or without mask filtration ($N = 5$; $^*P < 0.05$).

Results

Human coronavirus RNA in surgical smoke and the mask filtering effect

Pellets of HeLa-ACE2-TMPRSS2 cells infected with HCoV-229E, containing 7.9×10^{12} copies of viral RNA, were treated for 90 s with two types of surgical devices. The amounts of viral RNA in 1 mL of EMEM, which absorbed the surgical smoke generated by the electric or ultrasonic scalpel, were 6.3×10^{7} and 1.3×10^{8} copies, respectively (Figure 2).

Filtration of the surgical smoke through a surgical mask reduced the amount of viral RNA in the smoke by 99.90% (from 6.3×10^{7} to 6.3×10^{4} copies) and 99.80% (from 1.3×10^{8} copies to 2.5×10^{5} copies) for smoke generated by an electric scalpel and an ultrasonic scalpel, respectively (Figure 2). Thus, a surgical mask can filter out at least 99.80% of viral RNA.

In addition, to investigate the time-dependent change in the filtration efficacy of the mask during continued use, the viral removal rates were compared over time with a mask that repeatedly filtered surgical smoke and continued to inhale air. The chronological order in which the study was performed is shown in Figure 3A. Surgical smoke was generated for 3 min following the same method as described above and aspirated and passed through a surgical mask at intervals of 20 min. Furthermore, the smoke that was sucked in was adsorbed on to 1 mL of EMEM using the same method as described above, and specimens were collected at intervals of 60 min. Thereafter, the amount of viral RNA was measured by RT–qPCR. After collecting the sample, the equipment, except for the mask filter, was replaced. Aspiration continued, even after the surgical smoke generation was finished, as air was circulated during this experiment. The results of this experiment showed that the viral load passing through the mask did not change significantly during the 2 h (Figure 3B).

Although 10^{8-11} copies of viral RNA/g are excreted in sputum and faeces of COVID-19 patients, To *et al.* reported that the median amount of viral RNA in oropharyngeal saliva of patients was 1.5×10^{6} copies/mL [23,24]. Therefore, a series of target samples with varying amounts of virus was examined,

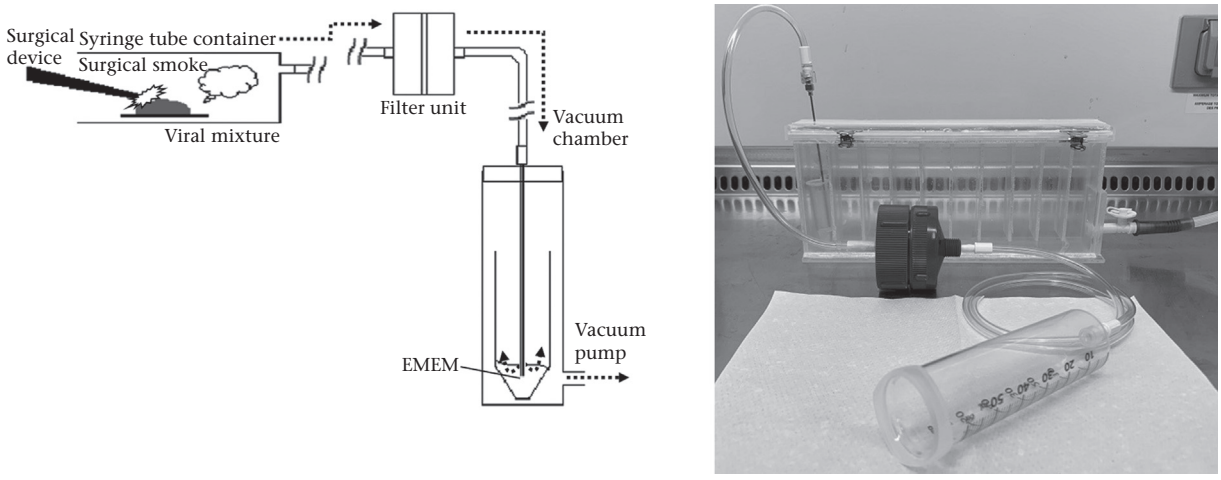

Figure 1. Sampling system for surgical smoke. Schematic and photograph of the sampling system.

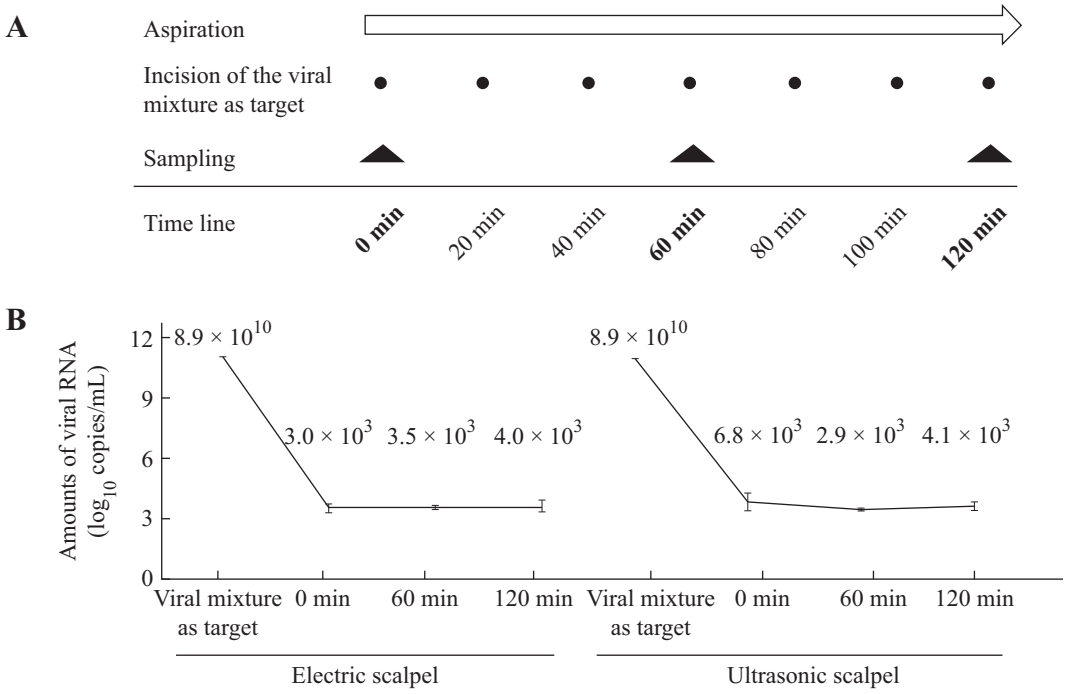

Figure 3. Time-dependent change of mask filtering effect. (A) Schematic of the study protocol in chronological order. (B) Viral load in surgical smoke generated by different types of surgical devices and filtered through a surgical mask for 2 h. The viral load that passed through the mask did not change significantly during the 2 h ($N = 3$).

and the transfer ratio between the viral RNA concentration in surgical smoke and the viral RNA concentration in the target was determined (Figure 4). When 1.9×10^8 copies of virus were used as the target, 1.1×10^3 and 9.2×10^2 copies of viral RNA were recovered in the surgical smoke generated by an electric scalpel and an ultrasonic scalpel, respectively. The transfer ratio was approximately $1/10^5$ to $1/10^6$, which was almost the same as the ratio when 2.5×10^{10} copies of virus were used as the target. However, for targets containing $\leq2.3\times10^6$ copies/mL of viral RNA, the viral RNA in surgical smoke was below the limit of detection (<10 copies). This result was expected because the transfer ratio from surgical smoke was expected to be less than $1/10^5$.

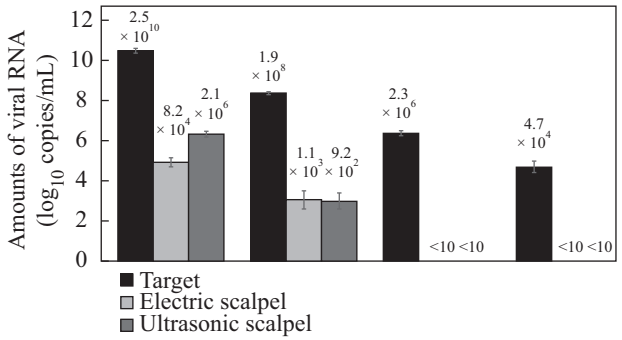

Figure 4. Amount of human coronavirus-229E RNA in surgical smoke generated from the targets containing different viral concentrations. The limit of detection was 10 copies/mL. The results are based on the mean of 10 samples. The error bars show the standard error.

Infectivity of human coronavirus in surgical smoke

The culture supernatant of infected HeLa-ACE2-TMPRSS2 cells contained 5.0×10^{10} copies/mL of viral RNA and 2.3×10^6 PFU/mL (plaque-forming units per mL) of infective virus. One PFU was equivalent to 2.2×10^4 copies of RNA (Figure 5). However, inoculation of EMEM containing surgical smoke with 1.3×10^8 copies/mL of viral RNA did not induce a visible cytopathic effect in cells on the microtitre plate (Figure 5). This suggests that infectivity of the virus was reduced considerably in surgical smoke.

After 4 days of incubation of the HeLa-ACE2-TMPRSS2 with 50 μL EMEM containing surgical smoke generated by the electric scalpel, the viral RNA in the medium was reduced by more than 97.50% from 3.2×10^6 to 7.9×10^2 copies. This reduction was substantially greater than the reduction obtained by incubation of intact HCoV-229E without cells under the same conditions, which was associated with a 32.00% reduction in viral RNA (Figure 6). These results suggest that treatment with an electric scalpel might degrade viral particles and render the viral RNA unstable.

By contrast, viral RNA in the culture supernatant with surgical smoke generated from the ultrasonic scalpel increased from 6.5×10^6 to 1.3×10^9 copies after 4 days of incubation with HeLa-ACE2-TMPRSS2 cells (Figure 6). Thus, infectious virus was retained in the surgical smoke generated by ultrasonic scalpel, although it was unable to induce visible plaques.

Discussion

This study showed that human coronavirus RNA is present in surgical smoke generated by incision of infected cell pellets,

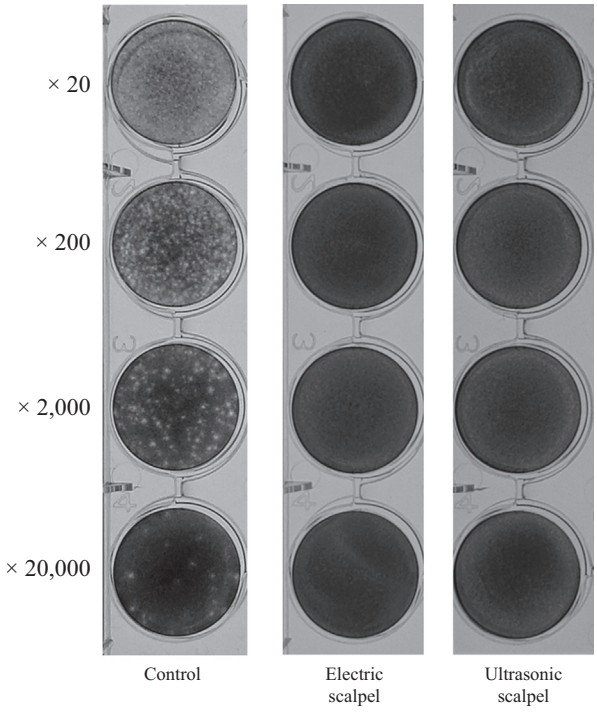

Figure 5. Plaques of human coronavirus-229E on HeLa-ACE2-TMPRSS2 cell monolayers. No plaques were observed in the plate incubated with the sample that had absorbed the surgical smoke.

with electric or ultrasonic scalpels using an experimental model.

Using a similar system, Johnson and Robinson demonstrated that HIV was transferred into surgical mist, and was infectious [18]. Several reports have further documented the presence of various viral genomes in surgical smoke [17–19]. However, to our knowledge this is the first demonstration of the potential hazard of human coronavirus in the operating room, generated by surgical devices. Treatment of the cell pellet containing $\sim 10^{13}$ copies of HCoV-229E viral RNA with surgical devices for 90 s generated smoke carrying 10^8 copies of viral RNA, with a transfer ratio of $1/10^5$. Surgical smoke generated from the sample with a lower amount of virus also contained viral RNA, with the same transfer ratio.

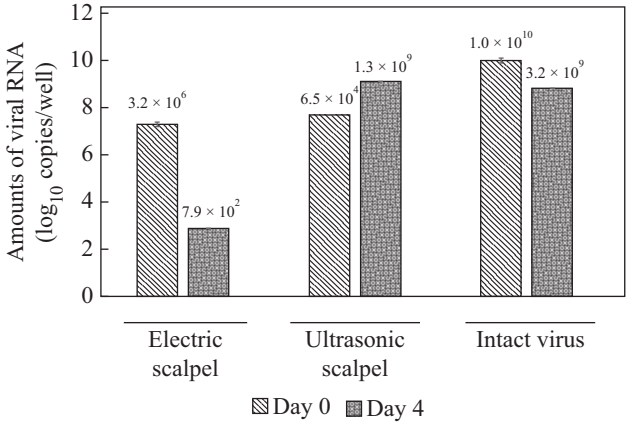

Figure 6. Amount of human coronavirus-229E RNA in the cultured cell supernatant after 4 days of incubation with smoke-containing media. The amount of RNA in the medium adsorbed with the smoke generated by an electric scalpel decreased by >97% after 4 days of incubation, while the amount of RNA incubation of intact virus without cells reduced the amount of viral RNA by approximately 33%. By contrast, the amount of RNA in the medium adsorbed with the smoke generated by an ultrasonic scalpel increased during incubation.

Weissleder *et al.* demonstrated that sputum and faeces in patients infected with SARS-CoV-2 contain 10^{8-11} copies of viral RNA/g [23]. Thus, incisions to the intestinal tract and upper respiratory tract may produce surgical smoke containing the same amount of virus as our experiment. Oropharyngeal saliva of COVID-19 patients has been reported to contain 1.5×10^6 copies/mL [24]. The surgical smoke with lower viral load, produced in such clinical settings, was below the limits of detection in our model system. However, assuming that the transfer ratio of viral RNA to surgical smoke is $1/10^6$ to $1/10^5$, surgical smoke should contain fewer than 100 copies of viral RNA per mL. Furthermore, major or prolonged surgery could generate surgical smoke with a larger amount of viral RNA.

Although a substantial amount of viral RNA was detected in the surgical smoke in our study, viral infectivity remained marginal. Freshly isolated viral samples from the culture supernatant of infected HeLa cells contained 5.0×10^{10} copies/mL HCoV-229E viral RNA and 2.7×10^6 PFU/mL infectious virus, while the medium that absorbed the surgical smoke contained 2.5×10^5 copies/mL viral RNA. However, the medium containing the surgical smoke could not induce plaque formation on target cells. Thus, treatment of specimens with surgical devices damages viral particles, resulting in loss of infectivity.

Specifically, 4-day incubation of the medium containing smoke generated by an electric scalpel diminished up to 99.90% of the viral RNA, whereas a mere 32.00% reduction in the amount of viral RNA occurred when intact virus was incubated under similar conditions. Thus, electric scalpel treatment may degrade viral particles, resulting in exposure of the RNA outside the viral structure.

By contrast, the surgical smoke generated by the ultrasonic scalpel retained human coronavirus RNA in the culture supernatant, with some infectivity observed. This was measured through production of viral RNA in the culture medium after 4 days with target cells, although no plaques were observed. This difference in infectivity of the surgical smoke generated by two different devices may potentially be related to the difference in temperature generated by these devices. The temperature of the tip of an ultrasonic scalpel ranges from 50 to 100°C, whereas that of an electric scalpel reaches 150–400°C [25]. In line with this observation, Zheng *et al.* hypothesized that the virus contained in the low-temperature aerosol generated from ultrasonic scalpels is not fully inactivated [26]. The higher temperature of the electric scalpel tip may destroy viral particles more effectively, thereby decreasing infectivity to a greater extent.

The efficacy of a surgical mask to prevent inhalation of human coronavirus from surgical smoke was also assessed, demonstrating that ≥99.80% of the viral RNA in surgical smoke could be removed by filtration through a surgical mask. The surgical mask used in this report has a bacterial filtration efficiency ≥98%. This indicates an ability to filter ≥98% of mist

with a diameter of 3 μm; the removal rates in our study were consistent with this.

Although in our model the mask filter was installed more tightly than it is worn in clinical settings, several studies have reported the general efficacy of masks to protect against transmission of SARS-CoV-2 [27,28].

In addition, to investigate the condition of this surgical mask after continued use, the viral removal rates were compared over time. The mask was not replaced throughout the experiment, was exposed to surgical smoke intermittently, and air was circulated through the mask constantly. As a result, the amount of viral RNA in the specimens tended to increase slightly; however, the difference was not significant, at least within the 2 h timeline.

To our knowledge, there is no recommendation regarding the maximum effective time for the use of a surgical mask after which it should be replaced. The viral removal rate of the mask was maintained, at least using this study's protocol. However, the viral adsorption rate of the mask may decrease in a time-dependent manner because of the influence of humidity on the mask. It may be necessary to change the mask during very long surgery or surgery that is associated with a lot of surgical smoke.

On the other hand, it has also been suggested that long-term use of N95 masks may increase blood CO_2 concentration and adversely affect the health of those wearing masks for long durations [29]. Overall, wearing a surgical mask is beneficial for surgeons; even though the virus present in surgical smoke loses its infectivity due to the heat generated by surgical devices, the mask provides sufficient additional protection against transmission of viruses that may still be infective, including damaged SARS-CoV-2.

In the present study, we used HCoV-229E as a representative of human coronavirus in place of SARS-CoV-2 that is currently of international concern. Although HCoV-229E is phylogenetically distinct from SARS-CoV-2, according to the World Health Organization, the stability of these viruses is likely similar [30,31]. Previous studies have also indicated that HCoV-229E may remain infectious for at least 5 days, whereas SARS-CoV-2 may remain infectious for 7 days on a common non-biocidal surface plastic [32,33].

In conclusion, this study shows the presence of viral RNA of HCoV-229E in surgical smoke, though its infectivity is substantially reduced. The residual infectivity of surgical smoke depends on the type of surgical device used, and the electric scalpel reduced the infectivity of surgical smoke considerably more than the ultrasonic scalpel. Furthermore, surgical mask filtration reduced the amount of viral RNA in surgical smoke by $\geq$99.80%. Thus, in a clinical setting, surgical mask filtration should be an effective means of additional protection against infection by coronaviruses, including SARS-CoV-2, via surgical smoke.

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Author contributions

T.Y. designed the study, carried out most experiments, analysed the data, and wrote the manuscript. T.O. and J.F. conceived and designed the virological study. T.O. also performed the plaque assay. Y.H. performed the literature search. M.K., J.F., and H.O. contributed to the conception of the study and approved the final paper.

Data availability statement

The datasets during and/or analysed during the current study are available from the corresponding author, T.Y., on reasonable request.

Conflict of interest statement

None declared.

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None.

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