ORIGINAL ARTICLE

Virus transmission by ultrasonic scaler and its prevention by antiviral agent: An in vitro study

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Abstract

Background: It is well recognized that dental procedures represent a potential way of infection transmission. With the COVID-19 pandemic, the focus of dental procedure associated transmission has rapidly changed from bacteria to viruses. The aim was to develop an experimental setup for testing the spread of viruses by ultrasonic scaler (USS) generated dental spray and evaluate its mitigation by antiviral coolants.

Methods: In a virus transmission tunnel, the dental spray was generated by USS with saline coolant and suspension of Equine Arteritis Virus (EAV) delivered to the USS tip. Virus transmission by settled droplets was evaluated with adherent RK13 cell lines culture monolayer. The suspended droplets were collected by a cyclone aero-sampler. Antiviral activity of 0.25% NaOCl and electrolyzed oxidizing water (EOW) was tested using a suspension test. Antiviral agents' transmission prevention ability was evaluated by using them as a coolant.

Results: In the suspension test with 0.25% NaOCl or EOW, the TCID50/mL was below the detection limit after 5 seconds. With saline coolant, the EAV-induced cytopathic effect on RK13 cells was found up to the distance of 45 cm, with the number of infected cells decreasing with distance. By aero-sampler, viral particles were detected in concentration ≤4.2 TCID50/mL. With both antiviral agents used as coolants, no EAV-associated RK-13 cell infection was found.

Conclusion: We managed to predictably demonstrate EAV spread by droplets because of USS action. More importantly, we managed to mitigate the spread by a simple substitution of the USS coolant with NaOCl or EOW.

KEYWORDS
antimicrobial(s), infection control, microbiology, non-surgical periodontal therapy, virology

1 | INTRODUCTION

It is well recognized that dental procedures represent a potential way of infection transmission by using air-water syringes, ultrasonic scalers (USS), high-speed handpieces, and lasers because of extensive generation of droplets and aerosol.[1,2] A concern was recently raised in medical literature, pointing to confusion in definition of aerosol and droplets, based on particle size threshold.[3,4] Interestingly, similar viewpoint on composition and terminology was raised in dental literature and the term "dental spray" was proposed for spatter, droplets, droplet-nuclei, and a true aerosol, created by saliva and water coolant combined with high-speed instrumentation.[5]

In the past, the spread of bacteria associated with dental procedures has already been extensively studied in real or

artificial working environment settings, reporting on large contamination to operator's face and head, patient chest and also surfaces up to 3 m from the patients' mouth.[1] To reduce bacterial load, preprocedural mouth-rinse was suggested despite the moderate level of evidence to support such action.[6] The bacterial content of aerosols was reduced 2 to 7 fold by using povidone-iodine and chlorhexidine gluconate as an ultrasonic coolant compared with water in an in-vivo study.[7] Although the SARS-CoV outbreak in 2004 was recognized in dental literature,[8,9] until recently,[10] no direct research data were available on virus transmissions associated with dental procedures.[11]

With the COVID-19 pandemic, the focus of dental procedure associated transmission has rapidly changed from bacteria to viruses. It is generally acknowledged that dental procedures represent a high risk of SARS-CoV-2 virus transmission[12–15] because of its presence in saliva,[16] gingival crevicular fluid,[17] nasopharyngeal, oropharyngeal, and bronchial excretions.[18] In addition to the respiratory droplets and contact routes,[19] SARS-CoV-2 virus may be transmitted by the dental spray that is generated from a mixture composed of virus-containing oral fluids and water from the dental unit water system. For the provision of safe dental care for dental team members and patients, numerous preventive measures were proposed and included in professional[12–15] and national[20] COVID-19 prevention guidelines. These range from pre-procedure mouth-rinse, use of personal protective equipment, high volume evacuation, air purification (ventilation, filtering, air disinfection), and surface disinfection. Personal protective equipment can protect the dental care providers; however, the contamination of clinical environments by sprays still necessitates periods of "fallow time" between appointments to protect patients and staff.[21] A special concern was raised for open plan clinic environments, reporting a safe distance of 5 m.[22] Although preprocedural mouth-rinse is included in most recommendations,[20] its effectiveness is questionable as no scientific clinical evidence exists for reducing the viral load.[23] So far, antiseptic mouth rinses containing cetylpyridinium chloride or povidone-iodine have shown the highest potential to reduce viral load in infected subjects.[24] Thus, there is a high need for effective prevention measures against virus transmission in dentistry.[25]

Although a simple replacement of cooling liquid with a virucidal agent might potentially reduce viral spread, this possibility was only recently evaluated by 0.5% hydrogen peroxide cooling spray used in air-turbine, demonstrating reduction of viral RNA below detection threshold on all tested sites.[10] Besides hydrogen peroxide, several agents were already found to be effective against coronaviruses

in as short as 30 seconds of suspension test.[26] Some of them, like sodium hypochlorite (NaOCl)[27] have already been evaluated for safe use as a daily oral rinse. Additionally, the electrolyzed oxidizing water (EOW) was proposed as an effective and safe biocidal agent for nasal[28] or ocular[29] application.

The aim of the study was twofold, (1) to develop an experimental model for evaluation of infective virus transmission by USS activity, and (2) to evaluate the reduction of USS viral transmission by replacement of cooling water with a virucidal agent pre-tested for antiviral activity in suspension test.

2 | MATERIALS AND METHODS

To evaluate our hypothesis, we have designed a virus transmission tunnel (VTT), thus simulating dental spray generating dental procedure in a controlled laboratory environment. It featured the operating USS in the presence of virus suspension and virus sampling device for a spill, settled droplets, and suspended droplets. It consisted of a clear acrylic box, resembling similar devices,[10,30] with dimensions of 120 × 50 × 40 cm (L × W × H) manufactured specifically for this purpose (Acrytech, Ljubljana, Slovenia).

At one end of the VTT, a platform for dental spray generation was positioned 25 cm above the floor. The piezo-electric USS (Varios 970, NSK Dental, Nakanishi Inc., Shimohinata, Japan) with the handpiece (VA2-LUX-HP) and a general prophylactic tip (G4) was firmly positioned in a mechanical holder. For all experiments, the USS was set to General mode, power level to 10 and the coolant level to 3 (flow rate between 12.5 and 17.5 mL/min). The USS tip was positioned into a semi-cylindrical groove, measuring 30 × 3 × 3 mm (L × W × D) (Figure 1). An experienced periodontology specialist (R.G.) precisely adjusted the USS tip to freely oscillate in the groove, assuring the constant and repeatable dental spray generation through the experiment. In the same groove, a virus suspension was delivered by an IV administration set (Normal set, Ferrari L., Verona, Italy) with a flow rate of 104 mL/h (1.73 mL/min) through a pre-curved, 20G blunt, rounded tip needle (0.9 × 42 mm) (Transcodent, Kiel, Germany), stably mounted with a connecting element (Figure 1).

2.1 | Virus suspension and sampling

For experiments, a laboratory Equine Arteritis Virus (EAV) strain was used. EAV was propagated in a 48 hours cell culture monolayer. A specific cytopathic effect (CPE) with cell
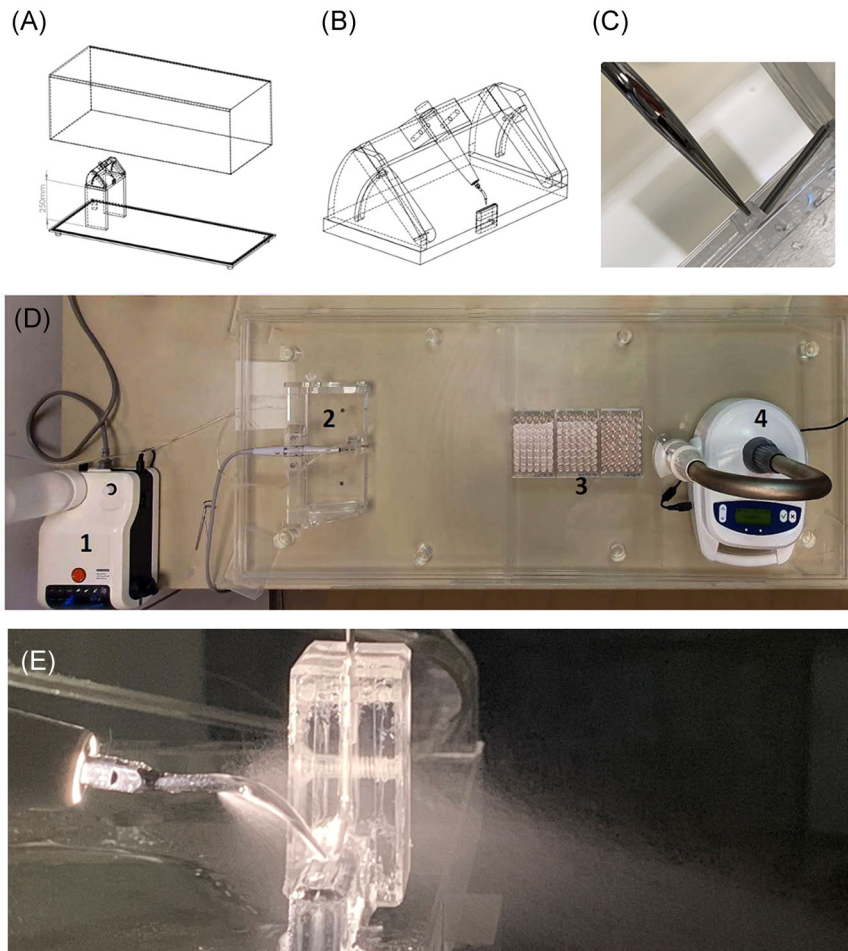
FIGURE 1 Experimental set-up for virus transmission evaluation. (**A**) The schematic diagram of the virus transmission tunnel (VTT). (**B**) Platform with ultrasonic scaler (USS) handpiece holder and groove. (**C**) Semi-cylindrical groove with USS tip, and blunt needle for virus suspension delivery. (**D**) Top view on the virus transmission tunnel, showing (1) USS device, (2) platform with USS handpiece and groove, (3) three consecutively positioned 48-well cell culture plates, and (4) air sampler. (**E**) USS tip, positioned in the groove, during the experiment

rounding and degeneration of cell monolayer is observed in 48 hours post-inoculation in 75 cm^2 cell culture flasks. After CPE reaches 90% of the monolayer, the cell culture flask was freeze-thawed two times, and finally, the whole-cell debris and media were transferred to 50 mL centrifugation tubes. Cell debris was removed by centrifugation 15 minutes at 1500×g. Clear virus suspension was stored at -80°C in 1 mL cryotubes.

One mL of clear virus suspension with a concentration of 1.33×10^6 TCID$_{50}$/mL (infective virus units: 50% Tissue Culture Infectious Dose per milliliter) was inoculated into 250 mL sterile saline solution (0,9% NaCl), which was delivered to the USS tip through infusion system. Virus transmission in the VTT was achieved by simultaneous activation of USS and flow of virus suspension in a duration of 10 minutes.

2.2 | Virus sampling

Virus sampling was performed at three sites: liquid sample (LIQ), settled droplet sample (SET_D), and suspended droplet sample (SUSP_D). At the end of the procedure, the LIQ was acquired from a mixture of virus suspension

and cooling liquid, flowing freely from the groove and collected in a glass jar placed directly under the platform. The SET_D samples were acquired during the procedure by the three 48-well standard cell culture plates (8.5 × / 12.8 cm) (Cell Culture Multiwell Plate, Cellstar) with adherent 48 hours cell culture monolayers that were positioned consecutively from the distance of 30 to 55 cm. In total, 108 wells, arranged in 18 lines, with six wells per line, were used for assessment of SET_D. After the procedure, plates were covered, removed, and incubated. The SUSP_D was collected by a cyclone air sampler (Coriolis Micro, Bertin Technologies, France), with airflow set at 100 L/min (Figure 1). The air inlet was positioned 60 cm from the USS tip and height of 25 cm. The experiment was repeated twice.

2.3 | Virus transmission evaluation

The virus was efficiently propagated in an adherent RK13 cell line (source: *Oryctolagus cuniculus*, kidney, epithelial). Eagle's Minimum Essential Medium (Sigma-Aldrich, St. Louis, MO) was used for cell culture medium, supplemented with final 10% fetal bovine serum (EuroClone, Italy) concentration.

TABLE 1 Chemical characteristics of antiviral coolants used in suspension test and virus transmission prevention test

	0.25% NaOCl	EOW
pH	11.2	7.34
Eh (mV)	507	765
Free chlorine (mg/L)	220	22

Virus concentration in LIQ and SUSP_D was determined with titration on cell monolayer in 96-well microtiter plate. Each 10-fold dilution was inoculated in eight replicates, including negative control. Briefly, cell culture media was removed from the cell monolayer, and 100 μL of virus suspension (LIQ or SUSP_D sample) was added to each well. The inoculated microtiter plate was incubated for up to 4 days in 37°C, 5% CO_2 atmosphere, and 95% humidity. After 4 days, cells were screened for CPE, and the number of infected wells was recorded.

Virus transmission in SET_D was evaluated by CPE formation in exposed microliter plates incubated for up to 4 days in 37°C, 5% CO_2 atmosphere, and 95% humidity. After 4 days, cells were screened for CPE by a microbiologist (A.S.) experienced in virology and cell culture virus propagation, and the number of inoculated wells in each row was recorded.

For the evaluation of specificity of observed CPE a real-time RT-PCR method was selected for viral RNA detection. After the development of CPE in cell culture wells of exposed microtiter plates, four cell culture wells with CPE and four wells without visible CPE were randomly selected. A 400 μL of supernatant was used for RNA extraction with MagNA Pure24 (Roche, Basel, Switzerland) Pathogen 200 protocol and 100 μL extracted RNA elution. Two microliters of extracted RNA was used for real time RT-PCR reaction using LightMix Modular EAV RNA Extraction Control primer-probes (TIB Molbiol) and LC Multiplex RNA Virus Master kit (Roche, Basel, Switzerland) following manufacturer instructions. For the detection a LightCycler 480 II was used (Roche, Basel, Switzerland), applying the fit points analysis.

2.4 | Coolant suspension evaluation

The virucidal ability of two coolants was evaluated with a suspension test. A 0.25% NaOCl and EOW (Table 1) were used. The pH, redox potential (Multi 3630 IDS, WTW, Weilheim, Germany), and free chlorine (Pocket Colorimeter II, Loveland, CO) were measured and recorded (Table 1). Virus suspension was exposed to the agent, and a reduction of infective virus particles was evaluated after a contact time of 5 s, 30 s, 1, 5, and 10 min.

2.5 | Virus transmission prevention evaluation

The virus transmission prevention test was evaluated with the same experimental setup as for the virus transmission test, except that the sterile saline (as a cooling agent) was replaced by agents evaluated in suspension tests: 0.25% NaOCl in the first and EOW in the second experiment. The coolant flow rate was the same as in the virus transmission test. For each coolant, the experiment was repeated twice.

2.6 | Statistical analysis

A 5% significance level was taken for the statistical analysis. The t-test was used for analyzing the suspension tests. For comparing saline, 0.25% NaOCl and EOW in the virus transmission and transmission prevention tests, Firth's bias reduced logistic regression was performed. Such a model was chosen because of a quasi-complete separation of the outcome variable, that is, no wells were infected when antiviral agents were used compared to successful infection with saline. All analysis was done in the R statistical software v4.0.3 by one of the coauthors (D.M.).

3 | RESULTS

3.1 | Suspension test

The suspension test results are shown in Table 2. For saline, the virus concentration was measured at time 0 and after 600 seconds. No reduction in the viral concentration was observed for saline after 600 seconds contact time and the difference (at 0 and 600 seconds) was not statistically significant ($P = 0.76$).

On the other hand, both antiviral agents (0.25% NaOCl and EOW) effectively reduced the virus concentration already after 5 seconds to a concentration below the detection limit (< 2.8 log10 (conc)/TCID50/mL). Even though the exact values after 5 seconds were not known, a t-test for the worst scenario (2.8 log10 (conc)/TCID50/mL) yielded a statistically significant decrease ($P = 0.003$) of viral concentration for both 0.25% NaOCl and EOW. Thus, both antiviral agents significantly decreased the viral concentration from 5 seconds onwards compared to the initial viral concentration (Table 2).

3.2 | Virus transmission and prevention tests

The estimated viral concentration in the saline generated LIQ sample, collected in the vicinity of the droplet-generating platform, was 3.25 log TCID$_{50}$/mL. In LIQ of

TABLE 2 Suspension test for the saline, 0.25% NaOCl and EOW coolants. The table presents the estimated mean log_10 $TCID_{50}$/mL viral concentration at different time points (0, 5, 30, 60, 300, 600 seconds after the start of the experiment). Saline was evaluated only at 0 and 600 seconds

	0 s	5 s	30 s	60 s	300 s	600 s
Saline	6.45					6.56
P-value						0.76
0.25% NaOCl	6.45	<2.8	<2.8	<2.8	<2.8	<2.8
P-value		0.003	0.003	0.003	0.003	0.003
EOW	6.45	<2.8	<2.8	<2.8	<2.8	<2.8
P-value		0.003	0.003	0.003	0.003	0.003

T-tests comparing the viral concentration at any time point to the one at time 0s have also been conducted. The corresponding *P*-values are given for every coolant.
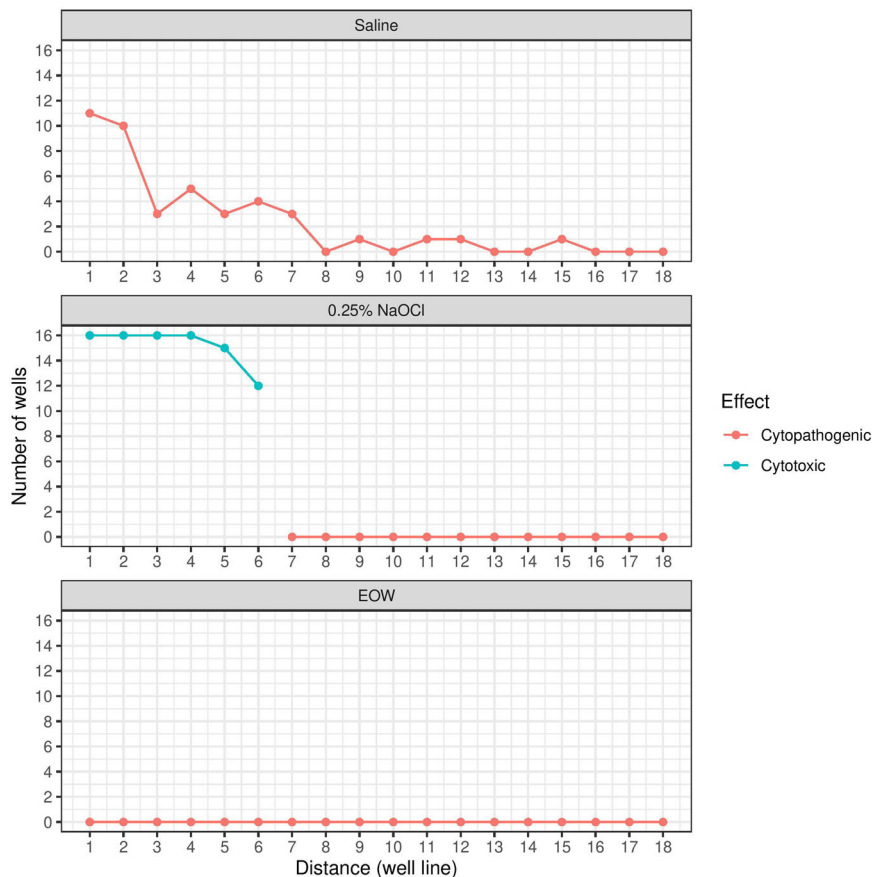

FIGURE 2 The cytopathogenic and cytotoxic ef in settled droplet sample, obtained from three different coolants: saline, 0.25% NaOCl, and EOW. When a cytotoxic effect was observed, the cytopathogenic could not be evaluated

both virucidal agents, no infective viral particles were detected. Importantly, a cytotoxic effect of NaOCl was found in LIQ for dilutions up to 10^5, whereas no cytotoxic effect was found for EOW.

In DSs collected in wells, CPE associated with viral infection was found up to the distance of the first 16 of 18 lines of cultivation plates. In SET_D sampled with 0.25% NaOCl used as a coolant (Figure 2), a cytotoxic effect was found in the first six lines, and thus the effect of infective viruses (CPE) could not be evaluated. In lines from 6 to 18, no CPE nor cytotoxic effect was found. With EOW as a coolant (Figure 2), nor CPE neither cytotoxic effect was found in any distance.

Although it is apparent (from Figure 2) that there are much more infected wells when saline is used compared to the two antiviral agents, we have also statistically evaluated this difference. Frith's logistic regression was used to assess the effect difference between both antiviral agents and the saline coolant. This revealed a statistically significant difference in the number of infected wells between the saline coolant and EOW ($P < 0.001$). At any given distance the odds of having an infected well using the saline coolant was 159.69 (95% CI [21.50, 20434.94]) higher compared to EOW. Compared to the baseline (and smallest) distance, odds ratios (ORs) for all further distances were smaller. The cytopathogenic effect of 0.25% NaOCl was compared

to the saline coolant from the seventh distance onwards because a cytotoxic effect was noticed up to this distance. We again observed a statistically significant difference in the number of infected wells between the saline coolant and 0.25% NaOCl ($P = 0.0065$). At any given distance from the 7th well onwards, the odds for having an infected well using saline as a coolant were 15.275 higher compared to 0.25% NaOCl (95% CI [1.85, 1985.55]). The effect difference between saline and 0.25% NaOCl was not significant with respect to the well distance ($P > 0.05$).

In one SUSP_D only, viral particles were detected in a very low concentration, which was ≤ 0.63 log $TCID_{50}$/mL. In SUSP_D, for both antiviral coolant experiments, no infective viral particles were detected. However, for 0.25% NaOCl cytotoxic effect was found at 10^{-1} dilution.

Virus specific real-time RT-PCR to test selected cell culture wells with clearly visible CPE revealed a strong positive result with a ct value between 13.00 and 15.00. In contrast, all tested wells without visible CPE and unaffected cell monolayer were negative with real-time PCR, which confirmed the absence of virus propagation and the viral RNA load below the limit of detection.

4 | DISCUSSION

The study results indicate that by USS activity EAV was transmitted mainly by settled droplets and up to the distance of 45 cm. The transmission of EAV by suspended droplets, however, yielded inconclusive results. More importantly, the virus droplet transmission was prevented by replacing saline with a virucidal agent that has already been tested as mouth-rinse for home oral care.

For our experiments, a non-human viral pathogen was selected, which is similar (by structure and genome) to SARS-CoV-2. An EAV is an animal pathogen, which is species-restricted to *Equidae* members. EAV is an enveloped virus with a single-stranded, positive-sense RNA genome. EAV is also a member of Baltimore's IV group and presents a similar structure to Coronaviruses, similar stability in the environment, and inactivation by general disinfectant as SARS-CoV-2.[31,32] Besides, EAV is also transmitted through respiratory route and aerosol transmission, although indirect or close contact is mandatory for infection.[31]

In the first part of the study, we designed an experimental setup to demonstrate the EAV spread by USS action and successful infection of RK13 cells. The EAV predominantly spread via larger droplets (splatter) that, according to ballistic laws, reach the near surrounding area, as infected droplets consistently infected cells on plates up to the distance of 45 cm (16th well) from the USS tip. These observations are in accordance with a recent review[21] stating that contaminants settle to a great extent on the operator's dominant arm, eyewear, and chest of the patient, and to a lesser extent on the non-dominant arm and chest of the operator and assistant. To the best of our knowledge, this is the first proof of the contribution of an USS action to the spread of infective virus via droplets to the environment. In a similar experiment, viral RNA transmission during air-turbine operation has already been demonstrated. However, it cannot be considered as direct evidence of infective virus transmission. Although we were able to detect low concentrations of EAV in suspended in the air, we failed to achieve the reproducible collection of the infectious virus from the air-sampler. This observation is in accordance with a recent review on air contamination by SARS-CoV-2 in hospital settings.[33] Air samples from the close patient environment were positive for SARS-CoV-2 RNA and viable viruses only in 17.4% and 8.6%, respectively. Identification and quantification of the virions from suspended droplets depends on air sampling equipment and several other important factors. Among the conventionally used sampling methods, cyclone-based air sampling is considered the simplest and most effective. As desiccation has always been a problem for viruses, the liquid collection medium in cyclones helps maintaining the viability of the virus and often can be used directly with analytical methods such as plaque assay.[34] Nevertheless, the sampling-associated microbial stress is important to consider when cultivation-dependent analysis is used. Physical damage resulting from the actions of cyclones can deactivate viruses, resulting in an underestimate of the collected infectious viruses. Enveloped viruses are more sensitive to physical or chemical treatments than non-enveloped viruses.[35] Despite lower physical sampling efficiency in comparison to a reference sampler, Coriolis (liquid) cyclone sampler, similar to the one used in our experiments demonstrated acceptably high biological sampling and yielded acceptable virion concentration in a limited sample time.[36] Nevertheless, in the clinical environment the exposure time is considerably longer than in our experiment and represent a higher cumulative viral load and increase probability of infection.[34] Another reason could be the low percentage of viral particles in suspended droplets. As the volume of a typical suspended droplet particle with a diameter of 1 μm is 5.23×10^{-13} mL, and 3.58×10^{11} of such particles may be generated from 1 mL, it is possible to calculate a fraction of particles actually harboring a virus (4.97×10^{-9}) by dividing the number of virus particles in 1 mL by the number of suspended droplets ($1.78 \times 10^{3}/3,58 \times 10^{11}$). However, to support such calculation, better characterization of dental spray particles would be needed.

In our experiments, the clear virus suspension with a concentration of log 4.12 ± 0.60 $TCID_{50}$/mL and flow

of 1.73 mL/min was diluted with coolant with flow between 12.5 and 17.5 mL/min. The concentration of log 3.25 ± 0.59 $TCID_{50}$/mL was measured in LIQ. This observation is in accordance with a recent paper, stating that dilution because of mixing of the introduced coolant with real saliva also requires consideration.[21] In our case, with virus titration control in saline suspension and splatter collected directly at the USS, the concentration reduction was 0.87 log, which is near 10x dilution and goes perfectly with the ratio of virus suspension and coolant flow. This observation confirms the importance of dilution, as noted in previous studies.[22,37]

In the second part of the experiment, we managed to prevent the droplet spread of the virus to the surroundings by changing the saline coolant with EOW or hypochlorite. Despite the promising results of our preliminary tests, in which 0.25% NaOCl did not show considerable cytotoxicity, such effect was always found in the wells reached by a higher number of settled droplets (the first six lines). Nevertheless, no cytotoxic effect was found in the wells at a larger distance from the USS tip. It should be noted that in the experiment with the saline, cell infection was apparent to the much greater distance (up to the 16th well). Therefore, the absence of CPE in wells 6 to 16 can be attributed to the effective inactivation of the virus by 0.25% NaOCl.

However, the effective infection prevention of plated cells was particularly interesting for the EOW experiment, as, despite the absence of cytotoxicity, we never found a single well with CPE suggestive of viral infection. Because all experiments were substantiated by suspension tests, well-controlled and performed sequentially, it is unlikely that the absence of signs of viral infection in these experiments resulted from a methodological error. We may consider our experiments as the first that have clearly shown the possibility of disinfecting the virus in spray generated by USS by replacing inert coolant with antiseptic coolant. On the other hand, the suspension test failed to prove virucidal capacity of 1% H_2O_2 (data not shown), which was even higher than the concentration that reduced coronavirus RNA spread by air-turbine.[10] As no suspension testing was reported and only reduced RNA levels were measured, successful transmission of infective virus by air-turbine as well its mitigation[10] may be questioned from different methodological aspects.

A low level of cytotoxicity and the high virucidal effect of EOW are optimal for highly effective potential disinfectants in dental procedures. EOW was found to have no systemic effects when it was provided to mice as water during a 2-week experiment, which served to the authors as indirect proof for safe usage as a mouthwash.[36] EOW was already proposed as an effective and safe biocidal agent for nasal[28] or ocular[29] application. Similarly, the 0.25% NaOCl has been proposed for oral rinse.[39,40] Because

SUSP_D from the NaOCl experiment exhibited cytotoxic effect already at 10^{-1} dilution, we could assume that NaOCl-containing suspended droplets were successfully collected during the experiment. This result could be attributed to the high throughput air sampler used in our case, as in 10 minutes, the whole volume of the VTT was filtered four-times. Nonetheless, the detection of infective virus particles was not reproducible in the first part of the experiment.

Besides direct infection by droplets, the proposed approach might also effectively prevent indirect transmission by contaminated surfaces or reduce viral load. It has been demonstrated that SARS-CoV-2 might be transmitted indirectly via contaminated surfaces as the virus can survive on surfaces like metal, glass, or plastic for up to a couple of days.[25,41]

To the best of our knowledge, this is the first dental procedure virus transmission study, performed with viable infective virus evaluation. The strength of proposed experimental setup is the possibility for evaluation of the other dental spray generating dental devices, such as air-water syringes, high-speed handpieces, and lasers. As EAV is incapable of infecting humans, the experiments can be safely performed using standard personal protective equipment with no risk for the researchers. However, the combination of EAV and RK13 cell line, representing kidney epithelial cells, does not precisely reflect clinical conditions, featuring SARS-CoV-2 and respiratory epithelial cells. The use of SARS-CoV-2 would require facilities with Biosafety Level 3 conditions, and with generating dental spray, the whole experiment procedure would not be possible.

It should be noted that our experiments were performed without any dental spray reduction device in order to maximize the contamination and to test the effect of coolant in the most challenging situation. Using a high vacuum evacuator or similar device, as they are usually used in a clinical setting, would considerably reduce the spread of dental spray.[42,43] Similar to a recent study on atomization from rotary dental instruments,[21] the generalization of our findings into the wide variety of clinical settings is difficult because of variability of USS devices and their operating parameters, including power setting tip selection and coolant flow rate. Even larger differences may be found between dental procedures.[44] Another limitation of this study a lack of quantitative characterization of dental spray particles and such evaluation would be necessary in the further research.[5,45]

In conclusion, by using the proposed experimental model, we managed to predictably demonstrate infective virus spread by droplets because of USS action. More importantly, we managed to mitigate the virus spread by a simple substitution of the coolant with clinically tested

virucidal agents, namely sodium hypochlorite or EOW. Future research using the proposed experimental model should include other dental spray generating dental procedures, additional agents, and ultimately the SARS-CoV-19 virus before clinical application. Nevertheless, the proposed principles for virus spread mitigation seems promising and warrant further evaluation.

ACKNOWLEDGMENTS

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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