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ORIGINAL ARTICLE

Significance of chlorine-dioxide-based oral rinses in preventing SARS-CoV-2 cell entry

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Abstract

Objective: This work aims to determine the efficacy of preprocedural oral rinsing with chlorine dioxide solutions to minimize the risk of coronavirus disease 2019 (COVID-19) transmission during high-risk dental procedures.

Methods: The antiviral activity of chlorine-dioxide-based oral rinse (OR) solutions was tested by pre-incubating with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pseudovirus in a dosage-dependent manner before transducing to human embryonic kidney epithelial (HEK293T-ACE2) cells, which stably expresses ACE-2 receptor. Viral entry was determined by measuring luciferase activity using a luminescence microplate reader. In the cell-to-cell fusion assay, effector Chinese hamster ovary (CHO-K1) cells co-expressing spike glycoprotein of SARS-CoV-2 and T7 RNA polymerase were pre-incubated with the ORs before co-culturing with the target CHO-K1 cells co-expressing human ACE2 receptor and luciferase gene. The luciferase signal was quantified 24 h after mixing the cells. Surface expression of SARS-CoV-2 spike glycoprotein and ACE-2 receptor was confirmed using direct fluorescent imaging and quantitative cell-ELISA. Finally, dosage-dependent cytotoxic effects of ORs were evaluated at two different time points.

Results: A dosage-dependent antiviral effect of the ORs was observed against SARS-CoV-2 cell entry and spike glycoprotein mediated cell-to-cell fusion. This demonstrates that ORs can be useful as a preprocedural step to reduce viral infectivity.

Conclusions: Chlorine-dioxide-based ORs have a potential benefit for reducing SARS-CoV-2 entry and spread.

KEYWORDS
chlorine dioxide, preprocedural oral rinses, SARS-CoV-2, viral entry, virus–host cell interactions

1 | INTRODUCTION

The airborne pathogen, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), can be transmitted from person to person by aerosolized droplets (Harrison et al., 2020; Meyerowitz et al., 2021; Morawska & Cao, 2020). Asymptomatic, pre-symptomatic, and symptomatic individuals can transmit the virus from the oral cavity via direct splashes and sprays in the form of infectious respiratory droplets and aerosol particles or via touching of mucous membranes with hands that have been cross-contaminated (Peng et al., 2020). After inhalation of aerosolized droplets or manipulation of contaminated surfaces, if infection of mucous membranes occurs, SARS-CoV-2 enters the host cell through the interaction between its spike glycoprotein to the host cell receptors (Bao et al., 2020; Clausen et al., 2020; Letko et al., 2020; Shang et al., 2020; Wang et al., 2020). Upon entering the cell, SARS-CoV-2 uses host cell machinery to

replicate its RNA genome. The translated structural proteins translocate into endoplasmic reticulum membranes and eventually transit through the Golgi intermediate compartment where interaction with N-encapsulated, newly produced, genomic RNA results in budding. The functional virions are secreted from the infected cell by exocytosis (V'kovski et al., 2021).

The proposed model of SARS-CoV-2 infectivity suggests that angiotensin-converting enzyme-2 (ACE2) receptors act as a major receptor for cell entry by interacting to the virus envelope spike glycoprotein (Bao et al., 2020; Letko et al., 2020; Wang et al., 2020). In addition to ACE-2, multiple other cell surface receptors and co-receptors have also been proposed to support SARS-CoV-2 entry (Gadanec et al., 2021; Sigrist et al., 2020; Tiwari, Beer, et al., 2020; Tiwari, Tandon, et al., 2020). The ACE2 receptors are highly expressed in nasopharyngeal tissues and the oral cavity (Okui et al., 2021; Sawa et al., 2021; Xu et al., 2020; Zhong et al., 2020). Interestingly, the expression levels of ACE2 receptors vary depending on age, gender, and certain predispositions (Drozdzik & Drozdzik, 2022; Okui et al., 2021; Peng et al., 2021). For instance, an increased expression of ACE2 receptors has been reported in the population with severe smokers, chronic obstructive pulmonary disease (COPD), diabetes, obesity, and Crohn's disease (Burgueno et al., 2020; Drucker, 2021; Yao et al., 2020), making compromised individuals with the above conditions at risk for SARS-CoV-2 infection. In addition, a recent increase in infection rates has been observed associated with the appearance of SARS-CoV-2 variants, including the more recently described lineage B.1.617.2 (Delta variant) and lineage B.1.1.529/BA.1 (Omicron variant) (Parra-Lucares et al., 2022; Tao et al., 2021). These variants have received the most scientific and clinical attention because they pose a higher affinity for ACE2 receptors compared with wild-type viruses, resulting in enhanced cell infectivity with broad host tropism (Cosar et al., 2022).

Given the fact that a significant number of patients visiting dental clinics may be compromised and/or unvaccinated, these patients are more likely to be vulnerable to infection and, if infected, may present a higher viral load, increasing risk to dental workers (Giudice, 2020; Volgenant et al., 2021). In addition, many devices used in the dental practice such as ultrasonic scalers, air-water syringes, and dental handpieces are known to generate water aerosols. Therefore, the above tools increase the risk of exposure with the infectious respiratory pathogens including SARS-CoV-2 to the patients and the healthcare workers (Anjum et al., 2019; Bidra et al., 2020). Hence, the strategies directed to minimize the risk of SARS-CoV-2 transmission by targeting the ability of viral particles to fuse with the host cells and/or by reducing the titers of SARS-CoV-2 in the saliva of infected patients seems a promising approach. This is particularly important in high-risk procedures such as dental treatment. In this regard, developing novel practices that either dismantle the virus and/or prevent the virus from infecting a new host provides valuable therapeutic interventions against SARS-CoV-2 (Koch-Heier et al., 2021; Paull et al., 2021; Takeda et al., 2021).The goal of this study was to investigate if the oral rinses (ORs) that contain stabilized chlorine dioxide (Unflavored, Ultrasensitive CloSYS) and/or freshly prepared chlorine dioxide (OraCare), affect SARS CoV-2 cell entry and spike glycoprotein-mediated cell-to-cell fusion. We hypothesize that the use of preprocedural ORs provides some protection against SARS-CoV-2 by compromising either the virus lipid membrane or having an affinity for the spike glycoprotein.

2 | OBJECTIVES

The aim of this study was to evaluate the effect of ORs against SARS-CoV-2 cell entry and SARS-CoV-2 spike glycoprotein-mediated cell-to-cell fusion.

3 | MATERIALS AND METHODS

3.1 | Oral rinses

The oral rinses utilized in this study are proprietary solutions. These include CloSYS (Rowpar Pharmaceuticals, Inc, Scottsdale, AZ, USA) and OraCare (Dentist Select, Bridgeport, WV, USA). CloSYS oral rinse came in a one-bottle solution with the active ingredient, sodium chlorite. OraCare oral rinse came in a two-bottle system where activation of the active ingredient, sodium chlorite, would be initiated after the combination of the two bottles in equal proportions. Dilutions of ORs were made by mixing freshly prepared OR with serum-free media.

3.2 | Cells, plasmids, and pseudoviruses

Human embryonic kidney epithelial cells expressing the ACE2 receptor (HEK293T-ACE2, Genecopoeia, Rockville, MD, USA) and Chinese hamster ovary cells (CHO-K1, ATCC, Manassas, VA, USA) were passaged and maintained according to the manufacturer's recommendations. The CHO-K1 cells were grown in Ham's F-12 medium containing 10% fetal bovine serum (FBS) and 100 U/mg/ml penicillin/streptomycin, while HEK293T-ACE2 cells were grown and cultured in Dulbecco's modified Eagle medium (DMEM) containing 100 U/mg/ml penicillin/streptomycin, 100 μg/ml hygromycin B, and 10% FBS. The mammalian expression plasmid encoding SARS-CoV-2 spike glycoprotein of 2019-nCoV (Cat # VG40589-UT, VG40589-ACG) and human ACE2 receptor (Cat # HG10108-UT, HG10108-ACR) were purchased from Sino Biological (Wayne, PA, USA). The mammalian luciferase reporter plasmids (pCAGT7 Pol and pT7EMCLuc) used in the cell-to-cell fusion assay were a gift from Professor Shukla's lab (University of Illinois at Chicago, USA). Luciferase-based pseudovirus entry studies were carried out using D614G variant of SARS-CoV-2 expressing spike glycoprotein on a pseudotyped lentivirus (Cat # SP003-100), which was purchased from Genecopoeia (Rockville, MD, USA). The imaging studies used a green fluorescent protein (GFP)-based baculovirus pseudotyped with a SARS-CoV-2 spike glycoprotein expressing D614G mutation (Cat# C1122G). This virus was purchased from Montana Molecular (Bozeman, Montana, USA).

3.3 | SARS-CoV-2 spike glycoprotein-pseudotyped lentivirus-based entry using luciferase reporter gene and GFP-tagged baculovirus pseudotyped with an SARS-CoV-2 spike glycoprotein for imaging analysis

HEK293T-ACE2 cells were grown to 70%–80% confluence in a 96-well plate in 150 µl DMEM supplemented with 10% heat-inactivated FBS. The following day, the volume equivalent of 2×10^8 RLU/ml pseudovirus per well was treated for 5 min with dilutions of each OR and/or mock treated with serum-free media. After treatment, the virus and OR preparations were diluted to 100 µl with complete media and were added to the existing 150 µl already present on the cells. The plate was incubated for 2 h at 4°C to enhance virus binding before being moved to 37°C for 48 h. After 48 h, the media was removed and 30 µl/well of reporter lysis buffer was added. The cells were scraped and freeze-thawed at −80°C to complete cell lysis. 20 µl of lysate was transferred to a white 96-well plate, 50 µl of luciferase assay reagent (Promega, Madison, WI, USA) was added, and luciferase activity was recorded using the EnSpire Multimode Plate Reader (PerkinElmer, Waltham, MA, USA) at a speed of 0.5 s per well. Luciferase based viral entry assay was performed in triplicate experiment with an *N* of at least 3 for each independent replicate. To visualize the direct effect of ORs on SARS-CoV-2 cell infectivity, HEK-293T-ACE2 cells were seeded on square cover glass in a 12-well plate to reach 40% confluence overnight. The following day, 2×10^8 VG/ml GFP-tagged baculovirus pseudotyped with an SARS-CoV-2 was treated with 50% dilutions of each OR and/or mock-treated with serum-free media for 5 min. Following treatment, the virus and OR were diluted in complete media containing 2 mM sodium butyrate and were added to the cells. After 24 h, wells were gently washed with phosphate buffer saline (PBS), and cells were fixed in 4% paraformaldehyde for 20 min. Following fixation, cells were washed 3× with PBS and were permeabilized with 0.2% triton x-100 in PBS for 5 min and washed an additional 3× in PBS. Cells were incubated with phalloidin (ThermoFisher, Waltham, MA, USA) 1:40 in blocking buffer for 30 min in a humidity chamber before washing 3× in PBS and a final wash with sterile water. The cover glass was mounted to slides using hardset mounting media without DAPI (Vector Laboratories, Burlingame, CA, USA). Slides were imaged using a Nikon A1R confocal microscope and images were processed using ImageJ (version 1.52a, NIH). Confocal imaging data were generated using triplicate experiments.

3.4 | SARS-CoV-2 spike glycoprotein-mediated cell-to-cell fusion assay

CHO-K1 cells were grown overnight on a 6-well plate for transient transfection the following day. After cells reached 80% confluence, effector cells were co-transfected with 2 µg of SARS-CoV-2 spike glycoprotein and 500 ng T7 RNA polymerase, and target cells were co-transfected with 2 µg ACE2 receptor and 500 ng luciferase using lipofectamine 2000 (Invitrogen, Waltham, MA, USA). As a negative control, a set of target wells were co-transfected with 2 µg empty vector pcDNA3.1 and 500 ng luciferase reporter to account for background fusion. Mock (PBS)-treated effector and the target cells that were cultured in serum-free media in the absence of CloSYS and/or OraCare were used as a positive control. Twenty-four hours post-transfection, the effector cells were lifted using trypsin and were centrifuged at 1500 rpm at 4°C for 10 min. The supernatant was removed, and the pelleted cells were resuspended in CloSYS, OraCare, or mock (PBS)-treated dilutions for 5 min. Following incubation, cells were pelleted again and resuspended in complete media. Target cells were lifted and combined in equal volumes with effector cells in a 24-well plate to allow fusion to occur. After 24 h, overnight media was removed, cells were washed with PBS and 60 µl/well of reporter lysis buffer (Promega, Madison, WI, USA) was added. The cells were scraped and freeze-thawed at −80°C to complete cell lysis. Next, 20 µl of cell lysate was combined with 50 µl luciferase assay reagent (Promega, Madison, WI, USA) in a clear culture tube and the luciferase signal was recorded using an FB12 single tube luminometer (Berthold, Bad Wildbad, Germany). Cell-fusion experiments were performed in triplicate experiments with an *N* of 9 for each independent replicate. To visualize the SARS-CoV-2 spike glycoprotein-mediated cell-to-cell fusion assay, GFP-tagged spike glycoprotein and RFP-tagged ACE-2 fluorescent constructs were used. Effector and target CHO-K1 cells were transfected, and the populations were combined as described above. After 24 h post mixing, the cells were fixed with 4% paraformaldehyde, washed 3x with PBS, and mounted to slides using hardset mounting media without DAPI (Vector Laboratories, Burlingame, CA, USA). The slides were then imaged using the Nikon A1R confocal microscope. The imaging data generated using transient transfection with SARS-CoV-2 spike glycoprotein and human ACE-2 receptor were derived from triplicate experiments.

3.5 | Expression of SARS-CoV-2 spike glycoprotein and ACE-2 receptor using Cell-ELISA

CHO-K1 and HEK-293T-ACE2 cells were grown in 96-well plates to reach 80% confluence overnight. The following day, CHO-K1 cells were separately transfected with 100 ng per well of SARS-CoV-2 spike glycoprotein, ACE2 receptor, or pCDNA3.1. After 24 h, the cells were washed with PBS and fixed with methanol for 5 min. The wells were washed 3× with tris buffered saline and blocked for 1 h with protein-free blocking buffer (ThermoFisher, Waltham, MA, USA). Cells were incubated with rabbit polyclonal antibodies against spike glycoprotein and ACE2 (Cat # TA890227, TA306149, OriGene Technologies, Rockville, MD, USA) diluted in blocking buffer for 2 h at room temperature. Plates were washed 3× with wash buffer (0.05% tween 20 in tris buffered saline) and incubated with 0.1 µg/ml peroxidase-conjugated goat anti-rabbit IgG (Cat #TA140003, OriGene Technologies, Rockville, MD, USA) diluted in wash buffer for 1 h at room temperature. Plates were washed an additional 3x in wash buffer before adding 50 µl 1-step ultra TMB substrate (ThermoFisher, Waltham, MA, USA) and absorbance was measured at 450 nm using the Multiskan FC

microplate photometer (ThermoFisher, Waltham, MA, USA). Cell-ELISA experiments were performed in triplicate with an *N* of 8 for each independent replicate.

3.6 | Lactate dehydrogenase cytotoxicity assay

Cell toxicity of the ORs was assessed using an lactate dehydrogenase (LDH) cytotoxicity assay kit (Pierce Biotechnology, Rockford, IL, USA) per the manufacturer's recommendations. CHO-K1 and HEK-293T-ACE2 cells were plated at 15,000 cells/well and grown overnight in complete media. To assess the toxicity of OR present in the cell-fusion experiment, cells were washed with PBS and subjected to a dosage-dependent treatment with CloSYS, OraCare, or mock (PBS)-treatment for 15 min at room temperature. To account for residual OR left behind during pseudovirus treatment, a separate experiment was performed in which HEK-293T-ACE2 cells were treated with the final diluted concentrations (11.4%–1.425%) of OR present during the infection for 48 h. Maximum LDH release (+) was measured by lysing a set of control wells, while spontaneous LDH release (–) was measured through mock (PBS) treatment. Following incubation, 50 µl supernatant was combined with 50 µl reaction mixture, and the plate was incubated at room temperature for 30 min. The LDH activity was determined by measuring the absorbance at 492 and 620 nm on a microplate photometer and subtracting the 620 nm values from the 492 nm values to remove background. To calculate percentage cytotoxicity, the following formula was used; (OR treated LDH activity − Spontaneous LDH activity)/(Maximum LDH activity − Spontaneous LDH activity) × 100%. The cell-fusion cytotoxicity experiments were performed in duplicate with an *N* of 4 for each cell type tested, while SARS-CoV-2 pseudovirus cytotoxicity experiments were performed in duplicate with an *N* of 8 in HEK-293T-ACE2 cells.

3.7 | Statistical analysis

GraphPad Prism 9 was used to analyze the data. A one-way analysis of variance (ANOVA) was used to determine the significance between the control and the experimental groups followed by Bonferroni's multiple comparisons test to determine significance compared with the positive control. In all figures, columns represent the mean of the data collected, and error bars represent SD. (****) signifies a *p*-value of <.0001.

4 | RESULTS

4.1 | Preincubation of SARS-CoV-2 with ORs inhibits viral entry in HEK293T-ACE2 cells

First, we tested the impact of the two selected ORs against SARS-CoV-2 cell entry using HEK293T-ACE-2 cells, which overexpress the human ACE2 receptor. Considering the evidence that spike glycoprotein of SARS-CoV-2 interacts with the human ACE-2 receptor to gain access to the cells, we took the advantage by using a luciferase-based reporter SARS-CoV-2 pseudovirus, which upon entry, generates a positive luciferase signal. In this experiment, the pseudo-SARS-CoV-2 virus was pre-incubated with each OR separately for 5 min in a dosage-dependent manner (100%–12.5%) before transducing the HEK293T-ACE2 cells. In parallel, the HEK293T-ACE2 cells transduced in the absence of ORs were used as a positive control, while the mock (PBS) infected HEK293T-ACE2 cells served as a negative control. As shown in Figure 1a,b, a dosage-dependent effect of both the ORs was observed in inhibiting SARS-CoV-2 cell entry.

4.2 | Imaging studies confirm the anti-SARS-CoV-2 activity of ORs

We next verified the anti-SARS-CoV-2 activity of ORs by visualizing the target cells transduced with the virus in the presence and absence of ORs under confocal imaging. In this experiment, the GFP-tagged reporter pseudo-SARS-CoV-2 virus was pre-incubated with the ORs for 5 min before challenging onto the HEK293T-ACE2 cells. In parallel, the HEK293T-ACE2 cells transduced with the GFP pseudovirus in the absence of ORs were used as a positive control, while the mock (PBS) infected HEK293T-ACE2 cells were considered as a negative control. Forty-eight hours post-transduction, the cells were washed, fixed, and stained for actin cytoskeleton using red phalloidin before imaging on confocal microscopy. As shown in Figure 2a, the cells challenged with GFP-tagged reporter SARS-CoV-2 pseudovirus in the absence of ORs had multiple green punctate spots throughout the cells confirming viral entry, while no green punctate spots were visible in the mock (PBS) challenged cells (Figure 2b). In contrast to the positive control, the HEK293T-ACE2 cells transduced with the pseudovirus pre-incubated with either CloSYS or OraCare had a significantly weaker GFP signal (Figure 2c,d). Taken together, the above data combined with quantitative viral entry data suggests that the ORs showed significant blockage in SARS-CoV-2 cell entry.

4.3 | Preincubation of effector cells expressing SARS-CoV-2 spike glycoprotein with ORs inhibits cell-to-cell fusion to the target cells expressing human ACE2 receptor

Since the antiviral effect of ORs was evident using pseudovirus viral entry assay, we rationalized to further test if ORs were able to impair virus-cell fusion. In this regard, we developed a luciferase-based, spike glycoprotein mediated, cell-to-cell fusion assay using CHO-K1 cells. CHO-K1 cells were selected for our cell fusion experiment because they lack endogenous cell surface receptors for Coronaviruses in general, including SARS-CoV-2, and are therefore resistant to infection and cell fusion (O'Donnell et al., 2020). In this assay, the effector CHO-K1 cells were generated by co-transfecting the

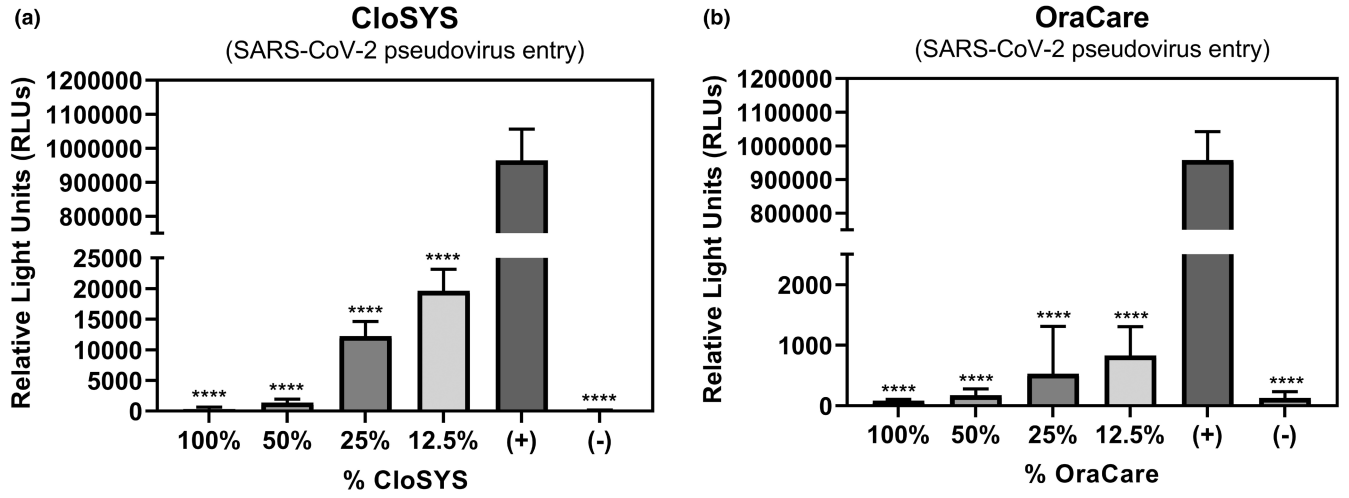

FIGURE 1 Effect of oral rinses (ORs) on SARS-CoV-2 cell entry using quantitative luciferase reporter-based pseudovirus assay. In this experiment, the SARS-CoV-2 pseudovirus was pre-incubated with each OR for 5 min and then transduced to HEK293T-ACE2 cells for 48 h at 37°C. The dosage-dependent treatment was performed in triplicate experiment using 100%–12.5% concentrations of either CloSYS (a) or OraCare (b). The HEK293T-ACE2 cells transduced with the untreated SARS-CoV-2 pseudovirus were considered as a positive control (+), while the un-transduced HEK293T-ACE2 cells were considered as a negative control (–). Asterisks (****) indicate a significant difference between the positive control and the cells infected with OR-treated pseudovirus ($p < .0001$)

SARS-CoV-2 spike glycoprotein along with the T7 RNA polymerase plasmids. In parallel, the target CHO-K1 cells were generated by co-transfecting CHO-K1 cells with the human ACE2 receptor plasmid with the firefly luciferase plasmid under the control of a T7 promoter. As shown in Figure 3, co-culturing of the effector cell together with the target cells resulted in cell-to-cell fusion, mimicking the virus-host cell membrane fusion. In contrast, the effector CHO-K1 cells co-cultured with the target CHO-K1 cell expressing an empty vector pCDNA3.1 in place of human ACE2 receptor had minimal cell-to-cell fusion, implicating no virus–cell interaction. Readouts from the cell-to-cell fusion assay are collected as relative light units (RLUs). When effector cells co-expressing spike glycoprotein/T7 RNA Polymerase fuse with target cells co-expressing ACE2/luciferase gene under the control of the T7 promoter, the luciferase enzyme is produced and catalyzes the reaction and produces light when the substrate is added (Tiwari, Tandon, et al., 2020). The effector CHO-K1 cells expressing SARS-CoV-2 spike glycoprotein pre-incubated with ORs for 5 min before co-culturing with the target CHO-K1 cells expressing human ACE2 receptor clearly showed a dosage-dependent inhibition in virus-cell fusion with both the CloSYS (Figure 3a) and the OraCare (Figure 3b). We also verified the expression of the viral ligand (SARS-CoV-2 spike glycoprotein) and the host cell receptor (human ACE-2) in our CHO-K1 cell-to-cell fusion assay using direct confocal imaging. Briefly, CHO-K1 cells cultured in 6-well plates were separately transfected with the mammalian expression plasmid encoding GFP-tagged spike glycoprotein and/or with the RFP-tagged human ACE2 receptor. As shown in the confocal imaging analysis data, both the effector (GFP positive; Figure 3c; panel i) and the target (RFP positive; Figure 3c; panel ii) cells demonstrated the expression of respective fluorescent proteins. In addition, upon mixing of the GFP-effector and the RFP-target, the co-cultured cells showed cell-to-cell fusion as evident from the positive co-localization signal and the formation of the multinucleated giant cell (Figure 3c, panel iii). We also verified the cell surface expression of SARS-CoV-2 spike glycoprotein and the ACE-2 receptor using quantitative cell-ELISA. As shown in the Supporting Information Figure S1, the transient transfection of SARS-CoV-2 spike glycoprotein and human ACE-2 receptor confirmed the cell surface expression of the target genes. In parallel, the CHO-K1 cells transfected with pcDNA3.1 along with the HEK-293T cells, which do not express ACE-2 receptor, were used as a negative control. The imaging analysis together with the viral entry and cell-to-cell fusion data clearly demonstrates that the ORs negatively impact SARS-CoV-2 entry.

4.4 | Quantification of cytotoxicity of the ORs using LDH assay

Since the blockage of SARS-CoV-2 cell entry in HEK293T-ACE2 and virus-cell fusion assay in CHO-K1 cells could also be due to the potential toxicity associated with the ORs, we next evaluated the potential side effect of the ORs by measuring the release of LDH—an enzyme, which is an indicator of cellular toxicity (Okui et al., 2021). In this experiment, we tested the impact of both the ORs separately on CHO-K1 and HEK293T-ACE2 cells using multiple dosages and the two critical time points (15 min and 48 h). Cytotoxicity was apparent in a dosage-dependent manner in both cell lines, but this toxicity occurred mostly in the higher concentrations and at a far lower level than the maximum LDH release positive control. In HEK293T-ACE2 cells, CloSYS (Figure 4a) and OralCare (Figure 4d) were found to have 25% and 24% associated cytotoxicity at the 100% OR concentration. The toxicity levels in subsequent dilutions were in range associated with spontaneous LDH release. In CHO-K1 cells, CloSYS (Figure 4b) was found to have a range of

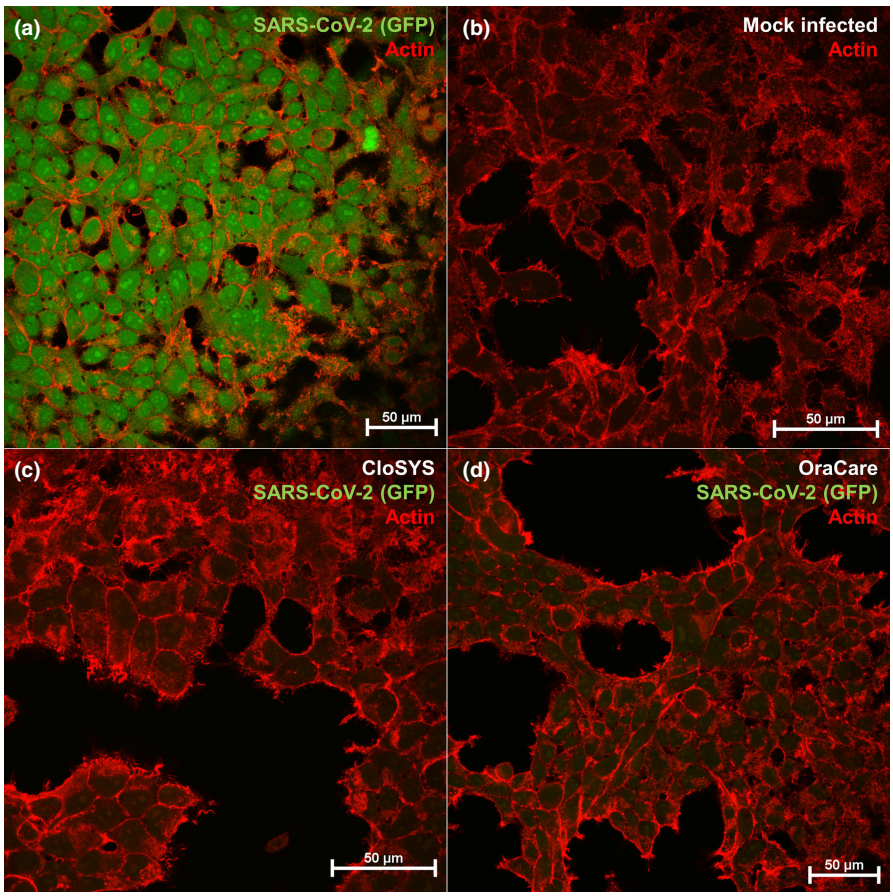

FIGURE 2 Confocal imaging of HEK293T-ACE2 cells transduced with GFP expressing SARS-CoV-2 pseudovirus in the presence and absence of the ORs. In this experiment, GFP-tagged SARS-CoV-2 pseudovirus was pretreated for 5 min with 50% dilution of ORs or mock (PBS)-treated at room temperature before introducing the cocktail to the cultured HEK293T-ACE2 cells. Shown are the representative images of GFP positive control cells transduced with SARS-CoV-2 in the absence of ORs (a), the mock un-transduced GFP-negative control cells (b), and the HEK293T-ACE2 cells transduced with green fluorescent protein (GFP)-tagged baculovirus pseudotyped SARS-CoV-2, which was pretreated with ORs (CloSYS; [c] and or with OraCare; [d]; at 50% dilution). The fixed HEK293T-ACE2 cells were finally stained with red-phalloidin for cell background. The SARS-CoV-2 pseudovirus generated a sharp green punctate signal was an indicator for successful viral entry into target cells (a). Confocal imaging was performed in triplicate experiment using Nikon A1R confocal microscope

9.5%–2.8% cytotoxicity at all concentrations tested. While in the case of OraCare (Figure 4e), this range was from 14% to 2.8% at all concentrations tested. Our results indicate that, in CHO-K1 cells, the OR treatment had slight toxicity at the tested concentrations. In contrast, in HEK293T-ACE2 cells the toxic effect of ORs was only observed at the undiluted concentrations, suggesting the effect of ORs could be cell-type-dependent. In addition, LDH release was measured for concentrations of OR present (11.4% to 1.425%) during the 48-hour infection utilized in the viral entry assay in HEK-293T-ACE2 cells (Figure 4c,f). At these concentrations, levels of LDH released were consistent with the spontaneous LDH release of untreated cells. Taken together, the concentrations of the ORs used in the SARS-CoV-2 entry and/or spike glycoprotein-mediated cell-to-cell fusion assays were nontoxic to cells. Although we did not test the effect of ORs on the cellular toxicity associated with the cells of the oral cavity, a previous study has shown the non-toxic nature of chlorine dioxide in ORs in human gingival fibroblasts (Parra-Lucares et al., 2022).

5 | DISCUSSION

SARS-CoV-2 constantly possesses a significant challenge in the clinic especially when providing dental care (Huang et al., 2021; O'Donnell et al., 2020), since ACE-2 receptor expressing oral cavity represents a robust port of entry site for SARS-CoV-2 infection (Huang et al., 2021). In fact, COVID-19 inflamed human gingival biopsies and tongue cells have shown an elevated levels of ACE-2 receptor expression (Altaie et al., 2021; Imai & Tanaka, 2021; Naqvi et al., 2021; Xu et al., 2020). Further, the recent emergence of SARS-CoV-2 variants with higher cell infectivity has also raised significant concern for the future rebounds of severe epidemics (Cosar et al., 2022; Gao et al., 2022; Hu et al., 2021; Parra-Lucares et al., 2022). Therefore, the preprocedural usage of ORs with proven anti-SARS-CoV-2 activity could be an effective strategy to reduce virus transmission from the oral route in clinical dental settings. To assess the benefits of ORs against D614G variant of SARS-CoV-2, we selected two types of ORs, which have similar active ingredients either in the form of

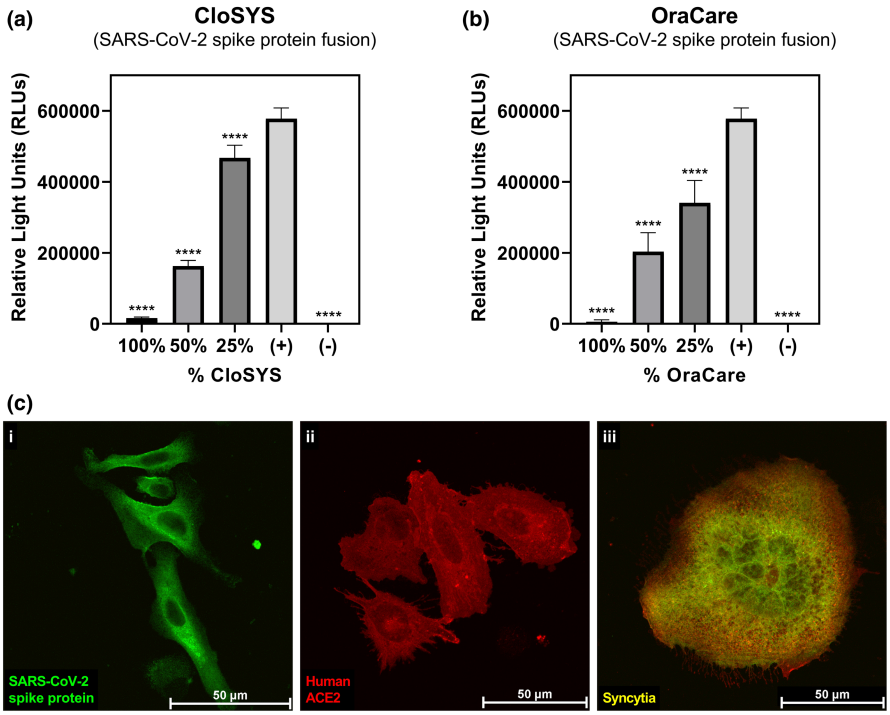

FIGURE 3 (a,b) Quantification of luciferase signal generated after co-culturing the SARS-CoV-2 spike glycoprotein expressing effector cell with the target cells expressing the human ACE-2 receptor in the presence and absence of the ORs. In this experiment, effector CHO-K1 cells co-transfected with SARS-CoV-2 spike glycoprotein with T7 RNA polymerase. In parallel, target CHO-K1 cells were co-transfected with human ACE2 expression plasmid and luciferase gene. Both the effector and target cell were mixed and co-cultured for additional 24 h before measuring the luciferase activity. Effector cells were pre-incubated with the CloSYS (a) or OralCare (b) in a dosage-dependent manner before mixing the effector cells with the target cells. Asterisks (****) indicate a significant difference between the positive control and the ORs treated cells ($p < .0001$). (c) Expression of SARS-CoV-2 spike glycoprotein, human ACE-2 receptor, in the effector and target cell respectively. The post-co-culture event demonstrating the co-localization and the formation of multinucleated giant cell (syncytia) using confocal imaging. In this experiment, CHO-K1 effector and target cells independently transfected with GFP-tagged SARS-CoV-2 (panel i), and or RFP-tagged human ACE-2 receptor (panel ii) respectively. A GFP-positive spike glycoprotein expressing effector cell co-cultured with RFP-positive ACE-2 expressing target cells imaged 24 h post-mixing shows co-localization and the formation of multinucleated giant cell (syncytia; panel iii). Confocal microscopy was performed in triplicate using Nikon A1R confocal microscope

stabilized chlorine dioxide, and or as a freshly prepared chlorine dioxide (Unflavored, Ultrasensitive CloSYS and OraCare, respectively). Chlorine dioxide is often used in the treatment of water and wastewater due to its virucidal effect as it causes disruption of viral glycoproteins including viral genome (Ge et al., 2021). It is also used as an alternative disinfectant to chlorine and has been shown to be effective against both enveloped and non-enveloped viruses (Ge et al., 2021; Totaro et al., 2021). However, limited studies have evaluated the effect of chlorine dioxide as an option to decrease the viral titers in the oral cavity. The formulation of the OraCare rinse takes advantage of a two-bottle system, which, when mixed, will cause the chlorine dioxide gas to be released to work as an oxidizer in the mouth. While the CloSYS OR utilizes a one-bottle system that contains stabilized chlorine dioxide. Both CloSYS and OraCare utilize the same base compound, 0.1% sodium chlorite to obtain chlorine dioxide, but the difference is the containment of the chlorine dioxide. To assess if the stabilized or freshly prepared chlorine-dioxide-based ORs would have the same effect against SARS-CoV-2 entry, both ORs were investigated. These ORs were selected because they are regularly used in the dental clinic (Drake & Villhauer, 2011).

Our study found the ORs have anti-SARS-CoV-2 activity as evident from the results generated from two independent assays determining viral entry and virus-host cell membrane fusion (Figures 1 and 3a,b). Although we tested the impact of ORs by pre-incubating with the pseudovirus and/or an effector cell expressing spike glycoprotein, we cannot ignore the possibility that ORs may have acted at multiple other steps in preventing SARS-CoV-2 infections. For instance, it is possible that ORs may have disrupted the viral lipid envelop, dismantling spike glycoprotein. In fact, the formulations in the commercially available ORs such as ethanol, chlorhexidine, cetylpyridinium chloride, hydrogen peroxide, and povidone-iodine are known to disrupt the SARS-CoV-2 lipid envelope, based on their usage concentrations (O'Donnell et al., 2020). Similarly, previous studies have performed virus neutralization assays by exposing ORs to cells that contained the virus for a set amount of time. An end-point dilution was used to determine the amount of active virus remained in the sample (Bidra et al., 2020; Carrouel et al., 2021; Tadakamadla et al., 2021).

The data generated from the SARS-CoV-2 spike glycoprotein-mediated cell-to-cell fusion assay suggest that ORs possibly act on

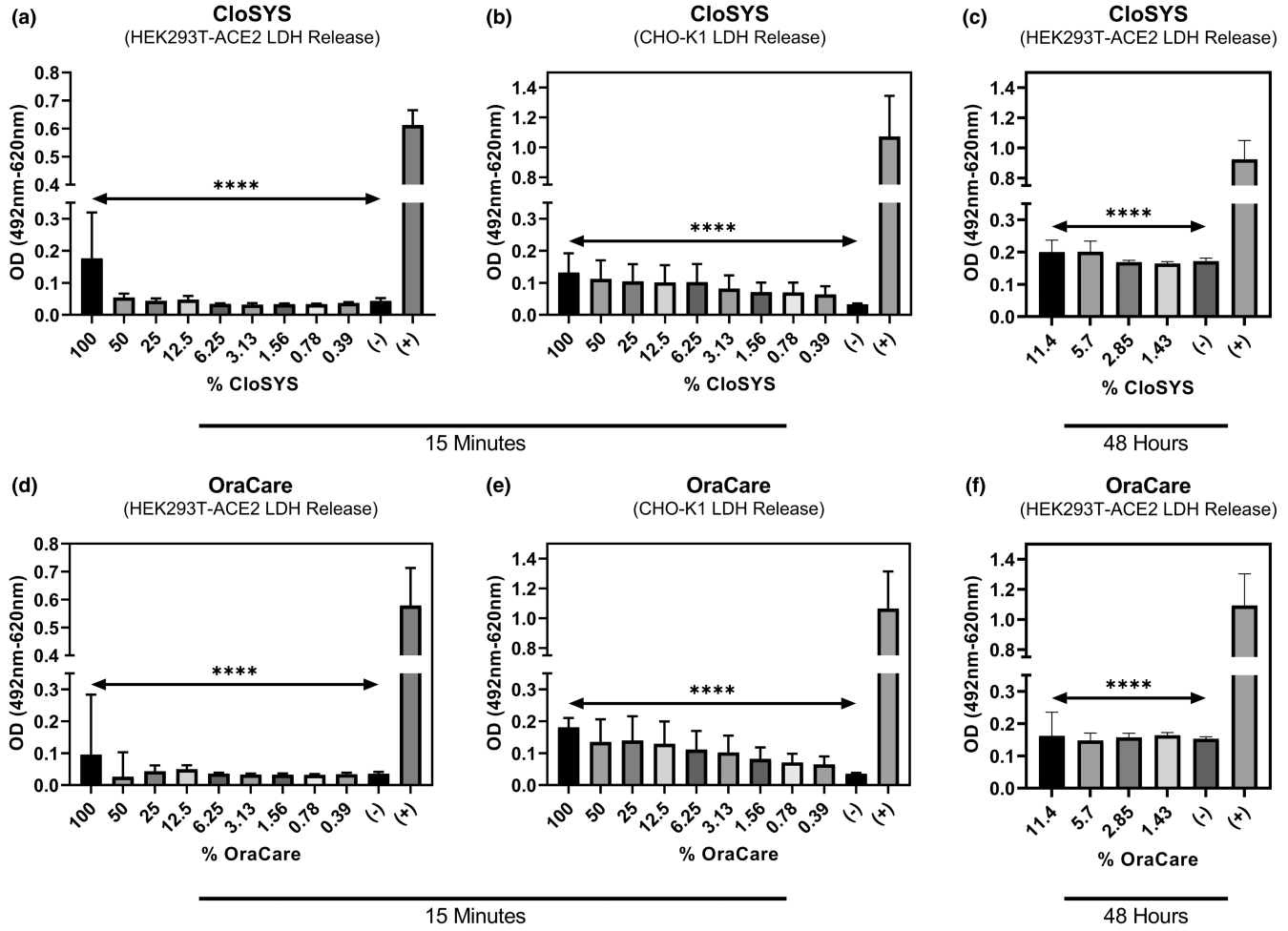

FIGURE 4 The effect of commercial ORs on cellular toxicity using LDH assay. The monolayers from HEK293T-ACE-2 and CHO-K1 cells were incubated in the presence or absence of ORs. Cytotoxicity was determined in the cells, which were pre-incubated with ORs at indicated dilutions ranging from 100% to 0.39% for 15 min (a–b and d–e), and 11.4% to 1.43% for 48 h (c and f) to mimic the conditions used during SARS-CoV-2 entry and cell-to-cell fusion assays. The cells treated with media alone represent the spontaneous LDH release as a negative control, while the cells lysed with detergent represent maximum LDH release as a positive control. Asterisks (****) indicate a significant difference between the maximum LDH release and the OR treated cells ($p < .0001$)

the virus-host cell membrane fusion, which involves interactions between the spike glycoprotein and the membrane-associated ACE-2 receptor. Since multiple co-receptors have been proposed in SARS-CoV-2 cell entry (Gadanec et al., 2021; Sakaguchi et al., 2020), it will be interesting to evaluate if anti-SARS-CoV-2 activity of ORs is receptor-specific or is applicable against multiple other cell receptor or co-receptors (Gadanec et al., 2021; Tiwari, Tandon, et al., 2020). Further, we also noticed the impact of ORs on syncytium formation; however, additional studies are needed establish the kinetics especially with the cells of the oral epithelium. In addition, our study was performed using ORs against the Delta (D614G) variant of SARS-CoV-2, therefore future additional studies are needed to evaluate if CloSYS and OraCare are equally effectively against the newly emerging SARS-CoV-2 variants such as Omicron and or Omicron BA.2 (Miller et al., 2022; Parra-Lucares et al., 2022; Tao et al., 2021).

Interestingly, the other benefit reported for OraCare and CloSYS is their oxidizing ability to generate potent antimicrobial substances, which target protein synthesis, alter microorganism's metabolic pathway, and/or destabilize the structural components of cell membranes (Drake & Villhauer, 2011; Eggers et al., 2018; Grootveld et al., 2001). Further, in the case of chlorine dioxide, it has been shown to have bactericidal activity against many oral pathogens (Drake & Villhauer, 2011). One related aspect that is not very clear is the modulating ability of SARS-CoV-2 in the oral microbiome. The later event may favor the opportunistic infections especially in the patients with the compromised oral health (Rhoades et al., 2021). In this regard, developing a unique broad-spectrum OR, which preferentially targets multiple pathogens in the oral cavity such as herpes simplex virus, human immunodeficiency virus, *Candida* species including plaque reduction, can be of high demand especially among high-risk population (Baqui et al., 2001; Nicolatou-Galitis et al., 2001). Similarly, the transient usage of such ORs may also have an additional benefit in reducing the potential risk associated with the aspiration pneumonia and/or the associated bloodstream

infections (Imai & Tanaka, 2021; Lugo-Flores et al., 2021; Ramos et al., 2020).

Overall, our results, obtained using highly sensitive molecular assays, show promising activity of ORs against SARS-CoV-2 cell entry and spike glycoprotein-mediated cell-to-cell fusion. These results support the usage of preprocedural ORs as a preventative strategy against SARS-CoV-2. Moreover, our findings are in line with recent reports suggesting the potential protective role of ORs against SARS-CoV-2 (O'Donnell et al., 2020). Finally, an innovative and logical aspect in oral care is to prioritize and commercialize plant-derived ORs (Lugo-Flores et al., 2021) as opposed to be relying entirely on the synthetic products, which have multiple side effects (Bhat et al., 2014; Gagari & Kabani, 1995; Goldstep, 2014). In this direction new formulations can also be developed by infusing ORs with the known natural compounds having potent broad-spectrum antimicrobial and anti-inflammatory activities (Tiwari et al., 2009, 2010).

6 | CONCLUSIONS

The tested chlorine-dioxide-based ORs significantly impacted SARS-CoV-2 infectivity by blocking viral entry and SARS-CoV-2 spike glycoprotein-mediated cell-to-cell fusion. Our results suggest that the preprocedural rinsing either with OraCare or with CloSYS during the COVID-19 pandemic may be beneficial for reducing SARS-CoV-2 infectivity and potential cell-to-cell spread. Future studies are needed to understand if the downstream signaling associated with the anti-SARS-CoV-2 activity of ORs may also have a host protective effect using transgenic mice expressing human ACE2 (K18-hACE2).

AUTHOR CONTRIBUTIONS

Briana Joy Travis: Formal analysis; funding acquisition; investigation; methodology; writing – original draft; writing – review and editing. **James Elste:** Data curation; formal analysis; investigation; writing – review and editing. **Feng Gao:** Data curation; formal analysis; investigation; methodology; supervision; writing – review and editing. **Bo Young Joo:** Data curation; formal analysis; investigation; writing – review and editing. **Maria Cuevas-Nunez:** Conceptualization; methodology; project administration; supervision; writing – review and editing. **Ellen Kohlmeir:** Data curation; investigation; visualization. **Vaibhav Tiwari:** Conceptualization; funding acquisition; methodology; project administration; supervision; writing – review and editing. **John C Mitchell:** Conceptualization; investigation; methodology; project administration; resources; supervision; writing – review and editing.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

PEER REVIEW

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REFERENCES

Altaie, A. M., Hamdy, R., Venkatachalam, T., Hamoudi, R., & Soliman, S. S. M. (2021). Estimating the viral loads of SARS-CoV-2 in the oral cavity when complicated with periapical lesions. *BMC Oral Health*, *21*, 567. https://doi.org/10.1186/s12903-021-01921-5

Anjum A, Hosein M, Butt SA, Fakhuruddin, Fawad B, & Abidi, F. (2019). Efficacy of two mouth rinses in reducing aerosol bacterial load during ultrasonic scaling. *Journal of Advances in Medicine and Medical Research*, *31*, 1–9. https://doi.org/10.9734/jammr/2019/v31i730311

Bao, L., Deng, W., Huang, B., Gao, H., Liu, J., Ren, L., Wei, Q., Yu, P., Xu, Y., Qi, F., Qu, Y., Li, F., Lv, Q., Wang, W., Xue, J., Gong, S., Liu, M., Wang, G., Wang, S., … Qin, C. (2020). The pathogenicity of SARS-CoV-2 in hACE2 transgenic mice. *Nature*, *583*, 830–833.

Baqui, A. A. M. A., Jabra-Rizk, M. A., DePaola, L. G., Falkler, W. A., & Meiller, T. F. (2001). In vitro effect of oral antiseptics on human immunodeficiency virus-1 and herpes simplex virus type 1. *Journal of Clinical Periodontology*, *28*, 610–616.

Bhat, N., Mitra, R., Oza, S., Mantu, V. K., Bishnoi, S., Gohil, M., & Gupta, R. (2014). The antiplaque effect of herbal mouthwash in comparison to chlorhexidine in human gingival disease: A randomized placebo controlled clinical trial. *Journal of Complementary and Integrative Medicine*, *11*, 11.

Bidra, A. S., Pelletier, J. S., Westover, J. B., Frank, S., Brown, S. M., & Tessema, B. (2020). Comparison of in vitro inactivation of SARS CoV-2 with hydrogen peroxide and povidone-iodine oral antiseptic rinses. *Journal of Prosthodontics*, *29*, 599–603.

Burgueno, J. F., Reich, A., Hazime, H., Quintero, M. A., Fernandez, I., Fritsch, J., Santander, A. M., Brito, N., Damas, O. M., Deshpande, A., & Kerman, D. H. (2020). Expression of SARS-CoV-2 entry molecules ACE2 and TMPRSS2 in the gut of patients with IBD. *Inflammatory Bowel Diseases*, *26*, 797–808.

Carrouel, F., Gonçalves, L. S., Conte, M. P., Campus, G., Fisher, J., Fraticelli, L., Gadea-Deschamps, E., Ottolenghi, L., & Bourgeois, D. (2021). Antiviral activity of reagents in mouth rinses against SARS-CoV-2. *Journal of Dental Research*, *100*, 124–132.

Clausen, T. M., Sandoval, D. R., Spliid, C. B., Pihl, J., Perrett, H. R., Painter, C. D., Narayanan, A., Majowicz, S. A., Kwong, E. M., McVicar, R. N., Thacker, B. E., Glass, C. A., Yang, Z., Torres, J. L., Golden, G. J., Bartels, P. L., Porell, R. N., Garretson, A. F., Laubach, L., … Esko, J. D. (2020). SARS-CoV-2 infection depends on cellular heparan sulfate and ACE2. *Cell*, *183*, 1043–1057.e15.

Cosar, B., Karagulleoglu, Z. Y., Unal, S., Ince, A. T., Uncuoglu, D. B., Tuncer, G., Kilinc, B. R., Ozkan, Y. E., Ozkoc, H. C., Demir, I. N., Eker, A., Karagoz, F., Simsek, S. Y., Yasar, B., Pala, M., Demir, A., Atak, I.

N., Mendi, A. H., Bengi, V. U., … Demir-Dora, D. (2022). SARS-CoV-2 mutations and their viral variants. *Cytokine and Growth Factor Reviews*, *63*, 10–22.

Drake, D., & Villhauer, A. L. (2011). An in vitro comparative study determining bactericidal activity of stabilized chlorine dioxide and other oral rinses. *Journal of Clinical Dentistry*, *22*, 1–5.

Drozdzik, A., & Drozdzik, M. (2022). Oral pathology in COVID-19 and SARS-CoV-2 infection—Molecular aspects. *International Journal of Molecular Sciences*, *23*, 1431.

Drucker, D. J. (2021). Diabetes, obesity, metabolism, and SARS-CoV-2 infection: the end of the beginning. *Cell Metabolism*, *33*, 479–498.

Eggers, M., Koburger-Janssen, T., Eickmann, M., & Zorn, J. (2018). In vitro bactericidal and virucidal efficacy of povidone-iodine gargle/mouthwash against respiratory and oral tract pathogens. *Infectious Diseases and Therapy*, *7*, 249–259.

Gadanec, L. K., McSweeney, K. R., Qaradakhi, T., Ali, B., Zulli, A., & Apostolopoulos, V. (2021). Can SARS-CoV-2 virus use multiple receptors to enter host cells? *International Journal of Molecular Sciences*, *22*, 992. https://doi.org/10.3390/ijms22030992

Gagari, E., & Kabani, S. (1995). Adverse effects of mouthwash use. A review. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics*, *80*, 432–439. https://doi.org/10.1016/s1079-2104(05)80337-3

Gao, S. J., Guo, H., & Luo, G. (2022). Omicron variant (B.1.1.529) of SARS-CoV-2, a global urgent public health alert! *Journal of Medical Virology*, *94*, 1255–1256.

Ge, Y., Zhang, X., Shu, L., & Yang, X. (2021). Kinetics and mechanisms of virus inactivation by chlorine dioxide in water treatment: A review. *Bulletin of Environmental Contamination and Toxicology*, *106*, 560–567.

Giudice R lo (2020). The severe acute respiratory syndrome coronavirus-2 (Sars cov-2) in dentistry. Management of biological risk in dental practice. *International Journal of Environmental Research and Public Health*, *17*, 3067. https://doi.org/10.3390/ijerph17093067

Goldstep, F. (2014). Oral rinses for a proactive intervention approach to periodontal health. *The Compendium of Continuing Education in Dentistry*, *35*, 546–553.

Grootveld, M., Silwood, C., Gill, D., & Lynch, E. (2001). Evidence for the microbicidal activity of a chlorine dioxide-containing oral rinse formulation in vivo. *Journal of Clinical Dentistry*, *12*, 67–70.

Harrison, A. G., Lin, T., & Wang, P. (2020). Mechanisms of SARS-CoV-2 transmission and pathogenesis. *Trends in Immunology*, *41*, 1100–1115.

Hu, B., Guo, H., Zhou, P., & Shi, Z. L. (2021). Characteristics of SARS-CoV-2 and COVID-19. *Nature Reviews Microbiology*, *19*, 141–154.

Huang, N., Pérez, P., Kato, T., Mikami, Y., Okuda, K., Gilmore, R. C., Conde, C. D., Gasmi, B., Stein, S., Beach, M., & Pelayo, E. (2021). SARS-CoV-2 infection of the oral cavity and saliva. *Nature Medicine*, *27*, 892–903.

Imai, K., & Tanaka, H. (2021). Sars-cov-2 infection and significance of oral health management in the era of "the new normal with COVID-19". *International Journal of Molecular Sciences*, *22*, 6527. https://doi.org/10.3390/ijms22126527

Koch-Heier, J., Hoffmann, H., Schindler, M., Lussi, A., & Planz, O. (2021). Inactivation of sars-cov-2 through treatment with the mouth rinsing solutions viruprox® and bacterx® pro. *Microorganisms*, *9*, 521. https://doi.org/10.3390/microorganisms9030521

Letko, M., Marzi, A., & Munster, V. (2020). Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses. *Nature Microbiology*, *5*, 562–569.

Lugo-Flores, M. A., Quintero-Cabello, K. P., Palafox-Rivera, P., Silva-Espinoza, B. A., Cruz-Valenzuela, M. R., Ortega-Ramirez, L. A., Gonzalez-Aguilar, G. A., & Ayala-Zavala, J. F. (2021). Plant-derived substances with antibacterial, antioxidant, and flavoring potential to formulate oral health care products. *Biomedicine*, *9*, 1669. https://doi.org/10.3390/biomedicines9111669

Meyerowitz, E. A., Richterman, A., Gandhi, R. T., & Sax, P. E. (2021). Transmission of sars-cov-2: A review of viral, host, and environmental factors. *Annals of Internal Medicine*, *174*, 69–79.

Miller, N. L., Clark, T., Raman, R., & Sasisekharan, R. (2022). Insights on the mutational landscape of the SARS-CoV-2 Omicron variant receptor-binding domain. *Cell Reports Medicine*, *3*, 100527.

Morawska, L., & Cao, J. (2020). Airborne transmission of SARS-CoV-2: The world should face the reality. *Environment International*, *139*, 105730.

Naqvi, A. R., Schwartz, J., Brandini, D. A., Schaller, S., Hussein, H., Valverde, A., Naqvi, R. A., & Shukla, D. (2021). COVID-19 and oral diseases: Assessing manifestations of a new pathogen in oral infections. *International Reviews of Immunology*, *41*, 423–437. https://doi.org/10.1080/08830185.2021.1967949

Nicolatou-Galitis, O., Dardoufas, K., Markoulatos, P., Sotiropoulou-Lontou, A., Kyprianou, K., Kolitsi, G., Pissakas, G., Skarleas, C., Kouloulias, V., Papanicolaou, V., Legakis, N. J., & Velegraki, A. (2001). Oral pseudomembranous candidiasis, herpes simplex virus-1 infection, and oral mucositis in head and neck cancer patients receiving radiotherapy and granulocyte-macrophage colony-stimulating factor (GM-CSF) mouthwash. *Journal of Oral Pathology and Medicine*, *30*, 471–480.

O'Donnell, V. B., Thomas, D., Stanton, R., Maillard, J. Y., Murphy, R. C., Jones, S. A., Humphreys, I., Wakelam, M. J., Fegan, C., Wise, M. P., & Bosch, A. (2020). Potential role of oral rinses targeting the viral lipid envelope in SARS-CoV-2 infection. *Function (Oxf)*, *1*, zqaa002. https://doi.org/10.1093/function/zqaa002

Okui, T., Matsuda, Y., Karino, M., Hideshima, K., & Kanno, T. (2021). Oral mucosa could be an infectious target of sars-cov-2. *Healthcare (Basel)*, *9*, 1068. https://doi.org/10.3390/healthcare9081068

Parra-Lucares, A., Segura, P., Rojas, V., Pumarino, C., Saint-Pierre, G., & Toro, L. (2022). Emergence of SARS-CoV-2 variants in the world: How could this happen? *Life*, *12*, 194.

Paull, J. R. A., Heery, G. P., Bobardt, M. D., Castellarnau, A., Luscombe, C. A., Fairley, J. K., & Gallay, P. A. (2021). Virucidal and antiviral activity of astodrimer sodium against SARS-CoV-2 in vitro. *Antiviral Research*, *191*, 105089.

Peng, J., Sun, J., Zhao, J., Deng, X., Guo, F., & Chen, L. (2021). Age and gender differences in ACE2 and TMPRSS2 expressions in oral epithelial cells. *Journal of Translational Medicine*, *19*, 358.

Peng, X., Xu, X., Li, Y., Cheng, L., Zhou, X., & Ren, B. (2020). Transmission routes of 2019-nCoV and controls in dental practice. *International Journal of Oral Science*, *12*, 9. https://doi.org/10.1038/s41368-020-0075-9

Ramos, K. J., Kapnadak, S. G., Collins, B. F., Pottinger, P. S., Wall, R., Mays, J. A., Perchetti, G. A., Jerome, K. R., Khot, S., Limaye, A. P., Mathias, P. C., & Greninger, A. (2020). Detection of SARS-CoV-2 by bronchoscopy after negative nasopharyngeal testing: Stay vigilant for COVID-19. *Respiratory Medicine Case Reports*, *30*, 101120.

Rhoades, N. S., Pinski, A. N., Monsibais, A. N., Jankeel, A., Doratt, B. M., Cinco, I. R., Ibraim, I., & Messaoudi, I. (2021). Acute SARS-CoV-2 infection is associated with an increased abundance of bacterial pathogens, including *Pseudomonas aeruginosa* in the nose. *Cell Reports*, *36*, 109637.

Sakaguchi, W., Kubota, N., Shimizu, T., Saruta, J., Fuchida, S., Kawata, A., Yamamoto, Y., Sugimoto, M., Yakeishi, M., & Tsukinoki, K. (2020). Existence of SARS-CoV-2 entry molecules in the oral cavity. *International Journal of Molecular Sciences*, *21*, 6000. https://doi.org/10.3390/ijms21176000

Sawa, Y., Ibaragi, S., Okui, T., Yamashita, J., Ikebe, T., & Harada, H. (2021). Expression of SARS-CoV-2 entry factors in human oral tissue. *Journal of Anatomy*, *238*, 1341–1354.

Shang, J., Wan, Y., Luo, C., Ye, G., Geng, Q., Auerbach, A., & Li, F. (2020). Cell entry mechanisms of SARS-CoV-2. *Proceedings of the*

National Academy of Sciences of the United States of America, *117*, 11727–11734.

Sigrist, C. J., Bridge, A., & le Mercier, P. (2020). A potential role for integrins in host cell entry by SARS-CoV-2. *Antiviral Research*, *177*, 104759.

Tadakamadla, J., Boccalari, E., Rathore, V., Dolci, C., Tartaglia, G. M., & Tadakamadla, S. K. (2021). In vitro studies evaluating the efficacy of mouth rinses on Sars-Cov-2: A systematic review. *Journal of Infection and Public Health*, *14*, 1179–1185.

Takeda, Y., Jamsransuren, D., Matsuda, S., Crea, R., & Ogawa, H. (2021). The sars-cov-2-inactivating activity of hydroxytyrosol-rich aqueous olive pulp extract (Hidrox®) and its use as a virucidal cream for topical application. *Viruses*, *13*, 232. https://doi.org/10.3390/v13020232

Tao, K., Tzou, P. L., Nouhin, J., Gupta, R. K., de Oliveira, T., Kosakovsky Pond, S. L., Fera, D., & Shafer, R. W. (2021). The biological and clinical significance of emerging SARS-CoV-2 variants. *Nature Reviews Genetics*, *22*, 757–773.

Tiwari, V., Beer, J. C., Sankaranarayanan, N. V., Swanson-Mungerson, M., & Desai, U. R. (2020). Discovering small-molecule therapeutics against SARS-CoV-2. *Drug Discovery Today*, *25*, 1535–1544.

Tiwari, V., Darmani, N. A., Yue, B. Y. J. T., & Shukla, D. (2010). In vitro antiviral activity of neem (*Azardirachta indica* L.) bark extract against herpes simplex virus type-1 infection. *Phytotherapy Research*, *24*, 1132–1140.

Tiwari, V., Shukla, S. Y., & Shukla, D. (2009). A sugar binding protein cyanovirin-N blocks herpes simplex virus type-1 entry and cell fusion. *Antiviral Research*, *84*, 67–75.

Tiwari V, Tandon R, Sankaranarayanan NV, Beer, J.C., Kohlmeir, E.K., Swanson-Mungerson, M., & Desai, U.R. (2020). Preferential recognition and antagonism of SARS-CoV-2 spike glycoprotein binding to 3-O-sulfated heparan sulfate. *bioRxiv*.

Totaro, M., Badalucco, F., Costa, A. L., Tuvo, B., Casini, B., Privitera, G., Menchini Fabris, G. B., & Baggiani, A. (2021). Effectiveness of disinfection with chlorine dioxide on respiratory transmitted, enteric, and bloodborne viruses: A narrative synthesis. *Pathogens*, *10*, 1017. https://doi.org/10.3390/pathogens10081017

V'kovski, P., Kratzel, A., Steiner, S., Stalder, H., & Thiel, V. (2021). Coronavirus biology and replication: Implications for SARS-CoV-2. *Nature Reviews Microbiology*, *19*, 155–170.

Volgenant, C. M. C., Persoon, I. F., de Ruijter, R. A. G., & de Soet, J. J. (2021). Infection control in dental health care during and after the SARS-CoV-2 outbreak. *Oral Diseases*, *27*, 674–683.

Wang, Q., Zhang, Y., Wu, L., Niu, S., Song, C., Zhang, Z., Lu, G., Qiao, C., Hu, Y., Yuen, K. Y., Wang, Q., Zhou, H., Yan, J., & Qi, J. (2020). Structural and functional basis of SARS-CoV-2 entry by using human ACE2. *Cell*, *181*, 894–904.e9.

Xu, H., Zhong, L., Deng, J., Peng, J., Dan, H., Zeng, X., Li, T., & Chen, Q. (2020). High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. *International Journal of Oral Science*, *12*, 8. https://doi.org/10.1038/s41368-020-0074-x

Yao, Y., Wang, H., & Liu, Z. (2020). Expression of ACE2 in airways: Implication for COVID-19 risk and disease management in patients with chronic inflammatory respiratory diseases. *Clinical and Experimental Allergy*, *50*, 1313–1324.

Zhong, M., Lin, B., Pathak, J. L., Gao, H., Young, A. J., Wang, X., Liu, C., Wu, K., Liu, M., Chen, J. M., Huang, J., Lee, L. H., Qi, C. L., Ge, L., & Wang, L. (2020). ACE2 and furin expressions in oral epithelial cells possibly facilitate COVID-19 infection via respiratory and fecal–oral routes. *Frontiers in Medicine (Lausanne)*, *7*, 580796. https://doi.org/10.3389/fmed.2020.580796

SUPPORTING INFORMATION

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