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## Scientific Research Report

## Effectiveness of Toothpastes on SARS-CoV-2 Viral Load in Saliva

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## ABSTRACT

**Introduction:** The effect of toothpastes on viruses, such as SARS-CoV-2, is unknown. This study investigated the short-term effect of toothpastes containing antimicrobial properties in patients with novel coronavirus disease 2019 (COVID-19) to determine whether they could reduce the SARS-CoV-2 salivary viral load.

**Methods:** Hospitalised patients with COVID-19 (n = 83) were instructed to perform toothbrushing with 1 of 3 arms: a toothpaste containing 0.96% zinc (zinc oxide, zinc citrate) in a silica base (Test 1), a toothpaste containing 0.454% SnF<sub>2</sub> in a silica base (Test 2), and a non-antibacterial toothpaste (control). Saliva was collected before intervention (T0), immediately after intervention (T1), and 30 (T2) and 60 minutes (T3) after intervention. The SARS-CoV-2 salivary viral load was measured using quantitative real-time polymerase chain reaction (qRT-PCR) assays. For Test 1 and Test 2 toothpastes, the fold reductions were normalised to baseline and to the control toothpaste at each time point after brushing. A fold change of  $\geq 2$  is considered clinically effective.

**Results:** Brushing with the Test 1 toothpaste reduced the SARS-CoV-2 salivary viral load by 4.06-fold at T1, by 2.36-fold at T2, and by 1.42-fold at T3. Similarly, brushing with a Test 2 toothpaste reduced the SARS-CoV-2 salivary viral load by 2.33-fold at T1, by 2.38-fold at T2, and by 0.77-fold at T3.

**Conclusions:** Immediately after brushing, the use of antimicrobial toothpastes reduced the salivary viral load of patients with COVID-19. The trial was registered on <https://clinicaltrials.gov/> (NCT04537962).

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## Introduction

The novel coronavirus disease 2019 (COVID-19) pandemic has mobilised the scientific community to establish antimicrobial protocols that are able to reduce or eradicate the novel coronavirus in human tissues and object surfaces. In patients with COVID-19, the severe acute respiratory syndrome

coronavirus 2 (SARS-CoV-2) virus is present in the oral mucosa and saliva.<sup>1</sup> The effectiveness of oral antiseptic products and their active ingredients against SARS-CoV-2 have been investigated in vitro<sup>2-9</sup> and in vivo.<sup>10-13</sup> However, only 2 of the in vitro studies and none of the clinical studies evaluated toothpaste formulations. A toothpaste slurry containing 0.05% cetylpyridinium chloride produced a 3.3-log<sub>10</sub> reduction in viral titre as reported by Komine et al,<sup>5</sup> and a fluoride toothpaste slurry produced a 2.26-log<sub>10</sub> reduction in viral titre as reported by Shewale et al.<sup>8</sup>

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The present study examined whether toothpastes with antimicrobial properties have a potential anti-SARS-CoV-2 effect in patients with COVID-19. This research was motivated by recent investigations that revealed the SARS-CoV-2 presence in the gingival crevicular fluid,<sup>14</sup> leading to the hypothesis that SARS-CoV-2 viral particles participate in oral biofilm colonisation in dental and periodontal tissues and that the dental plaque and saliva could be reservoirs of the novel coronavirus.<sup>15</sup>

Stannous fluoride inhibits the bacterial glycolysis and, in conjunction with fluoride, prevents the accumulation of supragingival dental plaque and calculus and reduces gingival inflammation.<sup>16</sup> A stannous fluoride toothpaste stabilised with zinc phosphate has been shown to significantly improve the control of dental plaque, calculus formation, dentinal hypersensitivity, and gingivitis.<sup>17</sup> In a toothpaste, the zinc oxide and zinc citrate combinations have been shown to produce enhanced antimicrobial effectiveness.<sup>18</sup> Zinc has been considered an important element for controlling COVID-19 infection due to its antiviral properties as well as immune system stimulation and oxidative stress inhibition, which have primarily been observed through the use of zinc supplements.<sup>19</sup>

Based on the antibacterial properties and potential antiviral effects of toothpastes containing metal ions, this study aimed to investigate the short-term effects of toothpastes composed of these elements on the SARS-CoV-2 salivary viral load of patients with COVID-19.

## Study population and methodology

### *Institutional review board approval*

This study was approved by the Ethical Committee of Human Research of Hospital Israelita Albert Einstein, Brazil (project no. 32018820.5.0000.0071), and was conducted according to the Declaration of Helsinki, as revised in 2013. After being informed regarding the proposed study's nature along with known benefits and risks, each patient provided informed consent.

### *Trial design*

This was a randomised, double-blind, single-centre clinical trial that involved hospitalised patients with COVID-19. The study involved 3 parallel intervention groups with a conceptual framework to assess the equivalence of interventions to reduce the SARS-CoV-2 salivary viral load.

### *Participants*

Patients diagnosed with COVID-19 and hospitalised in negative pressure rooms from December 2020 to May 2021 participated in the study. The inclusion criteria were both sexes, age >18 years, SARS-CoV-2 positivity confirmed by nasopharyngeal samples and real-time polymerase chain reaction (RT-PCR) performed in an interval of up to 4 days, recent hospital stay (up to 3 days), sufficient physical and mental conditions for the performance of adequate toothbrushing and saliva

collection, and oral mucosa with normal clinical aspect. The exclusion criteria were age <18 years; absence of SARS-CoV-2 positivity detected in nasopharyngeal swabs using RT-PCR; absence of signs and symptoms of COVID-19 at the moment of the recruitment; absence of cooperation during the intervention and saliva collection; reduced salivary flow that impeded saliva collection; presence of lesions in the oral cavity, including opportunistic infection; bleeding during saliva collection; and absence of SARS-CoV-2 positivity in saliva samples collected before intervention.

### *Interventions*

The interventions consisted of toothbrushing using 1 of 3 different commercial toothpastes:

- (a) Test 1: toothpaste containing 0.96% zinc (zinc oxide, zinc citrate), 1.5% L-arginine and 0.32% fluoride as sodium fluoride in a silica base (Colgate-Palmolive Company).
- (b) Test 2: toothpaste containing 0.454% SnF<sub>2</sub> in a silica base (Colgate-Palmolive Company).
- (c) Control: toothpaste containing 1.1% sodium monofluorophosphate in an abrasive base (Colgate-Palmolive Company).

After consent form signing, the patient performed the first toothbrushing of the day following the researcher's instructions. The patient received a toothbrush with soft bristles and a tongue and cheek cleaner that had been covered with a standardised quantity (1 g) of toothpaste. The toothbrush was previously prepared by the researcher and offered to the patient without toothpaste identification. The patient brushed for 2 minutes, applying their usual brushing technique. During this brushing, the tongue and cheek cleaner cleans the cheeks. The researcher controlled the toothbrushing time with a chronometer. After the procedure, the patient expectorated the toothpaste/saliva slurry and gently brushed the tongue dorsum using the tongue and cheek cleaner. The patient was instructed to place the tongue and cheek cleaner at the back of the tongue as far as they could comfortably reach and pull the cleaner forwards to the tip of the tongue. To clean the entire tongue, this motion was repeated several times. The patient was instructed to rinse the oral cavity with water and not use dental floss and mouthwash solutions during the study's evaluation periods.

### *Primary outcome*

The primary outcome was the fold reduction in SARS-CoV-2 salivary viral load after the interventions. This outcome was assessed by analysing the salivary viral load reduction percentage measured by salivary quantitative reverse transcription polymerase chain reaction (PCR) tests.

### *Sample size estimation*

The sample size was estimated based on a previous pilot study's standard deviation.<sup>11</sup> For the sample size estimation, the following parameters were set: minimal detection difference of one viral log reduction, 80% power, 95% confidence

interval, and 5% alpha. Based on this, 25 patients were required in each arm.

### Randomisation

For patient allocation, computer-generated randomisation was adopted. The computer also generated codes for each intervention and patient, which were accessed by the researcher who instructed the patient during the intervention and the laboratory staff. Throughout the study, allocation concealment was maintained. Two senior researchers (FPE and LMB) were responsible for the allocation sequence, patient enrollment, and intervention assignment. Recruitment and allocation were performed to achieve each arm's estimated sample size.

### Blinding

The patient and the laboratory staff who analysed the samples were blinded to the intervention until the end of statistical processing.

### Oral health conditions

Before the intervention, a general clinical evaluation was performed to determine the teeth and gingiva's oral health quality. Gingival inflammation and dental plaque accumulation were recorded following the modified gingival index<sup>20</sup> and plaque index.<sup>21</sup> These indexes were chosen for being noninvasive and not requiring a periodontal probe or plaque staining, techniques that are difficult to perform at the bedside and can increase the SARS-CoV-2 contamination risk. Based on individual scores attributed to each analysed tooth, an average score was obtained (1 anterior tooth and 2 posterior teeth in each dental arcade).

### Saliva collection

Unstimulated saliva (1 mL) was collected before intervention (T0), immediately after intervention (T1), and 30 minutes (T2) and 60 minutes (T3) after intervention. The researcher controlled the experimental times with a chronometer. The patient was instructed to expectorate saliva into a 50-mL sterile tube for 5 minutes.<sup>22</sup> Only samples with at least a 1-mL volume were included in the study. During sample collection, the samples were stored in ice and immediately sent to the laboratory for RNA extraction.

### Total RNA extraction and quantitative reverse transcription PCR analysis

SARS-CoV-2 viral load quantification was performed using quantitative reverse transcription polymerase chain reaction (qRT-PCR).<sup>23,24</sup> The saliva samples' nucleic acid was extracted using the QIASymphony DSP Virus/Pathogen kit (Qiagen) in accordance with the manufacturer's instructions. In all samples, the RNA amount and integrity was checked by Nanodrop at the moment of RT-PCR assay. cDNA was generated from the collected RNA using XGEN Master COVID-19 as per the manufacturer's instructions. Briefly, 5  $\mu$ L of extracted RNA sample was added to 15- $\mu$ L MIX CV19 which contained the enzymes,

probes, oligonucleotides, buffer, and deoxynucleotide triphosphates (in triplicate). The oligonucleotides amplify conserved regions of 2 genes coding for the viral nucleocapsid (N) and the polyprotein ORF1ab as well as the gene coding the human RNase P (internal control). For the qRT-PCR, a PCR reaction that did not contain an RNA template served as the negative control. Positive control qRT-PCR reaction consisted of viral RNA extracted from SARS-CoV-2 culture supernatant provided by the manufacturer.

To quantify the total number of viral particles present in the patient samples, a standard curve was used. The standard curve was generated by serially diluting the positive control RNA, previously described above, into a range of  $10^1$  to  $10^6$  copies/mL virus particles. Primer efficiency assays were performed using known viral cDNA standards to ensure proper amplification of the target genes. For the reverse transcription, the PCR cycling conditions were 45 °C for 15 minutes, followed by 95 °C for 2 minutes, then 45 cycles of 95 °C for 10 seconds and 60 °C for 50 seconds. The cycles were performed using the QuantStudio™ 6 Real-Time PCR System (ThermoFisher Scientific). Cases with a >40 cycle threshold (Ct) value were considered SARS-CoV-2 undetectable.

### Clinical data

Data about sex, age, COVID-19 signs and symptoms, presence of comorbidities, the extension of lung lesions, and oxygen saturation were collected from medical records. The researcher also noted the patients' reports about dry mouth and taste/smell changes.

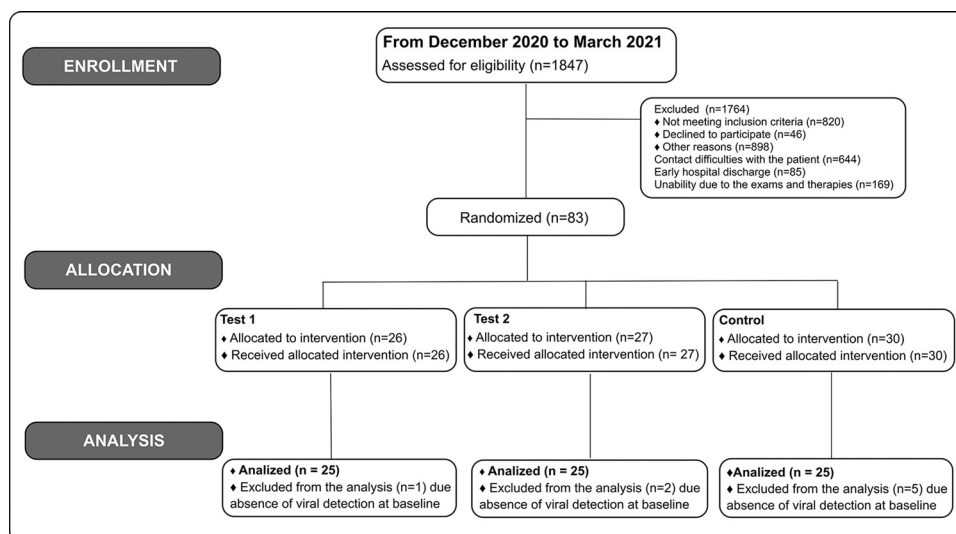
### Statistical analysis

Fold reduction was calculated against the control toothpaste with the difference to relative baselines used to quantify the reduction of SARS-CoV-2 level in the saliva. The control was considered as reference control (CtC), and baseline time (T0) was considered the experimental control (CtE).  $\Delta$ CtC was calculated by normalising the Ct values from the control at T1, T2, and T3 with those at T0.  $\Delta$ CtE was calculated by normalising the Ct values for the Test 1 and Test 2 toothpastes at T1, T2, and T3 to the corresponding values at T0.  $\Delta\Delta$ Ct (fold-difference) was then determined using the formula  $2^{-(\Delta$ CtC- $\Delta$ CtE)}. The fold reduction was calculated using  $1/\Delta\Delta$ Ct. A fold-change cutoff  $\geq 2$  was specified.<sup>25,26</sup> If a fold-change reduction is found to be equal to or greater than the specified cutoff, then the fold-change reduction at the time point is considered a significant reduction.

## Results

### Participant flow and recruitment

A total of 1847 patients who showed mild-to-moderate COVID-19 signs and symptoms were screened to assess study eligibility. From these patients, 83 were randomised into 1 of the 3 study arms. One, two, and five patients were excluded from Test 1, Test 2, and control arms, respectively, due to the absence of viral detection in the saliva at baseline. In total, 25 patients were analysed in each arm (Figure 1).



**Fig. 1 – Flowchart of patients enrolled in the study. Test 1: toothpaste containing 0.96% zinc (zinc oxide, zinc citrate), 1.5% L-arginine and 0.32% fluoride as sodium fluoride in a silica base; Test 2: toothpaste containing 0.454% SnF<sub>2</sub> in a silica base; and control: toothpaste containing 1.1% sodium monofluorophosphate in an abrasive base.**

### Baseline data

Table 1 shows the clinical data, including gingival health conditions, detected at baseline. Most of the 3 arms' patients were male, with a median age of 51 or 52 years and a range of 21 to 75 years across the study. Amongst the 3 groups, there were no statistically significant differences with respect to age or sex. Ten patients in each group had comorbidities and were at risk for COVID-19 complications. In each group, most patients exhibited fatigue, fever, headache, coughing, and dyspnoea. Smell and taste changes were detected in fewer than half of the patients. Dry mouth was reported by at least 60% of the patients in each group, but a sufficient amount of saliva was collected from all patients. Patients using the Test 1 toothpaste had a higher abdominal pain incidence than in the other groups. For oxygen saturation, the median values were either 94% or 95% with a range of 88% to 100% across all patients. There were no patients with extension of lung lesions by >50%; however, less than 50% of each group had lung lesions with extensions of up to 25%. There were no patients with caries activity and residual tooth roots. All patients had satisfactory oral health, with more than 80% of patients exhibiting no signs of gingival inflammation and more than 70% exhibiting no visible plaque. All other patients only had mild gingivitis, and only 2 patients exhibited a moderate amount of plaque.

### Fold reduction

Fold reduction (Figure 2) was calculated using the Ct mean values shown in Table 2. Based on double  $\Delta$ Ct value, which is an approximation method to determine relative gene expression with qRT-PCR experiments, and normalised for T0 (baseline) and the control toothpaste, brushing with the Test 1 toothpaste reduced the salivary viral load by 4.06-fold at T1, 2.36-fold at T2, and 1.42-fold at T3. Similarly, brushing with the Test 2 toothpaste reduced the salivary viral load by 2.33-

fold at T1, 2.38-fold at T2, and 0.77-fold at T3. Both Test 1 and Test 2 toothpastes met the minimum acceptance clinical criteria of at least a 2-fold reduction in viral bioload in the saliva at T1 and T2 when normalised to the sodium monofluorophosphate toothpaste and the baseline. At T3, neither toothpaste met the criteria.

### Viral load (expressed as log<sub>10</sub>)

The patient mean values for SARS-CoV-2 viral load (log<sub>10</sub>) are included as Supporting Information for all 3 test products at each time point.

### Adverse events

None of the patients experienced discomfort or side effects as an intervention result.

### Discussion

Toothpastes and their ingredients have demonstrated in vitro effectiveness against the SARS-CoV-2 virus,<sup>5,8</sup> the human influenza viruses,<sup>27</sup> and the human parainfluenza virus.<sup>28</sup> Using an in vitro assay, Tateyama-Makino et al<sup>29</sup> determined that 5 surfactants, commonly found in commercially available toothpastes and mouthwashes, exhibited inhibitory effects on the SARS-CoV-2 spike protein with ACE2 interaction and on the TMPRSS2protease activity. They further postulated that these ingredients could help in SARS-CoV-2 infection prevention.

This is the first clinical study to analyse the potential antiviral effects of toothpastes on SARS-CoV-2 in patients with COVID-19. Both Test 1 and Test 2 toothpastes demonstrated clinically relevant fold changes relative to baseline and the sodium monofluorophosphate toothpaste control at both T1



**Table 1 – Clinical variables related to COVID-19 and gingival health conditions.**

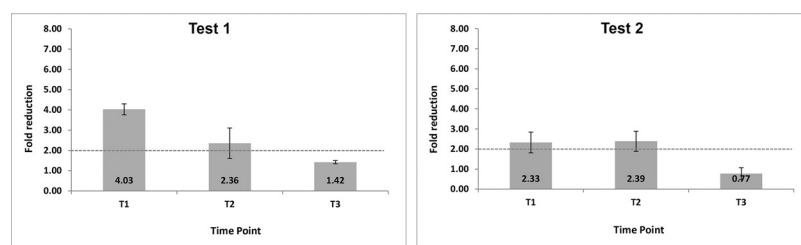
Variables	Test 1 (n = 25)	Test 2 (n = 25)	Control (n = 25)
Sex, n (%)			
Male	14 (56.0)	13 (52.0)	17 (68.0)
Female	11 (44.0)	12 (48.0)	8 (32.0)
Age (y), median (range)	52 (29-70)	52 (21-75)	51 (39-73)
Days of COVID-19 symptoms, mean (range)	6 (1-12)	5 (2-12)	7 (2-14)
Patients with comorbidities at risk for COVID-19 complications, n (%)	10 (40.0)	10 (40.0)	10 (40.0)
COVID-19 signs and symptoms, n (%)			
Fatigue	23 (92.0)	23 (92.0)	21 (84.0)
Fever	18 (72.0)	18 (72.0)	21 (84.0)
Headache	17 (68.0)	20 (80.0)	18 (72.0)
Cough	15 (60.0)	20 (80.0)	17 (68.0)
Nausea	9 (36.0)	8 (32.0)	8 (32.0)
Diarrhoea	7 (28.0)	5 (20.0)	3 (12.0)
Abdominal pain	7 (28.0)	1 (4.0)	1 (4.0)
Nasal congestion	14 (56.0)	15 (60.0)	11 (44.0)
Dyspnoea	16 (64.0)	16 (64.0)	20 (80.0)
Smelling changes	10 (40.0)	10 (40.0)	10 (40.0)
Taste changes	9 (36.0)	12 (48.0)	8 (32.0)
Dry mouth	15 (60.0)	16 (64.0)	16 (64.0)
Oxygen saturation, median (range)	94 (88-100)	95 (88-99)	95 (91-98)
% Extension of lung lesions, n (%)			
0%-25%	11 (44.0)	9 (36.0)	9 (36.0)
26%-50%	14 (56.0)	16 (64.0)	16 (64.0)
>50%	0 (0.0)	0 (0.0)	0 (0.0)
Gingival health conditions, n (%)			
Modified gingival index			
Grade 0	21 (84.0)	21 (84.0)	20 (80.0)
Grade 1 or 2	4 (16.0)	4 (16.0)	5 (20.0)
Grade 3 or 4	0 (0.0)	0 (0.0)	0 (0.0)
Plaque index			
Grade 0	18 (72.0)	19 (76.0)	19 (76.0)
Grade 1	6 (24.0)	6 (24.0)	5 (20.0)
Grade 2	1 (4.0)	0 (0.0)	1 (4.0)
Grade 3	0 (0.0)	0 (0.0)	0 (0.0)

Modified gingival index grades: 0, absence of inflammation; 1, mild inflammation in some regions of the gingiva (marginal gingiva or papilla); 2, mild inflammation in all regions of gingiva; 3, moderate inflammation (erythema, oedema, and hypertrophy); 4, severe inflammation with spontaneous bleeding, congestion, or ulceration. Plaque index grades: 0, no visible plaque; 1, thin plaque layer at the gingival margin, detectable only by scraping; 2, moderate layer of plaque visible to the naked eye; 3, abundant plaque along the gingival margin and in the interdental space. COVID-19, coronavirus disease 2019.

and T2. Zinc is considered an important element in the control of COVID-19 infection owing to its antiviral, immune system–stimulatory, and oxidative stress–inhibitory properties.<sup>19</sup> In the oral cavity, besides the prevention of dental plaque accumulation and gingivitis, toothpastes containing zinc have an anti-inflammatory effect, reducing the number

of polymorphonuclear cells in the saliva.<sup>30</sup> Moreover, toothpastes composed of zinc and arginine can improve the oral keratinocyte function against pathogenic microorganisms.<sup>31</sup>

For maintaining human microbiome homeostasis in patients with COVID-19, a good oral hygiene routine is essential, by preventing secondary intestinal and pulmonary



**Fig. 2 – Mean ± standard error of SARS-CoV-2 fold reduction in the saliva immediately after brushing (T1), 30 minutes after brushing (T2), and 60 minutes after brushing (T3). The fold reductions of each toothpaste are determined relative to the baseline and to the NaMFP toothpaste control at each timepoint. A fold reduction of  $\geq 2$  (dashed line) is considered significant.**

**Table 2 – Mean cycle threshold values in the saliva of COVID-19–positive patients treated with toothpaste in accordance with baseline, immediate postbrushing, 30 minutes postbrushing, and 60 minutes postbrushing time points.**

Treatment	n	Baseline mean (SD)	Immediate mean (SD)	30-minute mean (SD)	60-minute mean (SD)
Test 1	25	30.50 (3.06)	31.88 (2.94)	30.57 (4.00)	31.00 (5.20)
Test 2	25	30.34 (3.34)	30.92 (4.90)	30.42 (5.38)	29.95 (2.83)
Control	25	31.25 (3.70)	30.61 (4.15)	30.08 (5.44)	31.24 (5.06)

bacterial and other viral infections.<sup>32,33</sup> The oral biofilm has been implicated in COVID-19 pneumonia by aspiration of oral microorganisms and induction of dysbiotic communities in the lungs.<sup>15</sup> Oral microbiome changes can have a detrimental impact on oral health; thus, maintaining a balanced oral microbiome is crucial to maintaining oral health.<sup>34</sup> This aspect may be crucial in the management of patients with COVID-19, considering the multiple sites affected by COVID-19 infection and the intense dysbiosis caused by the virus.

The current study investigated a single toothbrushing with a 60-minute follow-up due to high contamination risk, discomfort of patients during saliva collection, and the patients' compromised systemic conditions. Increased reduction of SARS-CoV-2 salivary viral load might be achieved with repeated toothbrushings, and future investigations with these toothpastes should be designed to involve patients who are SARS-CoV-2–positive and asymptomatic, which could allow for multiple toothbrushings and a longer follow-up period. Most of the patients were in good oral health as evidenced by reduced levels of dental plaque and absence of gingivitis, limiting the extrapolation of the results to a population where dental plaque and gingivitis is more prevalent. Additional studies should be conducted using different populations, diverse oral health conditions, and other COVID-19 severity levels to understand the toothpastes' effectiveness in reducing the SARS-CoV-2 salivary viral load.

Therefore, establishing specific oral hygiene protocols is urgent for patients who are asymptomatic and symptomatic SARS-CoV-2–positive, focusing on the use of toothpastes and mouthwashes that contain metal ions to control the SARS-CoV-2 salivary viral load.

In conclusion, this study has demonstrated that brushing with antimicrobial toothpastes reduced the salivary viral load immediately after brushing in patients with COVID-19 as measured by fold changes relative to baseline and to the nonantibacterial fluoride toothpaste. This suggests that there may be merit in incorporating the toothpaste use with antimicrobial properties as part of a risk-mitigation strategy for patients infected with SARS-CoV-2. Although these toothpastes will not treat or prevent the disease, their use will temporarily reduce the viral load in the oral cavity.

## Clinical significance

### Scientific rationale for study

The effect of such toothpastes on viruses, like SARS-CoV-2, is unknown.

### Principal findings

Although these toothpastes will not treat or prevent the disease, their use will temporarily reduce the viral load in the oral cavity.

### Practical implications

There may be merit in adding the toothpaste use with antimicrobial properties as part of a risk-mitigation strategy for patients infected with SARS-CoV-2.

### Conflict of interest

None disclosed.

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### Author contributions

Fernanda de Paula Eduardo, Luciana Corrêa, Debora Heller, and Letícia Mello Bezinelli contributed equally to this work.

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### Supplementary materials

Supplementary material associated with this article can be found in the online version at [doi:10.1016/j.identj.2022.03.006](https://doi.org/10.1016/j.identj.2022.03.006).

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