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Preprocedural Mouthwashes for Reduction of SARS-CoV-2 Viral Load and Infectivity

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Following the emergence of SARS-CoV-2 and the outbreak of the COVID-19 pandemic in early 2020, specific infection control regimens were introduced to protect health care workers and patients in dental practices (Meng et al. 2020; Peng et al. 2020). Among other measures, the use of antiseptic mouthwashes prior to dental procedures has been specifically recommended to temporarily reduce intraoral viral load and infectivity in potentially SARS-CoV-2–positive individuals (Peng et al. 2020), although the underlying evidence base was sparse (Ortega et al. 2020; Carrouel et al. 2021).

Soon after, several in vitro studies reported virucidal effects of various antiseptics against SARS-CoV-2, such as povidone-iodine, cetylpyridinium chloride (CPC), benzalkonium chloride, or essential oils (Meister et al. 2020; Carrouel et al. 2021; Muñoz-Basagoiti et al. 2021; Anderson et al. 2022; Meister et al. 2022). It was postulated that these antiseptics primarily target the SARS-CoV-2 envelope, composed of a host cell–derived outer lipid membrane, rather than act on viral RNA (O'Donnell et al. 2020). Recently, we and others provided definitive experimental evidence that the antiviral effects of antiseptics such as povidone-iodine, CPC, or benzalkonium chloride against SARS-CoV-2 are exerted by disruption of the lipid membranes of the viral envelope (Muñoz-Basagoiti et al. 2021; Bañó-Polo et al. 2022; Meister et al. 2022).

Most clinical studies investigating the antiviral efficacy of antiseptic mouthwashes in SARS-CoV-2–positive individuals have examined intraoral viral load using methods based on reverse transcription quantitative polymerase chain reaction (RT-qPCR) (Gottsauner et al. 2020; Chaudhary et al. 2021; Ferrer et al. 2021; Guenezan et al. 2021; Huang and Huang 2021; Seneviratne et al. 2021; Alemany et al. 2022; Meister et al. 2022). RT-qPCR can detect viral RNA copies but gives no indication on the infectivity of the detected viral particles (Gottsauner et al. 2020; Ferrer et al. 2021; Alemany et al. 2022; Meister et al. 2022). Therefore, RT-qPCR seems an insufficient method for assessing the efficacy of antiseptic agents that target the viral envelope but not RNA (Alemany et al. 2022; Meister et al. 2022). Furthermore, previous studies have shown that SARS-CoV-2 could still be detected by RT-qPCR when COVID-19–related symptoms already had resolved for several weeks and/or no viral infectivity could be shown from the sample material in cell culture experiments (Gniazdowski et al. 2020; Wölfel et al. 2020). Accordingly, relatively small reductions in viral load of <1 $\log_{10}$ step (<90% reduction in viral RNA copies) seen by RT-qPCR after antiseptic mouthwashes (Gottsauner et al. 2020; Chaudhary et al. 2021; Ferrer et al. 2021; Seneviratne et al. 2021; Alemany et al. 2022; Meister et al. 2022) are likely due to mechanical effects during gargling rather than antiseptic action (Gottsauner et al. 2020; Ferrer et al. 2021; Meister et al. 2022).

A major challenge for clinical studies is to assess the levels of SARS-CoV-2 that remain infective following antiseptic treatment. The direct method is to rescue the virus in cell culture before and after treatment with mouthwash. Cells must then be cultured for several days in vitro to detect cytopathic effects and to calculate so-called tissue infective doses ($TCID_{50}$ [50% tissue culture infectious dose]) or plaque-forming units (Gottsauner et al. 2020; Meister et al. 2022). However, this method is labor, time, and cost intensive. In addition, successful virus rescue can be expected only from samples with high viral loads (i.e., at least 10^6 viral RNA copies/mL; Wölfel et al. 2020) and within the first few days after onset of COVID-19 symptoms (Bullard et al. 2020; He et al. 2020; Wölfel et al. 2020). This severely complicates the application of this method in a variety of clinical studies, including clinical trials on antiseptic mouthwashes, as there is a high probability of negative culture results even in baseline samples, especially when patients with long-standing infections or hospitalized patients are included (Gottsauner et al. 2020). Therefore, alternative methods are needed that can provide robust evidence on the efficacy of antiseptic mouthwashes against SARS-CoV-2 as surrogate for virus rescue studies.

In the current issue of the *Journal of Dental Research*, Alemany et al. (2022) describe a double-blind placebo-controlled randomized study on the virucidal efficacy of a CPC-containing mouthwash. In this multicenter clinical trial, the

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authors included 118 individuals with SARS-CoV-2 positivity who were treated as outpatients, 105 of whom could be included in the analysis, making this study the largest clinical trial on the topic in the literature to date. They examined viral load by RT-qPCR and, importantly, modified a commercially available ELISA to quantify the SARS-CoV-2 nucleocapsid protein as a measure of virus particle degradation. The nucleocapsid protein is localized inside the viral envelope and therefore can be detected only after lysis of the viral envelope. The authors modified the ELISA by omitting the membrane lysis step so that increased detection of nucleocapsid would indicate disruption of the viral envelope by the mouthwash or its active ingredient, CPC. Viral particles with a disrupted envelope are associated with decreased infectivity in vitro, as demonstrated by the authors and likely due to impeded entry into target cells. In addition, the authors showed that a decrease in viral infectivity was associated with an increase in nucleocapsid detection after treatment of SARS-CoV-2 with CPC in vitro. In the clinical study, they found an increase of nucleocapsid detection following the CPC-containing mouthwash but not following the placebo mouthwash, whereas the assessment of viral load resulted in no significant differences between groups at any of the investigated time points, reinforcing that RT-qPCR–derived data must be considered within its inherent limitations. Despite some limitations of this modified ELISA, such as a high variability in nucleocapsid detection from the clinical samples, these data provide the first clinical evidence that mouthwashes containing CPC could reduce the infectivity of SARS-CoV-2 in vivo. Nevertheless, these results need to be verified in future clinical trials that combine this modified ELISA for nucleocapsid detection with virus rescue in cell culture while investigating other variants of SARS-CoV-2, such as the still-circulating omicron variant. In addition, further work is needed to establish the link between the observed reduction in viral infectivity from CPC-containing mouthwashes and a clinically useful reduction in the risk of transmission of SARS-CoV-2. Ultimately, large-scale clinical studies will be needed to address this issue and identify the optimal approaches for delivering CPC or other antiseptics, while considering potentially detrimental ecologic shifts in the oral microbiota that may result from regular antiseptic use (Bescos et al. 2020; Mao et al. 2022).

Author Contributions

F. Cieplik, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; N.S. Jakubovics, contributed to conception, design, data analysis, and interpretation, drafted and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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