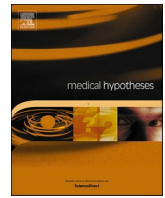




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Can preprocedural mouthrinses reduce SARS-CoV-2 load in dental aerosols?

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ABSTRACT

Dental professionals work closely with patients and present an increased risk of person-to-person transmission of SARS-CoV-2. Moreover, the use of ultrasonic scalers, air-water syringes, and slow and high-speed handpieces, which are common in the dental office, generate spatter and aerosol. The use of preprocedural mouthrinses has been proposed to reduce the viral load in saliva and oropharyngeal tissues, thus decreasing viral load in dental aerosol. Although some mouthrinses demonstrates an antiviral effect, there is limited evidence about the clinical efficacy of any mouthrinse in the reduction of SARS-CoV-2 in the dental aerosol. We hypothesized that mouthrinses may reduce SARS-CoV-2 viral load in the oropharynx and its fluids reducing viral load in dental aerosol. The potential use of mouthrinses is discussed, along with proposal of in vitro and clinical studies, in order to evaluate this hypothesis. If this hypothesis holds true, dental professionals and patients may benefit from the routine use of preprocedural mouthrinses.

Introduction

As of March 11, 2020, WHO declared COVID-19 outbreak a pandemic [1]. COVID-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [2], which can be transmitted by direct or indirect contact with infected persons [3]. Direct transmission may happen when infected secretions or droplets from a contaminated person reach the oral, nasal and/or conjunctival mucosa of a susceptible host when the former coughs, sneezes, breaths or talks. SARS-CoV-2 is primarily transmitted through respiratory droplets (particles > 5 µm in diameter), but recent findings suggest that virus transmission may be possible through aerosolized droplet nuclei (particles ≤ 5 µm in diameter) [3]. While droplets quickly settle to the ground or to surfaces and travel to shorter distances, droplet nuclei can be suspended in the air for longer times and may travel to longer distances [4]. Indirect transmission is possible through hand-mediated transfer of the virus from contaminated objects or surfaces (fomites) to the before mentioned mucosa [3].

Dental professionals work in close proximity to patients and present an increased risk of person-to-person transmission of SARS-CoV-2. Furthermore, some procedures such as ultrasonic scalers, air-water syringes and slow and high-speed handpieces are known for generating spatter and aerosol [5]. The contaminated dental aerosol may be associated with cross-contamination in the dental setting, via direct contact with the oral, nasal or conjunctival mucosa of dental personnel, or it may settle on surfaces in the dental office and result in contamination via indirect contact [6]. In the context of Covid-19 pandemic, some researchers have recently recommended a series of precautions for the dental team, before, during and after dental procedures. Among these precautions, the use of preprocedural mouthrinses have been proposed, aiming to reduce viral load in saliva and oropharyngeal tissues and thus reduce viral load in the dental aerosol [7–10]. There is some evidence from in vitro studies about the effect of mouthrinses against SARS-CoV-2 [11,12] and other enveloped virus, such as SARS-CoV, MERS-CoV [13] influenza A [14,15] parainfluenza, cytomegalovirus, hepatitis B, herpes simplex virus 1 [14], herpes simplex type 2 [16], human

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immunodeficiency virus-1 [17] and human papilloma virus (HPV) [18]. A recent pilot clinical study observed reduction of SARS-CoV-2 viral load in saliva after chlorhexidine rinse [19]. However, so far, there is limited evidence about the clinical effectiveness of mouthrinses in the reduction of SARS-CoV-2 in the dental aerosol [6].

Virus characteristics

Coronavirus is an RNA virus that belongs to the Coronavirinae subfamily of Nidovirales and its infection mechanism occurs through the action of S glycoproteins (Spike) present in the membrane [20]. Since the characteristics of the virus are beyond the scope of this paper, we recommend a review on this subject [21].

Transmission

SARS-CoV-2 may be transmitted between humans through respiratory droplets, aerosol, close proximity, fomites (contaminated surfaces), and possibly through fecal-oral from an infected subject. Respiratory droplets (particles > 5–10 µm in diameter) are propelled when speaking, coughing, breathing, or sneezing. Droplets do not remain suspended in the air for a long time, due to their size. They reach a short distance (≤1 m) and then settle on surfaces. SARS-CoV-2 may also be indirectly transmitted from fomites to individuals by contaminated hands [3]. When droplets dry out, they become droplet nuclei, which can be transported on airborne vectors as aerosols (particles ≤ 5 µm). These aerosolized particles can remain in the air for a long period and travel over long distances (>1 m) [3,4]. As a result, viruses in particles may be inhaled by susceptible individuals, or they can be transmitted via contact with nasal and/or conjunctival mucosa.

SARS-CoV-2 can be detected for prolonged periods, around 14 days, but some people have positive RT-PCR for up to 90 days [22]. After symptom onset, people with moderate levels of COVID-19 stay infectious for a maximum of 10 days. However, when individuals are most critically ill or severely immunocompromised, they may remain infected for about 20 days. Recovered people can still present SARS-CoV-2 detectable RNA in the upper respiratory tract for up to 90 days after the onset of the disease. However, the infectivity of the virus in these secretions is lower [6].

Oropharyngeal tissues and fluids as a reservoir of SARS-CoV-2

The transmembrane protein angiotensin-converting enzyme (ACE2) was identified as the main host cell receptor of SARS-CoV-2 and entrance portal of the virus into the cell. There is an abundant expression of ACE2 in different oral cavity mucosae, mainly in epithelial cells of the tongue, T cells, B cells, and fibroblasts of the oral mucosa [23–25]. There is also a high expression of ACE2 in salivary glands, especially in minor salivary glands. It has been suggested that salivary glands may act as reservoirs for COVID-19 asymptomatic infections and transmission [25].

There is evidence that the virus accumulates at the oral mucosae in the first 10 days of infection and at a subsequent time it will accumulate in the lungs [26]. Therefore, the oropharynx may be an important reservoir for SARS-CoV-2. The virus is detected in the saliva of the oral cavity and deep throat in high viral loads [2]. The magnitude of the viral load presented in saliva may be one of the factors which contribute to easy transmission, even when symptoms are mild [27].

Dental practice

Dental settings present a high risk of contamination for dentists and their team, as well as cross-contamination among patients because they are exposed to a very high quantity of contaminated spray (a combination of saliva and water coolant with high-speed instrumentation) produced by the standard clinical procedures inside the mouth where microorganisms and viruses may be found. As an infected patient has many viral particles in the saliva and on the back of the tongue [28], dental procedures which use high-speed turbines, air–water syringes, ultrasonic instruments and lasers [4,5] generate contaminated spray, spatter and aerosols and may spread a considerable load of virus in the

air, which may also settle on surfaces. Studies suggest that aerosolized SARS-CoV-2 can remain in the air for up to 3 h [29].

Due to the increased risk of COVID-19 infection among dental personnel, authors, associations and agencies have been recommending preventive measures to be adopted in the dental office, in order to minimize the risk of cross-contamination [6,8,19,30].

Mouthrinses

Among these recommendations, some guidelines advocate pre-procedural mouthrinses as a measure to decrease the risk of contamination among dental personnel [8,30,31]. According to the American Dental Association (ADA), there are two types of mouthrinses: cosmetic and therapeutic. Cosmetic mouthrinse do not have chemical or biological application beyond their temporarily control bad breath. Therapeutic mouthwash, which is more used and researched in dentistry, has active ingredients intended to help control or diminish conditions like bad breath, plaque, gingivitis, and dental caries [32]. A recent meta-analysis showed that mouthrinses with essential oils, chlorhexidine (CHX) and cetylpyridinium chloride significantly reduced the number of bacteria on the dental aerosol by 64.8% [5].

There is also evidence regarding the antiviral action of mouthrinses [14–17,33]. In addition, there are some studies that evidence the effectiveness of CHX [19], hydrogen peroxide (HP) [34], essential oils (OE) with ethanol [12] and povidone-iodine (PVP-I) [11,12] against SARS-CoV-2.

A study that evaluated the in vitro inactivation of SARS-CoV-2 using HP and PVP-I mouthrinses demonstrated that the virus was completely inactivated by PVP-I mouthrinse in vitro. HP at the recommended oral mouthrinse concentrations was minimally effective against the virus [11]. Another in vitro study demonstrated that PVP-I and EOs with ethanol significantly reduced viral infectivity to undetectable levels [12]. But these effects should be analyzed in vivo. The clinical study that analyzed effects of CHX mouthwash on SARS-CoV-2 viral load showed that the viral load in saliva decreased transiently for 2 h after using 15 mL of 0.12% CHX mouthrinse [19]. However, this was a small-scale study, without a control group and the viral load was measured using rRT-PCR only.

Rinsing and gargling with antimicrobial agents may reduce the viral load of some viruses in oropharyngeal tissues and fluids that is why there is a general recommendation is their use. Nevertheless, it is unlikely that mouthrinses are able to completely eliminate the viruses [16,35]. On the basis of these concepts, there is a clear need for a comprehensive investigation about the effectiveness of mouthrinses against SARS-CoV-2 in oropharynx and aerosols generated by dental procedures.

Chlorhexidine (CHX)

CHX is a synthetic cationic biguanide with a broad spectrum of antibacterial activity. CHX has a bactericidal effect against a wide variety of microorganisms, such as Gram-positive and Gram-negative bacteria, both aerobic and anaerobic. In addition, it has an effect on yeasts and viruses [33,36]. CHX presents high substantivity in the oral cavity, its molecules will be released in 8–12 h in active form, although it can be detected in saliva even after 24 h [37] or 48 h [38,39]. As a consequence, it has a long-lasting antimicrobial effect on oral microorganisms [36]. The most used concentrations are 0.12% and 0.2%, but there is no evidence that one concentration of chlorhexidine mouthrinse is more effective than another [36]. The most common adverse event related to CHX is tooth staining, followed by taste and mucosal alterations [40], but it is associated with CHX prolonged use. Although less common, CHX use may be also associated with cutaneous reactions [40], immediate type I hypersensitivity reactions mediated by IgE [41] and even anaphylaxis [42]. Allergic reactions are possible to happen after a single mouthrinse, which is the case of pre-procedural use.

An in vitro study using virucidal assays in tissue cultures showed that

CHX is effective against enveloped viruses (*herpes simplex virus 1 [HSV-1]*, *cytomegalovirus*, *influenza A*, *parainfluenza*, and *hepatitis B*) on the skin and in the oral cavity (0.12%) [14]. An anti-human immunodeficiency virus-1 (anti-HIV-1) and anti-HSV-1 effect were also demonstrated in an in vitro study by inhibition of the syncytia formation or the cytopathic effect for HIV-1 and by inhibition of the plaque formation for HSV-1 on Vero cell monolayers [17]. The probable mode of virus inactivation is by interaction with the virus lipid envelope, and the differences in effects in different virus types are based on differences in the physical and chemical structures of the virus envelope glycoprotein [14].

A recent study has evaluated the effect of chlorhexidine in the decontamination of inanimate surfaces demonstrated weak inactivation of coronavirus [34]. However, these findings cannot be directly extrapolated to the mucosal surface. Further, the concentration used (0.02%) was lower than the most commonly used in dentistry 0.12%. On the other hand, a recent clinical prospective study with two patients diagnosed with COVID-19 has shown that chlorhexidine mouthwash (0.12%, 15 mL) was effective in reducing SARS-CoV-2 viral load in saliva for 2 h after gargling [19]. Although the viral load increased again 2–4 h post-mouthwash, it may contribute to reduce the viral load during dental procedures and potentially reduce the cross-contamination in the dental office. Nevertheless, this study has limitations such as no comparison group and small sample size. Furthermore, although rRT-PCR was performed, no viral culture cell was conducted to determine the survival of the virus along the time and standardized plasmid DNA was not used [19]. Further studies are needed, with larger sample size and randomization into test (preprocedural mouthrinse) and comparison groups (no mouthrinse), in order to prevent selection bias and minimize confounding effects.

Essential Oils (EO)

EO mouthwash contains chemical compounds originally extracted from plants: eucalyptol 0,092%, menthol 0,042%, methyl salicylate 0,060% and thymol 0,064% [43]. Some investigations of antiviral effects in the oral cavity were documented [16,35]. Regarding adverse effects, prolonged EO mouthwash may be associated with a mild burning sensation, and reversible palatal erythema [40]. Considering a single use in pre-procedural mouthwash, systematic allergic reactions, allergic stomatitis, and chemical gastritis are possible, although rare [44].

EO shows potent virucidal effect in vitro against enveloped viruses such as HSV-1 and herpes simplex type 2 (HSV-2) [35,45], HIV-1 [17] and influenza, but limited evidence against non-enveloped viruses such as rotavirus and adenovirus [35].

One randomized clinical trial investigated the efficacy of EO in the reduction of HSV-1 and HSV-2 virus in saliva for at least 30 min after oral mouthrinse of 30 s [16]. Additionally, it was demonstrated that antiviral activity against enveloped viruses occurred rapidly, in just 30 s. Viruses attach to the host cell membrane by the layer surface glycoproteins present on its envelope. EO could act by preventing the attachment of the virus, affecting the glycoproteins of the envelope. Moreover, EO may prevent the penetration of virus by viral envelope rupture which does not allow the membranes fusion [35]. Besides, there is evidence that EO and their major components have exhibited potent antiviral activity to other coronaviruses, such as SARS-CoV, although the mechanism of action of these oils and their components were found to be mainly through inhibition of viral replication [46].

A recent in vitro study evaluated effects of EOs with ethanol mouthrinse and demonstrated that different SARS-CoV-2 strains can be effectively inactivated under conditions mimicking nasopharyngeal secretions. It supports the idea that a pre-procedural mouthrinse with EO may potentially reduce SARS-CoV-2 load in saliva [12]. However, this hypothesis needs to be tested in human clinical trials.

Cetylpyridinium chloride (CPC)

Cetylpyridinium chloride (CPC) is a quaternary ammonium compound from the group of surfactants soluble in alcohol and in aqueous solutions. It can act as a neutral pH detergent and antiseptic. It is classified by the US Food and Drug Administration (FDA) as a safe and effective antimicrobial, for the control of bacterial plaque-induced periodontal disease and is present in numerous mouthwashes [47,48].

Prolonged use of CPC mouthrinses may cause a burning sensation on the tongue, appearance of extrinsic stains due to the interaction with food dyes and increased calculus formation. Short-term and single use is not associated with adverse effects [40].

It is believed that CPC promotes virus inactivation. A randomized double-blind clinical study has reported that CPC spray effectively acts to prevent symptoms in upper respiratory infections by viral respiratory pathogens. In addition, it suggests a double mechanism of action that forms a barrier that prevents contact between the virus and the host mucosa and, also, destroying the capsid, by the lysosomotropic action, a common characteristic of CPC to the external viral membrane [49].

In this sense, other studies have shown CPC effectiveness related to the prevention of the HPV [18], oral HIV manifestations [50] and control of HSV-1 [51]. In addition, it was shown to be effective in reducing Influenza virus in vitro and in vivo with virucidal effect of CPC in 10 min, in concentrations between 5 and 20 µg/mL without viral resistance [15].

Povidone-Iodine (PVP-I)

PVP-I is a broad-spectrum disinfectant, rapid bactericidal, fungicidal, tuberculocidal, virucidal and sporicidal agent. Lipid enveloped viruses are more sensitive to its action than nonlipid viruses. Similarly to bacteria, it is likely that PVP-I affects the surface proteins of enveloped viruses, but it may also disrupt membrane fatty acids by reacting with unsaturated carbon bonds [33]. Moreover, PVP-I based mouthwash demonstrated to have strong virucidal activities against SARS-CoV and MERS-CoV after 15 s of exposure [13].

The most important adverse events related to PVP-I are temporary burning sensation, local irritation, and itching [52]. In addition, PVP-I has been associated with allergic reactions from minutes to hours after exposure and delayed allergic reactions, too. PVP-I is contraindicated in patients with allergy to active ingredients, with thyroid disease, with renal insufficiency on lithium therapy, in pregnant women and during breastfeeding [53]. Although these events are not frequent, they could be associated with PVP-I pre-procedural mouthwash and the patient's medical history must always be taken into account.

Some studies have demonstrated the antiseptic effect of PVP-I, at mouthwash concentration, against SARS-CoV-2. The virus on inanimate surfaces was effectively inactivated in 1 to 5 min by 1% PVP-I in vitro [34,54]. A recent in vitro study showed that PVP-I oral antiseptics at tested concentrations of 0.5%, 1% and 1.5%, completely inactivated SARS-CoV-2 within 15 s of contact. Moreover, 70% ethanol achieved the same result but at 30 s of contact time [11]. After 15 s and 30 s of contact time, PVP-I oral antiseptic mouthrinse at all 3 concentrations completely inactivated SARS-CoV-2. The minimal virucidal effect was shown by HP solutions at concentrations of 1.5% and 3.0%, after 15 s and 30 s of contact [55]. These studies demonstrated the superiority and rapid action of PVP-I in comparison with ethanol and HP. Likewise, Meister and colleagues (2020) showed that PVP-I after 30 s of contact significantly inactivated SARS-CoV-2 under conditions mimicking nasopharyngeal secretions [12].

Along the same lines, a case series study demonstrated that for patients with higher saliva viral load of SARS-CoV-2, PVP-I mouthwash was more effective in comparison with the other patients. The dosage used in this study was 15 mL of 1% PVP-I for 1 min [56]. Nevertheless, this study presented limitations such as no control group and a small number of participants. It is important to know that the study did not

mention any adverse effects of patients using PVP-I mouthwash.

As far as we know, there is only one clinical study of 315 patients regarding gargling with PVP-I, and 98% of them were comfortable with 0.5% PVP-I gargles and any allergy was reported [57]. Thus, it may be a good alternative to use PVP-I gargling/mouthwash to potentially diminish the viral load from oropharynx and saliva.

Moreover, a recent randomized controlled trial has evaluated the efficacy of PVP-I, CHX, CPC and water mouthrinses in reducing salivary SARS-CoV-2 viral load in 16 COVID-19 positive patients. Saliva samples were collected from all patients at baseline and 5 min, 3 h and 6 h after mouthrinse and subjected to RT-PCR analysis. CPC and PVP-I formulated commercial mouthrinses reduced SARS-CoV-2 viral load. The effect was sustained after 6 h of follow-up. A highly varied efficacy of CHX mouthwash on SARS-CoV-2 in saliva was observed. The small sample size may have influenced the results of this study [58].

Hydrogen peroxide (HP)

HP is a strong oxidizer, commercially available in variable concentrations. It is often used for disinfection, sterilization, and antiseptics, due to its broad spectrum against microorganisms. Produces hydroxyl free radicals (OH) which attack essential cell components, including lipids, proteins, and DNA [59]. Although HP can be toxic at high concentrations (>5%), its use in low concentrations (1–3%) is associated with few adverse effects [60]. Studies have shown the effectiveness of HP in inactivating some viruses, influenza virus, rabies and others [61,62].

SARS-CoV-2 is an enveloped virus that is overly sensitive to agents that disrupt lipid membranes. Some authors, based on the study of Kampf et al. (2020), started to recommend using HP preprocedural mouthrinses for reducing the salivary load of SARS-CoV-2 [34]. However, this study has some limitations, because the substance that was used was the Accelerated Hydrogen Peroxide® (AHP®), which contains HP combined with other ingredients used as disinfectant wipes [63]. Besides, the results were obtained from inanimate surfaces, and cannot be extrapolated to oral cavity.

An *in vitro* study, HP in the concentrations of 1.5 and 3%, presented limited effect as a virucidal agent against SARS-CoV-2, after contact times as long as 30 s [55]. This may be justified by the rapid inactivated HP due to the presence of host and bacteria-derived catalase activity in saliva and other endogenous peroxidases when hydrogen is present in the mouth [64]. Since it may be related to substantivity, there are no studies that show these actions in the use of HP.

In a recent pilot study, 10 subjects performed mouthwash and gargle with 1% hydrogen peroxide for 30 s. There was no significant reduction in the intraoral viral load of SARS-CoV-2 [65]. However, there was no control group. Randomized clinical trial is necessary to evaluate the effect of HP mouthrinses, with larger sample size and a control group.

Chlorhexidine-hydrogen peroxide combination

It is well known that CHX and HP are potent antibacterial agents and combined use of these substances has been recommended to benefit from the advantages of both, minimizing their side-effects and reducing the used concentration [66].

An *in vitro* study showed that combinations of certain concentrations of CHX and HP can increase their antibacterial effect compared with their individual antibacterial activity [66]. Another *in vitro* study, on cultured human periodontal ligament fibroblasts, demonstrated that HP affects the cytotoxicity of CHX in a variable concentration-dependent manner. The 2% CHX alone and in combination with either 1 or 3% HP is significantly more toxic than 0.2% CHX alone and in combination with 1 and 3% HP. A synergistic antimicrobial effect was also observed, and the authors have recommended using 0.2% concentration of CHX combined with 3% HP [67]. There is evidence that rising with HP after CHX prevents teeth staining and decreases plaque scores [68]. Nevertheless, as far as we have known, no study related to action against

viruses was done until now.

The synergistic mechanism of these substances is not known, but the hypothesis is that the CHX increases permeable bacteria cell wall which HP can penetrate easily and harm the intracellular organelles [69,70] and the same mechanism could be associated to action against enveloped viruses, such as SARS-CoV-2.

Justification

If mouthrinses are effective in reducing the viral load of SARS-CoV-2 in the oral cavity, it may reduce cross-contamination related to aerosol-generating dental procedures and be beneficial to dental professionals as well as patients. Many ongoing clinical trials aiming to evaluate the effect of the use of pre-procedural antiseptic mouthrinses on SARS-CoV-2 viral load in saliva and other fluids can be found on ClinicalTrials.gov. An example is the trial under the identifier NCT04409873 with the official title “Effect of Antiseptic Mouthwash/Gargling Solutions and Pre-procedural Rinse on SARS-CoV-2 Load (COVID-19)”. In this ongoing trial, authors are evaluating the effect of essential oils, hydrogen peroxide, cetylpyridinium chloride, and chlorine dioxide solutions compared to control (distilled water) in the reduction of SARS-CoV-2 load in saliva. However, so far, no published study or registered ongoing study aims to directly evaluate the efficacy of pre-procedural mouthwash on dental aerosol viral load.

Hypothesis

Mouthrinses with antiseptic substances, such as CHX, EO, CPC, PVP-I, HP may reduce the viral load of SARS-CoV-2 in the oral cavity. Thus, preprocedural mouthrinses may reduce the number of active aerosolized virus particles from the oral cavity and as consequence, reduce the risk of contamination by SARS-CoV-2 in the dental office.

Hypothesis assessment

We propose two studies in order to test the efficacy of preprocedural mouthrinse and gargling with antiseptic substances in the reduction of the viral load in the oropharyngeal and dental aerosol. In the first phase, an *in vitro* study testing the virucidal effect of different mouthrinses and a placebo should be conducted. Subsequently, a clinical prospective study should test the substances which achieved the greatest reduction of viral load in the oral cavity in a randomized controlled study. Furthermore, airborne infection and surface contamination in the dental office will also be evaluated.

In vitro study

The different substances will be added to a flat-bottomed 96-well microtiter plate. The virus suspension will be added to the product test solution for a specific time (30 s and 1 min). Different dilutions of culture infectious dose (TCID₅₀) will be used and added to the product test solution. TCID₅₀ will be previously determined by standard methods. Virucidal activity of the solution will be immediately suppressed by dilution with an ice-cold medium. Subsequently, 100 µL of each different dilution will be obtained and added to a sterile polystyrene flat-bottomed microtitre plate containing permissive cell suspensions.

The virucidal activity of mouthwashes will be assessed on Vero cells. Inoculated cultures will grow in a humidified 37 °C incubator in an atmosphere of 5% CO₂ and observe for cytopathic effects (CPEs) daily. The inoculated cultures will be observed for at least three days. CPEs will be verified using an Inverted microscope. The results will be analyzed by observing the degree of inhibition of the SARS-CoV-2 cytopathic effect by optical microscopy and by the analysis of cell viability by using a colorimetric assay [71]. The cytotoxicity will be calculated in analogy to the determination of virus titer [TCID₅₀/ml].

Viral culture

Vero cells (ATCC® CCL-81™) will be cultured in Dulbecco minimal essential medium (DMEM) supplemented with heat-inactivated fetal bovine serum (5% or 10%) and antibiotics/antimycotics (GIBCO) as described by other authors [72–74]. All tests should be conducted in two independent test runs on different days. Virus controls can be incorporated during all phases of the experiment. The procedures should be done in Biosafety level 3 area.

Randomized controlled trial

We propose a randomized, double-blind, parallel arms, placebo-controlled trial, aiming to assess the effect of 2 active mouthrinses and a placebo mouthrinse in the reduction of the viral load in the dental aerosol. The 2 active mouthrinses will be selected according to the results of the in vitro study. In order to guarantee environmental safety, this study will be conducted on a hospital setting, in a room with HEPA filters for air filtration. Further, dental team will wear all necessary personal protective equipment.

The inclusion criteria will be patients aged 18–50 years who tested positive for SARS-CoV-2, using the RT-PCR test, on the third day after the onset of symptoms or until the tenth day. Exclusion criteria will be patients who did not use medication to treat the disease (Chloroquine, Azithromycin, Ivermectin), smoking patients, patients with chronic diseases - diabetes, coronary artery disease, chronic obstructive pulmonary disease, and kidney failure. The sample will be collected after rinsing and gargling in four places: 1) oropharyngeal swab; 2) saliva; 3) on dental office surfaces; 4) in the air.

The primary outcome will be the SARS-CoV-2 viral load reduction in the oropharynx, by oropharyngeal swab samples measured as \log_{10} copies per mL and saliva samples measured as \log_{10} copies per mL. Secondary outcomes will be the quantification of the virus on the dental surfaces, measured as \log_{10} copies per mL and in the air, measured as \log_{10} copies per mL.

Oropharyngeal swab and saliva samples will be collected by an investigator in five moments: 1) 0 h (before gargling); 2) 30 min; 3) 1 h; 4) 2 h; and 5) 4 h after using the mouthwashes. After collecting the oropharyngeal mucosa cells, the swabs will be stored in the tube containing the viral transport medium and frozen in a -80° refrigerator. Furthermore, patients will be asked to spit saliva into the specimen container until the limit of 1 mL in a sterile plastic tube. Saliva samples should be processed in the same day.

To analyze the presence of the virus on the surface and in the air, patients will be submitted to a single ultrasonic scaling session, in order to generate dental aerosol [4, 5]. The internal environment will be previously cleaned with alcohol, detergent and sodium hypochlorite and the heating, ventilation and air conditioning systems will be mounted so that the air is expelled to the external environment. In addition, a 24-hour interval will be adopted between one patient and another since studies suggest that SAR-CoV-2 can remain in the air for 3 to 12 h and also may be transmitted by aerosols [29]. In this context, examiners will use personal protective equipment, including face shield, goggles and N95 respirators during all dental procedures and while in the dental room.

Air samples can be collected into a conical vial containing 5 mL Dulbecco's minimal essential medium (DMEM) with the Coriolis μ air sampler (Bertin Technologies, St-Berthely, France) which has a high flow rate (up to 300 L/min) and allow the virus particle collection in a few hours. The air sampler will be fixed on a tripod and set above floor level in the dental office approximately 1–5 m from the dental chair during the clinical procedure. After the collection, their liquid medium can be directly used in RT-PCR analysis.

On the other hand, aerosol samples deposited on the surface after the scaling (ultrasound frequency of 25 kHz and distilled water for 10 min) will be collected in the reflector, the bracket tray and the office bench in

three moments: 1) 1 min after the procedure 2) 10 min after the procedure 3) 30 min after the procedure and 4) 2 h after the procedure.

The collection will be made on the surfaces through a swab with a synthetic tip, and a plastic shaft applying pressure will be moving in at least two different directions while rotating the swab stick an area of 25 cm^2 . According to the WHO protocol, the swab specimen collection vials need to have 1–3 mL of the viral transport medium (e.g. protein stabilizer, antibiotics, and buffer solution) including neutralizing buffer to counteract the effects of any residual disinfectant (e.g. Tween 80), should be stored at -80°C and will be sent to the laboratory on the day of collection [75].

Thus, when the volunteer to be discharged the environment will be cleaned and disinfected with detergent, alcohol, and sodium hypochlorite and the next one was attended at least 3 h later [29]. As a negative control, a sample will be collected before each patient is seen.

SARS-CoV-2 rRT-PCR

Viral RNA will be extracted from clinical samples and the rRT-PCR will be performed using a real-time PCR detection system and a 2019-nCoV real-time PCR kit aimed at SARS-CoV-2 E and RdRP genes. If the value of the cycle threshold (Ct) exceeds 35 cycles, the sample will be defined as negative. The number of RNA copies will be calculated from the Ct values using the standard curve generated by diluting the plasmid's DNA [19].

Sample size calculation will be based on the primary outcome and on the expected difference between any of the active mouthrinses versus the placebo mouthrinse. The differences between the two active mouthrinses will be exploratory only. Based on an expected large effect size ($f = 0.40$), $\alpha = 5\%$, $\text{power} = 90\%$, 28 patients per group would be required. To compensate for losses to follow-up, we will recruit 30 patients per groups, which would result in a total of 90 patients.

Statistical analysis

Regarding the in vitro study, concentrations at which the substances reach the half-maximal virus inactivation effective concentration 50 (EC50) will be calculated using nonlinear regression using the robust fitting method on the normalized TCID50 data. The mean TCID50 of two individual experiments and the standard deviation of means will also be determined.

In relation to randomized clinical trial, analysis of variance (ANOVA) and post hoc Tukey will be used to compare groups regarding the primary outcome (SARS-CoV-2 viral load reduction). Repeated measures ANOVA will be conducted for exploratory analysis of the differences between groups and time point estimates. Level of significance will be set at 5%.

Consequences of the hypothesis

If preprocedural mouthrinses effectively reduce SARS-CoV-2 viral load in saliva and on dental aerosols, they could reduce the chances of cross-contamination through particles in saliva and aerosol in the dental office. It is important to emphasize that they should be used together with personal protective equipment, hand washing and other recommendations. Therefore, if our hypothesis holds true, dental professionals and patients may benefit from the routine use of preprocedural mouthrinses.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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