

Dental biofilm of symptomatic COVID-19 patients harbours SARS-CoV-2

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Abstract

Aims: SARS-CoV-2 RNA has been recovered from different sites in the human body, including the mouth. The present study aimed to investigate the presence of SARS-CoV-2 RNA in the dental biofilm of symptomatic patients who tested positive in nasopharyngeal and oropharyngeal (NASO/ORO) samples.

Materials & Methods: An observational clinical study of individuals with flu-like symptoms was conducted between July and September 2020. Dental biofilm (BIO) samples were collected and analysed using real-time quantitative polymerase chain reaction (RT-qPCR) to determine the virus's presence.

Results: Seventy participants (40 ± 9.8 years of age, 71.4% female) tested positive for SARS-CoV-2 RNA in NASO/ORO samples and were included in the study. Among them, 13 tested positive in BIO samples (18.6%; 95% CI: [9.5, 27.7]). The median and interquartile range of cycle quantification (Cq) for NASO/ORO and BIO samples were 15.9 [6.9] and 35.9 [4.0] ($p = .001$), respectively. BIO-positive participants showed a higher virus load in NASO/ORO samples ($p = .012$) than those testing negative (Cq = 20.4 [6.1]).

Conclusions: Dental biofilms from symptomatic COVID-19 patients harbour SARS-CoV-2 RNA and might be a potential reservoir with an essential role in COVID-19 transmission.

KEYWORDS

COVID-19, dental plaque, observational study, pandemics, SARS-CoV-2

Clinical relevance

Scientific Rationale: The dental biofilm can harbour SARS-CoV-2 RNA.

Principal Findings: About a fifth of the participants who tested positive for viral RNA in nasopharyngeal and oropharyngeal samples also have tested positive for SARS-CoV-2 in the dental biofilm.

Practical implications: The results show a not yet explored human habitat of the viral RNA. This finding might stimulate investigations about SARS-CoV-2 in dental biofilms, helping to promote virus containment strategies.

1 | INTRODUCTION

Since emerging at the end of 2019, coronavirus disease 2019 (COVID-19), caused by the SARS-CoV-2 virus, has affected millions of people worldwide, constituting one of the most challenging public health issues in human history. While many countries are still dealing with the so-called “first COVID-19 wave,” a “second wave” has been observed, raising questions about the scope of available control measures (Adhikari et al., 2020; de Brouwer et al., 2020; Xu & Li, 2020). Various interventions—such as social distancing, face masks, hygiene measures, massive diagnostic efforts, contact tracing and quarantines—remain the best option to try to interrupt the spread of the virus, thus lowering the risk of contagion between people until there are enough vaccines to prevent further spread of the virus (Lewnard & Lo, 2020; Wiersinga et al., 2020).

Understanding the sites within the human body capable of harbouring SARS-CoV-2 RNA is crucial for understanding the virus's points of entry and reducing its spread. A meta-analysis evaluating the presence of SARS-CoV-2 RNA in different clinical samples detected viral RNA in samples from nasopharynges and oropharynges, secretions from the lower respiratory tract, bronchoalveolar lavage fluid, rectal swabs, blood and faeces (Bwire et al., 2021). The literature also identifies saliva as one of the most critical routes of inter-individual SARS-CoV-2 transmission (Cheng et al., 2020; Li et al., 2020; Wang et al., 2020), and viral RNA was recently observed in the salivary glands (Huang et al., 2020).

However, saliva may not be the only intra-oral niche capable of harbouring viruses. Therefore, it is essential to investigate the presence of SARS-CoV-2 RNA in other intra-oral sites. The teeth, gingival sulcus, tongue, cheek mucosa, hard and soft palates, and tonsils are all niches that harbour several types of microorganisms (Dewhirst et al., 2010; Teles et al., 2013), including viruses (Slots, 2010; Baker et al., 2017). Recently, SARS-CoV-2 RNA was detected in crevicular gingival fluid (Gupta et al., 2021).

In addition, the tooth structure and adjacent tissues might represent a potential virus-harboring site in the context of the present pandemic. Since teeth have a non-shedding surface, they harbour microorganisms in surface biofilm, which may resist host inflammatory and immune defences and pharmaceutical treatments unless it is disrupted mechanically (Harrel & Molinari, 2004; Bjarnsholt et al., 2018). Furthermore, the process of biofilm formation and maturation presupposes a detachment phase, during which microorganisms can travel to other parts of the human body (Talsma, 2007). According to the literature, viruses from dental biofilm may also infiltrate the bloodstream (Slots, 2015).

SARS-CoV-2 depends on the presence of angiotensin-converting enzyme II (ACE2) receptors—which exhibit high expression on the epithelial cells of the oral mucosa (Xu et al., 2020)—to attach, enter and multiply, leading to infection. Alternatively, it can also remain at the virion stage (i.e. a complete viral particle constituting an infective form of a virus outside the host cell; Lodish et al., 2000). To

date, the presence of SARS-CoV-2 in dental biofilm has not been explored. The present study was developed to explore the presence of SARS-CoV-2 RNA in the dental biofilm of symptomatic patients who tested positive in nasopharyngeal and oropharyngeal (NASO/ORO) samples.

2 | MATERIALS AND METHODS

2.1 | Ethical considerations

An observational clinical study was conducted at Hospital de Clínicas de Porto Alegre (HCPA), which is affiliated with the Federal University of Rio Grande do Sul in Porto Alegre, Rio Grande do Sul, Brazil. The study protocol was approved on June 9, 2020, by the HCPA Ethics Committee (CAAE: 30801120.0.0000.5327). This report followed STARD statement guidelines.

2.2 | Sample selection and data collection

According to the hospital's protocol, all staff members with flu-like symptoms must be tested for SARS-CoV-2 at the institution's occupational medicine division (OMD). Those seeking consultation between July 14 and September 9, 2020, were recruited for the present study. Those who met the study eligibility criteria, signed the informed consent form and tested positive for viral RNA in the NASO/ORO samples were included in the study, composing a consecutive sample. Demographic data were collected to define the characteristics of all individuals enrolled in the study.

After being examined by the medical team, biofilm samples were collected from the participants by two trained dentists (SF and JF). Before sampling, subjects rinsed their mouths with water twice, for one minute each time. However, given the possibility that spitting out water could release particles into the environment, the participants were given a glass of mineral water to rinse their mouths with and were instructed to swallow when finished. Additionally, the cheeks, tongue and lips were retracted using a sterilized wooden spatula during the sampling, and cotton rolls were placed at the bottom of the labial and lingual vestibules. During biofilm sampling, the number of teeth and the presence of visible dental biofilm were noted. Sterilized dental swabs (regular size; KG Sorensen Brush, São Paulo, SP, Brazil) were used to sample the dental biofilm from the dental-gingival area; samples were collected from the buccal and lingual surfaces of all teeth (upper right and left vestibular; lower right and left lingual; lower right and left vestibular). A total of six swabs for each participant were pooled in a coded falcon tube, constituting one sample per individual, and stored at -80°C until being sent to the laboratory to conduct a blind analysis (Instituto de Ciências Básicas da Saúde, Federal University of Rio Grande do Sul).

2.3 | Laboratory analysis

2.3.1 | Preparation of samples

At the laboratory, 3 ml of saline solution was added to each tube, then the tubes were kept refrigerated until processing. All samples were handled in a security level II - B2 chamber following the recommendations for viral diagnosis set out by the Brazilian Ministry of Health. During processing, the samples were vortexed, and three 1 ml aliquots were extracted. Two of the aliquots were stored at -80°C as reserve samples, while the third was used to extract viral genetic material using a QIAamp Viral RNA Mini Kit (QIAGEN) following the manufacturer's instructions. Extracted RNA samples were also stored at -80°C . Before RNA isolation, 200 μl of buffer was added.

2.3.2 | Real-time quantitative polymerase chain reaction (RT-qPCR)

Determination of the presence of the SARS-CoV-2 virus was conducted using real-time quantitative polymerase chain reaction (RT-qPCR, also known as RT-PCR). The Charité protocol (Corman et al., 2020) was used along with the AgPath-ID One-Step RT-PCR Reagents kit (Thermo Fisher Scientific). Additionally, a control assay was run with ribonuclease P (RNase P) according to the protocol of the US Center for Disease Control and Prevention (CDC, 2020).

Samples that showed amplification with Cycle quantification (Cq) <40 for both viral and control genes were considered positive controls for SARS-CoV-2. Samples where only one of the viral genes was amplified were classified as inconclusive, and RT-qPCR was repeated. Samples without amplification of either gene were considered inadequate, and both RNA extraction and RT-qPCR were repeated. A total of three samples had to be repeated (once each).

2.4 | Data analysis

The mean (SD) or median (interquartile range) was calculated for each numeric variable, according to its distribution. Distribution frequencies were determined for categorical variables. The proportion of positive results for SARS-CoV-2 RNA was explored using 95% confidence intervals (https://istats.shinyapps.io/Inference_prop/).

The Wilcoxon matched pair signed rank test was used to compare Cq from NASO/ORO samples and Cq from positive BIO samples. The Mann-Whitney *U* test was used to compare the Cq values from NASO/ORO samples for participants who tested positive and negative in BIO samples.

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) for Windows version 18.0 (SPSS Inc.).

TABLE 1 Sociodemographic data, number of teeth, presence of visible dental biofilm and presence of systemic comorbidities for the total sample ($n = 70$) and for participants positives for SARS-CoV-2 in the dental biofilm (BIO; $n = 13$)

Indicator	Total sample	BIO positives
Age (years) ^a	40 \pm 9.8	42 \pm 10.6
Female participants ^b	50 (71.4)	10 (76.9)
Number of teeth ^a	28 \pm 3.1	27 \pm 3.2
Presence of dental biofilm ^b	28 (40.0)	5 (38.4)
Presence of comorbidities		
Diabetes ^b	5 (7.1)	1 (7.7)
Hypertension ^b	9 (12.9)	2 (15.4)
Cardiac disease ^b	1 (1.4)	0 (0.0)

^aMean \pm SD.

^bNumber (Percentage).

Analyses were conducted at the level of the individual and the significance was set at 5%.

3 | RESULTS

Medical records from staff members at the OMD showed that 70 individuals tested positive for SARS-CoV-2 in NASO/ORO samples, composing the present sample (40 \pm 9.8 years, 71.4% female). Of these, 13 individuals (18.6%) tested positive for SARS-CoV-2 in the dental biofilm (Table 1).

Sociodemographic data, number of teeth, presence of visible dental biofilm, and distribution of comorbidities for the total sample and the participants testing positive for SARS-CoV-2 RNA in BIO samples are reported in Table 1. Hypertension, the most common comorbidity, showed a similar distribution in the total sample (12.9%) and the positive BIO samples (15.4%).

The expression of viral RNA was higher in NASO/ORO samples ($p = .001$), as shown by the smaller Cq median, compared to BIO-positive samples (Table 2). In addition, the virus expression in NASO/ORO samples was significantly higher for those who tested positive for SARS-CoV-2 RNA in BIO samples compared to those who tested negative ($p = .012$).

Descriptive results related to flu-like symptoms and systemic markers for the total sample and for the participants who tested positive for SARS-CoV-2 RNA in the dental biofilm are shown in Table 3. A large proportion of the BIO-positive participants (46.2%) exhibited five flu-like symptoms (data not shown).

4 | DISCUSSION

The findings of the present study confirm, for the first time, the hypothesis that dental biofilms harbour SARS-CoV-2 RNA in patients with COVID-19 flu-like symptoms. This observation is important because it may impact COVID-19 control strategies to

TABLE 2 Cycle quantification (Cq) from the RT-qPCR reaction in nasopharyngeal and oropharyngeal (NASO/ORO) and dental biofilm (BIO) samples

	NASO/ORO ^a	BIO ^a	p-value ^b
Total sample (n = 70)	19.5 [7.4]	NA	
BIO positives (n = 13)	15.9 [6.9]	35.9 [4.0]	.001
BIO negatives (n = 57)	20.4 [6.1]	NA	
p-value ^c	.012		

^aMedian [Interquartile Range]; NA, not available.

^bWilcoxon matched pair signed rank test. Comparison between NASO/ORO samples C quantification (Cq) of participants testing positives in dental biofilms and BIO samples Cq.

^cMann-Whitney U test. Comparison of NASO/ORO samples Cq between participants that have tested positive and negative in BIO samples.

TABLE 3 Flu-like symptoms and systemic markers for the total sample (n = 70) and for the participants positives for SARS-CoV-2 RNA in the dental biofilm (BIO; n = 13)

	Total sample	BIO positives
Flu symptoms ^a		
Fever	31 (44.3)	10 (76.9)
Cough	23 (32.9)	8 (61.5)
Fatigue	29 (41.4)	6 (46.2)
Runny nose	33 (47.1)	7 (53.8)
Throat ache	20 (28.6)	10 (76.9)
Body ache	30 (42.9)	7 (53.8)
Headache	43 (61.4)	10 (76.9)
Loss of taste	23 (32.9)	2 (15.4)
Loss of smell	29 (41.4)	4 (30.8)
Systemic markers ^b		
Body temperature (°C)	36.5 ± 0.7	36.7 ± 0.7
Oxygen saturation	98.5 ± 1.7	99.1 ± 1.4

^aNumber (Percentage).

^bMean ± SD.

prevent the spread of the disease, as well as potential therapeutic measures.

The presence of viral RNA in 13 of 70 biofilm samples (18.6%) is concurrent with the literature, which shows that the prevalence of SARS-CoV-2 RNA varies widely depending on the sampling method, from 91.8% for bronchoalveolar lavage to 7.6% for oropharyngeal swabs (Bwire et al., 2021). Interestingly, SARS-CoV-2 RNA was detected in crevicular gingival fluid with 63.64% sensitivity (concerning nasopharyngeal findings) in a sample of 33 patients, where 14 (42.42%) were deemed to have gum disease upon further examination (Gupta et al., 2021).

It is known that the results from molecular tests, such as RT-qPCR, do not measure microorganism viability (Keer & Birch, 2003;

Polonyi et al., 2013; Burton et al., 2021) and that SARS-CoV-2 requires angiotensin-converting receptors to enter the cell and reproduce (Xu et al., 2020). However, SARS-CoV-2 virions can also survive in different environments and on various surfaces (van Doremalen et al., 2020).

RT-qPCR showed a median Cq of 35.9 [4.0] in BIO samples, which was significantly greater than that observed for NASO/ORO (15.9 [6.9]). This demonstrates a lower load of SARS-CoV-2 RNA in the dental biofilm. On the other hand, the high RNA virus expression for NASO/ORO samples, denoted by the lower Cq values for participants who tested positive in BIO samples, suggests an association between the NASO/ORO load and the presence of viral RNA in the dental biofilm. However, it is essential to highlight that the number of patients who tested positive for SARS-CoV-2 RNA in the BIO samples was too low to support reliable statistical analyses. Furthermore, due to substantial uncertainty regarding the viral load and its relation to the level of infectivity or disease severity (Walsh et al., 2020), no conclusions can be drawn about it.

The present study was not designed to determine the virus's mechanism for colonizing dental biofilm, the potential paths involved or the occurrence of cross-contamination from saliva, oropharynx exhalations or blood, thus warranting further investigations. However, the simple presence of SARS-CoV-2 in the dental biofilm, irrespective of the viral load, may define the oral cavity as a potential reservoir for the virus. In the future, researchers should always consider the oral cavity's role in the transmission of SARS-CoV-2 (Herrera et al., 2020), pay close attention to the richness of ACE2 receptors at the dorsum of the tongue (Xu et al., 2020), and continue to define colonization pathways and mechanisms.

To date, there have been very few recorded cases of viral transmission and infection in the dental clinic setting. In November 2020, Estrich and colleagues reported a prevalence rate of 0.9% among dentists in the United States, based on a cross-sectional study that included over 2000 practising dentists (Estrich et al., 2020). The authors concluded that adherence to the use of personal protective equipment, including N-95 masks, contributed to the low number of cases and made the clinical practice safe for both patients and dentists.

Although mouth rinses containing chemical agents (povidone-iodine, chlorhexidine, cetylpyridinium chloride, oxygen peroxide, essential oils, beta-cyclodextrin and citrox) are currently used to control SARS-CoV-2 load before clinical procedures, scientific proof of their effectiveness is still lacking (Burton et al., 2020; Gottsauner et al., 2020; Herrera et al., 2020; Mendez & Villasanti, 2020; Moosavi et al., 2020).

However, the use of mouth rinses combined with the mechanical disruption of the biofilm by the professional could help reduce the spread of the virus during clinical treatment. Another situation that has not yet been explored is that SARS-CoV-2 may be transferred from the dental biofilm to the oral cavity through routine dental hygiene measures such as tooth brushing or flossing. This suggests the

need for future studies about viral spread and containment strategies focusing on dental care procedures.

The literature suggests that fever, cough and fatigue are the main symptoms observed in positive patients (Adil et al., 2021). Herein, fever, throat ache and headache were the main flu-like symptoms reported by the participants. However, symptoms such as fever, cough and throat ache were around 2–3 times more prevalent in the BIO-positive group than in the BIO-negative group.

In conclusion, the presence of SARS-CoV-2 RNA in dental biofilm supports the hypothesis that the oral cavity can be a potential source of viral infection. This source should be considered when defining the appropriate preventive measures to stop the spread of the virus and reverse the increase in case numbers.

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CONFLICT OF INTEREST

The authors have stated that there are no conflicts of interest in connection with this article.

DATA AVAILABILITY STATEMENT

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

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REFERENCES

- Adhikari, S. P., Meng, S., Wu, Y.-J., Mao, Y.-P., Ye, R.-X., Wang, Q.-Z., Sun, C., Sylvia, S., Rozelle, S., Raat, H., & Zhou, H. (2020). Epidemiology, causes, clinical manifestation and diagnosis, prevention and control of coronavirus disease (COVID-19) during the early outbreak period: a scoping review. *Infectious Diseases of Poverty*, 9, 29. <https://doi.org/10.1186/s40249-020-00646-x>
- Adil, M. T., Rahman, R., Whitelaw, D., Jain, V., Al-Taani, O., Rashid, F., Munasinghe, A., & Jambulingam, P. (2021). SARS-CoV-2 and the pandemic of COVID-19. *Postgraduate Medical Journal*, 97, 110–116. <https://doi.org/10.1136/postgradmedj-2020-138386>
- Baker, J. L., Bor, B., Agnello, M., Shi, W., & He, X. (2017). Ecology of the oral microbiome: Beyond bacteria. *Trends in Microbiology*, 25, 362–374. <https://doi.org/10.1016/j.tim.2016.12.012>
- Bjarnsholt, T., Buhlin, K., Dufrene, Y. F., Gomelsky, M., Moroni, A., Ramstedt, M., Rumbaugh, K. P., Schulte, T., Sun, L., Åkerlund, B., & Rømling, U. (2018). Biofilm formation - what we can learn from recent developments. *Journal of Internal Medicine*, 284, 332–345. <https://doi.org/10.1111/joim.12782>
- Burton, J., Love, H., Richards, K., Burton, C., Summers, S., Pitman, J., Easterbrook, L., Davies, K., Spencer, P., Killip, M., Cane, P., Bruce, C., & Roberts, A. D. G. (2021). The effect of heat-treatment on SARS-CoV-2 viability and detection. *Journal of Virological Methods*, 290, 114087. <https://doi.org/10.1016/j.jviromet.2021.114087>
- Burton, M. J., Clarkson, J. E., Goulao, B., Glenny, A.-M., McBain, A. J., Schilder, A. G., Webster, K. E., & Worthington, H. V. (2020). Antimicrobial mouthwashes (gargling) and nasal sprays administered to patients with suspected or confirmed COVID-19 infection to improve patient outcomes and to protect healthcare workers treating them. *The Cochrane Database of Systematic Reviews*, 9, CD013627. <https://doi.org/10.1002/14651858.CD013627.pub2>
- Bwire, G. M., Majigo, M. V., Njiro, B. J., & Mawazo, A. (2021). Detection profile of SARS-CoV-2 using RT-PCR in different types of clinical specimens: A systematic review and meta-analysis. *Journal of Medical Virology*, 93, 719–725. <https://doi.org/10.1002/jmv.26349>
- CDC - Division of Viral Diseases. (2020). *CDC 2019-novel coronavirus (2019-nCoV) real-time RT-PCR diagnostic panel for emergency use only instructions for use*. Retrieved from <https://www.fda.gov/media/134922/download>
- Cheng, V.-C.-C., Wong, S.-C., Chuang, V.-W.-M., So, S.-Y.-C., Chen, J.-H.-K., Sridhar, S., To, K.-K.-W., Chan, J.-F.-W., Hung, I.-F.-N., Ho, P.-L., & Yuen, K.-Y. (2020). The role of community-wide wearing of face mask for control of coronavirus disease 2019 (COVID-19) epidemic due to SARS-CoV-2. *The Journal of Infection*, 81, 107–114. <https://doi.org/10.1016/j.jinf.2020.04.024>
- Corman, V. M., Landt, O., Kaiser, M., Molenkamp, R., Meijer, A., Chu, D. K., Bleicker, T., Brünink, S., Schneider, J., Schmidt, M. L., Mulders, D. G., Haagmans, B. L., van der Veer, B., van den Brink, S., Wijsman, L., Goderski, G., Romette, J.-L., Ellis, J., Zambon, M. ... Drosten, C. (2020). Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveillance: Bulletin Europeen Sur Les Maladies Transmissibles*, 25, 2000045. <https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045>
- de Brouwer, R., van Veldhuisen, D. J., & de Boer, R. A. (2020). Surviving the first COVID-19 wave and learning lessons for the second. *European Journal of Heart Failure*, 22, 975–977. <https://doi.org/10.1002/ejhf.1938>
- Dewhirst, F. E., Chen, T., Izard, J., Paster, B. J., Tanner, A. C. R., Yu, W.-H., Lakshmanan, A., & Wade, W. G. (2010). The human oral microbiome. *Journal of Bacteriology*, 192, 5002–5017. <https://doi.org/10.1128/JB.00542-10>
- Estrich, C. G., Mikkelsen, M., Morrissey, R., Geisinger, M. L., Ioannidou, E., Vujicic, M., & Araujo, M. W. B. (2020). Estimating COVID-19 prevalence and infection control practices among US dentists. *Journal of the American Dental Association*, 139(151), 815–824. <https://doi.org/10.1016/j.adaj.2020.09.005>
- Gottsauner, M. J., Michaelides, I., Schmidt, B., Scholz, K. J., Buchalla, W., Widbill, M., Hitzenbichler, F., Ettl, T., Reichert, T. E., Bohr, C., Vielsmeier, V., & Cieplik, F. (2020). A prospective clinical pilot study on the effects of a hydrogen peroxide mouthrinse on the intraoral viral load of SARS-CoV-2. *Clinical Oral Investigations*, 24, 3707–3713. <https://doi.org/10.1007/s00784-020-03549-1>
- Gupta, S., Mohindra, R., Chauhan, P. K., Singla, V., Goyal, K., Sahni, V., Gaur, R., Verma, D. K., Ghosh, A., Soni, R. K., Suri, V., Bhalla, A., & Singh, M. P. (2021). SARS-CoV-2 detection in gingival crevicular fluid. *Journal of Dental Research*, 100, 187–193. <https://doi.org/10.1177/0022034520970536>
- Harrel, S. K., & Molinari, J. (2004). Aerosols and splatter in dentistry: A brief review of the literature and infection control implications. *Journal of the American Dental Association*, 139(135), 429–437. <https://doi.org/10.14219/jada.archive.2004.0207>
- Herrera, D., Serrano, J., Roldán, S., & Sanz, M. (2020). Is the oral cavity relevant in SARS-CoV-2 pandemic? *Clinical Oral Investigations*, 24, 2925–2930. <https://doi.org/10.1007/s00784-020-03413-2>

- Huang, N., Perez, P., Kato, T., Mikami, Y., Okuda, K., Gilmore, R. C., Domínguez Conde, C., Gasmi, B., Stein, S., Beach, M., Pelayo, E., Maldonado, J., LaFont, B., Padilla, R., Murrach, V., Maile, R., Lovell, W., Walle, S., Bowman, N. M. ... Byrd, K. M. (2020). Integrated single-cell atlases reveal an oral SARS-CoV-2 infection and transmission axis. *MedRxiv: The Preprint Server for Health Sciences*. <https://doi.org/10.1101/2020.10.26.20219089>
- Keer, J. T., & Birch, L. (2003). Molecular methods for the assessment of bacterial viability. *Journal of Microbiological Methods*, 53, 175–183. [https://doi.org/10.1016/s0167-7012\(03\)00025-3](https://doi.org/10.1016/s0167-7012(03)00025-3)
- Lewnard, J. A., & Lo, N. C. (2020). Scientific and ethical basis for social-distancing interventions against COVID-19. *The Lancet Infectious Diseases*, 20, 631–633. [https://doi.org/10.1016/S1473-3099\(20\)30190-0](https://doi.org/10.1016/S1473-3099(20)30190-0)
- Li, Y., Ren, B., Peng, X., Hu, T., Li, J., Gong, T., Tang, B., Xu, X., & Zhou, X. (2020). Saliva is a non-negligible factor in the spread of COVID-19. *Molecular Oral Microbiology*, 35, 141–145. <https://doi.org/10.1111/omi.12289>
- Lodish, H., Berk, A., Zipursky, S. L., Matsudaira, P., Baltimore, D., & Darnell, J. (2000). *Viruses: Structure, function, and uses*. Retrieved from <https://www.ncbi.nlm.nih.gov/books/NBK21523/>.
- Mendez, J., & Villasanti, U. (2020). Use of povidone as a mouthrinse to decrease the viral load of Covid-19 before dental care: Review of the literature. *American Journal of Dentistry*, 33, 248–250.
- Moosavi, M.-S., Aminishakib, P., & Ansari, M. (2020). Antiviral mouthwashes: Possible benefit for COVID-19 with evidence-based approach. *Journal of Oral Microbiology*, 12, 1794363. <https://doi.org/10.1080/20002297.2020.1794363>
- Polonyi, M., Prenninger, N., Arweiler, N. B., Haririan, H., Winklehner, P., & Kierstein, S. (2013). Assessment of viable periodontal pathogens by reverse transcription quantitative polymerase chain reaction. *Journal of Periodontal Research*, 48, 671–676. <https://doi.org/10.1111/jre.12052>
- Slots, J. (2010). Human viruses in periodontitis. *Periodontology 2000*, 53(1), 89–110. <https://doi.org/10.1111/j.1600-0757.2009.00325.x>
- Slots, J. (2015). Periodontal herpesviruses: Prevalence, pathogenicity, systemic risk. *Periodontology 2000*, 69(1), 28–45. <https://doi.org/10.1111/prd.12085>
- Talsma, S. S. (2007). Biofilms on medical devices. *Home Healthcare Nurse*, 25, 589–594. <https://doi.org/10.1097/01.NHH.0000296117.87061.14>
- Teles, R., Teles, F., Frias-Lopez, J., Paster, B., & Haffajee, A. (2013). Lessons learned and unlearned in periodontal microbiology. *Periodontology 2000*, 62(1), 95–162. <https://doi.org/10.1111/prd.12010>
- van Doremalen, N., Bushmaker, T., Morris, D. H., Holbrook, M. G., Gamble, A., Williamson, B. N., Tamin, A., Harcourt, J. L., Thornburg, N. J., Gerber, S. I., Lloyd-Smith, J. O., de Wit, E., & Munster, V. J. (2020). Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. *The New England Journal of Medicine*, 382, 1564–1567. <https://doi.org/10.1056/NEJMC2004973>
- Walsh, K. A., Jordan, K., Clyne, B., Rohde, D., Drummond, L., Byrne, P., Ahern, S., Carty, P. G., O'Brien, K. K., O'Murchu, E., O'Neill, M., Smith, S. M., Ryan, M., & Harrington, P. (2020). SARS-CoV-2 detection, viral load and infectivity over the course of an infection. *The Journal of Infection*, 81, 357–371. <https://doi.org/10.1016/j.jinf.2020.06.067>
- Wang, W., Xu, Y., Gao, R., Lu, R., Han, K., Wu, G., & Tan, W. (2020). Detection of SARS-CoV-2 in different types of clinical specimens. *JAMA*, 323, 1843–1844. <https://doi.org/10.1001/jama.2020.3786>
- Wiersinga, W. J., Rhodes, A., Cheng, A. C., Peacock, S. J., & Prescott, H. C. (2020). Pathophysiology, transmission, diagnosis, and treatment of coronavirus disease 2019 (COVID-19): A review. *JAMA*, 324, 782–793. <https://doi.org/10.1001/jama.2020.12839>
- 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>
- Xu, S., & Li, Y. (2020). Beware of the second wave of COVID-19. *Lancet*, 395, 1321–1322. [https://doi.org/10.1016/S0140-6736\(20\)30845-X](https://doi.org/10.1016/S0140-6736(20)30845-X)

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