Brazilian Dental Science

unesp
UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Instituto de Ciência e Tecnologia
Campus de São José dos Campos

ORIGINAL ARTICLE

DOI: https://doi.org/10.4322/bds.2023.e3672

Antimicrobial efficacy of S-PRG containing toothpastes on *S. mutans* biofilm development

Eficácia antimicrobiana de dentifrícios contendo S-PRG sobre o desenvolvimento de biofilme de *S. mutans*

Manuela da Silva SPINOLA[1], Jacqueline Landi MENDONÇA[2], Maíra Terra GARCIA[3], Taciana Marco Ferraz CANEPPELE[2], Juliana Campos JUNQUEIRA[3], Carlos Rocha Gomes TORRES[2], Alessandra Buhler BORGES[2]

1 - Faculdade de Odontologia da Universidade Braz Cubas, Mogi das Cruzes, Brazil.
2 - Instituto de Ciência e Tecnologia da UNESP, Departamento de Odontologia Restauradora, São José dos Campos, Brazil.
3 - Instituto de Ciência e Tecnologia da UNESP, Departamento de Biociências e Diagnóstico Oral, São José dos Campos, Brazil.

How to cite: Spinola MS, Mendonça JL, Garcia MT, Caneppele TMF, Junqueira JC, Torres CRG, et al. Antimicrobial efficacy of S-PRG containing toothpastes on *S. mutans* biofilm development. Braz Dent Sci. 2023;26(1):e3672. https://doi.org/10.4322/bds.2023.e3672

ABSTRACT

Objective: to investigate the antimicrobial effects of toothpastes containing bioactive surface pre-reacted glass particles (S-PRG) on *S. mutans* biofilms adherence, initial colonization and maturation. **Material and Methods:** a reference UA 159 and a clinical *S. mutans* (SM6) strain were used. Bovine enamel specimens were randomly allocated into the groups (n=5): toothpastes containing 0%; 1%; 5%; 20%; 30% S-PRG; positive control dentifrice (NaF+triclosan); and negative control (distilled water). For biofilm development, samples were placed in a 24-well plate containing artificial saliva (4h), followed by adding 1mL of artificial saliva, BHI broth and 225μL of *S. mutans* suspension. Treatments with toothpastes were applied previously or after 4h and 24h of biofilm formation. Samples were incubated for 48h at 37°C in 5%CO2 and biofilm was detached and seeded in Petri dishes for determining the number of viable cells. Data were analyzed by ANOVA and Tukey test (5%). **Results:** significantly lower microorganisms' adherence ($p<0.05$) was obtained for all S-PRG toothpastes, with similar results to NaF+triclosan for SM6 and 20 and 30%S-PRG groups exhibiting higher inhibition effect than the NaF+Triclosan for UA159. Antibacterial effect on the early-stage biofilm was also observed for the S-PRG groups, but was not superior to the NaF+Triclosan toothpaste. For the mature biofilm, the effective antimicrobial potential of S-PRG toothpastes was observed only for the SM6 clinical strain, but was not higher than the positive control. **Conclusion:** experimental S-PRG toothpastes were effective to inhibit *S. mutans* biofilm growth by exhibiting antimicrobial activity, being promising agents to prevent cariogenic biofilm development.

KEYWORDS

Biofilm; Dental enamel; Giomer; *Streptococcus mutans*, S-PRG.

RESUMO

Objetivo: investigar o efeito de dentifrícios contendo S-PRG sobre a colonização inicial e maturação de biofilmes de *S. mutans*. **Material e Métodos:** uma cepa de referência (UA 159) e uma cepa clínica de *S. mutans* (SM6) foram utilizadas. Espécimes de esmalte bovino foram alocados nos grupos (n=5): dentifrícios contendo 0%; 1%; 5%; 20% e 30%S-PRG; controle positivo (NaF+triclosan); e controle negativo (água destilada). Os espécimes foram inseridos em uma placa de 24 poços contendo saliva artificial (4h), seguido por adição de 1mL de saliva artificial, BHI, 225μL de suspensão de *S. mutans* e foram tratados com suspensões de dentifrícios antes ou depois de 4 e 24h da formação do biofilme. Os espécimes foram incubados por 48h e o biofilme foi removido dos espécimes e semeado em placas de Petri para contagem de UFC/mL. Os dados foram analisados por ANOVA e teste de Tukey (5%). **Resultados:** houve diminuição na adesão de microrganismos para os grupos tratados com S-PRG ($p<0.05$). Para SM6, os dentifrícios contendo S-PRG apresentaram resultados semelhantes ao NaF+triclosan e para a cepa

UA159 o dentifrício com 30%S-PRG apresentou efeito superior. Efeito antimicrobiano no biofilme recém-formado (4h) foi observado para os grupos contendo S-PRG, mas não foi observado efeito superior ao NaF+Triclosan. Para o biofilme maduro, efeito antimicrobiano do S-PRG foi observado apenas para a cepa clínica, mas não superior ao efeito do NaF+Triclosan. **Conclusão:** dentifrícios contendo S-PRG foram eficazes na inibição do desenvolvimento de biofilmes de *S. mutans,* sendo promissores agentes para prevenir o desenvolvimento de biofilme cariogênico.

PALAVRAS-CHAVE

Biofilme; Esmalte dental; Giomer; *Streptococcus mutans*; S-PRG.

INTRODUCTION

Dental caries is a biofilm and sugar-dependent disease that results in mineral loss of tooth surface. The control of the disease is directly related to the biofilm monitoring, which can be achieved by means of a controlled diet, with less intakes of carbohydrates, and with efficient oral hygiene, to disaggregate the cariogenic biofilm formed over the tooth surface [1,2]. Although these methods are well established, and the prevalence and severity of dental caries have declined in recent years, the cariogenic biofilm tends to remain in regions that are difficult to access by brushing, such as the occlusal pit and fissures, and interproximal regions, making it still one of the most prevalent diseases at a world level [3].

Strategies to improve the control of the acidogenic microorganisms that are prevalent in the cariogenic biofilm using products with multiple biological effects such as antibacterial properties and biofilm formation inhibition have been tested [4,5].

The surface pre-reacted glass ionomer (S-PRG) filler is an innovative bioactive material composed by a 3-layer structure derived from a proprietary technology. A fluoroboroaluminosilicate multifunctional glass is covered with polysiloxane, creating a porous superficial silica-glassy layer. Then, a polyacrylic acid solution is sprayed on the particle and penetrates the surface porosities, reacting with the inner glass core. This creates a thin intermediate stable pre-reacted glass ionomer phase [6]. The dental products containing S-PRG fillers are named GIOMER, due to their "Glass IOnoMER" phase. The bioactive effect of these materials is exerted by the multi-ions release (fluoride, strontium, borate, sodium, aluminum, and silicate) derived from the multifunctional glass in the presence of moisture [7].

These fillers can be incorporated into preventive and restorative materials. The antimicrobial/anticaries/remineralizing/desensitizing effects have been shown with surface barrier materials, varnishes, and sealants [8-11]. Favorable results have been observed regarding the antimicrobial activity of restorative materials using Giomer technology [12,13], besides the ability to reduce the demineralization of dentin adjacent to restorations [14], and exert acid-buffering properties, which might reduce the susceptibility to secondary caries [15].

This motivated the development of toothpastes containing the S-PRG particles, providing the benefit of releasing the multi-ions into the oral environment, as an alternative for caries prevention. Studies have shown that experimental S-PRG based toothpastes were effective on the enamel demineralization prevention [16-18]. However, despite previous studies present promising results regarding the addition of S-PRG to dental products, little is still known about the ideal concentration of these particles in dentifrices concerning their antimicrobial efficacy on dental cariogenic biofilms, aiming to promote strong scientific evidence so that commercial products can be developed and be further available in the market. Thus, the present study reports the findings on the inhibitory effect of experimental dentifrices containing different concentrations of S-PRG on adherence, initial colonization and maturation phases of *S. mutans* biofilms. The null hypothesis tested was that there is no difference in the *S. mutans* biofilm inhibition among the toothpastes in the different phases tested.

MATERIAL AND METHODS

Ethical aspects

The study protocol was approved by the local Ethics Committee in Research (CAAE:005611/2017).

Microorganisms

In this study, one reference *S. mutans* strain (UA159) and one clinical strain were used. The clinical strain was previously isolated from the active cavities of a subject according to Carvalho et al. (2006) [19], was identified by PCR [20], and confirmed by automatic sequencing [21], named SM6.

Study design

Six independent experiments were conducted (three for the *S. mutans* UA159 strain, and three for the SM6 strain). Each experiment presented one factor under investigation (treatment toothpastes) at 7 levels (distilled water as negative control (DW); commercial NaF+Triclosan-based toothpaste as positive control; and toothpastes containing S-PRG: 0%; 1%, 5%, 20%, and 30%). For both strains, the following effects were investigated: the inhibitory effect on biofilm adherence, initial colonization and maturation (n = 5/group). UFC/mL was the response variable.

Specimens' preparation

Fresh, non-damaged bovine incisors were collected for this study. The crowns were separated from the roots and stored into 0.1% thymol solution at 4°C until required. Seventy cylindrical enamel specimens (6 mm in diameter) were obtained from the labial surface of the teeth, using a custom-made diamond-coated trephine mill adapted to a circular cutting machine. The enamel specimens were polished using SiC sandpapers in sequential grits of 1200, 2400, and 4000 (FEPA-P, Struers, Copenhagen, Denmark), under constant water irrigation for 10, 20 and 30 s, respectively, using an automatic polishing machine (Tegramin 25, Struers). After each paper grit change, specimens were kept in an ultrasonic bath for 5 min, to remove debris and abrasive grains. The specimens were examined under a stereomicroscope (Carl Zeiss – Stemi 2000 - 20X) to ensure the absence of cracks or other surface defects [22]. The specimens were autoclaved before the beginning of the experiments.

Preparation of the standardized suspension of *S. mutans*

The microorganisms were cultured in brain-heart infusion broth (BHI Himedia, Mumbai, India) at 37°C for 48 h (5% CO_2). The microbial cells in the culture were centrifuged (1300 rpm for 10 min), and the pellet was rinsed twice with 0.85% NaCl (Labimpex, Sao Paulo, Brazil). The cell suspensions were adjusted to 10^8 cells/mL using a spectrophotometer at a wavelength of 398 nm and optical density of 0.620 (B5B2, Micronal, São Paulo, Brazil) [23].

Groups division

For each experiment, testing both the reference and the clinical strain, the specimens were divided in seven groups (n = 5 each). The experimental toothpastes were prepared by Shofu Inc. (Kyoto, Japan), and contained silicic anhydride, sodium carboxymethylcellulose, glycerol, sorbitol solution, perfume, and 1 μm S-PRG particles: 0% (placebo), 1%, 5%, 20%, and 30% (in weight). The distilled water was the negative control and a commercial toothpaste containing NaF (1450 ppm F^-), Triclosan, PVM/MA copolymer, sodium lauryl sulfate, sorbitol, carrageenan, aroma, and hydrated silica (Colgate Total 12, Colgate-Palmolive, São Paulo, Brazil) was the positive control.

S. mutans biofilm formation

Specimens were placed in 24-well plates containing 2 mL of artificial saliva containing 0.33g KH_2PO_4; 0.34g Na_2HPO_4; 1.27g KCl; 0.16g NaSCN; 0.58g NaCl; 0.17g CaCl, 0.16g NH_4Cl; 0.2g urea; 0.03g glucose; 0.002g ascorbic acid and 2.7g mucin [24] and incubated for 4 h at 37°C to allow the formation of a surface pellicle. After 4h, specimens were rinsed twice with 2 mL of PBS solution with the use of a pipette and then 1 mL of BHI broth supplemented with 5% sucrose and 1 mL artificial saliva were added to each well. Then, 225 μL of the standardized *S. mutans* suspension was added to each well, and the plates were incubated for 24 h at 37°C (5% CO_2). The wells were washed three times with 2 mL PBS for the removal of weakly adhered cells with the use of a pipette, and 1 mL of fresh BHI broth supplemented with 5% sucrose and 1 mL of fresh artificial saliva was again added to each well. The plates were incubated for an additional 24 h at 37°C.

Antibacterial potential of the toothpastes on *S. mutans* biofilm development

Specimens were treated with dentifrices slurries prepared in artificial saliva (1:3) for

5 min in 3 different phases: before the immersion in artificial saliva to evaluate the effect of the treatments on the microorganisms adherence phase, after 4 h of biofilm growth to evaluate the antibacterial potential over the early-stage biofilm, and after 24 h of biofilm growth to evaluate the antibacterial potential over biofilm maturation.

Cell count for biofilm analysis

After final incubation, all specimens (treated with the slurries before, after 4h and 24h of biofilm growth) were transferred to Falcon tubes containing 4 mL PBS. The biofilm was then detached from the specimens using an ultrasonic homogenizer (Sonoplus KD2200, Bandelin Eletronic, Berlin, Germany) at 7 W for 30 s. Serial dilutions were prepared from the obtained solution and plated on Petri dishes containing BHI agar. The plates were incubated for 48 h at 37°C (5% CO_2) to determine the number of colony-forming units (CFU/mL) [23].

Statistical analysis

Data of CFU/mL were transformed in log10 and means ± standard deviation were calculated for each treatment. The Shapiro-Wilk and Brown-Forsythe tests were used to assess the normality and homoscedasticity of the data. They were then analysed by one-way ANOVA followed by Tukey's test with a p-value set at 5%, using Statistica for Windows software (StatSoft, Hamburg, Germany).

RESULTS

Potential of the toothpastes on *S. mutans* adherence

Figure 1a-1b show mean and standard deviation data for the tested groups, using the reference UA159 and the SM6 clinical strains, respectively. The results of ANOVA one-way indicated significant difference for the groups ($p<0.05$). The inhibiting effect was higher when more concentrated toothpastes were used, indicating a dose-dependent effect. According to Tukey's test, the toothpastes containing S-PRG were capable to inhibit *S. mutans* adherence when compared to the negative control ($p<0.05$) for both strains. For UA159 strain, both 20 and 30% S-PRG toothpastes showed significantly lower *S. mutans* adherence than the positive commercial toothpaste containing NaF and Triclosan. For SM6 strain, 1 to 30% were similar to the positive control. The 0% S-PRG toothpaste presented no significant *S. mutans* adherence inhibition potential compared to negative control ($p>0.05$).

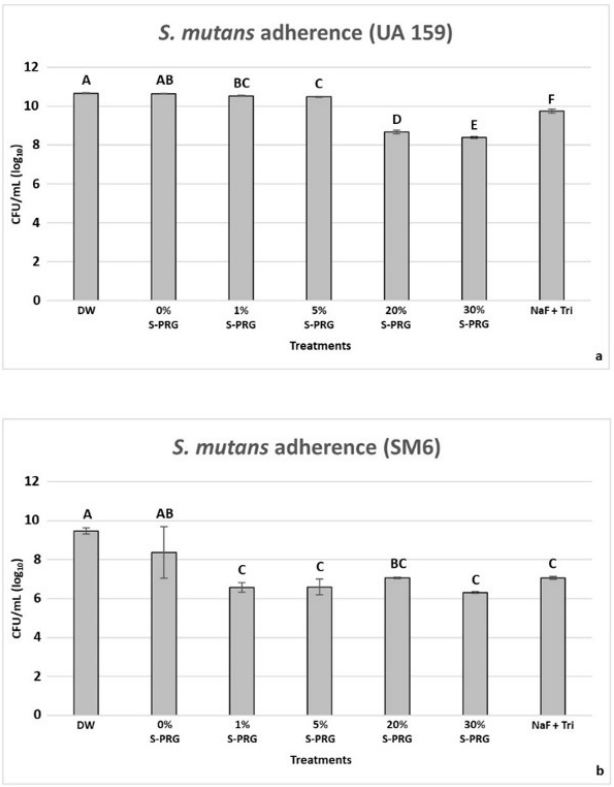
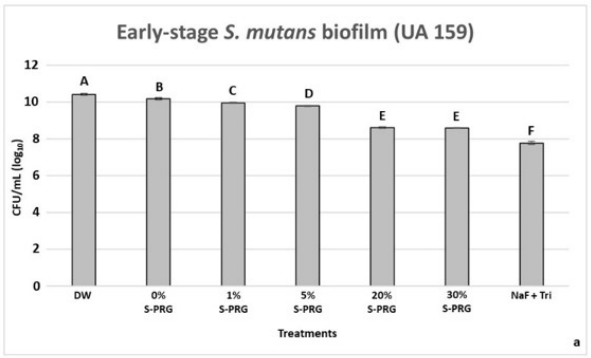


Figure 1 – Mean CFU/mL (log_{10}) and standard deviation data when treatments were applied previously to *S. mutans* adherence (before biofilm formation) over the UA159 reference (a) and SM6 clinical (b) strains. Different upper case letters mean significant statistical differences.

Antibacterial potential of the toothpastes on early-stage *S. mutans* biofilm

The mean and standard deviation data for the treatments applied after 4 h of reference and the clinical *S. mutans* strains biofilm formation are shown in Figure 2a-2b, respectively. ANOVA one-way indicated significant difference for the tested groups ($p<0.05$). According to Tukey's test results, all S-PRG containing toothpastes were significantly different from the negative control for both strains ($p<0.0001$). The most effective products were the 20% and 30% S-PRG toothpastes. Nevertheless, all were significantly different from the positive commercial product (NaF + Triclosan) that showed the higher antimicrobial effect against early-stage biofilms ($p<0.0001$).



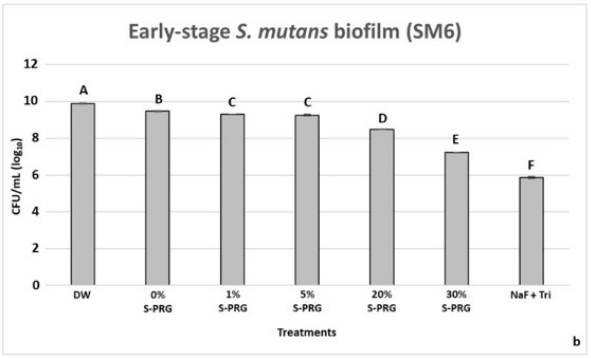
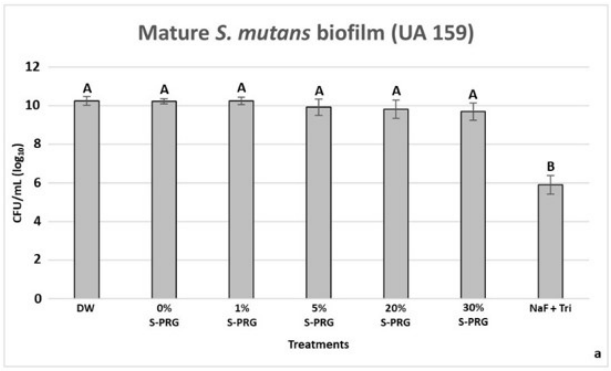


Figure 2 – Mean CFU/mL (log_{10}) and standard deviation data for the antibacterial potential of treatments over the early-stage *S. mutans* biofilms (after 4 h of biofilm growth) cultivated from UA159 reference (a) and SM6 clinical (b) strains. Different upper case letters mean significant statistical differences.



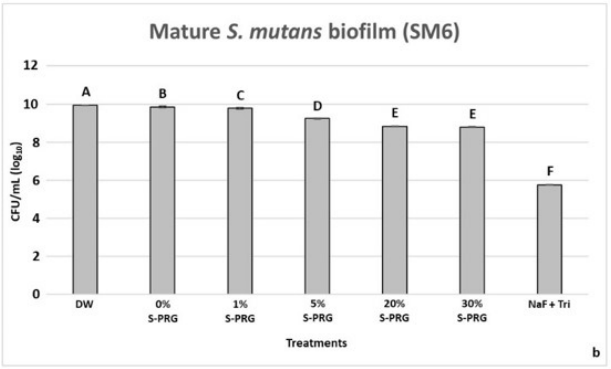


Figure 3 – Mean CFU/mL (log_{10}) and standard deviation data for the antibacterial potential of treatments over *S. mutans* biofilms maturation (after 24 h of biofilm growth) cultivated from UA159 reference (a) and SM6 clinical (b) strains. Different upper case letters mean significant statistical differences.

Antibacterial potential of the toothpastes on mature *S. mutans* biofilm

The mean and standard deviation data of cell counts for the treatments applied after 24 h of reference and clinical *S. mutans* biofilm formation are shown in Figure 3a-3b, respectively. ANOVA one-way showed significant difference for the groups ($p<0.05$). According to the Tukey's test, there were no significant differences for the S-PRG containing toothpastes compared to the negative control, when reference UA159 strain was tested ($p>0.05$). For the SM6 clinical strain, the experimental toothpastes exhibited significant antimicrobial effect compared to the negative control, with higher efficacy when 20 and 30% S-PRG were used, but they were not superior than the commercial NaF toothpaste used as positive control ($p<0.05$).

DISCUSSION

The effect of toothpastes containing different concentrations of S-PRG was evaluated at three distinct moments: before, after 4 h, and after 24 h of biofilm development, to represent the different clinical situations found in the oral cavity. The five minutes treatment time was chosen to favour the effect of the toothpastes in this in vitro model, since only one application was performed. A standard strain of *S. mutans* (UA 159) was used because this microorganism represents one of the most important microorganisms in the aetiology of caries disease [25]. Additionally, a clinical strain, previously isolated from dental caries (SM6), was used in order to extend the knowledge of the antimicrobial properties of the S-PRG toothpastes to a more real condition [26]. The model used here for biofilm growth has been widely used in the microbiology and cariology areas [23,27] to simulate the conditions of a cariogenic biofilm due to the presence of 5% sucrose added to the culture medium. The biofilm was cultivated over bovine tooth specimens. The use of bovine specimens in substitution of human in caries lesion studies is a viable alternative due to their similar mineral content [28] and biofilm cariogenicity pattern with both substrates [29]. Besides, they present great advantages, such as easier acquisition and the presence of a large and flat surface to obtain the specimens.

The null hypothesis was rejected, as the toothpastes containing S-PRG were able to inhibit the adherence of the microorganisms and all the concentrations tested reduced the development of the early-stage biofilm. The toothpastes containing 20% and 30% were superior to the positive control in inhibiting biofilm development for the standard strain, while toothpastes from 1 – 30% were had similar effect to the positive control in inhibiting biofilm development for the clinical strain. These results agree with previous studies, in which the ions released from S-PRG in composite materials [13,30] and its eluate [31,32] inhibited the growth of *S. mutans* biofilm. Inhibition of initial microorganisms is an important factor in prevention of the biofilm development because coaggregation influences the properties of plaque [33]. The observed inhibition of biofilm growth and effect on the newly formed biofilm are probably associated with the release of BO_3^{3-}, F^- and Sr^{2+} as it has been suggested that these ions are able to diffuse into the biofilm [34].

It is known that BO_3^{3-} presents great ability to prevent bacterial growth, because it acts by inhibiting quorum sensing inside bacteria [35]. Quorum sensing is characterized as a highly specific system that controls different bacterial activities such as the production of signalling molecules, transport, and perception of the surrounding environment. This system is responsible for the process of bacterial adhesion to surfaces, the production of the extracellular polysaccharide matrix, and the virulence of the biofilm [36,37].

The F^- ions are inhibitors of carbohydrate metabolism in oral Streptococci [38] because they can penetrate the cell and bind to enolase and ATPase, inhibiting these enzymes and leading to the reduction of carbohydrate metabolism [39-41]. The inhibition of enolase causes a reduction in phosphoenolpyruvate levels and, consequently, a reduction in glucose uptake by phosphoenolpyruvate phosphotransferase system [42,43] while the inhibition of ATPase causes ineffective extrusions of protons [44], leading to an acidification of cell cytoplasm and reduced metabolism and acid tolerance by the cells [41,45].

Regarding the Sr^{2+} released from the S-PRG fillers, the cariostatic activity caused by the presence of such ions in oral products is not completely understood, since it seems that its concentration in saliva and plaque is usually not able to exert an important antimicrobial effect [46]. Nevertheless, the release of Sr^{2+} might represent an important ally in products containing S-PRG, since its cariostatic properties may act synergistically to F^- to inhibit bacterial activity [47].

In mature biofilms (24h), S-PRG toothpastes had no effect on the standard strain, but all concentrations of S-PRG were able to decrease the number of bacteria in the biofilm cultivated from the clinical strain SM6. Nevertheless, this effect was lower than the NaF + triclosan toothpaste. Triclosan, in the concentration of 0.3% in the dentifrice used as the positive control, has well-established antimicrobial properties when added to toothpastes to act against dental plaque [48], although it has been replaced in the recently launched toothpastes due to its potential hormonal disfunction properties [49]. This active agent may be released in a higher concentration than the ions present in the S-PRG particle, to exert its antimicrobial effect on the mature biofilm, but this was not tested in the present study.

It was previously shown that the inhibitory effect of a S-PRG eluate was less pronounced when the eluate was applied in the post-logarithmic phase of biofilm growth, suggesting that the main action of these bioactive particles is related to the inhibition of *S. mutans* virulence and growth [32]. The fact that S-PRG could decrease the number of bacteria in the clinical strain, but not in the standard strain may be related to the fact that *S. mutans* standard strain UA 159 is known to be a highly cariogenic strain, besides being adapted to the culture medium since it is the strain of choice for many studies in cariology. The difference between the cariogenicity and adaptation potential of the standard strain UA159 and the clinical strain SM6 is clear when we observed that for the three types of biofilms tested, the products presented better results with the clinical strain.

These less favourable results observed with the mature biofilms can be explained by the fact that the dental products containing S-PRG particles, in general, are characterized as agents that constantly release the ions present in their composition. However, the amount of ions released from the experimental toothpastes

might not be high enough to act in the rupture of a mature biofilm. In addition, the short time of exposure to the treatments and the dilution of the products in artificial saliva prior to the treatment of enamel specimens, to simulate the dilution caused by saliva in the oral cavity, may also have influenced the efficacy of the products. Previous studies have shown that low concentrations of S-PRG in eluates, for example, were not effective on mature biofilm and that higher concentrations of these products were needed to cause a rupture in the mature biofilm when compared to the concentration to inhibit the biofilm development [31]. This may be due to the fact that the properties of a mature biofilm are completely different when compared to a newly formed biofilm. In a mature cariogenic biofilm, formed by the interaction between bacteria and sucrose, we can observe the presence of an extracellular polysaccharide matrix that is responsible for its virulence and that interferes with the physical and chemical properties of the biofilm [50,51].

The presence of the extracellular matrix promotes a support for the development of the biofilm because it encourages bacterial adhesion, besides hindering the diffusion of substrates from the medium to the interior of the biofilm [50], thus impairing the action of the ions inside the biofilm.

The results obtained with the experimental toothpastes containing S-PRG are promising, especially those with higher concentrations of particles in their composition. Nevertheless, more studies are needed to confirm the action of these agents on the dental biofilm in more relevant clinical conditions.

CONCLUSION

Experimental dentifrices containing S-PRG showed antimicrobial activity mainly on microorganisms' adherence and on early-stage *S. mutans* biofilm, being promising agents to prevent *S. mutans* biofilm growth and development.

Acknowledgements

The authors wish to express thanks to Shofu Inc., Japan for providing the materials for this study, and to CAPES and CNPq (processes 38882.434264/2019-01 and 51256/2019) for the scholarships granted.

Author's Contributions

MSP, JCJ, CRGT, ABB: Conceptualization. MSP, JLM, MTG, JCJ: Methodology. MSP, MTG, ABB: Formal Analysis. ABB: Resources. MSP, ABB: Writing – Original Draft Preparation. MSP, TMFC, JCJ, CRGT, ABB: Writing – Review & Editing. ABB: Supervision. ABB: Project Administration.

Conflict of Interest

No conflicts of interest declared concerning the publication of this article.

Funding

Part of this study was funded by CNPq (process 51256/2019).

Regulatory Statement

The study protocol was approved by the local Ethics Committee in Research and the approval code for this study is: CAAE:005611/2017.

REFERENCES

1. Takahashi K, Araujo HC, Pessan J, Munhoz FC, Jardim EG Jr, Cunha RF. Microorganisms related to early childhood caries in a sample of an oral preventive-educative program: a longitudinal study. Braz Dent Sci. 2019;22(2):267-74. http://dx.doi.org/10.14295/bds.2019.v22i2.1723.
2. Machiulskiene V, Campus G, Carvalho JC, Dige I, Ekstrand KR, Jablonski-Momeni A, et al. Terminology of dental caries and dental caries management: consensus report of a workshop organized by ORCA and Cariology Research Group of IADR. Caries Res. 2020;54(1):7-14. http://dx.doi.org/10.1159/000503309. PMid:31590168.
3. Frencken J. Caries epidemiology and its challenges. Monogr Oral Sci. 2018;27:11-23. http://dx.doi.org/10.1159/000487827. PMid:29794449.
4. Hickl J, Argyropoulou A, Sakavitsi ME, Halabalaki M, Al-Ahmad A, Hellwig E, et al. Mediterranean herb extracts inhibit microbial growth of representative oral microorganisms and biofilm formation of Streptococcus mutans. PLoS One. 2018;13(12):e0207574. http://dx.doi.org/10.1371/journal.pone.0207574. PMid:30540782.
5. Philip N, Bandara HMHN, Leishman SJ, Walsh LJ. Inhibitory effects of fruit berry extracts on Streptococcus mutans biofilms. Eur J Oral Sci. 2019;127(2):122-9. http://dx.doi.org/10.1111/eos.12602. PMid:30592324.
6. Nakatsuka T, Yasuda Y, Kimoto K, Mizuno MNN. Kabushiki Kaisha Shofu assignee. United States patent. Dental fillers. Alexandria: United States Patent and Trademark Office; 2003.
7. Fujimoto Y, Iwasa M, Murayama R, Miyazaki M, Nagafuji A, Nakatsuka T. Detection of ions released from S-PRG fillers and their modulation effect. Dent Mater J. 2010;29(4):392-7. http://dx.doi.org/10.4012/dmj.2010-015. PMid:20610878.
8. Moecke SE, Silva AGCS, Andrade ACM, Borges AB, Torres CRG. Efficacy of S-PRG filler varnishes on enamel caries

remineralization. J Dent. 2022;119:104074. http://dx.doi.org/10.1016/j.jdent.2022.104074. PMid:35218877.

9. Spinola MS, Moecke SE, Rossi NR, Nakatsuka T, Borges AB, Torres CRG. Efficacy of S - PRG filler containing varnishes on enamel demineralization prevention. Sci Rep. 2020;10(1):18992. http://dx.doi.org/10.1038/s41598-020-76127-w.

10. Shimazu K, Ogata K, Karibe H. Caries-preventive effect of fissure sealant containing surface reaction-type pre-reacted glass ionomer filler and bonded by self-etching primer. J Clin Pediatr Dent. 2012;36(4):343-7. http://dx.doi.org/10.17796/jcpd.36.4.n444r730r773un53. PMid:23019829.

11. Agulhari MAS, Giacomini MC, Rios D, Bombonatti JFS, Wang L. Giomer technology for preventive and restorative clinical management of erosive tooth wear: a case report. Braz Dent Sci. 2022;25(2):e3162. http://dx.doi.org/10.4322/bds.2022.e3162.

12. Saku S, Kotake H, Scougall-Vilchis RJ, Ohashi S, Hotta M, Horiuchi S, et al. Antibacterial activity of composite resin with glass-ionomer filler particles. Dent Mater J. 2010;29(2):193-8. http://dx.doi.org/10.4012/dmj.2009-050. PMid:20379030.

13. Miki S, Kitagawa H, Kitagawa R, Kiba W, Hayashi M, Imazato S. Antibacterial activity of resin composites containing surface pre-reacted glass-ionomer (S-PRG) filler. Dent Mater. 2016;32(9):1095-102. http://dx.doi.org/10.1016/j.dental.2016.06.018. PMid:27417376.

14. Shiiya T, Tomiyama K, Iizuka J, Hasegawa H, Kuramochi E, Fujino F, et al. Effects of resin-based temporary filling materials against dentin demineralization. Dent Mater J. 2016;35(1):70-5. http://dx.doi.org/10.4012/dmj.2015-135. PMid:26830825.

15. Kaga M, Kakuda S, Ida Y, Toshima H, Hashimoto M, Endo K, et al. Inhibition of enamel demineralization by buffering effect of S-PRG filler-containing dental sealant. Eur J Oral Sci. 2014;122(1):78-83. http://dx.doi.org/10.1111/eos.12107. PMid:24372898.

16. Amaechi BT, Kasundra H, Joshi D, Abdollahi A, Azees PAA, Okoye LO. Effectiveness of S-PRG filler-containing toothpaste in inhibiting demineralization of human tooth surface. Open Dent J. 2018;12(1):811-9. http://dx.doi.org/10.2174/1874210601812010811. PMid:30505361.

17. Iijima M, Kawaguchi K, Kawamura N, Ito S, Saito T, Mizoguchi I. The effects of single application of pastes containing ion-releasing particles on enamel demineralization. Dent Mater J. 2017;36(4):461-8. http://dx.doi.org/10.4012/dmj.2016-307. PMid:28367912.

18. Nakamura K, Hamba H, Nakashima S, Sadr A, Nikaido T, Oikawa M, et al. Effects of experimental pastes containing surface pre-reacted glass ionomer fillers on inhibition of enamel demineralization. Dent Mater J. 2017;36(4):482-90. http://dx.doi.org/10.4012/dmj.2016-303. PMid:28367910.

19. Carvalho FG, Silva DS, Hebling J, Spolidorio LC, Spolidorio DMP. Presence of mutans streptococci and Candida spp. in dental plaque/dentine of carious teeth and early childhood caries. Arch Oral Biol. 2006;51(11):1024-8. http://dx.doi.org/10.1016/j.archoralbio.2006.06.001. PMid:16890907.

20. Oho T, Yamashita Y, Shimazaki Y, Kushiyama M, Koga T. Simple and rapid detection of Streptococcus mutans and Streptococcus sobrinus in human saliva by polymerase chain reaction. Oral Microbiol Immunol. 2000;15(4):258-62. http://dx.doi.org/10.1034/j.1399-302x.2000.150408.x. PMid:11154412.

21. Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F, et al. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res. 2008;36(Suppl 2):W465-9. http://dx.doi.org/10.1093/nar/gkn180. PMid:18424797.

22. Borges AB, Santos LFTF, Augusto MG, Bonfiette D, Hara AT, Torres CRG. Toothbrushing abrasion susceptibility of enamel and dentin bleached with calcium-supplemented hydrogen peroxide gel. J Dent. 2016;49:54-9. http://dx.doi.org/10.1016/j.jdent.2016.03.009. PMid:27072568.

23. Garcia MT, Pereira AHC, Figueiredo-Godoi LMA, Jorge AOC, Strixino JF, Junqueira JC. Photodynamic therapy mediated by chlorin-type photosensitizers against Streptococcus mutans biofilms. Photodiagnosis Photodyn Ther. 2018;24:256-61. http://dx.doi.org/10.1016/j.pdpdt.2018.08.012. PMid:30157462.

24. Klimek J, Hellwig E, Ahrens G. Fluoride taken up by plaque, by the underlying enamel and by clean enamel from three fluoride compounds in vitro. Caries Res. 1982;16(2):156-61. http://dx.doi.org/10.1159/000260592. PMid:6951638.

25. Takahashi N, Nyvad B. The role of bacteria in the caries process: ecological perspectives. J Dent Res. 2011;90(3):294-303. http://dx.doi.org/10.1177/0022034510379602. PMid:20924061.

26. Terra-Garcia M, Souza CM, Gonçalves NMF, Pereira AHC, Barros PP, Borges AB, et al. Antimicrobial effects of photodynamic therapy with Fotoenticine on Streptococcus mutans isolated from dental caries. Photodiagnosis Photodyn Ther. 2021;34:102303. http://dx.doi.org/10.1016/j.pdpdt.2021.102303. PMid:33887495.

27. Pereira CA, Costa ACBP, Carreira CM, Junqueira JC, Jorge AOC. Photodynamic inactivation of Streptococcus mutans and Streptococcus sanguinis biofilms in vitro. Lasers Med Sci. 2013;28(3):859-64. http://dx.doi.org/10.1007/s10103-012-1175-3. PMid:22847685.

28. Edmunds DH, Whittaker DK, Green RM. Suitability of human, bovine, equine, and ovine tooth enamel for studies of artificial bacterial carious lesions. Caries Res. 1988;22(6):327-36. http://dx.doi.org/10.1159/000261132. PMid:3214846.

29. Ayoub HM, Gregory RL, Tang Q, Lippert F. Comparison of human and bovine enamel in a microbial caries model at different biofilm maturations. J Dent. 2020;96:103328. http://dx.doi.org/10.1016/j.jdent.2020.103328. PMid:32240676.

30. Hotta M, Morikawa T, Tamura D, Kusakabe S. Adherence of Streptococcus sanguinis and Streptococcus mutans to saliva-coated S-PRG resin blocks. Dent Mater J. 2014;33(2):261-7. http://dx.doi.org/10.4012/dmj.2013-242. PMid:24615002.

31. Suzuki N, Yoneda M, Haruna K, Masuo Y, Nishihara T, Nakanishi K, et al. Effects of S-PRG eluate on oral biofilm and oral malodor. Arch Oral Biol. 2014;59(4):407-13. http://dx.doi.org/10.1016/j.archoralbio.2014.01.009. PMid:24530472.

32. Nomura R, Morita Y, Matayoshi S, Nakano K. Inhibitory effect of surface pre-reacted glass-ionomer (S-PRG) eluate against adhesion and colonization by Streptococcus mutans. Sci Rep. 2018;8(1):5056. http://dx.doi.org/10.1038/s41598-018-23354-x. PMid:29568011.

33. Huang R, Li M, Gregory RL. Bacterial interactions in dental biofilm. Virulence. 2011;2(5):435-44. http://dx.doi.org/10.4161/viru.2.5.16140. PMid:21778817.

34. Kato K, Tamura K, Shimazaki Y. Oral biofilm uptake of mineral ions released from experimental toothpaste containing surface pre-reacted glass-ionomer (S-PRG) filler. Arch Oral Biol. 2020;117:104777. http://dx.doi.org/10.1016/j.archoralbio.2020.104777. PMid:32592930.

35. Dembitsky VM, Quntar AAA, Srebnik M. Natural and synthetic small boron-containing molecules as potential inhibitors of bacterial and fungal quorum sensing. Chem Rev. 2011;111(1):209-37. http://dx.doi.org/10.1021/cr100093b. PMid:21171664.

36. Nadell CD, Xavier JB, Levin SA, Foster KR. The evolution of quorum sensing in bacterial biofilms. PLoS Biol. 2008;6(1):e14. http://dx.doi.org/10.1371/journal.pbio.0060014. PMid:18232735.

37. Sifri CD. Quorum sensing: bacteria talk sense. Clin Infect Dis. 2008;47(8):1070-6. http://dx.doi.org/10.1086/592072. PMid:18781869.

38. Bibby B, Van Kesteren M. The effect of fluorine on mouth bacteria. J Dent Res. 1940;19(4):391-402. http://dx.doi.org/10.1177/00220345400190040601.

39. Marquis RE. Diminished acid tolerance of plaque bacteria caused by fluoride. J Dent Res. 1990;69(Suppl 2):672-5. http://dx.doi.org/10.1177/00220345900690S130. PMid:2138181.

40. Marquis RE, Clock SA, Mota-Meira M. Fluoride and organic weak acids as modulators of microbial physiology. FEMS Microbiol Rev. 2003;26(5):493-510. http://dx.doi.org/10.1111/j.1574-6976.2003.tb00627.x. PMid:12586392.

41. Van Loveren C, Hoogenkamp MA, Deng DM, Cate JMT. Effects of different kinds of fluorides on enolase and ATPase activity of a fluoride-sensitive and fluoride-resistant Streptococcus mutans strain. Caries Res. 2008;42(6):429-34. http://dx.doi.org/10.1159/000159606. PMid:18832829.

42. Kanapka JA, Hamilton IR. Fluoride inhibition of enolase activity in vivo and its relationship to the inhibition of glucose-6-P formation in Streptococcus salivarius. Arch Biochem Biophys. 1971;146(1):167-74. http://dx.doi.org/10.1016/S0003-9861(71)80053-X. PMid:5144023.

43. Hamilton IR. Effects of fluoride on enzymatic regulation of bacterial carbohydrate metabolism. Caries Res. 1977;11(Suppl 1):262-91. http://dx.doi.org/10.1159/000260304. PMid:318573.

44. Quivey RG Jr, Kuhnert WL, Hahn K. Adaptation of oral streptococci to low pH. Adv Microb Physiol. 2000;42:239-74. http://dx.doi.org/10.1016/S0065-2911(00)42004-7. PMid:10907552.

45. Sheng J, Marquis RE. Enhanced acid resistance of oral streptococci at lethal pH values associated with acid-tolerant catabolism and with ATP synthase activity. FEMS Microbiol Lett. 2006;262(1):93-8. http://dx.doi.org/10.1111/j.1574-6968.2006.00374.x. PMid:16907744.

46. Lippert F, Hara AT. Strontium and caries: a long and complicated relationship. Caries Res. 2013;47(1):34-49. http://dx.doi.org/10.1159/000343008. PMid:23051661.

47. Dabsie F, Gregoire G, Sixou M, Sharrock P. Does strontium play a role in the cariostatic activity of glass ionomer? Strontium diffusion and antibacterial activity. J Dent. 2009;37(7):554-9. http://dx.doi.org/10.1016/j.jdent.2009.03.013. PMid:19410352.

48. Valkenburg C, Van der Weijden FA, Slot DE. Plaque control and reduction of gingivitis: the evidence for dentifrices. Periodontol 2000. 2019;79(1):221-32. http://dx.doi.org/10.1111/prd.12257. PMid:30892760.

49. Zorrilla LM, Gibson EK, Jeffay SC, Crofton KM, Setzer WR, Cooper RL, et al. The effects of triclosan on puberty and thyroid hormones in male wistar rats. Toxicol Sci. 2009;107(1):56-64. http://dx.doi.org/10.1093/toxsci/kfn225. PMid:18940961.

50. Flemming HC, Wingender J. The biofilm matrix. Nat Rev Microbiol. 2010;8(9):623-33. http://dx.doi.org/10.1038/nrmicro2415. PMid:20676145.

51. Koo H, Falsetta ML, Klein MI. The exopolysaccharide matrix: a virulence determinant of cariogenic biofilm. J Dent Res. 2013;92(12):1065-73. http://dx.doi.org/10.1177/0022034513504218. PMid:24045647.

Taciana Marco Ferraz Caneppele
(Corresponding address)
Departamento de Odontologia Restauradora, Instituto de Ciência e Tecnologia da UNESP, São José dos Campos, Brasil.
Email: taciana.caneppele@unesp.br

Date submitted: 2022 Oct 02
Accept submission: 2023 Jan 21