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.e3767

The ability of different formulations of artificial saliva to protect dentin from erosive wear

Ação protetora de diferentes formulações de saliva artificial no desgaste erosivo

Graziela Ribeiro BATISTA[1], Rayssa Ferreira ZANATTA[2], Marina Gullo AUGUSTO[3], Gabriela Souza ARANTES[4], Alessandra Bühler BORGES[4], Carlos Rocha Gomes TORRES[4]

1 - A.T. Still University, Missouri School of Dentistry and Oral Health. City- Kirksville, Missouri, USA.
2 - Universidade de Brasília, Faculdade de Ciências da Saúde, Departamento de Odontologia. Brasília, Distrito Federal, Brazil.
3 - Centro Universitário de Cascavel. Cascavel, Paraná, Brazil.
4 - Universidade Estadual Paulista, Instituto de Ciência e Tecnologia, Departamento de Odontologia Restauradora. São José dos Campos, Brazil.

How to cite: Batista GR, Zanatta RF, Augusto MG, Arantes GS, Borges AB, Torres CRG. The ability of different formulations of artificial saliva to protect dentin from erosive wear. Braz Dent Sci. 2023;26(2):e3767. https://doi.org/10.4322/bds.2023.e3767

ABSTRACT

Objective: Evaluate the protective effect of artificial saliva formulations associated or not with mucin on dentin. **Materials and Methods:** Bovine dentin specimens were randomly allocated to 10 groups (n = 20) according to the artificial saliva tested and the presence or absence of mucin: Amaechi et al. (1998); Klimek et al. (1982); Vieira et al. (2005) and Eisenburger et al. (2001) and deionized water (control). Samples were submitted to an erosive cycle consisting of two immersions of 120 min in the saliva, followed by 1 min in hydrochloric acid solution, and new storage in saliva for 120 min. Surface loss (μm) was measured before and after the cycle. Data were analyzed using 2-way ANOVA and Tukey's test ($p < 0.05$). **Results:** A significant difference was observed for the saliva formulation but not for the presence of mucin. The deionized water provided the highest surface loss and the Eisenburger's saliva formulation the lowest. The groups testing the Amaechi, Klimek, and Vieira saliva did not present significant differences. **Conclusion:** Eisenburger's saliva formulation provides a higher protective effect against dentin erosion. The presence of mucin did not increase the erosion-preventive effect of artificial saliva formulations.

KEYWORDS

Artificial saliva; Dental erosion; Dental Wear; Profilometry; Surface loss.

RESUMO

Objetivo: Avaliar o efeito protetor de formulações de saliva artificial associadas ou não à mucina sobre a dentina submetida a erosão. **Material e Métodos:** Espécimes de dentina bovina foram alocados em 10 grupos (n = 20) de acordo com a saliva testada e a presença ou ausência de mucina: . Amaechi et al. (1998); Klimek et al. (1982); Vieira e cols. (2005), Eisenburger et al (2001) e agua deionizada (controle). As amostras foram submetidas a um ciclo erosivo composto por duas imersões de 120 min na saliva, seguidas de 1 min em solução de ácido clorídrico e novo armazenamento na saliva por 120 min. A perda de superfície (μm) foi medida antes e depois do ciclo. Os dados foram analisados usando ANOVA 2 fatores e teste de Tukey ($p < 0,05$). **Resultados:** Foi observada diferença significativa para a formulação de saliva, mas não para a presença de mucina. A água deionizada proporcionou a maior perda de superfície e a formulação de saliva de Eisenburger a menor. Os grupos que testaram a saliva Amaechi, Klimek e Vieira não apresentaram diferenças significativas entre si. **Conclusão:** A formulação de saliva de Eisenburger fornece o maior efeito protetor contra a erosão dentinária e a presença de mucina não aumentou o efeito preventivo de erosão de formulações de saliva artificial.

PALAVRAS-CHAVE

Erosão dental; Desgaste dental; Perda de estrutura; Perfilometria; Saliva artificial.

INTRODUCTION

Erosive tooth wear (ETW) is a multifactorial condition resulting from a chemical-mechanical process involving the dissolution of enamel and dentin by non-bacterial acids and modulated by several behavioral and biological aspects [1]. Dental surface loss due to erosive conditions is considered a frequent condition, mainly among children and young adults [2-4], enhancing the scientific community's interest in this topic and encouraging the development of preventive measures and treatment options.

Saliva is considered the main biological factor responsible for modulating the development of ETW lesions [5]. It acts as a reservoir of ions responsible for remineralizing the dental tissues softened by extrinsic or intrinsic acids. It also functions as a diluent of them and promotes their buffering, reducing surface loss [6,7]. Most of the evidence in dental erosion is obtained by *in vitro* studies. These set-ups allow standardization of the study variables under controlled situations, reduce costs compared to clinical studies, and enable a rapid assessment of products or treatments without considering ethical aspects [8].

Ideally, *in vitro* experiments should mimic the clinical conditions to produce results comparable to the clinical situations [9]; therefore, in 1982, Klimek et al. [10] described an artificial saliva formulation to be used in erosion experiments [10], and later, different formulations were proposed, such as the ones from Amaechi et al. (1998) [11], Vieira et al. (2005) [12] and Eisenburger et al. (2001) [13]. To make the artificial saliva consistency more similar to natural human saliva, some formulas add mucin to the mixture, an important component of the salivary pellicle and the main lubricant constituent of saliva [5]. This aims to increase the representativity of the *in vitro* experiments and facilitate the performance of such experiments since there would be no need to collect human saliva from volunteers. However, there are a few suggestions that using artificial saliva formulations and human saliva in *in vitro* experiments does not reflect the actual intraoral situation [8] and that the presence of mucin in the artificial saliva formulation may increase its remineralizing effect [14].

Therefore, this study aimed to evaluate the dentin erosion-preventive effect of artificial saliva formulations used in previous studies [11-13] with or without mucin. The null hypotheses tested were that the different artificial saliva formulation does not influence the dentin erosive surface loss and that the presence of mucin in the formulation does not improve its protective effect.

MATERIAL AND METHODS

Specimen preparation and allocation to the groups

Fresh and sound bovine incisors were collected for this study. The crowns were separated from the roots and stored in 0.5% thymol solution at 4°C until use [15]. Two hundred cylindrical dentin specimens (3 mm diameter) were obtained from the roots using a diamond-coated trephine mill. The specimens were embedded in acrylic resin (diameter: 6 mm, height: 3 mm; JET, Classico, Sao Paulo, Brazil). The external surfaces of the specimens were ground flat and polished using silicon carbide paper in sequential grits of 1200, 2400, and 4000 (Extec Corp, CT, USA) in a polishing device (DP-10, Panambra Industrial e Técnica SA, Sao Paulo, SP, Brazil) under water irrigation for 30, 60 and 120 s, respectively. After each paper grit change, specimens were kept in ultrasonic baths in distilled water for 10 min to remove debris and abrasive grains. The prepared samples were examined under a stereomicroscope (Discovery V20, Karl Zeiss, Jena, Germany) to ensure the absence of cracks or other surface defects. After preparation, the specimens were stored at 100% relative humidity at 4°C to avoid dehydration.

Artificial saliva formulations were prepared according to the descriptions in the previous studies: Amaechi et al. (1998) [11], Klimek et al. (1982) [10], Vieira et al. (2005) [12], and Eisenburger et al. (2001) [13]. Table I shows the composition of the artificial saliva formulations. Each formulation was made with and without mucin, as shown in Figure 1.

After preparing artificial saliva, the specimens were randomly assigned to ten groups (n = 20) according to the storage media (Figure 1). The control group was deionized water (negative control).

Surface loss assessment and erosive cycling

In each specimen, two parallel groves were made to provide a reference for the surface loss

Table I - Composition of tested artificial saliva formulations

Compound	Artificial saliva formulations			
	Klimek et al. [10]	Vieira et al. [12]	Amaechi et al. [11]	Eisenburger et al. [13]
$C_6H_8O_6$	2 mg/l			
$C_6H_{12}O_6$	30 mg/l			
NaCl	580 mg/l			
$CaCl_2$	170 mg/l			
NH_4Cl	160 mg/l			
KCl	1270 mg/l	11182.50 mg/l	624.73 mg/l	2236.50 mg/l
NaSCN	160 mg/l			
KH_2PO_4	330 mg/l		326.62 mg/l	544.36 mg/l
CH_4N_2O	200 mg/l			
Na_2HPO_4	340 mg/l			
$Ca(NO_3)_2.4H_2O$		60.12 mg/l		
NaF		0.066 mg/l		
$NaH_2PO_4.2H_2O$		160.19 mg/l		
$C_4H_{11}NO_3$ Tris Buffer		12114.00 mg/l		
K_2HPO_4			804.712 mg/l	
$CaCl_2.2H_2O$			166.130 mg/l	77.690 mg/l
$C_8H_8O_3$			2000 mg/l	
CMC-Na			10000 mg/l	
$MgCl_2.6H_2O$			58.96 mg/l	
$MgCl_2$				19.04 mg/l
$C_8H_{18}N_2O_4S$ HEPES				4766.20 mg/l
Mucin*	2700 mg/l	2700 mg/l	2700 mg/l	2700 mg/l
Deionized water	1000 ml	1000 ml	1000 ml	1000 ml
pH	6.4	7.0	6.75	7.0

*Only in the groups containing mucin.

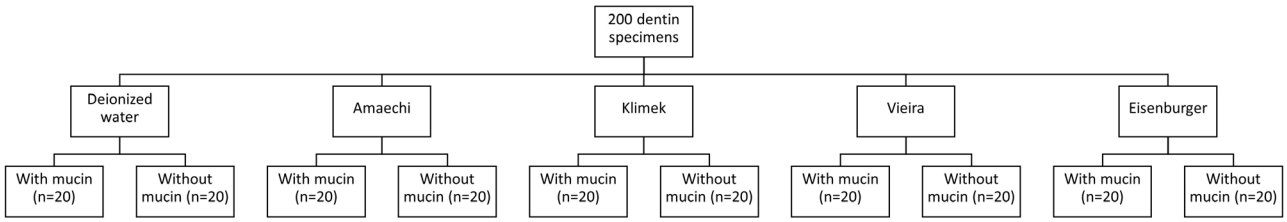

Figure 1 – Specimen allocation according to the storage media.

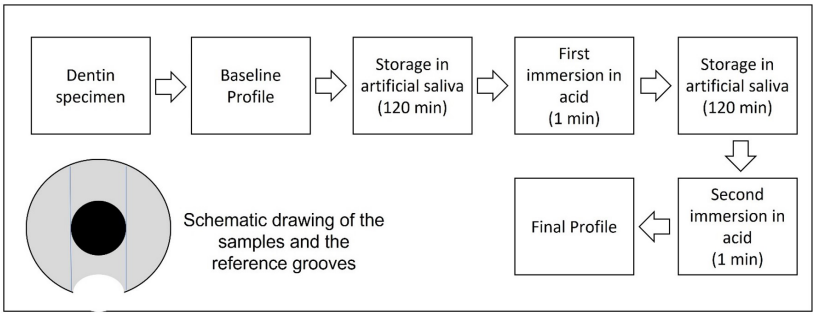

Figure 2 – Schematic chart of the steps performed in the study.

determination (profilometry), as shown in Figure 2. The baseline profiles of each specimen were obtained using a contact profilometer (MaxSurf XT 20, Mahr-Goettingen, Germany). The diamond stylus moved from the first groove in the acrylic resin to the second one (4.2 mm long). Three profile measurements were performed for each specimen at intervals of 0.25 mm.

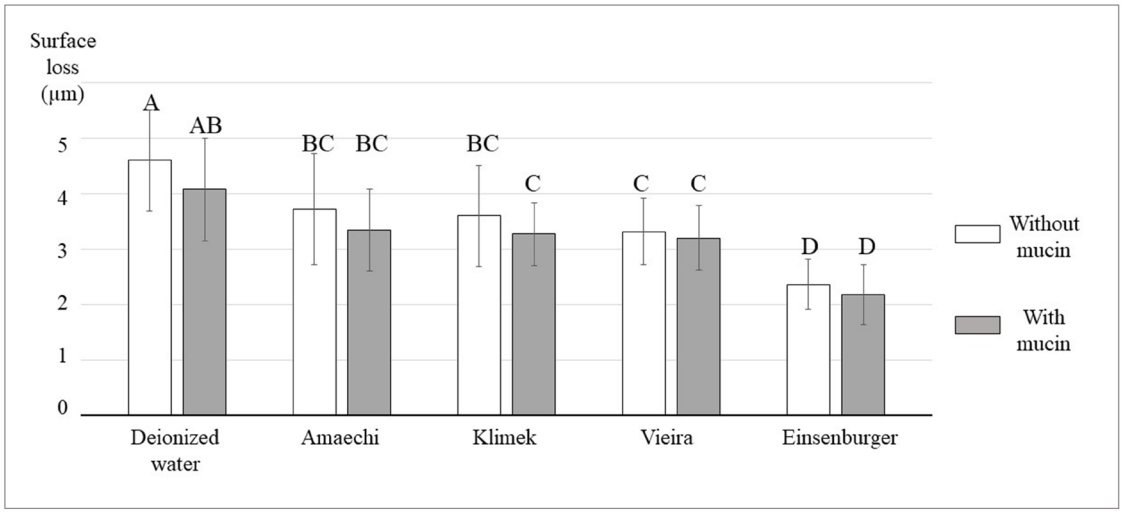

Figure 3 – The mean surface loss (µm), standard deviations, and results of Tukey's test for the artificial saliva, with or without the presence of mucin. Different letters mean significant difference among groups.

After obtaining the baseline profiles, the erosive cycling was performed under agitation (40 rpm) and at room temperature. The specimens of each group were stored in the respective artificial saliva for 120 min. Then, they were immersed for 1 min in a hydrochloric acid solution (2.5 mmol/l, pH = 2.6). The specimens were washed with deionized water (pH = 5.5) for 10 s. Additional storage in the respective artificial saliva (120 min) and erosive challenge (1 min) were performed [8]. After the second erosive challenge, the final profiles of each specimen were obtained using the same parameters previously described. Figure 2 shows a schematic chart illustrating the erosive cycle.

At the end of the cycling, a second profile reading was performed in each specimen using the same parameters described above. The initial and final profiles were overlapped, and the dentin loss was calculated by the difference in height between them using dedicated software (MarSurf XCR 20 4.50-07 SP3, 2011).

Statistical analysis

The assumption of normal distribution (Kolmogorov-Smirnov test) was checked for the variable tested. Descriptive and inferential statistical analyzes were performed using SigmaPlot 13 (Systat Software Inc, San Jose, CA, EUA). Two-way ANOVA was performed for profilometry analysis, followed by Tukey's test with 5% significance.

RESULTS

The results of 2-way ANOVA showed a significant difference ($p < 0.05$) for the different artificial saliva formulations ($p = 0.0001$) and the interaction between the two factors ($p = 0.040$), but not for the presence of mucin ($p = 0.360$). Tukey's test revealed that the deionized water without mucin (control group) provided the highest surface loss compared with the other formulations. The lowest surface loss was observed for the Eisenburger's saliva formulation, while the groups testing the formulas from Amaechi, Klimek, and Vieira presented similar surface loss. The mean surface loss (μm), standard deviations, and results of Tukey's test are shown in Figure 3.

DISCUSSION

This study showed that the different artificial saliva formulations significantly influenced the erosive surface loss but not by the presence of mucin. Hence, the first null hypothesis was denied, and the second one was accepted.

Hydrochloric acid is a strong inorganic acid, easily ionizable in an aqueous solution and resistant to buffering by saliva, making it a potent agent to erode dental tissues and cause surface loss. It was used in the present study in short erosive events (1 min) aiming to simulate patients with gastroesophageal disorders, such as GERD or bulimia [16]. Also, the storage in artificial saliva for 120 minutes aimed to optimize the remineralization process as proposed previously [17].

When acid comes into contact with dentin, it dissolves the mineral phase, but the organic part remains as a spongy and demineralized structure that acts as a physical barrier for all chemical processes, including active ingredients from preventive measures and remineralizing agents [18]. It is suggested that in *in vivo* situations, the exposed collagen matrix can be broken down by collagenases and proteolytic enzymes found in the oral fluids [18,19] or through abrasion such as toothbrushing. However, in this study, salivary collagenases and toothbrushing were not performed, so this collagen barrier resultant from the erosive process might have remained intact and helped to modulate the remineralization by the ions from some of the artificial saliva formulations. Still, it is suggested that the collagen fibrils of organic matrix exposed during erosive or carious events house some prenucleation mineral crystals that aggregate into larger amorphous calcium phosphate (ACP) nanoparticles and induce mineral formation [20] in the presence of remineralizing ions, such as calcium, phosphate, or magnesium.

From the solutions tested, the artificial saliva formulation described by Eisenburger presented the lowest values of surface loss, which might have been due to the presence of-HEPES ($C_8H_{18}N_2O_4S$), an organic chemical buffer known as zwitterionic sulfonic acid and classified as a good buffer [21], thus able to neutralize the H^+ ions from the dissociation of HCl and promote an anti-erosive effect. The groups testing the formulation from Amaechi, Klimek, and Vieira did not differ from each other, even though the last one also presented a buffer in its composition ($C_4H_{11}NO_3$ - Tris Buffer), and the possible explanation for that relies on the different degrees of saturation with respect of calcium phosphates in each solution [8].

Regarding the groups in which mucin was included, it has been reported that they contribute to a large extent to the protective effect of the acquired pellicle against enamel erosion [22]. However, this study showed that mucin did not significantly reduce dentin erosive surface loss. Even for the comparison between the control group (deionized water) with and without mucin, the absence of significant differences indicated that this protein was not able to protect dentin against erosion. This may be because the surface of eroded enamel presents high mineral content, whereas the mesh of collagen matrix from eroded dentin might have interfered with the deposition of mucin, thus reducing its protective effect against erosion. Although, a previous study found that the anti-erosive protection of the acquired pellicle in dentin is lower than in enamel [23]. Moreover, it is essential to consider that the behavior of the synthetic mucin used in *in vitro* studies may be different from the mucin found in human saliva. Its stability in the formulations and the combination with the other components may be able to cause differences from the natural human saliva.

Thus, the result from the present study suggested that the protection of dentin submitted to erosive challenges is affected by the formulation of the artificial saliva tested, and future studies, including the comparison with human saliva in *in situ* protocols, are encouraged, aiming to find the best suitable formula for in vitro analysis. It can be concluded that the protective effect of different artificial saliva against erosion must be considered to avoid incorrect inferences in *in vitro* studies. The presence of mucin did not provide a significant reduction in the erosive surface loss of dentin.

Author's Contributions

GRB: Conceptualization. GRB: Methodology. RFZ: Formal Analysis. GRB, MGA, GSA: Investigation. GRB, MGA, GSA: Data Curation. GRB, RFZ: Writing – Original Draft Preparation. ABB, CRGT: Writing – Review & Editing. ABB, CRGT: Visualization. ABB, CRGT: Supervision. CRGT: Project Administration. CRGT: Funding Acquisition.

Conflict of Interest

The author's disclosure any conflict of interest.

Funding

This research was supported by National Council for Scientific and Technological Development (CNPq).

Regulatory Statement

The authors have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

REFERENCES

1. Carvalho TS, Colon P, Ganss C, Huysmans MC, Lussi A, Schlueter N, et al. Consensus report of the European Federation of Conservative Dentistry: erosive tooth wear—diagnosis and management. Clin Oral Investig. 2015;19(7):1557-61. http://dx.doi.org/10.1007/s00784-015-1511-7. PMid:26121968.
2. Martignon S, Bartlett D, Manton DJ, Martinez-Mier EA, Splieth C, Avila V. Epidemiology of erosive tooth wear, dental fluorosis and molar incisor hypomineralization in the American continent. Caries Res. 2021;55(1):1-11. http://dx.doi.org/10.1159/000512483. PMid:33440378.
3. Salas MMS, Nascimento GG, Huysmans MC, Demarco FF. Estimated prevalence of erosive tooth wear in permanent teeth of children and adolescents: an epidemiological systematic review and meta-regression analysis. J Dent. 2015;43(1):42-50. http://dx.doi.org/10.1016/j.jdent.2014.10.012. PMid:25446243.
4. Salas MMS, Nascimento GG, Vargas-Ferreira F, Tarquinio SBC, Huysmans MCDNJM, Demarco FF. Diet influenced tooth erosion prevalence in children and adolescents: results of a meta-analysis and meta-regression. J Dent. 2015;43(8):865-75. http://dx.doi.org/10.1016/j.jdent.2015.05.012. PMid:26057086.
5. Buzalaf MAR, Hannas AR, Kato MT. Saliva and dental erosion. J Appl Oral Sci. 2012;20(5):493-502. http://dx.doi.org/10.1590/S1678-77572012000500001. PMid:23138733.
6. Attin T, Knofel S, Buchalla W, Tutuncu R. In situ evaluation of different remineralization periods to decrease brushing abrasion of demineralized enamel. Caries Res. 2001;35(3):216-22. http://dx.doi.org/10.1159/000047459. PMid:11385203.
7. Hara AT, Zero DT. The potential of saliva in protecting against dental erosion. Monogr Oral Sci. 2014;25:197-205. http://dx.doi.org/10.1159/000360372. PMid:24993267.
8. Batista GR, Torres CRG, Sener B, Attin T, Wiegand A. Artificial saliva formulations versus human saliva pretreatment in dental erosion experiments. Caries Res. 2016;50(1):78-86. http://dx.doi.org/10.1159/000443188. PMid:26870948.
9. Attin T, Wegehaupt FJ. Methods for assessment of dental erosion. Monogr Oral Sci. 2014;25:123-42. http://dx.doi.org/10.1159/000360355. PMid:24993262.
10. 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.
11. Amaechi BT, Higham SM, Edgar WM. Use of transverse microradiography to quantify mineral loss by erosion in bovine enamel. Caries Res. 1998;32(5):351-6. http://dx.doi.org/10.1159/000016471. PMid:9701660.
12. Vieira AEM, Delbem ACB, Sassaki KT, Rodrigues E, Cury JA, Cunha RF. Fluoride dose response in pH-cycling models using bovine enamel. Caries Res. 2005;39(6):514-20. http://dx.doi.org/10.1159/000088189. PMid:16251798.
13. Eisenburger M, Addy M, Hughes JA, Shellis RP. Effect of time on the remineralisation of enamel by synthetic saliva after citric acid erosion. Caries Res. 2001;35(3):211-5. http://dx.doi.org/10.1159/000047458. PMid:11385202.
14. Ionta FQ, Mendonça FL, Oliveira GC, Alencar CRB, Honório HM, Magalhães AC, et al. In vitro assessment of artificial saliva formulations on initial enamel erosion remineralization. J Dent. 2014;42(2):175-9. http://dx.doi.org/10.1016/j.jdent.2013.11.009. PMid:24269764.
15. Attin T, Meyer K, Hellwig E, Buchalla W, Lennon A. Effect of mineral supplements to citric acid on enamel erosion. Arch Oral Biol. 2003;48(11):753-9. http://dx.doi.org/10.1016/S0003-9969(03)00156-0. PMid:14550377.
16. Wiegand A, Bliggenstorfer S, Magalhães AC, Sener B, Attin T. Impact of the *in situ* formed salivary pellicle on enamel and dentine erosion induced by different acids. Acta Odontol Scand. 2008;66(4):225-30. http://dx.doi.org/10.1080/00016350802183401. PMid:18607835.
17. Alencar CRB, Mendonça FL, Guerrini LB, Jordão MC, Oliveira GC, Honório HM, et al. Effect of different salivary exposure times on the rehardening of acid-softened enamel. Braz Oral Res. 2016;30(1):e104. http://dx.doi.org/10.1590/1807-3107BOR-2016.vol30.0104. PMid:27737358.
18. Ganss C, Lussi A, Schlueter N. The histological features and physical properties of eroded dental hard tissues. Monogr Oral Sci. 2014;25:99-107. http://dx.doi.org/10.1159/000359939. PMid:24993260.
19. Schlueter N, Hardt M, Klimek J, Ganss C. Influence of the digestive enzymes trypsin and pepsin in vitro on the progression of erosion in dentine. Arch Oral Biol. 2010;55(4):294-9. http://dx.doi.org/10.1016/j.archoralbio.2010.02.003. PMid:20197186.
20. He L, Hao Y, Zhen L, Liu H, Shao M, Xu X, et al. Biomineralization of dentin. J Struct Biol. 2019;207(2):115-22. http://dx.doi.org/10.1016/j.jsb.2019.05.010. PMid:31153927.
21. Good NE, Winget GD, Winter W, Connolly TN, Izawa S, Singh RMM. Hydrogen ion buffers for biological research. Biochemistry. 1966;5(2):467-77. http://dx.doi.org/10.1021/bi00866a011. PMid:5942950.
22. Amerongen AVN, Oderkerk CH, Driessen AA. Role of mucins from human whole saliva in the protection of tooth enamel against demineralization in vitro. Caries Res. 1987;21(4):297-309. http://dx.doi.org/10.1159/000261033. PMid:3475175.
23. Hall AF, Buchanan CA, Millett DT, Creanor SL, Strang R, Foye RH. The effect of saliva on enamel and dentine erosion. J Dent. 1999;27(5):333-9. http://dx.doi.org/10.1016/S0300-5712(98)00067-0. PMid:10377607.

Graziela Ribeiro Batista
(Corresponding address)
A.T. Still University, Missouri School of Dentistry and Oral Health,
Kirksville, MO, USA.
Email: grazielabatista@atsu.edu

Date submitted: 2023 Jan 24
Accept submission: 2023 Apr 04