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ORIGINAL ARTICLE

Effect of Fluoridated Solution based on Silver Nanoparticles Associated with High Fluoride Dentifrice on Dental Erosion

Gabriela Torres Zanin[1], Edgar Pedreiro Ribeiro[1], Arilson de Matos[2], Rebeka Beatriz Silvério de Oliveira[3], Murilo Baena Lopes[1,3], Ricardo Danil Guiraldo[1,3], Sandrine Bittencourt Berger[1,2,3]

[1]University Anhanguera/Uniderp, Department of Restorative Dentistry, Campo Grande, MS, Brazil.
[2]University of Cuiabá, Department of Dentistry, Cuiabá, MT, Brazil.
[3]University of North Parana, Department of Dentistry, Londrina, PR, Brazil.

Corresponding author: Sandrine Bittencourt Berger **E-mail:** berger.sandrine@gmail.com

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ABSTRACT

Objective: To investigate the effect of fluoridated solution based on silver nanoparticles (AgNano) associated or not with toothpaste containing high fluoride concentration (TP) on dental erosion. **Material and Methods:** Ninety-six enamel blocks from bovine incisors were prepared and analyzed for microhardness and initial roughness. Then, divided into 8 experimental groups (n = 12): G1 - brushing with water (H_2O); G2 - brushing with TP; G3 - AgNano + TP; G4 - AgNano + H_2O; G5 - SDF + TP; G6 - SDF + H_2O; G7 - FV + TP; G8 - FV + H_2O. SDF, AgNano, and FV were applied and, after 24 hours, all samples were submitted to erosive challenge with 0.3% citric acid (pH 2.6) and brushing with TP or H_2O for 5 days. After treatments and erosion challenge, microhardness and roughness were again evaluated. The data were statistically analyzed by ANOVA followed by Tukey's test (microhardness) and Kruskal-Wallis test (roughness), $\alpha=0.05$. **Results:** There was no difference between treatments (AgNano, SDF, and FV), ($p>0.05$); however, brushing with toothpaste with high fluoride concentration significantly decreases loss of enamel microhardness. **Conclusion:** AgNano associated or not with toothpaste containing high fluoride concentration was not able to reduce dental erosion in the enamel, as well as SDF and FV.

Keywords: Tooth Erosion; Cariostatic Agents; Metal Nanoparticles; Tooth Remineralization.

Introduction

People's lifestyle has changed over the years, and, in recent decades, there has been a large increase in the consumption of acidic beverages by populations [1]. Thus, in addition to caries, another problem has attracted the attention of oral health professionals: tooth erosion. Dental erosion is the physical result of the pathological, chronic, and localized loss of dental hard tissues, which is chemically removed from the surface of teeth by acids or chelation, without bacterial involvement [2]. Acids that cause erosion can be of intrinsic or extrinsic in origin. Acids of intrinsic origin are those from recurrent vomiting and gastric disorders, such as reflux [3]. Acids of extrinsic origin are mainly represented by acidic foods and drinks [4].

Due to its usually quick and asymptomatic progression, erosion is usually diagnosed in advanced stages, when the dentin substrate is already exposed. Although there is currently no efficient treatment option, palliative care using products containing high fluoride content is performed [5], with fluorine concentrations varying between 1100 and 1450 ppm [6]. Toothpastes with higher fluoride concentration (5000 ppm) may have a more effective action [7], but Rios et al. [6] did not observe improvement in dental erosion with dentifrices containing 5000 ppm of sodium fluoride.

The use of fluoride varnish has been investigated as an option to avoid erosion [8]. A study showed that the application of fluoride varnish can prevent loss of dental structure when the substrate is submitted to at least 70 minutes of erosive challenge [9]. However, when erosion is combined with brushing, the varnish is partially removed and the protective effect is reduced by at least 50%, indicating that the product must be periodically applied or applied in multiple layers, in order to keep dental surfaces protected from erosive challenges [10].

Silver diamine fluoride (SDF) has been suggested by many researchers as a non-invasive procedure to stop dental caries in children. SDF has been shown to be effective in protecting teeth against new carious lesions [11] and has been used as a preventive measure in pediatric populations at high risk of developing caries, due to the bactericidal effect of SDF, as well as its ability to remineralize affected and softened tooth structures through the deposition and incorporation of fluoride ions [12].

Recently, Ainoosah et al. [13] evaluated the effect of SDF on tooth erosion, both enamel and dentin. However, the authors found that SDF was effective in eroding dentin, but not enamel. They reported that the process of preventing erosion in dentin and enamel was probably mainly driven by fluoride, but further studies testing erosion and the use of SDF in controlling tooth erosion are necessary.

Solutions based on silver nanoparticles have been studied, mainly due to the staining of the tooth structure caused by SDF [14,15]. The study by Scarpelli et al. [16] demonstrated that a solution based on silver nanoparticles was able to remineralize dental enamel after pH cycling to obtain initial caries lesion in 14%.

Thus, based on the above, the objective of this study was to evaluate the effect of fluoride solution based on silver nanoparticles associated or not with dentifrice with high fluoride concentration on the ability to minimize tooth erosion.

Material and Methods

Study Design

In this study, an experimental solution based on Silver Nanoparticles (AgNano) was tested in terms of effectiveness compared to silver diamine fluoride (SDF) (Cariestop, Biodinâmica Química e Farmacêutica, Ibiporã, PR, Brazil) and to fluoride varnish (FV) (Duraphat, Colgate-Palmolive Ind. e Com. Ltda., São Bernardo do Campo, SP, Brazil). Treatments with AgNano, SDF, and FV were associated or not with dentifrice with high fluoride concentration (5000 ppm –F, ClinproTM 5000, 3M do Brasil Ltda., Sumaré, SP, Brazil).

Table 1. Materials and their compositions.

Treatments / Treatment Agents	Composition
AgNano: solution based on 0.04% silver nanoparticles and 2% sodium fluoride (Biodinam, Ibiporã, PR, Brazil).	Vial 1: Colloidal solution of silver nanoparticles, ethylene glycol, polyvinylpyrrolidone, and water. Vial 2: sodium fluoride and water.
SDF 30: 30% silver diamine fluoride (Cariestop, Biodinama, PR, Brazil).	Hydrofluoric acid, silver nitrate, ammonium hydroxide, and deionized water.
FV: Sodium fluoride varnish (5% NaF) with high fluorine concentration (22,600 ppm –F) (Duraphat® – Colgate®).	Sodium fluoride, colophony, ethyl alcohol, shellac, mastic, saccharin, aroma, and white beeswax.
High fluoride toothpaste: 1.1% Sodium Fluoride toothpaste (5000 ppm–F, Clinpro™ 5000, 3M ESPE).	Water, uncrystallized sorbitol solution, precipitated synthetic amorphous silica (crystalline-free), amorphous silica, glycerin, polyethylene glycol, polyethylene-polypropylene glycol, sodium carboxymethylcellulose, sodium fluoride, sodium lauryl sulfate, sodium saccharin, titanium dioxide, flavorings, modified tricalcium phosphate.

Sample Preparation

For this study, 120 bovine incisors were selected, which had their crowns separated from the root with the aid of a double-sided diamond disc (KG Sorensen Indústria e Comércio, Barueri, SP, Brazil). Two-hundred and forty enamel/dentin blocks (4 × 4 × 2 mm) were obtained from crowns and roots (2 blocks from each tooth) using an automatic cutter (Isomet, Buehler, Lake Bluff, IL, USA). Blocks were embedded in a PVC pipe ring (3⁄4 - 1.5 cm) (Tigre Materiais e Soluções Para Construção Ltda., Castro, PR, Brazil) with chemically activated acrylic resin (Varidur, Buehler, Lake Bluff, IL, USA) and were flattened with abrasive Al2O3 discs under refrigeration, in the following granulation and time sequence: 400 (10 seconds), 600 (30 seconds), 1200 (60 seconds) in electric polisher (APL4, Arotec S/A Ind. e Com., Cotia, SP, Brazil). Blocks were polished with felt disk (Arotec S/A Ind. e Com., Cotia, SP, Brazil) and diamond paste in granulations of 1 and 0.25 µm (Arotec, Cotia, SP, Brazil), for 3 minutes each. Between each polishing paper, samples were submitted to ultrasonic bath (Ultrasonic Cleaner, Odontobrás Equipamentos Médicos e Odontológicos, Ribeirão Preto, SP, Brazil) in distilled water for 10 minutes to remove residues.

Sample Selection and Initial Microhardness

Samples were examined under stereoscopic loupe (Bel Photonics, Bel Microimage Analyser, Monza, Italy) to verify the presence of cracks and/or other defects. Samples showing defects and/or cracks were excluded from the study.

Sample selection was performed by reading the surface microhardness of enamel and dentin. The test was performed using a Shimadzu microhardness tester (HMV-G 21S, Shimadzu Corporation, Kyoto, Japan) coupled to a microcomputer, with specific software (HMV-G) using Knoop-type pyramidal diamond indenter (KHN) (HMV-G – Shimadzu Corporation, Kyoto, Japan), with a static charge of 50 grams, applied for 5 seconds. Three indentations were performed in each sample, 100 µm apart from the center, and the average indentation values of each sample represented the sample value at baseline. The calculation of the overall average was performed and samples with microhardness values 10% above or 10% below the overall average were excluded, which resulted in 96 enamel samples. Then, each substrate was divided into 8 experimental groups (n=12), as shown in Table 2.

All groups went through the erosive challenge process. As observed in Table 2, treatments were applied and brushing was performed with dentifrice or water in enamel blocks.

Table 2. Division of experimental groups according to treatments.

Group	Treatments	Dentifrice
G1	-	No
G2	-	Yes
G3	AgNano	Yes
G4	AgNano	No
G5	SDF	Yes
G6	SDF	No
G7	FV	Yes
G8	FV	No

AgNano: Fluoride solution based on silver nanoparticles; SDF: Silver Diamine Fluoride (Cariestop®); FV: Fluoride Varnish; D: Dentifrice with 5000 ppm –F (Clinpro 5000).

Initial Roughness Evaluation

Before treatment, the surface roughness of the samples was evaluated. This evaluation was carried out in three different directions (vertical, horizontal, and cross-sectional) using a rugosimeter (SJ-410, Mitutoyo Corporation, Kanagawa, Japan). The surface of each sample was analyzed at an extension of 0.25 mm, with speed of 0.01 mm/sec, three times. The mean surface roughness (Ra) in µm represented the arithmetic mean of the size of peaks and valleys found during the reading of the surface scanning of each sample. Subsequently, the mean of the three measurements representing the initial roughness (baseline) was calculated.

Treatment Application

Both SDF and AgNano were applied prior to the erosive challenge using a disposable applicator (KG Brush - KG Sorensen Indústria e Comércio, Barueri, SP, Brazil), for 1 minute on the enamel or dentin surface. Then, samples were washed with deionized water and stored in artificial saliva at 37ºC for 24 hours prior to the erosive challenge [13]. Fluoride varnish (Duraphat, Colgate-Palmolive Ind. e Com. Ltda., São Bernardo do Campo, SP, Brazil) was also applied prior to the erosive challenge. A thin layer was applied, following the manufacturer's recommendations, using a disposable applicator (KG Brush - KG Sorensen Indústria e Comércio, Barueri, SP, Brazil). Specimens were rinsed with deionized water and kept in artificial saliva for 24 hours at 37°C.

Erosive Challenge

The erosion challenge sequence followed the model proposed by Ainoosah et al. [13], described in Table 3. Each day, samples were immersed for 5 minutes in 0.3% citric acid solution, with an approximate pH of 2.6, followed by immersion in artificial saliva (pH 7.9) for 60 minutes. The artificial saliva formulation consists of 0.213 g/L of $CaCl_2*2H_2O$; 0.738 g/L of HH_2PO_3; 1.114 g/L KCl; 0.381 g/L NaCl; 12 g/L of Tris buffer, pH adjusted to 7 with concentrated HCl. This procedure was repeated 5 times a day for 5 days.

For brushing, an electric brush was used (Oral B Professional Care 5000 Oral B Schwalbacham Taunus, Germany) equipped with a pressure alert that signals when the pressure reaches a value of 2N [17,18], using toothpaste or water according to treatments described in Table 2, performing light abrasion. After brushing, samples were washed in running water for 30 seconds and then proceeded to the next step.

Table 3. Daily cycling sequence for the erosive challenge.

Daily Cycling*	Sequence	Procedures
1	Erosion challenge	5 minutes (0.3% citric acid)
	Remineralization	60 minutes (artificial saliva)
	Brushing	15 seconds
	Remineralization	60 minutes (artificial saliva)

2	Erosion challenge	5 minutes (0.3% citric acid)
	Remineralization	60 minutes (artificial saliva)
3	Erosion challenge	5 minutes (0.3% citric acid)
	Remineralization	60 minutes (artificial saliva)
4	Erosion challenge	5 minutes (0.3% citric acid)
	Remineralization	60 minutes (artificial saliva)
5	Erosion challenge	5 minutes (0.3% citric acid)
	Remineralization	60 minutes (artificial saliva)
	Brushing	15 seconds
	Remineralization	60 minutes (artificial saliva)

During the period between cycles, samples remained in an oven at 37°C; *Adapted from Ainoosah et al. [13].

Final Microhardness

After the treatments/erosion challenge, samples were analyzed again using the Knoop microhardness test, in the same way as previously evaluated, with 3 indentations 100 µm apart. Initial microhardness (IM) and final microhardness (FM) values were used to calculate the percentage change in surface microhardness (%M), using the formula: %M = 100 - [(100 x FM)/IM].

Final Roughness

After the treatments/erosion challenge, samples were again submitted to surface roughness reading, as previously described, and the final roughness values were compared with initial roughness values and the surface alteration (SA) calculated by the formula: SA = Initial roughness – Final roughness.

Statistical Analysis

Microhardness and roughness data were evaluated for normality using the Shapiro-Wilk test. Microhardness data showed normal distribution; thus, they were submitted to two-way Analysis of Variance (ANOVA) ($\alpha=5\%$), considering factors brushing (dentifrice and water) and treatment (AgNano, SDF, FV and without treatment), time (initial and final) and the interaction between factors (brushing x treatment x time). In addition, percentage microhardness change (%M) was evaluated considering factors treatment, brushing, and treatment x brushing. Roughness alteration values did not present normal distribution, being thus submitted to the Kruskal-Wallis test ($\alpha=5\%$).

Results

%M data were used to calculate the power test. The power test found was 0.97 (97%), considering 8 experimental groups, 12 samples per group, the greatest difference between groups of KHN = 22.65, and a standard deviation of 10.60.

For microhardness, ANOVA identified statistically significant differences between groups in terms of brushing ($p<0.001$), time ($p<0.001$), and interaction between them ($p<0.001$). For the other factors, no statistically significant differences were observed, treatment ($p=0.711$), brushing x treatment ($p= 0.600$), treatment x time ($p=0.587$), and treatment x time x brushing ($p=0.158$).

When %M was analyzed, a statistically significant difference was observed for the dentifrice factor ($p<0.001$); however, for the treatment factor or interaction between factors (treatment x dentifrice), no statistically significant difference was observed, $p=0.0756$ and $p=0.170$, respectively.

Figure 1 shows the effect on %M when toothpaste with high fluoride concentration was used, promoting the statistically smaller loss of microhardness when compared with water, regardless of treatment. For roughness, The Kruskal-Wallis test showed no statistically significant difference, both for treatment (p=0.274) and for brushing (p=1.00).

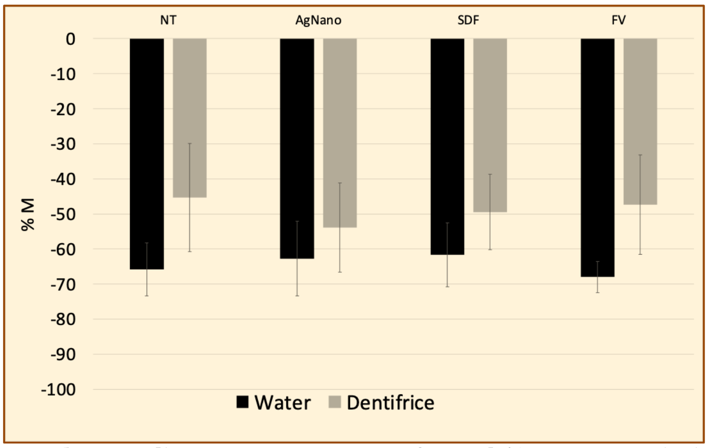
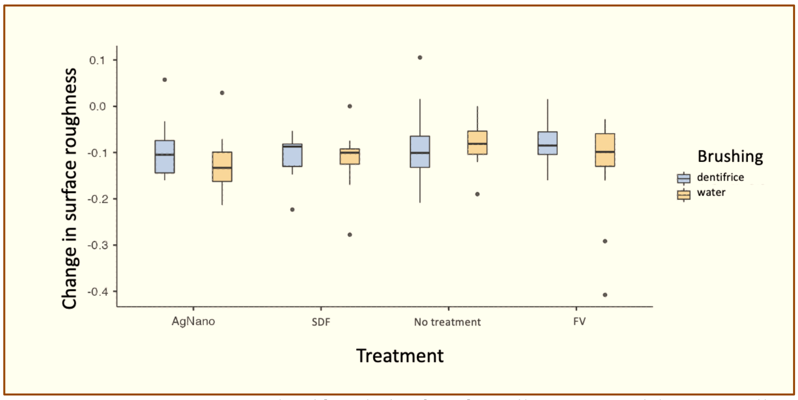


Figure 1. %M bar graph according to treatments performed (AgNano – solution based on silver nanoparticles, SDF – silver diamine fluoride, FV – fluoride varnish, NT – no treatment).

Figure 2 shows loss of tooth structure in all groups and no statistically significant difference was observed between them.



NT: No treatment; AgNano: Fluoride solution based on silver nanoparticles; SDF: Silver Diamine Fluoride (Cariestop); FV: Fluoride varnish (Duraphat®).

Figure 2. Box-plot graph of enamel roughness change values in µm according to treatments.

Discussion

This study evaluated the effect of fluoride solution based on silver nanoparticles (AgNano) associated or not with dentifrice with high fluoride concentration on reducing dental erosion in enamel. Groups that received the experimental solution based on silver nanoparticles were compared to groups that received SDF and fluoride varnish.

Ten Cate and Imfeld [2] stated that there are two ways to prevent dental erosion: weakening the erosive potential of acid challenges or increasing the resistance of the tooth surface. An option to reduce the erosive potential of acids would be the consumption of acidic drinks with the aid of a straw, for example [2], or making use of toothpastes with high fluorine content to increase the resistance of the dental substrate [5,7,19]. The present study showed that the loss of structure in enamel was quite significant after acid challenge, even in groups where protective solutions were applied, supposedly due to the combination of erosion/abrasion, resulting in greater wear than the erosion-only condition. According to Rios et al. [6], the erosive attack softens the tooth enamel, leading to increase in susceptibility to abrasive wear. It is also speculated that the short time of fluorine application on the surfaces of bovine blocks did not allow the deposit of a calcium-fluorine layer capable of preventing the erosive/abrasive attack [6].

Rios et al. [6] carried out an *in situ* study using bovine enamel, where they evaluated the effect of fluoridated dentifrices on dental erosion (1,100 ppm –F and 5000 ppm –F) and found no significant differences between dentifrices in terms of enamel wear; in contrast, Ren et al. [7] found positive results with the use of dentifrice with high fluoride content (5000 ppm –F) on tooth erosion. Ren et al. [7] found, in an *in situ* study using human teeth, that the periodic application of dentifrice containing 5000 ppm –F can be beneficial in individuals at risk of acid erosion associated with the consumption of soft drinks. This was also observed in this work, where dental enamel groups that used fluoridated dentifrice with 5000 ppm –F (ClinproTM 5000, 3M ESPE) showed positive results in microhardness in relation to groups that used water for brushing, showing that the high fluoride concentration, in this case, was able to reduce loss of tooth structure.

The use of fluoride has shown promising results in increasing resistance and decreasing the development rate of tooth erosion in enamel, when used at high doses [19]. In the present study, all groups had loss of tooth structure regardless of previous treatments. Imfeld [20] stated that fluoride varnish forms a film on the tooth surface, which could justify a possible mechanical protection superficially created; however, this layer can be easily removed by abrasion caused by brushing, requiring the development of a prevention product, combining ideal chemical and mechanical properties [10].

Silver diamine fluoride (SDF) is a solution commonly used to stop dental caries. Crystal and Niederman [21] highlight some advantages of using SDF, such as: retention of caries for deciduous teeth and root caries and its ease of use, low cost, and relative safety in interrupting the carious process without the need to remove the carious tissue. However, a very negative point is the fact that SDF leaves the carious lesion with a darkened color [14,15,21]. Solution containing silver nanoparticles, such as AgNano used in this study, does not compromise aesthetics, as it does not cause darkening of the region where it is applied, as it does not form oxides when in contact with oxygen in the environment [14,22,23] stopping the metabolism of bacteria [24], in addition to promoting an effect similar to SDF in enamel remineralization with an antimicrobial effect without cytotoxicity [25] and does not leave a metallic taste in the mouth [26].

In this study, due to its remineralizing potential, SDF was used as an agent for possible reduction of dental erosion. According to Ainoosah et al. [13], SDF was able to reduce tooth erosion after simulation of an erosion/abrasion model in dentin but was not effective in enamel. These results differ from those found in this study, where SDF was not effective in preventing tooth erosion in bovine enamel. However, although the erosive challenge was quite similar in both studies, the SDF concentration and the way of evaluating the loss of structure were different, which could explain the contradiction found in the results.

Fung et al. [27] compared two different SDF concentrations, 12% and 38%, and found that 38% SDF was more effective than 12% in stopping active caries in deciduous teeth. Mei et al. [28] also performed a study comparing different SDF concentrations with solutions containing silver and fluorine and concluded that SDF at three different concentrations (12%, 30%, and 38%) was able to inhibit the activity of cysteine cathepsins, an enzyme that contributes to the degradation of collagen in the caries progression. Ainoosah et al. [13] used SDF at 38% and reported that SDF was effective in preventing erosion in dentin agreeing with Gadallah et al. [29] who stated that SDF is effective in preventing tooth decay in primary teeth, as well as FV. In our study, SDF at 30% was used. The divergence in results can be attributed to the difference in SDF concentration and to the different substrates tested; however, there is a lack of studies comparing the effectiveness of different SDF concentrations on dental erosion.

Solutions based on silver nanoparticles have been gaining popularity due to their antibacterial, antifungal, and antiviral potential [30]. In this study, an experimental solution containing silver nanoparticles (AgNano) was tested as a possible solution to reduce dental erosion, and it was compared with silver diamine fluoride and fluoride varnish. The results show that AgNano was not effective against dental erosion in bovine enamel. Abo El Soud et al. [31] performed a comparative study on the effects of SDF and nanoparticulate silver fluoride on demineralized enamel. The authors concluded that nanoparticulate silver fluoride is more effective than SDF in remineralizing the surface of demineralized enamel. Remineralizing solutions were applied after the demineralization of specimens in the study by Abo El Soud et al. [31], unlike our study, where remineralizing agents were applied before the erosive challenge, which may explain the divergence of results, as well as differences in the composition of these solutions.

Chitosan is a cationic polysaccharide that forms acid-resistant multilayers [32]. Some authors incorporated chitosan into the silver nanoparticulate solution and obtained positive results in inhibiting dental caries [14,15], due to its antibacterial and remineralizing properties [22]. Targino et al. [15] used chitosan incorporated into the silver nanoparticle solution to improve the molecular weight and stabilize the compound, increasing the solution's adhesiveness. However, Beltrame et al. [5], who extensively studied the anti-erosion effect of chitosan, concluded that when compared to other fluoridated solutions, the solution presented limitations in reducing loss of dentin surface after erosive challenge.

Some limitations of this study must be reported. Bovine teeth showed greater susceptibility to erosion/abrasion compared to human teeth [6]. Furthermore, the oral environment, simulating dental remineralization, could not be fully reproduced. Further studies are needed, as the evidence is still limited.

Conclusion

AgNano associated or not with dentifrice with high fluoride concentration was not able to reduce tooth erosion, as well as SDF and FV. Dentifrice with high fluoride concentration significantly minimized the effects of enamel microhardness loss.

Authors' Contributions

GTZ	https://orcid.org/0000-0002-7802-7014	Conceptualization, Methodology, Validation, Investigation and Writing - Original Draft.
EPR	https://orcid.org/0000-0002-3263-0299	Conceptualization, Methodology and Resources.
AM	https://orcid.org/0000-0002-0695-402X	Methodology, Investigation and Writing - Review and Editing.
RBSO	https://orcid.org/0009-0007-4665-843X	Methodology, Resources and Writing - Review and Editing.
MBL	https://orcid.org/0000-0001-6198-7695	Writing - Original Draft, Writing - Review and Editing and Visualization.
RDG	https://orcid.org/0000-0002-1625-3120	Writing - Original Draft, Writing - Review and Editing and Visualization.
SBB	https://orcid.org/0000-0002-7915-3207	Writing - Original Draft, Writing - Review and Editing, Visualization, Supervision and Project Administration.

All authors declare that they contributed to critical review of intellectual content and approval of the final version to be published.

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Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

The data used to support the findings of this study can be made available upon request to the corresponding author.

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