Received: 17 February 2024 | Revised: 13 May 2024 | Accepted: 25 May 2024

DOI: 10.1111/jerd.13271

RESEARCH ARTICLE

WILEY

Effectiveness and safety of biosilicate-enhanced bleaching gels on enamel with early erosion lesion

Rafael Dascanio DDS, MS[1] | Rafael Antonio de Oliveira Ribeiro DDS, MS[2] | Camila Siqueira Silva Coelho DDS, MS, PhD[1] | Marina Trevelin Souza DDS, MS, PhD[3] | Matheus Kury DDS, MS, PhD[4] | Edgar Dutra Zanotto Eng, MS, PhD[3] | Carlos Alberto de Souza Costa DDS, MS, PhD[5] | Vanessa Cavalli DDS, MS, PhD[1]

[1]Department of Restorative Dentistry, Piracicaba Dental School, University of Campinas, Piracicaba, São Paulo, Brazil

[2]Department of Dental Materials and Prosthodontics, School of Dentistry, São Paulo State University (UNESP), Araraquara, Brazil

[3]Vitreous Materials Laboratory (LaMaV), Department of Materials Engineering, Federal University of São Carlos, São Carlos, Brazil

[4]Dental Research Division, School of Dentistry, Paulista University (UNIP), São Paulo, Brazil

[5]Department of Physiology and Pathology, School of Dentistry, São Paulo State University (UNESP), Araraquara, Brazil

Correspondence
Vanessa Cavalli, Department of Restorative Dentistry, Piracicaba Dental School, University of Campinas, Avenida Limeira 901, Piracicaba, São Paulo CEP: 13414-903, Brazil.
Email: cavalli@unicamp.br

Funding information
Coordination for the Improvement of Higher Education Personnel (CAPES), Grant/Award Number: 001; Institutional Program of Scientific Initiation Scholarships from the Brazilian National Council for Scientific and Technological Development (CNPq); São Paulo Research Foundation, FAPESP, Grant/Award Numbers: # 2020/06782-4, # 2023/03511-8

Abstract

Aim: This study evaluated the efficacy and cytotoxicity of 35% hydrogen peroxide (HP) gel incorporated with 10% (w/w) biosilicate (BioS) on sound enamel and early-stage enamel erosion lesions.

Methods: Discs of enamel/dentin were selected, subjected to erosive cycles (0.3% citric acid, pH 2.6), and treated with ($n = 8$): HP (35% HP, positive control); HP_BioS [carboxymethyl cellulose (CMC) + HP + BioS]; BioS (CMC + BioS); CMC (negative control). The discs were adapted to artificial pulp chambers with the enamel exposed for bleaching, and the dentin facing toward the culture medium (Dulbecco's modified Eagle's medium [DMEM]). Bleaching was performed in three 30-min sessions at 7-day intervals. After bleaching, the diffusion product (DMEM extract + diffused HP) was pipetted onto MDPC-23 odontoblastic cell line and inoculated. Color parameters (ΔL, Δa, Δb), color change (ΔE_{00}), and changes in whiteness index (ΔWI_D) were determined before (T_0) and after the last bleaching session (T_3). Cell viability (MTT, %), H_2O_2 diffusion (μg/mL), oxidative cell stress (OxS), and cell fluorescence (live/dead assay, in confocal microscopy) were assessed (ANOVA/Tukey; $\alpha = 0.05$).

Results: No difference in ΔL, Δa, Δb, ΔE_{00}, and ΔWI_D were found between HP and HP_BioS ($p > 0.05$). The incorporation of BioS decreased the HP diffusion into the substrates and mitigated oxidative stress in early-stage eroded enamel ($p < 0.05$). HP_BioS presented significantly higher cell viability compared with HP under erosion conditions. Live/dead assay indicated that BioS_HP maintained viability with larger clusters of viable cells.

Conclusion: Incorporating BioS into HP maintained bleaching effectiveness, favored cell viability, reduced the oxidative stress, and the cytotoxicity in teeth with early-stage erosion.

Clinical Significance: BioS formulation showed promising results for reducing cytotoxicity in patients seeking tooth bleaching and presenting undetectable early-stage erosion.

KEYWORDS
biosilicate, cytotoxicity, tooth bleaching

1 | INTRODUCTION

Dental erosion is a multifactorial pathological process characterized by the gradual loss of dental tissue due to enamel exposure to nonbacterial origin acids.[1] Intrinsic factors like gastroesophageal reflux and extrinsic factors, such as the consumption of acidic foods, may be implicated and capable of initiating mineral loss.[2] This process starts with the softening of the enamel surface, with a wear thickness ranging between 0.2 and 3 μm, followed by the continuous dissolution, layer by layer, of the enamel crystals, leading to a permanent loss of tooth volume.[3]

In recent years, the rapid and constant consumption of acidic products has contributed to the increase and severity of early-stage dental enamel erosion lesions.[2,4] During the early-stage erosion, where there is no visible loss of the tooth structure or associated sensitivity, patients often undergo esthetic treatments, such as bleaching, which could further compromise enamel mineral loss.[5] Besides mineral loss, studies reveal that highly concentrated hydrogen peroxide (HP) bleaching treatments can adversely affect cell viability and harm pulp cells.[6–8] From the clinical standpoint, the cytotoxicity effect of HP would be translated into tooth sensitivity, a common side effect that leads to treatment discontinuation in approximately 60% of patients.[9]

The cytotoxic effects of HP are intrinsically related to its transenamel and transdentinal diffusion within the dental structure. This direct relation is established because the reactive oxygen species (ROS), generated from the breakdown of HP, holds the ability to reach the pulp chamber, triggering a significant increase in oxidative stress in pulp cells.[6–8] Before reaching the pulp tissue, short-lived ROS cleave the organic compounds that stain dentin,[10–12] but due to the low molecular weight of ROS and unreacted HP, they can penetrate dental pulp.[13] As a result, cell mutation, enzymatic inactivation, protein degradation, and cell death or tissue necrosis may occur.[14] It is important to note that the magnitude of pulp damage is directly associated with the residual amount of HP capable of diffusing through enamel and dentin to reach the cells.[14]

Based on previous investigations, the diffusion capacity of HP is intrinsically linked to its concentration, frequency, and exposure time to the dental surface[15] and the thickness of the bleached substrate.[16] Indeed, a previous study conducted by Oliveira Duque et al.,[16] indicated that teeth with lower thickness, such as mandibular incisors, are more susceptible to the cytotoxicity of HP. Hence, it is plausible to infer that high concentrations of HP applied to enamel with initial erosion may induce more pronounced cytotoxic effects than on sound enamel, in addition to causing greater surface hardness loss, increased roughness, and an imbalance in the calcium/phosphate concentration in the enamel.

In this scenario, alternatives to counteract the damage to teeth with early-stage enamel erosion become necessary. Studies have demonstrated that using bioactive glasses and glass–ceramics resulted in a remarkable and beneficial interaction with hard tissues, forming in vivo a hydroxycarbonate apatite layer on their surfaces and promoting a robust interface and solid connections with bones and teeth. Biosilicate (BioS) is an example of highly bioactive glass–ceramic whose bioactivity index (BI) exceeds eight.[17–19] Initially, this biomaterial was proposed to address dentin hypersensitivity, presenting the best performance to control sensitivity compared with other agents in a randomized-controlled clinical trial.[20] Also, studies have shown that BioS can trigger tissue regeneration, control dental erosion, and reduce the progression of caries lesions.[21–23] A previous study highlighted the relevance of a bleaching gel formulation associated with biosilicate in improving postbleaching enamel microhardness.[24] Originating from the sealing effect of the dental surface, BioS would prevent or minimize external stimuli, avoiding pulp inflammation and reducing the sensation of pain.[25] In fact, the evaluation of pulp inflammation after the application on sound enamel of gels containing HP combined with BioS or only BioS before bleaching indicated that BioS could attenuate pulp damage.[26]

Previous studies have underscored the potential of BioS in reducing superficial loss and the development of erosion and caries lesions.[23] An aqueous suspension containing 10% BioS with distilled water, applied on enamel and dentin surfaces, has demonstrated efficacy in controlling the progression of these lesions. The results indicated that BioS could precipitate a homogeneous layer of hydroxyapatite carbonate (HCA) in just 24 h, covering the entire dentin surface.[23] In another study, a remineralization solution containing BioS was prepared by diluting 10% of the remineralizing agent powder in deionized water, applied on dentin after dental bleaching.[24] Dentin remineralization has proven to be an effective therapy for restoring damage caused by bleaching and prior acid conditioning before restoration. This approach not only increased dentin mineral compounds but also enhanced its chemical interaction capacity with the adhesive system.[24]

Although HP therapies are effective for bleaching purposes, the incorporation of 10% BioS into high-concentrated bleaching gel could minimize undesired effects without negatively affecting its optimal esthetic outcomes, especially on teeth with early-staged eroded enamel. Therefore, this study aimed to assess the efficacy and cytotoxicity of a bleaching gel containing 35% HP incorporated with 10% (w/w) biosilicate applied on sound enamel and enamel with initial erosion lesions. Thus, the null hypotheses postulated were that the experimental gels containing BioS (1) would not affect the bleaching outcome of 35% HP on sound enamel and enamel with initial erosion lesions and (2) would not decrease transenamel-dentin cytotoxicity on sound enamel and enamel with initial erosion lesions.

2 | MATERIALS AND METHODS

2.1 | Experimental design

Sixty-four bovine enamel/dentin discs were prepared and selected according to the initial mean lightness values (L^* parameter). Half of the discs were immersed in 0.3% citric acid (pH 2.3) for 5 min. Sound and early-stage eroded dental discs were fixed into artificial pulp chambers (APCs) and randomly submitted to the treatments ($n = 8$/group) with:

- Carboxymethyl cellulose (CMC, thickening gel containing 6 w/v% CMC and deionized water)—negative control.
- HP (commercial 35% HP) —positive control
- HP_BioS (35% HP + 10 wt% biosilicate combined with CMC)
- BioS (10 wt% biosilicate combined with CMC)

Bleaching was carried out in groups HP and HP_BioS (with or without early-stage erosion) during three sessions of 30 min each with a 7-day interval between them. Groups CMC and BioS were treated following the same protocol as the bleaching groups. Color (ΔE_{00}) and whiteness index for dentistry (ΔWI_D) changes were determined before (T_0) and after the last bleaching session (T_3). Viability of MDPC-23 cells (MTT, %), H_2O_2 transenamel/dentin diffusion (μg/mL), oxidative cell stress (OxS), and live/dead confocal microscopy fluorescence assay were assessed immediately after the first bleaching session (T_1).

2.2 | Specimen preparation

Bovine incisors with intact enamel, free of fractures and fissures, were selected. The teeth were cleaned and disinfected in a 0.4% thymol solution (Labsynth, Diadema, SP, Brazil). Subsequently, enamel/dentin discs (5 mm in diameter and 3 mm in thickness) were obtained from the central region of the bovine incisors using a bench drill (FSB 16, Pratika, Shulz, SP, Brazil). The dentin surface of the discs was smoothed with #600 grit sandpaper to ensure parallelism and achieve a standardized total thickness of 2.3 mm.[22] The enamel and dentin discs were stained with black tea with neutralized pH (7.0) for 24 h and then kept in artificial saliva for 48 h.

2.3 | Early-stage erosion protocol

The blocks underwent an erosion cycling adapted from a previous study.[26] Since the original protocol preconized 4 days of cycling, this would not represent an early-stage in vitro erosion model, so one day of cycling was used herein. This process was adapted from a previous study[27] and involved immersion in a 0.3% citric acid solution (pH 2.6, 2.5 mL/mm^2 of the exposed dental enamel area) for 5 min, followed by rinsing with distilled water only for 1 day. Subsequently, these blocks were immersed in artificial saliva (1.5 mM Ca, 0.9 mM PO4, and 150 mM KCl in a 20 mM Tris buffer solution at pH 7.0) for 24 h (2.5 mL/mm^2 of the exposed dental enamel area).

2.4 | Preparation of the gels

The negative control group consisted of CMC as a thickening agent and H_2Od (6 w/v%). The positive control was a commercial 35% HP gel treatment (Whiteness HP, FGM, Dental Industry and Commerce, Joinville, PR, Brazil), which was manipulated as described by the manufacturer (Figure 1).

The experimental HP_BioS gel consisted of CMC and H_2Od, 10 wt% of BioS particles, and 35% HP. The BioS particles were prepared following the conventional melting followed by the heat treatment process,[28] and the fabricated particles were incorporated into the CMC gel and homogenized in a specific mixer (Speed Mixer, Dac Iso 1. FVZ, Flack Teck, Inc., Hann, Germany) in 2 cycles of 4000 rpm for 2 min (Figure 1B). Later, 0.4 g of this gel was combined with 0.54 mL of 35% HP solution of the commercial Whiteness HP kit to create the HP_BioS. The BioS gel consisted of 10% BioS combined with CMC, but without 35% HP.

The pH of the BioS gel was determined in triplicate using a pH meter coupled to a microelectrode (DG-101SC, Mettler Toledo, Brazil). The mean values were 9.77, while the Whiteness HP (FGM, Dental Industry and Commerce, Joinville, PR, Brazil) exhibited mean pH of 6.75.

2.5 | Colorimetric analysis

A digital spectrophotometer (EasyShade, Vita Zahnfabrik, Bad Säckingen, Germany) determined the color parameters L^* (black–white axis), a^* (red green axis), and b^* (yellow blue axis). Color change was evaluated using the CIEDE2000 formula $[\Delta E_{00} = (\Delta L'/KLSL)^2 + (\Delta C'/KCSC)^2 + (\Delta H'/KHSH)2 + RT^*(\Delta C'/KCSC)(\Delta H'/KHSH)^{1/2}]$. The whiteness index was calculated using the following formula: $WI_D = (0.511 \times L) - (2.324 \times a^*) - (1.100 \times b^*)$. These equations were calculated after staining the specimens with black tea (T_0) and 24 h after the last bleaching session (T_3). ΔE_{00}, ΔWI_D, ΔL, Δa, and Δb refer to the final color measured between T_0 and T_3.

2.6 | Artificial pulp chambers

Dental discs were adapted into APCs using two silicone rings, sealed with utility wax to prevent any leakage of the bleaching gel into the pulp space, according to this previously described protocol.[6] The assembly, consisting of the chambers and disc, was previously sterilized using ethylene oxide (ACECIL—Industrial Sterilization Center Ltd., Campinas, São Paulo, Brazil). The disc-APC assemblies were placed in 24-well plates (KASVI Inc.). Only the dentin surface remained in contact with the culture medium, and the enamel surface was left exposed to undergo the bleaching protocol. Immediately after the bleaching procedures, the culture medium in contact with the dentin was collected, homogenized, and distributed into aliquots in 24-well plates (KASVI Inc.), where pulp cells had been previously cultured. The extracts were incubated for 1 h in contact with the cells and immediately analyzed.

2.7 | MDPC-23 cell culture

Immortalized MDPC-23 odontoblastic lineage cells, stored in liquid nitrogen at the Laboratory of Experimental Pathology and

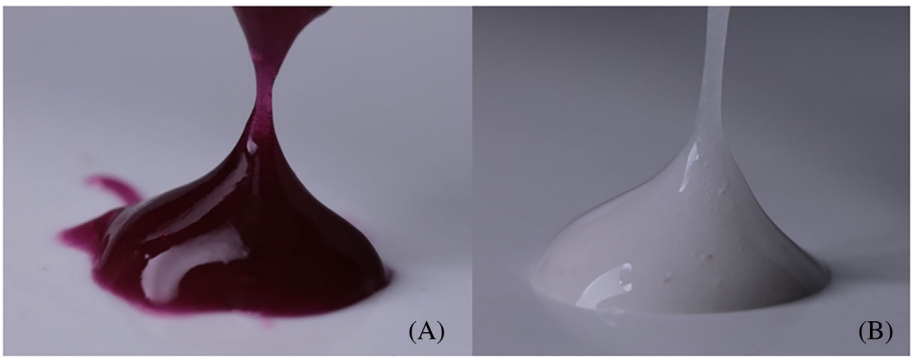

FIGURE 1 (A) Commercial bleaching gel (whiteness hydrogen peroxide [HP]). (B) The experimental gel containing BioS, carboxymethyl cellulose (10 wt%), and HP.

Biomaterials at the School of Dentistry in Araraquara—UNESP, were thawed and cultured in 75 cm^2 flasks (KASVI Inc., São José dos Pinhais, PR, Brazil). Subsequently, the pulp cells were maintained in culture media using Dulbecco's modified Eagle's medium (DMEM; GIBCO, Grand Island, NY, USA), supplemented with 10% fetal bovine serum (FBS; GIBCO), 100 IU/mL of penicillin, 100 μg/mL of streptomycin, and 2 mmol/L of glutamine (GIBCO). Plates containing the cells were kept in a humid atmosphere at 37°C with 5% CO_2 and 95% air concentration. The cells were plated and counted using an inverted light microscope and subcultured as needed until the required number of cells was obtained.

2.8 | Cellular metabolism (MTT)

The APC was secured onto 24-well acrylic plates, ensuring direct contact with the dentin surface submerged in the culture medium. Following the 30-min bleaching protocol, the bleaching gels were completely removed. Subsequently, cell viability was evaluated based on the ability of active mitochondria to convert a tetrazolium compound into an insoluble product. Viable cells with active succinate dehydrogenase enzyme activity produced blue formazan crystals. After bleaching, MDPC-23 cells adhered to the plate bottoms ($n = 8$) were incubated in a solution comprising 90 μL DMEM and 10 μL MTT solution. The formazan crystals were dissolved, and absorbance at 570 nm was measured (Synergy H1, Biotek). The absorbance value from groups without the bleaching treatment (CMC and BioS) was considered 100% cell viability, serving as reference for calculating cell viability in other experimental groups.

2.9 | Oxidative stress measurement

The cellular oxidative stress assay assessed the ROS production by the cells ($n = 8$) immediately after bleaching. For this purpose, prior to exposure to the extracts, MDPC-23 cells were subjected to a 30-min exposure to 10 μg/mL of carboxy-H2DCFDA dye (Invitrogen, San Francisco, CA, USA). This dye can permeate the cell membrane and generate fluorescence upon encountering ROS. Subsequently, following exposure to the extracts, the cells' emitted fluorescence intensity assessed at 592 nm with an emission wavelength of 517 nm using the Synergy H1 instrument. The data were normalized concerning the negative control group, enabling the computation of the proportional increase in fluorescence intensity for each experimental group in accordance with previous descriptions.[6]

2.10 | Quantification of HP transenamel/dentin diffusion

Aliquots of 100 μL from the extracts of each group ($n = 8$) were transferred to tubes containing 900 μL of acetate buffer solution (2 mol/L, pH 4.5), which stabilizes the pH. Subsequently, 500 μL of this solution was transferred to tubes containing water and leucocrystal violet dye (0.5 mg/mL; Sigma). After mixing the tubes, 50 μL of the solution containing horseradish peroxidase enzyme (1 mg/mL, Sigma) was added to these tubes, and the absorbance of the solutions was measured using a spectrophotometer at a wavelength of 596 nm. A standard curve of known amounts of HP was used to convert the optical density values obtained from the samples into μg of HP per mL of extract.

2.11 | Live/dead confocal fluorescence microscopy

This assay was performed with the Live/ Dead Cell Viability/ Cytotoxicity kit (Invitrogen) ($n = 4$), which uses the ethyl homodimer-1 (EthD-1) fluorescence probe that connects DNA bands only to cells with membrane ruptures. The second probe was Calcein AM (CA), hydrolyzed with cytoplasmic esterases in viable cells (Leica DM 5500B, Nussloch GmBH, Germany).

2.12 | Cell morphology

For this analysis, cells were seeded on glass coverslips placed at the bottom of 24-compartment plates ($n = 4$), and the same bleaching procedures described previously were performed. After the incubation in the extracts, cells were aspirated, and MDPC-23 cells that remain adhered to the glass coverslips were fixed for 1 h in 1 mL of 2.5% glutaraldehyde (VETEC Química Fina LTDA, Duque de Caxias,

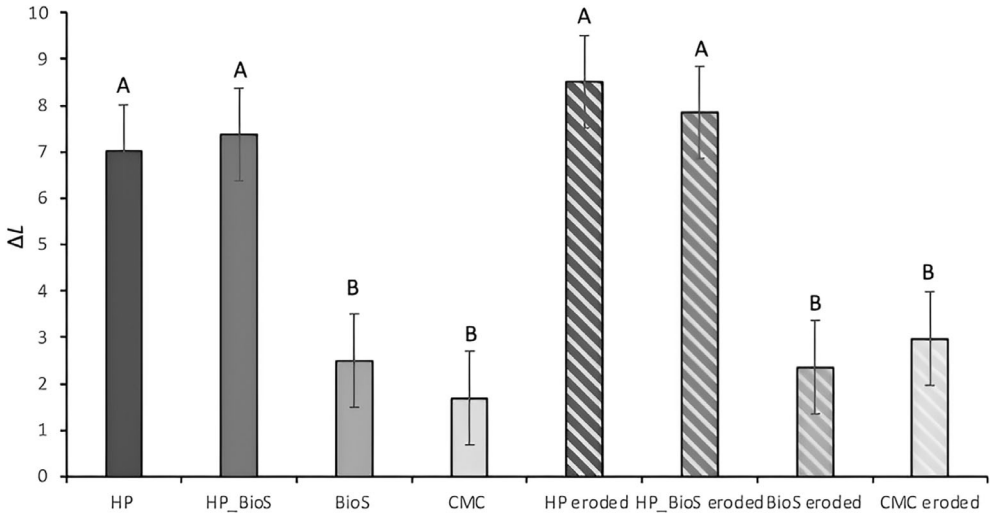

FIGURE 2 Lightness variation analysis. Bar chart with mean values and standard deviation for comparative analysis of groups with and without the addition of BioS. Different letters indicate a significant difference between groups (ANOVA; Tukey test; $p < 0.05$).

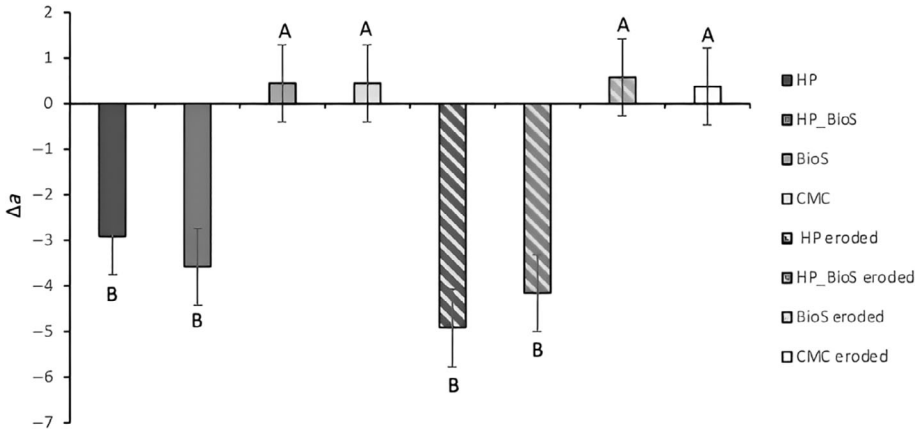

FIGURE 3 Analysis of the variation of the * axis (green-red). Bar chart with mean values and standard deviation for comparative analysis of groups with and without the addition of BioS. Different letters indicate a significant difference between groups (ANOVA; Tukey test; $p < 0.05$).

RJ, Brazil). After initial fixation, the glass coverslips with adhered cells were washed three times with 1 mL of PBS (5 min for each wash) and postfixed with 200 µL of 1% osmium tetroxide for 1 h. Cells were washed three times with 200 µL of HMDS (1,1,1,3,3,3,-hexamethyldisilazane; Sigma-Aldrich Corp., St. Louis, MO, USA) for 20 min. Finally, the coverslips were placed on metal stubs and kept in a desiccator (Laborquimi, Poá, SP, Brazil) for 72 h, followed by gold coating for scanning electron microscopy (SEM) analysis. Representative images were obtained at 300×, 500×, and 1500× magnifications (SEM, MEV-LEO 435 VP, LEO Electron Microscopy Ltd, Cambridge, UK).

2.13 | Statistical analysis

Data underwent an exploratory analysis of normality and were analyzed using SPSS 23 software (IBM Corporation—Armonk, NY, USA). After the normality was verified, ΔL, Δa, Δb, ΔE_{00}, ΔWI_D, cell viability, oxidative stress, and HP diffusion data were subjected to one-way ANOVA and Tukey tests. A significance level of 5% was established for all analyses.

3 | RESULTS

3.1 | Color change

For lightness variation, no significant differences were observed with or without adding BioS in the HP's composition, whether applied to sound or eroded enamel (Figure 2). Likewise, for the red-to-green axis (Δa) and the yellow-to-blue axis variation (Δb), no significant differences were found with the addition of BioS to the HP gel's composition, indicating no interference with the bleaching efficacy (Figures 3 and 4).

Figure 4 displays color change (ΔE_{00}), and Figure 5 displays the whiteness index (ΔWI_D) variation results. HP-containing groups exhibited the highest ΔE_{00} and ΔWI_D mean values among groups, and no significant differences in ΔE_{00} (Figure 5) and ΔWI_D (Figure 6) were observed between the experimental bleaching gels (HP_BioS) and the positive control group (35% HP), regardless of the enamel condition (sound or early-stage erosion) ($p \geq 0.05$). As expected, BioS and CMC exhibited lower ΔE_{00} and ΔWI_D among groups at both enamel conditions ($p < 0.05$).

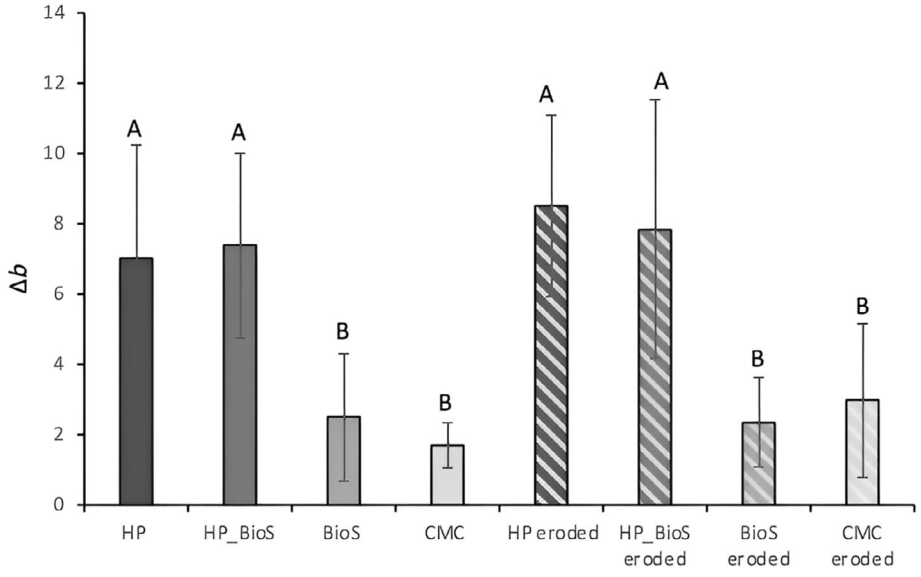

FIGURE 4 Analysis of the variation of the b^* axis (yellow-blue). Bar chart with mean values and standard deviation for comparative analysis of groups with and without the addition of BioS. Different letters indicate a significant difference between groups (ANOVA; Tukey test; $p < 0.05$).

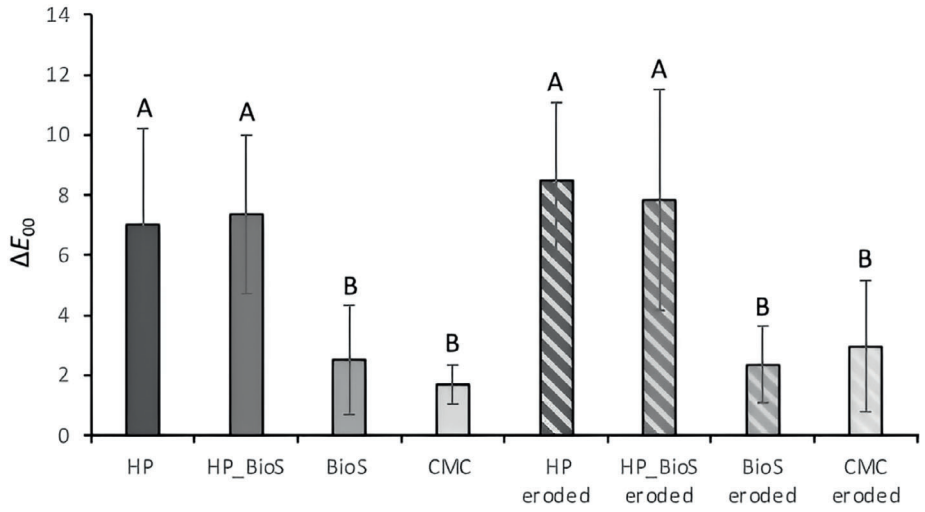

FIGURE 5 Color change analysis. Bar chart with mean values and standard deviation for comparative analysis of groups with and without the addition of BioS. Different letters indicate a significant difference between groups (ANOVA; Tukey test; $p < 0.05$).

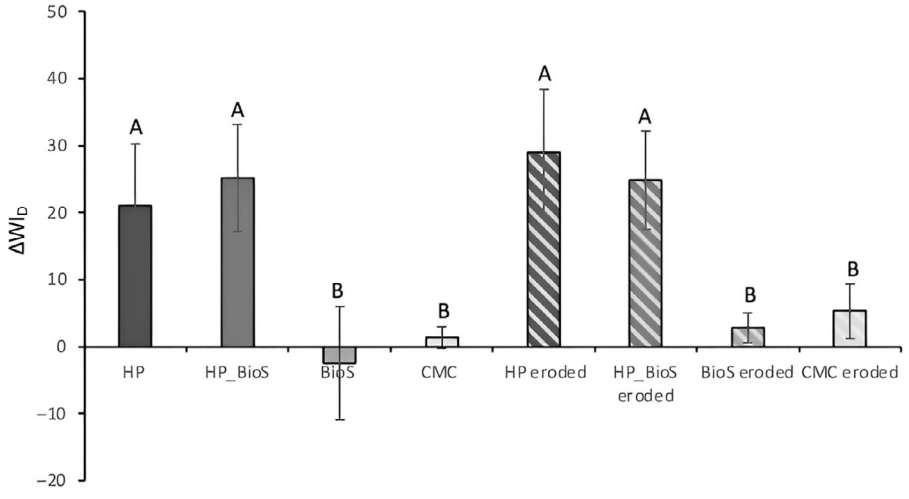

FIGURE 6 Whiteness Index variation analysis. Bar chart with mean values and standard deviation for comparative analysis of groups with and without the addition of BioS. Different letters indicate a significant difference between groups (ANOVA; Tukey test; $p < 0.05$).

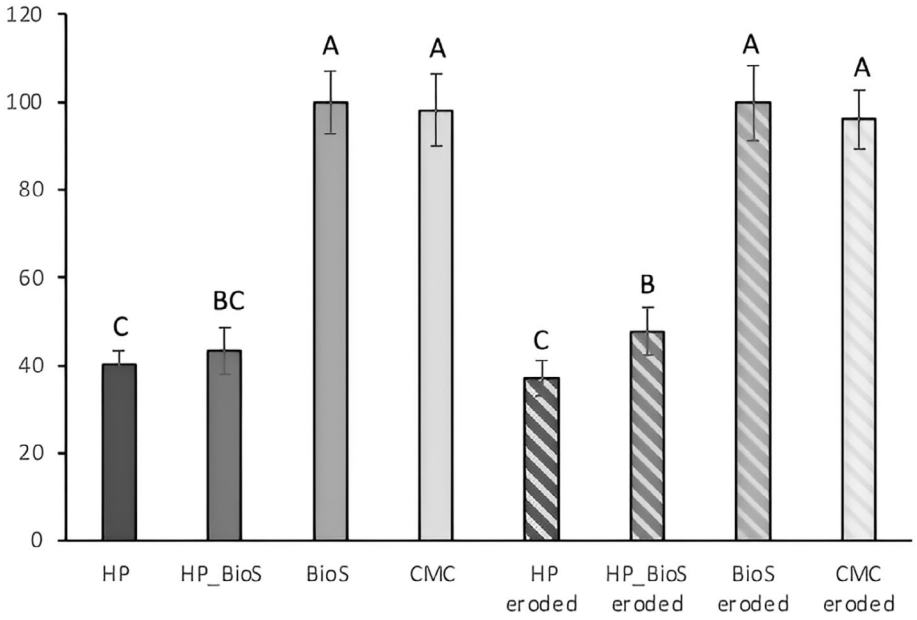

FIGURE 7 Cell viability analysis. Bar graph with mean and standard deviation values (in percent) of cell viability after the first bleaching session (T_1). Different letters indicate statistical differences, as determined by one-way ANOVA and Tukey's test ($p = 0.0009$).

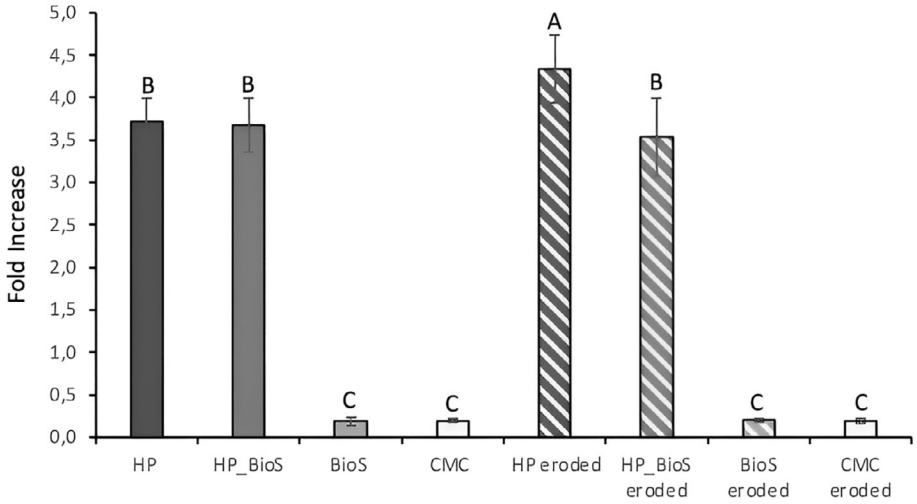

FIGURE 8 Oxidative stress analysis. Bar graph showing mean values and standard deviations of oxidative stress obtained in the first bleaching session (T_1). Different letters indicate statistical differences, according to one-way ANOVA and Tukey's HSD test ($p < 0.05$).

3.2 | Cell metabolism (MTT assay)

BioS and CMC treatments (no HP) exhibited the highest cell viability among the groups, regardless of enamel condition (sound/eroded). On the other hand, HP bleaching caused lower cell viability among the groups (Figure 7) either for sound or eroded enamel. The combination of HP and BioS (HP_BioS) displayed intermediate values among groups and higher viability on eroded enamel condition than HP ($p < 0.05$).

3.3 | Oxidative stress measurement

Figure 8 displays OxS in the cells, which was determined by fluorescence values. HP bleaching on eroded enamel caused the highest OxS among groups ($p < 0.05$), followed by HP bleaching on sound enamel and HP_BioS on sound and eroded enamel ($p < 0.05$). BioS and CMC displayed the lowest OxS among groups ($p < 0.05$), and no differences were found.

3.4 | Quantification of HP transenamel/dentin diffusion

Figure 9 displays the results of HP diffusion in the extracts. No differences in HP transenamel/dentin diffusion were observed among HP (sound and eroded enamel) and HP_BioS (sound enamel) groups ($p > 0.05$). However, HP_BioS applied on eroded enamel exhibited the lowest HP diffusion mean values among groups ($p < 0.05$).

3.5 | Live/dead confocal fluorescence microscopy

Figures 10 and 11 display confocal microscopy images of MDPC-23 cells submitted to HP, HP_BioS, BioS, and CMC treatments. Images of sound and eroded enamel specimens treated with HP revealed a decrease in viable cell clusters (green), an increase in necrotic cells (red), and an evident reduction in the number of cells in the culture

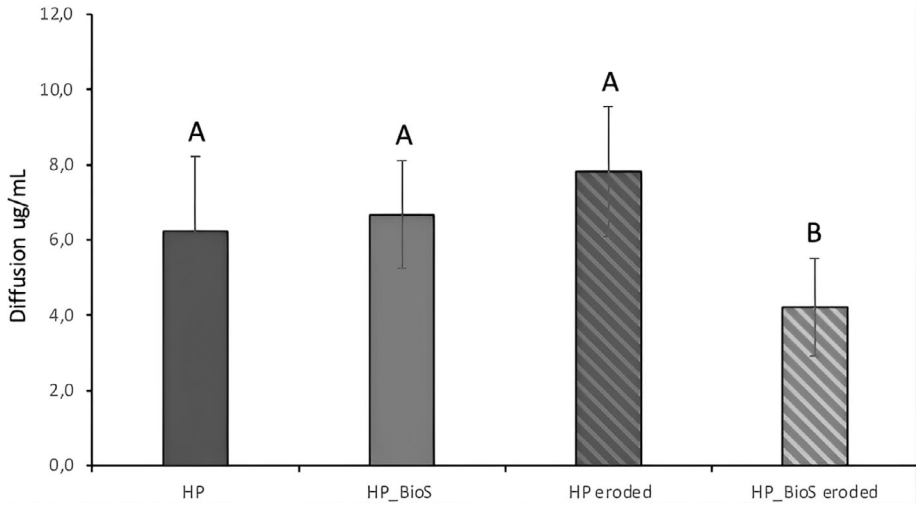

FIGURE 9 Mean values and standard deviations of HP transenamel/dentin diffusion (in micrograms per milliliter) in the first bleaching session (T_1). Different letters indicate statistical differences, according to one-way ANOVA and Tukey HSD test ($p < 0.00638$).

media for both sound and eroded enamel samples when compared with the other groups. The combination of HP_BioS displayed a noticeable preservation of cell viability with larger clusters of cells (green), higher concentrations of living cells, and lower necrotic process (red) in comparison with HP treatment. BioS and CMC groups (with no HP) exhibited mostly living cells (green), although higher amounts of dead cells (red) can be observed in CMC groups (sound and eroded enamel) in comparison with BioS.

3.6 | Cell morphology

SEM reveals (Figures 12 and 13) that BioS and CMC exhibited cells with extensive cytoplasm, spherical morphology (yellow star), and extensions from the membrane and cytoplasm (white star) on the glass substrate, for both sound and enamel early-stage eroded groups. The bleached groups (HP and HP_BioS) displayed extensive areas of the glass substrate (red star), considering that few cells exposed to the extracts remained adhered. The HP group showed cells with relevant morphological changes (blue arrows) regardless of initial erosion. HP_BioS, despite extensive areas of the glass substrate, exhibited a morphology closer to normality among the bleached groups, both for the sound enamel group and the early-stage eroded enamel group.

4 | DISCUSSION

The application of high concentrations of HP on enamel generates ROS and unreacted HP that diffuse into the pulp chamber, causing substantial biological damage to pulp cells.[6–8] In order to mitigate the presence of ROS, this study investigated the incorporation of a biomaterial known as biosilicate (BioS) with the aim of providing support to the structural integrity of sound dental elements or those with initial erosion lesions.

In this context, it is imperative that the addition of BioS does not compromise the bleaching potential of HP. According to the study results, no statistical disparities were found in luminosity (ΔL, Figure 1) between groups with HP, with or without BioS. This suggests that the presence of this biomaterial did not induce changes in bleaching effectiveness, as the groups not exposed to HP showed statistical differences compared with those exposed. For the red-to-green axis variation (Δa), all HP-containing groups, regardless of the presence of BioS, exhibited negative values with statistical similarity, indicating bleaching of the blocks. For the yellow-blue axis variation (Δb), the addition of BioS to HP did not influence the shift in the axis, being statistically similar ($p > 0.05$), indicating substrate bleaching. The groups without HP presented the lowest values, being statistically similar among themselves ($p > 0.05$).

The ΔE_{00} results demonstrate that adding BioS did not affect the bleaching efficacy of HP, as no differences were identified between the experimental gel and the positive control group. Additionally, these gels provided perceptibility (PT) and acceptability (AT) values far higher than the previously established threshold ($\Delta E_{00} > 0.8$ and 1.8, respectively).[28,29] Groups containing only BioS and CMC exhibited ΔE_{00} values of BioS on sound (2.50), early-stage eroded enamel (2.35), and CMC application on early-stage eroded enamel surface (2.97) were above the acceptability (AT) and perceptibility (PT) threshold limit of 1.8. This observation can be explained by the ionic deposition of BioS and its ion exchange with artificial saliva. These processes could alter the surface roughness, consequently impacting the ΔE_{00} variation. However, it is important to bear in mind that these ΔE_{00} were significantly lower than that obtained by HP and HP_BioS, regardless of the enamel substrate condition. To corroborate to this indication of bleaching efficacy, positive ΔWI_D values indicate the bleaching potential of groups containing HP, with or without BioS, surpassing the PT (0.7) and AT (2.6) limits. Additionally, BioS, in its remineralizing action, may contribute to longer-lasting bleaching results, as mineral-loss-free enamel is less susceptible to future staining and discoloration.[27] Thus, the first null hypothesis was accepted, as gels containing BioS did not affect the results of HP bleaching.

Various studies point to bleaching sensitivity as the main associated side effect of teeth whitening, largely attributed to the high

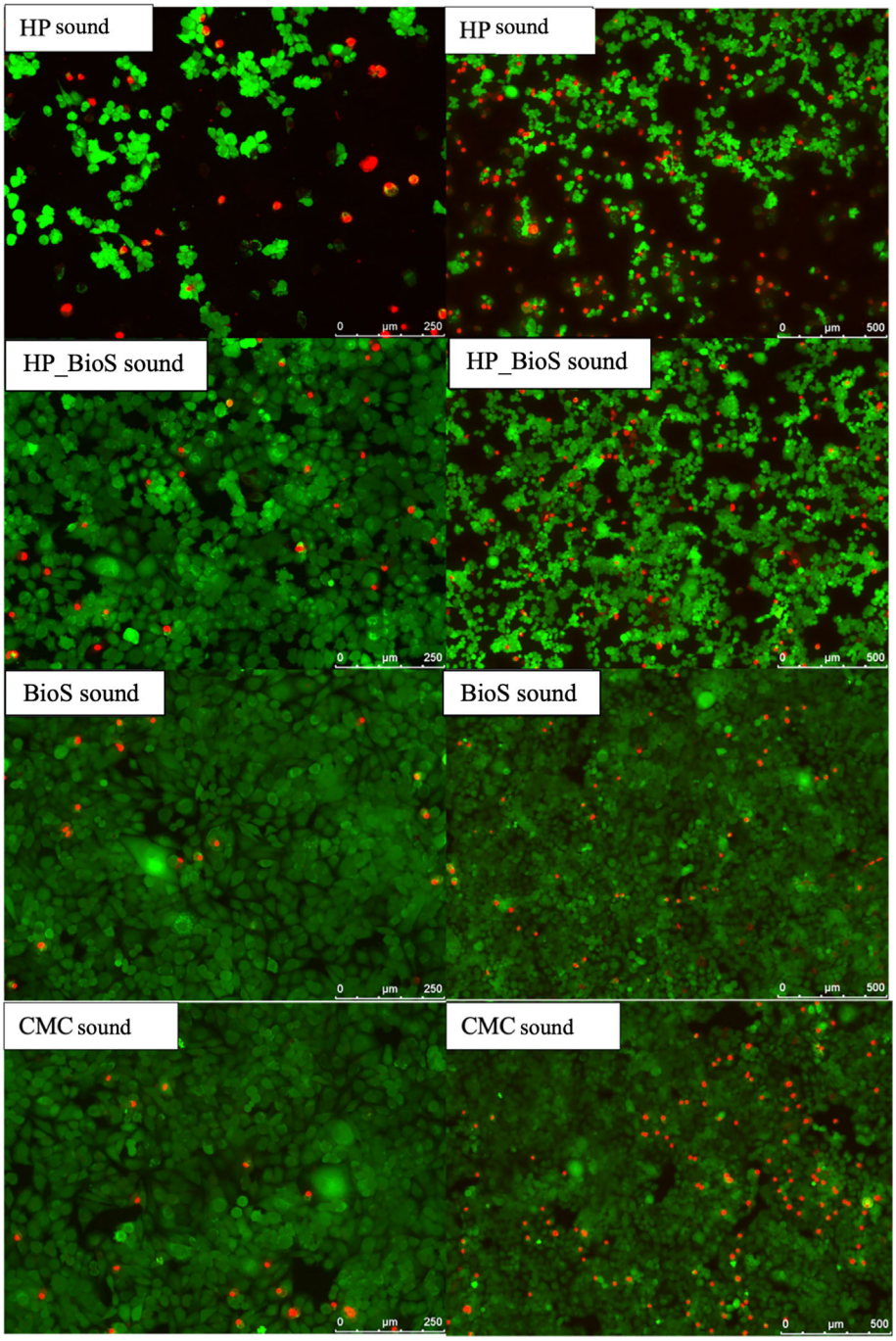

FIGURE 10 Confocal fluorescence live/dead microscopy of MDPC-23 cells submitted to treatments (HP, HP_BioS, BioS, and CMC) on sound enamel. Red fluorescence represents dead cells, and green cells represent viable ones. Scale: left column 250 µm and right column 500 µm.

oxidative stress induced by HP, resulting in irreversible damage to pulp cells.[6–8] In accordance with the literature, this study observed an increase in oxidative stress when HP was applied to sound enamel[6] and eroded enamel, with the highest value in the second one. These initial erosion lesions, potentially present in patients seeking dental bleaching, may alter the enamel permeability to substances, including HP, allowing greater surface wear[19,20] and increased ROS permeability to the pulp, as the protective enamel barrier is compromised. In this study, immortalized MDPC-23 cells, presenting an odontoblastic phenotype, were used to assess the potential transenamel and transdentinal cytotoxic effect of bleaching gels containing BioS. Odontoblasts are the first pulp cells to come into contact with components of dental materials that can diffuse through the hard tissues and reach the pulp.[6] Overall, this investigation showed that a number of MDPC-23 cells were irreversibly damaged when the HP bleaching gel was applied to enamel/dentin discs, both on sound enamel and eroded enamel. This is also evident in confocal fluorescence microscopy (Figures 10 and 11), where a lower quantity of viable cells, represented in green, and an increase in apoptotic or dead cells, represented in red, were observed compared with other groups with BioS and without HP. Clinical/histopathological studies with human teeth have shown that cell death after conventional in-office

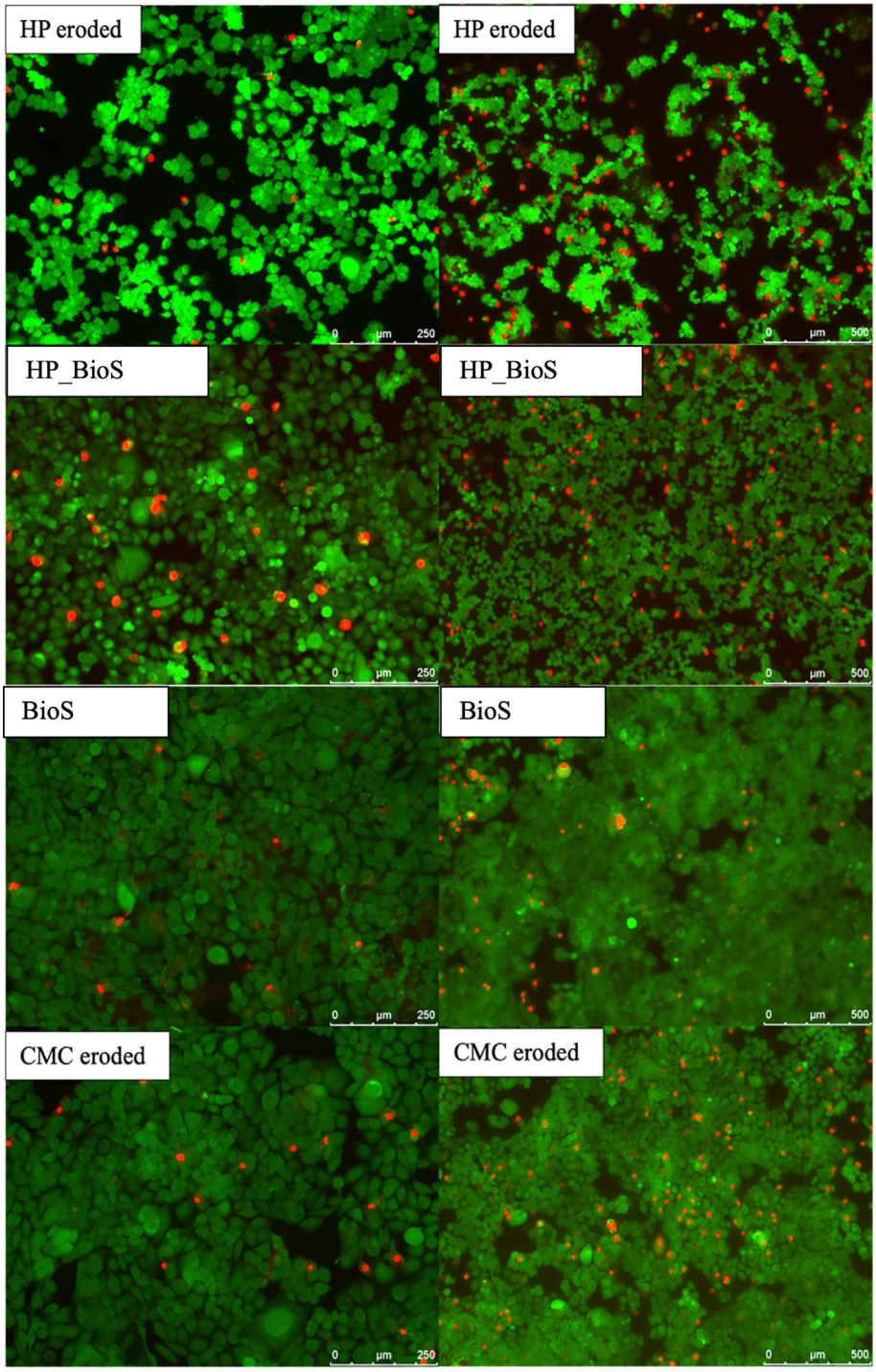

FIGURE 11 Confocal fluorescence live/dead microscopy of MDPC-23 cells submitted to treatments (HP, HP_BioS, BioS, and CMC) on early-stage eroded enamel. Red fluorescence represents dead cells, and green cells represent viable ones. Scale: left column 250 µm and right column 500 µm.

bleaching is not limited to the odontoblast monolayer lining the dentin internally[6] but becomes a trigger for acute inflammatory reactions in living tissues, resulting in the expression of tissue proteases and degradation of the extracellular matrix.[10–14] This inflammatory response can, in some cases, lead to tissue necrosis, releasing lysosomal enzymes and other intracellular components, causing substantial damage to the tissues.[13,14]

Incorporating BioS into the bleaching gel, when applied to enamel with initial erosion lesions, reduced oxidative stress statistically differing from the HP treatment. This can be justified by the bioactive compound's ability to catalyze ROS and HP residues. Furthermore, the surface reactivity of BioS could be taken into consideration, given that both the external surface and the walls of the internal nanopores of these glass ceramics increase the contact area with the environment, presenting antioxidant potential in cells and tissues.[25] This means that BioS could "capture" and neutralize excess HP, reducing the ROS that could come into contact with the pulp tissue.[26] Additionally, biosilicate can act as a buffer agent, supersaturating the medium with carbonated hydroxyapatite, contributing to maintaining an alkaline pH during bleaching.[18–20] This is important because an

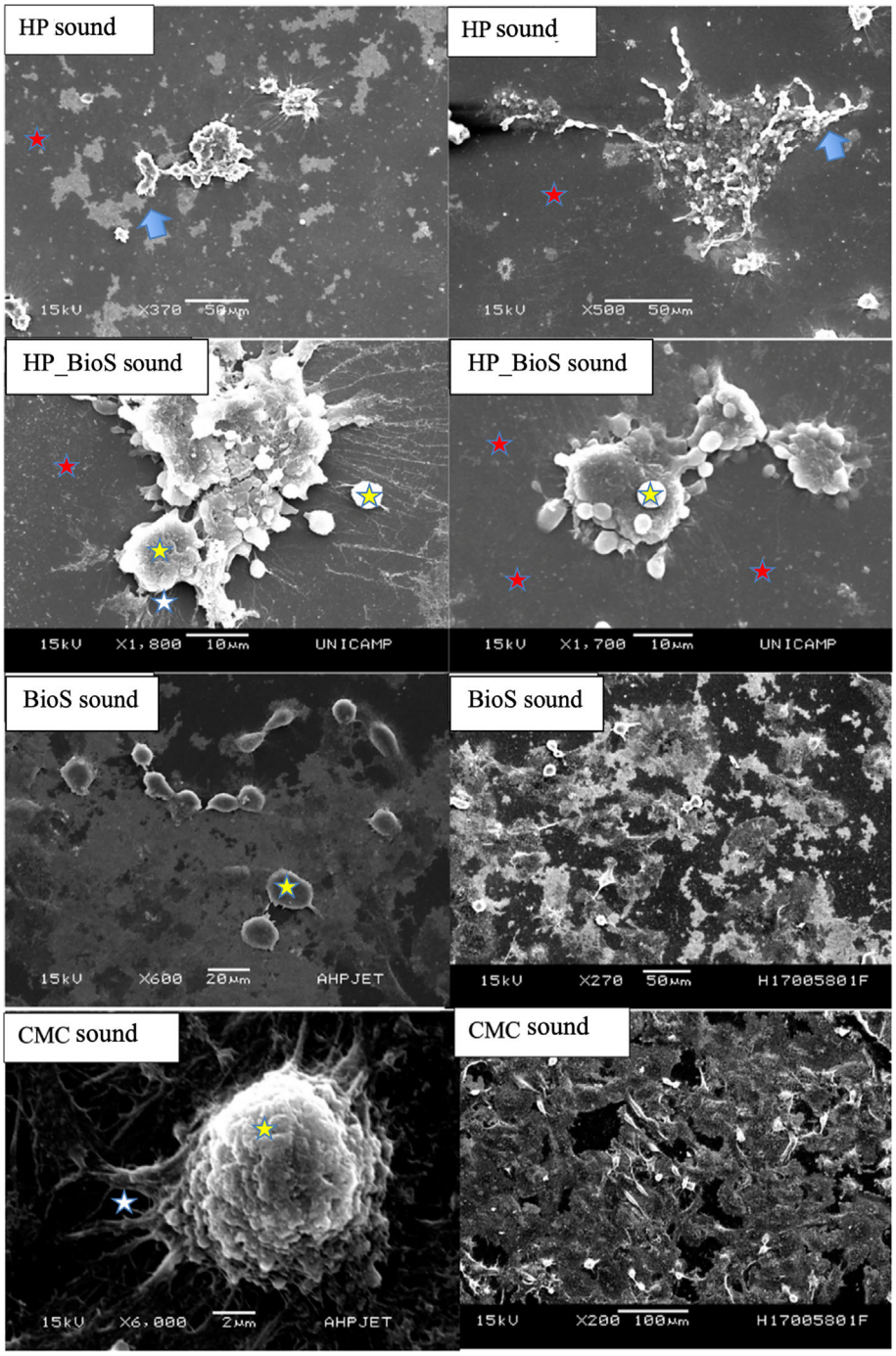

FIGURE 12 Scanning electron microscopy (SEM) images of MDPC-23 cells submitted to the transenamel/dentin diffusion at different magnifications in the bleaching protocol on sound enamel in SEM.

acidic pH can promote ROS generation, increasing oxidative stress.[13,14] Concomitantly, biosilicate can promote remineralization of dental enamel, making it less permeable to external substances, including ROS.[26] In this study, greater interaction between biosilicate and the enamel with initial erosion lesions was observed. This can be explained by the increased contact area between the bioactive material and the substrate, allowing greater ion exchange and hydroxyapatite precipitation.[18–20]

The results also showed a reduction in transenamel-dentin diffusion of HP when associated with BioS and subjected to enamel with initial erosion lesions. This phenomenon is attributed to the possibility that BioS, releasing ions to the medium, can form complexes with the eroded enamel, given its increased surface area.[25,26] This could potentially generate a layer of insoluble compounds, thus delaying the diffusion of residual ROS and unreacted HP toward the dental substrate. In this context, the research employed standardized enamel/dentine discs, simulating the characteristic thickness of human lower incisors, known for their particular susceptibility to the adverse effects of dental bleaching in a clinical setting,[10–14] which could be further exacerbated with initial erosion lesions.

In this investigation, the cytotoxicity data obtained showed a direct relationship between the cell viability rate and the presence of

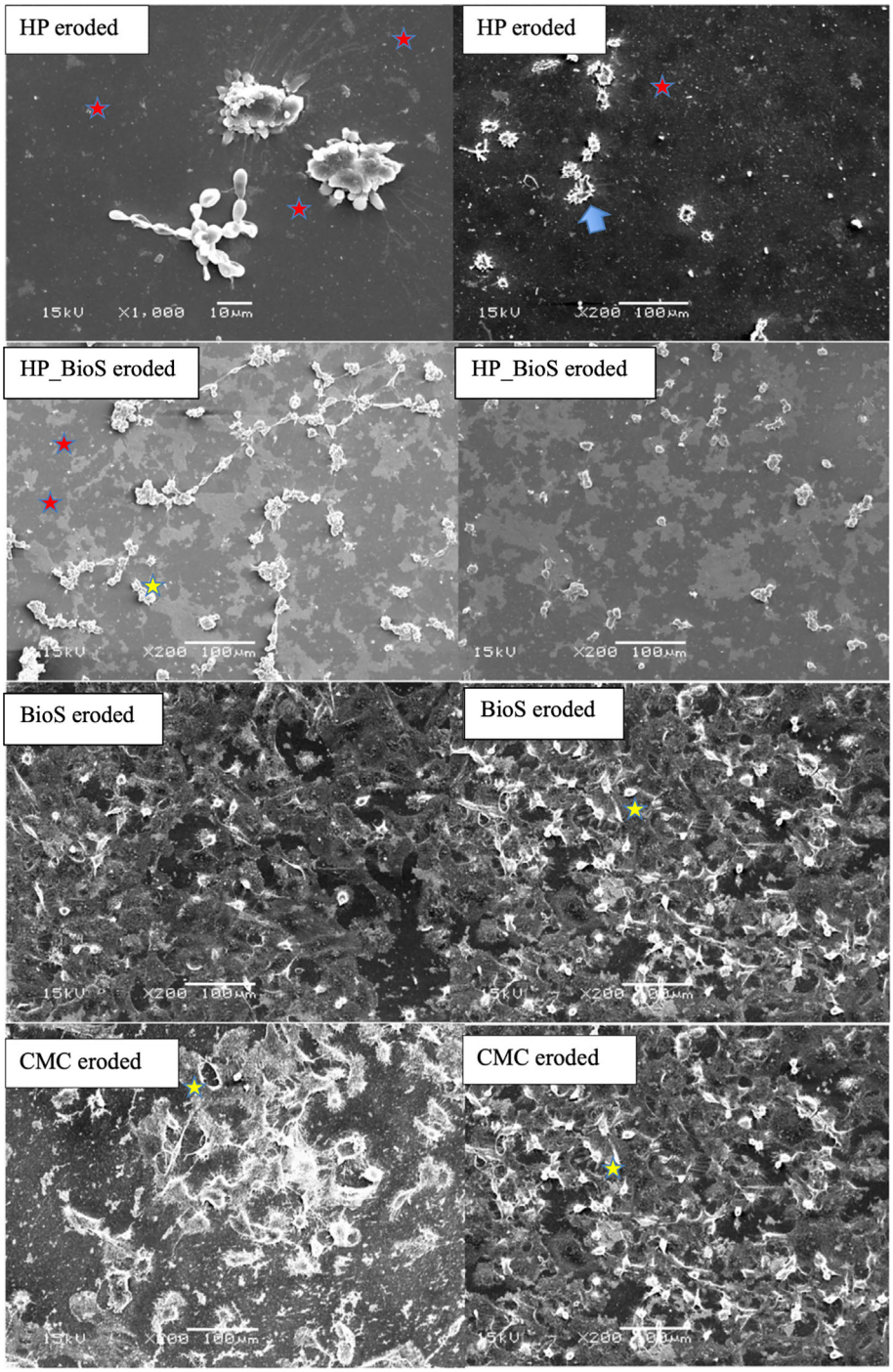

FIGURE 13 MDPC-23 cell morphology at different magnifications in the bleaching protocol on eroded enamel under SEM.

BioS in the bleaching gel. When a pure bleaching gel with HP was applied to enamel/dentine discs with initial erosion lesions, the cell viability index dropped to 37%, while adding BioS resulted in a 10% gain in cell viability compared with pure HP. This can also be observed in the assessment of cell morphology through SEM, where MDPC-23 cells, which naturally present a spherical shape when subjected to 35% HP, exhibit changes in their conformation with filaments, membrane ruptures, and cell clusters. Conversely, when BioS is added to the bleaching composition, there is a tendency to maintain cell shape. Thus, the second null hypothesis was rejected, as bleaching gels containing BioS reduced transenamel and transdentinal cytotoxic effects on enamel with initial erosion lesions. It is noteworthy that, even in the presence of BioS, the concentration of HP remains high (35%), thus maintaining itself as an extremely cytotoxic agent for pulp cells. Research has delved into reducing the concentration of HP while aiming to preserve whitening efficacy, incorporating catalysts and accelerators to minimize potential toxic effects on dental pulp.[30]

The intriguing results obtained in this in vitro investigation are quite promising. The formulation of bleaching products associated with BioS showed lower toxicity to cells and remarkable bleaching

efficacy. Its adsorption properties, buffering action, remineralization capacity, and antioxidant properties contribute to a safer environment during dental bleaching procedures, protecting the pulp tissue and reducing adverse effects related to oxidative stress.

However, despite these promising findings, existing literature reveals a significant gap in addressing the incorporation of BioS in bleaching treatments. Previous studies have investigated the use of a BioS-based gel before, during, or after bleaching with HP, and it was observed that the most favorable outcomes were achieved when BioS was mixed with 35% HP, showing similarity of the pulp histological condition with the negative control group (which did not receive HP) in all thirds of the coronal pulp. Additionally, after a 30-day period, it was found that the group treated only with HP exhibited greater deposition of tertiary dentin, followed by the group treated with the BioS and HP mixture. These findings suggest a promising interaction between BioS and HP. Furthermore, other studies indicate a potential applicability of BioS in the context of dental bleaching, such as applying BioS as a primer before the adhesive to bleached dentin, aiming to increase bond strength by enhancing the mineral matrix and dentin's chemical interaction with the adhesive. The analysis of these two BioS approaches in dental bleaching highlights the promising potential of this particle in the clinical context. The formulation of a whitening gel containing BioS aligns with this initiative to reduce damage to the pulp, as described in the current literature.

However, it is imperative to emphasize the need for additional research to comprehensively understand the mechanisms involved and the efficacy of BioS in specific clinical contexts. It is essential to understand the actual interaction between HP and BioS, and to evaluate the enamel surface and possible mineral content deposition promoted by BioS. These detailed analyses will allow for a more comprehensive understanding of the mechanisms of action of BioS along with HP, optimizing the bleaching gel formulation. Furthermore, the pulp of vital teeth, characterized as a specialized connective tissue with intrinsic regeneration potential, presents an aspect that cannot be fully replicated in the laboratory environment. This factor interferes with the intratubular diffusion of components released by dental materials toward the pulp tissue. Consequently, more significant damage to pulp cells is expected in in vitro tests of indirect cytotoxicity of dental materials. It is crucial to recognize that in vitro studies have intrinsic limitations that warrant attention. For instance, the absence of a complete biological environment may not accurately replicate the complex interactions observed in a real clinical setting. Additionally, the lack of elements, such as the host's immune response, can impact the results of in vitro studies.

As observed, BioS incorporated into a gel and combined with 35% HP did not affect its bleaching efficacy and perceptibility. However, when the potential for oxidative stress and HP diffusion to the substrate was evaluated, the BioS formulation showed promising results for the group with initial erosion lesions. Moreover, there is an increase in cell viability when BioS is incorporated into HP gel and applied on enamel with initial erosion lesions. Therefore, those patients seeking tooth bleaching and presenting undetectable early-stage erosion on enamel could benefit from this approach. However, due to the experimental nature of the tested bleaching gels, further analyses should be conducted to validate their good performance. Furthermore, future studies should evaluate the interaction process between BioS and HP, as well as their interaction with the tooth surface and adjacent tissues.

AUTHOR CONTRIBUTIONS

Conceptualization: Vanessa Cavalli, Edgar Dutra Zanotto, and Carlos Alberto de Souza Costa. *Formal analysis and methodology*: Rafael Dascanio, Camila Siqueira Silva Coelho, Rafael Antonio de Oliveira Ribeiro, Marina Trevelin Souza, Matheus Kury, Edgar Dutra Zanotto, Carlos Alberto de Souza Costa, and Vanessa Cavalli. *Writing—original draft*: Rafael Dascanio. *Writing—review and editing*: Rafael Dascanio, Camila Siqueira Silva Coelho, Rafael Antonio de Oliveira Ribeiro, Marina Trevelin Souza, Matheus Kury, Edgar Dutra Zanotto, Carlos Alberto de Souza Costa, and Vanessa Cavalli.

ACKNOWLEDGMENTS

This study was partially supported by the Coordination for the Improvement of Higher Education Personnel (CAPES)—001, the Institutional Program of Scientific Initiation Scholarships from the Brazilian National Council for Scientific and Technological Development (CNPq), and the São Paulo Research Foundation, FAPESP Grant # 2020/06782-4 and Grant # 2023/03511-8.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no financial interest in the companies whose materials are included in this article.

DATA AVAILABILITY STATEMENT

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

ORCID

Rafael Dascanio https://orcid.org/0000-0001-5590-6756
Matheus Kury https://orcid.org/0000-0002-9971-0568
Vanessa Cavalli https://orcid.org/0000-0002-9459-1926

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How to cite this article: Dascanio R, de Oliveira Ribeiro RA, Coelho CSS, et al. Effectiveness and safety of biosilicate-enhanced bleaching gels on enamel with early erosion lesion. *J Esthet Restor Dent*. 2024;1-14. doi:10.1111/jerd.13271