Resistance against Erosive Challenge of Dental Enamel Treated with 1,450-PPM Fluoride Toothpastes Containing Different Biomimetic Compounds

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Abstract

Objectives This *in vitro* study aimed to characterize the superficial and subsurface morphology of dental enamel treated with fluoridated gels containing different biomimetic compounds after erosive challenge.

Materials and Methods Bovine incisor teeth were sectioned to obtain enamel blocks (4 mm x 4 mm x 6 mm; $n = 5$) that were demineralized to create artificial caries lesions and treated by pH cycling interspersed with exposure to fluoridated toothpaste. During pH cycling (demineralization and remineralization for 2 and 22 hours, respectively) for 6 days, the enamel blocks were exposed to toothpaste slurries under agitation with one of the dental gels: Regenerate Enamel Science (NR-5 technology), Daily Regenerator Dental Clean (REFIX technology), and Sensodyne Repair & Protect (Novamin technology). The enamel blocks were subjected to an erosive challenge, immersed in 50% citric acid for 2 minutes, and then washed with plenty of distilled water. The surface and cross-sectional were assessed using scanning electron microscope (SEM). The elemental analyses (weight percentage) were determined with an energy-dispersive X-ray spectroscopy (EDS).

Results Enamel treated with the product containing REFIX technology presented a smoother surface morphology compared to the other treatments. The higher resistance to the erosive challenge can be attributed to a silicon-enriched mineral layer formed on the enamel induced by the REFIX-based toothpaste. This was not observed in the specimens treated with the other technology-containing toothpastes.

Conclusion The REFIX technology seemed to be the most promising compared to the Novamin and NR-5 technologies. In addition to forming a surface mineralized layer, the enamel treated with REFIX technology associated with the pH cycling resisted a subsequent erosive challenge.

Keywords

- enamel
- dentin
- tooth remineralization
- toothpaste
- scanning electron microscope

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Introduction

Dental erosion, defined as an irreversible chemical wear of the dental hard tissue without the involvement of bacteria, represents a tooth pathology that causes patient discomfort.¹ Enamel remineralized by natural saliva is not able to withstand the recurrent erosive attack to the tooth structure.² Therefore, preventive measures are indicated for preventing progressive, erosive tooth wear.³ Fluoride-containing oral care products used against enamel and dentin erosion might promote remineralization through apatite crystallization or replacement of the lost mineral.⁴ In fact, most of these products only reduce the hydroxyapatite dissolution to some extent.⁴ Recently, a new generation of biomimetic oral care products, using advanced technologies with stronger surface bioactivity, has been developed to optimize the interaction with the dental tissues.⁵ Different components or supplements associated with fluoride were added as ingredients in these products aiming to reproduce the natural process of dental tissue mineralization^{5,6} and boost the remineralization and regeneration potential of the hydroxyapatite.^{7,8}

Several promising biomimetic approaches to prevent tooth erosion have been investigated. One of the approaches comprises altering the dissolution properties of the hydroxyapatite with different foreign ions as substituents in the different sites of the hydroxyapatite molecule.^{9,10} Each ionic grouping of the hydroxyapatite molecule can be replaced by another of the same or different valence, either anionic or cationic.^{9,11} Changes in the solubility of hydroxyapatite may occur depending on the substitution at the calcium, phosphate, and/or hydroxyl sites.¹¹ The degree of substitution by foreign ions can vary from low substitution (such as fluoride, magnesium, and potassium) to a complete substitution (three sites).¹² The properties of the ion-substituted hydroxyapatite may vary according to crystallite morphology, crystallinity, particle size, and foreign ion substitute.⁹

A proprietary technology named REFIX was recently developed. It comprises a fluoride-containing toothpaste in association with phosphates and silica. According to the manufacturer, this association favors the formation of a fluoridated apatite and the deposition of silicon which was also incorporated deep into the hydroxyapatite and the open dentinal tubules.¹³ A recent *in vitro* study¹⁴ demonstrated that brushing teeth with the REFIX-containing toothpaste induced the formation of a silicon-enriched mineral layer on the enamel surface, proving the biomimetic mechanism of action of this fluoridated oral care product. To date, the effect of REFIX technology to prevent tooth erosive wear is unclear. This *in vitro* study aimed to characterize the mineral content and surface and cross-sectional morphology of enamel treated with fluoridated toothpaste containing REFIX technology after an erosive challenge.

Materials and Methods

Specimen Preparation of Dental Enamel

Enamel blocks (4 mm × 4 mm × 2 mm) were prepared from extracted bovine incisor teeth and stored in 0.08% thymol

solution. The specimens ($n = 5$) were embedded in self-cured acrylic resin circular molds 16-mm diameter and 3-mm deep. The outer enamel surface was ground flat with grit papers (600–1,500 grades) under water cooling and polished with 1- μ m diamond paste (Extec Corporation, Enfield, CT) in a rotating polishing machine PSK-2V (Skill-tec Comércio e Manutenção Ltda, São Paulo, SP, Brazil).

Caries-Like Lesion Formation

Following 5-min sonication in water using an ultrasonic device, one-third of the exposed enamel surface was covered with two layers of nail varnish (Risque; Niasi, Taboão da Serra, São Paulo, Brazil) as a reference sound area. Then, the specimens were demineralized to form an artificial caries lesion. Subsurface enamel demineralization was carried out using a modified model.¹⁵ Following 5-min sonication in water using an ultrasonic device, the enamel blocks were immersed individually in 32 mL of a demineralizing solution containing 1.3 mM/L $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 0.78 mM/L $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ in 0.05 M/L acetate buffer, 0.03 mgF/mL (NaF), pH of 5.0, 32 mL/specimen, during 16 hours at 37°C.

The pH Cycling

Before the remineralization pH cycling model,¹⁶ the enamel specimens had another one-third of its surface covered with two layers of nail varnish (Risque) as a reference for caries lesion area. The specimens were submitted to a pH cycling model at 37°C for 6 days. The blocks were immersed individually in a remineralization solution (1.5 mM.L-1 calcium, 0.9 mM.L-1 phosphate, 150 mM.L-1 potassium chloride in 0.02 mM.L-1 cacodylic buffer, pH = 7.0; 0.02 $\mu\text{gF/mL}$ and 1 mL/mm²), for 22 hours. The cariogenic challenge was performed by immersing the enamel blocks in a demineralization solution (2.0 mM.L-1 calcium and phosphate in 75 mM.L-1 acetate buffer, pH = 4.7; 0.03 $\mu\text{gF/mL}$ and 3 mL/mm²) for 2 hours per day (12–2 p.m.). Twice a day, at 10 a.m. and 2 p.m., the enamel blocks were exposed to toothpaste slurries (toothpaste: deionized water, 1:3 w/w; 2 mL/enamel specimen) for 1 minute, under agitation. Enamel blocks were then rinsed with deionized water between each step. Then, the enamel blocks were individually immersed in a remineralization solution at 37°C. The de and remineralizing solutions were changed daily. Between the steps, the specimens were water rinsed with deionized water for 5 seconds. Deionized water rinses were performed between each steps. In between treatments, each enamel block was individually immersed in remineralization solution at 37°C. The de- and remineralizing solutions were freshly changed every day. The toothpastes selected for the present study are described in ►Table 1.

Erosive Challenge

After the caries pH cycling, the blocks were then subjected to an erosive challenge. Enamel blocks were immersed in 50% citric acid for 2 minutes, and subsequently washed in abundant distilled water for at least 5 minutes.¹⁷ The results were compared to untreated control half blocks.

Table 1 Composition of the toothpastes selected for the study

Product	Ingredients	Active agents	Lot no. Expiry date
Regenerate Enamel Science ^a	Glycerin, calcium silicate, PEG-8, hydrated silica, trisodium phosphate, sodium phosphate, aqua, PEG'60, sodium lauryl sulfate, aroma, flavor, synthetic fluorophlogopite, sodium saccharin, polyacrylic acid, tin oxide, limonene, CI 77891 pH: 8.92 (Tomaz et al) ¹³	1,450 ppm F ⁻ (as sodium fluoride and sodium monofluorophosphate) NR-5 technology: calcium silicate and sodium phosphate	L72878CCApril, 2020
Regenerador + Sensitive DentalClean ^b	Glycerin, silica, sorbitol, sodium lauryl sulfate, aqua, aroma, PEG-12, cellulose gum, O-phosphoric acid, xylitol, sodium saccharin, triclosan, menthol, mica, sodium benzoate pH: 4.73 (Tomaz et al) ¹³	1,450 ppm F ⁻ (as sodium fluoride) REFIX technology Tetrasodium pyrophosphate	41531 May, 2021
Sensodyne Repair & Protect ^c	Glycerin, PEG-8, hydrated silica, pentasodium triphosphate, sodium lauryl sulfate, flavor, titanium dioxide, polyacrylic acid, cocamidopropyl betaine, sodium saccharin. pH: 8.63 (João-Souza et al) ⁴⁰	1,450 ppm F ⁻ (as sodium fluoride) Calcium sodium phosphosilicate 5% (Novamin)	BN 028E December, 2019

Abbreviations: PEG, polyethylene glycol; F⁻, fluoride.

^aManufacturer's information: Rabbit Corp., Londrina, PR, Brazil.

^bManufacturer's information: Unilever UK Limited, Leatherhead, Surrey, United Kingdom.

^cManufacturers' information: GlaxoSmithKline, Philadelphia, Pennsylvania, United States.

Characterization of the Enamel Surfaces by Scanning Electron Microscopy Imaging Observation and Energy-Dispersive X-Ray Spectroscopy

The morphological analysis of the specimens was performed using a scanning electron microscope (SEM; TESCAN VEGA3, LMU, Kohoutovice, the Czech Republic) operating at 15 kV. The blocks were first sputter coated with gold in a vacuum evaporator (MED 010; Balzers, Balzers, Liechtenstein) and then microscopically analyzed to obtain photomicrographs of the surface morphology of the treated specimens (×1,000 magnification). Representative images of selected regions of the specimens were obtained to characterize the morphological aspect of the surface. The energy-dispersive X-ray spectroscopy (EDS) point analysis (80 mm², silicon drift detector [SDD], Oxford Instruments, Concord, Massachusetts, United States) was performed to determine a qualitative elemental analysis of the specimens, operating in high vacuum mode with an accelerating voltage of 15 kV. Five points per sample were randomly selected (300 μm² per point), and the mean values were calculated.

Characterization of the Cross-Sections by Scanning Electron Microscope Imaging Observation

For the subsurface analysis, cross-sections of the bovine blocks were obtained by longitudinally sectioning the specimens under water cooling. Both half blocks were used for the SEM analysis. The halves were dehydrated in silica gel for 3 hours. The specimens were then gold-sputtered and evaluated using SEM.

Results

► **Fig. 1** shows the representative scanning electron micrographs of the enamel surfaces treated with the different toothpastes after the pH cycling (images above) and after the

erosive challenge (images below). After the erosive challenge, the extent to which the enamel surface presented a characteristic morphological aspect of eroded mineral tissues depended on the previous treatments (► **Fig. 1B**). The morphological aspect of the eroded enamel surfaces in the specimens treated with the toothpaste containing NR-5 technology (Regenerate Enamel Science) resembled the control group (► **Figs. 1D** and **1B**, respectively). Similarly, eroded morphology was observed for the specimens treated with the product containing Novamin (Sensodyne Repair & Protect; ► **Fig. 1H**), which also resembled the control group. On the other hand, the specimens treated with the product containing REFIX technology presented a smoother enamel surface morphology compared to the other treatments (► **Fig. 1F**).

► **Table 2** shows the elemental mapping of the enamel treated with the different toothpastes and after the erosive challenge. EDS detected different amounts of carbon, oxygen, silicon, phosphorus, and calcium before and after the erosive challenge. In the specimens treated with the REFIX technology, no changes in the chemical elements were observed after the erosive challenge, which agrees with the morphological analysis. Conversely, a reduction in the percentage weight of calcium was observed in the specimens treated with Novamin after the erosive challenge (from 34.11 to 29.33%). The percentage weight of calcium increased in the eroded specimens treated with NR-5 (from 28.07 to 30.39%). However, this increase may be not a real increase, instead the resulting of measuring the calcium content of the enamel layer exposed after the erosive challenge. This can be confirmed by the Ca/P ratio after the erosive challenge (2.11), which is similar to that found in untreated bovine hydroxyapatite (2.08).¹⁸ The percentage weight of silicon was similar after the erosive challenge for the specimens treated with the REFIX and Novamin technologies. Conversely, when the enamel was treated with the NR-5-containing toothpaste, a decrease in the percentage weight was observed (from 0.33 to

Fig. 1 A representative scanning electron micrograph of the enamel surfaces treated with the different toothpastes during the pH cycling (images above) and after the erosive challenge (images below). (**A, B**) The morphological aspect of the control (untreated area) before and after the erosive challenge of the control, untreated group; (**C, D**) Morphology of the enamel surface treated with toothpaste containing NR-5 technology (Regenerate Enamel Science), before and after the erosive challenge. (**E, F**) Representative image of the morphology of the enamel surface treated with the product containing REFIX technology before and after the erosive challenge. (**G, H**) Representative micrograph of the morphology of the enamel surface treated with a product containing Novamin (Sensodyne Repair & Protect), before and after the erosive challenge. Mag, magnification. WD, Working distance. EHT: Electron high tension. HV, high vacuum. WD, Working distance. CME-UFPR, Centro de Microscopia Eletrônica da Universidade Federal do Paraná. There is no need to spell out VEGA3, TESCAN. These words are specifications of the SEM model.

Table 2 Elemental mapping of the enamel treated with the different toothpastes and after erosive challenge

Toothpaste	C weight %		O weight %		Ca weight %		P weight %		Si weight %		Na weight %		Ca/P ratio	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
Regenerate Enamel Science	7.36	6.48	32.09	30.90	28.07	30.39	14.17	14.39	0.33	0.27	0.42	0.53	1.98	2.11
Regenerador Diário DentalClean	6.92	7.14	31.97	32.35	31.61	30.72	15.31	15.11	0.42	0.41	0.43	0.31	2.06	2.03
Sensodyne Repair & Protect	4.95	7.60	18.97	25.14	34.11	29.33	16.74	14.27	0.32	0.33	0.29	0.36	2.03	2.05

Abbreviations: C, carbon; Ca, calcium; Na, sodium; O, oxygen; P, phosphorus; Si, silicon.

0.27% weight). The highest percentage weight of silicon was found in the specimens treated with REFIX technology, before and after the erosive challenge (0.42 and 0.41%, respectively).

►Fig. 2 shows micrographs of cross-sectional areas of the enamel treated with the different toothpastes. A mineralized layer formed on the enamel surface after treatment with NR-5 (►Fig. 2A) and REFIX technologies (►Fig. 2B). A thicker mineral layer was observed for REFIX in all the specimens evaluated. Conversely, no mineralized surface layer was observed in the specimens treated with Novamin technology (►Fig. 2C). This toothpaste is known to induce the formation of a mineralized layer on the dentin and inside the dentinal tubules.¹⁹

►Fig. 3 shows micrographs of cross-sectional areas of the enamel treated with REFIX technology, in which a mineralized layer formed on the enamel surface is observed (►Fig. 3B), in comparison with the untreated enamel (►Fig. 3A). The product containing REFIX technology induced the formation of a mineralized layer when associated with pH cycling. This layer was approximately 6-µm thick (►Fig. 3B).

►Fig. 4 shows photomicrographs of the morphology of cross-sectional areas of the enamel treated with the REFIX-containing toothpaste comparing the effect of the erosive challenge with an intact, untreated enamel area. The

eroded area was around 15-µm deep in the intact enamel (►Fig. 4A). Conversely, in the specimens treated with the REFIX technology, virtually no erosion was observed and the mineralized layer after the erosion challenge (►Fig. 4B).

Discussion

As dental erosion may lead to irreversible loss of hydroxyapatite, it is of paramount importance to prescribe products with remineralizing potential that can assist in the mineral gain of the demineralized surface and reduce the solubility of the dental structure in recurrent acidic challenges.⁹ As previously pointed out, fluoride-containing products may have antierosive properties, but they are only able to repair smaller enamel lesions.^{20,21} Promising biomimetic approaches to erosion prevention have been developed.^{4,22}

The toothpaste with the proprietary technology called NR-5 contains, according to the manufacturer, sodium phosphate associated with calcium silicate. Also, according to the manufacturer, this technology was developed by combining calcium silicate, sodium phosphate salts, and fluoride. This technology was proposed to accelerate the mineralization

Fig. 2 Representative scanning electron micrographs of the enamel cross sections of the enamel treated with the different technology-containing fluoride toothpastes. (A) NR-5 technology (Regenerate Enamel Science); (B) REXIS technology (Regenerator + Sensitive DentalClean); (C) Novamin technology (Sensodyne Repair & Protect). MAG, magnification; SEM, scanning electron microscopes.

Fig. 4 Scanning electron micrographs of the morphological analysis of the cross-sectional areas of the enamel comparing the untreated control area (A) and the treated area (B) with the REXIS-containing fluoride toothpaste. Mag, magnification. WD, Working distance. EHT: Electron high tension.

processes provided by saliva, assisting the nucleation of hydroxyapatite and in the formation of minerals in the enamel, thereby remineralizing, protecting, and repairing the enamel. A previous *in vitro* study²³ investigated the repair and protective properties after the deposition of calcium silicate on acid-eroded enamel surfaces. That study demonstrated that calcium silicate could transform into hydroxyapatite and be deposited on both intact and eroded enamel surfaces, providing significant protection against erosive challenges. This technology seems to induce the formation of a mineralized layer on the enamel surface after treatment with NR-5, as demonstrated in the present study (►Fig. 2A). In another *in vitro* study¹³ the toothpaste with NR-5 technology favored the recovery of superficial enamel hardness more than 100% compared to the untreated control. Conversely, this effect was not observed at the enamel subsurface, demonstrating that this technology was less effective at remineralizing the enamel in depth. This helps to explain the reasons for not promoting an effective protection against the erosive challenge.

The other product containing Novamin technology (Sensodyne Repair & Protect) uses sodium and calcium

Fig. 3 (A, B) Scanning electron micrographs of the morphological analysis of the cross-sectional areas of the enamel showing the formation of a mineralized surface layer after treatment with the REXIS-containing fluoride toothpaste (B), in comparison to the untreated specimen (A). Mag, magnification; WD, Working distance; EHT, Electron high tension.

phosphosilicate (Bioglass) in the form of an amorphous inorganic compound.²⁴ According to the manufacturer, a series of chemical reactions occurs when Bioglass is in contact with an aqueous solution, leading to the formation of a layer of carbonated hydroxyapatite on the dentin that forms an insoluble mineralized layer on the surface. This technology may favor another mechanism of action in enamel, possibly altering the structure of the enamel hydroxyapatite, and reinforcing it without actually forming a superficial mineralized layer on the enamel. In the present study, it was not observed the formation of a mineralized layer on the enamel surface (►Fig. 2C), although the formation of a less-soluble surface hydroxyapatite, which is resistant to acid challenges, may occur.²⁵

In an *in vitro* study,²⁶ the protective effect of four commercial toothpastes containing antierosion agents was investigated. The authors found that the toothpaste containing Novamin was not effective in preventing the erosion effect caused by orange juice when applied either before or after the erosive challenge. The authors of a recent systematic review²⁷ searched for clinical evidence of the effectiveness of Novamin in publications, evaluating its action as a remineralizing agent. The analysis of the different studies led to the conclusion that Novamin had significantly less clinical evidence to demonstrate its effectiveness as a remineralization agent in treating both carious and noncarious lesions. The authors recommended better-designed clinical trials to make definitive recommendations about this technology.²⁷

The toothpaste containing proprietary REXIS technology, according to the manufacturer, represents a novel, multifunctional, phosphate-based dental gel technology in an acidified stabilized phosphate/fluoride complex which is established especially in saliva.¹³ The combination of toothpaste, saliva, and dental tooth structures favors the generation of new minerals containing calcium/phosphate/fluorine, promoting the enamel surface, and remineralizing within the subsurface carious lesion.¹³ This product presents an acid pH that may be the main reason for its effectiveness due to the formation of calcium phosphate crystals in an acidic environment.^{13,28}

In the present study, it was demonstrated that a mineralized layer was formed on the enamel surface after treatment with REXIS technology (►Figs. 2B and 3B), in comparison with the untreated enamel (►Fig. 3A). A previous study demonstrated the formation of a silicon-enriched mineral

layer on the enamel surface induced by the REFIX-based toothpaste was favored by the formation of complexes of the bioactive particles of calcium, phosphorus, and sodium.¹⁴ Substituting PO_4 with SiO_4 is believed to affect the mechanical properties of the silicon-enriched hydroxyapatite in a dose-dependent manner, decreasing hardness and the elastic modulus.²⁹ Conversely, the silicon content in the toothpaste formulation in association with fluorine and phosphate groups induces increased bioactivity and apatite-forming ability of hydroxyapatite, which is enhanced by the substitution of silicon, or silicate, into the remineralizing hydroxyapatite.^{30,31} In this manner, a protective effect is provided by inducing the formation of hydroxyapatite after its deposition onto the eroded surfaces.²³

The results of the present study can also be explained by the pH at which the remineralization processes occur (► **Table 1**). The biomimetic effect of the technology-containing fluoride toothpastes may induce the nucleation and growth of new enamel crystals by incorporation into the porous spaces of the lesion, and at later stages by means of the growth and fusion with the preexisting crystals. In this manner, a faster remineralization process may be expected when treating the enamel with these multifunctional toothpastes compared with conventional fluoride toothpastes. Fluorine ions, known to reduce hydroxyapatite solubility, can replace hydroxyl ions.³² The small-sized fluoride anions are able to diffuse throughout the enamel matrix in either acidic or basic pH, inducing the remineralization process using a nucleophilic attack on silicon, coordinating to it, and promoting subsequent reactions.³³ In an acidic pH, such as the REFIX-containing toothpaste, the remineralization process in the presence of silicon leads to the formation of a less porous hydroxyapatite structure (<2 nm).³⁴ In addition, the REFIX product contains 30% more silicon than the other products (► **Table 2**). Conversely, in a basic medium, such as the NR-5- and Novamin-containing toothpastes, there is a tendency to form a mesoporous enamel structure,³⁴ with porosity varying from 2 to 50 nm.^{35,36} This also helps to explain the differences in the resistance to the erosive challenge after treatment among the technology-containing fluoride toothpastes.

Limitations

Considering the limitations of the present *in vitro* study, the protocol used to evaluate the protective effectiveness of the selected fluoride toothpastes can be explained considering that both cariogenic and erosive challenges might simultaneously occur in the oral cavity, depending on different etiological factors.^{37,38} By treating the enamel with the fluoride toothpastes allowed changes in the hydroxyapatite structure, which ends up forming fluoridated apatite and the deposition and/or replacement of hydroxyapatite sites with other substitutes. This also seems to occur deeper into the hydroxyapatite.³⁹ Without this protocol, it would not be possible to evaluate the effectiveness of the toothpastes to promote a protective effect after exposure to the erosive challenges. This protective effect of the toothpastes containing different technologies may not only be

restricted to the enamel surface but also to the enamel subsurface. It is true that the erosive challenge used in the present study has also limitations, but it is a valid and well-established method.¹⁷ Another limitation relies on the fact that this morphologic evaluation is qualitative, and one may argue that the results are quite subjective. In spite of this fact, the images are clear to demonstrate the results when the treatments were compared.

The present study found that the protective effect against the erosive challenge was material dependent. The toothpaste containing 1,450 ppm of sodium fluoride with REFIX technology enabled the formation of a mineralized surface layer less affected by the erosive challenge. This outcome appears to be due to the formation of an acid-resistance silicon-enriched mineral surface layer on the enamel surface. Although the treatment with the toothpaste containing NR-5 technology also enabled the formation of a mineralized surface layer, an eroded enamel surface morphology was observed after the erosive challenge, similar to the untreated enamel control. Conversely, no mineralized surface layer was observed in the specimens treated with the toothpaste containing Novamin technology, and the enamel surface morphology was significantly affected by the erosive challenge.

Conclusion

The present study characterized the enamel surface and subsurface morphologies of specimens treated with different 1,450-ppm fluoride toothpastes containing different biomimetic technologies. These technologies were developed to accelerate the remineralization process or to minimize the demineralization process of dental tissues, especially in the event of repetitive erosive challenges. Despite the limitations of the present *in vitro* study, the preferred REFIX technology was the most promising compared to other Novamin and NR-5 technologies. In addition to forming a mineralized layer superficially on the enamel, the most important result was the ability to resist acid dissolution by the erosive challenge. Further studies are needed to investigate the performance of the dental gel containing the REFIX technology using *in situ* and *in vivo* studies on the effectiveness of the dental gel on dental substrates.

Conflict of Interest

None declared.

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