ORIGINAL ARTICLE

Surface Prereacted Glass Ionomer Varnish as a Multifaceted Anticaries Agent: Investigating its Inhibitory Effects on Demineralization and Biofilm Formation on Primary Tooth Enamel

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Abstract

Background: Dental caries remains a significant oral health concern, particularly in young children. With an increasing interest in preventive strategies, pediatric and preventive dentistry research is now more focused on developing newer materials and techniques to coat the primary teeth to prevent the onset of new carious lesions. While traditional preventive measures such as fluoride application and sealants have been effective in reducing caries incidence, there is still a need for innovative approaches.

Aim: To evaluate the effectiveness of surface prereacted glass ionomer (S-PRG) light-cured varnish in inhibiting demineralization of primary teeth enamel.

Materials and methods: In this study, primary teeth samples were randomly divided into two groups: the control group received no coating, while the test group received an S-PRG filler coat. The samples were allowed to demineralize, and various analyses, including Fourier transform infrared (FTIR) spectroscopy, scanning electron microscope (SEM), energy-dispersive X-ray analysis (EDX), and Vickers microhardness analysis, were conducted. Additionally, biofilms of *Streptococcus mutans* and *Enterococcus faecalis* were developed on solid surfaces such as microtiter plates, glass, and dentures, and the quantity of bacterial biofilm was measured using crystal violet assay and fluorescence microscopy.

Results: The study results showed that the primary teeth samples in both groups had a significantly greater calcium content than the controls. The S-PRG group demonstrated a significant reduction in the development of biofilms of *S. mutans* and *E. faecalis*, as well as bacterial attachment to glass and denture surfaces compared to the control group, as indicated by crystal violet assay and fluorescence microscopy.

Conclusion: The findings of this study suggest that S-PRG filler-containing coating materials have the potential to prevent demineralization and inhibit *S. mutans* and *E. faecalis* biofilm formation on primary tooth enamel.

Clinical significance: These results are promising and may have implications for the prevention of dental caries in young children.

Keywords: Biofilm, Coating material, Mineralization, Primary teeth, Surface prereacted glass ionomer.

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Introduction

A continuum of demineralization cycles can lead to dental caries, which is a chronic tooth disease affecting both young and older generations and has modifiable risk factors.[1] The prevalence of dental caries in young children has been reported to be as high as 85%.[2] To intercept or stop the ongoing caries process, several therapeutic methods such as fluoride mouthwashes, professional administration of fluoride gels, and antimicrobial varnishes are used as treatment for dental caries or as preventative therapies for young children.[3] Unfortunately, children between the ages of 2 and 8 are not able to adapt to significant developments in caries preventive materials that can prevent dental cavities caused by the production of biofilm by cariogenic bacteria or potential pathogenic microorganisms (PPMs).[4]

The oral bacteria, the biome of the teeth, and dietary variables interact to cause the beginning and progression of dental caries. *Streptococcus mutans* and *Enterococcus faecalis* account for the majority of the PPMs initiation and development of dental caries due to the associated interplay between the oral flora, teeth biome,

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Conflict of interest: None

and dietary factors.[5] *S. mutans* and *E. faecalis* take the larger portion of the PPMs, which adhere resolutely to the surface of the teeth with the production of enormous exopolysaccharides and are reported to be highly acidogenic as well as acid-tolerant.[6] *S. mutans* and *E. faecalis* are known to adapt to a peculiar behavior of sugar metabolism, which is used by the PPMs to develop extracellular polysaccharides responsible for forming resistant, irreversible biofilm formations that are associated with the development of dental caries. However, resistant biofilm formation in dental caries has been extensively reported, and the therapeutic factors into the depth were limited.[7] Replacing restorative materials and restoring dental cavities is always challenging because of issues with isolation, accessibility, and replacement of materials, particularly in young children. Fluoride-based medicinal materials are widely used but may have a negative impact on both microbiological and physiochemical parameters.[8] Several byproducts of fluoride-based therapeutics, such as mouth rinses, toothpaste, and topical varnishes, are used, but the preventive strategy is limited and temporary.[9] Therefore, a novel class of material, surface prereacted glass ionomer (S-PRG), which can be applied as a barrier coat, is highly trending in recent research and development of preventive dentistry. The preparation of S-PRG is simple, by acid-base reaction of fluoro-boroalumino-silicate glass and polyacrylic acid in an aqueous solution.[10] Interestingly, there occurs a ligand exchange mechanism in prereacted hydrogel, which enables subsidization of S-PRG barriers programmed to release and recharge fluoride. Accordingly, S-PRG is developed to manage multiple ions such as Al^{3+}, B^{3+}, Na^{+}, BO_3, and Sr^{2+} with competence in mineral induction.[11]

Surface prereacted glass ionomer barriers have shown potential in not only reducing demineralization of permanent teeth but also aiding in their remineralization. Moreover, S-PRG coatings have been utilized as preventive therapeutics for dental caries with enhanced mineralization.[12,13] However, the efficacy of S-PRG-filled coating material in preventing demineralization of primary tooth enamel in the presence of associated bacterial biofilm remains unclear. Therefore, the objective of this study is to evaluate the inhibitory effect of S-PRG barrier coatings on enamel demineralization of primary teeth and to control biofilm growth by oral pathogens such as *S. mutans* and *E. faecalis*.

Materials and Methods

Sample Collection and Sectioning

This study was approved by the Yenepoya Ethical Committee 2 (YEC2/188 dated 18.11.2019), and all protocols were conducted according to the approved methods. This observational study had an experimental setup that included a test group and a control group, each consisting of 26 specimens.

We collected 26 healthy primary teeth that were exfoliated or extracted due to physiologic preshedding mobility, without any initial carious lesions, white spot lesions, hypoplastic lesions, or visible caries on the tooth surface according to clinical examination. The teeth were sectioned by removing the roots so that the resulting crowns were divided into mesial and distal halves. The tooth surfaces were then finely processed using a 600-grit emery sheet and polished to a surface area of 4 × 4 mm.

Surface Prereacted Glass Ionomer Filler Coating to Enamel Samples

Surface Prereacted Glass Ionomer filler-containing barrier coat (PRG Barrier Coat Varnish Desensitizer Mini Kit Fluoride No HEMA Acetone/No Alcohol) was applied onto the enamel samples using previously reported methods with slight modifications.[14] The specimens, which were the mesial and distal halves of each tooth, were then randomly divided into two groups based on the material application: (1) 26 noncoated (non-S-PRG/control) and (2) 26 S-PRG-coated material group (S-PRG/test group). The S-PRG filler coating was applied onto the test group specimens as per the manufacturer's instructions and cured using an irradiation light activation unit (PanamaR LED Curing Light) for 10 seconds while maintaining a minimum distance of 6 mm from the coating (Fig. 1).

Demineralization Assay

The demineralization assay was carried out on the enamel surface of the teeth using previously reported methods with minor modifications.[15] The experimental setup consisted of a control group and a test group. Enamel specimens in the control group were left uncovered, while the test group specimens were coated with the S-PRG filler material. An acidic demineralizing buffer solution was prepared using 2.2 mM calcium chloride, 2.2 mM sodium hydrogen phosphate, and 50 mM acetic acid (pH 4.5). The enamel samples were individually immersed in 1 mL of the demineralization solution and incubated at 37°C for 3 days under static conditions (Fig. 2). The demineralization solution was changed every 24 hours,

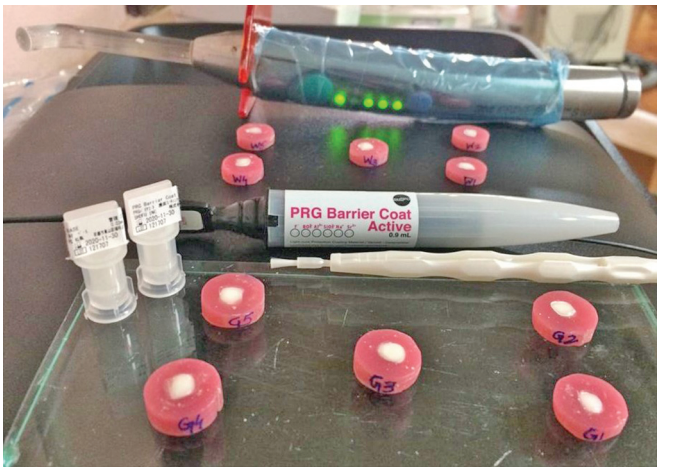
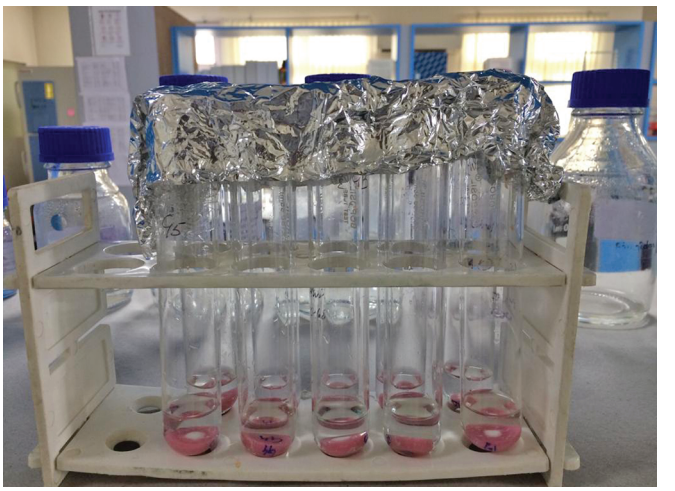


Fig. 1: Experimental setup exhibiting S-PRG barrier coat and enamel samples



Figs 2: Part of the experimental setup showing enamel samples immersed in demineralization solution

and the pH was recorded. After the 3-day incubation period, the S-PRG barrier coat was carefully removed from the tooth enamel, and the samples were further analyzed.

Vickers Microhardness Analysis

A digital Vickers hardness tester was utilized to measure the microhardness of the demineralized primary teeth surface. A load of 200 gm was applied for a dwell duration of 15 seconds using a Vickers pyramidal diamond indenter. Three random indentations were made in the center of each specimen's designated window. The Vickers hardness number was calculated using microhardness software analysis.[16]

Fourier Transform Infrared Characterization of Surface Prereacted Glass Ionomer Coatings

The internal structure of the S-PRG barrier coating was analyzed using Fourier transform infrared (FTIR) spectroscopy at room temperature, using a Shimadzu instrument from Japan. The coating was dissolved in a mixture of ethanol and distilled water in a 1:1 ratio at 25°C, and the absorption spectrum was measured and compared to that of the control and test specimens.

Scanning Electron Microscope Analysis

At the end of the 3rd day demineralization cycle, six specimens (three per group) were selected for morphological analysis by scanning electron microscope (SEM) (Zeiss, GeminiSEM 300). Prior to analysis, these specimens were covered with a gold filament for 60 seconds and then fixed to an aluminum plate with copper tape. Micrographs were obtained at a magnification of 5000× to observe the surface morphology and any changes in the enamel surface due to demineralization and S-PRG coating.

Energy-dispersive X-ray Analysis

The samples were dried using activated silica gel, and all the specimens were carbon-coated for energy-dispersive X-ray analysis (EDX). The atomic percentage of the elements was measured, and a specific region of interest was defined on the polished surface. Three fields in one diagonal line were defined for elemental analysis, and the test samples were compared with the control.

Bacterial Strains and Culture Media Preparation—The bacterial strains *Streptococcus mutans* MCC809 and *Enterococcus faecalis*

MCC2409 was procured from the National Centre for Microbial Resource, Pune, India. Both strains were maintained in Luria-Bertani (LB) broth at 37°C for 24 hours, and an inoculum suspension at OD600 containing 1×10^8 CFU/mL was prepared from a 16-hour fresh culture for the biofilm.

Quantitative Assessment of Biofilm Inhibition by Surface Prereacted Glass Ionomer Coating

The microtiter plate-based quantitative assessment of the biofilm formed by *S. mutans* and *E. faecalis* was carried out using previously reported methods with minor modifications.[17] The experimental setup involved coating the S-PRG onto the entire surface of the well covering a volume of 250 µL, which was taken as the test, and the noncoated wells were considered as the control. Overnight cultures of *S. mutans* and *E. faecalis* were seeded into both coated and uncoated wells containing LB media and incubated at 37°C for 48 hours. After the incubation period, planktonic cells were removed, and the absorbance of the growth was measured at 600 nm. Bound biofilm was fixed with 95% methanol. The biofilm attached was stained with 0.1% crystal violet for 15 minutes and measured at 590 nm spectrophotometrically.

Assessment of Antibiofilm Activity of Surface Prereacted Glass Ionomer Coating on Denture

The applicability of the S-PRG coating in the clinical industry was determined using previously reported methods with some modifications.[12,18] Briefly, a denture was coated with S-PRG, which was taken as the test, and a noncoated denture was considered as the control. The setup was carried out in 55 mm polystyrene Petri dishes with 10 mL of LB media; both the coated and noncoated dentures were immersed completely in the LB media. They were then seeded with overnight cultures of *S. mutans* and *E. faecalis* and incubated at 37°C for 48 hours under static conditions in separate settings. After the incubation period, the dentures were gently removed, washed with distilled water, and dried. The bound biofilm on the denture was fixed with 95% methanol and stained with 0.1% crystal violet for 5 minutes. The unbound crystal violet was removed by washing the denture with distilled water twice, and the surface of the coated denture with biofilm was treated with ethanol to detach the stain bound on the biofilm. The absorbance was measured at 590 nm spectrophotometrically.

Fluorescence Imaging of the Biofilm Inhibition by Surface Prereacted Glass Ionomer Coating

Fluorescence microscopy was done to analyze and understand the biomass inhibition of the biofilm formation by *S. mutans* and *E. faecalis*.[19] The glass slides were coated with S-PRG, which was taken as the test, and noncoated glass slides, which were taken as the control. Both the glass slides were immersed in 100 mm polystyrene Petri dishes containing 25 mL of LB media, inoculated with overnight cultures of *S. mutans* and *E. faecalis* in separate settings. The setup was incubated at 37°C for 48 hours under static conditions. After the incubation period, the slides were gently removed, rinsed with sterile distilled water, and air-dried. The biofilm adhered to the glass surface was fixed with 95% methanol for 30 seconds and stained with 0.1% acridine orange for 3 minutes. The slides were then washed with sterile distilled water and observed under a fluorescence microscope.

Statistical Analysis

Significant statistical differences in the atomic percentages of the elements and the Ca/P molar ratios between control and experiment were analyzed using the Sidak multiple comparisons test ($\alpha = 0.05$), performed in the GraphPad Prism software. One-way ANOVA was carried out for the relative comparison of the surface hardness. Tukey's test was performed within the groups, and the Wilcoxon test was carried out for the comparison of the hardness of the enamel samples before and after treatment.

Results

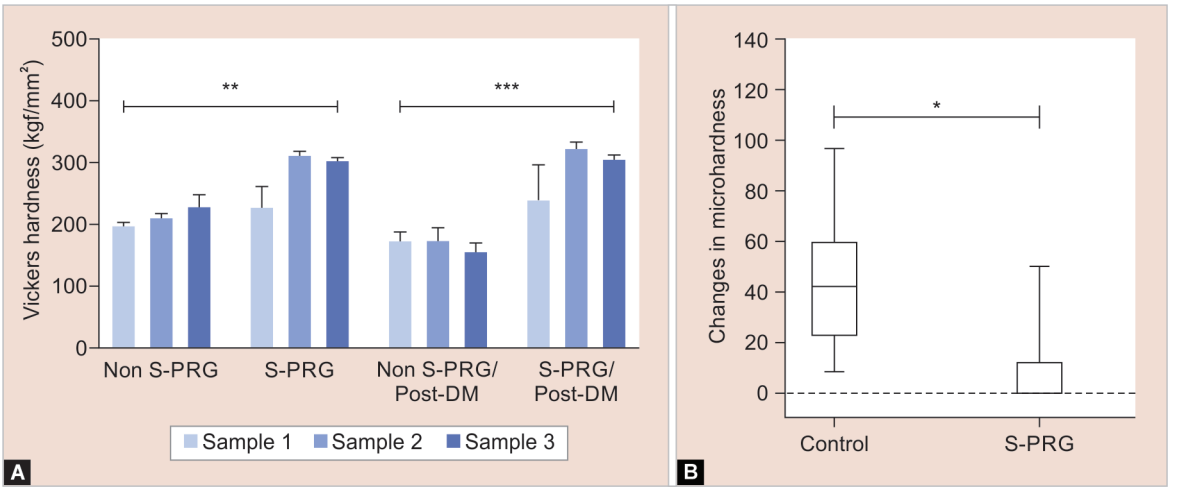
Microhardness Analysis of the Demineralized Samples

The first demineralization of the enamel has been quantitatively estimated in investigations using the microhardness of the enamel surface. This technique, which may also be tested nondestructively, reflects the mechanical characteristics of the tooth structure while being connected to the mineral composition of the enamel. When compared to enamel without S-PRG coatings, the microhardness was significantly reduced ($p < 0.001$) after demineralization treatment (Fig. 3A).

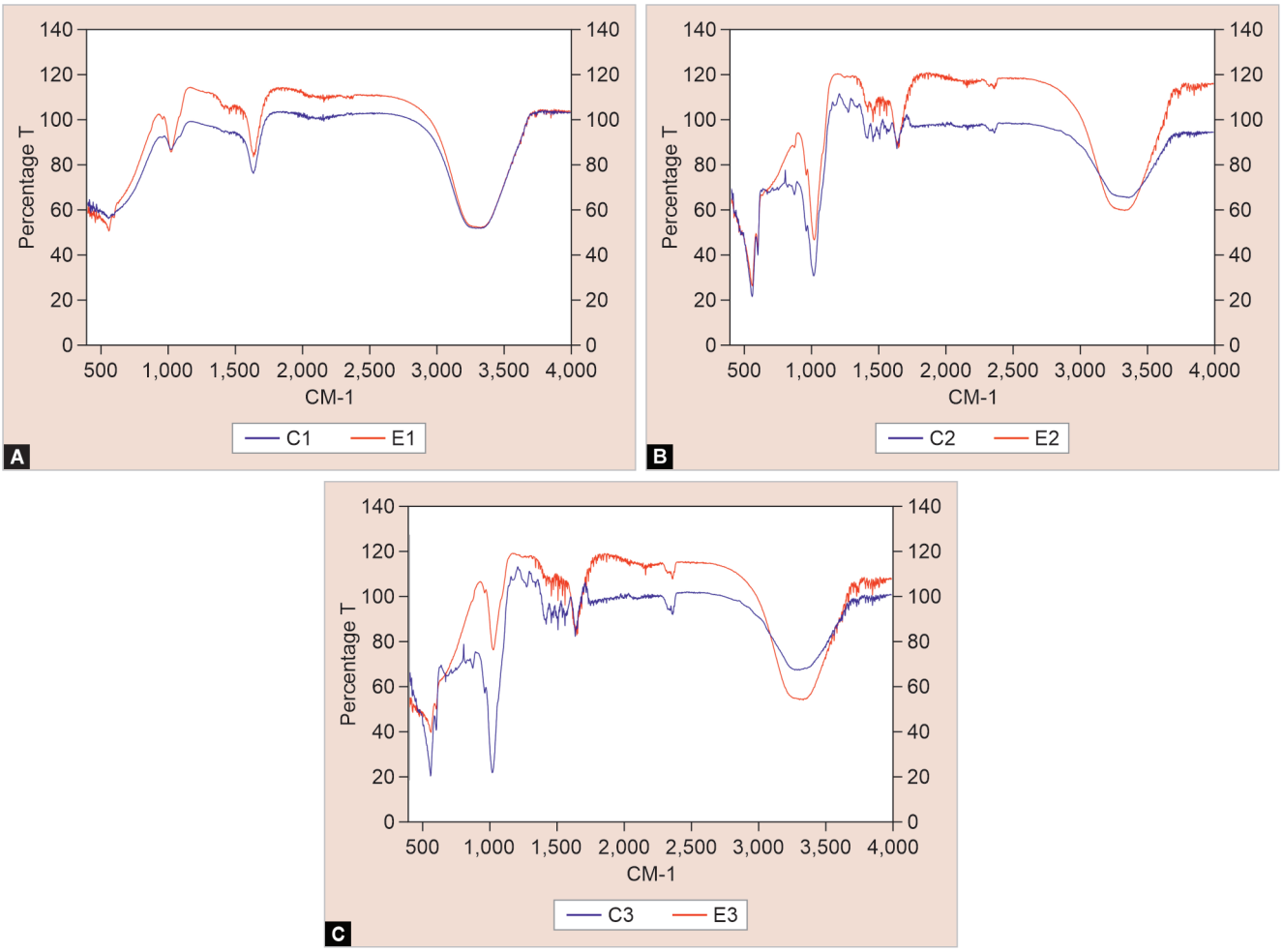
However, we found no significant changes in the hardness values for enamel with and without S-PRG treatment before demineralization immersion. Interestingly, the S-PRG coated enamel significantly recovered to its previous level even after demineralization treatment, indicating no surface loss (Fig. 3B).

Fourier Transform Infrared Analysis

The overall analysis shows that the functional groups found in the enamel samples are depicted in the wide scan FTIR spectrum (Fig. 4). The typical OH stretching band was observed from 3400 cm^{-1} to 3500 cm^{-1}. Regarding the phosphates, PO_4^{3-} was localized at



Figs 3A and B: Microhardness of primary teeth (samples 1–3) of non-S-PRG and S-PRG before ($p < 0.01$) and after demineralization (postdemineralization, $p < 0.001$) (A) Changes in microhardness of all samples show significant protection in the S-PRG group ($p < 0.05$); (B) No significant changes in microhardness observed in the control and S-PRG group prior to demineralization. However, S-PRG coated enamel shows recovery in surface microhardness even after demineralization, indicating no surface loss



Figs 4A to C: Fourier transform infrared spectroscopy analysis of primary teeth (samples 1–3) of non-S-PRG (C1, C2, and C3) and S-PRG (E1, E2, and E3) after demineralization

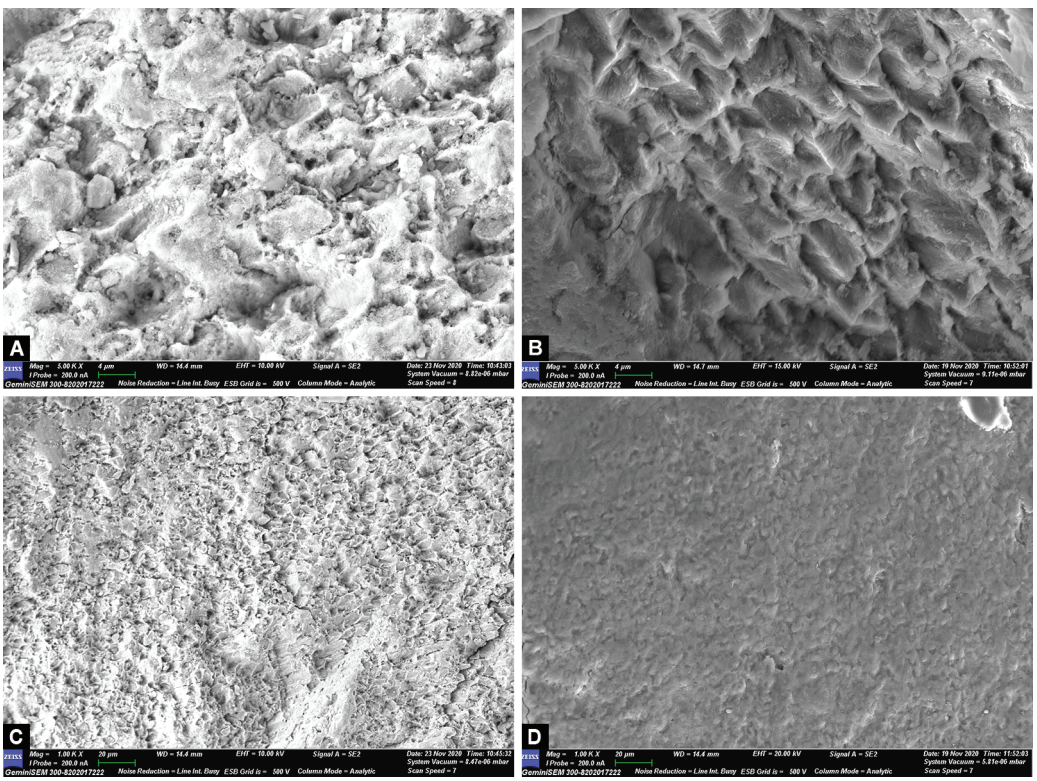
570 cm^{-1}, 950 cm^{-1}, and 1050 cm^{-1}, with a less intense band centered at 700 cm^{-1}. The major component of CO_3^{2-} was located at 1440 cm^{-1}, with another band at 1560 cm^{-1}. The amide vibration arising from C=O stretching was centered at the 1660 cm^{-1} band. The C=O stretching vibration had its major intensity at 2340 cm^{-1}.

Scanning Electron Microscope Image Analysis

Representative SEM images of an enamel surface restored with the S-PRG coatings are shown in Figure 5. The surface of the S-PRG coated enamel showed a rough and even texture, unlike the uncoated enamel. Comparing the demineralized enamel surfaces, both test groups exhibited a smoother surface than the uncoated enamel immersed in the demineralization solution. However, the surface of the S-PRG coated group showed significantly less distortion compared to the non-S-PRG control groups. SEM images of the test groups (S-PRG coated) revealed surface structure variance, highlighting the importance of PRG coatings. In the control (non-S-PRG), the extent of organic surface material loss was evident, with enamel erosive lesions on the surface and a relatively soft texture due to treatment with the demineralization solution. Additionally, control samples displayed a smooth surface with multiple microwear marks. This damage to the enamel surface in the control group was caused by the manifold chemical impacts of the demineralization solution. The demineralized enamel appeared smooth and brittle, particularly in vulnerable areas, and microscopically showed a distinctive well-like structure due to the chemical erosion effect. Interestingly, the tooth samples coated with S-PRG showed the enamel surface to be significantly better protected compared to control samples under the demineralization solution. The surface layer microstructure was well maintained in S-PRG coated enamel compared to the control samples. Furthermore, the S-PRG coating completely covered the enamel, clearly demonstrating the utility of PRG in surface coatings.

Energy-dispersive X-ray Analysis

The element atomic percentages and Ca/P molar ratios of the enamel surface showed a significant ($p < 0.05$) difference between the non-S-PRG and S-PRG coated samples as determined by EDX analysis (Table 1). The content of oxygen, phosphorus, and calcium in the sample under demineralization conditions was significantly higher than in the control. All measurements showed a wide range of variation for both element atomic percentage and Ca/P molar ratio. The Ca/P ratio ranged from 1.95 to 2.34 with respect to non-S-PRG and S-PRG coated samples.



Figs 5A to D: Representative SEM images of non-S-PRG (A and C) and S-PRG (B and D) after demineralization

Table 1: Mean values and standard deviations of elements atomic percentages and Ca/P molar ratios of enamel surface by energy-dispersive X-ray spectroscopy analysis

Elements	*Control*	*Experiment*	*p-value*
O	17.74 ± 0.19	28.85 ± 2.10	0.0015
P	22.41 ± 2.0	21.46 ± 1.27	0.9285
Ca	43.88 ± 2.47	50.24 ± 0.05	0.0248
Ca/*p*-value	1.95	2.34	0.9981

Bacterial Adhesion Test by Biofilm Quantification

The antibiofilm activity of the S-PRG coating was analyzed for both *S. mutans* and *E. faecalis*. In comparison to the non-S-PRG group, the S-PRG group demonstrated an excellent capacity to inhibit biofilm development and bacterial adherence to solid surfaces. Compared to the control and non-S-PRG groups, the S-PRG group exhibited significantly lower levels of bacterial adherence and biofilm development (Fig. 6) on microtiter well plate surfaces ($p < 0.001$).

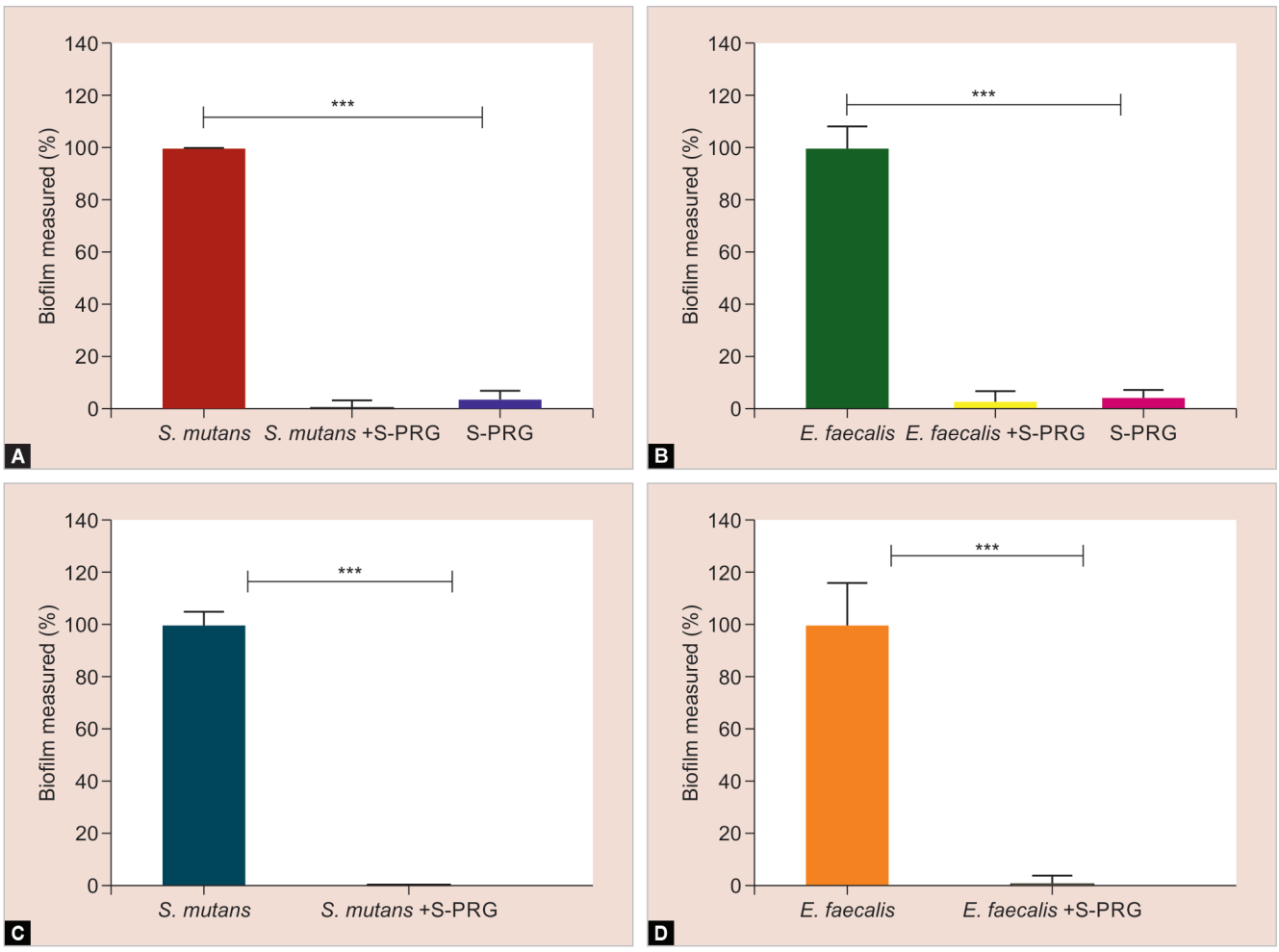
Furthermore, the clinical use of the S-PRG coating on the denture provided efficient support for both quantitative and qualitative assessment. Analyzing the results from both coated and noncoated dentures indicated a drastic reduction in biofilm for the S-PRG coated denture compared to the control (Fig. 6). This demonstrates the effective use of the S-PRG coating in clinical practice against *S. mutans* and *E. faecalis* biofilm-mediated infections (Fig. 7).

Fluorescence Microscopy Analysis

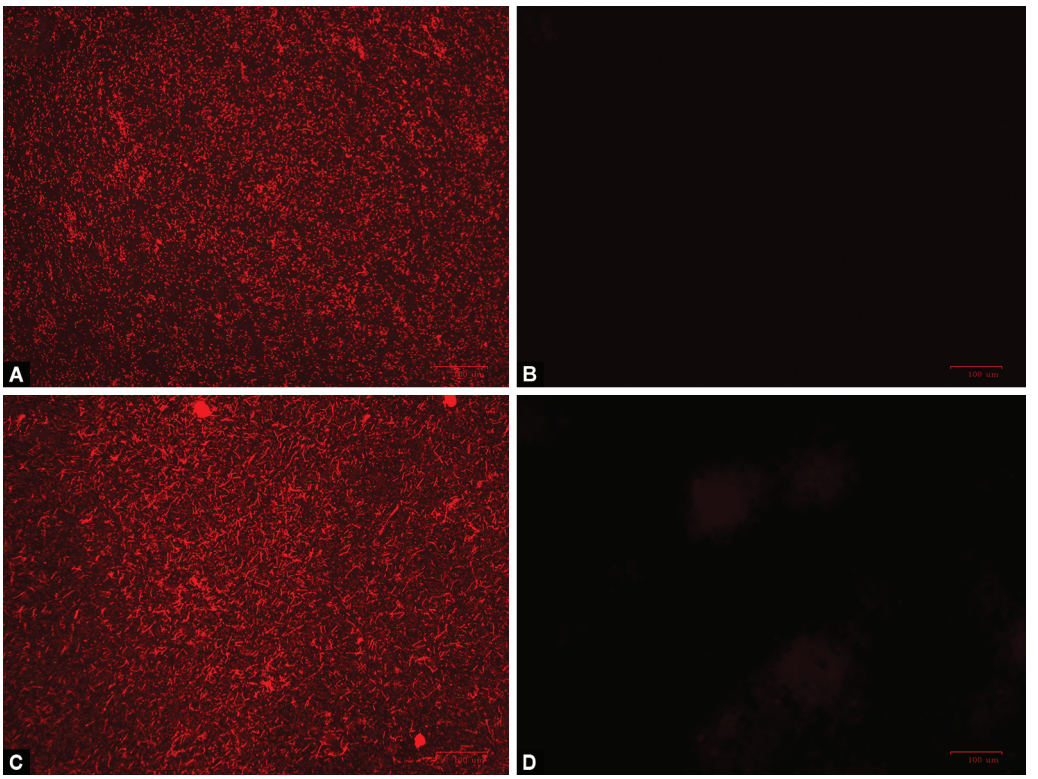
To support the quantitative analysis of the biofilm inhibition by the S-PRG coating against *S. mutans* and *E. faecalis*, fluorescence microscopic analysis was performed to reveal total biomass production and its inhibition. The noncoated slide showed efficient biofilm production by both organisms, whereas the glass slide coated with S-PRG demonstrated effective inhibition of biofilm formation, providing promising evidence for the quantitative assessment of the antibiofilm activity of S-PRG.

Discussion

The results of the current *in vitro* investigation, which attempted to imitate the complicated oral cavity, showed that the S-PRG barrier coat with a resin covering has an inhibitory impact on the demineralization of primary teeth enamel and biofilm of *S. mutans* and *E. faecalis*. S-PRG fillers help reverse the demineralization process which happens when the environmental acidity increases due to the presence of cariogenic plaque along with the presence of fermentable carbohydrates.[20] Microorganisms present in the oral cavity produce organic acids that lead to the decrease of minerals in the tooth structure. Recent studies have reported limitations on the efficacy of S-PRG filled coating material in inhibiting demineralization of primary tooth enamel in the presence of bacterial biofilm and linked acidic challenge.[12,13] Thus, the present study was conducted to evaluate the inhibitory effect of S-PRG filler-containing resin barrier coat on enamel demineralization of primary teeth and the control of biofilm development by oral pathogens like *S. mutans* and *E. faecalis*.



Figs 6A to D: Analysis of biofilm formation of *S. mutans* and *E. faecalis* on surfaces coated with S-PRG and non-S-PRG. S-PRG coating on microtiter plates showed inhibition of *S. mutans* (A) and *E. faecalis* (B) biofilm ($p < 0.001$). Analysis of biofilm inhibition of *S. mutans* (C) and *E. faecalis* (D) on denture surfaces ($p < 0.001$)



Figs 7A to D: Fluorescence microscopy analysis of bacterial biomass developed on glass surfaces with and without S-PRG coating against *S. mutans* (B) and *E. faecalis* (D). Control glass surfaces of *S. mutans* (A) and *E. faecalis* (C) show well-established biofilm upon 48-hour incubation

The comparison of non-S-PRG and S-PRG barrier coats suggests that there is a significant improvement in surface microhardness (SMH) of the primary teeth. At baseline, the mean SMH value for the S-PRG group was 281, which was higher than the control group at 210. Postdemineralization results showed that the mean SMH value for the S-PRG group was 291.3, significantly higher than the control group's mean SMH value of 180. Additionally, the mean surface microhardness values of the S-PRG group increased postdemineralization, indicating that the S-PRG coat not only prevented demineralization but also promoted remineralization to some extent.[21] Therefore, in young children, the S-PRG coat can be effectively used as a non-invasive caries preventive measure. Moreover, SEM analysis showed that the enamel surface appeared smoother and more regularly arranged in the S-PRG group compared to the non-S-PRG group. In contrast, the non-S-PRG group showed a rough enamel surface with surface defects such as porosities and wide fissures.[14,22]

Interestingly, it has been reported that the mean Ca/P ratio of primary teeth enamel is 2.00 ± 0.3.[23] The non-S-PRG group shows a mean Ca/P ratio of 1.95, suggesting that demineralization has occurred. However, the Ca/P ratio in the S-PRG group was 2.34, which presumably indicates a higher level of prevention of demineralization.[24,25] Furthermore, FTIR analysis confirmed the presence of a higher number of phosphate groups, major components of CO32-, OH bands, and amides in the S-PRG group. This demonstrates that spectral analyses are highly useful in probing the molecular structure of enamel.[26]

One of the major bacterial genera linked to dental caries, *S. mutans* and *E. faecalis*, can form biofilms and are transiently detected in the oral cavity.[27] When *S. mutans* generated organic acids are trapped in the glucan matrix, the pH around the surface of the tooth gradually decreases, which plays a major role in dental caries.[28] The presence of *E. faecalis* in the oral cavity is associated with endodontic failure and the formation of biofilm.[29] This adaptive lifestyle of both bacteria is linked to the multispecies biofilm and is the primary determinant of several bacterial pathogenicities. Therefore, inhibition of biofilm on a solid surface can reduce dental caries and may improve oral hygiene drastically. The reduction of biofilm formation by *S. mutans* and *E. faecalis* in the S-PRG group may be due to the presence of resin materials such as Sr^{2+}, Al^{3+}, Si^{4+}, BO_3^{3-}, and other S-PRG filler ions that prevent bacterial adhesion.[30] Moreover, by releasing Sr^{2+} or Na^{+} ions, the S-PRG fillers show impressive acid-buffering capacity, which may enable them to offset the effects of the acid medium and promote remineralization. Additionally, it is possible that the coating process itself protects against acid damage, which might account for the minimal damage in the non-S-PRG groups.[14]

Conclusion

S-PRG barrier coat is a promising solution for young children, especially those with high caries risk areas, such as partially or newly erupted molars, exposed root surfaces, white spots, areas of hypersensitivity, and difficult-to-brush areas surrounding orthodontic brackets, clasps, and crowded dental arches. This study's results have demonstrated the inhibitory effects of the S-PRG filler-containing resin coat on enamel demineralization of primary teeth, with a significant increase in tooth enamel microhardness and calcium/phosphorus ratio after the application of S-PRG varnish. This suggests that the S-PRG barrier coat not only aids in preventing

demineralization but also promotes remineralization of primary tooth enamel. Additionally, this study established the possibility of preventing the establishment of *S. mutans* and *E. faecalis* biofilm on the tooth surface with S-PRG coating, along with improved oral hygiene. However, more evidence is warranted to assess the solid surface-mediated biofilm prevention in clinical situations. Overall, the S-PRG barrier coat shows promise as a multifaceted solution for preventing dental caries and promoting oral health in young children.

Clinical Significance

S-PRG filler-containing coating materials may be utilized to prevent *S. mutans* and *E. faecalis* biofilm and prevent primary teeth demineralization.

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