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EVALUATION OF ENAMEL MICROHARDNESS AFTER APPLICATION OF THE PRE-REACTED GLASS IONOMER VARNISH IN PERMENANT TEETH (IN VITRO STUDY)

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ABSTRACT

INTRODUCTION: Surface pre-reacted glass ionomer varnish contains filler particles incorporated into resin material. It is promising in arresting pits and fissure caries of permanent teeth.
OBJECTIVES: Evaluate and compare the microhardness of enamel covered by pre-reacted glass ionomer varnish to pits and fissure sealant on extracted permanent teeth.
MATERIALS AND METHODS: Fifteen freshly extracted impacted third molars were selected. Baseline enamel microhardness was recorded for each tooth. Squares of self-adhesive labels (4×4 mm) were positioned on the buccal surface of both mesial and distal halves. Each tooth was split longitudinally into two halves, creating thirty specimens distributed randomly into two groups of 15 specimens each: Group I (test group) received S-PRG and Group II (control group) received Fisseal. Each specimen was incubated in an individual container and subjected to 5 pH cycles at 37ºC for 5 days. Immersion in a remineralizing solution was done during each cycle for 18 hours (pH = 7), followed by rinsing and re-immersion in a demineralizing solution for 6 hours. Evaluation of surface microhardness was conducted after pH cycling for both the test and control groups using Vickers microhardness indentation test.
RESULTS: A significantly higher microhardness value was recorded for Fisseal group (Gp II) compared to S-PRG (Gp I) after pH cycling.
CONCLUSION: Resin-based fissure sealant Fisseal, significantly enhanced the microhardness of underlying enamel compared to S-PRG varnishes. In this context, it is preferable to apply fluoride containing fissure sealants in order to increase enamel resistance to acid attacks.
KEYWORDS: S-PRG, Pre reacted glass ionomer varnish, PRG barrier coat, Pits and fissure caries, Fissure sealant.
RUNNING TITLE: Enamel microhardness evaluation following application of PRG.

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INTRODUCTION

Dental caries is a chronic, multifactorial, transmissible, and infectious disease that arises from a cycle of demineralization and remineralization of dental hard tissues, with several stages being either reversible or irreversible (1). A non-cavitated caries lesion is the initial clinical symptom of dental caries. Although research has shown that these lesions do not severely impact oral health-related quality of life (2), they can progress to a cavitated stage, especially in children who have already experienced caries (3). The eruption of permanent molars is a critical period where the prevention and management of initial caries are essential. Sealants and varnishes can be important prevention strategies (4).
The treatment of non-cavitated carious lesions is the focus of modern dentistry, emphasizing a minimally invasive approach that promotes remineralization to halt disease progression. Fluoride, in its various forms, is considered the cornerstone of this approach (5). Patient-centered care is a fundamental aspect of dental treatment and practice (5). To preserve as much sound tooth structure as possible, restorative treatment approaches have shifted from aggressive cutting to minimally invasive intervention (6). Advancing dental treatment and care require novel technologies, with bioactive materials being among the most promising innovations (7, 8). However, in dentistry, the concept of bioactive materials remains not fully defined. Some studies have focused on their functions, particularly in promoting (re)mineralization or the formation of hard tissue (9). These effects may have biological, chemical, or combined mechanisms, as outlined in the FDI World

Dental Federation's policy statement on bioactive restorative materials (10).

PRG Barrier Coat (Shofu Inc.) is a resinous coating material containing Surface Pre-reacting glass ionomer (S-PRG) filler that protects the enamel surface from demineralization caused by acidic attack (11-13). Ions produced by the S-PRG filler in the PRG Barrier Coat have acid-neutralizing properties near the coated surface. The uptake of fluoride and strontium released from the PRG Barrier Coat by the tooth substrate can be beneficial for inhibiting demineralization (14).

Dental pits and fissure sealant is a fluid material placed onto the occlusal pits and fissures of teeth prone to caries (15). The sealant material successfully penetrates and seals the tooth grooves (16). They halt dental caries by arresting bacterial growth on the occlusal surfaces of teeth (17). It is proven that the advancement of non-cavitated carious lesions is arrested by the use of sealants (18). This method showed a substantial reduction in the percentage of caries progression in sealed non-cavitated lesions in children, adolescents, and young adults for over five years after sealant placement compared to unsealed non-cavitated carious lesions (19).

To the best of our knowledge, no previous research has compared the enamel microhardness following the application of S-PRG with that of pit and fissure sealants. The aim of the present study is to compare the remineralizing potential of both agents, considering their distinct preventive mechanisms—S-PRG, which actively releases bioavailable ions (fluoride, strontium, boron) to enhance enamel remineralization, and Fisseal, which primarily acts as a physical barrier to prevent bacterial accumulation and caries formation. The null hypothesis proposed that there is no statistically significant difference in enamel microhardness between newly erupted permanent teeth treated with S-PRG and those treated with Fisseal.

MATERIALS AND METHODS

This in vitro experimental study was conducted in collaboration between multiple departments at Alexandria University. The Department of Pediatric Dentistry played a key role in specimen preparation and treatment application, while the Department of Dental Public Health contributed to study design and data analysis. The microhardness testing was performed at the Production Department, Faculty of Engineering, utilizing advanced testing equipment for precise measurements. The study protocol was reviewed and approved by the Ethics Committee of the Faculty of Dentistry, Alexandria University, Egypt (Approval No. 0591-01/2023).

Sample Size Estimation

Sample size was estimated based on the following assumptions: Estimation of sample size was carried out on the presumption that 5% alpha error and 80% study power. The mean (SD) percentage of microhardness was 30.0 (11.6) % for the 40% PRG, (20) and 43.27 (3.57) % for the dental sealant (21). In accordance with the difference between independent means choosing the highest SD=11.6 to achieve sufficient study power, a sample of 14 samples per group was required with an effect size of 1.143. This was raised to 15 samples in order to compensate for errors of processing. Total sample = Number per group x Number of groups x Number of measurements = 15 x 2 = 30 specimens.

A total of fifteen freshly extracted impacted third molar teeth were selected. The teeth were visually checked to fulfill the inclusion criteria for absence of any cavitations, white-spot lesions, restorations, developmental defects, and discoloration. A magnifying lens was used to ensure the absence of any cracks Meticulous cleansing of the teeth was carried out using a brush with fluoride-free pumice, after which they were stored in a saline solution. Baseline enamel microhardness was recorded for each tooth. Teeth were dried, and squares of self-adhesive labels (4×4 mm) were put on both the mesial and distal halves of the buccal surface of each tooth. All tooth surfaces were coated with acid-proof nail varnish. Each adhesive label was then removed, exposing only the underlying window of enamel. **(Figure 1)** Each tooth was sectioned longitudinally in a buccolingual direction through the center of the tooth to divide it into two equal halves (mesial half and distal half), resulting in 30 specimens.

Randomization and Grouping

- Teeth complying with the inclusion criteria were sectioned into two specimens then randomly assigned using a computer-generated list of random numbers to two groups. Specimens were equally allocated to the two groups depending on the type of material used (22).
- **Group I (test group)**: Specimens were treated with S-PRG varnish.
- **Group II (control group)**: Specimens were treated with pits and fissure sealant.

Allocation concealment

Each tooth was given a serial number. A duplicate of that number was kept in an opaque envelope indicating to which group the tooth belongs. The envelopes were kept by a trial independent individual who was assigned the role of opening it only at the time of intervention.

Blinding

The investigator wasn't blinded due to the different natures and application techniques of the materials. However, both evaluator and statistician were blinded to the allocation group.

Treatment procedure

For Group I, the S-PRG varnish was manipulated according to the guidelines of the manufacturer. The base container was snapped off, and the tab was twisted to open the container. A single drop of the active component was put in the base container and mixed using the manufacturer's applicator brush. The application of the prepared mixture was carried

out within 2 minutes of mixing to avoid the varnish becoming more viscous. Excess material was removed from the disposable brush by lightly pressing it against the edge of the container. One coat of the mixture was applied in a thin layer to the dried labial surface of the 15 specimens in the PRG group. The mixture was left undisturbed for 3 seconds and followed by irradiation curing using a unit of light activation at a light intensity [(LED) curing unit-woodbecker] of 1000 mW/cm² for 10 seconds, with a distance not greater than 6 mm between the light tip and the resin coat. The PRG varnish was removed from the specimens after 24 hours using a dental explorer.

For Group II, the buccal surfaces were acid-etched with 37% phosphoric acid gel (SDI) for 15 seconds, rinsed with water, and dried with compressed air until a matte white appearance was achieved. The surface was coated with Fisseal white sealant, which was left to set for 15 seconds before being light-cured for 20 seconds.

PH cycling (23)

A demineralizing solution was prepared containing Calcium Chloride (CaCl2) 2.2 mM, Potassium Dihydrogen Phosphate (KH2PO4) 2.2 mM, Acetic Acid (CH3COOH) 0.05 M and Potassium Hydroxide (KOH) 1 M. The pH of the solution was adjusted to 4.4 using a pH meter (24). For five days, each specimen was incubated in a separate container and exposed to five pH cycles at 37º C.

Fresh solutions (demineralizing and remineralizing) were provided for each cycle throughout the experimental period. After 18 hours of immersion in the remineralising solution (pH=7), each PH cycle involved rinsing with deionized water and then reimmersing for 6 hours in the demineralizing solution. It was rinsed once more with deionized water and the process was repeated throughout the test period, and finally prepared for evaluation.

Sealant and varnish removal

Sealant and varnish, Fisseal (fissure sealant) and S-PRG (varnish), were removed from specimens before measuring the microhardness of the underlying enamel using the following procedure:

Mechanical Removal

- A scalpel or sharp blade was carefully used to scrape off the sealant or varnish (25).

Evaluation of surface microhardness was conducted after pH cycling for both the test and control groups. **(Figure 2)** Dental resistance to abrasion, scratches, and indentation, as well as resistance to permanent curvature and deformation when force is applied, is referred to as surface hardness of enamel (26).

A diamond indenter (Vickers) applied a load of 50 kgf/mm² with a dwell time of 10 seconds to produce an indentation on each specimen's surface. After load removal, an optical microscope was used to measure the diagonals of the indentation at 40x magnification. The average microhardness (HV) of each specimen was measured using three indentations. The ratio of the load to the area of residual indentation is known as the hardness number.

The following Vickers hardness formula was used to calculate the Vickers hardness (HV):

The Vickers hardness (HV) is calculated using the following Vickers hardness formula :

HV = test force / surface area = F(kgf)/As(mm2)

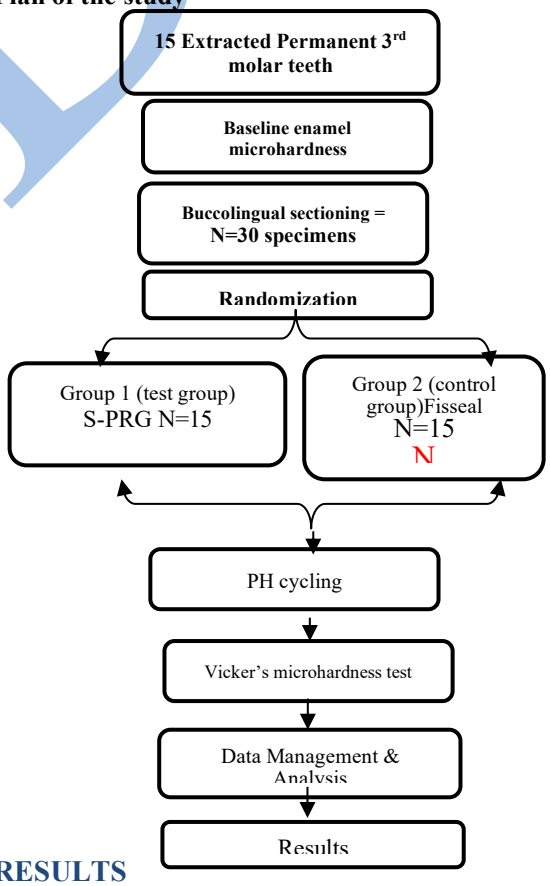
The study design was intended to measure enamel microhardness value for each tooth at baseline and after pH c ycling for both test and control groups using vicker's microhardness indenter, in addition to comparison of microhardness of both materials in reference to baseline readings.

Statistical analysis

Q-Q plots as well as Shapiro Wilk test was used to check normality of data. Microhardness values were normally distributed, but percent change was not normally distributed. The following formula was used to calculate the percent change: [(pH values – Baseline values) / Baseline values] x 100.

Data were presented using mean, standard deviation. 95% confidence interval (CI), median, inter quartile range (IQR), minimum and maximum values. In terms of comparison between the groups, it was carried out using independent t test. In mean time, comparison between baseline and values after pH was done using dependent t test. Whole tests were of two tailed type and the level of significance level was established at p value ≤0.05. IBM SPSS, version 23 for windows, Armonk, NY, USA was used to analyse the data.

Plan of the study



RESULTS

No significant difference was evident between the test and control group at baseline before pH cycling. (P=0.689). Nevertheless, a significant difference was recorded between group I (S-PRG group) and group II (pits and fissure sealant group) after Ph cycling, where Fisseal showed higher microhardness values compared to S-PRG (P<0.0001)

A significant difference in Fisseal microhardness values were also recorded before and after Ph cycling (P<0.0001). **(Table 1 and Figure 3)**

The graph shows a comparison of the mean of the two materials, S-PRG and Fisseal, before and after pH cycling. The p-values indicate the statistical significance of the differences between these groups:

- **Before pH**: The difference between S-PRG and Fisseal is not statistically significant (p = 0.123).
- **After pH**: The difference between S-PRG and Fisseal is highly significant (p < 0.0001), indicating a notable change in mean values after pH cycling.

The comparison between the "before" and "after" pH values for S-PRG shows no significant difference (p = 0.496), whereas the "before" and "after" comparison for Fisseal shows a highly significant difference (p < 0.0001). **(Table 2 and Figure 4)**

- **Median Percent Change:** The median percentage change is lower for S-PRG compared to Fisseal.
- **Statistical Comparison:** The p-value is 0.065, which is slightly above the typical threshold of 0.05 for statistical significance.

Figure (1): Specimen embedded in acrylic resin block and coated with acid-proof nail varnish, exposing a 4*4 mm2 window.

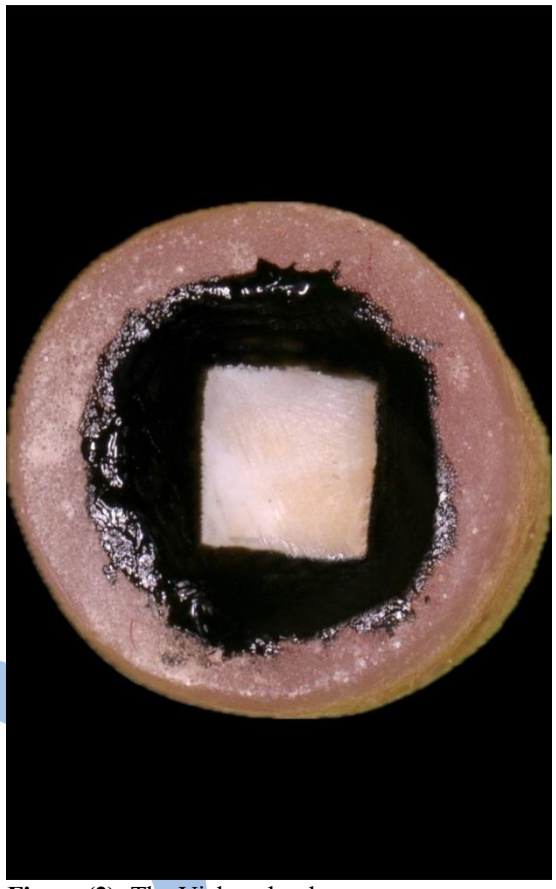


Figure (2): The Vickers hardness tester.

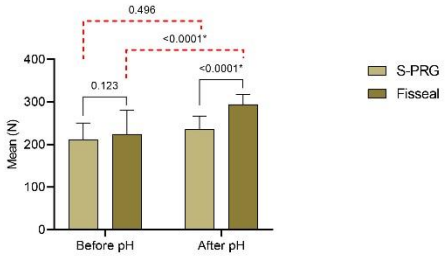


Figure (3): Comparison of microhardness between the study groups before and after pH cycling.

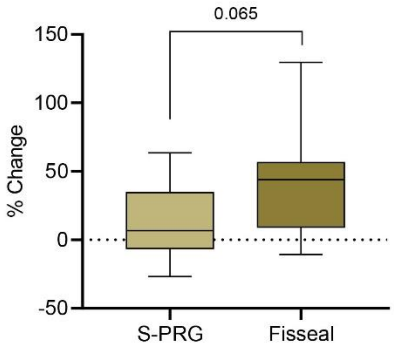


Figure (4): The percent increase in Fisseal was higher compared to S-PRG with no stastically significant difference between the two materials after treatment.

Table (1): Comparison of microhardness between the study groups before and after pH cycling.

		S-PRG (n=15)	Fisseal (n=15)	Test (*p* value)
Before pH	Mean ± SD	211.44 ± 39.05	223.71 ± 56.84	0.689 (0.496)
After pH	Mean ± SD	235.35 ± 31.39	293.96 ± 24.14	5.734 (<0.0001*)
Test (*p* value)		1.640 (0.123)	4.602 (<0.0001*)	

*Statistically significant difference at *p* value≤0.05

Table (2): Comparison of percent change in microhardness between the study groups before and after pH cycling.

		S-PRG (n=15)	Fisseal (n=15)	Test (*p* value)
Before pH	Median (IQR)	6.80 (41.03)	43.88 (47.47)	1.846 (0.065)
	Min - Max	-26.68 – 63.63	-10.82 – 129.60	

DISCUSSION

Two materials were selected for this study: S-PRG barrier coat, a promising bioactive fluoride varnish that actively contributes to the prevention of enamel demineralization while promoting remineralization through the release of various ions incorporated in its composition. The second material was a resin-based pit and fissure sealant (Fisseal), widely recognized for its role in preventing occlusal caries in young permanent first molars. To simulate the clinical scenario of newly erupted first permanent molars, freshly extracted impacted third molars were chosen as the test specimens, ensuring a comparable enamel condition suitable for evaluating the effects of these materials (27,28).

The pH cycling model was designed according to Malekafzali et al., (29) in order to exemplify as closely as possible the dynamic variations in mineral loss and gain of the acid cariogenic challenge during natural caries process in the oral cavity. To prevent the risk of reaching saturation threshold, remineralizing and demineralizing solutions were freshly prepared during the experimental period. Each solution was kept in a separate container to avoid cross reaction of solutions (29).

In this study, the remineralization potential was quantitatively assessed using Vickers Microhardness indentation after simulating the oral environment in laboratory conditions. A pH cycling model was implemented to replicate repeated acidic challenges, mimicking the oral cavity; however, due to the complex nature of remineralization—affected by factors such as saliva flow rate, buffering capacity, and composition—achieving complete simulation was not feasible. Since enamel surface integrity plays a crucial role in caries progression, evaluating microhardness changes is essential. The Vickers Hardness test was chosen for its high accuracy and quantitative precision, allowing for repeated measurements over time with various applied loads. Moreover, because the tested points were closely spaced and initial microhardness values showed minimal variation, each measurement was considered representative of the enamel surface hardness, ensuring reliable comparative analysis (30-34).

The total values of surface microhardness acquired in the current study prior to the application of the tested materials fell within the range of 217.2 kg/mm2, which aligns with values reported in the literature (35).

Our study findings revealed a significant increase in the microhardness of the underlying enamel beneath the fissure sealant Fisseal compared to S-PRG material. This result can be attributed to the superior sealing ability and mechanical properties of resin-based sealants, which not only protect the enamel from acid exposure but also enhance the microhardness of the enamel through effective barrier formation. In contrast, while S-PRG materials are known for their bioactive properties, including fluoride release and the ability to inhibit demineralization, their impact on the underlying enamel microhardness may be less pronounced due to their lower mechanical strength and different interaction with the enamel. The fluoride release from S-PRG, although beneficial, might not compensate fully for the lack of a robust physical barrier, which is critical in enhancing and maintaining enamel microhardness.

A systematic review reported that while both fissure sealants and fluoride varnishes effectively prevent decay, there was no clear consensus on whether one consistently outperforms the other in terms of enhancing enamel microhardness. However, combining resin-based sealants with fluoride varnish showed better results in preventing caries compared to using fluoride varnish alone (36).

Prior research has documented early caries lesion remineralization with products that contain S-PRG filler (6, 37). The increase in enamel microhardness after application of S-PRG was evident. This increase might be considered an accurate indicator of the effectiveness of treatment. The S-PRG varnish tested in this study was also found effective for enamel caries prevention in a study conducted by Spinola et al (4).

These findings are in line with those of Moecke et al. (21) who found that the varnish that proved efficiency for promoting reminerlization of enamel caries included 40% S-PRG fillers.

The buffering capacity of the S-PRG filler may contribute to the observed microhardness by promoting remineralization through increased pH levels and elevated mineral concentrations (5). Strontium, sodium, and aluminum ions are primarily responsible for the acid-neutralizing effect of S-PRG (7). Strontium plays a particularly crucial role as it combines with hydroxyapatite to form strontium apatite (38). Furthermore, the release of silicate ions facilitates the absorption of calcium and phosphate from the surrounding environment, thereby

promoting heterogeneous apatite nucleation and enhancing the process of remineralization (9).

The increased microhardness observed under the fissure sealant can be explained by the material's ability to more effectively isolate the enamel from the oral environment, thereby preventing demineralization and promoting remineralization processes. The protective barrier provided by the resin matrix of fissure sealants like Fisseal ensures that the enamel is shielded from cariogenic challenges, resulting in a harder and more resilient enamel surface over time (39).

These results were found to be in agreement with a study published by Salar et al., (10) who found that, in comparison to traditional non-fluoride-based sealants, the incorporation of fluoride with sealants enhanced demineralization inhibition.

However, the results of this study differed from those presented by Kantovitz et al., (40) who came to the conclusion that both fluoride and resin sealants without fluoride did not stop mineral loss and suggested that more preventative measures were needed. For 15 days, all groups underwent pH cycling. The Knoop microhardness scale was the method used. Moreover, they evaluated the mineral loss at different distances and depths from the sealant margin.

It was revealed by the results of the present study that the fluoride pit and fissure sealant (fisseal) possesses the potential to prevent demineralization compared to S-PRG in terms of microhardness. Accordingly, the null hypothesis was rejected.

These findings underscore the importance of material selection based on the specific clinical objective—whether it is maximizing fluoride release and bioactivity with S-PRG or achieving superior mechanical protection and enamel hardening with resin-based sealants. The observed increase in enamel microhardness under fissure sealants in our study supports the use of these materials in scenarios where long-term enamel protection and reinforcement are of paramount importance.

To the best of our knowledge, no previous studies compared the remineralizing effect of S-PRG to fluoride containing pits and fissure sealant on freshly erupted permanent molar teeth. Thus, the present study seems to fill a gap of knowledge, by adding to the limited evidence, the difference in remineralizing potential of the two materials on newly erupted teeth.

CONCLUSION

Resin based fissure sealant (Fisseal) significantly enhanced underlying enamel microhardness compared to S-PRG varnish.

In this context, it is preferable to apply fluoride containing fissure sealants in order to increase enamel resistance to acid attacks. Further in vivo research is recommended to confirm these results and guide clinical practice.

CONFLICT OF INTEREST

There was no conflict of interest in the following study.

FUNDING STATEMENT

No institutional funding was provided.

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