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Evaluation Of Micro-Hardness And Demineralization Resistance Of A New Adhesive Agent For Sealing Smooth Proximal Surfaces: An In Vitro Study.

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ABSTRACT

Prevention and management of proximal caries in primary teeth remains a challenge. This in vitro study aimed to evaluate the enamel-protective potential of a new adhesive agent (G-Coat Plus™) when used as a smooth surface sealant. Twenty extracted bovine permanent incisors were chosen, then randomly distributed into two equal groups according to the sealant material used: Group I, G-Coat Plus™; and Group II, Clinpro™. The baseline surface micro hardness (B-SMH) of each tooth was measured in the area of the centralized working window with a Vickers micro hardness testing machine. The mean micro hardness values were calculated from the measurements taken from three indentations created on each tooth. The teeth were then immersed in demine ralizing solution for 96 h and incubated at 37°C to produce artificial carious lesions. The calcium (Ca) and phosphorus (P) concentrations of the demine ralizing solutions were analyzed before and after tooth immersion. The mean micro hardness values for Groups I and II were statistically significant at baseline and after demineralization ($p = 0.007$ and $p < 0.001$, respectively). No significant difference in mean micro hardness was observed between the groups at baseline ($p = 0.055$) indicating that both materials had comparable results. However, the difference in mean micro hardness between the groups was statistically significant after demineralization ($p = 0.001$), indicating changes in the mineralization of the tooth samples. Statistical analyses demonstrated significant differences among the two groups regarding the mean Ca and P concentration values (Ca:, $p = 0.028$; P, $p < 0.001$). G-Coat Plus exhibited higher surface micro hardness than Clinpro. Both sealant materials released Ca and P ions, suggesting that additional preventive measures are necessary when using these materials as proximal sealants.

Keywords: biochemistry, calcium, proximal sealants, phosphorus, preventative dentistry.

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INTRODUCTION

Despite a general decline over years, caries is still one of the main communal health problems. [1] Growing interest regarding oral condition has shifted the focus of dental care from restorative to protective care, thereby increasing the demand for another agent that can help with fluoride to prevent caries. [2]Consequently, efforts for other protective policies and novel preventive provisions remain essential.

Dental caries affects more the proximal surfaces of teeth because of the broad contact between adjacent teeth that can be cleaned with difficulty via brushing.[3] Though dental flossing is primarily suggested for mechanical removal of biofilm from the interproximal areas,[4] proximal caries control with dental flossing alone has not been found to be effective;[5] this is likely because children have difficulties practicing correct dental flossing techniques, this is beside the poor acquiescence of both children and parents.[6] Therefore, controlling primary teeth proximal caries remains a challenge.

Sealants are first applied on occlusal surfaces years ago, then it became one of the best consistent and proficient techniques for preventing caries among children. Lately, the sealant technique has been experimented on teeth proximal surfaces; [7] the idea that the sealants create a barrier between dental biofilm and tooth's surface, thereby stopping caries origination and development. However, Alkizy et al. had an issue regarding proximal sealant application concept because the smooth proximal surfaces are somehow less retentive than occlusal pits and fissures.[8]

Sealants should be of appropriate resistance to cariogenic abilities to protect enamel. Schmidlin et al. stated that these demands are not met by unfilled resins, though met by filled flow able composite resins .[9] Various means and materials were tried for sealing proximal surface, including glass ionomers,[10] current anticipatory materials such as casein phosphopeptide-amorphous calcium phosphate and fluoride,[11] adhesives with antibacterial agents,[12] fluoride with adhesive agents, infiltrating agents ,[13] laser,[14] adhesives with bioactive glass,[15] and deproteinizing materials.[16] Low viscosity resins have been used to seal incipient caries in order to control proximal caries.[7] Though this technique has been successful, teeth need to be physically separated from proximal sides to apply resins. Besides, the neighboring teeth and adjoining soft tissue require protection during acid etching.[17]

Though early investigations reported that applying sealant material to proximal surfaces was useful, [18, 19] problems are often encountered, such as imperfect etching and formation of an incomplete sealant layer.[9] Advances in adhesive dentistry, boost greater trials to develop a more practical sealant material for preventing proximal caries. G-Coat Plus™ (GC America Inc. Patterson Companies, Inc., Old Cleveland Rd, South Bend, IN 46628, USA) is a recently introduced nano-filled, light-cured, self-adhesive protective coating that is formed of adhesive monomer and nano-fillers with uniform dispersion. According to the manufacturer, "One thin coat protects margins, prevents staining and provides a high gloss comparable to intense

polishing" [20] They also purport that use of G-Coat Plus, "Results in superior adhesion to enamel, dentin, composite, glass ionomer, resin modified glass ionomer, Bis-acryl and acrylic. Nanofiller particles are uniformly dispersed for balanced wear-resistance, providing a smoother, longer-lasting finish."[20]

Since protecting and sealing smooth tooth surfaces clinically necessitate testing in vitro setting first, the aim of this current study was to investigate the enamel protective potential of G-Coat Plus and Clinpro when applied as smooth surface proximal sealants. In view of that, the null-hypotheses tested *in vitro was*: g-coat plus as a smooth surface sealant increases enamel micro-hardness, increases proximal surface resistance to demineralization and decreases surface mineral loss.

MATERIALS AND METHODS

The ethical board at the Faculty of Dentistry of King Abdul-Aziz University (KAUFD) accepted the protocol for this in vitro study (0430318).

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Study sample

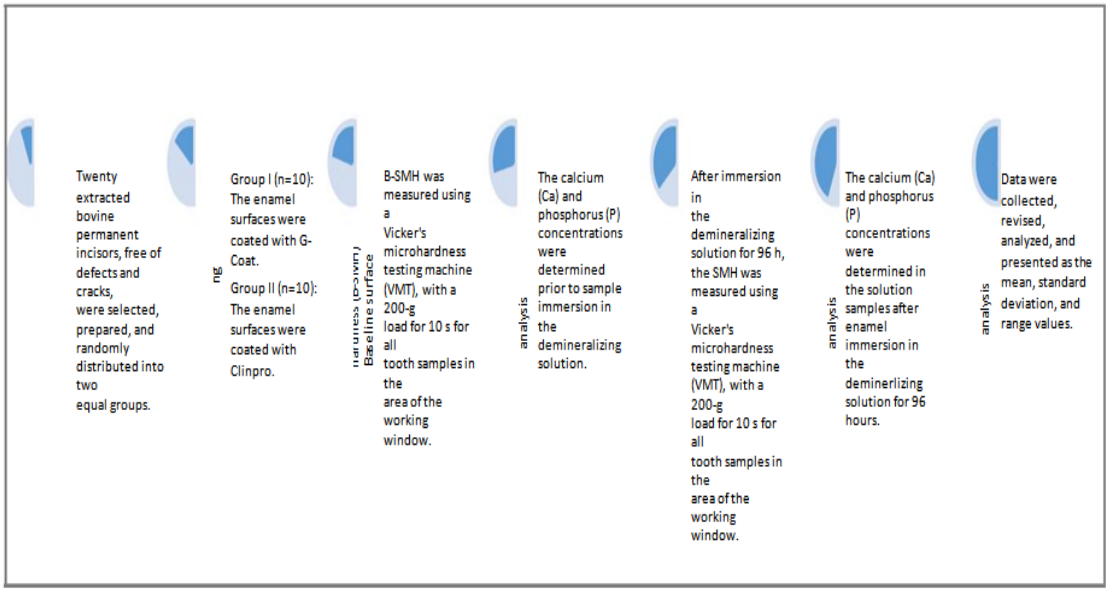
Bovine permanent incisors were chosen for this study where 20 incisors free of cracks and defects were used. After cleaning, the teeth were split at the cement-enamel join by a water cooling diamond disc (3M, SP., MN., U.S.A.), then stored in distilled water. Immediately prior to use, the sectioned teeth were washed away then cleaned with pumice using a rubber cup and conventional hand piece. Each tooth was inserted into plastic cylindrical molds filled with self-cured acrylic resin, then the resin was allowed to set.

Finishing discs made of silicon (grit #600 & #800; 3M, SP., MN., U.S.A.) were used to flatten enamel surfaces of the teeth before being polished with a low-speed hand piece. An acid-resistant nail varnish (DaniProTM, Ald. Associates, and LLC. P.O. Box 325 Closter, N.J. 07624) was used about the perimeter of the enamel surfaces, leaving a central, 4 mm × 4 mm square window (i.e., the working window) of untreated enamel. Teeth were divided randomly into 2 groups of 10 teeth each according to type of sealant material applied to the enamel window of the sectioned teeth following instructions of manufacturer: Group I: Teeth enamel surfaces were coated with G-Coat Plus Group II: Teeth enamel surfaces were coated with light cure resin-based sealant, Clinpro™ (3M ESPE, 300 Tartan Drive, London, Ontario, N5V 4M9) The composition and the manufacturers of materials used are presented in Table 1, and the experimental steps performed for all samples of the two groups are summarized in Figure 1.

Table 1: Characteristics and composition of the sealant materials used in this study

Trade name	Type	Composition			Filler type	Lot no.
G-Coat Plus™	Nanofilled,	Methyl methacrylate, colloidal			35–40 m	0908061
(GC Co., Tokyo, Japan)	self-adhesive, protective coating	silica, camphorquinone, urethane methacrylate, and phosphoric ester monomer			Nanofiller particles	
Clinpro™ (3M ESPE)	Resin infiltrate, Protective agent	Bisphenol A Diglycidylether methacrylate-(Bis-GMA) Matrix resin, triethylene glycol Dimethacrylate (TEGDMA) matrix resin, and tetra butylammonium tetra fluoroborate (TBATFB;fluoride releasing source)			Unfilled	N560346

Figure 1: Summary of the experimental steps performed in the two groups.



Baseline surface micro-hardness:

The baseline surface micro-hardness (B-SMH) was assessed by applying a load of 200-g to the working window area in the enamel surface of all tooth samples of group I and group II for 10 sec, using a Vickers Micro-hardness Digital Display Tester (Model HVS-50; Laizhou Huayin Testing Instrument Co., Ltd. China). [**21]** On the surface of each specimen, three equally spaced (≥ 0.5mm) indentations were prepared, then the micro-hardness was measured at each of the three indentations, where the mean value of each specimen was determined to avoid discrepancy.

Demineralizing solution:

The demineralizing solution was prepared by mixing analytical grade chemicals with deionized water. [**22]** The demineralizing solution composed of 2.2 mM sodium phosphate, 0.05 M acetic acid and 2.2 mM calcium chloride. The pH was adjusted to 4.4 using 1 M potassium hydroxide.

Artificial carious lesions:

The phosphorus (P) and calcium (Ca) content of the demineralizing solution samples were determined before being used. The ratio recommended to each 1 mm2 of exposed enamel is about 2 ml of demineralizing solution. Having the total enamel area exposed approximately about 16 mm2, tooth samples of group I and II were immersed in demineralizing solution (32ml) for about 4 days and incubated at 37°C to produce subsurface artificial lesions in enamel. [29]

Surface micro-hardness measurement:

Surface micro-hardness (SMH) was assessed by applying a load of 200-g to the working window area in the enamel surface of all tooth samples of group I and group II for 10 sec, using the Vickers micro-hardness tester (VMT), with a diamond indenter and a 20× objective lens. [**21, 23]** A built-in, scaled microscope measured the diagonal lengths of the indentations and the values obtained by Vickers tester were changed into micro-hardness values. These values were counted according to the equation (HV = 1.854 P/d2; HV = Hardness in kgf/mm2; d = Length of the diagonals in mm and P = Load in kgf).

Biochemical analysis

The P and Ca content of the demineralizing solutions were analyzed twice: prior to use, then again after 4 days immersion in the demineralizing solution. The Inductively Coupled Argon Plasma (ICAP) 6500 Duo (Thermo Scientific, England) was used for Biochemical analysis. For instrument standardization, a 1000 mg/l multi-element, certified standard solution (Merck, Germany) was used as stock solution.

Statistical analysis

The statistical analysis was conducted using Statistical Package for Social Science (SPSS) software (version 20); SPSS Inc., Chicago, IL, USA). The confidence interval (CI) was set at 95% and the accepted margin of error was set at 5%. The significance level was set at $P < 0.05$. The independent groups were compared using a two-sample t-test, while a paired t-test was used to compare between the paired groups. The data is presented as mean, standard deviation (SD), and range values.

RESULTS

Table 2 shows the mean micro-hardness (± SD) values at baseline and after 4 days immersion in demineralizing solution. Statistics revealed significant differences between the two sealant materials where group I showed statistically higher enamel micro-hardness values (305.3 ± 42.9 kgf/mm2 and 249.7 ± 10.7 kgf/mm2) compared to group II (273.9 ± 15.5 kgf/mm2and 165.8 ± 26.3 kgf/mm2) at baseline and after 4 days immersion in demineralizing solution ($p = 0.007$ and $p < 0.001$ respectively).

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Table 2: Mean microhardness values within the two groups.

Group	Time interval	Mean	SD	Standard error of the mean	Mean difference	p value
I	Baseline	305.3	42.9	14.3	55.60	0.007
	After 96 h of initial demineralization	249.7	10.7	3.55		
II	Baseline	273.9	15.5	5.17	108.09	< 0.001
	After 96 h of initial demineralization	165.8	26.3	8.76		

Group I, G-Coat Plus; Group II, Clinpro. Abbreviations: SD, standard deviation.

Table 3 summarizes a comparative analysis of micro-hardness between the two groups at baseline and after 4 days immersion in demineralizing solution. At base line, group I showed higher enamel micro-hardness values (305.3 ± 42.9 kgf/mm2) compared to group II (273.9 ± 15.5 kgf/mm2) without any statistical significant differences between the two groups (p = 0.055), showing comparable hardness values of the two materials.

Table 3: Comparisons of the mean microhardness values between the two study groups

Microhardness	Group	Mean	SD	Standard of the mean	95% CI for the mean error		*p* value
					Lower bond	Upper bond	
Baseline	I	305.3	42.9	14.3	272.37	338.32	0.055
	II	273.9	15.5	5.17	291.99	285.86	
After demineralization	I	294.7	10.7	3.55	241.54	257.94	< 0.001
	II	165.8	26.3	8.76	145.63	186.04	

Group I, G-Coat Plus; Group II, Clinpro. Abbreviations: CI, confidence interval; SD, standard deviation.

However, after 4 days demineralization group I samples demonstrated statistically higher enamel micro-hardness values (249.7 ± 10.7 kgf/mm2) compared to group II samples (165.8 ± 26.3 kgf/mm2 (p < 0.001), showing differences in the mineralization properties between the two groups.

Table 4 shows the mean (± SD) differences in each group between the amounts of P and Ca released in demineralizing solution after 4 days and the P and Ca content in the demineralizing solutions at base line. The mean (± SD) of P content in group I and II were 30.40± 18.77 and 253.20 ± 32.76 mM, respectively, indicating a greater P release from teeth treated with group II. On the other hand, the mean (± SD) of Ca content in group I and II were 169.60 ± 18.43 and 151.20 ± 15.83 mM, respectively, indicating that more Ca was released from teeth samples of group I. Statistics revealed significant differences between the two sealant materials regarding their mean concentration values (for Ca, p = 0.028; for P, p < 0.001).

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Table 4: Differences between the amounts of calcium and phosphorus released after immersion in the demineralizing solution, and the calcium and phosphorus concentrations of the demineralizing solutions.

Groups		Mean	SD	Standard error of the mean	95% CI of the difference		
							p value
					Lower	Upper	
	I	169.60	18.43	5.83	2.26	34.54	
Ca						0.028	
	II	151.20	15.83	5.01	2.23	34.57	
	I	30.40	18.77	5.94	-247.89	-197.71	
P						< 0.001	
	II	253.20	32.76	10.36	-248.35	-197.25	

Group I, G-Coat Plus; Group II, Clinpro. Abbreviations: Ca, calcium; CI, confidence interval; P, phosphorus; SD, standard deviation.

DISCUSSION

Inhibiting proximal caries by using proximal sealants is based on the theory of sealing high caries risk surfaces. Because of biofilm stagnation in contact parts that cannot be cleaned properly via brushing, and are reachable only using dental floss, proximal surfaces are considered as high risk areas for development of caries.24 Many approaches have been practiced to control proximal caries like, dental flossing and use of fluoride mouth rinses, but they are found to be of limited success because such techniques rely on patient's behavior and compliance to attain positive results. [25]

Recently, with novel advances in adhesive dentistry , strong efforts have been made to discover a practical and proficient proximal sealant.[8, 24] Though analyzing material micro-hardness delivers evidence regarding some physical characteristics as enamel rigidity and hardness in addition to demineralization potential, surface micro-hardness assessment and biochemical analysis testing, are tested to measure the properties and changes in hard dental tissues.26 Surface micro-hardness is a linear property of the local calcium content 27 that can be 10 used to directly measure mineral loss or gain as a result of demine ralization and remine ralization and as a proportional measure of hardness as well.28 This techniques was proven as effective and valid to assess the variations in enamel surface demineralization.29 Bovine teeth are selected for in this study because they are similar in their mineral composition and content to natural teeth, easily collected and they are analogous to human teeth regarding radio-density [30] The null hypothesis—that G-Coat Plus increases enamel micro-hardness — was accepted. The results revealed no significant difference statistically between the mean micro-hardness values of two materials at baseline. The G-Coat Plus group exhibited superior hardness values. Such finding maybe related to its composition. G-Coat Plus is a dental resin with distinctive nano-filler technology. Commonly, nano-fillers are used as clumped fillers, [31] however modern technology has enabled equal dispersion of the nano-sized filler component which consequently stiffens and reinforces the entire set cement.

The results revealed that G- coat plus showed statistically significant higher enamel hardness values than clinpro after immersion in demineralizing solution. This might be attributed to the novel technology of Nano filler system implemented in G-coat plus resin, that results in stiff dispersed hardened surface. The lower enamel surface micro-hardness values showed by clinpro group might be a result of its chemically aligned structure and composition of the unfilled resin matrix. The results go in agreement with Paris et al. who concluded that the lower penetration depth of the Bis-GMA resin resulted in reduced micro-hardness or low demineralization resistance. [32]

The other hypothesis assumed was —that G-Coat Plus increases proximal surface resistance to demineralization and decreases mineral loss —was rejected. Assessing P and Ca content in the demineralizing solutions was regarded as one of the indirect methods of determining mineral loss. Such method is supported by the study conducted by Jabbarifar et al.,

who found a direct relation between hardness property and mineralization degree.[33]

The results of the present study demonstrated that both groups released phosphorus and calcium ions with different degrees, which explains the variance in the mean micro-hardness values observed between the two sealants. Analyzing both G coat and Clinpro reaction after immersion in demineralizing solution, a significantly higher amount of Phosphorus was released in the Clinpro group than that in G-Coat Plus. Conversely, a significantly higher amount of Calcium was released in the G-Coat Plus group than that in Clinpro. This may be explained based on the notion that Clinpro holds soluble patented organic fluoride that is released by diffusion where hydroxide ions are exchanged for fluoride ions, resulting in formation of fluoro-apatite on the tooth surface which lessen the amount of calcium released in the demineralizing solution in the Clinpro group compared to G-Coat Plus group. This is supported by the results obtained by Shen et al., who concluded that Clinpro exhibited greater amount of calcium than the other control material after 48 h obsevation. [34] The anionic behavior of Fluoride and its higher affinity for positive ions, such as calcium, allows it to partially or completely substitute hydroxide ions in the hydroxyapatite framework, forming fluoro-hydoxyapatite or fluoro-apatite.

The phosphorus ions release after immersion in the demineralizing solution which was significantly higher in the Clinpro group than the G-Coat Plus group may be counted as a drawback for Clinpro material as this decreases fluoride bioavailability in the surrounding. According to Shen et al., the release of increased amounts of inorganic phosphorus ions affect or sometimes decrease fluoride reservoir retention and inhibit both CaF+2 and CaF formation as well as simultaneously enable development of more soluble fluoride stages.[34] The soluble fluoride stages decrease fluoride ion availability and promote calculus formation. The significantly lower amount of inorganic phosphorus released in the G-Coat Plus paralleled to that of the Clinpro group, as well as its superior hardness, may support recommending its use as a smooth surface sealant.

Though artificial enamel lesions have been used in many studies to mimic *in vivo* caries behavior setting, [35, 36] natural and simulated lesions are to some extent dissimilar. Natural caries develop after longer periods of demineralization. Therefore, the use of artificial enamel lesions may have been a limitation in this study; further studies utilizing longer demineralization periods are needed to check the effectiveness of G-Coat Plus. A correlation between P and Ca content and surface micro-hardness might be concluded; where high micro-hardness values associated with low ion release after exposing the tested material to the demineralizing solution supports the recommendation of using it as a sealant. Though, in the current study, the demineralization resistance of the two experimental materials differed when the study samples were exposed to demineralizing solution due to the alteration in the materials' bonding mechanisms and compositions. To the best of the authors' knowledge, preventive proximal sealant techniques have been evaluated by only few short- and long term studies; hence, further extensive research is required to recognize the precise bonding mechanism and system of each material.

To conclude, G-Coat Plus displayed high surface micro-hardness compared to Clinpro. Both sealant materials released P and Ca ions, signifying that utilizing additional preventive measures is necessary when these materials are used as proximal preventive sealants. The protective potential of fluoride incorporated in Clinpro sealant material requires further investigations to draw more definitive conclusions.

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