



Effectiveness of Silver Diamine Fluoride, Bioactive Glass and Casein Phosphopeptide Amorphous Calcium Phosphate in Comparison with Sodium Fluoride on Tooth Surface Roughness (An in Vitro Study).

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Abstract

Aims: Tooth surface roughness is a fundamental feature that impact on the foreign materials retention and caries progression, as well is considered an effective tool for checking the efficacy of various remineralizing agents and evaluating the activity of the carious lesion, so the aim of this study is to test the effectiveness of various remineralizing agents (varnishes) and compare their efficiency on tooth surface roughness. The main ingredient of the used varnishes was (BioActive Glass: BAG), (Casein Phosphopeptide Amorphous Calcium Phosphate: CPP-ACP), (Silver Diamine Fluoride: SDF), and (5% Sodium Fluoride: NaF). **Materials and Methods:** Samples used in the experiment were (50) maxillary primary canine teeth, which were distributed randomly in 5 groups, each group has 10 teeth. Groups were: Group A: SDF, Group B: BAG, Group C: CPP-ACP, Group D: NaF, Group E: Control (just preserved in artificial saliva). The measurement of the surface roughness was achieved at 3 intervals: baseline reading, the 2nd reading was after immersing for 3 days in demineralizing solution and the final measurement was after 2 weeks of varnishes application. **Results:** The results revealed that Group C significantly decrease the surface roughness, followed by D, B Groups, with insignificant effect in Group A. **Conclusions:** Based on the findings of this study, varnish made up of CPP-ACP was the most efficacious one in reducing the surface roughness. The addition of Potassium Iodide to Silver Diamine Fluoride could diminish its capacity for surface roughness improvement.

الخلاصة

الأهداف: خشونة سطح الأسنان هي سمة أساسية تؤثر على احتباس المواد الغريبة وتطور التسوس، كما أنها تعتبر أداة فعالة للتحقق من فعالية عوامل إعادة التمدن المختلفة وتقييم نشاط الآفة النخرية، اذن فهدف هذه الدراسة هو اختبار فعالية أنواع مختلفة من علاجات إعادة التمدن (الورنيش) ومقارنة كفاءتها على خشونة سطح الأسنان. المكون الرئيسي للورنيش المستخدم هو (الزجاج النشط بيولوجيًا)، (كازين فوسفو بيتيد فوسفات الكالسيوم الغير متبلور)، (ديامين فلوريد الفضة)، و (5% فلوريد الصوديوم). **المواد وطرائق العمل:** العينات المستخدمة في التجربة (50) من الأنياب العليا للأسنان اللبنية، وزعت عشوائياً على 5 مجموعات، كل مجموعة تتكون من 10 أسنان. كانت المجموعات: المجموعة أ: (ديامين فلوريد الفضة)، المجموعة ب: (الزجاج النشط بيولوجيًا)، المجموعة ج: (كازين فوسفو بيتيد فوسفات الكالسيوم الغير متبلور)، المجموعة د: (5% فلوريد الصوديوم)، المجموعة هـ: المجموعة الضابطة (محفوظة فقط في اللعاب الاصطناعي). تم قياس خشونة السطح على ثلاث فترات، القراءة الأولية، والقراءة الثانية كانت بعد الغمر لمدة 3 أيام في محلول إزالة المعادن. تم القياس النهائي بعد أسبوعين من تطبيق الورنيش. **النتائج:** أظهرت النتائج أن المجموعة (ج) تقلل بشكل كبير من خشونة السطح، تليها المجموعات (د)، (ب)، مع تأثير ضئيل في المجموعة (أ). **الاستنتاجات:** بناءً على نتائج هذه الدراسة، كان الورنيش المكون من: (كازين فوسفو بيتيد فوسفات الكالسيوم الغير متبلور) الأكثر فاعلية في تقليل خشونة السطح. يمكن أن تؤدي إضافة يوديد البوتاسيوم إلى ديامين فلوريد الفضة إلى تقليل قدرته على تحسين خشونة السطح.

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INTRDUCTION

Surface roughness is the enamel surface irregularity as a result of tooth demineralization. Although the enamel has a smooth surface clinically, it contains some microscopic structures that are a measure of surface texture, which in turn can affect how the surface wears away^[1].

Since high roughness enamel surface has a higher risk of demineralization than the undamaged surfaces and improve the colonization and retention of bacteria, so the management of the roughness of enamel surface plays an essential role in the prevention of caries^[2].

The profiler is the most widely used method for analyzing enamel surface roughness, it is considered a non-invasive method that provides an efficient quantitative evaluation and is calculated as an average value, so statistical analysis can be performed accurately^[3].

The current trend in the treatment of tooth decay is to make use of minimally invasive, tissue preservation, reachable and safe methods. At the same time, efforts have been made to develop more effective anti-caries agents, which are in great need^[4].

In last few years, researchers have agreed that fluoride varnish provides an effective means to remineralize the white spot lesions, which can not only prevent tooth decay, however additionally arresting the early carious lesion^[5].

BioActive materials are defined as materials that induce positive responses in the body, especially by adhering to the bone

tissue of the host and forming a layer of calcium phosphate on the surface of the material. Moreover, it promotes the remineralization of tooth enamel or decreases the demineralization process^[6].

Topical application of SDF can be regarded as a simple and non-invasive treatment for dental caries. Stable forms of silver fluoride such as silver Diamine Fluoride (SDF) have been used in Japan several decades ago to treat dentin hypersensitivity and dental caries^[4].

Due to their small size and ionic neutrality, CPP-ACP complexes can also diffuse into the subsurface enamel lesions. According to reports, in early carious lesions the nano-complexes diffuse through the pores into the lesion, where they release loosely bound calcium and phosphate ions, and then deposit in the crystalline cavity, around the hydroxyapatite. CPP were proven to preserve fluoride ions in solution, in addition to improve the remineralization effect of the fluoride agents^[7].

MATERIALS AND METHODS

Sample collection and surface preparation

The study included (50) maxillary primary canine teeth, which were collected from the specialized dental centers in Mosul city. The teeth samples were examined by 10X magnifying lens to ensure that the samples followed the inclusion criteria on intact crowns, without carious lesion, fractures, dental anomalies or cracks^[8], then the teeth were kept in solution of 0.1% thymol to

avoid microbial growth, and keeping moisture of the samples until use^[9].

When the experiment began, the teeth samples were cleaned and washed by

deionized water, then the teeth surfaces were polished by rubber cup and non-fluoridated pumice (figure 1).



Figure (1): Teeth samples cleaning and polishing

By using double ended diamond disc bur with copious amount of water; to avoid enamel destruction and damaging, the crowns were separated from the remaining roots at level 2 mm below the cement-enamel junction^[10]. For preparing the teeth surfaces in an appropriate and stable model for measuring of the surface roughness, the tooth specimens were embedded in plastic cylindrical rings which were filled with cold cure setting acrylic resin^[11], so that the measured target surface area; which was the middle part of the buccal surface; exposed to the top of the ring, (figure 2).



Figure (2): Teeth samples were prepared completely with exposing the labial surfaces

Sample grouping and measurements interval:

The samples of the current study were randomly divided into 5 experimental groups for each of the remineralizing

agents used, and each group included 10 teeth for surface roughness assessment.

The groups were:

Group A (SDF group): each specimen in the group was treated with a thin layer coating of Silver Diamine Fluoride SDF

varnish (Riva Star) with careful application following the manufacture instructions, applying a thin layer from the first gray capsule, then and immediately applying layer of the second green capsule Potassium Iodide (KI).

Group B (BAG group): a thin layer of (Fluoro dip Bioactive) varnish was applied for each tooth sample in the group in a hasty manner with a specific brush, then left for drying for 20 second as mentioned in the instruction leaflet.

Group C (CPP-ACP group): each tooth sample in the group was coated with a thin film of (CPP-ACP, MI varnish), the application setting and drying time was according to the instructions of the material using.

Group D (NaF group): the teeth sample in this group were treated with (5% NaF, Fluorodose) painted with thin covering layer as the instructions of the manufacture.

Group E (Control group): the specimens of this group were left without any treatment, just kept in artificial saliva, nothing else.

After treatment application, teeth samples of the whole groups later kept in daily refreshed artificial saliva.

The teeth sample roughness were recorded and measured 3 times during the experimental study, the first reading was the baseline or initial reading when the experiment started, then the second reading for the demineralization process, after immersing the samples within the demineralizing solution for 72 hours, the final measurement was done after 2 weeks

of the usage of the remineralizing agents once as manufacture instructions.

Solutions preparation protocol:

1- Artificial saliva: mixing the following substances with the specified concentrations: 10 gm. (Sodium carboxymethyl cellulose) + 1gm. of each of (Sodium chloride, Potassium chloride, Sorbitol) + 0.05gm. of each of (Calcium chloride, Magnesium chloride) + 0.04 gm Potassium phosphate + 0.01gm Potassium thiocyanate + 0.0002gm. Sodium fluoride). These ingredients were dissolved in (985.5 ml) of deionized water, after that Sodium carboxymethyl cellulose was dissolved in (100 ml) of boiling water and added to the previous mixture after it has been cooled. The pH of the solution was verified by portable pH meter and amended to 7^[12].

2- Demineralizing solution: this demineralizing protocol was accomplished to form an obvious enamel surface demineralization that similar to the enamel carious lesion, in which the following components were mixed: (50 mM acetic acid + 2.2 mM Ca(NO₃)₂ + 2.2 mM KH₂PO₄ + 5.0 mM Na₃N + 0.5 ppm NaF) and incubated for (72) hour in shaker with (37°C, 50 rpm/min). The pH was adjusted to the specific pH of the solution, which was 4.5 and checked by portable PH meter^[13].

Surface roughness measurements:

A profilometer (profile projector MITUTOYO, Japan) was employed for

measuring the tooth surface roughness with recommended magnification and cut off value (figure 3). The test was performed in Technical Institute/ Northern Technical University at Mosul city. The measurements were carried out for each of the experimental period: baseline,

demineralizing and remineralizing phase. For each tooth specimen, the average of 3 measurements was taken as a metric value which considered as a suitable quantitative tool for accurate assessment and used for statistical analysis^[14, 15].



Figure (3): Profilometer (profile projector).

With adjustment of reference length (cut off value) 0.8 mm, and amplification 50X (figure 4). The value of measurements was expressed in micrometer and calculated

within a central line of y direction as arithmetical average of surface highest and lowest lines of the examined area.^{[3][14]}



Figure (4): Surface roughness measuring of the teeth samples by profilometer

The surface roughness of the samples was measured by calculating the sum of the

distance between the lowest valley (R_v) and the highest peak (R_p) of the central line

in y direction, and the result value is maximum roughness height (Rt) which expressed in micrometer (μm), the formula as the follow: ($Rt = Rv + Rp$).^[15, 16]

Statistical analysis:

Data presentation and analysis was done by the usage of SPSS version 21 which denote a Statistical Package of Social Science. One-way Repeated Measurements Analysis of Variance (RM-ANOVA) was used to compare the surface roughness at a different measurement periods between the experimental groups.

Pairwise Comparisons: Post Hoc Bonferroni test that was used to determine if there is a significance among in each of the studied group and the different measurement periods. (P): level of significance: Not significant (>0.05), Significant (<0.05).

RESULTS

Roughness assessment among groups by levels:

- **Descriptive and statistical test:**

Assessment of surface roughness by descriptive and statistical test among groups by periods (table 1).

Findings showed that there was no significant difference between groups in both baseline and acid challenge phases regarding the roughness but in the remineralization stage, the lowest roughness value was in the CPP-ACP agent followed by NaF and BioActive and largest value in the control group followed by SDF with significant difference.

- **Multiple pairwise comparisons**

Multiple pairwise comparisons of roughness between groups in the Remineralization phase using Bonferroni Post Hoc test (table 2).

All results showed significant difference when comparing each group with the other, except when comparing SDF with Control, and BioActive with NaF, findings were not significant. When comparing each group with the control, the largest mean difference in roughness was found in CPP-ACP followed by NaF and BioActive, while the lowest was when compared with SDF. When comparing each agent with the other rather than control group, the largest mean difference was in (SDF - CPP-ACP) groups, followed by (SDF - NaF) and (SDF - BioActive).

Table (1): Descriptive and statistical test of roughness among groups by periods.

Periods	Statistics	Groups					F	P value	Effect size
		Control	SDF	BioActive	CPP-ACI	NaF			
Base Line Levene test=1.989 P value=0.112 NS	Minimum	0.390	0.390	0.390	0.380	0.400	0.962	0.438	0.079
	Maximum	0.460	0.460	0.460	0.460	0.430			
	Mean	0.422	0.423	0.423	0.409	0.410			
	±SD	0.024	0.029	0.024	0.023	0.012			
Demineralization Levene test=0.483 P value=0.748 NS	Minimum	5.900	6.100	5.600	5.700	5.800	2.356	0.065	0.057
	Maximum	6.700	6.800	6.300	6.300	6.500			
	Mean	6.280	6.460	5.960	6.025	6.160			
	±SD	0.278	0.217	0.217	0.187	0.232			
Remineralization Levene test=2.125 P value=0.090 NS	Minimum	5.700	5.100	3.050	1.300	2.900	750.055	0.000*	0.870
	Maximum	6.400	6.100	4.800	3.350	5.100			
	Mean	5.980	5.658	4.036	2.428	3.602			
	±SD	0.204	0.354	0.600	0.689	0.671			

*=significant at p<0.05.

Table (2): Bonferroni posthoc test for roughness comparisons between groups in the remineralization phase

Groups	Mean Difference (I-J)	P value
Control	SDF	0.322
	BioActive	1.944
	CPP-ACP	3.552
	NaF	2.378
SDF	BioActive	1.622
	CPP-ACP	3.230
	NaF	2.056
BioActive	CPP-ACP	1.608
	NaF	0.434
CPP-ACP	NaF	-1.174

*=significant at p<0.05

DISCUSSION

The roughness of the enamel surface is considered as a critical factor in detecting the progression rate of the caries activity, that affects the attachment of the bacteria and other foreign materials on the tooth surface, providing an appropriate environment for the bacterial retention and colonization, consequently could be used as a measure of assessment of the de- and remineralization processes [2].

The results of this study revealed that when comparing from demineralization to the

remineralization stage the largest significant difference (best one in reducing the roughness) showed in CPP-ACP, then NaF and BioActive, followed by SDF, finally in the artificial saliva which was not significant. The findings about the efficiency of CPP-ACP in improving the surface roughness was agreed with Heshmat et al., (2014) [17] in which they found that CPP-ACP was efficacious in decreasing the tooth surface roughness and led to somewhat a smooth surface, which

limit the bacterial adhesion and subsequently the carious activity.

The result of the current study was in agreement with the study of Jaina et al., (2019) ^[18] which stated that fluoride varnishes were significantly effective in reducing the enamel surface roughness and enhancing the remineralization. In their study Pandya and Diekwisch, (2019) ^[19] reported that BioActive glass have an important role in remineralization enhancement and improving the surface roughness, which come in accordance with the findings of this study. Whereas its efficiency could be explained by the formation of a tooth surface chemically bonded hydroxycarbonate apatite layer after contacting with saliva, which considered as a protective layer that enrich in phosphate and calcium ions ^[20].

The results of the current study about the ineffectiveness of the artificial saliva in reducing the surface roughness was in accordance with Heshmat et al., (2014)^[17] in which they reported that the roughness of the tooth surface was not alter or improved when immersed in the artificial saliva.

The findings of this study revealed that when multiple comparisons between groups, all are significant difference when comparing with each other, (except SDF with saliva), and (BioActive with NaF) are not significant.

Within limitation of this study, Fluoride was better and more efficient than BioActive Glass in surface roughness reduction and remineralization stimulating

but without a significant difference between them. These findings were aligned with other previous studies as Worschech et al., (2003) ^[16].

However, the results of this study indicated that NaF was better comparing to SDF in restoring normal surface texture and decreasing the surface roughness. On the contrary, these results disagree with Abdil-Nafaa and Qasim, (2020) ^[21], whose found that SDF was superior to fluoride varnish in this aspect. The difference in the results may contribute to many factors, as the variation in the enamel surface between different teeth and dentitions in addition to the mineral content^[22]. Furthermore, the possibility of the side effect of Potassium Iodide (KI) which was added to solve the discoloration problem as reported by Shimizui et al., (2021) ^[23].

In the study done by Jaina et al., (2019) ^[18] they stated that Fluoride varnish was more effective with significantly difference comparing to the artificial saliva in improving the tooth surface roughness, these results were in accordance with this study findings.

The results of the study done by Sayed et al., (2020) ^[24] revealed that the application of SDF with or without (KI) was significantly more effective in decreasing the tooth surface roughness than the artificial saliva, which was in disagreement with the findings of this study which stated that SDF was efficient in improving surface roughness; slightly more than artificial

saliva, but without a significant difference between them.

Ultimately, the difference in the effect of the remineralizing agents that utilized in this study on the tooth surface roughness could be related to the variation in the composition of each agent and its component beside the solubility, acidity, concentration and the chemical formula of each agent [25, 26]

CONCLUSION

Based on the findings of this study, the most efficacious varnish of the types used in the present study in decreasing the roughness of the demineralized tooth surface and restoring of the enamel surface smooth texture was CPP-ACP with an obvious effect, followed by (NaF and BAG) which showed an almost similar effect. The lowest influence was noticed in SDF Group, Its effect was roughly the same of artificial saliva in reducing the surface roughness, which may be referred to the utilization of KI, which could be the reason to diminish its capacity in surface roughness improvement.

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