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Research Article

Impact of Hydroxyapatite Toothpaste on Enamel Surface Roughness of Artificially Demineralized Permanent Teeth (An in vitro study)

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Abstract: The current study aimed to evaluate the impact of hydroxyapatite (HA) toothpaste, synthesized from chicken eggshell powder (CESP) on the surface roughness of artificially demineralized enamel, in comparison to sodium fluoride paste via profile projector machine. **Materials and Methods**: Fifty sound premolars had been split into five groups at random, and artificial caries lesion was made by soaking the samples in the demineralizing solutions, and demineralized surface was cured with: G1 (base line group n=10) without any thing, G2 (control negative group n=10) immersed in demineralizing solution only, G3 (HA paste group n=10) treated with HA paste, G4: (Naf n=10) treated with sodium fluoride paste as control positive, G5: (combination group n=10) treated with HA paste and Naf paste alternatively. The enamel surface roughness was evaluated (at base line, after demineralization, and after treatment. **Results**: All teeth samples exhibited increased surface roughness after demineralization, in comparison with surface roughness of baseline group, then all treatment groups showed decrease in surface roughness after treatment, except control negative where was deionized water used for storing teeth. **Conclusion**: HA paste, Naf toothpaste (Flurokin), and a combination of them have remineralizing ability; a decrease in the surface roughness of demineralized enamel surface reflects this.

Keywords: Demineralization, Hydroxyapatite, Toothpaste, Remineralization, Roughness

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INTRODUCTION

Dental caries develops when sugar consumption often changes the biofilm microbiota that typically maintains homeostasis in the oral cavity into an acidogenic, aciduric, and cariogenic population [1]. This shift may have clinically no effect or may cause a net loss of minerals in the tooth's hard structures, which would manifest as a visible carious lesion [2]. As a result, dental caries is seen as a dietary-microbial illness [3]. This requires the development of a cariogenic biofilm and repeated exposure of the body to fermentable carbohydrates (such as glucose and sucrose). Additionally, as demineralization is a reversible process, hydroxyapatite crystals in teeth that have undergone partial demineralization may re-mineralize and revert to their previous state under the right circumstances [4]. Surface roughness is characterized as closely spaced irregularities on the enamel surface that are quantified concerning the perfect surface form. The surface is rough when there is a lot of separation and smooth when the spacing is close [5]. A fundamental and significant characteristic of teeth is their roughness, which is related to the adhesion of plaque, stains, and foreign objects to their surfaces. Surface roughness has an impact on biofilm colonization, quality, and color. The limit of surface roughness for dental biofilm attachment that has been most frequently observed is 0.2 um; hence, a roughness increase over this limit enhances bacterial adhesion and colonization. Monitoring the roughness of the enamel surface plays a key function in avoiding tooth disease [6&7]. The results of the remineralization agent on the roughness of the enamel surface may have been affected by the many components and remineralization processes that make up each remineralizing agent.

MATERIALS AND METHODS

- Sodium fluoride toothpaste (Fluor kin) that contains (2500 ppm) fluoride ion and an expiration date of January 2025 \Made in Spain.
- The hydroxyapatite tooth paste (HA paste) was formulated according to [8], from Sodium Carboxyl Methyl Cellulose, Glycerol, and Nipagin from AVONCHEN UK, while hydroxyapatite powder extract from chicken eggshells by chemical precipitation method, (patent 6987, A61C13/08, A61L27/12) [9].
- The demineralization solution consists of $CaCl_2$ (2.2mM), KH_2PO_4 (2.2 mM), and acetic acid (0.05 M); pH was adjusted with (1M) KOH to (4.4), [10].
- De ionized water \Drug production factory in Nineveh \Iraq.
- Distilled water \Drug production factory in Nineveh \Iraq.
- Thymol powder \Flukachemie \Switzerland.

Sample collection.

Fifty human sound permanent premolars extracted for orthodontic purposes were collected from the private clinics in Mosul City. The teeth were washed with deionized water and kept at room temperature in a covered container with a 0.1 % thymol solution until the next step in the study. Each tooth that had a white spot lesion, a fracture, a hypo plastic lesion in the enamel, fluorosis, a restoration, or an intrinsic or extrinsic stain was excluded from the research.

Sample Preparation.

All accompanying soft tissue and calculus were removed from the tooth by hand scaling, and then the enamel surface was polished using a rubber cup and a slurry of non-fluoridated pumice (PD, Germany) using a contra-angled low-speed hand-piece [(11)]. For every tooth sample, the hand piece's speed and polishing duration remained consistent. Then, the roots were chopped at the intersection of the cement and enamel using a straight diamond bur (Nti, Germany) on a high-speed hand piece (continuous water cooling) to protect the enamel. After that, each tooth crown was inserted into an auto-polymerized cold-cure acrylic resin from (Veracril, Colombia), which was then poured into a cylindrical plastic ring with a 14 mm diameter and 16 mm depth. The buccal surface of the ring was then oriented upward and parallel to the floor. The enamel surfaces were ground wet polished one by one with a fine grit silicon carbide paper (1200 grit) to yield standardized flat enamel surfaces [(12)], and each sample's buccal surface had a circular 6x6 mm piece of adhesive tape applied in the center. After applying acid-resistant nail polish to the remaining surface, the tape was removed to show an enamel window [(13)]. Then, the exposed enamel window was polished with the universal polishing machine (Surf-Corder, Japan) as shown in figure **1**. After that, all samples were kept in deionized water until they were soaked in a demineralized solution.

Figure (1): Creation of a window on the buccal surface to standardize the area of measurement in the middle third of the tooth.

Creation of the initial Carious Lesion.

Every tooth in the study, except the baseline group, was soaked in a demineralizing solution for 48 hours (2 days) at 37 °C to produce subsurface enamel caries (white spot lesions without cavities) [10] Specimens were washed with deionized water and stored in deionized water, until subjected to remineralizing agents**.**

Synthesis of hydroxyapatite paste:

The hydroxyapatite paste (HA paste) was prepared according to Natsir N et al., 2020 [8], and formulated from Sodium Carboxyl Methyl Cellulose (Na-CMC), distilled water, Glycerol, Nipagin, and Hydroxyapatite powder extract from chicken eggshells by chemical precipitation method. The most frequently utilized concentration of hydroxyapatite in the in vitro studies and the optimal concentration to achieve the remineralization was 10%, according to [14]. So, we prepared 5 ml of 10%WV Hydroxyapatite paste daily for 7 days.

Infrared Spectroscopy Estimation of Important Bands

The prepared HA powder from CESP and the prepared HA paste components were characterized by using the FTIR-Alfa-Bruker spectrophotometer (Germany) in the region (400-4000 cm^{-1}). This measurement was carried out at the University of Mosul, College of Chemistry Sciences.

Grouping and application of remineralizing agents

The whole number of teeth samples in the main study were (50) teeth, which were haphazardly distributed into (5) clusters, (n=10) samples in each cluster.

Group A (10 teeth): baseline group without demineralization.

Group B (10 teeth): without treatment, demineralized teeth stored in deionized water only, as control negative group.

Group C (10 teeth): after demineralization, HA tooth paste applied by micro brush as shown in figure (2) then brushed by soft brush, applied once at mooring and once at evening for (2-3) minutes to simulate routine normal hygiene in vivo, and these samples called HA paste group.

Group D (10 teeth): Each tooth's exposed window on its enamel surface received a little coating of fluoridated toothpaste that contains (2500 ppm) fluoride ion (Flurokin) using a fine brush for 2-3 minutes as shown in figure (3). This procedure was performed twice daily to simulate normal oral hygiene instructions, then washed with deionized water and stored in deionized water changed after each application until complete one week, and these samples called as Naf paste group.

Group E (10 teeth): Combination group (HA paste + Naf paste) separately each one from the two pastes applied by micro brush once daily for 7 days, HA paste at morning and Naf paste at evening, and these samples called as combination paste group.

Figure (2): Application of hydroxyapatite toothpaste

Figure (3): Application of sodium fluoride toothpaste.

Surface Roughness

Each sample in the experimental groups had a surface roughness test to determine the enamel's surface texture. This test used a profilometer (Mitutoyo, Tokyo/ Japan) with a 50X magnification to evaluate the enamel's surface texture at baseline, after demineralization, and after treatment. the specimens' surface roughness was measured based on the parameter of maximum roughness height by calculating the distance between the highest peak and lowest valley in y-direction along the central line of the area and the obtained value was recorded in μm, each sample had it is surface roughness assessed three times, with the average of the three results being calculated [15].

RESULTS

Data of surface roughness of all groups were explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests and showed parametric (normal) distribution when sig> 0.05 as shown in table (1). The descriptive statistics, including means, number of tested groups, and standard deviation, are listed in table (2). We

noticed that negative control group (acid group) taken the highest mean value of surface roughness (0.65) when matched with baseline group, and this mean all teeth samples showed increase surface roughness after immersion in demineralizing solution in compare with roughness of base line group (0.33), and all three treatment groups exhibited reduction in surface roughness after treatments.

Table (1): Display test of normality of surface roughness values of all study groups

Tests of Normality						
Variables	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Roughness of baseline group	.184	9	.200*	.949		9.674
Roughness of negative control G	.194	9	.200*	.911	9	.325
Roughness of HA paste G	.133	9	.200*	.950	9	.691
Roughness of Naf paste G	.134	9	.200*	.978	9	.955
Roughness of combination G	.116	9	.200*	.956	9	.755

Table (2): Descriptive Statistics of Surface Roughness Measures Across Testing Groups.

Surface Roughness			
Groups	N	Mean	S.D.
Baseline group	10	0.3300	0 .05497
Control negative G	10	0 .6500	0.05462
Hydroxy apatite G	10	0 .4000	0 .04967
Sodium fluoride G	10	0.3556	0 .06710
Combination G	10	0 .4200	0.05312

Table (3) shed light on the comparison of enamel surface roughness mean values for (baseline, control negative, HA paste, Naf paste and combination paste) groups by one way analysis of variance (ANOVA) test, and the results exhibited that there were a highly statistically significant differences at $p \leq 0.01$.

ANOVA Test					
Roughness	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.658	4	0.164	52.706	0.000
Within Groups	0.140	45	0.003		
Total	0.798	49			

** $p \leq 0.01$, highly statistically significant.

Table (4) Duncan's Multiple Range test revealed that the mean surface roughness for baseline, control negative, HA toothpaste, Naf tooth, and combination paste) groups are highly significantly different from each other at $p \leq 0.01$. The acid group (control negative) had a mean surface roughness of 0.65, followed by the combination group (0.42), followed by HA paste G& Naf paste G with no significant difference between them, and lastly, the baseline group (0.33) as revealed in Figure 4).

Surface Roughness

Groups	N	Mean	S.D.
Baseline G	10	0.33 00 d	0.05497
Control negative G	10	0.6500 a	0.05462
Hydroxyapatite paste G	10	0.4000 bc	0.04967
Sodium fluoride paste G	10	0.3556 cd	0.06710
Combination G	10	0.4200 b	0 .05312

N: Number of the samples, S.D: Standard Deviation, Different Small letters in the same column indicate statistically significant variance.

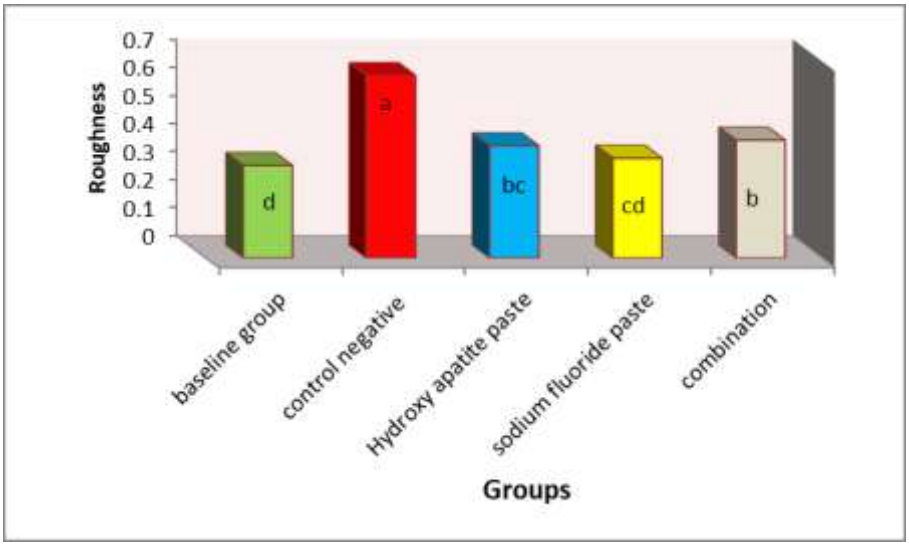

Figure (4): Duncan's multiple range test of surface Roughness. The mean value of surface roughness measurements for baseline group, control negative group, Hydroxyapatite paste group, Sodium fluoride toothpaste group, and combination paste group.

Table (5): The t-test analysis to determine significant difference between base line and control negative groups in Surface Roughness.

		T-test			
Roughness	N	Mean	t-value	sig	S.D.
Baseline G	10	0.3300		0.000	0.05497
Control negative G	10	0.6500	-13.058-	0.000	0.05462

** Highly statistically significant at $p \leq 0.01$.

We noticed that from mean values of surface roughness of the enamel teeth samples increased after immersion in a demineralizing solution, compared to roughness of the baseline group without anything.

DISCUSSION

The most effective way to assess an early enamel caries lesion and the effectiveness of a remineralizing agent was to measure the tooth's surface roughness. For standardization, all three treatment groups received two applications of the remineralizing agent on an artificially induced enamel lesion. Additionally, due to the similar demineralization methodology utilized in all treatment groups, the results for enamel surface roughness significantly increased after demineralization as compared to the baseline group's roughness values [16].

Following treatment, three treatment groups showed a significant decrease in enamel surface roughness; this would suggest that the three types of remineralizations can remineralized early caries lesions, but complete remineralization did not take place within seven days as concluded by [17].

The limit of surface roughness that is most usually discussed is below 0.2 um; increases in surface roughness above this value lead to the accumulation of dental biofilm, bacteria, and discoloration. Any modifications to the enamel's surface roughness encourage the development of plague [7] , and increases vulnerability to the development of dental decays, and raise in enamel brittleness [18], findings of the current study indicated that there was a large increase in surface roughness from roughness of base line group after demineralization followed by a considerable reduction after remineralization, therefore, demineralization hence increases the roughness of enamel's surface and maximizes plaque buildup, whereas remineralization replaces lost tooth mineral [19].

However, there were no statistically significant differences between the three treatment groups. The Combination group of HA+Naf toothpaste gives the highest mean for surface roughness after treatment. Then HA paste group, and sodium fluoride paste group. Combination group and HA tooth paste gives mean surface roughness higher than mean value of sodium fluoride can be explained by the HA particles adhering to the enamel's surface supplying a significant number of calcium and phosphate ions, which promote the development of existing crystals and provide a rougher surface, and this with agreement with the studies formed by Roveri [20] and Hassan [5], that it is said this adhere HA particles layer made the teeth rougher.

However, the results of the study may be different in case of prolong duration of treatment and use small size of HA particles which can pass through the porosities of

an enamel subsurface lesion and diffuse into the body of the lesion which revealed greater reminerlizaing effect and this led to significant reduction in surface roughness, and these results agree with [21&22]. While Naf toothpaste showed the lowest mean of surface roughness between the three treatment groups, this result was due to a higher concentration of fluoride that precipitates on the outer surface layer of the enamel, resulting in highly mineralized and this led to a significant reduction in roughness.

CONCLUSIONS

Within the constraints of the current study, it is possible to conclude that:

- Hydroxyapatite toothpaste synthesis from chicken eggshell, Sodium fluoride toothpaste (Flurokin), and a combination of both of them have remineralizing ability; this is reflected by the decrease in surface roughness of demineralized enamel surface,
- In terms of statistics, there was no discernible difference between the remineralizing agents. the highest mean of surface roughness is in the combination group, followed by the hydroxyapatite paste group, then sodium fluoride paste group.

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Ethical statement: The current study was conducted in the University of Mosul / College of Dentistry / Department of Pedodontic, Orthodontics, and Preventive Dentistry after getting approval from the research ethics committee. REC (UoM. Dent/ H.DM.78/22).

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript

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تأثير معجون تأثير معجون الأسنان هيدروكسيباتيت على خشونة سطح المينا للأسنان الدائمة المنزوعة المعادن بشكل اصطناعي (دراسة في المختبر)

ندى حسام الدين محمد رفيق , ساهر كصكوص

الملخص

الأهداف: هدفت الدراسة الحالية إلى تقييم تأثير معجون الأسنان هيدروكسيباتيت (HA) ، المُصنَّع من مسحوق قشر بيض الدجاج (CESP)على خشونة سطح المينا المنزوعة المعادن صناعيًا ، مقارنةً بمعجون فلوريد الصوديوم عبر جهاز بروجيكتور. **المواد وطرائق العمل**: تم تقسيم خمسين ضاحكًا سليمًا إلى خمس مجموعات بشكل عشوائي ، وتم عمل آفة تسوس صناعية عن طريق نقع العينات في محاليل التنقية ، وتم علاج السطح المنزوع المعادن بـــ) G1 :مجموعة الخط الأساسي (n = 10 بدون أي شيء ، G2 (مجموعة التحكم السلبية ن = 10) مغمورة في محلول إزالة المعادن فقط ،) G3مجموعة معجون HA ن = 10 (تعامل مع معجون HA، (Naf n = 10) :G4تعامل مع معجون فلوريد الصوديوم كمجموعة تحكم إيجابية ،) :G5المجموعة المركبة (n = 10 تعامل مع معجون HAومعجون Naf بدلاً من ذلك. تم تقييم خشونة سطح المينا (عند خط الأساس ، بعد ازالة المعادن، وبعد المعالجة. **النتائج**: أظهرت جميع عينات الأسنان زيادة خشونة السطح بعد التنقية ، مقارنة بخشونة السطح لمجموعة خط الأساس ، ثم أظهرت جميع مجموعات المعالجة انخفاضًا في خشونة السطح بعد المعالجة. **الاستنتاجات**: تركيبة معجون HA من CESP ومعجون الأسنان Naf (Flurokin)ومزيجهم لديهم القدرة على إعادة التمعدن، وينعكس ذلك من خلال انخفاض خشونة السطح لسطح المينا المنزوع المعادن.