Research Article

Comparing the effectiveness of using three different re-mineralizing pastes on remineralisation of artificially induced white spot lesion

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Abstract: Background: This study aimed to compare the surface microhardness (MH) and mineral content of white spot lesions(WSLs) after using bioactive glass (BAG)casein phosphopeptides-amorphous calcium phosphate(CPP-ACP),and nanohydroxyapatite(Nano-HAP) under pHcycling. Material and method:18 sound maxillary first premolar were used for the study.10 were selected for the vickers microhardness test, For Energy-dispersive X-ray spectroscopy analysis (EDX), the remaining 8 premolar teeth were used, 40 sections of enamel blocks (Four from each tooth) were produced from the middle part of the buccal and palatal surfaces of teeth for MH test while 48 sections of enamel blocks (Six from each tooth) were produced for EDX analysis. Enamel slabs were divided into four groups: control group that preserved in DDW, Nano-HAP, CPP-ACP and BAG group, then were demineralised using 0.1 M lactic acid and 8 wt.% carboxymethylcellulose gel to create artificial WSL. The specimens were subjected to a pH cycling regime for 20 days. The remineralisation potential of the specimens was studied by evaluating the surface MH, calcium (Ca), and phosphrous (P) at different stages: baseline, after production of WSLs, and after treatment with different materials. The gathered data were statistically analyzed using repeated measures one-way ANOVA test and the Bonferroni test. Results: The results showed that Nano-HAP had the highest mean values of MH (157.699 kg/ mm^2), Ca (50.108), and P (24.840) followed by BAG (MH=147.769 kg/ mm^2, Ca=47.408, P=22.285), and the lowest mean value was found in the control group (MH=52.299 kg/ mm^2, Ca=35.291, P=17.228). Bonferroni's and Tukey's HSD test showed higher significant difference ($p<0.05$) from demineralization to remineralization phase in all groups, except when compared control group with WSL (Demineralization) showed non-significant difference ($P>0.05$). Conclusion:all tested agents have highly significant remineralizing potential. Nano-HAP has the highest potential for remineralizing initial enamel caries lesions.

Keywords: White Spot Lesions, Nanohydroxyapatite, Enamel, Bioactive Glass, Remineralization.

Introduction

White spot lesions are the first sign of dental caries lesions which can be clinically detected with the naked eye (1). The current approach of conservative treatment focuses on a reinforcement of non-cavitated lesions for the longest functional ability (2). The use of agents to enhance remineralization is thus one of the methods to manage non-cavitated lesions (3). Examples of remineralizing agents include fluoride (4), CPP-ACP (5), and Nano-HAP (6).

Currently, several studies are focused on the combination of Nano-HAP in oral care products to prevent dental cavities, such as toothpaste (7). Accordingly, remineralization of the teeth can be expected and its effect will be increased when the particle size of hydroxyapatite can be reduced to less than that of the micron-size in existing toothpaste preparations [8]. In recent years, Nano-HAP has acquired wide acceptance in medicine and dentistry as one of the most biocompatible and bioactive materials. In terms of morphology and crystal structure, nanoparticles are identical to apatite crystals found in tooth enamel [9]. CPP-ACP functions by binding to the surface of the tooth and plaque. Due to the stability of amorphous calcium phosphate, this interaction maintains large concentration gradients of soluble calcium and phosphate ions, both of which play an essential role in remineralization [10]. BAG is marketed as a treatment for dentin sensitivity. Its success and mechanism of action as a sensitivity-reduction agent promoted interest as a remineralizing agent [11]. It's called "Bioglass" because it has a high level of bioactivity. Bioactivity is frequently defined as the ability to create a hydroxyapatite-like surface layer and bond to living tissues. The creation of an apatite-like layer bonds bioglass to tissues, and its bioactivity is regulated by a variety of parameters such as chemical composition, textural characteristics, and structural density [12, 13].

Toothpastes that contain new remineralization agents may be utilized because of their low cost, easy application, and capability of preventing the formation of the dental caries with their remineralizing characteristics [14].

This in-vitro study, the aim was to compare the MH and mineral contents of enamel in WSL after treatment with three commercial products: Apagard remineralizing paste (sangi, tokyo, Japan), tooth mousse (GC CORP, Japan) and BioMin Restore toothpaste (Dr. Collins Inc, USA). A comparative evaluation of those 3 re-mineralizing agents will be helpful to identify the most effective and potent agent in the treatment of the initial caries lesions in effective non-invasive manner, and also to eliminate the potential of fluoride toxicity and fluorosis by using remineralizing agents that are more safe. All three claim to have a good impact on enamel remineralization. The null hypotheses are Nano-HAP treatment neither improve the mineral content nor restore the MH of decalcified WSLs. There are no significant differences in the MH as well as mineral content of WSLs treated with the three different treatment modalities.

Materials and Methods

This in vitro study was conducted in the Department of Conservative Dentistry, University of Baghdad/Collage of Dentistry, Baghdad, Iraq, and it was approved by the scientific and the ethical committee at the Collage of Dentistry / University of Baghdad (Approval Number: 494522, Date:19-1-2022).

Sample size calculation

Using G power 3.1.9.7 (Program developed by Franz-Paul, Universitatit Kiel, Germany) with a research power of 95%, Probability alpha error equals 0.05, a correlation between time points is 0.5, the effect size of F is 0.40 (large effect size) , these values were obtained from a pilot study, with 4 groups and 3-time points, with all these conditions the sample size is 28 samples, thus 40 enamel slabs for MH was enough for this study. With the use of G power 3.1.9.7 (Program written via Franz-Faul, Universitatit Kiel, Germany) with power of study=85%, alpha error of probability=0.05, effect size of F is 0.60 (large effect size), with 6 groups, with all these condition the sample size for EDX analysis is 48 samples.

Preparation of specimens

Eighteen upper premolar teeth were extracted for the purpose of orthodontic treatment, washed and cleaned with tap water and polished with non-fluoridated pumice slurry for each sample using a rubber cup in a low-speed handpiece. The teeth were then placed in 0.1% thymol solution (as an anti-microbial solution for inhibition of bacterial growth) and stored for one week. Then placed in DDW until use [15]. The teeth were examined by magnifying lens (10X), any tooth had a visible fracture, crack, Decay, or Restorations was discarded. The roots were removed and the crowns were divided longitudinally into buccal and lingual sections [16]. 10 teeth were selected for the Vickers microhardness test. For EDX analysis eight premolar teeth were used to obtain ultrastructural information and measure the elemental composition of enamel [17].

The samples had comparable dimension by measuring the mesiodistal (M-D) and occlusogingival (O-G) lengths using a digital Vernier, the lengths of teeth were from 8.12 to 8.90 mm and width from 6.47 to 7.19 mm Four sections of enamel blocks (approximately 3 mm length _ 3 mm width _ 1.5 mm thick [18]) are produced from the middle part of the buccal and lingual surfaces of each tooth using XP precision sectioning saw (Pelco, USA) and were measured using an electronic digital caliper to obtain accurate dimensions. And six sections of enamel blocks, (approximately 3 mm length _ 1.5 mm width _ 1.5 mm thick) were produced from each tooth for EDX analysis, one used as baseline, one for WSL and the remaining four blocks with WSLs were distributed to one of the four different groups in order not to repeat the measurement on same enamel slabs because the EDX is a destructive method.

The samples were embedded in an acrylic resin using a silicone mold. After that, samples were polished with the help of a polishing machine (Dap-U, Struers, Denmark). The surface of the superficial enamel was flattened by grinding it with water-cooled carborundum discs (1200 grit for 10 s; Water Proof Silicon Carbide Paper, AL-ALAMAIN CHALIB, K.S.A) and polishing it with diamond paste (15 m Diamond Paste, Struers) [19]. The EDX samples were taken without further grinding or polishing in order to protect the microstructure [20]. All samples were stored in deionized water at 4C before lesion characterizations were carried out [21].

Demineralization procedure

Artificial white spot lesions were created on the enamel slab samples by subjecting the samples to an acidifying gel system for 7 days. The gel system composed of 8 wt.% carboxymethylcellulose gel and 0.1 M lactic acid (pH 4.5) [21].

Sample grouping

This study has four different groups; every specimen was taken from the same tooth was assigned to one of the four distinct groups. (n = 18/group):

1. Control group: enamel slabs with WSLs were placed in the pH cycle using DDW without treatment.

2. Nano-HAp group: enamel slabs with WSLs were placed in the pH cycle using Apagard remineralizing paste (Sangi, Tokyo, Japan).

3. CPP-ACP group: enamel slabs with WSLs were placed in the pH cycle using tooth mousse cream (GC corporation, japan)

4. BAG group: enamel slabs with WSLs were placed in the pH cycle using Biomin restore toothpaste (Dr Collins, USA).

Treatment agents' application

Because artificial saliva was utilized as a remineralizing solution, a freshly created slurry of each paste was mixed with it. Using a magnetic stirrer, a dilution of one part paste (9 g) to three parts remineralizing solution (27 mL) was completely mixed for 4 minutes in a beaker; 4.0 mL of dilution for each tooth was utilized. The samples were placed in the pH-cycling system as shown in table 1 for 20 days,. The remineralization solutions (artificial saliva) contained 1.5 mM $CaCl_2$, 0.9 mM KH_2PO_4, 130 mM KCl, 3.1mM NaN_3 and 20 mM HEPES pH 7.0, and the demineralization solution 1.5 mM $CaCl_2$, 0.9 mM KH_2PO_4, and 50 mM acetic acid adjusted to pH 5.0 [22].Each specimen's microhardness was determined using a Vickers microhardness testing machine at the baseline (sound enamel), after artificial WSL formation and after remineralization.A 500-g load was applied to the tooth surface using a Vickers diamond indenter for 30 seconds, creating a diagonal on the surface.

EDX analysis was performed using the EDX detector (TESCAN MIRA 3, France) operating at 15 kV accelerating voltage to measure the weight percentage of Ca and P at the surface of the enamel at the baseline, then after demineralization, finally after remineralization period for each sample/group [23].

Table 1: *Treatment schedule for the pH cycling phase [24].*

The following treatment schedule is not given on the first day rather the specimens are stored in saliva with constant gentle rotation to allow an artificial pellicle-like layer to form

Time	PH cycling phase
2 min (8:00 – 8:02 a.m.)	Test / Placebo treatment *
1 h (8:02 – 9:00 a.m.)	Artificial saliva (remineralization solution)
2 min (9:00 – 9:02 a.m.)	Test / Placebo treatment *
1 h (9:02 – 10:00 a.m.)	Artificial saliva
4 h (10:00 a.m. – 2:00 p.m.)	Acid challenge (demineralization)
1 h (2:00 – 3:00 p.m.)	Artificial saliva
2 min (3:00 – 3:02 p.m.)	Test / Placebo treatment *
1 h (3:02 – 4:00 p.m.)	Artificial saliva
2 min (4:00 – 4:02 p.m.)	Test / Placebo treatment *
Overnight (4:02 p.m. – 8:00 a.m.)	Artificial saliva

* = Nano-HAP, CPP-ACP, BAG or DDW

Statistical analysis

The recorded data was tested by Shapiro-Wilk test at p>0.05 which revealed that surface MH, Ca and P are normally distributed among groups. The statistical analysis was performed using Statistical Package for Social Science (SPSS version -22, Chicago, Illinois, USA), for MH General Linear model (Repeated Measure Factorial Analysis Of Variance) and Bonferroni posthoc test for comparison between the group were used. For EDX analysis, One Way Analysis Of Variance (ANOVA) and Tukey Honestly Significant Difference (Tukey's HSD) were used. Level of significance as: Not significant P>0.05, Significant P<0.05.

Results

The mean and standard deviation (SD) for MH and EDX analysis were shown in table 2 and 3. There was no statistical significant difference found in MH between samples at the baseline measurement (p=0.067). Surface MH is decreased from baseline to demineralization phase for each group, then it increased again from demineralization to remineralization phase. The mean of MH for control group was decrease from baseline (257.799 kg/ mm^2) to demineralization (51.967 kg/ mm^2) then increase at the remineralization phase (52.299 kg/ mm2). for Nano-HAP the mean value at baseline was (274.953 kg/ mm2) that decrease at demineralization phase (53.700 kg/ mm2) then increase after remineralization (157.699 kg/ mm2). The mean of CPP-ACP group at baseline (259.596 kg/ mm2) decrease to (55.929 kg/ mm2) at demineralization then increase at remineralization phase (139.078 kg/ mm2). for BAG the mean value at baseline was (272.677 kg/ mm2) that decrease at demineralization phase (55.922 kg/ mm2) then increase after remineralization (147.769 kg/ mm2).

Results of EDX showed that greater mean of Ca and P at remineralization phase found in the Nano–HAP (Ca=50.108, P=24.840) followed by the BAG (Ca=47.408, P=22.285) and CPP-ACP (Ca=43.485, P=19.945) and the lowest in the control (Ca=35.291, P=17.228) and WSL groups (Ca=34.541, P=16.481).

Multiple pairwise comparisons of MH and Ca, P% between phases by groups using Bonferoni post hoc test and Tukey's HSD showed that when compare each phase with other phase in each group there was significant increasing difference ($p<0.05$) from demineralization to remineralization phase in all groups except for the control group there was a non significant difference between the demineralization and re-mineralization phases.

Table 2: Descriptive test of Surface microhardness in (kg / mm2) at the three time periods for each remineralizing agent

Groups		Baseline	Demin.	Remin.
Control	Mean	257.799	51.967	52.299
	±SD	15.602	2.049	9.858
Nano-HAP	Mean	274.953	53.700	157.699
	±SD	20.114	5.981	3.874
CPP-ACP	Mean	259.596	55.929	139.078
	±SD	10.696	8.633	6.803
BAG	Mean	272.677	55.922	147.769
	±SD	13.166	6.728	6.724

Table 3: Descriptive test of Ca% and P% amongst the groups

		Mean	±SD
Ca%	Baseline	53.483	2.355
	WSL	34.541	1.197
	Control	35.291	2.003
	Nano-HAP	50.108	0.730
	CPP-ACP	43.485	0.854
	BAG	47.408	2.172
P%	Baseline	27.111	5.150
	WSL	16.481	0.960
	Control	17.228	0.676
	Nano-HAP	24.840	0.645
	CPP-ACP	19.945	0.928
	BAG	22.285	0.432

Discussion

The early enamel lesion appears white because the enamel has lost its usual translucency. Even while first enamel lesions have intact surfaces, they have a reduced mineral content at the surface layer compared to healthy enamel, resulting in a lower hardness rating (25, 26). Remineralization is a novel noninvasive management of early carious lesions (27).

To overcome the restricted bioavailability of calcium and phosphate ions for the remineralization process, many calcium phosphate-based remineralization systems are now commercially available (28). In the current study, three types of remineralizing agents were used and incorporated into toothpastes, namely Nano-HAP paste, CPP-ACP and BAG.

The study results show that the usage of Nano-HAP treatment improve the minerals content and restore the MH of decalcified WSLs. Also there is significant difference in MH and surface minerals values of the WSLs treated with the three different treatment modalities, accordingly, the null hypotheses were rejected.

enamel slabs were produced from each tooth, this enabled every tooth to be as its own control, reducing effects of the confounders that are associated with inherent differences in individual teeth composition and structure (29). In EDX analysis each tooth has been sectioned into six enamel slabs, to avoid the destructive effect of the device (the samples must be dried and coated with carbon or metal to decrease charging effects, and that SEM typically operates in a high vacuum environment).

Artificial white spot lesion are created by using carboxymethylcellulose gel and lactic acid to produce exact surface topography similar to the natural WSLs which is characterized by intact external surface with subsurface demineralization (30). Compared to individual demineralization and remineralization experiments, the pH-cycling model provided a more accurate simulation of the caries process and was closer to the oral environment (31).In the present research, Vickers hardness method was used to check MH because it was simple, Very reliable, Rapid, Economical as compared to other hardness tests (32). Modern prospective caries study requires the measurement of small changes in a tooth's mineral content. Hence, the EDX analysis is utilized (33).

Initial MH measurements in this investigation yielded values (257.79 – 274.95), which satisfies the VHN range of normal enamel tissue (250-350) (34). The results displayed that the MH and mineral content of demineralized enamel surfaces in all groups significantly decreased when compared to baseline MH, implying loss of minerals.

Analysis of the data revealed that all three treatments significantly increased enamel MH and mineral content Ca, P have been gained when compared to control group. The comparison between study groups in enamel MH and Ca, P weight % after remineralization showed that there was significant difference in values between groups of Nano-HAP, BAG and CPP-ACP.

Group treated with Nano-HAP showed highest enamel MH than other groups followed by the BAG group then CPP-ACP, however the lowermost mean of surface MH was found in the control group. These results supported by a similar study conducted by Geeta et al. (35) who compared Nano-HAP crystals, BAG, CPP-

ACP, and fluoride and showed that the surface MH value for Nano-HAP was significantly higher than the other groups. The intrinsic properties of Nano-HAP, such as size, chemical composition, and structural resemblance to enamel apatite, may play a key role in the remineralization process, as suggested by our study (36). According to a study done by Hemalatha et al. (37) Nano-HAP had the highest capacity for remineralization, followed by CPP-ACP; these findings were consistent with those of the current investigation.

AL-Dahan (38) compared the effects of Nano-HAP and CPP-ACP in preventing mineral loss from teeth after exposure to an acidic beverage and found that Nano-HAP has a more effective preventive effect than CPP-ACP.

This results disagree with Suryani et al (39) who reported that BAG and CPP-ACPF showed better remineralizing potential than Nano-HAP. This outcome might be due to the difference in experimental design and treatment regimens. The remineralizing materials that used in their study contained fluoride, also the pH cycle time was for 7 days and this will reduce the remineralizing effect of Nano-HAP. The period of pH cycling simulation also influence the amount of calcium and phosphate ions released from Nano-HAP, since the solubility of Nano-HAP was increased in acidic conditions. Increased free ions had positive effects on the remineralization of dental caries in both lesion depth and mineral volume, which ultimately may cause greater remineralization rather than demineralization (19).

When comparing BAG group to the group of CPP-ACP it was significantly higher, this comes in agreement with a previous in vitro study that compare various remineralizing agents including BAG and CPP-ACP, and reported that BAG is a more effective agent in the treatment of initial caries than CPP-ACP (40).This could be attributed to The bioactivity of bioglasses that results from their reactivity with tissue fluids and the subsequent production of a hydroxycarbonate apatite (HCA) layer on the glass surface (41).

On the other hand, the lower CPP-ACP MH values when compering it to other treatment groups could be attributed to its amorphous nature; which doesn’t adhere to the surface of the enamel, in contrast to BAG that attached to the tooth, therefore, it doesn't re-mineralize the surface of the tooth for longer time period to enhance its hardness (42). CPP-ACP showed significantly higher mean MH value than the demineralized enamel, The result was in agreement with the other earlier studies on CPP- ACP presenting their remineralization efficacy on artificial enamel carious lesions (43, 44). This could be clarified by the ability of Casien phosphopeptide to act as reservoir of bio-available calcium and phosphate, by the localization of amorphous calcium phosphate on the tooth surface, which buffers the free calcium and phosphate ion activities, the state of supersaturation with regard to the tooth enamel is maintained and remineralization is enhanced (45).

Based on the findings of the current study, none of the mentioned agents were able to increase the MH and EDX values to that of the sound enamel, Nano-HAP which showed better results compared to the others is recommended to be used as teeth remineralizing agent in treatment of white spot lesions.

Conclusion

Within the scope of this investigation, this study found that all tested agents may enhance the surface MH and restore minerals content of WSLs. Nano-HAP has sufficient potential for re-mineralizing the initial enamel caries lesions under the dynamic pH-cycling conditions, which are shown by maximum value of the average hardness and restoring mineral content that is followed by the BAG and CPP-ACP respectively.

Conflict of interest: The authors have no conflicts of interest to declare.

Author contributions

RHJ; study conception and design. SSR; data collection. RHJ ; Methodology. RHJ and SSR; statistical analysis and interpretation of results. SSR; original draft manuscript preparation. RHJ and SSR; Writing - article & editing. Supervision; RHJ. All authors reviewed the results and approved the final version of the manuscript to be published.

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تأثير استخدام ثلاث معاجين مختلفة لإعادة التمعدن على البقع البيضاء الاصطناعية

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المستخلص:

الخلفية: يشار إلى المرحلة المبكرة من تسوس الأسنان على أنها آفات أولية ، وإذا لم يتم إيقاف العملية في هذه المرحلة ، فقد تنتقل الآفات من التنقية إلى آفات غير مجوفة ثم إلى آفات مجوفة. عند استخدام مواد إعادة التمعدن للآفات النخرية المبكرة ، يمكن إيقاف العملية أو عكسها. هدفت هذه الدراسة المختبرية إلى مقارنة الصلادة السطحية (**MH**) والمحتوى المعدني لآفات البقع البيضاء (**WSLs**) بعد استخدام الزجاج النشط بيولوجيًا (**BAG**) ، وكازين فوسفو ببتيد-فوسفات الكالسيوم غير المتبلور (**CPP-ACP**) ، ونانو هيدروكسي اباتيت (**Nano-HAP**) تحت ظروف دورة درجة الحموضة.

المواد وطرق العمل: في هذه الدراسة ، تم استخدام 18 من أسنان الضواحك العلوية الاولى، وتم اختيار 10 أسنان لاختبار الصلادة، ولاختبار التحليل الطيفي للأشعة السينية المشتتة للطاقة ، تم استخدام الاسنان الثمانية المتبقية ، وتم إنتاج 40 قطعة من كتل المينا من الجزء الأوسط من الأسطح الشدقية والحنكية للاسنان. و48 قطعة من كتل المينا للتحليل الطيفي للأشعة السينية المشتتة للطاقة . تم تقسيم قطع المينا إلى أربع مجموعات: المجموعة المقارنة المحفوظة في المياه منزوعة الأيونات ، مجموعة النانو هيدروكسي اباتيت ، مجموعة الكازين فوسفو ببتيد - فوسفات الكالسيوم

غير المتبلور ومجموعة الزجاج النشط بيولوجيًا ، ثم تم نزع المعادن منها باستخدام 0.1 مولار من حمض اللاكتيك و 8٪ بالوزن كاربوكسيميثيل سلولوز جل لعمل بقع بيضاء صناعية. تعرضت العينات لدورة درجة الحموضة لمدة 20 يومًا. تمت دراسة إمكانية إعادة التمعدن للعينات من خلال اجراء فحص الصلادة والمحتوى المعدني لسطح العينات في مراحل مختلفة: قبل بدء التجربة ، بعد إنتاج البقع البيضاء و بعد المعالجة بمواد مختلفة. وقد تم تحليل البيانات التي تم جمعها إحصائيا بأستخدام **one way ANOVA** واختبار **Bonferroni**.

النتائج: أظهرت نتائج هذه الدراسة أن **Nano-HAP** كان لها أعلى متوسط قيم لـاختبار الصلادة (**157.699**) كجم / مم 2 و كاليسيوم (**50.108**) وفسفور (**24.840**) مقارنة بمجموعات المعالجات الأخرى. متبوعًا بـ **BAG** (**MH = 147.769**) كجم / مم 2 ، **Ca = 47.408**، **P = 22.285**) ، وتم العثور على أدنى قيمة متوسطة في المجموعة المقارنة (**MH = 52.299** كجم / مم 2 ، **Ca = 35.291**، **P = 17.228**). أظهر اختبار **Bonferroni** و **Tukey** فرقًا معنويًا متزايدا (**$p < 0.05$**) من مرحلة البقع البيضاء إلى مرحلة إعادة التمعدن في جميع المجموعات ، باستثناء عندما أظهرت مجموعة التحكم مقارنة مع **WSL** (التنقية) فرقًا غير مهم (**$P > 0.05$**).

الاستنتاج: جميع المواد المختبرة لها قدرة كبيرة على إعادة التمعدن. يمتلك النانوهيدروكسي اباتيت أعلى إمكانية لإعادة تمعدن آفات تسوس المينا الأولية.