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REMINERALIZATION ASSESSMENT OF EARLY ENAMEL CARIOUS -LIKE LESION AFTER TREATMENT WITH NEW NANO SILVER TOOTH PASTE (AN-VITRO STUDY)

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ABSTRACT

Objective: The objective of this study is to evaluate and comparing the effect of Coral Nano Silver, Biomin, and Novamin kinds of toothpaste on remineralization of demineralized enamel considering the time factor. **Materials and methods:** one hundred and ten enamel slabs were prepared from the buccal and palatal enamel surface of the human maxillary premolar teeth. The prepared enamel samples were randomly divided into five main groups according to the type of remineralizing toothpaste. Each main treated group was further subdivided into 3 equal subgroups (n=10) according to the remineralization periods (7, 14, and 28 days). Data were statistically analyzed using SPSS® statistics version 20, F-test, (ANOVA) was used for normally distributed quantitative variables, to compare between more than two groups, all tests are two- tailed, and the level of significance was set $P<0.05$. **Results:** After remineralization, all samples showed a decrease in surface roughness and an increase in surface microhardness, which was significantly higher with coral calcium Nano silver toothpaste. **Conclusion:** Coral calcium Nanosilver toothpaste is a promising remineralizing agent by reducing enamel surface roughness and increasing surface microhardness.

KEYWORDS: Surface roughness, Coral calcium, silver nanoparticles, Vickers microhardness test.

INTRODUCTION

Dental caries pathophysiology is not simply a continual cumulative loss of tooth minerals, but rather a dynamic process characterized by alternating periods of demineralization and remineralization[1].Minimally invasive dentistry necessitates the need for remineralizing early enamel carious lesions aiming to increase surface hardness and decrease surface roughness of defect area, there are various caries management strategies currently used, yet the cornerstone strategy is fluoride-mediated remineralization. However, fluoride oral care products carry some risks, such as fluorosis; thus, non-fluoride remineralizing approaches with innovative technologies, including regenerative and physiochemical mechanisms, are under development to help control caries [2,3].

Novel bioactive glass materials have been introduced that claim to dissolve more quickly in water under acidic conditions, thus raising pH levels faster, releasing calcium, phosphate, and fluoride more readily protecting by forming hydroxyapatite mimicking the tooth structure [4,5].

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Novamin (calcium sodium phospho-silicate) was the first bioactive glass to be included in toothpaste helping to promote bone regeneration [6], which is now being included in toothpastes to remineralize teeth. This is done by forming a crystalline hydroxyapatite layer for demineralized area of teeth, with the two main components being calcium and phosphorus for protection. This layer also acts as a reservoir of minerals to aid in remineralization. Soluble fluoride is also provided as an external component in the toothpaste [7, 8].

Biomin (fluoro-calcium phospho-silicate) was developed later after observing various bioactive glass formulations to further improve their characteristics. The formulation incorporates smaller particles for better penetration and lower abrasion, as well as the inclusion of fluoride in the bioactive glass chain itself, providing a more prolonged release of fluoride at lower levels compared to soluble fluoride[9]. With the development of nanotechnology, a major impact on materials science has been noted. In this century, the production of materials with nanostructures has gained significant attention for adsorption, catalytic, biomaterial, and optical application. [10] . Recently, Coral calcium is a new biomimetic agent, which has been the most common form of calcium on the planet [11]. A study showed the ability of coral calcium to enhance calcium deposition in bone and help to heal bone fractures. It has also been launched in the market as a dietary supplement to prevent osteoporosis [12]. Surface roughness is considered a predisposing factor for bacterial adhesion, and extrinsic stain cause plaque retention, which increases the demineralization rate and possibly results in many oral diseases [13]. A relation of enamel hardness values with the mineral content of the tissue is on a weight basis, which also accounts for the variation in enamel structure, and the correlation between initial enamel hardness and abrasion degree. [14]. However, the effect of coral calcium Nano silver topical application on enamel and its remineralizing capability on teeth is not yet adequately studied. Therefore, it is worthwhile to evaluate the remineralization effect of Coral Nano silver and compare it to Novamin and Biomin via assessment of surface roughness and microhardness of treated early carious lesions.

MATERIALS AND METHODS

Three different kinds of remineralizing toothpaste were selected.

1. Novamin toothpaste (Sensodyne repair and protect). (GlaxoSmithKlineCompany, England)
2. Biomin F toothpaste. (Biomin Technologies Limited Company,England)
3. Coral Nano Silver toothpaste.(Coral Limited Liability Company, Carson City USA)

Sample grouping: 110 enamel slabs were prepared from the buccal and palatal enamel surface (55 buccal and 55 palatal) of intact, free of defects in human maxillary premolar teeth.

TABLE (1) Sample grouping according to the remineralization period.

Remineralizing toothpaste	Period			No. of samples
	7 days	14 days	28 days	
Biomin F (C)	C1	C2	C3	30
Novamin (D)	D1	D2	D3	30
Coral (E)	E1	E2	E3	30
No. of samples	30	30	30	Grand total = 90

The selected teeth were cleaned to remove debris, calculus, and soft tissues, Then the teeth were polished with pumice and a rubber cap After that, the teeth were stored and disinfected in a 0.5% Chloramine T solution for one week [15,16], and Then the selected teeth were stored in a plastic bottle of 10% formalin solution at room temperature until use [15].

Sample preparation: [15]

Enamel samples were prepared from the buccal and lingual surfaces of the selected maxillary premolar teeth, by cutting the coronal portion mesiodistally, with a slow-speed diamond disc under copious water spray. Crowns were vertically sectioned mesiodistally, into two halves. Then, self-cured acrylic resin was poured into specially constructed plastic cylinder moulds. The buccal and palatal surface of each half was exposed, facing upward, and parallel to the horizontal axis when it was implanted in the resin, each half of the crown was embedded facing upward. Using abrasive paper with successive grits of 800, 1000, and 1200, the buccal surface was flattened and polished. Then, a piece of adhesive tape was put on the center of the buccal or palatal smooth surface, which was used to create a 3 mm x 3 mm window of exposed enamel in the center of the sample's surface, and a uniform coat of acid-resistant nail varnish was applied all around it. After the samples had sufficiently dried, an explorer removed the adhesive tape from the tooth surface, revealing a square area on the enamel surface.

Preparation of demineralizing solution:

The demineralization solution was prepared using analytical grade chemicals and deionized water as follows: 2.2 mm calcium chloride, 2.2 mm potassium phosphate, and 0.05 M acetic acid. The pH was adjusted to 4.4 using of 1 M sodium hydroxide (NaOH) [15].

Artificial demineralization process:

A total of 100 enamel samples were utilized during demineralization. The enamel samples in each group (group B - group E) were individually immersed in the demineralizing solution in glass bottles for 96 hours to produce artificial carious-like lesions (demineralization) in the enamel [15]. The glass bottles of demineralized samples that were subjected to remineralization with different kinds of remineralizing toothpaste (group C- group E) were randomly divided into nine glasses according to the remineralizing toothpaste (Biomin F, Novamin, and Coral) and time of observation (7,14, and 28 days), where each glass was labeled with different symbols.The enamel samples for groups "C-E" were stored individually in 25 ml glass bottles containing 10 ml of demineralizing solution for a period of 96 hours to produce artificial demineralization. This was done to create artificial carious lesions of approximate range between 200 to 250-micrometer depths [16], the enamel samples were removed from the demineralization solution, and was cleaned from the demineralizing medium by deionized water

Preparation of the artificial saliva: [15,16]

The solution was prepared at the Faculty of Pharmacy (Cairo, Boys), Al-Azhar University. It is similar to natural saliva in its inorganic composition as follows: A mixture of 2.20 g/L gastric mucin, 1.45 mmo1/L $CaCl_2 2H_2O$, 5.42 mmol/L KH_2PO_4, 6.50 mmol/L NaCl, 14.94 mmol/L KCl., The pH was adjusted to 7.0 using potassium hydroxide (KOH). During the study, the artificial saliva was kept in the refrigerator to avoid bacteria and fungi accumulation.

Application of remineralizing toothpaste: [15]

Artificial demineralized enamel specimens were then treated with respective remineralizing agents every 24 hours for 7,14 and 28 days. The specimens were rubbed with the different kinds of the remineralizing toothpaste, used undiluted (0.03g) twice daily with the help of a polishing cup attached to a low-speed contra-angle handpiece for 2 minute, then washed with deionized water for 1 minute, and then stored in 10 ml of artificial saliva which was renewed every 24 h just before immersion of freshly treated specimens.

Surface roughness testing using SJ-210 Surface roughness tester (Mitutoyo Japan) Each specimen is fitted to the specimen holder in which the surface is to be measured in the horizontal direction, then the specimen holder moves in a vertical direction up to the specimen surface just to touch the measuring tip. The measuring distance was 3 mm, Stylus with tip radius 2-micron, with a tip angle 60 degrees. The cut–off value was adjusted to act at 0.25μm and surface roughness was characterized by the arithmetical average of surface peak and valley heights found with a central line along the area assessed (Ra), in micrometers (μm). Five readings were recorded for each specimen at distance of 500 microns each.

Surface hardness testing using Tukon 1102 Wilson digital microhardness tester (Buehler Germany) with a magnification camera of 40X. Three indentations were performed on each specimen; the average was calculated to be the value of the Vickers surface hardness of the specimen, (50g) load is applied smoothly, without impact, forcing the indenter into the test specimen and, the indenter is held in place for (10) seconds. After the load is removed, the indentation is focused with the magnifying eyepiece and the two impression diagonals are measured, usually to the nearest 0.1µm with a micrometer, and then averaged. The Vickers hardness (HV) is calculated using: $HV = 1854.4L/d^2$

RESULTS

1. Changes in surface roughness of the tested materials with regard to time:

Means and standard deviation values (mean± SD) of surface roughness for the enamel samples in the different tested groups that were treated with the different kinds of toothpaste at different follow-up periods are symbolized in micrometer and shown in (Table 2), For each tested toothpaste in this present study, the statistical results displayed that there was a statistically significant difference (P<0.00001) between the surface roughness at different follow-up periods. Among the tested teeth samples that were treated with the Biomin toothpaste, the lower mean surface roughness was recorded in the teeth samples after 28 days (2.104±0.12μm), followed by the teeth samples after 14 days (2.561±0.10μm), and the higher surface roughness was recorded in the teeth samples after 7 days (3.245±0.14μm). Among the tested teeth samples that were treated with the Novamin toothpaste, the lower mean surface roughness was recorded in the teeth samples after 28 days (2.160±0.12μm), followed by the teeth samples after 14 days (2.593±0.09μm), and the higher surface roughness was recorded in the teeth samples after 7 days (3.271±0.14μm). Among the tested teeth samples that were treated with the Coral toothpaste, the lower mean surface roughness was recorded in the teeth samples after 28 days (1.626±0.09μm), followed by the teeth samples after 14 days (2.228±0.05μm), and the higher surface roughness was recorded in the teeth samples after 7 days (2.726±0.08μm). Among the groups, the Tukey HSD test revealed that there was a statistically significant difference between the different follow-up periods for all tested kinds of toothpaste (P<0.05), where the recorded means surface roughness decreased significantly with time.

TABLE (2) Means and standard deviation of Change in surface roughness among all tested groups along the study:

Variable	7 days	14 days	28 days	P-value
Biomin	$3.245{\pm}0.14^{A}$	$2.561{\pm}0.10^{B}$	$2.104{\pm}0.12^{C}$	<0.00001*
Sig. among the groups	P1=0.00000*, P2=0.00000*, P3=0.00003*			
Novamin	$3.271{\pm}0.14^{A}$	$2.593{\pm}0.09^{B}$	$2.160{\pm}0.12^{C}$	<0.00001*
Sig. among the groups	P1=0.00000*, P2=0.00000*, P3=0.00005*			
Coral	$2.726{\pm}0.08^{A}$	$2.228{\pm}0.05^{B}$	$1.626{\pm}0.09^{C}$	<0.00001*
Sig. among the groups	P1=0.00000*, P2=0.00000*, P3=0.00000*			

2. Changes in surface microhardness of the tested material with regard to time:

Means and standard deviation values (mean± SD) of surface microhardness for the enamel samples in the different tested groups that were treated with the different kinds of toothpaste at different follow-up periods are symbolized in (kg/mm^2) and displayed in (Table3). For each tested toothpaste in this present study, the statistical results displayed that there was a statistically significant difference ($P<0.00001$) between the surface microhardness at different follow-up periods. Among the tested teeth samples that were treated with the Biomin toothpaste, the higher mean surface microhardness was recorded in the enamel samples after 28 days (256.62±4.96 kg/mm^2), followed by the enamel samples after 14 days (225.6±4.61 kg/mm^2), and the lower surface microhardness was recorded in the enamel samples after 7 days (201.67±2.01 kg/mm^2).Among the tested enamel samples that were treated with the Novamin toothpaste, the higher mean surface microhardness was recorded in the enamel samples after 28 days (254.82±3.05 $kgmm^2$), followed by the enamel samples after 14 days (225.45±3.97 kg/mm^2), and the lower surface microhardness was recorded in the enamel samples after 7 days (197.05±3.60 kg/mm^2).

Among the enamel teeth samples that were treated with the Coral Nano silver toothpaste, the higher mean surface microhardness was recorded in the enamel samples after 28 days (271.43±4.88kg/mm^2), followed by the enamel samples after 14 days (237.28±2.62 kg/mm^2), and the lower surface microhardness was recorded in the enamel samples after 7 days (209.2±1.47kg/mm^2). Among the groups, the Tukey HSD test revealed that there was a statistically significant difference in the surface microhardness between the different follow-up periods for all tested kinds of toothpaste ($P<0.05$), where the recorded means surface microhardness decreased significantly with time for the enamel samples that were treated with different kinds of toothpaste.

TABLE (3) Means and standard deviation of surface microhardness among all tested groups along the study:

Variable	7 days	14 days	28 days	P-value
Biomin	201.67 ± 2.01^{C}	225.6 ± 4.61^{B}	256.62 ± 4.96^{A}	<0.00001*
Sig. among the groups	P1=0.00000*, P2=0.00000*, P3=0.00000*			
Novamin	197.05 ± 3.60^{C}	225.45 ± 3.97^{B}	254.82 ± 3.05^{A}	<0.00001*
Sig. among the groups	P1=0.00000*, P2=0.00000*, P3=0.00000*			
Coral	209.2 ± 1.47^{C}	237.28 ± 2.62^{B}	271.43 ± 4.88^{A}	<0.00001*
Sig. among the groups	P1=0.00000*, P2=0.00000*, P3=0.00000*			

DISCUSSION

Dental caries in its early stages (non-cavitated) can be remineralized, it is known that the caries process can be slowed down, stopped, or even reversed by the remineralization process via the restoration of lost minerals in hard dental tissues(17,18). Global research is being done on toothpaste types that can promote optimal remineralization, to avoid caries progression subsequently and reduce the need to remove healthy tooth structures during restorative dentistry procedures(19). Due to its cariostatic capability, fluoride has been identified as the primary factor in the decrease in dental caries(15). Despite having a significant impact in stopping the advancement of caries, it has some drawbacks. Fluoride does not completely help to prevent tooth decay. Furthermore, the tooth may suffer from a rise in fluoride concentration which may cause enamel fluorosis (15,20). Due to potential negative dental health implications, the fluoride-free dentifrice was not used as “a negative control” in this study; however, each toothpaste group functioned as its own control in this study. Furthermore, fluoride retention and net remineralization may be constrained by the availability of calcium and phosphate ions (15).

Many people who have diminished calcium, phosphate, and fluoride ions due to decreased salivary flow brought on by aging, certain medications, Sjogren's syndrome, diabetes, and radiation therapy may find this type of treatment to be helpful [18]. Additionally, women are more likely to develop caries due to low amounts of salivary calcium during several life stages[21].

In this study, three different kinds of toothpaste (Coral Nano silver, BiominF, and Novamin) based on bioactive glasses containing calcium phosphate were chosen to test their remineralization ability where the manufacturer claims that the glass particles release calcium and phosphate ions intra-orally to promote remineralization[19,22]. This is because the essential components (calcium and phosphate) needed by the oral environment for tooth remineralization can be supplied via bioactive glass[19]. In this present study, BiominF toothpaste was chosen as the tested remineralizing agent because according to previous studies it has the potential to remineralize the enamel with a significant increase in calcium and phosphate content compared to Novamin [19,23]. Moreover, it was reported that the efficacy of using calcium-fluoride-containing bioactive glasses (i.e. BiominF) in treating dental lesions, may enhance the formation of fluorapatite, which is expected to exhibit acid resistance to acidic cariogenic attacks[24]. The most prevalent kind of calcium on earth is coral calcium, which comes from fossilized Coral calcium sources. A study demonstrated Coral calcium's capacity to promote calcium deposition in bone and aid in the healing of bone fractures. In order to prevent osteoporosis, it has also been introduced to the market as a nutritional supplement[11]. However, Coral calcium topical application's impact on enamel and its potential to remineralize teeth have not yet been sufficiently researched. Therefore, the current study's objective is to contrast Coral Calcium's remineralization impact with that of two distinct toothpaste types that contain bioactive glass (BiominF and Novamin).

Enamel was selected as a study specimen because enamel lacks cellular repair mechanisms, and the events surrounding the development and reversal of caries are dependent upon physiochemical mechanisms at the tooth-pellicle interface [25]. Instead of employing carious teeth with varying amounts of demineralization, teeth were soaked in a standardized demineralizing solution to simulate the demineralized stage that could typically occur in the structure of the tooth and establish a baseline for the various specimens[18]. Consequently, a 4.4 intermediate pH solution was applied for 96 hours[15,18].

The remineralization periods were extended to 28 days, and the approximate lesion depth of the demineralized subsurface lesion induced in the current study exceeded 200 microns. This is because it was reported that subsurface enamel lesions of about 100 microns needed approximately 28 days to re-mineralize in artificial saliva, without being exposed to any demineralization challenges [23]. Artificial saliva used in this study has the potential to remineralize initial enamel lesions, which goes in accordance with the study done by Haung et al. [26].

Therefore, artificial saliva was selected as the storage medium in order to simulate the clinical condition. Moreover, it was found that during the remineralization process the availability of ionic calcium and phosphate from saliva is highly variable and depends on many factors like- basic salivary mineral contents, salivary pH, and buffering capacity of saliva [25]. In the present study, the pH of saliva was adjusted to 7.0 to simulate normal clinical conditions. If the acidogenic pH of the biofilm is buffered, the demineralization of crystals can be stopped. Due to its buffering ability and slightly alkaline pH at the tooth surface, normal healthy saliva plays a significant role in aiding in the restoration of a favorable pH for remineralization [27].

The experimental remineralizing agents were applied topically to the enamel specimens twice a day for a period of two minutes each, to simulate the normal recommended daily oral prophylaxis [15]. Qualitative evaluation for enamel remineralization in this present study was performed by surface roughness. This is because it was found that the demineralized enamel has higher surface roughness due to porosities while the remineralized enamel has a smooth enamel surface due to the ability of the remineralized agent to fill the porosities and produce a homogenous surface [15,28]. Surface roughness measurements were performed using a profilometer to evaluate the zones of porosities of caries-like lesions, which has the advantage of accurate and precise measurement of the surface roughness without the need for additional measurements [29].

In this study, it was found that the use of demineralizing agent resulted in a significant increase in surface roughness of the enamel specimens when compared to the intact enamel. This is because it was found that when the initial enamel lesion has been formed on the surface it shows significantly more porosity than the sound enamel due to the dissolution of the mineral content of the enamel [15,25] these results are in agreement with the results of Khoroushi et al**.** [30] who found that demineralized enamel has higher surface roughness than intact enamel. However, the results of this current study showed that the use of the three different remineralizing kinds of toothpaste resulted in a significant decrease in surface roughness, as well as a significant increase in microhardness of the demineralized enamel specimens. This is because the constituent's Ca^{2+} and $PO4^{3-}$ ions in these kinds of toothpaste were able to penetrate the enamel pores acting as a template in the precipitation process and attracting a large amount of Ca^{2+} and $PO4^{3-}$ from the remineralization solution to the enamel surface, to fill the vacant positions of the enamel calcium crystals [15,31]. However, the results of this study showed that the Coral Nanosilver toothpaste produced a smoother surface with higher surface hardness when compared to BiominF and Novamin. This could be attributed to the ability of Coral calcium to increase the alkalinity of the enamel surface and enhances the remineralization of initial enamel lesions [11]. The ability of the Coral calcium to remineralize the demineralized enamel may be due to the precipitation of calcium ions of calcium carbonate from the Coral toothpaste. Additionally, the high calcium concentration in Coral toothpaste changes the acidic pH to alkaline which favors the precipitation of the minerals[32]. Moreover, the presence of silica promotes the adsorption of the gel to the tooth structure [22]. Qais et al.[33] mentioned that These Smaller Ag-NPs interact with cell membranes and disorganize the lipid bilayer, causing an increase in the membrane permeability and bacterial lysis. Ag-NPs smaller than 30nm demonstrated strong anti-microbial activity against *Staphylococcus aureus* (*S. aureus*) and *Klebseilla pneumonia* (*K. pseumonia*), whereas Ag-NPs with sizes ranging from 5 to 20 nm have a vigorous antimicrobial activity against S. aureus. Additionally, as the demineralized enamel is more porosity than sound enamel, it may encourage more penetration of the gel ion components, also showed a larger surface area allowing better subsequent reaction response of the enamel mineral; these, factors allowed the precipitation of calcium from the Coral calcium gel on the enamel surface, as due to the high concentration of the calcium carbonate present in the coral calcium which leads to deposition of calcium ions by a concentration gradient[22]. This would probably block the surface pores and result in an increased in surface roughness and microhardness. Moreover, the result of this study revealed that the enamel specimens treated with BiominF have lower surface roughness and improved surface hardness when compared to Novamin. That may be due to the high phosphate content of Biomin, as the company claimed that it is three times that of Novamin. High phosphate content glasses form apatite considerably faster (within 6h) than low phosphate content glasses[5]. However, the insignificant difference between BiominF and

Novamin in this present study could be attributed to the existence of fluoride in both Biomin F (CaF_2) and Novamin (NaF), which could negatively affect the supersaturation condition and result thereby negatively affect the apatite deposition.[5,18]. The results of this study were also revealed that the surface roughness of the demineralized enamel specimens in decreased significantly with time for all tested kinds of toothpaste. Furthermore, the results of this study exhibited that the microhardness of the demineralized enamel specimens in all tested kinds of toothpaste increased significantly with time. These results agreed with the results of Balakrishnan et al. [34] who evaluated the remineralization potential of various dentifrices over a period of 30 days and concluded that the extent of remineralization achieved was dose-dependent. These results agreed with the results of Soares et al. [15] who concluded that the remineralizing values of the enamel specimens exhibited statistically significant differences with time. This is because the mineral starts to regain calcium and phosphate from saliva as well as from other topical sources (toothpaste) diffuses into the tooth, and with the help of remineralizing agent, builds on the surface when the pH is above the critical level (alkaline) [11,35].

CONCLUSION

Within the limitations of this in vitro study the following conclusions can be drawn:

1. Coral Nano silver is more effective than both BiominF and Novamin in the remineralization of demineralized enamel.
2. BiominF has reasonable remineralization ability on the demineralized enamel comparable to Novamin.
3. The use of bioactive glass kinds of toothpaste as remineralizing agents shows promising results in the treatment of early carious lesions, the remineralization ability of the bioactive glass kinds of toothpaste is time-dependent.

RECOMMENDATIONS

Based on the results of the present study, it can be recommended that:

1. The use of bioactive glass kinds of toothpaste as remineralizing agents in the treatment of early carious lesions.
2. The use of Coral Nanosilver toothpaste is recommended over BiominF and Novamin in the treatment of early carious lesions.

Further in-vitro and in-vivo investigations were recommended to support the results of this present study.

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