EGYPTIAN
DENTAL JOURNAL
www.eda-egypt.org

VOL. 71, 2885:2898, OCTOBER, 2025

PRINT ISSN 0070-9484 • ONLINE ISSN 2090-2360

ORTHODONTICS, PEDIATRIC AND PREVENTIVE DENTISTRY

Submit Date : 06-08-2025 • Accept Date : 16-08-2025 • Available online: 01-10-2025 • DOI : 10.21608/edj.2025.402882.3579

COMPARATIVE EVALUATION OF ENAMEL SUPERFICIAL MICROSTRUCTURE AND MICROHARDNESS FOLLOWING APPLICATION OF CORAL CALCIUM NANO SILVER MI PASTE PLUS TOOTH PASTE ON MANAGEMENT OF WHITE SPOT LESION (AN IN VITRO STUDY)

Aya Mohammed Obeid[*], Eman Sayed Elmasry[**] *and* Yasmin Mohamed Yousry [**]

ABSTRACT

Aim of the study: To compare remineralizing potential of Coral Calcium Nano Silver toothpaste versus MI paste plus toothpaste on primary molar white spot lesions, evaluating enamel superficial microstructure and microhardness.

Methods: Sixteen extracted human primary molar teeth were randomized into two groups (n=8), mounted in acrylic resin, and a 2×2 mm buccal enamel window was created. Artificial white spot lesions were induced via 72-hour demineralization (pH 4.6). Subsequently, Samples underwent a 14-day remineralization protocol with daily 3-minute agent application and artificial saliva storage. Surface morphology evaluation was done using Scanning Electron Microscopy (SEM) and surface microhardness by a Vickers Microhardness Tester at baseline, post-demineralization, and post-remineralization. Data were analyzed using a two-way mixed model ANOVA with Tukey's post-hoc comparisons ($p<0.05$).

Results: SEM analysis revealed that both groups exhibited favorable morphological alterations on the enamel surface post-remineralization. Microhardness values significantly varied across intervals ($p<0.001$), with post-remineralization being highest and post-demineralization lowest; baseline and remineralization values significantly exceeded demineralization. Baseline and remineralization values were significantly higher than demineralization values. While the Coral group showed numerically higher microhardness, no statistically significant difference was observed between the two materials. Only time demonstrated a significant effect on microhardness, while the effect of material and its interaction with time were not statistically significant.

Conclusion: Both Coral Calcium Nano Silver and MI Paste Plus Toothpaste are equally effective in remineralizing the enamel of the primary teeth. Although, Coral Calcium Nano Silver showed a higher microhardness, no significant differences were observed between the two toothpastes.

KEYWORDS: Enamel; white spot lesion; Remineralization; Surface Morphology; Microhardness

* Master Degree Candidate, Department of Pediatric Dentistry and Dental Public Health, Faculty of Dentistry, Cairo University, Cairo, Egypt
** Professor of Pediatric Dentistry and Dental Public Health, Faculty of Dentistry, Cairo University, Cairo, Egypt

INTRODUCTION

Dental caries is a prevalent disease that can result in demineralization, tooth decay, pain, and functional impairment, affecting both aesthetics and oral health. While caries is often viewed as a one-way progression towards demineralization, it is actually a cyclical process characterized by alternating phases of demineralization and remineralization. However, when demineralization exceeds remineralization, cavitation can occur [(1)].

White spot lesions (WSLs), characterized by white opacity, are caused by subsurface enamel demineralization on smooth tooth surfaces. Studies have shown that WSLs develop due to prolonged plaque accumulation resulting from inadequate oral hygiene. Acids from plaque diffuse into the enamel, leading to subsurface demineralization. Eventually, the intact enamel surface collapses, forming a cavity. These lesions can become visible within four weeks [(2)].

Enamel demineralization is characterized by subsurface mineral loss, driven by the inward migration of acidic ions from plaque and the outward diffusion of mineral ions. Conversely, remineralization, occurring optimally in near-neutral pH environments, involves the redeposition of calcium and phosphate ions from plaque and saliva, promoting the formation of larger, more acid-resistant hydroxyapatite crystals [(3)].

Fluoride is considered to inhibit dental caries through multiple mechanisms, including the suppression of microbial acid production, the inhibition of both intracellular and extracellular enzymatic activity, and the substitution of hydroxide ions in hydroxyapatite with fluoride ions, leading to the formation of fluorapatite crystals that exhibit enhanced resistance to acid dissolution. Despite its efficacy in caries prevention, fluoride may induce undesirable effects such as dental fluorosis and systemic toxicity at elevated concentrations, prompting intensified research into alternative anti-caries agents with reduced adverse effects [(4)].

Innovative bioactive glass materials have been developed with enhanced solubility in acidic environments. These materials lead to a more rapid increase in pH levels and accelerate the release of calcium, phosphate, and fluoride ions. This process promotes the formation of hydroxyapatite, a mineral closely resembling natural tooth structure [(5,6)].

MI plus Paste, a popular remineralization agent, has gained widespread use for treating WSLs. The active ingredient, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), is thought to stabilize and localize calcium, fluoride, and phosphate on the tooth surface. This action facilitates deeper remineralization of white spot lesions (WSLs). The remineralization potential of CPP-ACP has been substantiated by numerous in vitro and in situ investigations. Recent research further indicates a synergistic remineralization effect when CPP-ACP is combined with fluoride. A newer product, MI Paste Plus, which contains 900 ppm fluoride and casein phosphopeptide-amorphous calcium phosphate, is now commercially available [(7)].

Nanotechnology has significantly impacted materials science, particularly in the production of nanostructured materials. These materials have garnered considerable attention for their applications in adsorption, catalysis, biomaterials, and optics. Coral calcium, a recently discovered biomimetic agent, is the most abundant form of calcium on Earth. Research has shown its effectiveness in stimulating calcium deposition in bone and facilitating bone fracture healing. Additionally, coral calcium has gained popularity as a dietary supplement for preventing osteoporosis [(8)].

Enamel and dentin demineralization and remineralization are commonly assessed through various methods, including microradiography, iodine absorptiometry, polarized light, iodide permeability, light-scattering, and wet chemical analysis. In the present study, the Vickers hardness number (VHN) test was employed to measure the microhardness of the tooth surface. This method creates a pyramid-shaped indentation, enabling precise visual and digital measurements [6].

Given the limited research on the efficacy of fluoride-free coral calcium nano-silver toothpaste, this study aimed to compare its remineralization potential with fluoride-containing MI Paste Plus toothpaste in managing white spot lesions on primary molars. The study evaluated the enamel's superficial microstructure using scanning electron microscopy (SEM) and measured surface microhardness as an indicator of enamel demineralization and remineralization.

AIM OF THE STUDY

The aim of this in vitro study was **"**To compare remineralizing potential of Coral Calcium Nano Silver toothpaste versus MI paste plus toothpaste on primary molar white spot lesions, evaluating enamel superficial microstructure and microhardness."

MATERIALS AND METHODS

The protocol for this study adhered to established ethical standards and received formal approval from the Research Ethics Committee, Faculty of Dentistry, Cairo University (Approval No. 29-5-24).

Sample size calculation

A power analysis was performed to ensure adequate statistical power for a two-sided test of the null hypothesis, which proposed no difference between the experimental groups. An alpha level of 0.05 and a beta of 0.10 (resulting in a power of 90%) were adopted. The effect size (d) of 1.89 was calculated from the findings of **Abdelnabi et al. (2019)**[9]. The predicted sample size was determined to be 8 samples per group, yielding a total sample size of 16. Sample size calculations were performed using G*Power version 3.1.9.7 [10].

Sample Collection

A total of sixteen freshly extracted human primary molar teeth, obtained from normal exfoliation, were collected from the Department of Pediatric Dentistry and Dental Public Health at the Faculty of Dentistry, Cairo University. Teeth were meticulously cleaned with running tap water to remove organic debris. Subsequently, each tooth was examined under a stereomicroscope (Olympus SZ1145, Olympus Optical Co. LTD, Tokyo, Japan) at 40× magnification to confirm the absence of enamel decay lesions and cracks [11]. Only primary molars with sound buccal tooth surfaces free from cracks were included in the study [12].

Randomization of these sixteen teeth was carried out by the main investigator utilizing www.random.org. The samples were then randomly allocated into two equal groups, according to the remineralizing agent: Group I, teeth treated with Coral Calcium Nano Silver toothpaste (Coral Limited Liability Company, Carson City USA) (n=8); and Group II, teeth treated with MI Paste Plus (GC corporation, CO Tokyo, Japan) (n=8).

Samples preparation

The selected teeth underwent prophylactic cleaning and polishing using a polishing paste (Acro polish, Acrostone dental factory, Egypt). Teeth were initially disinfected with a 0.02% thymol solution for one week. Subsequently, they were stored in artificial saliva, which was renewed daily, at room temperature until their use, with a maximum storage period of one month [13,14].

The artificial saliva was prepared at the Faculty of Pharmacy, Cairo University. To prevent microbial contamination, the artificial saliva was refrigerated throughout the duration of the study [15].

The coronal sections of the teeth were separated from their roots at the dentino-enamel junction. This was achieved using a diamond disc (KG Sorensen, 7015, Barueri-SP, Brasil) operated by a micro motor straight handpiece (NSK, Nakanishi Inc., Japan), under continuous water cooling to prevent heat-induced damage.

Teeth mounting

Custom-fabricated cylindrical plastic molds were prepared and filled with auto-polymerized acrylic resin (Acrostone acrylic material, Acrostone Dental and Medical Supplies, Egypt). Each tooth crown was embedded in the resin, with the buccal surface oriented upward, exposed, and positioned parallel to the horizontal plane. The buccal surface of each sample was coated with an acid-resistant nail varnish (DentKist, Inc., South Korea), leaving a standardized 2 × 2 mm window at the center of the enamel surface exposed [(16)].

Preparation of artificial white spot lesions

The demineralization solution was prepared using analytical-grade reagents and deionized water. Its composition included 2.2 mM calcium chloride, 2.2 mM potassium phosphate, and 0.05 M acetic acid. The pH of the solution was adjusted to 4.6 with 1 M sodium hydroxide (NaOH) and maintained at 37°C for 72 hours. All solutions were prepared at the Faculty of Pharmacy, Cairo University.

Artificial white spot-like lesions were induced on the enamel surfaces. Each sample was individually immersed in a freshly prepared demineralizing solution, which was renewed daily for 72 hours to ensure the formation of uniform white spot lesions [(17)]. Following demineralization, the samples were thoroughly rinsed and subsequently stored in distilled deionized water.

Remineralization procedures

For a duration of 14 days, each group of samples was treated once every 24 hours with the assigned remineralizing agent (either GC MI paste plus or Coral calcium Nano silver toothpaste). Utilizing a disposable cotton tip applicator, the experimental agent was precisely applied to the exposed enamel surface within the designated window area for a duration of three minutes. Subsequent to this application, the samples underwent thorough rinsing with deionized water before being stored in artificial saliva at room temperature. Throughout the 14-day remineralization period, the artificial saliva solution was replenished every 24 hours prior to the re-immersion of the newly treated samples (16).

Outcome assessment

1 Surface morphology evaluation

The teeth from both groups were examined using a Scanning Electron Microscope (SEM) (FEI Inspect S, Netherlands) at a magnification of 4000× to evaluate surface morphology. A randomly selected sample from each group was mounted on a Scanning Electron Microscope (SEM) stub for analysis of its morphological features. Examinations were conducted at three stages: baseline, post-demineralization, and post-reminralization.

2 Microhardness testing

Surface microhardness was assessed at three time points: initially on sound enamel (baseline), following demineralization, and after remineralization. The surface microhardness of each sample was evaluated using a Digital Display Vickers Microhardness Tester (Wilson hardness tester model TUKON 1102, Germany). This specific instrument was equipped with a Vickers diamond indenter and utilized a 20× objective lens for the assessments.

For the Vickers hardness test, a 100-gram load was applied to the indenter onto the test sample for 10 seconds to ensure a smooth and impact-free indentation [(14)]. All measurements were conducted by a single examiner utilizing the same calibrated device to ensure consistency and reliability of the results.

The mean microhardness of each sample was calculated based on three separate indentations to ensure accuracy and minimize variability. The Vickers hardness number (HV) was calculated using the formula MHV = 1854.4L/d^2, where L denotes the applied load in gram-force (gf) and d represents the average diagonal length in micrometers (µm). The resulting hardness is expressed in gf/µm^2, although this unit is commonly omitted in reporting.

Statistical analysis

Quantitative data were comprehensively presented, detailing the mean, standard deviation (±SD), as well as the minimum and maximum recorded values. Subsequently, the critical assumptions of data normality and homogeneity of variances were rigorously assessed; this involved an initial examination of data distribution, followed by the formal application of the Shapiro-Wilk test for normality and the Levene test for homogeneity of variances. The data exhibited a normal distribution with homogeneous variances across all variables. A two-way mixed-model ANOVA was utilized for data analysis. Post hoc comparisons of the estimated marginal means were subsequently performed using the error term derived from the two-way model. Tukey's method was applied to adjust for multiple comparisons, with statistical significance set at $p<0.05$. All statistical analyses were performed using R statistical analysis software version 4.5.0 for Windows (R Foundation for Statistical Computing, Vienna, Austria) [18].

RESULTS

For baseline samples, the mean microhardness for Coral Calcium Nano Silver (Group I) was 285.89 (95% CI, 256.64 to 315.14), with a standard deviation of 47.20, a minimum value of 209.22, and a maximum value of 351.57. For MI Paste Plus (Group II, the mean was 278.30 (95% CI 251.69 to 304.91), the standard deviation was 42.94, the minimum value was 197.18, and the maximum value was 328.29 as presented in **Table (1)**.

For demineralized samples, the mean microhardness for Group I was 235.51 (95% CI 205.66 to 265.36), with a standard deviation of 48.16. The minimum value was 169.99, and the maximum value was 297.07. For Group II, the mean was 222.86 (95% CI 195.89 to 249.83), with a standard deviation of 43.51. The minimum value was 182.67, and the maximum value was 293.41 **Table (1)**.

For remineralized samples, Group I exhibited a mean microhardness of 315.63 (95% CI: 280.74–350.52), with a standard deviation of 56.30. The microhardness values for this group ranged from a minimum of 221.87 to a maximum of 378.51. In comparison, Group II demonstrated a mean microhardness of 297.32 (95% CI: 266.08–328.56), with a standard deviation of 50.40. The observed microhardness values in Group II ranged from 227.01 to 357.12 **Table (1)**.

TABLE (1) Descriptive statistics.

Interval	Group	Mean	95% Confidence Interval		SD	Minimum	Maximum
			Lower	Upper			
Baseline	**Group I**	285.89	256.64	315.14	47.20	209.22	351.57
	Group II	278.30	251.69	304.91	42.94	197.18	328.29
Demineralization	**Group I**	235.51	205.66	265.36	48.16	169.99	297.07
	Group II	222.86	195.89	249.83	43.51	182.67	293.41
Remineralization	**Group I**	315.63	280.74	350.52	56.30	221.87	378.51
	Group II	297.32	266.08	328.56	50.40	227.01	357.12

Effect of time

In both groups, microhardness values differed significantly across the evaluated time intervals (p<0.001). The highest mean values were recorded following remineralization, followed by baseline measurements, while the lowest values were noted after demineralization. Post hoc pairwise comparisons confirmed that baseline and post-remineralization values were significantly higher than those obtained after demineralization **Table (2).**

Effect of material

Regardless of measurement time, group I had higher mean microhardness values than group II; however, the differences were not statistically significant as shown in **Table (3)**.

Effect of different variables and their interaction

Only time had a significant effect on microhardness (p<0.001). However, the effects of material (p=0.437) and its interaction with time (p=0.911) were not statistically significant as shown in **Table (4)**.

TABLE (2): Comparisons and summary statistics of microhardness for different intervals.

Material	Microhardness (Mean±SD)			p-value	PES (95% CI)
	Baseline	Demineralization	Remineralization		
Group I	285.89±47.20[A]	235.51±48.16[B]	315.63±56.30[A]	<0.001*	0.372 (0.145 to 0.510)
Group II	278.30±42.94[A]	222.86±43.51[B]	297.32±50.40[A]	<0.001*	0.351 (0.126 to 0.492)

PES: Partial Eta Squared; CI: Confidence interval; Values with ***different superscripts*** *within the* ***same horizontal row*** *are significantly different; * significant (p<0.05).*

TABLE (3) Comparisons and summary statistics of microhardness for different materials.

Interval	Microhardness (Mean±SD)		p-value	PES (95% CI)
	Group I	Group II		
Baseline	285.89±47.20	278.30±42.94	0.727ns	0.003 (0.000 to 0.072)
Demineralization	235.51±48.16	222.86±43.51	0.561ns	0.008 (0.000 to 0.096)
Remineralization	315.63±56.30	297.32±50.40	0.401ns	0.016 (0.000 to 0.119)

PES: Partial Eta Squared; CI: Confidence interval; ns: not significant.

TABLE (4) Effect of different variables and their interactions on the microhardness.

Source	Sum of Squares	df	Mean Square	f-value	p-value	PES (95% CI)
Material	2476.84	1	2476.84	0.63	0.437ns	0.034 (0.000 to 0.229)
Time	62450.64	2	31225.32	20.32	<0.001*	0.530 (0.310 to 0.640)
Material * Time	287.60	2	143.80	0.09	0.911ns	0.005 (0.000 to 0.030)

df: degree of freedom; PES: Partial Eta Squared; CI: Confidence interval; * significant (p<0.05); ns: not significant.

Scanning Electron Microscopy (SEM) Evaluation

Before demineralization, the enamel in Group I appeared intact and morphologically sound, characterized by a smooth, compact surface exhibiting discernible perikymata and shallow surface depressions, consistent with physiological enamel formation processes. No evidence of surface porosity, prismatic disintegration, or structural breakdown was observed. Similarly, in Group II, the enamel surface exhibited a homogeneous mineralized architecture, indicating that the hydroxyapatite crystal structure remained unaltered and free from acid-induced dissolution, serving as the reference condition for evaluating subsequent demineralization and remineralization changes **(Fig 1A)** and **(Fig 2A).**

After demineralization, the enamel surface in both groups, following 72 hours of exposure to a demineralizing solution, exhibited clear signs of mineral loss and structural degradation. The surface showed pronounced porosity with a honeycomb-like pattern, irregular depressions, and disintegration of

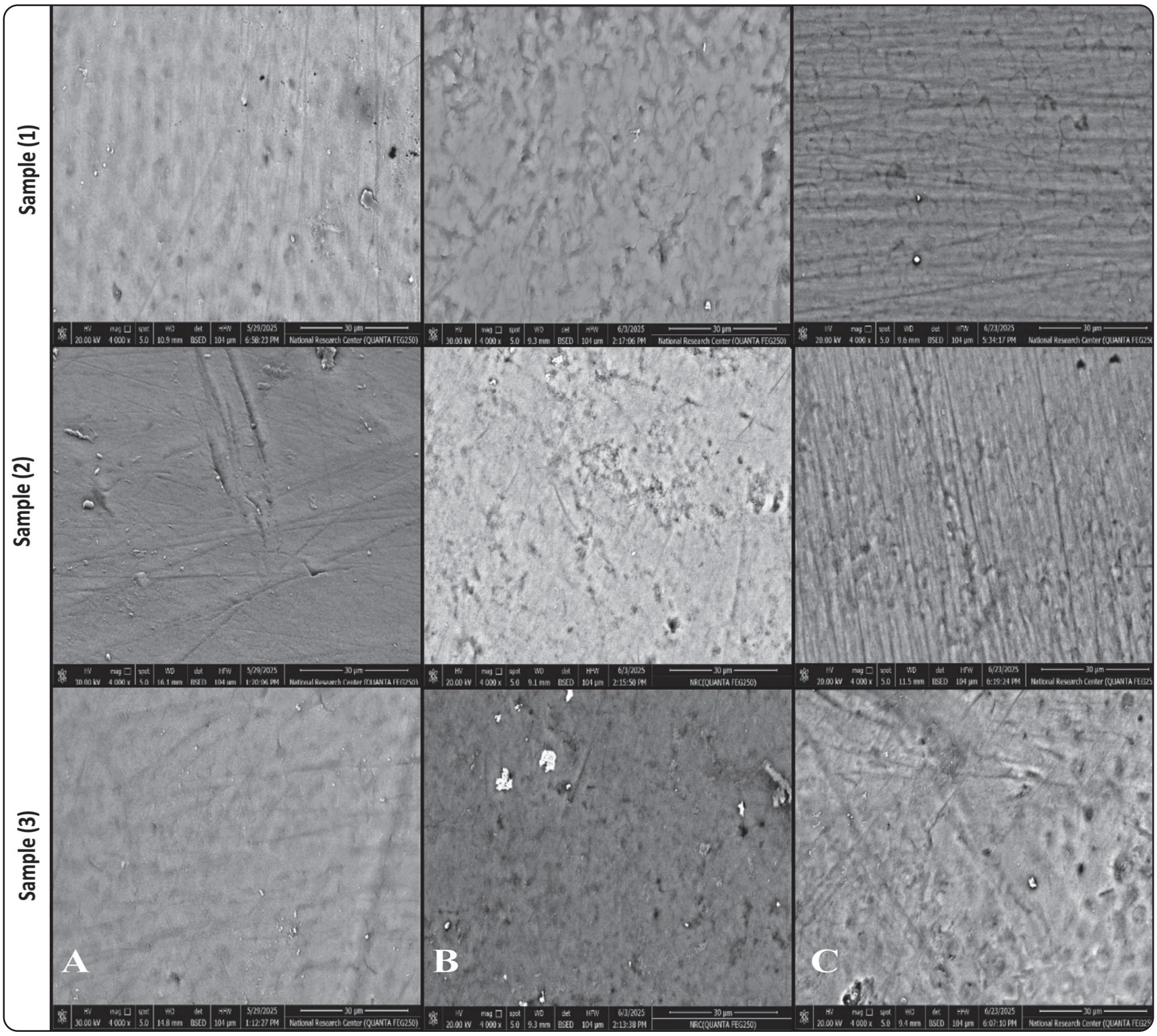

Fig. (1). SEM of Group I samples illustrating surface morphology changes across different treatment phases. A) Baseline SEM images of three distinct samples. B) Demineralization of the three samples. C) Remineralization of the samples.

enamel prisms. These morphological alterations confirm the successful induction of artificial enamel demineralization, indicating dissolution of hydroxyapatite crystals and a loss of surface integrity **(Fig 1B)** and **(Fig 2B).**

After 14 days of remineralization, Group I displayed notable morphological recovery compared to its demineralized state; previously observed porosities and irregularities were significantly reduced, with many microdefects appearing partially or fully occluded, indicating successful mineral deposition within the enamel structure **(Fig 1C).** Similarly, in Group II, the overall surface appeared more homogeneous and smoother, with a reduction in surface roughness and reestablishment of mineral integrity. Faint outlines of interprismatic enamel structures were still observable, suggesting partial subsurface remineralization, and these features collectively reflect the therapeutic action of the applied remineralizing agent, which likely facilitated crystal growth and the filling of porous lesions **(Fig 2C).**

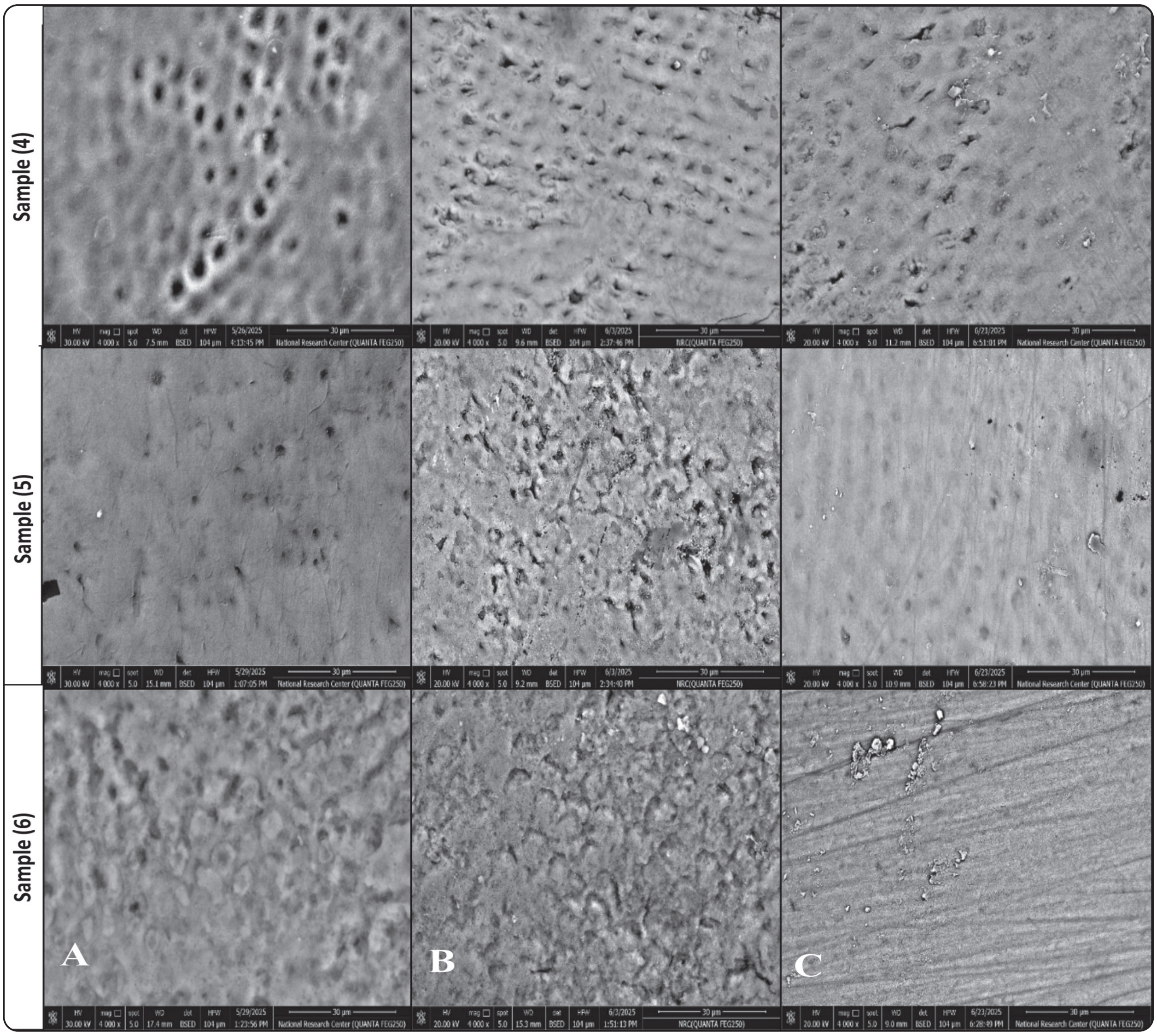

Fig. (2). SEM of Group II samples illustrating surface morphology changes across different treatment phases. A) Baseline SEM images of three distinct samples. B) Demineralization of the same three samples. C) Remineralization of the same samples.

DISCUSSION

Minimally invasive dentistry aims to preserve healthy dental tissue to the greatest extent possible, achieved either through caries prevention or the remineralization of early demineralized lesions [19]. Non-cavitated lesions can be effectively managed without invasive procedures by employing remineralizing agents, offering a significant advantage compared to conventional restorative approaches [20]. While fluoride therapy remains a cornerstone in the non-invasive management of caries and the remineralization of initial lesions, its efficacy is limited in high-risk populations, regardless of the administered dosage [21]. Furthermore, dental fluorosis, an adverse aesthetic outcome associated with fluoride-based preventive measures, has raised concerns regarding its clinical acceptability. Consequently, there is growing interest in exploring safer, naturally derived alternatives for caries management.

In this study, coral nanosilver incorporated into bioactive glass containing calcium phosphate was selected to assess its remineralization potential, as the manufacturer claims that these glass particles release calcium and phosphate ions intraorally to promote remineralization. Bioactive glass acts as a source of calcium and phosphate, which are essential for tooth remineralization within the oral environment [9,22].

Recent advancements highlight the growing importance of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) in the clinical approach to enamel caries management and prevention. Research indicates that CPP-ACP effectively stabilizes calcium phosphate within dental plaque adjacent to tooth surfaces. In the presence of acidic conditions, particularly after food consumption, CPP-ACP sustains a supersaturated mineral concentration favorable to dental enamel. This mechanism reduces demineralization and facilitates the remineralization of subsurface enamel lesions. MI Paste Plus, a formulation combining CPP-ACP with 900 ppm fluoride, has demonstrated superior remineralization efficacy for early enamel lesions compared to standalone sodium fluoride or CPP-ACP, highlighting the synergistic effect of fluoride incorporation [23].

Significant research efforts have focused on inhibiting dental caries progression. While clinical trials are considered the definitive standard, standardized in vitro models are widely utilized in cariology research to practically assess the efficacy of remineralizing agents in preventing caries development [24].

Considering the critical role of the surface layer in caries development, assessing changes within this region is essential. Accordingly, measuring surface microhardness represents a suitable approach for examining the demineralization–remineralization process, supporting its use in the present study [25,26].

Existing literature lacks sufficient exploration into the remineralization efficacy of topically applied coral calcium and MI Paste on enamel. Therefore, this study aimed to evaluate and compare the remineralizing potential of Coral Calcium Nano Silver toothpaste and MI Paste Plus in the management of white spot lesions on primary molars. This was achieved by assessing changes in enamel surface microstructure and microhardness.

Dental enamel was chosen as the research sample due to its absence of cellular regenerative capabilities, making the processes involved in caries formation and remineralization reliant on physicochemical interactions occurring at the pellicle-enamel interface [27].

To simulate subsurface demineralization beneath a preserved surface layer, samples in this study were immersed in a demineralizing solution and incubated at 37°C for 72 hours, consistent with the methodology described by **Lata et al. (2010)**[25]. This approach aimed to mimic the early stages of enamel lesions, which occur naturally in individuals with a high susceptibility to dental caries [28,29].

In this study, the pH of the artificial saliva was maintained at 7.0 to replicate physiological conditions. Neutralizing the acidic biofilm environment can prevent crystal demineralization. Additionally, healthy saliva contributes to remineralization by maintaining a slightly alkaline pH at the tooth surface and providing effective buffering, thereby promoting a favorable environment for mineral reminralization [(30)].

The efficacy assessment was conducted after a 14-day period, as this duration represents the minimum time required for a toothpaste or cream to demonstrate its intended therapeutic outcomes [(31)].

The enamel samples received topical applications of the experimental remineralizing agents twice daily, with each application lasting two minutes, to replicate the standard recommended oral hygiene regimen [(32)].

Application of the test compounds in this study was achieved using a paste formulation delivered with disposable cotton-tip applicators. This approach was selected to simulate clinical practicality, ensuring alignment with patient-friendly application methods such as toothpaste delivery via toothbrushes or cotton-tip applicators [(28,33)].

In the current study, SEM imaging indicated that the surface of samples treated with Coral Calcium Nano Silver displayed that notable morphological recovery compared to the demineralized state. The previously observed porosities and irregularities have been significantly reduced, with many of the microdefects appearing partially or fully occluded, indicating successful mineral deposition within the enamel structure. This suggest that the Coral Calcium Nano Silver acts mainly through superficial mineral precipitation.

In this study, the SEM reveled that the overall surface of samples treated with MI paste plus appeared more homogenous and enhanced the superficial mineral layer creation in comparison to Coral Calcium Nano Silver paste. This finding aligns with the results reported by **Poggio et al. (2016)**[(34)], who investigated the efficacy of several remineralizing agents (Remin Pro, Tooth Mousse, MI Paste Plus, and Profluorid Varnish) on bleached enamel. Their results indicated that MI Paste Plus formed a complete and uniform protective layer, demonstrating superior remineralization compared to other agents.

Similarly, **Jayarajan et al. (2011)**[(35)] observed that enamel treated with CPP-ACPF exhibited indistinct enamel rods and prismatic structures, with calcified deposits predominantly localized in porous regions. Furthermore, MI Paste Plus-treated enamel displayed a smoother, more homogeneous surface compared to MI Paste. Additionally, **Abd El Halim and Raafat (2017)**(36) investigated the impact of MI Paste Plus on acid-etched and laser-etched enamel using Environmental Scanning Electron Microscopy (ESEM), revealing that surfaces treated with acid-etching followed by MI Paste Plus and synthetic saliva rinsing exhibited a fine granular texture without noticeable porosities. These collective findings support the efficiency of CPP-ACP-based agents in enamel remineralization.

The Vickers hardness number (VHN) test is a widely recognized method for evaluating the surface microhardness of substrates such as enamel, owing to its fine microstructure and heterogeneous surface, which is susceptible to cracking [(37)]. Microhardness indentations offer a straightforward, noninvasive, and efficient approach for studying demineralization and remineralization processes. The Vickers test was preferred over the Knoop method due to its ability to produce indentations with more precise and measurable geometry. Consequently, Vickers hardness values serve as reliable indirect indicators of remineralization efficacy [(38,39)].

The findings from the surface microhardness assessment demonstrated a notable reduction in the microhardness measurements of demineralized enamel samples following the demineralization process when compared to initial baseline values. This decline can be attributed to the demineralizing solution's action, which induced enamel surface softening as a consequence of mineral loss [40]. While a significant increase was observed after the remineralization process, no statistically significant distinction was noted between the two groups. This suggests that the toothpastes in question exhibit comparable efficacy in the remineralization of incipient carious lesions.

The null hypothesis was accepted as the findings of this study demonstrated that Coral Nanosilver toothpaste yielded a greater surface hardness compared to MI Paste, but with no statistically significant differences. The greater surface hardness of samples treated with Coral Nanosilver may be linked to Coral calcium's capacity to elevate enamel surface alkalinity and facilitate the remineralization of early enamel defects [8]. The remineralization of demineralized enamel by coral calcium is likely due to the precipitation of calcium ions originating from the calcium carbonate present in the formulation. Furthermore, the elevated calcium content in Coral toothpaste shifts the acidic pH toward an alkaline environment, promoting mineral deposition[41]. The inclusion of silica in the formulation enhances gel adhesion to the tooth structure [9].

Research by **Qais et al. (2019)** [42] indicates that silver nanoparticles (Ag-NPs) smaller than 30 nm disrupt microbial cell membranes by destabilizing the lipid bilayer, increasing permeability, and inducing bacterial lysis. Specifically, Ag-NPs below 20 nm exhibit potent antimicrobial effects against Staphylococcus aureus and Klebsiella pneumoniae. Additionally, the porous nature of demineralized enamel facilitates deeper penetration of gel components, while its increased surface area enhances mineral reactivity. The elevated concentration of calcium carbonate within the Coral calcium gel facilitates the precipitation of calcium ions onto the enamel surface [9]. Consequently, this process may occlude surface pores, contributing to improved microhardness.

This study observed a significant time-dependent increase in the microhardness of demineralized enamel specimens across all evaluated toothpaste formulations. These observations align with the research conducted by **Elbakry et al. (2024)**[43], who assessed the remineralization efficacy of different dentifrices over intervals of 7, 14, and 28 days, reporting that bioactive glass-based toothpastes exhibited considerable potential in managing early carious lesions, with their remineralization capacity being time-dependent. In agreement with **Balakrishnan et al. (2013)**[44], our study supports the concept of dose-dependent remineralization, as previously investigated in their 30-day study on various dentifrices. Additionally, the data are consistent with the conclusions of **Elbakry et al. (2024)**[43] and **Soares et al. (2017)** [32], who noted statistically significant variations in enamel remineralization values with time. This can be attributed to the progressive remineralization of the tooth structure. Calcium and phosphate ions, derived from saliva and exogenous sources like toothpaste, infiltrate the enamel. When the pH surpasses the critical threshold, these ions, facilitated by remineralizing agents, deposit onto the enamel surface [8,45].

Consistent with the findings of **Samaha et al. (2020)** [46], the mean surface hardness of MI Paste samples was observed to be greater than that of the sound enamel group. Conversely, a previous study conducted by **Alattar et al. (2023)**[16] reported lower surface hardness for the MI Paste group compared to sound dental enamel. The observed outcome may be explained by the strong affinity of casein phosphopeptide in CPP-ACPF for biofilm, leading to an elevated localized concentration of calcium and phosphorus ions. These ions then permeate the enamel's crystalline lattice, resulting in increased hydroxyapatite crystal density [47,48].

The findings of this study, based on SEM analysis and surface microhardness measurements, demonstrate the remineralizing efficacy of Coral Calcium Nano Silver toothpaste and MI Paste Plus. Nevertheless, limited evidence currently exists to comprehensively evaluate their effectiveness as remineralizing agents. Thus, further research employing diverse analytical methodologies is needed to assess their remineralization potential and compare their performance with other established agents, such as fluoride and CPP-ACP.

While further research is necessary to assess the long-term effectiveness of these remineralizing agents—especially those replicating in vivo conditions for more definitive findings—the in vitro model used in this study offers the key advantage of tightly regulated single-variable analyses. This allows for the precise investigation of mechanistic factors that would be considerably more complex to evaluate within an in vivo system.

Moreover, the present study design did not account for all oral variables, such as the complex interactions at the tooth-pellicle-plaque-saliva interface, which were not replicated. Additionally, the demineralizing solution lacked bacterial presence also adds to the limitation. Consequently, future investigations should assess the remineralizing efficacy of these agents under more clinically representative settings, utilizing enamel slabs embedded in oral appliances to evaluate and compare their performance within the oral environment.

CONCLUSIONS

Within limitation of this in vitro study, both Coral Calcium Nano Silver and MI Paste Plus Toothpaste are equally effective in remineralizing the enamel of the primary teeth. Although, Coral Calcium Nano Silver have a higher microhardness, no significant differences were observed between the two toothpastes.

REFERENCES

1. Veiga NJ, Aires D, Douglas F, Pereira M, Vaz A, Rama L, et al. Dental caries: A review. Journal of dental and oral health. 2016;2(5):1–3.
2. Deveci C, Çınar Ç, Tirali RE. Management of white spot lesions. Dental Caries-Diagnosis, Prevention and Management2018. 2018;
3. Deswal R, Kukreja N, Chhabra S, Trivedi S, Sharma A, Thakur A. Comparative assessment of enamel microhardness using various remineralising agents on artificially demineralized human enamel. Cureus. 2022;14(10).
4. Piesiak-Pańczyszyn D, Zakrzewski W, Piszko A, Piszko PJ, Dobrzyński M. Review on fluoride varnishes currently recommended in dental prophylaxis. Polymers in Medicine. 2023;53(2):141–51.
5. Degli Esposti L, Zheng K, Piancastelli A, Ionescu AC, Adamiano A, Boccaccini AR, et al. Composite materials of amorphous calcium phosphate and bioactive glass nanoparticles for preventive dentistry. Ceram Int. 2024;50(1):593–602.
6. Zhong Y, Liu J, Li X, Yin W, He T, Hu D, et al. Effect of a novel bioactive glass–ceramic on dentinal tubule occlusion: an in vitro study. Aust Dent J. 2015;60(1):96–103.
7. Gonçalves FMC, Delbem ACB, Gomes LF, Emerenciano NG, dos Passos Silva M, Cannon ML, et al. Combined effect of casein phosphopeptide-amorphous calcium phosphate and sodium trimetaphosphate on the prevention of enamel demineralization and dental caries: an in vitro study. Clin Oral Investig. 2021;25:2811–20.
8. Abdelnabi A, Hamza MK, El-Borady OM, Hamdy TM. Effect of different formulations and application methods of coral calcium on its remineralization ability on carious enamel. Open Access Maced J Med Sci. 2020;8(D):94–9.
9. Abdelnabi A, Hamza NK, Othman MS. Evaluation of re-mineralization of initial enamel lesions using nanohydroxyapatite and coral calcium with different concentrations. Egypt Dent J. 2019;65(4-October (Fixed Prosthodontics, Dental Materials, Conservative Dentistry & Endodontics)):3713–8.
10. Faul F, Erdfelder E, Lang AG, Buchner A. G* Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods. 2007;39(2):175–91.

11. Ebadifar A, Nomani M, Fatemi SA. Effect of nano-hydroxyapatite toothpaste on microhardness ofartificial carious lesions created on extracted teeth. J Dent Res Dent Clin Dent Prospects. 2017;11(1):14.

12. Geeta RD, Vallabhaneni S, Fatima K. Comparative evaluation of remineralization potential of nanohydroxyapatite crystals, bioactive glass, casein phosphopeptide-amorphous calcium phosphate, and fluoride on initial enamel lesion (scanning electron microscope analysis)–An in vitro study. Journal of Conservative Dentistry and Endodontics. 2020;23(3):275–9.

13. Narayanaswamy M, Chikkanarasaiah N. Shear Bond Strength of Resin-modified Glass Ionomer Cement to Primary Teeth Dentin Pretreated with Silver Diamine Fluoride and Sodium Fluoride Varnish: An In Vitro Study. World. 2024;15(1):61.

14. Kamal D, Hassanein H, Elkassas D, Hamza H. Complementary remineralizing effect of self-assembling peptide (P11-4) with CPP-ACPF or fluoride: An in vitro study. J Clin Exp Dent. 2020;12(2):e161.

15. Elbakry ZI, Ahmed BS, Mohamed EK. Remineralization assessment of early enamel carious-like lesion after treatment with new Nano-silver toothpaste. Al-Azhar Journal of Dental Science. 2024;27(2):269–77.

16. Alattar W, Mostafa MH, Hashem SN. The effect of Remin Pro and MI Paste Plus on induced demineralized enamel in primary molars. Al-Azhar Dental Journal for Girls. 2023;10(1):239–46.

17. Rafiei E, Fadaei Tehrani P, Yassaei S, Haerian A. Effect of CO 2 laser (10.6 μm) and Remin Pro on microhardness of enamel white spot lesions. Lasers Med Sci. 2020;35:1193–203.

18. Team RC. RA language and environment for statistical computing, R Foundation for Statistical. Computing. 2020;

19. Shelke UR, Shiraguppi VL, Deosarkar B, Tayeeb S, Rathod S, Shah Y. Minimal Invasive Dentistry–A Systematic Respect For The Original Tissue. J Interdisciplin Dent Sci. 2020;9(2):29–36.

20. Chen Y, Chen D, Lin H. Infiltration and sealing for managing non-cavitated proximal lesions: a systematic review and meta-analysis. BMC Oral Health. 2021;21:1–21.

21. Amaechi BT, AbdulAzees PA, Alshareif DO, Shehata MA, Lima PP de CS, Abdollahi A, et al. Comparative efficacy of a hydroxyapatite and a fluoride toothpaste for prevention and remineralization of dental caries in children. BDJ Open. 2019;5(1):18.

22. Omran TM, Mostafa MH, Abd El-Raouf EM. Assessment of remineralizing effect of bioactive glass based toothpastes: An in-vitro comparative study. Al-Azhar Dental Journal for Girls. 2021;8(3–C):491–9.

23. Borges AB, Yui KCK, D'Avila TC, Takahashi CL, Torres CRG, Borges ALS. Influence of remineralizing gels on bleached enamel microhardness in different time intervals. Oper Dent. 2010;35(2):180–6.

24. Neto FCR, Maeda FA, Turssi CP, Serra MC. Potential agents to control enamel caries-like lesions. J Dent. 2009;37(10):786–90.

25. Lata S, Varghese NO, Varughese JM. Remineralization potential of fluoride and amorphous calcium phosphate-casein phospho peptide on enamel lesions: An: in vitro: comparative evaluation. Journal of Conservative Dentistry. 2010;13(1):42–6.

26. Soares R, De Ataide IDN, Fernandes M, Lambor R. Assessment of enamel remineralisation after treatment with four different remineralising agents: a scanning electron microscopy (SEM) study. J Clin Diagn Res. 2017;11(4):ZC136.

27. Vyavhare S, Sharma DS, Kulkarni VK. Effect of three different pastes on remineralization of initial enamel lesion: an in vitro study. Journal of Clinical Pediatric Dentistry. 2015;39(2):149–60.

28. Kamath P, Nayak R, Kamath SU, Pai D. A comparative evaluation of the remineralization potential of three commercially available remineralizing agents on white spot lesions in primary teeth: An: in vitro: study. Journal of Indian Society of Pedodontics and Preventive Dentistry. 2017;35(3):229–37.

29. Yadav S, Sachdev V, Malik M, Chopra R. Effect of three different compositions of topical fluoride varnishes with and without prior oral prophylaxis on Streptococcus mutans count in biofilm samples of children aged 2–8 years: a randomized controlled trial. Journal of Indian Society of Pedodontics and Preventive Dentistry. 2019;37(3):286–91.

30. Farooq I, Bugshan A. The role of salivary contents and modern technologies in the remineralization of dental enamel: a narrative review. F1000Res. 2021;9:171.

31. Baysan A, Whiley RA, Lynch E. Antimicrobial effect of a novel ozone–generating device on micro–organisms associated with primary root carious lesions in vitro. Caries Res. 2000;34(6):498–501.

32. Soares R, De Ataide IDN, Fernandes M, Lambor R. Assessment of enamel remineralisation after treatment with four different remineralising agents: a scanning electron microscopy (SEM) study. J Clin Diagn Res. 2017;11(4):ZC136.

33. Yamaguchi K, Miyazaki M, Takamizawa T, Inage H, Moore BK. Effect of CPP–ACP paste on mechanical properties of bovine enamel as determined by an ultrasonic device. J Dent. 2006;34(3):230–6.

34. Poggio C, Grasso N, Ceci M, Beltrami R, Colombo M, Chiesa M. Ultrastructural evaluation of enamel surface morphology after tooth bleaching followed by the application of protective pastes. Scanning. 2016;38(3):221–6.

35. Jayarajan J, Janardhanam P, Jayakumar P. Efficacy of CPP-ACP and CPP-ACPF on enamel remineralization-An in vitro study using scanning electron microscope and DIAGNOdent®. Indian journal of dental research. 2011;22(1):77–82.

36. Abd El Halim S, Raafat R. Chemical and morphological analysis of human demineralized enamel surface by acid and laser etching following remineralization by MI paste plus. Egypt Dent J. 2017;63(4-October (Fixed Prosthodontics, Dental Materials, Conservative Dentistry & Endodontics)):3351–9.

37. Joshi C, Gohil U, Parekh V, Joshi S. Comparative Evaluation of the Remineralizing Potential of Commercially Available Agents on Artificially Demineralized Human Enamel: An: In Vitro: Study. Contemp Clin Dent. 2019;10(4):605–13.

38. Valian A, Raeisi-Sarkhooni A, Moravej-Salehi E, Emamieh S. Effect of remin pro and neutral sodium fluoride on enamel microhardness after exposure to acidic drink. Journal of Iranian Dental Association. 2017;29(2):44–50.

39. Mehta AB, Kumari V, Jose R, Izadikhah V. Remineralization potential of bioactive glass and casein phosphopeptide-amorphous calcium phosphate on initial carious lesion: An: in-vitro: pH-cycling study. Journal of conservative dentistry. 2014;17(1):3–7.

40. Haghgoo R, Rezvani MB, Zeinabadi MS. Comparison of nano-hydroxyapatite and sodium fluoride mouthrinse for remineralization of incipient carious lesions. J Dent (Tehran). 2014;11(4):406.

41. Kumar VM, Govind GK, Siva B, Marish P, Ashwin S, Kiran M. Corals as bone substitutes. Journal of International Oral Health. 2016;8(1):96–102.

42. Qais FA, Shafiq A, Khan HM, Husain FM, Khan RA, Alenazi B, et al. Antibacterial Effect of Silver Nanoparticles Synthesized Using Murraya koenigii (L.) against Multidrug–Resistant Pathogens. Bioinorg Chem Appl. 2019;2019(1):4649506.

43. Elbakry ZI, Ahmed BS, Mohamed EK. Remineralization assessment of early enamel carious-like lesion after treatment with new Nano-silver toothpaste. Al-Azhar Journal of Dental Science. 2024;27(2):269–77.

44. Balakrishnan A, Jonathan R, Benin P, Kuumar A. Evaluation to determine the caries remineralization potential of three dentifrices: An: in vitro: study. Journal of Conservative Dentistry and Endodontics. 2013;16(4):375–9.

45. Jones EM, Cochrane CA, Percival SL. The effect of pH on the extracellular matrix and biofilms. Adv Wound Care (New Rochelle). 2015;4(7):431–9.

46. Samaha AE, Gomaa DHH. The effect of different remineralizing agents on laser bleached enamel. Egypt Dent J. 2020;66(1-January (Fixed Prosthodontics, Dental Materials, Conservative Dentistry & Endodontics)):469–83.

47. Cochrane NJ, Cai F, Huq NL, Burrow MF, Reynolds EC. New approaches to enhanced remineralization of tooth enamel. J Dent Res. 2010;89(11):1187–97.

48. Pallepati A, Yavagal PC. Enamel remineralization efficacy of three widely used remineralizing tooth pastes: An in-vitro study. International Journal of Applied Dental Sciences. 2020;6:392–6.