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REMINERALIZING POTENTIAL OF NANO-HYDROXYAPATITE VERSUS NANO-CHITOSAN ON ARTIFICIAL WHITE SPOT LESION IN PERMANENT TEETH

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Abstract

Background: This study aimed to evaluate and compare the remineralization potential of nano-hydroxyapatite (n-HA) and nano-chitosan (n-CS) on artificial white spot lesion under pH cycling condition. **Methods:** Eighty premolars were immersed individually in 10 ml of demineralizing solution in separate test tubes at 37^0C for 15 hours to create an artificial white spot lesion. The demineralized specimens were divided randomly into four groups: (a) n-HA; (b) n-CS; (c) fluoridated toothpaste (positive control); (d) negative control. The remineralization potential of the experimental pastes were evaluated by a quantitative measurement of mineral density using cone beam computerized tomography (CBCT) and a quantitative measurement of surface roughness using the ZYGO Maxim-GP 200 profilometer. The measurements were carried out at five time points: before surface treatment, after lesion preparation, and during treatment protocols at days 7, 14, and 21. The normal distribution of the data was analyzed using the Kolmogorov-Smirnov test. As the data exhibited a normal distribution, a one-way analysis of variance (ANOVA) test was applied to calculate mean differences and compare the study groups. Tukey's post-hoc test was used for pairwise comparisons. **Results:** CBCT evaluation revealed that n-HA paste gave the best results and recorded the highest mean values of mineral density compared to all groups. Regarding surface roughness results, n-HA paste was the most significant for reducing surface roughness compared to n-CS paste and fluoridated paste. **Conclusion:** n-HA paste has the highest remineralization potential compared to n-CS paste and fluoridated paste.

Keywords: Nano Hydroxyapatite, Nano Chitosan, White Spot Lesion, Remineralization.

BACKGROUND

Dental caries is a pandemic disease with a high prevalence worldwide, results from imbalance in a dynamic process characterized by alternating periods of demineralization and remineralization. The dominance of the demineralization process leads to the loss of minerals, then cavitation, discomfort, pain, and finally tooth loss (1).

Until recently, dental professionals treated carious teeth by surgical removal of all infected carious part of the tooth (5). Currently, there is shift in management of dental caries from surgical method to a novel approach called Minimal Intervention Dentistry (MID). It is a

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professional care philosophy that focuses on the first occurrence, earliest detection, and earliest possible cure of diseases on the micro molecular level to preserve as much of the tooth structure as possible by preventing disease and intercepting its progress (2, 3)

Additionally, one of the cornerstones of MID is remineralization of the demineralized tooth structure rather than the traditional method of complete surgical removal of all infected tissue (4). Numerous methods have been reported to remineralize the incipient enamel lesions (5).

Historically, fluoride therapy was the most commonly used remineralizing agent as it is able to prevent demineralization by binding to hydroxyapatite and forming fluorohydroxyapatite crystals, in the presence of calcium and phosphate ions (6). Thus, the deficiency of the later minerals can prevent the synthesis of fluorohydroxyapatite. Moreover, this fluoride-based remineralization occurs on the lesion surface causing clogging of pores and consequently hindering mineral ions penetration into the body of the lesion and remineralization of the underlying tissues (14).

These shortcomings of fluoride-based strategies in caries prevention and remineralization justify the search for new treatment strategies to overcome fluoride remineralization flaws resulting in emergence of biomimetic remineralization. Biomimetic remineralization is regenerative biomineralization therapy, wherein diseased dental tissue are replaced with biologically similar tissues (15). An exciting development in this field includes nanohydroxyapatite and nanochitosan (8, 9) .

In recent years, n-HA has achieved wider acceptability in dentistry as it resembles natural Hydroxyaptite (HA) in features, has a powerful affinity for demineralized surface enamel, and is believed to be a superior biocompatible and bioactive substance (14-16).

N-CS is another attractive biomimetic cationic polymer that has biocompatible, biodegradable, and cariostatic properties. Chitosan's amino group (NH_2) has a strong affinity for binding to cariogenic acid, which results in a decrease the disintegration of the Hydroxyaptite crystal. Additionally, the positive charge of chitosan improves its absorption via electrostatic force into negative-charged demineralized enamel. Furthermore, it acts as an ideal template for phosphate (Po_4^{3-}) and calcium (Ca^{2+}), promoting the regeneration of enamel (17-19).

Several studies were conducted to evaluate the remineralization potential of nano hydroxyapatite and nanochitosan on early enamel lesions; however, up till our knowledge, no study was conducted to compare the remineralization potential of n-HA and n-CS on white spot lesions, so the present study was conducted.

METHODS

Study setting:

This work was conducted as an experimental study; the steps of this study were conducted at the Faculty of Dentistry, Tanta University, and the evaluation of mechanical properties was conducted at the National Institute of Standards.

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Ethical consideration:

The Research Ethics Committee (REC), Faculty of Dentistry, Tanta University approved this study with code (PED-02-22-4) in accordance with the ethical guidelines outlined in 1964 Helsinki Declaration and its subsequent revisions.

The purpose of the present study was explained to the patients, and informed consents from the parents in addition to assent from their children were obtained to use their extracted teeth.

Eligibility criteria:

One hundred and twenty human permanent premolars freshly extracted for orthodontic purpose in the age range of 12-18 years; were gathered from outpatient clinic of maxillofacial surgery department, Faculty of Dentistry, Tanta University. The inclusion criteria of selected teeth were sound, devoid of cracks or white spot lesions and not previously restored. **However,** teeth have any of the following: Carious lesions, hypo calcification, fracture, restoration, cracks, and fluorosis were excluded after inspection underneath a stereomicroscope (x10). Figure (1) points out a flow chart that includes enrollment, allocation, assessment and sample analysis.

Sample size calculation and randomization:

G* Power (version 3.1.9.2, University Kiel, Germany. Copyright © 1992-2014) [20] was used to calculate the sample size. The estimated sample size (n) in this study was found to be 20 for each group based on the effect size d of 0.48, alpha (α) level of 0.05, and beta (β) level of 0.05, i.e., power = 95%. Samples were randomized using the computer-generated Research Randomizer software program (https://www.randomizer.org/)[21]. An independent person deposited randomization codes in consecutively numbered, secured, opaque wrappers to guarantee covert distribution into the four groups.

Teeth cleaning:

Eighty eligible teeth were cleaned using dental prophylactic cup through low speed micro motor to remove smear layer and surface debris, washed under running water, and then were disinfected with 0.1% thymol solution to avoid spread of infection[22]. The teeth samples were stored for a maximum of one month at 37 °C in weekly-changed deionized water (SIGALD, Sigma-Aldrich Chemie GmbH)[23].

Specimen preparation:

The roots of all specimens were embedded in a self-curing acrylic resin block 12mm in diameter. A layer of 3x3mm wax (Perfect Wax Base Plate, Turkey) was cut and stuck on the center of the middle third of the buccal surface of each specimen, then all enamel of both buccal and lingual surfaces were protected with two layers of transparent acid-resistant nail varnish and allowed to dry. Finally, the wax piece was removed, creating a 3mm x 3mm window in the center of the middle third of the buccal surfaces of all specimens [22]. All specimens were stored in artificial saliva (NaCl 0.08%, KCl 0.12%,

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$MgCl_2$ 0.01%, K_2HPO_4 0.03%, $CaCL_2$ 0.01%, CMC-Na 0.10% and Sterile deionized water 99.6% (pH 7) before, during and after experiments[(24)].

White spot lesion preparation:

All specimens were removed from artificial saliva and subjected to demineralizing solution; they were immersed individually in 10 ml of demineralizing solution (2.2 mMol calcium chloride, 2.2 mMol sodium phosphate, 0.05 M acetic acid, 1 M potassium hydroxide, (pH 4.2))[(25)] in separate test tubes at 37^0C for 15 hours [(16)]. The demineralizing solution was prepared in the Chemistry Department, Faculty of Pharmacy, Tanta University. The pH of solution was checked using digital PH meter and adjusted at 4.2[(26)]. The solution was freshly prepared and changed daily to avoid super saturation.

Specimens' grouping:

All demineralized samples were color coded and divided randomly into four groups as the following:

Group I (Nano hydroxyapatite): 20 specimens were treated with n-HA[1].

Group II (Nano Chitosan): 20 specimens were treated with n-CS[2].

Group III (fluoride toothpaste) (positive control)**:** 20 specimens were treated with Colgate toothpaste[3].

Group IV (negative control group): 20 specimens of this group were subjected to artificial saliva and demineralization pH cycling only.

Treatment protocol and pH cycling regimen:

In the present study, a pH-cycling model adapted from Karlinsey[(27)]was incorporated: Remineralizing pastes were applied on the wet enamel specimen for three minutes using a micro brush. After remineralizing pastes application, the specimens were immersed in artificial saliva for two hours. The specimens then were washed carefully by deionized water, followed by immersion in demineralizing solution for 4 hours. After demineralization, teeth were rinsed with deionized water, followed by another two-hour immersion in artificial saliva. Teeth were treated again with remineralizing agent for three minutes. Finally, teeth were stored in artificial saliva at 37^0C in an incubator with 100% humidity for the remaining of the day. Negative control group underwent the pH-cycling regimen. The mentioned regimen was repeated daily for 21 days.

Surface characterization and assessments of specimens:

Measurements were carried out at five time points; before surface treatment, after lesion preparation and during treatment protocols at days 7, 14 and 21. All teeth were examined by surface characterization regarding:

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A) Evaluation of the mineral density by Cone beam computerized tomography.

All specimens were scanned using Cone beam computerized tomography (CBCT). The type of CBCT was KaVo OP 3D Vision (Kavo Dental, Biberach, Germany) with the following specifications: standardized kilo voltage =120 kVP, 5mA, 80 ×50 mm field of view (FOV) with 0.125 mm voxel sizes for high resolution, and a scanning time of 0.4 sec & an exposure time of 0.1 sec.

Standardization of tomographing:

The samples were adjusted in linear pattern in a custom-made block of cork that was constructed with dimensions smaller than the CBCT machine's field of view (FOV). At each scanning, the teeth were repositioned in the identical preconstructed blocks in the same orientation with the same protocol and parameter settings.

CBCT image analysis:

The images analyzed using On Demand 3D software version 1.0 (build 1.0.10.7462), (× 64 Edition), copyright (c) 2004–2017 Cybermed, and license key 670094709. To maintain standardization, the images for each sample were fused in the same section and position using the fusion module.

Mineral density was measured by determining the Region of Interest (ROI) [area of approximately 3mm^2 in the center of the middle third of the buccal surface image of the tooth]. Then, the quantitative assessment of mineral density in ROI was made using the Hounsfield unit (HU) scale [(28, 29)].

B) Surface roughness testing:

The samples were scanned using the ZYGO Maxim-GP 200 profilometer, which utilizes non-contact coherence scanning interferometry (CSI) technology to provide accurate, quick, and perform quantitative surface metrology of form and roughness. After that, a three-dimensional (3D) image of the surface profile of the samples was created on a computer using a fixed magnification of 20X Mirau[(30)].

Analysis for surface roughness images:

The images were analyzed using MetroPro 7.3.2 analysis software [(31)], and the roughness was described by a colored scale indicating elevations and depressions.

Statistical analysis:

The obtained data were collected, tabulated, and statistically analyzed using the SPSS version 22.0 statistical package system (IBM Corp., Armonk, New York, USA). The normal distribution of the data was analyzed using the Kolmogorov-Smirnov test at the significant level alpha = 0.05, data exhibited a normal distribution, so parametric tests were used to analyze it. All data were presented in mean and standard deviation and the P value was adjusted at <0.05, one-way ANOVA test was applied to calculate mean differences and compare the study groups. Tukey's post-hoc test was used for pairwise comparisons.

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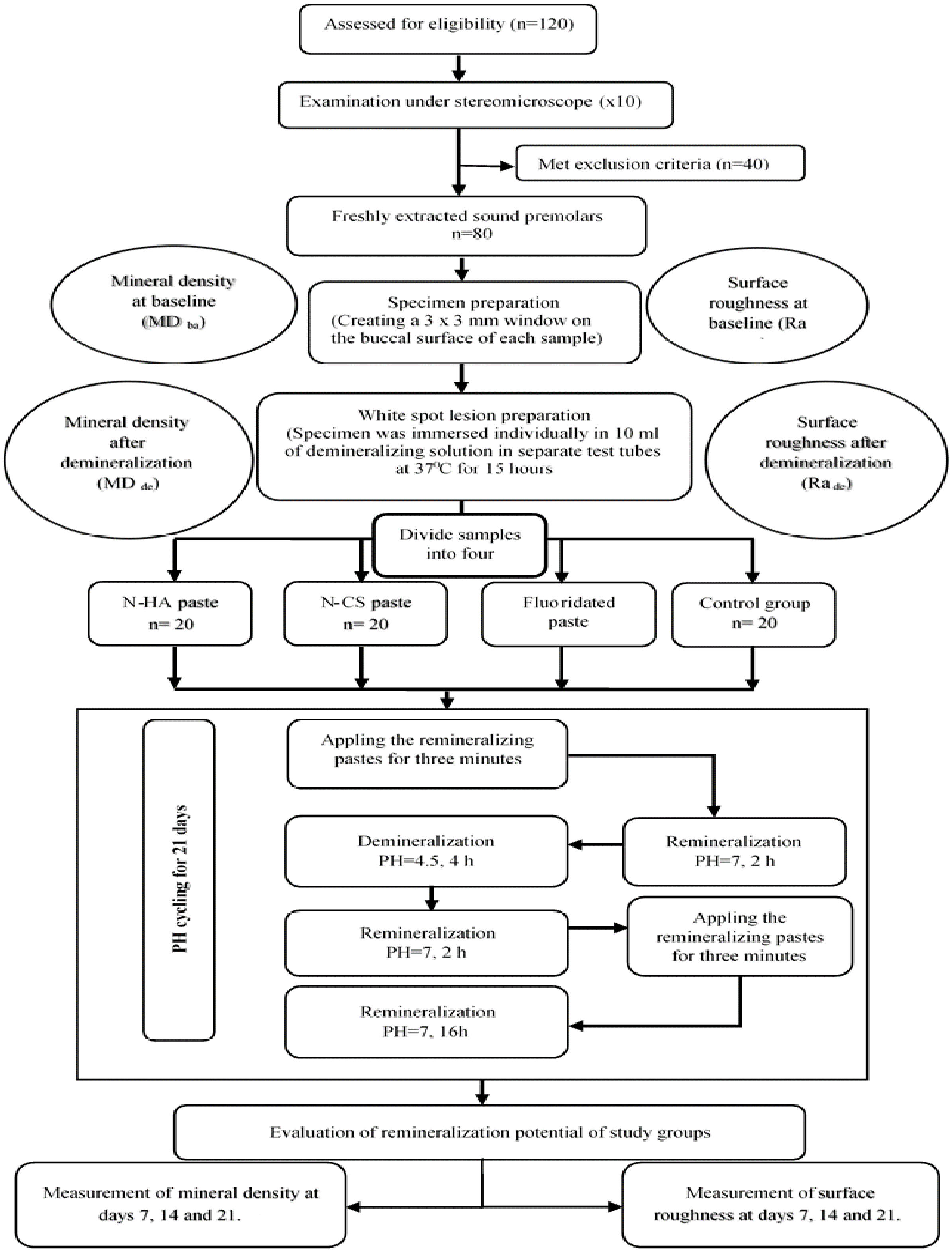

Fig 1: Schematic representation of in vitro study design

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RESULTS

Regarding **Table (1) and figure (2)**, at baseline and after demineralization, **statistical analysis by ANOVA test revealed** no statistically significant difference between study groups (P = 0.1343, P = 0.075 respectively). After 7, 14, and 21 days, group I had the highest mineral density, followed by groups II and III; however, group IV had the lowest mineral density, with a statistically significant difference between the mean values of the mineral density among the study groups (p < 0.05). Tukey's post hoc test highlighted that there was no statistically significant difference between the mean values of mineral density in groups II and III after 7 and 14 days (P > 0.05). After 21 days, there was a statistically significant difference between the mean values of mineral density between all groups (P < 0.05).

Concerning **Table (2) and figure (3)**, at baseline and after demineralization **ANOVA test displayed no statistically significant difference between the mean values of the surface roughness** among study groups (P > 0.05). After 7, 14, and 21 days, group I had the lowest surface roughness, followed by groups II and III; however, group IV had the highest surface roughness, with a statistically significant difference between the mean values of the surface roughness among the study groups (P < 0.05). Post-hoc Tukey's test disclosed that there was a statistically significant difference in the mean values of the surface roughness after 7, 14, and 21 days between all groups (P < 0.05), except between groups II and III only (P > 0.05).

Table (1): Comparison between the mean values of the mineral density among the study groups.

Study group \ Mineral density		Baseline	Post demineralization	7 days	14 days	21 days
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Group I		1733 ±273	1511 ±228	1634±247 [a]	1693 ±254 [a]	1712± 262 [a]
Group II		1503±203.2	1386±214.8	1457±200.8 [b]	1356±247.6 [b]	1260±249.8 [b]
Group III		1454±127.5	1347±118.1	1412±104.3 [b]	1283±137.3 [b]	1077±114.6 [c]
Group IV		1616±318	1497±329.1	1173±240.1 [c]	1029±223.8 [c]	872±177.2 [d]
ANOVA	F	5.275	2.389	17.06	30.74	36.83
	P value	0.1343	0.075	<0.05*	<0.05*	<0.05*

GP I= Hydroxyapatite Nano- paste group. Gp II= Chitosan Nano- paste group.

Gp III=fluoridated group. Gp IV= control group.

* The same superscripts in the same row indicate that there was no significant difference
* The different superscripts in the same row indicate that there was a significant difference.
* Significant < 0.05 level.

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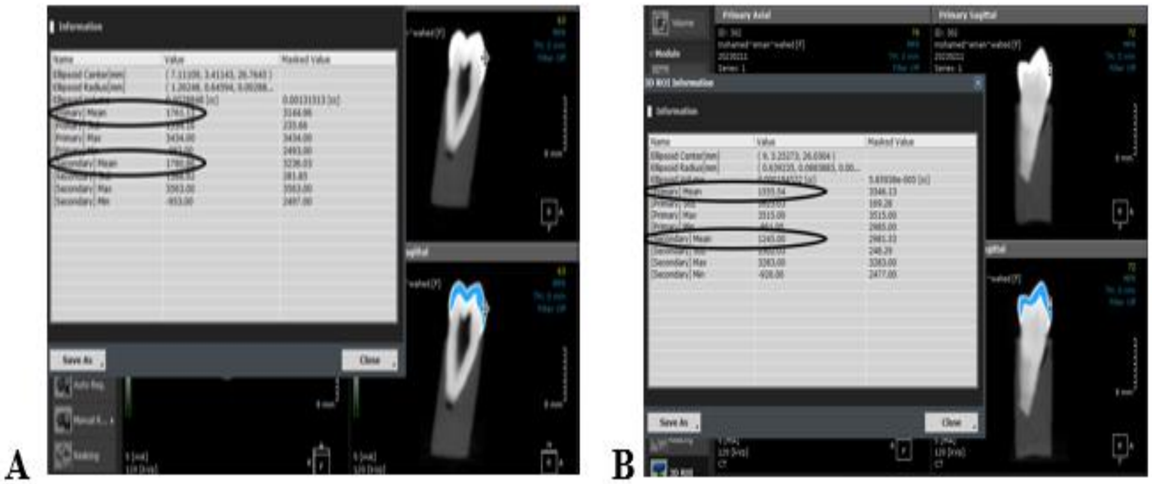

Fig 2: Evaluation of mineral density using On Demand 3D software. (A), n-HA (B), n-CS.

Table (2): Comparison between the mean values of the surface roughness among study groups.

Study group \ Surface roughness		Baseline	Post demineralization	7 days	14 days	21 days
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Group I		0.655±0.27	0.88±0.24	0.724±0.12 [a]	0.636±0.24 [a]	0.684±0.18 [a]
Group II		0.79±0.11	1.143±0.213	0.918±0.158 [b]	1.317±0.191 [b]	1.812±0.16 [b]
Group III		0.8013±0.19	0.976±0.23	0.9123±0.18 [b]	1.409±0.196 [b]	1.89±0.37 [b]
Group IV		0.678±0.167	0.866±0.041	1.083±0.101 [c]	1.75±0.043 [c]	2.614±0.08 [c]
ANOVA	F	2.976	4.619	21.73	130.7	247.3
	P value	0.063[ns]	0.0511[ns]	<0.05*	<0.05*	<0.05*

* GP I= Hydroxyapatite Nano- paste group. * Gp II= Chitosan Nano- paste group.

* Gp III=fluoridated group. * Gp IV= control group.

* The same superscripts in the same row indicate that there was no significant difference

* The different superscripts in the same row indicate that there was a significant difference.

* Significant < 0.05 level. *ns: no statistically significant difference.

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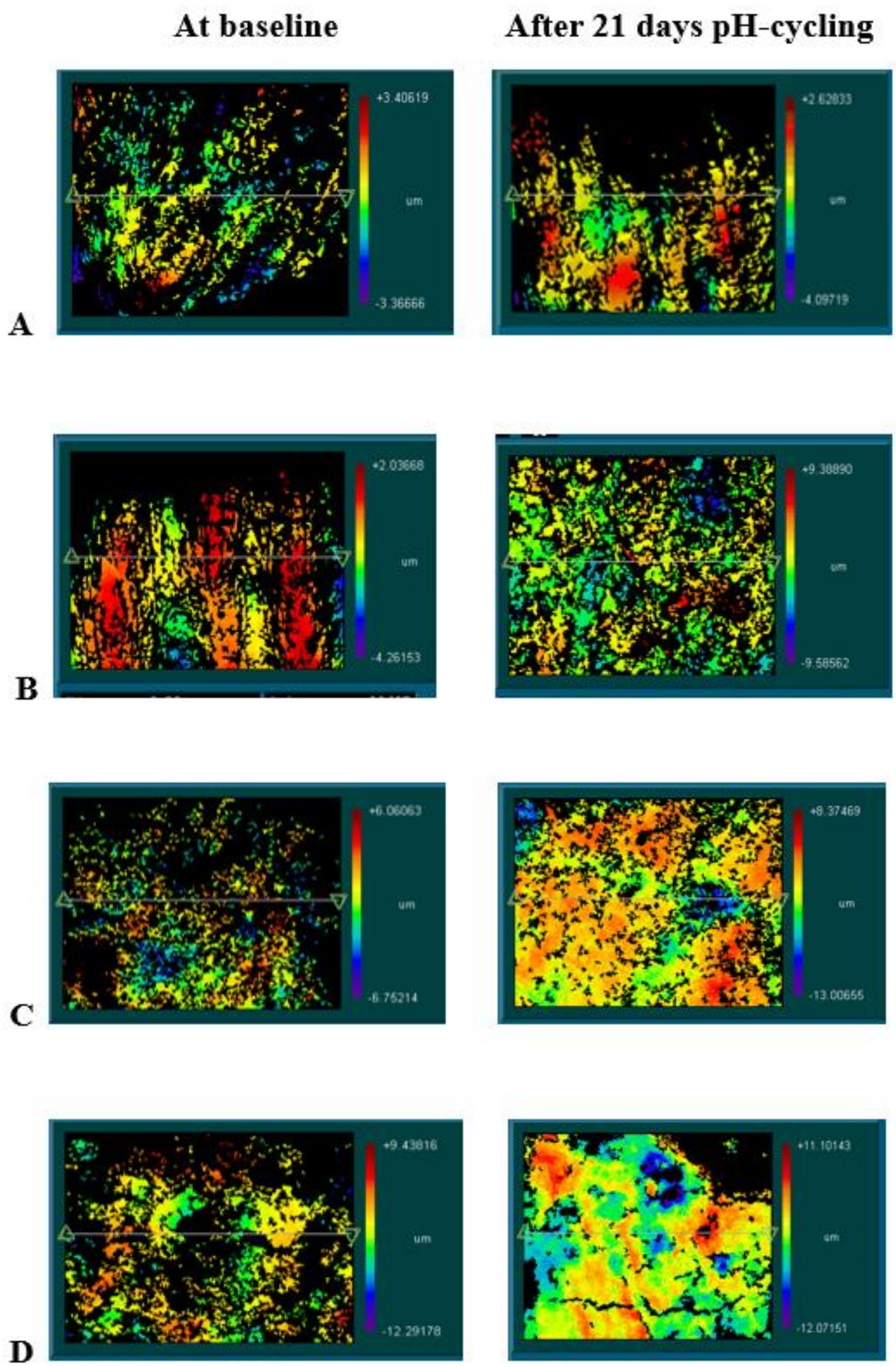

Fig 3: Three-dimensional (3D) images of the surface roughness from each group using ZYGO Maxim-GP 200 profilometer. (A), n-HA (B), n-CS (C) fluoride (D) control.

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DISCUSSION

Efforts have been directed in recent years to develop innovative biomaterials that can provide the best outcomes in enhancing the remineralization of early enamel lesions [16, 32]. Therefore, the aim of the present study was to evaluate and compare the remineralization potential of nano hydroxyapatite and nano chitosan on white spot lesion of perment teeth. According to the result of the study, the null hypothesis was rejected.

In this study, Premolars were selected not only for easy collection but also for the elimination of variables as possible, also the middle third of the buccal surfaces of the teeth was currently chosen to standardize the enamel thickness, type, and prism orientations [33, 34]. Moreover, these premolars were collected from patients in the age group 12-18 years because therapeutic management with fixed orthodontic appliances is usually implemented in this age group and to avoid age-related changes of enamel [35].

In the present study, artificial saliva was chosen as a storage medium to resemble oral circumstances [16]; this coincided with the findings of **Raum et al.** [38], who confirmed that artificial saliva had not changed the elastic properties of enamel slabs stored in it for 21 days using the quantitative time-resolved scanning acoustic microscopy.

In the current study, a modified pH cycling model was adapted from Karlinsey [27] rather than using the conventional pH-cycling approach; this study's pH cycling protocol was considered a good mimic of the pH changes occurring in the oral environment [39]. The demineralization solution persists for four hours in this pH-cycling model, which is approximately similar to the number of times an oral cavity is subjected to acid challenges over 24-hours [16]. Furthermore, the remineralizing paste was applied on the enamel surface in a circular motion using a micro brush for 3 minute to mimic the daily oral prophylaxis conducted by the individual[37].

A 21-day pH cycling protocol was chosen in this study to evaluate the remineralization potential of the remineralizing agents; this agreed with **Vashisht et al.**[40], **Soares et al.** [41], and **Khanduri et al.** [42], who assessed the remineralization potential of various remineralizing agents over a period ranging from 7 to 14 days and concluded that the extent of remineralization is dose-dependent and improved with the increase in exposure time.

CBCT was used to evaluate the mineral density of enamel in this study since CBCT is a reliable, non-invasive diagnostic imaging technique that provides definite, reproducible, highly accurate life-size images without causing damage to the specimens [43]. This is in accordance with **Yavuz et al.**, 2022 who used CBCT to measure the mineral density of mineralized dental tissues (enamel and dentin)[29].

In the present work, the measurement was carried out at five time points using CBCT and the HU scale to compare the enamel mineral density at baseline and after using the tested remineralizing pastes; this coincided with **Hayashi-Sakai et al.** [44] and **Carey** [45]. After demineralization, all groups showed a decrease in the mineral density of enamel, with no statistically significant difference between study groups; this was attributed to the creation

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of initial white spot lesions, which have less mineral content at the surface layer when compared to sound enamel. This is in line with **Huang et al.**[46] and **Mehta et al.** [39].

After 7days of remineralization, all experimental groups except the control group showed an increase in enamel mineral density when compared to the demineralization stage; this could be elucidated by the remineralizing abilities of the different agents used in the study. This is in concert with previous studies performed by **Golpayegani et al.**[47], **Swarup et al.** [48], and **Kiranmayi et al.** [49]. While this finding contradicts the results of **Al-Ward and Radhi** [50], who found an increase in the mineral density in all groups, even the control group, during the remineralization stage; this may be due to the use of distilled water in the negative control group.

The highest mean value of the mineral density was recorded in n-HA-paste group compared to all groups; this may be linked its smaller particles, which definitely assist in its ability to penetrate through the enamel's surface and continually fill the porosities of the artificial carious lesion [51]. This was in line with the findings of **Huang et al.**[46], **Nozari et al.**[15], and **Juntavee et al** [16] who evaluated the remineralization potential of n-HA on enamel and found that n-HA toothpaste had greater efficacy for enamel remineralization than amine fluoride.

On the contrary, the results of the present study disagree with **Itthagarun et al.** [14] **and Najibfard et al.**[52], who demonstrated that n-HA promotes mineral deposits on the external surface of the lesion rather than its interior. The highly mineralized upper surface may hinder mineral ion diffusion into deeper parts of a lesion. This could Tbe explained by the time span of the study carried out by **Itthagarun et al.** (10 days), which may be too short to determine the remineralization potential of n-HA. In addition, the difference in experimental design used by **Najibfard et al.** may explain this conflict as they carried out a randomized, double blind, crossover study.

Regarding n-CS paste group, the mean values of the mineral density were increased significantly than the demineralized stage; these findings coincide with **Effendi et al.**, 2019[17] , **Fitriani et al.**, 2020[18], and **Magalhes et al.**, 2021[19], who used n-CS as a biomimetic, effective remineralizing agent for enamel remineralization.

Concerning fluoridated paste group (positive control), the mean values of the mineral density were slightly less than the previous two groups but better than the negative control group; this may be explained by its ability to form less soluble fluorapatite, which reduces apatite dissolution but is unable to restore the lost mineral structure. This finding concurs with **Newby et al.**[53] and **Nobre-dos-Santos et al.**[54]. On the other hand, there was no statistically significant difference between the mean values of mineral density between fluoridated paste group and nanochitosan paste group after 7 and 14 days of remineralization. These findings are in a line with a previous study carried out by **Wassel** [55], who found no significant difference in surface hardness values among groups treated with nanochitosan and sodium fluoride.

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After 21 days of remineralization, there was a statistically significant difference between the mean values of mineral density between all groups; these findings are not in accordance with with **Vacharangkura et al.**, 2021 [56] who found no statistically significant differences between the effects of n-HA paste and fluoride paste on the early-demineralized enamel under simulated pH cycling. This conflict may be explained by the time span of Vacharangkura et al. study (14 days), the small sample size, and the different evaluation method.

In the present work, the surface roughness (Ra) characteristic was chosen to characterize the nano-scale mechanical properties of the enamel surface, provide an accurate assessment of the depth of the scratches present on the enamel's surface, and indirectly determine the progression of remineralization through MetroPro 7.3.2 analysis software [30].

In the current study, a non-contact technique employing a ZYGO Maxim-GP 200 profilometer was used to measure the surface roughness because it is a simple, reliable, non-damaging technique, completes scans faster, and evaluates the enamel's surface topography both quantitatively and qualitatively [30, 57, 58]. As well, **Salama et al.**, 2020 [59] and **Wakwak et al.**, 2021 [60] used the optical 3D profilometer to evaluate the effect of remineralizing agents on the surface roughness of enamel.

In the present study, all groups showed an increase in the surface roughness of enamel after demineralization with no statistically significant difference between study groups; this may be attributed to decrease mineral content and morphological alterations at the surface layer when compared to sound enamel. These findings are in accordance with **Mohammed et al.**[61] and **Majeed et al.** [62], who reported that the application of demineralization solution led to a significant increase in enamel's surface roughness.

After 7 days of remineralization, all groups except the negative control group showed a decrease in enamel surface roughness when compared to the demineralization stage; this could be clarified by the remineralization potential of the different agents used in the study that could increase the mineral content and decrease the porosity of the enamel surface. This was in line with **Ann** [63] and **Verma**[64].

The current study demonstrated that the most significant reduction in surface roughness reported in n-HA group compared to n- CS and fluoridated paste groups, which showed lower roughness reductions, respectively. Additionally, the surface topography of samples treated with nanohydroxyapatite mimics the surface topography of sound enamel. In contrast, profilometer images revealed that n- CS paste and Fluoridated paste groups exhibited an uneven surface roughness interspersed with smooth enamel areas compared to the uniform roughness seen in control specimens.

These could be explained by the fact that toothpastes containing n-HA fill the microscopic spaces and depressions on the enamel surface due to the tiny size of the particles that comprise the substance. These findings coincided with **Ann** [63], **Wakwak et al.** [60], and **Elmancy et al.** [65], who reported that n-HA paste gives the lowest value for surface roughness after remineralization compared to fluoride paste.

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In the n-CS group and the fluoridated group (positive control), there was no statistically significant difference between the mean values of surface roughness after 7, 14, and 21 days of remineralization. These findings agreed with a previous study carried out by **Magalhes et al.** (19), who found that the application of 0.05% sodium fluoride and nanochitosan suspension may have protected the enamel surface and reduced demineralization in smooth enamel areas, despite not being able to prevent the increase in surface roughness.

Regarding the limitations of the study, in vitro remineralization significantly differs from the dynamic, complex biological system that exists in the oral cavity in vivo. In addition, the pH-cycling model does not perfectly represent the oral circumstances, in which the pH frequently changes, as it depends on the person's eating habits, oral hygiene routines, fluoride usage, the quantity and quality of saliva, and biofilm. Furthermore, there is a lack of a bacterial model that replicates the biological oral conditions.

CONCLUSION

After 7 days of treatment, all the remineralizing agents used in this study were effective in the remineralization of artificially white spot lesions. After 14 and 21 days, only n-HA - paste still had a remineralizing effect. n-HA paste has the highest remineralization potential compared to n-CS paste and fluoridated paste.

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Authors' contributions:

S.W.E. participated in the study design, collected the data and drafted the manuscript. E.H.M. participated in designing the study, collecting data, and revised the manuscript. E.I.A. contributed to the study design, gathering data, and writing the manuscript. All authors reviewed and approved the final manuscript.

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Availability of data and materials

On reasonable request, the datasets utilized and/or analyzed during the present study are accessible from the corresponding author.

Declarations:

Ethical approval and consent to participate

Ethical approval for this study was obtained from the ethical committee (REC), Faculty of Dentistry, Tanta University (Code PED-02-22-4) in accordance with the ethical guidelines outlined in the 1964 Helsinki Declaration and its subsequent revisions. Informed written consent from parents was attained to use their children's extracted teeth in the research.

Consent for publication:

Not applicable.

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Competing interests

The authors declared no conflict of interest relevant to this article.

Abbreviations

n-HA: nano-hydroxyapatite

n-CS: nano-chitosan

MID: Minimal Intervention Dentistry

CBCT: cone beam computerized tomography

HU: Hounsfield unit

ROI: Region of Interest

CSI: coherence-scanning interferometry

ANOVA: a one-way analysis of variance

Footnotes:

1) Nano-Gate Company. Nasr city (Cairo, Egypt)
2) Nano-Gate Company. Nasr city (Cairo, Egypt)
3) Colgate Calci-Lock, Colgate-Palmolive Company New York City

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