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IMPACT OF ACCELERATED ARTIFICIAL AGING ON SURFACE MICROHARDNESS AND ROUGHNESS OF NATURALLY NANO-TREATED INITIAL CARIES-LIKE LESIONS: (AN IN-VITRO STUDY)

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ABSTRACT

Aim: This study investigated the effect of remineralization potential and surface roughness of nano chicken eggshell gel (CESG) or solution (CESS) on initial enamel caries lesion after thermocycling.

Materials and methods: A total of 50 caries-free human premolars were randomly divided into five groups (n=10): group(I) (positive control): No treatment was done to the sound enamel. Group (II):(negative control): The enamel surface underwent demineralization and received no treatment. Group III (GCM): After demineralization, the teeth were treated with (GCM). Group IV (CESG): After enamel demineralization, the teeth were treated with (CESG). Group V (CESS): After demineralization, the teeth were immersed in (CESS). The remineralizing agent was applied twice daily for two minutes each time for 30 consecutive days. All groups underwent 10000 cycles of thermocycling. Surface microhardness and surface roughness were measured for all groups before and after thermocycling.

Results: (CESG) recorded higher statistically significant microhardness values than (CESS), while it reported lower statistically significant surface roughness values than (CESS) after accelerated aging**.**

Conclusions: (CESG) exhibited a promising therapeutic potential in treating (WSLs) due to its natural biogenic composition. Remineralization using (CESG) improved and maintained the microhardness and surface roughness more effectively than (CESS).

Clinical significance: Nano chicken egg shell powder has a clinical potential to remineralize and restore the enamel smoothness of subsurface enamel lesions.

KEYWORDS: Nano-chicken egg shell, demineralized enamel, thermocycling, surface microhardness, surface roughness

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INTRODUCTION

Initial caries lesions (ICLs), commonly known as white spot lesions (WSLs), occur when cariogenic bacteria produce acids that reduce the pH level at the tooth surface. Acid ions penetrate deeply into the prism sheath porosities, creating subsurface lesions while leaving the surface intact, as remineralization occurs preferentially at the surface due to elevated quantities of Ca and HPO4 ions, fluoride ions, and buffering by salivary secretions.[1] The initial enamel caries lesions are nearly hard and may or may not have a similar surface texture compared to the neighboring sound enamel. Over time, the demineralization process compromises the hardness of the WSLs.[2]

Remineralization can be accomplished by replenishing minerals in demineralized enamel or forming amorphous mineral precipitates in the interrod and intercrystal spaces. Furthermore, remineralization can happen spontaneously through saliva or by utilizing therapeutic agents [3]

Bioactive substances like casein phosphopeptide–amorphous calcium phosphate (CPPACP), which comes from milk products, have been shown to successfully treat these early enamel carious lesions in vitro and in vivo by replenishing the calcium and phosphate ions that have been lost.[4] The CPP-ACP has been delivered through a variety of media, such as mouth rinses, sugar-free lozenges, chewing gum, topical creams, and water-based mousses.[5]

(CESP), which is regarded as an increased bioavailable calcium source that may help to reduce enamel demineralization and have remineralization potential for the (WSLs). Furthermore, the CESP was used to treat several human health issues, including osteoporosis.[6]In addition to its content of calcium carbonate (94%), it also includes trace amounts of magnesium carbonate(1%), calcium phosphate(1%), and organic materials(4%).[7,8]

Surface hardness is a mechanical property of teeth that is correlated with the mineral content of the dental tissue. It indicates resistance to piercing, scraping, and indentation. Surface hardness increases resistance to surface invasion via mineral content and micro integrity, and it is linked to enamel demineralization. The amount of calcium and phosphate in enamel can be estimated indirectly by enamel microhardness.[9,10] Surface roughness is defined as irregularities with a close spacing and coarse texture. Its relevance depends on the measurement scale.[11] Roughness is thought to be a risk factor for extrinsic stain and bacterial adherence. It has been noted to have a distinguished role in the formation of oral bacterial biofilms.[12]

Although multiple studies concluded that (CESP) could be a promising material for improving the mineral content and surface texture of demineralized enamel.[13–17]The effect of aging on the surface roughness and hardness of nano-treated initial caries lesions (ICLs) using (CESP) requires more research before a definitive conclusion can be drawn. Because prolonged exposure to environmental factors, including temperature, salivation, chewing force, and others, can cause the material to deteriorate.[18]The aging of tooth structure or the restorations has been simulated using artificial accelerated aging systems, which replicate the physical and chemical conditions that could partially mimic those found in clinical conditions.[19] Consequently, this study aimed to compare the microhardness and surface roughness of initial caries-like lesions before and after artificial accelerated aging using different remineralizing agents. The null hypothesis postulated that either the use of different remineralizing agents or the artificial accelerated aging did not affect the initial caries-like lesions regarding the surface roughness and the microhardness.

MATERIALS AND METHODS

All materials, Composition, Preparation, and Manufacturer were listed in Table (1).

TABLE (1) Materials, Composition, Preparation and Manufacturer.

Martial	Composition	Preparation	Manufacturer
Nano chicken egg shell solution	90-95% calcium carbonate, less than 1% calcium phosphate, less than 1% magnesium carbonate, 4% organic matrix, 0.01% to about 3% fat content and about 0.2% to 3% protein and distilled water.	15 grams of eggshell powder was dispersed in 500 ml of distilled water to prepare a 3% eggshell nanosuspension.	Nano Gate company. Egypt
Nano chicken egg shell gel	90-95% calcium carbonate, less than 1% calcium phosphate, less than 1% magnesium carbonate, 4% organic matrix, 0.01% to about 3% fat content and about 0.2% to 3% protein, 120 ml DH2O(deionized water), 12 gm of Hydroxypropyl methylcellulose.	3% eggshell nano-gel, 3.6 grams of eggshell nanopowder were dispersed in 120 ml of deionized water (DH_2O). Then, 12 grams of Hydroxypropyl Methylcellulose (Loba CHIME, India) were gradually and gently sprinkled over the solution while maintaining a mild temperature and stirring vigorously to achieve a homogeneous gel.	Nano Gate company. Egypt
CPP-ACP (GC mousse)	Pure water, glycerol, zinc oxide, phosphoric acid, flavoring, D-sorbitol, CMC-Na, propylene glycol, silicon dioxide, titanium dioxide, xylitol, RECALDENT (CPP-ACP) Guar gum, propyl and butyl p-hydroxybenzoates		GC Europe N.V. Lot number:2142148
Demineralizing solution	0.244 gm of calcium chloride, 3ml acetic acid, 0.343 gm sodium phosphate and 800 ml deionized water.(pH= 4.2).	CaCl2 – 0.244 gm of calcium chloride in 800 ml deionized water,NaH2PO4 - Sodium Hydrogen Phosphate -0.343 gm in 800 ml of deionized water and acetic acid - 3ml acetic acid in 800 ml deionized water.Then the pH is adjusted by adding sodium hydroxide until reaches 4.2 PH).	Laboratory prepared at faculty of dentistry, Ain shams University.

Sample Size Calculation:

Based on prior research findings, sample size was calculated using G*Power version 3.1.9.7.[18] A power analysis was conducted to ensure sufficient power to use a two-sided statistical test to reject the null hypothesis that there is no difference between groups. By using an alpha level of (0.05) and a beta level of (0.1), the power is 90%, and the effect size (d) is (1.025). The expected sample size (n) was (50), i.e., ten samples per group.

Preparation of materials:

Production and characterization of Nano (CESP)

By following the guidelines provided by the World Property intellectual organization, the calcination method produced the (CESP).[13] Twenty chicken eggs were utilized. Eggshells were cleaned with distilled water after removing the egg contents. Afterward, the egg shells were immersed in a hot water bath set at 100°C for 10 minutes to ease

the process of removing the membrane. Then the eggshells were smashed with a sterile mortar and pestle, followed by heating in a muffle furnace at 1200°C.[20] The dry powder was processed using a ball milling machine for 10 hours at 350 rpm at 3-minute intervals to produce nano-sized CESP with an average size of 300–450. Using a Thermo-Fisher F200i High-Resolution Transmission Electron Microscope operating at 200 kV, the crystalline phases present in the raw and calcined CESP were characterized. A 2θ range of 20° to 80° was covered by the XPERTPRO Powder Diffractometer device, which was also used to obtain an XRD pattern with a wavelength (Kα) of 1.54614° and a minimum step size of 0.001°. (Nano Gate; 25 Ibrahim Abou El Naga St., Abbas El Akkad extension, beside ENPPI, Nasr City)

Preparation of Gel form from the nano (CESP)

To prepare 60 ml of 3% eggshell nano-gel, 3.6 grams of eggshell nano powder were dispersed in 120 ml of deionized water (DH_2O). Then, 12 grams of Hydroxypropyl Methylcellulose (Loba CHIME, India) were gradually and gently sprinkled over the solution while maintaining a mild temperature and stirring vigorously to achieve a homogeneous gel.

Preparation of solution form from the nano (CESP)

To prepare a 3% eggshell nanosuspension, 15 grams of eggshell powder was dispersed in 500 mL of distilled water.

Specimens Preparation:

Fifty human premolar teeth that were extracted for orthodontic treatment were collected from the Ain Shams University Faculty of Dentistry's Department of Surgery. Before using their teeth, each patient gave their informed consent. To be eligible for inclusion, the teeth should be free of hypoplasia, cavities, enamel cracks, developmental anomalies, and restorations. To remove any soft tissue debris, the teeth were cleaned for six minutes using an ultrasonic cleaner. The labial enamel surfaces were cleansed with fluoride-free pumice and then ground flat using silicon carbide paper (grades 600–1200) while being constantly irrigated with water to preserve precision and prevent overheating them. Using a low-speed diamond saw (STRONGWT 90 micromotor / FTMM01/ Multipurpose micromotor-65W/ CHINA) with continuous water irrigation, the coronal and radicular sections of the collected teeth were separated. Coronal sections of each tooth were then placed in self-curing acrylic resin blocks, with the buccal sides pointing upward. The teeth were then

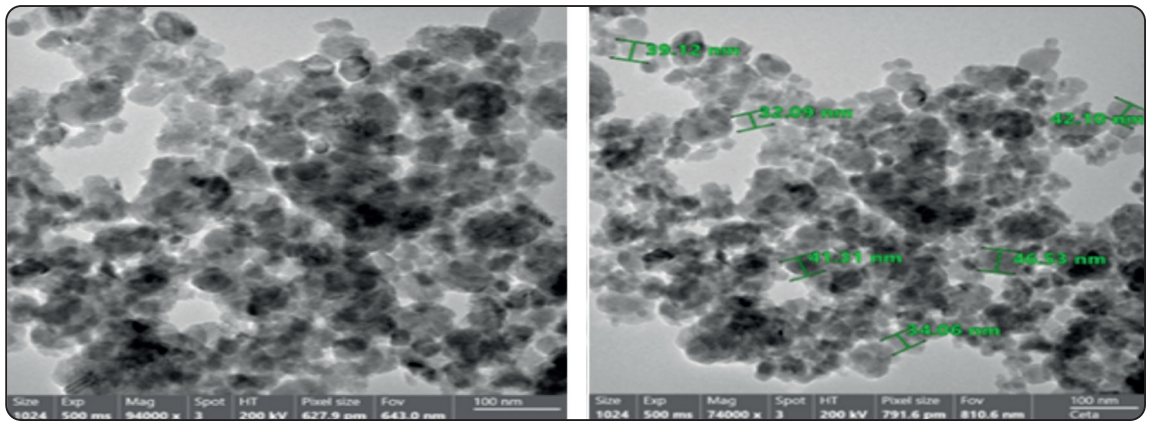

Fig. (1) TEM images of the eggshell's nanoparticles at different magnifications (74000x,94000x).

dried, and a window of (4x4 mm) was demarcated at the center of the buccal surfaces using self-adhesive labels. All surfaces were covered with acid-resistant nail polish, then the labels were removed to create a small, exposed enamel window. After preparation, the teeth were stored in distilled water at 4˚C not more than one month until use, with the water being changed weekly to prevent bacterial growth.[21]

Specimens grouping

Fifty human premolars were randomly divided into five groups (n=10) according to the surface treatment. G1: Positive control (sound enamel), G2: Negative control (ICLs), G3: (ICLs) treated with GC mousse (GCM), G4:(ICLs) treated with nano chicken egg shell gel (CESG), and G5: (ICLs) treated with nano chicken egg shell solution (CESS). Then, each group was further subdivided, according to the performed test, into two subgroups (n=5) (five specimens were used for the surface microhardness test, and the other five specimens were subjected to surface roughness testing).

Creation of artificial enamel caries-like lesions

Teeth of groups 2,3,4,5 were removed from the distilled water and placed individually in test tubes filled with 30 milliliters of freshly prepared demineralizing solution, which was changed every day to avoid saturation at 37°C for 96 hours.[22]Specimens were then removed, cleaned with deionized water for five seconds to counteract the effects of the demineralizing solution, and then placed back in an incubator (BTC / B1020 /621023080/EGYPT) set to 37 °C until the next step of the study. [23]

Application of remineralizing agents on (ICL)

Group 1 (positive control): The specimens received no treatment and were stored individually in deionized water (changed daily) in an incubator (BTC / B1020 /621023080/EGYPT) set to 37 °C for 30 days.

Group 2 (ICLs) (negative control): The specimens that underwent enamel surface demineralization and received no treatment were stored individually in deionized water (changed daily) in an incubator (BTC / B1020 /621023080/ EGYPT) set at 37 °C for 30 consecutive days.

Group 3 (GCM): After creation of (ICLs), the specimens were received CPP-ACP (GC Mousse) twice daily for two minutes each time for 30 consecutive days. After application, the samples were rinsed with deionized water for one minute and then stored individually in deionized water at 37°C

Group 4 (CESG): After creation of (ICLs), the specimens were received (CESG) twice daily for two minutes each time for 30 consecutive days. After application, the specimens were rinsed with deionized water for one minute and then stored individually in deionized water at 37 °C. A fresh batch of eggshell gel was applied daily.

Group 5 (CESS): After creation of (ICLs), the specimens were received the specimens were immersed in (CESS) twice daily for two minutes each time for 30 consecutive days. After immersion, the specimens were rinsed with deionized water for one minute and then stored individually in deionized water at 37 °C. A fresh eggshell solution was used daily.

Surface Microhardness Testing

Vickers microhardness testing was carried out utilizing a Wilson Tukon Microhardness Tester (Buehler, Germany). The test was carried out at a load of 100g for 20 seconds.Three indentations were made in the center of each specimen. To achieve the desired results, the physical quality of the indenter and the accuracy of the applied force must be carefully managed. After removing the load, concentrate the indentation with the magnifying eyepiece and measure the two impression diagonals to the nearest 0.1-μm with a micrometer, then average them. Vickers hardness (HV) is computed

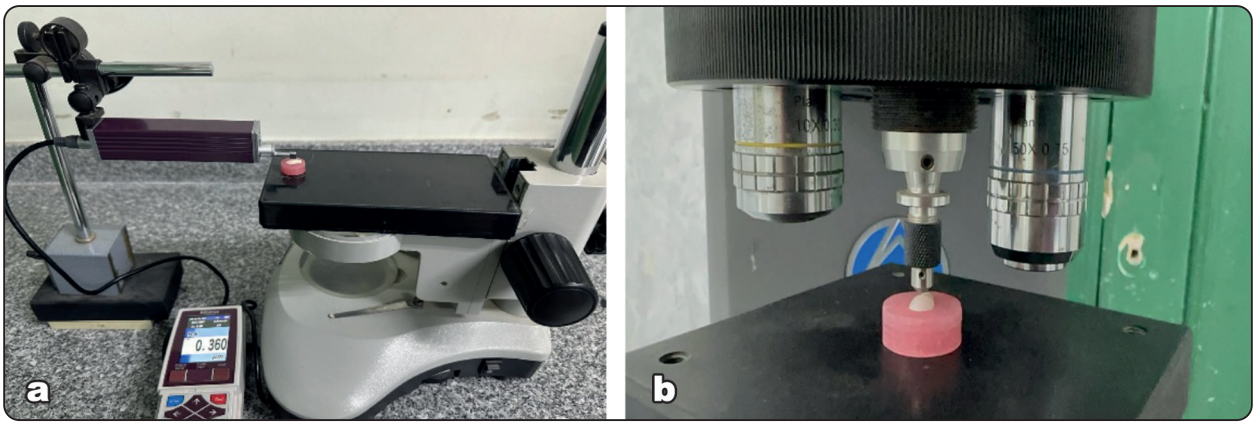

Fig. (2) (a) Surface roughness tester (b) Microhardness tester.

as HV = 1854.4L/d2, where the load L is in gf and the average diagonal d is in μm. This yields a hardness value of gf/μm2.In practice, numbers are presented without indicating the units.

Surface roughness Testing:

Surface roughness testing was done using a contact surface roughness tester (SJ- 210, Mitutyoyo Japan). Each specimen is fitted to the specimen holder in which the surface is to be measured in the horizontal direction, and then the specimen holder moves in a vertical direction up to the specimen surface just touch the measuring tip. Device calibration is done using the standard calibration specimen before use. Testing parameters: Measuring distance 12 mm, Measuring Speed 0.5 mm/s. Returning 1mm/ measuring force 0.75 mN, Stylus profile: tip radius 2 micron, tip angle 60-degree. Evaluation parameter Ra values expressed in microns. three readings are recorded for each specimen at a distance of 500 microns each.

Thermocycling

All specimens underwent thermocycling. Samples from each group were wrapped in gauze with color-coded strings. All groups underwent 10000 cycles of thermocycling (SD Mechatronic thermocycler, Germany), alternating between 30-second immersions in cold water at 5°C and hot water at 55°C, each with a 10-second dwell time. The surface microhardness and surface roughness were measured before and after the thermocycling.

Statistical analysis:

The recorded data were analyzed using the statistical software for social sciences, version 23.0 (SPSS Inc., Chicago, Illinois, USA). The quantitative data were given as median with inter-quartile range (IQR) (non-parametric data) and mean and standard deviation (parametric data). Kolmogorov-Smirnov and Shapiro-Wilk tests were used to determine the normality of the data. The following experiments were conducted: When comparing more than two means, a one-way analysis of variance (ANOVA) was used. A pairwise comparison between each two groups was performed using the Post Hoc test. Tukey's test was used to compare numerous variables in parametric data. The confidence interval was set at 95%, with a 5% acceptable margin of error. So, the p-value was considered significant as follows: Probability P-value ≤ 0.05 was considered significant

RESULTS

1. Surface Microhardness:

Mean, standard deviation (SD) values for the effect of different surface treatments on mean surface microhardness of artificial enamel lesions compared to sound enamel before and after

thermocycling are shown in Table (2). Before thermocycling, the (CESG) group recorded the highest statistically significant value compared to other treatments and showed an increase in the mean microhardness value closer to the sound enamel value. The (CESS) group showed the lowest statistically significant mean surface microhardness value. After thermocycling, both (CESG and GCM) recorded significantly higher values than (CESS) with no statistically significant difference between them.

The results of comparing the mean percentage change in surface microhardness of the tested groups after thermocycling were shown in table (3). There was a statistically significant difference between all groups after thermocycling. The highest statistically significant percentage change in surface microhardness values was recorded in (CESG) group, with no statistically significant with the sound enamel group. The lowest percentage change value was recorded in (GCM) group.

2. Surface roughness

Mean, standard deviation (SD) values for the effect of different surface treatments on mean surface roughness of artificial enamel lesions before and after thermocycling were shown in Table (4). Before thermocycling, there was no statistically significant difference between all groups. After thermocycling, the (GCM & CESS) groups recorded higher statistically significant values than (CESG) group, with no statistically significant difference between them.

The results of comparing the mean percentage of change in surface roughness of the tested groups after thermocycling are shown in (Table 5). The highest statistically significant value surface was recorded in the (GCM &CESS) groups, with no statistically significant difference between them. The (CESG) group showed the lowest value of surface roughness change with no statistically significant with the sound enamel group.

TABLE (2) Mean, standard deviation (SD) values for the effect of different surface treatments on mean surface microhardness of artificial enamel lesions before and after thermocycling.

Microhardness (HV)	(+ve control)	(-ve control)	GCM	CESS	CESG	F-test	p-value
Before	270.32±31.74A	192.23±22.26C	242.99±23.60B	198.76±24.19C	271.11±22.10A	34.454	0.001*
After	242.28±28.89A	174.75±18.55B	236.55±26.82A	184.80±23.13B	235.84±24.73A	25.706	0.001*

Data are expressed mean± and standard deviation. Different capital letters indicate significant difference at (p≤0.05) among means in the same row

TABLE (3) Mean, standard deviation (SD) values of change in surface microhardness after the thermocycling.

Microhardness (HV)	(+ve control)	(-ve control)	GCM	CESS	CESG	F-test	p-value
Mean±SD	28.39±4.39A	17.48±4.67B	8.10±1.90C	15.92±2.69B	35.43±4.79A	7.801	0.001*
Percentage (%)	10.33±1.46A	8.58±2.26B	3.44±0.81C	7.92±1.23B	13.02±1.63A	5.139	0.001*

Data are expressed mean± and standard deviation. Different capital letters indicate significant difference at (p≤0.05) among means in the same row

TABLE (4) Mean, standard deviation (SD) values for the effect of different surface treatments on mean surface roughness of artificial enamel lesions before and after thermocycling.

Roughness (mm)	(+ve control)	(-ve control)	GCM	CESS	CESG	F-test	p-value
Before	0.36±0.22	0.46±0.16	0.46±0.14	0.40±0.07	0.34±0.15	1.944	0.113
After	0.55±0.16B	0.64±0.21AB	0.79±0.25A	0.77±0.09A	0.46±0.17B	3.666	0.009*

Data are expressed mean± and standard deviation. Different capital letters indicate significant difference at (p≤0.05) among means in the same row.

TABLE (5) Mean, standard deviation (SD) values of change in surface roughness after thermocycling.

Roughness (mm)	(+ve control)	(-ve control)	GCM	CESS	CESG	F-test	p-value
Mean±SD	0.21±0.06B	0.19±0.05B	0.33±0.05A	0.37±0.03A	0.15±0.03B	4.55	0.003*
Percentage (%)	48.83±9.06B	37.50±8.10B	80.91±13.56A	98.39±10.40A	45.65±7.67B	6.825	0.001*

Data are expressed mean± and standard deviation. Different capital letters indicate significant difference at (p<0.05) among means in the same row

DISCUSSION

White spot lesions (WSLs), which appear as milky white opacities without cavitation on the smooth tooth surface, are subsurface enamel porosity caused by carious demineralization.[24] Modern dental treatment is constantly evolving towards non-invasive restorative treatments,[25,26] This conservative concept focuses on early diagnosis of carious lesions, remineralization of tooth surfaces, and preservation of the surrounding tooth structure.[13,27]

Fluoride has long been the cornerstone of noninvasive care of early enamel carious lesions, but its potential to enhance net remineralization is restricted, owing to its reliance on the availability of calcium and phosphorus ions. In recent years, a greater knowledge of physicochemical and biological mineralization mechanisms has encouraged the development of many remineralization strategies that go beyond fluoride-mediated remineralization.[28]

This prompted the quest for new remineralization technologies based on several agents that have demonstrated efficacy in this field, such as (CPP-ACP).[16] (CPP- ACP) is offered as a substitute remineralizing agent that remains supersaturated in the oral environment and stabilizes calcium phosphate. The high levels of calcium phosphate in the biofilm would therefore help the tooth structure and remineralization would take place.[29]

For cost-effectiveness and economic reasons, more natural calcium and phosphate sources are always being sought. As a result, earlier research was conducted to determine how to make recycling of chicken eggshells (CES), which are regarded as inexpensive, abundant, and safe when compared to current materials used for this purpose.[30] (CESP) has been identified as a promising biomimetic material, due to its well-documented efficacy in inducing enamel remineralization, as well as its broad use in several medical and dentistry sectors.[31]

In this study, premolars were collected with an age range (18-25 years old) that were extracted for orthodontic reasons to standardize the enamel thickness and tooth size. To standardize the type, thickness, and orientations of the enamel prisms, the middle third of the buccal surfaces of the teeth was selected.[32]

Replication of (ICLs) lesions while leaving the superficial enamel layer intact was achieved by using weak organic acetic acid, which was added to the demineralizing agent. Furthermore, the inclusion of calcium and phosphate in the demineralizing solution served to protect the superficial enamel layer while promoting mineral loss from the subsurface layer.[13] The samples were maintained in the demineralization solution for 96 hours at 37°C in an incubator to produce a depth lesion of approximately 150 μm.[15] Thermal cycling is a dynamic test that replicates the effects of cold and hot foods in the oral environment. The goal of this test is to induce unbalanced tension within the object by repeatedly changing the temperature, hence accelerating the material's aging process. To simulate one year of aging, a total of 10,000 cycles were used.[18]

The Vickers micro-hardness test was chosen to determine the hardness of the enamel surface. It is considered a non-destructive, simple, and rapid approach for assessing nonhomogeneous materials with microstructure and susceptibility to surface cracking.[31] It allows hardness determination in the same sample before and after the treatments, which decreases the experimental error.[33] Surface roughness measurements were performed using a contact surface roughness tester, which has the benefit of measuring surface roughness precisely and accurately without requiring extra measurements..

According to the results of this study, the null hypothesis was rejected as the results showed that different tested remineralizing agents and the artificial accelerated aging affected the enamel microhardness and surface roughness of (ICLs). Regarding Vickers micro-hardness measurements before thermocycling, the highest statistically significant value was reported for (CESG) group, while the (-ve control and CESS) groups showed the lowest values with no statistically significant difference between them, as shown in Table (2). This might be due to high alkaline pH and an abundance of bioavailable calcium of (CESG), which has the potential to improve remineralization. This elevated pH promotes the ionic activity of anions like hydroxyl and phosphate ions. As a result, more ions are available for remineralization of the enamel surface.[11,31]

The (ILCs) group (-ve control) showed the lowest statistically significant value.it could be explained that during the demineralization process, the minerals on the surface of enamel gradually dissolved and fell off, which led to a significant decrease in the surface microhardness.[18,27] Although (CESS&CESG) contain the same active ingredients and mineral concentration, the difference in dosage form; gel versus solution, resulted in enhancing remineralizing protentional of the gel over the solution one. The remineralizing nanoparticles in the gel form could be stabilized and maintained over the area to be treated during all treatment period. On the other hand, the nanoparticles in the solution could be aggregated and settled away from the demineralized area or the aggregated nanoparticles might have lower penetration and remineralizing efficacy to the depth of the lesion.

This finding was in agreement with **Abd el-monem M et al**, who concluded that the (CESG) group exhibited the statistically significant highest mean value of microhardness after PH cycling compared with other treated groups.[27]while it was inconsistent with **Mohamed et al**. who concluded that eggshell powder solution showed a higher statistically significant value than its control. **Elbahrawy et al**, suggested that the (CESP) solution or slurry had higher statistical significant value compared to demineralized enamel.[16] **Sarhan et al,** in randomized clinical trial found no significant difference between the effect of ESP and GC Tooth Mousse in remineralization of WSLs when assessed using DIAGNOdent.[30] Differences in results might be due to different methodology as differences in nanoparticle size, concentration, and treatment period, or different assessment tools.

After the accelerated aging, all groups showed significant change in microhardness results. The surface microhardness of the control group decreased by 10.33%, the demineralized group decreased by 8.58%, (GCM) group decreased by 3.44%, the CESS group decreased by 7.92% and the CESG group decreased by 13.02% as shown in Table (3). The lowest statistically significant change was observed in (GCM) group. This could be because CPP in (CPP ACP) stabilizes calcium and phosphate ions at the tooth surface, preventing them from transforming into crystalline form and functioning as a reservoir for ions released from nanocomplexes and deposited into apatite crystals, protecting minerals during accelerated aging.[11,20]

Regarding surface roughness measurement, the results in this study revealed no statistically significant difference among all tested groups before thermocycling, with notice that the (CESG) group restored the baseline sound enamel smoothness. this was consistent with **Malaghan and Nagaveni,** who concluded that there were no significant differences between both remineralizing agents used i.e (eggshell paste and CPP-ACP) on surface roughness of bleached enamel. [17] **Feroz et al.** stated that Eggshell powder (ESP) application reduced the enamel surface roughness of erosive enamel.[34] After thermocycling, the (CESG) group showed the lowest surface roughness values and the lowest percentage change compared to other treated groups. This might be due to the high remineralizing efficacy of supersaturated nano (CESG) and high ionic reactivity of calcium and phosphate ions that close all porosities, healing the demineralized enamel surface. Furthermore, it could resist dissolution after thermocycling, protecting the enamel surface integrity.

CONCLUSIONS

Within the limitations of this study, the following conclusions could be drawn:

1- Chicken egg shell gel (CESG) exhibited a promising therapeutic potential in the treatment of (WSLs) due to its natural biogenic composition and high calcium bioavailability.

2- The Chicken egg shell gel (CESG) was more effective in improving and maintaining the microhardness and surface roughness of initial caries lesions than the Chicken egg shell solution (CESS).

REFERENCES

1. 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. J Conserv Dent [Internet]. 2010 [cited 2025 Apr 14];13:42. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC2883807/

2. Chabuk MM, Al-Shamma AM. Surface roughness and microhardness of enamel white spot lesions treated with different treatment methods. Heliyon. 2023;9.

3. MN H, AR S. Salivary Proteins-A Barrier on Enamel Demineralization: An in vitro Study. Int J Clin Pediatr Dent [Internet]. 2017 [cited 2025 Apr 15];10:10–3. Available from: https://pubmed.ncbi.nlm.nih.gov/28377647/

4. Elkassas D, Arafa A. Remineralizing efficacy of different calcium-phosphate and fluoride based delivery vehicles on artificial caries like enamel lesions. J Dent [Internet]. 2014 [cited 2025 Apr 14];42:466–74. Available from: https://pubmed.ncbi.nlm.nih.gov/24412586/

5. Willmot D. White Spot Lesions After Orthodontic Treatment. Semin Orthod. 2008; 14:209–19.

6. A S, JJ van D, FA M, GJ H, I P, E van der V. Positive effects of a chicken eggshell powder-enriched vitamin-mineral supplement on femoral neck bone mineral density in healthy late post-menopausal Dutch women. Br J Nutr [Internet]. 2002 [cited 2025 Apr 14];87:267–75. Available from: https://pubmed.ncbi.nlm.nih.gov/12064336/

7. Shetty AK, Shivani S. Quantitative assessment of enamel remineralization after treatment with chicken eggshell powder: A scanning electron microscope (SEM) and energy-dispersive x-ray spectroscopy (EDX) study. In: AIP Conference Proceedings. American Institute of Physics Inc.; 2020.

8. Methili Singhal, Sonal Gupta, Vishakha Mittal, Manisha Kaushik. Effect of Chicken Egg Shell Powder and Gallic Acid as Remineralising Agent on Early Enamel Carious

LesionAn In Vitro Study. Indian Journal of Forensic Medicine & Toxicology. 2021;15:2850–7.

9. Alkattan R, Lippert F, Tang Q, Eckert GJ, Ando M. The influence of hardness and chemical composition on enamel demineralization and subsequent remineralization. J Dent [Internet]. 2018 [cited 2025 Apr 14];75:34–40. Available from: https://pubmed.ncbi.nlm.nih.gov/29738789/

10. Gutiérrez-Salazar MP, Reyes-Gasga J. Enamel Hardness and Caries Susceptibility In Human Teeth. Revista Latinoamericana de Metalurgia y Materiales [Internet]. 2001 [cited 2025 Apr 14];21:36–40. Available from: http://ve.scielo.org/scielo.php?script=sci_arttext&pid=S0255-69522001000200007&lng=es&nrm=iso&tlng=en

11. Hassan S, Moharam L. Effect of eggshell powder and nano-hydroxyapatite on the surface roughness and microhardness of bleached enamel. Contemp Clin Dent. 2023;14:62–7.

12. Attia R, Kamel M. Changes in surface roughness of bleached enamel by using different remineralizing agents. Tanta Dental Journal [Internet]. 2016 [cited 2025 Apr 16];13:179. Available from: https://journals.lww.com/tdoj/fulltext/2016/13040/changes_in_surface_roughness_of_bleached_enamel_by.3.aspx

13. Taher HM, Bayoumi RE. REMINERALIZATION OF INITIAL ENAMEL LIKE LESIONS WITH CHICKEN EGG SHELL POWDER SOLUTION VERSUS AMORPHOUS CALCIUM PHOSPHATE [Internet]. Vol. 64, DENTAL JOURNAL. 2018. Available from: www.eda-egypt.org

14. Mony B, Rajesh Ebenezar A V., Ghani MF, Narayanan A, Anand S, Mohan AG. Effect of chicken egg shell powder solution on early enamel carious lesions: An invitro preliminary study. Journal of Clinical and Diagnostic Research. 2015;9:ZC30–2.

15. Mohamed E, Elbahrawy S, Ibrahime El -Dosoky A, Elbahrawy S, Ibrahime El-Dosoky A. Remineralization Potential of Chicken Eggshell Powder in the Treatment of Artificially Induced Enamel Carious Lesion in Permanent Teeth [Internet]. Vol. 65, Dental Journal. 2019. Available from: www.eda-egypt.org

16. Elbahrawy E, El -Dosoky A. Remineralization Potential of Chicken Eggshell Powder in The Treatment of Artificially Induced Enamel Carious lesion in Permanent Teeth. Egypt Dent J. 2019;65:3581–93.

17. Malaghan A, NB N. In vitro comparative evaluation of remineralizing potential of chicken eggshell paste and casein phosphopeptide-amorphous calcium phosphate on surface roughness of bleached enamel surface. IP International Journal of Medical Paediatrics and Oncology [Internet]. 2024;10:87–91. Available from: https://ijmpo.com/article-details/23601

18. Su YW, Zhang C, Liu SS, Pang LY, Lin HC, Zhi QH. Effect of Thermal Cycling Aging on the Surface Microhardness and Roughness of Resin-Infiltrated Enamel Lesions. Sci Adv Mater. 2021;13:356–63.

19. Neres Y, Moda MD, Chiba EK, Briso ALF, Pessan JP, Fagundes TC. Microhardness and roughness of infiltrated white spot lesions submitted to different challenges. Oper Dent. 2017;42:428–35.

20. Bandekar S, Parkhi S, Kshirsagar S, Sathawane NR, Khan WA, Razdan P. The Remineralization Potential of Fluoride, Casein Phosphopeptide-Amorphous Calcium Phosphate, and Chicken Eggshell on Enamel Lesions: An In Vitro Study. Cureus [Internet]. 2025; Available from: https://www.cureus.com/articles/333769-the-remineralization-potential-of-fluoride-casein-phosphopeptide-amorphous-calcium-phosphate-and-chicken-eggshell-on-enamel-lesions-an-in-vitro-study

21. Ajami S, Pakshir HR, Babanouri N. Impact of nanohydroxyapatite on enamel surface roughness and color change after orthodontic debonding. Prog Orthod. 2016;17.

22. Yadav P, Desai H, Patel K, Patel N, Iyengar S. A comparative quantitative & qualitative assessment in orthodontic treatment of white spot lesion treated with 3 different commercially available materials - In vitro study. J Clin Exp Dent [Internet]. 2019 [cited 2025 Apr 14];11:e776–82. Available from: https://pubmed.ncbi.nlm.nih.gov/31636868/

23. Essa M, Mostafa El-Bolok A, Hassan Hammad H. Biomimetic Human Enamel Remineralization Using Calcium Extract from Nano Fish Bone and Eggshell: An In vitro Study. Egypt Dent J. 2023;69:1603–11.

24. Rajendran R, Hussain MS, Sandhya R, Ashik M, Thomas AJ, Mammen RE. Effect of Remineralization Agents on White Spot Lesions. J Pharm Bioallied Sci. 2022;14:S7–12.

25. Senestraro S V., Crowe JJ, Wang M, Vo A, Huang G, Ferracane J, et al. Minimally invasive resin infiltration of arrested white-spot lesions: a randomized clinical trial. J Am Dent Assoc [Internet]. 2013 [cited 2025 Apr 15];144:997–1005. Available from: https://pubmed.ncbi.nlm.nih.gov/23989837/

26. Heymann GC, Grauer D. A contemporary review of white spot lesions in orthodontics. J Esthet Restor Dent [Internet]. 2013 [cited 2025 Apr 15];25:85–95. Available from: https://pubmed.ncbi.nlm.nih.gov/23617380/

27. Abd el-monem MM, Niazy MA, El-Yassaky MA. Remineralization of Early Carious Lesion Using Natural Agents Versus Bioadhesive Polymers. Al-Azhar Dental Journal for Girls. 2021;8:553–60.

28. Grohe B, Mittler S. Advanced non-fluoride approaches to dental enamel remineralization: The next level in enamel repair management. Vol. 4, Biomaterials and Biosystems. Elsevier Ltd; 2021.

29. El Sayed H, Abo elezz A, Fahmy O. Effect of Three Treatment Modalities for White Spot Enamel Lesions on Enamel Surface Roughness and Micro-Hardness. Dental Science Updates. 2022;3:127–37.

30. Sarhan M, El Baz M, Hamza O. Remineralization Effect of Eggshell Powder On Post-Orthodontic White Spot Lesions Compared To Cpp-Acp: A Randomized Controlled Clinical Trial. Egypt Dent J. 2021;67:2701–10.

31. Helal MB, Sheta MS, Alghonemy WY. Comparing the remineralization potential of undemineralized dentin powder versus chicken eggshell powder on artificially induced initial enamel carious lesions: an in-vitro investigation. BMC Oral Health. 2024;24.

32. Bolty HA, El-Olimy GAE, Elbahrawy E. Efficacy of different natural remineralizing agents on treatment of artificially induced enamel caries (An in vitro comparative study). Tanta Dental Journal. 2023;20:307–18.

33. Kamh RA, Niazy MA, El-Yasaky MA. Clinical Performance and Remineralization Potential of Different Biomimitic Materials on White Spot Lesions. Al-Azhar Dental Journal for Girls. 2018;5:349–58.

34. Feroz S, Moeen F, Nisar Haq S. Protective Effect of Chicken Egg Shell Powder Solution (CESP) on Artificially Induced Dental Erosion: An in Vitro Atomic Force Microscope Study. International Journal of Dental Sciences and Research [Internet]. 2017 [cited 2025 Apr 14];5:49–55. Available from: https://www.researchgate.net/publication/319399727_Protective_Effect_of_Chicken_Egg_Shell_Powder_Solution_CESP_on_Artificially_Induced_Dental_Erosion_An_in_Vitro_Atomic_Force_Microscope_Study