International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2020): 7.803

Remineralizing Effect of Nanoegg Shell and Gc Tooth Mousse On Initial Caries Like Lesions in Young Permanent Teeth - An Invitro Study

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Abstract: ***Aim: To evaluate the remineralizing effect of NanoEgg shell powder and GC Tooth Mousse on initial caries like lesions in young permanent teeth-an invitro study. Methodology: Twenty-four extracted premolars were selected. Specimens were immersed in the demineralizing solution for 72 hours to produce artificial carious lesions on the exposed enamel surface. Each crown was sectioned longitudinally in a buccolingual direction through the center of the window into two equal halves: mesial half (control group) and a distal half (experiment group). Teeth were divided into two groups depending on the reminerlizing agent used, Group 1: Nanoegg shell, Group 2: GC Tooth Mousse. Each group was further divided into subgroups depending on the time duration for remineralisation at 7days and 14 days. Surface microhardness was evaluated before demineralization, after demineralization & after remineralisation using Vickers's Microhardness test. Statistical Analysis: Student's t tests, non-parametric, Mann-Whitney U test were used. Results: XRD Analysis showed the average crystallite size of eggshell was 53.26 nm. Samples treated with NanoEggshell and GC Tooth Mousse showed higher microhardness value on 14th day, however no significant difference was seen between 2 time intervals. Conclusion: Both Nanoeggshell and GC Tooth Mousse were effective for remineralization of initial caries-like lesions.***

Keywords: Remineralisation, Demineralisation, GC Tooth Mousse, Egg shell

1.Introduction

Dental caries is one of the most common diseases that has affected the oral health of people around the world. [1] It leads to discomfort and pain, compromising functionality & facial aesthetics. It is an energetic alternating cyclic mechanism which occurs naturally between demineralization and remineralization of the mineral phase on the tooth surface [2]. The predominance of the demineralization process can lead to tooth cavitation. Carious lesion can be progressed or reversed depending on the balance between pathologic factors, bacteria, salivary dysfunction, fermentable carbohydrates, and protection mechanism of antibacterial and remineralization agents in sufficient salivation [3]. The enamel demineralization process is slow in its progression, which permits the possible reversal of mechanism if the process is detected early and managed adequately. Recently, the conservative approach in dentistry has been applied to the remineralization process as an optimal method to reconstruct the tooth structure. Early identification of caries, together with a conservative treatment of the incipient carious lesion [4], can reduce a number of oral health care-related expenses. Recent research has turned to searching for advanced methods to detect caries at the earliest stage in order to make the use of a noninvasive treatment approach possible. Numerous remineralizing agents have been tried, with the intention to regenerate hard tissues.

Egg shell powder (ESP) is a natural source of calcium ions [5]. ESP contains 94% calcium phosphate, 4% organic matter, 1% magnesium carbonate and low concentration of strontium, fluoride, manganese, zinc and copper ions [8]. ESP has the ability to form high quality hydroxyapatite. The minerals of ESP when come in contact with enamel caries-like lesions; they diffuse into the superficial layer and obstruct the surface porosities.

With the development of nanotechnology, a major impact on materials science has been noted. In this century, the production of materials with nanostructures has gained great attention for adsorption, catalytic, biomaterials and optical applications.

Aim:

Thus, the aim of the study was to evaluate the remineralizing effect of NanoEgg shell powder and GC Tooth Mousse on initial caries like lesions in young permanent teeth-an invitro study

Objectives:

- To evaluate the effect of particle size of NanoEgg shell particles on remineralization using X-Ray diffraction Analysis-Diffractometer
- To evaluate the remineralizing effect of NanoEgg shell powder on initial caries like lesions in young permanent teeth by Vickers Microhardness test.
- To evaluate & compare the remineralizing effect of GC Tooth Mousse on initial caries like lesions in young permanent teeth by Vickers Microhardness test.
- To evaluate & compare the remineralizing effect of NanoEgg shell & GC Tooth Mousse with the time period of 7 days & 14 days.

2.Materials & Methodology

I. Preparation of Nanoegg Shell Particles:

Eggshells were ball milled by placing 30g of eggshell in a 500ml stainless steel jar.10 stainless steel balls of 10mm diameter were used to dry mill the eggshell in a planetary ball mill at 400rpm for 20min. The collected powder was sieved to a particle size <25um using a mechanical sieving shaker, 20g of fine egg shell powder were obtained. [6]

[A] Rotary ball mill

[B] Stainless steel balls used to dry mill

[C] Mechanical sieving shaker

3.Characterization of Nanoegg Shell Particles

X-ray Diffraction Analysis

The X-ray diffraction (XRD) analysis was performed to observe the possible changes in crystallinity. The XRD patterns were recorded using a diffractometer (PANalytical-Empyrean instrument; Co radiation: 1.54056 A°) and analyzed between 0 and 90° (2 theta). The voltage, current, and pass time used were 40 Kv, 40 mA, and 1 second, respectively [7]

Preparation of Nanoegg Shell Powder Paste

10% solution of nanoegg shell powder solution was prepared by dissolving 20gm of egg shell powder in 10ml of distilled water [8]

Preparation of Teeth Specimens

Twenty-four extracted premolars were collected from the Department of Oral and Maxillofacial Surgery, Krishnadevaraya College of Dental Sciences and stored in saline which were extracted for periodontal reasons. Each tooth was decoronated using a diamond disc mounted on a straight hand piece [Fig. D]. Teeth were dried; squares (windows) of self-adhesive labels measuring 5x5mm were stuck at the center of the buccal surface of each tooth. All teeth surfaces were coated with acid-poof nail varnish. Then, the adhesives were removed exposing only a small window of enamel. [Fig. E] Each crown was sectioned longitudinally in a buccolingual direction through the center of the window into two equal halves: mesial half (control group) and a distal half (experiment group). [9] [Fig. F]

Then the teeth were divided into two groups depending on the reminerlizing agent used.

Group 1: Nanoegg shell (n=12) **GROUP 2:** GC Tooth Mousse (n=12)

Each group were further divided into 4 subgroups (n=6) depending on the time duration for remineralisation at 7days and 14 days [Fig. G]

Group 1A: Control (7days)

Group 2A: Control (7days)

Group 1B: Nanoegg shell (7days)

Group 2B: GC Tooth Mousse (7days)

Group 1C: Control (14days)

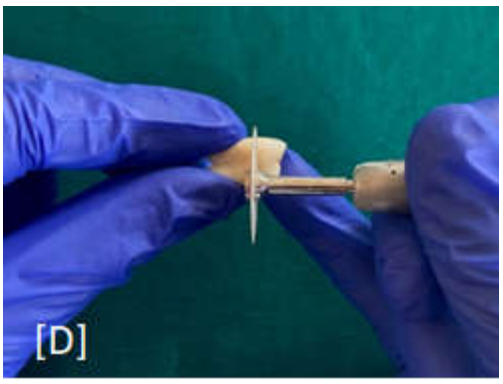
Group 2C: Control (14days)

Group 1D: Nanoegg shell (14days)

Group 2D: GC Tooth Mousse (14days)

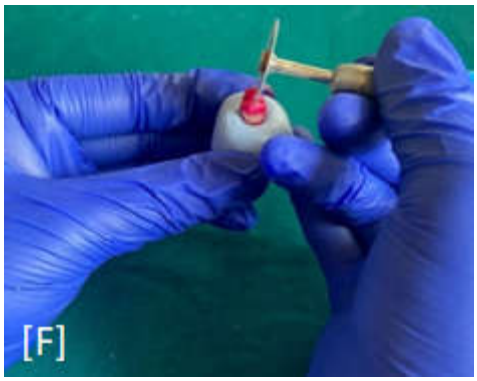
Baseline Surface Microhardness Measurement

All the specimens were subjected to Vickers microhardness test for Initial Baseline assessment. The buccal surface of each tooth specimen was set perpendicular to the long axis of the block. Baseline surface microhardness (B-SMH) was checked in the area of the working window to be recorded. The indentations were made at the rate of 25 g load for 5 s. [10] [Fig. H]

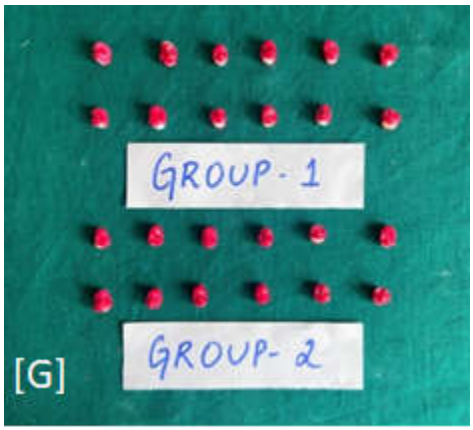


[D] Tooth was decoronated using a diamond disc mounted on a straight hand piece

[E] Small window of enamel measuring 5x5Cm



[F] Crown was longitudinally in a buccolingual direction



[G] Grouping of the samples

[H] Vickers microhardness test

Preparation of Demineralizing Solution

The buffered demineralizing solution was prepared using analytical grade chemicals and deionized water. The demineralizing solution contained 2.2 mM calcium chloride, 2.2 mM sodium phosphate, and 0.05 M acetic acid. It was adjusted to a pH 4.4 with 1 M potassium hydroxide [11]

Deminerlisation Phase for Inducing Artificial Caries

Each tooth specimen was immersed in the demineralizing solution at 37^0C for 72 hours without stirring in order to produce artificial carious lesion (white spot lesion without cavitation) [12]. After the lesion formation, teeth specimens were rinsed with distilled water and stored in artificial saliva for 7 days to simulate the oral condition. Subsequently, all the samples were subjected to Vickers Microhardness test and readings were recorded which is the second surface microhardness value after demineralization phase of the specimens.

Preparation of Remineralisation Solution (Artificial saliva)

The remineralising solution was prepared using 1.5 mM calcium chloride, 0.9 mM sodium phosphate, and 0.15 M potassium chloride, with a pH of 7.0. [11]

Remineralisation Phase

For subgroup 1A, 1C, 2A, 2C (Control): Each specimen was left untreated and stored in artificial saliva.

After 7 days, all specimens were rinsed with distilled water and prepared for final microhardness which will be the 3rd data collection.

For Subgroups 1B and 1D*:* Each specimen was treated with Nanoeggshell for 3 minutes. The gel was applied on demineralized enamel surface and slightly rubbed with a microbrush for a period of 3 minutes. Later on the specimens were stored in freshly taken artificial saliva and replenished every 24 hours. The same procedure was continued once a day for 7days & 14 days depending on Subgroup 1B & 1D.

For Subgroups 2B and 2D: GC Tooth Mousse was applied on demineralized enamel surface with a microbrush according to the manufacturer's instruction for 3 minutes. Later on the specimens were stored in freshly taken artificial saliva and replenished every 24 hours. The same procedure was continued once a day for 7days & 14 days depending on Subgroup 2B & 2D. All the specimens were subjected to Vickers microhardness test for final assessment post remineralisation. [13]

Statistical Analysis

The sample size has been estimated using the GPower software v.3.1.9.4 [(Franz Faul, Universität Kiel, Germany). Differences in enamel microhardness between test and control in each group were analyzed using

parametric test; Student's t test and the non-parametric test; Mann-Whitney U test. Meanwhile, differences between the two remineralizing agents were assessed by using ANOVA for normally distributed data and Kruskal Wallis H test for the not normally distributed data. The level of statistical significance was set at 0.05.

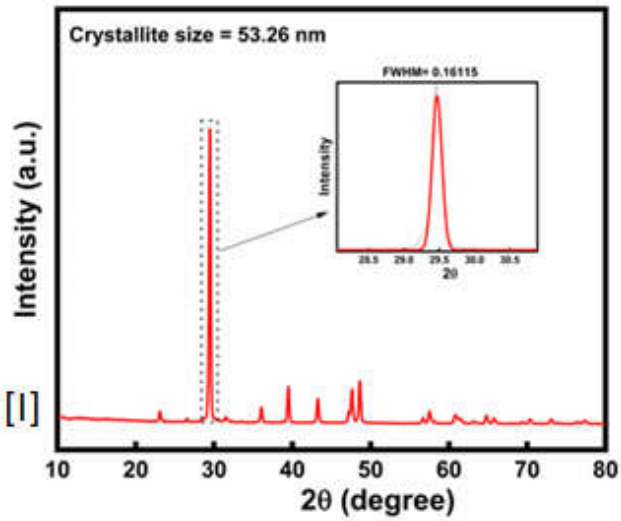
4.Results

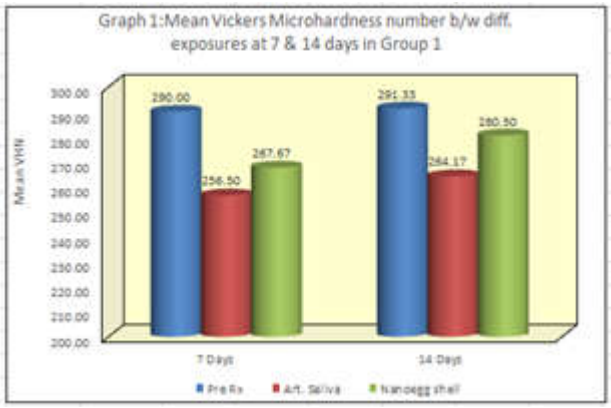
I. Characterisation of Nanoegg shell:

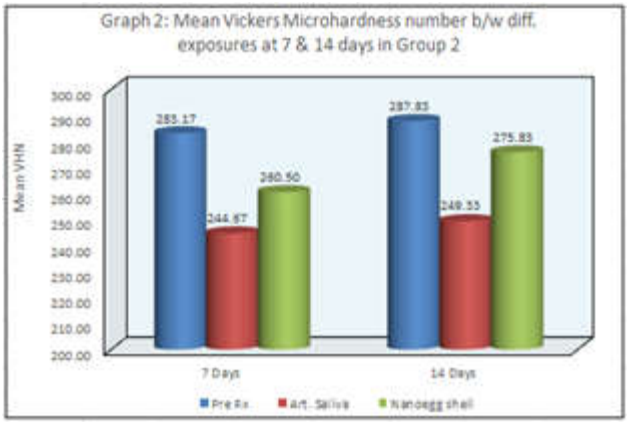
X-Ray Diffraction pattern of eggshell is shown in [Fig. I] which depicts that peaks were obtained at 2Θ values of 30°. The average crystallite size of the Nanoegg shell particles calculated using the Scherrer formula was 53.26 nm.

II. Vicker's microhardness test:

Samples which were Remineralized with Nano egg shell (Group 1B, 1D) on day 7 & 14 showed a mean micro hardness of 267.67 ± 27.77 and 280.50 ± 28.23 respectively and was relatively higher as compared to those samples of GC Tooth Mousse (Group 2B, 2D), which showed a mean micro hardness on day 7 & 14 with 260.50 ± 28.40 and 275.83 ± 24.13 respectively.
However, no significant difference was noted with respect to mean micro hardness between 2 groups on 7th & 14th day [Table 1]







[I] XRD pattern of eggshell shows that peaks were obtained at 2Θ values of 30°

TABLE 1 : Comparison of mean Vickers Micro hardness number b/w Group 1 & 2 at diff. exposures on 7 & 14 days using Mann Whitney Test

Treat	Time	Group	N	Mean	SD	Mean Diff	P-Value
Pre Rx	7 Days	Group 1	6	290.00	29.87	6.83	0.59
		Group 2	6	283.17	29.12		
	14 Days	Group 1	6	291.33	28.25	3.50	0.82
		Group 2	6	287.83	22.97		
Art. Saliva	7 Days	Group 1	6	256.50	27.56	11.83	0.42
		Group 2	6	244.67	27.41		
	14 Days	Group 1	6	264.17	31.06	14.84	0.26
		Group 2	6	249.33	26.40		
Remineralized	7 Days	Group 1	6	267.67	27.77	7.17	0.52
		Group 2	6	260.50	28.40		
	14 Days	Group 1	6	280.50	28.23	4.67	0.69
		Group 2	6	275.83	24.13		

TABLE 2: Comparison of mean Vickers Micro hardness number b/w 7 & 14 days at diff. exposures in Group 1 using Wilcoxon Signed Rank Test

Treat	Time	N	Mean	SD	Mean Diff	P-Value
Pre Rx	7 Days	6	290.00	29.87	-1.33	0.92
	14 Days	6	291.33	28.25		
Art. Saliva	7 Days	6	256.50	27.56	-7.67	0.60
	14 Days	6	264.17	31.06		
Nano egg shell	7 Days	6	267.67	27.77	-12.83	0.51
	14 Days	6	280.50	28.23		

TABLE 3: Comparison of mean Vickers Micro hardness number b/w 7 & 14 days at diff. exposures in Group 2 using Wilcoxon Signed Rank Test

Treat	Time	N	Mean	SD	Mean Diff	P-Value
Pre Rx	7 Days	6	283.17	29.12	-4.66	0.75
	14 Days	6	287.83	22.97		
Art. Saliva	7 Days	6	244.67	27.41	-4.66	0.83
	14 Days	6	249.33	26.40		
GC Tooth Moose	7 Days	6	260.50	28.40	-15.33	0.46
	14 Days	6	275.83	24.13		

The sample which was treated with Nano egg shell on 14^{h} day was relatively higher, 280.50 ± 28.23 as compared to 7th day [267.67 ± 27.77], however no significant difference was noted between 2 time intervals. [Table 2] The samples which was treated with GC Tooth Mousse on 14^{h} day was relatively higher, 275.83 ± 24.13 as compared to 7th day [260.50 ± 28.40], however no significant difference was noted between 2 time intervals [Table 3]

5.Discussion

The objective of current dentistry is to manage incipient caries lesions non-invasively to prevent disease progression and enhance aesthetics, strength, and function of teeth. [14] The development of a remineralization protocol that inhibits demineralization and encourages remineralization remains a challenge in this field. Hence, this study sought to assess and compare the enamel

remineralization potential of Nanoegg shell and GC Tooth Mousse on artificial caries.

In the present study, the quantitative evaluation of the enamel surface was carried out by measuring the surface microhardness. Microhardness measurement is appropriate for a material having fine microstructure, non-homogenous and prone to cracking like enamel. Surface microhardness indentation provides relatively a simple, non-destructive, rapid method. [15]

XRD pattern of eggshell [Fig. I] depicts that peaks were obtained at $2\Theta = 30^{o}$, indicating that calcite ($CaCO_3$) is a major phase of the eggshell.

The average crystallite size of the Nanoegg shell particles calculated using the Scherrer formula was 53.26 nm. The characterization results confirm that the particle sizes obtained were in nano-dimension. This finding is consistent with the results of a study by Tsai et al [16] who stated that planetary ball milling reduces particles to fine powder through the imposition of impact and frictional forces. More importantly, the presence of carbonate structure confirmed that the ball milling did not negatively impact on the carbonate composition of the Eggshell powder. [7]

Crystals of NanoEgg shell on to the enamel surfaces act as a template in the mineral precipitation process. The template further attracts large amounts of Ca^{2+} and PO^{3-} ions continuously from the remineralizing solution to fill up the defects and micropores on the demineralized enamel surface. This facilitates crystal growth and integrity, thereby promoting remineralization. According to Huang et al the effect of remineralization is increased when the size of the HA particle is reduced to nanometric range. [17]

The test results demonstrated that the Group 1B (NanoEgg Shell) on day 7 showed a relative increase in the mean micro hardness number as compared to Group 1A (Control). Such similar findings were observed on 14th day as well (Group 1D). However, no significant difference was noted on day 7 & 14. [Graph 1].

In recent years, chicken eggshell has attaining importance in numerous fields [18, 19]. According to Mony et al in 2015 (15), eggshell powder has the potential to favor remineralization because of its high pH and rich bioavailable calcium content. Besides, Eggshells are rich in calcium carbonate and also has a high percentage of bioavailable calcium (39%); relevant amounts of Mg, P, and Sr; and low levels of toxic metals such as Pb, Al, Cd, and Hg. This composition makes eggshell an attractive source of calcium. [20] The results were also backed by Haghoo et al. in 2016 [8] who revealed that eggshell solution can be used as a remineralizing agent for incipient enamel carious lesions and it is as effective as nano-hydroxyapatite for enamel remineralization. Additionally, Yaberi and Haghoo in 2018 [21] reported that microhardness of the enamel significantly increased after treatment with eggshell extract solution.

In this study, the dynamic cycles of de-and re-mineralization are simulated by sequentially immersing the teeth in acidic (demineralizing) and supersaturated (remineralizing) buffer solutions. According to Cate and Duijsters, these solutions approximate the mineral ion composition and supersaturation of saliva. [22]

Over many years the cornerstone of the non-invasive management of incipient carious lesions was fluoride, but its ability to promote net remineralization is limited mainly because its action is confined by the availability of Calcium and Phosphate ions. [23] This lead to the search for new remineralization technologies utilizing different agents which have proven success in this field, as casein phosphopeptide amorphous calcium phosphate (CCP-ACP), dicalcium phosphate (DCP), tricalcium phosphate (TCP), bioactive glass and nanohydroxyapatite. [24]

Furthermore, the test results demonstrated that the Group 2B (GC Tooth Mousse) on day 7 showed a relative increase in the mean micro hardness number as compared to Group 2A (Control). Whereas on 14th day (Group 2D), the samples showed a mean micro hardness number of 275.83 ± 24.13 which was significantly higher than the baseline value. This difference in the mean micro hardness number on day 14 was statistically significant at P=0.04 [Graph 2].

The increased enamel remineralisation of GC Tooth Mousse found in this study was consistent with previous studies showing the anti-cariogenic and remineralisation potential of CPP-ACP and the proposed mechanism of the localisation of amorphous calcium phosphate on the tooth surface by the CPPs, depressing enamel demineralisation and enhancing remineralisation. GC Tooth Mousse can be considered a valid product with anti-caries effect. [25-30] And acts as reservoir of Ca and Po4 and helps the dental plaque to maintain a state of ion supersaturation, thus depressing demineralization and enhancing remineralization. [31]

CPP in CPP-ACP stabilizes calcium and phosphate ions at the tooth surface in a bioavailable state and prevents them from transforming into a crystalline phase. This reservoir of calcium, phosphate, and fluoride ions released from the nanocomplexes of CPP-ACP diffuse down concentration gradients across demineralized zones and deposit themselves into voids in apatite crystals. This promotes crystal growth in the form of fluoride-containing apatite, thereby achieving remineralization. [32, 33] Yengopal and Mickenautsch conducted a systematic meta-analysis and concluded that there is sufficient clinical evidence demonstrating enamel remineralization and caries prevention by regular use of products containing CPP-ACP. [34] These results are in accordance with other studies, [35-37] which also found that enamel mineral loss was significantly reduced when CPP-ACP was applied.

Likewise, Talaat and Mahmoud evaluated the effect of acid on enamel subsurface lesions that were previously treated with CPP-ACPF and found that CPP-ACPF was able to remineralize the enamel subsurface lesions, due to

the first demineralization, and to protect them against further acid attack. [38]

Finally it is worth mentioning that remineralization of initial lesion should be targeted to enhance shifting from destructive drill and fill policy to a more conservative one. Therefore, the rich bioavailability of calcium along with the high concentration of phosphates present in EggShell particles coupled with its increased pH may be responsible for remineralization. [39]

6.Conclusion

Within the limitation of this in vitro study, the following conclusions were derived:

1. Both NanoEgg shell and GC Tooth Mousse can be used as remineralizing agents.
2. NanoEgg shell particles has a promising future in treating initial enamel surfaces lesion due to its natural source and easy bioavailability and can be considered as an optimal alternative to the commercial ones.

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