Original Article

Assessment Of The Power Of The Chicken Eggshell Powder In Remineralization Of Induced Caries Like Lesions In Primary Teeth; An In-Vitro Study

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Abstract

Purpose to evaluate the remineralization effect of the Chicken egg shell powder on primary teeth with induced caries-like lesions. **Materials and methods** the current experimental in-vitro study was performed on twenty dentin specimens prepared from twenty fresh extracted deciduous and stored in simulated body fluid. EDTA treated the dentin specimens as a demineralized agent for two hours, washed them with water, dried them, and analyzed them by SEM and EDX. After that, the dentin specimens were immersed in the Chicken egg shell powder solution as a remineralizing agent and finally analyzed by SEM and EDX. **Results** By a Comparison of atom mass percent in the different observation times (blank sample, after demineralization process, and after remineralization process, it was revealed that the main value of the calcium of the blank dentin samples was significantly decreased after demineralization by EDTA (3.48±1.33), (p=0.000) then significantly increased after remineralization by the Chicken egg shell powder (25.9±5.82), (p=0.000). **Conclusion** the Chicken egg shell powder has a great power of remineralization as it deposits Calcium, phosphorous, and magnesium minerals on the demineralized dentin surface.

Keywords: Chicken egg shell powder, EDTA, Remineralization, demineralization, induced caries-like lesion.

Introduction

One of the most common harmful diseases that affect the teeth is the dental Caries, which is characterized by demineralization and cavitation of normal tooth structure that finally lead to painful sensation, decreasing rate of function, and imbalanced facial harmony [(1)]. The bacterial infection can be transferred to the children either by direct contact to the mothers or by salivary contamination of affected primary teeth at 6 to 30 months of life [(2,3)].

The release of minerals from the tooth structure is the main feature of the demineralization process that is combined with substitution with acid ions that is released from dental plaque surrounding the tooth structure [(4)]. The ethylene diamine tetra acetic acid (EDTA gel) with 19% concentration at 37°C for 72 hours acts as one of the demineralizing agents used in induced artificial carious diseases (white spot lesions on enamel surface) [(4,5)].

Dentine loss significantly affects the integrity of the tooth structure, so it should be substituted by an artificial filling material, which can restore the physiological integrity of the entire tooth structure [(6)]. Glass-Ionomer Cement (GIC) materials although can use as a substitute for dentine loss in the coronal part, such as in case of deep carious lesions, but with its limitation of not stimulating any reparative dentin formation on its own [(7,8)].

Fluoridated and Non-fluoridated agents cause the remineralization of the carious lesions [(9,10)]. Remineralizing agents that contain fluoride can increase caries resistance that may arise from both systemic and topical applications of fluoride and can be divided as the following - increased enamel resistance to caries, increased rate of tooth maturation, remineralization of induced caries, interference with bacteria and improved tooth morphology [(11-13)].

One of the calcium carbonate structures is the Chicken eggshell element, which mainly contains 98.2% calcium carbonate, 0.9% magnesium, and 0.9% phosphate approximately, depending on its components, it ac as rich source of calcium [(14-18)]. Based on this fact there were previous literatures used it as a bone substitution, treatment of bone diseases, also in current days is recommended to be used in the treatment of white spot lesions of enamel [(19- 23)].
Thus, the aim of the current experimental in vitro study, therefore, was to evaluate the possible remineralization of demineralized dentin surface lesions using Chicken eggshell powder in primary teeth.

Materials and Methods

Study setting
This current in-vitro study was conducted in Pedodontics and Public health department at the faculty of Dental Medicine for girls, Al-Azhar University. The present study was conducted on twenty dentin samples prepared from freshly extracted twenty primary lower molar teeth.

Ethical consideration
The current in-vitro experimental study was approved by the Ethical committee of the faculty of dental medicine, Cairo, girl's branch, Al-Azhar University. The ethical code is REC-PE-23-01.

Sample size calculation
Sample size calculation was based on the observed average effect size derived from a previous article addressing "Evaluating the mechanical properties and calcium and fluoride release of glass ionomer cement modified with chicken eggshell powder " (8). According to previous studies, a sample size of 20 has a 95% confidence interval and an 80% power to detect a difference between means of 10.48 with an alpha level of 0.05 (two-tailed). The results will be considered statistically significant when the P value in 80% (the power) of those trials is less than 0.05 (two-tailed) "statistically meaningful 20 dentin samples were used in the current study. So the present study was conducted on 20 dentin specimens.

Intervention steps
1. **Teeth selection**

A total number of twenty (20) freshly extracted non-carious human deciduous lower molar teeth were used in this study. All teeth were obtained from patients who had already signed the consent form describing their approval of using their biological samples. The teeth were thoroughly washed, scrubbed, and scaled to remove surface debris and contaminants by scaler (Woodpecker-China). The teeth were free from cracks or developmental defects.

2. **Dentin samples preparation**

Totally twenty Dentin specimens were obtained from freshly extracted lower molar deciduous teeth by the following steps; Firstly, we start to remove the enamel of occlusal surface by using a low-speed diamond disk saw (IsoMet; Buehler, Lake Bluff, IL, USA), this was performed by using water cooling to obtain clear dentin surfaces. The exposed dentin surfaces were then polished with polishing discs (Sof-Lex, 3M ESPS, St. Paul, USA) for 30 s. In order to get the standardized occlusal dentin, samples of 1.5 ±1 mm thickness will be obtained from occlusal dentin by using water cooled low–speed diamond saw (Fig. 1). Then those dentin specimens were embedded in acrylic-resin molds to facilitate analysis by scanning Electron Microscope (SEM) and Energy Dispersive X-ray (EDX) (Fig. 2).

Figure (1) Longitudin … n of sodium hypo-chloride.

Figure (2) dentin specimens embedded in acrylic resin molds.

3. **Storage of dentin specimens**

After sample preparation, all dentin specimens should be immersed in artificial saliva; simulated body fluid (SBF). Based on Ringer's solution, this solution was made from analytical reagent grade (type of artificial saliva). Typically, the solution includes 9g of NaCl, 0.24g of $CaCl_2$, 0.43g of KCl, and 0.2g of $NaHCO_3$ dissolved in 1 L of water. [24]. The dentin samples were stored in the simulated Body fluid solution for (2 weeks & 1 month) and SBF was changed every 3 days (Fig. 3).

Figure (3) (A) Simulating body fluid**, (B)** prepared dentin specimens acrylic resin blocks immersed within artificial saliva.

4. **Demineralization stage (Caries-like lesion formation)**

To create artificial carious lesions, all dentin samples were demineralized (surface treated) using ethylene diamine tetra acetic acid (EDTA gel)* at a 19% concentration for 72 hours at 37°C (white spot lesions without cavitation). Teeth samples were washed with distilled water after lesion formation and kept in fake saliva for seven days [10] (**Fig. (4**).

Figure (4) Dentin samples covered by EDTA gel.

5. **Remineralization stage**

Preparation of Chicken eggshell powder (CESP)

CESP was prepared in the Department of Chemistry, Faculty of Science, Boys, Cairo, Al-Azhar University by calcination process [25] to produce pure, pathogenic free calcium oxide powder [26]. Chicken eggs were cleaned with sterile distilled water and, then boiled in a hot water bath at 100°C for about 10 min to facilitate their internal white membranes removal, then kept in furnace muffle (Thermolyne 47900, Model F4791; Kerper Boulevard, Iowa, USA) at a temperature of 1200°C for about one h. The obtained mass from firing was crushed several times again in a sterile mortar and pestle to ensure homogeneous particle size (27) at the National Research Center, Cairo, Egypt. Then the powder was analyzed for its elemental composition percentage by weight using Fourier Transform Infra-Red spectroscopy (FTIR).

Production of Eggshell Powder Solution

A ten grams of chicken egg shell powder were dissolved in 100 ml of distilled water to create a 10% solution of egg shell powder. The pH of the clear liquid, which is gathered at the top, was then measured using a pH metre (Deluxe deep vision, model no. 101, California, USA), and it was 11.7. [28] (Fig. 5).

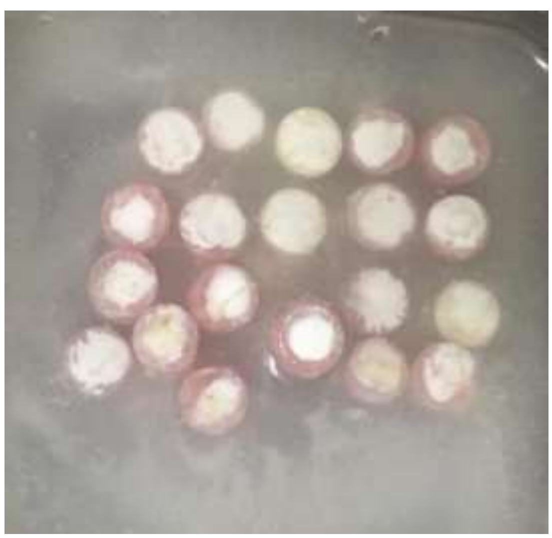

Figure (5) immersion of dentin specimen acrylic resin blocks in chicken egg shell powder solution.

Protocol of remineralization phase

After the demineralization step, the dentin specimen was washed with distilled water and dried by air for subsequent analysis by SEM and EDX. Then dentin specimens were stored in chicken eggshell solution for 21 h for fourteen consecutive days for remineralization. For every 24 h, the fresh chicken eggshell solution was prepared, and the samples were washed twice with distilled water. Then the dentin samples were dried by air and scanned again by SEM and EDX to determine the atom's mass percentage after the remineralization phase by Chicken egg shell powder solution [(29)].

SEM and EDX analysis

The dentin specimens were examined with SEM and EDX to evaluate calcium, phosphate, magnesium, carbon, and oxygen atoms mass percentage at three-time intervals; before demineralization (blank sample) (Fig. 6), after demineralizationphase (Fig. 7) and after remineralization phase (Fig. 8). Dentin specimens at all steps were stored in simulated body fluid.

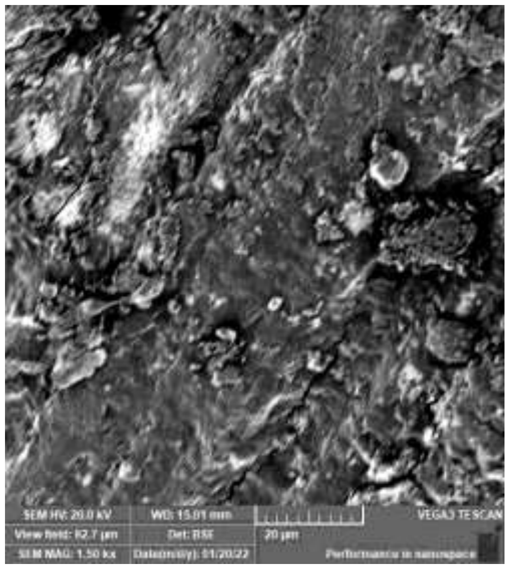

Figure (6) photograph showing dentin blank sample by SEM.

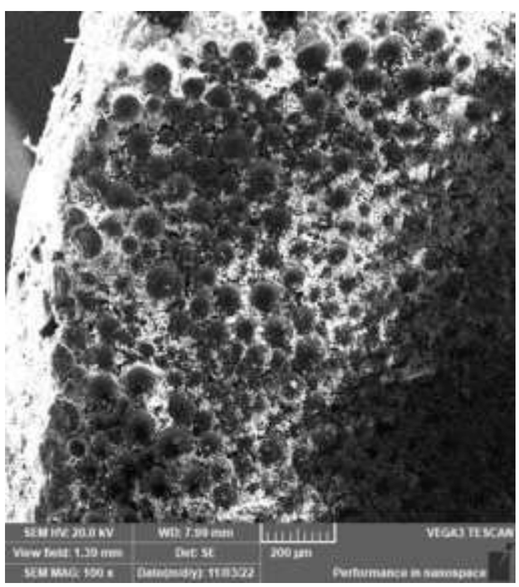

Figure (7) photograph showing dentin sample after demineralization phase by SEM.

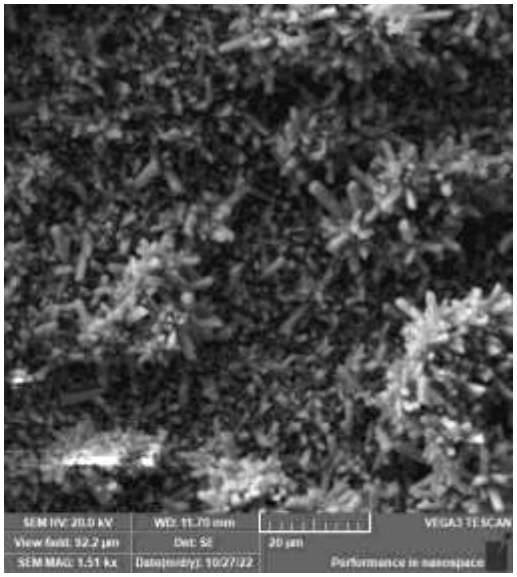

Figure (8) photograph showing dentin sample after remineralization phase by SEM.

Results

Results are summarized in tables (1) and (**fig. 9**)

Comparison of atom mass percent in the different observation times

1. Calcium: The mean value in the blank sample was (21.26±7.02), then decreased to (3.48±1.33) after demineralization, then increased to (25.9±5.82) after remineralization. ANOVA test showed a significant difference between these values (p=0.000). Post hoc test revealed a significant difference between each of these values.
2. Phosphorus: The mean value in the blank sample was (8.15±3.27), then decreased to (1.12±0.35) after demineralization, then increased to (6.74±4.37) after remineralization. ANOVA test showed a significant difference between these values (p=0.000). Post hoc test revealed no significant difference between theblank and after-remineralization values.
3. Magnesium: The mean value in the blank sample was (0.3±0.19), then decreased to (0.05±0.03) after demineralization, then increased to (0.28±0.19) after remineralization. ANOVA test showed a significant difference between these values (p=0.000). Post hoc test revealed no significant difference between theblank and after-remineralization values.
4. Carbon: The mean value in the blank sample was (23.34±12.18), then increasedto (48.23±17.74) after demineralization, then decreased to (17.63±10.30) afterremineralization. ANOVA test showed a significant difference between these values (p=0.000). Post hoc test revealed no significant difference between theblank and after remineralization values.
5. Oxygen: The mean value in the blank sample was (48.01±18.52), then decreased to (40.88±16.04) after demineralization, then further decreased to (37.64±12.17) after remineralization. ANOVA test showed no significant difference betweenthese values (p=0.114).

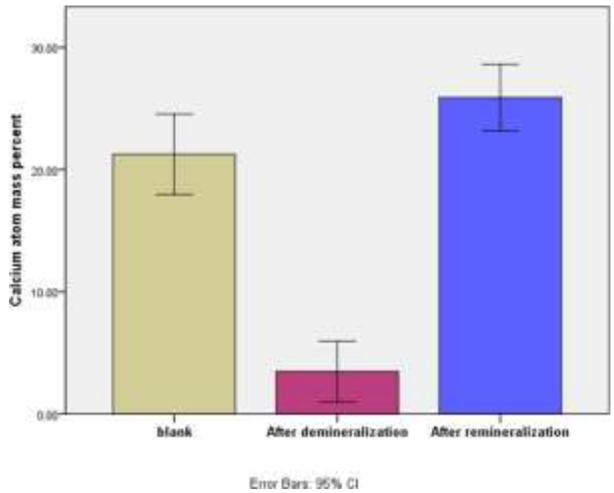

Figure (9) Bar chart illustrating mean atom mass percent of calcium in blank sample, after demineralization and after remineralization

Table (1) Descriptive statistics of atom mass percent in blank samples, afterdemineralization and after remineralization (ANOVA test)

		Mean	Std.Dev	95% Confidence Interval for Mean		Min	Max	F	P
				Lower Bound	Upper Bound				
Calcium	blank	21.26^{b}	7.02	17.97	24.54	8.80	31.46	**75.36**	**.000***
	After demineralization	3.48^{c}	2.33	.98	5.97	.00	17.64		
	After remineralization	25.90^{a}	5.82	23.18	28.62	10.41	32.38		
Phosphorus	blank	8.15^{a}	3.27	6.62	9.68	.87	12.60	**23.50**	**.000***
	After demineralization	1.12^{b}	0.35	.02	2.22	.00	8.00		
	After remineralization	6.74^{a}	4.37	4.70	8.79	.00	12.62		
Magnesium	blank	$.30^{a}$	.19	.21	.38	.00	.75	**14.66**	**.000***
	After demineralization	$.05^{b}$	.03	.02	.09	.00	.27		
	After remineralization	$.28^{a}$	.19	.19	.37	.00	.80		
Carbon	blank	23.34^{b}	12.18	17.64	29.04	6.78	46.52	**27.91**	**.000***
	After demineralization	48.23^{a}	17.74	39.93	56.53	10.63	73.20		
	After remineralization	17.63^{b}	10.30	12.81	22.45	5.75	46.52		
Oxygen	blank	48.01^{a}	18.52	39.34	56.67	20.38	85.24	**2.256**	**.114 ns**
	After demineralization	40.88^{a}	16.04	33.37	48.39	19.20	72.85		
	After remineralization	37.64^{a}	12.17	31.94	43.33	12.01	57.70		

Significance level p≤0.05, *significant, ns=non-significant
Post hoc test: within the same element, means sharing the same superscript letter are notsignificntly different

Discussion

The present experimental in-vitro study was conducted in Pedodontics and Public health department at the faculty of Dental Medicine for girls, Al-Azhar University. This current study was conducted on twenty dentin specimens that were prepared from twenty fresh extracted lower deciduous molars.

In the current study, we used EDTA, which was preferred over other demineralizing agents like phosphoric acid and citric acid, as it has a high power of penetration that help to remove any smear layer and mineral contents of dentinal tubules; almost it leads to complete removal of dentin in-organic elements precisely as it occurs in caries lesion, so it is called inducer for caries like lesion, while other demineralizing agents like phosphoric acid lead to the removal of smear layer and partial removal of in-organic content of dentin structure, so it is not effective as EDTA (30). Using EDTA helps us assess the pure effect of the used re-mineralizing agent (deposition of new minerals contents).

Chicken Eggshell Powder (CESP), one of calcium carbonate, is a complex and highly structured calcite bio-ceramic and water molecule in the lattice. CESP is usually the waste from the community and can be developed as a source of calcium carbonate in the future. As expected, depending on the tremendous remineralizing effect of the chicken egg shell powder in previous studies, in the current study, after the addition of the chicken egg shell solution to the demineralized dentin samples, it leads to deposition of its mineral contents; mainly calcium and phosphate onto the demineralized dentin surface and can restore its in-organic dissolved elements, and these results come in agreement with previous literature (27-29).

In the current study, we use scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM/EDX) for the evaluation of differential changes of minerals content of dentin structure after demineralization and remineralization process, as we know, there are different methods for assessment of remineralization effect of chicken egg shell powder. Still, the present study depended on SEM and EDX as The information on a sample's chemical composition, including the elements present as well as their distribution and concentration, can be swiftly generated by the researchers.

Our results also show that the SEM examination of demineralized dentin samples revealed many circular radiolucent spaces surrounded by an outer radiopaque circle line. This image reflected the effect of EDTA as a demineralizing agent in dissolving in-organic content of the dentinal tubules, while SEM examination after remineralization revealed the presence of radiopaque tangled threads and filaments; this image can explain the significant effect of the Chicken egg shell powder in the deposition of calcium onto the dentin outer surface and within the dentinal tubules.

By a Comparison of atom mass percent in the different observation times (blank sample, after demineralization process, and after remineralization process, it was revealed that the primary value of the calcium of the blank dentin samples was significantly decreased after demineralization by EDTA (3.48±1.33), (p=0.000) then significantly increased after remineralization by the Chicken egg shell powder (25.9±5.82), (p=0.000), as EDTA lead to dissolving and release of calcium content. In contrast, the Chicken egg shell powder deposited the calcium onto the surface of the dentin samples and within dentinal tubules, as revealed by the scanning electron microscope (SEM); these results are similar to the results revealed by previous studies (26-29).

Also, the main value of phosphorus of the blank dentin samples were significantly decreased after demineralization by EDTA (1.12±0.35), (p=0.000) Then significantly increased after remineralization by the Chicken egg shell powder (6.74±4.37), (p=0.000), as EDTA led to dissolving and release of minerals content while the Chicken egg shell powder deposited the phosphorus onto the surface of the dentin samples and within dentinal tubules, as revealed by the scanning electron microscope (SEM), these results are similar to the results revealed by previous studies (26-29). On the other hand, few studies revealed a significant increase of phosphorus after the demineralization process as they used phosphate-buffered saline (PBS) solution as a storage media that led to increased percentage of phosphorus after demineralization (23,24).

As a result of the Chicken egg shell powder acting as a source of minerals, calcium, phosphorus, and magnesium, the current study revealed that the main value of the magnesium at blank samples was significantly decreased (0.05±0.03), (p=0.000) after demineralization and significantly increase after remineralization (0.28±0.19), (p=0.000) these results com in concomitant with another study (25).

In contrast, the main value of the carbon in the blank dentin samples was significantly increased after demineralization process (48.23±17.74), (p=0.000), and significantly decreased after remineralization by the

Chicken egg shell powder (17.63±10.30), (p=0.000). This result can be explained as Carbon content plays a crucial role in deciding the rate of demineralization. The carbonate ion substitutes the phosphate ion and produces defects in calcium deficit areas.

In enamel, one out of ten phosphate ions are replaced with carbon and in dentin, one out of five. Therefore, the mineral loss in dentin is three times greater than in enamel due to high carbonate content [26]. These results come in contact with previous literatures [26-29].

Regarding the main value of the oxygen in the blank dentin samples, it decreased both after demineralization and remineralization as free oxygen ions come in contact with other free ions resulting from the demineralization and remineralization process to form new compounds.

Conclusion

The Chicken egg shell powder (CESP) is a reservoir for calcium and an excellent remineralizing agent. It deposits and releases free ions of minerals on demineralized dentin structure and within its dentinal tubules.

Recommendation

1. Use a large number of dentin samples in future studies to obtain more clear statistical results.
2. Addition of the Chicken egg shell powder on different restorative material such as glass ionomer and biodentin as it act as a reservoir for calcium.
3. Using the Chicken egg shell powder directly to the demineralized enamel surface of permanent teeth as, in white spot lesions, has a great remineralizing effect.

Conflict of Interest

No conflict of interest.

Funding

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