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Evaluation of the remineralizing effect of the chicken eggshell paste after removal of the fixed orthodontic appliance: An *in vitro* study

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Abstract

BACKGROUND: Demineralization of the enamel surface, which appears as white spot lesions during and after removal of the fixed orthodontic appliance, is the most common disadvantage of the orthodontic treatment course. Using the remineralizing agents during and after orthodontic treatment helps to avoid those enamel defects.

OBJECTIVE: The present study aims to assess the remineralizing effect of the chicken eggshell powder on the demineralized enamel surfaces after debonding the orthodontic bracket system.

MATERIALS AND METHODS: The current study was performed on 80 prepared premolar crowns embedded into acrylic molds. The samples were prepared to receive routine steps of the bonding process for the bracket system. The paste of the chicken eggshell powder was added to the samples after the debonding process. Scanning electron microscopy (SEM) and energy-dispersive X-ray (EDX) were used to evaluate the remineralization effect of the chicken eggshell powder. Also, the Vickers microhardness tester was used to assess the enamel surface microhardness.

RESULTS: It was found that the mean value of the Ca/P ratio for the samples before bonding of the orthodontic bracket system was (4.17 ± 2.2). This value significantly decreased to (2 ± 1.3) after debonding of the orthodontic bracket system and then showed a significant increase to (4.79 ± 2.65) after remineralization. These results were assured by the values of the Vickers microhardness tester.

CONCLUSION: The chicken eggshell powder has an excellent remineralization effect for the demineralized enamel surface after debonding the orthodontic enamel surface.

Keywords:

Chicken eggshell powder, demineralized enamel surface, remineralizing agent, SEM and EDX, white spot lesion

Introduction

One of the most common drawbacks that can interfere with the esthetic objective of orthodontics and appear during and after the completion of orthodontic treatment is white spot lesions that present as a result of demineralization of the enamel surface. About one-third of orthodontic patients have at least one to two teeth affected by the demineralized enamel surface.[1-3] The demineralized part of the tooth surface ranges from 5% to 85% of the total enamel surface.[4-6]

Mechanics and the components of fixed orthodontic appliances can directly affect the prevalence of white spot lesions as they help in more retention of food debris and plaque bacteria on the tooth surface,

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so it is recommended to remove any composite excess surrounding orthodontic attachments; also, the method of ligation of the orthodontic archwire is critical; use of a ligature wire is preferable than elastic rings.[7-9]

The best approach for the prevention of white spot lesions during orthodontic treatment is to carry out good oral hygiene measurements. Occasionally, the occurrence of partial enamel demineralization during orthodontic treatment, even after treatment completion, is self-treated by a normal remineralization process depending on the mineral contents of saliva, mainly hydroxyapatite crystals.[10,11]

Different approaches for treating the white spot lesions after orthodontic treatment use different remineralizing agents such as fluoride, casein phosphopeptides, and amorphous calcium phosphate (CPP-ACP).[12-14] The advantages of these remineralizing agents are as follows: they have high power of the remineralizing effect and are easy to be prepared. Their disadvantages are as follows: they are not natural sources for calcium; also, some previous studies were against using high doses of remineralizing agents as fluoride because it leads to the arrest of physiological demineralization and remineralization processes by saliva and advocates using low doses through using different mouth rinses.[15-18]

The chicken eggshell powder is one of the greatest and richest natural sources of calcium and phosphate elements as it is mainly composed of 95% calcium carbonate; it also contains other minerals such as phosphate, magnesium, fluoride, and strontium.[19-21]

Surface hardness is a mechanical property of a tooth, and it is related to the mineral content of the dental tissue. It represents the resistance to penetration, indentation, and scratching. Surface hardness is related to enamel demineralization, affecting the resistance to surface invasion through mineral content and micro-integrity. Enamel microhardness is an indirect indicator of enamel calcium and phosphate contents.[22-24]

For the evaluation of an anisotropic material such as the tooth enamel, the Vickers microhardness tester is more suitable than the Knoop indenter device, which requires a perfectly smooth surface.[25] The enamel is organized into prisms, and this orientation determines anisotropic performance and affects the surface microhardness.[26]

Due to the excellent calcium contents of chicken eggshell powder, it has been used in different medical fields as a remineralizing agent, treatment of osteoporosis, and remineralization of the carious dental enamel and dentin, and it can be used as a bone graft material and a bio-ceramic material.[27-33] So, the aim of the current study is to assess the remineralizing effect of the chicken eggshell powder on the demineralized enamel surfaces, white spot lesions that present after debonding of the orthodontic bracket system, and completion of orthodontic treatment.

Materials and Methods

Sample size calculation

The sample size was calculated depending on the effective size derived from previous literature.[34] A sample size of 80 has a 95% confidence interval (CI) and an 80% power to determine a mean difference of 10.48 with an alpha level of 0.05 (two-tailed). So, the current study applied to 80 extracted teeth.

Sample selection

A total number of 80 freshly extracted premolar teeth for the purpose of orthodontic treatment were used in the current study. The teeth were collected using the following inclusion criteria: intact teeth free from cracks, caries, fluorosis, fractures, fillings, and demineralization with no root canal treatments. These criteria were determined by visual inspection using a stereomicroscope (Leica EZ4HD, Leica Microsystems, Switzerland) before and after disinfection.

Sample preparation

The samples are prepared at the National Research Centre in Cairo. The extracted teeth were disinfected by immersion in a 0.1% thymol solution for 24 hours. This was followed by putting them deionized water to clean them. Any traces of soft tissue or deposits were removed with a sharp hand scaler. Teeth were gently brushed with a soft toothbrush (Sensodyne Advanced Repair and Protect, GlaxoSmithKline, UK) and finally rinsed with deionized water.

The root of each tooth was removed using Isomet Saw (Buehler, Lake Bluff, IL, USA) under water cooling. Then, the crown was sectioned mesiodistally to obtain a test specimen with a 3 mm thickness measured from the buccal surface. The tooth segment was embedded in acrylic resin cylinder molds with 1.5 cm height and 1.5 cm diameter, leaving the buccal surface fully exposed and parallel to the bottom of the molds to facilitate analysis by scanning electron microscopy (SEM) and energy-dispersive X-ray (EDX) and also to test the surface microhardness.

After sample preparation, the acrylic molds were stored in simulated body fluid (SBF) for 2 weeks at room temperature, and the solution was changed every 3 days. The artificial saliva is based on Ringer's solution; this solution was made from analytical reagent grade (type of artificial saliva). Typically, the solution contains 9 g of NaCl, 0.24 g of $CaCl_2$, 0.43 g of KCl, and 0.2 g of $NaHCO_3$ dissolved in 1 L of water. After that, the samples were analyzed using an SEM and EDX and Vickers microhardness tester (Wilson hardness tester model Tukon 1102, Germany).

Bonding of the bracket system

After storage of prepared samples within simulated body fluid for 2 weeks, the extracted premolar teeth were prepared to receive routine steps of the bonding process for the bracket system (Oramco, USA), starting by application of the acid etching gel (37% phosphoric acid, Meta Biomed, England) on selected areas of the enamel surface for 20 seconds; this is followed by rinsing the surface by water and drying by air. The second step is application of a bonding agent. The third step is application of a bonding agent and orthodontic composite on the base of the bracket system and holding it directly to the selected position on the enamel surface and using light cure for 30 seconds. The adhesive remnants on the tooth surface were removed by using surgical scalpel blade No. 11 (Swann-Morton, England B.S). Samples were stored again in the artificial saliva for 2 weeks. After that, the bracket system was debonded from the samples by ultrasonic instrumentation, which also helped remove bond remnants on the demineralized enamel surface. After the debonding step, the samples were analyzed using a SEM and EDX and Vickers microhardness tester (Wilson hardness tester model Tukon 1102, Germany).

It is worth mentioning that the debonding process of the orthodontic bracket system not only leads to enamel cracks but also may result in the appearance of white spot lesions as a result of the demineralization of the enamel surface as a basic step for the bonding process.[4-6,9]

Remineralization procedures

First, the chicken eggshell powder was prepared by the calcination process[35]. To make it easier to remove the internal white membranes from chicken eggs, they were cleaned with sterile distilled water, boiled in a hot water bath for about 10 minutes at 100°C, and then kept in a furnace muffle (Thermolyne 47900, Model F4791; Kerper Boulevard, Iowa, USA) at a temperature of 1200°C for about an hour. At the National Research Centre in Cairo, Egypt, the fired mass was repeatedly crushed in a sterile mortar and pestle to achieve uniform particle sizes. The powder was then subjected to a Fourier transform infrared (FTIR) spectroscopic analysis to determine its elemental composition percentage by weight.

Second, to make a 20% paste of eggshell powder, 20 grams of chicken eggshell powder was dissolved in 100 ml of distilled water. A pH meter (Deluxe deep vision, model no. 101, California, USA) was then used to determine the pH of the clear liquid at the top.[36]

Third, the prepared 20% paste of eggshell powder was added to the previous bracket position of the debonded enamel surface of the study samples gently by a bonding brush step by step until it covers the selected demineralized area; then they were stored in the artificial saliva for 2 weeks; every 24 hours, a new paste of the eggshell powder was added after washing the samples with distilled water. Also, the artificial saliva was changed every 3 days. After the remineralization stage, the samples were washed with distilled water and dried to analyze them again using a SEM and EDX and Vickers microhardness tester (Wilson hardness tester model Tukon 1102, Germany) [Figure 1].

SEM and EDX analysis

SEM and EDX examined the prepared samples to evaluate calcium and phosphate percentages at three time intervals: before bonding of the bracket system, after the debonding process, and after the remineralization stage.

After drying, the samples were mounted on aluminum stubs using double-sided carbon-coated adhesive tape. Then, using an EMS-350 sputter coater (Electron Microscopy Sciences, Hatfield, PA, USA) set at 20 Ma for 90 s, samples were coated with gold–palladium.

At the National Research Centre in Cairo, the samples were examined using SEM (TESCAN VEGA 3, JEOL USA Inc., USA) at an accelerating voltage of 10 kV and a working distance of 5–10 mm. The Aztec EDS system (Oxford Instruments, Abingdon, UK) with computer-controlled software was used to analyze the samples using an energy-dispersive X-ray detector.

Micrographs of the material surface were taken at magnifications of 1000x. Samples were then subjected to EDX quantitative chemical analyses at the same magnification. Representative regions for each stage were analyzed, and the mineral atom mass % was computed.

Microhardness testing

The samples were tested by using a Vickers microhardness tester (Wilson hardness tester model Tukon 1102, Germany) that was equipped with objectives for 100x and 500x magnifications and integrated with a camera system (Minuteman Security Technologies, USA). Testing procedures were conducted in accordance with the International Organization for Standardization (ISO). A load of 0.49 N (equivalent to a mass of 50 g) was applied smoothly without impact, forcing the indenter into the buccal surface. The indenter was held in place for 15 seconds. Three different indentations were performed (spaced a minimum distance apart of 0.2 mm), and the mean was calculated for each

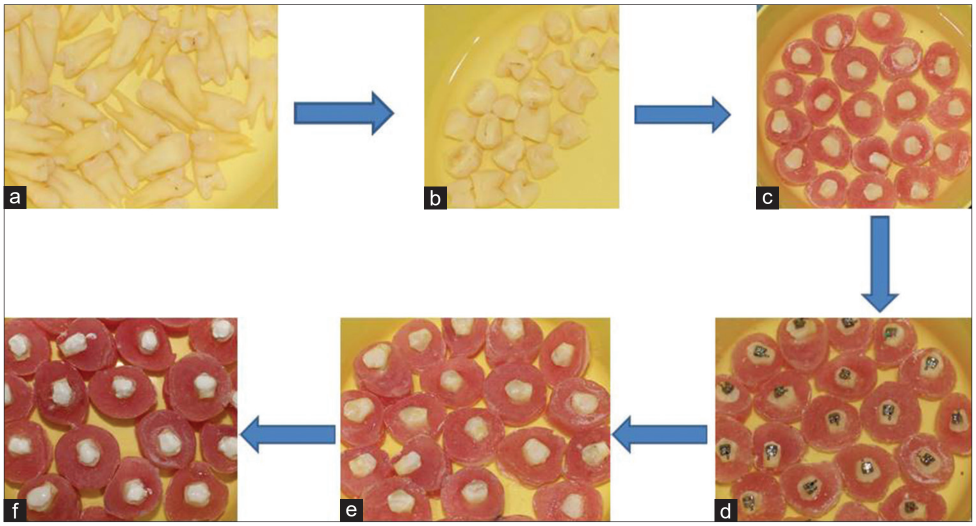

Figure 1: Intervention procedure; (a) Sample disinfection; (b) Samples after removing the root; (c) Samples within acrylic blocks; (d) Samples with bonded bracket system; (e) Samples after debonding of bracket system; (f) Samples after addition of paste of egg shell powder

sample. The length of the two impression diagonals was measured with a micrometer and averaged. The microhardness value (Vickers hardness number, VHN) was calculated using this formula VHN = 1854.4 P/d2, where (P) is the load (g) and (d) is the mean of diagonals of indentation (µm) [Figure 2].

Statistical analysis

Data management and statistical analysis were performed using the Statistical Package for Social Sciences (SPSS) version 20. Numerical data were summarized using mean, standard deviation, median, and range. Data were explored for normality by checking the data distribution and using Kolmogorov–Smirnov and Shapiro–Wilk tests.

Comparisons between groups with respect to normally distributed numeric were performed using repeated measures ANOVA test, followed by Bonferroni *post hoc* test for pairwise comparisons. All *P* values are two-sided. *P*-values ≤ 0.05 were considered significant.

Results

After analysis of the samples by EDX in three different time intervals to evaluate the calcium/phosphorus ratio [Figure 3], the results showed the following:

Calcium, phosphorus, and Ca/P ratio

- Calcium (Ca): The mean value of blank samples (before bonding of the orthodontic bracket system) was (26.78 ± 8.8). This value significantly decreased to (2.11 ± 2.94) after the debonding of the orthodontic bracket system and then showed a significant increase to (27.35 ± 7.03) after remineralization. The *post hoc* test revealed no significant difference between values recorded in blank samples and after remineralization. The value recorded after debonding of the orthodontic bracket system was significantly lower than the other two observations (*P* = 0.000) [Table 1 and Figure 4].

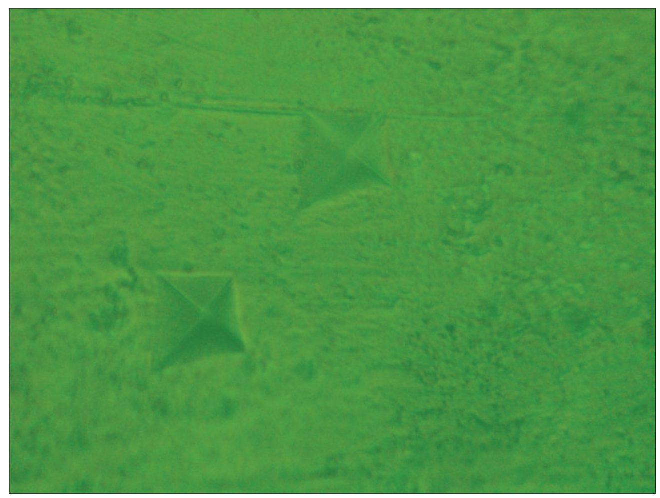

Figure 2: Image of Vickers indentations on the surface of the sample

- Phosphorus (P): The mean value of blank samples (before bonding of the orthodontic bracket system) was (8.3 ± 3.49). This value significantly decreased to (1.46 ± 0.67) after debonding of the orthodontic bracket system and then showed a significant increase to (7.6 ± 3.85) after remineralization. The *post hoc* test revealed no significant difference between values recorded in blank samples and after remineralization. The value recorded after debonding of the orthodontic bracket system was significantly lower than the other two observations (*P* = 0.000) [Table 1 and Figure 4].
- Ca/P ratio: The mean value of blank samples (before bonding of the orthodontic bracket system) was (4.17 ± 2.2). This value significantly decreased to (2 ± 1.3) after debonding of the orthodontic bracket

Table 1: Descriptive statistics of Ca, P, and Ca/P ratio in blank samples (before bonding of the orthodontic bracket system), after debonding of the orthodontic bracket system, and after remineralization and comparison between different observations (repeated measures ANOVA test)

	Mean	Std. Dev	95% Confidence Interval for Mean		Min	Max	*F*	*P*
			Lower Bound	Upper Bound				
Calcium								
Blank	26.78[a]	8.80	25.19	28.37	9.80	41.46	551.350	0.000*
After debonding	2.11[b]	2.94	1.58	2.64	0.00	7.64		
After remineralization	27.35[a]	7.03	26.08	28.62	10.41	35.46		
Phosphorus								
Blank	8.30[a]	3.49	7.67	8.93	1.64	12.10	149.625	0.000*
After debonding	1.46[b]	0.67	0.97	1.94	0.00	8.00		
After remineralization	7.60[a]	3.85	6.90	8.29	1.64	12.10		
Ca/P Ratio								
Blank	4.17[a]	2.20	3.59	4.75	2.05	12.45	40.599	0.000*
After debonding	2.00[b]	1.30	1.76	2.24	0.00	4.51		
After remineralization	4.79[a]	2.65	4.31	5.27	2.49	8.89		

Significance level $P \leq 0.05$, *Significant. *Post hoc* test: means with different superscript letter are significantly different

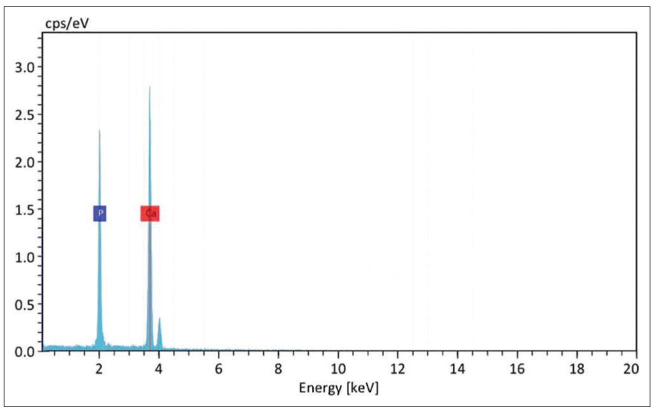

Figure 3: Sample analysis by EDX

system and then showed a significant increase to (4.79 ± 2.65) after remineralization. The *post hoc* test revealed no significant difference between values recorded in blank samples and after remineralization. The value recorded after debonding of the orthodontic bracket system was significantly lower than the other two observations ($P = 0.000$) [Table 1 and Figure 4].

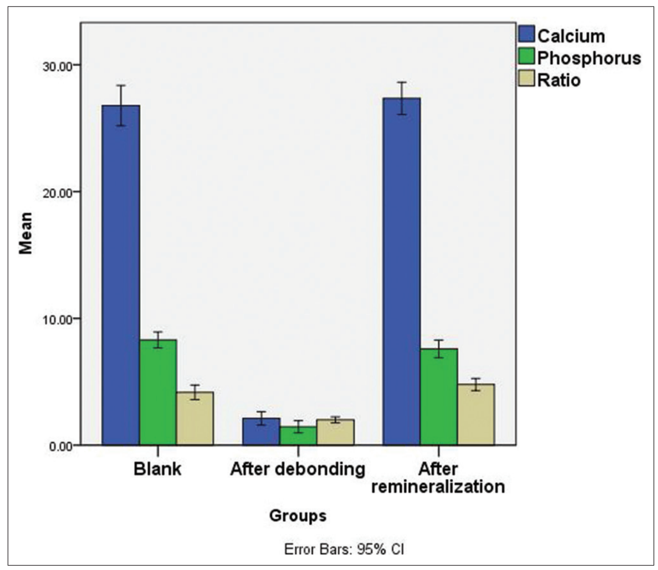

Figure 4: Bar chart illustrating mean value of Ca, P, and Ca/P ratio in blank samples (before bonding of the orthodontic bracket system), after debonding of the orthodontic bracket system, and after remineralization

SEM examination

Figures of SEM examination showed an intact solid surface before bonding of the orthodontic bracket system [Figure 5], while after the debonding step, it showed minute fractures on the surface [Figure 6]. At the same time, SEM examination of the enamel surface of the prepared samples after the remineralization stage by a paste of the chicken eggshell powder showed the presence of radiopaque tangled threads and filaments [Figure 7].

After analysis of the samples using a Vickers Micro-hardness Tester in three different time intervals to evaluate the level of the microhardness at each stage, the results showed the following:

Vickers Micro-hardness Tester

The mean value of blank samples (before bonding of the orthodontic bracket system) was (219.64 ± 24.82). This value significantly decreased to (75.62 ± 12.91) after the debonding of the orthodontic bracket system and then showed a significant increase to (210.83 ± 24.10) after remineralization. The *post hoc* test revealed no significant difference between values recorded in blank samples and after remineralization. The value recorded after debonding of the orthodontic bracket system was significantly lower than the other two observations ($P = 0.000$) [Table 2 and Figure 8].

Percentage of surface microhardness recovery (SMHR)

SMHR was calculated according to a previous study.[37] Using the formula (SMH remineralized-SMH

Table 2: Descriptive statistics of surface microhardness Kg/mm² VHN in blank samples (before bonding of the orthodontic bracket system), after debonding of the orthodontic bracket system, and after remineralization and comparison between different observations (repeated measures ANOVA test)

	Mean	Std. Dev	95% Confidence Interval for Mean		Min	Max	*F*	*P*
			Lower Bound	Upper Bound				
Blank	219.64[a]	24.82	215.15	224.12	133.80	257.60	1720.00	0.000*
After debonding	75.62[b]	12.91	73.29	77.96	49.20	99.10		
After remineralization	210.83[a]	24.10	206.48	215.19	105.20	256.20		

Significance level $P \leq 0.05$, *Significant. *Post hoc* test: means with different superscript letters are significantly different

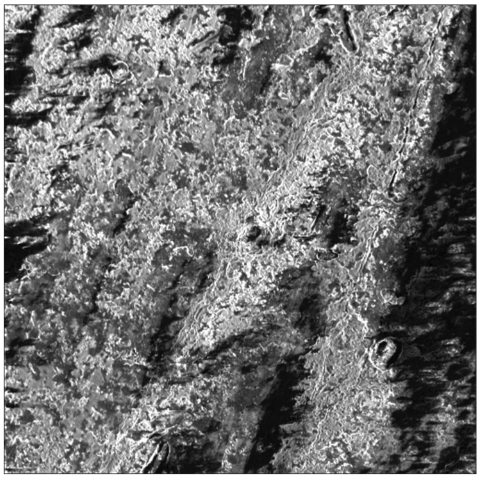

Figure 5: Sample by SEM before bonding of the bracket system; arrows refer to an intact solid surface

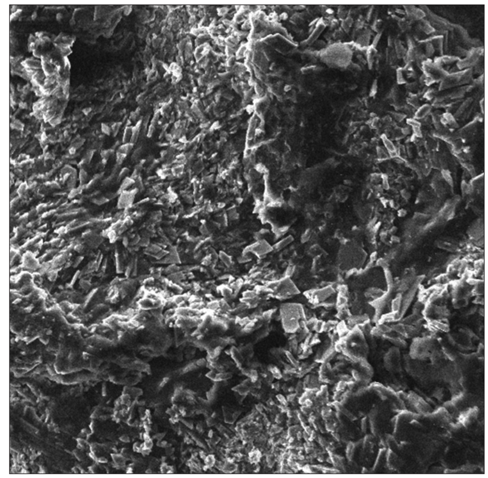

Figure 6: Sample by SEM after debonding of the bracket system; arrows refer to minute fractures

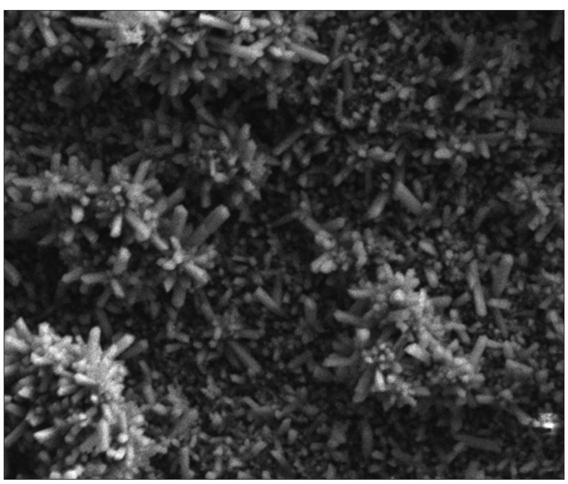

Figure 7: Sample by SEM after remineralization stage by paste of chicken egg shell powder; arrows refer to radiopaque tangled threads and filaments

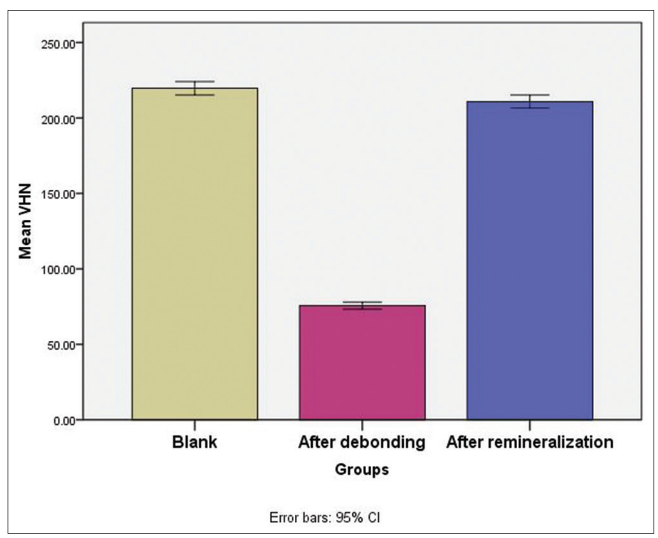

Figure 8: Bar chart illustrating the mean value of surface microhardness Kg/mm² VHN in blank samples (before bonding of the orthodontic bracket system), after debonding of the orthodontic bracket system, and after remineralization

demineralized)/(SMH baseline-SMH demineralized) X100), the mean percentage of Surface microhardness recovery (SMHR) was 95.72 ± 26.17%, with minimum 22.95 and maximum 200.97%.

Discussion

The current *in vitro* study was conducted on 80 freshly extracted premolar teeth for the purpose of orthodontic treatment as it was revealed from sample size calculation to obtain more accurate results.

As artificial saliva (simulated body fluid) maintains a simulated PH level and nearly identical contents to normal saliva, it serves as an excellent environment and medium for storing the study samples. This allows researchers to balance their samples' inorganic and organic contents while preventing the loss of any inorganic components. It is also important to note that the simulated body fluid should be replaced every 3 days to prevent any substance from leaking out or being swapped, which could change the ph.[38]

The main cause of the occurrence of white spot lesions during and after orthodontic treatment is that the bonding process in orthodontics requires surface preparation and etching of the enamel surface that ends in demineralization and release of calcium and phosphorous ions from the superficial surface.[3] The affected part of the tooth surface ranges from 5% to 85% of the total enamel surface.[4,5]

It is worth mentioning that frequent use of organic acids and solutions can affect the normal balance of demineralization and remineralization of enamel surfaces and rapidly lead to the dissolution of the enamel surface (3 to 4 weeks) by plaque microorganisms that result in the appearance of the first sign of enamel caries, white spot lesions.[6]

There are many remineralizing agents as fluoride, casein phosphopeptides, and amorphous calcium phosphate (CPP-ACP), but they have disadvantages as they are not natural sources for calcium; also, some previous studies were against using high doses of remineralizing agents as fluoride because it leads to the arrest of physiological demineralization and remineralization processes by saliva and advocates using low doses through using different mouth rinses.

The ultrasonic procedure is the gentle debonding method that was used in our current study to avoid occurrence of any cracks or fractures by other methods of debonding that affect negatively on the end results of the remineralizing effect of the chicken egg shell paste.

The chicken eggshell powder (CESP) is a complex calcium carbonate with highly organized calcite bio-ceramic and water molecules in the lattice. CESP is often municipal garbage, although it has the potential to be transformed into a calcium carbonate supply in the future. According to expectations based on the significant remineralizing effects of chicken eggshell powder in earlier studies,[20,21] the addition of chicken eggshell paste to the samples following the debonding of the orthodontic bracket system causes the mineral contents, primarily calcium and phosphate, to deposit onto the enamel surface and can restore its in-organic dissolved elements. These findings are consistent with earlier literature.[27,28]

SEM/EDX was used in the present study for the assessment of the differential changes of mineral content of the enamel surface at three different time intervals, before the bonding process, after debonding of the bracket system, and after remineralization stage, as it reveals an accurate report about a sample's chemical composition, including the elements present as well as their distribution and concentration.[38]

The EDX results revealed that the Ca/P ratio for the prepared samples before bonding of the orthodontic bracket system (blank samples) was (4.17 ± 2.2). This value significantly decreased to (2 ± 1.3) after the debonding of the orthodontic bracket system due to the decreasing of minerals, mainly calcium and phosphorus, during enamel surface conditioning by 37% phosphoric acid as a step for bonding of the orthodontic bracket system. The Ca/P ratio showed a significant increase to (4.79 ± 2.65) after the remineralization stage; this was owing to the effect of the paste of the chicken eggshell powder as it deposited calcium and phosphorus inside and around enamel prisms. These results were compatible with the results of previous studies.[20,21] Also, there was no significant difference between values recorded in blank samples and after remineralization due to the high mineral recovery rate.

In the current study, figures of SEM examination showed an intact solid surface before bonding of the orthodontic bracket system that revealed intact enamel prisms. In contrast, after the debonding step, it showed minute fractures on the surface that explained the effect of the etching step during the bonding process of the orthodontic bracket system as it led to the dissolution of the prism cores of the enamel surface and its boundaries.

As for SEM examination of the enamel surface of the prepared samples after the remineralization stage by a paste of the chicken eggshell powder, it showed the presence of radiopaque tangled threads and filaments; this image can explain the significant effect of the paste of the chicken eggshell powder in the deposition of calcium and phosphorus elements onto the prism core and its boundaries of the enamel surface. These results are similar to those revealed by previous literature.[30,38]

The results of EDX were assured by the results of the Vickers Micro-hardness Tester as that was performed in previous studies,[25,26] which revealed that the mean value of the samples before bonding of the orthodontic bracket system (blank sample) was significantly decreased to (75.62 ± 12.91) after debonding of the orthodontic bracket system due to mineral dissolution; then it showed a significant increase to (210.83 ± 24.10) after remineralization due to the significant effect of the chicken eggshell paste.

There was no significant difference between microhardness values recorded in blank samples and after remineralization due to the high minerals recovery rate, which was measured by the percentage of SMHR, which showed 95.72 ± 26.17%, with minimum 22.95 and maximum 200.97%.[37]

Conclusion

1. The chicken eggshell powder has a great remineralization effect for the demineralized enamel surface after debonding of the orthodontic enamel surface as it is one of the greatest and richest natural sources of calcium and phosphate elements that are deposited onto the defected enamel surface.
2. SEM and EDX are reliable methods to evaluate the remineralization effect of the chicken eggshell powder; this can be assured by using the Vickers microhardness tester to assess the surface microhardness.
3. The Vickers Micro-hardness Tester revealed a significant increase in its value after application of the chicken eggshell paste, which indicates an excellent remineralizing effect.

Recommendations

1. It is a very important step to apply the paste of the chicken eggshell powder during orthodontic treatment to avoid the appearance of white spot lesions after removal of the fixed orthodontic appliance.
2. It is recommended to combine the chicken eggshell powder with glass ionomer cement and with an orthodontic bonding agent, which help to avoid demineralization of the enamel surface.
3. Also, it is recommended to use the chicken eggshell with manufacturing of the mouth wash to be easy in application by the orthodontic patient.

Ethical statement

This study was approved by the Ethical Committee of the Faculty of Dental Medicine, Cairo, Boys, Al-Azhar University.

Patient consent

This type of study does not require informed consent.

Highlights

1. The chicken egg shell powder has a great remineralization effect for the demineralized enamel surface after debonding of the orthodontic bracket system as it is one of the greatest and richest natural sources of calcium and phosphate elements that are deposited onto the defected enamel surface.
2. Scanning electron microscopy (SEM) and energy-dispersive X-ray (EDX) are reliable methods to evaluate the remineralization effect of the chicken egg shell powder.
3. The Vickers microhardness tester is helpful to assess the enamel surface microhardness.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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