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Research Article

Comparative Evaluation of Remineralizing Potential of Chicken Eggshell Paste and Casein Phosphopeptide-Amorphous Calcium Phosphate on Surface Hardness of Bleached Enamel Surface: An *Invitro* Study

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Received: November 21, 2024; **Published:** December 27, 2024

Abstract

Background: Eggshells are the rich sources of minerals since it contains calcium, phosphorous, magnesium, strontium and fluoride. Hence, it can be used as remineralizing agent.

Aim and Objectives: To evaluate the effect of Chicken eggshell paste on surface microhardness of bleached enamel surface in comparison with commercial CPP-ACP paste using Vickers microhardness test.

Materials and Methodology: 96 extracted permanent anterior teeth were decoronated at CEJ and embedded into self-cure acrylic with the labial enamel surface exposed. All samples were randomly divided into the following groups: Group A: No bleaching was done for samples. Group B: Bleaching was done by using 35% Hydrogen peroxide. Group C: Bleaching was done with 35% Hydrogen peroxide followed by application of CPP-ACP. Group D: Bleaching was done with 35% Hydrogen peroxide followed by application of Chicken Eggshell paste. All the samples including those of Group A were stored in artificial saliva at 37°C. The baseline surface microhardness was measured, followed by surface microhardness measurement of posttreatment done at the end of 2 weeks. Data was analyzed using Kruskal Wallis test and Wilcoxon Signed Rank test.

Results: Statistically significant differences were observed between the group B followed by bleaching which decreased microhardnesss values. There were no significant differences between both remineralizing agents used i.e. eggshell paste and CPP-ACP.

Conclusion: Chicken eggshell paste can be used as an alternate to commercially available remineralizing agents.

Keywords: *Bleaching; Chicken Eggshell; CPP-ACP; Microhardness; Remineralization*

Introduction

There is an increasing awareness among patients regarding dental esthetics. Discoloration of teeth resulting from various reasons poses a major obstacle in achieving desirable esthetics [1]. Tooth bleaching technique is one of most conservative, esthetic and ideal solution which has gained high patient acceptance in improving the tooth discoloration. The use of high concentration of peroxide does not cause macroscopic changes but cause microscopical alterations of enamel surface in the form loss of fluoride, increased susceptibility

to erosion or caries, increased surface roughness, reduced enamel micro tensile strength, decreased abrasion resistance of bleached dental hard tissue [2]. Bleaching agents have generated microstructural changes on the bleached enamel surface. Studies demonstrated demineralization, degradation, and changes on surface microhardness and roughness of sound enamel surface but these adverse effects of the bleaching procedures on enamel surface defects like roughness and microhardness can be overcome by saliva, artificial saliva or remineralizing agents [3]. One of the attractive natural sources that can be used to provide minerals is Chicken eggshell powder [4]. Chicken eggshell powder (CESP) has been investigated in various fields regarding its potential use and is known for its Calcium source. Remineralizing agents such as fluoride, calcium, amorphous calcium phosphate (ACP), Casein phosphopeptide- ACP, hydroxyapatite, nanohydroxyapatite have shown promising results in various studies [1]. Ideal remineralizing material should diffuse or deliver calcium and phosphate into the lesion or boost the remineralization properties of saliva and oral reservoirs and remineralization ability of fluoride is considered to be the gold standard against others [4]. Studies have shown that the efficacy of nanohydroxyapatite derived from chicken eggshell in combination with 2% sodium fluoride in dentinal tubule occlusion demonstrated the effective depth of penetration of this combination into dentinal tubules and studies also have proven that the remineralization potential of CPP-ACP is capable of repairing initial enamel caries lesions [1].

Till date, there are no studies done comparing efficacy of Chicken eggshell paste and CPP-ACP on surface microhardness and surface roughness of enamel following bleaching procedure.

Aim of the Study

This study aimed to evaluate the effect of Chicken eggshell paste on surface microhardness of bleached enamel surface in comparison with commercial CPP-ACP paste using Vickers microhardness test.

Methodology

Source of data

A total of 96 permanent anterior teeth needed for the study were collected from Department of Oral and Maxillofacial surgery, College of Dental Sciences, Davangere, Karnataka after obtaining an informed consent from the patient.

Study design: *Invitro* experimental study.

Duration of the study: 6 months.

Procedure for making of chicken eggshell powder

The CESP was obtained by the process of calcination following the protocol given by World property intellectual organization (W0/2004/1055912: Method of producing eggshell powder) [5]. This Calcination process was done to obtain pure powder free from pathogens and to increase the alkalinity of the powder. Twenty chicken eggs were obtained and the contents were removed and the shells were cleaned in distilled water. The eggshells were then kept in hot water bath to facilitate the membrane removal. These eggshells were then crushed. The crushed particles were heated at 1200°C in a muffle furnace for one hour and finally powdered5 and this powder was sieved to collect the fine powder through 150 micron I.S sieve.

Procedure for chicken eggshell paste

Chicken eggshell powder and Xanthan gum was mixed as a dry powder with the help of spatula. Then a hot solution of Glycerol, water and Sodium saccharine were added slowly to the dry powder. After this, solution of peppermint oil and sodium lauryl sulphate was added and mixed to form homogenous paste. 5g of paste was dispensed from the container in a 20 ml beaker and 5 ml of freshly boiled and

cooled water was added to make 50% aqueous suspension and stirred well to make a thorough suspension. Determination of pH was done within 5 minutes, using a pH meter [6].

Specimen preparation

The teeth were decoronated and were positioned in a plastic mould and embedded using a self- curing acrylic resin with the labial enamel surfaces exposed. The enamel surfaces of the teeth were grounded into a flat surface.

Grouping

Group A: (Positive control group)

No bleaching was done for samples in group A.

Group B: (Negative control group)

Bleaching was done by using 35% hydrogen peroxide.

Group C: (Experimental group)

Bleaching was done with 35% hydrogen peroxide followed by application of CPPACP (GC Tooth Mousse; GC America Inc., USA).

Group D: (Experimental group)

Bleaching was done with 35% Hydrogen peroxide followed by application of Chicken Eggshell paste.

Procedure: The teeth were randomly divided into four groups based on remineralizing agent used. In Group B, C, D bleaching was done by allowing application of HP solution to remain in contact with the labial enamel surface for 30 minutes, following which will be rinsed off. In Group C, D application of remineralising agents was done using a micro brush and was allowed to remain on the enamel surface for 5 minutes. After remineralizing treatment, all the specimens including those of Group A were stored in artificial saliva at 370C. This procedure was repeated every day for 14 days [1].

Microhardness test using Vickers testing machine was done at initial and posttreatment values were taken at 14th day.

Figure 1: *Armamentarium.*

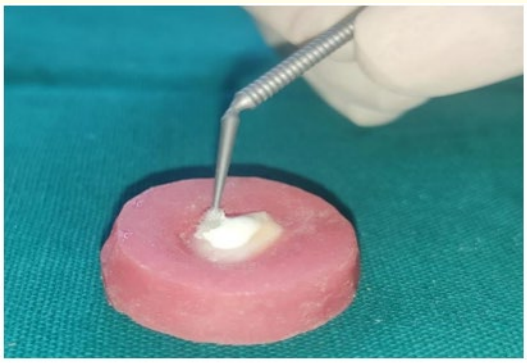


Figure 2: *Application of CESP.*

Figure 3: *Vickers testing of samples.*

Figure 4: *Making of eggshell paste.*

Statistical analysis was done using Windows Version 22.0 Released 2013. Armonk, NY: IBM Corp.

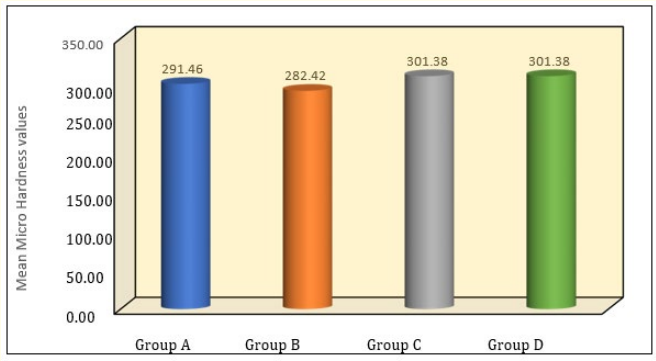
Inferential statistics

Kruskal Wallis Test followed by Dunn's post hoc test was used to compare the mean micro hardness values between 4 groups at baseline and 14-days' period. Wilcoxon Signed rank test was used to compare the mean micro hardness values between baseline and 14-days' period in each group. The level of significance was set at $P < 0.05$.

Results

The results obtained along with their statistical analysis have been explained in section of tables and graphs. Detailed explanation of each is as follows.

Graph 1 shows comparison of mean micro hardness values b/w 4 groups at baseline period using Kruskal Wallis test. It was noticed that the mean micro hardness value at baseline period for Group A was 291.46 ± 37.03, Group B was 282.42 ± 38.12, Group C was 301.38 ± 40.94 and Group D was 301.38 ± 36.46. These differences in the mean micro hardness between 4 groups at baseline period were not statistically significant p = 0.24.



Graph 1: *Mean micro hardness values b/w 4 groups at baseline period.*

Table 1 shows comparison of mean micro hardness values b/w 4 groups at 14 days period using Kruskal Wallis test. It was observed that the mean micro hardness value at 14-days period for Group A was 291.33 ± 36.81, Group B was 260.83 ± 40.34, Group C was 287.04 ± 39.64 and Group D was 279.88 ± 41.13. These differences in the mean micro hardness between 4 groups at 14 days period was statistically significant at p = 0.02.

Groups	N	Mean	SD	Min	Max	p-value
Group A	24	291.33	36.81	245	356	0.02*
Group B	24	260.83	40.34	202	350	
Group C	24	287.04	39.64	236	360	
Group D	24	279.88	41.13	200	345	

Table 1: *Comparison of mean micro hardness values b/w 4 groups at 14 days period using Kruskal Wallis test.*

Table 2 shows comparison of mean micro hardness values b/w baseline and 14 days period in each group using Wilcoxon Signed Rank test. The mean micro hardness in Group 1 at Baseline period was 291.46 ± 37.03 and at 14 Days period was 291.33 ± 36.81 and the mean difference between 2-time period was not statistically significant. In Group B, Group C and Group D the mean micro hardness at 14 days period was significantly reduced [260.83 ± 40.34, 287.04 ± 39.64, 279.88 ± 41.13] as compared to baseline period [282.42 ± 38.12, 301.38 ± 40.94, 301.38 ± 36.46] and the differences between 2-time intervals in each group was statistically significant at $p < 0.001$.

(I) Groups	(J) Groups	Mean Diff. (I-J)	95% CI for the Diff.		p-value
			Lower	Upper	
Group A	Group B	30.50	0.65	60.35	0.004*
	Group C	4.29	-25.55	34.14	0.63
	Group D	11.46	-18.39	41.30	0.36
Group B	Group C	-26.21	-56.05	3.64	0.02*
	Group D	-19.04	-48.89	10.80	0.04*
Group C	Group D	7.17	-22.68	37.01	0.67

Table 2: *Multiple comparison of mean difference in micro hardness values b/w 4 groups at 14 days period using Dunn's Post hoc test.*

Discussion

As many studies have been reported regarding the effects of bleaching on enamel surface texture and morphological changes of the enamel [7]. However there is great difference between the outcome of the studies regarding these changes on tooth surface, this might be due to differences in study design, microhardness test (Knoop vs. Vickers hardness), storage conditions between bleaching intervals (no remineralizing solution vs. artificial saliva vs. human saliva), fluoridation measures (applied vs. not applied) [2].

In the present study, one of the in-office bleaching agents was tested with relatively higher concentration, 35% hydrogen peroxide, and the study aimed to compare and evaluate remineralising potential of chicken eggshell paste and casein phosphopeptide-amorphous calcium phosphate on microhardness on bleached enamel surface.

Microhardness of enamel varies depending on its degree of mineralization, local variations in its structure resulting from the presence of enamel rods, and tufts or porosities near the dentino-enamel junction and it was observed that bleaching agents demineralize enamel to a depth of up to 50 μm. Hence, this mineral loss indicates changes in the hardness of teeth. So, microhardness tests is considered to be appropriate to evaluate the adverse effects of bleaching agents on enamel [1]. In the present study, Vickers hardness test was adopted as the basis for investigation over Knoop's because the square shape of indent obtained in Vickers is more accurate to measure. Even the minute changes in the square shape indent obtained after the test can be easily detected [8]. The Vickers hardness values obtained during the baseline measurements in the present study were not statistically significant. However, the surface mean micro hardness values for each group of the enamel specimens reduced following bleaching regimen except for positive control Group A and these differences in the mean micro hardness between 4 groups at 14 days' period was statistically significant at $p = 0.02$. These results coincide with those of various other studies [9-13] which have shown that bleached enamel is less hard than normal enamel, as bleaching would reduce strength of enamel. Some of the contradicting studies by different authors showed no changes were seen in the enamel surface after bleaching [14,15]. In 2000, Potocnik., *et al*. [16] found no significant changes in the enamel microhardness after treatment with 10% carbamide peroxide. Sulieman., *et al*. [17] in 2004, evaluated the effects of high concentrations of hydrogen peroxide used in office on the enamel and dentin. The results of this study showed no change in abrasion, hardness and topography in the enamel and dentin. These contradictory

studies regarding the microhardness alterations can be explained by the fact that surveys have different methodologies, such as using different bleaching agents (with different concentrations, application times and methods of application), different forms of hardness evaluation (Knoop, Vickers, weight and length indentation), pH level and storage method of the specimens [18].

Reductions in the microhardness and roughness of tooth enamel can result in adverse effects, such as pain, an increased risk of dental caries, increased vulnerability to fracture, as well as sensitivity of teeth. Therefore, the application of materials that stimulate the remineralization of teeth, which contains calcium, phosphate, and fluoride, can be necessary to reduce negative effects [13]. Therefore in the present study, we tested chicken eggshell paste and CPP-ACP to determine its ability to inhibit demineralization and promote the remineralization [1].

In the current study, the application of CPP-ACP after bleaching (Group C) increased enamel microhardness with the value showing 301.38 ± 40.94 significantly when compared to samples that received no additional treatment after bleaching. In comparison to the other group that is chicken eggshell paste which was also applied post bleaching (Group D) showed the value 301.38 ± 36.46 which increased microhardness in comparison to the samples that received no additional treatment after bleaching. Though values in both Group C, D did not reach the baseline values. As there were no significant differences seen between both remineralizing agents tested after 14 days, but slightly higher values were seen in Group C which was not statistically significant when compared to Group D. A systematic meta-analysis conducted by Yengopal and Mickenautsch concluded that there was sufficient clinical evidence demonstrating enamel remineralization and caries prevention by regular use of products containing CPP-ACP [19]. These results are supported with other studies [9,10] which also found that enamel mineral loss was significantly reduced when CPP-ACP was applied.

Micro-hardness results of this study revealed that the topical treatment with CESP significantly enhanced the micro-hardness of samples following bleaching which did not reach baseline values and indicated the process of remineralization. These present findings can be supported by Feroz., *et al*. [20] who recorded enhancement on the hardness and decrease on the surface roughness following the use of CESP. Also, the pH of a CESP was high enough to which it was favourable to increases the ion activity of anions such as hydroxyl ions and presence of high calcium content significantly helps in remineralization process.

The effect of commercially available CPP-ACP (GC tooth mousse) and eggshell paste on improving the microhardness were nearly comparable. There was no significant difference found in their remineralizing effect. As each one of them has its own manner of remineralization, further *in vivo* studies have to be conducted in order to evaluate the concentration and application time to reduce adverse effects of bleaching agents.

Conclusion

The present *invitro* study has been done to evaluate the effect of chicken eggshell paste on surface microhardness of bleached enamel surface in comparison with commercially available CPP-ACP paste using Vickers microhardness test.

It was observed that there was statistically significant difference seen using chicken eggshell paste which reduced surface roughness followed by bleaching.

It was observed that there was statistically significant difference seen using CPP-ACP paste which reduced surface roughness followed by bleaching.

In comparison with both the remineralizing agents, it was observed that negligible differences were seen following the application postbleaching in terms of surface roughness values.

Thus, it is proved that CESP possesses remineralizing ability which is comparable to that of CPP-ACP. Hence, CESP can be as an alternative to other commercially available remineralizing agents.

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Volume 24 Issue 1 January 2025