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Original Research Article

In vitro comparative evaluation of remineralizing potential of chicken eggshell paste and casein phosphopeptide-amorphous calcium phosphate on surface roughness of bleached enamel surface

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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 roughness of bleached enamel surface in comparison with commercial CPP-ACP paste using Surface Profilometer.
Materials and Methods: Ninety six 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 37^0C. The baseline surface roughness were measured, followed by surface roughness and 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 increased surface roughness 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.

1. 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] There are different ways to achieve this, such as composite fillings, ceramic coating, porcelain laminates, crowns and so on which are more expensive and time consuming.[2] Tooth bleaching technique is one of most conservative, esthetic and ideal solution which has gained high patient acceptance in improving the tooth discoloration.

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] Remineralizing agents such as fluoride, calcium, amorphous calcium phosphate (ACP), Casein phosphopeptide- ACP, hydroxyapatite, nanohydroxyapatite

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have shown promising results in various studies.[1] 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. Studies also has shown that chicken eggshell powder not only contains calcium but also other elements such as fluoride (F) and strontium which have a positive effect on bone and dental metabolism.[4]

Casein phosphopeptite (CPP) is derived from the main protein in the milk and plays a role in the stabilization of calcium, phosphate and fluoride ions. CPP-ACP can be connected to the biofilm, plaque, bacteria, hydroxyl-appetite, and mucous tissues. Its role in delaying tooth caries and also its low aptitude to induce caries has been proved.[2] Till date, there are no studies done comparing efficacy of Chicken eggshell paste and CPP-ACP on surface roughness of enamel following bleaching procedure. Hence, this study aimed to evaluate the effect of Chicken eggshell paste on surface surface roughness of bleached enamel surface in comparison with commercial CPP-ACP paste using Surface Profilometer.

2. Materials and Methods

The present study is an invitro experimental study, in which a total of 96 permanent anterior teeth needed for the study were collected from the source of data after obtaining an informed consent from the patient. The study was carried out for a period of six months. The sample size for the groups was calculated using the formula $n=z^2pq/e^2$.

The Chicken Eggshell powder (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 at 1000c for 10 minutes to facilitate the membrane removal. These eggshells were then crushed. The crushed particles were heated at 1200^0C in a muffle furnace for one hour and finally powdered.[5] To obtain chicken eggshell paste, the CESP powder and Xanthun 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 min, using a pH meter.[6] In the next step, the specimens were prepared by decoronating the teeth at the cemento-enamel junction (CEJ) and teeth were positioned in a plastic mould and embedded using a self-curing acrylic resin with the labial enamel surfaces exposed.

2.1. Group A: (Positive control group)

No bleaching was done for samples in group A.

2.2. Group B: (Negative control group)

Bleaching was done by using 35% Hydrogen peroxide.

2.3. Group C: (Experimental Group)

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

2.4. Group D: (Experimental Group)

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

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 37^0C. This procedure was repeated every day for 14 days.[1]

Surface roughness test using Profilometer was done at initial and posttreatment values were taken at 14^{th} day. Statistical analysis was done using Windows Version 22.0 Released 2013. Armonk, NY: IBM Corp.

2.5. Inferential statistics

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

3. 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:

Table 1 Comparison of mean Surface Roughness values b/w 4 groups at Baseline period using Kruskal Wallis Test.

The mean surface roughness value at baseline period for Group A was 0.5341 ± 0.3364, Group B was 0.6803 ± 0.4104, Group C was 0.6053 ± 0.4138 and Group D was

Table 1: Comparison of mean surface roughness values b/w 4 groups at Baseline period using Kruskal Wallis Test.

Groups	N	Mean	SD	Min	Max	p-value
Group A	24	0.5341	0.3364	0.112	1.23	0.48
Group B	24	0.6803	0.4104	0.248	1.542	
Group C	24	0.6053	0.4138	0.125	1.54	
Group D	24	0.6484	0.4085	0.125	1.658	

0.6484 ± 0.4085. These differences in the mean surface roughness between 4 groups at baseline period was not statistically significant [p=0.48].

Table 2 Comparison of mean Surface Roughness values b/w 4 groups at 14 days period using Kruskal Wallis Test.

The mean surface roughness value at 14-days period for Group A was 0.5320 ± 0.3368, Group B was 0.8504 ± 0.4279, Group C was 0.5883 ± 0.4155 and Group D was 0.6309 ± 0.4124.

These differences in the mean surface roughness between 4 groups at 14- days period was statistically significant at p=0.01.

Table 3Comparison of mean Surface Roughness values b/w Baseline & 14 days period in each group using Wilcoxon Signed Rank Test

The mean surface roughness in Group 1 at Baseline period was 0.5341 ± 0.3364 and at 14 days period was 0.5320 ± 0.3368 and the mean difference between 2-time period was not statistically significant. In Group B, the mean surface roughness at 14 days period was significantly increased [0.8504 ± 0.4279] as compared to Baseline period [0.6803 ± 0.4104] and the difference was statistically significant at p=0.02. The mean surface roughness in Group C & D was significantly lesser [0.5883 ± 0.4155, 0.6309 ± 0.4124] as compared to Baseline period [0.6053 ± 0.4183, 0.6484 ± 0.4085] and the differences between 2-time intervals in each group was statistically significant at p<0.001.

4. Discussion

As many studies have been reported regarding the effects of bleaching on enamel surface texture and morphological changes of the enamel.[7] In the present study, one of the in-office bleaching agents was tested with relatively higher concentration, 35% hydrogen peroxide, which is a commercially available and the study aimed to compare and evaluate remineralising potential of chicken eggshell paste and casein phosphopeptide-amorphous calcium phosphate on microhardness and surface roughness on bleached enamel surface.

The present study analyzed the surface roughness because it is possible to assess the alteration of the dental structure quantitatively that shows loss or gain of mineral of the dental tissues following bleaching therapy[8] and the roughness measurements were performed using profilometer, which has an advantage of accurate and precise measurement of the surface roughness without the need of additional measurements. The profilometric method was considered by many studies as an effective quantitative evaluation,[9] hence the same was used in our study. According to results of our study the mean surface roughness value at baseline period in all the four groups was not statistically significant, but there was a significant increase in the enamel surface roughness after application of bleaching agent. The mean differences of surface roughness between 4 groups at 14-days interval was statistically significant which shows p=0.01 and in Group B the mean surface roughness at 14 days interval was significantly increased [0.8504 ± 0.4279] as compared to baseline period [0.6803 ± 0.4104] and this difference was statistically significant at p=0.02. These results of our study were supported by authors with different studies[10–13] this might be due to the bleaching agent which is of high concentration reacts non-selectively with organic structures of dental tissues causing porosity in tooth also leading to exposure of enamel prisms following bleaching. regimen.[14] In contrast, many studies found that there were no significant changes seen in enamel roughness after hydrogen peroxide and carbamide peroxide application even after using a high concentration of hydrogen peroxide.[15–17] This might be due to the fact of change in ph, duration of the bleaching procedure and method of testing roughness.

Reductions in the 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.[18] Therefore in the present study, we tested chicken eggshell paste and CPP-ACP to determine its ability to inhibit demineralization and promote the remineralization process.

Surface roughness results of this study revealed that the topical treatment with CESP significantly reduced the mean surface roughness following bleaching which did not reach baseline values and indicated the process of remineralization. These present findings can be supported by Feroz et al[19] 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.

Table 2: Comparison of mean surface roughness values b/w 4 groups at 14 days period using Kruskal Wallis Test

Groups	N	Mean	SD	Min	Max	p-value
Group A	24	0.5320	0.3368	0.112	1.23	0.01*
Group B	24	0.8504	0.4279	0.405	1.703	
Group C	24	0.5883	0.4155	0.118	1.525	
Group D	24	0.6309	0.4124	0.118	1.648	

Table 3: Comparison of mean Surface Roughness values b/w Baseline & 14 days period in each group using Wilcoxon Signed Rank Test

Groups	Time	N	Mean	SD	Mean Diff	p-value
Group A	Baseline	24	0.5341	0.3364	0.0020	0.85
	14 Days	24	0.5320	0.3368		
Group B	Baseline	24	0.6803	0.4104	-0.1701	0.02*
	14 Days	24	0.8504	0.4279		
Group C	Baseline	24	0.6053	0.4138	0.0170	<0.001*
	14 Days	24	0.5883	0.4155		
Group D	Baseline	24	0.6484	0.4085	0.0175	<0.001*
	14 Days	24	0.6309	0.4124		

As there was significant reduction in surface roughness followed by application of CPP-ACP (Group C), this result was supported by studies done by different authors.[20,21] This may be due to the small size of the CPP-ACP nanocomplex which can enter the porosities of an enamel subsurface lesion and diffuse along its concentration gradient into the body of the lesion

The effect of commercially available CPP-ACP (GC tooth mousse) and eggshell paste on the reduction of enamel surface roughness were nearly comparable. There was no significant difference found in their remineralization 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.

5. Conclusion

Based on the results obtained from the present in-vitro study, it was observed that there was statistically significant difference seen in both groups such as chicken eggshell paste and 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 roughness values.

6. Source of Funding

None.

7. Conflict of Interest

None.

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