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COMPARATIVE EVALUATION OF TWO REMINERALISING AGENTS TO PREVENT STAIN ABSORPTION ON FRESHLY BLEACHED ENAMEL : AN IN VITRO STUDY

Dental Science

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ABSTRACT

An in vitro study was conducted to assess the efficacy of Tricalcium phosphate(β- TCP) and Casein phosphopeptide –amorphous calcium phosphate(CPP-ACPF)to prevent tea stain absorption on the enamels of thirty freshly bleached teeth .The specimens were bleached using 10% Carbamide Peroxide and divided into three groups 1,2 and 3, receiving no surface treatment, β- TCP and CPP-ACPF respectively. All specimens were then exposed to tea solution and the colour change in all the groups were analysed using digital photography software(Adobe CS5). The results indicated that both β- TCP and CPP-ACPF were effective($p<0.05$) in preventing stain reabsorption after exposure to tea solution with β-TCP being superior(Group 2) to Group 1 (Control) and Group 3(CPP-ACPF).

KEYWORDS

Carbamide Peroxide, Bleaching, B- Tcp, -acpf, Adobe Cs5

INTRODUCTION

Vital tooth bleaching with Carbamide Peroxide (CP) gels is becoming more and more popular showing good clinical long-term results and great satisfaction of the patients.But many studies in the literature have investigated the effects of bleaching on enamel morphology and the surface texture morphological alterations(Singh, 2010) (Attin, 2003). It has been observed that bleaching with 10% CP may result in a decrease of the calcium,phosphate and fluoride content in enamel leading to microscopic alterations such as increased surface roughness and formation of shallow erosions (Singh, 2010). Since this damage leads to more staining susceptibility after vital bleaching, it was conceivable that it must be possible to compensate for this problem by employing re-mineralizing agents like casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), or Tri-calcium phosphate(TCP) after bleaching to reduce the microstructural defects to increase the longevity and quality of bleaching (Rezvani,2015) (Hegde,2012). It was thought if the enamel of freshly bleached tooth was surface treated with remineralising agents, along with the effect of reduced sensitivity, it may reduce the absorption of stains and therefore maintain the effect of bleaching for a longer time. Therefore this invitro study was conducted to assess the efficacy of tricalcium phosphate and casein phosphopeptide –amorphous calcium phosphate to prevent stain absorption on freshly bleached enamel.

Materials instead of materils

This in-vitro study was undertaken in the study was conducted in the Department of Conservative Dentistry and Endodontics of a reputed Dental College in Western India. Thirty freshly extracted human upper central incisors and lateral incisors (extracted for peri-odontal reasons) were selected for the study.Other materials used were the remineralising agents viz, Casein phosphopeptide-amorphous calcium phosphate fluoride(GC Tooth Mousse Plus), B- Tricalcium phosphate(3M ESPE, Clinpro Tooth Crème), Carbamide peroxide bleaching agent(Opalescence 10% tooth whitening system),artificial saliva(prepared in house),tea solution (commercially available). The stain absorption was recorded using still digital photography (Nikon 3303 Camera) and measured using Adobe CS5 software. Only sound non-carious human maxillary central and lateral incisors were included in the study. Teeth with visible caries, cracks, defects, evident crown fracture and teeth on which previous root canal treatment was done were excluded. Extracted teeth were cleaned of calculus and debris with the help of ultrasonic scaler. Teeth selected were stored in 5% normal saline at room temperature (22degC) until use. The teeth were mounted on putty blocks such that only root was embedded till the crown enamel junction and the crown portion was visible and were numbered from 1 to 30. An initial photograph of all the specimens were taken before the procedure from a fixed distance of 50 cms from the putty blocks in a complete dark chamber. For exposure metering, a circular punch of the gray card was put behind each sample and the same automatic exposure mode of normal setting was selected for the all samples. The same standards were maintained throughout the conduct of the study.The labial surface of the samples were coated with one coat of 10% carbamide peroxide (Opalescence) bleaching agent in gel form and were left in place for 8 hrs. Subsequently,the samples were stored in artificial saliva for the next 16 hrs. This cycle continued for 8 successive days and photographs were taken after the completion of the bleaching and were stored in artificial saliva. The samples were randomly divided in to three groups in each group containing 10 teeth The groups were as follows for surface treatment.

- Group 1 (1-10): Control group- no surface treatment
- Group 2 (11-20): Specimens were treated with β TCP
- Group 3 (21-30): Specimens were treated with CPP-ACP

All the samples in Group 1 were taken out of the artificial salvia and immersed in tea solution for 10 minutes. No surface treatment was done for the specimens in the control group. After removing the specimens from the tea solution all the specimens were placed again in the artificial salvia for 24 hours and the treatment cycle was continued for 5 days. For specimens in Group 2 , the dried labial surface of these samples were coated with one coat of 10% β tricalcium phosphate (3M ESPE, Clinpro Tooth Crème) for 3 minutes using a brush. The specimens were placed again in artificial saliva for one hour and subsequently immersed in freshly prepared tea solution for 10 minutes before storing them in artificial saliva for 24 hours.The treatment cycle continued for 5 days.The same procedure cycle was employed in the specimens of Group 3 ,but using Casein phosphopeptide-amorphous calcium phosphate (GC Tooth Mousse Plus) as the re-mineralising agent. Digital photographs of the specimens were taken after this procedure. The photographs of the specimens were given the suffices ‘a’ and ‘b’ for denoting the specimens before and after the application of the remineralising agents viz, 1a and 1b for group 1 and 2a and 2b for group 2 and 3a and 3b for group 3.Adobe photoshop software CS5 was used to analyze the photographs. First, the global color cast of the images was eliminated according to the piece of gray card in the pictures. In order to compare the color, Commission International de l’Eclairage (CIE) system in the form of L*, a*, and b* obtained by the software. In the CIE system, L* characterizes the lightness and can range from 0 (dark) to 100 (light). The value of “a” represents the red (+) green (-) spectrum and “b” represents the yellow (+) blue (-) spectrum. Subsequently, L, a, and b values of the selected area were metered and histogram information was obtained. The Photoshop L, a and b values were transformed in to the CIE L*, a* and b* values using the following formulas:

$$L^* = \frac{(L \times 100)}{250}$$

$$a^* = \frac{((a -128) \times 240)}{250}$$

$$b^* = \frac{((b -128) \times 240)}{250}$$

Ultimately, ΔE* that represents the total color difference in CIE system was calculated as $\Delta E^* = [(\Delta L^*)^2 \ (\Delta a^*)^2 \ (\Delta b^*) 2]^{1/2}$(Rezwani, 2015). The method of colour analysis of the specimens using Adobe Photoshop CS5 is as depicted in Figure 1

Figure 1

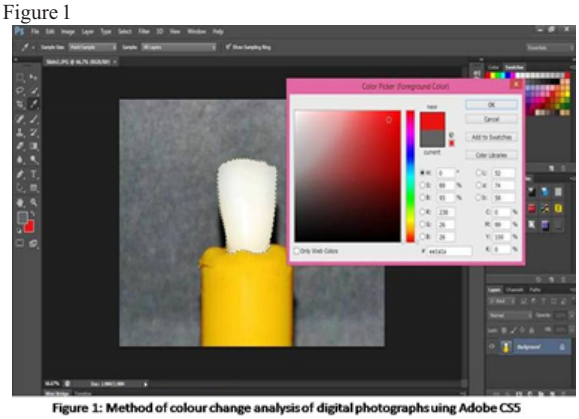

Figure 1: Method of colour change analysis of digital photographs uing Adobe CS5

Results

In all the three groups Control group(Group1), Group 2(B TCP)and Group 3(CPP-ACP) , the comparison of the L value(lightness) in terms of {Mean (SD)} between the groups before and after the surface treatment showed that there was statistically significant difference using the unpaired t test , (p value: < 0.001) as shown in Table 1,2 and 3 respectively.

Table 1: Comparison of difference in Lightness(L*) after bleaching (a) and dipping in tea solution(b)

Group1	*N*	*Mean*	*S.D*	*t value*	*p- value*
1a	10	78.80	8.753	4.324	<0.05*
1b	10	61.90	8.724		* Significant

Table 2:Comparison of difference in Lightness(L*) after bleaching (a) and dipping in tea solution(b) in Group 2

Group 2	*N*	*Mean*	*S.D*	*t value*	*p- value*
2a	10	75.80	7.569	4.715	<0.05* * Significant
2b	10	62.50	4.720		

Table 3:Comparison of difference in Lightness(L*) after bleaching (a) and dipping in tea solution(b) in Group 3

Group 2	*N*	*Mean*	*S.D*	*t value*	*p- value*
3a	10	74.70	3.561	2.409	<0.05*
3b	10	66.40	10.298		* Significant

On measuring the total colour difference before and after the surface treatment using the Delta E values of the CIE system it was found that in all the groups 1,2 and 3 there was a statistically significant colour difference in the respective specimens after dipping in tea solutions and the least colour change being in Group 2 ,where Tricalcium Phosphate was used as the remineralising agent(ANOVA Test) as depicted in Table 4(p value: <0.05)

Table 4: Comparison of difference in colour (DeltaE) after bleaching (a) and surface treatment in all three groups

Group	*N*	*Mean*	*Std. Deviation*	*P value*
Control	10	412.36	0.13	<0.05*
TCP	10	119.17	5.12	* Significant
CPP	10	223.85	1.31	

Discussion

Aesthetics have propitiated the development and improvement of techniques that satisfy beyond the cosmetic function of tooth appearance. Tooth bleaching has become an increasingly popular procedure; it is minimally invasive and highly effective. Most commonly used bleaching agents are hydrogen peroxide and carbamide peroxide.The mechanism of tooth bleaching is that the strong oxidative action of hydroxyl radicals generated from hydrogen peroxide or carbamide peroxide acts on the organic components of dentine and the pigmented materials, resulting in bleaching (Dahl,2003). Due to low molecular weight, hydroxyl radicals quickly and easily penetrate into the enamel and dentin porosities and cleave and weak bonds between stain molecules and organic matrix into smaller, less complex molecules. Carbamide peroxide, breaks down into hydrogen peroxide and urea in presence of water or saliva. Hydrogen peroxide and urea further break down into water, oxygen, carbon dioxide and ammonia resulting in the drop in pH causing enamel demineralization, surface alterations, decrease in micro hardness and loss of dental hard-tissue volume (Kelleher,1999). It was observed that bleaching with 10% CP may result in a decrease of the calcium and phosphate content, and also of the fluoride amount in enamel. Slight alterations of the enamel surface which could be noticed on scanning electron microscopic pictures or result in a hardness decrease of the bleached surface as a possible result of these interactions of the bleaching agent with the tooth surface (Attin,2003). Foods and drinks (like tea, coffee) consumed after the bleaching treatment (either immediately or after a while) can stain the bleached enamel (Addy,1979). A rough enamel surface with the pores or superficial defects after these changes can discolor easily. For these reasons, it is essential that the damaged enamel surface should be recovered after bleaching for a lasting bleaching effect. Remineralization of demineralized enamel is a gradual process that may occur partially or incompletely, thus the application of agents that accelerate this process is recommended following tooth bleaching. Remeralizing agents like fluoride, casein phosphopepide amorphous calcium phosphate and tricalcium phosphate recovers the damaged enamel surface, prevents staining, decreases post operative tooth hypersensitivity and increases the durability of bleaching treatment. The casein phosphopeptides (CPP) contain multiphosphoseryl sequences with the ability to stabilize calcium phosphate in nanocomplexes in solutions like amorphous calcium phosphate (ACP). Through their multiple phosphoseryl sequences, CPP binds to ACP in metastable solution preventing the dissolution of calcium and phosphate ions. The ACP-CPP also acts as a reservoir of bio-available calcium and phosphate, and maintains the solution supersaturated, thus facilitating remineralization (Baryak,2015). Tricalcium phospahate is a new hybrid material created with a milling technique that fuses beta tricalcium phosphate (β-TCP) and sodium lauryl sulfate or fumaic acid. This blending results in a “functionalized” calcium and a “free” phosphate, designed to increase the efficacy of fluoride remineralization. This in vitro study was carried out to compare and evaluate the efficacy of various remineralizing agents on bleached enamel in resisting stain absorption (Rezwani,2015). The remineralizing agents used were CPP-ACP and TCP.10% carbamide peroxide was used as the bleaching agent. The main factors that determine the tooth whitening efficacy are the peroxide concentration and its time of application(Meireles,2002). The present study was conducted with 10% carbamide peroxide and an eight-hour period of bleaching was chosen to simulate the condition of wearing bleaching tray overnight, followed by placing the samples in freshly prepared artificial saliva for 16 hours.Saliva was chosen as the storage medium to simulate the oral environment. To standardize the conditions in the study,freshly prepared artificial saliva was used since the pH of human saliva varies from person to person . In the present study, digital photography was used under standard conditions described above and the numeric values of colour differences were noted using Adobe CS5 software akin to previous studies existing in literature (Bentley , 1999). Each specimen was subjected to Adobe Photoshop CS5 and the tooth to be measured was selected. L, a, b, their mean and standard deviation values of the selected area were metered by clicking the image and noting on the histogram values. The Photoshop *L*, *a* and *b*values were transformed into the Commision Internationale de l’Eclairage (CIE) *, *a**, and **b* values using the following formulas as discussed in the materials and method. The mean and standard deviation were calculated and test of significance were done for each group,similar to methods used in existing literature(Rezwani,2015). On comparing the L value of Group I, there was a statistically significant difference (p<0.05;unpaired t-test) in the 1b stage(78.80) and the initial 1a (61.90).Similarly the L values in Group II(TCP) also showed a statistically significant difference on comparing the 2b (75.80) and 2a (62.50). Group III also showed similar changes on comparing the 3b (74.70) and the 3a (66.40). The total colour difference (ΔE) was calculated using the CIE formula to note which agent showed minimum stain absorption. The (ΔE) values of Groups I, II and III were 412.36,119.17 and 223.85 respectively. It is evident that Group II

(TCP) had the minimum stain absorption and the difference was found to be statistically significant using the ANOVA test ($p<0.05$) One of the most interesting outcomes of the current research was the stronger effect of β-TCP compared to CPP-ACPF. In our study, group II that is the β-TCP group showed least stain absorption than the control group and the CPP-ACPF group. TCP was found more effective than other groups. Higher concentration of calcium ion in TCP might have led to better remineralization capacity than CPP-ACPF(Karlinsey , 2010).Thus from the results obtained from this study it can be suggested that TCP is more effective in resisting stain on freshly bleached enamel as compared to CPP-ACPF. Hence, our study reported the efficacy of stain absorption in order of β-TCP > CPP-ACPF > Control group.

However this is an in-vitro study and although the potential of TCP and CPP-ACPF are promising, clinical trials and more studies and further research are required to prove its efficacy in boosting remineralization. One must bear in mind that remineralization in-vitro may be quite different when compared with dynamic complex biological system, which usually occurs in oral cavity in vivo.

Conclusion

Within the limitations of this in-vitro study it can be concluded that both the remineralizing agents viz, β- TCP and CPP-ACPF were found to be effective in inhibiting the demineralization caused by tea solution and β- TCP was found to be more effective than CPP-ACPF.

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