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RESEARCH ARTICLE

EFFECT OF DIFFERENT PEROXIDE BLEACHING REGIMENS AND SUBSEQUENT REMINERALIZATION ON THE HARDNESS OF HUMAN ENAMEL

[1,*]Dr. Kavita Sharma and [2]Dr. Shahjad Pathak

[1] Reader, Department of Pedodontics and Preventive Dentistry, BRS Dental College and Hospital, Barwala, Panchkula, Haryana, India

[2]Reader, Department of Conservative Dentistry and Endodontics, Luxmi Bai Institute of Dental Sciences, Patiala

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**Corresponding author:*

ABSTRACT

Aim: The purpose of this study was to evaluate the effect of different peroxide bleaching regimens and subsequent remineralization on the hardness of human enamel. **Materials and Methods:** One hundred fifty extracted human teeth were taken and divided into four groups i.e. group I- control and group II, III and IV- experimental groups bleached with 10% carbamide peroxide, 30% carbamide peroxide and 30% hydrogen peroxide respectively. After bleaching experimental groups were further divided into three subgroups, based on remineralization: subgroup A-stored in artificial saliva, subgroup B-application of CPP-ACP (Tooth mousse) and subgroup C-Reminpro respectively. The effects of each product were evaluated by calculating the change in average microhardness (AMH) of these groups after their application. **Statistical analysis:** It was performed by using paired t-test for intragroup comparisons and ANOVA for intergroup comparisons followed by Post-hoc Tukey's test and Bonferroni for groupwise comparisons. The significance level was taken at p value 0.05.**Results:** When comparing the effects of bleaching agents on enamel, all of the three regimens showed statistically highly significant decrease of mean AMH with statistically significant higher difference for in office bleaching regimens as compared to at home regimens. On comparing the effects of remineralizing agents on post-bleached enamel, CPP-ACP and Reminpro revealed statistically significant recovery of baseline values of mean AMH in all three experimental groups. No statistically significant differences were found between CPP-ACP and Reminpro. **Conclusions:** Demineralization produced by bleaching agent is dependent on concentration of peroxide and use of remineralizing product after bleaching is an imperative step.

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INTRODUCTION

With the barge of the audio-visual media and availability of internet in recent years of this information age, dental knowledge is increasing among the human population. Consequently, parents and the news media request information on dental whitening for children and adolescents with increasing frequency (AAPD guidelines). Currently, vital and nonvital bleaching techniques employ oxidizing agents such as hydrogen peroxide or hydrogen peroxide releasing agents to brighten teeth (Christensen, 1997). It has been reported in literature that bleaching treatment may cause morphological alterations in mineralized structures (Lewinstein *et al*., 2004; Zalkind, 1996; Kwon, 2002) and inorganic composition of dental structures (Hegedus *et al*., 1999) Changes in organic and inorganic contents after bleaching treatment can be measured by means of micro hardness tests (Featherstone, 1986). To overcome the decrease in micro hardness due to alteration in surface morphology and mineral contents it was proposed that the presence of fluoride may act as a remineralizing agent, by forming a calcium fluoride layer on enamel that inhibits demineralization or a decrease in micro hardness values (Mazzaoui, 2003). The purpose of this study was to evaluate the effects of different bleaching materials on the microhardness of human enamel and to see whether loss of hardness, if any can be reversed by different remineralizing solutions like CPP-ACP (tooth mousse) and reminpro.

MATERIALS AND METHODS

150 human permanent non carious teeth with intact enamel were collected. Carious teeth, hypo calcified teeth or teeth with any developmental anomaly were excluded from the study. The teeth were thoroughly cleaned and polished with rubber cup and pumice. All samples were stored in normal saline at room temperature till further use. The crowns were separated from the roots using a diamond disc using low speed micro

motor straight hand piece with water spray. These enamel sections were then embedded in acrylic resin blocks of dimensions 25mm (l) x 20mm (w) x 15mm (h). The outer enamel surface of teeth was kept exposed. These exposed enamel surfaces were made flat and smoothened by using abrasive grit papers of Silicone Carbide (Kemet International, Maidstone, UK). The samples were then polished with diamond paste (shofu) and super snap buffs (shofu). A window of 4mm x 6mm dimension was exposed on each enamel sample by covering the rest of sample area with nail varnish. 15 samples were taken as control and labeled Group I. The rest of 135 samples were equally divided into 3 experimental groups. Each of the three experimental groups was further subdivided equally into 3 subgroups of 15 teeth each depending on the remineralizing agent used.

The various groups and subgroups were:

Group I – Control group (n=15)
Group II - 10% carbamide peroxide group (n=45)
Sub-Group II A- stored in artificial saliva(n=15)
Sub-Group II B- remineralized with CPP-ACP(n=15)
Sub-Group II C-remineralized with Fluoride+ Hydroxyapatite+Xylitol (n=15)
Group III - 30% carbamide peroxide group(n=45)
Sub-Group III A- stored in artificial saliva(n=15)
Sub-Group III B- remineralized with CPP-ACP(n=15)
Sub-Group III C-remineralized with Fluoride+ Hydroxyapatite+Xylitol(n=15)
GROUP IV - 30% hydrogen peroxide group (n=45)
Sub-Group IV A- stored in artificial saliva(n=15)
Sub-Group IV B- remineralized with CPP-ACP(n=15)
Sub-Group IV C-remineralized with Fluoride+Hydroxyapatite+Xylitol(n=15)

For microhardness testing the, "Mitutoyo Hardness Testing Machine" was used for measuring Vickers hardness score. Tests were carried out according to the manufacturer's instructions. 3 readings were recorded for each sample and average was taken. For all of the 150 samples Baseline microhardness was recorded. After baseline readings the specimens in Control group i.e. Group I (n=15) were stored in artificial saliva without any further intervention.

Application of bleaching agents in Experimental groups: Bleaching of the specimens in 3 experimental groups was done as follows:

Group II- Forty-five enamel samples of this group were bleached with 10% Carbamide peroxide.

Group III- Two applications of 30% Carbamide peroxide gel were made on the 45 enamel samples of this group.

Group IV- Two applications of 30% Hydrogen peroxide gel were made on the 45 enamel samples of this group.

Post-bleaching microhardness assessment: After bleaching the experimental group (Group II, III and IV), post bleaching micro hardness values were measured in the same way as mentioned earlier for the baseline scores. The experimental groups were then divided into subgroups as explained earlier.

Application of remineralizing agents on Post-bleached specimens: Immediately after post-bleaching testing, specimens of different subgroups were subjected to different remineralizing agents as follows:

Subgroups IIA, IIIA and IVA- stored in artificial saliva.
Subgroups IIB, IIIB and IVB- Tooth mousse was applied.
Subgroups IIC, IIIC and IVC- ReminPro was applied.

Statistical analysis was performed by using paired t-test for intragroup comparisons and ANOVA for intergroup comparisons followed by Post-hoc Tukey's test and Bonferroni for group wise comparisons. The significance level was taken at p value 0.05.

DISCUSSION

Cosmetic dentistry has become an integral part of contemporary dental practice. Improvement of the appearance of discolored teeth by whitening (bleaching) systems has been an extraordinary advancement in modern esthetic dentistry (Mielczareka, 2008).Depending on application mode and peroxide concentration vital tooth whiteners are classified into three categories (Cavalli, 2004): For professional use only: contain high concentrations of carbamide peroxide (30–37%) and hydrogen peroxide (30–35%) solutions; Patient applied tooth whiteners for at home bleaching: contain up to 20% carbamide peroxide and 10% hydrogen peroxide; dispensed and monitored by dentist. Over-the-counter products: available directly over-the-counter; used at home by consumers however, its safety is questionable (Haywood, 1992). Bleaching agents might result in the change in phosphate, calcium and fluoride content of enamel. Thus, a remineralization system should supply stabilized bioavailable calcium, phosphate and fluoride ions as all of these minerals may be lost after bleaching (Wrigth, 2002). Thus, the addition of fluoride to bleaching agents or its use after bleaching procedures has been investigated in attempt to increase mineralization of bleached enamel. Attin *et al* in 2007 found that the addition of fluorides into bleaching agent can support the rehardening of bleached enamel with a shorter period needed for hardness to recover compared to gels without fluoride (Attin, 2007).

Research has shown that adding ACP (Acidulated Calcium Phosphate) to whitening gels will reduce tooth sensitivity after bleaching via remineralization. It has also been reported to enhance the natural healing remineralization process of saliva. However, ACP system stabilized by CPP, otherwise known as CPP-ACP, provides a higher reservoir of bioavailable calcium and phosphate ions in comparison with ACP only, leading to increased remineralization potential (Wrigth, 2002). Xylitol is able to form complexes with calcium ion and prevent decalcification by inhibiting the translocation of dissolved Ca^{2+} and PO_4^{3-} ions from the lesions (by lowering the diffusion coefficients of calcium and phosphate ions) (Chunmuang, 2007). The remineralizing agents used in the present study were Tooth Mousse and ReminPro. Tooth Mousse is topical cream with bioavailable Ca and phosphate. It binds to bacteria, plaque, biofilms, hydroxyapatite and soft tissue. By composition, it contains CPP-ACP and Xylitol. ReminPro combines three components for effective protection against demineralization and erosion: hydroxyapatite, fluoride, and xylitol. Microhardness measurement of tooth material can be done in three different ways like in Knoop's hardness number (KHN), Vickers's hardness number (VHN) and Brinnel's hardness number (BHN). KHN gives a rhomboid shaped indentation, VHN a square shaped and BHN a circle shaped. In the present study Vicker hardness number was chosen because a square

OBSERVATIONS AND RESULTS

Table-1: Mean Average Microhardness(AMH) Of Control (I) & Experimental Groups (II,III & IV) At Baseline

GROUP	n	Mean	S.D.	S.E.
Group I (Control)	15	296.700	19.4938	5.0333
Group II (10% CP)	45	297.502	18.3603	2.7370
Group III (30% CP)	45	300.871	19.9671	2.9765
Group IV (30% HP)	45	295.836	18.6491	2.7800

Table-1a. Inter and Intra-group comparison of Mean Average Microhardness(AMH) At Baseline

ANOVA Baseline					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	617.585	3	205.862	.567	.638
Within Groups	52997.425	146	362.996		

Table 2. Post-Bleaching Mean Average Microhardness(AMH) of Experimental Groups (II,III & IV)

Experimental Group	n	Mean	S.D.	S.E.
Group II (10% CP)	45	277.614	17.2239	2.5676
Group III (30% CP)	45	265.789	19.6203	2.9248
Group IV (30% HP)	45	249.684	18.5778	2.7694

Table-2a. Inter and Intra-group comparison of Post-Bleaching Mean Average Microhardness(AMH)

ANOVA Post-bleaching					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	17689.298	2	8844.649	25.843	<.001**
Within Groups	45177.156	132	342.251		

Table-3: Inter-group Comparison of Post-Bleaching Mean Average Microhardness(AMH) values of Experimental groups (II,III & IV)

POST-HOC TEST (Tukey HSD)				
Group (I) value	Group (J) value	Mean Difference (I-J)	S.E.	Sig.
10% CP 277.614 ± 22.22	30% CP 265.789 ± 19.62	11.825	4.2518	.007**
	30% HP 249.684 ± 18.58	27.930	4.2518	<.001**
30% CP 265.789 ± 19.62	10% CP 277.614 ± 22.22	-11.825	4.2518	.007**
	30% HP 249.684 ± 18.58	16.1044	4.2518	.001**
30% HP 249.684 ± 18.58	10% CP 277.614 ± 22.22	-27.930	4.2518	<.001**
	30% CP 265.789 ± 19.62	-16.1044	4.2518	.001**

Table-4. Intra-group Comparison of Change in Baseline & Post-bleach Mean AMH values in Experimental Groups (II,III & IV)

Paired Samples- t test										
group		n	Mean	S.D.	S.E.	Paired Differences			t	Sig. (2-tailed)
						Mean of Difference between PB-B	S.D.	S.E.		
Group II (10% CP)	BASELINE	45	297.502	18.360	2.737	19.8880	1.459	.217	91.473	<.001**
	POST-BLEACH	45	277.614	17.224	2.568					
Group III (30% CP)	BASELINE	45	300.871	19.967	2.977	35.0822	2.333	.348	100.866	<.001**
	POST-BLEACH	45	265.789	19.620	2.925					
Group IV (30% HP)	BASELINE	45	295.836	18.649	2.780	46.1511	1.030	.154	300.533	<.001**
	POST-BLEACH	45	249.684	18.578	2.769					

Table-5. Mean Values of Average Microhardness(AMH) of Experimental Groups after Remineralization with Various Agents

Group	Subgroup	n	Mean	S.D.	S.E.
Group I (Control)	Artificial Saliva	15	307.073	20.145	5.201
Group II (10% CP)	Subgroup A (Artificial Saliva)	15	282.613	17.825	4.602
	Subgroup B (CPP-ACP)	15	301.400	16.903	4.364
	Subgroup C (ReminPro)	15	308.747	20.727	5.352
Group III (30% CP)	Subgroup A (Artificial Saliva)	15	282.180	23.652	6.107
	Subgroup B (CPP-ACP)	15	304.667	18.945	4.892
	Subgroup C (ReminPro)	15	302.893	19.863	5.129
Group IV (30% HP)	Subgroup A (Artificial Saliva)	15	271.360	21.978	5.675
	Subgroup B (CPP-ACP)	15	295.533	17.152	4.429
	Subgroup C (ReminPro)	15	297.840	19.382	5.004

Table-6. Inter & Intra Subgroup Comparison of post-remineralization Mean AMH Values of Bleached Experimental Samples

ANOVA(Post-remineralization)						
Group	Comparison	Sum of Squares	df	Mean Square	F	Sig.
Group II	Between Subgroups	5449.317	2	2724.659	7.912	.001**
(10% CP)	Within Subgroups	14462.775	42	344.352		
	Total	19912.092	44			
Group III	Between Subgroups	4689.185	2	2344.593	5.358	.008**
(30% CP)	Within Subgroups	18380.067	42	437.621		
	Total	23069.252	44			
Group IV	Between Subgroups	6454.306	2	3227.153	8.398	.001**
(30% HP)	Within Subgroups	16139.945	42	384.284		
	Total	22594.251	44			

Table 7. Intra group Comparison of Post-Remineralization Mean AMH of Experimental Subgroups

POST-HOC TEST (Tukey HSD)					
Group	Subgroups (I) value	Subgroups (J) value	Mean Difference (I-J)	S.E.	Sig.
Group II (10% CP)	SubGroup A (Artificial Saliva)	CPP-ACP	-18.7867	6.776	.022*
		ReminPro	-26.1333	6.776	.001**
	SubGroup B (CPP-ACP)	Artificial Saliva	18.7867	6.776	.022*
		ReminPro	-7.3467	6.776	.529
	SubGroup C (ReminPro)	Artificial Saliva	26.1333	6.776	.001**
		CPP-ACP	7.3467	6.776	.529
Group III (30% CP)	SubGroup A (Artificial Saliva)	CPP-ACP	-22.4867	7.639	.014*
		ReminPro	-20.7133	7.639	.026*
	SubGroup B (CPP-ACP)	Artificial Saliva	22.4867	7.639	.014*
		ReminPro	1.7733	7.639	.971
	SubGroup C (ReminPro)	Artificial Saliva	20.7133	7.639	.026*
		CPP-ACP	-1.7733	7.639	.971
(Group IV) 30% HP	SubGroup A (Artificial Saliva)	CPP-ACP	-24.1733	7.158	.004**
		ReminPro	-26.4800	7.158	.002**
	SubGroup B (CPP-ACP)	Artificial Saliva	24.1733	7.158	.004**
		ReminPro	-2.3067	7.158	.944
	SubGroup C (ReminPro)	Artificial Saliva	26.4800	7.158	.002**
		CPP-ACP	2.3067	7.158	.944

Table-8. Comparison of Baseline, Post-bleach & Postremineralization Mean AMH Values at Subgroup level

ANOVA						
Subgroups	Comparison	Sum of Squares	df	Mean Square	F	Sig.
Subgroup II A	Between Groups	3085.107	2	1542.554	4.874	.012*
(10% CP:SALIVA)	Within Groups	13291.871	42	316.473		
	Total	16376.978	44			
Subgroup II B	Between Groups	5303.750	2	2651.875	9.962	.001**
(10% CP:CPP-ACP)	Within Groups	11180.781	42	266.209		
	Total	16484.531	44			
Subgroup II C	Between Groups	5635.914	2	2817.957	7.072	0.002**
(10% CP:REMINPRO)	Within Groups	16736.151	42	398.480		
	Total	22372.065	44			
Subgroup III A	Between Groups	9193.011	2	4596.506	8.780	.001**
(30% CP:SALIVA)	Within Groups	21988.645	42	523.539		
	Total	31181.656	44			
Subgroup III B	Between Groups	13125.490	2	6562.745	19.285	.001**
(30% CP:CPP-ACP)	Within Groups	14292.400	42	340.295		
	Total	27417.890	44			
Subgroup III C	Between Groups	13917.297	2	6958.649	17.728	.001**
(30% CP:REMINPRO)	Within Groups	16486.395	42	392.533		
	Total	30403.692	44			
Subgroup IV A	Between Groups	15863.566	2	7931.783	17.999	.001**
(30% HP:SALIVA)	Within Groups	18508.025	42	440.667		
	Total	34371.591	44			
Subgroup IV B	Between Groups	22155.714	2	11077.857	38.279	.001**
(30% HP:CPP-ACP)	Within Groups	12154.839	42	289.401		
	Total	34310.552	44			
Subgroup IV C	Between Groups	22136.617	2	11068.309	29.428	.001**
(30% HP:REMINPRO)	Within Groups	15796.895	42	376.117		
	Total	37933.512	44			

Table 9. Comparison of Post-remineralization mean AMH values with Baseline & Post-bleach mean AMH values of all Subgroups

Subgroups	Mean value (I)	Mean value (J)	Mean Difference (I-J)	S.E.	Sig.
Subgroup II A	PR (282.613)	B (294.513)	-11.9000	6.4959	.222
(10% CP:SALIVA)		PB (274.34)	8.2733	6.4959	.629
Subgroup II B	PR (301.4)	B (295.453)	5.9467	5.9577	.972
(10% CP:CPP-ACP)		PB (275.98)	25.4200	5.9577	.001**
Subgroup II C	PR (308.747)	B (302.54)	6.2067	8.7261	1.000
(10% CP:REMINPRO)		PB (282.52)	26.2267	8.7261	.001**
Subgroup III A	PR (282.18)	B (301.18)	-19.0000	8.3550	.084
(30% CP:SALIVA)		PB (266.213)	15.9667	8.3550	.189
Subgroup III B	PR (304.667)	B (301.627)	3.0400	6.7359	1.000
(30% CP:CPP-ACP)		PB (267.013)	37.6533(*)	6.7359	.001**
Subgroup III C	PR (302.893)	B (299.807)	3.0867	7.2345	1.000
(30% CP:REMINPRO)		PB (264.14)	38.7533(*)	7.2345	.001**
Subgroup IV A	PR (271.36)	B (297.14)	-25.7800(*)	7.6652	.005**
(30% HP:SALIVA)		PB (251.267)	20.0933(*)	7.6652	.036*
Subgroup IV B	PR (295.533)	B (294.093)	1.4400	6.2118	1.000
(30% HP:CPP-ACP)		PB (247.76)	47.7733(*)	6.2118	.001**
Subgroup IV C	PR (297.84)	B (296.273)	1.5667	7.0816	1.000
(30% HP:REMINPRO)		PB (250.027)	47.8133(*)	7.0816	.001**
Control	PR (307.073)	B (296.7)	10.3733	7.2451	.479
(Group I)					

*Significant
** Highly Significant

shape of indent obtained in VHN was easy and more accurate to measure. The baseline values of the present study are similar with the earlier studies performed by Ryges and Foley (1961). (Ryge, 1961) The average hardness value for enamel is in the range from 250 to 360 VHN. The results are also consistent with previous studies performed by Lopes *et al* (2002)[16] and Wongkhantee *et al* (2006). When paired t-test was applied, the Post-bleach values of all experimental groups showed statistically significant difference as compared to Baseline values of respective groups (Group II to Group IV). This evaluation was in accordance with Chen *et al*. (2008), Akal *et al*. (2001) and Rodrigues *et al*. (2005) who also found significant decrease in microhardness after application of 10% CP bleaching agent. Rodrigues *et al*. (2005) and Maleki pour (2012) found significant decrease in microhardness for office bleaching agents with 37% and 35% carbamide peroxide respectively. Similarly, Jiang *et al*. (2008) with 30% hydrogen peroxide, Abreu *et al*. (2011) and Ulukapi (2007) with 35% hydrogen peroxide reported significant decrease in microhardness. The loss of mineral content and organic matrix decreased enamel microhardness (Pinto *et al*., 2004). The mean AMH values of subgroups of experimental groups after remineralization were highest for Subgroup C followed by Subgroup B and lowest for Subgroup A in Group II and IV. Whereas, the values were highest for Subgroup B followed by Subgroup C and Subgroup A in Group III. The values were highest for Group I (Control) which was not bleached but kept in artificial saliva for a total of 12 days. When intra-group comparison was done between various Remineralizing agents, Subgroup A showed statistically significant difference as compared to Subgroup B and Subgroup C in each of the 3 experimental groups. No statistically significant difference was found between Subgroup B and Subgroup C. Borges *et al* (2010) found significant differences of microhardness for bleached groups that received no remineralizing gel but stored in artificial saliva as compared to bleached groups treated with remineralizing gel post-bleaching (Clark, 1993). Post-remineralization mean AMH values compared with baseline & post-bleach mean AMH values for Subgroup II A and III A revealed no statistically significant difference. Like the results of our study, Chen *et al* (2008) also showed partial recovery of microhardness in group bleached with 10% CP and then kept in artificial saliva for 7 days. Costa *et al* (2007) similarly found no significant difference in microhardness after storage in artificial saliva of 10% CP bleached group. Basting *et al*. (2003) revealed that the immersion of specimens in artificial saliva for 14 days, after bleaching treatment with different concentrations of carbamide peroxide, promoted an increase in enamel microhardness, but the baseline values were not recovered. For Subgroup II B, II C, III B, III C, IV B and IV C Post-remineralization AMH showed statistically highly significant increase as compared to Post-bleach Mean AMH. But these readings were comparable to baseline scores and no statistically significant difference was found. Although artificial saliva was used in our study, it was not able to reestablish baseline enamel microhardness values without application of CPP-ACP or Reminpro. Also in this in vitro study, teeth were not brushed with toothpaste. In vivo, if the patient brushes with fluoridated toothpaste, it might help with remineralization process. The oral environment provides conditions for enamel remineralization because of some important factors, such as salivary flow, buffering capacity of saliva, oral hygiene and the use of topical fluorides that may increase the remineralization of bleached enamel (Basting, 2003). However, these results illustrate the need for at home whitening agents to be used with professional supervision, to ensure proper application of the bleaching agents. Also our study highlights the beneficial effects of using remineralizing products after bleaching for both at home and in office bleaching protocols.

Conclusion

When comparing the effects of bleaching agents on enamel, all of the three regimens showed statistically highly significant decrease of mean AMH. Also the decrease in mean AMH was statistically significant higher for in office bleaching regimens as compared to at home regimens. On comparing the effects of remineralizing agents on post-bleached enamel, CPP-ACP (Tooth mousse) and Reminpro revealed statistically significant recovery of baseline values of in all three experimental groups. No statistically significant differences were found between

CPP-ACP (Tooth mousse) and Reminpro. So, we can conclude that demineralization produced by bleaching agent is dependent on concentration of peroxide and use of remineralizing product after bleaching is an imperative step.

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