ORIGINAL ARTICLE

COMPARATIVE EFFECT OF DIFFERENT REMINERALIZING AGENTS ON THE MICROHARDNESS OF BLEACHED ENAMEL- AN IN VITRO STUDY

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ABSTRACT:

Background: This study was undertaken to investigate the effect of McInnes bleaching agent on the microhardness of enamel before and after bleaching and to evaluate the effect of G C Tooth Mousse Plus and Toothmin on the bleached enamel surface for its microhardness using Vickers microhardness indenter. **Materials and methods:** Twenty freshly extracted, sound central incisors were selected and their roots were removed. Twenty samples were divided into 2 groups; n=10 (Group 1for tooth mousse plus and 2 for Toothmin). Microhardness was measured after seventh (1st remineralization cycle) and fourteenth day (2nd remineralization cycle) respectively. **Results:** Baseline microhardness values of GC Toothmousse and Toothmin when compared with first cycle of bleaching decrease by 1.07% ($P<0.001$) and .96% ($P<0.001$) respectively, which is highly significant and a decrease of 2.45% ($P<0.001$) and 2.56% ($P<0.001$) after second bleaching cycle which is also highly significant. But significant difference was found between baseline microhardness values and remineralization values in case of GC Toothmouse Plus while in case of Toothmin, it was not significant. **Conclusion:** McInnes bleaching agent causes a decrease in the microhardness of enamel by causing enamel demineralization. GC Tooth mousse plus and toothmin used in this study caused increase in microhardness of the bleached enamel. Toothmin application led to more remineralization as compared to GC Tooth Mousse Plus even though it could not reach baseline level.

Key words: Bleaching agent, demineralisation, microhardness, remineraslisation.

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INTRODUCTION

One of the main reasons for which patient seek esthetic dental treatment is for real or perceived discoloration of anterior teeth. Several methods are available to restore the esthetics of discoloured teeth. Laminates, porcelain jacket crowns, bonding and mechanical or chemical stain removal, bleaching are the options for esthetic treatment. Laminates and full coverage crown involves more tooth structure removal. And hence, it is more invasive option. [1]Bleaching is most popular amongst these as it is simple , less invasive and least expensive option available to dentist to lighten discoloured tooth and eliminate or diminish any stains in both vital and non vital teeth.[2]

Bleaching was first described in 1877 as whitening of teeth. Later in 1916, Dr. Walter Kane used hydrochloric acid to remove fluorosis stains. In 1937, Ames used an alternative technique using the hydrogen peroxide instead of hydrochloric acid to remove fluorosis stains.[3,4] McInnes solution has been successfully used to bleach the teeth with endemic fluorosis, wherein five parts 30% hydrogen peroxide, five parts 36% hydrochloric acid and one part ethyl ether were used.[5,6] Increased frequency of acid exposure in bleaching tends to alter the total demineralization/ remineralization amounts, resulting in significantly greater amounts of mineral loss.[2,5] Different methods have been utilized to determine tooth demineralization/remineralization including

microradiography and indirect methods like iodine permeability and surface microhardness.[7] Until today, fluoride therapy has remained the most popular of all caries prevention and remineralization methods. Fluoride is generally known to promote remineralization, but its remineralization process relies on calcium and phosphate ions from saliva. Therefore, several new remineralizing agents have been introduced to supplement and enhance the ability of fluoride to restore tooth minerals.[9,10] Lately, dairy products (milk, milk concentrates and cheeses) have received a lot of attention for their anticariogenic effect in animal and human in situ caries models.[8] CPP-ACP has a potential to remineralize the enamel.[11] CPP contains the cluster sequence of phosphoseryl residues from casein and CPP-ACP is reported to have topical anticariogenic effects due to its ability to stabilize calcium and phosphate in an amorphous state.[12]The proposed mechanism for the CPP-ACP is to localize ACP in dental plaque, which buffers the free calcium and phosphate ion activities, thereby helping to maintain a stage of supersaturation with respect to tooth enamel depressing demineralization and enhancing remineralization.[13]

GC Tooth Mousse Plus contains 10% CPP-ACP and 0.2% NaF. Combined together in GC Tooth Mousse Plus they form CPP-ACPF (casein phosphopeptide – amorphous calcium phosphate fluoride). When applied in the mouth CPP-ACPF binds to tooth, pellicle, plaque and soft tissue surfaces localising bioavailable calcium, phosphate and fluoride. The addition of fluoride in CCP-ACP enhances its remineralization potential and increases hardness of enamel.[14]

Recently, a new product by the name of Toothmin (abbott) has been launched. It consists of a formula known as Anticay which aids in remineralization. Anticay is a mixture of calcium salts of sucrose phosphate esters, complexed with inorganic calcium orthophosphate. It is composed of 10-12% calcium and 8-10% phosphorous by weight. Calcium and phosphate in aqueous media tend to form insoluble precipitates. Anticay is colorless, odorless, has a bland neutral flavour, is stable across a wide pH ranges at high temperatures and can be easily incorporated into product formulations.

The present study was undertaken to investigate the effect of McInnes bleaching agent on the microhardness of enamel before and after bleaching and to evaluate the effect of G C Tooth Mousse Plus and Toothmin on the bleached enamel surface for its microhardness using Vickers microhardness indenter.

MATERIALS AND METHODS

Various materials and equipments used in study are shown in table 1.

Twenty freshly extracted, sound central incisors were selected and their roots were removed. Then, each tooth crown was embedded in self cured acrylic resin molds, with the labial surface leveled on top and lying flat and parallel to the horizontal plane. Twenty samples were divided into 2 groups; n=10 (Group 1for tooth mousse plus and 2 for Toothmin). Mounted samples were stored in artificial saliva to prevent dehydration. The baseline microhardness measurements were taken on the labial surface by means of a Vickers indenter with 100 g of force for 30 s by focusing at × 10 objective lens [Figure 1], two indentations on each sample away from edge were made and the average of two readings were taken as baseline microhardness measurement.

Figure 1: Vickers indenter to measure microhardness

Bleaching agent preparation and its application

Freshly prepared McInnes bleaching solution consists of a mixture of 1 ml of 36% hydrochloric acid, 1 ml of 30% hydrogen peroxide and 0.2 ml of anaesthetic ether which is mixed in the ratio of 5:5:1. It was applied to the enamel surface using a cotton applicator for 5 minutes (1st cycle of bleaching). Then, the samples were washed with de-ionized water and blotted dry using absorbent paper and subjected to the microhardness of the enamel surfaces with the Vickers indenter as

previously. Then again the samples were stored in artificial saliva for next 24 hr to prevent dehydration. Again after 24 hours, the second application of bleaching agent was carried out (2nd cycle of bleaching) as described earlier and the microhardness values were recorded. Then the samples were applied with remineralizing agents, using cotton applicator, with a small pea-sized amount of remineralizing agent which was applied on each sample and then left for 5 minutes. After 5 minutes, all samples were washed with deionized water and blotted dry using absorbent paper. This application was started within 12 hours of demineralization cycle and repeated 12 hourly for fourteen days. All samples were stored in artificial saliva in between the application procedure. Microhardness was measured after seventh (1st remineralization cycle) and fourteenth day (2nd remineralization cycle) respectively.

TABLE 1: Materials and equipments used for study

Material used in the study	Equipment used for testing	Material used for preparing artificial saliva
Mcinnes bleaching solution	Vickers microhardness tester (Vaiseshika)	Calcium chloride 0.22 g/L
GC Tooth Mousse Plus		Sodium phosphate 1.07 g/L
Toothmin(abbott)		Sodium bicarbonate 1.68/L
Artificial saliva		Sodium azide 2 g/L
Deionized water		Distilled water 1 L

Application of GC Tooth Mousse Plus and Toothmin

GC Tooth Mousse Plus and Toothmin (figure 2) were applied with cotton applicator tips (figure 3) on ten samples of each group respectively on the post bleached samples, everyday for seven days with minimum application time of 5 minutes. The samples were then washed under deionized water, stored in artifi cial saliva for seven days (first cycle of remineralization) after which the samples were tested for microhardness and the values were recorded as described earlier. Following this, GC Tooth mousse Plus and Toothmin were applied for seven more days and at the end of fourteen days (second cycle of remineralization) the samples were subjected for microhardness testing using the same procedure as described earlier. The recorded values are subjected to statistical analysis.

Figure 2: GC Tooth Mousse Plus and Toothmin

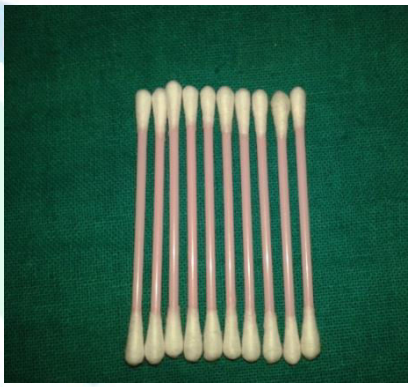

Figure 3: Cotton applicator tips

RESULTS

The data obtained from the following test were subjected for statistical analysis. A "P" value of 0.05 or less was considered for statistical significance. The changes in microhardness at different times of assessment were analysed using "ONE WAY ANOVA".

In this study these comparisons were done between the groups:

1. In the first comparison baseline was compared with bleaching and remineralisation cycles (Table 2).
2. The next comparison was done between the 1st cycle and 2nd cycle of bleaching (Table 3).
3. The third comparison was done between the 2nd cycle of bleaching and cycles of remineralization (Table 4).
4. The next comparison was donr between the 1st and 2nd cycle of remineralisation (Table 5).

The recorded values were subjected to statistical analysis.

Table 2: It shows that baseline microhardness values of GC Toothmousse and Toothmin when compared with first cycle of bleaching decrease by 1.07% (P<0.001) and .96% (P<0.001) respectively, which is highly significant and a decrease of 2.45% (P<0.001) and 2.56% (P<0.001) after second bleaching cycle which is also highly significant. But significant difference was found between baseline microhardness values and remineralization values in case of GC Toothmouse Plus while in case of Toothmin, it was not significant.

Groups		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
G C Tooth Mousse Plus	baseline	10	310.3100	36.26780	11.46888	284.3656	336.2544	251.25	347.85
	after 1st bleaching cycle	10	307.0300	36.48630	11.53798	280.9293	333.1307	247.05	345.35
	after 2nd bleaching cycle	10	302.6000	34.51833	10.91565	277.9071	327.2929	245.75	333.85
	1st cycle of remineralization	10	308.8350	35.88664	11.34835	283.1632	334.5068	249.50	346.45
	2nd cycle of remineralization	10	309.6600	34.34311	10.86025	285.0924	334.2276	250.95	348.05
Toothmin	baseline	10	304.4045	39.40911	12.46226	276.2129	332.5961	255.71	357.45
	after 1st bleaching cycle	10	301.4500	38.88394	12.29618	273.6341	329.2659	253.65	351.50
	after 2nd bleaching cycle	10	296.6250	38.89752	12.30048	268.7994	324.4506	248.80	346.80
	1st cycle of remineralization	10	295.7350	39.19488	12.39451	267.6967	323.7733	245.95	346.55
	2nd cycle of remineralization	10	297.4100	38.19619	12.07870	270.0861	324.7339	250.10	348.20

Table 3: It shows comparison of microhardness between the first and second cycle of bleaching which showed 2.45% and 2.56% decrease for GC Toothmousse and Toothmin respectively, which is significant (P<0.05).

Groups		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
G C Tooth Mousse Plus	after 1st bleaching cycle	10	307.0300	36.48630	11.53798	280.9293	333.1307	247.05	345.35
	after 2nd bleaching cycle	10	302.6000	34.51833	10.91565	277.9071	327.2929	245.75	333.85
Toothmin	after 1st bleaching cycle	10	301.4500	38.88394	12.29618	273.6341	329.2659	253.65	351.50
	after 2nd bleaching cycle	10	296.6250	38.89752	12.30048	268.7994	324.4506	248.80	346.80

Table 4: It shows .46 %and .13% increase in Vicker's hardness number and was significant after 1st remineralization cycle but not significant after 2nd remineralization cycle for GC Toothmousse Plus. For Toothmin increase in hardness was 2.87% and 2.27% after 1st and 2nd remineralization cycle respectively which was highly significant.

Groups		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
G C Tooth Mousse Plus	after 2nd bleaching cycle	10	302.6000	34.51833	10.91565	277.9071	327.2929	245.75	333.85
	1st cycle of remineralization	10	308.8350	35.88664	11.34835	283.1632	334.5068	249.50	346.45
	2nd cycle of remineralization	10	309.6600	34.34311	10.86025	285.0924	334.2276	250.95	348.05
Toothmin	after 2nd bleaching cycle	10	296.6250	38.89752	12.30048	268.7994	324.4506	248.80	346.80
	1st cycle of remineralization	10	295.7350	39.19488	12.39451	267.6967	323.7733	245.95	346.55
	2nd cycle of remineralization	10	297.4100	38.19619	12.07870	270.0861	324.7339	250.10	348.20

Table 5: It shows an increase in the VHN by .1% and 2.3% for GC Toothmouse Plus and Toothmin respectively, when comparison was done between first and second cycle of remineralization.

Groups		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
G C Tooth Mousse Plus	1st cycle of remineralization	10	308.8350	35.88664	11.34835	283.1632	334.5068	249.50	346.45
	2nd cycle of remineralization	10	309.6600	34.34311	10.86025	285.0924	334.2276	250.95	348.05
Toothmin	1st cycle of remineralization	10	295.7350	39.19488	12.39451	267.6967	323.7733	245.95	346.55
	2nd cycle of remineralization	10	297.4100	38.19619	12.07870	270.0861	324.7339	250.10	348.20

DISCUSSION

Treatment of teeth with endemic fluorosis has been specifically recommended by using McInnes bleaching agent which has become popular in clinical setup owing to its quality of being less expensive, superficial nature and less expensive.[4,5] Bleaching or tooth whitening dates back to the 18th century. In early nineteenth century, hydrogen peroxide alone and in combination with other materials, was used as bleaching agent.[15] Enamel microhardness is affected by bleaching which is related to their pH and there is alteration of organic matrix of enamel under the chemical action of hydrogen peroxide. The strong oxidizing effect of hydrogen peroxide on the organic matrix of teeth plays a predominant role in the alterations observed after bleaching.[16,17] As hydrogen peroxide diffuses through the enamel and dentin , it releases reactive oxygen molecule, free radicals and hydrogen peroxide ions that converts long chain dark colored chromophores in to light colored chromophores. This effect can be increased by low pH of the bleaching agent, which subsequently cause alteration in mineral composition, decreasing enamel and dentin microhardness.[18]

Various direct and indirect methods have been utilized for analysing tooth demineralization and remineralization. The key in utilization of indirect methods is the realization that they measure properties, which may change with mineral content variations, but are not equivalent to mineral gain or loss.

Several methods can be used for analysing tooth demineralisation and remineralisation which includes spherical, Knoops or Vickers indenters. In the Vickers hardness test, a diamond in the shape of a square based pyramid is pressed into the polished surface of a material under a specific load. The average hardness value for enamel is in the range from 270 to 350 KHN range or from 250 to 360 VHN range. In this study, the microhardness values were in the range from 285 to 317 VHN, which was within the standard range.

One of the factors, which may alter the hardness, was preparation of specimens, because any tilt or not flat surface may yield a too large indentation and thus a smaller Vickers hardness measurement. Hence two indentations were made to avoid any operational bias and then the average of these two indentations was taken for statistical analysis.

Artificial saliva was used for storing the specimens in between bleaching cycles as studies have shown that by doing this there is slight increase in microhardness after demineralisation.[19,20] Baking soda, prophylactic paste containing fluoride, acidulated phosphate fluoride gel and use of copious amount of water have been used for neutralizing the effect of bleaching.[21] In the present study GC Tooth mousse Plus and Toothmin has been used, which is commercially available.

In our study, there was not much decrease in microhardness after first cycle of bleaching. However, significant difference in microhardness was observed after 2nd cycle of bleaching. With the use of GC Tooth mousse Plus, there was a considerable increase in microhardness. This higher amount of remineralization can be attributed to synergistic effect of fluoride in CPP-ACP (0.2% or 900 ppm of NaF). The fluoride ions are adsorbed onto the surface of enamel crystals, inhibiting dissolution and increasing remineralization. But, in case of Toothmin increase in microhardness after 2nd cycle of remineralization was not upto the baseline level but more as compared to the 2nd cycle of remineralisation as in GC Toothmousse plus.

In summary, McInnes agent used in this study caused a significant decrease in microhardness in all samples 302.600± 3.45(standard deviation=3.45) for GC Toothmousse plus and 296.625± 3.88 (standard deviation= 3.88) when compared with the baseline values i.e. 310.310± 3.62 (SD=3.62) for GC Tooth mousse plus and 304.404± 3.94 (SD=3.94) for Toothmin. However, subsequent remineralisation with GC Tooth mousse plus for 14 days caused recovery in microhardness 309.660± 34.34 (SD=34.34) while is almost equal to the baseline value. But in case of toothmin, application for 14 days caused recovery in microhardness upto 297.410± 3.81 which is not upto the baseline level.

This study was conducted with a small number of samples utilizing in vitro conditions. Application to the general population requires further research and analysis.

CONCLUSION

From the above study it can be concluded that,

- McInnes bleaching agent causes a decrease in the microhardness of enamel by causing enamel demineralization
- GC Tooth mousse plus and toothmin used in this study caused increase in microhardness of the bleached enamel. But, increase in microhardness was upto baseline level in case of GC Toothmousse plus which was not upto baseline level in case of toothmin group.
- Toothmin application led to more remineralization as compared to GC Tooth Mousse Plus even though it could not reach baseline level.

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