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Research Article

COMPARATIVE EVALUATION OF SHY-NM AND REMIN PRO ON THE MICROHARDNESS OF BLEACHED ENAMEL: AN IN VITRO STUDY

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ABSTRACT

The current study was done to evaluate the remineralising effect of SHY-NM and Remin Pro tooth pastes on the microhardness of bleached enamel. Fortyhuman maxillary central incisors were divided into four groups (n=10).The Group A consisted of intact enamel which is the positive control group and Group B consisted of bleached enamel samples as the negative control group. The samples from Group B, Group C and Group D were subjected to bleaching procedure with Pola Office bleaching agent (35% hydrogen peroxide, SDI, Australia) for 8 minutes and the procedure was repeated for three times consecutively as per manufacturer's instructions. The samples of Group C and Group D were then subjected to remineralisation procedure using the agents SHY-NM and Remin Pro twice daily for 5 minutes for 20 days consecutively with a cotton applicator. All the samples were then subjected to surface microhardness testing using Vickers microhardness tester and the data obtained were analysed using one way ANOVA and group wise comparison was done using Post-Hoc Tukey's test with a significancelevel set at $P<0.05$. The results revealed a significant decrease in enamel microhardness after bleaching procedure. The application of Remin Pro showed a significant increasein enamel microhardness whereas SHY-NM showed a marginal recovery in microhardness. The study concluded that Remin Pro exhibited a good recovery in enamel microhardness and can be ensured as a promising material for remineralisation of bleached enamel.

INTRODUCTION

Teeth play an immense role in emphasising the beauty of smile. In the present era, tooth discoloration is considered to be one of the most alarming concerns of the individual due to social and psychological factors. Although there are numerous methods to enhance the aesthetics of teeth, thein-office bleaching technique is one of most popular and non-invasive conservative method of treating tooth discolorationby the superoxide free radical which is the most likely cause for the oxidation of stained pigments (Borges *et al*., 2009).

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The growing demand for teeth bleaching as an esthetic improvement has led to considerable developments in bleaching products. However these bleaching agents cause undesirable effects like enamel demineralisation, roughness and alteration of surface microhardness (Borges *et al*., 2009; Darshana *et al*., 2014; Kamath *et al*., 2013). Several studies have reported with sensitivity following bleaching due to the removal of mineral content from enamel and dentin by demineralization (Mogadham *et al*., 2013; Martin *et al*., 2013). The post treatment sensitivity can be related to small microscopic enamel defects and subsurface pores allowing the bleaching agent to penetrate into the dentinal tubules and ultimately the pulp (Mogadham *et al*., 2013).

Table 1. Products used in the study

Product	Manufacturer	Ingredient
SHY-NM	Group pharmaceuticals, India	Glycerine, PEG400, silica, calcium sodiumphosphosilicate, sodiumlaurylsulphate, titaniumoxide, flavour, carbomer, potassiumacesulfame
Remin Pro	VOCO, Germany	Hydroxyapatite, Fluoride (1450 ppm sodium fluoride),Xylitol

Table 2. Grouping of samples according to bleaching and remineralisation procedure

Group A (positive control)	No treatment (intact enamel)
Group B (negative control)	Bleaching done with Pola Office(SDI, Australia; 35% hydrogen peroxide) for 8 minutes and the procedure repeated thrice
Group C	Bleaching done with Pola Office (SDI, Australia; 35% hydrogen peroxide) for 8 minutes and the procedure repeated thrice + SHY-NM (Group pharmaceuticals, India) application with a cotton applicator on the enamel surface for five minutes twice daily for 20 days.
Group D	Bleaching done with Pola Office(SDI, Australia; 35% hydrogen peroxide) for 8 minutes and the procedure repeated thrice + Remin Pro (VOCO Germany) application with a cotton applicator on the enamel surface for five minutes twice daily for 20 days

Table 3. Intergroup comparison of mean values of microhardness (VHN)

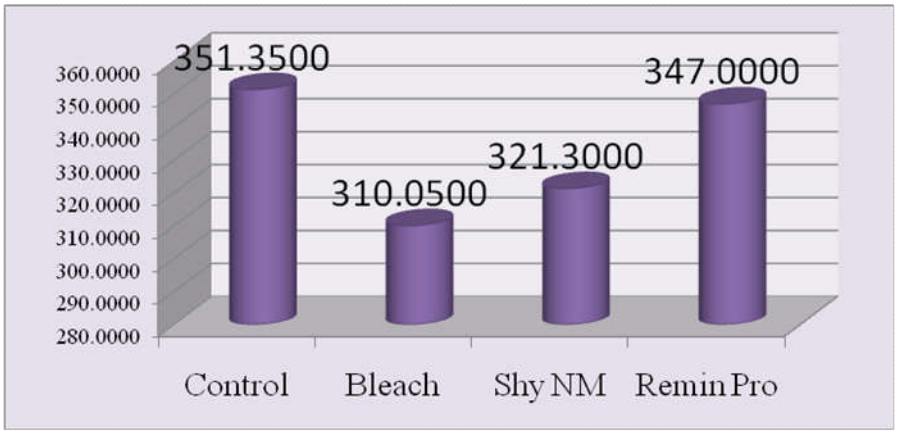
Groups	N	Mean	Std. Deviation	95% Confidence Interval for Mean		F value	p value
				Lower Bound	Upper Bound		
Group A	10	351.3500	14.38759	341.0577	361.6423	29.56	<0.001
Group B	10	310.0500	9.68231	303.1237	316.9763		
Group C	10	321.3000	9.65574	314.3927	328.2073		
Group D	10	347.0000	12.04159	338.3860	355.6140		

One way analysis of variance; VHN –Vickers hardness number

Table 4. Groupwise comparison of mean VHN

Group	Vs. Group	Mean Difference (I-J)	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
Group A	Group B	41.30000	<0.001	27.3188	55.2812
	Group C	30.05000	<0.001	16.0688	44.0312
	Group D	4.35000	.836	-9.6312	18.3312
Group B	Group A	-41.30000	<0.001	-55.2812	-27.3188
	Group C	-11.25000	.152	-25.2312	2.7312
	Group D	-36.95000	<0.001	-50.9312	-22.9688
Group C	Group A	-30.05000	<0.001	-44.0312	-16.0688
	Group B	11.25000	.152	-2.7312	25.2312
	Group D	-25.70000	<0.001	-39.6812	-11.7188
Group D	Group A	-4.35000	.836	-18.3312	9.6312
	Group B	36.95000	<0.001	22.9688	50.9312
	Group C	25.70000	<0.001	11.7188	39.6812

Post Hoc Tukey's test; VHN –Vickers hardness number



Bar Graph 1. Showing the mean microhardness value (VHN) for all the four groups

The changes in the organic and inorganic content of the enamel can be evaluated by the Vickers microhardness test (Melo *et al*., 2014). The various remineralising agents like Tooth Mousse (casein phosphopeptide-amorphous calcium phosphate; CPP-ACP), SHY-NM (bioactive glass), Clinpro tooth crème (3M ESPE; 950ppm fluoride; functionalized tri-calcium phosphate) and Remin Pro with different composition and action hasbeen used to promote remineralisation (Darshana *et al*., 2014; Mehta *et al*., 2004; Shetty *et al*., 2014; Balakrishnan *et al*., 2013). Remin Pro (VOCO, Germany) is a newer remineralising water based cream which has a unique formulation of hydroxyapatite, fluoride and xylitol. The hydroxyapatite fills the eroded enamel, fluoride seals the dentinal tubules and the xylitol acts as an antibacterial agent. It is recommended for the management of dentinal hypersensitivity, enamel demineralisation and to promote remineralisation of enamel subsurface lesions (Kamath *et al*., 2013). SHY-NM (Group pharmaceuticals, India) is a bioactive glass that acts as a biomimetic mineralizer, matching the body's own mineralising traits and contains calcium sodium phosphosilicate. This has been used in the treatment of dental hypersensitivity and enamel remineralisation, which was originally developed as a bone regeneration material These materials are reactive when exposed to body fluids and deposit hydroxycarbonate apatite, a mineral that is chemically similar to natural tooth minerals (Mehta *et al*., 2014; Balakrishnan *et al*., 2013). However, there is no consensus and limited data available regarding the efficacy of these agents in promoting remineralisation. Hence the purpose of this invitro study was to compare the efficiency of the remineralising agents SHY- NM and Remin Pro on bleached enamel surface by microhardness testing.

MATERIALS AND METHODS

Sample selection

Forty freshly extracted human maxillary central incisors were collected from Department of Conservative Dentistry and Endodontics, Sri Siddhartha dental college, Tumkur after obtaining patient consent. Ethical clearance from the institutional ethical committee was also obtained. The teeth selected were free from dental caries, restoration or developmental defects. The teeth were cleaned and external debris removed using ultrasonic scaler and stored in 10% formalin for two weeks as per Centre for Disease Control (CDC) guidelines. Teeth were then stored in deionized water to which 0.1% thymol was added.

Sample preparation and grouping

Forty teeth were equally divided into four groups and the specimen were prepared by sectioning the tooth at cementoenamel junction separating the coronal portion from the radicular portion using diamond disc in a high speed air turbine hand piece (NSK, Japan) using air water spray. The pulp tissue was removed using endodontic instruments and the root canal openings were sealed with utility wax. The sectioned teeth were then embedded in self-cured acrylic resin moulds with the outer labial surface of the teeth exposed. The enamel surfaces were ground into flat surfaces using #600, #1200 and #2400 grit silicon carbidepapers. The seprocedures were conducted to form planar surfaces, which are fundamental for microhardness testing. The specimens were then randomly divided into four groups according to the procedure (Table 2).

Microhardness measurements

Vickers microhardnesswas determined using a microhardness tester (HIGHWOOD DMH7 – TTC unlimited INC–Japan, Model HWMMT-X7 Digital Micro Hardness Tester) at a load of 100g and with an indentation time of 20 seconds. Two indentations were performed on the surface of each specimen and themean value was considered as the final hardness value.

Statistical analysis

The intergroup comparison was statistically analysed with one way ANOVA test and groupwise comparisonwas done using Post-Hoc Tukey's test with a significant difference set at $P<0.05$ levels.

RESULTS

Measurements of Vickers microhardness of the specimen surfaces are presented in Bar graph 1.The intergroup comparison of microhardness values revealed significant difference between groups (Table III).The microhardness value for the positive control group (Group A) was 351.35±14.38 VHNwhich reduced to 310.05±9.68 VHN after bleaching treatment in Group B which is statistically significant ($p<0.001$).The application of remineralising agent Remin Pro(Group D) resulted in significant increase in microhardness values (347±12.04 VHN; $p<0.001$) as shown in Table IV. The use of remineralising agent SHY-NM (Group C) although showed an increase in microhardness value to 321.30±9.65 VHN was not statistically significant ($p=0.152$) as shown in Table IV The order of hardness values which was statistically analysed by Post Hoc Tukey's test as shown in Table IV.

Group A>Group D>Group C>Group B

DISCUSSION

Bleaching is considered to be one ofthe most conservative and non-invasive treatment to manage discoloured teeth when compared to resin bonded composite, porcelain veneers or crowns. However adverse effect of the in-office bleaching treatment which include enamel demineralisation, roughness and alteration in the surface microhardness were reported in several studies resulting in significant alterations in enamel matrix (Borges *et al*., 2009; Darshana *et al*., 2014). Vickers microhardness test was used in the present study to check the enamel microhardness as it is more suitable for determining the hardness of very brittle material like tooth structure. In the Vickers hardness test, a diamond in the shape of a square based pyramid is pressed into polished surface of material under a specific load (Kamath *et al*., 2013). According to results of the present study, the Pola office bleaching agent caused a significant decrease in the enamel microhardness (310.05 VHN) when compared to control group (351.35 VHN).

This microhardness alterations are attributed to the reaction of bleaching agent on the enamel structure due to strong oxidising effect of hydrogen peroxide and enhanced by lowpH (5.5)of the bleaching agent (Martin *et al*., 2013; Melo *et al*., 2014). The hydrogen peroxide due to its low molecular weight diffuses through enamel and dentin, exerting its oxidative effect on the subsurface enamel resulting in further decrease in its hardness and releasing free radicals.

These reactive oxygen molecules and hydrogen peroxide ions convert long chained dark coloured chromophores into light coloured chromophores thereby causing a bleached effect (Kamath *et al*., 2013; Kaur *et al*., 2015). The hydrogen peroxide has the potential to diffuse into the tooth and dissolve the carbonated hydroxyapatite, resulting in demineralized porous enamel resembling carious lesion (Heshmat *et al*., 2014). The free radicals of oxygen are non-specific and react with organic structures of dental tissues thereby degrading the stains on tooth surface (Rajan *et al*., 2015). However subsequent application of SHY-NM and Remin Pro resulted in the recovery of enamel microhardness. The Remin Pro showed a significant improvement in enamel microhardnesswhen compared to SHY-NM, which only showed a marginal recovery. In the present study, the extracted teeth were stored in 10% formalin, because it resists demineralization by fixing proteins present in the organic pellicle attached to the surface of teeth and also act as a best disinfecting agent (Rajan *et al*., 2015). The outer enamel surface is harder than the inner surface due to high concentration of phosphorous and calcium on the surface layer of enamel. Therefore the evaluation of changes in this region is considered to be relevant (Sandeep *et al*., 2015). There is a direct correlation between the mineral content and the microhardness values, therefore microhardness testing can also be a direct measure of mineral gain or loss as a result of demineralisation and remineralisation (Mahmoud *et al*., 2009). In the present study, the microhardness values are in the range from 329 VHN to 376VHN, which is within the standard range. The presence of non-flat surface alters the hardness value which was avoided by rendering the surface flat with abrasive paper. Two intendations were made to avoid any operational bias and average of these intendations was taken for statistical analysis.

The present study revealed a considerable increase in microhardness after the use of Remin Pro on bleached enamel and the values were almost comparable to the control group. This is in accordance with Kamath U *et al*, on the effect of Remin Pro on bleached enamel microhardness which exhibited a statistically significant increase in the hardness even after short time application for 7 days. In the present study the application time was increased to20 consecutive days to enhance the remineralising effect (Kamath *et al*., 2013). The considerable increase in the microhardness values after remineralising with Remin Pro in the present study may be attributed to the presence of more amount of fluoride i.e. 1450ppm which contributes the precipitation of flourapatite layer on enamel surface and rendering it resistant to acid attack. The use of fluorides following bleaching has reported to restore the surface hardness of softened bleached enamel (Borges *et al*., 2009). The remineralisation efficiency of bioactive glass (SHY-NM) on artificially induced carious lesion has shown to increase enamel microhardness due to the formation of hydroxycarbonate apatite layer suggesting SHY-NM as an effective remineralising agent (Mehta *et al*., 2014; Balakrishnan *et al*., 2013). In the aqueous environment around the tooth, sodium ions from the bioactive glass particles rapidly exchange with hydrogen cations which brings about the release of calcium and phosphate ions from glass. A localised, transient increase in pH occur during initial exposure of material to water due to release of sodium .This increase in pH helps to precipitate the extra calcium and phosphate ions provided by bioactive glass to form a calcium phosphate layer which crystallizes to hydroxycarbonate apatite (Gjorgievska *et al*., 2011). But in the present study there is no statistical significant difference in the mean hardness values with regard to bleached group and SHY-NM treated group whereas there is only a marginal gain in microhardness. The results of the present study are in contrast to other reports which revealed a high remineralising potential for the enamel samples treated with SHY-NM on artificially induced carious lesions and direct comparison is not possible as there are no reports regarding the effect of SHY NM on bleached teeth (Mehta *et al*., 2014; Balakrishnan *et al*., 2013). The buffering potential of saliva and its remineralisation effect also play an important role in clinical scenario and in the present study it was unable to simulate a clinical situation (Klaric *et al*., 2013). In this study deionized water was used as storage media in order to avoid the mineral uptake that could happen while using agents that are rich in minerals (Justino *et al*., 2004). The limitation of the present study is attributed to the fact that remineralisation in the oral cavity is a complex procedure involving a change in the pH and replenishing of calcium and phosphate elements which cannot be simulated in the laboratory set up (Borges *et al*., 2010). Further clinical studies are recommended to validate the present study results.

Conclusion

Within the limitation of the present study it was concluded that;

- The Pola office bleaching agent cause a decrease in enamel microhardness
- Remin Pro and SHY-NM improved the enamel microhardness.
- Remin Pro exhibited an increase in enamel microhardness compared to SHY-NM and can be considered as effective remineralising agent

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