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Comparative Evaluation of Four Different Remineralising Toothpastes on the Microhardness and Stain Susceptibility of Bleached Enamel Surface: An In Vitro Study

Fathima Farhana[1], K Harish S Shetty[2], Anita Precilla Dsouza[3]
[1](MDS, Department of conservative dentistry and endodontics, Yenepoya dental college, Yenepoya (deemed to be university), Deralakatte, Mangalore, Karnataka, India - 575018)
[2](MDS, Professor and Head of the Department, Department of conservative dentistry and endodontics, Yenepoya dental college, Yenepoya (deemed to be university), Deralakatte, Mangalore, Karnataka, India - 575018)
[3](MDS, Department of conservative dentistry and endodontics, Yenepoya dental college, Yenepoya (deemed to be university), Deralakatte, Mangalore, Karnataka, India - 575018)

Abstract:

Introduction: *Hydroxyapatite is the crystalline form of calcium and phosphorus found in the enamel, dentin, cementum, and bone. Bleaching is a common procedure and has been known to alter the enamel matrix because of the strong oxidizing effect of hydrogen peroxide and can cause significant mineral loss and decrease in surface hardness. It creates surface porosities making it more susceptible to stains. The microscopic loss of tooth structure resulting from this procedure is reversible, and remineralization can be attempted. The study aimed to compare the effectiveness of four different remineralising toothpastes in increasing enamel microhardness and making the enamel less susceptible for stains.*

Materials and method: *Freshly extracted premolars were cut sagittally and reduced into fragments measuring 4mm X 4mm. These were impregnated in self cure acrylic resin with the buccal surface of the crown facing upwards. Specimens were kept in artificial saliva to prevent dehydration. Teeth were randomly divided into six groups. 30-40% Hydrogen peroxide will be used to bleach the teeth in group B, C, D ,E and F. Teeth in group C(n=12) were treated with Gc tooth mousse,Teeth in group D (n=12) were treated with Enafix, teeth in group E(n=12) were treated with Aclaim, teeth in group F (n=12) were treated with Vantej daily for seven days . All 6 groups were checked for microhardness of enamel surface. Specimen subjected to microhardness were further checked for dye uptake . Teeth were immersed in a 2% methylene blue solution. Afterwards, specimens were dissolved in nitric acid and prepared for the spectrophotometric analysis.*

Result*: **The mean microhardness values in descending order**: Positive control > Aclaim > GC Tooth mousse > Enafix > Vantej > Negative control.*

The mean stain uptake values in descending order*: Negative control > Unbleached > GC tooth mousse > Vantej > Enafix > Aclaim.*

Conclusion*: All remineralizing agents showed improved surface mineralization. Out of all the agents Aclaim exhibited the highest surface remineralisation and made the samples least susceptible to stains.*

Keywords*: Bleaching, Novamin , Bioactive glass, Casein phosphopeptide- amorphous calcium phosphate, Calcium sucrose phosphate, Vickers microhardness testing machine, Spectrophotometric analysis.*

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I. Introduction

Bleaching is a simple method to treat discolouration of teeth. However it has been known to alter the enamel matrix because of the strong oxidizing effect of hydrogen peroxide and can cause significant mineral loss.[1] It also causes porosity of enamel surface and thereby increasing its stain susceptibility. [2] Remineralising treatment is well recognized and recieved lot of attention by both clinician and researchers. [3]

This study was conducted to compare the effectiveness of four different remineralising tooth pastes namely GC TOOTH MOUSSE (Recaldent), ENAFIX (Group pharmaceuticals), ACLAIM (Group pharmaceuticals) ,VANTEJ (Dr Reddy's) in increasing enamel microhardness and making the enamel less susceptible for stains. It was conducted since the constituents in these toothpastes are different. Active ingredient in GC tooth mousse is Casein phosphopeptide amorphous calcium phosphate (CPP-ACP)[4], and in Enafix it is calcium sucrose phosphate[5], Aclaim has nanohydroxyapatite[6] and Vantej has bioactive glass

(calcium phosphosilicate)[7]. These agents promote restoration of altered enamel structure thus increasing its hardness and make it less susceptible to stains

II. Materials And Methods

DIVISION OF GROUPS

Group A:Unbleached teeth
Group B:Bleached teeth ,not treated with any remineralising agent.
Group C:Bleached teeth , treated with Gc tooth mousse
Group D:Bleached teeth ,treated with Enafix
Group E:Bleached teeth , treated with Aclaim
Group F:Bleached teeth ,treated with Vantej

Teeth were randomly divided into six groups. 35% Hydrogen peroxide (Pola Office) was used to bleach the teeth in group B, C, D ,E and F. Teeth in group C(n=12) were treated with Gc tooth mousse ,Teeth in group D (n=12) were treated with Enafix,teeth in group E(n=12) were treated with Aclaim, teeth in group F (n=12) were treated with Vantej daily for seven days .

Evaluation of Surface Micro Hardness after 1 week

After cycle of remineralization, the microhardness of the specimens was determined using Vickers hardness testing machine. A load of 100g was exercised gradually to the surface of specimens for 15s using Vickers elongated diamond pyramid indenter under a 40x objective lens. Two indentations were placed on the surface and the average value was considered for each specimen.

Evaluation of stain uptake using spectrophotometer

Specimen subjected to microhardness were further checked for dye uptake . The samples were coated with nail varnish all over the surface except for a small area measuring 4x4 on the buccal surface , area of indentation were also varnished. Teeth were immersed in a 2% methylene blue solution. Afterwards, specimens were dissolved in 65% nitric acid for 72 hours and prepared for the spectrophotometric analysis. Standard solution of 65% nitric acid was prepared and used as blank during analysis and the sample solutions were subjected to spectrophotometric analysisusing a filter of 630nm.

III. Results

MICROHARDNESS

The statistical parameters such as mean and SD of microhardness of samples were obtained for each group as shown in Table 1.The mean for positive control was the highest 332.717± 2.813 MPa, while that of negative control was the lowest 240.342± 5.245 MPa

In case of groups in which remineralisisng materials were used , ACLAIM indicated highest mean 311.658± 3.544 MPa, followed by CPP-ACPF (GC TOOTH MOUSSE PLUS) with a mean value 303.467 ± 4.890 MPa and ENAFIX with a mean value of 286.167±5.425, Bioactive glass NovaMin (Vantej) with the least mean value of 279.779 ± 3.344 MPa.

Analysis of variants was highly significant with the $P=0.000 \leq 0.001$.

Further Post hoc or Pairwise analysis by TUKEY HSD test was carried out as shown in (Table 2**)** .

STAIN SUSCEPTIBILITY

The statistical parameters such as mean and SD of absorption of samples were obtained for each group as shown in Table 3.The mean for (GROUP E – ACLAIM) was the LOWEST 0.0013 ± 0.0010 , while that of negative control (GROUP B -BLEACHED TEETH) was the HIGHEST 0.0132 ± 0.050

Analysis of variants was highly significant with the $P=0.000 \leq 0.001$.

Further Post hoc or Pairwise analysis by TUKEY HSD test was carried out as shown in (Table 4**)** .

Table 1: Comparison of level of hardness (MPa) in different groups

GROUPS	N[a]	Mean	Std. Deviation	Minimum	Maximum
Unbleached	12	332.717	2.813	328.500	337.550
Bleached	12	240.342	5.245	233.700	246.850
GC	12	303.467	4.890	292.900	309.700
ENAFIX	12	286.167	5.425	279.250	293.300
ACLAIM	12	311.658	3.544	306.150	318.700
VANTEJ	12	279.779	3.344	273.750	284.400
Total	72	292.355	29.446	233.700	337.550

a. F=643.428 p<0.001 vhs

Table 2: Pairwise analysis of different groups (microhardness)

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) group	(J) group	Mean Difference (I-J)	p
microhardness	Unbleached	Bleached	92.3750	.000
		GC	29.2500	.000
		ENAFIX	46.5500	.000
		ACLAIM	21.0583	.000
		VANTEJ	52.9375	.000
	Bleached	GC	-63.1250	.000
		ENAFIX	-45.8250	.000
		ACLAIM	-71.3167	.000
		VANTEJ	-39.4375	.000
	GC	ENAFIX	17.3000	.000
		ACLAIM	-8.1917	.000
		VANTEJ	23.6875	.000
	ENAFIX	ACLAIM	-25.4917	.000
		VANTEJ	6.3875	.007
	ACLAIM	VANTEJ	31.8792	.000

Table 3: Comparison of absorption in different groups

WL630.0

	N[a]	Mean	Std. Deviation	Minimum	Maximum
Unbleached	12	.0040	.0031	.0000	.0100
Bleached	12	.0132	.0050	.0050	.0220
GC	12	.0095	.0032	.0020	.0140
ENAFIX	12	.0013	.0012	.0000	.0040
ACLAIM	12	.0013	.0010	.0000	.0030
VANTEJ	12	.0023	.0027	.0000	.0080
Total	72	.0053	.0054	.0000	.0220

a. F=32.126 p<0.001 vhs

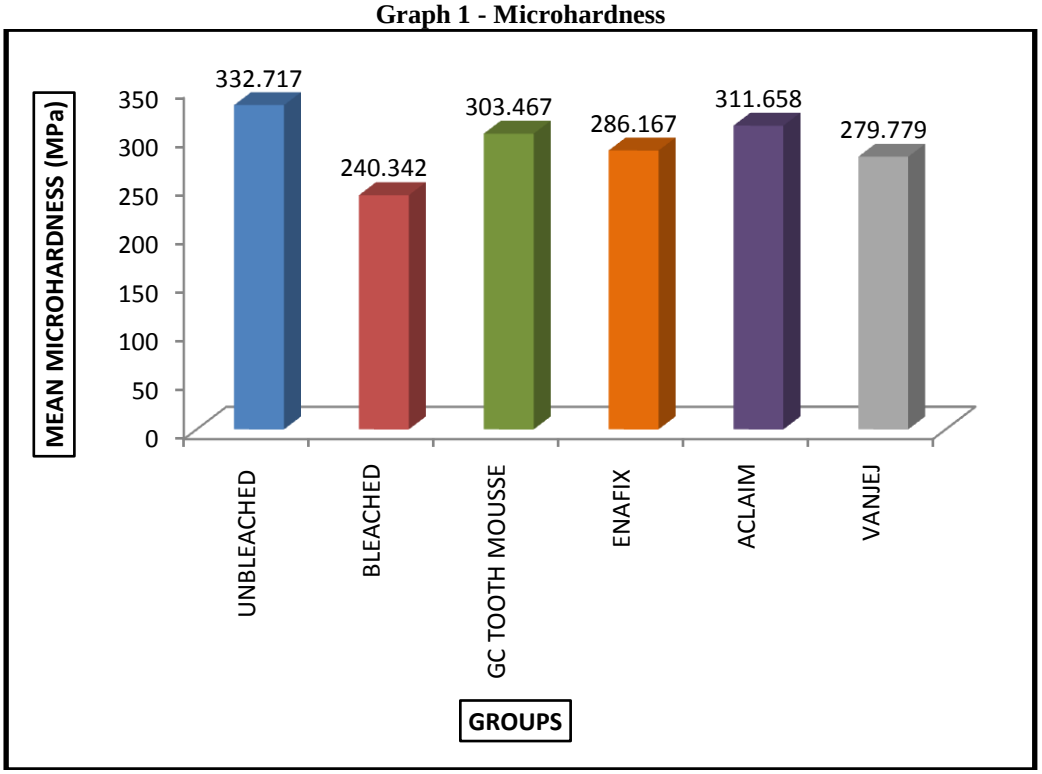
Table 4: Pairwise analysis of different groups(absorption)

Multiple Comparisons

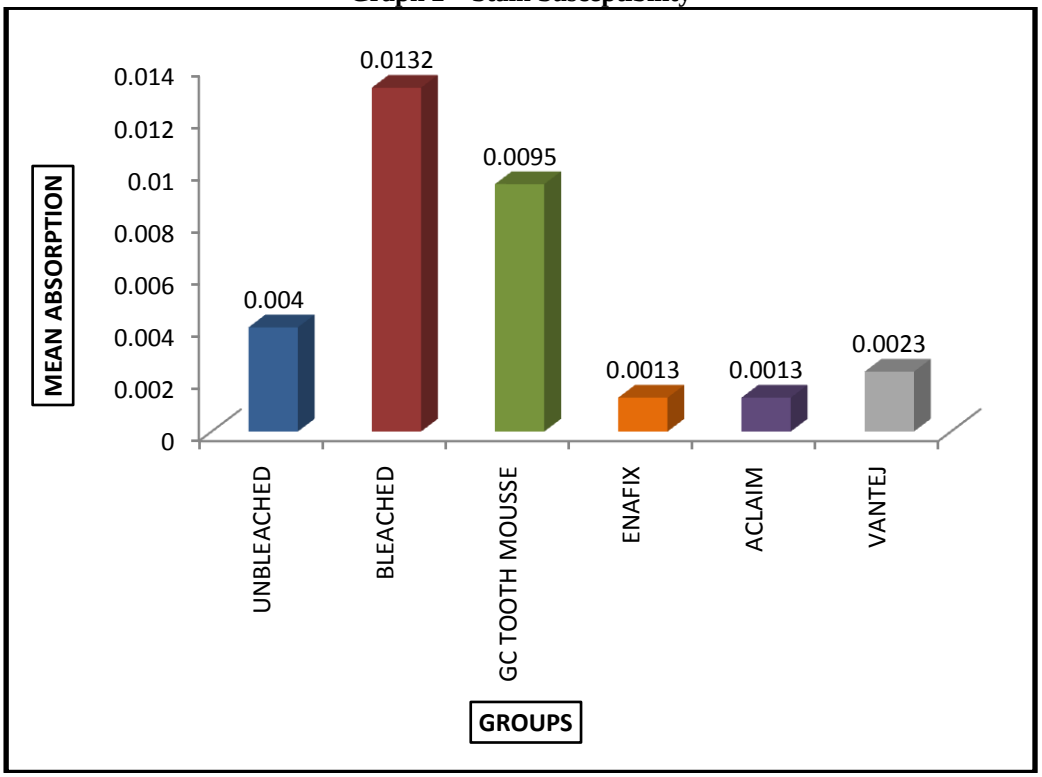
Tukey HSD

Dependent Variable	(I) group	(J) group	Mean Difference (I-J)	p
WL630.0	Unbleached	Bleached	-.0092	.000
		GC	-.0055	.000
		ENAFIX	.0027	.269
		ACLAIM	.0028	.238
		VANTEJ	.0017	.755
	Bleached	GC	.0037	.045
		ENAFIX	.0118	.000
		ACLAIM	.0119	.000
		VANTEJ	.0108	.000
	GC	ENAFIX	.0082	.000
		ACLAIM	.0083	.000
		VANTEJ	.0072	.000
	ENAFIX	ACLAIM	.0001	1.000
		VANTEJ	-.0010	.965
	ACLAIM	VANTEJ	-.0011	.950

Graph 1 - Microhardness



Graph 2 – Stain Susceptibility



IV. Discussion

Bleaching is a most common method to treat discolouration of teeth. However the use of highly oxidizing agents can cause surface loss of enamel and crfeate porosities.[1] It also causes porosity of enamel surface and thereby increasing its stain susceptibility [2] During the process of demineralization calcium, phosphate, sodium, magnesium gets displaced from the enamel surface to the exterior result the formation of crystal voids.[2] One of the common method which can be adapted is theapplication of remineralising agent after

bleaching..[3] There are numerous remineralising agents available in the market. Post bleaching such agents should be applied to the bleached teeth by the dentist and at home patients can incorporate remineralising tooth pastes to their oral hygiene practice.

This study was conducted to compare the effectiveness of four different remineralising tooth pastes namely GC TOOTH MOUSSE (Recaldent), ENAFIX (Group pharmaceuticals), ACLAIM (Group pharmaceuticals) ,VANTEJ (Dr Reddy's) in increasing enamel microhardness and making the enamel less susceptible for stains. It was conducted since the constituents in these toothpastes are different. Active ingredient in GC tooth mousse is Casein phosphopeptide amorphous calcium phosphate (CPP-ACP)[4], and in Enafix it is calcium sucrose phosphate[5], Aclaim has nanohydroxyapatite[6] and Vantej has bioactive glass (calcium phosphosilicate)[7]. These agents promote restoration of altered enamel structure thus increasing its hardness and make it less susceptible to stains. The key ingredient or active ingredient in these tooth pastes are responsible for the remineralising effect on demineraised enamel.

The ACP CPP also acts as reservoir of bio-available calcium and phosphate, and maintains the solution supersaturated, thus facilitating remineralization.[8]

The CPP-ACP complex acts as a vehicle for calcium and phosphate,transporting it to the tooth surface and localizing it in plaque and salivary pellicles. CPP-ACP maintains saturation levels of minerals, especially calcium and phosphate, at the tooth surface thereby depressing demineralization and enhancing remineralization process.[9] CPP–ACP works effectively as a remineralizing agent at acidic pH levels, as well as in neutral and alkaline phosphate range.[10] Casein phosphopeptide CPP-ACP has also been proposed to have anticariogenic properties. Casein phosphopeptides are used alone as CPP-ACP, or it can be further complexed with fluoride as, CPP-ACFP (casein phosphopeptides with amorphous calcium fluoride phosphate).[11]

Bioactive glass (Bioglass®) was invented by Dr. Larry Hench in1960s. Bioactive glass materials have been introduced in many fields of dentistry and are considered as a breakthrough in remineralizing technology.[12] This is because the current standard treatment for tooth remineralization and prevention of decay is slow acting and is dependent on adequate saliva as a source of calcium and phosphorus.[13] This unique material has numerous novel features, most important of which are its ability to act as a biomimetic mineralizer matching the body's own mineralizing traits while also affecting cell signals in a way that benefits the restoration of tissue structure and function.[14] Bioactive glass material is a multi- component inorganic compound made up of elements such as silicon, calcium, sodium and phosphorus.[15] The active ingredient is amorphous calcium sodium phosphosilicate. Novamin a trade name for bioactive glass, manufactured by Novamin technologies showed that remineralization of enamel is relatively promising.[16] The active ingredient of NovaMin is calcium sodium phosphosilicate. [17] Antibacterial effect of NovaMin toothpaste has been documented against several periodontal pathogens. [18]

Hydroxyapatite is the mineral crystalline form of calcium and phosphorus found in the enamel, dentin, cementum, and bone. It is widely used in biology, medicine, and dentistry due to its optimal characteristics, such as similarity to the mineral structure of hard tissues, biocompatibility, and low solubility.[19] HA is expected to significantly enhance remineralization of initial enamel and dentin caries, and NHA is believed to have a higher efficacy than HA for this purpose due to its nano-size particles.[20,21] NHA has hydrophilic and wetting characteristics and is capable of producing a thin but tightly bound layer on the tooth surface, resulting in higher surface hardness and remineralization. Nano hydroxyapatite (NHA), due to its higher surface/ volume ratio, is more effective than large HA particles. Recent advances in nanotechnology resulted in smaller sized particles of highly bioactive calcium phosphate compounds that have higher potential for penetration into the porosities of the decalcified area as remineralizing agents.[22]

Calcium sucrose phosphate is a remineralizing agent that releases calcium, phosphate and sucrose phosphate ions. Sucrose phosphate absorbs on to enamel provides high concentrations of calcium & phosphate ions.[23] Calcium Sucrose Phosphate (CaSP) is a mixture of calcium sucrose mono and diphosphate, disucrose monophosphate and inorganic calcium phosphate that contains 11% calcium, 9.5% organic phosphate and 2.5% inorganic phosphate [24]. It reduces the rate of dissolution of hydroxyapatite in acid buffers and decreases enamel demineralization. It creates an environment conducive for remineralization of tooth surface and inhibits the formation of plaque.[25]

In dental research, microhardness indentation measurements have been employed to study remineralization. A relationship of enamel hardness values with mineral content of the tissue is on weight basis, which also accounts for the variation in the enamel structure. Thus, assessing the remineralizing potential through microhardness is an accurate tool. However, a comparison of the aforementioned formulations that adopt the micro-indentation technique has not been conducted. The most widely used microhardness tests for evaluating remineralization are Vickers microhardness test and Knoop microhardness test.[26] As this study evaluates of surface microhardness of enamel, Vickers surface microhardness test was used. Spectrophotometric analysis is a suitable method to study the stain uptake as it quantitatively measures the dye penetration. [27,28]

This study was conducted to compare the effect of bioactive glass, nanohydroxyapatite crystals and

caesin phosphopeptide amorphous calcium phosphate effect on enamel surface microhardness after bleaching using vickers microhardness tester, and stain susceptibility using spectrophotometric analysis.

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