ORIGINAL RESEARCH PAPER

Volume - 13 | Issue - 04 | April - 2024 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

COMPARATIVE EVALUATION OF THE EFFECT OF REMINERALIZING AGENTS ON THE SURFACE MICROHARDNESS OF BLEACHED ENAMEL: AN IN VITRO STUDY

Dentistry

Dr. Prachi Choudhari*	Junior Resident, Ranjeet Deshmukh Dental College And Research Center, Nagpur, India. *Corresponding Author
Dr. Pratima Shenoi	Professor And Head Of Department, Ranjeet Deshmukh Dental College And Research Center, Nagpur, India.
Dr. Rajesh Kubde	Professor, Ranjeet Deshmukh Dental College And Research Center, Nagpur, India
Dr. Gautam Badole	Associate Professor, Ranjeet Deshmukh Dental College And Research Center, Nagpur, India
Dr. Mohit Gunwal	Associate Professor, Ranjeet Deshmukh Dental College And Research Center, Nagpur, India
Dr. Shriya Shahu	Senior Lecturer, Ranjeet Deshmukh Dental College And Research Center , Nagpur, India.

ABSTRACT

Aim and background: Patients seek cosmetic dentistry care for the elimination of any and all perceptions of discoloration in their teeth. Tooth bleaching is one of the most frequently used cosmetic dental treatments to improve the appearance of discolored teeth. Vital tooth bleaching is used for the removal of extrinsic stains due to tea, coffee, and tobacco etc. Bleaching chemicals cause a decrease in enamel microhardness due to the oxidation and reduction processes, which disintegrate organic and inorganic tooth components (demineralization). Studies have proven that remineralization treatments with a remineralizing agent are capable of reestablishing lost enamel surface's hardness and alterations caused by dental bleaching. In the present study, changes in microhardness are analyzed upon the application of a remineralizing agent after subjecting the enamel surfaces to bleaching. Aim of the study is to evaluate the surface microhardness of bleached enamel after treating with Calcium sucrose phosphate (Toothmin), and CPP-ACP (GC Tooth mousse).**Methods:** Twenty six freshly extracted, sound premolars were collected and their roots were removed. Samples were divided into two experimental groups; n=13 (Group 1for Tooth mousse and 2 for Toothmin). Microhardness was measured at baseline and after bleaching and remineralization. **Results:** The application of bleaching agents resulted in a significant decrease in enamel microhardness as compared to baseline. The application of remineralizing agents significantly recovered the initial microhardness of the enamel as before bleaching treatment. The recovery of surface microhardness after remineralization was significantly greater in Group 2 (Toothmin) as compared to Group 1 (CPP-ACP) ($p<0.05$). **Conclusion:** Tooth mousse and Toothmin both are efficient in reestablishing surface microhardness of enamel which decreased after vital bleaching. Comparatively, Toothmin showed more potential for restoring surface microhardness than Tooth mousse.

KEYWORDS

surface microhardness, bleached enamel, remineralizing agents

INTRODUCTION

The field of aesthetics delves into the study of beauty and serves as a significant factor in the development of various corrective treatment methods aimed at enhancing the visual appeal of individuals' teeth. The increasing public interest coupled with the better technical expertise of the clinicians, has paved the way for improving smiles by lightening the tooth stains. There are various methods available for the management of discolored teeth, such as bleaching, veneers, microabrasion, and macroabrasion. Among these, bleaching is the most frequently used method for managing discolorations, as it seems to be less invasive and cost-effective at the same time. The American Dental Association defines bleaching as 'the treatment, usually involving an oxidative chemical, that alters the light-absorbing and/or light-reflecting nature of the material structure, thereby increasing its value (whiteness) [1]. The history of bleaching dates back to 1877. Since then, bleaching has evolved as a non-invasive treatment option for the management of discolored teeth.

However, the utilization of bleaching agents has been associated with a range of adverse effects, including hypersensitivity, gingival irritation, increased sensitivity, demineralization, and alterations in the surface of the enamel [2-3]. These alterations manifest as increased porosity, crater-like formations, depressions, changes in enamel microstructure, and an uneven enamel surface characterized by peaks and valleys [4]. These changes are largely attributed to the acidic nature and oxidative impact of bleaching products, which reduce the amount of hydroxyapatite crystals and proteins in enamel [5]. Consequently, this can result in reduced microhardness [6], variations in surface structure [7], and decreased resistance to wear [8]. Furthermore, changes in enamel morphology can have implications for its optical characteristics and its susceptibility to bacterial adhesion [9].

To counteract the detrimental impact of bleaching agents on enamel, numerous innovative remineralization substances are being researched. These include alternatives such as sugar substitutes, ozone therapy, fluorides, calcium sucrose phosphate, inorganic calcium phosphate, calcium phosphate remineralization methods, and casein phosphopeptide amorphous calcium phosphate fluoride, which have been explored with promising outcomes. Among these, Mousse is one of the most commonly used remineralizing agents in clinical practice.

Tooth Mousse (CPP-ACP, or casein phosphopeptide amorphous calcium phosphate) has demonstrated its ability to effectively stabilize the levels of calcium, phosphate, and fluoride ions on the surface of the tooth by binding to the salivary pellicle. This serves as a reservoir for calcium and phosphate, adhering to dental plaque and tooth surfaces. When subjected to an acidic environment, the CPP-ACP that is bound to these surfaces releases calcium and phosphate ions, consequently mitigating demineralization and promoting the remineralization of enamel [10]. Simultaneously, Toothmin (Abott) has been recently introduced as a remineralizing agent. Toothmin is calcium sucrose phosphate, which is a white, non-hygroscopic powder with a bland, neutral taste and high water solubility. It contains calcium sucrose and inorganic calcium phosphate, which has 10% to 12% calcium and 8% to 10% phosphorus by weight. When dissolved in water, it breaks down into calcium, phosphorus, and sucrose phosphate ions, resulting in the formation of insoluble precipitates. This process involves the adsorption of sucrose phosphate ions onto the enamel surface, leading to a reduced rate of acid-induced dissolution of hydroxyapatite and promoting remineralization [11].

There is limited literature available comparing the efficacy of GC Tooth Mousse and Toothmin as remineralizing agents to restore the microhardness of bleached enamel. Therefore, the present study was designed to investigate and comparatively evaluate the effect of CPP-ACP and Toothmin on the surface microhardness of bleached enamel.

MATERIALS AND METHODS

This study was designed in the Department of Conservative and Endodontics, and ethical clearance (EC/NEW/INST/2020/687) was obtained.

In the present study, 26 freshly extracted premolars without any stains, enamel cracks, fractures, or other defects were included. The teeth were thoroughly cleaned to remove any debris and soft tissues adhering to their surfaces. They were then placed in a solution of 0.5% thymol to safeguard against fungal and bacterial growth until they were needed for the study. A minimum sample size of 8 was calculated considering the difference in the mean microhardness in the two groups as main outcome measures as per a similar study conducted by Manju Krishna EM et al(2019) [12]. As a total sample size of 8 was too small, a total of 26 samples were included in the study (Figure 1).

Figure 1 – Total samples

For the preparation of samples, a decoronation procedure was carried out, and each tooth crown was subsequently embedded in an acrylic resin mold with the labial surface positioned upwards, lying flat and parallel to the horizontal plane. These mounted samples were then preserved in an artificial saliva solution (NANOCHEMAZONE, Aritech Chemazone, Pvt. Ltd., India) to prevent desiccation.

The total samples were randomly allocated into two experimental groups, i.e., Group 1 (Tooth Mousse) and Group 2 (Toothmin), with each group comprising 13 samples. Initial microhardness measurements were obtained from the labial surface using a Vickers microhardness tester (Figure 2), applying a force of 100 g for 30 seconds with the magnification set to ×10 under an objective lens. Two indentations were made on each sample away from the edge, and the average of these two readings was considered the baseline microhardness measurement.

Figure 2 – Vickers microhardness tester

For the bleaching procedure, after the prophylaxis with pumice was carried out, one drop of bleaching agent from the bleaching kit (Pola Office, SDI, Victoria, Australia) (Figure3) was deposited on the labial surface of each sample, left for 8-minutes, and then rinsed with water as per the manufacturer's instructions. After bleaching is completed, microhardness testing is carried out again on each bleached sample.

Figure 3 – Pola office bleaching kit

For the remineralization procedure, Tooth Mousse (Figure 4) and Toothmin (Figure 5) were applied with a cotton applicator tip to the bleached samples in Group 1 and 2, respectively, every day for 14 days, with a minimum application time of 5 minutes as proposed by the manufacturer (Figure 6).

Figure 4 – Tooth Mousse

Figure 5– Toothmin

Figure 6 – Application of experimental remineralizing agents on bleached enamel surface

Samples were washed with distilled water and stored in artificial saliva. Again, the microhardness testing was carried out on each sample in both groups.

RESULTS

The recorded values were subjected to statistical analysis. Statistical analysis was done using Statistical Product and Service Solution (SPSS) version 22 software. To assess the variations in microhardness at various assessment time intervals, ONE WAY ANOVA" was employed. An unpaired t-test was utilized for the intergroup comparison between the two groups and, a "p" value of 0.05 or less was considered statistical significance.

Surface microhardness was measured in the form of Vickers hardness number (HV) between the two groups at three different study time intervals, i.e., at baseline, after bleaching, and after treatment with remineralizing agents (Table1). On using an unpaired t test, microhardness in both groups was found to decrease from baseline values after the bleaching, and an increase in microhardness was observed after treatment with both remineralization agents.

Table 1 – Comparison between change in microhardness at three different study intervals

Microhardness	Baseline Mean (SD)	After Bleaching Mean (SD)	After Remineralization on Mean (SD)
Group 1 (Tooth mousse)	310.5 HV(27.89)	259.04 HV (30.67)	312.0 HV(11.28)
Group 2 (Toothmin)	312.5 HV(27.68)	265.33 HV(18.0)	339.03 HV (17.34)
Unpaired t test	t = -0.125	t = -0.433	t = -3.188
P value, Significance	p =0.903 (NS)	p =0.674 (NS)	p =0.01

Comparison between change in microhardness values between both groups (Table *2*) showed significantly better($p<0.05$) results for Group 2 (Toothmin) as compared to Group 1 (Tooth Mousse).

Table 2 – Comparison between change in microhardness between two experimental groups (* statistically significant p value < 0.005)

Change in mean microhardness	Change in microhardness after bleching and after remineralization Mean (SD)	Change in microhardness from baseline to post remineralization Mean (SD)
Group 1 (Tooth mousse)	52.95 HV (30.07)	1.5 HV(25.81)
Group 2 (Toothmin)	73.7 HV (26.97)	26.53 HV (16.96)
Mann Whitney U test	U = 10.0	U = 5.0
P value, Significance	p =0.048*	p = 0.041*

DISCUSSION

Tooth discoloration can unfavorably affect an individual's aesthetic appearance. Among the array of therapeutic modalities employed for the management of discoloration, the technique of bleaching stands out as the most frequently used treatment option. This can be attributed to its perceived qualities of minimal invasiveness and cost-effectiveness. The substances accountable for inducing discoloration on tooth surfaces are composed of organic compounds that are characterized by the presence of a single or double-chained carbonyl and a phenyl ring, collectively referred to as chromophores [13]. The bleaching procedure involves the interaction of hydrogen peroxide with these chromophores, causing its decomposition into water and unbound oxygen species. These oxygen species, comprising unstable free radicals such as OH, OOH, and O2, enact a reaction leading to the fragmentation of the chromophore bonds into smaller constituents. Consequently, this process results in the lightening of the tooth structure [14-15].

Nonetheless, the utilization of bleaching agents has the potential to induce deleterious alterations in the microstructure of surface enamel. These alterations cause increased porosity in the external enamel structure, accompanied by a process of demineralization. The organic matrix undergoes degradation along with changes in the calcium-phosphate ratio [16-17]. In the current study, a bleaching agent containing 10% carbamide peroxide was deployed. The presence of peroxide within bleaching agents causes the liberation of free radicals, reducing the pH of the bleaching formulation. This, in turn, negatively influences the microhardness of the surface enamel. Notably, the propensity for complications increases proportionally with increased concentrations of peroxide. After bleaching the samples with carbamide peroxide, microhardness measurements for the experimental groups were documented as 259.04 and 265.33, respectively, using Vickers microhardness tester. These values exhibited a noteworthy reduction when contrasted with the initial baseline values of 311.50 and 310.50. This outcome concurs with the findings of a study conducted by C.F. Pinto et al. (2001), who observed a decrease in surface enamel microhardness consequent to the application of a 10% carbamide peroxide bleaching agent [18]. To support these findings, multiple investigations have demonstrated alterations in the composition and structural attributes of enamel, along with a decrease in microhardness [19] and fracture toughness [20], after bleaching with a 10% carbamide peroxide solution. The majority of the observed alterations were attributable to the acidic pH and oxidative properties inherent in bleaching agents, leading to a reduction and change in the hydroxyapatite crystals of enamel, which subsequently resulted in decreased surface microhardness [21].

To counteract these adverse effects after bleaching, many remineralizing agents are being studied. Fluoride has remained a preferred agent for dental remineralization for the past six decades. Application of fluoride results in the formation of a protective layer on the enamel surface resembling calcium fluoride. It serves to shield the enamel from subsequent acid attacks and reduces the ensuing mineral loss. However, it was found to be most effective only at the surface and causes the blockage of pores in hydroxyapatite crystals, thereby reducing the physiological ion exchange activity of surface enamel [22]. Therefore, in recent years, many new remineralizing agents have been introduced and are being researched, such as sugar substitutes, ozone treatment, calcium sucrose phosphate, inorganic calcium phosphate, calcium phosphate-based remineralization methods, and fluoride compounds containing casein phosphopeptide amorphous calcium phosphate (CPP-ACPF). In the present study, GC Tooth Mousse and Toothmin were used, as these are commonly used in clinical practices.

CPP-ACP, a complex nano milk protein, possesses the ability to remineralize teeth by forming amorphous nano complexes. E.C. Reynolds (1993) documented the formation of nano complexes between casein phosphopeptides (CPPs) and amorphous calcium phosphate (ACP) approximately three decades ago. These nano complexes were observed to stabilize calcium and phosphate ions, with subsequent release to the pellicle and diffusion into the enamel and dentin. In a study conducted by Bayrak (2009), it was demonstrated that CPP-ACP exhibited a notable increase in enamel hardness following the application of a bleaching agent containing 38% hydrogen peroxide, surpassing the effects of fluoride gel [23]. Similar observations were made in the present study, as the enamel microhardness values were found to increase from 259.04 to 312.0 after treatment with CPP-ACP. This effect of CPP-ACP can be attributed to its potential for the deposition of calcium and phosphate on the tooth surface from the saliva [24]. Also, it enhances the strength of tooth enamel structure for seven days by maintaining the calcium-phosphate ratio. Additionally, when used for a week following a bleaching procedure, it is reported to counteract the potential side effects associated with the bleaching treatment [25].

Toothmin, which is a calcium sucrose phosphate compound, was developed by Abbott in India as a dental cream with 'anticay' properties. When dissolved in water, calcium sucrose phosphate breaks down into calcium, phosphorus, and sucrose phosphate ions, resulting in the formation of insoluble precipitates. This process involves the adsorption of sucrose phosphate ions onto tooth enamel, leading to a reduced rate of acid-induced dissolution of hydroxyapatite [26]. In the current investigation, we observed an improvement in enamel surface microhardness following Toothmin treatment, which aligns with the findings reported by TN Raghu et al. in 2016. They assessed the remineralizing properties of Toothmin using energy-dispersive X-ray spectroscopy (EDX) and noted enhanced enamel surface smoothness after two weeks of Toothmin brushing [27]. The favorable impact of Toothmin on enamel microhardness can be attributed to its water solubility, which allows it to provide significantly elevated concentrations of free calcium and phosphate ions, exceeding the typical levels found in saliva by several magnitudes.

In the present study, the mean values of microhardness obtained after remineralization with Tooth Mousse and Toothmin were 312 and 339.3, respectively, which suggests that both remineralizing agents can be effective in re-establishing the surface microhardness of enamel that is lost after bleaching.

A comparative analysis was also carried out between Tooth Mousse and Toothmin for their potential to increase the surface microhardness of bleached enamel. Microhardness values obtained after remineralization with Tooth Mousse and Toothmin were 312 and 339.05, respectively, which were increased when compared to baseline values, i.e., 310 and 312. This result suggests that Toothmin can be more effective than tooth mousse. Esfahani et al. (2015) stated that tooth mousse requires sustained application for a longer period to be effective. As in our study, both remineralizing agents were applied for an equal time period, i.e., 5 minutes for 7 days, which could be insufficient for Tooth Mousse to be as effective as Toothmin [28].

Our study has some limitations. Given that the present investigation is conducted in vitro, it is essential to recognize that the process of remineralization within the oral cavity is complex, entailing alterations in pH levels and enzyme-mediated processes. These factors may not be entirely replicable within the controlled conditions of an in vitro study. Therefore, it is prudent to conduct further research employing specific assessment parameters, including energy-dispersive X-ray spectroscopy (EDX) and scanning electron microscopy (SEM) analyses, while also increasing the sample size. These measures are necessary before extrapolating the study's findings to clinical scenarios.

CONCLUSION

Within the confines of the existing investigation's limitations, it is plausible to infer that bleaching agents can reduce the surface microhardness of enamel however, both the experimental remineralizing agents, Tooth Mousse and Toothmin demonstrate efficacy in the restoration of enamel surface microhardness subsequent to a bleaching procedure. Notably, Toothmin exhibited a greater propensity to reestablish surface microhardness in comparison to Tooth Mousse. This suggests that Toothmin can be a prospective remineralization agent for mitigating the unfavorable consequences of enamel bleaching.

REFERENCES:

1. Jyothi Kashi Nanjundasetty , Mohammed Ashrafulla: Efficacy of desensitizing agents on postoperative sensitivity following an in-office vital tooth bleaching: A randomized controlled clinical trial. J Conserv Dent.. 2016, 19:207-211. 10.4103/0972-0707.181927
2. Mohammed Q. Alqahtani: Tooth-bleaching procedures and their controversial effects: A literature review. Saudi Dent J.. 2014, 26:33-46. 10.1016/j.sdentj.2014.02.002
3. Kaur G., Sanap A., Aggarwal S., Kumar T: Comparative evaluation of two different remineralizing agents on the microhardness of bleached enamel surfaces: results of an in vitro study. Indian journal of dental research. 2015, 26:176-9. 10.4103/0970-9290.159154
4. Darshan HE, Shashikiran ND: The effect of McInnes solution on enamel and the effect of tooth mousse on bleached enamel: an in vitro study. J Conserv Dent. 2008, 11:86-91. 10.4103/0972-0707.44058
5. Mcevoy SA: Chemical agents for removing intrinsic stains from vital teeth. II. Current techniques and their clinical application. Quintessence Int. 1989, 20:379-84.
6. Singh N.,Ranjan D.,Mahreen S., et al.: A Comparative Evaluation of the Effects of Three Remineralizing Agents on Bleached Enamel: A Scanning Electron Microscopy (SEM)

Analysis.. Cureus15.4. 2023, 15:10.7759/cureus.37240
7. Sun L., Liang S., Sa Y., et al.: Surface alteration of human tooth enamel subjected to acidic and neutral 30% hydrogen peroxide. J Dent. 2011, 39:686-92. 10.1016/j.jdent.2011.07.011
8. Silva Costa SX, Becker AB, and de Souza Rastelli AN, de Castro Monteiro Loffredo L, de Andrade MF, Bagnato VS: Effect of four bleaching regimens on color changes and microhardness of dental nanofilled composites. Int J Dent. 2009, 313845:10.1155/2009/313845
9. Miranda CB, Pagani C, Benetti AR, and Matuda F da S: evaluated the bleached human enamel by scanning electron microscopy. J Appl Oral Sci. 2005, 13:204-11. 10.1590/s1678-77572005000200021
10. Borges BCD, Borges JS, de Melo CD, et al.: Efficacy of a novel at-home bleaching technique with carbamide peroxides modified by CPP-ACP and its effect on the microhardness of bleached enamel. Oper Dent. 2011, 36:521-8. 10.2341/11-013
11. Chaudhury T, Ananthakrishna S, Kumari RV, Kour S, and Syam: A. A comparative study of the remineralization potential of three different remineralizing agents on demineralized enamel using light fluorescence and a confocal fluorescence microscope: an in vitro study. International Journal of Preventive and Clinical Dental Research. 2018, 5:45. 10.5005/jp-journals-10005-1715
12. Manju Krishna EM, Robin Theruvil, Jain Mathew et.al: Evaluation of the Effect of Remineralizing Agent on Bleached Enamel Surfaces Subjected to Erosion: An In Vitro Study. Cons Dent Endod J. 2019, 4:1-5. 10.5005/jp-journals-10048-0045
13. Joiner A: Review of the effects of peroxide on enamel and dentine properties. Journal of Dentistry. 2007, 35:889-96. 10.1016/j.jdent.2007.09.008
14. Rodrigo S. Lacruz, Stefan Habelitz, J. Timothy Wright, and Michael L. Paine: Dental Enamel Formation and Implications for Oral Health and Disease. Physiol Rev. . 2017, 97:939-993. 10.1152/physrev.00030.2016
15. Joshi SB: An overview of vital teeth bleaching. Journal of Interdisciplinary Dentistry. 2016, 6:3. 10.4103/2229-5194.188155
16. Kwon, So Ran, and Philip W. Wertz: Review of the mechanism of tooth whitening. Journal of esthetic and restorative dentistry. 2015, 27:240-257. 10.1111/jerd.12152
17. Yildirim E, Vural UK, Cakir FY, and Gurgan S: Effects of Different Over-the-- Counter Whitening Products on the Microhardness, Surface Roughness, Color, and Shear Bond Strength of Enamel. Acta Stomatol Croat. 2022, 56:120-31. 10.15644/asc56/2/3
18. Cristiane Franco Pinto, Rogério de OliveiraII, Vanessa Cavalli et.al.: Peroxide bleaching agent effects on enamel surface microhardness, roughness and morphology. Braz Oral Res. 2004, 18:306-11. 10.1590/s1806-83242004000400006
19. Sa Y., Sun L., Wang Z., et al.: Effects of two in-office bleaching agents with different ph on the structure of human enamel: an in situ and in vitro study. Oper Dent. 2013, 38:100-10. 10.2341/11-173-L
20. Abouassi T, Wolkewitz M, and Hahn P: Effect of carbamide peroxide and hydrogen peroxide on enamel surface: an in vitro study. Clin Oral Investig. 2011, 15:673-80. 10.1007/s00784-010-0439-1
21. Xu B, Li Q, and Wang Y: Effects of pH values of hydrogen peroxide bleaching agents on enamel surface properties. Oper Dent. 2011, 36:554-62. 10.2341/11-045-1
22. Amaechi B.T: Remineralization Therapies for Initial Caries Lesions. Curr Oral Health Rep . 2015, 2:95-101. 10.1007/s40496-015-0048-9
23. Sule Bayrak , Emine Sen Tunc, Isil Saroglu Sonmez et.al.: Effects of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) application on enamel microhardness after bleaching. Am J Dent. 2009, 22:393-6. 10.2341/11-013-L
24. D Gurunathan, S Somasundaram, Sa Kumar: Casein phosphopeptide-amorphous calcium phosphate: a remineralizing agent of enamel. Aust Dent J. 2012, 57:404-8. 10.1111/adj.12006.
25. Melo M, Fioresta R, Sanz JL, Pecci-Lloret MP, and Llena C: Effect of highly concentrated bleaching gels on enamel microhardness and superficial morphology and the recovery action of four remineralizing agents. BMC Oral Health. 2022, 22:645. 10.1186/s12903-022-02693-2
26. Gomes MN, Rodrigues FP, Silikas N, and Francci CE: Micro-CT and FE-SEM enamel analyses of calcium-based agent application after bleaching. Clin Oral Investig. 2018, 22:961-70. 10.1007/s00784-017-2175-2
27. Raghu TN, Ananthakrishna S: Remineralization Potential of Calcium Sucrose Phosphate on Demineralized Enamel: Results of an In Vitro Study. Journal of International Oral Health. 2016, 8:704. 10.2047/jioh-08-06-12
28. Esfahani KS, Mazaheri R, and Pishevar L: Effects of Treatment with Various Remineralizing Agents on the Microhardness of Demineralized Enamel Surfaces. Journal of Dental Research, Dental Clinics, and Dental Prospects. 2015, 9:239. 10.15171/joddd.2015.043