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Evaluation Of The Change In Enamel Microhardness After Use Of Different Remineralizing Agents On Bleached Enamel Surfaces Subjected To Erosion: An In Vitro Study.

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Abstract:
Aim: *The aim of this study is to evaluate change in enamel microhardness on a bleached enamel surfaces treated with re-mineralizing agents on teeth subjected to erosion.**Settings and Design:** An in vitro study done in dept of conservative dentistry and endodontics, S B Patil college of dental science and research.*
Methods and Material:
Thirty-six enamel specimens were randomly allocated into three groups (n = 12):
- ***Group I** – Treated with 35% carbamide peroxide followed by 1% citric acid*
- ***Group II** – Treated with 35% carbamide peroxide, BioEnamel remineralizing agent, then 1% citric acid*
- ***Group III** – Treated with 35% carbamide peroxide, Colgate Sensitive Plus®, then 1% citric acid*

*All samples were subjected to **Vickers microhardness testing**, and **percentage surface hardness loss (%SHL)** was calculated to assess erosion resistance.*
Statistical analysis used: The statistical analysis for this study includes descriptive statistics (mean, standard deviation) for initial hardness, hardness after erosion and percentage surface hardness loss across three group. A one-way ANOVA was likely used to determine significant differences between the groups.
***Results**: Colgate sensitive plus showed reduced surface hardness loss when compared to Bioenamel and the control group.*
***Conclusions:** The results of the present study demonstrated that **Group III (Colgate Sensitive Plus®)** exhibited the most favorable outcomes in preserving enamel surface microhardness following bleaching, indicating its superior remineralization potential. In contrast, **Group II (BioEnamel)** recorded the highest percentage of surface hardness loss, raising concerns regarding its efficacy in safeguarding enamel integrity post-bleaching.*
Key Word: *Intrathecal; Bupivacaine; Buprenorphine; Nalbuphine; Postoperative analgesia.*

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I. Introduction

The pursuit of aesthetically pleasing dentition has significantly elevated the emphasis on tooth colour, thereby establishing dental bleaching as a cornerstone in contemporary cosmetic dentistry. Bleaching entails the chemical degradation of chromogenic compounds—whether extrinsic or intrinsic to the dental substrate—through oxidative mechanisms. Owing to its non-invasive approach and relatively lower cost compared to alternative esthetic interventions, bleaching is widely favoured for managing tooth discoloration and enhancing patient satisfaction.[1]

Among the most extensively utilized bleaching agents are hydrogen peroxide and carbamide peroxide, both of which exert their effect by oxidizing chromophores responsible for pigmentation.[2] Notably, carbamide peroxide, a mainstay in tray-based at-home bleaching protocols, decomposes into urea and hydrogen peroxide, thereby enabling a sustained release of the active oxidizing agent.[2]

A noteworthy advancement in bleaching technologies is embodied in Prevest DenPro's '24 Carat' gel—a high-viscosity, high-adhesion formulation integrating a carbamide peroxide–glycerin complex within a pH-adjusted medium. This proprietary "zero sensitivity" composition has been engineered to mitigate post-operative hypersensitivity while significantly reducing treatment duration, thereby enhancing patient compliance and comfort.

The bleaching process generates free radicals that penetrate the enamel interprismatic regions and dentinal tubules, initiating redox reactions with organic chromophores. However, this interaction is not without consequence; the oxidative challenge extends to both the organic and inorganic matrices of the tooth, resulting in enamel demineralization, diminished microhardness, reduced structural integrity, and compromised fracture resistance. The acidic pH of bleaching agents, coupled with by-products such as urea—known to destabilize enamel proteins and their associated mineral complexes—further exacerbates these deleterious effects.[3]

Moreover, it is well established that the consumption of acidic foods and beverages in the post-bleaching period can further predispose the enamel to erosion, thereby amplifying the risk of surface degradation. In response to these concerns, the incorporation of remineralization therapies post-bleaching has garnered increasing clinical interest for their potential to restore mineral content and reinforce enamel resilience.

Remineralizing agents are specifically formulated to replenish depleted minerals and enhance enamel resistance to acid challenges. Among these, *Bio Enamel Remineralizing Gel* (Prevest DenPro Ltd., Jammu, India) has demonstrated considerable promise. It is indicated for a range of clinical scenarios, including post-bleaching hypersensitivity, enamel demineralization, non-carious cervical lesions, and as a prophylactic adjunct following professional dental cleaning.

Complementing traditional remineralizing agents, the present study also investigates the efficacy of an arginine-based complex. Arginine, a semi-essential amino acid prevalent in salivary peptides and proteins, has been recognized since the 1980s for its biofilm-buffering properties, primarily through its capacity to elevate biofilm pH. Subsequent studies have validated its remineralization potential, particularly when formulated in conjunction with fluoride and bicarbonate in dentifrices.

Mechanistically, the arginine complex enhances remineralization by binding to calcium carbonate reservoirs on the tooth surface, facilitating a gradual and sustained release of calcium ions. This mechanism supports the replenishment of mineral content and fortification of the enamel surface. This formulation is commercially available under the brand *Colgate Sensitive Plus.*

Aim of the Study

This study aims to evaluate and compare the effects of two remineralizing agents—*Bio Enamel Remineralizing Gel* and *Colgate Sensitive Plus*—on the surface characteristics of bleached enamel subjected to erosive insult, with an emphasis on their potential to restore enamel integrity post-bleaching.

II. Material And Methods

Sample Size Calculation:

Sample size calculated using the formula mentioned below:

$$n=\frac{2\alpha^2(Z\alpha/2+Z\beta)^2}{\Delta}$$

Where,

- n = sample size per group
- Standard deviation (σ\sigmaσ) around **6.38** for initial hardness.
- The estimated **sample size per group** is **6**, leading to a **total sample size of 18** for the study.

Selection and preparation of enamel blocks:

For the preparation of specimens, 9 intact human premolars that were extracted for orthodontic reasons were chosen. The crowns were attached to acrylic resin blocks, and the cemento-enamel junction (CEJ) was sectioned using a water-cooled diamond disc. Each crown was divided vertically to obtain two enamel samples per tooth. A total of 18 enamel samples were collected and randomly assigned into three groups, with each group containing six samples.

Before conducting the baseline surface microhardness evaluation, all samples were carefully rinsed under running water to eliminate any debris and then stored in a humid environment to avoid dehydration.

Assessment of initial surface hardness:

A diamond penetrator weighing 100 grams was used for a duration of 10 seconds. To measure the hardness values (Vickers hardness number [vhn]), two indentations were made in the center of each sample, with a separation of 100 µm. The hardness of the samples was determined by calculating the average of these two values.

After obtaining the initial microhardness, the samples were randomly divided into three groups (n = 6) based on the application of a remineralizing agent.

Group I: Bleaching with 35% carbamide peroxide, samples exposed to erosion using 1% citric acid. (figure 1 and 4)

Group II: Bleaching with 35% carbamide peroxide followed by application of BioEnamel, exposed to erosion with 1% citric acid. (figure 2 and 4)

Group III: Bleaching with 35% carbamide peroxide followed by application of arginine bicarbonate-based Colgate Sensitive Plus toothpaste, which was then subjected to erosion with 1% citric acid. (figure 3 and 4)

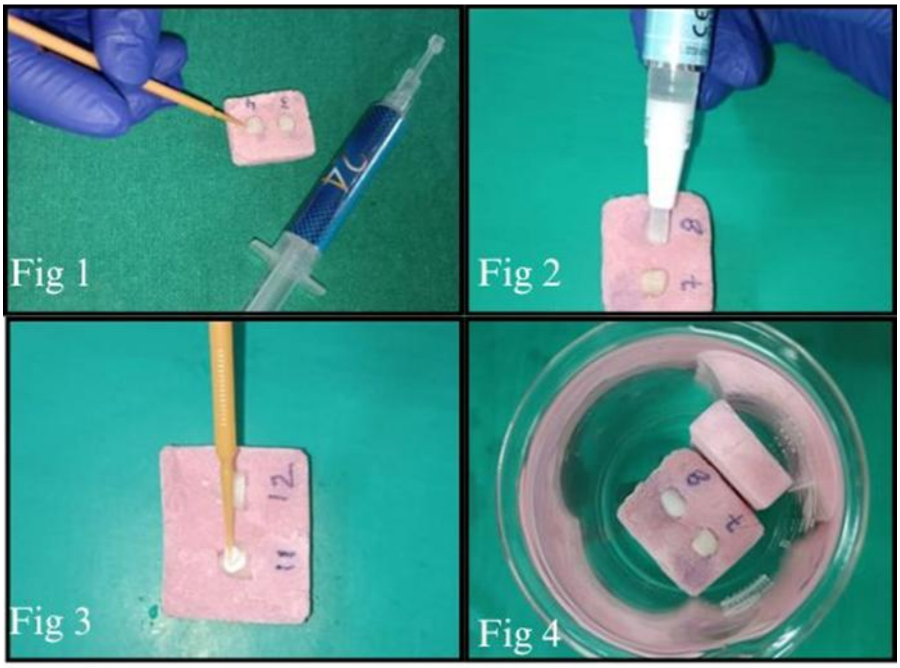


Fig1:Application of bleaching agent for 30 mins followed by rinsing with water for 5 mins; Fig 2:Application of Bioenamel for 5 minutes in a 15 days period, twice daily; Fig 3:Application of colgate sensitive plus for 5 minutes in a 15 days period, twice daily;Fig 4:Subjected to erosion(citric acid) for 5 minutes, twice daily for 3 days

Surface microhardness analysis was conducted in a similar manner as it was done for the baseline assessment.

Statistical analysis: The statistical analysis for this study involves calculating descriptive statistics, such as the mean, standard deviation, for initial hardness, hardness after erosion, and the percentage surface hardness loss across three different groups. A one-way analysis of variance (ANOVA) was likely employed to identify significant variations between the groups.

III. Result

- Enamel hardness reduction (table 1 & figure 5). All groups exhibited a substantial decrease in enamel hardness after erosion ($p < 0.05$), validating the erosive potential. Group iii exhibited the least decrease, suggesting superior resilience.
- Table 2 & Figure 6: Percentage Hardness Loss Group II exhibited the highest surface hardness loss (10.16%), followed by Group I (7.94%). Group III showed minimal loss (0.13%), indicating its effectiveness in providing protection.
- Post hoc analysis (table 3 & figure 7). All group comparisons were statistically significant ($p = 0.000$). Group III outperformed groups I and II in terms of hardness retention, while group II exhibited a much higher loss, confirming its inferior performance.

Group	Initial Hardness (Mean ± SD)	Hardness After Erosion (Mean ± SD)	P value
Group I	394.62 ± 0.00	363.25 ± 0.00	< 0.001
Group II	367.79 ± 0.00	330.41 ± 0.00	< 0.001
Group III	359.83 ± 0.00	359.36 ± 0.00	0.034

TABLE 1

Group	% Surface Hardness Loss (Mean ± SD)	P value
Group I	7.94 ± 0.0	0.000
Group II	10.16 ± 0.00	
Group III	0.13 ± 0.00	

TABLE 2

Comparison	Mean Difference	p-value
Group I vs. Group II	+2.22	0.000
Group I vs. Group III	-7.81	0.000
Group II vs. Group III	-10.03	0.000

TABLE 3

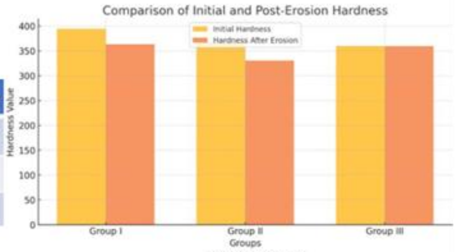
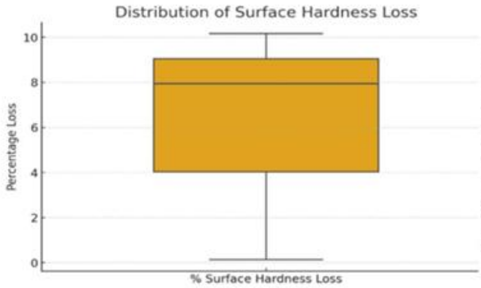


FIGURE 5



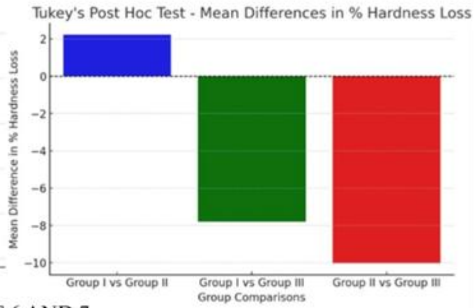


FIGURE 6 AND 7

Table 1: Initial and Post-Erosion Hardness Values with Statistical Significance.
Figure 5: Comparison of Initial and Post-Erosion Hardness
Table 2: Percentage Surface Hardness Loss with Statistical Significance
Table 3: Tukey's Post Hoc Test for % Surface Hardness Loss Between Groups
Figure 6: Box Plot of % Surface Hardness Loss Across Groups,
Figure 7: Tukey's Post Hoc Test - Mean Differences in % Surface Hardness Loss

IV. Discussion

Tooth bleaching is widely recognized as a conservative and effective modality for managing dental discoloration. The choice of bleaching protocol is dictated by clinical considerations and may vary in terms of agent composition, concentration, delivery method (in-office vs. at-home), activation strategy (e.g., light-assisted), and application site (external, internal, or combined).[6]

In the present study, a commercially available bleaching agent—*24 Carat* (Prevest DenPro)—was utilized. This formulation comprises carbamide peroxide as the principal active ingredient, supplemented with potassium nitrate, EDTA, citric acid, propylene glycol, carbopol, glycerine, and spearmint oil. The inclusion of desensitizing agents and pH buffers is designed to attenuate the adverse effects typically associated with peroxide-based bleaching systems.

Carbamide peroxide undergoes hydrolysis to yield hydrogen peroxide and urea. According to Basheer et al., urea may destabilize structural enamel proteins such as amelogenin and enamelin, potentially altering enamel ultrastructure and enhancing permeability.[7] Nonetheless, due to its alkalizing nature, urea may mitigate demineralization relative to hydrogen peroxide alone. Additionally, carbamide peroxide formulations offer superior chemical stability and extended shelf life—particularly under refrigerated storage—which further enhances their oxidative efficacy.[8]

By contrast, hydrogen peroxide, although a potent bleaching agent, is inherently unstable and requires stringent storage conditions—typically in a cool, dark environment—to maintain efficacy. Its clinical application necessitates immediate preparation and use to avoid degradation.[9]

Despite their widespread use and clinical efficacy, peroxide-based agents can exert detrimental effects on enamel, including demineralization, increased surface roughness, erosion, and post-operative hypersensitivity. These effects are exacerbated by dietary acids and are often attributed to the low pH of certain formulations. Such observations underscore the growing consensus on the necessity of adjunctive remineralization therapies following bleaching.[10]

The pathophysiology underlying peroxide-induced surface degradation has been elucidated by Gabriel et al., who proposed that oxidative disruption of the enamel's organic matrix results in localized dissolution and the emergence of surface irregularities. The accumulation of nitrogen and oxygen residues in bleached enamel further corroborates this mechanism.[11]

Remineralizing agents, such as CPP-ACP (casein phosphopeptide-amorphous calcium phosphate) and bioactive glass, have demonstrated efficacy in reversing the morphological and microhardness changes induced by bleaching.[4] In the present investigation, *Colgate Sensitive Plus®*, containing the proprietary Pro-Argin™ formula, exhibited the lowest percentage surface hardness loss (SHL), followed by the control group and then the *BioEnamel* group.

The superior performance of the Pro-Argin™ group can be attributed to its formulation, which includes arginine, calcium carbonate, and sodium monofluorophosphate. Chandru et al. demonstrated that the synergistic interaction of arginine and fluoride within a calcium carbonate base enhances fluoride uptake and facilitates the development of acid-resistant enamel, thereby promoting greater recovery in enamel microhardness.[12] Similarly, Alsubhi et al. reported that *Colgate Sensitive Pro-Relief* significantly improved enamel microhardness when compared to untreated controls.[13]

BioEnamel Remineralizing Gel, comprising glycerine, potassium nitrate, bioglass, and carbopol, also exhibited a favorable remineralization effect and contributed to a reduction in post-bleaching sensitivity. The presence of bioglass initiates the formation of octacalcium phosphate (OCP), a precursor to hydroxyapatite. OCP facilitates mineral deposition and promotes the incorporation of fluoride, ultimately forming fluoridated apatite—a structure known for its superior acid resistance. Furthermore, *BioEnamel* has the capacity to bind to dentin surfaces and form a hydroxycarbonate apatite layer that occludes dentinal tubules, thereby mitigating sensitivity.[14]

However, the comparatively higher surface hardness loss observed in the *BioEnamel* group may be explained by the absence of fluoride in its formulation, compounded by the lack of salivary fluoride in the in vitro experimental setting. This limitation may have hindered the formation of fluoridated apatite and attenuated the full remineralization potential of the material.

V. Conclusion

The results of the present study demonstrated that **Group III (Colgate Sensitive Plus®)** exhibited the most favorable outcomes in preserving enamel surface microhardness following bleaching, indicating its superior remineralization potential. In contrast, **Group II (BioEnamel)** recorded the highest percentage of surface hardness loss, raising concerns regarding its efficacy in safeguarding enamel integrity post-bleaching.

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