Journal of Carcinogenesis
Covering Genesis to Therapeutics
Journal of Carcinogenesis, ISSN: 1477-3163
2025; Vol 24: Issue 8s, https://carcinogenesis.com/

# Effectiveness of Bioactive Glass Based Dentifrice With and Without Chitosan Nanoparticles in Restoring Enamel Microhardness Post Bleaching: An In Vitro Study

**Jinitha G.C.[1], Priya K.C.[*2], Tony Daniel[3], Teena Jerry[4], Afzal A[5], Vineet R.V.[6]**

[1]MDS Student, Department of Conservative Dentistry and Endodontics, PMS College of Dental Science and Research
Email ID: jinitha456gc@gmail.com

[2]MDS Student, Department of Conservative Dentistry and Endodontics, PMS College of Dental Science and Research

[3]MDS Student, Department of Conservative Dentistry and Endodontics, PMS College of Dental Science and Research
Email.ID: tonydaniel98@gmail.com

[4]MDS Student, Department of Conservative Dentistry and Endodontics, PMS College of Dental Science and Research
Email.ID: teenajerry90210@gmail.com

[5]Professor, Department of Conservative Dentistry and Endodontics, PMS College of Dental Science and Research, PMS College of Dental Science and Research
Email ID: afzalabdulsalim@gmail.com

[6]Professor, Department of Conservative Dentistry and Endodontics, PMS College of Dental Science and Research
Email ID: dr.vrv@rediffmail.com

***Corresponding Author:**
Priya K.C.
Email ID: drpriyachandrabanu@gmail.com

## ABSTRACT

**Aim:** To evaluate the effectiveness of a bioactive glass-based dentifrice, with and without the addition of chitosan nanoparticles, in restoring enamel hardness after bleaching with 37.5% hydrogen peroxide.

**Material And Methods:** Fifteen extracted human maxillary central incisors were divided into three groups: control (no treatment), treatment with bioactive glass-based dentifrice and treatment with bioactive glass-based dentifrice infused with 0.2% chitosan nanoparticles. Enamel microhardness was measured at baseline, post-bleaching, and post-remineralization using a Vickers microhardness tester.

**Results:** All study groups showed significant enamel demineralization post-bleaching. Remineralization using both formulations improved enamel hardness, with chitosan-infused dentifrice showing slightly superior recovery.

**Conclusion**: These findings suggest that bioactive glass-based dentifrices, particularly when combined with chitosan nanoparticles, offer a viable strategy for mitigating the deleterious effects of dental bleaching.

***Keywords:*** *Bioactive Glass, Bleaching, Enamel Microhardness, Remineralization*

**How to Cite:** Jinitha G.C., Priya K.C., Tony Daniel, Teena Jerry, Afzal A, Vineet R.V., (20yy) Effectiveness of Bioactive Glass Based Dentifrice With and Without Chitosan Nanoparticles in Restoring Enamel Microhardness Post Bleaching: An In Vitro Study, *Journal of Carcinogenesis*, *Vol.24, No.8s*, 845-850

## 1. INTRODUCTION

Tooth whitening through external bleaching is a widely utilized non-invasive aesthetic dental procedure, often perceived as a conservative alternative to restorative approaches such as veneers or crowns. Hydrogen and carbamide peroxide have been using successfully for many years as bleaching agents. Hydrogen peroxide oxidises a wide variety of organic and inorganic compounds. The mechanisms of these reactions are varied and dependent on the substrate, the reaction environment, and catalysis [1]. With the growing trend of repeated and aggressive bleaching regimens, there is increasing concern about the long-term impact on enamel health. One of the disadvantages, post bleaching can be that of dental sensitivity, so several remineralizing agents have been introduced in the market to be availed before and after bleaching or in combination with bleaching gels [2].Consequently, strategies to re-mineralise and restore enamel following bleaching are imperative for preserving tooth integrity. A variety of compounds for remineralisation are currently available such as fluoride, novamin, tricalcium phosphate, sodium monofluorophosphate, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), etc. [3].

Novamin is calcium sodium phosphosilicate bioactive glass (BAG) which when exposed to body fluids deposit hydroxycarbonate crystals, a mineral that is analogous to natural tooth mineral [4]. Chitosan, a cationic polysaccharide derived from the deacetylation of chitin found in crustacean exoskeletons, is gaining attention as a multifunctional biomaterial.It has been utilized as a dentin pretreatment agent because the covalent immobilization of chitosan on dentinal collagen causes the exposed demineralized dentin structure to remineralize [5].

Given the potential synergistic effect of combining bioactive glass with chitosan nanoparticles, this study aims to investigate the comparative remineralization efficacy of bioactive glass-based dentifrice, with and without chitosan nanoparticle incorporation, on enamel microhardness following bleaching using 37.5% hydrogen peroxide.

## 2. MATERIALS AND METHODS

### 2.1 Sample Selection

Fifteen extracted human maxillary central incisors, free of caries, cracks, restorations, or developmental defects, were selected and stored in 0.9% NaOCl solution prior to use.

### 2.2 Sample Preparation

Each tooth was embedded in acrylic resin with the buccal surface exposed and polished using silicon carbide paper as shown in figure 1. Baseline enamel hardness was recorded using a Vickers microhardness tester (load: 300g, dwell time: 10s).

### 2.3 Bleaching Protocol

Enamel surface was dried with cotton pellet and specimens were treated with Pola Office Plus ((Hydrogen Peroxide 37.5%SDI Limited Bayswater, Victoria, Australia), applied at a 1 mm thickness for 8 minutes, and light-cured. This procedure was repeated four times, after which microhardness was measured again.

### 2.4 Group Allocation

Samples were randomly divided into three groups:

Group A (Control): No remineralizing treatment

Group B: Treated with bioactive glass dentifrice (SHY-NM paste) (Shy-NM, Group Pharmaceuticals Ltd.

Group C: Treated with SHY-NM containing 0.2% chitosan nanoparticles

### 2.5 Remineralizing Agent Preparation And Preocedure

Group B: 100g of dentifrice containing bioactive glass was weighed in a precision balance.

Group C: 0.02g of chitosan nanoparticle was mixed with 100g of dentifrice to obtain the 0.2% chitosan.

### 2.6 Procedure

The two groups were subjected for remineralization for four minutes twice daily, once in the morning and later in the afternoon for a period of 5 days. Group A (n=) is control group specimens which undergone bleaching only. In group B SHY NM tooth paste is applied using an applicator tip. After 4 minutes the agent is rinsed off with air water spray. This cycle was repeated for 5 days. In group C chitosan infused dentifrice was applied on the enamel surface for four minutes twice daily for a period of 5 days. In between the remineralization the specimen was kept in artificial saliva.

## 3. STATISTICAL ANALYSIS

Data were analysed using SPSS 26.0. Normality was assessed using the Shapiro-Wilk test. Intergroup and intragroup differences were analysed using one-way ANOVA followed by Bonferroni post hoc tests. Significance was set at $p < 0.05$.

## 4. RESULTS

### 4.1 Microhardness measurement

Enamel hardness was tested using Vickers hardness testing machine with a load of 300g for 10 seconds. Indentation were made on the surface of enamel as shown in Figure 2. Each specimen was tested for 3 times such as before bleaching, after bleaching and after remineralization.

### 4.2 Pre-Bleaching Hardness

Baseline microhardness values ranged from 309.12HV to 334.06HV, with no significant differences among groups ($p = 0.07$).

### 4.3 Post-Bleaching Hardness

A significant reduction in microhardness was observed across all groups, ranging from 231.96 to 246.66. Differences were not statistically significant ($p = 0.68$), indicating uniform demineralization.

### 4.4 Post-Remineralization Hardness

Significant improvements were observed in Groups B (284.14 ± 23.10) and C (285.50 ± 11.43), compared to Group A (245.84 ± 20.84), with $p = 0.01$. No significant difference was found between Groups B and C ($p = 1.000$).

### 4.4 Intragroup Comparisons

Group A showed minimal recovery post-remineralisation. Groups B and C demonstrated significant recovery, though not to baseline values.

**Table 1 showing difference in the enamel microhardness between the groups at baseline, post bleaching and remineralization**

| | | N | Mean (HV) | Std. Deviation | 95% Confidence Interval for Mean | | Minimum | Maximum | P Value One way anova |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | | |
| Before bleaching | Without remineralizing agent | 5 | 320.600 | 11.5804 | 306.221 | 334.979 | 304. | 336.2 | 0.07 |
| | with chitosan | 5 | 309.120 | 19.9737 | 284.319 | 333.921 | 291.2 | 341.2 | |
| | without chitosan | 5 | 334.060 | 12.8413 | 318.115 | 350.005 | 319. | 350.4 | |
| After bleaching | Without remineralizing agent | 5 | 231.960 | 26.2993 | 199.305 | 264.615 | 194.6 | 255.2 | 0.68 |
| | with chitosan | 5 | 239.740 | 34.0824 | 197.421 | 282.059 | 183.6 | 271.5 | |
| | without chitosan | 5 | 246.660 | 15.8622 | 226.965 | 266.355 | 228.8 | 268.6 | |
| After remineralizing agent | Without remineralizing agent | 5 | 245.840 | 20.8375 | 219.967 | 271.713 | 214.6 | 262.2 | 0.01* |
| | with chitosan | 5 | 284.140 | 23.0969 | 255.461 | 312.819 | 246.4 | 306.4 | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **without chitosan** | **5** | **285.500** | **11.4320** | **271.305** | **299.695** | **267.4** | **296.6** | |

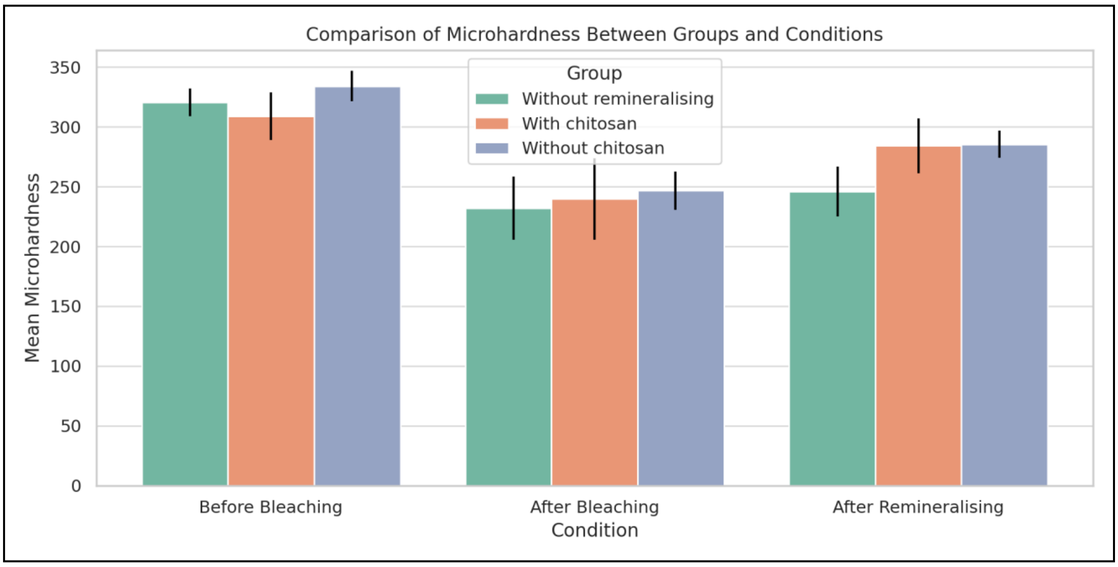


**Figure 3 depicting changes in the microhardness of enamel before bleaching, after bleaching and post remineralization with and without chitosan**

## 5. DISCUSSION

This in vitro investigation demonstrates that high-concentration hydrogen peroxide bleaching significantly compromises enamel microhardness across all experimental groups, corroborating existing literature that identifies demineralization and subsurface porosity as key consequences of oxidative bleaching.

Previous studies by **Li Y.et al highlighted** key safety concerns associated with tooth bleaching, particularly focusing on tooth sensitivity, gingival irritation, and potential effects on enamel and pulp tissue. While most effects are reversible and mild, they emphasize the importance of professional supervision and appropriate use to mitigate risks [6]. **Basting RT et al.** evaluated enamel microhardness after exposure to various carbamide peroxide bleaching agents over time and demonstrated a significant reduction in enamel microhardness with prolonged use, suggesting that bleaching may weaken enamel integrity depending on agent concentration and duration [7].

The present study observed reduction in Vickers hardness reflects loss of mineral content, primarily hydroxyapatite, which forms the bulk of enamel's mechanical resilience. This is in accordance with the previous study by C P Ernst **et al.** *Effects of hydrogen peroxide bleaching on enamel surface texture* using scanning electron microscopy and the study found that hydrogen peroxide bleaching can alter the enamel surface, leading to increased porosity and roughness [8]. These morphological changes support concerns about the physical impact of bleaching agents on enamel structure.

Post-bleaching treatment with remineralizing agents yielded statistically significant improvements in enamel hardness, particularly in the groups receiving bioactive glass-based interventions (Groups B and C). This reinforces the findings of previous studies which concluded that Bioactive glass deposits were found on the enamel surface of all the specimens, suggesting that they may act as a reservoir of ions available for remineralization at sites of possible demineralization [9], [10].While both remineralizing formulations showed comparable outcomes in terms of microhardness recovery, the chitosan-enhanced group (Group C) exhibited a numerically higher recovery. This can be attributed to chitosan's cationic nature, due to its positive zetapotential, chitosan electrostatically binds to surfaces with negative ions, such as the enamel surface enhancing the adhesion and retention of calcium and phosphate ions [11], [12].The ability of chitosan to chelate trace minerals and facilitate prolonged ionic release may contribute to a more robust and stable apatite layer, as supported by Huang et al. and He et al., who observed increased remineralization and structural regeneration in enamel lesions treated with chitosan-based nanocomposites [13].

Although the statistical analysis in this study did not reveal a significant difference between the two active groups ($p = 1.000$), the trend suggests a potential clinical advantage worth exploring in larger-scale or in vivo studies. The limited recovery in the control group (Group A) further emphasizes the detrimental and persistent effects of bleaching on enamel when not followed by remineralization therapy. This aligns with findings from ten Cate and Duijsters, who demonstrated that remineralization is both time-dependent and highly influenced by the surrounding environment and availability of mineral ions [14].

From a clinical perspective, integrating remineralizing agents into post-bleaching care protocols may serve as an essential preventive strategy. The use of dentifrices containing bioactive glass, especially when enhanced with chitosan, presents a biocompatible, minimally invasive approach to restoring enamel integrity and reducing post-bleaching sensitivity.

Limitation of the present study is that it was an in vitro design which does not fully replicate the dynamic conditions of the oral environment. Also, the remineralization phase was conducted over just five days. Longer observation periods may provide more accurate insights into the sustained efficacy of the remineralizing agents. Future studies should consider dynamic pH cycling, longer observation periods, larger sample size and in vivo models to validate these findings and assess long-term outcomes, including resistance to secondary caries and overall oral health impact.

## 6. CONCLUSION

This study demonstrates that bleaching with hydrogen peroxide significantly compromises enamel hardness. Remineralization with bioactive glass dentifrice effectively mitigates this effect, with enhanced outcomes observed upon the addition of chitosan nanoparticles. Although further in vivo research is warranted, these findings highlight the potential of such formulations in post-bleaching enamel care.

**Financial support and sponsorship**

Nil

**Conflicts of interest**

There are no conflicts of interest.

## REFERENCES

[1] A. Joiner, “The bleaching of teeth: a review of the literature,” Journal of Dentistry, vol. 34, no. 7, pp. 412-417, 2006.

[2] S. Vats, D. J. Sinha, S. Singh, R. Rathi, S. Jha and I. Singh, "Effect of remineralizing agent on laser and nonlaser bleached enamel surfaces subjected to erosion: An in vitro study.," Journal of conservative dentistry and endodontics, vol. 26, no. 4, pp. 388-394, 2023.

[3] J. S. Reddy, S. V. Reddy and D. K. Sharma, "A comparative evaluation of human enamel remineralization ability of biomimetic nacre against casein phosphopeptide-amorphous calcium phosphate: An in vitro study.," Conserv Dent Endod., vol. 27, no. 9, pp. 954-961, 2024.

[4] S. Taneja, M. Kumar, P. M. Agarwal and A. S. Bhalla, "Effect of potential remineralizing agent and antioxidants on color stability of bleached tooth exposed to different staining solutions," Journal of conservative dentistry, vol. 21, no. 4, pp. 378-382, 2018.

[5] R. Meher, R. R. Mallick, P. Sarangi, A. Jena, S. Suman and G. Sharma, "Optimization of chitosan nanoparticle dentin pretreatment with different concentrations and application times to improve bonding at resin-dentin interface," Journal of conservative dentistry and endodontics, vol. 28, no. 3, pp. 248-252, 2025.

[6] Y. Li, "Safety Controversies in Tooth Bleaching," Dental Clinics of North America, vol. 55, no. 2, pp. 255-263, 2011.

[7] R. T. Basting, A. L. J. Rodrigues and M. C. Serra, "The effects of seven carbamide peroxide bleaching agents on enamel microhardness over time.," Journal of the American Dental Association (1939), vol. 134, no. 10, pp. 1335-1342, 2003.

[8] C. P. Ernst, B. B. Marroquín and B. Willershausen-Zönnchen, "Effects of hydrogen peroxide-containing bleaching agents on the morphology of human enamel," Quintessence international, vol. 27, no. 1, pp. 53-56, 1996.

[9] E. Gjorgievska and . J. Nicholson, "Prevention of enamel demineralization after tooth bleaching by bioactive glass incorporated into toothpaste," Australian Dental Journal, vol. 56, no. 2, pp. 193-200, 2011.

[10] . A. B. Mehta , V. Kumari , . R. Jose and V. Izadikhah, "Remineralization potential of bioactive glass and casein phosphopeptide-amorphous calcium phosphate on initial carious lesion: An in-vitro pH-cycling study," Journal of conservative dentistry, vol. 17, no. 1, pp. 3-7, 2014.

[11] N. I. Pavesi Pini, M. R. Piccelli, W. . F. Vieira-Junior, L. . N. Ferraz, . F. H. B. Aguiar and . D. A. N. Leite Lima, "In-office tooth bleaching with chitosan-enriched hydrogen peroxide gels: in vitro results," Clinical oral investigations, vol. 26, no. 1, p. 471–479, 2022.

[12] C. Ganss, A. Lussi, O. Grunau, J. Klimek and N. Schlueter, "Conventional and anti-erosion fluoride

toothpastes: effect on enamel erosion and erosion-abrasion," Caries research, vol. 45, no. 6, p. 581–589., 2011.

[13] S. Huang, S. Gao, L. Cheng and H. Yu, "Remineralization potential of nano-hydroxyapatite on initial enamel lesions: an in vitro study," Caries research, vol. 45, no. 5, pp. 460-468, 2011.

[14] J. M. ten Cate and P. P. Duijsters, "Influence of fluoride in solution on tooth demineralization. I. Chemical data," Caries research, vol. 17, no. 3, pp. 193-199, 1983.