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COMPARATIVE EVALUATION OF INCORPORATING THREE DIFFERENT NANO BIOMATERIALS IN HYDROGEN PEROXIDE GEL ON MICROHARDNESS OF BLEACHED ENAMEL AN IN-VITRO STUDY

Dental Science

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ABSTRACT

BACKGROUND: A good aesthetic smile is one of the most important factors to attain positive life style. Among various aesthetic treatment modalities available, bleaching gained immense response in aesthetic enhancement. But in some cases, bleaching alone does not fulfill the aesthetic demands which require additional procedures like composites restorations, laminates, veneers etc. However, some of the studies reported that bleaching can alter the microhardness of the tooth, resulting in deficient bonding and demineralisation. The current study was to evaluate the change in microhardness of enamel specimens bleached with 40% hydrogen peroxide gel incorporated with three different nano remineralising agents.

MATERIALS AND METHODS : 30 enamel specimens (5x5x4mm) were obtained from 15 molars and incorporated in acrylic resin mold which were divided into 5 groups (6 per group).After attaining baseline Knoop microhardness values enamel specimens were subjected to bleaching treatment .Group 1 – control(with no bleaching) ,Group 2 – Bleaching with 40 % hydrogen peroxide gel, Group 3 – Bleaching with 40 % hydrogen peroxide gel incorporated with Nano calcium chloride, Group/ 4 - Bleaching with 40 % hydrogen peroxide gel incorporated with Nano sodium fluoride, Group 5 - Bleaching with 40 % hydrogen peroxide gel incorporated with Nano hydroxyapatite .Post treatment knoop microhardness values are evaluated and difference between baseline and post treatment microhardness values were obtained.

STATISTICAL ANALYSIS: Statistical analysis was performed using one-way analysis of variance and post hoc Tukey's test.

RESULTS: By the obtained values results have been showed that Significant differences were observed between bleaching groups of with and without incorporation of remineralizing agents . The enamel microhardness change in Groups 1, 3, 4, and 5 were significantly lower than that of Group 2 ($p < 0.001$)

CONCLUSION: Within the limitations of this study, it can be concluded that incorporation of each one of the three tested biomaterials as remineralizing agents might be effective in decreasing enamel microhardness changes subsequent to in-office bleaching

KEYWORDS

Bleaching; Enamel; Micro Hardness; Hydroxyapatite ; Calcium Chloride , ;sodium Fluoride

INTRODUCTION

The evolutions of bleaching products and improved techniques have made dental whitening the most sought-after esthetic treatment because it is effective, simple and minimally invasive when compared with other restorative treatments. 1

Currently agents like hydrogen peroxide & carbamide peroxide are used as bleaching agents in various forms like paste, gel, chewing gums, paint on strips and in various formats like vital & non vital or in office & home bleaching techniques.2

The development of high-concentrations of hydrogen peroxide (HP) gel formulations and the use of gingival barriers to simultaneously protect the gingival tissue of both arches have made in-office bleaching procedure easier and faster. This technique is especially indicated for scenarios of severe discolorations, discoloration of an individual tooth, absence of patient compliance, and to fulfill fast outcome expectations by patients .1

Although in-office bleaching has been overall reported as a safe and effective procedure, high-concentrated agents are potentially aggressive to dental tissues. Several studies have investigated the effects of bleaching agents on enamel and controversial results have been obtained. While some authors observed a decrease in enamel microhardness after bleaching procedures, and its association with the low pH of bleaching gels, this negative effect was not reported by others.1

Dental enamel is composed of a huge number of highly mineralized prisms, and its hardness is due to a high percentage of inorganic matrix (95%) made of hydroxyapatite crystals (calcium phosphate) and low percentage of organic protein nature matrix (0.36 to 2%) .3 The enamel permeability is low, but it acts as a semipermeable membrane allowing the diffusion of water and some ions.4

Some bleaching gels are presented in low pH solutions to ensure the stability of hydrogen peroxide, so these bleaching. Solutions with acidic pH, may promote microstructure and chemical changes in the enamel.5 Changes in chemical composition of enamel may reflect on modified values of mechanical properties, resulting in undesirable effects such as affecting the structural components like microhardness and strength .6

Assuming that enamel is mainly composed by calcium phosphorus, oxygen and carbon$Ca_{10}(PO_4)_6((OH))_2$, in vitro assays designed to identify these components are desirable to reveal possible alterations of enamel's chemical structure. Some studies showed that dental bleaching alters the enamel chemically and have shown decreased calcium values in enamel.6

Dental enamel is evaluated by the Knoop hardness number (KHN) test, which provides data regarding the loss or gain of components in the enamel. The KHN test is nondestructive in nature, as several indentations can be made in the same specimen without causing changes in the enamel during bleaching. The loss of enamel components is indicated by an increase in the size of the diagonal dental impressions produced by a diamond indenter, whilst those diagonals become smaller upon the gain of components, as it is more difficult for the indenter to penetrate the surface.7

In order to minimize such demineralization after contact with bleaching agents, some biomaterials such as fluoride, hydroxyapatite, calcium ions and amorphous calcium have been added to the composition of those whitening agents. Beneficial effects of combining these substances with bleaching agents have been verified in previous studies, which concluded that such association may protect the enamel from demineralization and also may avoid hypersensitivity during the procedure.8 However, fluoride and calcium addition to the bleaching agents were not sufficient to increase the microhardness of enamel.9so recently trends of incorporating remineralizing agents into bleaching materials are being carried out which will benefit the patient by preventing tooth demineralization . 10

The current study was to evaluate the change in microhardness of enamel specimens bleached with 40% hydrogen peroxide gel incorporated with three different nano remineralising agents (Nano calcium chloride, Nano sodium fluoride, Nano hydroxyapatite)

2.METHODS AND MATERIALS: -

A total of 15 extracted human molars (figure 1) were collected and stored in 0.2% thymol solution at 40c , teeth were subsequently stored in distilled water for 24 hours for thorough removal of thymol residues. The crowns were separated from the roots (figure 2) and crowns of each tooth was sectioned along the central line with a slow-speed water-cooled diamond disks(figure 3) . The enamel surfaces were ground and polished sequentially with 600, 800, 1,500, and 3,000 grit silicon carbide abrasive papers .These procedures were carried out to achieve parallel planar surfaces, considered fundamental for microhardness testing.10 30 enamel specimens were obtained at a respective dimensions (5 × 5 × 4 mm) (figure 4) from all the tooth i.e. samples were selected, samples tooth embedded in acrylic resin totally except exposing the enamel surface .

The specimens were randomly divided into five groups,(figure 5) according to the bleaching procedure (n = 6).Baseline microhardness values are evaluated for all samples.(figure 6) In Group 1, samples did not undergo any bleaching procedure (control) and were only stored in distilled water. In Group 2, samples were bleached with a 40% HP gel (Opalescence Xtra Boost, Ultradent Products Inc.).(figure 7) In Groups 3, 4, and 5, samples were bleached with a 40% HP gel modified by incorporation of Nano calcium chloride,(figure 8) Nano sodium fluoride(figure 9) and Nano hydroxyapatite (figure 10) respectively.

Remineralizing agents ground and filtered to particles up to 50 nm were used. Then, remineralizing agents were mixed with distilled water in a ratio of 2 g powder to 1 mL liquid. After that, they were mixed with 1 mL of hydrogen peroxide 40% .(figure 11) The bleaching agents were applied for 60 minutes (figure 12). After bleaching, the specimens were thoroughly rinsed with air-water spray for 15 seconds. The procedure was repeated twice a week for 2 weeks. All the samples were stored in distilled water at 37 degree centigrade between procedures.

Knoop microhardness value was determined using a microhardness tester under a load of 100 g and an indentation time of 20 seconds.(figure 13) . Three indentations were placed on the surface of each specimen, 100 µm apart from each other. The obtained values were averaged as the baseline microhardness value before treatment. After the treatment procedures, the specimens were tested for final microhardness measurements. The difference between baseline and final values was calculated. The differences in enamel surface microhardness values before and after treatment in each group were analyzed using one-way ANOVA, followed by post hoc Tukey tests at a 5% significance level.

FIGURE 1 : MANDIBULAR MOLARS

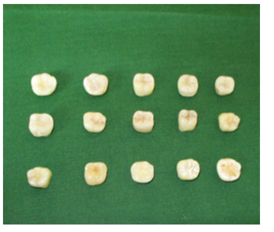

FIGURE 2 : DECORONATED MOLARS

FIGURE 3 : SECTIONING TOOTH INTO TWO HALVES

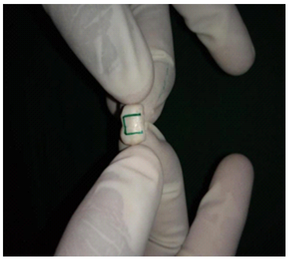

FIGURE 4 : MARKING SAMPLES TO THE RESPECTIVE DIMENSIONS

FIGURE 5 : PLACING SAMPLES IN RESIN MOLD

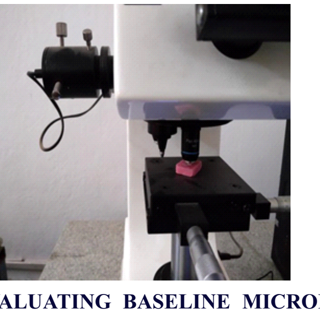

FIGURE 6: EVALUATING BASELINE MICROHARDNESS VALUES

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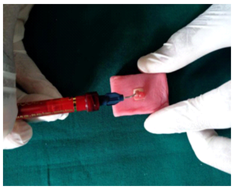

FIGURE 7 : GROUP 2 : ONLY BLEACHING (WITHOUT INCORPORATIONS)

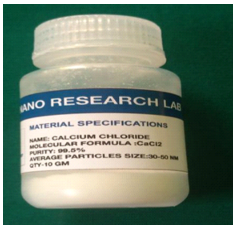

FIGURE 8 : NANO CALCIUM CHLORIDE

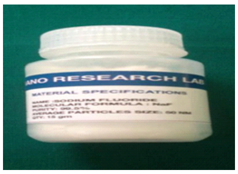

FIGURE 9 : NANO SODIUM FLUORIDE

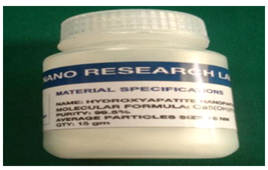

FIGURE 10 :NANO HYDROXYAPATITE

FIGURE 11 : INCORPORATING RESPECTIVE

Remineralizing Agents(Nano Calcium Chloride ,nano Sodium Fluoride Nano Hydroxyapatite) With Hydrogen Peroxide 40%

FIGURE 12 : APPLYING MIXED BLEACHING AGENT ON ENAMEL SAMPLE

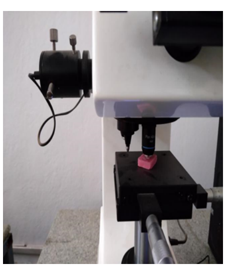

FIGURE : 13 : EVALUATING POST BLEACHING MICRO HARDNESS VALUES

MICROHARDNESS CHANGE OBSERVED AFTER ANOVA AND POST HOC TEST IN BASELINE AND POST BLEA CH ING VALUES

GROUPS	MH [B]	MH [F]	MICROHARDNESS CHANGE
GROUP 1 [CONTROL]	321.46	319.86	1.61 ± 1.37
GROUP 2 [HP 40 %]	327.07	246.82	80.24 ± 18.44
GROUP 3 [HP 40 % + NANO CALCIUM CHLORIDE]	320.36	240.87	34.49 ± 11.45
GROUP 4 [HP 40 % + NANO SODIUM FLOURIDE]	319.49	297.13	25.36 ± 16.51
GROUP 5 [HP 40 % + NANO HYDROXYAPATITIE	323.52	303.22	20.30 ± 15.49

DISCUSSION :

Enamel is the superficial and highly mineralized tissue on the tooth structure forming a very hard, thin, translucent layer of calcified tissue that covers the entire anatomic aspects of crown .[11] Its composition mainly constitutes of 96%mineral, 3% water, and 1% organic matter by weight. [11]The outermost layer of enamel is rich in mineralized content therefore, it is more resistant to demineralization .Enamel thickness and hardness varies from tooth to tooth and from person to person . [11]

Many studies in the literature have investigated the effects of bleaching on enamel morphology and the surface texture . Bleaching resulting in increased porosity , demineralization , decreased protein concentration, organic matrix degradation, modification in the calcium: phosphate ratio, and calcium loss thereby supporting the hypothesis that bleaching agents are chemically active components potentially able to induce substantial structural alterations in human dental enamel . [12]

In the present study, baseline and post bleaching microhardness values are evaluated for all smaples and in order to minimize variations among teeth, the superficial layer of enamel samples were minimally abradred and polished to achieve a uniform mirror-like surface that is considered an appropriate surface for microhardness test. [10]

Based on the results, the use of each one of the three remineralizing agents in nano composition including sodium fluoride ,calcium chloride , and hydroxyapatitie during bleaching procedures with 40% hydrogen peoxide resulted in higher mean enamel microhardness values, compared to the Group 2 without any remineralizing agents. This result was significant for Group 5, in which hydroxyapatite was incorporated into the bleaching gel, resulting in sustaining the microhardness compared to group 2, 3 & 4 (only bleaching , nano calcium chloride ,nano sodium fluoride) . This might be attributed to the fact that the microporosities formed on the subsurface enamel due to bleaching gives rise to the areas conducive to re-deposition of these materials similar to that taking place in arrested caries .[10]

while bleaching along with incorporation of remineralizing agents it is proved that ionic exchange is facilitated, resulting in greater absorption of minerals to replace those lost during bleaching.[10] Two previous studies have suggested that the combined use of Bioactive glass , CPP-ACP, and bleaching agents did not interfere in tooth whitening efficacy.In addition, incorporation of hydroxyapatite into toothpastes resulted in a marked increase in tooth whitening.[10] In the present study, three remineralizing agents were used in nano sizes. An increase in remineralization effect has been reported when the particle size is reduced down to the nano-metric levels because the interaction between the nanoparticles and dentin and enamel becomes more effective due to an increase in surface-to-volume ratio.[10]

We used three different remineralizing agents during bleaching procedure in this study and among the three , hydroxyapatite might serve as a promising biomimetic adjunct in bleaching procedures to prevent/restore enamel damage induced by bleaching agents. Many studies are avaialable in literature concluding that the hydroxyapatite acts as efficient remineralizing agent . [12]

Nano sodium fluoride used as remineralizing agent in this study because the fluoride content acts a main component which get incorporated into the hydroxyapatite crystals of enamel and convert it into flourapatite which protects against demineralization and coverts enamel into less soluble medium. [13]

AB borges et al conducted a study on incorporating sodium flouride in bleaching gel and concluded that bleaching with an only acidic agent resulted in a significant lowering of enamel microhardness compared to the control group; bleached enamel micro hardness was enhanced with the use of remineralizinggel . [14]

Nano calcium chloride used as another biomaterial in this study is a essential component available in tooth paste acts as a remineralizing agent . calcium chloride is a water soluble salt which is safe to use in oral cavity and mainly it contains calcium ions which aids in remineralization of tooth structure subsequent to demineralization .[15] vanessa cavelli et al stated that even though experimental bleaching agents with Ca or F reduced mineral loss for both sound and demineralized enamel surfaces, these agents were unable to reverse the enamel subsurface demineralization . [8]

Hydroxyapatite is a calcium phosphate similar to the human hard tissues in morphology and composition. Particularly, it has a hexagonal structure and a stoichiometric Ca/P ratio of 1.67, which is identical to bone apatite .Important characteristic of hydroxyapatite is its stability when compared to other calcium phosphates and thermodynamically, hydroxyapatite is the most stable calcium phosphate compound under physiological conditions as temperature, pH and composition of the body fluids.[16]

Nano-hydroxyapatite is attracting interest as a biomaterial for use in remineralizing applications due to its similarity in size, crystallography and chemical composition with human hard tissue outstanding properties Biocompatibility, Bioactivity, Osteoc on ductivity[16].

Main reason for efficient remineralizing activity of hydroxyapatite in this study is when it is incorporate with hydrogen peroxide , it increases the pH of hydrogen peroxide solution from 3.2 to 5.4, making it less acidic. In addition, even adherence of hydroxyapatite particles to the enamel surface resulted in the formation of a protective layer for the underlying enamel, decreasing the direct contact of hydrogen peroxide with enamel surface. Furthermore, the solution around the enamel surface might soon become supersaturated with respect to enamel apatite. All these effects of hydroxyapatite might give rise to more significant decrease in enamel demineralization induced by hydrogen peroxide .[10] Hydroxyapatite facilitates the bleaching procedure by promoting the release of free radicals from hydrogen peroxide .Therefore, hydroxyapatite nanoparticles incorporated into hydrogen peroxide might improve the biocompatibility of the final product and increase the safety of the bleaching processs when compared with other two agents .[10] Recnetly a study conducted by the use of 6% hydrogen peroxide with 2% nano-hydroxyapatite resulted in significantly less sensitivity than the bleaching product without nano-hydroxyapatite . In the present study the microhardness change between the Groups 1 and 5 was not significantly different.[17]

In the present study, there was a significant sustainability in microh ardness is noticed in Group 5 (nano hydroxyapatite incorporation in hp 40%) compared with group 2 (only bleaching) . Hydroxyapatite might improve the mineralization [and] decrease the change in microhardness during the bleaching process. However, further studies are necessary to determine the most effective concentration of the material and the incorporation procedure should be evaluated more precisely.

Withing the limitations of the study , It should be emphasized that the study was carried out *in vitro*, and further studies are required to show that remineralizing agents can prevent a decrease in microhardness when they are used in association with high concentration bleaching agents.

CONCLUSION :

By this in vitro study it can be concluded that all the three agents (nano sodium flouride ,nano calcium chloride ,nano hydroxyapatite) showed remineralizing capacity in sustaining tooth microhardness Among the three agents nano hydroxyapatite proven as best remineralizing material by creating least amount of change in microhardness.

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