EVALUATION OF THE EFFECT OF REMINERALIZING AGENT ON HOME AND PROFESSIONALLY BLEACHED ENAMEL SURFACES SUBJECTED TO EROSION- AN *IN VITRO* STUDY

By

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled "Evaluation of the Effect of Remineralizing Agent on Home and Professionally Bleached Enamel Surfaces Subjected to Erosion - An *In Vitro S*tudy" is a bonafide and genuine research work carried out by me under the guidance of **Prof. Dr. Robin Theruvil**, Department of Conservative Dentistry and Endodontics, St Gregorios Dental College, Chelad, Kothamangalam.

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ABSTRACT

Tooth bleaching improves the appearance of discoloured teeth. Unfortunately, the use of acidic food can lead to increased erosion after bleaching. A remineralizing treatment with a remineralizing agent is capable of re-establishing the lost enamel surface hardness after bleaching. In this study, changes in micro hardness and surface roughness were analysed upon the application of remineralizing agent after subjecting the enamel surface to bleaching and erosive challenge.

Aim:

To evaluate the effect of remineralizing agent on bleached enamel surfaces subjected to erosive challenge.

Materials used

1) 10% Carbamide peroxide (Opalescence, Ultradent)-bleaching agent

2) 35% carbamide peroxide (Opalescence, Ultradent)-bleaching agent

3) Remin Pro –Remineralizing agent

4) 1% citric acid solution-Erosive agent

Methodology:

The sample size was calculated using statistical package G*power (3.1.5) with a level of significance of 0.05. The sample size obtained was n=120.

The 120 Samples selected were divided into 6 Groups. Each group had 20 samples. Given below are the treatments that each Group was subjected to.

Group 1 - Treated with 10% Carbamide peroxide and then treated with 1% citric acid solution.

Group 2 - Treated with 10% carbamide peroxide and a remineralizing agent followed by 1% citric acid solution.

Group 3 - Treated with 35% Carbamide peroxide and then treated with 1% citric acid solution.

Group 4 - Treated with 35% carbamide peroxide and a remineralizing agent followed by 1% citric acid solution.

Group 5 - (Negative Control) without bleaching.

Group 6 - (Positive Control) without bleaching with 1% citric acid solution.

G1, G2, G3, G4, G5, G6 were subjected to Vickers micro hardness analysis. Initial micro hardness was calculated. Microhardness after bleaching in G1, G2, G3 and G4 were calculated. Microhardness after erosion in G1, G2, G3, G4 and G6 were calculated. Loss of surface hardness loss (% SHL) after bleaching and after erosion was analysed. Surface profilometry analysis and SEM analysis were done in G1, G2, G3, G4, G5 and G6.

Results:

After the samples were subjected to bleaching, it was observed that the % SHL value was significantly higher in G3 in comparison with the other groups. After the erosive challenge, an increase of the % SHL values was observed in all the groups. G2 showed the lowest % SHL after the erosive phase when compared with G1, G3, G4 and G6. The profilometry analysis results showed that all the groups of treatment (G1, G2, G3 and G4) and G6 did not differ statistically between themselves but differed from G5. The images captured by Scanning Electron Microscopy revealed that there were morphological alterations in the superficial structure of enamel resulting from erosion either associated with the bleaching treatment, with or without a remineralizing agent as well as the erosive challenge. The eroded areas were less visible in G2 compared with the other groups because of prominent interprismatic structures. Impact of erosion after bleaching was calculated from the hardness after the bleaching phase with the hardness after the erosion phase. The real impact of erosion was observed to be higher in G3 (% SHL 63.21) and G4 (% SHL 62.7), which were different from themselves and from the other groups.

Conclusion:

Application of remineralizing agent post bleaching helped to decrease the effect of erosion that occurred as a consequence of bleaching.

Keywords:

Tooth bleaching, remineralizing agent, erosion, surface hardness loss

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INTRODUCTION

Aesthetics gained considerable importance in Dentistry and in our society in the past few years. People have become increasingly aware and are keen on maintaining an aesthetic appearance in addition to maintaining a healthy environmental condition. As put forth by Arens **"Aesthetics, the science of beauty is that particular detail of an animate or inanimate object that makes it appealing to the eye".**[1]

Discoloured teeth are frequently one of the main complaints in the dental office. It is a major challenge to dentists nowadays. It can be limited to one tooth in an arch or to many teeth. There are several options for managing discoloured teeth. Scaling and polishing of the teeth can improve the situation in some conditions. But more extensive procedures are needed for achieving good results in most cases. Treatment options in these conditions include vital and non-vital bleaching, micro abrasion, composite, porcelain veneers and porcelain crowns.[2]

The public are influenced by the portrayal of perfect smiles through various Medias. Colour quality of electronic and print media raised self-awareness in people. By the introduction of home based and professionally applied tooth whitening products, people have become more aware in wanting a perfect white smile.[3]

Night guard vital bleaching (NGVB) was introduced in 1989. This technique involves the use of 10% carbamide peroxide applied in a custom mouth guard for a certain number of hours per day or night for 2 to 6 weeks. 10% carbamide peroxide can readily be used safely. Under professional supervision, NGVB is safe as any other routinely used dental treatment.

The advantage of mouth guard bleaching over in-office bleaching is in its ability to bleach the posterior most teeth also in the arch. Dentin and enamel can change colour as a result of easy passage of peroxide and urea through the teeth. Heavy nicotine stains and tetracycline stains can be removed through night guard vital bleaching.[4]

Many studies have further proven the efficiency and safety of home bleaching techniques. This fact encouraged the manufacturers to produce and market higher concentrations of at-home bleaching agents that can range up to 35% carbamide peroxide to be used for shorter periods of time. It increases the patient comfort as it can be used for up to 30 minutes for effective bleaching when compared to 8 hours of night guard bleaching.[5]

Studies have shown that similar colour change was recorded using higher and lower concentrations of carbamide peroxide.[6] Studies have shown that higher concentrations of bleaching agents may jeopardize the enamel and dentin structures.[7] These lead to a major concern for dentists as well as patients with regard to vital bleaching.

Researchers have found that bleaching treatment may be associated with changes in enamel microhardness, decrease in calcium and phosphorous concentrations and with increasing the surface roughness.[8,9] There are a lot of controversies associated with regard to different concentrations of bleaching gels on enamel surface.[6,10]

Many researchers have reported that longer the contact time of bleaching agents with lower concentration such as 10% carbamide peroxide, may negatively affect the enamel surface. Others have proposed that bleaching with higher concentrations such as 35% carbamide peroxide produced more intense side effects than 10% carbamide peroxide.[11]

In order to overcome the potential side effects with the use of bleaching agents, there have been several attempts that have been put forth by many researchers such as adding minerals to bleaching agents e.g. tin oxide and calcium ions.[12]

Several researchers have also investigated the use of remineralizing pastes such as MI paste containing CPP-ACP (Casein Phosphopeptide-amorphous calcium phosphate) on bleached enamel surfaces. They found that post-operative application of remineralizing tooth paste for 2 weeks could decrease the side effects related to bleaching agents.[13, 14]

Major concern with regards to aesthetics has led people to over use bleaching agents. In addition, the increased intake of acidic food and beverages after bleaching cause more dental erosion.[15]

Bleaching can lead to decrease in microhardness and mineral loss of enamel leading to bleached surface becoming more susceptible to acid erosion. Erosion is loss of enamel surface due to acidic chemicals that are non-bacterial in origin. This became more pronounced after bleaching. [16, 17]

Studies have shown that bleaching performed with low concentrations such as 10% carbamide peroxide, did neither increase the enamel surface roughness nor made the tooth more susceptible to erosion. [18, 19] There are a few studies which determine the effects of different concentrations of bleaching agents on enamel susceptibility to erosion after bleaching. However, there is lack of studies that determine the effects of remineralizing agents on bleached enamel surfaces that are subjected to erosive challenges after bleaching. Dental surface associated with erosive challenges that simulate the action of acidic substances present in food and beverages available for human consumption are evaluated by this study.

Enamel surface microhardness tests were used as parameters to evaluate the mineral alteration followed by bleaching. Studies have reported that microhardness test perfectly detect the demineralization or remineralization of a substrate, thus providing indirect evidence of mineral loss or gain in enamel.[20]

Studies have also confirmed that the use of microhardness for analysing erosion should be complemented with profilometry to quantify the tissue lost from the eroded area. [21]

The images from the Scanning Electron Microscopy can be used as a measure to determine the difference in the morphology of dental enamel after erosion as per earlier studies.[15]

Hence the present study was done to evaluate and compare the changes in surface microhardness and surface roughness upon the application of remineralizing agent after subjecting the enamel surface to bleaching and erosive challenge.

AIM AND OBJECTIVES

AIM

To evaluate the effect of remineralizing agent on professionally applied and home bleached enamel surfaces subjected to erosive challenge.

OBJECTIVES

- To compare the effect of erosion on home and professionally bleached enamel surface.

- To evaluate the effect of remineralizing agent on the micro hardness and surface roughness on home and professionally bleached enamel surface when subjected to erosion.

REVIEW OF LITERATURE & BACK GROUND

Fitch CP et al (1861)[22] reported that the practitioner must identify the type of discolouration, diagnose the cause and then define the appropriate treatment plan. Tooth bleaching is not a new technique in dentistry. It was reported more than a century ago.[22]

Ames et al (1937)[23] did a technique using a mixture of hydrogen peroxide and ethyl ether on cotton and then heated with a metal instrument for 30 minutes and applied over 5 to 25 visits to treat mottled enamel.[23]

McInnes J et al[24] in the year 1966 combined the use of hydrochloric acid and hydrogen peroxide which was promoted to remove brown stains from mottled teeth.[24]

Nutting EB, Poe GS et al. (1967)[25] introduced the walking bleach technique, in that they used 35% hydrogen peroxide and sodium perborate for whitening nonvital teeth. In 1968, Klusmeier developed a technique using Gly-Oxide a 10% carbamide peroxide oral antiseptic, which he placed in the orthodontic positioners of some patients to improve gingival health. He noted that it whitened teeth and improved the tissue as a result. He switched to Proxigel, which also contained 10% carbamide peroxide, in a custom-fitted night guard in 1972 because the viscosity of the Proxigel allowed it to stay in the tray. [25]

Cohen S, Parkins FM et al (1970)[26] published a procedure for whitening tetracycline discoloured dentin of the teeth of young adults treated for cystic fibrosis. It was the first publication indicating that there is a chemical penetration of hydrogen peroxide to the dentin to whiten teeth.[26].

Berman LH (1982)[27] reported that there are varieties of methods which have been put forth for the management of discolouration and it can vary depending upon the underlying etiological factors. Accordingly, professional cleaning by scaling followed by polishing to remove extrinsic stains, micro abrasion of enamel, internal bleaching for non-vital discoloured teeth and external bleaching for vital teeth are the alternatives.[27]

Adams et al (1987)[28] reported the use of hypochloric acid to treat fluorosis.[28]

Haywood VB, Heymann HO (1989)[29] investigated that home bleaching otherwise known as Night guard vital bleaching typically uses a relatively low level of bleaching agent applied to the teeth via a custom fabricated mouth guard and is worn at night for at least 2 weeks. Wearing time varies according to the concentration of bleaching agents. He published the first clinical study on tooth whitening using Proxigel in vacuum-formed custom trays. This technique known as ‘‘night guard vital bleaching’’ is in common use today. Haywood and Heymann conducted laboratory and clinical investigations of this technique in 1989. They reported night guard vital bleaching using 10% carbamide peroxide.[29]

Night guard vital bleaching was custom fabricated to hold the whitening gel in contact with the enamel surface. Currently available peroxide containing tooth whitening materials include professionally dispensed and supervised products for use by patients at home, professional use in-office products and over-the-counter products for sale directly to consumers.[29]

Darnell DH et al (1990)[30] reported that the first commercially available 10% carbamide peroxide which was developed and subsequently marketed by Omni International in 1989 was based on the findings of Munro who used a 10% carbamide peroxide solution to control inflammation after root planing in a vacuum-formed plastic splint.[30]

Bitter NC et al[31] in the year 1992 reported that tooth whitening has an effect on enamel and dentin. It was found that whitening agents were capable of removing the smear layer from dentin, but produced little or no change in enamel. Additional studies concluded the same result in enamel but others have shown changes in porosity and surface morphology of enamel.[31]

Gegauff AG, Rosenstiel SF, Langout KJ, Johnson WM (1993)[32] concluded that hydrogen peroxide (H_2O_2) was the active ingredient in most of the commonly used whitening products and was delivered either as hydrogen peroxide or Carbamide peroxide.[32]

Patzer GL et al (1994)[33] reported that aesthetics of the teeth is of great importance to many patients. Public demand for tooth whitening has increased in recent years.

Patient interest in whitening and articles on whitening in popular magazines suggest that tooth colour is a significant factor in the attractiveness of a smile. An attractive smile plays a major role in the overall perception of physical attractiveness.[33]

Haywood VB et al (1994)[34] conducted numerous studies assessing the safety of hydrogen peroxide and carbamide peroxide for tooth whitening. It was indicated that 10% carbamide peroxide, which is equivalent to 3.6% hydrogen peroxide, is safe when applied in the night guard vital bleaching techniques.[34]

Joiner A et al[35] in the year 1995 investigated that successful whitening treatment depends on the correct diagnosis by the practitioner regarding the type, intensity and location of tooth discolouration. It is imperative to determine if the discolouration is extrinsic, which is associated with the absorption of materials such as tea, red wine, certain medications, iron salts, tobacco and foods, onto the surface of the enamel and in particular, the pellicle coating or intrinsic, where the tooth colour is associated with the light-scattering and absorption properties of the enamel and dentin such as seen in tetracycline staining, amelogenesis and dentinogenesis imperfecta, hypoplasia, erythroblastosis fetalis and porphyria. Additionally, discolouration can also result from aging process. As teeth ages, more secondary dentin is formed and more translucent enamel layer thins. The combination of less enamel and darker, opaque dentin creates an older-looking, darker tooth.[35]

Josey AL, Meyers IA, Romaniuk K, Symons AL (1996)[36] investigated that morphological changes have been observed in the enamel with erosion, craters and porosity being reported by various authors.[36]

McCracken MS, Haywood VB (1996)[37] reported that 35% Carbamide peroxide is recommended for daily use for 30 minutes. There is no noticeable difference in sensitivity, using either a hydrogen peroxide or Carbamide peroxide product. Products with Carbamide peroxide have a slightly longer shelf life than those with hydrogen peroxide, although when refrigerated, all peroxide products have an increased shelf life.[37]

Dunn J et al[38] in the year 1998 confirmed the importance of attractiveness on perceived success and self-esteem.[38]

Barghi N et al (1998)[39] in their study concluded that compared with restorative treatment modalities, tooth bleaching, is the most conservative treatment for discoloured teeth. Public demand for a whiter smile and improved aesthetics has made tooth whitening a popular and often requested dental procedure, since it offers a conservative treatment option for discoloured teeth. Whitening often enhances the treatment and encourages patients to seek further aesthetic treatment.[39]

Mokhlis GR, Matis BA, Cochran MA, Eckert GJ(2000)[40] concluded that reduced tooth sensitivity can be expected using carbamide peroxide since lower concentration of hydrogen peroxide is available. Among the products available in the market, carbamide peroxide-based whiteners with concentrations as high as 35% are preferable for at-home bleaching procedures. Theoretically, a 35% Carbamide peroxide bleaching agent has the same bleaching effect as another agent with approximately 12% hydrogen peroxide that could be used in-office with reduced chair-time.[40]

Watts A, M. Addy (2001)[1] investigated that the colour of the tooth can vary depending on the quality of the incident light and the amount of reflected light. Enamel is translucent and hence any alteration in the underlying dentin and pulp can change the external appearance of the tooth. Tooth discolouration occurs due to extrinsic, intrinsic or internalised stains. Extrinsic tooth discolouration is caused either due to the incorporation of polyphenolic compounds that provide colour to the food into the pellicle causing a change in appearance or due to a chemical interaction of the chromogens with the tooth structure.[1]

There are many local and systemic factors responsible for intrinsic staining. Systemic factors can be due to either systemic diseases or drug induced systemic diseases like alkaptonuria, congenital erythropoietic porphyria, congenital hyperbilirubinaemia, amelogenesis imperfecta or dentinogenesis imperfecta. Drug which causes discolouration includes tetracycline and ciprofloxacin. Fluorosis of the tooth can also result in intrinsic discolouration. Local factors responsible for tooth discolouration include pulpal haemorrhagic products, root resorption and ageing.[1] Causes of internalised discolouration are extrinsic stain incorporation into the tooth during developmental stages. Many developmental defects like enamel hypoplasia and acquired defects like tooth wear and gingival recession cause internalised discolouration.[1]

David C. Sarrett et al (2002)[41] reported that tetracycline staining had proved to be difficult or impossible to remove through traditional bleaching methods. Recent report indicated that using 10 percent carbamide peroxide in custom trays for three to six months can be effective in removing tetracycline staining. The adverse effects of long-term bleaching are not well established; however, if the only alternative to bleaching is restoring the teeth with ceramic veneers or crowns, it seems reasonable to attempt bleaching first. According to various authors, hydrogen peroxide whitens teeth and was safe with minor side effects when compared with carbamide peroxide which was less effective than hydrogen peroxide but had fewer side effects.[41]

Roberta et al (2003)[42] compared the microhardness of enamel after bleaching with seven different bleaching agents containing CP at different bleaching times. Authors analysed seven bleaching agents and a placebo. The agents were applied on the surface of human dental fragments for 8 hours per day for 42 days. Microhardness of each group was tested at baseline, 8 hours on 7, 14, 21, 28, 35 and 42 days preoperatively and 7 and 14 days postoperatively. The results showed that the microhardness values decreased from the baseline with the use of different concentrations of bleaching agents and placebo. They concluded that CP with higher concentration of fluoride can bleach tooth in a shorter period with lesser detrimental effect on enamel mineral content.[42]

Cavalli et al (2004)[43] evaluated the enamel surface roughness, surface staining and morphology after in-office bleaching using high concentrations of CP gels. 30 flat enamel samples were chosen and carbamide peroxide at a concentration of 35% and 37% were selected as bleaching agents along with untreated enamel as control group. The bleaching agent was applied on the respective group for 30 minutes which was repeated 4 times with 72-hour interval between individual applications. The samples were kept in artificial saliva for the remaining hours. Profilometer was used to test the surface microhardness which was calculated from the mean value of 3 readings taken at 3 different directions. Staining test was done for 8 samples per group by treating with 2% methylene blue and later ground into powder and treated with absolute alcohol. The other 2 samples from each group were prepared for SEM evaluation.

From the observations, the authors concluded that the 35% CP produced greater surface roughness on the enamel. Unbleached enamel surface was more stain resistant and there was no statistical difference between the bleaching agents tested with regard to staining characteristics and morphological changes.[43]

Cristiane Franco Pinto et al (2004)[44] investigated the effect of bleaching on enamel surface roughness, microhardness and morphology. 43 molars were selected for the studies which were sectioned into two equal halves. Seventy-seven samples were selected from the 86 which were then divided into 7 equal groups including the control group (n=11). The samples were treated with different bleaching agents containing CP at concentrations of 10%, 35% and 37% and HP at concentrations of 7.5% and 35% onto the respective samples at specified time interval. After the specified time period, the samples were subjected to microhardness testing to evaluate the Knoop hardness. The surface roughness was determined with profilometer in which reading was taken at 3 different directions. SEM evaluation was done to determine the changes in the enamel structure. The alterations were classified as no alterations, mild or slight alterations and altered surfaces. From the results they concluded that every bleaching procedure causes a significant decrease in microhardness and significant increase in surface roughness. In this study the alterations were higher for groups treated with 35% HP. [44]

Sulieman M (2004)[45] concluded that tooth whitening lightens the colour of a tooth. Whitening of teeth can be achieved by physical removal of the stain or a chemical reaction. Chemical degradation of the chromogens within or on the tooth is called as bleaching. Bleaching of vital teeth can be done by different methods depending on the bleaching agent used, their concentration, mode and time of application and the sources used for activation. There are three fundamental bleaching approaches, namely in-office or power bleaching, mass market bleaching products and dentist supervised night guard bleaching. Over usage of bleaching agents can cause many deleterious effects on teeth. Over usage of bleaching with high concentrations of hydrogen peroxide office-based products cause enamel softening, surface roughness and an increase in the susceptibility of the tooth to demineralization, based on *in vitro* findings. Indiscriminate use of these whitening agents may negatively affect the hard and soft tissue of the oral cavity, causing morphological changes on the tooth surface such as pores, erosion and increase in surface roughness, mineral alterations and reduction in microhardness. These effects may be related to the composition and concentration of the agent, instructions for use of the product, exposure time and pH values.[45]

Neslihan et al (2005)[46] investigated the demineralisation effect of 10% CP containing bleaching agent on enamel and dentin using micro CT. 6 upper second molars were selected and 12 tooth rods of specified dimension were prepared from the buccal one third of the crown of each tooth. The samples were stored in a cell culture well with artificial saliva to mimic the oral environmental conditions. The bleaching gel containing 10% CP were applied onto the enamel surface for 8 hours and incubated in artificial saliva for the remaining 16 hours. The bleaching cycle was continued for 15 consecutive days. The micro CT measurement was done to evaluate the change in mineral content before and after bleaching procedure. From the results, the authors concluded that in vitro application of CP on enamel for two weeks caused demineralization of the enamel extending to a depth of 50µm below the enamel surface.[46]

Carolina Baptista Miranda et al (2005)[47] did SEM evaluation of human enamel bleached with 35% CP and 35% light activated HP at varying time intervals simulating in-office bleaching procedure. 20 third molars were selected and decoronated at the level of CEJ and the crowns were divided into buccal and lingual segments. The buccal segments were selected for bleaching and were divided into 4 groups with 5 samples in each including the control group. Group 1 served as the control group. Group 2 and 3 received 35% CP application for 30 minutes and 2 hours which was repeated weekly for 4 weeks. Group 4 received two 20 minutes application of the bleaching agent with a total of 14 minutes light activation. SEM evaluation was done to evaluate the changes in the surface characteristics. Results showed severe alterations in enamel surface characteristics with depression and crater formation and exposure of enamel prisms with loss of aprismatic layer irrespective of the bleaching agent used. Authors concluded that in-office treatment with higher concentration of bleaching agent can adversely affect the enamel surface properties. [47]

Christensen GJ et al[3] in the year 2005 stated that public was influenced by the portrayal of perfect white smiles in the media. Colour quality of television, movies, electronic and print media had raised self-awareness of discoloured teeth. In the late 1980‘s, several companies introduced both home-based products and professionally applied tooth whitening products into the U.S. market place. These have gained popularity with the public who demand whiter, more perfect smiles.[3]

Pretty IA, Edgar WM, Higham SM (2005)[21] reported that nowadays tooth bleaching is one of the most popular cosmetic dental treatments which help to improve the appearance of discoloured teeth. Mainly vital tooth bleaching is used for the removal of extrinsic stains caused by tea, coffee and tobacco as well as for the reduction of intrinsic stains within the dentin. Some of these studies have found that aggressive whitening treatments can change the surface integrity, microstructure of enamel crystals and susceptibility to demineralization. The mechanism suggested for the surface demineralization is that during the bleaching procedure the pH of the whitening agent becomes more acidic and the hydrogen ions attack the enamel crystals, freeing calcium and phosphate ions from the enamel surface.[21]

Mandel L. (2005)[48] investigated that the bleaching gel typically contained between 10% and 44% Carbamide peroxide, which was roughly equivalent to 3% to 16% hydrogen peroxide concentration. 35% Carbamide peroxide resulted in significant improvement in tooth colour. Studies have shown that Carbamide peroxide appeared to produce slightly more dramatic results at first, ultimately products containing equivalent amounts of Carbamide peroxide and hydrogen peroxide produced exactly the same results.[48]

Joiner A. (2006)[49] concluded that higher concentrations of bleaching products produced greater surface roughness on the enamel. It caused a significant decrease in microhardness and significant increase in surface roughness. SEM evaluation showed severe alterations in enamel surface characteristics with depression and crater formation and exposure of enamel prisms with loss of aprismatic layer irrespective of the bleaching agent used. Authors concluded that in-office treatment with higher concentrations of bleaching agent could adversely affect the enamel surface properties. The degradation of high molecular weight organic substances that reflect a specific wavelength of light into low molecular weightless complex structures that reflect less light help in tooth whitening. Colour producing materials on a surface were mainly organic compounds that possessed extended conjugated chains of alternating single or double bonds and often included hetero atoms, carbonyl and phenyl rings in the conjugated system, often referred to as chromophore.[49]

Fearon J. (2007)[50] concluded that when compared with home bleaching, in office bleaching had greater advantages in terms of clinician control, quick whitening results, reduced treatment time and avoidance of material ingestion and discomfort from wearing trays. Today’s dentists have an array of treatment options to harmonize a smile with discoloured vital teeth. Wide variety of treatment ranges from invasive options such as crowns, veneers and placement of direct restorations to minimally invasive therapies such as macro abrasion, micro abrasion and bleaching to mere oral prophylaxis.[50]

Braun A, Jepsen S, Krause F (2007)[51] concluded that bleaching with carbamide peroxide was a well-accepted treatment modality for discoloured teeth. The active ingredient in most whitening products is hydrogen peroxide (H_2O_2) which is delivered as hydrogen peroxide or carbamide peroxide. Carbamide peroxide is a stable complex that broke down in contact with water to release hydrogen peroxide. As carbamide peroxide released hydrogen peroxide, the chemistry of most tooth whitening is that of hydrogen peroxide.[51]

Amparo et al (2007)[52] evaluated the effects of two bleaching products containing 10% CP and 3.5% HP on the enamel by means of an in vivo study. 20 patients were selected and divided into 2 equal groups based on the bleaching agents used. Bleaching agent was applied for 2 hours daily using application tray with reservoir for 2 hours and 3 hours in CP group and HP group respectively. The bleaching process was continued for 28 - 33 days. Replicas were taken before and after treatment and were analysed in SEM. The results showed that neither of the products negatively affected the enamel surface and there was normal perikymata pattern and the authors concluded that low concentrations of bleaching gel can cause significant alteration in the shade without affecting the morphological characteristics of enamel.[52]

Thomas Attin et al (2007)[53] concluded that external bleaching by application of carbamide peroxide containing agents was a popular method to whiten discoloured teeth. It is debated whether carbamide peroxide or peroxide containing agents might induce a negative impact on dental hard tissues. Studies found that surface softening, mineral loss, increased susceptibility to erosion or caries, reduced fracture stability or a decrease in abrasion resistance of bleached dental hard tissues occured after bleaching. On the other hand, there are other studies, which did not confirm these observations. It was shown that the loss of microhardness in bleached enamel could be reduced when fluoride compounds were administered after the bleaching steps or during the bleaching period.[53]

Kihn PW. (2007)[54] concluded that carbamide peroxide was commonly used for at-home bleaching using a tray-based technique and dissociated into hydrogen peroxide (approximately a third of its former concentration) and urea, which further broke down into water and ammonia.[54]

Faraoni-Romano JJ, Turssi CP, Serra MC. (2007)[20] reported that the results obtained with microhardness tests perfectly detect the demineralization or remineralization of a substrate, thus providing indirect evidence of mineral loss or gain in human teeth.[20]

Wiegand A, Drebenstedt S, Roos M, Attin T et al (2008)[55] investigated that the colour change of enamel, dentine and combined enamel and dentin of bleached tooth samples found that the colour was not stable over time with regard to lightness. However, yellowness did not return to baseline within 1 year.[55]

Tay LY, Kose C, Loguercio AD, Reis A et al[56] in the year 2009 reported that in recent years, tooth discolouration had become a common cosmetic complaint. A large number of patients requested dental treatment for tooth whitening procedures. In-office bleaching and dentist-prescribed home-applied bleaching were the two most commonly utilised whitening procedures. When compared with home bleaching, however, in-office bleaching had advantages in terms of clinician control, quick whitening results, reduced treatment time and avoidance of material ingestion and discomfort from wearing trays. [56]

Shereen S. Azer et al (2009)[57] reported that enamel surface hardness was not adversely affected by bleaching with 10% carbamide peroxide solutions or when over bleached for 70 hours (fivefold) in excess of clinical recommendations. Seghi and Denry, however, reported that although the surface hardness was not affected, enamel fracture toughness was reduced by 30% after 12 hours bleaching. Cavalli et al noticed that the use of higher concentrations of carbamide peroxide (35 and 37%) altered the enamel and produced surface roughness.[57]

Similarly, several scanning electron microscopic studies confirmed the enamel surface roughness resulting from bleaching and also the formation of surface porosity with more susceptibility for dissolution by phosphoric acid etching. In contrast to earlier hardness studies, recent investigations on enamel characteristics following bleaching have shown reduction in enamel microhardness, modulus of elasticity, surface toughness, as well as shear bond strength to composite resin. Enamel microhardness was noticeably affected immediately after in-office bleaching (more than home bleaching) using high concentrations of carbamide peroxide, however, baseline hardness values were restored after fluoride application.[57]

Previous investigations have reported that the hardness of enamel was not affected after being treated with whitening agents containing 1.5–7.5% hydrogen peroxide, or 10–20% carbamide peroxide. In contrast, however, other studies reported that whitening products containing hydrogen peroxide at concentrations of 5.3–35%, or 10–37% carbamide peroxide reduced enamel micro hardness and induced surface alterations, in addition to causing more surface susceptibility to dissolution by phosphoric acid etching compared to non-bleached enamel.[57]

Souza RO et al (2010)[10] reported that physical and chemical changes of enamel surface after vital teeth bleaching are of a major concern to dentists as well as patients. Researchers have proposed that bleaching treatments may be associated with changes in enamel microhardness, decrease in calcium and phosphorus concentrations and increasing of surface roughness. However, controversial results were published concerning the influence of different concentrations of bleaching agents on enamel surface. Some researchers have reported that longer contact time of bleaching gels even with low peroxide concentration such as 10% CP may negatively affect the enamel surface.[10]

Goldberg M, Grootveld M, Lynch E et al (2010)[58] reported that risks commonly associated with tooth whitening include increased tooth sensitivity and mild gingival irritation. Degree of these side effects is directly related to the concentration of the peroxide bleach component, duration of the treatment and the non-bleach composition of the product used. Tooth sensitivity usually occurs at the time of treatment and can last several days; gingival irritation begins within a day of the treatment and can also last several days. There are many risks that have been reported from *in vitro* studies which include tooth erosion, tooth mineral degradation, increased susceptibility to demineralization and pulpal damage. The ultimate endpoint for tooth whitening is dependent upon the tooth itself, with common wisdom telling us that all treatment regimens will eventually arrive at the same whitening endpoint. This was not exactly true as some very aggressive regimens can damage the tooth through dehydration and demineralization such that the tooth temporarily appears whiter.[58]

Abouassi T, Wolkewitz M, Hahn P. (2011)[59] concluded that carbamide peroxide bleaching agents are more preferred for home bleaching procedures nowadays. Carbamide peroxide bleaching gels are more effective than hydrogen peroxide bleaching gels in treating teeth discolouration.[59]

Shi XC, Ma H, Zhou JL, Li W et al (2012)[60] conducted a study on the effect of cold-light activated bleaching treatment on enamel surfaces because there is concern that either heat (infrared) or ultraviolet light exposure of teeth and gingiva during in office bleaching could cause pulp and / or gingival tissue damage.

In that study the crystal and surface microstructure of dental enamel was evaluated after 35% hydrogen peroxide bleaching with cold-light irradiation; 35% hydrogen peroxide bleaching without cold-light irradiation; a control group treated with what is identified as a silica dioxide catalysing agent as a peroxide-free bleaching agent (control group); and a control group treated with the cold-light irradiation alone (control group L). The authors reported that no colour change or enamel crystal size change was observed in the two control groups, indicating that the cold-light treatment alone or the silica dioxide agent had no effect on the tooth structure.

However, the groups that had 35% hydrogen peroxide (with or without cold-light) did result in significant colour change as well as significant decrease in crystal size and crystallinity. There was no significant difference in the degree of colour change between the cold-light activated hydrogen peroxide and the hydrogen peroxide only groups.[60]

Additionally, surface roughness was observed to increase in all groups except the cold-light only control group. The authors concluded that the 35% hydrogen peroxide solution had a major demineralization effect on enamel surface and the cold-light had no significant increase or decrease effect on the demineralization or colour change. The mechanism suggested for the surface demineralization is that during the bleaching procedure the pH of the whitening agent became more acidic and the hydrogen ions attack the enamel crystals, freeing calcium and phosphate ions from the enamel surface.

Several important deductions can be made from this study:

1. Aggressive bleaching can lead to surface demineralization and reduction of enamel crystallinity;

2. Cold-light activation seems to have little effect on colour change or tooth structure;

3. Hydrogen peroxide reduces the pH on the tooth surface during treatment, leading to surface demineralization.

It is difficult to predict the persistence of bleaching regimens because the patient may routinely expose their dentition to food or beverages which are known to stain teeth and would experience re-staining within a month. If the teeth are not exposed to chromogens such as coffee, red wine, cigarette smoke, then it would be reasonable to assume that whitened teeth could persist or up to a year.[60]

Alessandra B. Borges et al (2012)[61] reported that dental bleaching treatments are mainly based on the action of hydrogen peroxide, which is able to penetrate the tooth structure and release free radicals, oxidizing the chromophore molecules. Such molecules are mainly organic, although inorganic molecules can also be affected by these reactions. The free radical reaction is not specific and it may also alter the organic component of enamel. Since the organic content contributes to the integrity of enamel, different adverse effects on both mineral and organic parts of bleached enamel have been observed. Alterations in enamel surface morphology, chemical composition, and microhardness values after bleaching were previously reported.

Some changes in bleached enamel were also described as slight erosive effects promoted by the bleaching agent. Nevertheless, some authors claim that the erosive pattern on the surface of bleached enamel only occurs when bleaching gels with low pH are used.[61]

De Oliveira et al observed that no significant increase of bleached enamel microhardness when calcium and fluoride were added to 10% carbamide peroxide gel. On the other hand, Borges et al observed a significant increase of enamel microhardness after bleaching with 35% hydrogen peroxide agent with the addition of calcium and fluoride. Chen et al also reported a less distinct erosion pattern on the surface of enamel bleached with fluoridated gels. The association between the bleached enamel surface alterations and the subsequent susceptibility to erosive lesions resulting from the contact of bleached enamel with demineralizing solutions has been discussed.[61]

As per previous studies, the application of carbamide peroxide gel rendered enamel more susceptible to demineralization. While in other studies, at-home bleaching technique did not increase the susceptibility of enamel to erosion. Bleaching gels with high concentration of HP could increase the susceptibility of enamel to erosion; the addition of remineralizing ions into bleaching gels would be beneficial for preventing a further enamel demineralization. Considering various study designs, it can be concluded that the application of 35% HP based bleaching agents did not alter the enamel susceptibility to erosion and the addition of calcium to the bleaching gel improved erosion resistance of the bleached enamel.[61]

Da Costa JB, McPharlin R, Hilton T, Ferracane JL, Wang M. (2012)[62] concluded that hydrogen peroxide (H_2O_2) is the active ingredient in most commonly used in whitening products and is delivered as either hydrogen peroxide or Carbamide peroxide.[62]

Li Y, Greenwall et al (2013)[63] concluded that light activation offers no benefits for amount of whitening achieved, persistence of the whitening treatment, or avoidance of tooth sensitivity from the whitening treatment.

Night guard or Home-based bleaching (following manufacturer's instructions) results in less tooth sensitivity than in-office bleaching.

The optimal regimen to obtain persistence of tooth whitening is to follow an in-office treatment with monthly home-based touch-up treatments using over the counter products.

Aggressive bleaching with high concentrations of hydrogen peroxide office-based products causes enamel softening, surface roughness, and an increase in the susceptibility of the tooth to demineralization, based on *in vitro* findings.

Dental restorations are susceptible to unacceptable colour change even when using the home-based over the counter systems.

In-office bleaching using a 35% hydrogen peroxide product caused tooth sensitivity in all cases. Teeth with restorations have a significantly greater chance of becoming sensitive and result in a greater degree of pain when exposed to whitening regimens.[63]

Ogura K, Tanaka R, Shibata Y, Miyazaki T, Hisamitsu H et al (2013)[64] conducted a new *in vitro* study in Japan that evaluated the susceptibility of a tooth to subsequent demineralization after a home based 10% carbamide peroxide bleaching regimen was compared to a more aggressive office-based 35 % hydrogen peroxide photo activated regimen. In the extracted teeth of colour, Vita shade A3 were bleached following manufacturer's instructions until the shade was lightened to Vita shade A2 for all teeth. The teeth were then demineralized for two weeks and evaluated for mineral density loss. This study found that the surface integrity of the home-based treated teeth was altered such that significantly greater demineralization occurred in the home-based treated teeth than in the office-based treated teeth. The authors postulated that the peroxide in the office-based gel penetrates deeper into the enamel before it was activated by photo irradiation.[64]

Bonafé E, Bacovis CL, Iensen S, Loguercio AD, Reis A, Kossatz S et al (2013)[65] conducted a randomized clinical trial which showed that in-office bleaching with 35% hydrogen peroxide product caused tooth sensitivity in all cases. There was significantly greater tooth sensitivity pain for teeth that had restorations than for sound teeth. It was concluded that in-office bleaching with 35 % hydrogen peroxide was effective for patients with restored teeth, however that a higher degree of pain was found for these patients, especially associated with the upper lateral incisors.[65]

Yu H, Li Q, Wang YN, Cheng H. et al (2013)[66] in their study reported that aggressive tooth bleaching can cause increased tooth sensitivity, changes in tooth microstructure, and restoration changes. Aggressive bleaching can chemically react with composite restorations, glass ionomer cements, sealants and ceramic crowns, thus reducing their stability.[66]

Alkhtib A, et al (2013)[10] proposed that post-operative application of CPP-ACP for two weeks on bleached enamel surfaces is able to decrease the bleaching agents related adverse effects; re-establishing the baseline enamel hardness and the reduced modulus of elasticity.[10]

Soares DG et al (2013)[11] proposed that bleaching gels with a relatively higher concentration produced more intense side effects compared with 10% CP.[11]

Tompkins AM, Coleman SS, Carey CM. et al [67]in the year 2014 reported that the risk of tooth structure damage has received attention by researchers who have evaluated the effects of aggressive tooth bleaching treatments on tooth structure and susceptibility to demineralization. These studies have found that aggressive whitening treatments can change the surface integrity, microstructure of enamel crystals, and susceptibility to demineralization. In an *in vitro* study five different bleaching products were tested for erosion of human enamel. The five products included strips at 6.5 % hydrogen peroxide and gels at 10 % and 22 % carbamide peroxide, 35 % hydrogen peroxide and a sodium hypochlorite containing gel system. They followed the manufacturer's instructions and measured the loss of enamel surface by polarized light microscopy. They found that although some surfaces were softened, no enamel erosion was found.[67]

Clifton M. Carey et al[68] (2014) in their study found that there are several different methods for whitening teeth, each with their own mechanism of action. Efficacy of these different methods is dependent upon the particular tooth discoloration that is being treated. Causes of tooth discoloration can be categorized into two groups: intrinsic and extrinsic staining. Intrinsic staining, sometimes called internal staining, can be attributed to factors such as genetics, age, antibiotics, and high levels of fluoride and developmental disorders that can start before the tooth has erupted. After the eruption of the tooth some restorations can cause tooth staining. Extrinsic staining, sometimes called external staining, is largely due to environmental factors including smoking, pigments in beverages and foods, antibiotics and metals such as iron or copper. Coloured compounds from these sources are adsorbed into acquired dental pellicle or directly onto the surface of the tooth causing a stain to appear. Home-based bleaching (following manufacturer's instructions) results in less tooth sensitivity than in-office bleaching. Optimal regimen to obtain persistence of tooth whitening is to follow an in-office treatment with monthly home-based touch-up treatments using OTC products. Aggressive bleaching with high concentrations of hydrogen peroxide office-based products causes enamel softening, surface roughness and an increase in the susceptibility of the tooth to demineralization, based on *in vitro* findings.[68]

Heshmat H, Ganjkar MH, Jaberi S, Fard MJ (2014)[69] concluded that remineralization treatments with a remineralizing agent are capable of re-establishing lost enamel surface hardness and alterations caused by dental bleaching. Bleached tooth treated with remineralization agents have produced a positive effect on enamel morphology and micro hardness recovery. Remin Pro (VOCO, Germany) remineralizing paste contains calcium, phosphate in the hydroxyapatite form along with Fluoride and Xylitol. It promotes remineralization by filling porous areas, forming a protective film on the tooth surface and thereby impeding the adhesion of bacterial plaque.[69]

Alqahtani MQ (2014)[70] concluded that different forms of acids like hydrochloric acid and oxalic acid were used for bleaching procedures in earlier days. The harmful effects of using acids on the tooth structure led to the use of hydrogen peroxide. People are more concerned about their aesthetic appearance due to influences of various Medias. Although tooth bleaching procedures are conservative, effective and cost-effective, like all other treatment modalities, they are associated with some side effects. The risk of tooth structure damage has received considerable attention by researchers who have evaluated the effects of aggressive tooth bleaching treatments on tooth structure and their susceptibility to demineralization.[70]

Thakur R et al[7] in the year 2015 reported that that higher concentrations of at-home bleaching agents ranging up to 35% CP can be used for a shorter period of time within the bleaching tray. However, higher concentrations of bleaching agents may jeopardize enamel and dentin structures. Currently available bleaching systems are composed of hydrogen peroxide (HP) or carbamide peroxide as one of its precursors. Although the appropriate mechanism of action is not relevantly clear, the superoxide free radical is the most likely cause for the oxidation of stained pigments. In spite of the favourable results of bleaching therapy, the documented possible side effects of the various bleaching agents used include alteration of the enamel surface morphology and mineralization level. About 15-78% of patients reported experiencing hypersensitivity in the teeth after undergoing vital tooth bleaching. This led to the present trend of advocating the application of remineralizing agents to control demineralisation and promote remineralization following the bleaching procedures.[7]

Universally remineralizing [RML] agents available contain calcium phosphate with or without fluoride which creates a supersaturated environment around the early lesion; thus, preventing mineral loss and forcing calcium and phosphate ions in the vacant areas. This leads to the fact that using peroxide not only bleaches the enamel surface but also affects the interprismatic and intraprismatic portion by degrading some of the proteins around the enamel rods and crystallites. Thus, any mineral element associated with the enamel protein is also removed, which would explain the loss of calcium and phosphorous in these areas; thus, contributing to the occurrence of microstructural damage and possibly even to changes in microhardness.[7]

Enamel mainly consists of rods or prisms of highly organized and tightly packed mineral crystallites separated by organic material and water. Mature enamel composed of different calcium phosphate salts (mostly hydroxyapatite) representing

88–90% of volume and 95–96% of its weight; while the organic matter accounts for approximately 1–1.5% of enamel weight. The presence of these small quantities of protein and water in enamel has an important softening or plasticizing effect.[7] The peroxide based bleaching agent caused a significant decrease in the enamel microhardness, when compared with the surface hardness of the intact unbleached group. Subsequent treatment with different remineralizing agents showed a relative recovery in the microhardness values in comparison to the unbleached group. Within the limitation of this study, it can be concluded that bleaching results in statistically significant loss of minerals from enamel surface when compared to the intact sound enamel. All the remineralizing agents tested were found that effective in remineralizing the bleached enamel surface in the following order: HA, CPP-ACPF and β-TCP. Among the remineralizing agents tested in the study, Hydroxyapatite based cream was found to be marginally more effective than the CPP-ACPF and β-TCP based products which was not statistically significant.[7]

Cvikl B, Lussi A, Moritz A, and Flury S (2016)[6] concluded that controversial results were published concerning the influence of different concentrations of bleaching agents on enamel surface. Some researchers have reported that longer contact time of bleaching gels even with low peroxide concentration such as 10% CP may negatively affect the enamel surface.[6]

Attia RM, Kamel MM (2016)[71] reported that hydrogen peroxide is analogous to carbamide peroxide as it is ultimately released when the stable complex is in contact with water. This means that most of the tooth whitening occurs due to hydrogen peroxide. When diffuses into the tooth, hydrogen peroxide acts as an oxidising agent that breaks down to produce unstable free radicals. In the spaces between inorganic salts in tooth enamel, these unstable free radicals attach to organic pigmented molecules resulting in small, less heavily pigmented components. Reflecting less light, these smaller molecules will create a "whitening effect.[71]

Heshmat H et al (2016)[72] investigated the effect of remineralizing pastes such as: MI paste (casein phosphopeptide-amorphous calcium phosphate; CPP-ACP) on bleached teeth surfaces. Basically, these agents are topically applied to remineralize damaged teeth by replacing lost calcium and phosphate.[72]

Joiner A, Luo W. (2017)[73] reported that present days of home bleaching agent commonly contains carbamide peroxide in varying concentrations of 10% to 20%. 10% CP in bleaching gel will break down into 3.35% HP and 6.65% urea. 15% and 20% CP will yield 5.4 and 7% HP respectively.
Studies show that Hydrogen peroxide is the main component of most bleaching agents. Hydrogen peroxide acts as an oxidant as well as an oxygenator and interacts with the chromophores causing the cleavage of double bond into single bond thereby converting yellow pigment (xanthopterin) into white (leucopterin). This creates a lighter appearance of the tooth. HP eventually deteriorates by releasing oxygen free radicals which are a very reactive species capable of changing many surface properties of teeth.[73]

Manal H et al (2017)[5] reported that with the increase in marketing of new vital teeth bleaching products, *in vitro* and *in vivo* studies are still needed to support their safety and efficacy. It is well accepted that safety is of a prime concern when it comes to cosmetic procedures such as vital teeth bleaching. With respect to the two, dentists supervised bleaching techniques; professionally administered (in-office bleaching system) and professionally dispensed (at-home bleaching system), both have proven effectiveness in teeth whitening.

For at home bleaching technique, a variety of concentrations of carbamide peroxide bleaching agents are available ranging from 10%, 15%, 16%, 20%, up to 35% CP. Different concentrations of CP break down into different concentrations of HP and urea. 10% CP breaks down into 3.5% HP, whereas, 35% CP produces about 10.6% HP. Carbamide peroxide bleaching gels also contains carbopol which is added to decrease the releasing time of HP, hence extending the bleaching potential over a longer period of time. However, both concentration and time of exposure for CP bleaching agents have a major influence on the efficacy of teeth whitening as well as their side effects.[5]

There are available evidences that support the safety of using 10% CP bleaching agent used for at-home bleaching treatment; however, concerns are still present with the use of at-home bleaching agents with higher HP concentrations. The results of the current study recorded that there was significant decrease in enamel microhardness after bleaching with 35% CP alone but not for those bleached with 10% CP. [5]

They concluded that 10% CP can decrease enamel microhardness, however, not significantly. Soares et al stated that bleaching agents with higher concentration (16% CP) can produce more side effects than lower concentration (10% CP). However, such a result is not in accordance with those reported in earlier studies as they concluded that bleaching gels with relatively high concentration of peroxide and shorter application time might be less harmful to enamel than agents with lower concentration and longer contact time. [5]

Several researchers have evaluated the influence of application of MI paste on the enamel surface after the bleaching regimens. However, using these agents separately after the bleaching treatment for additional two weeks needs more time and compliance by the patients. However, other studies reported that remineralizing agents when blended with the bleaching agents or used after the bleaching treatment could increase microhardness of bleached enamel. [5]

Attin et al. in their review of the effect of bleaching agents on enamel microhardness reported that the use of artificial saliva in *in vitro* or human saliva in *in situ* studies may highly affect the results. These storage media may reduce the risk of decreasing enamel microhardness due to bleaching treatments, by the remineralization action of saliva. Following enamel demineralization by the bleaching agents, a greater absorption of minerals by ionic exchange is produced by replacing those lost during bleaching. [5]

Analysis of SEM photographs of the enamel surfaces bleached with both CP bleaching agents showed minor morphological changes showing areas of shallow erosions on the enamel which were more obvious for those bleached with 35% carbamide peroxide. Similar results were reported with other studies supporting that granule accumulations induced by remineralizing agents could protect the bleached enamel surface from potential morphological changes. It has been proposed that micro porosities formed by the bleaching agents provide susceptible areas for redeposition of the remineralizing agents, similar to that which occurs in arrested caries. [5]

For at-home vital teeth bleaching system, 35% CP bleaching agent is the highest concentration with the shortest application time used within the bleaching trays. Its use is convenient for those patients unable to wear the bleaching trays overnight with 10% CP. However, in respect to the safety issue, it is recommended to be used with a remineralizing agent to overcome the potential side effects to the enamel surface. Similar teeth colour changes can be achieved after two weeks of bleaching with 10% and 35% CP when used as recommended by the manufacturer's instructions with and without the addition of MI paste plus (CPP-ACPF). Enamel microhardness was significantly decreased after home bleaching with 35% CP but not with the use of 10% CP. [5]

Peixoto AC, Vaez SC, Pereira NAR, Santana CNDS.(2018)[74] concluded that carbamide peroxide is commonly used for at-home bleaching using a tray-based technique and dissociates into hydrogen peroxide (approximately a third of its former concentration) and urea, which further breaks down into water and ammonia.[74]

Karima Lubbadeh et al (2018)[75] investigated that bleaching did not increase the enamel susceptibility to erosion or abrasion regardless of the bleaching gel concentration or frequency of application. Bleaching gels containing fluoride, calcium or potassium nitrate might have offered protection against enamel erosion and abrasion. Although it may not be of clinical significance, in-office bleaching caused more surface loss than at-home bleaching. Professional bleaching gels are not harmful to the enamel surface, especially if they contain fluoride, potassium nitrate and calcium. Patients should be advised to use low-abrasive fluoride dentifrices after bleaching.[75]

At-home bleaching gels may be favoured over in-office treatments for patients already at risk of erosive tooth wear. Bleaching agents released free radicals that can cause degradation of the enamel matrix. Thus, loss of tooth minerals, such as calcium and phosphorus, may occur. This can lead to a decrease in enamel microhardness, especially when high concentrations are being used and when treatments were repeated. It was noticed that bleaching products based on carbamide peroxide were more damaging due to longer application times. [75]

Also, in-office bleaching resulted in more surface softening compared to at-home bleaching. Some studies concluded that home bleaching with low peroxide concentrations did not affect enamel micro hardness. Using fluoride-supplemented bleaching products or topical fluorides immediately after bleaching decreased this adverse side effect and enhanced enamel remineralization. Interestingly, the addition of amorphous calcium phosphate (ACP) or calcium to bleaching gels was not found to be successful in reversing the demineralizing effects of bleaching in both sound and carious enamel. [75]

Studies have also shown morphological changes on the enamel surface after bleaching, leading to increases in enamel surface roughness and porosity and perhaps leading to more rapid discolouration in the future. Nevertheless, morphological changes were not considered to be significant with home bleaching products using 10% and 15% carbamide peroxide. On another hand, in situ studies suggested that there were no structural differences between bleached and unbleached teeth. That could be due to the continuous influx of fluoride and other minerals from saliva and topical oral care products to the bleached teeth that helped to restore their mechanical and chemical properties that the bleaching might have altered. Due to decreased microhardness and mineral loss, bleached enamel can become more susceptible to acid erosion. [75]

Erosion, known to be the loss of enamel surface due to exposure to acidic chemicals that are non-bacterial, became more pronounced after bleaching, especially when bleaching materials with a lower pH were being used. Bleaching performed with low peroxide concentrations, such as 10% carbamide peroxide, did not increase enamel surface roughness, nor did it make tooth enamel more susceptible to erosion or brushing abrasion. There were contradictory results among studies assessing the harmful effects of bleaching gels, which necessitated further investigation. Many variables had to be taken into consideration to reconcile gaps. The present study was novel as it addressed the effect of in-office and at-home bleaching products, their frequency of use, combined with erosion and abrasion in a comprehensive manner. [75]

The current study demonstrated that bleaching, regardless of the bleaching treatment concentration, did not increase enamel surface loss with erosion or erosion/abrasion challenges. Other studies showed that similar results, concluded that surface loss occurred with erosion or tooth brushing abrasion and not with bleaching. Bleaching treatments, especially home bleaching with low carbamide peroxide concentrations did not have deleterious effects. Similarly, other studies concluded that bleaching gels did not alter calcium and phosphate content of dental enamel and therefore did not cause demineralization matter how excessively they were used. [75]

In addition, studies that analysed surface roughness showed no statistically significant difference between bleached and unbleached enamel when using home bleaching agents. Also, some studies have shown that using bleaching gels with concentrations such as 10% and 16% carbamide peroxide for long durations resulted in decreasing enamel micro hardness, decrease in calcium and phosphate content and increase in surface roughness. Similarly, it was found that bleaching increased enamel demineralization. Also, studies found an increase in enamel susceptibility to acid erosion and mineral loss after bleaching. [75]

It is important to remember that these studies were conducted *in vitro* where the remineralizing effect of saliva is often absent. *In situ* studies showed that no significant differences between bleached and unbleached enamel whether mechanical, chemical or morphological properties being considered. In these studies, bleaching was performed intra-orally with continuous exposure to saliva. [75]

In this study, overnight remineralization was performed in artificial saliva after each bleaching cycle and 60 minutes of remineralization was allowed between treatment challenges during cycling, to mimic salivary remineralization that would take place in the oral cavity. Repeated bleaching and bleaching treatment concentrations did not have a significant effect on surface loss. Other studies proved that higher bleaching gel concentrations led to more intense adverse effects on enamel, more softening, mineral loss and increased roughness. Similarly, studies showed more adverse effects and morphologic changes with longer periods of bleaching application. [75]

Interestingly, in-office treatments caused more surface loss than at-home treatments. This difference was consistent with erosive and abrasive challenges and whether bleaching periods were repeated or not. Similar results were shown in a study investigating changes in enamel surface microhardness. The safety of at-home bleaching gels was also demonstrated by showing that their use in different concentrations and different time periods did not cause any statistically significant changes in surface roughness and morphology. This may be due to various factors, such as chemical composition, method, and mechanism of application. There was no effect of bleaching gel peroxide concentration between in-office or at home bleaching products, which warrants further, more longitudinal studies. [75]

Although a potential difference may not be of any clinical significance, since bleaching did not have a harmful effect overall, it may serve as a guideline when choosing the bleaching treatment for patients with certain risk factors, such as pre-existing erosive-abrasive lesions, patients having more acidic diet, patients with tooth grinding habits or more sensitive teeth. Tooth bleaching, regardless of the bleaching agent concentration and frequency of application, did not increase enamel surface susceptibility to erosion and abrasion. Agents such as fluoride, potassium nitrate and calcium, when added to bleaching gels, offered protection to enamel surface against erosion and abrasion. In-office bleaching caused more surface loss than at-home bleaching, which may not be of clinical significance, but can be used as a guideline when bleaching is prescribed to patients with increased risk to erosive and/or abrasive surface loss. [75]

Taneja S et al (2018)[76] reported that the irregularities of the enamel are further boosted with bleaching which could also confer to staining after bleaching. It was noticed that the susceptibility to staining tends to be greater when the tooth is exposed to 35% hydrogen peroxide. Teeth exposed to a pH <5.5 for enamel and 6.0 for dentin for an extended period of time can lead to demineralization and erosion of enamel. The cola soft drink (pH 2.60) and pomegranate juice (pH 3.20) used in this investigation are extremely acidic solutions in comparison to turmeric (pH 6.30), showing that the low pH of these solutions had a major effect on the bleached teeth.[76]

Mounika A, Mandava J, Roopesh B, Karri G(2018)[77] concluded that among the number of vital bleaching techniques currently available to the clinicians, home bleaching and in-office bleaching were widely used in dental practice. At-home and in-office bleaching procedures are equally effective in producing tooth whitening. Colour evaluation after 3 and 6 months showed that more colour decline for in-office bleaching procedure. In-office procedure recorded higher sensitivity compared to home bleaching. Both the bleaching procedures are equally effective in producing tooth whitening. In-office bleaching recorded that higher level of tooth sensitivity and greater colour rebound than home bleaching.[77]

Recent investigations on enamel characteristics following bleaching have shown that reduction in enamel microhardness, modulus of elasticity, surface toughness occurs after bleaching. Enamel microhardness was noticeably affected immediately after in-office bleaching (more than home bleaching) using high concentrations of carbamide peroxide. However, baseline hardness values were restored after fluoride application.
Bleached tooth treated with remineralization agent have produced a positive effect on enamel morphology and micro hardness recovery. Over usage of bleaching agents will create micro structural changes on the enamel surface resulting in demineralization, degradation, alteration on surface micro hardness and roughness of sound enamel surface.[77]

RELEVANCE

The demand for a beautiful smile is higher than ever nowadays. The growing importance of aesthetics is placing more emphasis on the colour of the teeth. The influence that media has, has led to an increased demand for whiter and more beautiful teeth which are important components of an attractive smile. Thus, tooth bleaching which is a simpler method for gaining white teeth has gained popularity. Various bleaching agents are available in the market today and this has led to the over usage of bleaching agents for attaining white teeth.

Erosion, which is caused by the loss of enamel surface due to exposure to acidic chemicals that are non-bacterial in origin, has become more pronounced after bleaching, especially when the bleaching material has a very low pH. Increased intake of acidic foods and beverages after bleaching can lead to deleterious effects on the tooth structure.

Studies have reported that the use of remineralizing agents help in reducing the loss of tooth mineral after bleaching. Studies have also reported the effects of varying concentrations of bleaching agents on the tooth structure. There has been an increase in the erosion pattern subsequently after bleaching. Hence this study aims at evaluating the application of remineralizing agents on the effects of erosion occurring as a consequence of bleaching. The study also aims at analysing the effects of different concentrations of bleaching agents on erosion pattern with or without the application of remineralizing toothpaste.

MATERIALS AND METHODS

60 Freshly Extracted Caries Free Upper First Premolars were selected.

⇨

Teeth were sectioned at the level of the CEJ and divided into 2 equal halves to obtain Enamel Specimens of 3×3 mm dimension to get 120 enamel samples.

⇩

All the samples (N = 120) were subjected to an initial surface microhardness test with Microdurometer with a Vickers Type Diamond Penetrator.

⇦

The Samples were divided into 6 groups (N = 20) based on the concentration of the bleaching agent, the use of Remineralizing agent and Erosive treatment.

⇩

GROUP 1
Treatment with 10 % Carbamide Peroxide, followed by treatment with 1% Citric Acid Solution

GROUP 2
Treatment with 10% Carbamide Peroxide followed by application of Remineralizing agent, then treated with 1 % Citric acid solution

GROUP 3
Treatment with 35 % Carbamide Peroxide then treated with 1 % Citric acid Solution.

GROUP 4
Treatment with 35 % Carbamide Peroxide followed by application of remineralizing agent, and then treated with 1 % Citric acid solution.

GROUP 5
Negative Control without bleaching

GROUP 6
Positive Control (without bleaching with 1 % Citric acid solution).

The following measurements, percentages, analyses were conducted:

MEASUREMENT OF FINAL SURFACE MICROHARDNESS AFTER BLEACHING

⇩

MEASUREMENT OF FINAL SURFACE MICROHARDNESS AFTER EROSION

⇩

PERCENTAGE OF SURFACE HARDNESS LOSS AFTER BLEACHING AND EROSION CALCULATION

⇩

SURFACE PROFILOMETRY ANALYSIS

⇩

SEM ANALYSIS

⇩

STASTISTICAL ANALYSIS

STUDY SETTING

The study was conducted in the Department of Conservative Dentistry and Endodontics, St. Gregorios Dental College Kothamangalam; National Institute of Technology, Calicut and Amrita Institute of Medical Science, Ernakulum.

SAMPLE SIZE

Sample size is calculated using statistical package G*power (3.1.5) with level of significance of 0.05.

The sample size obtained was n=120 which was divided into 6 Groups with 20 samples per group.

The materials and methods used for this study are described under the following headings.

1. Selection of specimens
2. Materials used for the study
3. Specimen preparation
4. Vickers microhardness test
5. Surface profilometry analysis
6. Scanning Electron microscopy analysis
7. Statistical Analysis

SELECTION OF SPECIMENS (FIG 1)

Human premolars extracted for orthodontic purposes were collected from the Department of Oral and Maxillofacial Surgery, St. Gregorios Dental College, Kothamangalam and St Mary's Dental Clinic, Thodupuzha.

INCLUSION CRITERIA

Non carious teeth
Teeth with complete root formation

EXCLUSION CRITERIA

Fractured teeth
Teeth with resorption
Teeth with developmental anomalies
Teeth with hypoplastic defects

ARMAMENTARIUM (FIG 2)

Bleaching agents - 10% carbamide peroxide, 35% carbamide peroxide, ReminPro remineralizing toothpaste, 1% citric acid solution, Glass rod, Glass jar, Artificial saliva.

MATERIALS USED FOR THE STUDY

Bleaching agents

1. 10% Carbamide Peroxide (Opalescence, Ultradent) (FIG 3)
2. 35% Carbamide peroxide (Opalescence, Ultradent) (FIG 4)

Remineralizing agent

Remin Pro (VOCO, Germany) (FIG 5)

Erosive agent

1% citric acid solution (Freshly prepared from Department of Biochemistry, St. Gregorios Dental College) (FIG 6)

METHODOLOGY

60 freshly extracted human upper first premolars were selected. Each tooth was fixed on an acrylic block such that the tooth was visible from the CEJ to the occlusal surface (FIG 7). The teeth were sectioned at the level of the CEJ and divided into 2 equal halves (a buccal and a palatal half) to obtain enamel specimens of 3 × 3 mm dimensions. So a total of 120 enamel samples were obtained from 60 teeth. These 120 samples were then divided into 6 Groups with 20 samples per group (FIG 8). The samples were washed in running water and stored in artificial saliva (FIG 9). All the samples (n=120) were subjected to an initial surface micro hardness test with microdurometer with a Vickers type diamond penetrator (FIG 10).

<u>Assessment of Initial Surface Microhardness (Baseline)</u>

All the samples (n=120) were submitted to an initial surface microhardness test. For this purpose, a Microdurometer with a Vicker's type diamond penetrator (Matzusawa

Japan) was used. Two indentations spaced at 100 micrometer distance from one another were made in the centre of each sample, thus obtaining the hardness values (VHN). The mean of these two values represented the hardness of the samples.

The Enamel blocks were then subjected to two phases of treatment: a bleaching phase and an erosive phase. The samples were then randomly divided into 6 Groups (n=20) based on the concentration of the bleaching agent, the use of remineralizing agent and erosive treatment.

Table 1:

GROUPS	BLEACHING PHASE	EROSIVE PHASE
G1	Treatment with 10% carbamide peroxide (n=20)	Treatment with 1% citric acid solution
G2	Treatment with 10% Carbamide peroxide followed by application of remineralizing agent (n=20)	Treatment with 1% citric acid solution
G3	Treatment with 35% Carbamide peroxide(n=20)	Treatment with 1% citric acid solution.
G4	Treatment with 35% Carbamide peroxide followed by application of remineralizing agent(n=20)	Treatment with 1% citric acid solution
G5	Negative control (without bleaching) (n=20)	
G6		Positive control (without bleaching with 1% citric acid solution solution)

BLEACHING PHASE

In the bleaching phase, G1 and G2 were subjected to prophylaxis with pumice and water with a rubber cup for 1 minute. One drop of 10% carbamide peroxide bleaching gel was deposited on the surface of each sample for a period of 8 hours (FIG 11). The Samples were then washed with distilled water for 5 minutes to remove the bleaching agent and stored in artificial saliva for the remaining hours. This procedure was repeated for 7 days to reproduce the procedure as in home bleaching.

In G3 and G4, professional bleaching treatment were done with 35% carbamide peroxide. After prophylaxis with pumice and water, one drop of bleaching gel was deposited on the surface of the samples for a period of 30 minutes. After this period, all the samples were washed with distilled water for 5 minutes and stored in artificial saliva.

After the bleaching treatment, on G2 and G4, remineralizing agent was applied to the samples during a 15-day period for 5 minutes, twice daily (FIG 12). The Samples were then washed with distilled water and stored in artificial saliva.

Surface Micro hardness Analysis after Bleaching Phase

G1, G2, G3, G4 were then subjected to surface micro hardness analysis. After bleaching, two indentations equidistant at 100 micrometres and parallel to that of the initial readout (baseline) were made, following the same pattern as that of the initial analysis. After the surface microhardness readout of all the blocks were made, the percentage of mineral loss (% SHL) was obtained in the different experimental groups, according to the following equation:

$$\%SHL = \frac{Microhardness\ after\ bleaching - Initial\ microhardness\ (baseline)}{Initial\ microhardness\ (baseline)} \times 100$$

EROSIVE PHASE

The samples of G1, G2, G3, G4 and G6 were immersed in a 1% citric acid solution of pH 3.6 under agitation for 5 minutes (FIG 13). This sequence was performed twice daily for 3 consecutive days, totalling 30 minutes of exposure to the acid solution. The samples were then kept in artificial saliva. The Surface microhardness analysis after erosive phase was calculated. Two indentations were made following the same pattern as that of the previous analysis. The percentage of surface hardness loss (%SHL) was obtained according to the following equation:

$$\% \text{ SHL} = \frac{Microhardness\ after\ erosion - Initial\ microhardness\ (baseline)}{Initial\ microhardness\ (baseline)} \times 100$$

SURFACE ROUGHNESS ANALYSIS

After the two phases of treatments and micro hardness analyses, the surface roughness of all the groups (G1, G2, G3, G4, G5 and G6) was measured by means of volumetric profilometry analysis (3D) and linear profilometry analysis. Two blocks from each group were taken to the noncontact optical 3D Profilometer (FIG 14) (Alicona, model: Infinite focus G5).

ANALYSIS BY SCANNING ELECTRON MICROSCOPY

Two samples from each group (G1, G2, G3, G4, G5 and G6) were subjected to Scanning Electron Microscopy analysis (SEM) (FIG 15). These samples were sputter coated with gold. After this, they were transported to the microscope where they underwent the process of pressurization under vacuum for the readout. The image was captured at 1000x and 5000x magnifications, by a software program (EDAX) and transmitted to a computer coupled to the SEM.

FIG 1: TOOTH SAMPLES

FIG 2: ARMAMENTARIUM

FIG 3: 10% CARBAMIDE PEROXIDE (OPALESCENCE, ULTRADENT)

FIG 4: **35% CARBAMIDE PEROXIDE (OPALESCENCE, ULTRADENT)**

FIG 5: REMIN PRO (VOCO, GERMANY)

FIG 6: 1 % CITRIC ACID SOLUTION

FIG 7: TOOTH EMBEDDED IN ACRYLIC BLOCK

FIG 8: TOOTH SPECIMEN PREPARED,20 SAMPLES PER GROUP

FIG 9: ARTIFICIAL SALIVA

FIG 10: MICRODUROMETER WITH A VICKER'S TYPE DIAMOND PENETRATOR

FIG 11: BLEACHING GEL APPLICATION

FIG 12: REMINERALIZING AGENT APPLICATION

FIG 13: SAMPLES WERE IMMERSED IN 1% CITRIC ACID SOLUTION

FIG 14: SURFACE PROFILOMETER

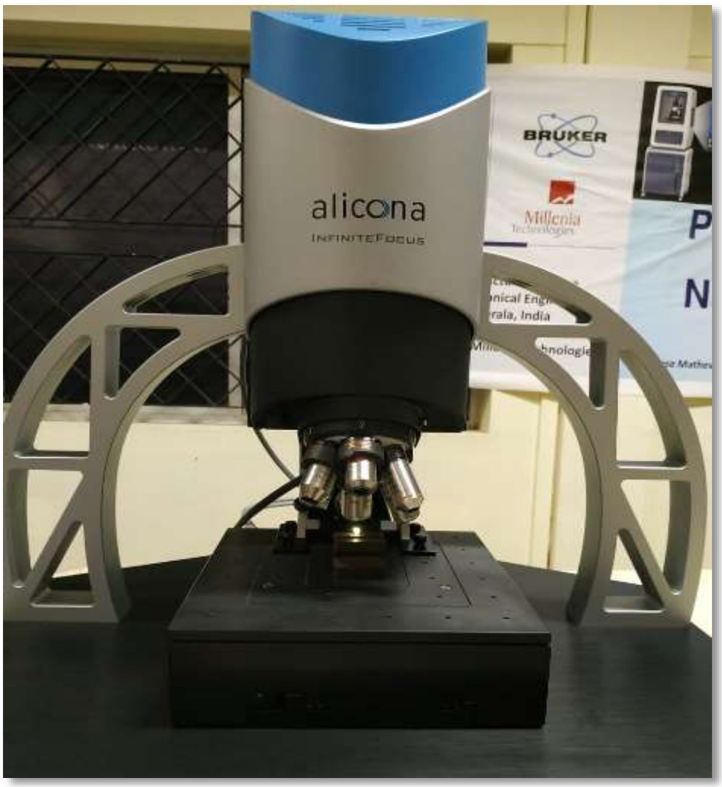


FIG 15: SCANNING ELECTRON MICROSCOPE

STATISTICAL ANALYSIS

TABLE 2 - Initial microhardness of the groups:

GROUPS	INITIAL MICROHARDNESS	
	MEAN	SD
G1	347.73	1.075
G2	286.46	1.311
G3	332.285	1.981
G4	338.335	1.523
G5	298.1	48.957
G6	375.125	8.09

TABLE 3 - Average of initial micro hardness and (%SHL) after bleaching phase

GROUPS	INITIAL MICROHARDNESS		AFTER BLEACHING		%SHL
	MEAN	SD	MEAN	SD	
G1	347.73	1.075	254.21	1.697	26.89
G2	286.46	1.311	200.83	2.266	29.89
G3	332.285	1.981	175.6	1.236	47.15
G4	338.335	1.523	194.56	1.452	42.49
ANOVA					0.0001*

*P<0.05 is statistically significant

ANOVA –POST HOC ANLAYSIS

Group comparison	P VALUE
G1 vs G2	0.937
G1 vs G3	**0.001***
G1 vs G4	**0.001***
G2 vs G3	**0.001***
G2 vs G4	**0.001***
G3 vs G4	0.924

*P<0.05 is statistically significant

TABLE 4 - Average of (%SHL) of each group after erosive challenge

GROUPS	INITIAL MICROHARDNESS		AFTER EROSION		%SHL
	MEAN	SD	MEAN	SD	
G1	347.73	1.075	143.015	1.051	59.55
G2	286.46	1.311	154.305	1.730	46.13
G3	332.285	1.981	122.215	1.677	63.21
G4	338.335	1.523	126.16	1.308	62.7
G6	375.125	8.09	176.523	1.555	52.9
Anova					0.001*

***P<0.05 is statistically significant**

ANOVA –POST HOC ANLAYSIS-TUKEY HSD TEST:

Group comparison	P VALUE
G1 vs G2	**0.004***
G1 vs G3	0.847
G1 vs G4	0.772
G1 vs G6	0.635
G2 vs G3	**0.001***
G2 vs G4	**0.001***
G2 vs G6	0.576
G3 vs G4	0.965
G4 vs G6	**0.03***

***P<0.05 is statistically significant**

Table 5 -
Linear surface (Ra) & volumetric (Sa) roughness values of each Group

GROUPS	LINEAR ROUGHNESS(Ra)		VOLUMETRIC ROUGHNESS(Sa)	
	MEAN	SD	MEAN	SD
G1	0.265	0.102	1.8	0.121
G2	0.222	0.034	1.9	0.113
G3	0.321	0.054	2.61	0.104
G4	0.194	0.022	1.8	0.141
G5	0.141	0.135	0.894	0.116
G6	0.254	0.231	1.362	0.102
ANOVA	**0.002***		**0.0001***	

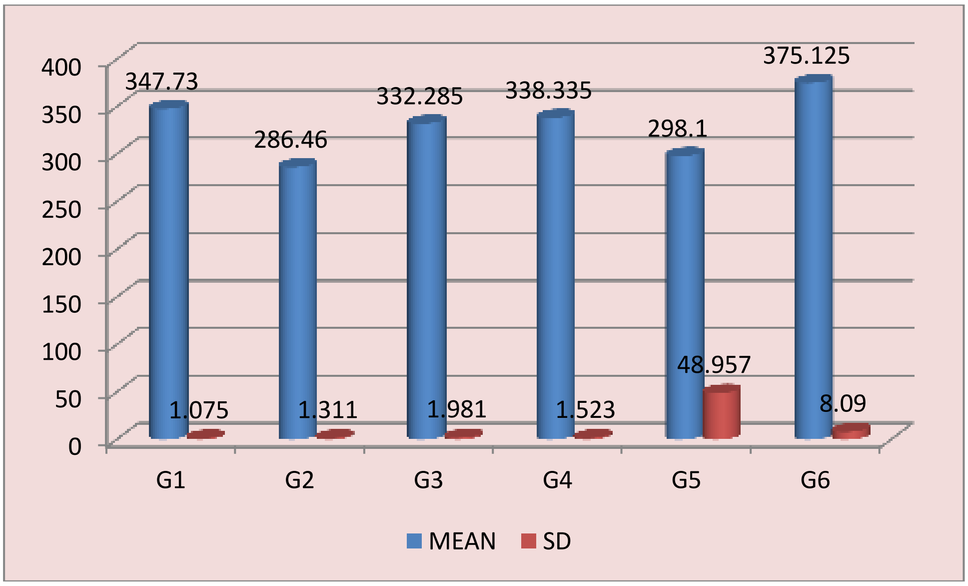
***P<0.05 is statistically significant**

ANOVA-POSTHOC ANALYSIS-TUKEY HSD TEST

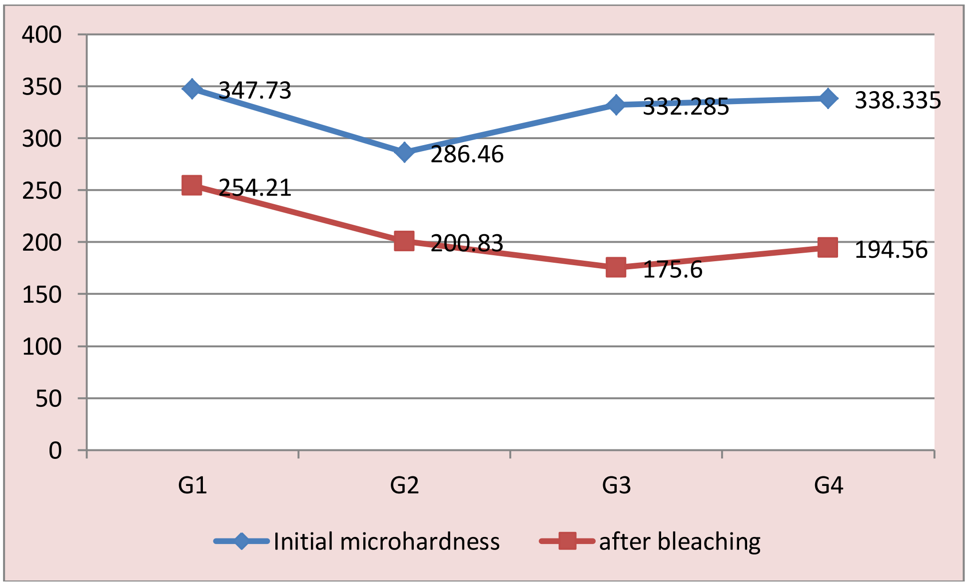
Ra (Linear roughness)	P VALUE	Sa (Volumetric roughness) P VALUE
G1 VS G2	0.867	0.082
G1 VS G3	0.681	**0.001***
G1 VS G4	0.426	**0.001***
G1 VS G5	**0.017***	**0.001***
G1 VS G6	0.999	**0.001***
G2 VS G3	0.104	**0.001***
G2 VS G4	0.976	0.082
G2 VS G5	0.278	**0.001***
G2 VS G6	0.958	**0.001***
G3 VS G4	**0.013***	**0.001***
G3 VS G5	**0.001***	**0.001***
G3 VSG6	0.492	**0.001***
G4 VS G5	0.729	**0.001***
G4 VS G6	0.613	**0.001***
G5 VS G6	**0.040***	**0.001***

***P<0.05 is statistically significant**

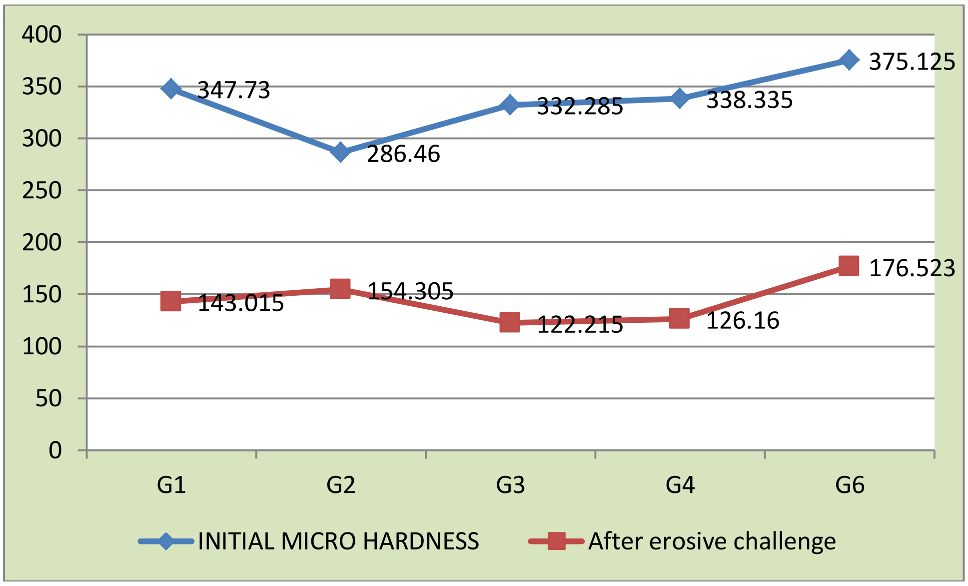
Graph 1 - Initial Micro Hardness



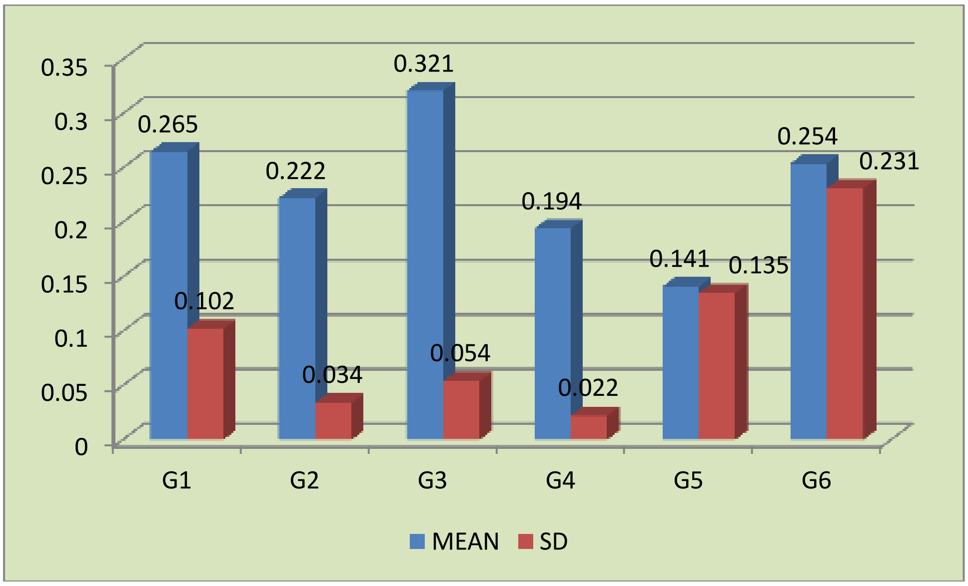
Graph 2 - Bleaching



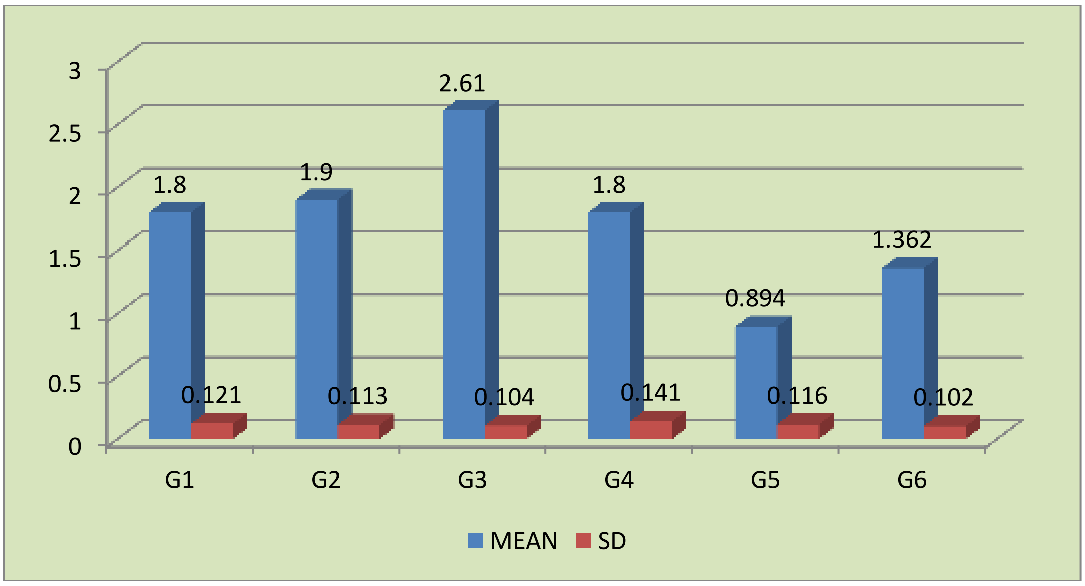
Graph 3 - After Erosive Challenge



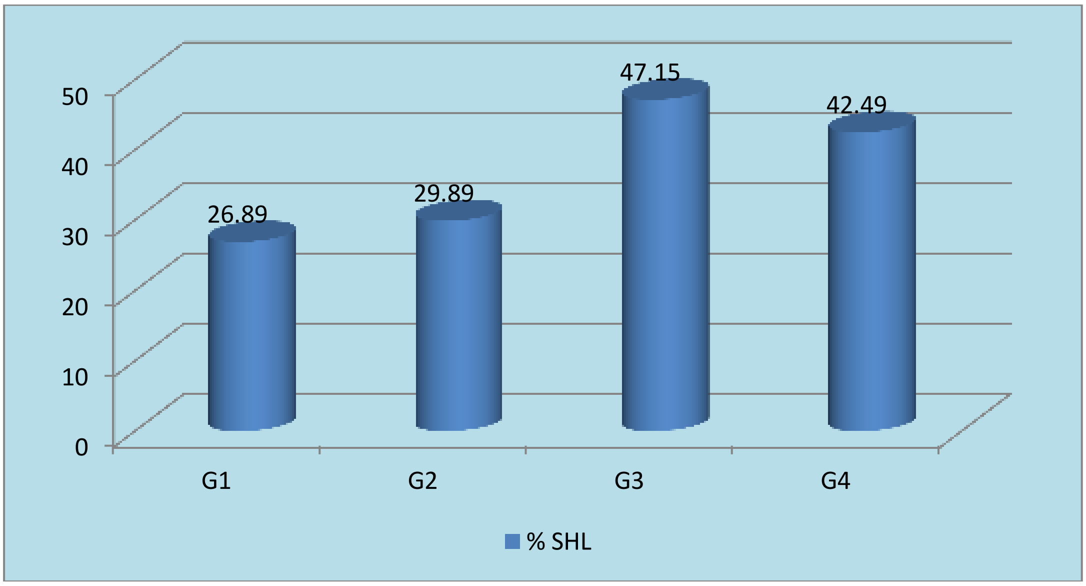
Graph 4 - Linear Roughness (Ra)



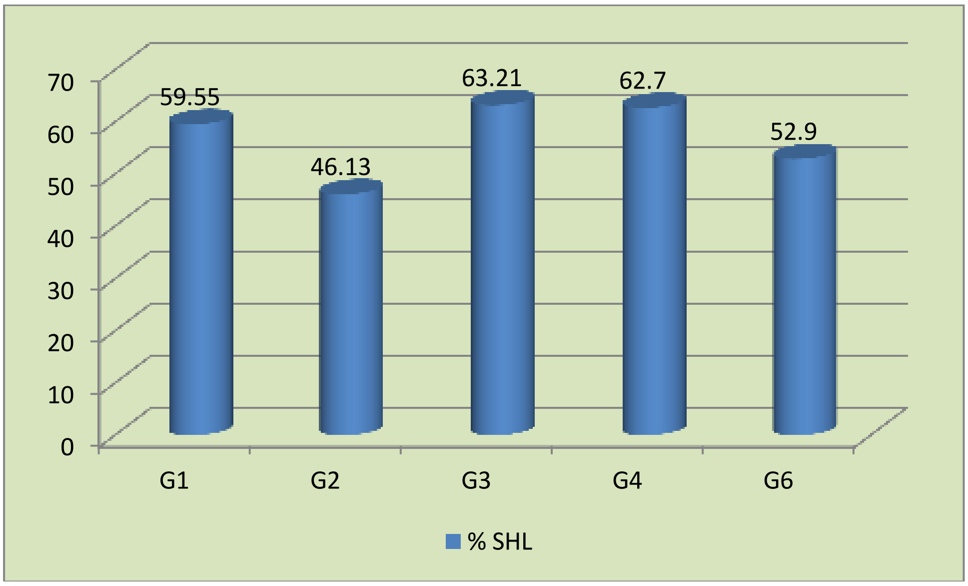
Graph 5 - Volumetric Roughness (Sa)



Graph 6 - % SHL After bleaching



Graph 7 - % SHL After Erosive Challenge



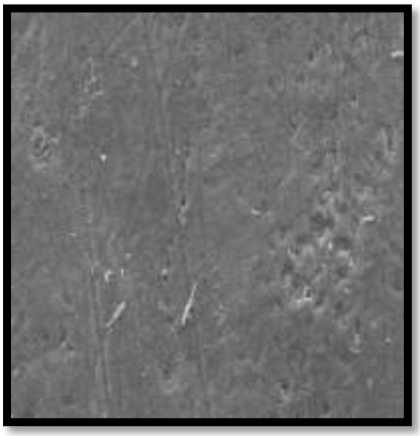
RESULTS

After the samples were subjected to bleaching, it was observed that the % SHL value was significantly higher in G3 in comparison with the other groups of treatment (Table 3). A difference in the % SHL was also found between G3 and G4, bleached with 35% carbamide peroxide without and with remineralizing toothpaste, respectively, in which G4 presented a significantly lower % SHL value than G3. G1 versus G3, G1 versus G4, G2 versus G3, G2 versus G4 were statistically significant. ($P<0.05$). Whereas, after erosive challenge, an increase of the % SHL values for all groups of treatment ($p<0.05$) was observed. However, G2 showed the lowest % SHL after the erosive phase ($p<0.05$) when compared with G1, G3, G4 and G6 (Table 4). In the profilometry analysis results, all the groups of treatment (G1, G2, G3, G4) and G6 did not differ statistically among them, but differed from G5 ($p<0.05$) (Table 5).

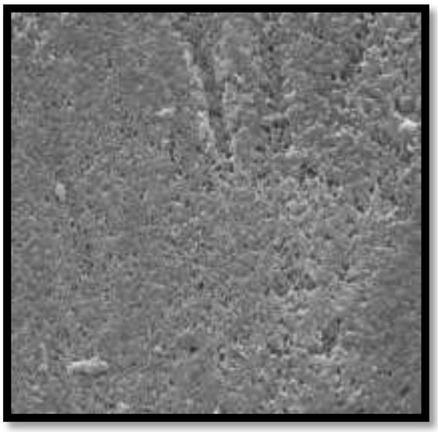
Also, the images captured by SEM (FIG.16) revealed morphological alterations in the superficial structure of enamel resulting from erosion either associated with bleaching treatment, with or without remineralizing agent and erosive challenge. The eroded areas were less visible in G2 compared with the other groups, since the interprismatic structure is more prominent in this group. The impact of erosion after bleaching was calculated from the hardness after bleaching phase compared with the hardness after erosion phase. Thus, the real impact of erosion was higher as observed in G3 (% SHL 63.21) and G4 (% SHL 62.7), which were different from themselves and from the other groups.

FIG 16: SEM Analysis

G1- Samples treated with 10% carbamide peroxide followed by erosive agent application

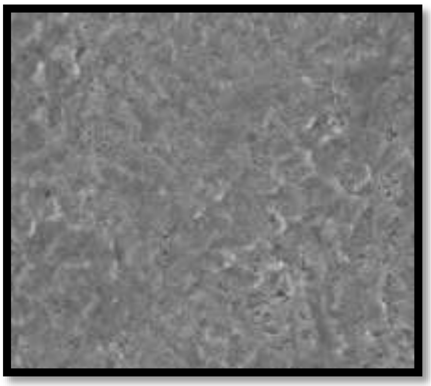


1000x magnification

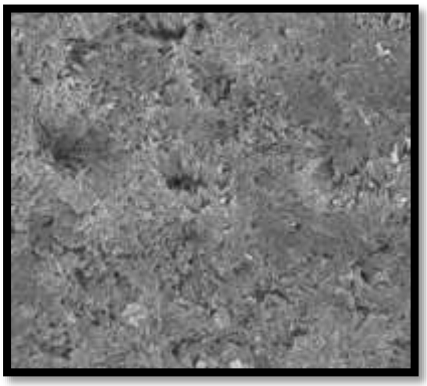


5000x magnification

G2 - Samples treated with 10% carbamide peroxide, Remin Pro remineralizing tooth paste followed by erosive agent application

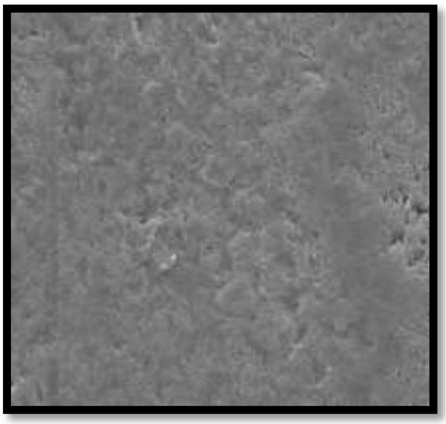


1000x magnification

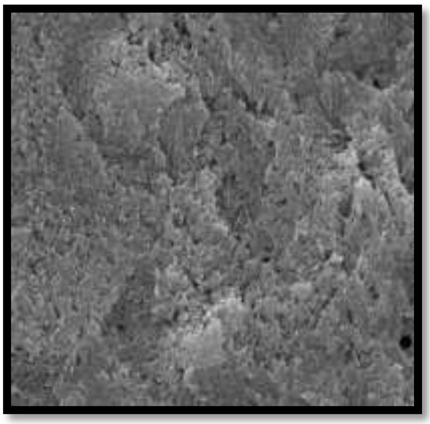


5000x magnification

G3 - Samples treated with 35% carbamide peroxide followed by erosive agent application

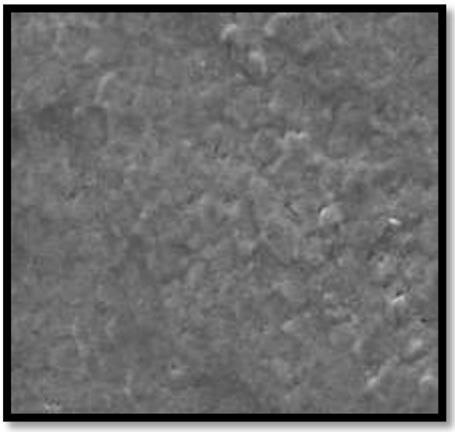


1000x magnification

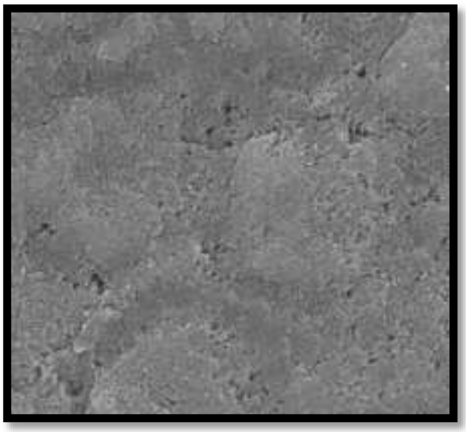


5000x magnification

G4 - Samples treated with 35% carbamide peroxide, Remin Pro Remineralizing tooth paste followed by erosive agent application

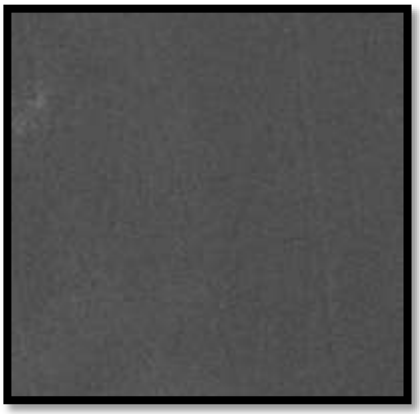


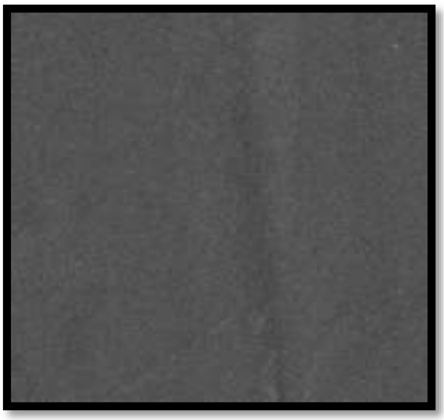
1000x magnification



5000x magnification

G5 - Negative control-without bleaching

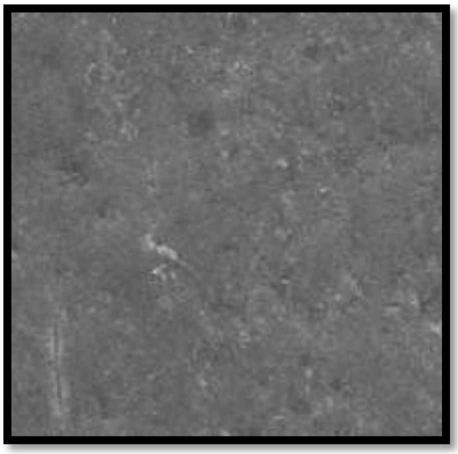


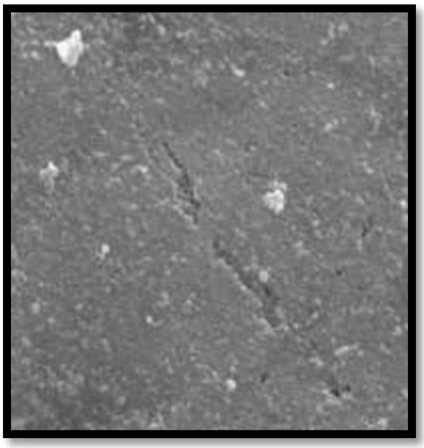


1000x magnification

5000x magnification

G6 - Positive conrol-without bleaching with erosion





1000x magnification

5000x magnification

DISCUSSION

Vital tooth bleaching procedures are the most opted conservative and effective treatment for discoloured tooth.[78] Chemical degradation of the chromogens within or on the tooth is termed as bleaching.[51] Use of peroxide was found to be safer and required the least time compared to other bleaching agents.[63] Hydrogen peroxide (H_2O_2) is the active ingredient in commonly used whitening products and is delivered as either hydrogen peroxide or Carbamide peroxide.[62] Hydrogen peroxide is analogous to Carbamide peroxide as it is ultimately released when the stable complex is in contact with water. Carbamide peroxide appeared to produce slightly more dramatic results at first, ultimately products containing equivalent amounts of Carbamide peroxide and hydrogen peroxide produced exactly the same results.[48] 35% Carbamide peroxide is recommended daily use for 30 minutes.[37] Carbamide peroxide dissociates into hydrogen peroxide and urea, which further breaks down into water and ammonia.[74]

35% Carbamide peroxide bleaching agent has the same bleaching effect with approximately 12% hydrogen peroxide that could be used in-office with reduced chair-time.[40] Peroxide present in the bleaching agent will diffuse into the enamel and dentin.[79] 10% carbamide peroxide (CP) bleaching agent within a night guard to bleach vital teeth was done in 1989 by Haywood & Heymann.[29] This technique is known as at-home bleaching technique.[75] This fact encouraged manufacturers to market higher concentrations of at-home bleaching agents ranging up to 35% CP to be used for a shorter period of time within the bleaching tray. However, higher concentrations of bleaching agents may jeopardize enamel and dentin structures.[7]

Tooth bleaching are associated with some side effects. Bleaching results in demineralization, degradation, and alteration on surface micro hardness and roughness of sound enamel surface.[80] Morphological changes have been observed in enamel, with erosion, craters and porosity being reported by various authors.[82]

In this study enamel surface microhardness tests were used as the parameter to evaluate the mineral alteration that occurred in this enamel after bleaching with carbamide peroxide at different concentrations, with and without remineralizing agent application after bleaching.

According to Faraoni-Romani and colleagues, the results obtained with microhardness tests perfectly detect the demineralization or remineralization of a substrate, thus providing indirect evidence of mineral loss or gain in human teeth.[77]

Controversial results were published concerning the influence of different concentrations of bleaching agents on enamel surface. Some researchers have reported that longer contact time of bleaching gels even with low peroxide concentration such as 10% CP may negatively affect the enamel surface.[76, 63]

However, others proposed that bleaching gels with a relatively higher concentration produced more intense side effects compared with 10% CP. [61] According to Manal H et al the results of the study recorded significant decrease in enamel microhardness after bleaching with 35% CP alone but not for those bleached with 10% CP.[64]

In this study bleaching agents with higher concentration produced highest percentage of surface hardness loss than with lowest concentration. (G1<G2<G4<G3). G1 versus G3, G1 versus G4, G2 versus G3 and G2 versus G4 were statistically significant.

Pinto and colleagues and Amaral and colleagues, in in vitro studies, have shown that hydrogen peroxide in the concentration of 35% produces a greater destructive effect on the tooth surface than it does in other concentrations, thereby altering the microhardness and surface roughness of enamel. The authors suggested in their studies that the concentration of bleaching agents used influences enamel microhardness. [80]

In the present study, the results obtained after bleaching the tooth fragments showed that the changes caused in enamel by the action of 10% carbamide peroxide was not worse than those of the action of 35% carbamide peroxide. The action of 35% carbamide peroxide without remineralizing agent (G3) was the one that generated the highest loss of surface hardness, showing that the concentration of the agent was determinant in increasing the changes in enamel microhardness.

In this study, tooth bleached with 35% carbamide peroxide produced higher percentage of surface hardness loss compared to 10% carbamide peroxide. (G3=47.15, G4=42.49)

On the other hand, several researchers have investigated the effect of remineralizing pastes such as: MI paste (casein phosphopeptide-amorphous calcium phosphate; CPP-ACP) on bleached teeth surfaces. Basically, these agents are applied topically to remineralize decay-damaged teeth by replacing lost calcium and phosphate.[72]

Researchers have proposed that post-operative application of CPP-ACP for two weeks on bleached enamel surfaces is able to decrease the bleaching agents related adverse effects; re-establishing the baseline enamel hardness and the reduced modulus of elasticity. [10]

Bleached tooth treated with remineralization agents have produced a positive effect on enamel morphology and micro hardness recovery. Remin Pro (VOCO, Germany) remineralizing paste contains calcium, phosphate in the hydroxyapatite form along with Fluoride and Xylitol.[69]

It promotes remineralization by filling porous areas, forming a protective film on the tooth surface and thereby impeding the adhesion of bacterial plaque.[69]

In this study, bleached tooth treated with remineralizing tooth paste produced lowest percentage of surface hardness loss compared to teeth that were not treated with remineralizing toothpaste (G4=42.49< G3=47.15).

Analysis of SEM photographs of the enamel surfaces bleached with both CP bleaching agents showed minor morphological changes showing areas of shallow erosions on the enamel which were more obvious for those that were bleached with 35% carbamide peroxide. Similar results were reported with other studies supporting granule accumulations induced by remineralizing agents that could protect the bleached enamel surface from potential morphological changes.

It has been proposed that micro porosities formed by the bleaching agents provide susceptible areas for redeposition of the remineralizing agents, similar to that which occur in arrested caries. For at-home vital teeth bleaching system, 35% CP bleaching agent is the highest concentration with the shortest application time used within the bleaching trays. Its use is convenient for those patients unable to wear the bleaching trays overnight with 10% CP. However, with respect to the safety issue, it is recommended to use it with a remineralizing agent to overcome the potential side effects to the enamel surface.

The reduction in the incidence of dental caries and high consumption of acid food and beverages have led to more attention being paid to the risk of dental erosion.[5]

According to Alessandra B. Borges et al, some changes in bleached enamel were also described as slight erosive effects promoted by the bleaching agent. Nevertheless, some authors claim that the erosive patterns on the surface of bleached enamel only occur when bleaching gels with low pH are used. In other studies, at-home bleaching technique did not increase the susceptibility of enamel to erosion. Considering the possibility that bleaching gels with high concentration of HP could increase the susceptibility of enamel to erosion, the addition of remineralizing ions into bleaching gels would be beneficial for preventing a further enamel demineralization.[81]

According to Karima Lubbadeh et al because of the decreased microhardness and mineral loss, bleached enamel can become more susceptible to acid erosion. Erosion, known to be the loss of enamel surface due to exposure to acidic chemicals that are non-bacterial, became more pronounced after bleaching, especially when bleaching materials with a lower pH were being used. Bleaching performed with low peroxide concentrations, such as 10% carbamide peroxide, did not increase enamel surface roughness, nor did it make tooth enamel more susceptible to erosion or brushing abrasion.[75]

In the present study, effects on the dental surface associated with erosive challenges, which simulate the action of acid substances present in food and beverages available for human consumption is analysed. In this study 1% citric acid which commonly present in acidic food and beverages was used as an erosive agent.

According to Karima Lubbadeh et al, overnight remineralization needed in artificial saliva after each bleaching cycle and 60 minutes of remineralization was allowed between treatment challenges during cycling, to mimic salivary remineralization that would take place in the oral cavity.[75]

In this study also the samples were kept in a solution simulating artificial saliva before the beginning of the bleaching treatment for 12 h, and during the intervals between bleaching gel applications.

In this study, bleached tooth treated with remineralizing agent produced lowest percentage of surface hardness loss after erosion than tooth bleached and treated without remineralizing tooth paste. Among the groups compared, the teeth treated with 10% carbamide peroxide with remineralizing agent produced lowest percentage of surface hardness loss after erosion than tooth bleached with 35% carbamide peroxide with remineralizing agent after erosion. Tooth surfaces treated with remineralizing agent produced lowest percentage of surface hardness loss after erosion than tooth surface treated without remineralizing agent (G2=46.13< G6=52.9< G1=59.55< G4=62.7< G3=63.21). G1 vs G2**,** G2 vs G3, G2 vs G4, G4 vs G6 were statistically significant.

Pretty and colleagues affirmed that the use of microhardness for analysing erosion should be complemented with profilometry in order to quantify the tissue lost from the eroded area.[21] In this study, roughness results showed that the groups submitted to an erosive challenge presented statistically higher values compared with the negative control (G5). In the present study, groups subjected to bleaching and erosive challenges differed from the negative control (G5). The highest linear roughness (Ra) (.321), volumetric roughness (Sa) (2.61) value obtained was for G3.

In the profilometry analysis results, all the groups of treatment G1, G2, G3, G4 and G6 did not differ statistically amongst themselves, but differed from G5 ($p<0.05$)

(Table 5). Linear profilometry analysis showed that G1 vs G5, G3 vs G4, G3 vs G5 and G5 vs G6 were statistically significant. In volumetric profilometry analysis showed that G1vs G3, G1 vs G4, G1 vs G5, G1 vs G6, G2 vs G3, G2 vs G4, G2 vs G5, G2 vs G6, G3 vs G4, G3 vs G5, G3 vs G6, G4 vs G5, G4 vs G6 and G5 vs G6 were statistically significant.

SEM studies have shown that tooth bleaching causes exaggerated enamel prism peripheries and some (described as mild to moderate) prism core.[21]

In this study, the images captured by SEM revealed morphological alterations in the superficial structure of enamel resulting from erosion either associated with bleaching treatment, with or without remineralizing agent and erosive challenge. The eroded areas were less visible in G2 when compared with the other groups, since the interprismatic structure is more prominent in this group. The impact of erosion after bleaching was calculated from the hardness after bleaching phase compared with the hardness after erosion phase. Thus, the real impact of erosion was higher observed in G3 (% SHL 63.21) and G4 (% SHL 62.7), which were different from themselves and from the other groups ($p<0.05$).

In this study, bleached tooth treated with remineralizing agent resulted in the lowest percentage of surface hardness loss after erosion when compared to bleached tooth treated without application of remineralizing agent. By the application of remineralizing agent we can reduce the effect of erosion occurring after bleaching. Among the bleaching agents, the highest concentration of bleaching agents produced more deleterious effect than the lowest concentration of bleaching agent. Hence within the limitation of the study it can be concluded that post bleaching application of remineralizing agent helps to decrease the effect of erosion occurring as a consequence of bleaching.

CONCLUSION

Within the limitation of this in vitro study, the following conclusions can be drawn.

1. Highest percentage of surface hardness loss after bleaching occurred in teeth bleached with 35% carbamide peroxide without application of remineralizing agent.
2. Among the two bleaching agents 35% carbamide peroxide produced the highest surface hardness loss than 10% carbamide peroxide.
3. By the application of remineralizing agent, the loss of surface hardness can be reduced.
4. The lowest percentage of surface hardness loss after erosion occurred in the group treated with 10% carbamide peroxide with remineralizing agent.
5. Erosion which usually occurs as a consequence of bleaching can be minimised by the application of remineralizing agent after bleaching.
6. The micro hardness values in all samples after bleaching showed significant reduction when subjected to an erosive challenge. Hence, use of acidic food and beverages must be reduced post bleaching and the same should be advised to the patient.

SUMMARY

One of the most popular cosmetic dental treatments for discoloured teeth is tooth bleaching. Vital tooth bleaching is a very conservative and effective treatment for intrinsic and extrinsic tooth discolouration. Studies have showed that bleaching gels containing carbamide peroxide are more effective than hydrogen peroxide. However aggressive whitening treatments can change the surface integrity and microstructure of the enamel crystals as well as increase the susceptibility to demineralization.

Increased intake of acidic food and beverages after bleaching can lead to increased erosion. Enamel surfaces that had undergone bleaching are more susceptible to acid erosion and this has been proved by various studies. Remineralization treatments with remineralizing agents like Remin Pro are capable of re-establishing the lost enamel surface hardness and alterations on the tooth surface caused by dental bleaching. The different concentrations of bleaching agents influence the effect of erosion that occurs after bleaching.

This study was done to evaluate and compare the changes in the surface microhardness and surface roughness upon the application of a remineralizing agent after the enamel surface was subjected to a bleaching and erosive challenge. 60 upper first premolar teeth were selected. These were then cut at the CEJ and then divided into 2 equal halves to make 120 sample size. These 120 samples were further divided into 6 groups of 20 samples each. These were then subjected to bleaching and erosion treatment. G1 and G2 were treated with 10% CP; G3 and G4 were treated with 35% CP. G2, G4 were then treated with Remin Pro remineralizing toothpaste for 15 days. G5 was kept as the negative control (without bleaching) and G6 was kept as a positive control (without bleaching with erosion).

G1, G2, G3, G4, G6 were then immersed in 1% citric acid solution for erosive challenge. G1, G2, G3, G4, G5, G6 were subjected to Vickers micro hardness analysis. Initial micro hardness was calculated. Microhardness after bleaching in G1, G2, G3, and G4 were calculated. Microhardness after erosion in G1, G2, G3, G4 and G6 were calculated. Loss of surface hardness loss (% SHL) after bleaching and after erosion was analysed. Surface profilometry analysis and SEM analysis were done in G1, G2, G3, G4, G5, and G6. Statistical analysis was done using One –Way ANOVA.

Results of the study showed that the highest % SHL after erosion occurred in samples treated with 35% CP without remineralizing agent. The lowest % SHL occurred in samples treated with 10% CP with remineralizing toothpaste. Surface profilometry analysis showed that the results did not differ much among the treated groups, but differed from the negative control. SEM analysis showed that G2 showed the lowest surface alteration than other groups.

Thus, the result of the study shows that the application of a remineralizing agent on teeth subjected to bleaching helps to reduce the effect of erosion which occurs after bleaching.

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ANNEXURE

ST.GREGORIOS DENTAL COLLEGE

UNDER THE MANAGEMENT OF MJSCE TRUST, PUTHENCRUZ

CHELAD, KOTHAMANGALAM, ERNAKULAM DIST, KERALA - 686681

ETHICAL CLEARANCE CERTIFICATE

SGDC/152/2017/1733/5

Date:- 20-10-2017

To,

Dr. Manju Krishna E M
St. Gregorios Dental College
Chelad, Kothamangalam

Dear Dr. Manju Krishna E M,

Subject:- Ethics Committee Clearance Reg.

Protocol -Evaluation of the effect of remineralizing agent on home and professionally bleached enamel surfaces subjected to erosion: An *in vitro* study

After the Institutional Ethics Committee (IEC) held on 20th of October 2017, this study was examined and discussed. After the consideration, the committee had decided to approve and grant clearance for the aforementioned study.

The members who attended the meeting at which the protocol was discussed were:-

1. Dr.CKK Nair - Former BARC scientist
2. Dr.Ommen Aju Jacob - Dean, St. Gregorios Dental College, Chelad
3. Dr.Cinu Thomas A - Scientist, Senior Lecturer, Department of Pharmaceutical Sciences Centre for Professional and Advanced Studies
4. Rev. Fr. Shanu K. Paulose
5. Lissy Jose – Former Member Women's Welfare Association
6. Adv. Jose Aranjani - Advocate
7. Dr.Sauganth Paul - Senior Lecturer, Department of Biochemistry, St.Gregorios Dental College
8. Dr.Eapen Cherian - Secretary
9. Dr.Jain Mathew - Principal and Head of the Department, Department of Conservative Dentistry and Endodontics.
10. Dr.George Francis - Head of the Department, Department of Prosthodontics Crown & Bridge
11. Dr.Binnoy Kurian - Head of the Department, Department of Orthodontics & Dentofacial Orthopaedics

Dr.CKK Nair
Chairman Institutional Ethics Committee
St.Gregorios Dental College, Chelad

Dr.Eapen Cherian
Secretary

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Initial surface microhardness (VHN) of the samples in kg/mm^2

Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
349	287.1	334.6	338.2	349.2	378.8
348.5	285.4	333.5	336.7	242.7	376.7
347.6	286.7	331.1	337.3	247.3	377.1
346.6	287.7	331.6	340.4	225.4	379.4
348.3	285.8	329.4	336.2	326.2	340.6
347.5	288.1	330.6	339.1	287.1	378.4
346.7	284.1	335	340.4	282.4	376.8
349.2	284.6	330.2	338.3	239.3	377.6
348.7	286.4	329.8	339.1	324.1	375.8
346.5	286.6	330.6	336.7	334.7	376
345.6	285.3	331.3	340.2	338.2	374.2
348.9	287.8	333.1	341.8	299.8	378.8
347.6	287.6	331.7	338.1	348.1	373.1
346.5	288.8	332.2	336.6	236.1	378.6
346.9	288.1	330.5	337.4	334.6	376.4
348.1	286.4	336.4	338.6	239.4	374.6
348.9	284.3	334.7	339.8	254.6	378.3
346.7	286.4	335.4	337.5	323.8	378.7
347.4	285.2	332.2	336.6	389.6	376.2
349.4	286.9	331.8	337.7	339.4	376.4

Final surface microhardness of the samples (VHN) after bleaching in kg/mm^2

Group 1	Group 2	Group 3	Group 4
255.43	199.4	176.7	194.7
253.83	203.3	175.8	195.9
254.74	198.8	174.9	195.7
253.52	197.7	173.5	196.2
256.35	201.4	174.3	194.3
258.43	202.5	175.2	193.4
252.43	203.3	175.4	193.6
253.32	201.2	176.3	195.6
254.97	204.5	176.2	194.53
253.72	199.72	174.9	193.52
254.82	198.97	177.8	196.25
256.32	197.46	176.7	193.51
256.46	201.75	173.6	193.61
252.52	202.5	174.5	194.31
253.75	204.8	176.3	196.32
253.43	198.46	174.4	192.61
252.5	197.75	176.3	191.71
253.8	202.46	178.2	197.81
252.9	201.82	176.1	194.32
251.1	198.91	174.9	193.4

Final microhardness of the samples (VHN) after erosion in kg/mm^2

Group 1	Group 2	Group 3	Group 4	Group 6
143	155.3	122.8	126.1	177.35
142.9	154.2	121.3	125.2	174.8
141.7	153.7	124.2	124.7	177
144.3	156.7	120.8	125.3	175.2
145.4	154.3	119.6	127.4	174.2
142.8	153.4	123.8	126.2	176.3
141.6	152.9	123	127.4	178.4
143.4	149.4	124.1	123.3	177.3
141.9	156.7	123.4	125.7	179.7
144.7	153.2	121.6	125.8	176.2
141.8	153.2	121.2	126.1	177.8
143.2	154.1	119.5	127.6	174.1
141.9	155.6	123.4	126.4	176.6
141.9	156.4	122.7	124.6	178.4
142.9	154.3	121.4	128.8	173.7
143.4	153.2	124.3	124.8	176.2
142.8	154.2	119.1	125.6	175.5
142.6	153.4	120.7	127.8	177.8
143.7	157.4	124.8	127.6	177.6
144.4	154.5	122.6	126.8	176.3

Surface Roughness Values

Ra (Linear surface roughness values in micrometer)

G 1	0.265	0.260
G 2	0.222	0.226
G 3	0.321	0.333
G 4	0.194	0.198
G 5	0.141	0.136
G 6	0.254	0.258

Sa (Volumetric surface roughness values in micrometer)

G 1	1.8	1.783
G 2	1.9	1.98
G 3	2.6119	2.78
G 4	1.8049	1.85
G 5	0.894	0.9
G 6	1.3626	1.432

LIST OF ABBREVIATIONS USED

(In Alphabetical Order)

No.	Abbreviations	Descriptions
1	ANOVA	Analysis of Variance
2	CEJ	Cemento-Enamel Junction
3	CP	Carbamide Peroxide
4	CT	Computerized Tomography
5	DEJ	Dentino Enamel Junction
6	HP	Hydrogen Peroxide
7	SHL	Surface hardness loss
8	SEM	Scanning electron Microscopy
9	VHN	Vickers Hardness Number