THE EFFECT OF VARYING CONCENTRATIONS OF HYDROGEN PEROXIDE ON BOVINE ENAMEL AND DENTINE

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ABSTRACT

The effect of varying concentrations of hydrogen peroxide on bovine enamel and dentine

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Background: Bleaching has become a popular treatment option for aesthetic management of discolored teeth. Bleaching is a conservative approach in aesthetic dentistry compared to other invasive restorative treatment modalities such as indirect veneers and crowns. While bleaching is successful and effective, several studies have documented structural changes such as increased roughness, decreased microhardness, and decreased enamel strength, as well as a decrease in mineral content (calcium, phosphate, and fluoride) and the emergence of clinical symptoms such as dentin hypersensitivity and gingival irritation.

Aim: The aim of this study was to investigate the effects of varying concentrations of hydrogen peroxide (HP) on bovine enamel and dentine in term of calcium, magnesium, and phosphorous ion release.

Materials and Methods: Bovine enamel and dentine discs were sectioned, prepared and treated with different concentrations of carbamide peroxide (CP) including 10%, 15%, and 20% CP, and 35% HP. Negative control group was saline solution, and the positive control group was vinegar (N=5). All solution samples were analyzed for ion release using a calcium, magnesium and phosphate meter.

Results: Treatment with 35% HP resulted in statistically significant increase in the amount of calcium and magnesium ions released from both enamel and dentin samples ($p<0.05$). Treatment with CP 10-20% caused statistically significant increase in the amount of magnesium and phosphate ions released from enamel and dentin samples ($p<0.05$).

Conclusions: From the results of this study, it can be concluded that tooth whitening with CP (10-20%) and high concentrations of HP (35%) can have detrimental effects on the structure of both enamel and dentin by increasing the dissolution of their mineral components.

DEDICATION

My dedication with a special feeling of gratitude to my husband, whose words of encouragement and push for tenacity ring in my ears. You have been my best cheerleader.

I dedicate this work and give special thanks to my loving Kids, who have supported me throughout this entire journey. I will always appreciate all you have done. You deserve a trip in Disney cruise ship!

I also dedicate this dissertation to my parents, my wonderful siblings and my grandmother for being there for me throughout the process.

DECLARATION

I declare that all the content of this thesis is my own work. There is no conflict of interest with any other entity or organization

Name: Nouf Alnaqbi

Signature: 

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1. INTRODUCTION

Tooth discoloration causes a slew of cosmetic issues, and the dental community and the general public spend a lot of time and money in an attempt to improve the esthetics of discolored teeth. Bleaching has been around since the late 1800s, and dentists have been experimenting with different approaches to whiten teeth and alter the shape of teeth. In 1961, one of the most widely known whitening procedures was suggested, but it could only be used on teeth that had undergone root canal treatment. The "walking bleach technique" was described as putting sodium perborate with water into the tooth and then covering it with a temporary restoration. Nowadays, dentists typically use a mixture of hydrogen peroxide and sodium perborate in a modified "rolling bleach" technique. The most popular whitening agents are sodium perborate, hydrogen peroxide, and carbamide peroxide. Since they are non-invasive and relatively easy to perform, the use of a variety of bleaching techniques has attracted the interest of the profession. Modern bleaching systems are mainly based on hydrogen peroxide or one of its precursors, most notably carbamide peroxide, and are often used in conjunction with an activating agent like heat or light. Bleaching agents may be used on the teeth externally (vital bleaching) or within the pulp chamber (internal bleaching) (non-vital bleaching). Both procedures tend to bleach the chromogens within the dentine, resulting in a change in the tooth's body color.

On the other hand, the effects of dental bleaching on the physical properties of enamel and dentin are still a source of debate.

2. REVIEW OF THE LITERATURE

2.1 Tooth Structure

The tooth is made up of 4 tissues, that are enamel, dentin, cementum, and pulp. The enamel, dentin, and cementum are relatively hard since they contain minerals, especially calcium, so they are calcified. Pulp is soft, and often not calcified or mineralized in the pulp cavity. The pulp cavity has a coronal portion (pulp chamber) and a root portion (pulp canal or root canal). Nerves and blood vessels enter the pulp through apical foramina.[1]

2.2 Structure of Enamel

Enamel is the hardest tissue in the human body. It is derived from the epithelium and forms the anatomical crown of a tooth. It is composed of 92–96% inorganic matter, and 4% of organic material and water. The mineral phase (inorganic matter) consists mainly of calcium phosphate in the form of hydroxyapatite crystals, which are carbonated or fluoridated. Enamel is highly mineralized, acellular, and avascular tissue.[2] Enamel thickness varies on the dental crown, being thickest on the buccal surfaces (about 2.5 mm) and thinner toward the cervix. Enamel is translucent and varies in color from yellowish to grayish white. The enamel microstructure consists of crystals arranged in prisms or rods, which run approximately perpendicular from the dentine-enamel junction towards the tooth surface. The interfacial area between prisms is proteinrich, and termed interprismatic enamel. Ameloblasts, or enamel-forming cells, eventually disappear as the development completes. Accordingly, enamel cannot be repaired or remodeled. An enamel defect or chipping/spalling damage is permanent. [3,4]

2.3 Structure of Dentine

Dentin may be described in two ways: extracellular dentin consists of a mineralized organic extracellular matrix, while functional dentin includes predentin and dentin-forming cells (odontoblasts) with their cytoplasmic processes penetrating mineralized dentin, and dentinal fluid (the biological entity). The mineral phase comprises approximately 70% of the weight percentage and 45% of volume, and the organic matrix about 20% and 33%, respectively, the remaining fraction being water. However, since water is located primarily in dentinal tubules, and the tubule diameter increases significantly from the dentin–enamel junction toward the pulp, these percentages are only average values. The water content—or wetness— of dentin is not uniform, but varies approximately 20-fold from superficial to deep dentin.[4 5]

2.4 Composition of enamel and dentine

Up to 40 elements have been reported to be present in enamel and dentine. The concentrations of most elements are found to be higher in enamel than dentin, which is making sense due to the high organic content of dentin, because the minor ionic components are often form the apatite crystal structure.[2] The main elements are Ca, Na, Cl, Zn and P. The calcium and phosphorus (as phosphate) content of the teeth range 34–39% and 16–18% by weight, respectively. Various cations and anions are incorporated into cationic (Ca^{2+}) and anionic centers (OH^-, $PO4^{3-}$) of the hydroxyapatite matrix. Sodium (Na^+), potassium (K^+), and magnesium (Mg^{2+}) can substitute in the calcium position, fluoride (F^-) and chloride (Cl^-) in the hydroxyl position and carbonate ($CO3^{2-}$) in the hydroxyl and phosphate positions. [5 6 7 8]

2.5 Tooth color change

Tooth color is closely associated with the structure of teeth, the total color effect resulting from absorbed and reflected light. An ideal method for color measurement should be reliable, show a high level of precision, be simple to use and of a reasonable cost for widespread clinical and laboratory use. Both the subjective and objective approaches for tooth color measurement have their advantages and limitations.[9] Visual shade matching is quick and simple to use, but the major disadvantage is the variation in results between different assessors. Visual stain assessment using various indices also has limitations due to its subjectivity.[10] With adequate knowledge and training, colorimetry and spectrophotometry methods are fairly easy to use and the assessment conditions such as lighting can be controlled. However, a disadvantage of these methods is the variability of results when measuring curved and translucent tooth surfaces. These methods usually entail measuring small sample areas and so may not give an adequate measure of the color of the whole tooth surface. Digital image analysis offers advantages, and this non-contact approach can objectively measure large surface areas of a tooth. Using this method images can be stored to allow subsequent analysis and repeat measurement. [4,11 12]

2.6 Tooth Discoloration

There has been a recent increase in interest in the treatment of tooth staining and discoloration. The correct diagnosis for the cause of discoloration is important as, invariably, it has a profound effect on treatment outcomes. It would seem reasonable, therefore, that dental practitioners have an understanding of the etiology of tooth discoloration in order to make a diagnosis and carry out the appropriate treatment. [13]

The methods available to manage discolored teeth range from removal of surface stain, bleaching or tooth whitening techniques and operative techniques to conceal the underlying discoloration, such as crowns and veneers.

Tooth discoloration has been classified according to the location of the stain, which may be either intrinsic or extrinsic. It may also be of good to consider one more category of internalized stain or discoloration.[14]

2.6.1 Intrinsic discoloration

Intrinsic discoloration occurs following a change to the structural composition or thickness of the dental hard tissues. The normal color of teeth is determined by the blue, green and pink tints of the enamel and is reinforced by the yellow through to brown shades of dentine underneath. [13]

Intrinsic discoloration can be classified into:

1. Metabolic causes
    a. Congenital erythropoietic porphyria
2. Inherited causes
    a. Amelogenesis imperfecta
    b. Dentinogenesis imperfecta
3. Iatrogenic causes
    a. Tetracycline staining
    b. Fluorosis
4. Traumatic causes
    a. Enamel hypoplasia
    b. Pulpal hemorrhagic products
    c. Internal root resorption
5. Idiopathic causes

6. Ageing causes

2.6.2 Extrinsic discoloration

Extrinsic staining can be direct or indirect. Direct staining occurs by compounds merged into the pellicle layer and producing a stain as a result of the basic color of the chromogens. Direct staining has a multi-factorial aetiology with chromogens derived from dietary sources or habitually placed in the mouth. These organic chromogens are taken up by the pellicle and the colour imparted is determined by the natural colour of the chromogen. Tobacco smoking and chewing are known to cause staining, as are particular beverages such as tea and coffee. The colour seen on the tooth is thought to be derived from polyphenolic compounds which provide the colour in food.[13]

Indirect staining occurs where there is chemical interaction at the tooth surface with another compound that produces the stain. Indirect extrinsic tooth staining

associated with cationic anti-septics and metal salts. The agent is without colour or a different colour from the stain produced on the tooth surface.

Extrinsic tooth discolouration has usually been classified according to its origin, whether metallic or non-metallic. Non-metallic stains: The non-metallic extrinsic stains are adsorbed onto tooth surface deposits such as plaque or the acquired pellicle. The possible aetiological agents include dietary components, beverages, tobacco, mouthrinses and other medicaments. Chromogenic bacteria have been cited in children. Metallic stains: Extrinsic staining of teeth may be associated with occupational exposure to metallic salts and with a number of medicines containing metal salts. The characteristic black staining of teeth in people using iron supplements and iron foundry workers is well documented. Copper causes a green stain in mouthrinses containing copper salts and in workers in contact with the

metal in industrial circumstances. A number of other metals have associated colours such as potassium permanganate producing a violet to black colour when used in mouthrinses; silver nitrate salt used in dentistry causes a grey colour, and stannous fluoride causes a golden brown discoloration.

The most commonly used procedure to treat extrinsic stains is by a professional hygiene treatment and by polishing tooth surfaces with prophylactic cups and more or less aggressive abrasive pastes. [13]

2.6.3 Internalized discoloration

Internalized discoloration is the incorporation of extrinsic stain within the tooth substance following dental development.[13] It occurs in enamel defects and in the porous surface of exposed dentine. The ways by which pigments may become internalized are:

1. Developmental defects
2. Acquired defects
    a) Tooth wear and gingival recession
    b) Dental caries
    c) Restorative materials

2.7 Bleaching

Over the past three decades, bleaching has become one of the most popular esthetic dental treatments. Since the 1800s, the initial focus of dentists in this area was on in-office bleaching of non-vital teeth that had discolored as a result of trauma to the tooth or from endodontic treatment. By the late 1980s, the field of tooth whitening dramatically changed with the development of dentist-prescribed, home bleaching

(tray bleaching) and other products and techniques for vital tooth bleaching that could be applied both in the dental office and at home. [15,16]

2.7.1 Vital bleaching

Vital tooth bleaching refers to the clinical application of a chemical solution to a tooth surface in order to achieve a lightening effect.[17] It has been found to be effective, however, relapse does occur. The literature suggests that bleaching agents may have transient effects on the tooth itself and may affect some dental materials. [18]

In vital dental bleaching, *Hydrogen peroxide (*HP) is the active ingredient, which is commonly delivered as HP or *Carbamide Peroxide* (CP) in commercial agents. CP is a stable complex that breaks down in contact with water to release HP and urea and thus, the chemistry of these agents is that of HP. [19,20]

2.7.2 Non-vital bleaching

Various methods to bleach non-vital teeth have been considered. Three techniques were described through the literature: the walking bleach technique, the thermocatalytic technique, the inside/outside bleach technique and the in-office bleaching procedure. The most recommended one is the walking bleach technique since it is simple, safe, with lower risks and suitable for patients and dentists.[21]

Different bleaching agents were used such as sodium perborate, hydrogen peroxide and carbamide peroxide with various concentrations.[22 23]

Walking Bleach Technique

In the walking bleach technique, the mixture of sodium perborate and water should be left in the pulp cavity for a few days, and the access cavity can be sealed with

provisional cement. The use of 30% hydrogen peroxide instead of water can improve the bleaching effectiveness of the mixture. There are numerous studies that have reported on the successful use of the walking bleach technique for correction of severely discolored teeth.[24] [23]

Thermocatalytic Technique

This technique has been proposed for many years as the best technique to bleach nonvital teeth because of the strong interaction between hydrogen peroxide and heat. Moreover, a common clinical technique is to use 30%–35% hydrogen peroxide placed in the pulp chamber between appointments. [25]

Preparation of the access cavity in this technique is the same as all the preparation procedures when using the walking bleach technique. However, this technique involves placement of 30%–35% hydrogen peroxide in the pulp chamber followed by heat application by electric heating devices or specially designed lamps. [23]

In-office Technique

Some authors have described the successful clinical use of external bleaching of nonvital root-filled teeth with carbamide peroxide gels or hydrogen peroxide at high concentrations (15%–35%). The whitening gel is applied by means of a bleaching tray and is placed directly on the tooth, which is isolated with rubber dam or with other techniques, without an access opening. The in-office procedures can also be used when the walking bleach technique does not produce satisfactory results after 3– 4 applications.[26-28] [23]

Inside/outside bleach technique

In this technique, the bleaching gel is placed on the internal and external aspects of the discolored root-filled tooth. The access cavity is left open during treatment so that the 10% carbamide peroxide can be easily and regularly changed. A custom-made bleaching tray keeps the bleaching agent in and around the tooth.[29] [23]

2.8 Mechanisms of bleaching

Basically, the bleaching process involves oxidation, which consists of a chemical process where the organic materials are converted to carbon dioxide and water. The pigments are complex compounds, with large amounts of carbon molecules, which are broken and converted into intermediate compounds (smaller carbon chains), resulting in lighter teeth.[30]

The mechanism of action of bleaching agents is based on the release of reactive forms of oxygen, as a function of the interaction of hydrogen peroxide with tooth structure. During the bleaching the carbon chains are transformed into CO_2 and H_2O, being gradually released together with the nascent oxygen. The point of saturation is the moment in which the maximum bleaching occurs, from that stage the pigments are no longer bleached. This fact is of high clinical relevance because the excessive use of high concentrations and prolonged times can cause undesirable damages to the dental structure. It can cause degradation of the crystalline structure of the enamel, which occurs when the bleaching agent begins to act on other carbon compounds, such as enamel matrix proteins. [20]

The bleaching agents that are most commonly used for whitening teeth are hydrogen peroxide, carbamide peroxide, and sodium perborate. Hydrogen peroxide (H_2O_2) is the active ingredient in currently used tooth bleaching materials, can be used at different concentrations from 5%–35%. The most common concentration of

hydrogen peroxide is 35%. It might be applied directly or can be produced by a chemical reaction from carbamide peroxide or sodium perborate. [31]

Hydrogen peroxide is an oxidizing agent that produce free radicals (H_2O+O_2), which are very reactive, and somehow acidic. The result is the perhydroxyl (HO_2) that is the most potent free radical. To be able to promote the formation of the ion perhydroxyl, hydrogen peroxide needs to become alkaline, which means that the pH should be about 9.5 to 10. In the ionization of hydrogen peroxide buffered by this pH, a large amount of H_2O perhydroxyl free radicals are found, which result in a higher bleaching effect in the same amount of time. [4]

At a high concentration hydrogen peroxide is caustic, burns tissues on contact, and can release free radicals. High-concentration solutions must be handled with care because they are thermodynamically unstable and might explode unless refrigerated and kept in a dark container. Because of its low molecular weight, this substance can penetrate dentin and can release oxygen that breaks the double bonds of the organic and inorganic compounds inside the dentinal tubules. The breakdown of hydrogen peroxide into active oxygen is accelerated by application of heat, the addition of sodium hydroxide, or light. [32]

Carbamide peroxide is an organic white crystalline compound and is formed by urea and hydrogen peroxide and used in different concentrations. It is decomposed in a hydrophilic environment into hydrogen peroxide (10% carbamide peroxide produces 3.6% hydrogen peroxide and 7% urea). Currently, the most popular commercial bleaching preparations containing carbamide peroxide usually also include Carbopol or glycerin base at different concentrations because this makes it more chemically stable compared with hydrogen peroxide. 10% carbamide peroxide bleaching agents also demonstrated higher antibacterial effect than a 0.2% chlorhexidine solution.

Sodium perborate is an oxidizing agent available as a powder. It is stable when dry; however, in the presence of acid, warm air, or water, it breaks down to form sodium metaborate, hydrogen peroxide, and nascent oxygen. Sodium perborate is easier to control and safer than concentrated hydrogen peroxide solutions.

Hydrogen peroxide decomposes over time, especially in the presence of catalytically active compounds like metal ions, noble metals (Pt), and enzymes (catalase), according to.

(I) $H_2O_2 \rightarrow H_2O + {}^{1}/_{2}O_2$.

The released oxygen molecule can act as an oxidant. However, the normal oxidative action of hydrogen peroxide depends on the pH value:

(II) $H_2O_2 + 2H^+ + 2e^- \rightarrow 2H_2O$, or

(III) H O $+2e^- \rightarrow 2OH^-$. 22

This also happens after hydrolysis of peroxide-precursor compounds that release hydrogen peroxide:

(IV) $MgO_2 + 2\ H_2O \rightarrow Mg\ (OH)_2 + H_2O_2$ (magnesium peroxide).

(V) $CaO_2 + 2\ H_2O \rightarrow Ca\ (OH)_2 + H_2O_2$ (calcium peroxide).

(VI) $Na_2(CO_4) + H_2O \rightarrow Na_2CO_3 + H_2O_2$ (sodium percarbonate).

(VII) $CO(NH_2)_2 \cdot H_2O_2 \rightarrow CO(NH_2)_2 + H_2O_2$ (carbamide peroxide, an adduct of urea and hydrogen peroxide, contains about 36 wt% hydrogen peroxide).

1.8.1 The effect of hydrogen peroxide on the enamel and dentine.

The literature showed that a longer application time, reapplication, and light activation increases HP diffusion, but the concentration of the product is not correlated to the amount of diffused HP.[33]

Majority of studies indicate that HP and CP containing products have no significant deleterious effects on enamel and dentine surface morphology and chemistry, Surface microhardness SMH, subsurface enamel and dentine microhardness or ultrastructure, even if one of the highest concentrations of HP or CP are used. [34,35]

Also, in vitro studies indicate that HP and CP containing products have no significant clinically relevant effects on subsequent enamel and dentine loss caused by acidic erosive challenges, toothpaste abrasion or caries lesion formation. [35,36 37]

2.8.2 Ions release and how to measure it.

It's known that the main forming elements of the crystals of hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$]. The chemical characterization of dental tissues can be investigated by either Energy Dispersive X-Ray Spectroscopy (EDS) Tool which is a chemical microanalysis technique used in conjunction with Scanning Electron Microscopy (SEM), or calcium, magnesium and phosphate meter (portable photometer). The EDS technique detects x-ray emitted from the sample during bombardment by an electron beam to characterize the elemental composition of the analyzed volume. On the other hand, the photometer offers a superior optical system that utilizes a reference detector and narrow band interference filters for extremely fast and repeatable measurements, and it is used for the measurement of specific eleme4nts in water.[38]

3. AIM

The aim of this study was to investigate the effects of varying concentrations of hydrogen peroxide (HP) on bovine enamel and dentine.

Specific objective of the work was to investigate:

Calcium, magnesium, and phosphorous ion release from bovine enamel and dentine following exposure to varying concentrations of HP along with negative (Saline) and positive (white vinegar) controls.

4. MATERIALS AND METHODS

4.1. Dentin and enamel specimen preparation

Intact bovine incisors were extracted, washed and cleaned prior to storage in distilled water for 3 months. The source of bovine incisors was a bull skull obtained from butcher shop (figure 1). The teeth were randomly selected for ion release measurement. Each tooth was sectioned, and the enamel and dentine were separated using a dental diamond bur (figure 2). From each tooth 5 enamel and 5 dentine samples each measuring 2mm × 2mm × 1.5mm were cut and prepared. A total of 30 enamel samples divided into six equal groups. Each group contained a total of five samples (N=5). The 30 dentine samples were divided in the same way.

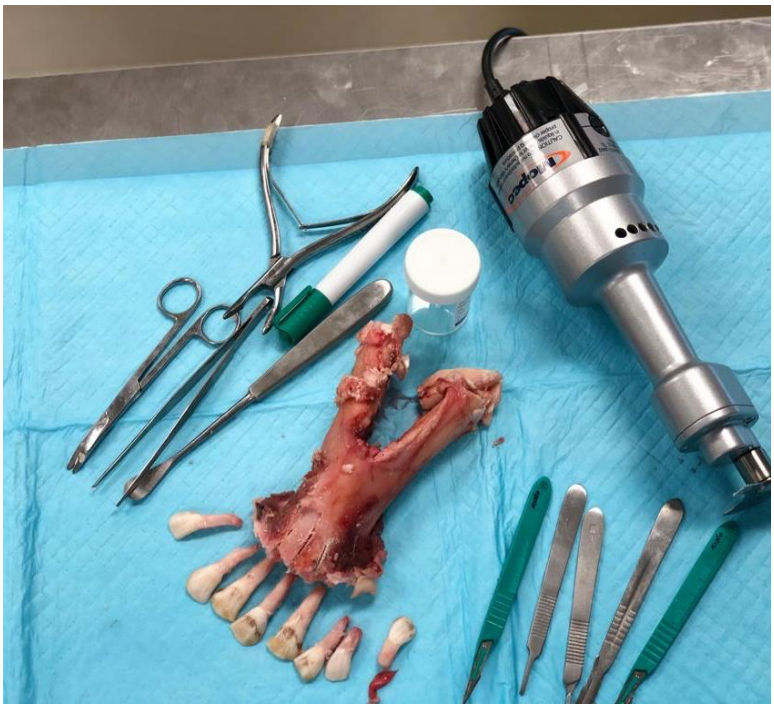


Figure 1: Teeth after extracted from a bull jaw.

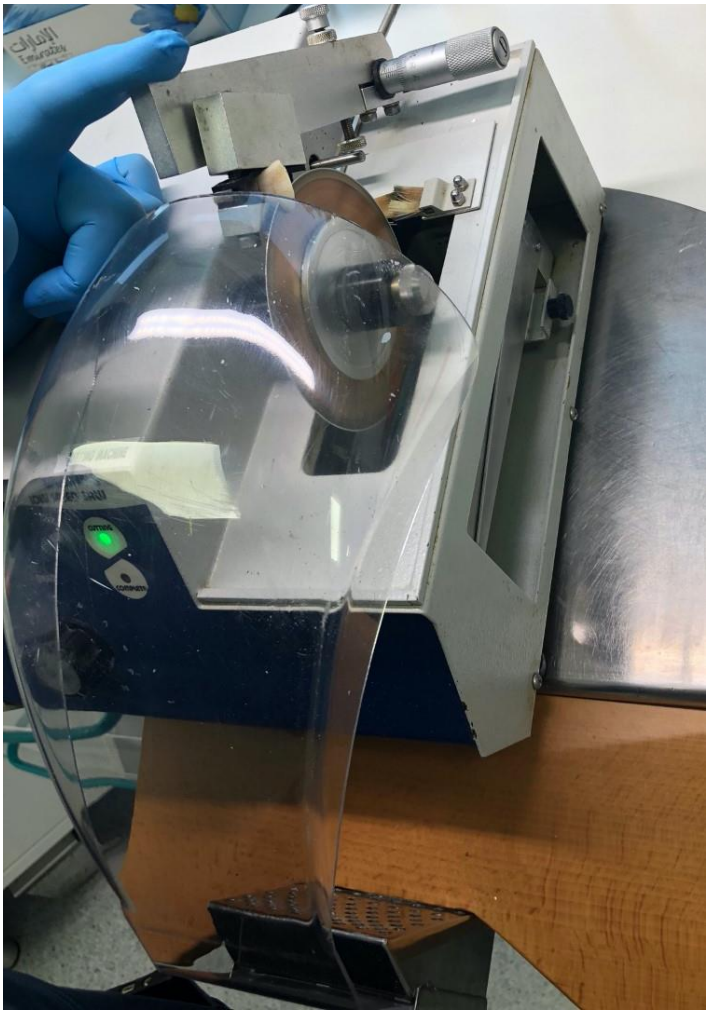


Figure 2: Teeth sectioning using dental diamond bur.

4.2. Bleaching protocol

Commercially available carbamide peroxide (CP) bleaching gels (Opalescence PF Melon Patient Kits USA) of different concentrations (10%, 15%, 20%) as well as a 35 % HP gel (Opalescence Endo Kit USA) were used for this work. Normal saline was used as the negative control and white vinegar was used as the positive control (figure 3).

The samples were immersed in 0.5ml of 10% CP, 15% CP, 20% CP or 35 % HP for 24 hours at 37°c in a labelled centrifuge tube with all surfaces exposed (figure 4). Negative control group was normal saline solution, and the positive control group was vinegar. After 24 hours, 0.5ml of each bleaching gel sample was diluted with 14.5ml deionized water to prepare a total of 15ml solution that was required for the analysis by the ion meters. For the calcium, 3ml of the sample was required; for the magnesium, 0.5ml; and for phosphate measurement, 10ml of the sample was required. Accordingly, each acquired readings for the samples in bleaching gel groups was multiplied by 30 to represent the actual amount of ions released into 0.5ml solution.

Figure 3: Opalescence PF Melon Patient Kits and Opalescence Endo Kit.

Figure 4: Incubator.

4.3. Ion release measurement

All samples were analyzed for ion release into the solution using a calcium, magnesium and phosphate meter (Hanna portable photometer II 1, Woonsocket, Rhode Island, USA) (figure 5). For each analysis, the instrument performed five measurements. Thus, with the five samples tested in each group, the total number of measurements recorded per element was 25 for enamel and 25 for dentine samples.

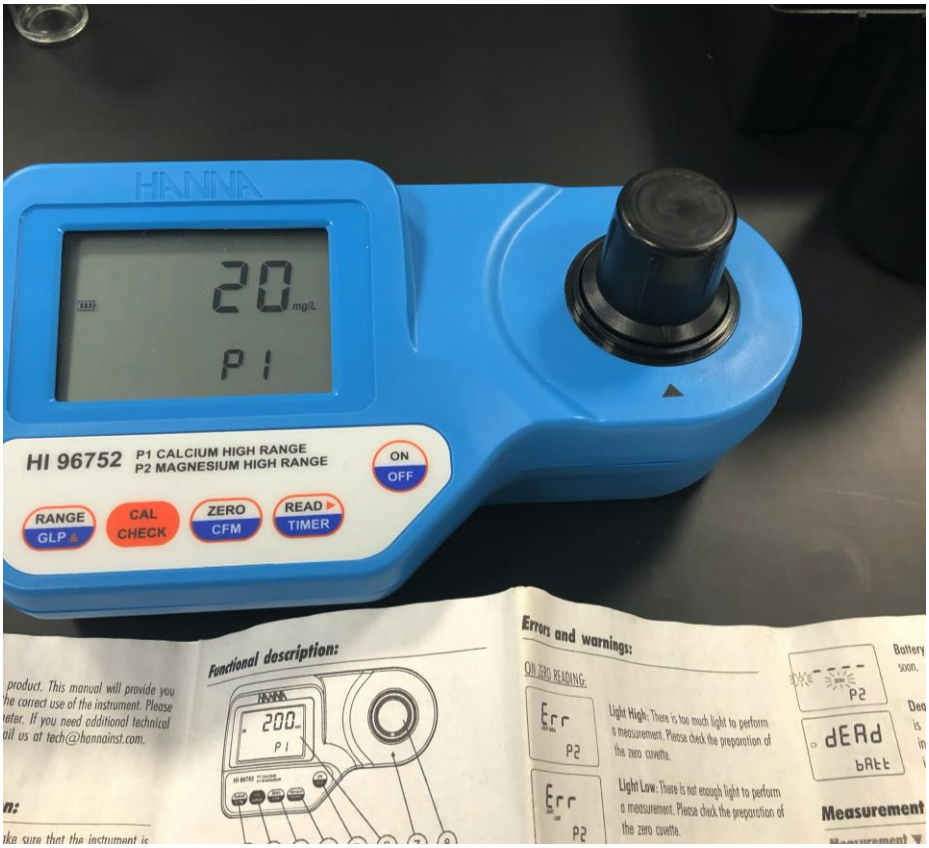


Figure 5: Hanna portable photometer II 1

4.4. Statistical Analysis

Data was entered into the computer using IBM-SPSS software for Windows version 25.0 (SPSS Inc., Chicago, IL). Kolmogorov-Smirnov test was used to test the normality of continuous variables. The continuous measurements in different groups were described by Median and Interquartile. When comparing the means between more than two groups Kruskal-Wallis test was used. A *p*-value of less than 0.05 was considered significant in all statistical analyses.

5. RESULTS

5.1. Enamel Results

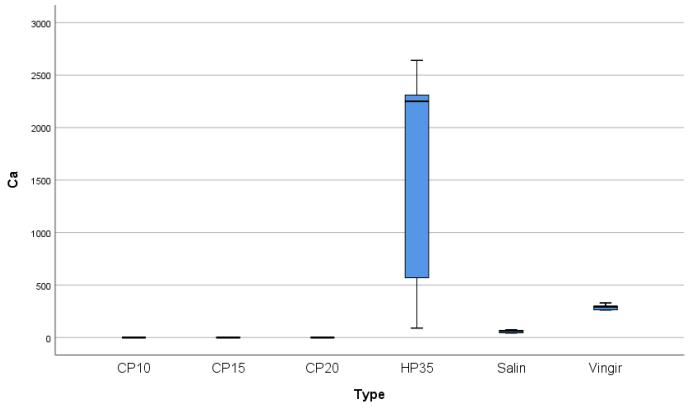
The results of ion release from the enamel sample groups treated with different concentrations of CP and HP have been summarized in Table 1 and graphically demonstrated in Figures 1 and 2.

Treatment with 35% HP resulted in statistically significant increase in the amount of calcium and magnesium ions released from enamel samples ($p<0.05$). Treatment with CP 10-20% caused statistically significant increase in the amount of magnesium and phosphate ions released from enamel samples ($p<0.05$).

Table 1: Comparison of Calcium (Ca), Magnesium (Mg), and Phosphate (P) release in different concentrations of HP in addition to negative and positive controls

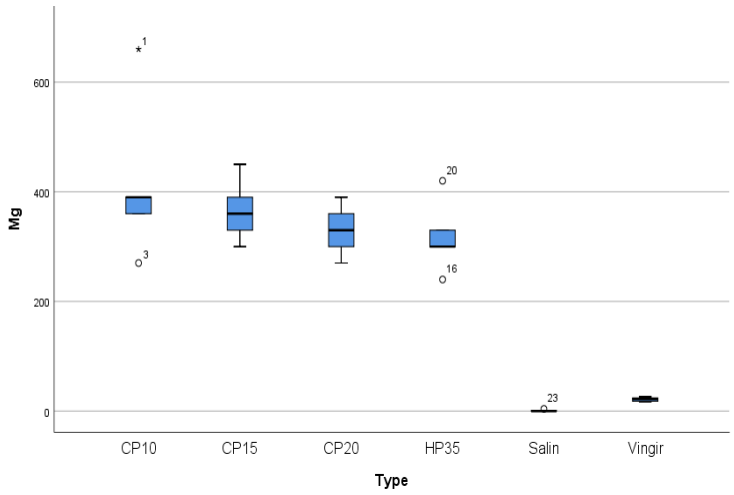
	Ca (mg/L)	Mg (mg/L)	P (ppm)
Concentrations	Median [IQ]	Median [IQ]	Median [IQ]
CP10	0	390[315-525]	37.8 [17.7-42.9]
CP15	0	360 [315-420]	34.8 [22.65-40.8]
CP20	0	330 [285-375]	32.7 [14.4-38.7]
HP35	2250 [330-2475]	300 [270-375]	1.8 [0.6-8.85]
Saline	63 [44-70]	0 [0-2.5]	0 [0-0.015]
Vinegar	294 [263-314.5]	22 [17.5-25]	0 [0-0.035]
P-value	< 0.001	0.001	0.004
Total	21.5 [0-271.5]	300 [21-367.5]	4.5 [0-35.85]

Ca (mg/L)



Box plot : In descriptive statistics, a boxplot, also known as a box-and-whisker diagram or plot, is a convenient way of graphically depicting groups of numerical data through their five-number summaries (the smallest observation, lower quartile (Q1), median (Q2), upper quartile (Q3), and largest observation).

Mg (mg/L)



Phosphate (ppm)

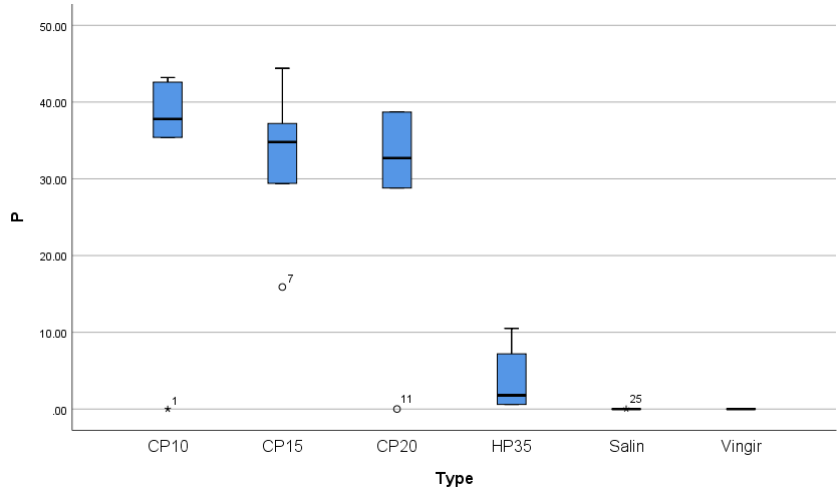


Figure 6: Error graphs of the distribution of Ca, Mg, P release in different concentrations of HP in addition to negative and positive controls

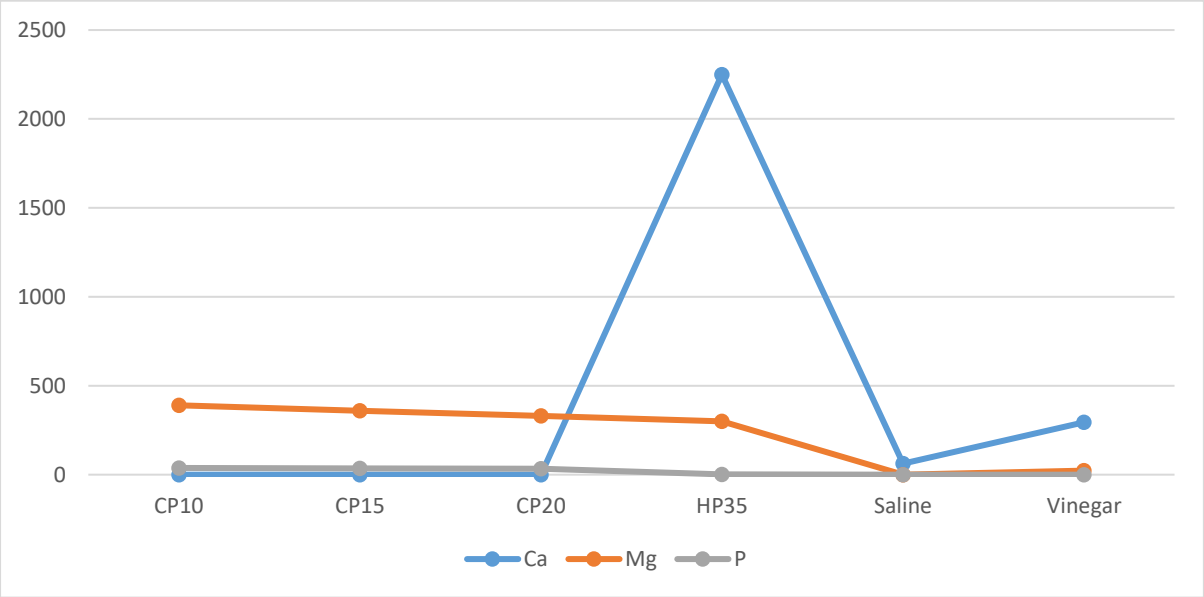


Figure 7: Trend of Ca, Mg, P release in different concentrations of HP in addition to negative and positive controls. X axis displays the different concentrations of CP (10%, 15%, 20%) & HP (35%) in addition to the controls (saline & vinegar), whereas the Y axis displays the amount of each ion released (Ca (mg/L), Mg (mg/L), & P (ppm)).

5.2. Dentin Results

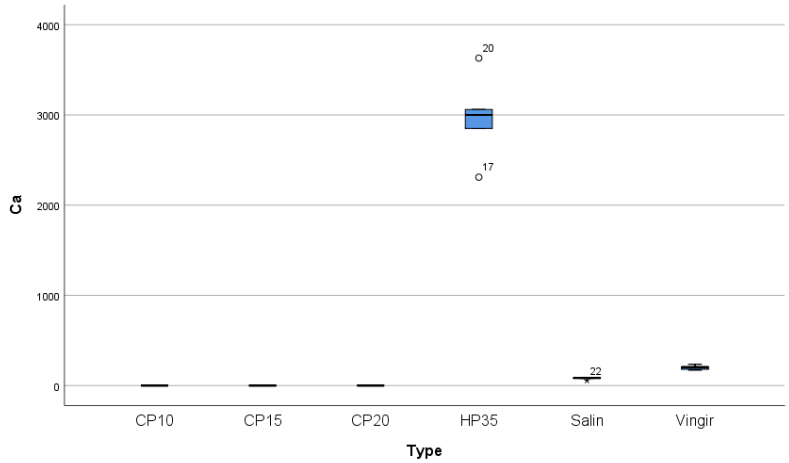
The results of ion release from the dentin sample groups treated with different concentrations of CP and HP have been summarized in Table 2 and graphically demonstrated in Figures 3 and 4.

Treatment with 35% HP resulted in statistically significant increase in the amount of calcium and magnesium ions released from dentin samples ($p<0.05$). Treatment with CP 10-20% caused statistically significant increase in the amount of magnesium and phosphate ions released from dentin samples ($p<0.05$).

Table2: Comparison of Ca, Mg, P release in different concentrations of HP in addition to negative and positive controls

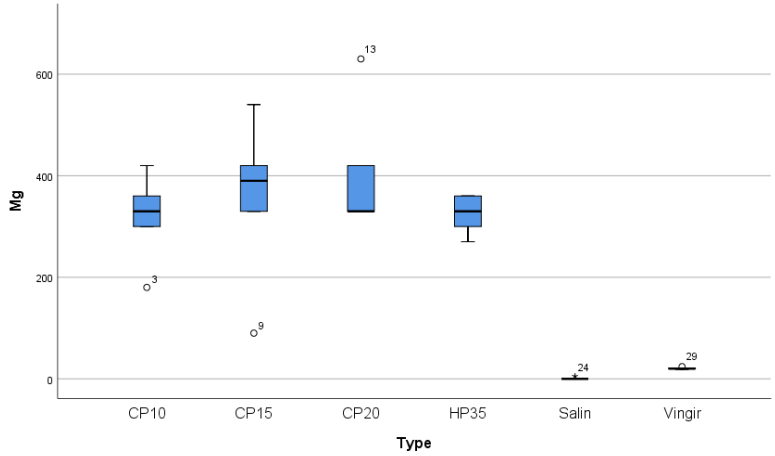
	Ca (mg/L)	Mg (mg/L)	P (ppm)
Concentrations	Median [IQ]	Median [IQ]	Median [IQ]
CP10	0	330 [240-390]	44.64 [39-51.6]
CP15	0	390 [210-480]	47.7 [43.05-51.3]
CP20	0	330 [330-525]	44.4 [40.95-47.1]
HP35	3000 [2580-3345]	330 [285-360]	0 [0-4.95]
Saline	84 [68-87]	0 [0-2.5]	0 [0-0.01]
Vinegar	204 [176-221.5]	20 [19.5-22.5]	0 [0-0.025]
P-value	<0.001	0.001	<0.001
Total	28 [0-204.75]	315 [20-360]	21.45 [0-45]

Ca (mg/L)



Box plot : In descriptive statistics, a boxplot, also known as a box-and-whisker diagram or plot, is a convenient way of graphically depicting groups of numerical data through their five-number summaries (the smallest observation, lower quartile (Q1), median (Q2), upper quartile (Q3), and largest observation).

Mg (mg/L)



Phosphate (ppm)

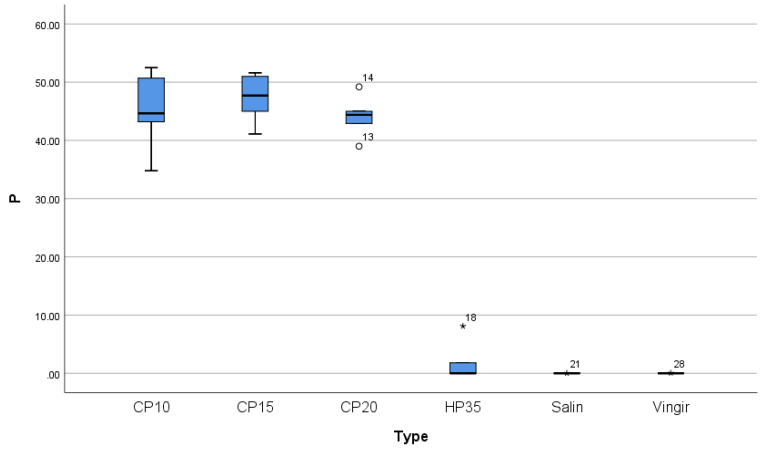


Figure 8: Error graphs of the distribution of Ca, Mg, P release in different concentrations of HP in addition to negative and positive controls

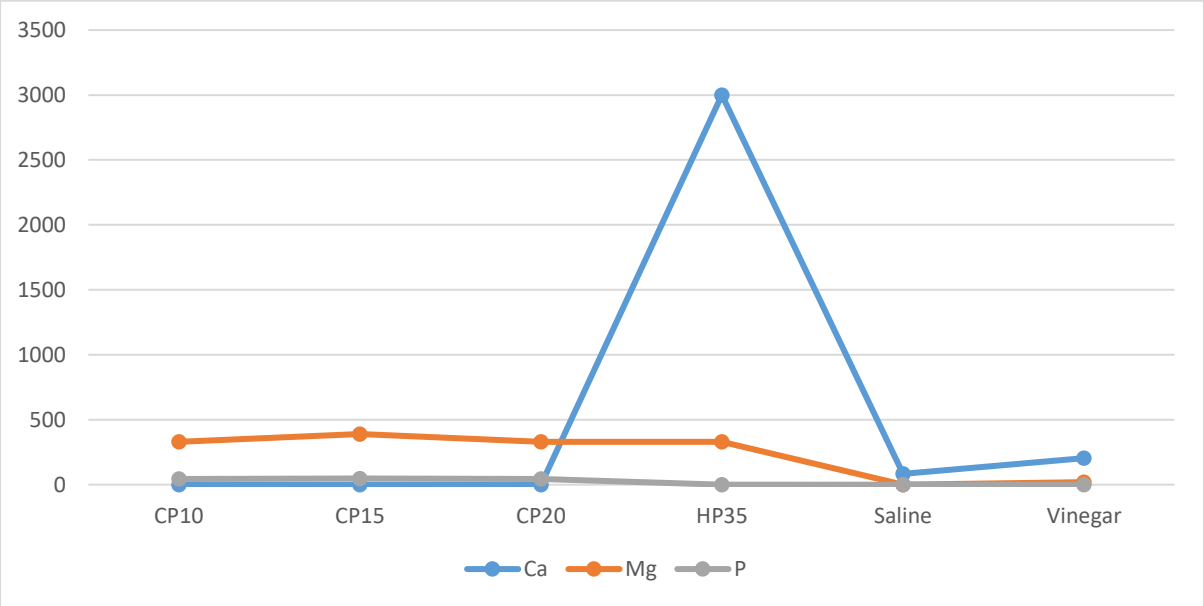


Figure 9: Trend of Ca, Mg, P release in different concentrations of HP in addition to negative and positive controls. X axis displays the different concentrations of CP (10%, 15%, 20%) & HP (35%) in addition to the controls (saline & vinegar), whereas the Y axis displays the amount of each ion released (Ca (mg/L), Mg (mg/L), & P (ppm))

6. DISCUSSION

The bovine tooth model used in this research is a reliable tool in the scientific community and has been used in many studies as a replacement for human teeth. In several comparative studies of the most varied analyzes, the physical, chemical, and ultrastructural properties of bovine and human enamel were evaluated, and structural similarity was stated. [7] [39] [40]

This study aimed to evaluate the effects of varying concentrations of hydrogen peroxide on bovine enamel and dentine in term of Ca, Mg, and P ions release. This is the first study to compare the release of these specific ions after treatment of dentin and enamel with different concentrations of commercially available CP and HP.

In our study, treatment with 35% HP resulted in statistically significant increase in the amount of calcium and magnesium ions released from both enamel and dentin samples ($p<0.05$). Treatment with CP 10-20% caused statistically significant increase in the amount of magnesium and phosphate ions released from enamel and dentin samples ($p<0.05$).

Absence of Ca ion detection in the groups treated with different concentrations of CP (10%, 15%, & 20%) can be explained by either no actual Ca release, or Ca binding with the components of the bleaching gels which will require further investigation in the future studies.

The impact of tooth bleaching on enamel and dentine properties is a contentious subject. While some studies [41] [42] [43] found no major changes in the enamel and dentine properties, other studies [34] [44] found that bleaching causes changes in the enamel surface and bulk properties. The majority of the recorded changes are most

likely due to the low pH and oxidative effect of bleaching products that decrease the hydroxyapatite and proteins quantityes.[45] Microhardness[46] [47], surface morphology[44] [46], and wear resistance[34] all suffer as a result of these changes. Optical properties and bacterial adhesion can also be affected by morphological changes in enamel.

Even when the highest concentrations of HP or CP are used, the vast majority of studies show that HP and CP-containing products have no substantial negative effects on enamel and dentine surface morphology and chemistry, SMH, subsurface enamel and dentine microhardness, or ultrastructure. Furthermore, in vitro studies show that HP and CP-containing products have no clinically important effects on subsequent enamel and dentine degradation caused by acidic erosive challenges, toothpaste abrasion, or the development of caries lesions.

Because of the action of its by-products, such as urea and oxygen, carbamide peroxide can cause changes in the mechanical properties of the enamel. Free radicals of hydrogen peroxide, one of the components that degrades carbamide peroxide, have no specificity and react with and degenerate dental tissues[44] [45]. Other tests that used CP 10% and followed the manufacturer's guidelines or the normal application time confirmed improvements in the bleached enamel's microhardness values. The decrease in microhardness values is due to peroxide action on the enamel organic matrix, which degrades and makes the enamel more fragile[46] [48]. As a result, repeated use of the bleaching gel for a prolonged period induces a greater deterioration of the organic matrix of the enamel, weakening the structure and making it more fragile.

Many studies have looked at the impact of dental bleaching on enamel chemical composition by evaluating changes in constituent enamel elements.[49-56] Al-Salehi et al. (2007) discovered that tooth-bleaching agents can harm tooth structure by

demonstrating that ion release from both enamel and dentin increased with increasing hydrogen peroxide concentrations, and that enamel microhardness decreased significantly with bleaching. Furthermore, Efeoglu et al. (2005) evaluated the impact of 10% carbamide peroxide applied to enamel using micro-computerized tomography. According to the findings, this causes demineralization of the enamel to a depth of 50 lm below the enamel surface. As a result, they suggested that the use of bleaching agents in patients who are vulnerable to caries and tooth wear be carefully considered. Rotstein et al. (1996) and Tezel et al. (2007) showed that a concentrated bleaching agent induced a substantial loss of calcium from the enamel surface in two additional studies. Cakir et al. (2011) concluded in a more recent study that the use of home bleaching agents (10%, 20%, and 35% carbamide peroxide) could influence the chemical composition of dental hard tissues, whereas the carbamide peroxide concentration of the bleaching systems used had no effect on the chemical composition of enamel and dentin.

Goo et al. (2004), on the other hand, showed that mineral degradation caused by dental bleaching does not pose a risk to teeth. Furthermore, Lee et al. (2006) found that the amount of calcium lost from teeth after a 12-hour bleaching procedure was equivalent to that lost from teeth exposed for a few minutes to a soft drink or juice. Changes in the chemical composition of enamel were found to be minor and not clinically relevant in these studies.

In comparison to enamel, little research has been done on the effect of dental bleaching on dentin structure. SEM was used by Zalkind et al. (1996) to demonstrate alterations in dentin surface morphology.[57] Pecora et al. (1994) discovered that after 72 hours of exposure to a 10% carbamide peroxide agent, dentin microhardness decreased.[58]

Basting et al. (2003) found that the thickening agent (carbopol and/or glycerin), not only the 10% carbamide peroxide, was responsible for the reduction in dentin microhardness.[59] Furthermore, Tam et al. (2005) found that direct exposure to 10% carbamide peroxide reduced the flexural intensity and flexural modulus of bovine dentin significantly.[60] Faraoni-Romano et al. (2008) investigated the effects of low and high-concentration bleaching agents on the microhardness and surface roughness of bovine enamel and root dentin, concluding that although bleaching had no impact on enamel microhardness or surface roughness, it did affect root dentin microhardness, which appeared to be dependent on the bleaching agent used.[61] Furthermore, Lewinstein et al. (1994) discovered that dentin's microhardness decreased after exposure to a 30% hydrogen peroxide solution at pH 3, while Tam et al. (2007) showed that dentin's in vitro fracture resistance was reduced after repeated use of bleaching products applied directly to dentin.[36,62] Engle et al. (2010) investigated the impact of the relationship between bleaching, corrosion, and dentifrice abrasivity on enamel and dentin in another study.[63] They reported that bleaching enamel with 10% carbamide peroxide did not increase erosive or abrasive wear. However, depending on erosive and abrasive challenges, it can alter dentin abrasive wear.

Reis et al (2011) conclude that a 35% hydrogen peroxide gel for in-office bleaching preferably should be applied in three 15-minute applications because 1 x 45 minutes reduces the bleaching speed and slightly increases the intensity of tooth sensitivity.[64]

Kelly et al (2019) found that as the bleaching agent application time increased, Mg levels decreased. This element's reduction can be attributed to the fact that it is one of the first elements to be extracted during the peroxide reaction with the dental surface.[38] As a consequence, this event may suggest a demineralization phase in

bleached enamel.[50] There was also a decline in Ca levels, but there was no statistically significant difference. A statistically significant decrease in P was observed, suggesting a decrease in the levels of these two elements. A decrease in Ca concentration levels, as well as the Ca and P ratio, was observed for bleached enamel in another study using CP 10%.[65] The Ca-P relationship is a significant predictor of the remineralization phase.[50] The drop in the levels of the two elements could result in an irreversible change, preventing the remineralization process.[19]

The limitations of this study included limited sample size and the fact that only one short-term storage time with the bleaching agent was tested in this study.

It is recommended for the future studies to investigate extended bleaching times and protocols and assess the release of other elements such as fluoride, as well as to investigate the effects of other protective agents that may have potential to reduce and prevent the ion release following tooth bleaching.

7. CONCLUSIONS

From the results of this study it can be concluded that tooth whitening with CP (10-20%) and high concentrations of HP (35%) can have detrimental effects on the structure of both enamel and dentin by increasing the dissolution of their mineral components. It is important that dentists have a thorough understanding and knowledge of the effects of different bleaching agents and protocols on the tooth structure and therefore, take appropriate precautions and use appropriate and safe methods of tooth whitening to minimize the risk of damage to the tooth structure during aesthetic treatment.

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