Comparative Evaluation of Enamel Mineral Content after Bleaching by in-Office, at-Home and Combined Techniques- Energy Dispersive X-Ray Analysis

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Abstract: ***This study compares the enamel mineral content after bleaching using In-Office, At-Home and Combined Techniques, utilizing a Scanning Electron Microscope (SEM) equipped with Energy Dispersive X-Ray Analysis (EDX). Fifty-six human premolar teeth were divided into four groups, each subjected to different bleaching techniques. Results indicated that In-Office bleaching led to the most significant loss of calcium and phosphorus, while At-Home and Combined techniques showed less mineral reduction. Statistical analysis confirmed significant differences in mineral content across the groups***

Keywords: Combined Bleaching Technique; 25% hydrogen peroxide; 10% carbamide peroxide; SEM-EDX; enamel mineral content

1. Introduction

Patients' demands for esthetic treatments have increased over the recent years. Bleaching is the most conservative treatment for discolored teeth compared to porcelain veneers, resin-bonded composites and crowns, because of its simple technique, clinical efficacy and non-invasive nature, requiring no sound dental structure removal. Dental bleaching can be performed using In-Office, At-Home Protocols, Over the counter products and Do- it- yourself whitening.

In-Office bleaching employs highly concentrated Hydrogen peroxide (HP) gels (20%-40%) or 35% Carbamide Peroxide (CP), which requires 2 or 3 clinical sessions with a duration of 30-50 minutes each. It may or maynot be used in conjunction with a light or heat source. The dentist controls the procedure and can stop it when the desired shade or effect is achieved. This technique has advantages including better control of the clinician, avoidance of material ingestion and faster whitening results.However,this technique is expensive , has increased risk of tooth sensitivity and gingival irritation and greater color rebound which may be caused due to dehydration.[1]

Tray delivered At-Home bleaching usually employs CP in concentrations ranging from10% -22% or HP at low concentrations (5%-10%). This technique has several advantages: self- administration by the patient at home with the use of custom-made trays under the direction of a dentist, less chair-side time, high degree of safety, fewer adverse effects, and low cost..The trays with the bleaching agent can be used according to the patients convenience.This technique has greater color stability and lesser risk of tooth sensitivity.However,the response is slow.Satisfactory whitening results can be achieved in 2-6 weeks only.

To obtain results which are less aggressive to the pulp tissue and more durable with respect to the longevity of the bleaching, and in an attempt to decrease the number of in-office bleaching sessions, some authors proposed the combined bleaching technique.[2] In combined protocol, a single in-office bleaching session is performed at the beginning of the treatment and then followed by tray-delivered, at-home bleaching.[2] Studies have proved that the use of low concentrations of hydrogen peroxide gel for the in-office bleaching phase would have the advantage of reduced pulpal and gingival irritation, and therefore, could also minimize the risk and intensity of bleaching-induced tooth sensitivity while achieving satisfactory results in terms of whitening .[3]

It is hypothesized that the bleaching effect is a result of degradation of complex organic molecules being responsible for the colour of the teeth to less complex molecules and results in reduction or complete elimination of discoloration. Hydrogen peroxide is an oxidizing agent that, as it diffuses into the tooth, dissociates to produce unstable free radicals which are hydroxyl radicals (HOH), perhydroxyl radicals (HOOH), perhydroxyl anions (HOO–), and superoxide anions (OOH–), which will attack organic pigmented molecules in the spaces between the inorganic salts in tooth enamel by attacking double bonds of chromophore molecules within tooth tissues (Minoux and Serfaty, 2008). The change in double-bond conjugation results in smaller, less heavily pigmented constituents, and there will be a shift in the absorption spectrum of chromophore molecules; thus, bleaching of tooth tissues occurs.[4] Carbamide peroxide (CP) decomposes into HP and urea ,the latter causes denaturation of enamelin and amelogenin, which are proteins present in the matrix component found between the enamel prisms. This could increase enamel permeability and thus induce microstructural changes.[5] On the contrary, urea induces alkalinization, which may result in reduced demineralization.[5] The pH of the product, temperature, the activation procedure used, or the presence of certain transition metal elements all modify the type of reaction produced.

The effects of dental bleaching on the physical properties of enamel and dentin is controversial. Some studies show that bleaching agents have adverse effects on the enamel surface morphology and texture such as increased porosity of the superficial enamel structure, demineralization and decreased protein concentration, organic matrix degradation, modification in the calcium:phosphate ratio, and calcium loss. HP has been associated to morphological changes, as well as to variations in microhardness, and in the mineral component of enamel and dentin ; also changes in the dentin-enamel junction and in the elasticity and mechanical properties of dentin, as well as to alterations of their organic components. The /oxidation-reduction effects of peroxides are known to cause the dissolution of both the organic component and the mineral component of teeth.[6]

However, many authors have observed no relevant changes in enamel and dentin after whitening treatments.[7,8] A factor that may account for the variation in the effect of bleaching agents on the enamel mineral content and morphology is the pH of the agents used. In this regard, some authors consider the pH of the bleaching agent to be more important than the concentration in conditioning the changes in morphology and roughness.[9]

Regarding the concentration of the products, some authors have only described morphological changes or variations in the mineral component when using high concentration gels,[10] while others have recorded changes even with products at low concentrations.[11] In turn, some investigators have observed no changes when using HP at a concentration of 38% or CP at a concentration of 35 %.[16]

For investigating the morphological ,chemical and structural changes on the prepared samples, it is advisable to use techniques, such as environmental scanning electron microscopy (ESEM), electron probe micro analyzer (EPMA) and X-ray diffraction (XRD). [12] Quantitative measurement of changes in mineral content can be assessed using Scanning electron microscope (SEM) equipped with Energy dispersing x ray analysis (EDX).[13] SEM gives the topographical pictures .It is used to assess the surface changes seen on enamel . EDX is a microanalytical technique that can be employed to estimate quantitatively the amount of minerals in a given tooth sample.

The observed variations in morphological changes and in mineral component of the enamel after bleaching by inoffice and at home techniques point to the need for further research in this field. Though studies have been done to assess the enamel mineral content changes after bleaching by In-office and At-Home techniques, few have been done to evaluate the enamel mineral content changes after bleaching by combined Technique which has been proposed by some authors to be safe and effective.[2,3,14] Hence, the aim of this study was to evaluate and compare the enamel mineral content after bleaching by In-office, At -Home and Combined Techniques.

Aim

To compare the Enamel mineral content after bleaching by In-Office, At-Home and Combined Techniques using Scanning Electron Microscope (SEM) equipped with Energy Dispersive X-Ray Analysis (EDX).

Significance of the Study

Combined bleaching technique has been proposed recently to obtain results which are less aggressive to the pulp tissue and more durable with respect to the longevity of the bleaching, and in an attempt to decrease the number of in-office bleaching sessions. In this technique a single in-office bleaching session is performed at the beginning of the treatment; followed by tray-delivered, at-home bleaching. Studies have proved that the use of low concentrations of hydrogen peroxide gel for the in- office bleaching phase would have the advantage of reduced pulpal and gingival irritation, and therefore, could also minimize the risk and intensity of bleaching-induced tooth sensitivity while achieving satisfactory results in terms of whitening . Compared to In-Office Technique, Combined Technique may have minimal effect on changes in Enamel Calcium and Phosphorus during bleaching which inturn would reduce bleaching induced sensitivity.

This study is significant as it provides clinical insights into the effects of various bleaching techniques on the enamel mineral content, guiding dental professionals in selecting safer and more effective treatments.

2. Methodology

Materials and Methods

Armamentarium

1) Fifty- six human premolars
2) Normal Saline 0.9% w/v (infutec healthcare limited)
3) Artificial saliva (Wet Mouth, ICPA Health Products Limited)
4) 37.5% Hydrogen Peroxide (Pola Office+ , SDI Limited, Australia)
5) 10% Carbamide Peroxide (Opalescence PF, Ultradent Products, Inc. South Jordans)
6) 25% Hydrogen Peroxide (24 Carat Plus 25%, Dental A2Z Limited, UK)
7) Diamond disc, low speed straight hand piece and micromotor (NSK, JAPAN)
8) Vinyl polysiloxane impression material (Zhermack elite P&P, Italy)
9) Scanning electron microscope and Energy dispersive X-ray analysis (JEOL, JSM-840 A, Tokyo, Japan)

Methodology

Approval of the study was obtained from Azeezia Ethics Committee (AEC/REV/2017/45). Fifty six sound human premolars, extracted for orthodontic reasons, were collected.

The samples were cleaned of calculus and soft tissues, and then stored in normal saline. The teeth were embedded in elastomeric impression material (Zhermack elite P&P Vinyl polysiloxane impression material). The roots of the teeth were immersed into the elastomeric impression material; 2mm apically to the cementoenamel junction, maintaining the long axis of the tooth perpendicular to the horizontal plane. The specimens were stored in artificial saliva for 7 days. The samples were then distributed into 4 groups to receive the bleaching treatments:

Table 1: Descriptive details of all Groups tested

Group No	Group Name	Sample Size
Group 1	Control Group	14
Group 2	In-Office Bleaching–37.5% Hydrogen Peroxide (Pola Office +)	14
Group 3	At-Home Bleaching- 10% Carbamide Peroxide- (OpalescencePF)	14
Group 4	Combined Technique - 25% Hydrogen Peroxide (24 Carat Plus), followed by 10%Carbamide Peroxide (Opalescence PF)	14

Bleaching Procedure

In In-Office Technique, 3 clinical sessions, each session intercalated by a 7-day immersion in artificial saliva were performed. Three 8-minute applications of the product were made using an applicator tip. After 8 minutes the bleaching agent was wiped off with cotton before the next application. After the third application (total treatment time: 24 minutes), the specimens were kept in artificial saliva for 7 days, after which a second treatment comprising three successive 8-minute applications was carried out. The specimens were again kept in artificial saliva for the next 7 days, after which a third treatment comprising three successive 8-minute applications was carried out. The treatment was completed in 15 days. After treatment, the specimens were placed in distilled water and subjected to analysis after 24 hours.

For At Home Technique, the bleaching agent was applied for 6hrs daily for a total of 15 days. The 14 experimental specimens in the At Home Group were treated with 10% carbamide peroxide, applied to the surface of the enamel using an applicator tip, and were kept for 6 hours a day. After this 6-hour period, the specimens were washed with distilled water and stored in artificial saliva until the next application. After the 15 days of treatment, the specimens were placed in distilled water and subjected to analysis after 24 hours.

For the Combined Technique, the samples were first subjected to a single clinical session of In-Office bleaching with 25% HP. Three 8-minute applications of the product were made using an applicator tip. After the third application (total treatment time: 24 minutes), the specimens were kept in artificial saliva. The next day, the specimens were treated with 10% carbamide peroxide, applied to the surface of the enamel using an applicator tip, and were kept for 6 hours a day, until completing 14 days of At-Home treatment. After this 6-hour period, the specimens were washed with distilled water and stored in artificial saliva until the next application. After the 15 days of treatment, the specimens were placed in distilled water and subjected to analysis after 24 hours.

The Control group was stored in artificial saliva throughout. Teeth in each group were stored separately in bottles containing artificial saliva for 15 days, changing the solution every 2 days. The teeth were dried with cotton prior to daily application of bleaching agents.

Table 2: Bleaching Technique, Composition, pH and Protocol of Use

Bleaching Technique	Commercial Brand/Manufacturer (Country)	Composition (According to the Manufacturer)	pH	Time Application in Each Session /Total No.of Sessions or Total Days of Treatment
In-office	Pola Office+, SDI Limited, Australia	37.5% Hydrogen Peroxide ,5% potassium nitrate	7	8 min x3=24 min/3 sessions
Combined	24 Carat Plus 25%, Dental A2Z Limited, UK.	25% Hydrogen Peroxide, thickener, 5% potassium	7	8 min x3=24 min/1 session;
	Opalescence PF, Ultradent Products, Inc. South Jordans	10% carbamide peroxide, polyacrylic acid (<10wt%), sodium hydroxide (<5 wt%), sodium fluoride 0.25 wt%) nitrate	6.64	6hrs/14 days
At-Home	Opalescence PF, Ultradent Products, Inc. South Jordans	10% carbamide peroxide, polyacrylic acid (<10wt%), sodium hydroxide (<5 wt%), sodium fluoride 0.25 wt%)	6.64	6hrs/15 days

Preparation of the specimen

The teeth were sectioned horizontally using a diamond disc, with a slow speed straight handpiece at 15,000 rpm at the level of the CEJ, separating the crown part of the tooth. The cusp and the occlusal surface of the crown were then removed following the same technique . 56 enamel specimen of 4x4x1mm in size were prepared from the buccal surfaces.

The samples were then subjected to Scanning Electron Microscope and Energy Dispersive X-ray Analysis. The operating parameters were: Working distance of 10µm, acceleration voltage 15 kV, acquisition time 30 sec, temperature 10ºC ,100µA illuminating current and magnification x5000.

In each specimen, analysis of atomic percentage of Calcium (Ca) phosphorus(P) and Oxygen (O) content of the enamel were carried out. The values obtained were tabulated and subjected to statistical analysis.

International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Figure 1: Armamentarium

Figure II: Fifty Six Extracted Premolars

Figure III: Application of Pola Office Plus -Inoffice Bleaching (Group II)

Figure IV: Application of Opalescence PF 10% –at Home Bleaching (Group III)

Figure V: Application of 24 Carat +, Followed by Opalescence PF 10%–Combined Bleaching (Group IV)

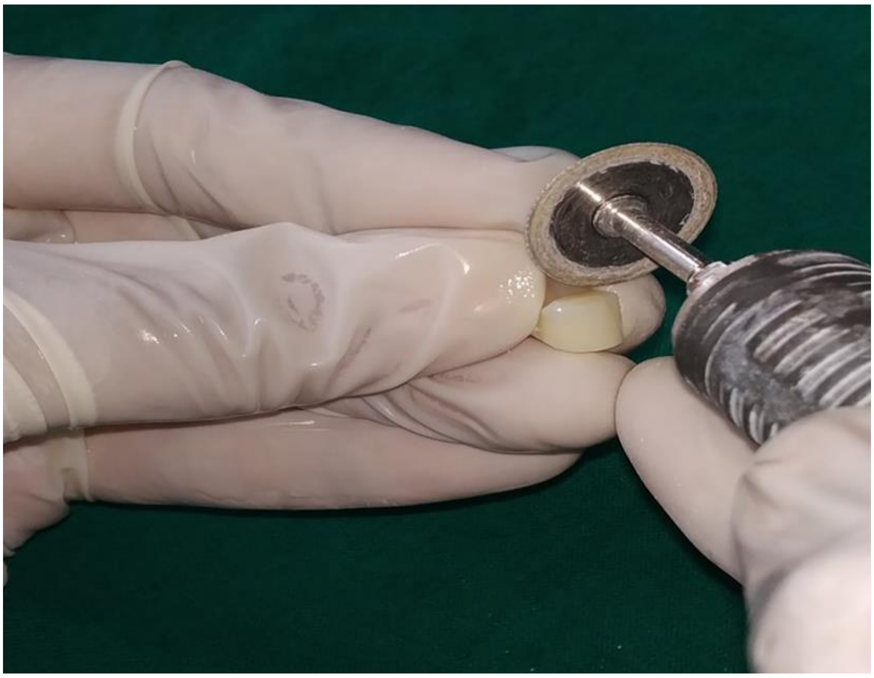


Figure VI: Sectioning the Samples

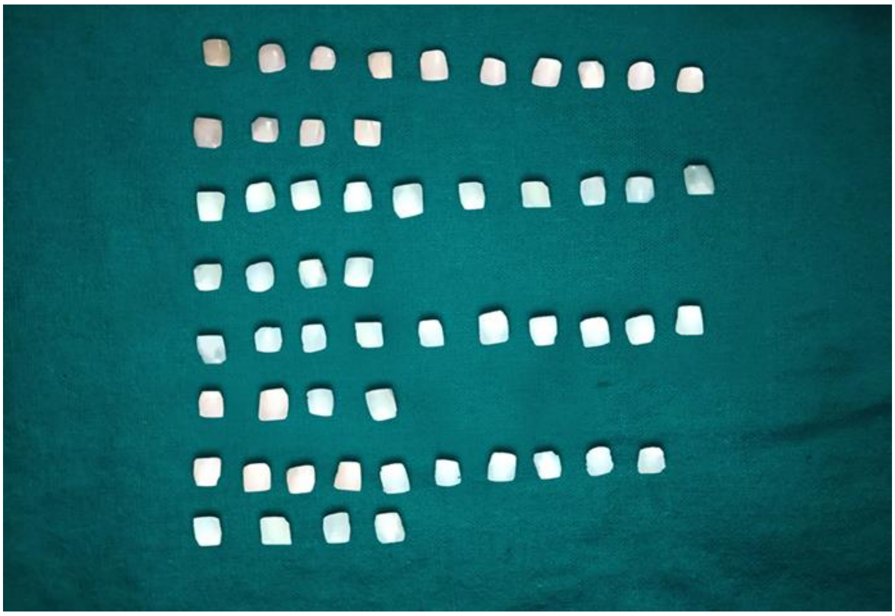


Figure VII: Sectioned Sample Specimens

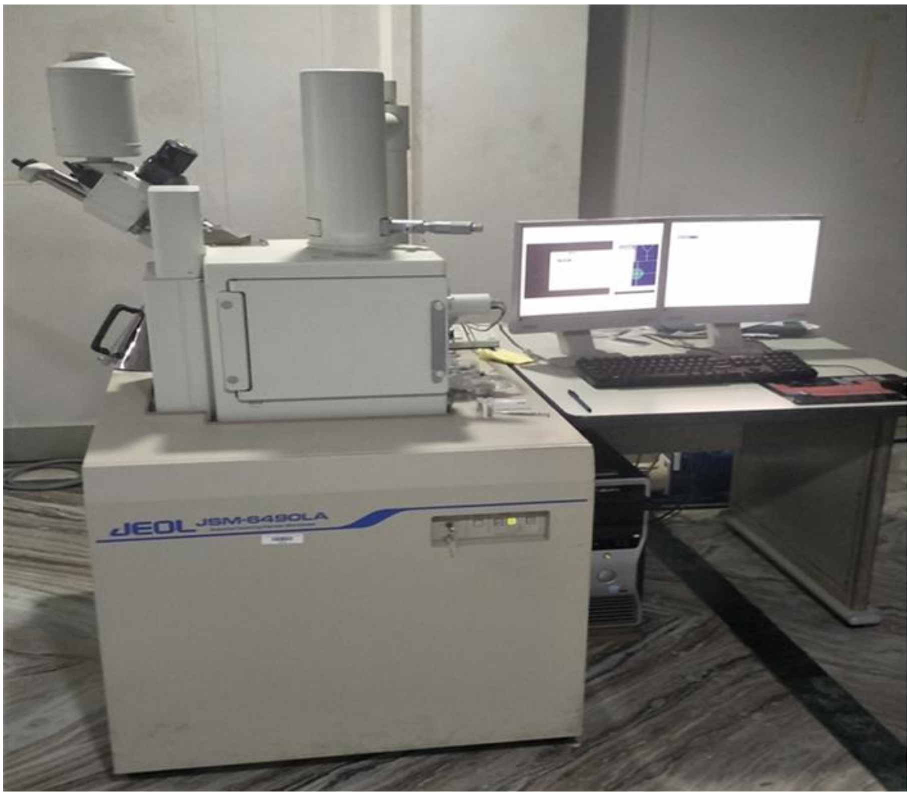


Figure VIII: Scanning Electron Microscope Equipped with Energy Dispersive Xray Analysis

3. Results

The micro photographic images of prepared specimen using scanning electron microscope

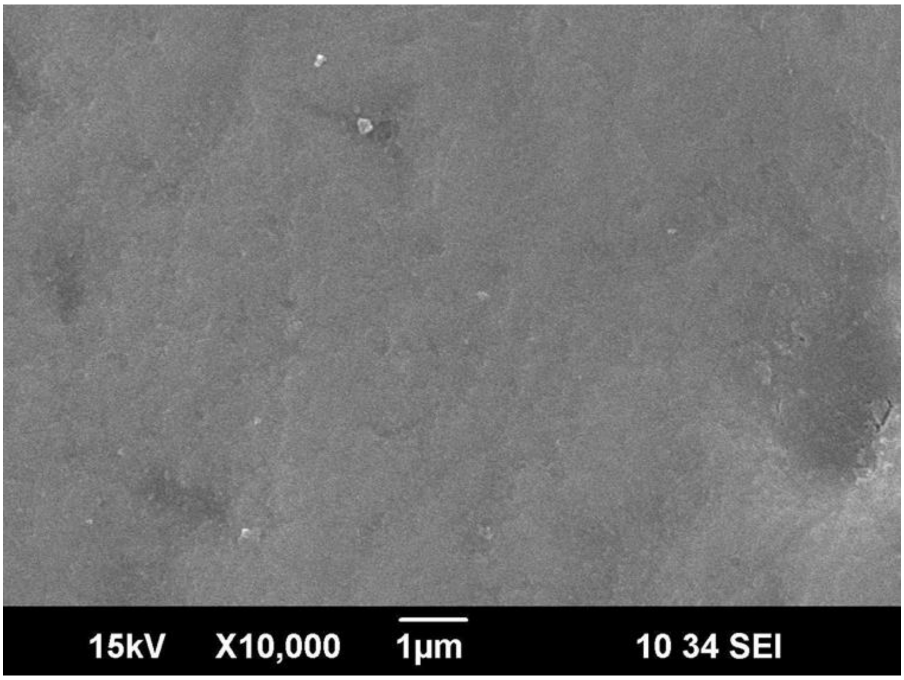


Figure IX: Scanning microscopic image of group I (Control)

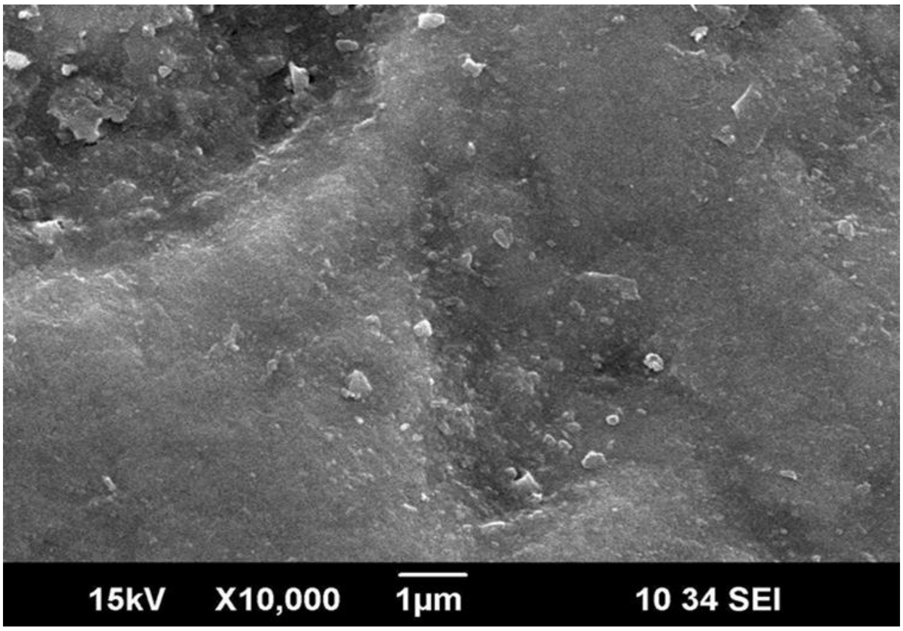


Figure X: Scanning Microscopic Image of Group II (In-Office Bleaching Technique)

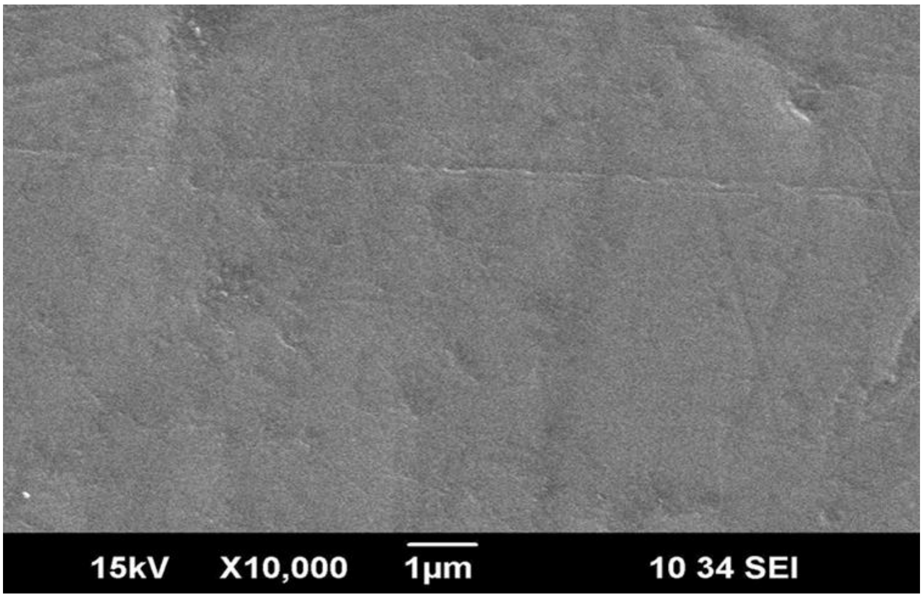


Figure XI: Scanning Microscopic Image of Group III (At- Home Bleaching Technique)

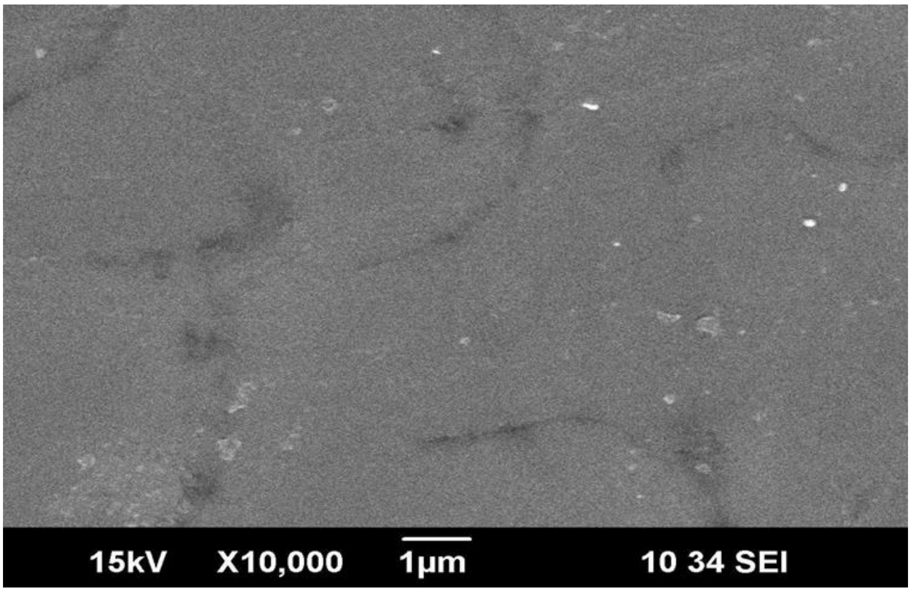


Figure XII: Scanning Microscopic Image of Group IV (Combined Bleaching Technique)

ISSN: 2319-7064
SJIF (2022): 7.942

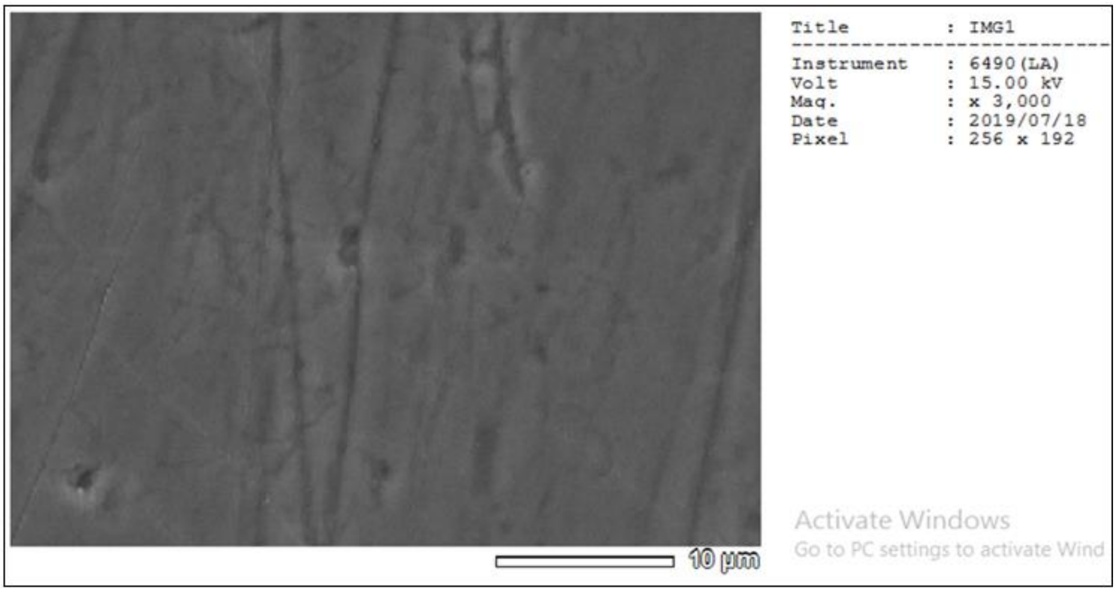


Figure XIII: Energy Dispersive Xray Analysis of Group I (Control)

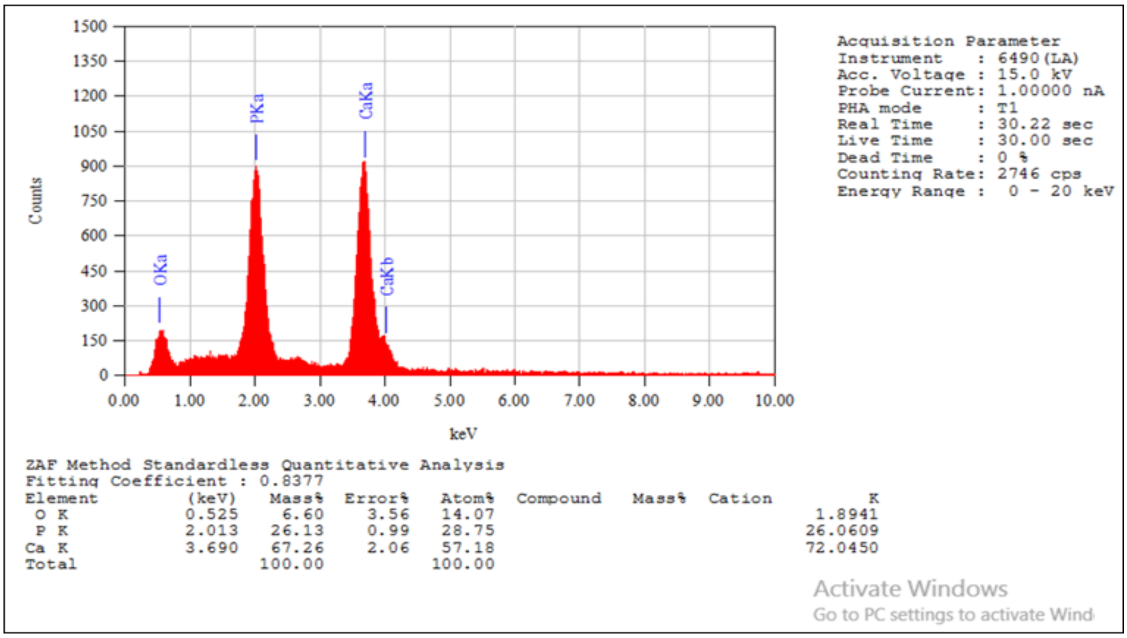
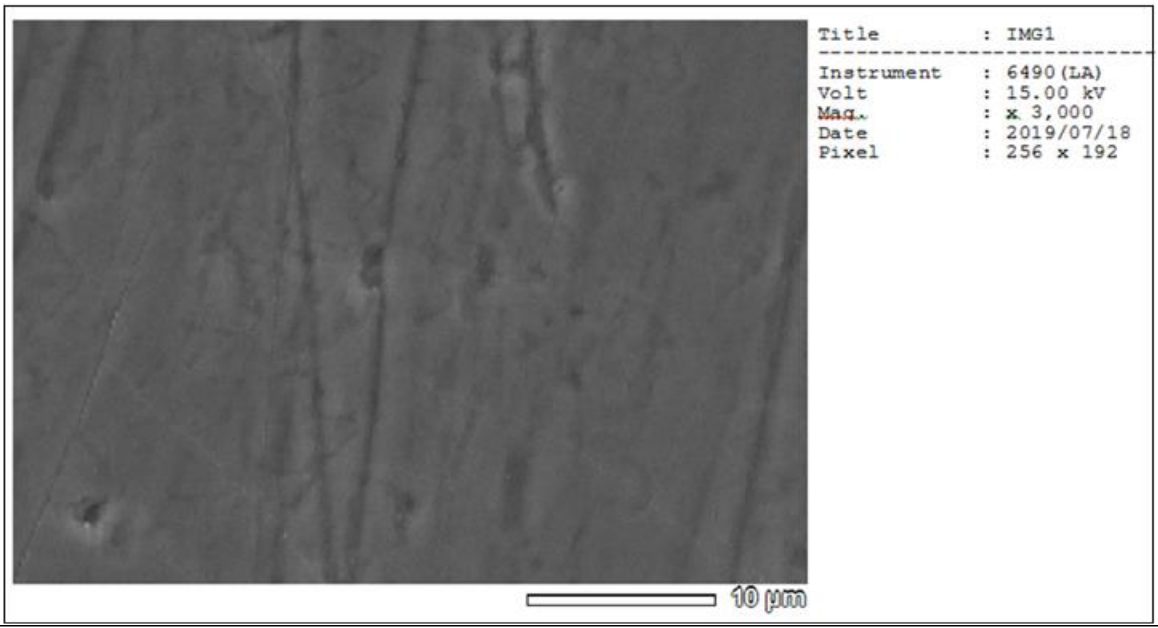
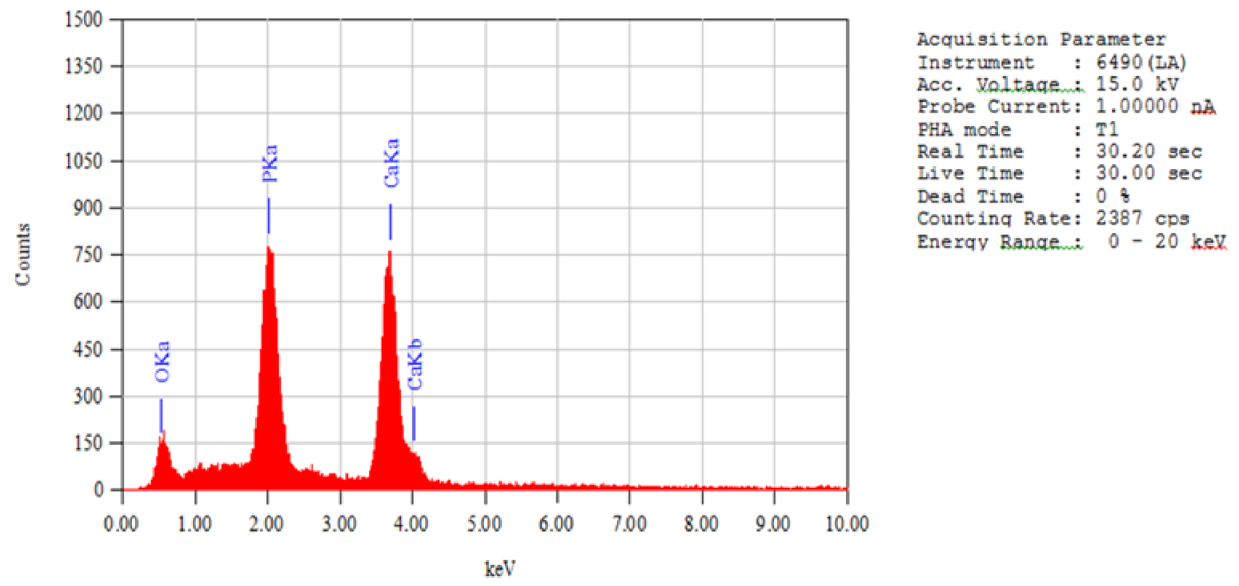


Figure XIV: Energy Dispersive Xray Analysis of Group II (In-Office Bleaching)

ISSN: 2319-7064
SJIF (2022): 7.942





ZAF Method Standardless Quantitative Analysis
Fitting Coefficient : 0.8886

Element	(keV)	Mass%	Error%	Atom%	Compound	Mass%	Cation	K
O K	0.525	16.51	3.75	31.52				5.4868
P K	2.013	21.65	1.13	21.35				23.0000
Ca K	3.690	61.84	2.33	47.13				71.5132
Total		100.00		100.00				

Figure XV: Energy Dispersive Xray Analysis of Group III (At-Home Bleaching)

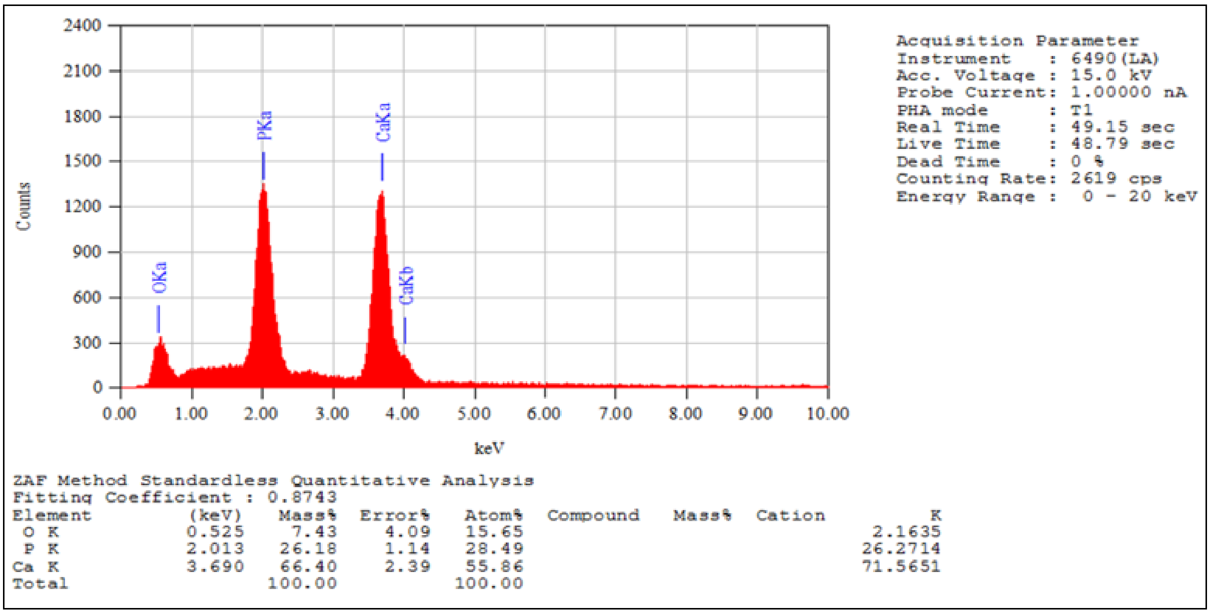
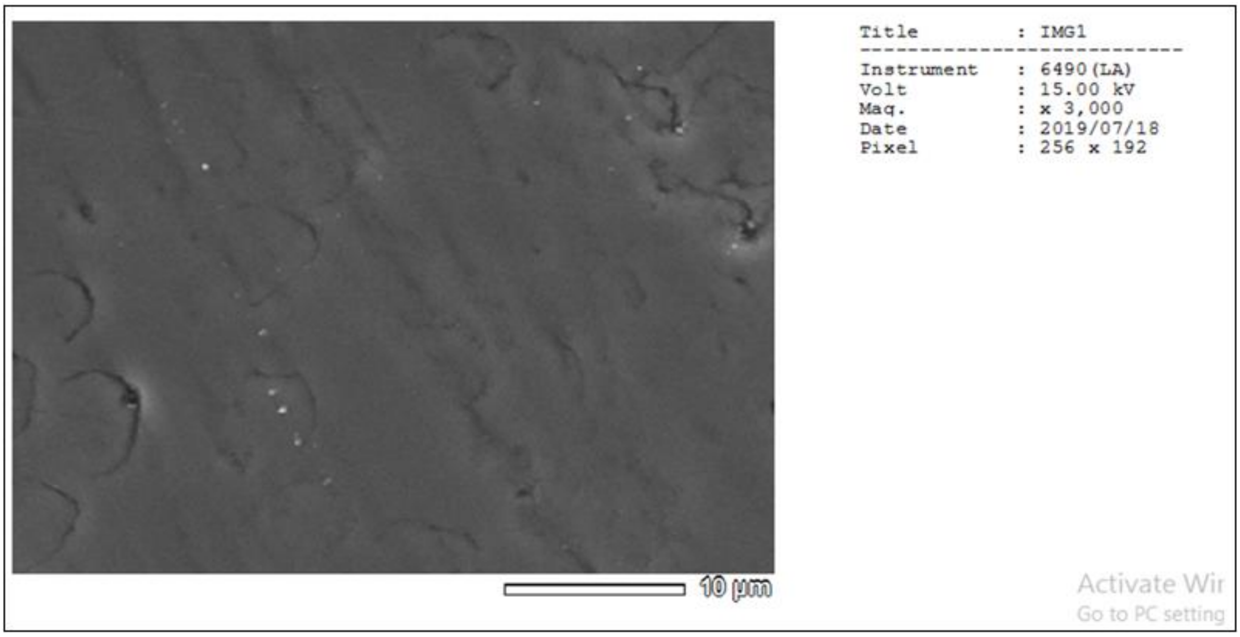
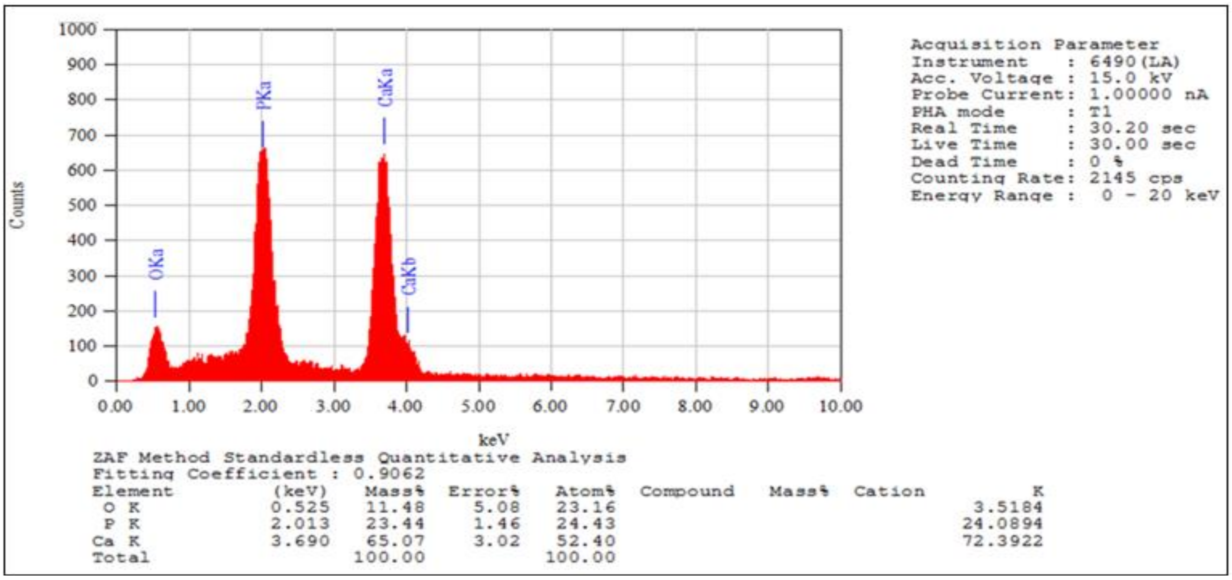


Figure XVI: Energy Dispersive Xray Analysis of Group IV (Combined Technique)





Statistical Analysis

Data was entered into Microsoft excel data sheet and was analyzed using SPSS for Windows (Statistical Presentation System Software, SPSS Inc.) version 17.0.

Continuous data was represented as mean and standard deviation. Data was found to be normally distributed using ***shapiro wilk test***, hence parametric test was used. **One way ANOVA** was used for comparison of four groups. For pair wise comparison Tukey post hoc test was used.

Graphical representation of data: MS Excel and MS word was used to obtain various types of graphs such as bar diagram.

p value (Probability that the result is true) of <0.05 was considered as statistically significant after assuming all the rules of statistical tests.

Statistical software: MS Excel**,** SPSS version 17.0 was used to analyze data.

Table III: Descriptive details of Mean Calcium and Phosphorus across four groups

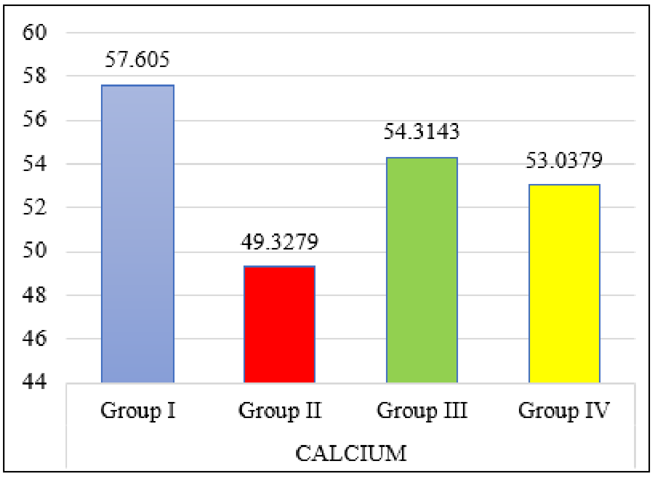
Variable	Group	N	Minimum	Maximum	Mean	Std. Deviation
Calcium	Group I	14	55.18	58.92	57.6050	1.11349
	Group II	14	45.45	52.73	49.3279	2.72809
	Group III	14	52.40	55.91	54.3143	1.06647
	Group IV	14	51.53	55.18	53.0379	1.22845
Phosphorous	Group I	14	27.43	30.81	29.1843	1.13508
	Group II	14	18.12	24.31	21.5136	2.04504
	Group III	14	25.01	31.57	26.6964	1.53032
	Group IV	14	24.40	28.93	27.5993	1.09159

Table IV (a): Comparison of calcium among the four groups

Variables	Groups	N	Mean	Std. Deviation	F-value	P-value
Calcium	Group I	14	57.6050	1.11349	57.85	0.001*
	Group II	14	49.3279	2.72809		
	Group III	14	54.3143	1.06647		
	Group IV	14	53.0379	1.22845		

*significant at the 0.05 level using one way ANOVA.

There is a significant difference (P<0.001) in mean calcium level between the four groups. Group I having the highest mean calcium level (57.6 +/- 1.11) and Group II having the lowest mean calcium level (49.32 +/- 02.72).



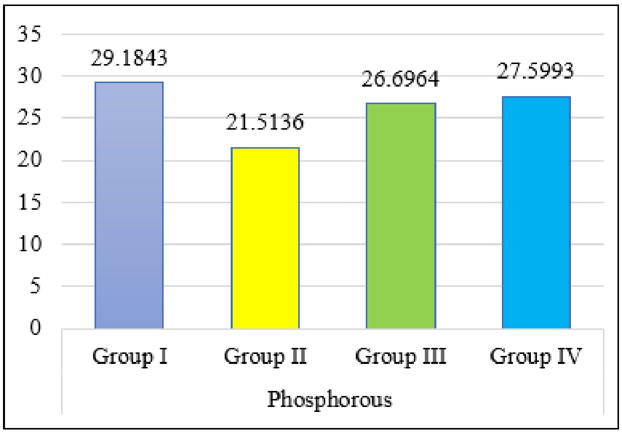
Graph 1: Graphical Representation of Mean Calcium

Table V: Comparison of phosphorous among the four groups

Variables	Groups	N	Mean	Std. Deviation	F-value	P-value
Phosphorous	Group I	14	29.1843	1.13508	68.54	0.001*
	Group II	14	21.5136	2.04504		
	Group III	14	26.6964	1.53032		
	Group IV	14	27.5993	1.09159		

*Significant at the 0.05 level using one way ANOVA.

There is a significant difference (P<0.001) in mean phosphorous level between the four groups. Group I having the highest mean phosphorous level (29.1 +/- 1.13) and Group II having the lowest mean phosphorous level (21.51 +/- 2.04).



Graph 2: Graphical Representation of Mean Phosphorus

4. Discussion

Dental bleaching can be performed using In-Office, At-Home or Combined protocols. Combined bleaching technique is less aggressive to the pulp tissue and more durable with respect to the longevity of the bleaching, and decreases the number of in-office bleaching sessions.

Several studies in the literature have investigated the effects of bleaching on enamel morphology and the surface texture morphological alteration of the enamel surface. The effects of the bleaching agents on the dental structure including alterations in microhardness ,surface roughness,chemical composition and bond strength of resins to the bleached enamel have been reported [19] .The results of research on changes in the morphologic pattern of the dental enamel after the use of bleaching agents are controversial because of the variations of methodologies and products used, concentrations, pH, difference in evaluation time and commercial brands analysed , sometimes the product used is not specified clearly.[15]

Abouassi et al found that application of carbamide peroxide and hydrogen peroxide showed only small quantitative and qualitative differences on the enamel surface. In addition, they found that the influence of the bleaching procedure on the morphology and hardness of the enamel surface depended on the concentrations of the active ingredients. Surfaces treated with carbamide peroxide showed somewhat less alterations compared to hydrogen peroxide. The main effect of bleaching on the morphology of enamel surface was thought to relate to the oxidation and subsequent partial lysis of organic material within the enamel.[17]

Ben-Amar et al. found that bleaching with 10% carbamide peroxide in a mouth guard for 8 hours daily for 21 days created some enamel pitting because, since it contains hydroxyl radicals which are highly reactive, unstable, and will attack most organic molecules to achieve stability, the agent removed some of the organic components of the enamel, and thus could lead to changes in the mechanical properties of the enamel, such as changing the abrasion resistance of the enamel surface .[15]

Some studies have reported that bleaching did not significantly affect the enamel surface. A study, by Smidt et al. found that enamel surfaces showed no mechanical, morphologic, or chemical changes after bleaching with any of the three different carbamide peroxide agents (16%), and this may be attributed to the protective effects of saliva, which provided dilution, buffering capacity, and a supply of Ca and P ions for tooth remineralization [8].

Simone Deliperi et al in 2004 clinically evaluated a combined in-office and take-home bleaching system .They found that when combined with 10 percent carbamide peroxide at-home applications, use of both 35% and 38% hydrogen peroxide resulted in significant tooth whitening. They concluded that using the combined technique clinicians can reduce the time required to complete toothwhitening treatment and reduce gingival and tooth sensitivity, thus increasing safety . Rezende M et al in 2016 evaluated the efficacy, color stability, risk, and intensity of tooth sensitivity (TS) of combined bleaching techniques performed with 20% or 35% hydrogen peroxide for an in-office protocol.They found that the combined bleaching technique using at-home bleaching associated with in-office bleaching was effective and stable over the course of 12 months, regardless of the concentration of the hydrogen peroxide used for in-office bleaching. However, the protocol with 20% hydrogen peroxide produced lower risk and intensity of TS [3] .

Various methods of analysis can be applied to evaluate changes in dental substrate in terms of mineral content, such as Fourier Transform infrared spectroscopy, energy-dispersive X-ray spectroscopy, Fourier Transform Raman spectroscopy, atomic absorption spectroscopy, surface microhardness, induced plasma mass spectrometry, total reflection X-Ray fluorescence, and spectrophotometric or colorimetric analysis [20, 21].

EDAX stands for energy dispersive X-ray analysis. It is sometimes also referred to as EDS or EDX analysis .The principle is based on the energy emitted in the form of xray photons ,when electrons from the external sources collide with the atoms in a material ,thus generating characteristic x-rays of that particular element . During EDAX, the specimen is bombarded with an electron beam inside the SEM. The bombarding electrons collide with the specimen atoms, knocking off some of them in the process. A position vacated by an ejected inner shell electron is eventually occupied by a higher-energy electron from an outer shell. However, the transferring electron must give up some energy by emitting an X-ray. The output of an EDX analysis is an EDX spectrum. The EDX spectrum is just a plot of how frequently an X-ray is received for each energy level. An EDX Spectrum normally displays peaks corresponding to the energy levels, for which most of the xrays had been received. Each of these peaks is unique to the atom and, therefore, corresponds to a single element. The higher the peak in the spectrum, the more concentrated the element is in the spectrum. The EDX provides a very precise quantitative measurement of the mineral content [22].EDX has been used for elemental analysis at the ultra structural level .It is a microanalytical technique used in conjunction with SEM wherein SEM does the structural analysis and the elemental analysis is done by EDX.

In the present study, the specimens were evaluated for changes in the calcium and phosphate content of the enamel. The specimens in In-office, At-Home and Combined technique Groups were compared with the control group. In this study, we followed the whitening protocols recommended by the manufacturers. By doing so, we were able to assess the effect of bleaching on the mineral content of the enamel when the treatment would be done as per the recommended protocols. In the present study, 10% carbamide peroxide (Opalescence PF) bleaching gel was opted for the at-home technique because this product has been extensively studied and its safety and effectiveness are well documented in the literature.[18,23] Carbamide peroxide disintegrates into Hydrogen peroxide and urea, and so there is a sustained `release of Hydrogen peroxide during the long hours the patient wears the tray with the bleaching gel overnight. The respective brands of 37.5% and 25% HP based bleaching agents were selected for inoffice bleaching due to their neutral pH and almost similar composition of Potassium Nitrate desensitizers. The pH values of 10% carbamide peroxide used in the study is almost neutral, 6.64, and 7 for hydrogen peroxide based bleaching agents, thus contributing to balancing the demineralization and remineralization processes.

The hydrogen peroxide based bleaching agents were applied as per the manufacturers instructions .In this study for the In-office Technique, the 37.5% HP bleaching agent was applied for a total of 24 minutes(3 x 8 minutes) per session for all 3 sessions.Similarly ,for combined technique, the 25% HP bleaching agent was applied for 24 minutes (3 x 8 minutes) .This was to prevent the excessive adverse effects of the high concentration hydrogen peroxide based bleaching agents that would occur due to prolonged duration of treatment.Generally the duration for at-home bleaching is 4-8 hrs /day and is applied in trays overnight during sleep. In the present study, the At-Home bleaching agent (10% CP) was applied for 6 hrs per day as it is the average sleep time of a person.

The specimens were stored in artificial saliva between treatment sessions. This medium, in turn, was replaced every two days to simulate the clinical conditions as far as possible. Although remineralization produced by saliva is expected to occur in clinical practice, some in situ and in vivo studies have reported a decrease in microhardness produced by loss of the mineral component secondary to demineralization; however, other in vivo studies have evidenced no changes in either enamel surface morphology or roughness with HP or CP treatment at different concentrations [24]. Artificial saliva may simulate the buffering capacity of natural saliva due to same pH. Hence, it was preferred over distilled water and saline as storage medium. The saliva in the oral cavity acts in the formation of the acquired pellicle. However natural saliva, being a reservoir of proteins and minerals such as calcium, phosphorus and fluoride might offer a greater protection and remineralization than artificial saliva due to the ability to form salivary pellicle that restricts the acid diffusion and transport of ions in and out of the enamel surface and by protein regulation of calcium and phosphate concentrations [25,26]. Therefore, under clinical conditions, the demineralization due to bleaching may be less pronounced compared to the conditions used in the current study. Another fact that may compromise the remineralization after bleaching by the artificial saliva is the presence of carboxymethylcellulose (CMC). CMC is added to some artificial saliva formulations to increase its viscosity. However, this component may reduce remineralization by forming complexes between calcium and/or phosphate ions, resulting in the unavailability of these ions for mineral replacement. In addition, the increase of artificial saliva viscosity promoted by CMC can decrease the rate of diffusion of minerals into the tooth structure.[25] Hence in this study use of artificial saliva as storage medium for bleached teeth had an added advantage of enabling us to evaluate the extent of demineralization caused by the bleaching agents without causing significant remineralization.

In the present study, there was a significant difference (P <0.001) in the mean atomic percentage of calcium between the four groups. Group I (Control)having the highest mean calcium level (57.6 +/- 1.11) and Group II (In –office Gp) having the lowest mean calcium level (49.32 +/- 02.72). The mean calcium level for group III (At Home Gp) was 54.31 +/- 1.07 and that for group IV (Combined Gp) was 53.04 +/- 1.23. Similarly, there was a significant difference (P<0.001) in the mean atomic percentage of phosphorous between the four groups. Group I (Control Gp) having the highest mean phosphorous level (29.1 +/- 1.13) and Group II (In-Office Gp) having the lowest mean phosphorous level (21.51 +/- 2.04). The mean phosphorus level for group III (At-Home Gp) was 26.69 +/- 1.53 and that for group IV (Combined Gp) was 27.59+/- 1.09.

It can be inferred that the Group I (control group) which was not subjected to bleaching had the highest calcium and phosphorus levels. Whereas, Group II (In-office Gp) which was subjected to bleaching with 37.5 % Hydrogen Peroxide had lowest calcium and phosphorus levels.

Between the groups, it was found that there was no significant difference between Group III (At-Home Group)and Group IV(Combined Group) in Calcium and Phosphorus levels (P=0.199 for calcium), (P=0.197 for phosphorus).Among the experimental groups,Gp III and Gp IV showed almost similar values of calcium and phosphorus levels which were higher as compared to the Group II (In-office group).This may be due to the lower concentration of hydrogen peroxide gel (25% HP) used during the in-office session of the combined technique(Group IV) and the use of lower concentration of carbamide peroxide gel (10%CP) in the at-home session of group IV as well as in Group III.

Hence, this study shows that bleaching with higher or lower concentrations of hydrogen peroxide or carbamide peroxide reduces the mineral content of the enamel.

According to the results of the present study, Inoffice bleaching technique with high concentration of hydrogen peroxide gel results in greater decrease in dental enamel calcium and phosphorus. This alteration has been correlated to the oxidative action of HP on the inorganic and organic phase of this hard tissue In this study, we observed no significant morphological changes in At Home bleaching technique, though a significant decrease in Ca and P was recorded with respect to the control.

The results of the present study show that Combined bleaching technique with a lower concentration of Hydrogen peroxide (25%) with one in-office session caused less demineralization effect. This was almost similar to demineralization caused by At-Home bleaching.The demineralization caused was much less compared to In-Office bleaching. As the compositions and pH of both hydrogen peroxide gels used in this study for in-office and combined bleaching were identical except for the HP concentration, it may be suggested as the reason for the greater demineralization and surface irregularities caused by In-Office bleaching technique with 37.5% HP gel in the present study. Only one in-office session (8x3 minutes) of bleaching was done with 25% hydrogen peroxide in the combined technique as against the 3 In-office sessions [(8 x3 minutes) x 3 sessions] done with 37.5 % hydrogen peroxide. Another reason for the lesser demineralization in combined technique could be due to the At-Home session with 10% carbamide peroxide. Carbamide peroxide disintegrates into hydrogen peroxide and urea. Urea has an alkalanization effect as it increases pH and hence reduces demineralization.

Enamel alterations were recorded in the SEM images of the specimens subjected to Inoffice Bleaching Technique. This may be due to disruption of the enamel organic matrix which results in loss of the crystalline material sketched out of this matrix, leaving zones of erosion intercalated with areas of intact enamel, resulting in a rough and irregular surface [26]

The high-concentration HP gels appear to promote greater alteration on dental structure, resulting in a more intense trans-enamel and trans-dentinal HP diffusion [27]. More alteration of the mineralized dental tissues, allow a higher influx of HP into tooth structure, resulting in increased toxicity. This hypothesis is confirmed by clinical studies, which demonstrated a significantly higher prevalence of tooth sensitivity for patients who used 35 % HP gel compared with 20% HP gel [5]

5. Limitations and Future Prospects

1) The specimens were stored in artificial saliva during the course of bleaching treatment. However, natural saliva might offer a greater protection than artificial saliva due to the ability to form salivary pellicle that restricts the acid diffusion and transport of ions in and out of the enamel surface Therefore, under clinical conditions, the demineralization due to bleaching may be less pronounced compared to the conditions used in the current study.
2) Only short-term effect of the different bleaching techniques on the mineral content of the enamel was studied. Long term effect may be influenced by the protective effects of the saliva.
3) 10% Carbamide peroxide bleaching agent used in the study have Sodium fluoride in its composition. This may have masked the demineralizing effect in specimens subjected to at home and combined bleaching techniques compared to those subjected to in-office bleaching.

In this invitro study, the bleaching protocols were carried out under standardized conditions and so we could evaluate the extent of enamel demineralization after bleaching without the influence of external factors. The purpose of the study was to evaluate the actual loss of calcium and phosphorus which would be more pronounced in artificial saliva. The relative effect of the bleaching techniques on the enamel would help provide a safe and effective treatment in the clinical scenario.

Future Invitro and invivo studies are required to further assess the effect of the combined bleaching technique on the enamel mineral content, microhardness, surface roughness along with the bleaching effect

6. Conclusion

In conclusion, this study demonstrates that In-Office bleaching results in the most significant reduction in enamel calcium and phosphorus content compared to At Home and Combined techniques. These findings suggest that using lower concentrations of bleaching agents, as seen in At - Home and Combined techniques, minimizes the adverse effects on enamel mineral content.

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