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Assessing the effects of In-Office, At-Home and Over-The-Counter bleaching agents on nano-hardness, roughness and chemical composition on dental enamel

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1. Introduction:

Over the last several decades, there has been an increased concern for esthetics. The demand for whiter teeth is higher than ever. Due to this demand, in the 1980s, Dental professionals popularized many enamel tooth-bleaching options for patients.[1] Tooth bleaching now represents the most common elective dental procedure globally [2]. Most tooth bleaching methods today use peroxide compounds at varying concentrations. [3] Treatments vary from low concentration (7-10% peroxide) over-the-counter products, to high concentration (up to 35% peroxide) dentist prescribed bleaching systems. There is a direct correlation with increased peroxide concentration and tooth whitening effect. According to a study by Kwon et al, "the high concentration of bleach itself can quickly produce enough radicals that react with pigments." [2]

Hydrogen peroxide can be used directly, or produced in a chemical reaction using hydrogen carbamide that produces hydrogen peroxide and water. Hydrogen peroxide has a molecular weight of 43.1 g/mol, and is small enough to penetrate through enamel to reach organic decomposed pigment compounds with double bonds. As an oxidizing agent, hydrogen peroxide breaks down into reactive oxygen and breaks these double bonds inside enamel; this renders a colourless organic enamel structure. [4] Hydrogen peroxide moves through enamel tissue by a physical and a chemical gradient. Hydrogen peroxide physically penetrates enamel according to Fick's second law of diffusion, which predicts how diffusion causes the concentration to change with time. The hydrogen peroxide moves through the enamel proportionally to the concentration of hydrogen peroxide and surface area of enamel through a chemical gradient. [2] This mechanism used for tooth-whitening treatment has remained unchanged since the early 1900's.

Hydroxyapatite is the largest mineral constituent of enamel. The remaining parts include organic matrix proteins and water. Structurally, enamel is composed of millions of enamel rods and sheaths. The inter-rod substance may be the increased distance between differently oriented crystallites, where the head portion meets the tail portion. These areas are filled with organic material. [5] It is postulated that in hydrogen peroxide enamel bleaching, the organic material is stripped from the inter-rod structure and the mineral content of the enamel rods and sheaths are removed

Recently, there has been an increase in the amount of scholarship reporting tooth bleaching's adverse effects on tooth surfaces [3]. In a study by He et al, the effects of common bleaching agents used in esthetic dentistry were determined. Hydrogen peroxide concentration, and each treatment modality of the products were compared for it's adverse effects on tooth structure. [4]The chemical, physical and structural effects of dental enamel in third molars were analyzed before and after application of 35% in-office hydrogen peroxide, 9.5% at-home dentist prescribed hydrogen peroxide and a 7.5% hydrogen peroxide over-the-counter product.

In this project we propose to study how three different kind of bleaching agents can affect the physicochemical properties of the enamel surface at the nanolevel by using several characterization techniques. The null hypothesis to be tested is that there will be no difference in the roughness, nanohardness and chemical composition changes among the three vital tooth-bleaching systems.

2. Materials & Methods

The whitening systems used in the study are indicated in Table 1. In order to investigate the changes in enamel, the following experimental conditions were used: 1) a control group without bleaching, 2) an in office (InO) bleaching system, 3) an at home (AtH) bleaching system, and 4) an over-the-counter (OTC) bleaching kit. Prepared samples were used instead of experimental participants to minimize differences in environmental and physiological intraoral constraints.(a) The controls were stored in deionized water at 22°C as a negative control. The samples were subjected to Atomic Force Microscope (AFM), then Nano-indentation and finally, X-ray Photoelectron Spectroscopy (XPS). The findings were used to classify each enamel bleaching system for various characteristics.

Table 1

	Market Name	Manufacturer	Active Ingredient	Lot No.
AtH	Poladay Gel	SDI limited; Sydney Australia	7.5% hydrogen peroxide	122159
InO	Pola-Office	SDI limited; Sydney Australia	35% hydrogen peroxide	122314
OTC	Crest 3D WhiteStrips: Intense Professional Effects	Procter & Gamble; Cincinnati, Ohio	9.5% hydrogen peroxide	JD-G-141- 12

2.1 Specimen Preparation

36 samples were produced for the 12 experimental conditions (n=3). Third molars extracted from patients under twenty years of age for orthodontic purposes were selected for specimen preparation. The buccal surfaces of the selected third molar samples were cut with refrigeration using a diamond round blade with a dremel hand piece into 3mm x 3mm squares. Each tooth produced four samples, one for each experimental condition in order to reduce the variation of sampling composition. The

enamel was isolated and the dentin was removed from the samples. After acid etching using 37% phosphoric acid etch on the internal side of the samples, a base of 2 mm of composite resin-based material was built using a composite resin and bonding system (Esthet-x HD and Prime&Bond NT; Dentsply, York, Philadelphia, USA). The samples were stored in deionized water at 22°C upon preparation. Each sample was examined for enamel abnormalities and deformations. Any samples with morphologic abnormalities were discarded and a new sample within normal limits was prepared. Samples were subjected to the three selected tooth bleaching treatments following manufacturer's instructions, conducted by one clinician to minimize procedural variations.

2.2 Sampling Groups:

In-Office: Pola-Office (SDI limited, Sydney, Australia)

Pola-Office (SDI limited, Sydney, Australia) was used as the in-office bleaching treatment. The samples (n=3) were cleaned with a flour pumice and dried. A small amount of gel was dispensed on a mixing pad until an evenly mixed gel was extruded. A thin layer of gel was applied in a continuous motion to prevent the gel from running. The product was left on each sample for 8 minutes and was light cured for 8 seconds to simulate in-office techniques. After 8 minutes, the bleach was suctioned with an aspirator. This process was repeated four times, once a week for two weeks.

At Home: Poladay Take Home Teeth Whitening Treatment (SDI limited, Sydney, Australia)

Poladay Take Home Teeth Whitening Treatment (SDI limited, Sydney, Australia) was used for the at home bleaching treatment. The Poladay gel was dropped onto the enamel samples with the syringe provided in the package and left undisturbed for 30 minutes. The sample was rinsed with warm deionized water post treatment. This process was repeated once a day for two weeks.

Over-the-counter: Crest 3D Whitestrips-Intense Professional Effects (Procter & Gamble, Cincinnati, USA)

Crest 3D Whitestrips-Intense Professional Effects (Procter & Gamble, Cincinnati, USA) were applied as the over-the-counter bleaching agent. Each strip was cut within the samples sizes, placed onto the enamel sample and left undisturbed for 30 minutes. After 30 minutes, the Whitestrip was removed and any remaining peroxide was wiped off with sterile gauze squares. The Crest Whitestrips were administered once a day for 30 minutes for 10 days.

All samples were treated with 4% sodium fluoride varnish to simulate a common practice prescribed by dentists post tooth bleaching. The significance and legitimacy of this practice was characterized.

2.3 Data Collecting Methods

Three forms of data analyses were used to effectively analyze changes in physical and chemical composition.

a) Roughness by Atomic Force Microscopy

For AFM, the experimental conditions were investigated during three time periods: one week (w1), two weeks (w2) and after fluoridation. The samples were placed in the AFM with magnetic sample holders. The atomic force microscope (Veeco Dimension 3100 D3100 with NanoScope IVa controller; Plainview, NY, USA) measured height, porosity and hardness to acquire a 3-dimensional image to investigate the change in enamel structural density and erosion, and to calibrate micro-hardness. Analysis was done at room temperature. Diamond cantilevers were used at $w \sim 250\text{kHz}$. The lateral resolution was 1-5 nm and the vertical resolution was 0.05 nm. Images were captured at 10 nm / 10 nm. The AFM software used was Nanoscope IVa (Veeco – Digital Instruments, Santa Barbara, CA, USA). Roughness was quantified analyzing the arithmetic mean value (Ra) and the quadratic mean value (Rq). Three-dimensional images were obtained of the specimens for visual analysis of changes in topography. Variations in enamel roughness (Ra) were plotted on scatter plots and analyzed by ANOVA two ways ($p < 5\%$).

b) Nanoindentation

A self-contained Hysitron TI Series standalone Nanoindenter (Hysitron, Minneapolis, MN, USA) was used to study mechanical changes in nano-hardness by calibrating depth changes in the nano-landscape of enamel samples. A filament rod indenter was used for the samples, which offers more accuracy in determining hardness than a diamond rod. The probe applied and removed a finite maximum force of 9000 μN at a maximum depth of 2000 nm into the enamel samples. Hardness is measured through force-displacement theory. Force was increased linearly into the enamel surface. Stiffness of the material is calibrated upon unloading of the rod using the load-depth curve of enamel with a standard hardness sample to achieve the area function. Errors in hardness calibrations were minimized using the Oliver-Pharr method. Baseline hardness and modulus of elasticity were determined for the various tooth enamel samples. Samples were analyzed after bleaching and fluoridation.

c) X-Ray Photoelectron Spectroscopy

X-Ray Photoelectron Spectroscopy was used on these samples in order to study the changes in chemical composition of enamel. Samples were analyzed after two weeks of bleaching and one week after fluoridation. XPS analyses were performed using a Kratos Axis, (Manchester, UK). An Al monochromatic radiation source was used, producing photons at an energy of 1486.6 eV; the samples were probed at a base pressure of $<1 \times 10^{-9}$ Torr. Carbon was normalized at 285 eV. The depth probed was 4-5 nm. High-resolution spectra were obtained using a passing energy of 20 eV and 0.05 eV steps, at a perpendicular takeoff angle. Etching was performed using an argon ion gun, at either 10 nm or 30 nm steps. As the samples were etched, the presence of argon, as detected by the $\text{Ar}2p_{3/2}$ spectral peak, became noticeable. The surface area analyzed was performed in 3 different regions per sample. After Shirley background removal, the peaks were analyzed by CasaXPS software. XPS high resolution peak data was analyzed for elemental changes of calcium, carbon, oxygen and fluoride in the enamel samples. Ratio of the amount of calcium to phosphorus (Ca/P) were obtained by the peaks of $\text{Ca}2p$ at 347 eV and $\text{P}2p$ at 135 eV. Composition changes (CC) were also calculated for Ca and P composition, according to the following formula:

$$\text{CC} = [(C_{\text{bleaching}} - C_{\text{control}}) / C_{\text{control}}] \times 100$$

Where $C_{\text{bleaching}}$ represents the atomic concentration after bleaching and C_{control} represents the atomic concentration before bleaching. The signs (-) or (+) in the percentage reveal the loss or gain of atoms.

3. Results:

3.1 Atomic Force Microscopy

Atomic Force Microscopy uses a high resolution scanning probe microscope that can measure optical refractions and deflections in nanometers. AFM captures the surface morphology of various materials from an atomic resolution along the x, y, z-axes. This quantifies the topographic landscape of a surface atomically, acting like a 'map' of the material.¹ Figure 1 displays AFM results of the samples after bleaching. It provides insight into the 'texture' of the enamel surface throughout each bleaching treatment by calibrating the fine irregularities of the surface, which occur from exposure to the chemical agents.

Figure 1 AFM results from enamel surfaces after bleaching agent’s application.

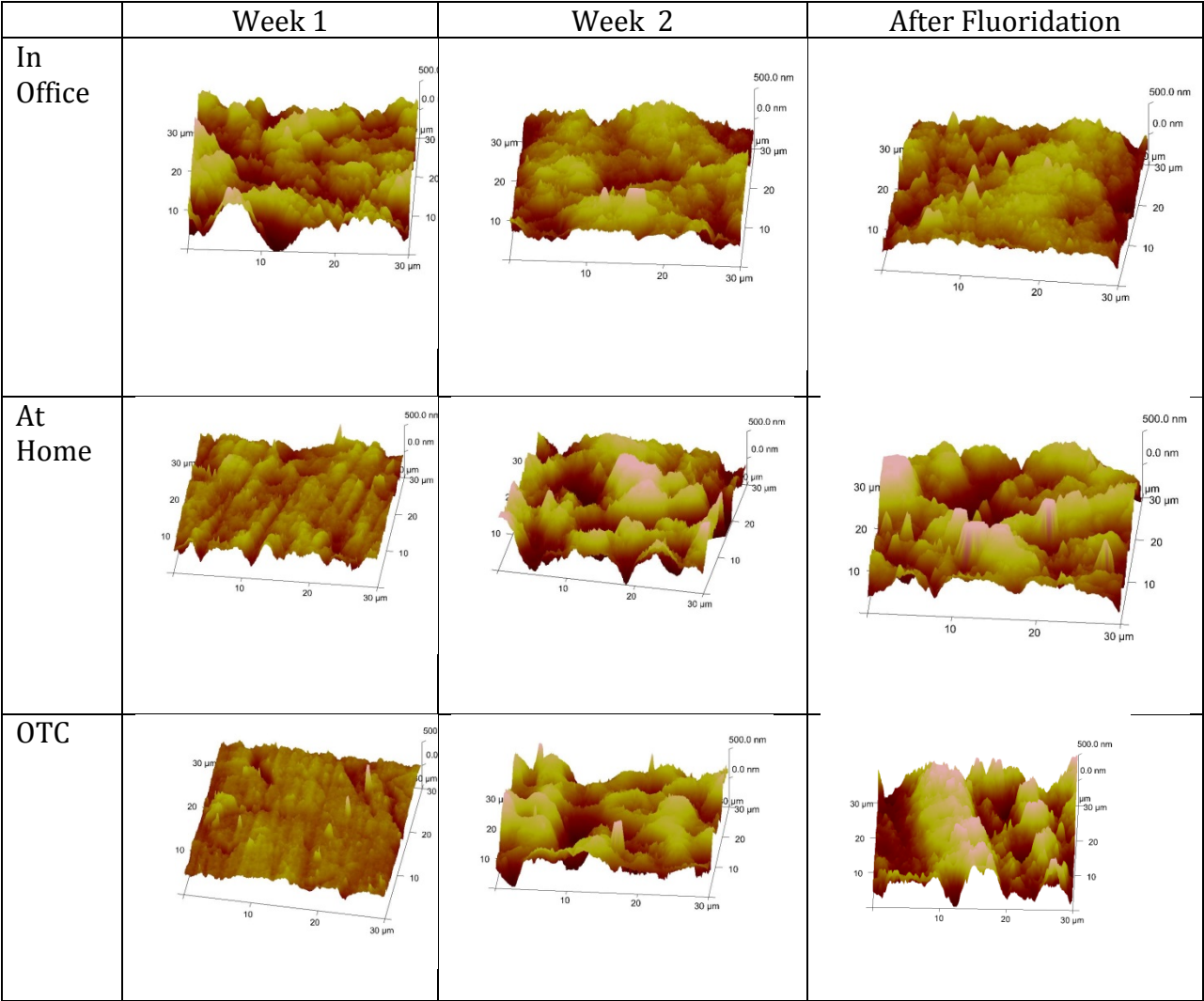


Figure 2 shows the average enamel surface roughness (Ra) of each bleaching agent in contrast to the control sample average. The control average surface roughness (Ra) was calculated to be 72.6 (± 10.3) nm.

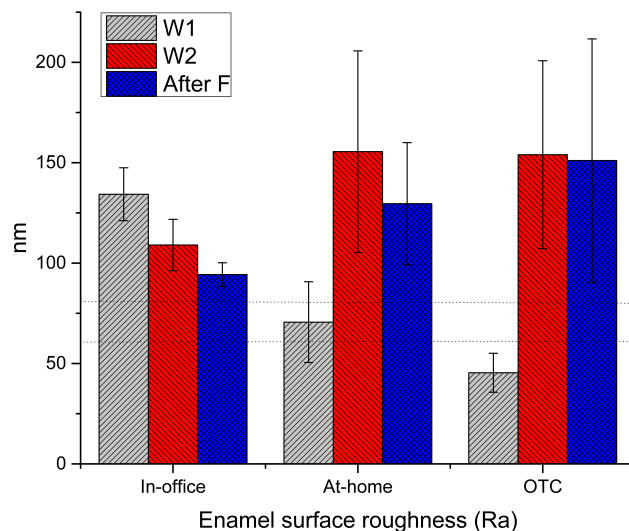


Figure 2. Mean and standard deviation of the roughness from AFM according to the experimental conditions. The horizontal dash lines show standard deviation of control group.

The average Ra for the in-office product after one week was 134.3 (± 13.2) nm. After two weeks of treatment, the in-office treated sample Ra decreased to 110.6 (± 12.8) nm. The in-office samples were then treated with a sodium fluoride varnish. Upon fluoridation, the AFM analysis rendered a decreased roughness of 94.3(± 2.9) nm.

The AtH specimens rendered 70.6 (± 20.1) nm in roughness after week one, with a significant increase in roughness after week two at 155.5 (± 50.2) nm. The at-home samples were then administered with fluoride, rendering a decrease in roughness at 129.6 (± 30.4) nm.

The over-the-counter strips (OTC) showed an average surface roughness of 45.4 (± 9.7) nm after week one, and 154.0 (± 46.8) nm of average surface roughness after week two. The OTC specimen demonstrated the largest increase in enamel roughness at an average difference of 103.6 nm from week one to week two. However, this product also demonstrated the smallest Ra difference from control to week one of treatment. The samples were treated with sodium fluoride varnish. The fluoridated over-the-counter samples showed little decrease in roughness, measuring at 151.1 (± 60.6) nm post treatment.

3.2 XPS

XPS survey analysis can provide the chemical composition of the outermost atomic layer of the enamel surface samples. In this study, three different depths had their composition probed and analyzed: at surface level (0 nm), 10 nm and 30 nm. At 0 nm, a large amount of adventitious carbon was calibrated. This was expected due to the high atmospheric carbon levels. High adventitious carbon levels mask the real chemical composition of the sample. For this reason, only the chemical compositions after Ar etching (at 10 nm and 30 nm) will be taken into consideration.

The Ca/P (calcium: phosphate ratio) was obtained from each sample, and the average and standard deviation were calculated for each experimental condition. Figure 3 shows the Ca/P ratio at 10 and 30 nm. Control samples showed a very constant ratio of ~ 1.73 (± 0.03), which is near the stoichiometric ratio of 1.67 found by França et al. [6] Figure 3 depicts chemical variations found in the samples of the three different experimental conditions. As shown, there are large differences in the Ca/P ratio between 10 nm and 30 nm. These differences are most profound with the in-office chemical composition and the over-the-counter chemical composition.

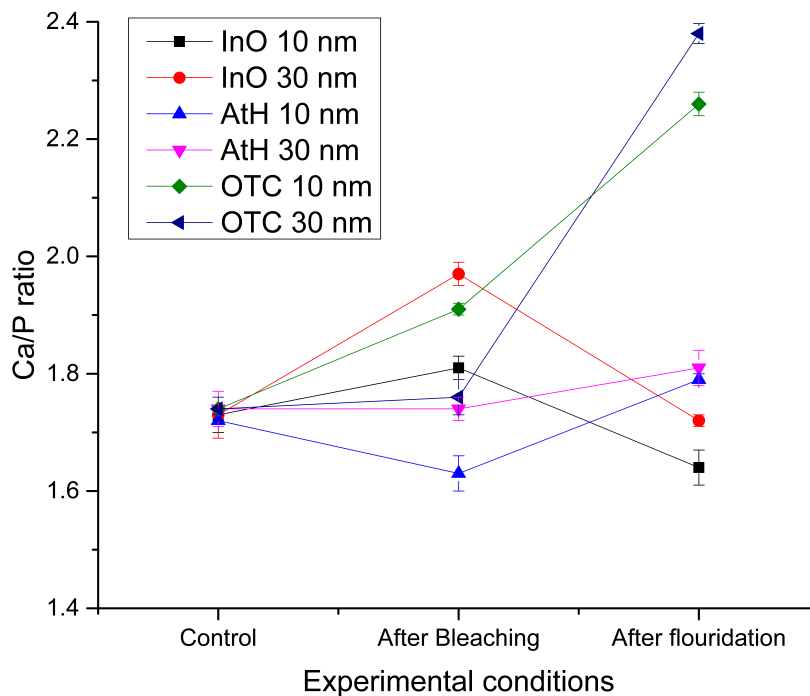


Figure 3. Ratio between and phosphorus according to XPS results, with two different layers analyzed at 10 nm and 30 nm.

In order to understand why these variations occurred, it is important to analyze the concentration changes of Ca and P throughout the study. Figure 4 shows the percentages of Ca and P in the three different tooth bleaching experimental conditions (Polaoffice, Poladay and Crest 3D) at 10 and 30 nm. The percentages were calculated using the previously shown formula:

$$CC = [(C_{\text{bleaching}} - C_{\text{control}}) / C_{\text{control}}] \times 100$$

Where $C_{\text{bleaching}}$ represents the atomic concentration after bleaching and C_{control} represents the atomic concentration before bleaching. The signs (-) or (+) in the percentage reveal the loss or gain of atoms.

In theory, if no concentration change takes place, the result would be 100%. This means that the sample has the same composition as the untreated control samples. If atoms were lost, the result would render < 100%. If atoms were gained, the result would be > 100%. Noting changes in specific ion concentrations is also significant – it is important to observe if bleaching agents have preference for one of the ions. These nuances show if the wear or chemical degradation of bleaching on enamel is selective.

As noted in Figure 4, the In-office product was shown to have major discrepancies in Ca to P concentration.

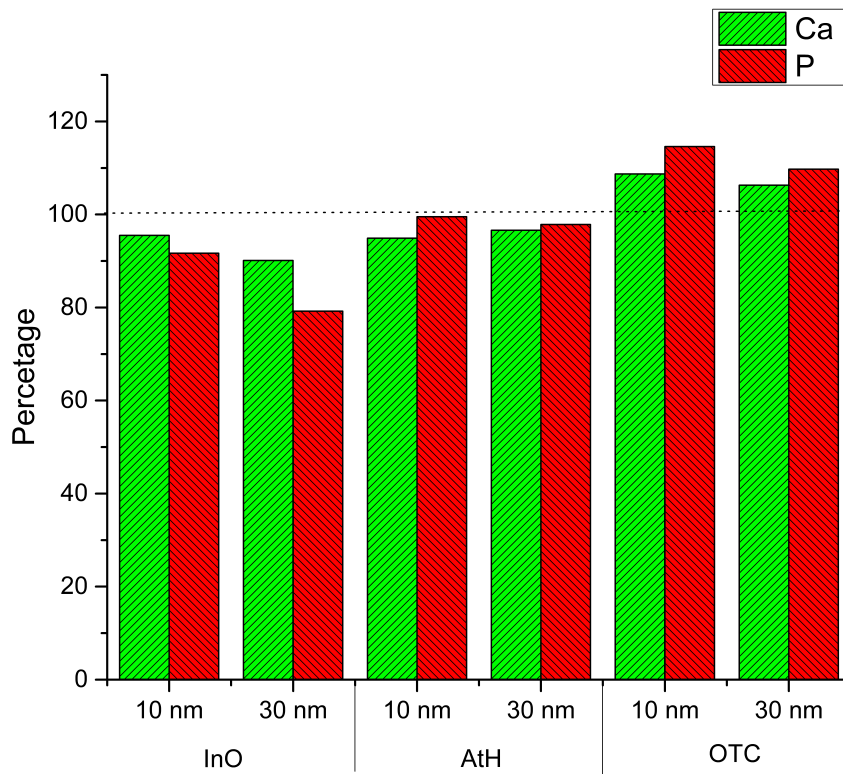


Figure 4. Percentage of ion calcium and ion phosphorus at 10 nm and 30 nm from the surface of enamel.

For example, at 30 nm deep, the phosphate concentration was 79.2%. This shows a remarkable preferential effect for phosphate degradation with the in-office bleaching agent at deeper enamel layers, with less preferential effect at superficial levels. An atomic phosphate gain in the OTC treated samples is also of note.

Figure 5 depicts changes in phosphate ions before and after fluoridation. Upon interpretation, we see that the post-treatment fluoride application was most significant with the InO samples. Increased levels of phosphate ions were found in their chemical composition, particularly profound in the internal layers (30 nm). These ions may be indicative of favourable potential for enamel remineralisation post enamel bleaching treatment.

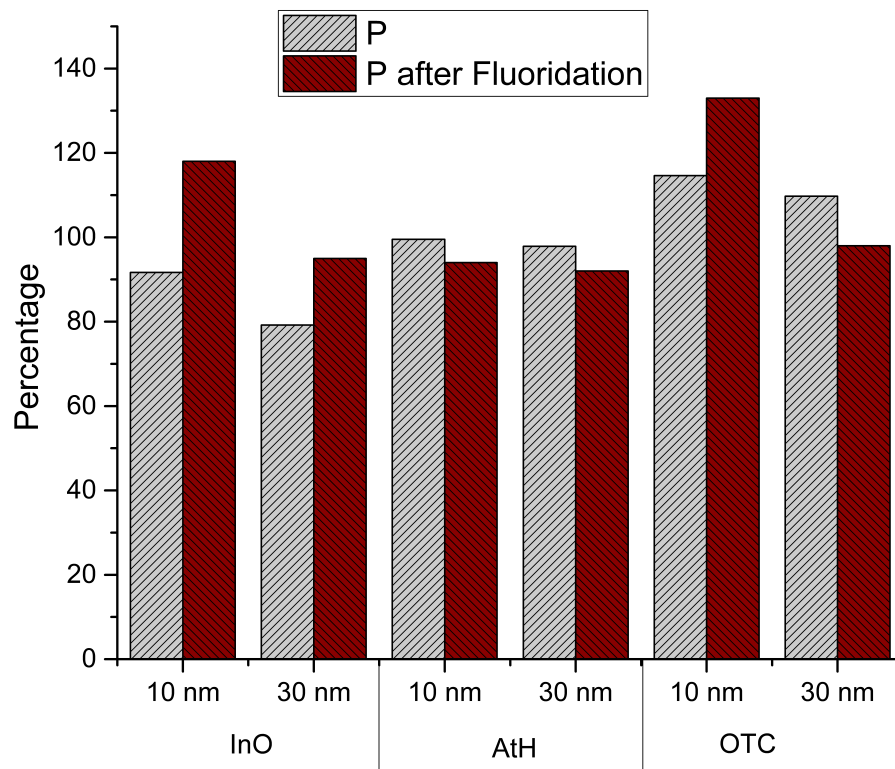


Figure 5. Percentage of the ion phosphorus at 10 nm and 30 nm from the surface of enamel before and after fluoride application.

3.3 Nanoindentation

The nanoindentation results are shown in Figure 6. The dashed horizontal lines depict the standard deviation of the control samples.

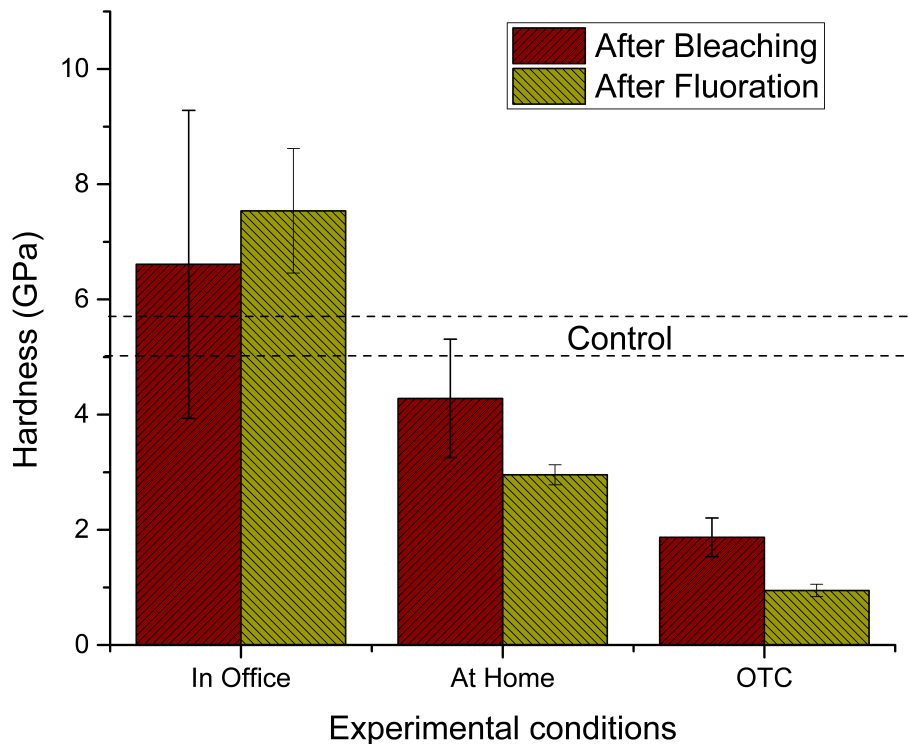


Figure 6. Nanohardness results from enamel after bleaching agent and fluoride application

4. Discussion:

4.1 Demineralization

Analyzing our XPS results, the largest changes in Ca/P ratio were seen with the in-office (InO) and over the counter (3D white) treatments. The change in the mineral content of enamel is due to enamel softening in the presence of acid down its concentration gradient. These changes in Ca/P ratio may have been greater due to the storage medium used. Our study was done in-vitro, using deionized water as the storage medium. The storage medium has a large impact on the mineral content of enamel [7, 8, 9,] Deionized water does not contain any of the aqueous minerals that would be naturally occurring in human saliva. A past study by Borges et al, done in-vivo, using vital teeth in each subject's oral cavity. Therefore, natural, mineral-rich saliva was used as the storage medium. The study found that hydrogen peroxide treatment had little morphologic and chemical effects on enamel surface throughout the bleaching treatment. [10] This was likely due to the Ca and P levels found naturally in saliva. These free ions have potential for enamel remineralization from hydrogen peroxide treatment. Some storage mediums, such as Hank's Balanced Salt Solution (HBSS), have been known to remineralize softened enamel. These storage mediums also have an

effect on enamel remineralization because of their high concentration of calcium (2.6 mM) and electrolytes [10]. The stripped enamel surface may have been restored due to the high mineral content found in saliva.[10] Our study found that hydrogen peroxide bleaching systems have deleterious effects on enamel quality penetrating beyond the enamel surface at the nanometric level. These effects were evident when looking at the large Ca/P ratio of post bleach treated samples throughout our study. Evidence of significant mineral loss was found at 10 nm beyond the surface and to a lesser extent, at 30 nm below enamel surface. Physiologic variability likely has an effect on the buffering capacity, hydrogen peroxide dilution and natural remineralization process that occurs within the oral cavity.[9, 10, 11] In our study, we found this mineral loss favoured phosphate as seen in Figure 3. However, it is likely that teeth in the natural oral environment can partially restore the mineral content of enamel.

In one study by Vargas-Koudriavtsev et al, there was a relationship between bleaching treatment and enamel phosphate loss when using Raman spectroscopy. The study showed statistically significant levels of phosphate demineralization in 14% hydrogen peroxide and 38% hydrogen carbamide treated enamel samples. Vargas et al showed that the more concentrated 38% hydrogen peroxide exhibited greater loss of phosphate concentration. 38% hydrogen peroxide led to 50% loss of phosphate concentration compared to the 20% phosphate concentration loss in the 14% hydrogen peroxide.[8] The study demonstrated that the largest mineral loss occurred after week one of treatment, with insignificant changes after week two, three and four. Our results show similar findings, as we saw the lowest levels of calcium and phosphate in the most concentrated in-office treatment. Our study shows a direct relationship between demineralization and nanohardness. The treated enamel samples with the highest degree of phosphate loss (InO; 35% hydrogen peroxide) also had the largest decrease in enamel nanohardness.

The same article found a surprising *increase* in calcium to phosphate ratio after week one of treatment using the 9.5% hydrogen peroxide DayWhite product. This result is likely due to the presence of *amorphous calcium phosphate* in this product. This finding is corroborated with our findings. We saw an increase in the Ca/P ratio with the over-the-counter (OTC) product. This change in Ca/P ratio indicated that a new structure was being formed. However, looking at Figure 3 the Ca/P ratio of bleached enamel was no longer 1.67, therefore it is unlikely hydroxyapatite. It is likely that the product formed is a structure with different levels of calcium and phosphorous, such as dicalcium phosphate, amorphous calcium phosphate or monocalcium phosphate.[6] The sodium fluoride product also contained phosphate, a pyrophosphate which likely attributed to the increased phosphate seen in Figure 3 post fluoridation.

Within our study, we also found that post treatment mineral application was important in rejuvenating mineral content. Fluoride ions were able to adhere to the peroxide treated enamel samples when treated with 4% sodium fluoride varnish past the surface. [9, 16] An increase in phosphate concentration was found to penetrate the enamel, at 10 nm and 30 nm. The presence of fluoride ions from the fluoride application help the remineralization process. Ions or minerals are able to penetrate beyond the surface

because they incorporate into enamel through a concentration gradient. Remineralization potential of teeth subjected to hydrogen peroxide should be further investigated in order to improve bleaching practices. [9] The addition of a high mineral varnish as a post treatment step in bleaching systems should be further explored. The applications of post treatment fluoride was seen to increase Ca/P and penetrated just beyond the surface.

4.2 Nanohardness

Nanoindentation was used to detect the presence of a change in the nano-topography of bleached enamel samples. We found that there was a decrease in hardness from week one of treatment to week two of treatment, in both the in-office (InO) treated enamel samples and the at-home (AtH) treated samples. These findings were in agreement with an in-vitro study done by Azer et al [13, 16] that found more decrease in enamel nanohardness as hydrogen peroxide concentration increased. This is confirmed when looking at our atomic force microscopy. We saw that as nanohardness decreased, there was an increase in erosion of the enamel surface. The enamel sheaths depicted were severely compromised and appeared eroded. The erosion seen would decrease the nanohardness.[8] Within hydroxyapatite, part of the crystalline structure is removed and part of the structure stays, altering the composition. In relation to the minerals within enamel, we saw that there was a decrease in nanohardness as the Ca/P ratio deviated from the natural stoichiometric ratio of 1.67. [6] There is a direct correlation to inorganic content and the mechanical properties of enamel. As there was a decrease of inorganic matter in the enamel, we saw a reduction in enamel nanohardness. [8] The increase in enamel hardness in the over-the-counter bleaching product may have been due to mineral recovery of the enamel process due to the lower concentration of hydrogen peroxide, having more affinity for remineralization. Higher concentration hydrogen peroxide products would inhibit enamel nanohardness recovery. [9] This finding is in agreement with our findings. The lower concentrated hydrogen peroxide product (over-the-counter 9.5% hydrogen peroxide) increased in nanohardness throughout the bleaching process. [9]

The storage medium used has an effect on nanohardness. A dry medium would exhibit less resistance than a wet medium. A storage medium with high mineral concentration should recover the surface from voids and loss of material, which would increase its nanohardness. In our study, the use of deionized water may have weakened the enamel structure. [8]

The study done by Chen et al showed that fluoride application of in-vitro enamel samples that were treated with hydrogen peroxide structurally bound more fluoride to their apatite crystals to form fluoroapatite than untreated samples. The use of high concentration mineral treatments post bleaching may potentiate mineral absorption. [9, 13, 17] A different study conducted by Chen et al found that hydrogen peroxide eroded enamel may recover from the acid attack. This study found that nanohardness returned

close to pretreatment levels and postulated that loss of nanohardness from bleaching procedures is reversible. [9]

Some nanoindentation samples showed a large, abnormal range of porosity, which is likely attributed to the natural irregular crystalline structure of enamel prisms and the anatomical shape of teeth. In a conversation with Dr. C Deng from the Faculty of Engineering (personal communication), due to the curved nature of the enamel samples, changes in topographic elevation of the material, without changing the force, caused small 'noise' in curves and analysis.[14] Deviations in the hardness curve are referred to as "bursting," known to be a disturbance in the enamel surface due to natural porosity or 'noise' that ranged from 2 microns–2000 nanometers. This results in a break in the curve. Bursts and outliers in hardness scale could be due to audible noise and physical movements, or disturbances within the building. A foreign material such as dust and residual moisture from the distilled water may also contribute to noise.

Another cause for 'bursts' in the hardness curve would be dislocation of the needle from different compositions within the material. The bleached enamel samples may have become heterogeneous in composition with an area of hardness then a sudden change to an area of softness.

4.3 Roughness

Looking at Fig.2, we see that the InO samples showed the largest changes in roughness are the difference from week one and week two of treatment compared to the control. Roughness is similar from week two to post fluoride treatment. The AtH samples showed no change in roughness after week one of treatment, but after a second week of treatment the sample showed a significant increase in roughness in comparison to both week one and controls. The OTC displayed a continuous corrosion of the enamel surface. After analyzing our samples for changes in roughness, we are able to analyze whether the erosion is selective or non-selective. A selective erosion process would show a more roughened surface exposing more surface area. This is because if an acid were selectively erosive, it would strip some elements while leaving others. This selective process would result in an irregular landscape and roughened surface. If the acid was non selective, the entire surface would be eroded without leaving any residual elements on the surface, resulting in a smoothed and flattened appearance. In Fig 2., we see that the AtH and the OTC products result in more roughness from week one to week two of treatment. This leads us to believe that these products are selectively erosive. The InO product decreased in roughness and therefore is likely non selectively erosive. This can be explained by the concentration of the hydrogen peroxide solutions used. The InO product was 35% hydrogen peroxide where as the AtH and OTC products were both below 10%. From our analysis, it is likely that a higher concentration of hydrogen peroxide resulted in a non-selective erosive process while a lower concentration of hydrogen peroxide preserved some elements of the hydroxyapatite structure.

Looking at Figure 6, we can see that the increase in roughness. We see a correlation between the loss of phosphate concentration in the enamel as seen in the AtH specimen in Figure . The AtH showed a decrease in phosphate concentration as indicated by an increase in Ca/P ratio with a decrease in hardness. In our study, we believe that phosphorus is the important to understand the mechanical properties of hydroxyapatite. With reduced concentration of phosphorus, there is a decrease in enamel's mechanical properties. Phosphorus acts as a structural link between the calcium ions.

4.4 Chemical Composition

Looking more closely at Figure 3, we are able to see that all specimens started with a Ca/P ratio of 1.7. We can see that at 10nm and 30 nm, the InO product Ca/P ratio after bleaching increased. This is because of a decrease of phosphate in the enamel structure at 10 nm and 30 nm, resulting in a higher Ca/P ratio. [9, 12] When looking at the InO product again post fluoride treatment, we see that the Ca/P ratio decreased close to original pre-bleaching values. This is likely due to the influx of phosphate as pyrophosphate in sodium fluoride varnish that recharges the phosphate levels in hydroxyapatite. [8, 9] The OTC product Ca/P ratio did not return to pretreatment levels, the phosphate levels did not return back to normal. This is likely because after fluoridation, there is amorphous calcium phosphate forming.

4.5 Micro-topography

Using atomic force microscopy, it became evident that hydrogen peroxide treated enamel samples suffer stripping of the enamel prisms. We found that the higher the hydrogen peroxide sample, the more extreme the level of topographic range, showing higher levels of erosion. We observed in all bleach-treated samples that that enamel appeared more irregular, with peaks and grooves appearing deeper from week one to week two of treatment. The loss of mineral elements as seen in our spectroscopy was gradually eroded away from the enamel surface. Acid dissolves some components of enamel while leaving other parts undissolved, causing porosity. It was also evident that the mineral loss penetrated beyond the surface, as there was a change in chemical and topographic composition at 10 nm and 30 nm [15]. Looking at Fig 1, we see the sharp ranges from peaks and troughs can be attributed to erosion of collagen fibers, while the enamel prism structure remained. During week one to week two, we analyzed that the roughness decreased in the In-office product, likely due to the high concentration of hydrogen peroxide (35%) that obliterated more of the inorganic enamel material. It is likely that the high concentration hydrogen peroxide stripped away a higher volume of enamel surface, leaving behind a smoother surface. The InO (In-Office) product and the over-the-counter (OTC) roughness increased from week one to week two. The irregularities seen in post bleach treated enamel samples are due to the complex composition of enamel. There could also be a variation in sizes of the elements. For instance, calcium has greater atomic mass, and is larger while hydroxide ions are smaller, causing porosities. This phenomenon can be compared to rusting metal,

causing the structure to become brittle and fall apart. However, after the 4% sodium fluoride application, the roughness of the in-office treatment system and the over-the-counter system was reduced. This is likely due to the remineralization that occurred from fluoride application on the enamel surface. Some studies have found that the remineralization potential of fluoride has led clinicians to apply fluoride prior to bleaching treatment. The protective effects of mineral application prior to, or post treatment should be further explored.

With Atomic Force Microscopy, the structural changes in morphology and roughness can be studied visually. AFM can be used to capture enamel changes and demineralization effectively, because AFM measures and depicts different material hardness and softness compositions.

5. Conclusion

Several analytical modalities were used to follow the chemical, physical and structural changes in enamel structure as three popular on-the-market tooth-bleaching treatments were used on standardized samples.

We concluded that hydrogen peroxide bleaching products might decrease hardness and increase roughness of enamel surfaces. Higher concentrations of hydrogen peroxide cause more severe damage to enamel than lower concentrations of hydrogen peroxide products. The null hypothesis was rejected. The in-office product (35% hydrogen peroxide) compromised enamel integrity the most.

Nanoindentation was successful in detecting mechanical changes in hardness and increases in porosity, while Atomic Force Microscopy is effective in visualizing physical changes in enamel topography. X-ray Photon Spectroscopy analyzes the chemical changes in enamel composition. Further studies should be conducted on pre or post mineral treatments to reduce loss of mineral content in enamel. In self-administered bleaching kits, a second step may be implemented as a means to recuperate lost enamel structure and increased porosity. Multiple samples of each bleaching product with enamel from differing sources would be more applicable for determining the generalized conclusion in the further study. Although the comprehensive trials studied the change in hardness and nano-topography of the enamel, further investigation on the volume of enamel removed from hydrogen peroxide bleaching treatment should be undertaken.

6. Acknowledgements

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