

University of Debrecen

Clinical Centrum

Department of Biomaterials and Prosthodontics

Effects of 40% Hydrogen-peroxide in two methods of application and the effect of GC Tooth Mousse on bleached enamel

By:

Tamir Ordeman

5th year Dentistry student



Research Leader:

Tamás Bistey D.M.D Ph.D.

Assistant Professor at Faculty of Dentistry

Approved by:

Csaba Hegedűs, M.D., L.D.S., Ph.D.

Professor and Dean of Faculty of Dentistry

Barna Kelentey D.M.D C.Sc.

Associate Professor at Faculty of Dentistry.

Supervisor of research program

Debrecen, 2014

Table of Contents

Abstract:	3
Introduction:.....	4
Aims:	7
Materials and Methods:.....	8
Results:.....	10
Discussion:	16
Conclusions:.....	19
Acknowledgement:	20
References:.....	21

Abstract

Objectives: The goal of this study was to examine micro-changes in human enamel after exposure to 40% hydrogen peroxide treatment with and without light, and then to test the effects of amorphous calcium-phosphate containing mousse on bleached enamel, using FT-IR spectroscopy. It is hypothesized that infrared spectroscopy is capable of showing alterations in human enamel after peroxide treatment and the alteration in enamel is proportional to peroxide concentration.

Methods: The effects of 40% hydrogen peroxide solution on human enamel, with and without focused light during treatment and the effect of amorphous calcium phosphate on bleached enamel were tested. Forty non-carious human teeth, extracted for periodontal reasons, were used in this study. They were divided into 4 groups. Group 1 and 2 were subjected to two treatments with 40% hydrogen peroxide each for a duration of 20 minutes without light, group 2 was given a treatment with amorphous calcium-phosphate containing mousse for 5 minutes in between and after the bleaching treatments. Group 3 was treated with 40% hydrogen peroxide and light for a total of 8 minutes and was then subjected to a 5 minute treatment with amorphous calcium-phosphate containing mousse. Group 4 was left untreated in isotonic salt solution that was kept in a stable 37°C and tested after two weeks and four weeks and was used as a control group. All teeth were separated from their roots, hemisected coronally, and embedded in epoxy-resin for infrared spectroscopic analysis.

Results: Infrared spectroscopic analysis showed two distinct bands (apatite PO_4 ν_1 and ν_2) that were capable of describing the alterations in enamel structure. On comparing the infrared spectra of non-treated and treated specimens, of pre-treatment and post-treatment, and after application of the amorphous calcium phosphate mousse, structural changes were evident in the superficial enamel. The alterations in enamel were evident post-treatment with 40% hydrogen peroxide concentration. Amorphous calcium-phosphate containing mousse has shown to have a significant effect and can possibly partially compensate the effects of bleaching. The numerical analysis of the IR spectra revealed that upon second treatment using hydrogen peroxide solutions, the alterations in the spectra were more pronounced. The spectra taken in 1 week after treatment in the bleached group showed a continuous deterioration of the enamel structure suggesting that 40% hydrogen peroxide might have a long lasting effect even after its superficial removal.

Conclusion: 40% hydrogen peroxide containing bleaching agents are capable of causing alteration in enamel. Changes are directly proportional to the duration of treatment. Amorphous calcium phosphate containing mousse has a significant effect and can possibly partially compensate for the structural alterations that are caused by the bleaching. 40% hydrogen peroxide has long lasting effect.

Introduction

In the recent decades, the appearance of teeth and their color became of great importance to patients as it became a measure representing the socio economic status of the individual, in addition tooth color may have great effects on self-esteem and generally the social life of the patient ^[64]. The mentioned and other reasons are thought to be the reason that an increasingly amount of patients are in seek for esthetic treatments, and especially treatments aimed at dental discoloration.

The visible tooth color is determined by a combination of different optical and microstructure characteristics of enamel, dentin and the pulpal tissue^[1].

Discoloration of teeth may vary, and is usually etiology dependent; other factors such as appearance, localization of the tooth or tooth surface, and the adherence of pigmenting agent to tooth structure play a role as well in the final appearance. Products that are known for their tooth discoloration effect are known as chromogenic agents, they include certain medication such as tetracycline, coffee, tea, tobacco and red wine.

Tooth discoloration can be categorized as intrinsic or extrinsic. While extrinsic discoloration can be removed with the use of prophylactic products and cleaning procedure, such as, at home brushing or tooth surface cleaning by a dental professional. Intrinsic staining entails the use chemical bleaching for its resolution.

Bleaching using chemical approach has been accepted as the least aggressive method for treating discolored teeth, as other methods, such as crown or laminate placing, require the removal of healthy tooth structure in order to cover the discolored tooth with an esthetic cover.

Chemical bleaching process is based on the process in which radicals cause an oxidative reaction in this reaction, oxygen-free radicals, with the help of their low molecular weight, creep in through the enamel and dentinal hard tissues of the tooth ^[2, 15]. The product releases free radicals that break up chemical bonds that conjugate the pigment to the tooth structure either by cleaving or adding to carbon double bond by reacting with causing a shift of electrons ^[4]. Once the bond is broken, the color molecules no longer show in the visible range of the electromagnetic spectrum and thus have no effect on esthetics.

Tooth bleaching has become one of the most popular esthetic dental treatments and is the fastest growing part of private dental practices with a 25.1% growth rate ^[66]. 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 home-applied bleaching and the development of vital tooth bleaching techniques that are applied both in the dental office and at home.

The tooth whitening market has evolved into four categories: By professional in the dental clinic, prescribed for at home-use, over-the-counter bought and self-applied by patients, and other options that are not dental related. It is important to add that professional in office bleaching materials are sometimes used at home after the bleaching in the dental clinic in order to maintain and improve whitening results^[67].

Most commonly used method for vital tooth at-home bleaching, was proposed in 1989, by Haywood and Heymann^[65], in this technique an individual tray was made and in it 10% carbamide peroxide was used by the patient during night for approximately 2 to 5 weeks. This technique presents a number of advantages such as easy application, lower peroxide concentration, and that less time needed to be spent at the dental office and its price was not high. However this method also presents a few disadvantages, as the long duration of treatment and the lack of control given by a professional when using the material and lastly patient compliance issues^[56-58].

For vital tooth bleaching^[59-61] three fundamental approaches remain, namely, at-home bleaching (night guard) as mentioned above, in-office bleaching, and over the counter (OTC) bleaching products^[17,57].

At present, there is a broad range of bleaching agents available, containing various concentrations of carbamide peroxide, hydrogen peroxide and sodium perborate. Among these three approaches, in-office bleaching utilizes between 25% and 40% of hydrogen peroxide in a gel solution this helps it remain stable on the targeted areas, as of such the in-office approach utilizes highest concentration of hydrogen peroxide as it's active ingredients^[65].

This method aids to overcome the disadvantages of at home bleaching as it provides with an especially short treatment time, full professional control of the whole duration of the procedure and overcomes lack of compliance from the patient.

In-office bleaching has been proven to be highly efficient and rapid treatment which provides the patient with satisfactory results^[62], on the other hand, the safety of this technique on enamel structure has been and still is a frequent question asked by dental professionals and patients alike.

Numerous literature on the topic is available today, researchers attempted to reveal the causes for the possible adverse effects of bleaching agents. Majority of these researches were focused on the concentration, the application modes or the treatment duration of the active bleaching agents and a few other studies aimed their focus on the acidity of the materials applied (pH values)^[1,63].

In this research, the focus to be investigated and discussed will be the usage of Opalescence® Boost PF 40%, containing 40 percent hydrogen-peroxide concentrations as the active ingredient, with and without focused light, and its effects in regard the structural integrity of human tooth enamel.

After bleaching and the results assembled, the second step of this research was to investigate the reparative potential and effect of applying GC tooth mousse calcium phosphate containing gel on the bleached enamel surface.

GC tooth mousse is a casein phosphopeptide–amorphous calcium phosphate containing mousse we chose to examine its effects on bleached enamel as many researchers have shown that it has a large potential in facilitating remineralization of enamel. Examples are in cases of erosion^[68] and remineralization of initial state caries lesions^[70].

Obtaining accurate results on the microstructural alterations of tooth mineralized enamel is strenuous, for the examination of the enamel microstructure in this research we have chosen to use the laser infra-red spectrum scanner, FT-IR spectroscopy, as this method only requires limited specimen preparation thus allowing for an accurate scan with minimal alteration to the original enamel structure.

The working hypotheses for this research stood as 1. FT-IR spectroscopy is accurate and sufficient to demonstrate changes after bleaching agent treatment in human enamel 2. Alterations to the mineralized structure of the enamel are proportional to the concentration of the bleaching agent 3. GC tooth mousse treatment will help remineralization and repair of the bleached enamel surface.

Finally, it is paramount to state, due to the abovementioned facts, that this scan results alone are polemical and further investigations are needed on the effects of bleaching agents on enamel and the restorative potential of application of fluoride post treatment.

Aims

This research aims were initially, examining changes made to the microstructure of the superficial human enamel after treatment with 40% hydrogen peroxide with, and without exposure to light. After bleaching treatment the second aim was to investigate the effects of GC tooth mousse on the enamel.

Our research hypothesis was that bleaching with 40% hydrogen peroxide does cause structural changes to superficial enamel. GC tooth mousse treatment will have an effect on the bleached enamel structure.

Materials and Methods

Sample selection and preparation:

Our examination required 30 extracted human teeth, free of pathological changes in the enamel, which were removed due to periodontal, orthodontic or oral surgical needs. The teeth were cleaned with the use of sterile gauze pads and physiological salt solution, the roots were removed, then the molar and premolar teeth's crowns were separated into two parts in a mesio-distal direction by air driven turbine using water-cooled diamond rotary burs. As a result, we gained 40 samples that were suitable for examination; the samples were stored in physiological salt solution at 37°C until the beginning of the treatment.

Embedding:

The samples were embedded in epoxy resin. In preparation for embedding, little pieces were cut from a double-sided adhesive tape, which were pasted to the base of the embedding container. The sample was placed on the other side of the adhesive tape so that the enamel covered surface faced the container's base. The embedding was made with the use of Epoxi Kit (Stuers, Denmark), embedding solution being kept to the mixing ratio. The Citovac device (Stuers, Denmark) was used for the embedding, this device created a vacuum environment during the pouring process of the epoxy mixture. The embedding solution was kept in embedding containers for 24 hours. After 24 hours, samples were removed from their containers.

Samples polishing:

The samples underwent polishing to achieve a perfect placement of the enamel to the spectroscope's crystal. For this LaboPol-35 (Stuers, Denmark) grinder was used with abrasive paper by SiliciumCarbid (Struers, Denmark). The samples were polished by "500" marked paper with 2,5 N clamping force, 1 round at 50rpm, followed by 2 rounds at 100rpm, second round of polishing was performed by the "1200" marked paper with 2,5 N clamping force, rotating 3 rounds at 50rpm, the samples were constantly cooled by water.

The available samples were randomly divided into 4 groups of 10, according to the treatment they will endure.

Recording:

Each of their infrared spectra were scanned and recorded by the use of Thermo Scientific Nicolet 6700 FT-IR spectroscope (Thermo Electron Scientific Instruments LLC, WI, USA) (Figure 1), which using an infra-red laser calculates the relative absorbance and transmittance of each sample. First scan that was made provided the native control before tooth whitening treatment in every sample's case (first measurement of all 4 groups).

Storage:

The samples were kept between scans in a physiological salt solution at 37°C using an incubator (Labnet Mini Incubator, Labnet International, Inc, USA)

Treatments:

The following were used on each group's samples, in the beginning of the experiment Group 1, 2 and 3 were all subjected to a treatment using 40% hydrogen peroxide (Opalescence Boost PF 40% Ultradent, Utah, USA) groups 1 and 2 each for a duration of 20 minutes. Group 3 was exposed to the treatment for 8 minutes while under focused polarized light (Radi-plus with full arch bleaching LED, SDI, Australia). Following the treatment, spectra were taken from all three groups. Next, group 2 and 3 were treated with GC tooth mousse, a casein phosphopeptide - amorphous calcium phosphate containing mousse (GC corporation, Japan), each for a duration of 5 minutes. After cleaning and washing of samples of Groups 2 and 3, using distilled water, spectra were taken again. The following day, groups 1 and 2 were exposed to a second treatment using the 40% hydrogen peroxide kit, and their post bleach spectra were recorded. After spectra were taken, group 2 underwent an additional treatment with the "GC tooth mousse" for 5 minutes then was cleaned with the use of distilled water and its spectra scanned. Group 1 was kept in isotonic salt solution at 37°C for a week after which we scanned it and recorded the spectra. Group 4 served as a control group and was kept untreated, in isotonic salt solution for four weeks at 37°C this group was scanned at the beginning of the research, at two weeks, and at four weeks' time.



Figure 1; Thermo Scientific NICOLET 6700 FT-IR infrared wavelength spectroscope

Results:

Native scan of human enamel specimens were performed in all groups prior to treatment. As seen in Figure 2, a characteristic infrared spectrum can be seen with two distinct peaks, representing the hydroxyapatite structure: apatite PO_4 v1 at wavelength between $680\text{-}1110\text{ cm}^{-1}$ and PO_4 v2 at wavelength in between $1350\text{-}1550\text{ cm}^{-1}$, these findings are typical for the enamel structure. At $876\text{-}892\text{ cm}^{-1}$, a secondary peak can be seen, which represents the carbonate apatite ($\text{v}_2\text{ CO}_3$) phase of enamel. The characteristic shape of the biological PO_4 v2 was doubled between 1410 and 1460 cm^{-1} .

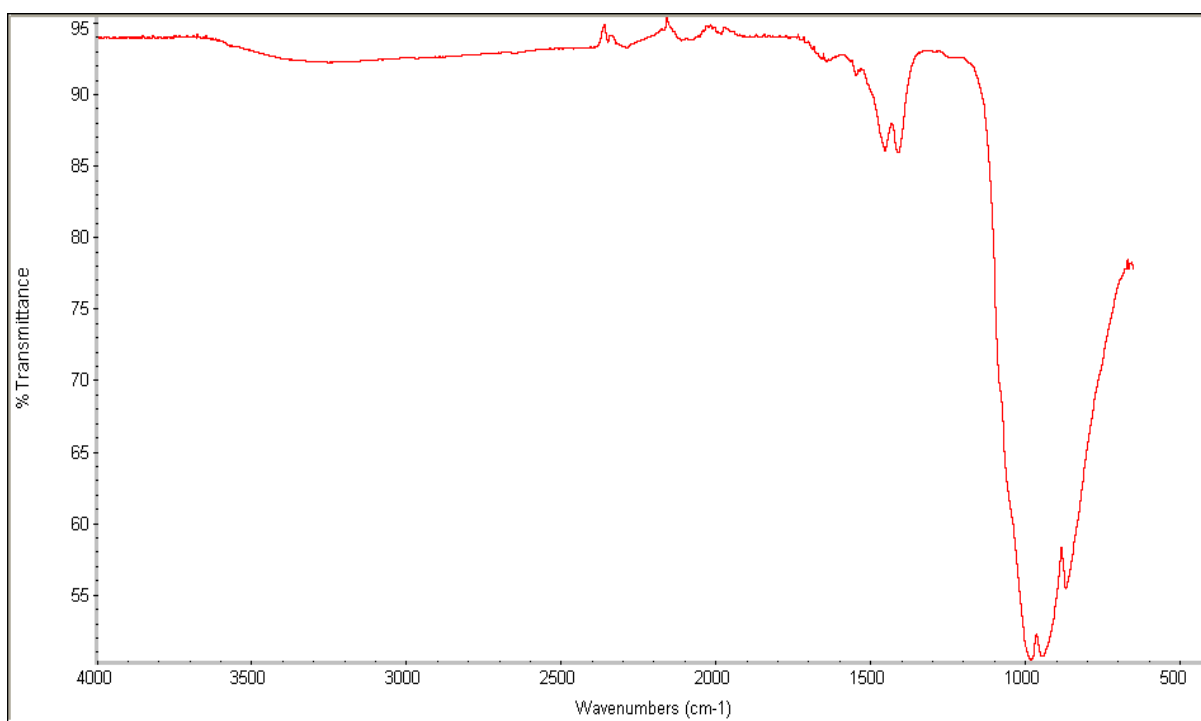


Figure 2; Native scan showing infra-red spectrum of human enamel specimen

Group 4 was used as a control group. Scans were made in the beginning of the experiment, after two and four weeks. In Figure 4, the spectral results can be seen. We can deduct from the results that during the four week period no major changes were occurred in the spectra of the superficial enamel.

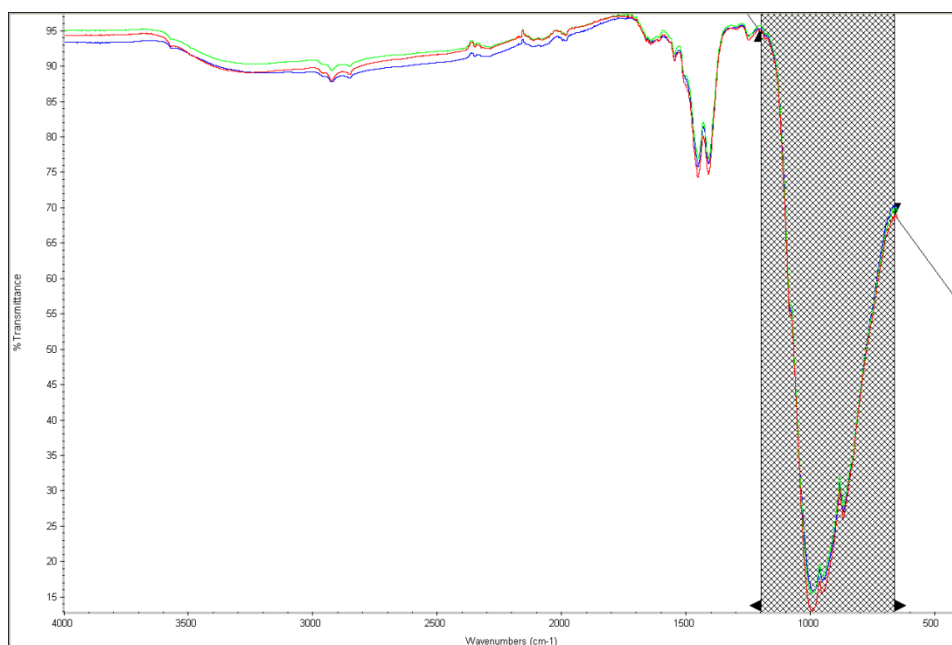


Figure 3; Human enamel IR spectra depicting measurement of ν_1 area under curve

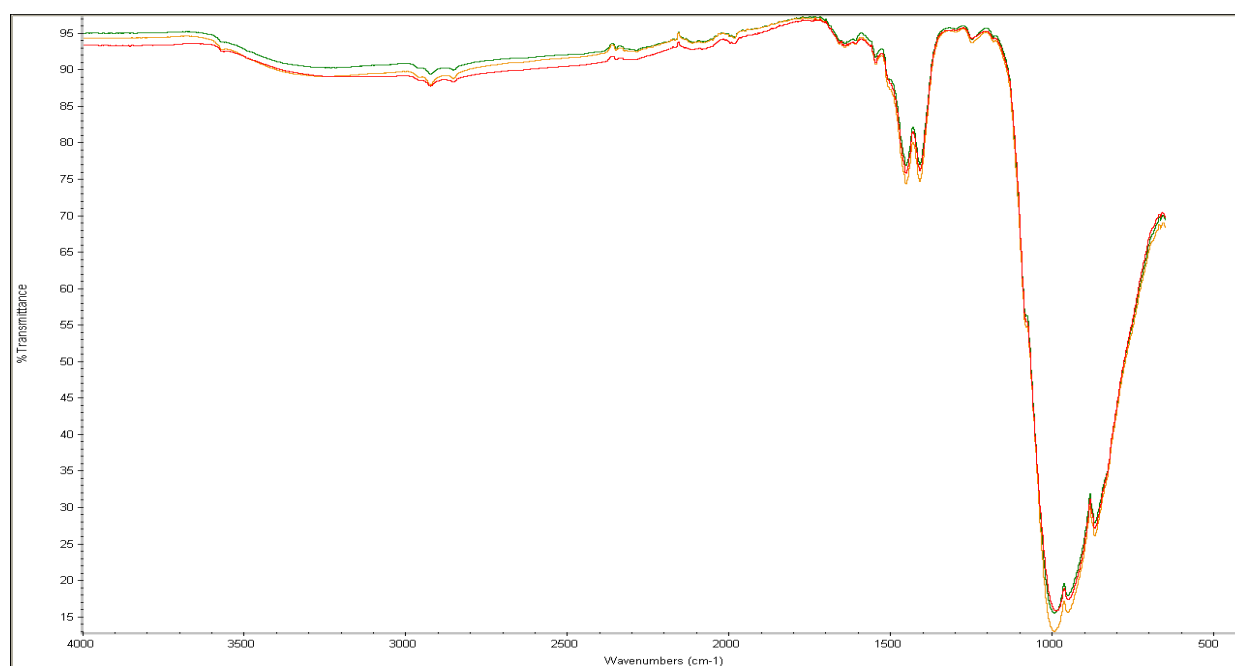


Figure 4; Human enamel IR spectra; red: native scan; orange: 2nd week scan; green: 4th week scan

Group 1 has been treated with 40% hydrogen peroxide for 20 minutes twice, within a two day period; after each treatment a scan was made. In Figure 5 distinct changes can be seen after each treatment, these changes show a continuous increase in size of the spectrum after each treatment. Another scan was made after a week of incubation in isotonic salt solution at 37°C, the spectrum suggests that the effect of the bleaching continued even when the specimens were not exposed to the active agent.

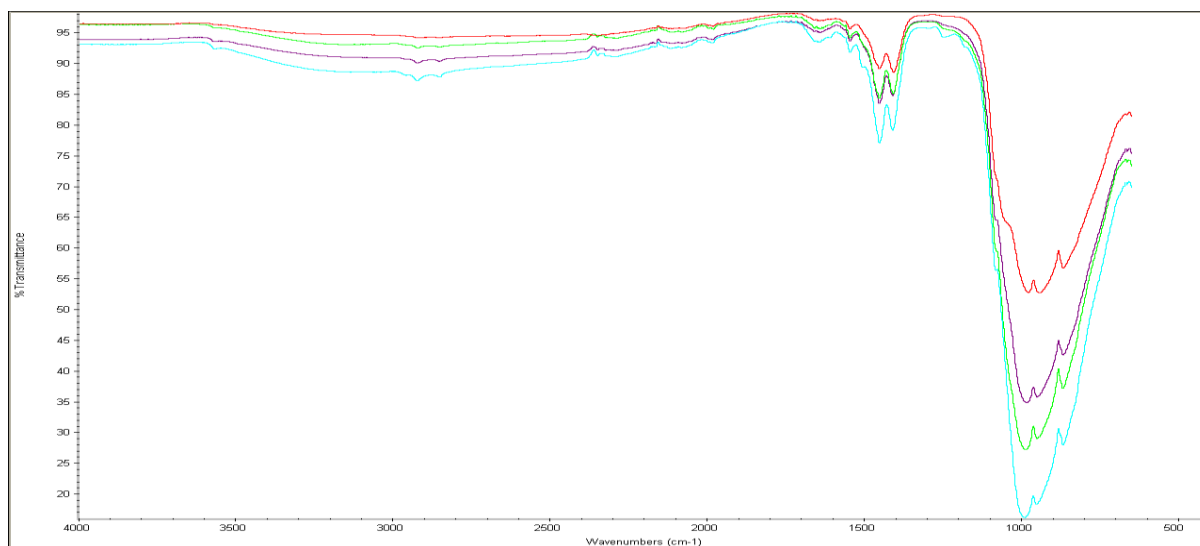


Figure 5; Group 1 human enamel IR spectra; red: Native scan; purple: 1st bleach treatment; green: 2nd bleach treatment; light blue: 1 week control

Our results, seen in Figure 6, show, as expected, the spectra of specimens of group 2 widen after the first bleaching treatment, and a distinctive decrease in area of the spectrum right after the first treatment with the tooth mousse. Second bleaching treatment did not produce a large increase in size of the spectrum. Second tooth mousse treatment caused for an additional decrease in area of the spectrum even beyond the native spectrum.

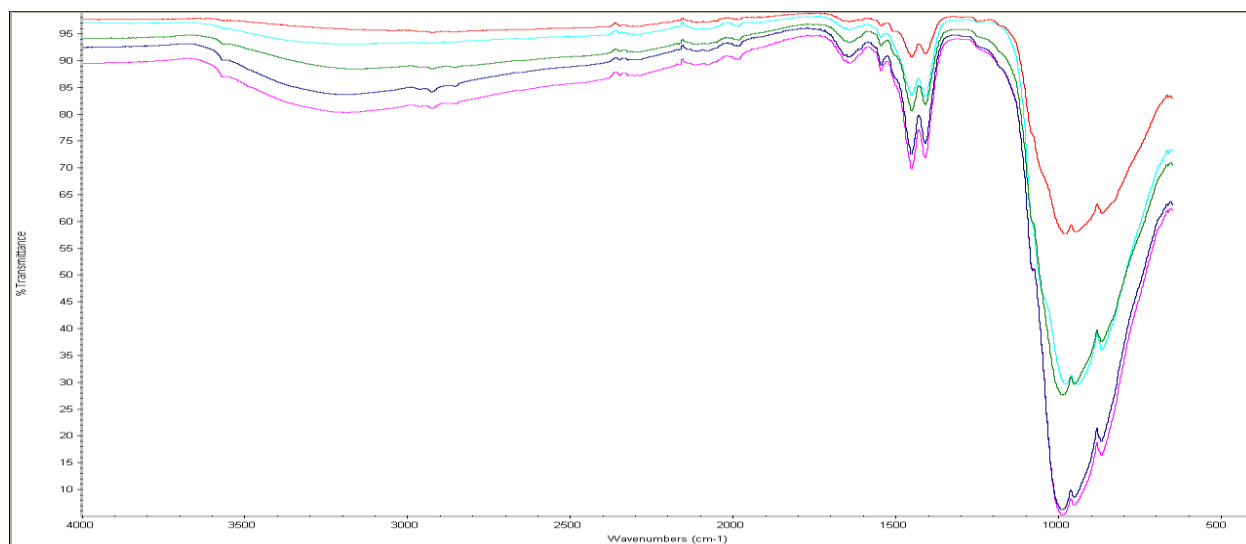


Figure 6; Group 2 human enamel IR spectra; light blue: native scan; pink: 1st bleach treatment; dark blue: 1st tooth mousse treatment; green: 2nd bleach treatment; red: 2nd tooth mousse treatment

Spectra seen in Figure 7, show a consistent increase in size of the spectrum after first bleaching treatment with light, and a decrease in size of the spectrum following the treatment with amorphous calcium phosphate containing toothmousse.

As seen in Figures. 6-7, after treatments with GC tooth mousse, the spectrum provides evidence that the tooth mousse can partially counter the effects of bleaching on the superficial enamel, this was observed as the spectrum was reduced in area width after the treatment, and so, more resemble the native spectrum. In case of Group 2, when bleached for the second time, the graph did not show a significant characteristic changes as was seen after the first bleaching treatment (Figure. 6). In Group 1 (Figure. 5), an increased spectrum area can be seen following each 40% hydrogen peroxide treatment and a continuous effect of the peroxide can be suggested since at the 1 week control additional increase in size of spectrum was detected. When Group 1 was subjected to a second GC tooth mousse treatment the graph showed to regress to area of width smaller than the original value scanned at the native scan.

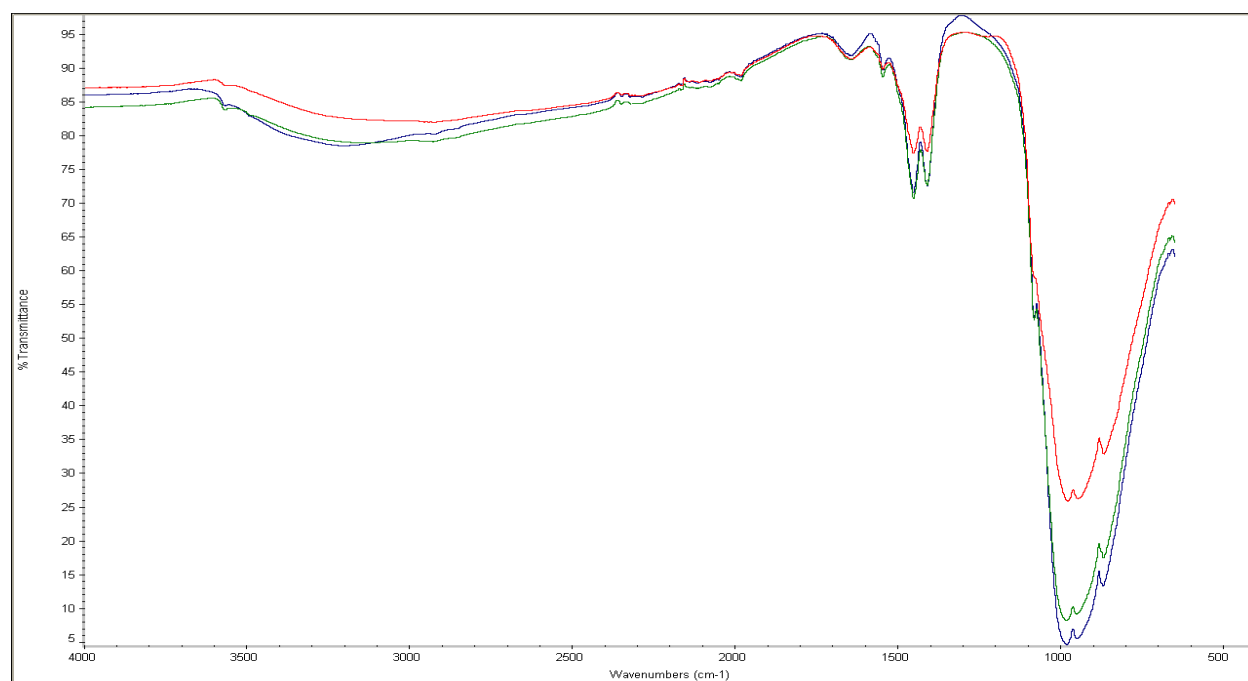


Figure 7; Group 3 human enamel IR spectra; red: native scan; purple: 1st bleach treatment; green: tooth mousse treatment

All the three groups of teeth that were exposed to 40% hydrogen peroxide treatment showed distinct alterations in the IR spectra in the superficial enamel which consisted of increase in area of the spectrum; these can be seen in Figure 8-10. In order to further clarify changes, spectral integrals (Figure 8-11) were made.

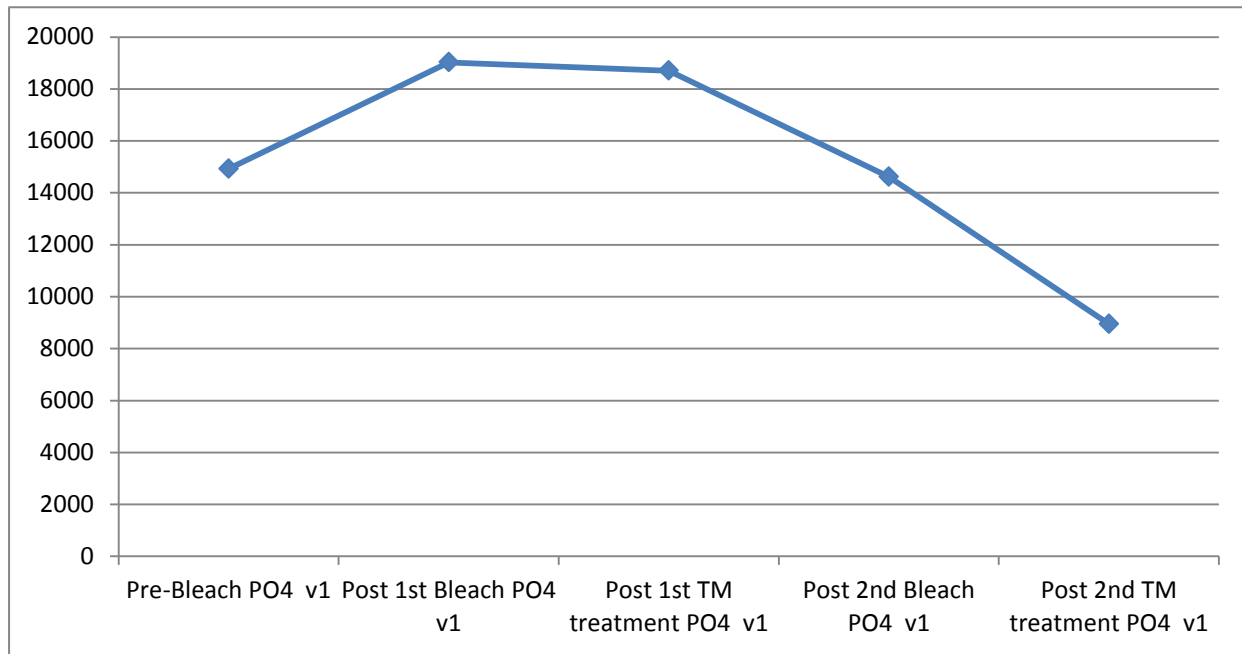


Figure 8; Integral representing area of PO₄ v1 area for group 2

As can be seen in Figure 8, area of apatite PO₄ v1 grew after bleaching treatment. After treatment with GC tooth mousse the area reduced in size.

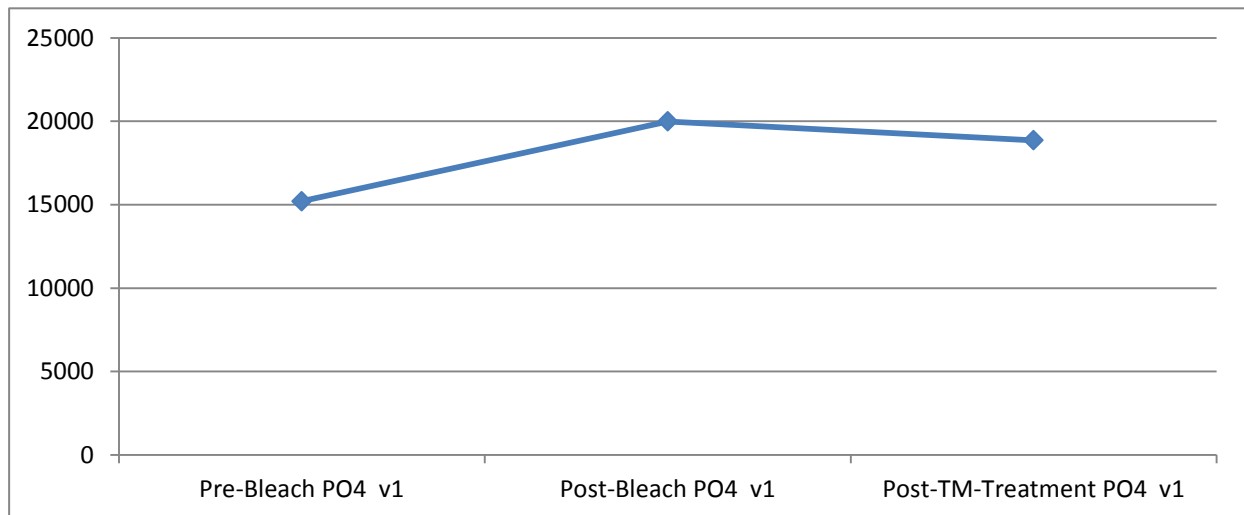


Figure 9; Integral representing area of PO₄ v1 area for group 3

Results shown in Figure 9 are similar to those seen in other groups in regard to an increase of area of the apatite PO₄ v1 area after first bleaching and a subsequent decrease in area after the GC tooth mousse treatment.

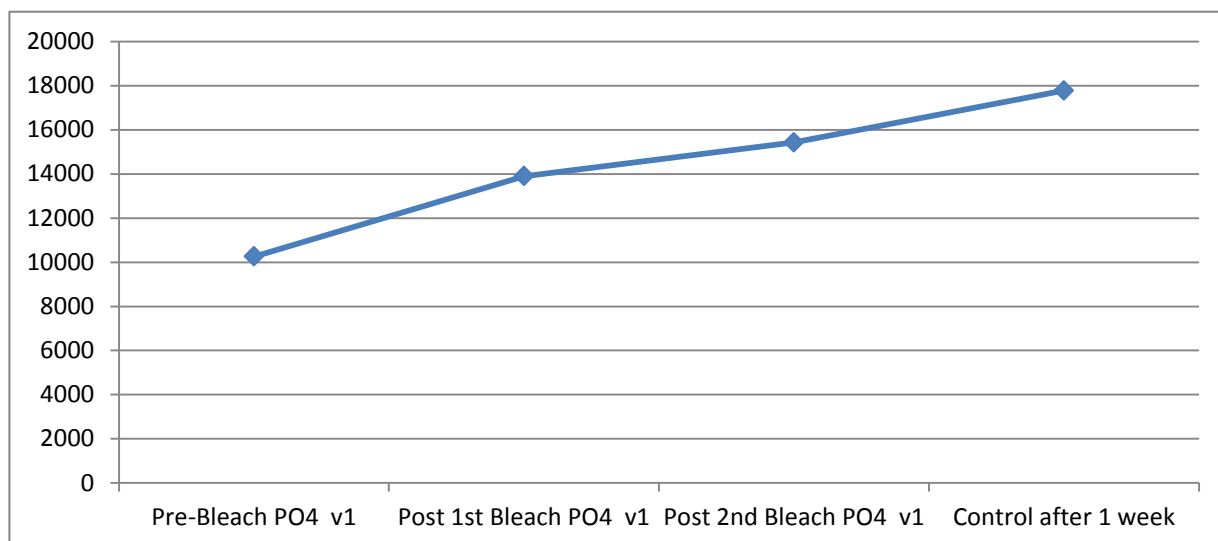


Figure 10; Integral representing area of PO₄ v1 area for group 1

In Figure 10 we can see a gradual increase with each bleaching treatment, continues increase in area can be seen at the control measurement done after 1 week.

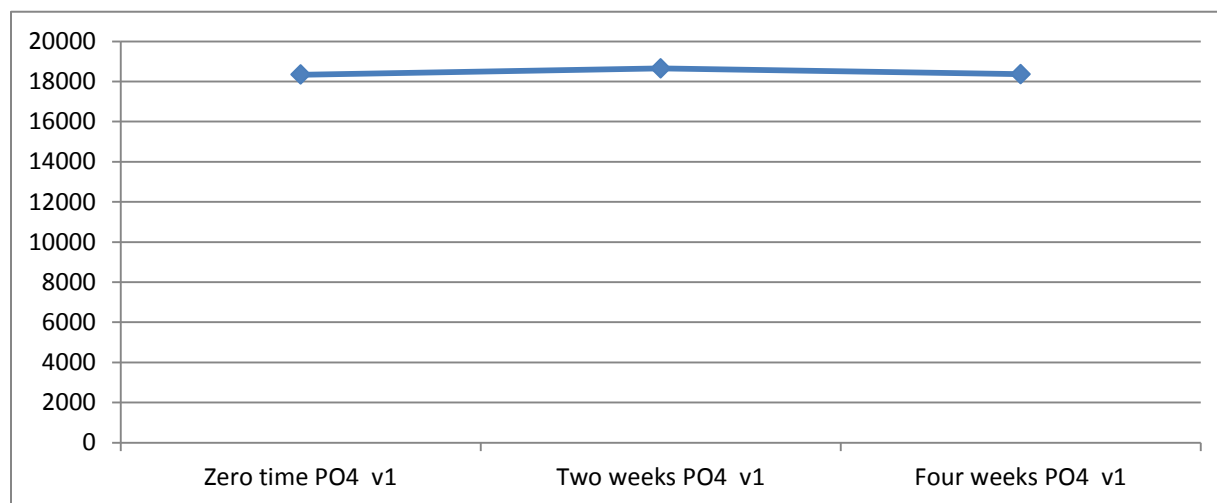


Figure 11; Integral representing area of PO₄ v1 area for group 4 (control group)

In Figure 11, we can see that without treatment, no major changes to the PO₄ v1 area could be detected.

Discussion:

In spite of the widespread use of the tooth whitening systems there is still confusion as to the impact of bleaching agents on the structure of hard dental tissues. Office treatments in particular involve application of high-concentrations of peroxide. To remain stable, these peroxide treatments must be formulated at extremely low pH-value^[18].

Dental enamel is the hardest mineralized biological tissue in the human body, containing approximately 96% mineral, 3% water and 1% organic matter by weight. Bleaching is a widely used conservative treatment of pigmented or discolored teeth; hydrogen peroxide is one of the primary agents used.^[3] Hydrogen peroxide has a low molecular weight, and degrades readily to form oxygen based free radicals ($\bullet\text{OH}$ and $\bullet\text{OOH}$), or the per-hydroxyl anion ($-\text{OOH}$), these radicals penetrate the enamel and move along the dental structure reaching the dentin and even the pulp^[53]. The chemical mechanisms involved in bleaching teeth causes alteration or destruction of the enamel organic matrix, allowing peroxide radicals to diffuse throughout enamel toward dentin and decolorize chromophores. This is done by disrupting the electron conjugation which can cause a change in the absorption energy of the organic molecules in the enamel^[11]. These disrupted molecules reflect less visible light, thus making the tooth appear lighter in shade^[2-4,10].

The hydrogen peroxide concentration of commercial products varies from 3% to 40%^[1, 5]. Previous studies show that the effectiveness of bleaching is directly related to exposure period and concentration of the bleaching agent. An increase in concentration and exposure time will cause the oxygen radical effect to become stronger, which then weakens the surface of the enamel^[12, 13] though a much faster effect is obtained with high concentration gels^[6] the end result can be achieved in all concentrations^[6-8]. In another study by Bistey et al.^[54] postulated that at higher concentration the deleterious effect on superficial enamel is more pronounced, for that reason in this study we chose to examine the effects of 40% hydrogen peroxide.

Commonly used techniques for measurement of the structure of enamel like transversal microradiography (TMR)^[69] and cross sectioned microhardness methods can be used. However, both these methods are destructive to the enamel structure^[14]. In our study IR spectroscopy was chosen as the method of analysis of alterations in superficial enamel as this method allows for a non-destructive measurement of the human enamel's superficial layer, before and after hydrogen peroxide treatments.

Besides the whitening effect of peroxides, side effects can also take place during or after bleaching treatment. The wide use of peroxide radical containing tooth whitening materials requires further investigation. Hydrogen peroxide is the only active ingredient in the most frequently applied bleaching products. That is why specifically the effects of this substance were studied in this research.

Studies show a conflicting approach to the extent of structural damage in relation to bleaching treatment, while some studies such as Lili Sun et al.^[15] which used AFM investigation, Microhardness test, color investigation, ATR-FTIR spectroscopy and Raman spectroscopic detection concluded that, with high concentration hydrogen peroxide satisfactory tooth whitening results were achieved with minimal to none deleterious effects in case of non-acidic bleaching. Other studies such as the study by Oltu, and Gurgan et al.^[16] that used IR absorption spectroscopy and X-ray diffraction analysis, concluded that hydrogen peroxide at a lower (10–16%) concentration, did not have a major effect on the enamel structure. In conclusion, the vast majority of studies indicate that hydrogen peroxide and carbamide peroxide containing products have only a minor deleterious effects on enamel and dentine surface morphology, chemistry, microhardness and microstructure, even if one of the highest concentrations of hydrogen peroxide or carbamide peroxide are used ^[17].

It is important to note that due to the fact that methods are not standardized in measuring effects of bleaching on enamel surface, literature generally reports findings considering the effects of high-concentration treatments on enamel structure which test for surface hardness, ultrastructure, permeability, tensile strength and chemical ratios of calcium phosphate. Many studies suggest that there is a potential deleterious effect on enamel and dentin ^[19–41]. While on the other hand, there are just as many other studies that suggest limited, minor or recoverable effects to the tooth hard tissues ^[15-17,42–52].

Our study consisted of two parts; the first examined the effect of *in vitro* 40% concentration hydrogen peroxide treatment, with, and without light, on human enamel. The second aimed to show the effects of amorphous calcium-hydroxide containing mousse (GC tooth mousse) application on the bleached superficial enamel. Results were measured using IR-spectroscopy show alterations following treatment with 40% hydrogen peroxide. These alterations were recorded in a form of transmittance pattern graph for apatite $\text{PO}_4 \nu_1$ and $\text{PO}_4 \nu_2$, after bleaching the graph became sharper at the peak and wider at the base suggesting an increased penetration of the laser through the superficial enamel (Figure. 2). These findings were exhibited and increased with each treatment using the 40% hydrogen peroxide. It was also observed that after samples were stored in an isotonic solution at 37 ° C., transmittance pattern kept on changing in Group 1 when scanned after a week. This suggested to us that hydrogen peroxide might have a long term effect even after bleaching treatment has ceased.

An important factor in tooth whitening is the reversibility of the inner or surface alterations in tooth hard tissues. The second half of our study examined the spontaneous reversibility and the effects of GC tooth mousse on the changes in enamel after peroxide treatment. While one group of specimens were stored in isotonic salt solution at 37 ° C., between the treatments and spectra measurements, the other was subjected to treatment with amorphous calcium hydroxide containing mousse for five minutes following each bleaching treatment. While IR spectral analysis showed a deleterious process, which continued after the second bleaching treatment and has been shown a week following the last treatment to still be active in Group 1; tooth mousse treated groups showed partial reversibility of the alterations both at the

apatite PO_4 v1 and at the PO_4 v2 spectral bands. According to the above, it can be suggested that amorphous calcium hydroxide treatment following bleaching is beneficial and can partially reverse the effects of bleaching on the enamel surface.

To describe the changes in enamel a quantitative analysis was performed by calculating the area of the apatite PO_4 v1 and at the PO_4 v2 spectral bands. In all groups when treated with 40% hydrogen peroxide an increase area of both bands was measured in each treatment. Following treatment with the amorphous calcium hydroxide mousse, the area of both bands decreased suggesting a partial reversibility to the alterations caused by bleaching. The hypothesis that these alterations were done by the bleaching treatment and not by preparation and storage were supported by the results measured with the control group.

Although more studies are needed in the matter, in accordance to the results of this study we recommend that tooth whitening treatment should be followed by a treatment with GC tooth mousse in order to minimize the negative effects on the superficial enamel.

Conclusions

Using FT-IR spectroscopy changes in the structure of superficial human enamel were seen in all specimens following treatment with 40% hydrogen peroxide with or without light *in vitro*. Application of GC tooth mousse has a significant effect on bleached enamel. GC tooth mousse can partially compensate the deleterious effect of bleaching. 40% hydrogen peroxide treatment has a long lasting effect even when the bleaching material is removed and washed away from tooth surface. Changes to the superficial enamel are proportional to the duration of treatment.

Acknowledgement

I would like to thank my research leader and teacher Dr. Bistey Tamás, for the kind assistance along every step of the research and his teachings to me personally, as I will cherish them along my career. I would also like to thank Dr. Hegedűs Csaba Dean of Dentistry for his support of my research and kind and wise advice. A special thank you goes to Bakó József, and all the laboratory employees, for your acceptance, help and the kindness. I would like to acknowledge the department of dentistry of Debrecen University for opening its doors for the support of my research.

I would like to thank my dear and beloved father and mother for their financial and spiritual support along the many years of my study, my brother and sister and my friends who became like a second family to me both at home and in Hungary.

Thank you.

References:

1. A. Joiner, The bleaching of teeth: a review of the literature, *J Dent*, 34 (2006) 412-9.
2. G. Plotino, L. Buono, N.M. Grande, C.H. Pameijer, F. Somma, Nonvital tooth bleaching: a review of the literature and clinical procedures, *J Endod*, 34 (2008)394-407.
3. M.E. Bittencourt, M.S. Trentin, M.S. Linden, Y.B. de Oliveira Lima Arsati, F.M. Franca, F.M. Florio, et al., Influence of in situ postbleaching times on shear bond strength of resin-based composite restorations, *J Am Dent Assoc*, 141 (2010) 300-6.
4. R.A. Feinman, G. Madray, D. Yarborough, Chemical, optical, and physiologic mechanisms of bleaching products: a review, *Pract Periodontics Aesthet Dent*, (1991) 32-6.
5. Toledano, M. Yamauti, E.Osorio, R. Osorio, Bleaching agents increase metalloproteinases-mediated collagen degradation in dentin, *J Endod*, 37 (2011) 1668-72.
6. C. Hannig, D. Lindner, T. Attin, Efficacy and tolerability of two home bleaching systems having different peroxide delivery, *Clin Oral Investig*, 11 (2007) 321-9.
7. R.F. Mondelli, J.F. Azevedo, A.C. Francisconi, C.M. Almeida, S.K. Ishikiriyama, Comparative clinical study of the effectiveness of different dental bleaching methods - two year follow-up, *J Appl Oral Sci*, 20 (2012) 435-43.
8. L.Y. Tay, C. Kose, D.R. Herrera, A. Reis, A.D. Loguercio, Long-term efficacy of in office and at-home bleaching: a 2-year double-blind randomized clinical trial, *Am J Dent*, 25 (2012) 199-204.
9. B.A. Matis, H.N. Mousa, M.A. Cochran, G.J. Eckert, Clinical evaluation of bleaching agents of different concentrations, *Quintessence Int*, 31 (2000) 303-10.
10. Goldberg M, Grootveld M, Lynch E: Undesirable and adverse effects of tooth-whitening products: A review. *Clin Oral Invest* 14:1-10, 2010.
11. Haywood VB, Berry TG. Natural tooth bleaching. In: Summit JB, Robbins JW, Hilton TJ, Schwartz RS, Dos Santos J, editors. *Fundamentals of Operative Dentistry-A Contemporary Approach*. 3rd ed. Illinois: Quintessence Publishing Co Inc; 2006. pp. 437–62.
12. Bitter NC. A scanning electron microscope study of the long term effect of bleaching agents on the enamel surface *in vivo*. *Gen Dent*. 1998;46:84–8. [PubMed: 9667168]
13. Braun A, Jepsen S, Krause F. Spectrophotometric and visual evaluation of vital tooth bleaching employing different carbamide peroxide concentrations. *Dent Mater*. 2007;23:165–9. [PubMed: 16504281]
14. White DJ, Faller RV, Bowman WD. Demineralization and remineralization evaluation techniques-added considerations. *Journal of Dental Research* 1992;71:929–33.
15. Lili Suna , Shanshan Liang a , Yue Sa, Zhejun Wang, Xiao Ma, Tao Jiang*, Yining Wang* Surface alteration of human tooth enamel subjected to acidic and neutral 30% hydrogen peroxide *journal of dentistry* 39 (2011) 686-692
16. Oltu U., Gurgan S. Effects of three concentration of carbamide peroxide on the structure of enamel. *Journal of Oral Rehabilitation* 2000;27:332–40.

17. Andrew Joiner. Review of the effects of peroxide on enamel and dentine properties journal of dentistry 35 (2007) 889-896.
18. Price RB, Sedarous M, Hiltz GS. The pH of tooth-whitening products. J Can Dent Assoc 2000;66:421-6.
19. Attin T, Muller T, Patyk A, Lennon AM. Influence of different bleaching systems on fracture toughness and hardness of enamel. Oper Dent 2004;29:188-95.
20. Attin T, Vollmer D, Wiegand A, Attin R, Betke H. Subsurface microhardness of enamel and dentin after different external bleaching procedures. Am J Dent 2005;18:8-12.
21. Chng HK, Yap AU, Wattanapayungkul P, Sim CP. Effect of traditional and alternative intracoronal bleaching agents on microhardness of human dentine. J Oral Rehabil 2004;31:811-816.
22. Wiegand A, Otto Y, Attin T. *In vitro* evaluation of toothbrushing abrasion of differently bleached bovine enamel. Am J Dent 2004;17:412-6.
23. Cavalli V Arrais CA, Giannini M, Ambrosano GM. High-concentrated carbamide peroxide bleaching agents effects on enamel surface. J Oral Rehabil 2004;31:155-9.
24. Rodrigues JA, Marchi GM, Ambrosano GM, Heymann HO, Pimenta LA. Microhardness evaluation of in situ vital bleaching on human dental enamel using a novel study design. Dent Mater 2005;21:1059-67
25. Chng HK, Palamara JE, Messer HH. Effect of hydrogen peroxide and sodium perborate on biomechanical properties of human dentin. J Endod 2002;28:62-7.
26. Cullen DR, Nelson JA, Sandrik JL. Peroxide bleaches: effect on tensile strength of composite resins. J Prosthet Dent 1993;69:247-9.
27. Bitter NC. A scanning electron microscopy study of the effect of bleaching agents on enamel: a preliminary report. J Prosthet Dent 1992;67:852-5.
28. Bitter NC, Saunders JL. The effect of four bleaching agents on the enamel surface: a scanning electron microscopic study. Quintessence Int 1993;24:817-24.
29. Bitter NC. A scanning electron microscope study of the long-term effect of bleaching agents on the enamel surface *in vivo*. Gen 1998;46:84-8.
30. Hegedus C, Bistey T, Flora NE, Keszthelyi G, Jenei A. An atomic force microscopy study on the effect of bleaching agents on enamel surface. J Dent 1999;27:509-15.
31. Heling I, Parson A, Rotstein I. Effect of bleaching agents on dentin permeability to *Streptococcus faecalis*. J Endod 1995;21:540-2.
32. Kodaka T, Toko T, Debari K, Hisamitsu H, Ohmori A, Kawata S. Application of the environmental SEM in human dentin bleached with hydrogen peroxide *in vitro*. J Electron Microsc 1992;41:381-6.
33. Lewinstein I, Fuhrer N, Churaru N, Cardash H. Effect of different peroxide bleaching regimens and subsequent fluoridation on the hardness of human enamel and dentin. J Prosthet Dent 2004;92:337-42.
34. McGuckin RS, Babin JF, Meyer BJ. Alterations in human enamel surface morphology following vital bleaching. J Prosthet Dent 1992;68:754-60.

35. Oltu U, Gurgan S. Effects of three concentrations of carbamide peroxide on the structure of enamel. *J Oral Rehabil* 2000;27:332–40.
36. Titley K, Torneck CD, Smith DC. Effect of concentrated hydrogen peroxide solution on the surface morphology of cut human dentin. *Endod Dent Traumatol* 1988;4: 32–6.
37. Titley K, Torneck CD, Smith DC. The effect of concentrated hydrogen peroxide solutions on the surface morphology of human tooth enamel. *J Endod* 1988;14:69–74.
38. Lai YL, Yang ML, Lee SY. Microhardness and color changes of human dentin with repeated intracoronal bleaching. *Oper Dent* 2003;28:786–92.
39. Rotstein I, Lehr Z, Gedalia I. Effect of bleaching agents on inorganic components of human dentin and cementum. *J Endod* 1992;18:290–3.
40. Yurdukuru B, Akoren AC, Unsal MK. Alterations in human enamel surface morphology following the use of an office bleaching agent and consecutive application of 37% phosphoric acid *in vivo*. *J Clin Dent* 2003;14:103–7.
41. Al Qunaian TA. The effect of whitening agents on caries susceptibility of human enamel. *Oper Dent* 2005;30:265–70.
42. Bishara SE, Sulieman AH, Olson M. Effect of enamel bleaching on the bonding strength of orthodontic brackets. *Am J Orthod Dentofacial Orthop* 1993;104:444–7.
43. Ernst CP, Marroquin BB, Willershausen-Zonnchen B. Effects of hydrogen peroxide-containing bleaching agents on the morphology of human enamel. *Quintessence Int* 1996;27:53–6.
44. Langsten RE, Dunn WJ, Hartup GR, Murchison DF. Higher-concentration carbamide peroxide effects on surface roughness of composites. *J Esthet Restor Dent* 2002;14: 92–6.
45. Lee CQ, Cobb CM, Zargartalebi F, Hu N. Effect of bleaching on microhardness, morphology, and color of enamel. *Gen Dent* 1995;43(158–60):162.
46. Park HJ, Kwon TY, Nam SH, Kim HJ, Kim KH, Kim YJ. Changes in bovine enamel after treatment with a 30% hydrogen peroxide bleaching agent. *Dent Mater* 2004;23:517–21.
47. Pretty IA, Edgar WM, Higham SM. The effect of bleaching on enamel susceptibility to acid erosion and demineralisation. *Braz Dent J* 2005;198:285–90.
48. Ruse ND, Smith DC, Torneck CD, Titley KC. Preliminary surface analysis of etched, bleached, and normal bovine enamel. *J Dent Res* 1990;69:1610–3.
49. Sulieman M, Addy M, Macdonald E, Rees JS. A safety study *in vitro* for the effects of an in-office bleaching system on the integrity of enamel and dentine. *J Dent* 2004;32:581–90.
50. Spalding M, Taveira LA, de Assis GF. Scanning electron microscopy study of dental enamel surface exposed to 35% hydrogen peroxide: alone, with saliva, and with 10% carbamide peroxide. *J Esthet Restor Dent* 2003;15:154–64.
51. Tam LE, Lim M, Khanna S. Effect of direct peroxide bleach application to bovine dentin on flexural strength and modulus *in vitro*. *J Dent* 2005;33:451–8.
52. Tong LS, Pang MK, Mok NY, King NM, Wei SH. The effects of etching, micro-abrasion, and bleaching on surface enamel. *J Dent Res* 1993;72:67–71.

53. Berger SB, Tabchoury CP, Ambrosano GM, Giannini M. Hydrogen peroxide penetration into the pulp chamber and dental permeability after bleaching. *Gen Dent*. 2013 May-Jun;61(3):e21-5.
54. Tamás Bistey, Istvan P. Nagy, Anett Simo, Csaba Hegedus *In vitro* FT-IR study of the effects of hydrogen peroxide on superficial tooth enamel *journal of dentistry* 35 (2007) 325-330.
55. Berga-Caballero A, Forner-Navarro L, Amengual-Lorenzo J. At-home vital bleaching: a comparison of hydrogen peroxide and carbamide peroxide treatments. *Med Oral Patol Oral Cir Bucal*. 2006;11:E94-9.
56. Haywood VB. History, safety, and effectiveness of current bleaching techniques and applications of the nightguard vital bleaching technique. *Quintessence Int*. 1992;23:471-88.
57. Haywood VB, Heymann HO. Nightguard vital bleaching. *Quintessence Int*. 1989;20:173-176.
58. Dahl JE, Pallesen U. Tooth bleaching—a critical review of the biological aspects. *Critical Reviews in Oral Biology & Medicine* 2003;14:292–304.
59. Lee KH, Kim HI, Kim KH, Kwon YH. Mineral loss from bovine enamel by a 30% hydrogen peroxide solution. *Journal of Oral Rehabilitation* 2006;33:229–33.
60. Ma X, Li R, Sa Y, Liang S, Sun L, Jiang T, et al. Separate contribution of enamel and dentine to overall tooth colour change in tooth bleaching. *Journal of Dentistry* 2011;39: 739–745.
61. Buchalla W, Attin T. External bleaching therapy with activation by heat, light or laser—a systematic review. *Dental Materials* 2007;23:586–96
62. Attin T, Schmidlin PR, Wegehaupt F, Wiegand A. Influence of study design on the impact of bleaching agents on dental enamel microhardness: a review. *Dental Materials* 2009;25:143–57.
63. L.G Davis, P.D Ashworth, L.S Spriggs Psychological effects of aesthetic dental treatment *Journal of Dentistry* Volume 26, Issue 7, September 1998, Pages 547–554
65. Marshall K, Berry T, Wollum J: Teeth whitening: Current status. *Compend Contin Educ Dent* 31:486-495, 2010
66. <http://www.thehistoryof.net/the-history-of-teeth-whitening.html> 21st of January 2014
67. ADA. Tooth Whitening/Bleaching: Treatment Considerations for Dentists and Their Patients, ADA Council on Scientific Affairs, revised November 2010. American Dental Association.
68. Ranjitkar S., Rodriguez J.M., Kaidonis J.A., Richards L.C., Townsend G.C., Bartlett D.W. The effect of casein phosphopeptide–amorphous calcium phosphate on erosive enamel and dentine wear by toothbrush abrasion *Journal of Dentistry* Volume 37, Issue 4, Pages 250-254, April 2009
69. <http://www.inspektor.nl/dental/tmrmain.htm> February 02, 2014
70. Ruchi Vashisht, Rajamani Indira, S Ramachandran, Anil Kumar, Manali Ramakrishnan Srinivasan. Role of casein phosphopeptide amorphous calcium phosphate in remineralization of white spot lesions and inhibition of *Streptococcus mutans*?. *J Conserv Dent*. 2013 Jul-Aug; 16(4): 342–346.

Appendix

In this research I performed by myself

1. Cutting the roots of the teeth
2. Hemisected the crown of the teeth
3. Embedding in epoxy resin
4. Polishing the epoxy in preparation for scanning
5. Bleaching treatments
6. GC tooth mousse treatments.

With the help of Bistey Tamás and Bakó József the following were done

1. Scanning using the IR-spectroscope
2. Collection of data and interpretations
3. Reaching conclusions about data collected