Title:

Clinical Evaluation of Enamel Microhardness Using At-Home and InOffice Tooth Whitening Agents

NCT#

07165366

Study Protocol:

May 13, 2024

Protocol of a Scientific Research Project to the UIC Bioethics Committee Therametric Technologies, Inc.,

May 13, 2024.

1- PRINCIPAL INVESTIGATOR:

FULL NAME: _____

INSTITUTION: Ultradent Products, Inc. and Therametric Technologies, Inc.

GRADUATION: Case Western Reserve University School of Dentistry, DDS, 1971.Indiana University School of Dentistry, Master's Degree, 1983.

ADDRESS FOR CORRESPONDENCE: 7141 Creekwood Est; Brownsburg, IN 46112.

COUNTY: Hendricks.

CITY: Brownsburg.

TELEPHONES: LANDLINE: 1-317-201-2089. OFFICE: 1-317-851-2139.

EMAIL:

HIGHEST DEGREE: Master of Science in Dentistry.

ADDRESS ONLINE CURRICULUM: None.

RESEARCH GROUP/NETWORK: None.

2- TITLE OF THE PROJECT:

Effect on the Enamel of At-Home Tooth Whitening Agents With and Without Potassium Nitrate and Fluoride and an In-Office Tooth Whitening Agent: *In-Vivo*.

AREA OF KNOWLEDGE: Effect of teeth whitening, oral surgery, orthodontics, fluoride.

SUBAREA OF KNOWLEDGE: Demineralization, appearance, profilometer, nano hardness.

SPECIALTY: Dental surgery, surgery, orthodontics, preventive dentistry.

KEYWORDS: Teeth whitening, oral surgery, orthodontics, demineralization.

3- PROJECT IDENTIFICATION:

Month/date and year of project completion: August to December 2024.

There are no links to school academic credits. This project will be conducted at the orthodontics specialty and oral surgery clinic.

4- LIST OF PROJECT TEAM MEMBERS:

NAME:

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Personal information:

- Maximum qualification: Master of Science in Dentistry, 1983.
- Phone:1-317-201-2089.
- Curriculum: (Attachment 1).

NAME:

-

Personal information:

- Maximum qualification: Master of Science in Dentistry, 2001.
- Phone:1-206-708-0429.
- Curriculum: (Attachment 2).

NAME:

-

Personal information:

- Maximum qualification: Master of Science in Dentistry, 2011.
- Phone: 55-3032-2376.
- Curriculum: (pending as Attachment 3).

ULTRADENT AND THERAMETRIC

NAME:

-

Maximum qualification: Doctor of Dental Surgery.

Title: Vice President of Clinical Affairs at Ultradent Products, Inc.

Curriculum: (pending as Attachment 4).

NAME:

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Maximum qualification: Master of Science in dentistry.

Title: Senior Vice President of Global Supply Chain at Ultradent.

Curriculum: (pending as Attachment 5).

NAME:

-

Maximum qualification: Bachelor of Science in biochemistry and biology.

Title: Director General de Therametric Technologies, Inc.

Curriculum: (Attachment 6).

5- PLACE OF INVESTIGATION:

STAGE 1: The Postgraduate Department of Orthodontics of the UIC in Mexico City will identify patients who need the extraction of a premolar from each of the four quadrants of the oral cavity and meet the inclusion/exclusion criteria. Those premolars will be marked on a dental model, and the patient will be referred to Dr. Fugaro. The doctor will explain the research study to the patients (and at least to one parent if the patient is under 18 years of age), ensure that they qualify for the research study, and ask for their consent. This will happen at the Universidad Intercontinental (UIC) in Mexico City. A clinical area will be used in the facility where the patient is referred to Dr. Fugaro, which has high and low-speed handpiece capabilities and where, privately, patients can sign the consent statement. All tooth whitening procedures will be performed in the UIC dental area or at the subject's home. Once the whitening treatment is completed, as described in the project methodology, the extractions will be performed on the patient at the UIC oral surgery clinic. The facility needs to include a sterilization center, clinic, laboratory, and a dental intraoral scanner.

STAGE 2: Evaluation of the collected samples will take place at Therametric Technologies, Inc. (TTI) in Noblesville, Indiana, USA.

6- GENERAL SUMMARY OF THE PROPOSAL:

The research project aims to determine what changes, if any, occur in the outer layers of enamel in microhardness, profilometer scanning, and SEM (Scanning electron microscopy) when tooth whitening agents are used intraorally. The samples will be collected from subjects being treated in orthodontic clinics at UIC, extracted at the UIC oral surgery clinic in Mexico City, and evaluated at the TTI dental laboratory in Indiana, USA.

The objective is to evaluate structural changes (microhardness and decalcification) in the enamel after an At-Home whitening agent with Opalescence 10% Carbamide Peroxide (CP) with and without PF (potassium nitrate and fluoride) or Opalescence Boost 40% Hydrogen Peroxide (HP), an In-Office tooth whitening agent and Ultra-Etch, a 35% phosphoric acid are used as recommended by the manufacturer. All are Ultradent Products, Inc., and in common use in dental practices, worldwide.

The methodology described in detail in field number 14 is summarized. The four premolars due to be extracted for orthodontic reasons will have at least a 1 x 1 millimeter surface flattened with a 169L bur. That surface will be polished with fine and extra fine sandpaper discs. This will improve the evaluation in the dental laboratory, especially of Vickers hardness. A maxillary premolar will be whitened at home with Opalescence 10% CP with PF in 30 sets of subjects and in 10 sets with Opalescence 10% CP without PF for 14 nights. The opposite maxillary premolar will be used as a control (no treatment). The mandibular and collateral premolar of the one that was whitened At-Home it will be whitened in all 40 sets at the office with Opalescent Boost PF 40% HP for a total of 60 minutes (three consecutive times of 20 minutes each). The opposite mandibular premolar to the control in three of the 30 sets of premolars that were treated with PF and in three of the 10 sets of premolars that were bleached without PF will have two narrow bands of Block-Out resin placed on a premolar. The area between the bands will be etched for 15 seconds with Ultra-Etch, a 35% phosphoric acid on the day of the extraction appointment. The extractions will be accomplished 14 days after finishing the whitening At-Home and In-Office procedures. The collected samples will be delivered to TTI for evaluation.

The *in-vivo* treatment of the teeth and the extractions will be performed at UIC. Treatment effects will be evaluated at TTI in Zionsville, Indiana, USA.

7- Keywords:

Demineralization, Teeth whitening, Profilometer, Scanning electron microscope (SEM), Vickers hardness, *in-vivo*.

8- INTRODUCTION OF THE PROJECT:

Matis et al. in 1996 established that teeth whitening was safe and effective. The concern of some patients and dental practitioners was whether the use of teeth whitening agents harms the teeth. One of the most commonly cited concerns is "Does it soften teeth?" A review of enamel microhardness has been published that identified 55 studies and 166 hardness measurements performed directly after bleaching and after post-treatment (Attin et al. Influence of study design on the impact of bleaching on dental enamel microhardness: A Review Den Mat 25;143;2009). The published article concluded: “The review shows that in those studies, which simulated intraoral conditions as closely as possible (use of human saliva and fluoridation measures, and evaluation after a post-treatment phase), the risk of enamel microhardness decrease due to bleaching treatments seems to reduce as compared to the remaining studies. Nevertheless, more *in-situ/in-vivo* studies are needed to verify the observation”. No *in-vivo* designed study has shown that there is a loss of microhardness when bleaching is accomplished. An *in-vivo* designed study published using 15% Carbamide Peroxide as a whitening agent with and without fluoride (Metz et al. Clinical Assessment of 15% Carbamide Peroxide on the Surface Microhardness and Shear Bond Strength of Human Enamel, Op Dent 32:427;2007) stated: “Additionally, there was no statistically significant difference between the treatment specimens compared to the controls in terms of SMH (Surface Microhardness)”. Another *in-vivo* published study used 38% hydrogen peroxide as a whitening agent (Cardenaro et al. Effect of Two In-office Whitening Agents on the Enamel Surface *In-Vivo*: A Morphological and Non-contact Profilometric Study Op Dent 33:127;2008), concluded: "No morphological changes were found on the enamel surfaces using non-contact profilometer or SEM analysis". Some researchers have found differences when evaluating enamel surfaces. Therefore, the intention of this study is to determine if there are changes in the enamel surfaces when treating premolars with tooth-whitening agents *in-vivo*.

A common concern with the use of teeth whitening agents is sensitivity to the agents and possible pulpal damage. Research has shown that between 50 and 75% of subjects have some sensitivity.

The American Academy of Pediatric Dentistry has published a document on tooth whitening that recommends that this treatment be performed by a dentist (Academy of Pediatric Dentistry, 2023). If the soft tissue or tooth becomes sensitive, it has been shown that with the application of potassium nitrate (Opalescence UltraEZ), this sensitivity usually decreases (Tam L.. Effect of potassium nitrate and fluoride on carbamide peroxide bleaching Quintessence Int 32(10):766-70; 2001). Fugaro JO et al. in 2004 concluded that the use of 10% CP can cause minimal short-term

pulpal reactions in about a third of the intact teeth of young people who used the whitening agent; however, if such changes occurred, they were completely reversible. Furthermore, no clinical sensitivity was reported during the two-week treatment period. Also, Fugaro OJ et al. in 2005 concluded that with the use of a 10% CP tooth whitening agent, no increase in the expression of neuropeptides associated with inflammation (substance-P and/or Peptide related to the calcitonin gene) was reported.

9- JUSTIFICATION OF THE PROJECT:

This research proposal will be a confirmatory research study of that published by Metz et al. in 2007 and Cardenaro et al. in 2009, documenting that there is no loss of microhardness when whitening occurs *in-vivo*. It was also recommended by Attin et al., in 2009, in their conclusion that "Nevertheless more *in-situ* and *in-vivo* studies are needed to verify this observation" that enamel microhardness loss decreases the more intraoral conditions are simulated. Metz et al. in 2007 published that there were no statistically significant differences between treatment samples compared to controls in terms of SMH (surface microhardness). Cardenaro et al. in 2008, concluded in her *in-vivo* study that no morphological changes were found on the enamel surfaces using a non-contact profilometer or SEM analysis. There are no known studies, to our knowledge, of the effect of PF compared with non-PF treated surfaces on the physical characteristics of enamel.

The identification, treatment, and extraction of the teeth to be whitened will be performed at the UIC and other approved entities. TTI will study the collected *in-vivo* samples from Mexico City and will also perform simultaneous studies *in-situ*.

10- PROJECT HYPOTHESIS:

The null hypothesis is that there will be no change in enamel microhardness when treatment is performed with the whitening agents used in this *in-vivo* study and no changes in enamel characteristics when using potassium nitrate. An alternative hypothesis is that there will be a significant loss of microhardness and in the physical characteristics of the treated premolars, *invivo*.

11- MAIN OBJECTIVE:

The main objective of this study is to determine if there are changes in the microhardness of the enamel surfaces when treating 40 sets of premolars with tooth-whitening agents in an *in-vivo* designed study of premolar teeth treated and extracted from orthodontic patients at UIC.

12- SECONDARY OBJECTIVES:

The secondary objective is to see what changes in enamel surfaces occur with profilometric analysis, and SEM analysis in the premolars submitted to and analyzed at TTI. Potassium nitrate and fluoride are added to tooth-whitening agents as preventive measures to decrease sensitivity and strengthen the enamel. With questionnaires and microhardness measurements, we will be able to determine if agents containing PF are effective and cause physical changes in enamel surfaces.

13- THEORETICAL BASIS:

Some teeth whitening agents are acidic, and pH values below 5.5 have been shown to cause demineralization of tooth structure. This may be a reason some studies have shown that a loss of microhardness occurs when using some acidic tooth-whitening products. In a study by Attin et al. 2009, when samples were kept in a “non-demineralizing solution”, 47% of studies showed no reduction in sample microhardness; when they were kept on “artificial saliva,” 46% of studies showed no reduction; when kept in “human saliva,” 78% of studies showed no reduction. However, after post-treatment, 100% of studies showed no reduction in microhardness when samples were kept in “human saliva.” When studies were conducted "fluoride-free" and microhardness measurements were accomplished, 46% showed no reduction in microhardness; when “fluoride” was used as part of the conditions of the study, 86% of the studies showed no reduction in microhardness. When the study design was *in-vitro*, 48% of the studies showed no reduction in the microhardness; when the study design was *in-situ*/*in-vivo*, 64% showed no reduction in microhardness. When whitening agents have a neutral pH and enamel samples are maintained in human saliva, there is no loss of hardness or changes in the enamel surface, according to a systematic review and meta-analysis accomplished in New Zealand. (Zanolla J. et al. Influence of tooth whitening on the microhardness of tooth enamel: a systematic review and meta-analysis. Aust Dent Jour 62:276-282;2017).

14- PROJECT METHODOLOGY:

Methods and materials

This study consists of 40 patients requiring the extraction of four permanent first or second premolars for orthodontic reasons. Patients will be recruited from the Universidad Intercontinental (UIC) Graduate Program in Orthodontics, the Universidad Autonoma de Mexico (UNAM) Graduate Program in Orthodontics, and private orthodontist dental offices in the city of Mexico. Patients who identify as needing extraction of the four first or second permanent premolars and who meet the following inclusion criteria will be invited to participate in the study.

Inclusion criteria:

- Have the four premolars in different quadrants that their orthodontist recommends be extracted.
- The premolars to be extracted should have prominent facial surfaces.
- Willing to have the four premolars slightly flattened and smoothed.
- Willing to have all four premolars extracted for orthodontic reasons.
- Between 12 and 35 years of age.
- Willing to attend four appointments.
- Willing to wear a custom-made whitening tray during the evening when they sleep.
- Willing to have their premolars bleached with In-Office and At-Home bleaching agents and one of their teeth that will be extracted treated with phosphoric acid.
- Willing to sign a consent form (parents must co-sign if they are under 18).
- Willing to discontinue active orthodontic treatment during whitening treatment.
- Willing to brush at least twice a day with fluoridated toothpaste provided during the study.

Exclusion criteria:

- Restorations or visible cavities in the teeth to be extracted.
- The presence of visible intraoral structural defects or pathology.
- Use of over-the-counter or professionally prescribed whitening agents in the past six months.
- Teeth with tetracycline staining.
- History of any medical illness that may interfere with the study or require special considerations.
- Pregnant or lactating women.
- Not willing to do the blood tests (biometrics hematic, prothrombin, and thromboplastin) prior to extractions (a legal requirement of the Oral Surgery Department of the UIC).

Treatment plan:

The study will be approved by Ultradent Products, Inc. and the Health Sciences Divisional Area Bioethics Committee, which is the equivalent of the Institutional Review Board (IRB) of UIC before any patients will be evaluated for eligibility to enter the study.

At the first appointment (selection appointment), the absence of evident cavities or restorations in the four premolars will be clinically verified with an intraoral mirror. Root formation will be confirmed with the panoramic radiograph from the patient's orthodontic chart. The treatment will be explained, and the patient will be allowed to ask questions and discuss any concerns. Subjects will be informed that the study will consist of 4 appointments. If the patient qualifies and agrees to be part of the study, they will be asked to sign an approved Consent Form. Parents or guardians will be asked to sign the consent form for patients under 18 years of age. They will be given a copy of the signed Consent Form. The requirements are that the four teeth that will be extracted for orthodontic reasons will be flattened and polished to produce a one-by-one millimeter surface on the facial or most prominent surface. This will be done with a finishing carbide bur and fine and extra-fine sandpaper discs. Additionally, they must agree to use a whitening tray every night for 14 nights with Opalescence 10% CP with or without PF (potassium nitrate and fluoride) on an upper right premolar (UR) or on the upper left premolar (UL) (the tooth that has the best alignment). The contralateral lower right (LR) or lower left (LL) premolar will be treated with 40% hydrogen peroxide (HP) Opalescence Boost PF at the dental clinic for three 20minute periods according to the manufacturer's instructions**. Thirty of the 40 subjects will treat their UR or UL premolar treated with Opalescence 10% CP with PF. The remaining 10 subjects will treat the UR or UL premolar, with Opalescence 10% CP without PF.

A digital scan impression (Dentsply Sirona Scanner) of each subject’s dentition will be taken. Study models will be fabricated. On the UR or UL premolar of the model, a 0.5 mm thick veneer of unfilled Block-Out Resin (Ultradent Products Inc.) will be placed. The veneer will be applied on the facial surface or, by exception, on the lingual surface (the most prominent) of the tooth at 1.0 mm mesial, distal, and incisal, and 1.5 mm from the gingival margin. The resin veneer will be lightcured for 15 seconds using a standard visible curing light unit. Bleaching trays will be fabricated from 0.035-inch plastic sheets (Sof-Tray; Ultradent Products Inc.) on each subject's digital model and appropriately trimmed in the UIC laboratory and/or in a private practice laboratory. The trays will be trimmed with micro scissors, 0.5 mm from the gingival margin. Excess tray material will be cut off, and the tray will be carefully removed from the digital model. To reduce gingival trauma, the trays will be trimmed with micro scissors 0.5 mm from the gingival margin. If the patient has started the placement of braces and/or orthodontic bands, the trays will be modified with “windows” to accommodate the brackets, if necessary, and the tray material

over the second molars will be removed to allow a better adaptation (no movements or orthodontic treatments will be allowed in any of the four premolars to be extracted).

The second appointment will occur at least 24 hours after the first appointment. The subject will receive the maxillary whitening tray with a single reservoir for the UR or UL premolar, a tube of Opalescence 10% CP containing or not containing PF whitening gel, and a container for tray storage, when tray is not in use. The tray will be placed in the mouth to ensure a proper fit without interference. During this appointment, the color of that premolar will be taken with VITA Bleachedguide 3-D MASTER shade guide (VITA, Zahnfabrik, Germany). The tray will be placed in the mouth to ensure a proper fit without interference. The subject will be instructed to place a small amount of whitening gel into the individual reservoir for the UR or UL premolar each evening, then carefully place the tray over the teeth, and wipe off the excess gel. The subject will be instructed to wear the bleaching tray for at least 8 hours every night, for 14 days, after brushing their teeth. Each morning after using the tray, the subject will be instructed to brush off the used gel, rinse the tray, store it in the container provided, and brush their teeth with the toothpaste provided. During the 14 days of whitening at home, the subject will be contacted twice by phone or text message to make sure they are using the tray with the whitening gel properly.

The third appointment will occur on day 15. The subject will return the maxillary whitening tray and will be encouraged to report any concerns with the bleaching treatment or tray usage. During this appointment, the LR or LL premolar and collateral to the one that was bleached at-home will be isolated with OpalDam (Ultradent Products, Inc.) and will be treated with Opalescence Boost PF 40%. The treatment will consist of the application of the gel three separate times, each will remain on the one premolar for 20 minutes, according to the manufacturer's instructions*. If the soft tissue or tooth is sensitive, a soothing gel will be applied to relieve discomfort (Opalescence UltraEZ). Tissue sensitivity usually lasts about thirty minutes and tooth sensitivity usually decreases in two or three days. This appointment will last approximately 90 minutes. During this appointment, an orthodontic separator band or elastic band of 2.5 to 4.5 oz / ⅛” to ¼” (depending on interproximal access) will be placed in the gingival area of each premolar to create bone resorption and facilitate an atraumatic extraction. The final color will be taken of the premolar that received 14 days of whitening at-home, as well as the initial and final color of the premolar that will be bleached in-office with VITA Bleachedguide 3-D Master (VITA, Zahnfabrik, Germany); this is to corroborate and document that the teeth were successfully whitened. At this appointment, the subject will be reminded to complete the blood tests required by the UIC oral surgery department since, without these lab tests, the extractions cannot be performed. Therefore, the compensation would not be completed. Also, the subject will be reminded to

continue documenting on the log the brushing at least twice a day for at least one minute each time and will be asked to follow these instructions for the next 14 days.

The fourth and final appointment will take place on day 29, in other words,14 days after the third appointment and 14 days after they have already finished their whitening treatment at-home and in-office. At this appointment, 6 subjects, three of the 30 subjects that used 10% CP with PF and three of the 10 subjects that used 10% CP without the PF, will have their mandibular second control tooth etched with 35% phosphoric acid (Ultradent Products, Inc.) for 15 seconds. These 6 subjects will be chosen to be treated before extractions with the etchant at a time convenient to the oral surgeon and Dr. Fugaro. Extractions will be performed during this appointment for all subjects. Four to five UIC maxillofacial surgeons will perform the extractions. Local anesthetics will be at the discretion of the oral surgeon and the patient's needs. Extractions will be performed with a simple elevator forceps technique as described in the attached protocol**. Immediately after extractions, the teeth will be cleaned to remove any debris with a dental curette without touching the treated area. Each tooth will be placed in a miniature vial with hinged cap (ColePalmer) with a wet sponge (sodium-free drinking water) and 0.12% chlorhexidine at the bottom of the bottle and snapped closed. Each vial will be labeled with the date of extraction, a subject identification number, the subject's initials, the tooth number, and how the tooth was treated (athome, in-office, etching, or control) and forwarded for testing to Therametric Technologies, Inc.

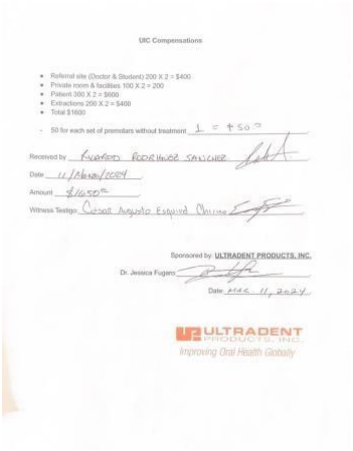
*Instructions for in-office whitening Opalescent Boost PF 40% HP are attached.

**Extraction protocol is attached.

Research risks, data collection instruments in addition to the ethical aspects of the research:

Risks include sensitivity to teeth whitening agents and infection during or after surgery. To decrease the chance of sensitivity, this study will use bleaching agents on 30 subjects to which potassium nitrate and fluoride (PF) have been added, which has been shown to reduce sensitivity. If necessary, the 10 subjects that will be bleached without PF will also receive a potassium nitrate syringe to reduce further sensitivity, if it occurs. There is the possibility of infection during surgery due to the use of instruments (which is unlikely since all instruments will be previously and adequately sterilized) or due to a lack of correct post-surgery follow-up of the subject. Subjects will be reminded to follow the recommendations of the maxillofacial surgeon and the instructions sheets they are given.

Compensations related to this project will be made directly with the general administration department of the School of Dentistry in conjunction with the Orthodontics specialty department of the UIC. The university will be responsible for any treatment and follow-up care required from the extractions. Ultradent Products Inc. will be responsible for any treatment that the subjects require directly related to the whitening treatment. Compensation logs will be signed by every person who receives compensation *(example below).*



UIC Compensations

- Referral site (Doctor & Student) 200 X 2 = $400
- Private room & facilities 100 X 2 = 200
- Patient 300 X 2 = $600
- Extractions 200 X 2 = $400
- Total $1600

- 50 for each set of premolars without treatment 1 = $50

Received by EVARISTO RODRIGUEZ SANCHEZ

Date 11/26/2024

Amount $1650

Witness Testigo Cesar Augusto Esquivel Chilino

Sponsored by ULTRADENT PRODUCTS, INC.

Dr. Jessica Fugaro

Date NOV 11, 2024

ULTRADENT PRODUCTS, INC.

Improving Oral Health Globally

Compensation log.

The UIC School of Dentistry will provide a clinical area where Dr. Fugaro can privately consent to subjects and use the dental scanner, clinic, laboratory, and will provide sterilizing support for the instruments used in the study.

Any supplies or additional expenses by the study sponsor, Ultradent Products, Inc. will need to be pre-approved.

15- EXPECTED RESULTS:

Since a similar study has been done *in-vivo*, this is a confirmatory study. No changes in microhardness are expected in this *in-vivo* designed study on the subjects' enamel, whether they were bleached in-office for 60 minutes or used bleaching agents with or without PF at-home for 14 days.

16- BIBLIOGRAPHICAL REFERENCES:

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Cardenaro M, Breishi L, Nucci C & Antoniolli, F et al. Effect of Two In-office Whitening Agents on the Enamel Surface In Vivo: A Morphological and Non-contact Profilometric Study. Oper Dent 33:127-134;2000.

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Matis BA, Cochran MA, Wang G & Eckert GJ. A Clinical Evaluation of Two In-Office Bleaching Regimens With and Without Tray Bleaching. Oper Dent 34:142-149;2009.

Matis BA, Cochran MA, Franco M, Al-Ammar W et al. Eight In-Office Tooth Whitening Systems Evaluated *Live*: A Pilot Study. Op Dent 32:332-327;2007.

Metz MJ, Cochran MA, Matis BA, Gonzalez, C . et al. Clinical Evaluation of 15% Carbamide Peroxide on the Surface Microhardness and Shear Bond Strength of Human Enamel. Oper Dent 32:427436;2007.

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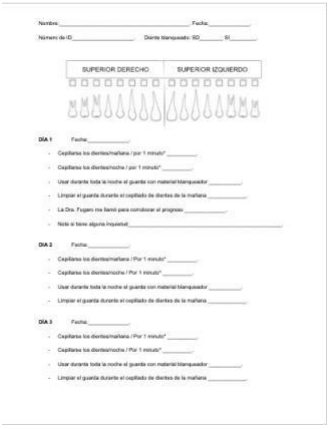
Tam L. Effect of potassium nitrate and fluoride on carbamide peroxide bleaching Quintessence Int 32(10):766-70; 2001).

17- IMPLEMENTATION PROGRAM:

Each subject will begin the study when the UIC Department of Orthodontics or another referral source determines the optimal time to proceed. Each subject, once enrolled, will have the following appointment schedule after the Bioethics Committee approves the study:

The first appointment will be scheduled to present the study to the patient (if under 18 years of age, a parent or guardian will be included), allow the patient to ask questions, and determine if the patient meets the inclusion/exclusion criteria. If so, the patient will be asked to sign the informed consent statement and given a copy. During this appointment, a 1 mm x 1 mm area on the facial area (or most prominent surface) of the four premolars to be extracted will be flattened with a 169L bur and the area further polished with fine and extra-fine sandpaper discs. Also, a digital impression of the maxillary arch will be taken with a dental scanner.

During the next appointment, a custom bleaching tray previously made for the subject will be placed and adjusted if necessary. Instructions and materials for at-home whitening will be given. The subject will be shown how to use the bleaching gel and tray and will also be given a personalized writing log to document day by day the use of the whitening agent at home on the treated teeth. They must brush their teeth at least twice a day for at least one minute each time. They are to clean and store the bleaching tray when not in use in the container provided *(example below)* and follow the instructions given to them. This appointment will be done at least 24 hours after the initial appointment.



Subject log.

On day 15, that is, 14 days after finishing whitening at-home, the subject will return the maxillary whitening tray and will be allowed to report any adverse events that have occurred. During this appointment, one of the mandibular premolars to be extracted will be treated with whitening agents in-office for a total of 60 minutes. The subject will be reminded to continue documenting the log of brushing the teeth at least twice a day for at least one minute each time, and you will be asked to do so for the next 14 days. In this appointment, an orthodontic separator band or elastic band of 2.5 to 4.5 oz / ⅛" to ¼" (depending on interproximal access) will be placed in the gingival area of each premolar to create bone resorption and facilitate an atraumatic extraction.

On day 29, 14 days after the at-home whitening treatment was ended and 14 days after the inoffice whitening treatment was accomplished, the subject will return to the UIC oral surgery clinic. Six randomly chosen subjects will have two strips of Block-Out Resin placed on one of their "Control" premolars and the tooth will be etched with 35% phosphoric acid for 15 seconds. On that day, all subjects will return to the clinic, where the maxillofacial surgeon(s) will extract the four premolars that the orthodontist prescribed on all subjects.

The implementation program will be performed on 40 subjects between August and December 2024 in blocks of 5 to 10 subjects at a time.

18- RESEARCH SUSPENSION CRITERIA:

If the subjects decide to withdraw from the research study at any time during the study or develop very uncomfortable tooth sensitivity and decide to stop the study, they may do so without any adverse consequences. However, the subject will not receive the compensation previously

stipulated directly with the administrative department of the UIC's School of Dentistry. Until the final protocol is accepted and authorized by Ultradent, this research study may be suspended in its entirety, or the methodology and/or the number of subjects required may be modified. Any modification of this protocol after it has been approved will be cleared with, and an amendment submitted to, the Bioethics Committee of UIC.

19- Explain the Responsibilities of the researcher, the institution, and the funders:

The investigator must verify that the subject meets the Inclusion and Exclusion Criteria to enter the study. The UIC Department of Orthodontics or other referral entity is responsible for identifying patients who may qualify for the research study by identifying one premolar in each of the four quadrants that require extraction due to relief of crowding to allow orthodontic treatment to continue. The sponsor is responsible for funding all necessary materials used to perform the tooth whitening procedures by the subjects, to conduct the research, and accomplish any follow-up treatment required due to the whitening agents.

20- PROJECT BUDGET:

Ultradent Products, Inc. will fund all necessary materials used to perform the teeth whitening procedures as agreed upon. It is responsible for 1) funding the extractions and compensating for visits to accomplish the tooth whitening procedures by the subjects, 2) compensating the researchers involved in the study, and 3) compensating the university or other referring entities for the use of their facilities and services. Additional materials necessary to accomplish the research must be pre-approved by Ultradent Products, Inc. or Dr. Fugaro.

Prepared by:

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Dr.

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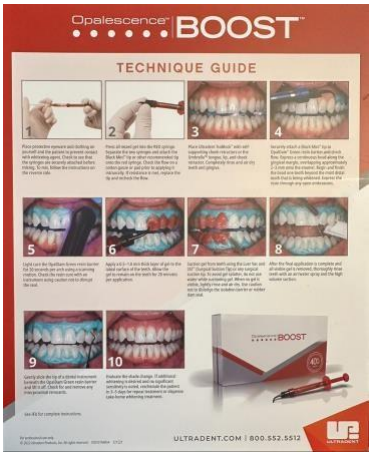
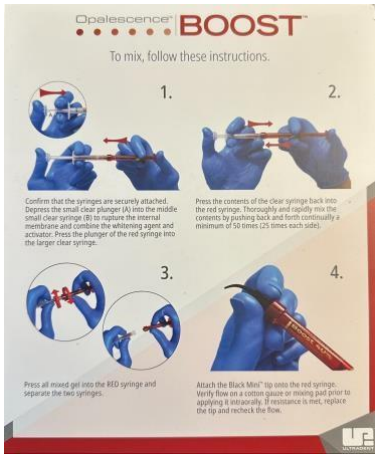




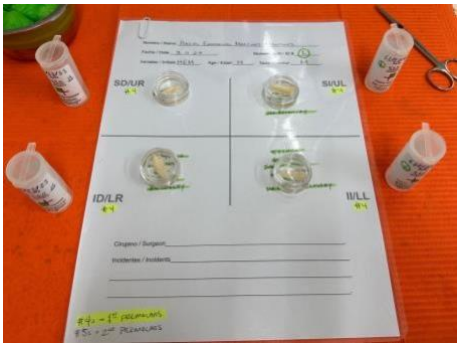
****Extraction protocol**

- At least one week prior to the extractions, an orthodontic spacer band or an elastic band of 2.5 to 4.5 oz / ⅛" to ¼" (depending on interproximal access) will be placed on the gingival area of each premolar to create bone resorption and facilitate an atraumatic extraction. The orthodontic separator band or elastic will not have contact with the clinical crown, either with the treated enamel area or the proper adaptation of the whitening tray *(photograph 1)*.
- On the day of the extractions, a small portion of a tube of Nelaton latex or a small portion of a nitrile glove (depending on interproximal access) will be placed on top of each premolar's tooth crown to protect the treated area during extractions.
- Local anesthetics will be at the discretion of the oral surgeon and the patient's needs.
- Separate the ligament with the appropriate instrument, taking care not to contact the clinical crown or the treated enamel surface. It can be buccal or lingual. It will depend on where is localized the surface more prominent that was treated in case of severe rotation. This will be previously identified before the oral surgeon extracts the teeth to avoid confusion.

- Luxate the tooth with an elevator or appropriate instrument without damaging or scraping the clinical crown or the treated enamel surface of the tooth.
- At the discretion of the surgeon, bone structure can be removed without damaging the treated enamel to facilitate tooth extraction without forceps if necessary.
- It is recommended, if possible, to perform extractions only with elevators *(photograph 2)*.
- If final tooth extraction is performed with forceps, care must be taken to protect the clinical crown to avoid damaging the treated enamel of the tooth. Forceps can engage the tooth below the gingival margin along the root surface if necessary. It is recommended to place a portion a small portion of a nitrile glove around the clinical crown to minimize the possibility of trauma *(photographs 3 and 4)*.

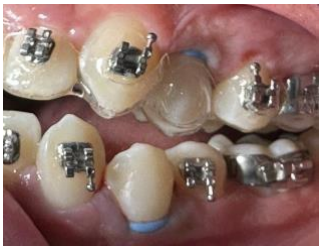
The oral surgeon will determine and choose the type of anesthesia and all instruments for extractions based on their professional needs and without putting the patient's health at risk.

A personalized clinical diagram will be created for each patient during extractions to avoid confusion about which tooth has been extracted and to ensure that surgeons document any possible incidents during extractions *(example below)*.

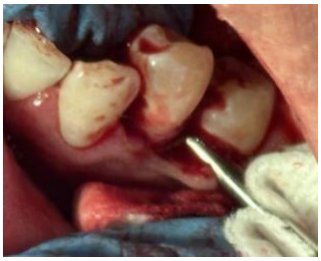


Extraction chart.

Photograph 1



Photograph 2



Photographs 3 and 4