International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 8; Issue 10 (C); October 2019; Page No.20224-20226
DOI: http://dx.doi.org/10.24327/ijcar.2019.20226.3943

Case Study

SURFACE CHARACTERISTIC ASSESSMENT OF BLEACHED, UNBLEACHED AND REMINERALISED ENAMEL- A SCANNING ELECTRON MICROSCOPE STUDY

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A R T I C L E I N F O

Article History:
Received 06th July, 2019
Received in revised form 14th August, 2019
Accepted 23rd September, 2019
Published online 28th October, 2019

Key words:

Bleaching, Recaldent, Scanning Electron Microscope

A B S T R A C T

Aim: To compare and assess change in morphology of human enamel after bleaching and post treatment with different remineralising agents using scanning electron microscope
Methodology: Adequate number of premolars indicated for extraction (for orthodontic purpose) were taken into consideration. Informed consent was obtained from the patients participating in the study. The teeth to be extracted were randomly divided into three groups Group I: 37.5% Hydrogen peroxide, Group II: 37.5% Hydrogen peroxide + 30 minutes in natural saliva, Group III: 37.5% Hydrogen peroxide + remineralising agent (CPP-ACP), Group IV: Untreated group. Bleaching treatment followed by different remineralising treatments were carried out for the three groups. All the specimens were decoronated, the samples were then sectioned longitudinally to obtain buccal and palatal/lingual halves. Scanning electron microscope analysis were performed on the samples. Statistical analysis using chi square test and Fisher's exact probability test was done.
Results: Results indicated that there was considerable amount of surface alteration after bleaching treatment and the best remineralisation results were obtained with 30 minutes exposure to saliva. It may be concluded in-office bleaching agent affected human enamel morphology.

INTRODUCTION

The current search for a whiter and brighter smile has drawn an increased number of patients in dental office for tooth bleaching procedures. Literature has several reports confirming that bleaching gels, in various concentrations, are effective for tooth whitening. Bleaching agents release nascent oxygen in dental structures, which, due to the low molecular weight and associated dental permeability, may diffuse through enamel and dentin substrates, acting on pigments.[1] These molecules, by means of redox reactions, can break macromolecules into smaller molecular chains, which are totally or partially removed from dental structure by diffusion. Many studies have reported that bleaching products may cause enamel mineral loss at different levels and alterations in surface morphology and other mechanical and chemical properties over a period of time. [2,3,4] These alterations may vary depending on the product concentration, time duration of application , pH of the product, environmental conditions, such as temperature, pH, ultraviolet (UV) light, and the presence of some ions like perhydroxyl anion.[5]

The present study was conducted to find out the changes in enamel properties specifically change in enamel surface morphology after exposure to hydrogen peroxide bleaching gel, also alteration in results within the groups where natural saliva and casein phosphopeptide and amorphous calcium phosphate (CPP-ACP) is used as remineralising agent was observed. Most of the studies conducted till date have been conducted post bleaching in-vitro and have reported conflicting results.

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Aim

The aim of the study is to compare any change in surface morphology of human enamel and to assess any change in the bleached enamel surface post treatment with different remineralising agents.

Objectives

- To compare the surface morphology of unbleached and bleached enamel surface.
- To assess the difference in surface morphology of bleached enamel exposed to natural saliva for 30 minutes.
- To assess the difference in surface morphology of bleached enamel after remineralising agent (CPP-ACP) application.
- To subject the results so obtained under statistical analysis.

Research Hypothesis

In the proposed study differences in enamel surface morphology and descriptive chemical analysis is expected to

be found with the bleached and unbleached teeth samples. It is expected that there will be more surface alterations post bleaching. It is also expected that natural saliva and recaldent (CPP-ACP) application will also result in difference in outcome.

Materials

The composition of the materials used are enlisted in table 1.

Table 1 Enlisted below are the materials used in the study

Material	Product	Composition
In-office bleaching agent	Pola-Office Plus	• Hydrogen peroxide 37.5% • Sodium hydroxide < 0.5% • Desensitizing agents
Recaldent	GC Tooth mousse	• 10% CPP-ACP • 0.2%Sodium Fluoride (NaF)
Gingival Barrier	SDI Limited	• 83.95% Methacrylic ester • 16% Silica • 0.01% Butylated hydroxy toluene

Armamentarium

The armamentarium used in the study is enlisted in table 2.

Table 2 Enlisted below are the armamentarium used in the study.

Armamentarium	Product	Manufacturer
Sputter coater system	Hitachi E 1010	Hitachi, Tokyo
Scanning Electron Microscope fitted with an EDS system	Hitachi S-3400 N	Hitachi, Tokyo

Inclusion Criteria

Patients were selected based on the following criteria:

- between sixteen and twenty one years of age
- orthodontic treatment indicated , requiring extraction of premolars
- caries-free vital premolar teeth
- devoid of restorations
- good oral hygiene
- free of periodontal disease and gingival irritation
- non-smokers
- free of cervical lesions and painful symptoms

Exclusion Criteria

Teeth with any fracture, decay, restoration or attrition

METHODOLOGY

Institutional Ethical Committee clearance was obtained for the study. Informed consent was taken from the participating patients. Ten patients participating in the study were divided randomly into following groups:

Group I 37.5%Hydrogen peroxide (Pola Office Plus)
Group II 37.5% Hydrogen peroxide + 30 minutes in natural saliva
Group III 37.5 % Hydrogen peroxide + Remineralising agent (CPP- ACP)
Group IV Untreated / Control group

Bleaching Treatment

Cheek retractors were placed on all the patients and the exposed surfaces were covered with petroleum gel. Gingival barrier was applied and light curing was done for ten-twenty seconds in a fanning motion until it was cured. A thin layer of gel was applied to the teeth using the nozzle as a guide. Base line shade was assessed using Vita-shade guide. Bleaching gel was left on for eight minutes (according to manufacturer's instructions). The gel was suctioned off using a surgical aspirator tip. Two-three cycles of bleaching treatment was performed, till the treated tooth became atleast one shade lighter which was evaluated with the same shade guide. After the last application, the gel was suctioned off and rinsed with water.

Remineralising Treatment

Group II patients were made to wait for thirty minutes prior extraction with the bleached tooth surface exposed to the patient's natural unstimulated saliva to assess the remineralising capacity of saliva.

Group III patients were treated with CPP-ACP (recaldent) application post- bleaching for three minutes (according to manufacturer's instructions).

Sample Preparation

The treated teeth were then extracted and twenty teeth samples were obtained. Any remaining debris, blood etc were cleaned from the teeth samples. The samples thus obtained were collected in distilled water at 37 ^{0}C temperature. All the specimens were decoronated and sectioned longitudinally in a mesiodistal direction to obtain two halves buccal half and lingual/palatal half. The buccal half was used for laboratory analysis and the palatal half was discarded. Laboratory analysis was done as soon as possible (within 7 days of collection of sample).

SEM Analysis

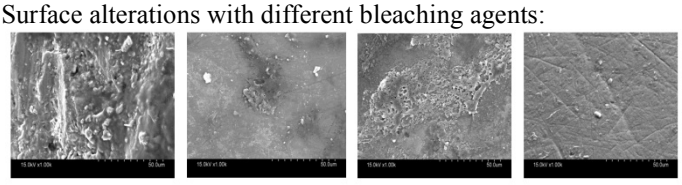
The specimens to be examined were dehydrated in ascending grades of ethanol (20 minutes in 50%, 60%, 70%, 80% and 90%, followed by 60 minutes in100%). Crowns werw be fixed on aluminium stubs and sputter coated with gold-palladium for 2 minutes. Enamel surface was examined at 500 X and 1000 X magnification.

SEM analysis was done for all the groups, and the entire surface was scanned and graded as follows:

A - Mild alterations, No loss of enamel prisms.
B - Moderate alterations, loss of enamel prisms.
C - Severe alterations, deep irregularities with loss of prisms

RESULTS

Surface alterations with different bleaching agents:



Group I Group II Group III Group IV

Groups	A	B	C
Group I	0	6	4
Group II	8	2	0
Group III	6	4	0

Statistical analysis using chi square test and Fisher's exact probability test was done. Comparison of enamel surface between experimental groups.

Groups	p value
II and III	0.39
I and III	0.001
I and II	0.0004

DISCUSSION

Many dental procedures, including bleaching, are performed empirically and without thorough knowledge of what their biological effects may be. This lack of understanding may give rise to unpredictable results. The results of this study showed that following bleaching, the enamel surface showed morphological alterations ranging from mild, moderate to severe, which is in accordance with previous studies.[6,7]

This study provides in vivo data on the widespread but not fully understood vital tooth-bleaching procedure. It was a maiden experiment with unique methodology comparing time dependent remineralising capacity of saliva and remineralising capacity of CPP-ACP post bleaching with in-office bleaching agent.

The demineralization of enamel under acidic conditions essentially happens due to the dissolution of the hydroxyapatite component. Previous study suggested that the acidic condition of bleaching agents attacked the enamel surface and caused demineralisation. When demineralization occurs, calcium and phosphorous the main mineral component of the enamel, has shown to decrease. Remineralization and demineralisation has a vital impact on the strength and hardness of dental enamel. [9]

In the present study, Pola Office Plus 37.5% HP agent was used which is known to have a neutral pH. The standard bleaching methods recommended by manufacturers were used in this study.

A pronounced increase in the depth of enamel grooves, morphological alterations on enamel surface, describing enamel patterns similar to type II acid etching, after 37.5% hydrogen peroxide bleaching was observed.[8]

Fewer alterations were observed in GROUP II, due to the buffering capacity of saliva. Saliva is essential to equilibrate the demineralization and remineralization processes. [9,10] Considering the reparative effect of human saliva, the present study showed that 30 minutes of salivary exposure led to remineralisation enamel which was in accordance with a previous study. [10]

Several remineralising agents have been used to aid in remineralisation post bleaching like bioactive glass, hydroxyapatite and nanohydroxy apatite based agents etc. But CPP-ACP is the most widely studied material with favourable results.

CPP-ACP maintains a supersaturated mineral environment by releasing calcium and phosphate ions, and thereby helps in enhancing remineralization of enamel. Hence less morphological changes were observed.

In this study, SEM was used to examine the enamel surface after the tooth bleaching treatments.

Many studies have employed this tool to characterize the physical properties of enamel after tooth whitening. SEM has traditionally been considered the gold standard in the studies of enamel surfaces treated with several different agents.

In general, it seems that bleaching treatment produced irregularities on enamel surface, including partial removal of the aprismatic layer, increased depth of enamel grooves, and exposure of the enamel prisms. An increased number of porosities, intermittent depressions of various depths ,craters and shallow erosions was also observed.

CONCLUSION

Within the limitations of this study, it may be concluded in-office bleaching agent affected human enamel morphology. These defects were well distributed for each group and affected the enamel surface at various degrees.

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How to cite this article:

Paromita Mazumdar and Sampurna Dutta Gupta (2019) 'Surface Characteristic Assessment of Bleached, Unbleached and Remineralised Enamel- A Scanning Electron Microscope Study', *International Journal of Current Advanced Research,* 08(10), pp. 20224-20226. DOI: http://dx.doi.org/10.24327/ijcar.2019.20226.3943

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