In vitro Assessment of the Effect of Opalustre Bleaching System on Dental Enamel

GALINA PANCU, SIMONA STOLERIU*, NICOLETA TOFAN, IRINA NICA, CRISTINA ANGELA GHIORGHE, GIANINA IOVAN, ANTONIA MOLDOVANU, GEORGIANA MACOVEI, SORIN ANDRIAN

Grigore T. Popa University of Medicine and Pharmacy, Faculty of Dental Medicine, 16 Universitatii Str., 700115, Iasi, Romania

The study was performed to investigate the changes induced by mechanical-chemical action of a bleaching product on dental enamel and to assess the effect of some remineralization products containing calcium, phosphate, and fluoride applied in conjunction with the bleaching product by surface roughness analysis using profilometry. Forty tooth samples were randomly divided in three study groups and a control group. In control group (group IV) the samples were maintained in artificial saliva. The study groups were as follows: group I-samples submitted to bleaching procedure using Opalustre (3 times a day, 1minute each), group II-samples submitted to remineralization and then to whitening, group III-samples submitted to remineralization, whitening and then remineralization. The surface roughness was determined in relation to a reference surface using Portable Surface Roughness Measuring Tester SJ-210 (Mitutoyo, America Corporation, USA). The bleaching system Opalustre increased the roughness of enamel and the product that contains calcium, phosphate and fluoride applied before and after bleaching improved the roughness parameters.

Keywords: bleaching, microabrasion, surface roughness, mineralisation

Nowadays dental bleaching represents one of the most demanded procedures in the dental offices. The pressure of the modern world, with beauty parameters related to a perfect dental smile, makes difficult the task of the dental practitioner [1-5]. Choosing a successfull solution is very difficult due to the complexity of the teeth morphology, of the ethiopathogenical factors of the various forms of dyschromia evolution, as well as to the multitude of bleaching methods and products [6-12].

The bleaching procedure changed along time due to the inovations and improvements. In the last decade new bleaching techniques and products were introduced in dental practice [8-14]. Therapeutic possibilities are directly related to the ethiology and the clinical aspects of dyschromia [5, 14-21].

Whitening system by microabrasion is indicated to ammeliorate the esthetic effect of dyschromia produced by extrinsec discoloration (tabacco, coffee, black tea) and especially by intrinsec discolorations (hypoplasia, amelogenesis imperfecta, fluorosis, non-cavitary caries [14, 16, 19, 22-27]. Fluorosis and amelogenesis imperfecta are associated to poor quality and structure of dental hard tissues [25, 27-29], and the action of a chemical abrasive product might change the tissues structure. It is important to know the type and the severity of the dental structural changes, in order to give optimal reccomendations to the patients.

The enamel microabrasion was initially used to remove fluorosis white spots by using hydrochloric acid 36%. Later, Croll, and Cavanaugh in 1986, and Sundfeld et al. in 1990 indicate the microabrasion technique to remove superficial enamel discoloration, using hydrochloric acid 18% and microgrit pumice stone [16, 22-24, 30-35]. By using this technique the esthetic effects are significantly high and the surface enamel loss is low. The disadvantages of this technique are related to the toxic and caustic effects of hydrochloric acid 18%. Thus full attention and caution of dental team is needed during bleaching procedure [26, 28, 34-37].

Looking for a safer and more easy to be used bleaching product, the producers introduced dental materials with lower concentrations of hydrochloric acid and with finer abrasive granulations [34, 35, 37]. The product Prema (Premier Dental Company, Philadelphia, PA, United States), containing clorhidric acid 10%, was one of the first bleaching products from this category. Newer products (for example, Opalustre, Ultradent Products Inc., South Jordan, UT, United States) contain lower concentrations of hydrochloric acid (6.6%). Both bleaching products use silicon carbide with different grit size as abrasive component, dispersed in a water soluble gel. Most studies that analyze the bleaching products containing peroxides demonstrated the absence of significant qualitative and quantitative phisyco-chemical changes of enamel and dentine [36, 37]. Regarding microabrasion technique, the results are controversial [2, 16, 29, 32-35].

The aim of the study to investigate the changes induced by mechanical-chemical action of a bleaching product on dental enamel and to assess the effect of some remineralization products containing calcium, phosphate, and fluoride applied in conjunction with the bleaching product by surface roughness analysis using profilometry.

Experimental part

The study followed all the regulations imposed by Ethics and Research Committee of Dental Medicine Faculty, University of Medicine and Pharmacy Gr.T.Popa Iasi and in accordance to some published models [38]. The study was performed on healthy, unaffected bicusps, extracted from orthodontic reasons (n = 20). Immediately after extraction the teeth were washed to remove the soft tissue fragments, blood, bacterial biofilm, and were examined to detect possible fissures or defects (incipient caries, hypoplasia). The teeth with visible defects were excluded. Until the beginning of the research, the teeth were stored in sodiun chloride, at room temperature. The teeth were cut using diamond discs at low speed (Extec Corp, Enfield, CT, USA), under water cooling to obtain enamel samples having the

* email: stoleriu_simona@yahoo.com

lenght of 4 mm, the width of 4 mm, and the thickness of 2mm were obtained from the buccal and the oral surfaces of the teeth. All the surfaces were examined using an optical stereomicroscope SMZ 1500 m (Nikon Instech, Kanagawa, Japan). Any sample with defects was excluded from study. The samples were embedded in epoxydic resin in metalic cylinders. On top of their surfaces adhesive band was applied (Tape Case, Elk Grove Village, Ill), leaving exposed only the central area with a surface of 1×4 mm^2.

The samples were randomly divided in three study groups (I, II, III) and a control group (IV). In control group (group IV) the samples were maintained in artificial saliva. In group I the samples were submitted to bleaching procedure using Opalustre product (3 times a day, 1minute each), in group II the samples were submitted to remineralization and then to whitening, in group III the samples were submitted to remineralization, whitening and then remineralization. Opalustre bleaching product (6.6% hidrocloric acid) was used for one minute, in three successive applications, simulating a bleaching procedure. Recaldent MI Paste Plus product was applied in the morning, before bleaching, for the group II, and applied twice daily, for the group III, simulating the remineralization sessions in the morning and in the evening. Details about the products used in the study are presented in table 1 and some steps in working protocol are presented in figure1.

The experimental protocol was runs during 24 h and included the steps presented in table 2. After each immersion, the samples were washed in deionized water (10 s) and then maintained in artificial saliva (30 mL) (*p*H = 6.7), changed twice daily [39]. After 24 h the surface roughness of the samples was evaluated by profilometry, using Portable Surface Roughness Measuring Tester SJ-210, Mitutoyo, America Corporation, USA, presented in figure 2. For each sample roughness parameters as Ra, Rq, and Rz were recorded.

Statistical analysis of the results was performed using ANOVA, SPSS v.17 program.

Results and discussions

The mean values, standard deviation and the results of statistical test for roughness parameters (Ra, Rq, Rz) in all three study groups (I, II, III) and in control group (IV) are presented in tables 3-5.

The results in all three study groups showed that the surface roughness was higher in group I, followed by group

Nr.	Product	Active ingredients	Manufacturer
1.	**Opalustre**	6.6% hydrochloric acid, abrazive-silicon carbide, (particle size 20-160 mm)	Ultradent Products Inc., South Jordan, UT, United States
2.	**Recaldent Mi Paste Plus**	Casein Phosphopeptides (CPP) and Amorphous Calcium Phosphate (ACP) and Fluoride 0,2% w/w (900ppm)	GC, America Inc, United States
3.	**Artificial Saliva**	1.5g KCl 1.5g $NaHCO_3$ 0.5g NaH_2PO_4 0.5g KSCN 0.7g $C_3H_6O_3$	Formula proposed by Christopher M.A. Brett, Florin Trandafir [37].

Table 1
PRODUCTS INCLUDED IN STUDY

Fig. 1. Samples of the study group (I, II, III) and control group (IV)

Reference information:
- roughness standard - evaluation based on ISO1997
- evaluation profile – R type
- filters – Gaussian filters
- the cut-off length (λc) - 0.25μm for high samples, 0,08μm for small samples
- number of sampling length (N) – 5
- traversing speed – 0.5 mm/s
- measuring range – AUTO
- parameters – Ra, Rq, Rz

Fig. 2. Surface Roughness Measuring Tester SJ-210, Mitutoyo

Time (h)	GROUP I	GROUP II	GROUP III	GROUP IV
9.00	S	S	S	S
10.00	S	**R**	**R**	S
10.30	S	S	S	S
15.00	**O**	**O**	**O**	S
15.30	S	S	S	S
18.00	S	S	**R**	S
18.30	S	S	S	S
O = application of bleaching product Opalustre (3x1min)→ imerssion in saliva R = remineralization with MI Paste product (30 min)→ immersion in saliva S = imerssion in saliva				

Table 2
STEPS OF WORKING PROTOCOL

Ra (mm)	Means	StDev
Group I	1.395	0.278
Group II	0.969	0.334
Group III	0.657	0.086
Group IV	0.208	0.051
Means with the same letter within each column are not significantly different at p≤0.05 St.Dev = Standard deviation		

Table 3
MEAN VALUE; STANDARD DEVIATION, AND STATISTICAL TEST RESULT FOR Ra ROUGHNESS PARAMETER

Rq (mm)	Means	StDev
Group I	1.693	0.299
Group II	1.184	0.389
Group III	0.815	0.109
Group IV	0.262	0.065
Means with the same letter within each column are not significantly different at p≤0.05 St.Dev = Standard deviation		

Table 4
MEAN VALUE; STANDARD DEVIATION, AND STATISTICAL TEST RESULT FOR Rq ROUGHNESS PARAMETER

Rz (mm)	Means	StDev
Group I	6.707	0.963
Group II	4.343	1.48
Group III	3.507	0.512
Group IV	0.943	0.214
Means with the same letter within each column are not significantly different at p≤0.05 St.Dev = Standard deviation		

Table 5
MEAN VALUE; STANDARD DEVIATION, AND STATISTICAL TEST RESULT FOR Rz ROUGHNESS PARAMETER

II, and group III (table 3-5). For all study groups the mean values of roughness parameters were higher than control.

The highest value of Ra parameter was recorded in group I, submitted to bleaching (1.395 μm), followed by group II, submitted to remineralization before bleaching (0.969 μm), followed by group III (0.657 μm), submitted to exposure to Recaldent before and after bleaching. In study group IV the lowest Ra value was recorded (0.208 μm). Regarding Rq parameter, the mean values were 1.693 μm in group I, higher than group II (1.184 μm) and group III (0.815 μm). The lowest value was recorded in group IV (0.262 μm). Also, Rz parameter values were higher in study groups when comparing to control: group I (6.707 μm), group II (4.343 μm), group III (3.507 μm), and 0.943 μm in group IV.

The changes of surface roughness seems to be a complex mechanism including both a *chemical attack* due to hydrochloric acid action and a mechanical micro-abrasion by bleaching product application on teeth surfaces.

The clinical procedures indicate to solve the intrinsic or extrinsic discolorations using chemical products based on peroxide or carbamide (in the composition of the bleaching products), mechanical procedures (polishing, finishing), mixed chemical-mechanical systems (microabrasion system Opalustre) and indirect or direct restorations (ceramic or zirconium esthetic veneers) [5, 6, 9, 18-19]. Clinical situation might impose an association of methods to obtain a long-term esthetic effect, without determining significant loss of dental hard tissues, or altering its structure or composition [1, 20, 28].

To assess the changes induced by the bleaching products in current practice, the researchers proposed various methods and techniques to analyze the changes induced on the dental hard surfaces by acidic attackand by mechanical action [15, 36]. These methods include profilometry, microscopic technique, microradiography, optical microscopy, scanning electron microscopy, scanning 3D microscopy, spectroscopy EDX, atomic force microscopy, microhardness tests, white light interferometry, optic coherence tomography (OCT) and other.

The studies focused on the erosive effects of hydrochloric acid 18% [16, 23, 32] and demonstrated that due to the high toxic risk special caution from the dentist during bleaching protocol is needed. Croll et al. evaluated several acids having different concentrations with many types of abrasives to find the ideal, safe and easy-to-use bleaching product [16, 23, 24]. The acids evaluated included citric acid, hydrochloric acid, nitric acid and phosphoric acid [41-47] and the abrasive particles tested were dental laboratory pumice, synthetic diamond powder, aluminum oxides and silicon carbide.

The measurement of surface roughness of dental hard tissues submitted to bleaching by profilometry is an accurate method to assess the erosive effecton dental hard tissues [37]. It is a simple technique and it offers useful data regarding the changes induced by bleaching and the influence of bleaching on the mechanical properties of the enamel and dentine. Also, the influences on the organic component and/or the loss of the mineral content can be evaluated [15, 37]. The assessment of enamel surface by profilometry is performed by scanning the sample with a laser fascicule or a stylus (metal, diamond) having the diameter of 2-20μm, which is loaded with a specific force. The scanning generates a map of the investigated surface and thus stylus tip produces scratches on the analysed surfaces, and atomic force microscopy can be used to quantitative measure (nm) the depth of these scratches.

The results of our study support the data in the literature [22, 37] and confirm the changes induced by the action of bleaching products on the surface status of dental hard tissues, but contradict the results of other studies that concluded that the bleaching method by microabrasion is inoffensive for dental tissues [34, 35].

In the studies applied in dental medicine that use profilometry Ra value is most used roughness parameter [40, 48-55]. This parameter is limited in bidimensional plan, providing only informations regarding the roughness height and is not related to the roughness profile. In our study Rq

and Rz parameters were included to improve the accuracy. Rq and Rz represent the height parameters on vertical axe (depth of degradation areas). The combination of quantitative and qualitative data allows obtaining tridimensional qualitative values of the tested surfaces.

Because this study is an in vitro study, further in vivo studies are requested to evaluate the effects the intraoral conditions and to obtain more accurate results, taking in account the temperature variations, the oral pH dinamic changes, the presence of the acquired pellicle, and other conditions.

Despite the favourable results obtained for microabrasion bleaching technique over a periode of 10-18 years [32-35], in vitro analysis of physical-chemical parameters reccomends a careful evaluation of the clinical case [32, 33].

Any external action of a bleaching product on dental hard tissues, even at a minimal invasive level, especially when the dental tissues are affected by fluorosis or dental caries, must be careful planned. Taking in account all the collateral associated factors, with a detailed analysis of the clinical case, a proper diagnostic, will ensure a favorable therapeutic result [14, 18, 25, 31, 55]. It can be recommended that the application of bleaching gel to be performed mostly on the dental surfaces that are not associated with the exposure of root surface. It is important to avoid (by proper isolation) the contact of the bleaching product with, the cervical area where sometimes dentine can be exposed and dentine hypersensitivity can be associated. Also, all the bleaching procedures must be performed under the guidance of the dental practitioner, by respecting the indications and the time recommended by the producer, and taking in account the possible concurrent dental pathology.

Conclusions

The bleaching system Opalustre increases the enamel surface roughness. The application of remineralization product that contains calcium, phosphate and fluoride applied before bleaching improves the roughness parameters, providing a good protection against the acid-abrasive effect of bleaching product. A significantly improvement of roughness parameters is obtain by using remineralization product before and after bleaching product application.

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