Influence of Bleaching Agents on Surface Roughness of Sound or Eroded Dental Enamel Specimens

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ABSTRACT

Purpose: The aim of the present in vitro study was to assess the effect of bleaching agents on eroded and sound enamel specimens.

Materials and Methods: Enamel specimens prepared from human permanent anterior teeth were incubated with different bleaching agents containing active ingredients as 7.5 or 13.5% hydrogen peroxide or 35% carbamide peroxide, ranging in pH from 4.9 to 10.8. The effect of the tooth whitening agents on surface roughness was tested for sound enamel surfaces as well as for eroded enamel specimens. To provoke erosive damage, the enamel specimens were incubated for 10 hours with apple juice (pH = 3.4). Afterwards, pretreated and untreated dental slices were incubated with one of the bleaching agents for 10 hours. The surface roughness (R_a) of all enamel specimens ($N = 80$) was measured using an optical profilometric device. A descriptive statistical analysis of the R_a values was performed.

Results: The study demonstrated that exposure to an acidic bleaching agent (pH = 4.9) resulted in a higher surface roughness ($p = 0.043$) than treatment with a high peroxide concentration (pH = 6.15). If the enamel surface was previously exposed to erosive beverages, subsequent bleaching may enhance damage to the dental hard tissue.

Conclusion: Bleaching agents with a high concentration of peroxide or an acidic pH can influence the surface roughness of sound or eroded enamel.

CLINICAL SIGNIFICANCE

Patients with erosive defects who wish to receive a tooth bleaching treatment must be informed about possible complications and damage to the tooth surface. However, extrapolation of in vitro results to clinical situations is limited.

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INTRODUCTION

In esthetic dentistry, tooth whitening with different concentrations of hydrogen peroxide (HP) is a commonly performed technique that can be done at home or by means of in-office whitening procedures.[1] Growing awareness of the patients and effective advertising in the media have supported this trend. In all age groups, special interest is focused on the

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visible area of the front teeth because in this particular region all discolorations, including those related to aging, are considered to be especially unattractive.

There are various causes of dental discolorations. In addition to carious defects, inadequate restorations, loss of tooth vitality, habitual pigmentations caused by various food and beverage pigments, exposure to certain drugs as well as generalized or localized hereditary mineralization disorders can lead to stained teeth. In the past, discolorations that could not be removed by means of simple prophylactic techniques were mostly an indication for extensive restorative therapy. As an alternative, noninvasive methods for removing tooth discoloration, the controlled internal or external application of a bleaching agent, have been further developed over the last decades. The internal bleaching technique is a method to remove discolorations from nonvital teeth.[2] This procedure consists of the insertion of a bleaching agent, a mixture of sodium perborate and water (or 3% HP), into the trepanation cavity where it remains for 3 to 5 days.[3,4] For the whitening of the vital teeth, external methods using carbamide peroxide (CP) and HP in different concentrations for home and in-office bleaching are available.[1,5] For home bleaching, concentrations of 10 to 20% CP are recommended. The in-office products contain higher concentrations of up to 35% HP or CP. In the dental office, the whitening process can be affected and also accelerated using different types of lamps, heat, or laser beams.[6]

Depending on the contents, the concentrations, and exposure time of the agent, the effectiveness of the treatment can differ; also, it must be considered that there are various side effects possible. The radicals and a low pH value were regarded to be the causes for tissue damage.[7] Furthermore, it was possible to demonstrate that higher concentrated gels led to hypersensitivities because of slight alterations of the enamel surface.[8] When using external bleaching techniques, low concentrations of HP could be detected in the pulpal tissue.[9]

Several in vitro studies, using HP or CP for external bleaching on human or bovine enamel, showed alterations of the surface structures, for example, in microhardness, fracture toughness, or surface roughness.[10–13] However, other investigations with CP alone or in combination with HP found no changes.[14–17] In an in vivo study, the enamel surface roughness was evaluated when using in-office agents containing 38% HP or 35% CP and found no alterations on enamel surface roughness after multiple applications.[18]

Although the majority of investigations were performed on sound enamel, Basting and colleagues[19] compared the effect of a 10% CP bleaching agent on sound and demineralized enamel. They found that after bleaching, the demineralized enamel specimens had statistically significant higher values for surface roughness than sound enamel specimens. The increasing consumption of acidic beverages can, according to frequency and form of intake and to exposure time of enamel to low pH, produce varying extents of demineralizations on enamel surfaces.[20–23] Whitening products can alter such surfaces to a considerably higher degree. The aim of the present in vitro study was to assess the effect of bleaching agents, containing different active ingredients, on human enamel specimens from sound enamel surfaces as well as from those pretreated with an acidic beverage to produce erosive damage.

MATERIALS AND METHODS

The experimental design was descriptive. The erosive potentials of commercially available tooth whitening products were studied in an in vitro setting, using extracted human permanent anterior teeth. Twenty-two products were purchased, and the pHs were

TABLE 1. SELECTION OF COMMERCIALLY AVAILABLE AGENTS FOR IN-OFFICE AS WELL AS HOME BLEACHING.

Product designation	Concentration and effective agents (%)	pH
Poladay	3 HP	5.87
Simply White	5.9 HP	4.5
Vivastyle Paint on	6 CP	7.84
Poladay	7.5 HP	5.94
Visalys	**7.5 HP**	**10.78**
Polapaint	8 CP	5.28
Poladay	9.5 HP	5.96
Illuminé home	10 HP	5.92
Polanight	10 CP	6.11
White Smile	10 CP	5.04
Platinum Daytime	10 CP	5.87
Visalys	**13.5 HP**	**6.15**
Opalescence PF	15 CP	6.53
Illuminé home	15 CP	5.75
Polanight	16 CP	6.06
White Smile	16 CP	5.05
Contrast pm plus	20 CP	6.51
Opalescence PF	20 CP	6.67
Polanight	22 CP	6.01
White Smile	22 CP	5.11
Opalescence Quick	35 CP	6.53
White Smile	**35 CP**	**4.94**

The agents in bold letters are used in this study.
HP = hydrogen peroxide; CP = carbamide peroxide.

measured. Three tooth whitening products with different pH values and different concentrations of the bleaching agent were then selected to study possible erosive effects on enamel surfaces. Enamel samples were obtained from intact, caries-free human permanent teeth (premolars and molars) from male and female patients (aged 40–65 years), which had been removed because of periodontal disease. The patients were informed about this study and consented to the use of their teeth. After extraction, the teeth were stored in a 1% chloramine B hydrate solution.

In total, 35 extracted teeth were collected, visually examined, and 15 teeth with irregularities of mineralization or other defects were excluded from the study. The teeth were cleaned, any soft tissue and debris was removed with a slurry of pumice, and then the teeth were stored in a 0.9% sodium chloride solution to prevent them from drying out. The crowns of the 20 suitable teeth were separated at the cementoenamel junction and the roots were discarded. The flat vestibular enamel surfaces of the dental crowns of the teeth were polished with silicon carbide and aluminum oxide disks (Shofu Dental Corporation, San Marcos, CA, USA) of decreasing particle size to obtain a uniform enamel surface and to eliminate already existing slight damage of the tooth surfaces arising from normal wear.

Then the 20 teeth were evenly divided into four slices (average surface area: 16 mm^2, thickness: 3–4 mm) per tooth and placed into 12 multi-well plates (Greiner Bio-One, Frickenhausen, Germany). The samples ($N = 80$) were washed and again stored in a 0.9% sodium chloride solution.

The pHs of 22 commercially available tooth whitening products were determined with a device (EC-pHTestr 20, Eutech Instruments, Novodirect, Kehl, Germany), equipped with an integrated microelectrode, resistant to oxidizing agents and suited for pH measurements in gels (Table 1). Three characteristic bleaching agents were chosen from this selection with diverse pH values and concentrations of bleaching agent for incubation with enamel specimens for 10 hours to simulate a prolonged

exposure. They were two HP-containing agents—one home (Visalys, 7.5%, pH = 10.8, Kettenbach, Eschenburg, Germany), one in-office agent (Visalys, 13.5%, pH = 6.15, Kettenbach), and one in-office agent containing 35% CP (pH = 4.9, White Smile, Birkenau, Germany).

First, the effects of the three selected bleaching agents on sound enamel surfaces were studied. Five teeth were sectioned into four slices ($N = 20$), and each experiment was performed five times. The samples were then either incubated with physiological sodium chloride solution (0.9% NaCl, pH = 6.5) and served as controls ($N = 5$) or they were covered with one of the three bleaching agents ($N = 15$). After 10 hours of exposure at room temperature, the specimens were washed with tap water and dried.

In a second set of experiments, the effects of the same three agents were to be tested on sound enamel surfaces and on enamel surfaces previously exposed to an erosive beverage (100% apple juice, pH = 3.4, Albi, Bühlenhausen, Germany). Fifteen teeth were sectioned into four slices ($N = 60$), and each experiment was performed five times. Again, one specimen per tooth served as control ($N = 15$), one specimen was incubated with 2.5 mL of apple juice ($N = 15$), another with one of the three bleaching agents ($N = 15$; 5 per agent), both for 10 hours; the fourth specimen was incubated first with 2.5 mL of apple juice for 10 hours, and after removal of the juice, it was exposed to a bleaching agent for another 10 hours ($N = 15$; 5 per agent). After exposure, the specimens were cleaned and dried as described previously.

After the prolonged exposure of 10 hours of the enamel samples ($N = 80$) to saline (controls, $N = 20$), apple juice ($N = 15$), one of three tooth whitening products ($N = 30$; 10 per product), or to apple juice first and then one of three tooth whitening products ($N = 15$; 5 per product), the specimens were cleaned and dried.

Surface roughness (R_a) of all enamel specimens was measured using an optical profilometric device (PRK, Perthen, Göttingen, Germany). In order to position the specimens correctly for horizontal measurements, they were embedded in a dental composite material (Venus, Heraeus Kulzer, Dormagen, Germany), leaving an enamel window of 10×10 mm^2. Ten measurements, each of a length of 1.75 mm in randomly chosen areas, were performed for each specimen and evaluated by means of the MarSurfX20 software (Mahr GmbH, Göttingen, Germany).

Statistical analyses of the R_a (μm) values of the enamel surfaces were conducted using the SPSS program (version 12, SPSS Inc., Chicago, IL, USA). Descriptive statistics were calculated, and values are given as medians or shown as boxplots. A descriptive statistical analysis of the R_a values (nonparametrical test for independent samples: Mann–Whitney test; for paired samples: Wilcoxon test; $p < 0.05$) was performed.

RESULTS

A variety of commercially available bleaching agents for home and in-office bleaching techniques were chosen for this study. The pH of a selection of 22 bleaching agents varied between 4.5 and an alkaline value of 10.8. It was noteworthy that 6 of the total 22 examined bleaching agents showed an acidic pH that was below the critical value of 5.5 for enamel surfaces. The concentrations of the chemical substances most commonly contained in the bleaching agents ranged from 3 to 13.5% HP and from 6 to 35% CP (Table 1).

To study the effect of selected tooth whitening products on the micromorphology of sound and eroded dental enamel specimens, three bleaching agents with different pH values and peroxide concentrations were chosen (Visalys, 7.5% HP, pH = 10.8; Visalys, 13.5%, HP, pH = 6.15; White

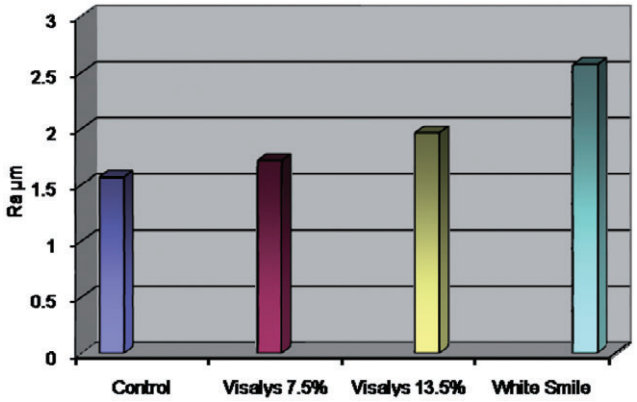

Figure 1. Median values for the surface roughness (R_a µm) of sound human dental enamel (N = 20), untreated (control, N = 5) or treated with Visalys 7.5% (HP, pH = 10.8; N = 5), Visalys 13.5% (HP, pH = 6.15; N = 5), or White Smile 35% (CP, pH = 4.9; N = 5). CP = carbamide peroxide; HP = hydrogen peroxide.

Smile, 35% CP, pH = 4.9). Enamel specimens, prepared exclusively from human permanent anterior teeth, were used in this study, because these teeth constitute the main target group for whitening procedures. After the exposure of the enamel specimens to the three whitening products, different values for the mean surface roughness were found. The control specimen showed the lowest surface roughness, with a median R_a of 1.57 µm; the treatment with the bleaching agent containing 7.5% HP led to an R_a value of 1.71 µm; the bleaching product with 13.5% HP caused a median value of 1.96 µm; and the whitening agent with 35% CP caused a median value of 2.57 µm. However, the comparison of the values obtained for R_a did not yield statistically significant differences when compared with the controls. Only the difference in values of R_a obtained after treatment with the acidic product containing 35% CP or with the one containing 13.5% HP was statistically significant ($p = 0.043$) (Figure 1).

To simulate erosive damage, the enamel specimens were first incubated for 10 hours in an acidic fruit juice (apple juice, pH = 3.4), then rinsed thoroughly with physiological sodium chloride solution, and afterwards they were exposed to the bleaching agents. The exposure of a sound enamel surface to one of the three bleaching agents caused in all cases a slight, but not statistically significant, increase in surface roughness when compared with the controls. Treating the specimens with an acidic apple juice produced a change in the microstructure of the individual teeth that was not uniformly reflected in a higher surface roughness. When the dental enamel surfaces were treated with a bleaching agent after exposure to an erosive beverage, no additional effect on roughness was observed with the products containing 7.5% HP or 35% CP. However, the treatment of a demineralized surface with the bleaching agent with the highest concentration (13.5%) of HP caused a considerable and statistically significant ($p = 0.043$) increase in surface roughness (median: 5.3 µm).

Figure 2 shows (as boxplots) the values for the surface roughness R_a of the controls and the specimens treated with a bleaching agent (Visalys, 13.5% HP, pH = 6.15), exposed to an acidic beverage and to the combination of acidic beverage first and then the whitening product. The most homogeneous results (median: 2.0 µm; range: 1.95–2.4 µm) were obtained for the treatment with an acidic beverage, which suggests a rather uniform loss of hard dental material.

DISCUSSION

For this in vitro study, enamel specimens were prepared exclusively from sound human anterior teeth and polished to obtain uniformly smooth, flat surfaces for the profilometric analysis. The decision

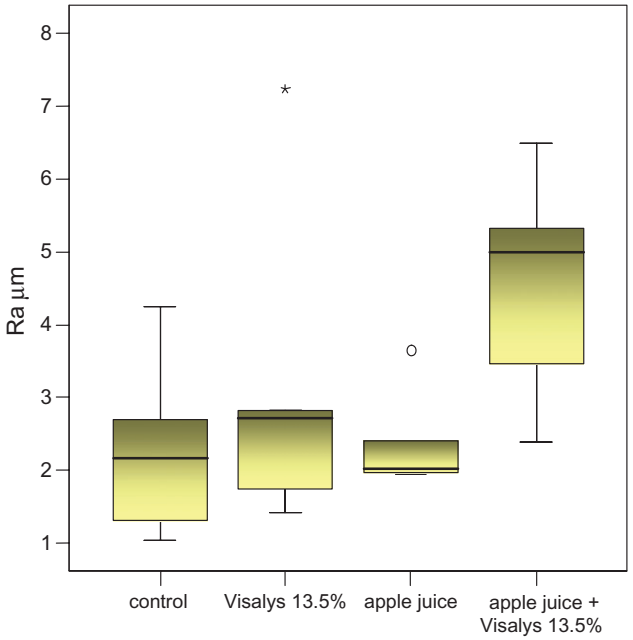

Figure 2. Surface roughness (R_a µm) of human dental enamel (N = 20), untreated (control, N = 5), treated with Visalys 13.5% (hydrogen peroxide, pH = 6.15; N = 5), with apple juice (pH = 3.4; N = 5), or with apple juice and then with Visalys 13.5% (N = 5).

to select only anterior teeth was made because, for esthetic reasons, these teeth are preferentially treated with bleaching agents. The superficial layer of enamel had to be removed because it is most affected by chemical processes taking place in the oral cavity after eruption.[22] However, the mean surface roughness (R_a) of the control specimens still showed considerable variability and its range (0.5–14.5 µm) corresponds to the data reported by Hosoya and colleagues.[24] Therefore, in order to allow for a comparison between the different treatments, for each experiment a dental slab was sectioned into four parts of equal size, to serve as control or experimental specimens.

Agents used for vital tooth bleaching contain (as active ingredient) either HP or CP, from which HP and urea are produced when dissolved in water. The pH from a selection of commercially available products showed a range of 4.5 to 10.8, which was similar to the values for various tooth whitening products (3.67–11.13) reported by Price and colleagues.[25] Three products were chosen for the present study according to different concentrations of active ingredient and to pH (acidic, almost neutral, or basic). Because bleaching agents are normally in direct contact with the enamel especially when applied by means of a custom tray, and only a single treatment took place, saliva was not included in the experimental setup. A bleaching time of 10 hours was chosen, similar to the setup in the in vitro study by Seghi and Denry,[26] as exposure time, especially for the products for home bleaching, are usually several hours or they are even worn over night. Long bleaching treatment times (e.g., 1 hour per day for 21 days for a product containing 7.5% HP) were also chosen by Faraoni-Romano and colleagues[27] for their study on the effect of bleaching agents on dental microhardness and surface roughness. Mielczarek and colleagues[28] found that long-term treatments with bleaching agents containing concentrations of HP comparable to the materials in the present study showed no significant effect on the enamel surface microhardness and roughness. However, it has to be noted that in the study by Mielczarek and colleagues,[28] a fluoride dentifrice was administered to the enamel specimens before and after the bleaching procedures. This treatment might influence and modify the composition and structure of the enamel surface and diminish the possible effects of bleaching agents.

The treatment of sound enamel with bleaching agents, containing 7.5 or 13.5% HP or 35% CP, led only to a slight increase in mean surface roughness. This is confirmed by Pinto and colleagues,[13] who reported that in vitro treatment with agents containing 7.5% HP or 35% CP caused only a mild intraprismatic dissolution, as was observed by scanning electron microscopy. This was reflected in a slight increase in surface roughness, and their R_a values were also not statistically significantly higher than those of the control group. Cadenaro and colleagues[18] reported in their in situ study that, after bleaching with a 35% CP product, the surface roughness of the enamel was not altered at all. This could be because of the fact that the product they employed had an almost neutral pH of 6.5, whereas the one used in the present study was more acidic (pH = 4.9). It has been reported that bleaching agents with a low pH value can cause a softening of the dental hard tissue.[29]

However, both Pinto and colleagues[13] and Maia and colleagues,[30] in an in situ study using a product with 7.5% HP, found a decrease in enamel microhardness after bleaching treatments. Santini and colleagues[31] studied the effect of a 10% CP bleaching agent on enamel at a molecular level using Raman spectroscopy and observed a decrease in the concentration of the phosphate group representing the mineral phase of enamel. It has been shown that, when compared with calcium/phosphorus analyses, profilometric analyses underestimated the loss of minerals from dental enamel[32,33] because subsurface loss of softened layers cannot be detected with this technique.

Although the susceptibility of bleached enamel to acid erosion has been studied,[29,34] little is known about the effect that tooth whitening might have on enamel with erosive lesions.

The effect of noncarbonated, fruit juice containing soft drinks or of fruit juices on sound human enamel specimens was studied in vitro.[22,35] In the present study, some enamel specimens were incubated for 10 hours with an apple juice with a low pH of 3.4 and high total acid content to simulate regular exposure to an acidic beverage for about 6 months.[36] It has been shown by means of electron probe microanalysis, by confocal laser microscopy, and chemical analysis that apple juice can cause a loss of minerals from sound human dental enamel that resulted in an increase in surface roughness.[23] In the present study, as it was the case with the control specimens, there was considerable variability in the values for the mean surface roughness in the specimens exposed to apple juice, and the slight increase observed in most cases was not statistically significant. The effect of tooth whitening agents and orange juice on enamel microhardness and surface topography was studied by Ren and colleagues.[37] In their study, enamel discs were incubated with a whitening gel with 6% HP with a pH of 5.5 or with an orange juice (pH 3.8) for five 20-min cycles. The authors found a significant increase of enamel surface area roughness only after orange juice incubation but not after the bleaching procedure.

Applying as bleaching agent the products with 7.5% HP or 35% CP to the enamel specimens previously exposed to the acidic beverage had little effect on surface roughness. Basting and colleagues,[19] on the other hand, have shown in an in situ study that demineralized enamel was more affected by a bleaching agent containing 10% carbamide and that its surface roughness increased to a higher degree than was the case with sound enamel. However, in the present study, the in-office bleaching material with the highest concentration of HP (13.5%) did lead to a considerable and statistically significant increase in surface roughness.

An additional issue to consider is that in the study by Hosoya and

colleagues,[24] *Streptococcus mutans* was capable of colonizing enamel specimens bleached with an agent containing 35% CP in higher numbers than control specimens, and that prior treatment with an etching gel increased bacterial adhesion. Therefore, the bleaching of partially demineralized enamel might make it more conducive to colonization by cariogenic bacteria.

CONCLUSION

In the present study, no effects of the bleaching agents on the morphology of sound enamel surfaces were detected using noncontact profilometric analysis. If the enamel surface was previously exposed to erosive agents, subsequent bleaching with 13.5% HP enhanced damage to the dental hard tissue. However, individual factors like salivary composition, dietary habits, and enamel structure of the individual teeth need to be considered to determine the clinical significance of these findings. Nevertheless, patients with erosive defects who wish to receive a tooth bleaching treatment should be informed about possible complications and potential alterations of the enamel surface.

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