The effect of in-office bleaching materials with different pH on the surface topography of bovine enamel

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This study evaluated the alterations of surface topography of the bovine enamel caused by different pH of in-office bleaching agents. 23% H2O2 with pH 5.5, 7.0 and 8.5 were applied on the bovine tooth specimens (n=10) and photo-irradiated for 10 min. The bleaching procedure was repeated three times and specimens were subjected to linear surface roughness (Ra) and Vickers microhardness (VHN) test (VHN) at baseline and after three consecutive applications. The morphological alterations were observed before and after third bleaching application. Data were analyzed by two-way ANOVA followed by Tukey's HSD. The pH of the bleaching agent significantly affects the Ra and VHN (p<0.05). Low pH yielded a significant increase in Ra and decrease in VHN. All the groups showed morphological alterations and profound effect was found in pH 5.5 group. It was concluded that the pH of the bleaching agent can affect Ra, VHN and surface morphology.

Keywords: pH, Bleaching agent, Surface roughness, Microhardness, Morphology

INTRODUCTION

Tooth bleaching treatment helps to enliven the smile disfigured by the discoloration. For most of the in-office bleaching agents, the active ingredient is hydrogen peroxide (H2O2) with high concentrations (25%–40%)3. H2O2 is a strong oxidizing agent and it produces free radicals, reactive oxygen molecules and hydrogen peroxide anions4. The tooth bleaching mechanism can be subdivided into three phases: the bleaching agent firstly goes into the tooth structure; then it interacts with the chromogen; and third, the tooth structure surface gets affected, alter the structure result in reflects light differently. Consequently, the tooth color is changing after bleaching with H2O2 with the advantages of low molecular weight. Thus, the higher penetration ability through the enamel and dentin substrate compared to the other bleaching agents. Ideal bleaching agent must have maximum bleaching potentials while minimizing the concurrent damage to the dental tissues. Moreover, the type of discoloration and the physical and chemical environment presented at the time of action can influence the bleaching reaction5. Thus, the bleaching agent concentration, heat, light, pH, catalysts, application times6-14 and other conditions should be justified.

The enamel is composed of inorganic material contains 97% of its total weight and essentially constituted of hydroxypatite, 1% of organic material; primarily proteins such as amelogenin and enamelin, and 2% of water15. The organic layer is a semi-permeable membrane that allows hydrogen peroxide to penetrate the tooth structure and react chemically with tooth discoloration and the tooth structure16. Enamel hardness is related to the mineral and protein content.

The organic content of the enamel plays an important role in the bleaching process. It is assumed that the reaction between peroxide and organic materials on the enamel surface or sub-surface can cause morphological alterations during the bleaching process17.

The importance of understanding the effects of pH of bleaching agent on the tooth enamel especially microhardness, surface roughness and morphology can help to determine the more safer bleaching material with maximum preservation of the tooth structure. Additionally, the effect of bleaching on the tooth enamel is still controversial. Some studies have reported the adverse effects associated with an increase in surface roughness, a decrease in microhardness and major morphological changes18, whereas several studies showed no such differences in the bleached enamel19. These controversial results are might be due to the type, concentration, pH and the formulation of bleaching agent used, bleaching protocol, alongside the variations in the study design such as the sample size and type of tissue (human or bovine enamel), method of specimen preparation (polished or sound enamel), type of storage medium, and method of data analysis20-23.

Most of studies focused on the concentration of bleaching agents20,24,25 and bleaching protocol26. But different bleaching agents have distinct pH values ranging from acidic pH to alkaline pH and it was revealed that the alkaline pH shows a higher bleaching effect than the acidic pH21,27. Since the pH of in-office bleaching agent plays an important role in the bleaching action, it is necessary to evaluate the effects of pH on dental tissues as well.

While high effectiveness of bleaching agent is desirable, its alterations for the substrate in terms
of surface roughness, microhardness and surface morphology should be taken into account. There are few publications evaluate the effect of pH on tooth enamel\textsuperscript{11,12}, but the preparation of bleaching agents was not standardized which could be a confounding factor in the evaluation. Therefore, the purpose of this \textit{in-vitro} study was to assess the effect of different pH of bleaching agents on dental enamel by evaluating surface roughness, microhardness and the surface morphology of bovine dental enamel using the different pH of bleaching agents with the same composition. The null hypothesis was that the pH of the bleaching agent will not affect the surface roughness, microhardness and morphology of the bovine enamel.

**MATERIALS AND METHODS**

**Specimen preparation for assessment of surface roughness and microhardness**

The labial enamel surface of bovine incisors were ground with 280-2000 grit silicon carbide papers (Sankyorkikagaku, Saitama, Japan) under running water to leave flat enamel surfaces of approximately 1-mm in thickness. Two specimens were obtained from each flattened surface (5×5×1 mm). The cut specimens were embedded in a plastic mold (Fig. 1) by self-curing clear acrylic resin (Unifast III, GC, Tokyo, Japan). Final polishing was done with diamond pastes from 6 µm up to 0.25 µm (DP-Paste P, Struers, Ballerup, Denmark). Finally, the specimens were cleaned ultrasonically for five minutes to remove any trace of the polishing materials.

**Preparation of bleaching material**

The materials and preparation formula to create different pH of bleaching agents are listed in Table 1. 30% H\textsubscript{2}O\textsubscript{2} was mixed with sodium carbonate (Na\textsubscript{2}CO\textsubscript{3}), carboxymethyl cellulose sodium (CMCS) and distilled water according to a pre-calculated formula. The pH was adjusted by Na\textsubscript{2}CO\textsubscript{3} and CMCS acts as the thickener. Finally, 23% H\textsubscript{2}O\textsubscript{2} with different pH 5.5, 7.0 and 8.5 were prepared according to a previous study\textsuperscript{28}. During the mixing of materials, the pH-conditioner was added just before usage, as the degradation of H\textsubscript{2}O\textsubscript{2} is accelerated in higher pH media. The pH of the bleaching agent was measured using a portable pH meter (LaquaTwin compact pH meter, Horiba, Kyoto, Japan) immediately before application.

**Bleaching procedure**

The thirty specimens were randomly divided into three treatment groups (n=10) based on the pH of the bleaching agents (pH 5.5, 7.0 and 8.5). The bleaching agent was applied evenly on the enamel surface of the specimens in a 2-mm-thick layer using a plastic spatula. Photo-irradiation was performed for 10 min by an arch type violet-LED light unit for tooth bleaching (Cosmo Blue, GC, Tokyo, Japan). The peak wavelength of the light unit was 405 nm and its intensity was 55 mW/cm\textsuperscript{2}. Then, the specimens were washed with running water and dried with mild air blow. The bleaching procedure was repeated three times. The leaner surface roughness (Ra) and Vickers microhardness (VHN) were measured after every bleaching application.

**Assessment of surface roughness**

All the specimens of three experimental groups were subjected to assessment of Ra using a confocal laser scanning microscope (CLSM; VK-X 150 series, Keyence, Osaka, Japan) under 20X magnification. Ra was measured before the bleaching application as the base line data. The measurement was repeated three times for each specimen as 250 µm apart horizontal lines and the average Ra was used to represent the surface roughness of each sample. Ra was measured after each bleaching application.

### Table 1  Ingredients of bleaching agent of each group and manufacture

| Ingredient | Manufacturer | pH 5.5 | pH 7.0 | pH 8.5 |
|------------|--------------|--------|--------|--------|
| H\textsubscript{2}O\textsubscript{2} (mL) | Wako Pure Chemicals, Tokyo, Japan | 10 | 10 | 10 |
| DW (mL) | Wako Pure Chemicals | 5 | 5 | 5 |
| CMCS (g) | Wako Pure Chemicals | 0.35 | 0.35 | 0.35 |
| Na\textsubscript{2}CO\textsubscript{3} (g) | Wako Pure Chemicals | 0 | 0.015 | 0.75 |
Assessment of microhardness
The VHN of the specimen was measured using a microhardness tester (HM-102, Mitutoyo, Yokohama, Japan) for the three experimental groups mentioned earlier. For each specimen, three indentations were performed on the enamel surface, with a distance of 200 µm between them. The measuring load was 100 g with dwelling time of 15 s. The VHN measurements were made in each specimen before and after every bleaching application.

Assessment of morphological changes
Three specimens were randomly selected from each experimental group to assess the changes of the morphology of bleached enamel. Further, three samples were prepared to assess the unbleached enamel (control). The observing surface of the specimens were sputter-coated with gold (300-µm-thick layer). Enamel surface was examined at 700× and 2,500× magnifications with scanning electron microscope (SEM; JSM-IT 100, JEOL, Tokyo, Japan), under operating condition of 20 kv. A scanning of the entire surface of the specimen was done and most critical areas were selected for photomicrographs.

Statistical analysis
The Kolmogorov-Smirnov and Shapiro-Wilk tests were performed to confirm the normal distribution of the data prior to the data analysis. The data of Ra and VHN were statistically analyzed using two-way ANOVA followed by Tukey’s HSD for multiple comparisons with the factors of pH of the bleaching agent and application time (α=0.05) (IBM SPSS Statistics for Windows, Version 27.0, Armonk, NY, USA).

RESULTS
Surface roughness
Figure 2 shows the mean and standard deviation of Ra of all the experimental groups. The baseline Ra was similar in all the experimental groups. Two-way ANOVA revealed that pH of the bleaching material and the number of application times (p<0.05) significantly affect the Ra. For the effect of pH, pH 5.5 showed significantly higher Ra value than pH 8.5 group (p<0.05). But comparatively there is no significant difference of Ra of pH 5.5 group with pH 7.0 (p>0.05). The Ra of all the experimental groups were significantly influenced by number of bleaching applications (p<0.001).

Microhardness
The mean and standard deviation of the VHN of all the experimental groups were shown in Fig. 3. Two-way ANOVA showed the VHN of the specimens in all experimental groups were significantly influenced by the pH of the bleaching agent and number of application times (p<0.001). On the other hand, an insignificant interaction between the pH and application times resulted (p>0.05). Tukey’s HSD showed that there is significant reduction of microhardness between pH 5.5 and pH 8.5 (p<0.001), as well as between pH 7.0 and pH 8.5 (p<0.05). But there is no significant difference between pH 5.5 and pH 7.0 groups (p>0.05). All the bleaching application times showed a significant reduction on the microhardness compared to baseline evaluation (p<0.05). Conversely, no difference between the bleaching steps resulted (p>0.05).
Surface morphology

The specimens belong to all the experimental groups showed different morphological changes after the bleaching procedure. The representative photomicrographs (Figs. 4a and b) of unbleached specimens did not show any remarkable morphological changes. The surface was smooth and uniform. Morphological changes were randomly distributed in most of the areas of the specimens in the experimental groups and more pronounced in pH 5.5 group (Figs. 4c and d). Surface erosions, depressions, increase depth of enamel grooves and increased porosity were detected.

Figures 4e, f and Figs. 4g, h showed the changes of pH 7.0 and pH 8.5 groups respectively. The morphological alterations were comparatively less prominent in pH 7.0 and 8.5 groups respectively. Erosions were seldomly detected. Comparatively the surface alterations were much more significant in pH 7.0 group than pH 8.5 group. The grooves that appeared to be an eroded enamel rod sheath were visible on the whole surface. Narrow gaps are observed in the enamel rod sheath region of the head of enamel prisms. Increased porosity can be detected.

DISCUSSION

The present study assessed the effect of pH of tooth bleaching agent on the enamel surface. The results of all the experimental groups showed a different degree of changes in Ra, VHN and surface morphology after bleached with different pH of bleaching gels, so the null hypothesis had to be rejected.

When using the high concentration of H₂O₂ for in-office bleaching, the formed free radicals from the oxidation of peroxide will act on both organic and inorganic matrix and cause the alterations of enamel surface even after single application. These alterations are due to the decrease in the quantity of hydroxyapatite and proteins by the synergistic effect of both oxidation and demineralization action of H₂O₂ and its low pH.²⁹,³⁰ Although H₂O₂ itself does not have any deleterious effects on the enamel, but the low pH of bleaching agent can adversely affect the enamel integrity.²⁵ The higher pH prevents the demineralization of dental surface as pH of the bleaching agent with higher than pH 6.0 can prevent the damage to dental tissues.³¹ The average pH of in-office bleaching gels available in the market is around pH 5.5. But the many bleaching gels with a lower pH between 3.6 and 6.5 also available.³² It is below the critical pH for enamel dissolution (pH 5.5–6.5).²⁰ The reaction for enamel dissolution occurs because H⁺ ions release from the acidic medium react with OH⁻ ions and form H₂O³³. Therefore, due to decreased OH⁻ concentration the reaction mentioned below is shifted to the right and more Ca²⁺ and PO₄³⁻ ions form to maintain the new state of equilibrium. As the result, the enamel loss takes place according to the following equation:

\[
\text{Ca}_{10} (\text{PO}_4)_6 (\text{OH})_2 \rightleftharpoons 10 \text{Ca}^{2+} + 6 \text{PO}_4^{3-} + 2\text{OH}^{-}
\]

Surface roughness is an important parameter that can influence the dental esthetics and health.³⁴ Among various parameters used to assess the surface roughness, we used in our present study the Ra, which measures on a single line on a sample. This parameter describes the overall roughness of a surface. It is the arithmetic average of all absolute distances of the roughness profile from the centerline within the measuring length.³⁵ The shape and size of irregularities on the surface can affect the quality and performance of the surface. Generally, the increase surface roughness can increase the food particles accumulation and biofilm formation causing periodontal diseases and tooth discoloration.³⁶
Therefore, one of the purposes of this study was to evaluate the surface roughness after the application of bleaching agents with different pH. In this study Ra was significantly increased in all the experimental groups, although the base line Ra of these groups had been similar. The average baseline Ra was 0.056±0.006 (mean±SD) and maximum Ra reported as 0.079±0.006. The pH 5.5 group exhibits highest Ra even after first application. Comparatively pH 7.0 and 8.5 groups yielded a low Ra. Enamel dissolution can take place when it is treated with bleaching agents with lower pH below the critical pH for enamel dissolution\textsuperscript{20}. As pH 5.5 is slightly acidic, it can be the reason for dissolution of enamel and increased Ra. Some studies have reported that the pH of bleaching agent has a direct relationship with the surface roughness\textsuperscript{36,37}. Acidic bleaching gels can demineralize the enamel and cause increase of surface roughness\textsuperscript{36,37}. Previous studies have revealed that 30–35% H\textsubscript{2}O\textsubscript{2} can increase surface roughness and reduce the calcium-phosphorus ratio\textsuperscript{38–40}. To date, a fewer studies related to direct effect of pH of bleaching agent on alterations of surface roughness available in the literature.

The changes in microhardness of enamel is related to its loss or gain of minerals and the changes of the organic content. Decreased microhardness can affect the mechanical properties of the enamel. To assess the minor changes resulted after mineral loss or gain, microhardness test was chosen as a fast and simple test with reliable outcome\textsuperscript{22}. In this study all the experimental groups showed a significant reduction of microhardness in relation to the base line values of them (The average baseline VHN was 236.83±9.3 and maximum reduction was 209.5±15.6). pH 5.5 group showed maximum reduction of microhardness after third application time. The pH 7.0 group showed comparatively intermediate reduction and pH 8.5 group showed a slight reduction of microhardness. The decrease in microhardness is related to the loss of minerals by the bleaching agent. The effect of the pH of bleaching agent on enamel is controversial. One study has reported that bleaching gel with pH 7.0 did not affect and pH 5.5 may cause the reduction of microhardness\textsuperscript{41}. Another study has noted that high concentration of bleaching agents with acidic pH were more effective on tooth bleaching while it caused reduction of microhardness. They have found that 30% neutral H\textsubscript{2}O\textsubscript{2} (pH=7.0) caused less detrimental effects compared to the acidic H\textsubscript{2}O\textsubscript{2} (pH=3.6) with the same efficiency of tooth bleaching\textsuperscript{42}.

The morphological alterations of the enamel could happen due to the reaction between peroxide and organic materials on the surface or in the subsurface of the enamel\textsuperscript{17}. That changes of enamel morphology and microstructure as the result of tooth bleaching have an impact on the physical and mechanical properties of the enamel. The enamel alterations are most probably due to an initial process of enamel demineralization with the loss of calcium in teeth when exposed to peroxide\textsuperscript{40,48}. Additionally, the higher solubility and lower resistance of dental hard tissues after the tooth bleaching is due to the modification of the organic and inorganic ratio of the dental tissues\textsuperscript{46}. Regarding the present study, SEM photomicrographs showed different degree of the enamel alterations in all the experimental groups. The pH 5.5 group showed some changes throughout the entire surface while pH 7.0 and pH 8.5 groups showed minor alterations randomly. That changes include surface erosions, depressions, increase depth of enamel grooves and increased porosity. The grooves around the eroded enamel rod sheath were visible in all experimental groups. The narrow gaps which consistent with the distribution pattern of organic substances in the enamel are observed in the enamel rod sheath region of the head of enamel prisms. They are probably created by decomposition and removal of the organic substances. The narrow gaps in the prism sheath and between crystals were created by dehydration and removal of the organic substances\textsuperscript{45}. In agreement with one study, in-office bleaching agents with pH 6.4 (35% carbamide peroxide) and pH 3.8 (35% H\textsubscript{2}O\textsubscript{2}) showed porosities, depressions, craters, increased depth of enamel grooves, and partial removal of enamel prisms\textsuperscript{49}. More severe enamel alterations could be expected after in-office bleaching. It consists of increased porosity\textsuperscript{46,47}, enamel erosion with crater formation\textsuperscript{48,49}, depressions\textsuperscript{49}, removal of the aprismatic layer and exposure of the enamel prisms\textsuperscript{17,49,50}. Conversely, the same concentration of 35% H\textsubscript{2}O\textsubscript{2} and 35% carbamide peroxide did not produce any morphological alterations on enamel in other studies\textsuperscript{39,51}.

The present study used bovine teeth model that has been verified as a good alternative for human teeth for tooth bleaching study\textsuperscript{2,36,41}, because the chemical and physical properties in terms of composition\textsuperscript{51}, heat capacity, hardness, dentinal tubule density\textsuperscript{52} and permeability\textsuperscript{53} are similar to the human teeth. The bleaching agent was photo-irritated although it did not contain a photocatalyst. As a result, the temperature of the bleaching gel was increased by converting the energy into heat. A violet LED light for tooth bleaching was used due to its effectiveness and the safety. As the violet LED light is having a short wavelength and high-vibration frequency, less penetrate through the dental tissues and remain greater amount of energy on the tissue surface. Because of this feature it can breakdown the large chromogenic molecules with lower heating. As well as it can use to enhance the bleaching outcome with various concentration of bleaching gel without causing any damage to its molecular structure\textsuperscript{54}.

Few studies have evaluated the direct effect of pH of bleaching gel on the enamel surface\textsuperscript{12,27}. But the content of each experimental group was different from each other. They have shown the difference in either type of pH-conditioner included or the concentration of the H\textsubscript{2}O\textsubscript{2}. But for the purpose of comparison using same concentration of H\textsubscript{2}O\textsubscript{2} and same composition of bleaching gel is essential. This is the unique feature of this study when compared with the previous studies. We have used the bleaching gels with same concentration of H\textsubscript{2}O\textsubscript{2} and same ingredients, only changed the quantity of the pH-conditioner in each group to eliminate the factor
of composition.

The adverse effects caused by low pH of bleaching materials can be transient. Because, the action of saliva can reverse the transient damage of the enamel by wash out and acid-buffering effect. This is happening in the healthy patients with normal salivary secretion. But there can be many patients with reduced salivary secretion due to aging, medications and various systemic disorders who are seeking tooth bleaching treatment. Under these conditions the enamel damage cannot be reversed by saliva6. Therefore, it is important to consider about using an alkaline pH of bleaching agent to avoid damage to the enamel.

According to this study acidic pH (pH 5.5) showed increased surface roughness, decreased microhardness and more pronounced morphological alterations. The neutral (pH 7.0) and alkaline pH (pH 8.5) showed comparatively less alterations of enamel surface. While having the advantages of alkaline pH there are some adverse effects due to the extremes of pH values of the bleaching agent. OECD (Organization of Economic Co-operation and Development) has published the guidelines correlated with pH and the degree of thermal irritation. According to them the safest pH range is pH 2.0–11.5 and extremes of pH may cause strong local effects as corrosion of skin or mucosa39. In our study design we have included the experimental groups with different pH within the safety range. Considering the findings of previous studies related to the effect of pH on effectiveness of the tooth bleaching and based on the results of the present study the alkaline pH is the most effective and safest pH for tooth bleaching among our experimental groups. Further studies with extracted human teeth and in-vivo study are necessary to evaluate the actual effect of pH of bleaching gel on tooth bleaching and the tooth structure, as well as to evaluate the degree of recovery after the damage. It will be helpful to development of new tooth bleaching agents that provide maximum bleaching effect with minimum damage to the dental enamel.

CONCLUSION

In conclusion, we could demonstrate that the bleaching agents with low, neutral and alkaline pH can cause increased surface roughness, decreased microhardness and morphological alterations in the enamel surface at different degree with repeated bleaching applications. Acidic bleaching agent showed more evident surface changes of the enamel compared to neutral and alkaline one.

CONFLICT OF INTEREST

The authors do not have any financial interest in the companies whose materials are included in this article.

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