In-office bleaching for the remineralization of enamel lesions filled with organic components of red wine

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ABSTRACT: Purpose: To investigate the effects of in-office bleaching on the remineralization of enamel lesions filled with organic components of red wine. **Methods:** Enamel specimens were exposed to 0.1% NaF solution for 1 minute immersed in red wine for 5 days at 37°C, and subjected to in-office bleaching followed by remineralization in 1.5 mM $CaCl_2$, 0.9 mM KH_2PO_4, 130 mM KCl, 20 mM HEPES, pH 7.0, at 37°C for 28 days. The presence of organic substances on the enamel surface was detected by Raman spectroscopy. The specimens were also subjected to transverse microradiography (TMR). **Results:** Raman spectroscopy of baseline lesions showed characteristic peaks at 1,300-1,600 cm^{-1} which disappeared in bleached specimens. TMR showed that red wine formed subsurface lesions with surface content at approximately 22 mineral volume %. The integrated mineral loss (IML) was significantly lower in unbleached remineralized specimens than at baseline ($P< 0.05$). The IML of bleached remineralized specimens was lower than that of unbleached specimens, although not significantly ($P> 0.05$). Lesion depth was significantly lower in the bleached than in the unbleached group ($P< 0.05$). (*Am J Dent* 2018;31:13-16).

CLINICAL SIGNIFICANCE: In-office bleaching can enhance the remineralization of enamel lesions filled with organic components of red wine.

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Introduction

There are some reports that highly pigmented beverages with a low pH (e.g. black tea and red wine) cause extrinsic tooth discoloration.[1,2] In addition, it has been shown that highly pigmented, acidic beverages cause both tooth discoloration and dissolution of hard tooth structures.[3-6] Wine is acidic in nature (pH 3.0-4.0) and contains organic acids with high erosive potential, such as tartaric, malic, and lactic acids, as well as smaller amounts of citric, succinic, and acetic acids.[7,8] Also, red wine contains direct extrinsic staining organic agents,[9] such as polyphenols as typified by tannin, which may affect dental enamel.[10] Because wine induced decalcified enamel surfaces of teeth may become softer and more susceptible to wear by masticatory forces and tooth brushing,[11] it is necessary to remineralize such lesions that contain organic components from wine. However, in the presence of saliva, tannin has been reported to suppress hydroxyapatite transformation from amorphous calcium phosphate,[12] and the tannins absorbed by teeth[13] may hamper remineralization. In-office bleaching is a safe and effective system,[14] and is currently used for whitening in dental offices worldwide. The active ingredient of in-office bleaching agent is 30-35% hydrogen peroxide, which generates free radicals. Free radicals can degrade the unsaturated double bonds of pigment molecules in teeth.[14] This study investigated the effects of in-office bleaching agent on the degradation of organic components of red wine that infiltrated enamel lesions and on the enhancement of remineralization in vitro.

Materials and Methods

Specimen preparation - Twenty-one extracted bovine incisors were sectioned at the cement-enamel junction using a low-speed water-cooled diamond saw (Isomet[a]). A 4 × 4 mm enamel block was cut from each crown using a diamond instrument with air-turbine handpiece (PRESTO AQUA II[b]). Enamel block surfaces were sequentially flattened using 1,500 and 2,000 grit waterproof abrasive paper (Fuji Star[c]). Subsequently, the specimens were ultrasonically cleaned (US-2R[d]) in deionized water for 5 minutes to remove residues of the polishing procedure. Three specimens each were placed in a plastic container[e] and seven such sets were prepared by fixing specimens to the bottom with wax and coating the edges with acid-resistant varnish, exposing an approximately 3 × 2 mm window. One set of three specimens was subjected to Raman analysis, and six sets containing 18 specimens were allocated into three groups.

Table 1. Composition of the bleaching agent used in this study.

Syringe A	35 wt% hydrogen peroxide
Syringe B	30 wt% carbamide peroxide, propylene glycol, vinyl polymer
Reactor	Nitrogen-doped titanium dioxide, ethanol, purified water

Baseline subsurface lesions filled with organic components were generated by exposing each specimen to 5 ml of 0.1% NaF solution[f] for 1 minute, rinsing with tap water for 5 seconds and with deionized water for 10 seconds, and immersing the specimen in 10 ml of red wine (Sunrise Cabernet Sauvignon[g]) for 5 days at 37°C. The pH of the red wine was 3.4, as measured with a pH meter (D-51[h]) fitted with a pH electrode (9621-10D[h]). After immersion in wine, the specimens were again rinsed with tap water and deionized water. Six specimens in two containers were allocated to the "Baseline lesion" group, and the other 12 specimens were subjected to remineralization procedure with or without in-office bleaching.

Bleaching procedure - The composition of the bleaching agent is shown in Table 1. The six specimens were treated with this in-office bleaching agent (TiON In Office[f]), according to the manufacturer's instructions. Briefly, the reactor was applied to each experimental surface using a disposable brush, with any

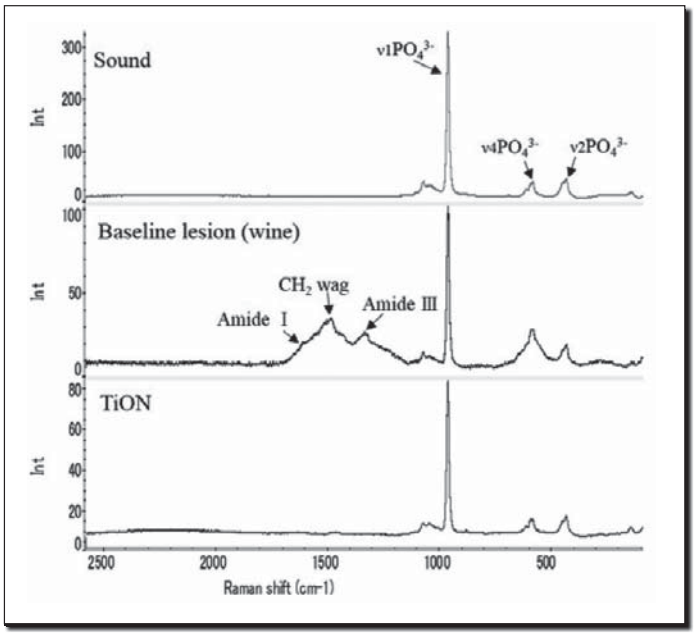

Fig. 1. Raman spectroscopic profiles. Three organic peaks of baseline lesion disappeared in bleached lesion.

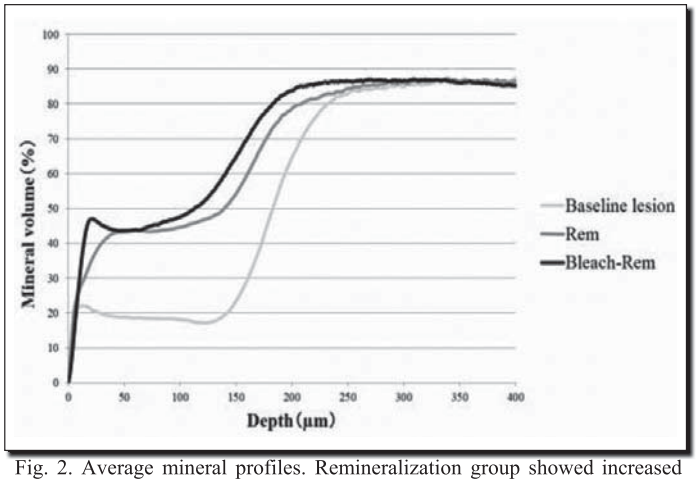

Fig. 2. Average mineral profiles. Remineralization group showed increased mineral volume percentages. Especially, mineral volume % of Bleach-Rem group increased in deeper region.

Table 2. Integrated mineral loss (IML) values and lesion depth (Ld).

Groups	IML (vol% × μm)	Ld (μm)
Baseline lesion	13,107.9 ± 1,501.7	238.2 ± 27.9
Rem	7,972.5 ± 1,331.3	225.8 ± 19.5
Bleach-Rem	6,707.1 ± 1,217.2	186.9 ± 15.4

Data are the mean ± SD of the six samples. Vertical lines mean significant differences with P< 0.05.

excess removed with air. The A and B syringes were connected and mixed, and the resulting gel applied to each experimental surface and activated with a light-curing unit (Demi Ultra[j]) for 1 minute, followed by incubation for 4 minutes. The gel was removed, and the entire treatment was repeated three times, with the specimens rinsed with deionized water after the third treatment.

Confocal laser Raman microspectroscopy - To detect changes in organic components, three specimens (sound, baseline lesion, and bleached enamel) were subjected to Raman analysis. The enamel surfaces of these specimens were analyzed by confocal laser Raman microspectroscopy (Nicolet Almega XR Dispersive Raman microspectroscopy system[j]), with a laser at 780 nm and a grating of 360 lines/mm.

Remineralization and transverse microradiography (TMR) - Twelve specimens were subjected to the remineralization procedure, six in "Bleach-Rem" group and another six in "Rem" group. Specimens were remineralized in a solution containing 1.5 mM $CaCl_2$, 0.9 mM KH_2PO_4, 130 mM KCl, and 20 mM HEPES, pH 7.0, at 37°C for 4 weeks, with remineralization solutions replaced at the end of each week. After remineralization, all specimens were successively dehydrated in a series of 70% to 100% ethanol, embedded in low-temperature curing epoxy (Low-Viscosity Embedding Kit[k]), and allowed to cure for 8 hours. Two 150-μm sections were cut from each embedded specimen using a diamond-coated wire sectioning machine (Well Diamond Wire Saw[l]) and placed between thin sealed polyester sheets with aluminum step wedges, consisting of 13 layers each 25 μm-thick aluminum foil.[m] Sections were radiographed on high-resolution glass film plates[n] with a nickel-filtered Cu-Kα source at 15 mA and 25 kV for 20 minutes (PW 3830[o]). Radiographic images of sections and aluminum step wedges were analyzed using a microscope/video camera/microcomputer, and software (TMR2000[p]).[15,16] Single scans of each section were obtained at the center of each lesion, and values of three parameters, the mineral content profiles of lesions, integrated mineral loss (IML, vol% × μm), and lesion depth (Ld, μm) were averaged.

Statistical analysis - Between-group differences in IML and Ld were evaluated by analysis of variance, followed by Tukey's HSD multiple comparison test. Statistical significance was defined as P< 0.05. All statistical analyses were performed using SPSS software (IBM SPSS Statistics Version 21[q]).

Results

Raman analysis - Raman spectroscopy of sound enamel, baseline lesions demineralized with red wine, and bleached TiON samples showed three phosphate peaks. The baseline lesions demineralized with red wine also showed three organic peaks (Amide I, Amide III, and CH2wag) between 1,300 and 1,600 cm^{-1}, all of which were absent from bleached TiON samples (Fig. 1).

TMR profiles, IML, and Ld - Figure 2 shows the average mineral profiles in the three experimental groups. Red wine generated lesions with a surface content of approximately 22 mineral volume %. The remineralization groups (Bleach-Rem and Rem) showed increased mineral volume percentages, much higher than in the baseline lesion group. In particular, the mineral volume % in the Bleach-Rem group showed a conspicuous increase at the lesion front. IML was significantly lower in remineralized than in baseline lesions (P< 0.05; Table 2). The mean IML was lower in the Bleach-Rem than in the Rem group, although not significantly. In contrast, lesion depth was significantly shallower in the Bleach-Rem than in the Rem group (Table 2).

Discussion

Several studies[7,17-20] have reported dental lesions due to long-term excessive wine consumption, or exposure among

professional wine tasters. In vitro studies[18,19] reported that extracted teeth incubated in wine show significantly reduced enamel microhardness within 2 minutes. Softened teeth, with a lower mineral/matrix ratio, may be susceptible to further dental attrition and toothbrush abrasion.[21,22] In addition, wine pigments can be taken up by subsurface enamel.[10] Effective remineralization strategies are therefore required for severe lesions filled with organic components.

The current study found that lesion depth was significantly lower in the Bleach-Rem than in the Rem group. In addition, IML was lower in the Bleach-Rem than in the Rem group, although this difference was not statistically significant. Remineralization of enamel lesions has been reported to occur at lesion fronts with decreasing lesion depth,[23] with a significant reduction in lesion depth indicative of remineralization, even if IML did not differ significantly.[24] The present study showed that in-office bleaching can enhance remineralization of enamel lesions by removing entrapped organic substances of red wine.

In a previous study,[25] application of an in-office bleaching agent to enamel subsurface lesions did not aggravate these lesions. Furthermore, a remineralization solution or natural human saliva can restore demineralization resulting from the application of in-office bleaching agents.[26,27] These findings indicate that in-office bleaching may be a safe method as a remineralization strategy. In the present study, enamel lesions with slight surface mineral content were generated by immersion in red wine. In contrast to previous findings,[28] Raman analysis revealed that the in-office bleaching agent did not have a marked effect on inorganic substances in the lesions. This may have been due to the pH of the TiON in-office bleaching agent used in this study, which at around six,[29] is higher than the critical pH needed for enamel demineralization.

Bovine enamel has been routinely used to evaluate the potential of cariogenic and anticariogenic substances[30,31] because the components of bovine teeth are similar to those of human teeth.[32] Human teeth are subjected to various fluoride containing substances, such as toothpaste. To generate similar tooth characteristics, bovine incisor samples were immersed in 0.1% fluoride solution before immersion in red wine in the present study.

In conclusion, in-office bleaching can degrade pigments and organic materials introduced by red wine, which is followed by much more efficient remineralization than without bleaching agent.

a. Buehler, Rosemont, IL, USA.
b. NSK, Tochigi, Japan.
c. Sankyo Rikagaku Co. Ltd., Saitama, Japan.
d. AS ONE, Osaka, Japan.
e. Becton Dickson, Franklin Lakes, NJ, USA.
f. GC, Tokyo, Japan.
g. Mercian, Tokyo, Japan.
h. Horiba, Kyoto, Japan.
i. Kerr, Orange, CA, USA.
j. Thermo Fisher Scientific, Inc., Waltham, MA, USA.
k. Polysciences, Inc., Warrington, PA, USA.
l. Walter Ebner, Mannheim, Germany.
m. Soekawa Chemicals, Tokyo, Japan.
n. Konica Minolta, Tokyo, Japan.
o. Spectris, Surrey, UK.
p. Inspektor Research Systems, Amsterdam, The Netherlands.
q. IBM, Tokyo, Japan.

Disclosure statement: The authors declared no conflict of interest.

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