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Full Length Research Paper

Effect of 10% sodium ascorbate on *Streptococcus mutans* adherence to bleached bovine enamel surface

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Sodium ascorbate has been suggested to modify bleaching agents' side effects especially on composite resin bonding to dental hard tissues. The aim of the present study was to evaluate the effect of 10% sodium ascorbate on *Streptococcus mutans* adherence to bleached enamel surfaces. Sixty enamel slabs from bovine incisors were used. After sterilization of the intact enamel surfaces with UV light, the specimens were randomly divided into the following treatment groups: (1) immersion in normal saline containing 2%NaN_3; (2) bleaching of enamel surfaces with 10% carbamide peroxide; (3) bleaching of enamel surfaces with 10% carbamide peroxide followed by 10% sodium ascorbate treatment. Adherence of *S. mutans* to enamel surfaces was determined bacteriologically. Data was analyzed using one-way ANOVA and post hoc Tukey tests ($P < 0.05$).10% sodium ascorbate after bleaching (Group 3) caused a significant increase in surface adherence of *S. mutans* compared to groups 1 and 2 ($P < 0.001$). Because of bacterial adherence subsequent to use of sodium ascorbate to bleached enamel caries risk may be increased.

Key words: Sodium ascorbate, *Streptococcus mutans*, carbamide peroxide.

INTRODUCTION

Adherence of bacteria to dental hard tissues and restoration is an important factor in dental caries (Mor et al., 1998) and periodontal diseases (Aren, 2003). Adherence mechanisms of oral bacteria to hard tissues are influenced by dental surface texture and the adherence properties of bacteria (Mor et al., 1998). Adherence of saliva and bacteria to tooth surfaces is an important step in plaque formation and factors such as electrostatic and hydrophobic reactions, binding sites and sucrose-related mechanisms influence the process (Steinberg et al., 1999). Bleaching agents are potent chemical agents which can influence bacterial adherence to restorations and tooth structures (Steinberg et al., 1999). Enamel roughness and adherence of *Streptococcus mutans* (as the most important cariogenic microorganism) to enamel surfaces subsequent to bleaching has been confirmed (Hosoya et al., 2003). However, clinical studies have yielded contradictory results concerning the inhibitory effect of bleaching agents on plaque formation: a study has reported that hydrogen peroxide-containing mouthwashes have no specific therapeutic role in preventing plaque formation and gingivitis (Jones et al., 1990). However, another study has shown the role of peroxide mouthwash in inhibiting bacterial colonization (Wennestrom and Lindhe, 1979). Sodium ascorbate is a potent reducing agent and its use has recently been suggested after bleaching to modify the side effects of bleaching agents, especially in improving bond strength to enamel (Kimyai and Valizadeh, 2006). Vitamin C and its salts are nontoxic and are widely used as antioxidants in food industries (Lai et al., 2002; Radcliffe et al., 2003). It is possible that this antioxidant can change surface texture and influence bacterial adherence to bleached enamel surfaces. The aim of the present study was to evaluate the effect of 10% sodium ascorbate on *S. mutans* adherence to enamel surfaces bleached with 10% carbamide peroxide.

MATERIALS AND METHODS

A total of 60 sound extracted bovine incisors were used in the

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Table 1. Mean (SD) of bacterial colony counts ($x10^5$ cfu/mL) in the experimental groups.

Group	No.	Mean (SD)	Minimum	Maximum
Control (1)	17	41.71 (13.86)	22	71
10% Carbamide peroxide (2)	17	68.53 (17.62)	32	98
10% Carbamide peroxide + 10% Sodium ascorbate (3)	17	137.65 (49.63)	71	215

present study. The teeth were stored in 2% chloramin T solution immediately after extraction. All tooth surfaces were examined by explorers and under a stereomicroscope (Nikon SMZ 1000; Tokyo; Japan) for any softness, scrapings or any other defects. A diamond disk was used to separate roots from crowns at CEJ.

Preparation of enamel specimens

Primary enamel blocks were prepared from the mid-buccal surfaces of the teeth using a diamond flat-end taper bur (MANI, Nakaa Kustn, Japan) under air and water spray; a diamond disk was used to prepare 60 enamel slabs measuring 5 × 2 × 2 mm. New diamond burs and disks were used after preparing four slabs. All the cut surfaces of the specimens were coated with two layers of nail varnish. Before division, all the specimens were immersed in distilled water for 24 h and then were divided into three groups, using simple random sampling method. In group 1 (control) all the specimens were immersed in normal saline containing 2% NaN_3 (storage media) during the study period. In group 2 the enamel specimens were placed in 10% carbamide peroxide gel (Opalescence, Ultradent, South Jordan, USA) after retrieval from distilled water by sterile tweezers. The treatment protocol was to immerse the specimens in the gel for 8 h daily for one week (Kaya and Turkun, 2003). After 8 h the specimens were retrieved from the gel by sterile tweezers and were rinsed for 30 s under running water to remove remnants of 10% carbamide peroxide. Specimens were immersed in 2% NaN_3 storage media until the next daily treatment to prevent microbial growth. In group 3 the enamel specimens were treated with bleaching agent in the same manner as described for group 2 and then immersed in 10% sodium ascorbate for 8 h (Kaya et al., 2008) followed by rinsing them under running water and preserve them in 0.2% NaN_3 storage media until the bacteriologic test. 10% Sodium ascorbate solution (pH = 7.8) was prepared by dissolving of pure sodium ascorbate powder (99% L-Ascorbic acid sodium salt, Fluka Swiss Product) in distilled water. All storage media were changed daily. Three specimens from each group were randomly selected for surface morphology evaluation under scanning electron microscope before the bacteriologic procedures. The other 17 enamel specimens were subjected to UV light for 1 h for sterilization; and then were immersed in storage media until the bacteriologic procedure began.

The bacteriologic procedure

S. mutans (Type A, No. 1683, ATCC NO. 35668, PTCC, Tehran, Iran) was used as the test organism. Isolated *S. mutans* was maintained on blood agar (Merk, Munich, Germany) for 48 h and then cultured in nutrient broth (MRVP, Merk, Munich, Germany) for 24 h. After thorough mixing, 0.1 ml of the suspension was spread homogenously on the blood agar surface and incubated at 37°C for 18 h in a candle jar (5% CO_2). Enamel specimens were separately placed on these culture plates with sterile tweezers so that the treated surface of the specimens came into contact with the bacteria. After incubating the specimens at 37°C 5% CO_2 for 24 h each slab was inserted in a test tube containing 10 ml of sterile phosphate buffer saline (pH 7.2) and sterile glass beads and stirred at Vortex (LABTORN Co, Iran) at maximum speed for one minute to free the bacteria attached on the surface of each enamel specimen. The (10^{-1}) dilution of the microbial suspension was used to produce 10^{-2}, 10^{-3} and 10^{-4} dilutions and 0.1 ml of the last dilution was again inoculated with a standard loop and incubated for 24 h (Gurgan et al., 1997). The tests were duplicated and the number of bacteria per specimen was calculated using the formula: final number of adhered bacteria to each specimen in each group (cfu/ml) = number of colonies × 10^5.

Evaluation of surface morphology of specimens

Three specimens from each group were selected for surface morphology evaluation under a scanning electron microscope (TESCAN/VEGA, USA). The specimens were gold sputtered in vacuum and were blindly evaluated at ×5000 magnification under the scanning electron microscope. One-way ANOVA was used to compare means of colony counts in the experimental groups; a post hoc Tukey test was used for the two-by-two comparison of the groups. Statistical significance was defined at $p < 0.05$.

RESULTS

Table 1 summarizes means and standard deviations obtained in the experimental groups. According to the Table, the highest and lowest bacterial colony counts were observed in groups 3 and 1, respectively. One-way ANOVA analysis demonstrated statistically significant differences in mean colony counts between the groups ($P < 0.001$). Two-by-two comparison of the groups by a post hoc Tukey test demonstrated that 10% carbamide peroxide (group 2) caused a significant increase in surface adherence of *S. mutans* compared to group 1($P = 0.04$), 10% sodium ascorbate after bleaching (group 3) caused a significant increase in surface adherence of *S. mutans* compared to groups 1 and 2 ($P < 0.001$). Scanning electron micrographs revealed that there were surface topographic changes in the group 2 compared to normal enamel (Figures 1 and 2), in addition complex networks including adsorbed sodium ascorbate were visible on the bleached enamel in the group 3 (Figure 3).

DISCUSSION

Considering the scarcity of human enamel and structural similarities between human enamel and bovine enamel (Sanches et al., 2009), bovine enamel was chosen as a substitute for human enamel in the present study. In addition, *S. mutans* was chosen as the test organism to evaluate the effect of 10% carbamide peroxide and sodium

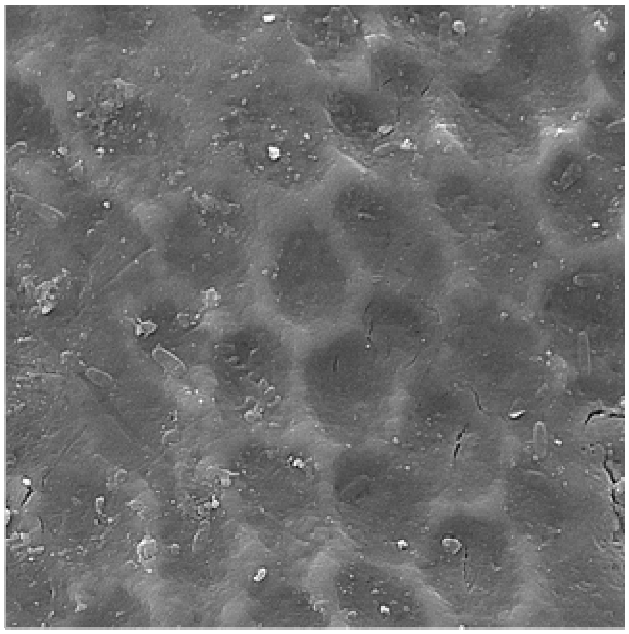

Figure 1. Scanning electron micrograph of intact bovine enamel in the control group (× 5000).

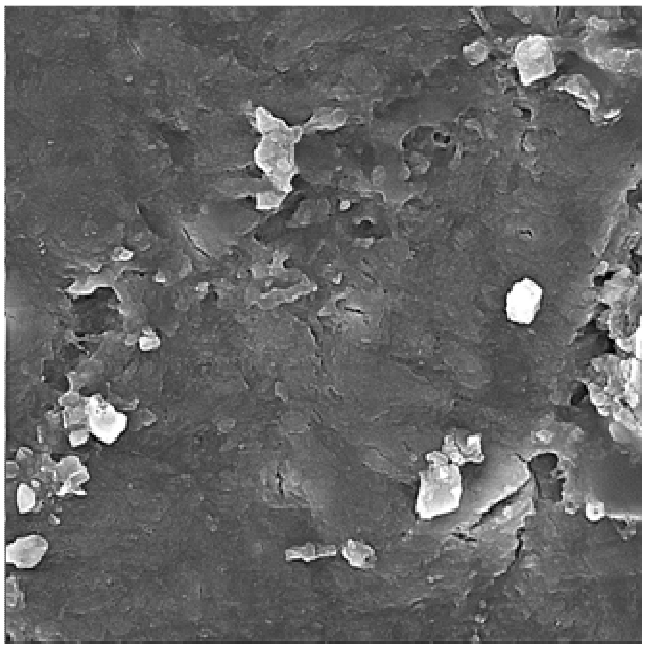

Figure 2. Scanning electron micrograph of bovine enamel bleached with 10% carbamide peroxide (× 5000).

ascorbate on bacterial adherence to bovine enamel, because of relationship between dental caries in humans and laboratory animals and this organism (Gurgan et al., 1997). The results of the present study demonstrated that bacterial adherence to enamel surfaces significantly increases with the use of carbamide peroxide, which is consistent with the results of studies carried out by Hosoya et al. (2003) and Gurgan et al. (1997).

Various mechanisms influence bacterial adherence to dental tissues, including type of bacteria and surface texture of the target (Steinberg et al., 1999). A non-specific process mediated by hydrophobic or electrostatic reactions is responsible for bacterial adhesion during the first steps (Steinberg et al., 1999). Specific adherence of oral bacteria is mediated by membrane binding sites (Van Houte, 1980). Bleaching agents are reactive chemicals which can result in alterations on the surface of dental hard tissues through their oxidizing properties (Steinberg et al., 1999). Rotstein et al. (1996) showed that the use of bleaching agents alters Ca/P ratio on enamel surfaces, which have been reported to occur up to 10 nm from the enamel surface (Covington et al., 1990). In addition to chemical changes, morphologic and topographic characteristics of enamel surface might be influenced by bleaching agents. Although Haywood et al. (1991), Gurgan et al. (1997) and Hosoya et al. (2003) did not demonstrate any significant changes in enamel surface texture following the use of 10% carbamide peroxide, scanning electron microscope studies have confirmed topographic changes on bleached enamel (Covington et al., 1991; Ernest et al.,1996; Chen et al., 1989), which is consistent with the electron microscopic findings in the present study. Mor et al. (1998) demonstrated that bleaching produces some micro-surface characteristics on dental hard tissues in the oral cavity, paving the way for *S. mutans* adherence, although Gurgan et al. (1996) reported the antibacterial effect of 10% carbamide peroxide bleaching agents. It seems that micro structural changes on enamel surface following the use of bleaching agents lead to an increase in *S. mutans* adherence to enamel surface.

The important result of present study was the highest bacterial adherence in the group 3. Ascorbic acid and its salts are well-known antioxidants, which have the capacity to reduce oxidative compounds, such as free radicals (Buettner, 1993; Rose and Bode, 1993). Sodium ascorbate has drawn attention in dentistry due to its reductive capacities and its ability to increase strength of adhesive bond to bleached enamel (Kaya and Turkun, 2003). Sodium ascorbate is oxidized to dehydroascorbate in two phases after contact with nascent surface oxygen molecules (Pournaghi-Azar and Ojani, 1995). According to electrochemical and topographic techniques, sodium ascorbate is adsorbed by smooth surfaces, polymers and hydroxyappatite crystals and prepares the surface for the adherence of other particles by producing porous physical networks (Akkermans et al., 1998). This is consistent with electron micrographs of the present study that is indicative of the presence of sodium ascorbate ($C_6H_7O_6Na$) or its derivatives on the surface, which have not been removed by conventional rinsing of the specimens under running water. It seems that the cumulative effect of the use of the bleaching agent and sodium ascorbate has led to a maximum amount of *S. mutans* adherence to the surface of enamel specimens. Therefore, it can be concluded that

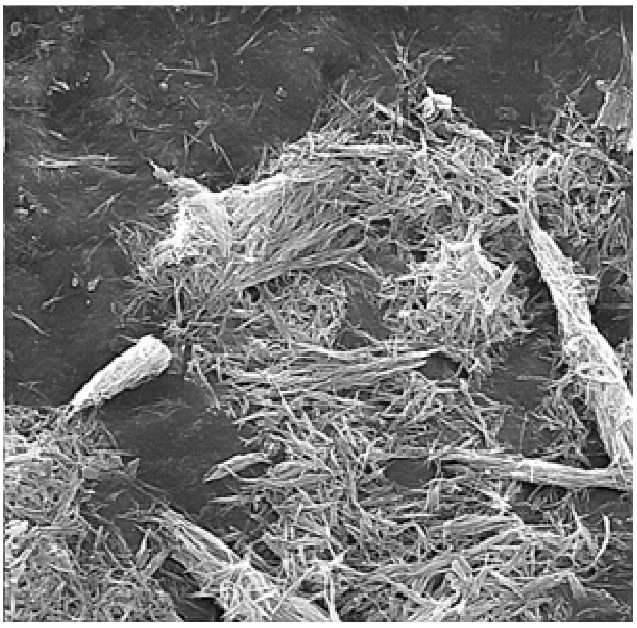

Figure 3. Scanning electron micrograph of bovine enamel treated with 10% sodium ascorbate subsequent to bleaching with 10% carbamide peroxide (× 5000).

alterations in surface topography can have a direct influence on bacterial adherence to bleached enamel surfaces. Although an increase in bacterial adherence and selective adherence of different kinds of bacteria to hard tissues in the oral cavity can have an important role in the formation of bacterial plaque and dental caries, however, it should be kept in mind that the oral cavity is constantly washed by salivary flow which is rich in proteins, carbohydrates, lipids and some other components, which might influence bacterial adherence in a manner different from that *in vitro* conditions. Considering the limited number of studies and based on the results of the present study it is suggested that adherence mechanisms of other bacterial species involved in caries be evaluated under conditions as similar to clinical conditions as possible with different concentrations, different application times and different irrigation methods of sodium ascorbate.

Conclusion

According to the results of the present study it can be concluded that because of bacterial adherence subsequent to use of sodium ascorbate to bleached enamel caries risk may be increased.

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