

Effects of Hydrogen Peroxide-containing Bleaching on Cariogenic Bacteria and Plaque Accumulation

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Objective: To evaluate the effects of a commercial bleaching agent containing 36% hydrogen peroxide on the clinical plaque index and *Streptococcus mutans* and *Lactobacilli* counts in dental plaque and saliva *in vivo*.

Methods: Twenty volunteers were recruited in the present study. Evaluation of plaque index and bacteria counts in plaque and saliva was performed before bleaching the teeth, and 3 days, 2 weeks and 4 weeks post-treatment.

Results: Quigley-Hein Plaque Index and Modified Navy Plaque Index were significantly lower on day three after bleaching compared to that before bleaching and the other time points after bleaching. The total number of bacteria in the plaque on day three after bleaching was also less than that before bleaching and the other time points after bleaching. The *Streptococcus mutans* counts in the plaque and saliva four weeks after bleaching were decreased significantly compared to that before bleaching.

Conclusion: Hydrogen peroxide bleaching can decrease plaque accumulation and cariogenic bacteria for a short period of time.

Key words: cariogenic bacteria, peroxide, plaque

Tooth discolouration has been a major concern nowadays due to modern high aesthetic demands. Bleaching, performed at home or in office, has been regarded as a safe and effective means to deal with intrinsic and extrinsic stains on anterior teeth¹. Most studies have failed to show significant changes of the enamel surface after vital bleaching procedures with 10% carbamide peroxide²⁻⁵. Some reports have shown a loss of calcium and alterations in enamel structure after bleaching⁶⁻⁷. Furthermore, other reports have suggested that both surface roughness and adhesion of *Streptococcus mutans* to the enamel surface were increased after bleaching *in vitro*⁸⁻¹⁰. Despite bleaching agents being widely used, the present knowledge of possible adverse effects remains controversial.

Al-Qunaian found that the caries susceptibility of the tooth surface did not change after bleaching². Actually, hydrogen peroxide was shown to have a bacteriostatic effect on *S. mutans* and *Lactobacilli*, which may suggest its potential usefulness as an anti-cariogenic agent¹¹⁻¹². Other researchers found that urea peroxide and hydrogen peroxide were highly effective in reducing plaque accumulation and caries incidence in rats¹³. Kraigher found the depth of caries lesions were smaller in bleached teeth than in the non-bleached control teeth in Wistar rats¹⁴.

However, most of the previous studies about the effects of bleaching on cariogenic bacteria were performed *in vitro* or *in vivo* on animal teeth; there is a lack of *in vivo* studies on human teeth. The oral environment (with the continuous flow of saliva and many other factors) is quite different from that *in vitro*^{12,15}. Caries is a specific site-related disease. The plaque on the specific site and its cariogenic bacteria were important for the development of caries. *S. mutans* and *Lactobacilli* in the dental plaque or saliva has been shown to be the most important bacteria in the pathogenesis of dental caries¹⁵⁻¹⁹. However, there are few clinical studies demonstrating the effects of a higher concentration of

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hydrogen peroxide-containing tooth bleaching on the cariogenic bacteria in plaque²⁰.

This study investigated the effect of bleaching with a higher concentration of hydrogen peroxide on plaque accumulation and cariogenic bacteria (*S. mutans* and Lactobacilli) in the plaque on bleached teeth surfaces, and on the salivary level in volunteers.

Materials and methods

Bleaching agent

The bleaching gel used in the present study contained 36% hydroxide peroxide and other components, including hydrogen peroxidase, 1,2-propanediol, glycerol, deionised water and sodium hydroxide, but without fluoride (BEYOND™ Professional Dental Whitening Kit, Beyond Technology, TX, USA).

Subjects

Twenty adult volunteers (17 females and three males) aged 22 to 44 years (average age 29.85) were recruited in this study. The protocol was approved by the Ethical Committee of Peking University Health Science Center. Subjects were informed of the study's protocol and aims, and informed consent was obtained. All subjects were medically fit, with no underlying chronic illness, and had not been taking antibiotics for at least 3 months before the study. Enamel surfaces on the labial aspect of maxillary incisors and canines were clinically sound.

Exclusion criteria were: subjects who had caries lesions, enamel defects, dentine sensitivity, fillings in the anterior teeth, fixed partial denture, removable prosthesis or orthodontic appliances. Smokers, expecting or breastfeeding mothers, and patients taking medicines that would decrease salivary flow were also excluded.

Clinical procedure

All subjects underwent a full-mouth examination, scaling and prophylaxis 1 month before the bleaching procedure. The tooth colour and shade were assessed using a Vita classic shade guide according to Marson's study²¹, and oral photographs were recorded to provide comparison of colour improvement between initial and final visit. The bleaching procedure followed the instructions provided by the manufacturer. After placement of protective eye goggles, cheek retractor and protective dam, the teeth involved were dried and an approximately 2 mm-thick layer of whitening gel was applied on the teeth surfaces.

The light source was positioned 10 mm perpendicularly from the tooth surface. The duration taken for one cycle was 8 minutes and the gel from the teeth surface was then removed. The subjects were informed to refrain from teeth brushing for 3 days before the next appointment.

Plaque and saliva samples

Samples were collected at four different time points: before bleaching (baseline); 3 days post-bleaching; 2 weeks post-treatment; and 4 weeks post-treatment. All subjects were instructed to refrain from tooth brushing for 3 days before the samples were collected. At each time point, the plaque indexes, TPI (Quigley-Hein Plaque Index) and MNI (Modified Navy Plaque Index), were recorded according to previous studies²²⁻²⁴. Plaque and saliva were sampled for further bacteriological investigation.

Plaque samples were collected 2 hours after breakfast by the method described by Sansone²⁵. Six maxillary anterior teeth were isolated with cotton roll and dried by short blasts of compressed syringe air. Plaque samples from labial surfaces and proximal surfaces of the teeth were collected by a sterile dental explorer. The samples were placed in 1.5 ml RTF (Reduced Transport Fluid, Oxoid, Cambridge, UK) on ice, and transported to the laboratory within 1 hour.

The collection of 'unstimulated' saliva was performed under resting conditions between 8:30 am and 10:30 am, at least 1 hour after eating, following standard procedures²⁶. Subjects were instructed to sit passively and expectorate into a sterile glass container for 5 minutes, as the saliva accumulated in the floor of the mouth. Usually about 2 ml of saliva was collected for each patient; the collected samples were put in an ice container, and transported to the lab within 1 hour.

Bacteriological assessment

The total number of cultivable bacteria and the numbers of *S. mutans* and Lactobacilli in the plaque and saliva were quantitatively evaluated.

The plaque samples were first dispersed with a glass stick. Then, the plaque suspension of each sample was put in an ultrasonic sonifier (BioSonic UC100, Coltène/Whaledent, USA) for 20 seconds, and vortexed for 30 seconds. Samples were serially diluted in RTF at dilutions of 10:1, 10:2, 10:3, 10:4 and 10:5. The saliva samples were dispersed by vortexing for 60 seconds, then diluted in RTF solution at 10:1, 10:2, 10:3, 10:4 and 10:5.

Aliquots (50 μ L) of appropriate dilutions of the 10-fold serially diluted suspensions were spread in duplicate on blood agar for enumeration of the total cultivable flora, on Rogosa SL agar for Lactobacilli, and on Mitis salivarius agar (Becton Dickinson Microbiology, MD, USA) supplemented with two IU of bacitracin/mL (Oxoid), 1% potassium tellurite and 15% sucrose for *S. mutans* specifically²⁷. All media were incubated anaerobically (85% N₂, 5% CO₂, 10% H₂) for 5 days at 37°C. Colonies of the target bacteria were counted on the plates with fewer than 250 colonies.

All isolates were identified presumptively on the following features: colony morphology, cell morphology, Gram staining reaction and catalase reaction. A stereoscopic microscope (Opton, Germany) was used to verify the presence of colony-forming units resembling *S. mutans* on the plates. They were distinguished by their molar and granular frosted glass-like formation, adherent growth in agar and production of glucans surrounding colonies.

For bacteria that were unable to be determined by colony morphology and Gram-stained cell morphology, API 20 Strep and 50 CHL commercial kits (bioMerieux, Marcy-l'Étoile, France) were used for further identification, according to the instructions by the manufacturer²⁸⁻²⁹.

Statistics methods

SPSS 10.0 software was used for the statistical analysis. Means, standard deviations and confidence intervals (95% CI) were determined for all data. The total

Table 1 Plaque index (TPI and MNI) at different time points and total numbers of bacteria in the plaque

	TPI	MNI	Total numbers of bacteria in the plaque (log[CFU/ml+1])
Pre-bleaching	2.9965	5.2885	7.1254
3 days after bleaching	1.8055*	3.4455*	6.7869*
2 weeks after bleaching	2.4350*	4.8350	7.1461
4 weeks after bleaching	2.5220	5.0360	7.1483

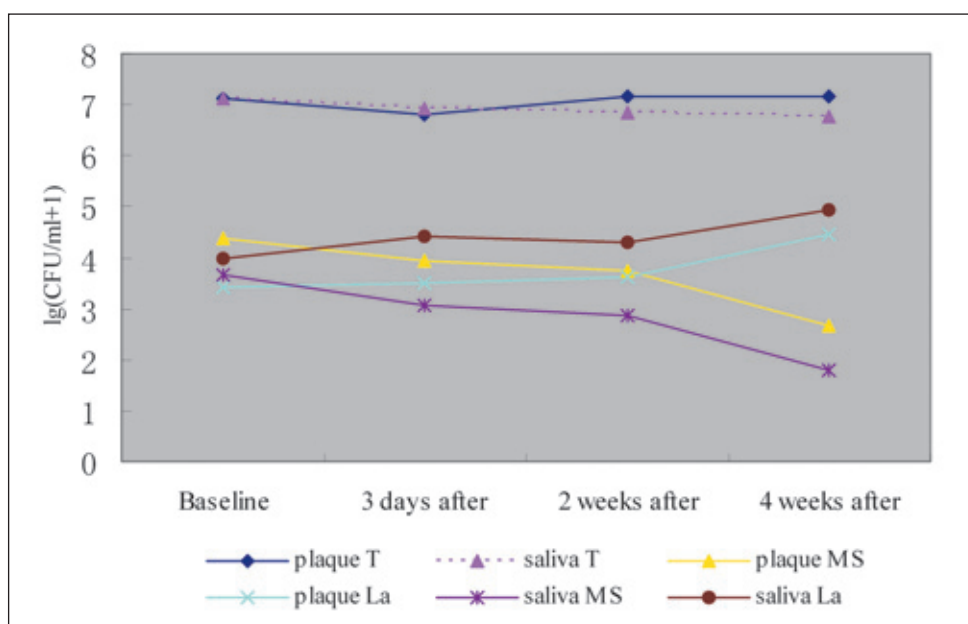
* ANOVA test ($P < 0.05$)

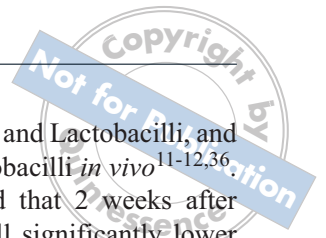
bacteria, *S. mutans* and Lactobacilli levels in CFU/ml of plaque and saliva were converted into logarithm (log[CFU/ml+1]) for statistical analyses. ANOVA and Mann–Whitney non-parametric analyses were used to compare the plaque index and bacteriological counts at different time points.

Results

Plaque index results (TPI and MNI) on day three after bleaching were significantly lower than that of before bleaching and the other two time points after bleaching ($P < 0.05$). However, TPI (2.4350) on week two after bleaching was still lower than that of baseline (2.9965, $P < 0.05$) (Table 1).

Fig 1 Linear diagram of numbers of bacteria recovered from the plaque and saliva at different time points. The number of *S. mutans* both in the plaque and saliva was statistically significantly lower on week four after bleaching compared with that of before bleaching ($P < 0.05$). Plaque T: total bacteria in plaque; Saliva T: total bacteria in saliva; Plaque MS: *S. mutans* in plaque; Plaque La: Lactobacilli in plaque; Saliva MS: *S. mutans* in saliva; Saliva La: Lactobacilli in saliva.





The total number of bacteria in plaque on day three after bleaching showed a decreasing trend compared to that of the pre-bleaching, and the other two time points ($P < 0.05$) (Table 1). However, the total number of bacteria in saliva was not changed after bleaching until week four after treatment (Fig 1).

The number of *S. mutans* both in the plaque and saliva decreased on week four after bleaching, compared with that before bleaching ($P < 0.05$). The number of Lactobacilli both in plaque and saliva did not change until week four after bleaching.

Discussion

Dental bleaching therapy has become increasingly popular because the procedures involved are simple and efficient. However, some studies have found that hydrogen peroxide could cause demineralisation of enamel, and cause microscopic alterations to the enamel and dentine surfaces³⁰⁻³³. Other studies reported that after bleaching, no significant micro-morphological changes on enamel surfaces were found using the scanning electron microscope method^{4,34-35}. Furthermore, some researchers speculated that the amount of calcium loss was not clinically significant, and not harmful to the tooth structure^{5,7}. The controversies on enamel surface morphology can be explained by the discrepancy in the study designs, concentration of hydrogen peroxide used, sample sizes, application times and enamel morphology evaluation techniques.

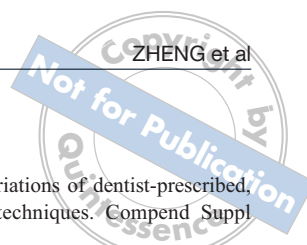
The enamel surface roughness could be an important factor for the accumulation of the plaque³⁶ due to the natural ability of the bacteria to adhere on to the tooth surface. After bleaching, the enamel surface could become much rougher, and the adhesion of *S. mutans* to the enamel surface could be increased *in vitro*⁹. However, the accumulation of bacteria on the bleached enamel surface *in vivo* remains unknown. The results of the present study showed the plaque index (TPI and MNI) 3 days after bleaching was significantly lower than the other three time points. The total number of bacteria in the plaque 3 days after bleaching decreased significantly, which is consistent with the plaque index. The results from the present study were in accordance with Guroy's report, in which the plaque index 3 days after bleaching was significantly lower than before treatment²². The possible explanation might be that there was some trace hydrogen peroxide left on the surface structure. Hydrogen peroxide has a bacteriostatic effect, which may cause the amount of plaque to be decreased¹¹.

It was reported that 10% carbamide peroxide could minimise the adherence of *S. mutans*, provide a bacteri-

ostatic effect *in vitro* for *S. mutans* and Lactobacilli, and reduce the salivary levels of Lactobacilli *in vivo*^{11-12,36}. The present results demonstrated that 2 weeks after bleaching, the TPI index was still significantly lower than that of pre-treatment. Four weeks after treatment, the TPI index was slightly higher than that of pre-treatment, which may be due to the residual hydrogen peroxide that was leaching out steadily from the enamel. The bleached enamel may leach out peroxide absorbed by enamel and re-establish the superficial morphology after 3 weeks, and there was significant sustainability of hydrogen peroxide on the tooth surface after bleaching³⁷. In the present study, the results also indicated that, in a short period of time, the bacteriostatic effect of the hydrogen peroxide could reduce the amount of plaque *in vivo*. The possible alterations on the enamel surface structure could not increase the accumulation of plaque. With the gradual decline of the residual hydrogen peroxide in the enamel, the amount of plaque could recover as before, or even more than before, due to the possible alterations on the enamel surfaces²².

The total number of bacteria in the saliva had no change at any time point. This could be the reason that the residual hydrogen peroxide in saliva was easy to clear out immediately, as it was reported that the concentration of hydrogen peroxide in saliva increased during bleaching therapy and could not be detected after 30 minutes. Therefore, the hydrogen peroxide could not have much effect on the salivary bacteria³⁸⁻³⁹.

Caries is an infectious disease and microorganisms are responsible for the development of caries^{15,40}. In the ecological plaque hypothesis, caries is the result of a shift in the balance of the resident microflora due to a change in local environmental conditions, e.g. frequent sugar intake favours the growth of species such as *S. mutans* and Lactobacilli⁴¹. Caries may start when the balance between demineralisation and remineralisation on the enamel surface under the plaque has been destroyed⁴⁰⁻⁴¹. *S. mutans* and Lactobacilli have been regarded as the most important cariogenic bacteria because of their acidogenicity and aciduricity in the development of caries^{19,41-42}. *S. mutans* is acidogenic and has a capacity to adhere to tooth surfaces, so it had been closely associated with the development of caries^{17-18,43}. Lactobacilli are also a very important group of bacteria related to the development of caries^{15,40}. Years of research have shown that the reduction in the numbers of these bacteria has been related to the control and prevention of caries. The number of these bacteria in plaque and saliva was even regarded as an index to predict the patient's susceptibility to caries^{19,40,44}. So, the authors decided to investigate the changes in the



numbers of *S. mutans* and Lactobacilli in the present study to evaluate the possible changes of the caries susceptibility of the patients. In this study, the number of *S. mutans* in the plaque decreased significantly 1 month after bleaching. It may have been the effect of the residual hydrogen peroxide¹¹. The same result could be found in saliva, which may be due to the fact that most of the *S. mutans* in saliva comes from plaque⁴⁵⁻⁴⁶. On the other hand, the subjects might be more conscientious about their oral hygiene during the study period. The numbers of Lactobacilli showed no change, both in the plaque and saliva. Some species of Lactobacilli may be more resistant to hydrogen peroxide than other bacteria^{39,47-49}. Kraigher made a possible explanation for less susceptibility to caries after enamel bleaching. The bleaching agent is bactericidal to cariogenic bacteria, thus may reduce the cariogenic activity of plaque. The mineral outflow from subsurface layers of enamel caused by peroxide may help to create a diffusion barrier against caries attack, since the saliva pH is alkaline in 15 minutes after application of bleaching gel. More importantly, the caries initiation is a biofilm-driven result of de- and remineralisation processes, different from the mineral loss induced by bleaching¹⁴. This *in vivo* study demonstrated that tooth bleaching could reduce caries susceptibility for a short period of time, but the long-term effect needs further investigation.

Conclusion

The plaque accumulation decreased significantly 3 days after bleaching with 36% hydrogen peroxide compared to pre-treatment. The total number of bacteria in the plaque 3 days after bleaching declined significantly compared to 4 weeks after bleaching. The numbers of *S. mutans* both in the plaque and saliva four weeks post-bleaching were significantly less than the numbers of pre-treatment.

In conclusion, the antibacterial effect of bleaching gel is short, and the long-term effect needs further investigation.

Clinical significance

Bleaching agent with 36% hydrogen peroxide can reduce plaque accumulation and *S. mutans* counts in the plaque on bleached teeth surfaces and in the saliva for a period of time, when used for teeth whitening.

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