

Study of The Adherence of *St. mutans* on Bleached and Fluoridated Tooth Surfaces. (An In Vitro Study)

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الخلاصة

الأهداف: لتقييم التصاق جرثومة المكورات السحبية الطافرة على سطح المينا بعد عملية التبييض ب(٧,٥٪ بيروكسيد الهيدروجين، ١٦٪ بيروكسيد الكارباميد) مع او بدون الفلورايد الموضعي. **المواد وطرائق العمل:** ستون عينة تم الحصول عليها من ثلاثين ضاحك علوي تم تنظيفها و صقلها و فحصت للتأكد من عدم وجود اضرار سطحية. ثم قطعت جذور الاسنان و قسمت التيجان الى قسمين ووزعت العينات عشوائيا الى ست مجاميع، عرضت الى واحد من انظمة تبييض الاسنان (٧,٥٪ بيروكسيد الهيدروجين، ١٦٪ بيروكسيد الكارباميد) مع او بدون استخدام الفلورايد الموضعي. المجموعة الضابطة السالبة تم حفظ عيناتها في الماء منزوع الايونات، بينما المجموعة الضابطة الموجبة تم حفظها في اللعاب الاصطناعي. وان عدد التصاق جرثومة المكورات السحبية الطافرة على سطح المينا تم تحديدها بالاختبار البكتريولوجي، كما وتم تحليل البيانات إحصائيا باستخدام طريقة التباين الأحادي واختبار(توكي). **النتائج:** اظهرت النتائج زيادة ملحوظة في عدد جرثومة المكورات السحبية الطافرة المتصقة على سطح المينا بعد عملية التبييض ب(٧,٥٪ بيروكسيد الهيدروجين، ١٦٪ بيروكسيد الكارباميد)، والتي قلت بشكل ملحوظ احصائيا مع استخدام تقنية الفلورايد الموضعي بعد عملية تبييض الاسنان. **الاستنتاجات:** تبين أن للعلاج الموضعي بالفلورايد بعد عملية تبييض الأسنان تأثير نافع في تقليل الجرثومة المتصقة على سطح المينا والتي قد يعزى إليه إمكانية تقليل احتمالية تعرض الأسنان للتسوس .

ABSTRACT

Aims: To evaluate *Streptococcus mutans* adherence on bleached enamel surface with (7.5% hydrogen peroxide , 16% carbamide peroxide) with or without topical fluoride therapy. **Materials and Methods:** Sixty specimens obtained from thirty maxillary premolars extracted for orthodontic reasons, cleaned, polished, and examined for any surface structural damage. The roots were discarded and crowns sectioned in two pieces (mesiodistally) using diamond separating disc in low speed hand piece. The specimens randomly divided into six groups and exposed to one of the bleaching agents (7.5% hydrogen peroxide , 16% carbamide peroxide) with or without topical fluoride application. The negative control group specimens stored in deionized water. The positive control group specimens stored in artificial saliva. Adherence of *Streptococcus mutans* count to enamel surfaces was determined bacteriologically. The data analyzed statistically using one way ANOVA and Tukey test ($p \leq 0.05$). **Results:** there is significant increasing in *Streptococcus mutans* adherence on enamel surface after bleaching procedure, which was decreased significantly when used fluoride application technique after bleaching. **Conclusions:** Topical fluoride therapy has beneficial effect on reducing the *Streptococcus mutans* adherence on enamel surface after bleaching regimens which attributed in reducing the possibility of dental caries.

Key words: enamel surface, *Streptococcus mutans*, vital bleaching.

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INTRODUCTION

Adherence of bacteria to dental hard tissues and restoration is an important factor in dental caries⁽¹⁾ and periodontal diseases.⁽²⁾ Adherence of saliva and bacteria to tooth surfaces is an important step in

plaque formation.⁽³⁾ Bleaching agents are chemical agents which can influence bacterial adherence to restorations and tooth structures.⁽³⁾ Enamel roughness and adherence of *Streptococcus mutans* (as the most important cariogenic mechanism) to

enamel surfaces subsequent to bleaching has been confirmed.⁽⁴⁾

The adverse effects of bleaching agents are not entirely known, some studies have shown an absence of deleterious effects on bleached enamel surfaces^(5,6). Others revealed topographic changes, decalcifications, increase porosity, loss of surface hardness, and increase roughness.^(7,8)

However, few studies investigated the use of fluoride to minimize the increase in surface changes following bleaching, which would be a major concern with regard to bacterial colonization.^(4,9) Moreover, although a considerable number of bleaching agent brands exhibit fluoride in their compositions, some studies reported that these products cannot prevent mineral loss from bleached dental enamel.^(7,10)

The objective of the present in vitro study was to evaluate *Streptococcus mutans* adherence on enamel surfaces bleached with (7.5% hydrogen peroxide ,16% carbamide peroxide) bleaching regimens with or without topical fluoride therapy.

MATERIALS AND METHODS

Samples Preparation:

Thirty extracted maxillary premolars, removed for orthodontic reasons, were used in this study. These teeth cleaned with a slurry of pumice and water and polished then examined under magnification (20X) for any surface structural damage. The roots discarded, and crowns sectioned in two pieces, in the mesiodistal direction using diamond separating disc in low-speed cutting handpiece under water cooling (new diamond discs used after sectioning of ten crowns). All the cut surfaces of the specimens coated with two layers of nail varnish. Then, all the specimens immersed in distilled water for 24 h at 37°C. After that, specimens randomly divided into 6 groups with 10 specimens in each group as follows:

Group I: Specimens stored in deionized water (- ve control).

Group II: Specimens stored in artificial saliva (+ve control), the composition of artificial saliva was 0.058 ppm fluoride, 1.55 mmol calcium and 0.92 mmol phosphate, according to the formulation by

Featherstone *et al.*⁽¹¹⁾

Group III: Specimens treated with 7.5% hydrogen peroxide bleaching regimen (Home Peroxide II, D.M.C. Equipamentos LTDA, Brazil) on the enamel surface of each sample for 1 hour per day for two weeks (according to manufacturer instruction).

Group IV: Specimens treated with 16% carbamide peroxide bleaching regimen (Home Peroxide, D.M.C. Equipamentos LTDA, Brazil) on the enamel surface of each sample for 4 hour per day for two weeks (according to manufacturer instruction).

Group V: Specimens bleached as in group III, with weekly fluoride topical technique on enamel surface, consisted of 5 minute application of acidulated phosphate fluoride gel containing 1.23% fluoride ion (TopEx, Sultan health care, Inc, U.S.A.) then washed with distilled water , placed in fresh artificial saliva, this procedure performed once a week for 2 weeks of bleaching.

Group VI: Specimens bleached as in group IV, with weekly fluoride topical technique as in group V.

Each day when specimens removed from the bleach, washed in running distilled water with soft brush for 30 sec. and placed in fresh artificial saliva until the next daily treatment.

After bleaching and fluoridation procedures completed, all specimens sterilized by autoclave at 121°C for one hour.

Bacteriological method:

In this experimental study , tested bacteria was *Streptococcus mutans* (*S.mutans*) isolated from clinical dental caries. Isolated *S.mutans* maintained on Elliker agar (culture media for *S. mutans*. Compositions: ascorbic acid 0.5 g/L, casein enzymic hydrolysate 20 g/L, dextrose 5 g/L, gelatin 2.5 g/L, lactose 5 g/L, saccharose 5 g/L, sodium acetate 1.5 g/L, sodium chloride 4 g/L, and yeast extract 5 g/L. pH 6.2±0.2 (25°C)), which prepared according to Harrigan *et al* ⁽¹²⁾ for 24 h, and then culture one colony on sterile screw capped vial containing 5 ml of brain heart infusion broth (BHI) and incubated at 37°C for 18 h. After thorough mixing, 0.1 ml of the bacterial suspension spread over Elliker agar plate with sterile cotton swab and incubat-

ed at 37°C for 18 h. Then, enamel specimens separately placed on these culture plates with sterile tweezers so that, the treated enamel surfaces of the specimens came into contact with the bacteria. After incubation of the specimens at 37°C for 24 hrs., each slab inserted separately in a sterile screw capped vial containing 5 ml of phosphate buffer saline (pH 7.2) and stirred at vortex shaker (Wiggen Hauser, VM, Malaysia) at maximum speed (2800 rpm) for one minute to free the bacteria attached on the surface of each enamel specimen. After that, 0.1 ml inoculum was taken from inoculated phosphate buffer saline and inoculated into Elliker agar plates, streaked and incubated at 37°C for 24 h. Then, the number of bacterial colonies per specimen calculated using the formula: final number of adhered bacteria to each specimen in each group (cfu/ml)= number of colonies×10⁵ (13).

RESULTS

One way analysis of variance (ANOVA) and Tukey post Hoc multiple range tests ($P \leq 0.05$) performed to evaluate the differences on count of *S. mutans* adher-

ence among tested groups. This is shown in (Tables 1, 2) and (Figure 1, 2).

One ANOVA analysis demonstrated statistically significant differences in mean colony count surface adherence among the tested groups.

Tukey test showed that the bleached groups with 16% carbamide peroxide and 7.5% hydrogen peroxide produce a significant increase in surface adherence of *S. mutans* from (+ve and -ve) control groups. However, there was a significant difference between the two bleached groups, but there was no significant difference between (+ve and -ve) control groups on to bacterial colony count surface adherence.

Results also found that fluoride therapy of enamel surfaces bleached with 16% carbamide peroxide and 7.5% hydrogen peroxide caused a decrease in the bacterial count adherence, which were significantly different from bleached groups without fluoride therapy but not different from (-ve and +ve) control groups. There is no significant difference between fluoridated groups .

Table (1): One way analysis of variance for the differences on the colony count surface adherence of *S. mutans* among different tested groups.

	Sum of Squares	df	Mean Squares	F	P-value *
Between Groups	39456.28	5	7391.25	58.86	0.000
Within Groups	7238.64	54	134.04		
Total	46694.93	59			

* $P \leq 0.05$ mean significant different exist.

Table (2): Tukey test for the differences on the colony count surface adherence of *S. mutans* among different tested groups.

Tested groups	<i>S. mutans</i> Colony Counts Adherence cfu/ml (×10⁵) Mean±SD
-ve control	44.70±6.44 A*
+ve control	49.20±5.22 AB
7.5% hydrogen peroxide	179.20±7.52 C
16% carbamide peroxide	220.44±10.6 D
Fl. of 7.5% hydrogen peroxide	52.45±12.19 AB
Fl. of 16% carbamide peroxide	58.62±5.5 B

Fl.: fluoride application with bleaching; *Different letters indicate significant differences.

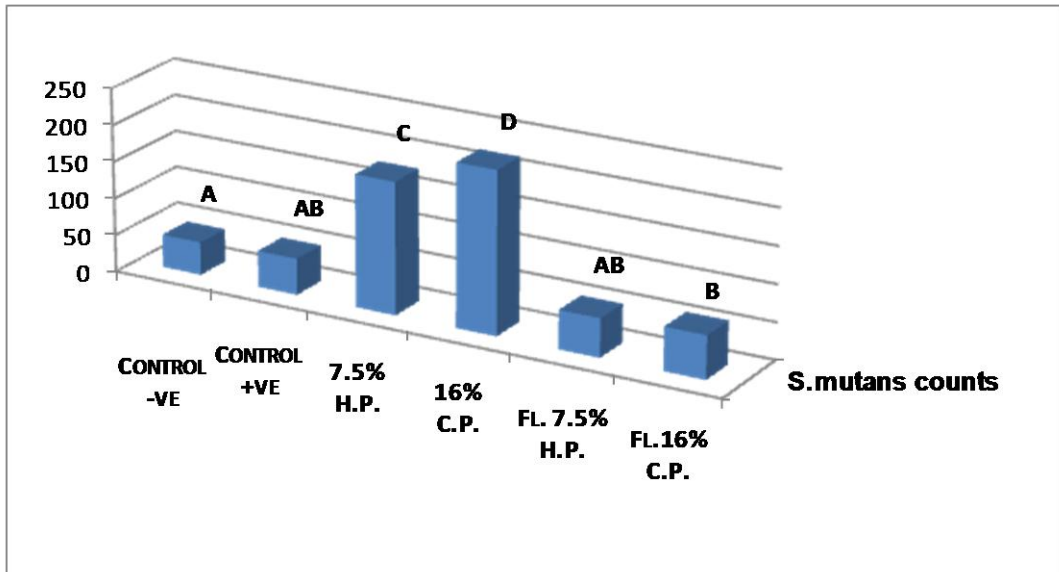


Figure (1): *S. mutans* adherence count on enamel surface among different groups. H.P.: hydrogen peroxide, C.P.: carbamide peroxide. Fl.: fluoride application with bleaching.

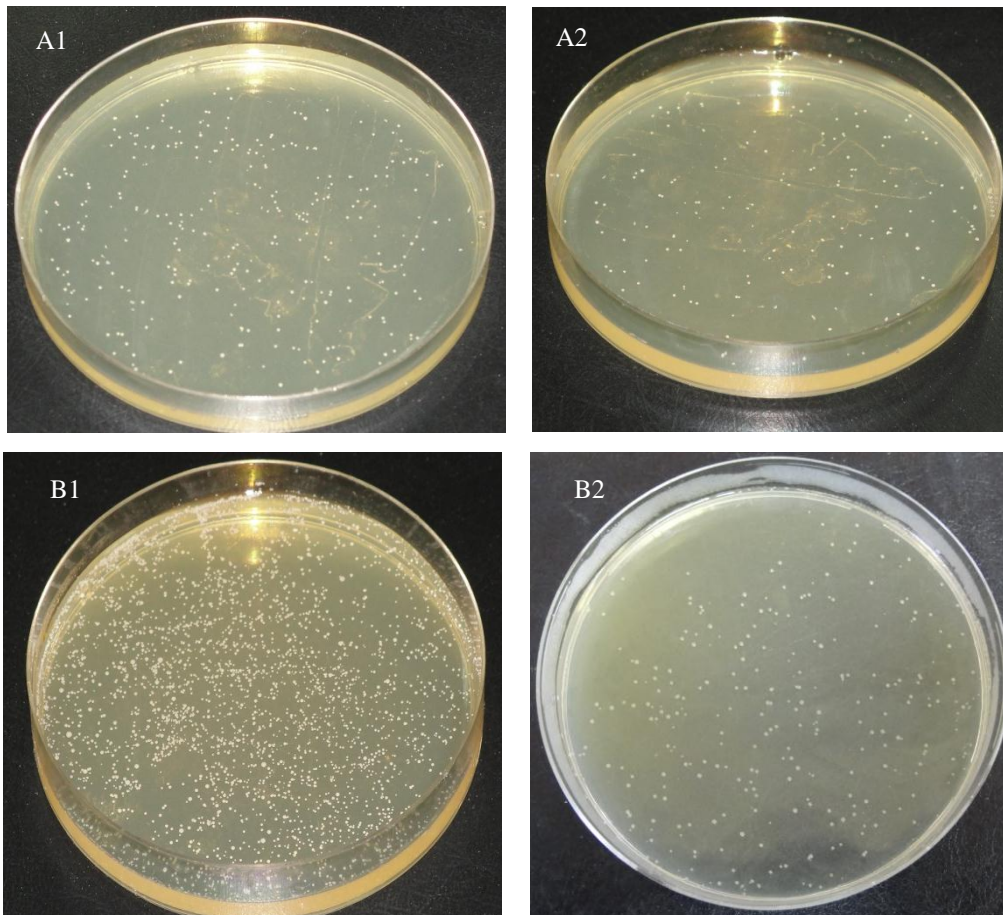


Figure (2): The colony count surface adherence of *S. mutans* among different tested groups. A1:(+ve control), A2:(-ve control), B1: bleached with 7.5% hydrogen peroxide, B2: B1 with fluoride application.

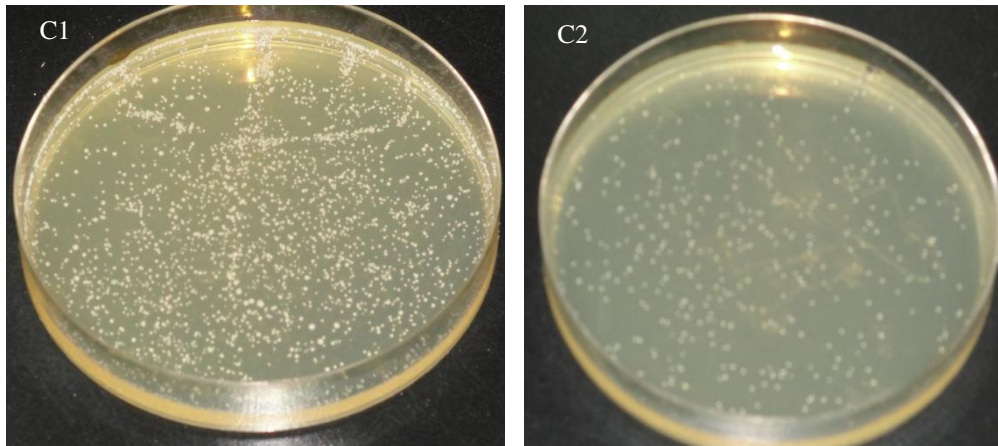


Figure (3): The colony count surface adherence of *S. mutans* among different tested groups. C1: bleached with 16% carbamide peroxide, C2: C1 with fluoride application.

DISCUSSION

The results of the present study demonstrated that bacterial count adherence to enamel surfaces significantly increases with the use of bleaching agents, which is consistent with the results of studies carried out by Hosoya *et al*⁽⁴⁾, Gurgan *et al*⁽⁹⁾ and Oskoe *et al*⁽¹³⁾ which concluded the changing characteristics of enamel surfaces with bleaching and covered with *S. mutans*, it may be much easier for such surface to capture the additional *S. mutans*.

Various mechanisms influence bacterial adherence to dental tissues, including type of bacteria and surface texture of the target.⁽³⁾ Specific adherence of oral bacteria is mediated by membrane binding sites.⁽¹⁴⁾ The bleaching agents are reactive chemicals which can result in alterations on the surface of dental hard tissues through their oxidizing properties, and alters Ca/P ratio up to 10 nm from the enamel surfaces.^(3, 15) Bleaching agents penetrate the tooth structure, denature the proteins, increase the permeability of the tissues, and allow the passage of hydrogen ions to the tooth, causing alteration in the dental hard tissues.⁽¹⁶⁾

The 16% carbamide peroxide investigated the highest count of bacterial adherence, this result is in agreement with Hosoya *et al*⁽⁴⁾ who showed that the acidic properties of bleaching agent, its concentration, as well as the exposure time, frequency, and low PH of the product have been indicated as potential factors in surface changes and bacterial adherence on enamel structure.

Because of the extensive knowledge of regarding the effect of fluoride on the remineralization process of dental tissues, the use of fluoride topical applications concomitant with bleaching procedures could be helpful to saliva in remineralizing damaged dental tissues after bleaching⁽¹⁷⁾, as observed in the count of *S. mutans* adherence which showed no significant differences between the two bleaching groups after fluoridation and reflect the reducing in bacterial adherence to be not significant differences with (+ve control) group. This result is in agreement with the result of Martin *et al*⁽¹⁸⁾ who indicated that the specimens received topical fluoride therapies obtained images closest to those in the positive control group.

The non significance in count of bacterial adherence between two control groups may be attributed to the enamel surface of specimens were normal, standard, and not exposed to any surface treatment.

Therefore, we can be concluded that alteration in enamel surface topography have a direct influence on bacterial adherence to bleached enamel surfaces, so an increase in bacterial adherence to hard tissue in the oral cavity have an important role in the formation of bacterial plaque and dental caries, in regardless the role of salivary flow in the oral cavity which is rich in proteins, carbohydrate, lipids and some other components which might influence bacterial adherence in a manner may be different from that in vitro study.⁽¹³⁾

CONCLUSIONS

Both bleaching regimens (7.5% hydrogen peroxide, 16% carbamide peroxide) increased *S. mutans* surface adherence to dental enamel. The 16% carbamide peroxide showed the highest count of bacterial adherence. Weekly topical fluoride technique concomitant with bleaching procedures decreasing the bacterial adherence .

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