INTRODUCTION

There’s nothing that radiates health, happiness and even success like a sparkling white smile. So it’s no wonder many people choose whitening to improve the appearance of their teeth. In fact, whitening is one of the least expensive cosmetic remedies available to enhance a faded smile. It can be done at home or at your dentist’s office, using a variety of products and techniques. The application technique of home vital tooth bleaching systems depends on a mouth guard to keep the bleaching agent in contact with tooth surfaces to be bleached. Home vital bleaching commonly utilize carbamide peroxide to deliver a more stable form of hydrogen peroxide, the active bleaching agent. Intraorally, carbamide peroxide dissociates into hydrogen peroxide and urea. Hydrogen peroxide is

ANTIBACTERIAL EFFECT OF TWO BLEACHING AGENTS: IN VIVO STUDY

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ABSTRACT

Objective: To investigate the antibacterial effect of two commercially available bleaching agents in vivo.

Materials and Methods: 30 volunteers were divided into 3 groups, a control group (chlorhexidine) and 2 treatment groups (Nite White ACP and Nite White ACP Turbo bleaching gels). Stimulated whole saliva samples were collected at baseline, after 1 and 3 applications and 14 days post-treatment. Total count of aerobic bacteria was determined.

Results: For all groups there was a statistically significant decrease in mean count of aerobic bacteria compared to baseline through all periods of the study. After 1 application and 3 applications, there was no statistically significant difference between mean percentage reductions in bacterial counts of the three groups. After 14 days, Nite White ACP Turbo showed the statistically significantly highest mean percentage reduction, followed by Nite White ACP group, then the control group.

Conclusion: Bleaching agents can reduce the total count of aerobic bacteria in saliva in vivo.
a non toxic, non allergic antibacterial agent eligible of killing a broad range of microorganisms. Urea is a non toxic bacteriostatic agent capable of dissolving necrotic tissue, healing wounds more quickly. Chlorhexidine is one of the most topical antibacterial agents used in dentistry. It has the ability to reduce plaque and affect cariogenic bacteria. It is a wide spectrum antimicrobial agent, acting against both aerobic and anaerobic bacteria. Chlorhexidine has the property of substantivity which consists of maintaining its antibacterial action for a long lasting period when adhered to anionic substrates, being slowly released as its concentration decreased.

Applying the bleaching agents can have benefits other than whitening the teeth. Their antibacterial effect can help shifting the bacterial ecology to a more favorable one thus promoting oral health. Since it is widely used it is important to study its side effects as well as its benefits. Many studies have been conducted to determine the effect of bleaching on enamel and dentine mineral content. However, few research reports have been found that focus the effect of bleaching agents on oral microbiota. Hence, this study was carried to assess the antibacterial effect of two bleaching agents.

**MATERIALS AND METHODS**

**Materials**

Two bleaching materials, Nite White ACP, Discuss Dental (16% carbamide peroxide, potassium nitrate, fluoride, and amorphous calcium phosphate) and Nite White ACP Turbo, Discuss Dental (6% hydrogen peroxide, potassium nitrate, fluoride, and amorphous calcium phosphate). Chlorhexidine, Hexitol mouthwash, The Arab Drug Company (0.125% chlorhexidine HCl) was used as control. Blood agar (Heart muscle, Infusion from (solids), pancreatic digest of casein, yeast extract, sodium chloride, agar). Fluoride free dentifrice Sensodyne Original, GlaxoSmithKline (10% strontium chloride hexahydrate).

**Methods**

1. **Volunteers selection:**

   The protocol of this in vivo study was approved by the ethics committee of Cairo Dental Faculty and with the informed consent of patients. Thirty volunteers were enrolled in the study. The volunteers who were candidates for bleaching treatment were dental students, making a homogenous sample with regard to adequate oral hygiene and motivation to oral health. Each volunteer was informed about the goals, benefits, and possible risks associated with this experiment. At the screening visit all the volunteers were evaluated to decide if they agree the inclusion/exclusion criteria.

2. **Inclusion/ exclusion criteria:**

   **Inclusion Criteria:**
   
   - Caries-free and unrestored facial surfaces of the six anterior teeth and the first premolars.
   - Age range from 20-25 years.
   - The will to complete the study and have their teeth whitened.
   - Ability to return for periodic examination and saliva collection.
   - The will to refrain from chewing gum.
   - The ability to avoid snacks in between meals during the study period.

   **Exclusion criteria:**
   
   - Medical history that contraindicates dental treatments.
   - Evidence of dry mouth or salivary gland disorders.
   - Systemic disease known to affect oral health.
   - Taking antibiotics or any drug that affect salivary flow rate or oral microbiota three months prior to the start of the study.
• Pregnant or nursing women.
• Allergy to chlorhexidine and/or hydrogen peroxide.
• Significant periodontal disease.
• Sensitivity to air blast from air-water syringe (dentine sensitivity).
• Fixed or removable dentures or orthodontic appliances.
• Tetracycline stained teeth.
• Smokers.

3. General instructions:

Two weeks before the experiment all volunteers were refrained from using all oral hygiene products including fluoride containing tooth-paste, and throughout the whole study period. They were instructed to brush their teeth twice a day, in the morning and evening using fluoride free tooth paste (Sensodyne Original) to prevent the fluoride antibacterial effect that may overlie the effect of bleaching agents and the Bass dental hygiene technique to standardize tooth brushing method. One week prior to the initiation of the active treatment a thorough professional oral prophylaxis was done. This phase lasted for two weeks. The volunteers were instructed to avoid snacks in between meals and chewing gum during the study period.

4. Volunteers grouping:

They were divided to 3 groups of ten each. For the control group volunteers rinsed before bedtime with 15 ml Hexitol mouthwash for 30 seconds and expectorate. This was repeated for 3 successive nights. While for the other 2 test groups volunteers, they used Nite White ACP or Nite White ACP Turbo bleaching gels in custom made bleaching trays for both arches according to manufacturer instructions overnight for 3 successive nights.

5. Saliva collection:

Prior to starting the treatment (baseline), whole saliva samples were collected in sterile calibrated screw-capped containers from each of the volunteers after stimulation in the morning, in order to assess the total level of aerobic bacteria in saliva. The volunteers were asked to avoid any food or drink (except water) or take any medication in the morning prior to sampling. The volunteers chewed on a piece of unflavored paraffin wax until it attained soft consistency (about 1 minute). They swallowed the first portion of saliva, then they chewed for additional 5 minutes, using both sides of the mouth and intermittently spit saliva into the calibrated sterile screw-capped containers. This was done before bleaching application after the first and third bleaching applications and 14 days post treatment. Four saliva samples were collected from each volunteer, so 40 samples were collected from each group.

6. Microbiological study:

The total count of aerobic bacteria in the saliva was determined before, during and after treatment using blood agar plates. After incubation the colonies on the plates were counted using magnifying glass, the count of total aerobic bacteria was expressed as the number of colony forming units per milliliter (CFU/ml) of saliva. All samples were processed directly after collection.

7. Culturing procedures:

A pilot study was conducted to determine the suitable dilution to obtain countable plates using 10-fold serial dilutions method. The dilution 1/1000 and 1/10000 produced countable plates, so the two dilutions were used.

8. Inoculation procedures:

For each sample the 2 dilutions were cultured in duplicate and the mean CFU/ml saliva was calculated for each sample. From each dilution,
100µl were transferred by an automatic micropipette from the test tube onto the agar surface to be inoculated. The samples were dispersed on the agar surface using sterile bent glass rods, which were used to provide smooth surface without scratching the agar surface giving a homogenous growth. All the cultivation procedures were done away from air currents and in area no more than 10 cm away from a glowing torch.

9. Incubation procedures:
After inoculation all plates were incubated aerobically at 37°C for 24-48 hours.

10. Bacterial count:
The number of viable cells was counted by viable count technique using the following equation:

\[
\text{Number of colonies/ml (CFU/ml)} = \text{Number of colonies counted} \times \text{Inverse of dilution} \times \text{Inverse of the cultured volume (ml)}.
\]

For each sample we had four counts (2 for each dilution 1/1000 and 1/10000), a mean count was obtained for each dilution.

Statistical analysis:
Data were presented as mean and standard deviation (SD) values. ANOVA (Analysis of Variance) was used to compare between the three groups. Tukey’s post-hoc test was used for pair-wise comparison between the means when ANOVA test is significant. Paired t-test was used to study the changes by time in each group. The significance level was set at \(P \leq 0.05\). Statistical analysis was performed with SPSS 16.0 (SPSS, Inc., Chicago, IL, USA), Statistical Package for Scientific Studies for Windows.

RESULTS
The results of this study showed a statistically significant decrease in the mean count of aerobic bacteria for the three groups through all periods, compared to baseline at \(P \leq 0.05\). Table (1) and figure (1) represents the mean, standard deviation (SD) values, results of ANOVA and Tukey’s tests for comparison between percentage reductions in the count of aerobic bacteria of the three groups. However, the difference became statistically significant at 14 days after treatment, where Nite White ACP Turbo group showed the statistically significant highest mean percentage reduction. This was followed by Nite White ACP group, chlorhexidine group showed statistically significant lowest mean percentage reduction. The difference in percentage reduction in bacterial count was considered significant at \(P \leq 0.05\).

The percentage reduction was calculated as:

\[
\frac{\text{Count (before)} - \text{Count (after)}}{\text{Count (before)}} \times 100
\]

TABLE (1) The mean, standard deviation (SD) values, results of ANOVA and Tukey’s tests for comparison between percentage reductions in bacterial counts of the three groups:

<table>
<thead>
<tr>
<th>Period</th>
<th>Group</th>
<th>Control</th>
<th>Nite White</th>
<th>Nite white Turbo</th>
<th>P- Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean%</td>
<td>SD %</td>
<td>Mean%</td>
<td>SD%</td>
<td>Mean%</td>
</tr>
<tr>
<td>Baseline- 1 application</td>
<td>44.3</td>
<td>7.5</td>
<td>45.9</td>
<td>5.4</td>
<td>49.4</td>
</tr>
<tr>
<td>Baseline- 3 applications</td>
<td>66.1</td>
<td>8.6</td>
<td>65.9</td>
<td>4.6</td>
<td>70.4</td>
</tr>
<tr>
<td>Baseline- 14 days</td>
<td>13.6(^a)</td>
<td>5.2</td>
<td>21.1(^b)</td>
<td>7.9</td>
<td>32.3(^a)</td>
</tr>
</tbody>
</table>

* Significant at \(P \leq 0.05\), means with different letters are statistically significantly different according to Tukey’s test
After 1 application and 3 applications, there was no statistically significant difference between mean percentage reductions in bacterial counts of the three groups.

After 14 days, Nite White Turbo group showed the statistically significant highest mean percentage reduction. This was followed by Nite White group. Control group showed the statistically significant lowest mean percentage reduction.

DISCUSSION

The results of this in vivo study showed that bleaching gels used in custom-fitted tray and the chlorhexidine mouth rinse reduced the total count of aerobic bacteria in saliva, after 1, 3 and 14 days post-treatment.

These results were in agreement with Benley et al., who found that 10% carbamide peroxides solutions reduced the levels of Lactobacilli in vivo, but did not affect the levels of Mutans Streptococci. In addition, Amaechi et al. found that carbamide peroxide is capable of killing bacteria harboring non-cavitated caries lesions. It was also in agreement with Kraigher et al. and Lazarchik et al. who claimed that 10% carbamide peroxide bleaching agents had an antimicrobial effect on cariogenic bacteria as a result of the direct chemical effect of hydrogen peroxide.

In opposition, Alkamin et al., found that different bleaching agents did not change the oral cavity Mutans Streptococci counts. Also, Haak et al. found that 9% hydrogen peroxide gel application does not reduce the quantity of Mutans Streptococci and Lactobacilli in saliva. In the two studies the volunteers used bleaching agents for a short period at each application; they wear the bleaching trays for 1 hour in the first study and for 30 minutes in the second study. In this study each application lasted for at least 6 hours, as the volunteers were instructed to wear the bleaching trays over night.

For the control group the reduction of the bacterial count was due to the antimicrobial activity of chlorhexidine. The antimicrobial effect is mediated by several mechanisms. It binds electrostatically to negatively charged sites on bacteria (phosphate groups on bacterial cell wall). By attaching to the bacterial cytoplasmic membrane, chlorhexidine causes loss of the osmotic balance, resulting leakage of intracellular material. It also binds to hydroxyapatite and soft tissues, changing their electric field to compete with bacterial binding. The unique advantage of chlorhexidine is its capability to bind to salivary pellicle thus prolonging its retention in the oral cavity.

For the test groups the reduction in the total count of aerobic bacteria may be due the antibacterial effect of hydrogen peroxide which might have been released into saliva during bleaching. Previous studies supported the release of hydrogen peroxide in saliva during the application of nightguard vital bleaching.

CONCLUSION

Nightguard vital bleaching may have the ability to reduce the total count of aerobic bacteria during and up to 14 days after their application. Bleaching gels may have an antibacterial effect in addition to their whitening effect.
REFERENCES


