In vitro evaluation of the efficacy of two bleaching procedures

Gonzalo Llambés 1, Carmen Llena 2,3, José Amengual 1, Leopoldo Forner 2

1 Associate Professor. Department of Stomatology. University of Valencia (Spain)
2 Professor. Department of Stomatology. University of Valencia (Spain)
3 Dentist. Valencian Health Service. Valencia (Spain)

Abstract
Objective: In vitro comparison of the efficacy of two bleaching procedures, one based on carbamide peroxide (CP) and the other on hydrogen peroxide (HP), simulating clinical conditions.
Study Design: Two groups of 20 teeth in each group were selected. Group A: 22% CP, one hour a day for 21 consecutive days. Group B: 37.5% HP, in 2 treatment sessions with an one week interval between each session. At each session the product was applied three successive times for eight minutes. Colour was recorded before treatment, when it was finished and one week after finishing it, with the Vita EasyShade spectrophotometer. CIEL*a*b* and ∆E were established at each moment in the study. Intra-group data was compared using the paired t-test and inter-group data with the independent groups t-test. Scores from the Vita Classical guide provided with the device were recorded and the colour improvement percentage was calculated.
Results: In both groups significant whitening was achieved by the end of treatment. Lightness remained significantly high when treatment was finished and one week after in both groups. The percentage of bleaching was significantly higher in group A.
Conclusions: Both 22% CP and 37.5% HP were effective for bleaching teeth. Bleaching effect was greater in CP group.

Key words: Dental bleaching, hydrogen peroxide, carbamide peroxide, dental whitening, dental colour.
patient’s mouth for 30 min to 2 hours while the patient is in the dental office (1).

In-office bleaching procedures seem to be an appropriate alternative to home bleaching applications with trays, strips or gels, specially in the case of very severe discolorations, discolorations of single teeth, lack of patient compliance or if a rapid treatment is desired. In-office bleaching could also be applied as a kind of boost therapy, thereby initiating the bleaching process, which might be continued afterwards by home-bleaching procedures (2). Most descriptions in the literature of successful applications of in-office bleaching therapies are case reports or studies in which no comparisons to well approved methods, such as home-bleaching procedures, have been done (3). Auschill et al. (4) were able to show that both home-bleaching procedures (10% CP gel tray application or 5.3% HP impregnated strips) and in-office-bleaching with 35% HP without heat activation may be used to brighten teeth six shades on the vita color scale. In the in-office procedure this change was achieved after only 3.1±0.5 applications of 15 min each, whereas 31.8±6.6 applications of 30 min each were needed with the strip. 7.1±1.9 applications for 8 h each were necessary when using the carbamide peroxide gel.

Professionally supervised at-home tooth bleaching has become a popular method used to treat tooth discoloration. The popularity of this method is related to its quick esthetic improvement, low incidence of side effects and ease of technique with reduced chair time (5). Until recently, the most common and widely accepted at-home tooth whitening method has been the one first proposed by Haywood and Heymann, in which a custom tray with CP is used by the patient for a select number of hours (6). Manufacturers have introduced different concentrations of CP (5% to 22%) (7,8) or HP (3% to 14%) for at-home whitening (9,10). In 2006, the American Dental Association (ADA) (11) published a new program guidelines for the acceptance of dentist dispensed home bleaching products that assure the safety and efficacy of tray applied 10 ± 1% CP based on published clinical trials. Few controlled clinical trials (12,13) have observed the improved efficacy of at-home whitening when increasing concentration of the bleaching agent. Additionally, an increase in side effects has been detected (12-14).

The available literature is not conclusive over whether higher product concentrations provide better short and medium term results, although there is consensus among most authors that higher concentrations provide favorable results with a shorter application time (4). Furthermore, the use of products with high concentrations of HP increases risks, such as burns to the oral mucosa or hypersensitivity and requires the use of rigorous oral tissues protection procedures such as rubber dam isolation or alternatives such as specially designed light-curing isolating pastes (15).

Based on the above, our study, carried out in vitro, aims to evaluate the bleaching efficacy of two procedures, one based on the use of 22% CP, and the other based on 37.5% HP.

Materials and Methods

For this study, 40 teeth were selected (premolars and molars) all caries free, without structural enamel defects or previous restorative treatments. The teeth had been extracted for periodontal or orthodontic reasons and their initial colour was greater than or equal than B2 (Vita Classical guide - VITA Zahnfabrik, Bad Säckingen, Germany). They were randomly distributed into two groups (A and B) of 20 teeth each. From extraction until use and during the treatment process the teeth were kept in physiological saline solution.

Group A was treated with 22% carbamide peroxide (Norblanc Home, Normon, Madrid, Spain), a household product, applied in trays for dentist supervised use. In our study it was applied one hour a day for 21 days (in total, 21 hours of treatment). Group B was treated with 37.5% hydrogen peroxide (Pola Office +, SDI, Bayswater, Victoria, Australia), a chemically activated product for in-office application. Two treatment sessions were carried out with an interval of one week between them. At each session the teeth were given 3 applications lasting eight minutes (a total of 48 minutes of treatment). These are two commons concentrations for at-home and in-office respectively dental bleaching techniques (high concentrations for each kind). A 22% CP is equivalent to a 7.3% HP, as CP [CO(NH2)2.H2O] breaks down into approximately 3% HP (H2O2) and 7% urea (16). Clinical procedures used are those generally applied for dental bleaching with the mentioned peroxide concentrations (17). To apply the bleaching agents, the teeth were mounted in groups of five teeth, CP was applied with a custom tray like those used in home bleaching treatments.

In order to make the trays, the 5 teeth were stabilized in a silicone block. An alginate impression was taken and a plaster model was obtained. The labial model teeth surfaces were covered with 1mm thick spacer up to 1 mm of the cervical margin. A vacuum forming machine was used to make a 0.8 mm thick custom tray (17).

HP was placed directly on all teeth surfaces, in a 2 mm thick layer in order to get enough material to produce active bleaching agent (17).

Tooth colour was recorded using a spectrophotometer (VITA EasyShade -VITA Zahnfabrik, Bad Säckingen, Germany-). In order to standardize the position of colour recording at all times, a custom positioning tray was made for each group of teeth (Fig. 1). The tip of the spectrophotometer was positioned perpendicular...
Dental bleaching to the tooth surface in the space created by the positioning tray. Colour was recorded before beginning of treatment again when treatment finished and lastly, one week after treatment had finished. The value showed by the spectrophotometer was determined in reference to the Vita Classical guide and CIELAB L*, a*, b* scores, both provided by the device. This last system was defined by the International Commission on Illumination in 1967 (18); L* represents the value (lightness or darkness), a* is a measure of redness (positive a*) or greenness (negative a*); b* is a measure of yellowness (positive b*) or blueness (negative b*). Difference between colour coordinates is calculated as ΔE* = [(ΔL*)^2 + (Δa*)^2 + (Δb*)^2]^{1/2}. Tooth whitening mainly occurs as a reduction in yellowness (lower b*) and an increase in lightness (higher L*) (19).

The Vita Classical guide was organized in relation to value -lightness- (Table 1). The number of shade tab changes for each tooth was determined from the pretreatment situation to the immediate posttreatment situation. Colour was assessed again one week after finishing the treatment. In addition, the bleaching percentage was calculated, taking into account the difference between the initial colour and the maximum bleaching a tooth can achieve, considering that a tooth achieves 100% improvement in colour when it reaches colour B1, so for teeth which did not reach B1, the bleaching percentage was determined by calculating a proportion according to the number of shade tab changes.

L*, a*, b* and ΔE parameters were also compared between the pretreatment situation, end of treatment and one week later.

The data records were checked for normal distribution using the Kolmogorov Smirnov test. Intra-group variables were compared with the paired t-test and inter-group variables using the independent groups t-test. Confidence level for all the analyses was 95% (α=0.05).

**Results**

At the end of treatment (immediately after the last bleaching agent application), 15% of the specimens in group A showed no bleaching, whereas after one week after the end of the treatment, 100% of the teeth had experienced some degree of change. In group B, 15% of the specimens showed no improvement in colour at the end of the treatment. 75% of the sample showed a bleaching percentage equal to 21% both at the end of treatment and one week later (Fig. 2).

Table 2 shows the mean values for each of the parameters in each group. It can be seen that in group A, L* lightness increased at the end of treatment and remained high one week after treatment, in both cases with
Table 2. Comparison of intra-group measurements for \( L^* \), \( a^* \), \( b^* \), \( \Delta E \), at the start, end and one week after treatment. Comparison of the shade tab changes and bleaching percentage between the end of treatment and one week later.

<table>
<thead>
<tr>
<th></th>
<th>Group A (CP)</th>
<th></th>
<th>Group B (HP)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>p</td>
<td>Mean</td>
</tr>
<tr>
<td>( L^* ) Initial</td>
<td>75.66</td>
<td>9.41</td>
<td>&lt;0.001</td>
<td>75.68</td>
</tr>
<tr>
<td>( L^* ) Final</td>
<td>94.62</td>
<td>6.12</td>
<td>&lt;0.001</td>
<td>87</td>
</tr>
<tr>
<td>( a^* ) Initial</td>
<td>2.81</td>
<td>3.65</td>
<td>&lt;0.001</td>
<td>3.44</td>
</tr>
<tr>
<td>( a^* ) Final</td>
<td>0.07</td>
<td>2.53</td>
<td>&lt;0.001</td>
<td>3.38</td>
</tr>
<tr>
<td>( b^* ) Initial</td>
<td>36.06</td>
<td>6.96</td>
<td>0.531</td>
<td>40.21</td>
</tr>
<tr>
<td>( b^* ) Final</td>
<td>35.27</td>
<td>6.69</td>
<td>&lt;0.001</td>
<td>44.01</td>
</tr>
<tr>
<td>( L^* ) Initial</td>
<td>75.66</td>
<td>9.41</td>
<td>&lt;0.001</td>
<td>75.68</td>
</tr>
<tr>
<td>( L^* ) Week</td>
<td>89.16</td>
<td>6.53</td>
<td>&lt;0.001</td>
<td>86.72</td>
</tr>
<tr>
<td>( a^* ) Initial</td>
<td>2.81</td>
<td>3.65</td>
<td>&lt;0.001</td>
<td>3.44</td>
</tr>
<tr>
<td>( a^* ) Week</td>
<td>-1.31</td>
<td>1.65</td>
<td>&lt;0.001</td>
<td>5.59</td>
</tr>
<tr>
<td>( b^* ) Initial</td>
<td>36.06</td>
<td>6.96</td>
<td>&lt;0.001</td>
<td>40.21</td>
</tr>
<tr>
<td>( b^* ) Week</td>
<td>27.23</td>
<td>6.80</td>
<td>&lt;0.001</td>
<td>47.84</td>
</tr>
<tr>
<td>( L^* ) Final</td>
<td>94.62</td>
<td>6.12</td>
<td>&lt;0.001</td>
<td>87</td>
</tr>
<tr>
<td>( L^* ) Week</td>
<td>89.16</td>
<td>6.53</td>
<td>&lt;0.001</td>
<td>86.72</td>
</tr>
<tr>
<td>( a^* ) Final</td>
<td>0.07</td>
<td>2.53</td>
<td>&lt;0.001</td>
<td>3.38</td>
</tr>
<tr>
<td>( a^* ) Week</td>
<td>-1.31</td>
<td>1.65</td>
<td>&lt;0.001</td>
<td>5.59</td>
</tr>
<tr>
<td>( b^* ) Final</td>
<td>35.27</td>
<td>6.69</td>
<td>&lt;0.001</td>
<td>44.01</td>
</tr>
<tr>
<td>( b^* ) Week</td>
<td>27.23</td>
<td>6.80</td>
<td>&lt;0.001</td>
<td>47.84</td>
</tr>
<tr>
<td>( \Delta E ) Final</td>
<td>19.96</td>
<td>6.11</td>
<td>&lt;0.001</td>
<td>12.40</td>
</tr>
<tr>
<td>( \Delta E ) Week</td>
<td>9.85</td>
<td>3.95</td>
<td>&lt;0.001</td>
<td>5.20</td>
</tr>
<tr>
<td>Shade tab changes Final</td>
<td>5.40</td>
<td>3.34</td>
<td>0.061</td>
<td>2.70</td>
</tr>
<tr>
<td>Shade tab changes Week</td>
<td>7.10</td>
<td>4.50</td>
<td></td>
<td>2.35</td>
</tr>
<tr>
<td>% of bleaching Final</td>
<td>44.65</td>
<td>28.99</td>
<td>0.016</td>
<td>20.40</td>
</tr>
<tr>
<td>% of bleaching Week</td>
<td>65.10</td>
<td>32.99</td>
<td></td>
<td>18.60</td>
</tr>
</tbody>
</table>


Fig. 2. Bleaching percentage obtained with CP and HP at the end of treatment and one week later for sample percentiles 25, 50 and 75. HP: hydrogen peroxide, CP: carbamide peroxide.
significant differences in relation to the initial situation. However, comparing the situation at the end of treatment with the situation one week after, lightness decreased significantly, a* (variation between red and green), displaced significantly towards green with time, b* (variation between yellow and blue), displaced towards blue and ∆E* dropped by two units from the end of treatment until one week later. Shade tab changes increased from the end of treatment until one week after treatment from 5.4 to 7.1. Bleaching percentage increased significantly (p=0.016) from end of treatment to the measurement taken one week later.

For group B, L* increases significantly at the end of treatment and remained significantly higher one week afterwards in comparison to the initial point, whereas between the end moment and one week after treatment there are no significant differences. a* tends to displaced towards red significantly between the end of treatment and one week later whereas b* displaces significantly towards yellow. ∆E* is higher after one week but there are no significant differences in relation to the data obtained immediately after the treatment. The same occurs with bleaching percentages.

Table 3 is a comparison of both study groups. At the start, both groups showed no significant differences in L*, a*, b*. It was observed that ∆L*, ∆a*, ∆b*, are significantly different in both groups at the end of treatment and one week later. Group A (CP) was found to achieve greater lightning at the end of treatment and one week later; group A achieved 3 scores more ∆E than group B in the comparison between the end of treatment and one week later. Shade tab changes and bleaching percentage one week after finalizing the treatment was significantly higher for the group treated with CP than for the group treated with HP.

**Discussion**

Two chemically activated bleaching agents were chosen (one based on HP and the other on CP) in order to eliminate the influence of other factors such as light or other
activation methods. In both cases, it was attempted to use a procedure similar to that used in-office with these products. The HP used in the study is presented in a double syringe with a self-mixing vial, which improves stability of the active agent and favors appropriate handling with a homogenous mix of the components. The concentrations chosen were: 22% for CP and 37.5% for HP; both are the highest percentages within the range of concentrations used in clinical procedures in-office and at-home. Recently published studies report that concentrations between 10 and 30% showed no significant differences in terms of short-term bleaching capacity, although higher concentrations are recommended for short treatment periods (20), as in our study.

Colour change was evaluated firstly using CIELAB L*a*b* parameters to provide an objective measurement of colour change (21) and secondly, the equivalences given by the spectrophotometer for Vita Classical guide values, more understandable from a clinical perspective. The definitive record was taken one week after treatment had ended in order to evaluate the stabilized colour (because colour can show changes during the first week after having finish the treatment) (22). ΔE assessment made it possible to establish colour differences before and after treatment. Under experimental conditions, the human eye can perceive ΔE of 1 or higher (23) but under clinical conditions differences must be 3.7 or above to be detectable (24). However, equal values of ΔE can correspond to different degrees of colour perception, so ΔE is not a valid indicator for comparing different teeth. ΔE does make it possible, however, to follow the evolution of colour in each tooth in a convenient, simple way, thus making it a suitable indicator for this purpose (16). In our study, based on teeth which showed no significant differences in ΔE at the start of the study, ΔE higher than values acceptable under clinical conditions in both groups were achieved, both at the end of treatment and one week later. We observed, however, that comparing the situation at the end of treatment and a week later, in both groups, there was a significant 10 and 7.20 point decrease (groups A and B, respectively).

In a study by Mokhlis et al., which evaluated CP at a similar concentration to that used in this study two weeks after treatment with custom trays and 20% CP (Opalescence 20 PF, Ultradent Products Inc.), ΔE was 9.22 and 7.67 at the end of the treatment and two weeks after treatment had finished, respectively (25). In our study, with a similar concentration in CP and a treatment time of 21 hours, ΔE values at the end of treatment and one week later were 19.96 and 9.85 respectively. It must be taken into account that our study was carried out in vitro, which could modify the capacity of free radicals to penetrate the dental structures and therefore these results may be influenced by the different conditions of the treated teeth (26).

**Conclusion**

Under our study conditions, both 22% CP and 37.5% HP were effective for bleaching teeth. Greater bleaching effect was achieved both at the end of treatment and one week later with CP. 3 unit differences in ΔE were found between the values obtained at the end of treatment and one week later, within the range of imperceptible to the human eye under clinical conditions.

**References**