



**University of  
Zurich**<sup>UZH</sup>

**Zurich Open Repository and  
Archive**

University of Zurich  
University Library  
Strickhofstrasse 39  
CH-8057 Zurich  
[www.zora.uzh.ch](http://www.zora.uzh.ch)

---

Year: 2008

---

## **12-Month color stability of enamel, dentine, and enamel-dentine samples after bleaching**

Wiegand, Annette ; Drebenstedt, S ; Roos, Malgorzata ; Magalhães, A C ; Attin, Thomas

DOI: <https://doi.org/10.1007/s00784-008-0195-7>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-6393>

Journal Article

Accepted Version

Originally published at:

Wiegand, Annette; Drebenstedt, S; Roos, Malgorzata; Magalhães, A C; Attin, Thomas (2008). 12-Month color stability of enamel, dentine, and enamel-dentine samples after bleaching. *Clinical Oral Investigations*, 12(4):303-310.

DOI: <https://doi.org/10.1007/s00784-008-0195-7>

## **12-months colour stability of enamel, dentine and enamel-dentine samples after bleaching**

### **Abstract**

The study aimed to quantify the colour regression of enamel (E), dentine (D) and combined enamel-dentine (ED) of differently bleached enamel-dentine specimens over a period of 12 months in vitro. Two enamel-dentine samples were obtained from the labial surfaces of bovine teeth and prepared to a standardized thickness with the enamel and dentine layer each 1 mm. The ED samples were distributed on 4 groups (each n = 80), in which the different bleaching products were applied on enamel (1: Whitestrips, 2: Illuminé 15%, 3: Opalescence Xtra Boost) or dentine surfaces (4: mixture of sodium perborate/distilled water). Eighty ED samples were not bleached (control). Colour ( $L^*a^*b^*$ ) of ED was assessed at baseline, subsequently after bleaching and at 3, 6 and 12 months of storage after bleaching (each 20 samples/group). E and D samples were prepared by removing the dentine or enamel layer of ED samples to allow for separate colour analysis. Bleaching resulted in a significant colour change ( $\Delta E$ ) of ED specimens. Within the observation period,  $\Delta L$  but not  $\Delta b$  declined to baseline.  $L^*$ -values of E and D samples also declined and were not significantly different from control samples after 12 months, while  $b^*$ -values did not decrease to baseline. Generally, no differences between the bleaching agents could be observed. Colour change of enamel, dentine and combined enamel-dentine of in vitro bleached tooth samples is not stable over time with regard to lightness. However, yellowness did not return to baseline within one year.

### **Key words**

bleaching, colour, enamel, dentine

## **Introduction**

Bleaching of vital and non-vital teeth has become an essential component of conservative esthetic dentistry as it is a non-restorative treatment for whitening of discoloured teeth. The current bleaching mechanisms are based on the application of hydrogen peroxide-releasing agents on external tooth surfaces (in-office or at-home-bleaching methods) or in the pulp chamber (only for non-vital teeth, walking-bleach-technique) to penetrate the tooth and produce free radicals that oxidize organic stains.

Several laboratory and clinical studies have demonstrated carbamide peroxide or hydrogen peroxide bleaching solutions to be effective in whitening of discoloured teeth [2, 4, 6, 16, 26, 27]. Thereby, efficacy might be related to the concentration of the active bleaching ingredient, the application time of the agents and the potential to penetrate dental hard tissues [10, 15, 22, 23]. The substantial increase of lightness and decrease of chroma observed directly after bleaching is, however, often followed by a decrease of whitening in the post-bleaching period indicating that the bleaching effect might not be stable over time. After an initial reduction of lightening possibly due to rehydration processes in the first weeks after bleaching [17, 19, 26] a further colour regression towards baseline shades might occur. Two-year evaluation of non-vital tooth whitening revealed a 19% reduction compared to tooth colour directly after bleaching [4]. Long-time follow-up of nightguard vital bleaching revealed colour regression in 37% of patients after 3 years and 58% after 7 years [14].

Due to the numerous products used and the great variation in application protocols, comparison of the results of clinical studies with regard to bleaching efficacy and especially

colour retention is difficult. While the short-term efficacy of different bleaching agents and techniques have been compared in vitro under standardized conditions [5], the colour stability of different bleaching techniques has not been investigated systematically as yet. However, to allow for comparison and assessment of the different treatment modalities, efficacy of bleaching has to be analysed not only directly after completion of bleaching but also after several months [5]. Therefore, the present study aimed to analyse the colour retention after simulation of different bleaching techniques (home-bleaching, in-office-bleaching, walking-bleach-technique) using established agents in the 12 months post-treatment period. As the efficacy of bleaching is not only related to the whitening of the superficial dental hard tissue, but also to the potential to penetrate the tooth and bleach the subsurface enamel or dentin [5, 24], the colour regression of enamel (E), dentine (D) and combined enamel-dentine (ED) was investigated. The null hypothesis tested were: a) the colour change of E, D and combined ED samples of differently bleached enamel-dentine specimens over a period of 12 months is not stable over time b) there were no significant differences among the different bleaching techniques in the colour stability.

## **Methods**

### **Sample preparation**

Two-hundred freshly extracted, non-damaged bovine intact incisors were stored in 0.1% thymol solution at room temperature. The labial surfaces were carefully cleaned with pumice and polished with prophylaxis paste (RDA 40, CCS AB, Borlänge, Sweden) using a polishing brush (Hawe Neos, Biggio, Switzerland) which was mounted in a contra angle (1200 rpm). Two enamel-dentine (ED) samples (3 mm in diameter) were prepared from the labial surface of each tooth by means of a trephine mill (Komet, Lemgo, Germany). The labial and pulpal sides of each ED specimen were ground flat and polished with water-cooled carborundum

discs (500, 800, 1000, 1200, 2400 and 4000 grit, Water Proof Silicon carbide Paper, Stuers, Erkrat, Germany) in a polishing device (DP-U3, Struers, Denmark). Thereby, the ED samples were trimmed to a thickness of 2 mm, with the enamel and dentine section each 1 mm thick.

Baseline L\*-values of both ED specimens of each tooth were assessed under standardized ambient conditions according to the CIE-Lab\*-system using a dental colorimeter (Shade Eye<sup>®</sup>, Shofu, Kyoto, Japan). Mean L\*-value of each two ED specimens was used for stratified allocation among five groups with groups 1 to 4 (1: Whitestrips, 2: Illuminé 15%, 3: Opalescence Xtra Boost, 4: Sodium perborate, Table 1) each n = 80 (40 x 2 ED) samples and the control group n = 80 (40 x 2 ED) samples. Within each group 1 to 4, the samples were divided into 4 subgroups (A to D, corresponding to the timepoint of measurement, see below) with each n = 20 (10 x 2 ED) specimens. After preparation, the specimens were stored in artificial saliva.

To allow for separate colour measurement of enamel and dentine sections within the experiment, removal of the dentine layer of the first and the enamel layer of the second ED specimen of each tooth was necessary. Therefore, after colour measurement of the ED specimens, the dentine or enamel layer, respectively, was completely removed by grinding and polishing resulting in one enamel sample (E) and one dentine sample (D). Samples E and D of the control group were prepared accordingly.

#### Bleaching treatment and post-bleaching period

Specimens of the control group were not bleached and prepared for separate colour evaluation of E and D specimens at the respective timepoints.

Eighty ED specimens of each group 1 to 4 were submitted to a 21-day whitening procedure for simulation of external (1: Whitestrips, 2: Illuminé 15 %, 3: Opalescence Xtra Boost) or internal (4: sodium perborate mixture) bleaching treatment. The active bleaching ingredients of the products under evaluation and their application protocol are described in Table 1.

For simulation of external bleaching procedures, the samples were treated with different home-bleaching (groups 1 and 2) and in-office-bleaching (group 3) agents according to manufacturers' instructions for 21 days. The bleaching agents were applied in a humid atmosphere at 37 °C on the enamel surfaces of the ED samples:

1: Whitestrips. The enamel surfaces were covered with a strip twice daily for 30 min each with storage time in saliva of 12 h in between treatments.

2: Illuminé 15%. The specimens were covered with a layer of 2 mm of the whitening gel for 2 h each day and were stored in artificial saliva between treatments.

3: Opalescence Xtra Boost. The enamel surfaces were treated with a 2 mm thick layer for 30 min on days 1, 5, 10, 15 and 20 and were stored in artificial saliva between treatments.

In group 4, sodium perborate mixed with distilled water in a ratio of 2 g of powder to 1 ml of liquid was applied on the dentine surfaces of the ED specimens to simulate internal bleaching treatment of devital teeth. The mixture was applied as 2 mm thick layer in a humid atmosphere at 37° C. This layer remained on the surface during the whole bleaching period and was renewed on day 5, 10, 15 and 20. The hydrogen peroxide concentration of the sodium perborate mixture was determined photometrically as described in detail previously [7, 8]. The method is based on the reaction of 4-aminoantipyrin and phenol with hydrogen peroxide catalysed by peroxidase. Inorganic peroxide is oxidized by peroxidase, releasing oxygen which oxidise achromatic chromogenic hydrogen donors. Absorbance of the colouring product (chinonimin) was measured at 510 nm. The pH-value of the sodium perborate bleaching agent was measured subsequently after mixing using pH-indicator strips (Merck, Darmstadt, Germany).

For whitening treatment, the ED specimens were embedded in Teflon moulds (3 mm diameter) which allow only the enamel (groups 1 to 3) or dentine (group 4) surfaces to be covered by the bleaching agent, while the respective bottom of the specimen (groups 1-3: dentine surface, group 4: enamel surface) was placed in contact to artificial saliva in order to

avoid dehydration. Following treatment, the specimens were washed with tap water and re-immersed in artificial saliva. The artificial saliva was prepared according to the formulation of Klimek et al.[11] and was renewed each 10 days. In the 12-months post-bleaching period, the samples were stored in artificial saliva and alternately exposed to dark conditions or 12 h daylight for 14 days, respectively. The control teeth were not bleached but stored in artificial saliva for 21 days and also alternately exposed to dark conditions and daylight for 14 days in the post-bleaching period.

### Colour measurement

Prior to the experiment, baseline colour of each ED specimen was assessed using a dental colorimeter (Shade Eye<sup>®</sup>, Shofu, Kyoto, Japan). Post-bleaching measurement was performed subsequently after bleaching (groups 1A to 4A) and 3 months (groups 1B to 4B), 6 months (1C to 4C) and 12 months (1D to 4D) after the bleaching treatment. Colour of the 20 ED specimens of each subgroup was evaluated as described below. Thereafter, the dentine or enamel layer of the ED specimens was removed as described above resulting in 10 enamel (E) and 10 dentine (D) samples, which were submitted to colour measurement.

The samples were carefully dried (not desiccated) and placed into individually prepared silicone moulds (President, Coltène/Whaledent AG, Altstätten, Switzerland) which allow for exact and repeatable positioning of each specimen and colour measurement under standardized ambient conditions. The colorimeter's light sensor was set at right angles to the samples' surfaces and could be fixed directly on the enamel surface as the diameter of the samples corresponds to the diameter of the colorimeter head. To maintain repeatable orientation of the test surface with the colorimeter, a line was scratched on the margin side of each sample to correspond with a line on the colorimeter head. Colour of each sample was measured five times and averaged. The results of colour measurement were quantified in terms of three coordinate values ( $L^*$ ,  $a^*$ ,  $b^*$ ) established by the Commission International de

l'Eclariage (CIE), which locate colour of an object in a three dimensional colour space. The L\* axis represents the degree of lightness within a sample and ranges from 0 (black) to 100 (white). The a\* plane represents the degree of green/red colour, while the b\* plane represents the degree of blue/yellow within the sample.

Colour of ED samples at the respective time points was calculated in reference to baseline values ( $\Delta L$ ,  $\Delta a$  and  $\Delta b$ ), with the following colour definitions of the respective positive (+) and negative (-) value:  $\Delta L = (+)$  white,  $(-)$  black;  $\Delta a = (+)$  red,  $(-)$  green;  $\Delta b = (+)$  yellow,  $(-)$  blue [7]. For determination of the overall colour change  $\Delta E$  the following formula was used:  $\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$ .

As the respective baseline colour of the E and D samples could not be assessed, L\* a\* b\* values of the E and D samples of the unbleached control group served as reference and was evaluated at baseline (0 months) and after 3, 6 and 12 months.

### Statistical analysis

Mean differences in L\* a\* b\* of baseline and the respective post-bleaching values (subgroups A-D) of ED samples in groups 1 to 4 were calculated and used for determination of  $\Delta E$ . For E and D specimens, L\* a \* b\* values were submitted to the statistical analysis and compared with the reference values of the control group. The data of the ED specimens were statistically analysed by one-way ANOVA followed by Bonferroni-adjusted post-hoc tests. For statistical analysis of E and D specimens, one-way ANOVA followed by Dunnett-adjusted and Bonferroni-adjusted post-hoc tests were applied. The overall level of significance was set at  $p < 0.05$ .

### Results

The mean overall colour change  $\Delta E$  ( $\pm$  standard deviation) of ED specimens in groups 1-4 is presented in Figure 1. Bleaching treatment led to a significant colour change of ED specimens



compared to baseline values. Thereby,  $\Delta E$  values of groups 1-4 amounted to 8.9 to 15.9 subsequently after bleaching (Fig. 1, time 0). In all groups, a significant increase of  $L^*$ -values (Fig. 2a) and decrease of  $b^*$ - values (Fig. 2b) could be observed indicating a shift in the direction of white and less yellow. With regard to  $\Delta a$ , only small changes amounting to 0.2 to 2.7 could be observed after bleaching treatment. After 12 months,  $a^*$ -values fell below the baseline values in all groups. As the variance of  $a^*$ -values has a minor influence on total colour change, the results of the  $a^*$ -values were not put into graphs.

Within the observation period,  $L^*$ -values of ED samples declined towards baseline. After 12 months,  $L^*$ -values of group 1 and 2 ( $p > 0.09$ ) were not significantly different compared to baseline, while lightness of groups 3 and 4 was still slightly increased ( $p < 0.01$ , Fig. 2a). In contrast,  $\Delta b$  was still significantly decreased at the end of the observation period in all groups ( $p < 0.001$ , Fig. 2b). Compared to baseline, the overall colour change of ED specimens did also not achieve the baseline values ( $p < 0.001$ ), but declined and then rebounded within the observation period (Fig 1).

$L^*$ - and  $b^*$ -values of the separated E and D samples are shown in Fig. 3 and 4 compared to the respective control values. Statistical analysis of the E and D control samples found no significant differences of  $L^*$  and  $b^*$ -values after 0, 3, 6 and 12 months.

With regard to E samples (Fig. 3), bleaching treatment resulted in significantly higher  $L^*$ - and lower  $b^*$ -values compared to the control. In all groups, colour regression was characterized by a drop of  $L^*$ -values towards the control values within the observation period.  $L^*$ -values were not significantly different from the controls after 12 months, with exception for  $L^*$ - values of groups 1 and 4. In contrast,  $b^*$ -values of all groups were significantly lower from the  $b^*$  value of the enamel control throughout the whole observation period. Bleaching treatment led to small changes of the  $a$ -values (1.1 to 1.6 units) compared to the control. After 12 months,  $a^*$ -values of all groups decreased to or below the control group.

Colour analysis of D samples (Fig. 4) revealed significantly higher L\* of bleached specimens compared to the unbleached controls at the beginning of the observation period. Except for group 3 (Opalescence Extra Boost), bleaching also resulted in significantly lower b\*-values compared to the control. After 12 months, L\*- and b\*-values of bleached samples decreased to or below the control values, except for b\*-values of group 1 (Whitestrips) and 4 (sodium perborate), which still showed a significant shift into less yellow. As for the ED and E samples, bleaching treatment led to small changes of the a-values (0.6 to 2.7 units) of the D samples compared to the control. After 12 months, a\*-values of all groups decreased to or below the control group.

The results of the between-group comparisons of colour values at the beginning (0 months) and the end (12 months) of the observation period are presented in Tables 2-4. Overall, the external (Whitestrips, Illuminé 15% and Opalescence Xtra Boost) and internal (sodium perborate mixture) bleaching agents performed similar regarding to bleaching efficacy and colour stability. While the statistical analysis of ED samples (Tab. 2) revealed some significant differences between the groups 1-4, between-group comparison of E and D specimens mostly showed no differences (Tab. 3 and 4).

## **Discussion**

To allow for a systematic analysis of bleaching efficacy and colour stability under standardized conditions, an in vitro model was applied using the pre-existing colour of bovine teeth [10]. The use of bovine teeth allowed the preparation of samples having a standardized size, which is compatible to the dimension of the colorimeter, and an anatomically relevant tissue thickness. As found by Harris and Hicks [9] and Murray et al. [20] the average thickness of enamel and dentine in human maxillary incisors amounted to approximately 1 mm each. Due to the size of the bovine crown, two ED samples could be obtained from each tooth, which allow for preparation of each one enamel (E) and dentine (D) sample from the same

origin. The bleaching agents were applied according to the manufacturers' information and in order to the techniques (at-home, in-office, walking-bleach-technique) to be simulated. Therefore, groups 1 and 2 aimed to simulate at-home bleaching techniques with the application of whitening strips (group 1, 6% H<sub>2</sub>O<sub>2</sub>) or tray-based 15% carbamide peroxide gels (group 2) on enamel surfaces. In group 3, the highly concentrated hydrogen peroxide gel was applied 5 times for 30 min imitating chairside in-office bleaching treatment. For simulation of the walking-bleach technique (group 4) the mixture of sodium perborate and distilled water was applied on dentine surfaces as recommended by Attin et al.[1]. Between the treatments and during the post-treatment observation period, the samples were stored in artificial saliva and exposed alternately to dark conditions or daylight to simulate clinical conditions in the oral cavity. As the L\* a\* b\* values of the control samples remained unchanged throughout the whole observation period, the in vitro model used represents a reliable tool for long-time determination of bleached teeth. However, it has to be taken into consideration that the results obtained from In-vitro-studies can not be directly applied to the clinical situation.

In all groups, bleaching treatment resulted in a significant overall colour change ( $\Delta E$ ) of ED samples distinctly above the limit of visible discrimination of colour differences, which is reported to be from one to three units [12, 21]. As expected, the most pronounced changes could be observed in L\*- and b\*-values, which reflected the increase of lightness and reduction in yellowness by bleaching treatment. With regard to a\*-values, only minimal changes in the red-green direction occurred within the observation period. This is consistent with the results of Lenhard [13], indicating that the variance in a\*-values had a minor influence on total tooth colour change. The overall colour change of ED samples is also reflected by the colour change of E and D samples, which also showed an increase of lightness and a reduction in yellowness. As the external (groups 1-3) and internal (group 4) bleaching techniques performed similar with regard to whitening effects of enamel (E) or

dentine (D) samples, respectively, it might be assumed that the ED specimens were penetrated and bleached uniformly irrespective of the agents' hydrogen peroxide concentration and whether the agent was applied on enamel or dentine.

Within the observation period, the colour changes of ED, E and D samples due to bleaching treatment were significantly reduced. Colour regression might be the result of the previously oxidized substance which might become chemically reduced and causes the samples to reflect the old coloration of enamel or dentine. In vitro, organic substances of the artificial saliva might contribute to the colour regression. In the clinical situation, external chromogens (coffee, wine, nicotine, metallic ions) might contribute to the colour regression of whitened teeth. This might explain the results of some in vivo studies, which showed that long-term colour retention of bleached teeth is less stable. Matis et al. [18] found a mean  $\Delta E$  reversal of 65% after 6 weeks [18] and of 50% after 6 months post-bleaching [17]. In contrast, other studies reported only slight colour changes of bleached teeth in the 6 months post bleaching period [3, 25].

Remarkably, in E and D samples, the decrease of yellowness due to bleaching remained nearly constant over time, even resulting in a persistent overall colour change  $\Delta E$  of ED samples above baseline values. After 12 months,  $\Delta E$  of ED samples was decreased compared to colour at the beginning of the observation period, but still increased above the limit for visible discrimination compared to the pre-bleaching values. It might be speculated that bleaching led to an irreversible degradation of organic substances contributing to the reduction of yellowness of the teeth.

$\Delta E$  and especially  $\Delta b$  of ED samples showed some fluctuations within the observation period, which can not be explained by information of the current literature. Maybe, bleaching treatment induces some alterations within the dental hard tissues, which in turn are not stable but subjected to continuous reorganisation over time. However, as the colorimeter was

calibrated accordingly to the manufacturers' recommendation prior to each measurement and the control samples did not change over time, an error in measurement can be excluded.

However, for most instances, colour regression of enamel, dentine and combined enamel-dentine was characterized by a decrease of lightness. Thus, the results indicate that colour regression of bleached teeth (combined enamel-dentine) is mostly provoked by the reversal of enamel and dentine lightness.

Moreover, the bleaching techniques applied and the differently concentrated bleaching agents seem to be equally effective under standardized in vitro conditions. However, it can not be excluded that differences in bleaching efficacy or colour stability might occur when bleaching would be performed on stained samples or when samples were stored in a staining solution in the post-bleaching period.

## **Conclusion**

The whitening effects of in vitro bleached tooth samples are not stable in the 12-months observation period. Colour change of enamel, dentine and combined enamel-dentine is mostly characterized by a decrease of lightness. However, even after 12 months, overall colour change  $\Delta E$  did not achieve baseline values of unbleached samples. Bleaching efficacy of the different bleaching agents or bleaching techniques, respectively, was not different under standardized in vitro conditions. Thus, the formulated null hypotheses were accepted.

## References

1. Attin T, Paqué F, Ajam F, Lennon AM (2003). Review of the current status of tooth whitening with the walking bleach technique. *Int Endod J* 36:313-329.
2. Auschill TM, Hellwig E, Schmidale S, Sculean A, Arweiler NB (2005). Efficacy, side-effects and patients' acceptance of different bleaching techniques (OTC, in-office, at-home). *Oper Dent* 30:156-163.
3. Brunton PA, Ellwood R, Davies R (2004). A six-month study of two self-applied tooth whitening products containing carbamide peroxide. *Oper Dent* 29:623-626.
4. Deliperi S , Bardwell DN (2005). Two-year clinical evaluation of nonvital tooth whitening and resin composite restorations. *J Esthet Restor Dent* 17:369-378.
5. Dietschi D, Rossier S, Krejci I (2006). In vitro colorimetric evaluation of the efficacy of various bleaching methods and products. *Quintessence Int* 37:515-526.
6. Hannig C, Lindner D, Attin T (2007). Efficacy and tolerability of two home bleaching systems having different peroxide delivery. *Clin Oral Investig* 11:321-329.
7. Hannig C, Willenbücher S, Becker K, Attin T (2006). Recovery of peroxides in saliva during home bleaching - influence of smoking. *J Oral Rehabil* 33:533-541.
8. Hannig C, Zech R, Henze E, Dorr-Tolui R, Attin T (2003). Determination of peroxides in saliva--kinetics of peroxide release into saliva during home-bleaching with Whitestrips and Vivastyle. *Arch Oral Biol* 48:559-566.

9. Harris EF , Hicks JD (1998). A radiographic assessment of enamel thickness in human maxillary incisors. *Arch Oral Biol* 43:825-831.
10. Joiner A (2006). The bleaching of teeth: a review of the literature. *J Dent* 34:412-419.
11. Klimek J, Hellwig E, Ahrens G (1982). Fluoride taken up by plaque, by the underlying enamel and by clean enamel from three fluoride compounds in vitro. *Caries Res* 16:156-161.
12. Kuehni FG , Marcus RT (1979). An experiment in visual scanning of small color differences. *Color Res Appl* 4:83-91.
13. Lenhard M (1996). Assessing tooth colour change after repeated bleaching in vitro with a ten percent carbamide peroxide gel. *J Am Dent Assoc* 127:1618-1624.
14. Leonard RH (2000). Nighguard vital bleaching: dark stains and long-term results. *Compend Contin Educ Dent Suppl* 28:S18-27.
15. Leonard RH, Sharma A, Haywood VB (1998). Use of different concentration of carbamide peroxide for bleaching teeth: an in vitro study. *Quintessence Int* 29:507.
16. Lim MY, Lum SO, Poh RS, Lee GP, Lim KC (2004). An in vitro comparison of the bleaching efficacy of 35% carbamide peroxide with established intracoronal bleaching agents. *Int Endod J* 37:483-488.
17. Matis BA, Cochran MA, Eckert G, Carlson TJ (1998). The efficacy and safety of a 10% carbamide peroxide bleaching gel. *Quintessence Int* 29:555-563.
18. Matis BA, Cochran MA, Franco M, Al-Ammar W, Eckert GJ, Stropes M (2007). Eight in-office tooth whitening systems evaluated in vivo: a pilot study. *Oper Dent* 32:322-327.

19. Matis BA, Mousa HN, Cochran MA, Eckert GJ (2000). Clinical evaluation of bleaching agents of different concentrations. *Quintessence Int* 31:303-310.
20. Murray PE, Stanley HR, Matthews JB, Sloan AJ, Smith AJ (2002). Age-related odontometric changes of human teeth. *Oral Surg Oral med Oral Pathol Oral Radiol Endod* 93:474-482.
21. Ruyter IE, Nilnu K, Moller B (1987). Color stability of dental composite resin materials for crown and bridge veneers. *Dent Mater* 3:246-251.
22. Shin DH, Summitt JB (2002). The whitening effect of bleaching agents on tetracycline-stained rat teeth. *Oper Dent* 27:66-72.
23. Sulieman M, Addy M, MacDonal E, Rees JS (2004). The effect of hydrogen peroxide concentration on the outcome of tooth whitening: an in vitro study. *J Dent* 32:295-299.
24. Wiegand A, Vollmer D, Foitzik M, Attin R, Attin T (2005). Efficacy of different whitening modalities on bovine enamel and dentin. *Clin Oral Investig* 9:91-97.
25. Zantner C, Derdilopolou F, Martus P, Kielbassa AM (2006). Randomized clinical trial on the efficacy of 2 over-the-counter whitening systems. *Quintessence Int* 37:695-706.
26. Zantner C, Derdilopolou F, Martus P, Kielbassa AM (2006). Randomized clinical trial on the efficacy of a new bleaching lacquer for self-application. *Oper Dent* 31:308-331.
27. Ziebolz D, Helms K, Hannig C, Attin T (2007). Efficacy and oral side effects of two highly concentrated tray-based bleaching systems. *Clin Oral Investig* 11:267-275.



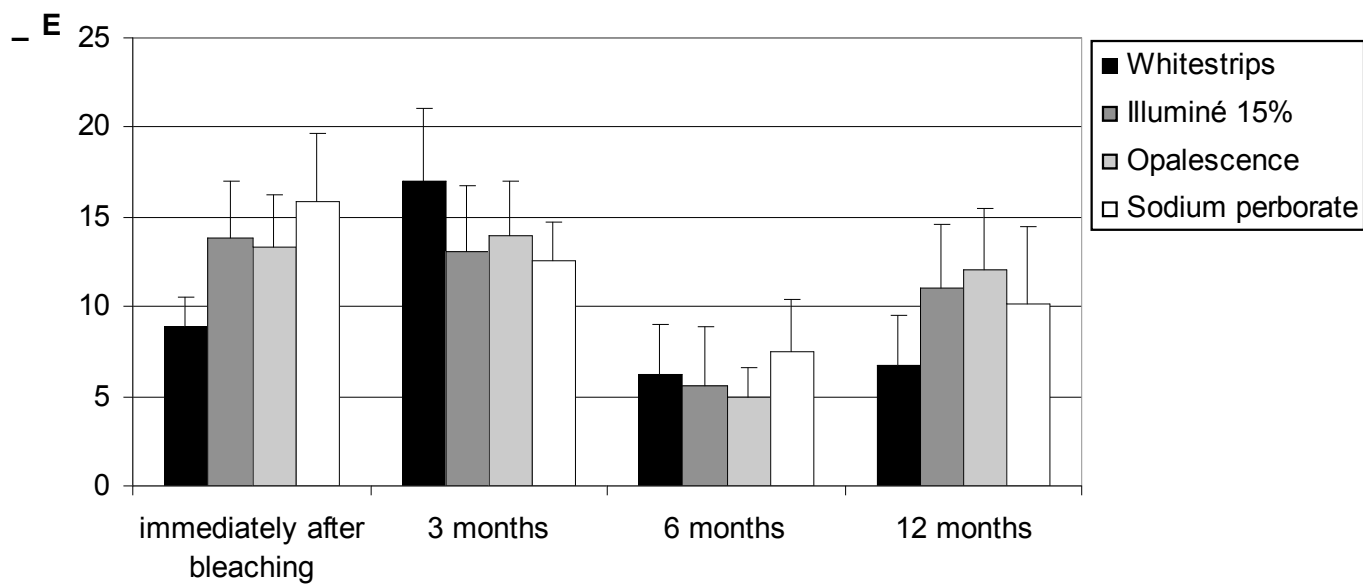


Fig 1

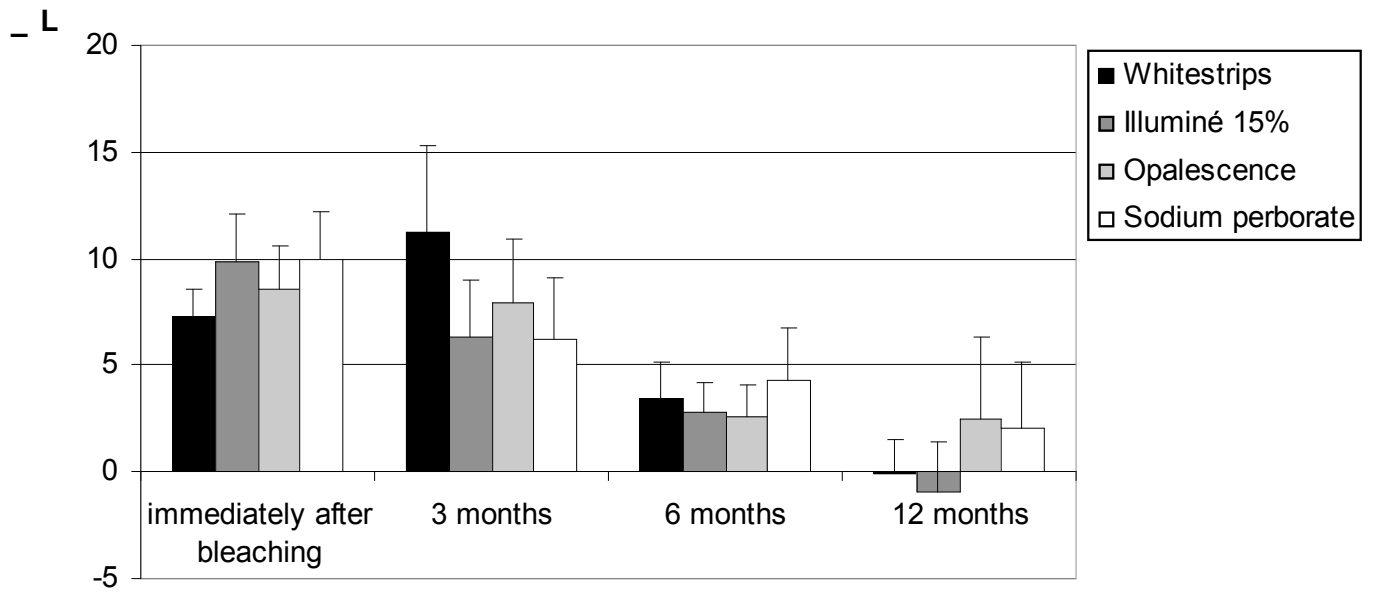


Fig. 2a

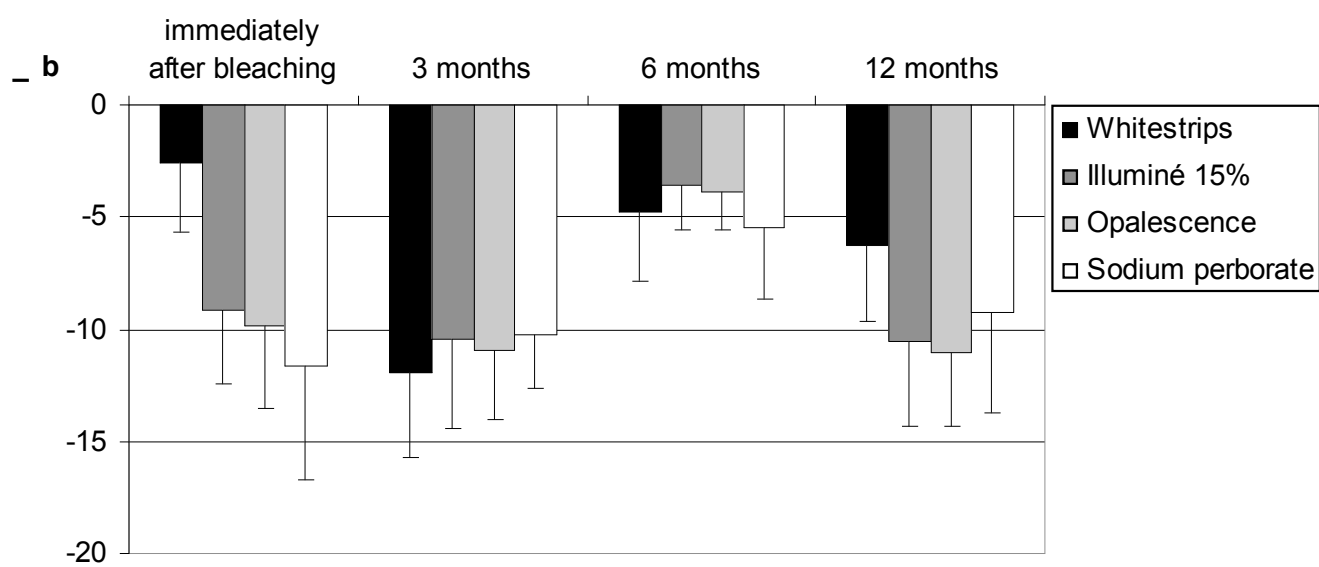


Fig. 2b

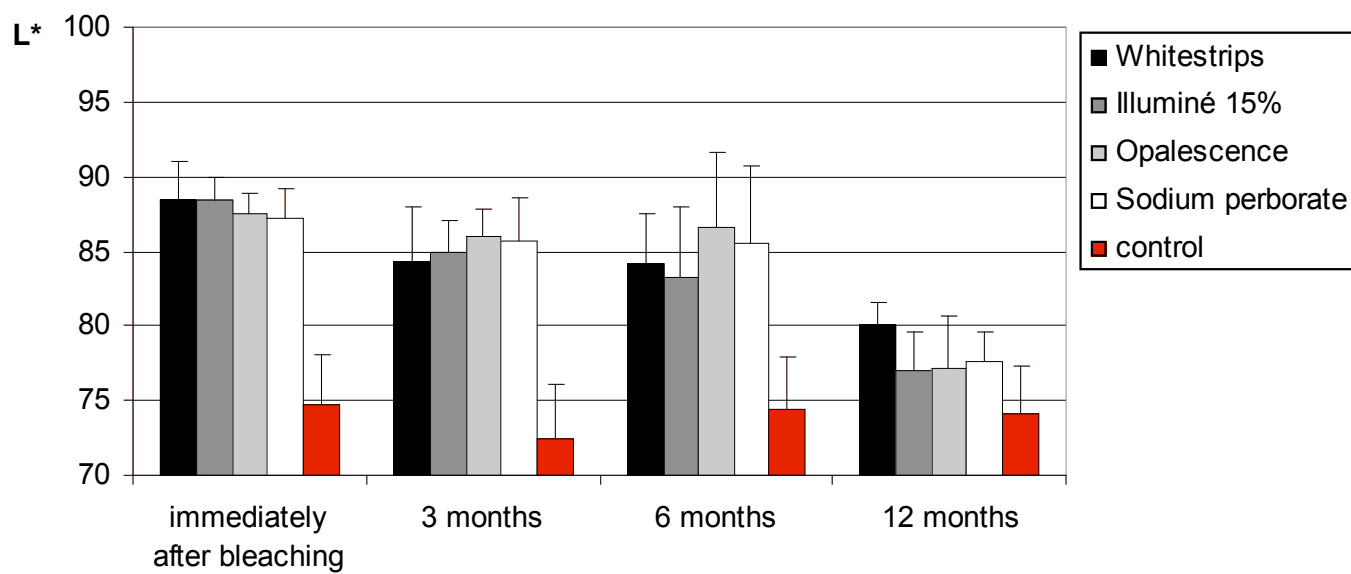


Fig. 3a

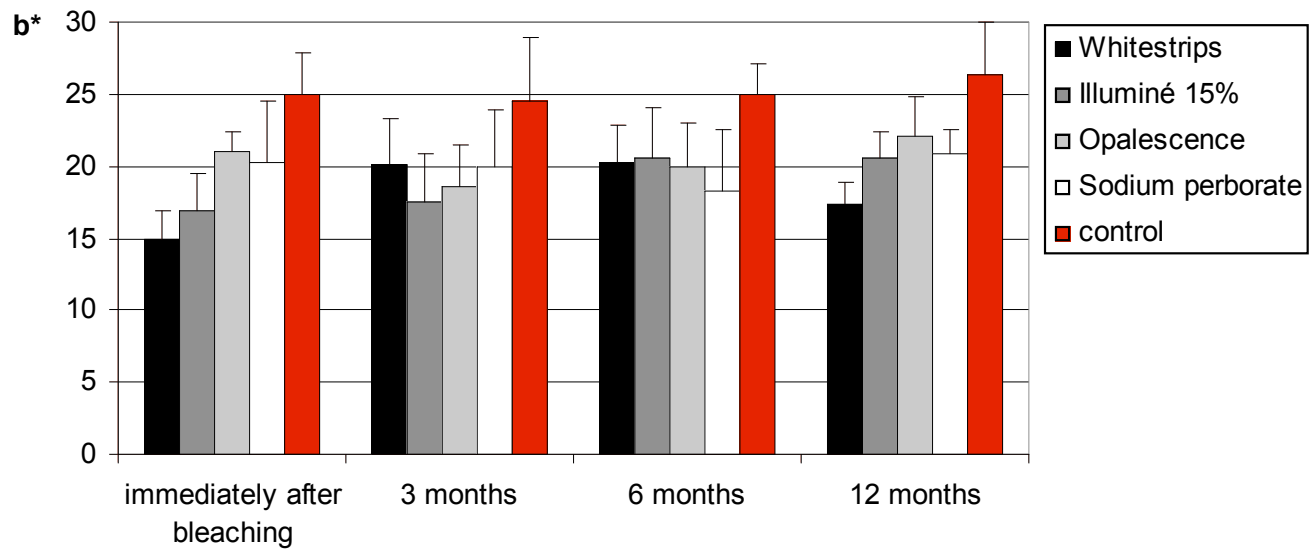


Fig. 3b

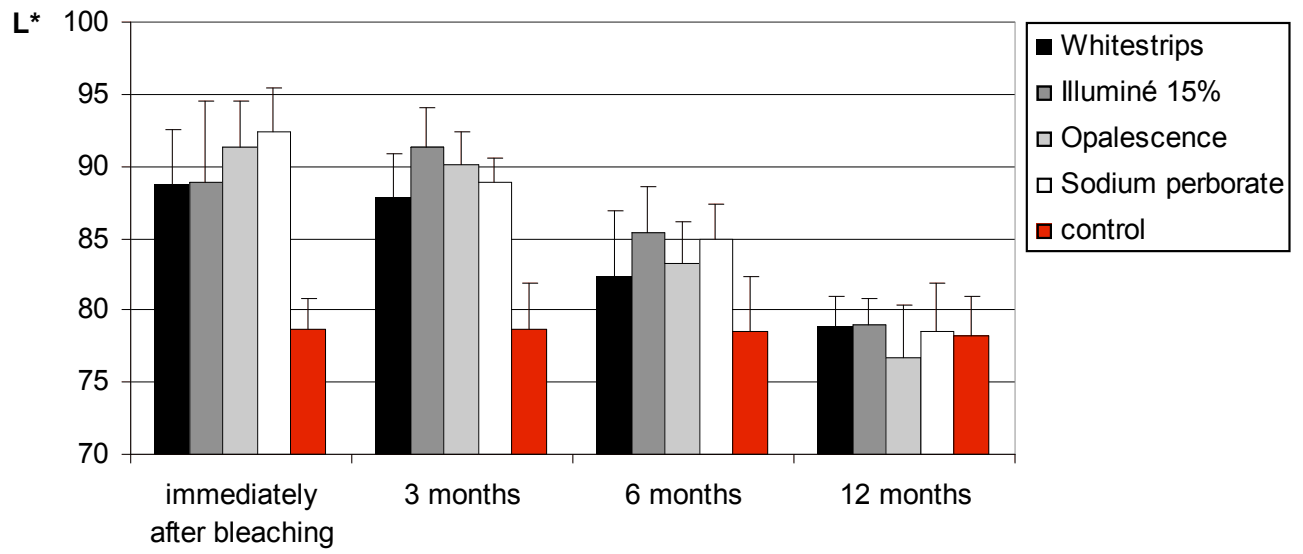


Fig. 4a

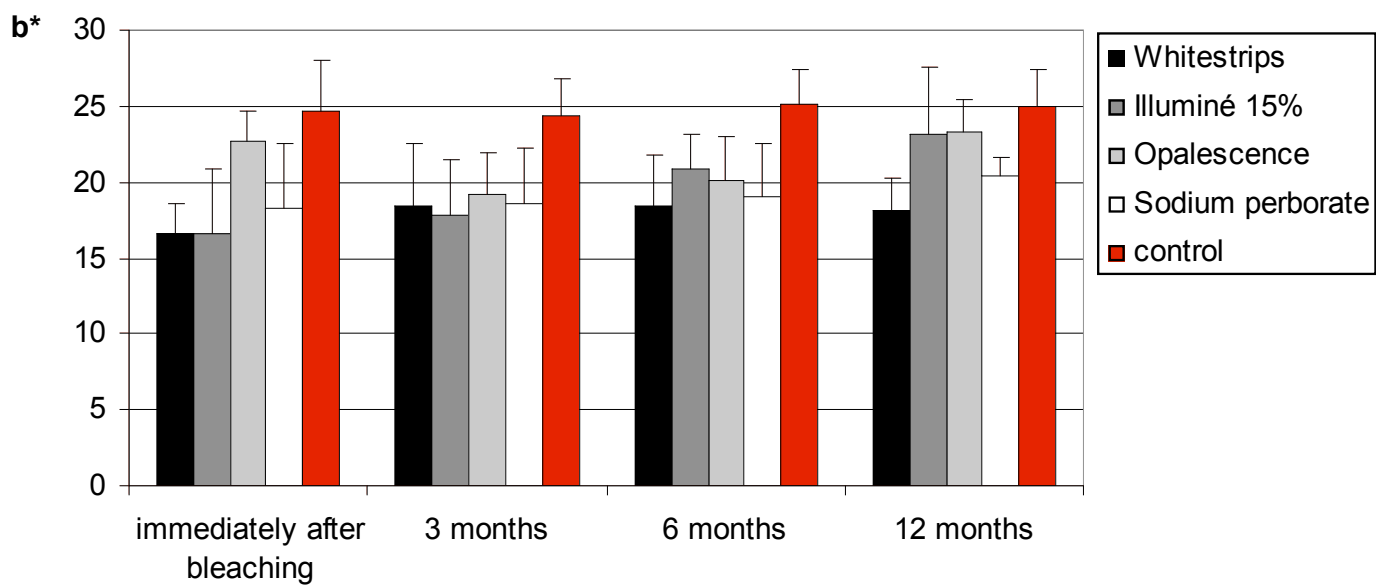


Fig. 4b

## Legends to figures

### Figure 1

Overall colour change (mean  $\pm$  SD) of enamel-dentine (ED) samples within the observation period. Colour values at time “0” were obtained subsequently after 21-day bleaching treatment (baseline).

### Figure 2 a-b

Changes of L\*- and b\*-values (mean  $\pm$  SD) of enamel-dentine (ED) samples within the observation period. Colour values at time “0” were obtained subsequently after 21-day bleaching treatment (baseline).

### Figure 3 a-b

L\*- and b\*-values (mean  $\pm$  SD) of enamel (E) samples within the observation period compared to the respective control. Within the control samples, no significant differences in colour values after 0 (subsequently after bleaching treatment), 3, 6 and 12 months could be observed. At the end of the observation period, L\*-values of groups 2 and 3 and b\*-values of group 1-4 were still significantly different compared to the control values.

### Figure 4 a-b

L\*- and b\*-values (mean  $\pm$  SD) of dentine (D) samples within the observation period compared to the respective control. Within the control samples, no significant differences in colour values after 0 (subsequently after bleaching), 3, 6 and 12 months could be observed. At the end of the observation period, only b\*-values of groups 1 (Whitestrips) and 4 (sodium perborate) were significantly decreased compared to the controls.



<b>Group</b>	<b>Bleaching agent/ manufacturer</b>	<b>Active bleaching ingredient</b>	<b>Hydrogen peroxide concentration</b>	<b>pH- value</b>	<b>Frequency and duration of application</b>
1	Whitestrips™ <i>(Procter&amp;Gamble, Egham, UK)</i>	6% H <sub>2</sub> O <sub>2</sub>	6% H <sub>2</sub> O <sub>2</sub>	6.4	Twice 30 min, each day on enamel surfaces
2	Illuminé 15% <i>(Dentsply DeTrey, Konstanz, Germany)</i>	15% carbamide peroxide	5.4% H <sub>2</sub> O <sub>2</sub>	6	2 h each day on enamel surfaces
3	Opalescence Xtra Boost <i>(Ultradent Products, South Jordan, Utah, USA)</i>	38% H <sub>2</sub> O <sub>2</sub>	38% H <sub>2</sub> O <sub>2</sub>	7	30 min on day 1, 5, 10, 15 and 20 on enamel surfaces
4	Sodium perborate/ distilled water (2g/1ml)	-	16.3% H <sub>2</sub> O <sub>2</sub>	11-12	Remained on the dentin surface during the whole experiment, but changed on days 5, 10, 15 and 20

Table 1: Active bleaching ingredients, hydrogen peroxide concentration, pH-value and application protocol of the bleaching agents. The data of products 1 to 3 are given according to the manufacturers information. The hydrogen peroxide concentration and the pH-value of the sodium perborate mixture (4) were evaluated as described in the methods section.

Colour value Observation period/ months	$\Delta E$		$\Delta L$		$\Delta b$	
	0	12	0	12	0	12
Whitestrips – Illuminé 15%	<0.0001*	0.0004*	<0.0001*	0.3176	<0.0001*	<0.0001*
Whitestrips – Opalescence Xtra Boost	<0.0001*	<0.0001*	0.0643	0.0058*	<0.0001*	<0.0001*
Whitestrips – Sodium perborate	<0.0001*	0.0046*	<0.0001*	0.0187	<0.0001*	0.0135
Illuminé 15% - Opalescence Xtra Boost	0.6466	0.3502	0.0358	0.0002*	0.5585	0.6884
Illuminé 15% - Sodium perborate	0.0269	0.4323	0.9067	0.0010*	0.0450	0.2746
Opalescence Xtra Boost – Sodium perborate	<0.0081*	0.0878	0.0271	0.6651	0.1509	0.1370

Table 2: Results of the Bonferroni-adjusted post-hoc tests (p-values) for between-group comparisons of enamel-dentin (ED) colour values after 0 (subsequently after bleaching) and 12 months. Comparisons in this table are not significant unless the corresponding p-value is less than 0.0083. Significant differences were marked by \*.

Colour value	L		b	
	0	12	0	12
Whitestrips – Illuminé 15%	0.9923	0.0321	0.1748	0.0107
Whitestrips – Opalescence Xtra Boost	0.4519	0.0333	<0.0001*	0.0003*
Whitestrips – Sodium perborate	0.3015	0.0773	0.0005*	0.0055
Illuminé 15% - Opalescence Xtra Boost	0.4462	0.9872	0.0055	0.2202
Illuminé 15% - Sodium perborate	0.2970	0.6915	0.0226	0.8052
Opalescence Xtra Boost – Sodium perborate	0.7763	0.7033	0.5907	0.3254

Table 3: Results of the Bonferroni-adjusted post-hoc tests (p-values) for between-group comparisons of enamel (E) colour values after 0 (subsequently after bleaching) and 12 months. Comparisons in this table are not significant unless the corresponding p-value is less than 0.005. Significant differences were marked by \*.

<b>Colour value</b>	<b>L</b>		<b>b</b>	
	<b>0</b>	<b>12</b>	<b>0</b>	<b>12</b>
Whitestrips – Illuminé 15%	0.9642	0.9276	0.9455	<0.0001*
Whitestrips – Opalescence Xtra Boost	0.1440	0.1335	<0.0001*	<0.0001*
Whitestrips – Sodium perborate	0.0353	0.8768	0.1893	0.0350
Illuminé 15% - Opalescence Xtra Boost	0.1564	0.1122	<0.0001*	0.9520
Illuminé 15% - Sodium perborate	0.0391	0.8058	0.2128	0.0100
Opalescence Xtra Boost – Sodium perborate	0.5017	0.1771	0.0009*	0.0085

Table 4: Results of the Bonferroni-adjusted post-hoc tests (p-values) for between-group comparisons of dentin (D) colour values after 0 (subsequently after bleaching) and 12 months. Comparisons in this table are not significant unless the corresponding p-value is less than 0.005. Significant differences were marked by \*.