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# Effect of Different Fluoridation Regimes on the Microhardness of Bleached Enamel

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### Clinical Relevance

Fluoridation is effective in preventing microhardness loss due to bleaching, but additional fluoride gel supplementation is not superior to toothpaste fluoridation alone.


## SUMMARY

**Purpose: This *in vitro* study evaluated the effects of toothpaste fluoridation and toothpaste plus gel fluoridation and influence of the time period of fluoride gel application on the microhardness of bleached enamel.**

**Methods: Ninety bovine enamel samples were distributed among nine groups (A-I), each having 10 samples. Half of each surface was bleached with 10% carbamide peroxide gel (8 hours/daily) for 14 days, while the remaining surface was not bleached and served as the control. Groups A-H were fluoridated with toothpaste twice daily throughout the experiment (42 days) and assigned to fluoride gel treatment during the pre-bleaching period (14 days), during the bleaching period and/or during the post-bleaching period (14 days): A: prior, B: during, C: post, D: prior+during, E: during+post, F: prior+post, G: prior+during+post, H: no gel. Group I was neither fluoridated by toothpaste nor gel. The Knoop microhardness (KHN) of each specimen was determined at baseline, after pre-bleaching (day 14), after bleaching (day 28) and after post-bleaching (day 42). Statistical analysis of the percentage of change at baseline KHN was performed by ANOVA and *t*-test ($p<0.05$).**

**Results: Bleaching led to a significant decrease of KHN in Group I (unfluoridated) compared to Groups A-H, where microhardness did not fall below baseline values. Fluoridation treatment in Groups A-H increased microhardness in bleached**



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**and unbleached samples, but additional supplementation of fluoride gel in Groups A-G was not superior to toothpaste fluoridation only (H). After the post-bleaching period, the microhardness of the bleached and unbleached surfaces was not significantly different in Groups A-H.**

**Conclusion: Regular toothpaste fluoridation prevents microhardness loss due to bleaching treatment *in vitro*. The additional supplementation of fluoride gel did not enhance the beneficial effect of toothpaste fluoridation, and microhardness was not influenced by the time period of gel fluoridation.**

## INTRODUCTION

The effectiveness of external bleaching by applying hydrogen or carbamide peroxide to discolored vital teeth has been frequently reported in the literature.[1-4] The application of carbamide peroxide gels at a concentration of 10%-15% is still one of the most popular at-home bleaching techniques and is suggested as an efficient, simple procedure for tooth whitening. With regard to potential adverse effects, carbamide peroxide bleaching is not associated with macroscopically or clinically visible damage to dental hard tissue; however, concerns have been expressed regarding surface alterations in enamel topography.

By scanning electronic microscopic analysis, it has been shown that morphological alterations of the superficial enamel surface, such as erosion, decalcification and porosity, might occur following exposure to 10%-15% carbamide peroxide.[5-6] The application of carbamide peroxide gels has been reported to affect calcium and phosphate content and, as a result, might also decrease the surface microhardness of the enamel layer.[7-9] Thereby, the loss of surface microhardness is not limited to the outermost enamel but can also be detected in the subsurface enamel layer.[10]

Despite the protective and remineralizing potential of human saliva, when bleaching is performed for six to eight hours daily for two to three weeks, microhardness loss is sometimes evident under *in situ* conditions.[11-13] As demonstrated by Basting and others,[9] saliva increases the microhardness of bleached enamel by providing calcium and phosphate ions, but rehardening might not always be complete. Fluoride might also contribute to the repair of the microstructural defects of bleached enamel. Attin and others[14] and Lewinstein and others[7] showed that the loss of microhardness could be reversed by a post-bleaching period in which fluoride gels or rinses were administered. Thereby, fluoridation with a pure amine fluoride gel was shown to be more effective in increasing the microhardness of bleached enamel than when bleaching was performed using a fluoridated carbamide peroxide gel.[15] Generally, the fluoridation of bleached enamel is frequently recommended for improving microhardness[7,14] and, thus, increasing the abrasion resistance of softened enamel.[16-17] However, there is no general agreement on the optimal time point of fluoridation during the bleaching regime. On one hand, the application of fluoride gels for several days prior to the bleaching treatment might be advisable, as fluoride increases the demineralization resistance of enamel and, therefore, might prevent microhardness loss during the subsequent bleaching treatment. On the other hand, fluoride uptake in enamel is known to be higher in demineralized enamel compared to sound enamel. It is assumed that bleaching might render enamel porous, which, in turn, might allow for better diffusion and penetration of the applied fluoride.[15] It is therefore of interest to analyze whether a fluoride gel should be applied several days prior to bleaching treatment, during bleaching treatment or in the post-bleaching period for the best hardening or complete rehardening of bleached enamel.

## METHODS AND MATERIALS

### Preparation of Enamel Specimens

Ninety freshly extracted bovine intact incisors were stored in 0.9% NaCl solution at room temperature until required. The crowns were separated from the roots using a water-cooled diamond bandsaw (Exakt, Norderstedt, Germany) and embedded in acrylic resin (Technovit 4071, Heraeus Kulzer, Wehrheim, Germany). The exposed labial surfaces 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). The thickness of the removed outermost enamel layer amounted to approximately 200 µm and was controlled with a micrometer (Digimatic, Micrometer, Mitutoyo, Tokyo, Japan). The initial surface microhardness of each sample was determined as a criterion for stratified allocation of the samples among nine groups (A-I) with 10 samples each. Prior to the experiment, the specimens were stored in artificial saliva (formulated according to Klimek & others).[18]

### Experiment

The enamel specimens of Groups A-I were submitted to a 14-day bleaching treatment *in vitro*, which was flanked by an individual fluoride regime during bleaching and in the pre- and post-bleaching period (Table 1).

Bleaching was performed by applying 10% carbamide peroxide gel (Illuminé home 10%, Dentsply DeTrey, Konstanz, Germany) according to the manufacturers' instructions for eight hours per day in a humidified atmosphere at 37°C. During bleaching, half of each sample's surface was covered with adhesive tape and served as the control (unbleached). After eight hours, the bleaching agent was washed away under running

Table 1: *Fluoridation Regime in Groups A-I During the Pre-bleaching Period, the Bleaching Period and the Post-bleaching Period*

| Group | Pre-bleaching Period (day 1-14) | Bleaching Period (day 15-28) | Post-bleaching Period (day 29-42) |
|---|---|---|---|
| **A** | F-toothpaste<br>**F-Gel** | F-toothpaste<br>- | F-toothpaste<br>- |
| **B** | F-toothpaste<br>- | F-toothpaste<br>**F-Gel** | F-toothpaste<br>- |
| **C** | F-toothpaste<br>- | F-toothpaste<br>- | F-toothpaste<br>**F-Gel** |
| **D** | F-toothpaste<br>**F-Gel** | F-toothpaste<br>**F-Gel** | F-toothpaste<br>- |
| **E** | F-toothpaste<br>- | F-toothpaste<br>**F-Gel** | F-toothpaste<br>**F-Gel** |
| **F** | F-toothpaste<br>**F-Gel** | F-toothpaste<br>- | F-toothpaste<br>**F-Gel** |
| **G** | F-toothpaste<br>**F-Gel** | F-toothpaste<br>**F-Gel** | F-toothpaste<br>**F-Gel** |
| **H** | F-toothpaste<br>- | F-toothpaste<br>- | F-toothpaste<br>- |
| **I** | - | - | - |

tap water and the tape was removed. During the remaining daily time (16 hours), the enamel samples of each group remained in 400 ml artificial saliva at 37°C. The artificial saliva was changed daily.

The groups were assigned to a specific fluoride treatment prior to bleaching (pre-bleaching period, 14 days), during bleaching (bleaching period, 14 days) and/or after bleaching treatment (post-bleaching period, 14 days), respectively (Table 1). Thereby, the fluoride agents were applied on both the bleached and the control enamel surface of each sample.

Groups A-H were fluoridated twice daily using fluoridated toothpaste (1250 ppm, Elmex, GABA, Lörrach, Germany) throughout the experiment (42 days) to simulate regular oral hygiene treatment. For toothpaste fluoridation, a mixture containing artificial saliva and fluoridated toothpaste in a ratio of 1:3 was applied for 60 seconds every 12 hours. Additionally, enamel samples from Groups A-G were fluoridated with a highly fluoridated acidulated fluoride gel (12,500 ppm, Elmex Gelée, GABA, Lörrach, pH: 4.8) at different time-periods of the experiment (Table 1). Gel fluoridation in the respective periods was performed once daily for 60 seconds subsequent to bleaching treatment. Therefore, the fluoride gel was diluted by artificial saliva (ratio 1:3).

Specimens of Group H were fluoridated by toothpaste slurry only. Samples of Group I were not fluoridated (either by toothpaste or gel).

### Microhardness Measurement

The Knoop microhardness (KHN) of each specimen was determined prior to the experiment and served as baseline value. Therefore, five indentations were performed in the center of each specimen using a microhardness tester (Leica VMHT Auto, Leica Microsystems, Wetzlar, Germany) and averaged. The Knoop indenter was applied for 30 seconds with 100gf. KHN of the bleached and the control surface of each specimen were evaluated again after the pre-bleaching period (day 14), after the bleaching period (day 28) and after the post-bleaching period (day 42). Five indentations were made at each surface area with a 50 µm distance between each measurement.

In each group, the mean KHN was calculated after the different experimental periods and presented as the percentage change of the initial hardness.

### Statistical Analysis

Three-way ANOVA was used to analyze possible differences in KHN of Groups A-I at the end of the respective experimental periods. Thereby, fluoride gel applications in the pre-bleaching period, bleaching period and post-bleaching period were considered to be dependent variables. Differences in KHN between Groups A-H and Group I (unfluoridated) at the end of the experiment were analyzed by *t*-test. The overall level of statistical significance was set at 0.05. All analyses were done using SAS 9.1 (SAS Institute Inc, Cary, NC, USA).

## RESULTS

The microhardness of bleached and control enamel surfaces in Groups A-I is presented in Figures 1 and 2 as the percentage change of initial KHN. Baseline microhardness amounted to 350 KHN.

ANOVA revealed significant differences in KHN of Groups A-I in both bleached and unbleached enamel surfaces. Groups A-H were found to be significantly different from Group I (unfluoridated) at day 28 (end of

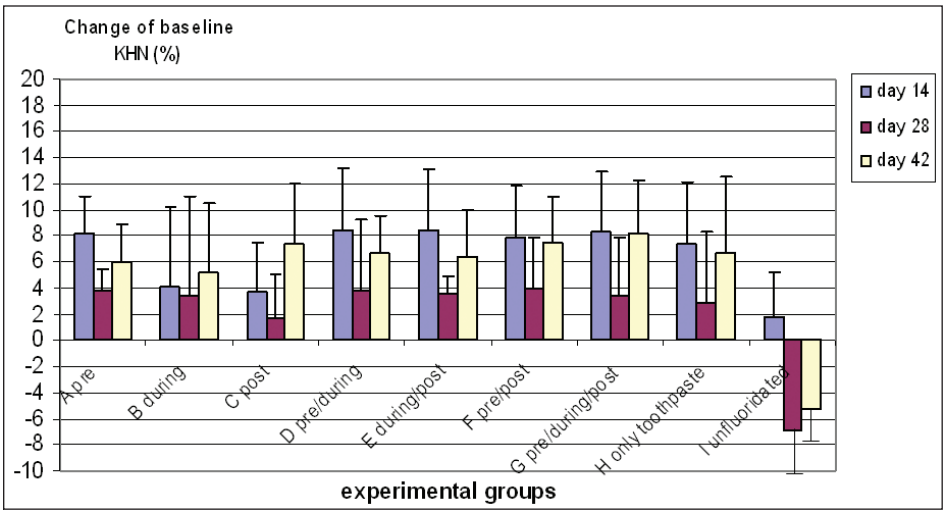


Figure 1: *Change of baseline KHN (mean ± standard deviation [%]) of bleached enamel surfaces in Groups A-I after the pre-bleaching period (day 14), the bleaching period (day 28) and the post-bleaching period (day 42). Groups A-H were fluoridated with toothpaste during the entire experiment and A-G were additionally supplemented by gel (pre-, during or post-bleaching).*

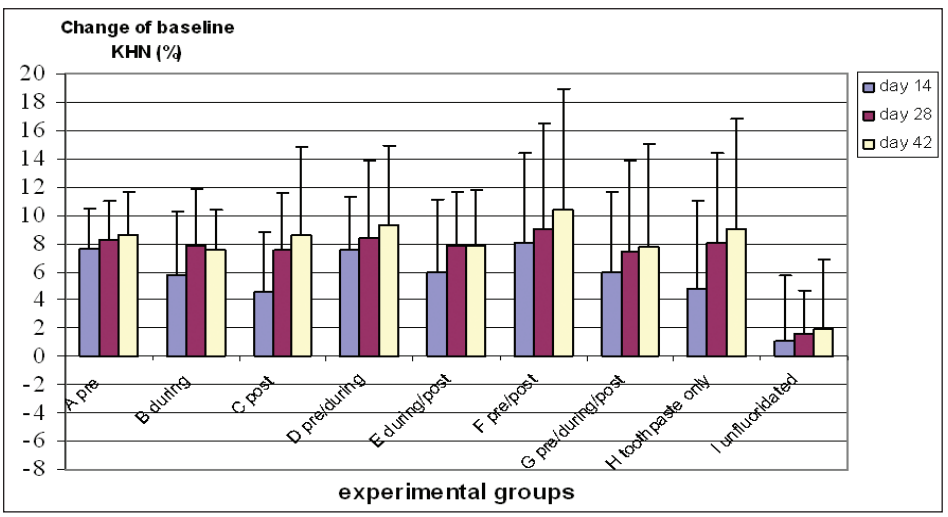


Figure 2: *Change of baseline KHN (mean ± standard deviation [%]) of unbleached enamel surfaces (control surfaces) in Groups A-I after the pre-bleaching period (day 14), the bleaching period (day 28) and the post-bleaching period (day 42). Groups A-H were fluoridated with toothpaste during the entire experiment and A-G were additionally supplemented by gel (pre-, during or post-bleaching).*

the bleaching period) and 42 (end of the post-bleaching period).

Generally, fluoridation treatment prevented microhardness loss during bleaching, but there were no significant differences between specimens treated with fluoridated toothpaste (Group H) and samples treated with fluoridated toothpaste and fluoride gel (Groups A-G). Therefore, microhardness at the end of the experiment was not significantly influenced by the time-period of gel fluoridation in Groups A-H of bleached and unbleached surfaces. Irrespective of the fluoride regime used, microhardness did not fall below baseline values during bleaching in Groups A-H.

After the bleaching (day 28) and post-bleaching period (day 42), statistical analysis revealed no significant differences in change of KHN between bleached and unbleached surfaces except for Group I (unfluoridated).

## DISCUSSION

From the results of this *in vitro* study, it is obvious that bleaching with 10% carbamide peroxide led to a slight but significant decrease in enamel microhardness when no fluoride agents were applied. This finding is in accordance with a number of previous studies that also showed a decrease in microhardness during bleaching treatment.[7,9,13,19-20] However, other studies reported that 10% carbamide peroxide gel did not affect enamel surface hardness.[21-23] These contradictory findings among different studies might be due to differences in method, such as frequency and time of bleaching treatment, storage media or the use of human or bovine teeth.

This study aimed to simulate clinical conditions. Therefore, the incubation time of carbamide peroxide and temperature during bleaching were performed in accordance with conditions of the overnight bleaching of vital human teeth. Also, frequency and duration of toothpaste and gel fluoridation of the samples were adjusted to the recommendations for regular oral hygiene measures. In contrast to a study by Lewinstein and others,[7] who also analyzed fluoridation effects on the hardness of bleached enamel, the samples were immersed in artificial saliva, instead of distilled water. Artificial saliva was used as the calcium and phosphate source for the remineralization of bleached enamel. However, it might be speculated that the beneficial effects of fluoridation shown in the current *in vitro* investigation might be more pronounced in the *in vivo* situation, as the presence of natural saliva might support further rehardening.

Fluoridation was shown to be effective in increasing the microhardness of enamel samples and preventing microhardness loss during bleaching. At the end of the bleaching and post-bleaching period, the microhardness of bleached and unbleached surfaces in Groups A-H was not significantly different. Similar effects were reported by Attin and others,[14] who found a complete rehardening of bleached enamel after the application of highly concentrated fluoride. However, treatment with lower concentrated fluoride (0.05% fluoride solution) led to a complete rehardening of the bleached enamel.[7] Therefore, rehardening is attributed to the incorporation of fluoride into the tooth surface, thus forming a calcium-fluoride rich layer.[15,24]

In the current study, the fluoridation treatment of bleached surfaces impeded microhardness loss below baseline values. Thereby, fluoridation by toothpaste slurry was shown to be as effective as fluoridation by a combined application of fluoridated toothpaste and fluoride gel. Because gel and toothpaste fluoridation had no additional beneficial effects on microhardness when compared to toothpaste fluoridation only, it is obvious that the time-period of gel fluoridation is also of minor importance. Therefore, it can be assumed that the additional use of fluoride gel in the pre- or post-bleaching period or during bleaching treatment with 10% carbamide peroxide gel is not necessary if toothpaste fluoridation is performed at least twice daily. However, the additional application of fluoride gel might be beneficial when toothpaste fluoridation is performed less than twice daily or at irregular intervals, respectively. Moreover, fluoride gels might be effective when the bleaching treatment is performed with highly concentrated carbamide peroxide or hydrogen peroxide gels. This assumption has to be verified in future studies.

## CONCLUSIONS

Due to the results of this study, it can be concluded that the regular application of fluoridated toothpaste is sufficient to avoid enamel microhardness loss during bleaching. The additional supplementation of fluoride gel is not superior to toothpaste fluoridation alone. Moreover, the time-period of fluoride gel application seemed to be of minor importance. In conclusion, fluoridation measures are effective in preventing microhardness loss due to 10% carbamide peroxide bleaching.

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