

## ORIGINAL ARTICLE

**Effect of home-bleaching gels modified by calcium and/or fluoride and the application of nano-hydroxyapatite paste on *in vitro* enamel erosion susceptibility**LUCIANA FLORIANI THIVES FREITAS SANTOS<sup>1</sup>, CARLOS ROCHA GOMES TORRES<sup>1</sup>, TACIANA MARCO FERRAZ CANEPELE<sup>1</sup>, ANA CAROLINA MAGALHÃES<sup>2</sup> & ALESSANDRA BUHLER BORGES<sup>1</sup><sup>1</sup>Department of Restorative Dentistry, Institute of Science and Technology, Univ Estadual Paulista UNESP, São José dos Campos, SP, Brazil, and <sup>2</sup>Department of Biological Sciences, Bauru School of Dentistry, USP, Bauru, SP, Brazil**Abstract**

**Objective.** This *in vitro* study compared the effect of bleaching agents modified by the addition of calcium and/or fluoride and the application of a nano-hydroxyapatite paste after bleaching, on the susceptibility of enamel to erosion. **Materials and methods.** Bovine enamel cylindrical samples (3 mm diameter) were assigned to six groups ( $n = 20$  specimens/group) according to the bleaching agent: no bleaching (C-control), 7.5% hydrogen peroxide gel (HP), HP with 0.5% calcium gluconate (HP+Ca), HP with 0.2% sodium fluoride (HP+F), HP with calcium and fluoride (HP+Ca+F) and HP followed by the application of a nano-hydroxyapatite agent (HP+NanoP). The gels were applied on the enamel surface (1 h) followed by cyclic erosive challenges (Sprite Zero<sup>®</sup>-2 min), for 14 days. The paste was applied after bleaching for 5 min (HP+NanoP). The enamel surface alteration was measured by contact profilometry ( $\mu\text{m}$ ) (after 7 and 14 days). **Results.** C-control (mean  $\pm$  SD:  $2.29 \pm 0.37$  at 7 days/ $4.86 \pm 0.72$  at 14 days) showed significantly lower loss compared to the experimental groups. HP+Ca ( $3.34 \pm 0.37/6.75 \pm 1.09$ ) and HP+F ( $4.49 \pm 0.92/7.61 \pm 0.90$ ) presented significantly lower enamel loss than HP ( $4.18 \pm 0.50/10.30 \pm 1.58$ ) only for 14 days and HP+Ca+F ( $4.92 \pm 1.03/8.12 \pm 1.52$ ) showed values similar to the HP+F group. The HP+NanoP ( $5.51 \pm 1.04/9.61 \pm 1.21$ ) resulted in enamel loss similar to the HP after 14 days. **Conclusions.** It was found that 7.5% hydrogen peroxide increased the susceptibility of enamel to erosion. The addition of calcium or fluoride to the bleaching gel reduced the erosion effect, while the nano-hydroxyapatite agent did not provide any protective effect.

**Key Words:** Enamel, erosion, tooth bleaching, hydrogen peroxide, tooth wear**Introduction**

Hydrogen peroxide is considered to be an effective dental bleaching agent, capable of whitening teeth with minimal expense. It is able to diffuse through the interprismatic and periprismatic enamel spaces [1]. Due to its inherent instability, it decomposes and produces free radicals. These unstable and unspecific released radicals oxidize the macromolecular chains of chromogens present inside dental tissues, altering their chemical structure by means of degradation of the molecular moieties responsible for absorbing visible electromagnetic radiation. This increases the total reflectance of the tooth substrate, resulting in a brighter appearance [2,3].

However, previous studies have reported that peroxides are associated with alterations of enamel surface morphology [4], microhardness values [5,6] and chemical composition [7,8]. Besides, erosion-like changes have been observed in enamel after bleaching treatments [9,10], especially when low pH and high-concentrated peroxides are used [11,12]. The reduction in microhardness can be associated with a higher susceptibility to erosive loss, since loss of surface hardness is the first step of erosion. Thus, it might be assumed that the bleaching agents could contribute to further erosion development, particularly in high-risk individuals, since, after each daily bleaching procedure, the teeth will almost certainly be exposed to demineralizing solutions, such as acidic beverages

[13]. Although home-bleaching treatment involves use of low-concentrated gels, it is performed over some weeks and there is concern that home-bleaching procedures may represent an additional risk factor for enamel erosion development and progression. Potentially additive harmful effects of bleaching and acid exposure on enamel have been reported, with different results observed according to the bleaching concentration, the study protocol [14,15] as well as the acid exposure regimen [13,16].

Remineralizing agents, such as fluoride and calcium, have been incorporated into bleaching gels, or applied on enamel surfaces directly after bleaching, as attempts to minimize the potential adverse effects to enamel during the bleaching treatment [12]. The novel bleaching gels have shown favorable results in reducing dental sensitivity and demineralization, as well as supporting remineralization [5,6,9]. Nevertheless, the protective effect of these remineralizing agents against potential detrimental effects of bleaching of enamel, associated with normal daily erosive challenges, is not properly investigated.

Recently, synthetic nano-hydroxyapatite (nano-HAP) has been considered as an interesting biocompatible and bioactive material. Studies have demonstrated that synthetic nano-sized hydroxyapatite particles have similar morphology, structure and crystallinity to dental apatite [17]. Recent reports have shown that nano-HAP has good potential to remineralize enamel carious lesions [18], but no information is available with respect to its effect when applied after bleaching on enamel erosion susceptibility.

In this study, the null hypotheses tested were that: (a) the bleaching agent will not increase enamel susceptibility to erosion after acid beverage exposure; and (b) the addition of calcium and/or fluoride in the bleaching gel and the application of nano-hydroxyapatite paste after bleaching will not reduce the erosion progression on enamel exposed to further acid challenges. Thus, the aim of this *in vitro* study was to evaluate the effect of 7.5% hydrogen peroxide gel alone and modified by the addition of calcium and/or fluoride and of a nano-hydroxyapatite paste applied after bleaching on the *in vitro* susceptibility of bleached enamel to erosion.

## Materials and methods

### *Experimental design*

This study tested two experimental factors: bleaching treatment with six levels (not-bleached and five different bleaching treatments) and time with two levels (7 days and 14 days), in an erosive-remineralizing cycling model using bovine enamel specimens ( $n = 20$ ). The response variable was surface loss (in  $\mu\text{m}$ ) measured by stylus profilometry.

### *Enamel specimens preparation*

Freshly extracted and intact bovine incisors were stored until required in a 0.1% thymol solution, refrigerated at 4°C. Cylindrical enamel samples (3 mm in diameter) were prepared from the labial surface of the tooth using a trephine mill.

The specimens were then positioned in a *silicon* mold with a cavity 6 mm in diameter and 3.1 mm in depth. On the bottom of the mold there was a second level cavity (3 mm in diameter and 0.1 mm in depth). The specimens were placed inside the internal cavity with the enamel surface directed towards the bottom of the mold, which was then filled with low viscosity composite resin (Opallis Flow, FGM, Joinville, SC, Brazil). On the side of the mold there was a projection in the shape of a line, which produced a lateral groove on the specimen. This helped to achieve the correct position at the time of surface profile measurements (before and after tests).

The specimens were attached to a metal holder and the enamel surface was polished using sequential aluminum oxide abrasive papers (1200, 2400 and 4000 grit x96 FEPA-P; Struers, Ballerup, Denmark) in a polishing device (DP-10, Panambra, Sao Paulo, SP, Brazil). The specimens were immersed in an ultrasonic bath with deionized water for 5 min (Ultrasonic Cleaner, Odontobras, Ribeirao, Preto, Brazil) for the removal of all waste. The prepared specimens were examined under the stereomicroscope (Carl Zeiss - Stemi 2000-20X Tokyo, Japan) to certify the absence of cracks or other surface defects and then stored in deionized water to avoid dehydration.

After polishing, the enamel specimens were selected from the average of the surface microhardness measured using a microhardness tester (FM-700, Future-Tech, Tokyo, Japan - Knoop tip, average of three indentations, under 25 g-load for 10 s) with an allowable variation within 20% of the mean. Microhardness average of each specimen was used for stratified allocation among six groups ( $n = 20/\text{group}$ ), so that the microhardness average of the groups was similar.

In order to maintain the reference surfaces for lesion-depth determination (profilometry) and allow exact replacement, two parallel grooves were marked as guides on the sides of the composite resin surface using a scalpel blade. The baseline profiles of the enamel surface were measured using a contact profilometer (MarSurf GD 25, Mahr, Göttingen, Germany). The diamond stylus moved from the first reference (composite resin) to the exposed area and then over to the other composite resin reference area (4.2 mm long). Three profile measurements were performed for each specimen at intervals of 0.25 mm.

### *Bleaching procedure and erosive challenge*

The specimens were divided in groups according to bleaching agent used: C (control), no bleach; HP,

Table I. Mean enamel wear ( $\mu\text{m}$ ) and standard deviation ( $\pm\text{SD}$ ) for each experimental group ( $n = 20/\text{group}$ ), after 7 and 14 days.

Bleaching treatment	7 days*	14 days*
C	2.29 ( $\pm 0.37$ ) <sup>a</sup>	4.86 ( $\pm 0.72$ ) <sup>cd</sup>
HP+Ca	3.34 ( $\pm 0.37$ ) <sup>b</sup>	6.75 ( $\pm 1.09$ ) <sup>e</sup>
HP	4.18 ( $\pm 0.50$ ) <sup>bc</sup>	10.30 ( $\pm 1.58$ ) <sup>g</sup>
HP+F	4.49 ( $\pm 0.92$ ) <sup>cd</sup>	7.61 ( $\pm 0.90$ ) <sup>ef</sup>
HP+Ca+F	4.92 ( $\pm 1.03$ ) <sup>cd</sup>	8.12 ( $\pm 1.52$ ) <sup>f</sup>
HP+NanoP	5.51 ( $\pm 1.04$ ) <sup>d</sup>	9.61 ( $\pm 1.21$ ) <sup>g</sup>

\* Values followed by different letters imply significant differences among the groups ( $p < 0.001$ ).

C, control, no bleaching; HP, 7.5% hydrogen peroxide gel; HP+Ca, hydrogen peroxide gel with 0.5% calcium gluconate; HP+F, hydrogen peroxide gel with 0.2% sodium fluoride; HP+Ca+F, hydrogen peroxide gel with calcium gluconate and sodium fluoride; HP+NanoP, hydrogen peroxide gel followed by nano-hydroxyapatite paste application.

bleached with 7.5% hydrogen peroxide gel (pH 5.62); HP+Ca, 7.5% HP gel with the addition of 0.5% calcium gluconate (pH 5.60); HP+F, 7.5% HP gel with the addition of 0.2% sodium fluoride (pH 5.65); HP+Ca+F, 7.5% HP gel with the addition of 0.5% calcium gluconate and 0.2% sodium fluoride (pH 5.66); and HP+NanoP, bleached with 7.5% HP gel followed by the application of remineralizing and desensitizing agent (NanoP) containing 10% nanoparticles of HAP, 0.2% NaF and 2% potassium nitrate (pH 8.60) (experimental formulation manipulated by the manufacturer, for daily use) (FGM, Joinville, SC, Brazil). The experimental gels were modified by the manufacturer (FGM), by adding the remineralizing agents in a 7.5% HP-based bleaching gel, without adjustments of pH.

A 2 mm thick layer of the bleaching gel was applied to the enamel surfaces for 1 h. After this period, the specimens were rinsed with deionized water to remove the bleaching agent (20 s) and immersed in artificial saliva for 2 h (pH 7.0). For the group HP+NanoP, after removal of the bleaching gel, the nano-HAP paste was applied on the enamel surface, rubbed in for 1 min with a micro-disposable applicator and left in place for an additional 5 min. Thereafter, the excess was removed from the surface with cotton pellets and the specimens were placed in artificial saliva (2 h). Artificial saliva was prepared according to Gohring et al. [19]: 22.1 mmol/l  $\text{NaHCO}_3$ ; 16.1 mmol/l KCl; 14.5 mmol/l NaCl; 2.6 mmol/l  $\text{KH}_2\text{PO}_4$ ; 0.8 mmol/l  $\text{H}_3\text{BO}_3$ ; 0.7 mmol/l  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ; 0.4 mmol/l KSCN; and 0.2 mmol/l  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  and adjusted to pH 7.0 with HCl solution.

After the described procedures, the specimens were subjected to cyclic erosive challenges. The cycles consisted of immersion in unstirred soft drink (20 ml/sample, Sprite Zero, Companhia Fluminense de Refrigerantes, Porto Real, RJ, Brazil), pH 2.6,

four-times per day for 2 min each time [20]. After each challenge the specimens were immersed in unstirred artificial saliva (pH 7.0, 20 ml/sample), at room temperature, for 2 h. The samples were kept in artificial saliva overnight. The beverage was replaced at the end of each challenge, and artificial saliva was renewed daily.

#### Profilometric analyses

After 7 and 14 days, enamel loss was measured with a contact profilometer, with the same specifications as performed in baseline profilometric measurements. The specimens were placed in a custom-made setting device in order to allow the exact replacement of the samples after the experimental procedures. The enamel loss was calculated after matching the baseline and post-treatment profiles, using the previously described grooves as guides. The depth of the bleached and eroded area for each specimen was calculated based on the subtraction of the two profiles, using a software (MarSurf -XCR 20 4.50-07 SP3, 2011).

#### Statistical analysis

Statistical assumptions were evaluated before statistical analysis. The results indicated that the data were normally distributed and, by plotting against predicted values, the uniformity was checked. None of the ANOVA assumptions were violated and the mean enamel loss ( $\mu\text{m}$ ) was calculated for each group. Data were analyzed using Repeated Measures (RM) ANOVA and *post-hoc* Tukey tests. Statistics for Windows software v. 8.0 (Statsoft, Tulsa, OK) was used for the calculations, with a significance level of 5%.

#### Results

The mean overall enamel losses ( $\mu\text{m}$ ) of the specimens for all experimental conditions are shown in Table I. RM ANOVA showed significant differences for both bleaching treatment and time, as well as for the interaction bleaching treatment vs time ( $p < 0.05$ ). All the bleaching treatments (with or without potential remineralizing agents) increased mean enamel loss, since the control group (no bleach) showed significantly lower mean overall enamel loss compared to the experimental groups at both 7 and 14 days. After 7 days, group HP+Ca exhibited the lowest mean enamel loss among the experimental groups and group HP+NanoP exhibited the highest mean enamel loss. After 14 days, groups HP+Ca and HP+F presented intermediate mean enamel loss. Group HP+Ca+F showed significantly higher mean enamel loss than HP+Ca, but similar to HP+F. Groups HP and HP+NanoP exhibited significantly

higher mean enamel loss compared to all other groups.

## Discussion

The results of this study showed significantly higher mean enamel loss in bleached and eroded groups compared to the control group subjected to erosion only after 7 and 14 days. Therefore, the first null hypothesis, that the enamel erosion susceptibility would not be affected by bleaching, was rejected. It can be speculated that the accumulated enamel microstructural effects related to the use of the bleaching agent itself, such as mineral alterations and erosion-like characteristics [9,10], cannot be completely repaired by artificial saliva exposure, leaving areas susceptible to further erosive damage [4,21]. Thus, after extended treatment, bleaching agents can affect the enamel surface and subsurface [22] to a point that intensifies the severity of the erosion induced by a soft drink on enamel.

Hydrogen peroxide diffuses into enamel, mainly along enamel proteins (amelogenin and enamelin), as the mineralized phase is much more compact, as suggested by Hegedus et al. [23]. As HP is decomposed, it releases free radicals that can become entrapped in the inner enamel structure, exerting a prolonged effect and, consequently, reacting with the organic components of enamel [23,24]. Given that the organic content is essential to maintain enamel integrity, this oxidizing effect can consequently affect the mineral phase of enamel [22], resulting in loss of carbonate [24] and increased porosity [23]. These porosities could, likewise, promote a fast spread of erosive agents inside enamel, resulting in faster demineralization over time [21].

The association between bleaching and erosion was investigated in previous studies and distinct outcomes were obtained. Engle et al. [25] did not find increased loss when 10% carbamide peroxide bleaching gels were applied on an enamel surface (10 h) followed by three erosion-remineralization-abrasion cycles/day consisting of immersion in 1% citric acid (2 min), brushing (40 strokes) and exposure to artificial saliva (60 min) for 5 days. Similarly, Pretty et al. [26] exposed enamel to 20 cycles of different concentrations of carbamide peroxide gels (2 h) and brushing (2 min), followed by immersion in 0.1% citric acid (14 h). Both authors observed no increased risk of enamel to demineralization. It is important to have in mind that both studies simulated the advanced erosion phase and possibly did not show any further effect of bleaching because abrasion might have removed the softened layer, minimizing the differences among the groups. Although brushing/abrasion is related to enamel loss and fluoride toothpastes are generally used on a daily basis, we did not include these variables in our study, as they could represent

confounding factors when the potentially remineralizing agents were investigated.

Fluoride and calcium have been added to bleaching gels in attempts to reduce enamel solubility, promote its remineralization and reduce tooth sensitivity. The saturation of bleaching gels with these ions could make their uptake by enamel possible, decreasing the deleterious effect and further demineralization [4,6].

The results indicate that, after 7 days of bleaching, enamel loss values were not significantly reduced with modified HP gels compared to HP alone and the nano-HAP did not prevent erosion at all. However, after 14 days of bleaching, HP with added calcium, fluoride or calcium/fluoride reduced enamel loss after exposure to the acid beverage by 34%, 26% and 21%, respectively, for groups HP+Ca, HP+F and HP+Ca +F compared to HP alone. Thus, the second null hypothesis was only proven to be incorrect for these groups and not for the HP+nano-P group.

The efficacy of bleaching gels has been shown to be unaffected by the addition of potentially remineralizing agents [4,27]. This supplementation is an attempt to reduce the mineral loss caused by the peroxide action on enamel [4] and could eventually minimize the potential erosive effect by subsequent acids contacting bleached enamel surface, as observed in the present study and in previous investigations, with different bleaching and remineralizing agents [16,21,28].

Although calcium and phosphate are naturally present in saliva, the addition of calcium gluconate in the bleaching gel could represent an extra source of these ions in the mouth. In the present study, HP supplemented with Ca was shown to be the most effective modified gel tested. Different forms of calcium have been added to bleaching gels, in an attempt to increase the chemical properties of bleached enamel [5,29]. The presence of calcium in the gel is expected to maintain the enamel calcium saturation during bleaching [6]. This may reduce changes produced by the bleaching itself and, consequently, the susceptibility to further erosion, by means of inhibiting the softening of the enamel surface. A similar mechanism is observed in the erosion modifying effect of calcium supplements to acidic preparations or solutions [30,31].

The protective effect of calcium compounds in enamel erosion has also been related to the deposition of calcium crystals on demineralized enamel [32,33], since a softened enamel structure can probably be re-hardened by either mineral precipitation on top of the surface or mineral deposition within the porous zone [34]. Nevertheless, this process is not able to restore the enamel original surface structure completely [34].

Additionally, the low amount of available calcium in the surrounding saliva may represent a limiting

factor in the enamel potential remineralization action of fluoride [35]. Thus, calcium has been added to fluoride-based preventive agents, as an attempt to increase their remineralizing potential. Accordingly, the present study tested the supplementation of bleaching gel with Ca+F. Although the HP gel modified by Ca+F decreased enamel loss compared to HP only gel, this effect was limited and did not provide any additional protection compared to HP +Ca. This might be attributed to a possible premature reaction between calcium and fluoride in the HP gel, limiting the ions available for reacting with the enamel surface. Indeed, the contact between these two ions should be avoided until their application on the tooth surface, in order to improve their action [36].

The use of hydroxyapatite preparations has been investigated in initial enamel caries remineralization models [18,37,38]. In addition to its desensitizing effect, these products were developed to act in the remineralization process, by providing calcium, phosphate and fluoride to dental tissues. It is reported that the size of the hydroxyapatite particles influences the remineralization ability [18]. The nano size tends to potentiate the chemical, biological and physical behavior of the nano-structured particles, due to the higher surface area, the hydration ability and to wetness and solubility properties.

It is hypothesized that nano-hydroxyapatite penetrates the enamel porosities of demineralized teeth, acts as a template allowing the precipitation process and attracts calcium and phosphate ions from the remineralizing solution, filling the pores of enamel crystals [37]. However, in the present study, the application of the Nano-P paste after the bleaching procedure did not significantly reduce the enamel loss compared to the unmodified HP group. This may be related to the high pH of the paste tested (8.60). It was previously demonstrated that an increase of pH from 4.0 to 7.0 resulted in a decrease of calcium and phosphate released from nano-hydroxyapatite [18].

Additionally, *in vitro* experimental conditions cannot simulate all the complexities of the *in vivo* oral environment. Therefore, in further studies, the effect of the Nano-P paste must be tested *in situ* or *in vivo*, to check whether the reaction between the paste and the enamel surface would be better in the presence of human saliva and acquired pellicle, which could stabilize the HAP particles on the enamel surface. Also, while calcium added in the bleaching gel inhibits the softening of the surface, the hydroxyapatite preparation is applied after bleaching, in order to achieve enamel re-hardening, which is a different mode of action, and can also explain the difference in the results.

Thus, it is suggested that the supplementation of bleaching gels with calcium or fluoride is of interest to minimize the erosion susceptibility of bleached enamel, especially considering the accumulated

detrimental effect of HP. It has to be noted, however, that this *in vitro* study used a bleaching/erosion/remineralization model and that *in vivo* there are additional factors that may interfere in both de- and remineralization dynamics, such as the presence of saliva and enzymes which could dilute or modify the bleaching gel, the protective effect of pellicle [39] and a possible effect of fluoride toothpaste abrasion [40]. Hence, caution should be taken to extrapolate results to the clinical scenario. Within the limits of this *in vitro* study, 7.5% hydrogen peroxide increased the enamel susceptibility to erosion over time and the addition of calcium or fluoride to the bleaching gel reduced the surface loss of bleached enamel after acid beverage exposure. The use of a remineralizing paste with nano-hydroxyapatite after bleaching did not offer protection against enamel erosion.

### Acknowledgments

This study was supported by Sao Paulo State Research Foundation (FAPESP 10/50637-7). The authors thank FGM Produtos Odontologicos for manufacturing the experimental bleaching agents.

**Declaration of interest:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

### References

- [1] Pignoly C, Camps L, Susini G, About I, Camps J. Influence of in-office whitening gel pH on hydrogen peroxide diffusion through enamel and color changes in bovine teeth. *Am J Dent* 2012;25:91-6.
- [2] Kawamoto K, Tsujimoto Y. Effects of the hydroxyl radical and hydrogen peroxide on tooth bleaching. *J Endod* 2004;30:45-50.
- [3] Dannacher JJ. Catalytic bleach: Most valuable applications for smart oxidation chemistry. *J Mol Catal* 2006;251:159-76.
- [4] Chen HP, Chang CH, Liu JK, Chuang SF, Yang JY. Effect of fluoride containing bleaching agents on enamel surface properties. *J Dent* 2008;36:718-25.
- [5] Borges AB, Samezima LY, Fonseca LP, Yui KC, Borges AL, Torres CR. Influence of potentially remineralizing agents on bleached enamel microhardness. *Oper Dent* 2009;34:593-7.
- [6] Cavalli V, Rodrigues LK, Paes-Leme AF, Soares LE, Martin AA, Berger SB, et al. Effects of the addition of fluoride and calcium to low-concentrated carbamide peroxide agents on the enamel surface and subsurface. *Photomed Laser Surg* 2011;29:319-25.
- [7] McCracken MS, Haywood VB. Demineralization effects of 10 percent carbamide peroxide. *J Dent* 1996;24:395-8.
- [8] Lee KH, Kim HI, Kim KH, Kwon YH. Mineral loss from bovine enamel by a 30% hydrogen peroxide solution. *J Oral Rehabil* 2006;33:229-33.
- [9] Pinheiro HB, Cardoso PE. Influence of five home whitening gels and a remineralizing gel on the enamel and dentin ultrastructure and hardness. *Am J Dent* 2011;24:131-7.
- [10] Xu B, Li Q, Wang Y. Effects of pH values of hydrogen peroxide bleaching agents on enamel surface properties. *Oper Dent* 2011;36:554-62.

- [11] Borges AB, Yui KC, D'Avila TC, Takahashi CL, Torres CR, Borges AL. Influence of remineralizing gels on bleached enamel microhardness in different time intervals. *Oper Dent* 2010;35:180–6.
- [12] Sun L, Liang S, Sa Y, Wang Z, Ma X, Jiang T, et al. Surface alteration of human tooth enamel subjected to acidic and neutral 30% hydrogen peroxide. *J Dent* 2011;39:686–92.
- [13] Ren YF, Amin A, Malmstrom H. Effects of tooth whitening and orange juice on surface properties of dental enamel. *J Dent* 2009;37:424–31.
- [14] Ushigome T, Takemoto S, Hattori M, Yoshinari M, Kawada E, Oda Y. Influence of peroxide treatment on bovine enamel surface-cross-sectional analysis. *Dent Mater J* 2009;28:315–23.
- [15] Abouassi T, Wolkewitz M, Hahn P. Effect of carbamide peroxide and hydrogen peroxide on enamel surface: An in vitro study. *Clin Oral Investig* 2011;15:673–80.
- [16] Borges AB, Torres CR, de Souza PA, Caneppele TM, Santos LF, Magalhaes AC. Bleaching gels containing calcium and fluoride: Effect on enamel erosion susceptibility. *Int J Dent* 2012;2012:347848.
- [17] Vandiver J, Dean D, Patel N, Bonfield W, Ortiz C. Nanoscale variation in surface charge of synthetic hydroxyapatite detected by chemically and spatially specific high-resolution force spectroscopy. *Biomaterials* 2005;26:271–83.
- [18] Huang S, Gao S, Cheng L, Yu H. Remineralization potential of nano-hydroxyapatite on initial enamel lesions: An in vitro study. *Caries Res* 2011;45:460–8.
- [19] Gohring TN, Zehnder M, Sener B, Schmidlin PR. In vitro microleakage of adhesive-sealed dentin with lactic acid and saliva exposure: A radio-isotope analysis. *J Dent* 2004;32:235–40.
- [20] Magalhaes AC, Stancari FH, Rios D, Buzalaf MA. Effect of an experimental 4% titanium tetrafluoride varnish on dental erosion by a soft drink. *J Dent* 2007;35:858–61.
- [21] Attin T, Kocabiyik M, Buchalla W, Hannig C, Becker K. Susceptibility of enamel surfaces to demineralization after application of fluoridated carbamide peroxide gels. *Caries Res* 2003;37:93–9.
- [22] Jiang T, Ma X, Wang Y, Tong H, Shen X, Hu Y, et al. Investigation of the effects of 30% hydrogen peroxide on human tooth enamel by Raman scattering and laser-induced fluorescence. *J Biomed Opt* 2008;13:13–9.
- [23] Hegedus C, Bistey T, Flora-Nagy E, Keszthelyi G, Jenei A. An atomic force microscopy study on the effect of bleaching agents on enamel surface. *J Dent* 1999;27:509–15.
- [24] Sato C, Rodrigues FA, Garcia DM, Vidal CM, Pashley DH, Tjäderhane L, et al. Tooth bleaching increases dentinal protease activity. *J Dent Res* 2013;92:187–92.
- [25] Engle K, Hara AT, Matis B, Eckert GJ, Zero DT. Erosion and abrasion of enamel and dentin associated with at-home bleaching: An in vitro study. *J Am Dent Assoc* 2010;141:546–51.
- [26] Pretty IA, Edgar WM, Higham SM. The effect of bleaching on enamel susceptibility to acid erosion and demineralisation. *Br Dent J* 2005;198:285–90; discussion 280.
- [27] Borges AB, Dantas RL, Caneppele TM, Borges AL, Rocha Gomes Torres C. Effect of remineralizing agents on the bleaching efficacy of gels. *Gen Dent* 2013;61:67–71.
- [28] Burgmaier GM, Schulze IM, Attin T. Fluoride uptake and development of artificial erosions in bleached and fluoridated enamel in vitro. *J Oral Rehabil* 2002;29:799–804.
- [29] Borges BC, Borges JS, de Melo CD, Pinheiro IV, Santos AJ, Braz R, et al. Efficacy of a novel at-home bleaching technique with carbamide peroxides modified by CPP-ACP and its effect on the microhardness of bleached enamel. *Oper Dent* 2011;36:521–8.
- [30] Hara AT, Zero DT. Analysis of the erosive potential of calcium-containing acidic beverages. *Eur J Oral Sci* 2008;116:60–5.
- [31] Magalhaes AC, Moraes SM, Rios D, Buzalaf MA. Effect of ion supplementation of a commercial soft drink on tooth enamel erosion. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess* 2009;26:152–6.
- [32] Tantbirojn D, Huang A, Ericson MD, Poolthong S. Change in surface hardness of enamel by a cola drink and a CPP-ACP paste. *J Dent* 2008;36:74–9.
- [33] Ranjitkar S, Rodriguez JM, Kaidonis JA, Richards LC, Townsend GC, Bartlett DW. The effect of casein phosphopeptide-amorphous calcium phosphate on erosive enamel and dentine wear by toothbrush abrasion. *J Dent* 2009;37:250–4.
- [34] Eisenburger M, Addy M, Hughes JA, Shellis RP. Effect of time on the remineralisation of enamel by synthetic saliva after citric acid erosion. *Caries Res* 2001;35:211–15.
- [35] Reynolds EC, Cai F, Cochrane NJ, Shen P, Walker GD, Morgan MV, et al. Fluoride and casein phosphopeptide-amorphous calcium phosphate. *J Dent Res* 2008;87:344–8.
- [36] Sullivan RJ, Masters J, Cantore R, Roberson A, Petrou I, Stranick M, et al. Development of an enhanced anticaries efficacy dual component dentifrice containing sodium fluoride and dicalcium phosphate dihydrate. *Am J Dent* 2001. 14 Spec No: 3A–11A.
- [37] Huang SB, Gao SS, Yu HY. Effect of nano-hydroxyapatite concentration on remineralization of initial enamel lesion in vitro. *Biomed Mater* 2009;4:034104.
- [38] Tschoppe P, Zandim DL, Martus P, Kielbassa AM. Enamel and dentine remineralization by nano-hydroxyapatite toothpastes. *J Dent* 2011;39:430–7.
- [39] Wiegand A, Bliggenstorfer S, Magalhaes AC, Sener B, Attin T. Impact of the in situ formed salivary pellicle on enamel and dentine erosion induced by different acids. *Acta Odontol Scand* 2008;66:225–30.
- [40] Magalhaes AC, Rios D, Delbem AC, Buzalaf MA, Machado MA. Influence of fluoride dentifrice on brushing abrasion of eroded human enamel: An in situ/ex vivo study. *Caries Res* 2007;41:77–9.