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Editorial Board

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Editorial

Dental Erosion

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Dental erosion is a condition defined as tooth substance loss by exogenous or endogenous acids without bacterial involvement. The most important sources are dietary acids and gastric acid in patients with regurgitation and reflux disorders. Dental erosion is a multifactorial condition, where different chemical, biological, and behavioral factors contribute to its development. Progressive erosive loss may lead to functional and aesthetic limitations, as well as hypersensitivity, and extensive restorative treatment often becomes necessary.

As there are signs that the prevalence of erosion is increasing in several countries, research in the field of dental erosion has expanded over the last few years. In this special issue, we have invited investigators to contribute with original research as well as review manuscripts that will stimulate the continuing efforts in the understanding of the development of erosive lesions. Thirteen papers were submitted, from which 5 were finally accepted (approval rate: 38.5%).

A. Mulic and coworkers present an investigation about dentists' general experience, knowledge about diagnosis, and treatment of dental erosive wear in young adults in Norway. They show that dentists are relatively up-to-date with respect to the clinical recording, diagnosis, and treatment of tooth erosive wear. However, dietary and salivary analyses were not given priority during the diagnosis process, and early and preventive treatment was lacking.

On the other hand, laboratory researchers have made concerted efforts to identify protective agents against erosion. Another paper tests if the in vitro application of fluoride (NaF), with or without hydroxyapatite (HAP), or casein phosphopeptide-amorphous calcium phosphate alone, were able to reduce early enamel erosion using nanohardness as the response variable. A. Z. Abdullah and coworkers demonstrate that 4500 mg/L fluoride, regardless of the presence of HAP, significantly increases the nanohardness of previously eroded enamel and reduces the progression of erosion.

However, not only the concentration, but also the type of fluoride salt might influence its protective effect against dental erosion. Accordingly, L. P. Comar and coworkers show a better in vitro effect of toothpastes containing TiF_4 or SnF_2 compared to NaF against enamel and dentin erosion and abrasion. These results are in accordance with previous studies showing that metal fluoride compounds performed better than conventional sodium fluoride in the prevention of tooth wear.

A. B. Borges and coworkers raise the question of whether bleached enamel is more susceptible to erosive wear. They tested the effect of adding calcium and fluoride to 35% hydrogen peroxide (HP) bleaching gel on the susceptibility of enamel to erosion provoked by a soft drink in vitro. While the bleaching gel alone does not increase the enamel susceptibility to erosion, the addition of calcium gluconate to the bleaching gel even results in a reduced susceptibility of the enamel to extrinsic erosive challenges.

Finally, use of sealants as a minimally-invasive approach to prevent the progression of erosive lesions is analysed with special regard to the effect of light-curing time (F. J. Wegehaupt and coworkers). The in vitro results show that given a constant energy density, light-curing time does not have an influence on the permeability and stability of the sealants.

We hope that this special issue makes interesting reading, and that these studies will encourage further research on the diagnosis, prevention, and therapy of dental erosion.

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Research Article

Effect of NaF, SnF_2, and TiF_4 Toothpastes on Bovine Enamel and Dentin Erosion-Abrasion In Vitro

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The aim of this study was to compare the effect of toothpastes containing TiF_4, NaF, and SnF_2 on tooth erosion-abrasion. Bovine enamel and dentin specimens were distributed into 10 groups ($n = 12$): experimental placebo toothpaste (no F); NaF (1450 ppm F); TiF_4 (1450 ppm F); SnF_2 (1450 ppm F); SnF_2 (1100 ppm F) + NaF (350 ppm F); TiF_4 (1100 ppm F) + NaF (350 ppm F); commercial toothpaste Pro-Health (SnF_2—1100 ppm F + NaF—350 ppm F, Oral B); commercial toothpaste Crest (NaF—1.500 ppm F, Procter & Gamble); abrasion without toothpaste and only erosion. The erosion was performed 4×90 s/day (Sprite Zero). The toothpastes' slurries were applied and the specimens abraded using an electric toothbrush 2×15 s/day. Between the erosive and abrasive challenges, the specimens remained in artificial saliva. After 7 days, the tooth wear was evaluated using contact profilometry (μm). The experimental toothpastes with NaF, TiF_4, SnF_2, and Pro-Health showed a significant reduction in enamel wear (between 42% and 54%). Pro-Health also significantly reduced the dentin wear. The toothpastes with SnF_2/NaF and TiF_4/NaF showed the best results in the reduction of enamel wear (62–70%) as well as TiF_4, SnF_2, SnF_2/NaF, and TiF_4/NaF for dentin wear (64–79%) ($P < 0.05$). Therefore, the experimental toothpastes containing both conventional and metal fluoride seem to be promising in reducing tooth wear.

1. Introduction

Erosion is a tooth lesion caused by acids that is currently receiving a lot of attention from researchers and clinicians. This fact is due to the reports of its increasing prevalence [1] and also to the increasing knowledge about erosion etiology and diagnosis. The erosion lesion can be divided in two phases: erosion (in that there is only softening) and erosive wear (advanced phase with tooth surface loss) [2, 3].

In modern societies extrinsic acids are becoming the most important source of erosive attacks through the increased consumption of acid drinks (mainly soft drinks) [4]. The acid attack, in the advanced phase, leads to both wear and softening of the enamel surface. The remaining softened layer, especially for enamel, presents low wear resistance, thus rendering it more susceptible to the effects of mechanical forces, such as tooth-brushing abrasion [5, 6].

Some studies have shown that the delay of 1 h of tooth-brushing after consuming acidic foods or drinks could minimize the deleterious effects on the eroded tooth surface [5–8]. However, this recommendation can be complicated in practice because it can interfere with the patient's routine. Therefore, new, more viable alternatives have been sought to prevent the effects of brushing on the eroded surface.

Tooth-brushing abrasion of eroded enamel and dentin is mainly influenced by the abrasivity of the toothpaste, and to a lesser extent by the toothbrush itself [9, 10]. Despite the possible abrasive effect of the toothpaste on eroded tooth surfaces, it plays an important role in maintaining general oral health (caries and periodontal diseases), and the daily fluoride exposure provides at least a slight protection against erosive demineralization from everyday exposure to acidic foods and drinks [11].

Most commercial toothpastes have NaF as a fluoride agent. The beneficial effect of this conventional fluoride is associated with the formation of a CaF_2-like precipitate on the tooth surface, which acts mainly as a fluoride reservoir and can partially behave as an acid-resistant layer [12, 13]. However, NaF presents limited beneficial effects compared to nonfluoridated toothpastes on the abrasion of eroded teeth [11, 14–19]. Based on this, recent studies have focused on fluoride compounds that might have a higher efficacy due to surface precipitation or incorporation of ions into demineralized tissue.

Accordingly, metal fluoride, such as SnF_2 or TiF_4, has been tested. SnF_2 or $SnCl_2$/NaF solution has shown a promising erosion-inhibiting effect both in vitro and in situ [20–22]; however, there are only limited results published about the effects of toothpastes containing SnF_2 on erosion-abrasion [11, 23, 24]. In vitro and in situ studies have also demonstrated a considerable erosion-protection ability of the TiF_4 solutions/varnish [25–30]. However, there is no study testing the effect of TiF_4 toothpaste.

Therefore, the present in vitro study aimed to compare the effect of TiF_4, NaF, and SnF_2 toothpastes on enamel and dentin erosion and abrasion. The null hypothesis tested was that there is no significant difference among the performance of the fluoridated and nonfluoridated toothpastes to protect the eroded enamel and dentin against abrasion.

2. Materials and Methods

2.1. Preparation of the Specimens. One hundred and twenty enamel and dentin specimens (4 mm × 4 mm × 3 mm) were prepared from the labial surface of the bovine crown (one specimen/tooth, total: 120 teeth) and the labial/lingual outer surfaces of the cervical portion of bovine roots (two specimens/tooth, total: 60 teeth), respectively. The teeth were stored in 0.1% buffered thymol solution (pH 7.0) at 4°C. The specimens were cut using an ISOMET low-speed saw-cutting machine (Buehler Ltd., Lake Bluff, IL, USA) with two diamond disks (Extec Corp., Enfield, CT, USA), which were separated by a 4 mm thickness spacer. The specimens' surfaces were ground flat using water-cooled silicon carbide discs (320, 600, and 1200 grades of Al_2O_3 papers; Buehler, Lake Bluff, IL, USA) and polished using felt paper wet with diamond solution (1 μm; Buehler). After the polish, the specimens were cleaned in an ultrasonic device with deionized water for 5 min. The removal of the cement from the root dentin by polishing was checked using a microscope (×40 magnification). Then, 2/3 of the outer surface (control, without treatment) specimens were covered with nail varnish in order to create control areas on both sides of a central band of enamel or dentin.

2.2. Experimental Groups, pH and F Analysis. Each 12 enamel and 12 dentin specimens were randomly allocated to each of 10 groups: placebo toothpaste without fluoride; experimental toothpaste with NaF (1450 ppm F); TiF_4 (1450 ppm F); SnF_2 (1450 ppm F); SnF_2 (1100 ppm F) + NaF (350 ppm F); TiF_4 (1100 ppm F) + NaF (350 ppm F); commercial toothpaste Pro-Health (SnF_2—1100 ppm F + NaF 350 ppm F, pH 5.7, Oral B); commercial toothpaste Crest (NaF—1500 ppm F, pH 6.9, Procter & Gamble); abrasion without toothpaste; only erosion.

The experimental toothpastes were prepared by a Brazilian pharmacy (Farmácia Específica, Bauru, SP, Brazil). The experimental toothpastes presented the same composition except for the presence or not of fluoride and the type of fluoride salt.

The fluoride concentrations and pH of the toothpastes were further checked. The pH was analyzed in duplicate using an electrode and digital pH meter, after checking the standards (pH 4 and pH 7). The total fluoride (TF), soluble fluoride (SF), and ionic fluoride (IF) were evaluated following the study of Bardal et al. [31].

To analyze the TF, 0.25 mL of 2 M HCl was added to 0.25 mL of the suspension of each toothpaste (0.5 g toothpaste/50 mL deionized water). This was kept at 45°C/1 h and 0.50 mL of 1 M NaOH and 1.0 mL of TISAB II were added. To assay SF and IF, after centrifugation, the supernatant was used, and the same steps described above were followed for SF. The analysis of IF was made by adding 0.25 mL of the supernatant to 1.0 mL of TISAB II, 0.5 mL of 1 M NaOH, and 0.25 mL of 2 M HCl. The analysis was done in duplicate using an ion-specific electrode (Orion 96-09) coupled to a potentiometer. The standard curve (0.5–4 ppm F) was prepared in triplicate using a standard fluoride solution (NaF, 100 ppm, Orion no. 940907). The potential (mV) was converted in μg F using the standard curve with an $r \geq 0.99$.

2.3. Treatment, Erosive, and Abrasive Challenges. All specimens were submitted to a 7-day erosive de- and remineralization cycling. Erosion was performed with a freshly opened bottle of the drink Sprite Zero (Coca-Cola Company Spal, Porto Real, RJ, Brazil, pH 2.6, 30 mL/specimen, unstirred, 25°C) four times daily for 90 s each. After each demineralization, the specimens were rinsed with deionized water (10 s) and transferred into artificial saliva (pH 6.8, 30 mL/specimens, unstirred, 25°C) for 2 h. After the last daily erosive treatment, the specimens were also stored in artificial saliva overnight. The artificial saliva was renewed daily and consisted of 0.2 mM glucose, 9.9 mM NaCl, 1.5 mM $CaCl_2 \cdot 2H_2O$, 3 mM NH_4Cl, 17 mM KCl, 2 mM NaSCN, 2.4 mM K_2HPO_4, 3.3 mM urea, 2.4 mM NaH_2PO_4, and 11 μM ascorbic acid (pH 6.8) [32].

Twice a day (after the first and the last erosive challenges), the treatment was carried out with the slurries of the toothpastes (1 toothpaste: 3 deionized water, v = 0.5 mL/specimen) associated with abrasion, for 15 s each. The toothbrushes were fixed in the constructed device that allowed the heads of the toothbrushes to be aligned parallel to the surface of the specimens. The tooth-brushing head was weighted by a precision scale (Pesola, Switzerland) and the weight was converted to power (1 kg—9.80665 N, F = 1.5 N) [33]. After the tooth brushing, the specimens were rinsed in water for 10 s before being immersed in artificial saliva.

Table 1: Mean (±sd) of fluoride concentration (ppm or μg/g) and pH of the experimental toothpastes.

Toothpastes	pH	Ionic fluoride	Soluble fluoride	Total fluoride
Placebo	5.6	31.1 ± 6.2	52.5 ± 13.9	48.6 ± 5.6
NaF 1450 ppm F	6.0	1324.5 ± 70.4	1323.3 ± 111.0	1324.5 ± 70.4
TiF_4 1450 ppm F	3.8	1403.9 ± 31.4	1606.2 ± 31.8	1443.2 ± 8.1
SnF_2 1450 ppm F	3.7	1307.2 ± 14.6	1406.5 ± 3.9	1323.6 ± 66.6
SnF_2 (1100 ppm F) + NaF (350 ppm F)	3.9	1474.9 ± 12.4	1522.7 ± 46.9	1567.0 ± 109.5
TiF_4 (1100 ppm F) + NaF (350 ppm F)	4.4	1275.3 ± 57.1	1203.6 ± 76.1	1207.3 ± 81.1

2.4. Profilometric Measurement. Enamel and dentin wear (μm) was quantitatively determined by a contact profilometer MSW 250/GD25 (Mahr Perthometer, Göttingen, Germany) after the experiment. For profilometric measurement, the nail varnish was carefully removed using a scalpel and acetone solution (1 : 1 water). During the measurement, the specimens were maintained in water (100% humidity) to avoid shrinkage of the dentin. The diamond stylus (2 μm in diameter, angulation 90°) moved from the first reference to the exposed area and then over to the other reference area (2 mm long and 1.5 mm wide). Four profile measurements were performed for each specimen at intervals of 0.5 mm. The vertical distance between the horizontal line drawn on the reference areas and the horizontal line drawn on the experimental area was defined as tooth wear using the software (Software MahrSurf XT20, 2009). The values were averaged (μm) and submitted to statistical analysis. The detection limit for the contact profilometry (MahrSurf XT20) is about 0.5 μm of tooth wear. The standard deviation of repeated analysis (the reproducibility of the method) of a given sample, without removing it from the holder, was 0.2 μm (mean wear of 2.8 μm for enamel) and 0.5 μm (mean wear of 3.4 μm for dentin).

2.5. Statistical Analysis. The software GraphPad InStat (GraphPad Software, San Diego, CA, USA) was used. The assumptions of equality of variances and normal distribution of data were checked for all the variables tested, using the Bartlett and Kolmogorov-Smirnov tests, respectively. As the equality of variances was satisfied for enamel, the differences among the treatments were analyzed using ANOVA followed by Tukey's test. For dentin, the equality of variances was not satisfied, and the differences among the treatments were analyzed using Kruskall-Wallis followed by Dunn tests. The level of significance was set at 5%.

3. Results

Table 1 shows the mean values and standard deviation of the fluoride concentration and pH of the experimental toothpastes.

Table 2 shows the values of enamel and dentin wear. The enamel wear caused by erosion only was similar that caused by erosion plus abrasion without toothpaste. However, the enamel erosive wear was lower than the erosive and abrasive wear in the presence of the placebo toothpaste. The experimental toothpastes with NaF, SnF_2, and TiF_4, as well as the SnF_2/NaF commercial toothpaste (Pro-Health), showed similar effect, reducing the enamel wear between 42% and 54% compared to the placebo toothpaste. The toothpastes containing SnF_2/NaF and TiF_4/NaF presented the best results regarding the enamel wear reduction (62–70% compared to the placebo toothpaste). Additionally, the combination of fluoride salts significantly reduced enamel wear compared to erosion only. The NaF-commercial toothpaste (Crest) was not significantly different than the placebo toothpaste.

The dentin wear caused by erosion was also similar to that caused by erosion plus abrasion (with or without the placebo toothpaste). Both experimental and commercial NaF toothpastes were unable to reduce dentin wear. The commercial SnF_2/NaF toothpaste (Pro-Health) significantly reduced dentin wear compared to the placebo, but not compared to tooth brushing without toothpaste or erosion only. The experimental toothpastes containing TiF_4 and SnF_2 significantly reduced dentin wear (64–73% compared to the placebo toothpaste). The toothpastes containing SnF_2/NaF and TiF_4/NaF also significantly reduced dentin wear (70–78% compared to the placebo toothpaste). Additionally, the metal fluoride alone or in combination in the experimental toothpastes significantly reduced dentin wear compared to erosion only.

4. Discussion

The present in vitro study assessed the effect of the experimental fluoridated toothpastes against tooth erosive and abrasive wear. The in vitro models are particularly well suited to experiments whose objective is to test a single process in isolation, where a more complex situation with many variables may confound the data. In this particular case, the present study followed the guidelines for the development and application of models for erosion research previously discussed by Shellis et al. [3]. We used bovine as a substitute for human teeth, due to the facility to obtain and prepare the samples from the former teeth. Although accurate data regarding the comparative properties of human and bovine hard dental tissue remain scarce, it is acceptable to use bovine as a substitute for human enamel and dentin [34, 35]. However, possible morphological and chemical property differences between bovine and human teeth substrates should be considered when the data are extrapolated to the clinical conditions.

In the present study, samples from the outer surface of the cervical root region were submitted to the erosive and

TABLE 2: The erosion-abrasion wear in the presence of the different toothpastes (μm).

Group	Enamel wear mean (±sd)	Dentin wear median (min–max)
Placebo	5.0 ± 0.9^{a}	$10.8\ (7.5–13.5)^{a}$
NaF 1450 ppm F	2.9 ± 0.7^{c}	$5.6\ (3.6–9.1)^{abcd}$
TiF_4 1450 ppm F	2.3 ± 0.7^{cd}	$2.9\ (1.3–6.2)^{de}$
SnF_2 1450 ppm F	2.8 ± 1.1^{c}	$3.9\ (2.1–6.9)^{cde}$
SnF_2 (1100 ppm F) + NaF (350 ppm F)	1.7 ± 0.7^{d}	$2.3\ (1.2–3.7)^{e}$
TiF_4 (1100 ppm F) + NaF (350 ppm F)	1.5 ± 0.4^{d}	$3.2\ (1.0–5.8)^{de}$
Pro-Health (Oral B)	2.9 ± 1.0^{c}	$4.6\ (3.0–7.9)^{bcde}$
Crest (Procter & Gamble)	4.1 ± 0.9^{ab}	$7.1\ (4.3–10.0)^{abc}$
Without toothpaste	4.1 ± 0.9^{ab}	$7.7\ (6.1–11.4)^{ab}$
Only erosion	3.1 ± 0.9^{bc}	$9.7\ (4.7–12.9)^{ab}$

Different letters indicate statistically significant differences among the groups (comparison within the same column, $P < 0.0001$).

abrasive challenges, since this region is the first one exposed in patients suffering from periodontal recession.

The contact profilometry is a method widely applied for analysis of tooth erosive/abrasive wear [36]. However, this method has some limitations, as it can damage the surface of the specimen, and in the case of dentin it can press or even penetrate into the demineralized organic layer in some extension, which is related to the moisture of the tissue. When it is important to consider the organic layer in the measurement of the dentin wear, the optical profilometry might be a more suitable method; however, when the loss of mineralized tissue is desirable, the organic layer should be removed prior to the use of contact profilometry [37, 38]. In the present study, to overcome partially this limitation, the dentin was kept in water during the measurement.

Accordingly, the results of dentin wear in the present study must be interpreted taking into account the presence of the hydrated organic material. However, it should be kept in mind that the stylus may have penetrated into the demineralized organic layer, so some inaccuracy in the measurement might be expected. Considering this limitation, great variation on dentin wear was found. Therefore, any lack of effect of the treatments on dentin must be carefully interpreted [22].

The abrasive wear of eroded dental hard tissues is considered an adverse side effect of tooth brushing, and it is mainly determined by toothpaste abrasivity and pH [9, 10]. The present study confirmed this finding for enamel, in which the association between erosion and abrasion (in the presence of a placebo toothpaste) caused a higher wear than erosion only. However, this was not true in case of dentin, in accordance with Ganss et al. [39], who showed that significant effects of brushing were only found on the demineralized organic fraction (the toothbrush pressed the layer of collagen fibrils), but the mineral loss was unaffected.

In the presence of conventional fluoride (experimental NaF toothpaste), the enamel and dentin wear was reduced; however, the preventive effect was less pronounced than with the other fluoride salts, especially for dentin. The commercial version of NaF toothpaste (Crest) was unable to protect against tooth erosion and abrasion. The reason for the lack of effect of NaF commercial toothpaste might be related to its higher abrasivity. The fluoride effects might be partly masked by the toothpaste abrasivity, as shown by Moron et al. [40] and Hara et al. [41]. Generally, toothpastes with NaF have shown no or partial effect against tooth wear [11, 14, 15, 18, 19], which might be explained by the low acid resistance of the CaF_2 layer, especially in case of in vitro models [13].

Following the results, the experimental TiF_4 and SnF_2 toothpastes, as well as the commercial Pro-Health toothpaste, were able to similarly reduce tooth wear, which was more pronounced in the case of dentin. This result showed that the effect of both metal fluorides is similar using this experimental model. Furthermore, even some differences in the abrasivity might be found between the experimental and commercial toothpastes containing SnF_2, though this seems to be of less impact considering the presence of this metal fluoride.

One of the first studies conducted testing SnF_2 toothpastes against tooth erosion showed that SnF_2 toothpastes markedly reduced the dissolution of teeth in vivo, whereas NaF provided no protection [23]. Recently, Ganss et al. [11] and Faller et al. [42] have confirmed the superiority of SnF_2 compared to NaF toothpastes to prevent dental erosion; however, for erosion-abrasion SnF_2 toothpaste had no effect in vitro [11]. Contrary, Huysmans et al. [24] showed that SnF_2-containing toothpastes significantly reduced erosive and abrasive wear compared to the NaF toothpaste using in situ protocol. Considering all findings, it seems that SnF_2 is an effective fluoride salt to be incorporated into toothpaste for the prevention of tooth erosion and abrasion.

For dentin, the SnF_2 has the advantage of reacting differently with the surface regardless of the presence of the collagen layer compared to the effect of NaF, which is dependent on the presence of the organic layer [43]. In cases in which the organic matrix is preserved, phosphoproteins might attract the tin ion, which is then retained in the organic matrix to some extent but also accumulates in the underlying mineralized tissue. In cases in which the organic matrix is removed, tin reacts with the mineral by precipitating different salts [44]. Furthermore, the inclusion of SnF_2 into toothpaste could also minimize some side effects such as tooth staining and astringent taste, which were found for solutions containing high tin concentrations [21].

In respect to TiF_4, there is no study testing the inclusion of this metal fluoride into toothpaste. Considering previous findings for the TiF_4 solution and varnish, the positive effect found in the present study might be related to the precipitation and formation of an acid-resistant layer (glaze) by the interaction of titanium and phosphate from the tooth [45]. With respect to the interaction between TiF_4 and dentin, it is not known if the reaction with a dentin surface might change in the presence or absence of a demineralized organic layer. Previous studies have shown that titanium tetrafluoride solution induces some coating on dentin surfaces, which partly covered dentinal tubules [46]. However, its protective potential did not exceed the efficacy of NaF or AmF [29, 46, 47].

The present study was the first that associated a conventional and metal fluoride into experimental toothpastes, especially in the case of TiF_4 and NaF. The toothpastes with SnF_2/NaF and TiF_4/NaF presented the best results in reducing tooth wear, even though they were similar to toothpastes containing only metal fluorides. Furthermore, the wear presented by the samples abraded using TiF_4/NaF and SnF_2/NaF toothpastes was also reduced compared to erosion only, which might be related to the surface precipitation (CaF_2 plus metallic layers) or to the incorporation of ions (tin and Ti) into the demineralized tissue, making it more resistant not only to abrasion, but also to further erosive challenges.

The effect of adding metal and conventional fluorides into toothpaste should be confirmed using in situ models. The stability and the abrasivity (REA/RDA) of the experimental products, as well as the mechanism of action, should be further investigated. Based on the findings, it can be concluded that the association between conventional and metal fluorides in toothpastes might be a promising strategy to reduce tooth wear provoked by the synergic effect of erosion and abrasion in vitro.

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Research Article

Bleaching Gels Containing Calcium and Fluoride: Effect on Enamel Erosion Susceptibility

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This *in vitro* study evaluated the effect of 35% hydrogen peroxide (HP) bleaching gel modified or not by the addition of calcium and fluoride on enamel susceptibility to erosion. Bovine enamel samples (3 mm in diameter) were divided into four groups ($n = 15$) according to the bleaching agent: control—without bleaching (C); 35% hydrogen peroxide (HP); 35% HP with the addition of 2% calcium gluconate (HP + Ca); 35% HP with the addition of 0.6% sodium fluoride (HP + F). The bleaching gels were applied on the enamel surface for 40 min, and the specimens were subjected to erosive challenge with Sprite Zero and remineralization with artificial saliva for 5 days. Enamel wear was assessed using profilometry. The data were analyzed by ANOVA/ Tukey's test ($P < 0.05$). There were significant differences among the groups ($P = 0.009$). The most enamel wear was seen for C ($3.37 \pm 0.80\,\mu$m), followed by HP ($2.89 \pm 0.98\,\mu$m) and HP + F ($2.72 \pm 0.64\,\mu$m). HP + Ca ($2.31 \pm 0.92\,\mu$m) was the only group able to significantly reduce enamel erosion compared to C. The application of HP bleaching agent did not increase the enamel susceptibility to erosion. However, the addition of calcium gluconate to the HP gel resulted in reduced susceptibility of the enamel to erosion.

1. Introduction

Tooth whitening is a highly desirable esthetic treatment, as the tooth color is one of the most important factors related to the patients' satisfaction with their appearance [1].

Dental bleaching treatments are mainly based on the action of hydrogen peroxide, which is able to penetrate the tooth structure and release free radicals, oxidizing the chromophore molecules. Such molecules are mainly organic, although inorganic molecules can also be affected by these reactions [2]. Nevertheless, the free radical reaction is not specific and it may also alter the organic component of enamel [3]. Since the organic content contributes to the integrity of enamel, different adverse effects on both mineral and organic parts of bleached enamel have been observed [3].

Alterations in enamel surface morphology [3–6], chemical composition [7–10], and microhardness values [3, 11, 12] after bleaching were previously reported. Furthermore, some changes in bleached enamel were also described as slight erosive effects promoted by the bleaching agent [6, 13]. Nevertheless, some authors claim that the erosive pattern on the surface of bleached enamel only occurs when bleaching gels with low pH are used [14, 15].

Attempts to minimize the adverse effects of bleaching treatments by increasing enamel remineralization have been conducted, however, the results are contradictory. De Oliveira et al. [16] observed no significant increase of bleached enamel microhardness when calcium and fluoride were added to 10% carbamide peroxide gel. On the other hand, Borges et al. [12] observed a significant increase of enamel microhardness after bleaching with 35% hydrogen peroxide agent with the addition of calcium and fluoride. Chen et al. [6] also reported a less distinct erosion pattern on the surface of enamel bleached with fluoridated gels.

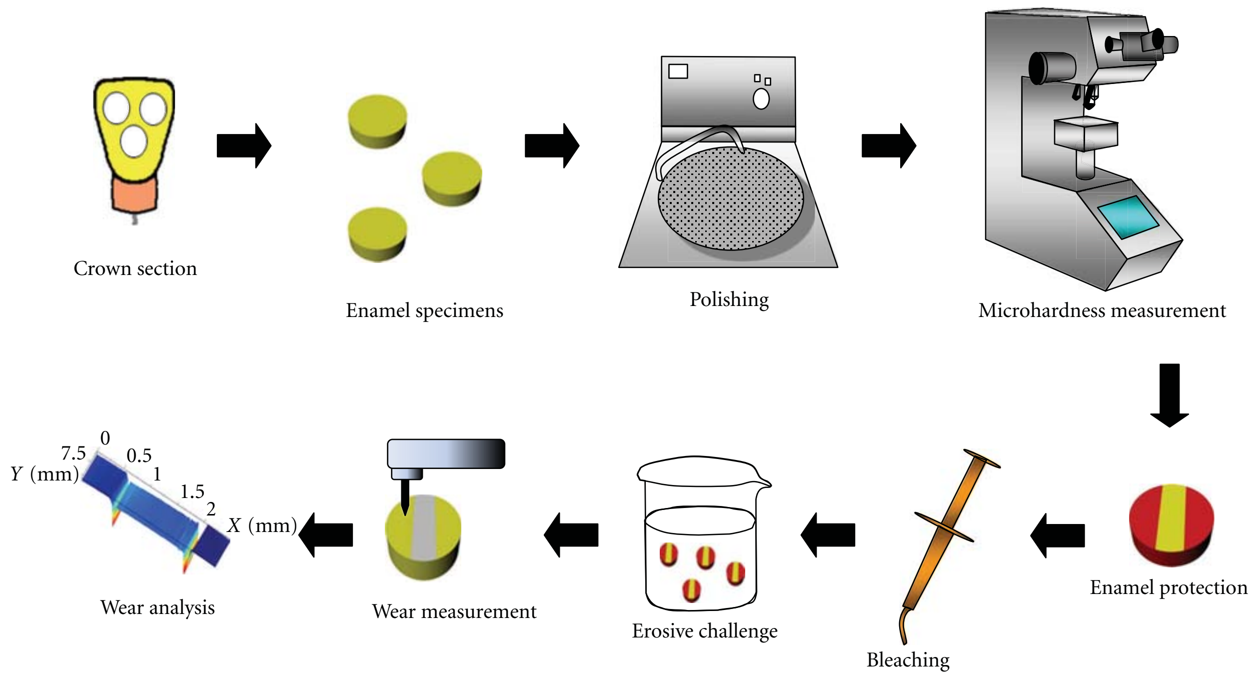

Figure 1: Schematic representation of sample preparation and analysis.

The association between the bleached enamel surface alterations and the subsequent susceptibility to erosive lesions resulting from the contact of bleached enamel with demineralizing solutions has been discussed. In a previous study, the application of carbamide peroxide gel rendered enamel more susceptible to demineralization [17]. In other studies, at-home bleaching technique did not increase the susceptibility of enamel to erosion [18, 19]. However, the effect of at-office bleaching agent (35% HP) on the enamel susceptibility to erosion has not been properly discussed.

Considering the possibility that bleaching gels with high concentration of HP could increase the susceptibility of enamel to erosion, the addition of remineralizing ions into bleaching gels would be beneficial for preventing a further enamel demineralization [6].

Therefore, the objective of this *in vitro* study was to evaluate the effect of bleaching agents based on 35% hydrogen peroxide modified or not by the addition of calcium or fluoride on the susceptibility of enamel to erosion. The null hypotheses tested were (1) 35% HP bleaching agent does not increase the susceptibility of enamel to erosive challenges and (2) there is no difference in erosion susceptibility of enamel that has been treated with hydrogen peroxide compared to enamel treated with hydrogen peroxide supplemented with calcium or fluoride.

2. Materials and Methods

2.1. Preparation of the Samples. Freshly extracted nondamaged and intact bovine incisors were stored in a 0.1% thymol solution and refrigerated at 4°C, until required. Cylindrical enamel samples (3 mm in diameter and 1 mm height) were prepared from the labial surface of the tooth using a trephine mill (Dentoflex, São Paulo, SP, Brazil). The enamel surface was ground flat and polished with water-cooled silicon carbide (SiC) paper discs (1200, 2500, and 4000 grit; Fepa-P, Panambra, São Paulo, SP, Brazil), thereby removing approximately 200 μm of the outermost layer as verified with a micrometer (Micromar 40EXL, Mahr-Goettingen, Germany).

The specimens were immersed in deionized water and placed in an ultrasonic bath for 10 min (Ultrasonic Cleaner, Odontobras, Ribeirao Preto, Brazil) for the removal of all waste, and then stored in thymol solution at 0.1% for rehydration.

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 4 groups ($n = 15$), so that the average microhardness for each group was similar. In order to maintain the reference surfaces for lesion-depth determination (profilometry), 2 layers of nail varnish were applied on 2/3 of the surface of each sample (1/3 of each side) exposing a central band area (see Figure 1).

2.2. Bleaching Procedures. The groups were divided as follows: C (control)-nonbleached, HP-bleached with 35% hydrogen peroxide gel without addition of remineralizing agents (pH = 6.35), HP + Ca-bleached with 35% hydrogen peroxide gel with the addition of 2% calcium gluconate (pH = 7.99), and HP + F-bleached with 35% hydrogen peroxide gel modified by the addition of 0.6% sodium fluoride (pH = 8.11).

The experimental groups were subjected to 35% hydrogen peroxide-based bleaching agents and modified by the manufacturer (based on Whiteness HP Blue formulation-FGM Dental Products, Joinville, SC, Brazil). The pH of the agents was measured using a pH meter (Digimed DM-20-Digicrom Analítica Ltda., São Paulo, Brazil) fitted with an electrode (DME-Digimed CV8) that was calibrated using solutions with pH 4.01 and 6.86.

A layer of approximately 2 mm of the bleaching gel was applied on the enamel surface for 40 minutes. The gel was periodically shaken to remove the air bubbles formed during the procedure. After this period, the specimens were rinsed with deionized water to remove the bleaching agent (20 s).

2.3. Erosive Challenge. After the bleaching procedures, the enamel samples were immersed in artificial saliva for 2 h and then subjected to erosive challenges for 5 days. The erosive cycles consisted of immersion in unstirred soft drink (20 mL/sample, Sprite Zero, Companhia Fluminense de Refrigerantes, Porto Real, RJ, Brazil), pH 2.8, four times per day for 2 min each time [20], followed by a remineralizing period of 2 h between erosive challenges (immersion in unstirred artificial saliva, pH 7.0, 20 mL/sample), at room temperature in small containers. The samples were kept in artificial saliva overnight. The composition of the artificial saliva was the same as used by Göhring et al. [21]: hydrogen carbonate (22.1 mmol/L), potassium (16.1 mmol/L), sodium (14.5 mmol/L), hydrogen phosphate (2.6 mmol/L), boric acid (0.8 mmol/L), calcium (0.7 mmol/L), thiocyanate (0.4 mmol/L), and magnesium (0.2 mmol/L). The pH was adjusted to 7.0 using concentrated HCl.

The beverage was replaced at the end of each challenge, and artificial saliva was renewed daily.

2.4. Surface Wear Assessment. Profiles were obtained from the enamel surfaces with a profilometer (MaxSurf XT 20, Mahr-Goettingen, Germany) after the erosive challenge. To determine the change in the surface profile after the experiment, the nail varnish was carefully removed with a spatula and a solution of acetone (1 : 1-acetone : water) to clean the surface. The diamond stylus moved from the first reference to the exposed area and then over to the other reference area (2.5 mm long and 1.0 mm wide). The vertical distance between the horizontal line drawn on the reference areas and the horizontal line drawn on experimental area was defined as tooth wear using the software (Software Mahr Surf XT20, 2009). Five profile measurements were performed for each specimen at intervals of 0.25 mm and the values were averaged (μm).

A schematic representation of the sample preparation and analysis is presented in Figure 1.

2.5. Statistical Analysis. Assumptions of normal distribution of error and equality of variance (Kolmogorov-Smirnov and Bartlett's tests) were checked for all the variables tested. Since the assumptions were justified, one-way ANOVA followed by post hoc Tukey's test were used to compare the different bleaching agents in relation to the susceptibility of enamel to erosive wear. Statistical analysis was performed with the software Statistica for Windows (Statsoft, Tulsa, OK, USA), with a significance level of 5%.

Table 1: Mean (standard deviation) of the erosive enamel wear (μm) for the different tested groups.

	Mean (standard deviation)	Homogeneous Sets*
C	3.37 (0.80)	a
HP	2.82 (0.98)	ab
HP + F	2.72 (0.64)	ab
HP + Ca	2.31 (0.92)	b

*Groups with different letters showed significant differences between them ($P < 0.05$).

3. Results

One-way ANOVA revealed significant differences between the groups ($P = 0.009$). Table 1 shows the results of post hoc Tukey's test. The bleaching groups HP, HP + F, and HP + Ca, presented similar enamel wear values among them (n.s.). However, specimens from group HP + Ca had significantly less mean enamel wear compared to the control after the erosive challenges, while the groups HP and HP + F did not differ from the control.

4. Discussion

Although in-office bleaching is considered a short-term treatment, it should not represent an additional risk for subjects susceptible to erosion. In the present study, the exposure of 35% HP-bleached enamel to the acid beverage did not cause higher enamel surface wear compared to control group (unbleached). Therefore, the first null hypothesis tested was accepted. It may be suggested that possible microstructural changes caused by the bleaching treatment did not predispose the enamel to greater erosive wear. These microstructural changes may have been repaired by the adsorption and precipitation of salivary calcium and phosphate, when immersed in artificial saliva for 2 h before the erosive challenge [17]. The ability of saliva to reharden and replenish lost minerals from bleached enamel has been demonstrated previously [22, 23].

Although the adverse effects of bleaching agents on enamel are mainly attributed to the concentration and pH of the bleaching gel [14, 24], some surface alterations are reported even with low-concentrated and nonacidic agents [4, 5, 9]. In fact, the demineralization of bleached enamel can occur as a result of mineral and organic content alterations [4, 5, 25]. Even though these factors are considered reversible with exposure to saliva [22], it might be speculated that when the bleached enamel is exposed to acid, these minimal changes could promote a greater spread of erosive agents inside enamel, leading it to a more pronounced demineralization [17].

Previous studies have shown that bleached enamel exposed to 37% phosphoric acid, which is applied before bonding procedures, presented a higher acid dissolution, with increased amount of Ca^{2+} extracted from enamel and an uneven etched surface, compared to the unbleached enamel [26, 27]. Nevertheless, other authors only reported this increased decalcified effect when high-concentrated hydrogen peroxide was used [28].

The association between carbamide peroxide bleaching and erosion and abrasion were previously investigated. Pretty et al. [18] applied 20 cycles of bleaching (2 h) using carbamide peroxide gels, from 10 to 22% concentration, and brushing (2 min), on enamel surface. The specimens were then immersed in 0.1% citric acid for a total of 14 h. The authors found no increased risk of enamel to acid dissolution. Engle et al. [19] also failed to demonstrate a significant increase in the susceptibility of bleached enamel to erosion/abrasion after a 5 days-bleaching treatment with 10% carbamide peroxide (10 h/day) associated with erosive and abrasive challenges. Although the bleaching agent tested in this study was more concentrated, which could increase the adverse effects, the unique application time was shorter (40 min) and, thus, similar results were achieved compared to control.

This study simulated only one session of bleaching treatment, however, multiple appointments are needed for obtaining optimal bleaching outcomes [29]. Therefore, the subsequent erosive wear of bleached enamel could be more evident after successive bleaching, as more severe chemical alterations have been observed in enamel bleached with high-concentrated hydrogen peroxide for long periods of exposure [30]. On the other hand, it might be speculated that the alterations provoked by the bleaching agents could be relevant only for the susceptibility of the enamel to initial erosive lesions ("erosion" phase), in which enamel softening, but not wear, is seen, as observed previously [17].

This experiment was conducted to simulate the effect of the intake of soft drinks (Sprite Zero that has no potential to stain the bleached enamel) after a session of in-office bleaching treatment using a high-concentrated hydrogen peroxide gel. The use of bovine enamel is considered a convenient substitute for human enamel in erosion studies, as they are easier to obtain, to handle, and standardize. Nevertheless, it has to be considered that these two substrates can behave in different physical and chemical manners and it was shown that the demineralization occurs faster in bovine enamel, due to its greater porosity [31–33].

The addition of potentially remineralizing agents in bleaching gels was also investigated in this study. The calcium and fluoride were added to the thickener phase of the gel, resulting in a concentration of 2% and 0.6%, respectively, after the final mixture, as these concentrations were compatible with the chemical formulation of the gel, without impairing its thickness. It would be conceivable that the bleaching gels containing remineralizing agents could act not only as whitening agents but also as remineralizing agents [34]. Supplements of fluoride and calcium in the bleaching gels have been shown to minimize the deleterious effects on enamel. It is supposed that the saturation of these ions in the gel allow their incorporation into the enamel apatite, increasing the resistance to demineralization [12, 35].

It was previously reported that the application of fluoride gel after bleaching resulted in increased resistance of the enamel against erosive attacks, compared to bleached/unfluoridated enamel [36]. However, when fluoride was added to the bleaching gel, the protection against demineralization was reported to be limited compared to fluoride only, since peroxide reduced the fluoride uptake by bleached enamel [34].

In the present study, the erosive challenges tested were frequent (4 cycles of 2 min each per day, for 5 days); therefore, the presence of fluoride into gel might have not been able to provide protection against enamel wear. As a reduced formation of KOH-soluble fluoride was observed in enamel bleached with fluoridated agents [34], it can be speculated that any "calcium fluoride-like" precipitation was readily dissolved by the subsequent acid challenges [37].

In addition, it has to be considered that fluoride dentifrices are frequently used in the daily practice and this additional source of fluoride can eventually contribute remineralizing the enamel surface after the bleaching procedures and erosive challenges. Nevertheless, the fluoride dentifrice was not included in this experimental model as this study attempted to analyze the effect of remineralizing agents added to the bleaching gel. Furthermore, fluoride dentifrice application is usually associated with a brushing procedure, which may increase the enamel erosive wear [38]. Due to the fact that the focus of this study was only on enamel susceptibility to erosion, brushing with fluoride dentifrice was not included in the pH cycling model.

In contrast to the effect of adding fluoride into the bleaching gel, the addition of calcium gluconate to the bleaching gel resulted in a protective effect against the erosion; thus, the second null hypothesis was rejected. In a previous study, deposits of calcium on the surface of the enamel bleached with calcium-added agent were shown using scanning electron microscope analysis [23]. These deposits may have acted as a physical barrier, minimizing the contact of the acid to enamel, or providing additional mineral to be dissolved during the acid challenge before the underlying enamel was attacked. Another forms of calcium combined with bleaching agents have been previously investigated, such as the casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) [39, 40] and calcium chloride [12], resulting in increased mechanical properties of bleached enamel.

Nevertheless, future studies should be conducted to investigate the solubility of different presentation forms of calcium salts, as well as their interaction with enamel surface, so that the mechanism of action of calcium in improving the resistance of bleached enamel to erosion can be established in different phases of its development (erosion and erosive wear).

5. Conclusion

Considering the experimental design, it can be concluded that the application of 35% HP-based bleaching agents did not alter the enamel susceptibility to erosion, and the addition of calcium to the bleaching gel improved erosion resistance of the bleached enamel.

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Research Article

Influence of Light-Curing Mode on the Erosion Preventive Effect of Three Different Resin-Based Surface Sealants

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Objectives. To investigate if reducing the light-curing time (while maintaining similar energy density) of resin-based surface sealants influences their erosion-preventive potential and mechanical stability after thermomechanical loading. *Methods*. Dentine samples were treated as follows: group 1—untreated, groups 2–4—Seal&Protect, groups 5–7—experimental sealer, and groups 8–10—Syntac Classic system. Groups 2, 5 and 8 were light-cured for 10 s (1000 mW/cm^2), groups 3, 6 and 9 for 7 s (1400 mW/cm^2), and groups 4, 7, and 10 for 3 s (3200 mW/cm^2). After water storage (7 d), first measurement was performed to evaluate baseline permeability of the sealants. After a thermomechanical loading (5000 cycles, 50/5°C, 12000 brushing strokes) a second evaluation of permeability was conducted (measurement 2). Permeability was tested by storing the samples in HCl (pH 2.3; 24 h) and measuring the dentine calcium release by atomic absorption spectroscopy. *Results*. For the first and second measurements, no influence of light-exposure time on permeability was observed (ANOVA: $P > 0.05$). No significant difference in the stability of the respective sealants was observed when light-cured for different durations. *Conclusion*. Shortening the light-curing time, while maintaining energy density constant, has no influence on permeability and stability of the investigated sealants.

1. Introduction

In industrialised countries a significant decrease of the caries prevalence has been observed during the last decades [1]. In the last few years, studies reporting a high prevalence (up to 32%) of erosion in certain patient groups (children or patients with gastrooesophageal reflux disease) have been published [2, 3]. Despite these studies, it is not clear if the prevalence of dental erosion is truly increasing or if only the increasing awareness about erosion results in a more precise diagnosis and more pronounced perception erroneously interpreted as an increased prevalence.

In order to prevent erosive enamel and/or dentine wear, different preventive approaches such as strengthening the chemical resistance of the dental hard tissues [4] or rehardening of erosively softened enamel or dentine have been discussed. Many of these preventive approaches based on the application of different fluoride compounds and formulations, namely, amine fluoride, sodium fluoride, sodium monofluorophosphate, titanium tetrafluoride, and stannous fluoride on enamel or dentine. Such preventive approaches act as therapeutics, which increase the acid resistance of the so treated dental hard tissues *in situ* and *in vitro* [5, 6].

Further, the erosive loss of dental hard tissue could be prevented by hampering the contact of the erosion causing substrates with the dental hard tissues by means of a mechanical barrier on the enamel or dentine surfaces [7]. In 2000, Brunton et al. [8] suggested a coating of dentine with a resin-based dentine adhesive to prevent erosive/abrasive wear. In a recent study [9], Seal&Protect (DENTSPLY DeTrey GmbH, Konstanz, Germany) showed a good long-term protective effect against enamel erosive wear. As Seal&Protect contains triclosan, for which possible negative side effects like induction of antibiotic resistances and accumulation in human milk were reported [10, 11], an experimental sealant without triclosan should be also tested in the present study. Furthermore, the Syntac Classis system should be tested as a representative of adhesive systems like suggested by Brunton et al. [8].

According to the manufactures' instructions these sealants must be light-cured at least two times for 10 s per tooth surface to be sealed, when using common polymerisation device settings (approximately 1000 mW/cm^2 output intensity). This fact might be regarded as disadvantageous, since it might be too time consuming, when multiple tooth surfaces have to be sealed.

Therefore, the aim of the present study was to investigate whether shortening the light-curing time has an influence on the erosion prevention potential of surface sealants.

As shortening the light-curing time may result in an unfavourable lower degree of polymer conversion [12] with inferior mechanical properties [13, 14] and higher cytotoxicity [15] of resin-based materials, it might be of interest to compensate the shorter light-curing time by increasing the light intensity. It is known that increasing the light intensity results in an increased degree of conversion [16], however also higher shrinkage stress was observed when resin-based materials were light-cured with higher intensities [17, 18]. This higher shrinkage stress might provoke an insufficient sealing of the dentine by the so cured surface sealants (shorter curing-time but higher curing intensity). Furthermore, it has to be taken in to consideration that increasing the light intensity may cause an increase of temperature in the pulp chamber [19].

The primary hypothesis of the present study was that shortening the light-curing time while simultaneously increasing light intensity results in a reduced erosion-preventing efficacy of the surface sealants. The secondary hypothesis was that surface sealants provide an erosion preventing effect and that effect will last under thermomechanical loading.

2. Materials and Methods

2.1. Sample Preparation. For the study, 120 dentine samples were prepared from freshly extracted bovine (age under 36 months) lower incisors. The teeth were sectioned at the cementum-enamel junction with a water-cooled diamond-coated disc. The pulp tissue was removed from the roots with endodontic files.

From the distal and mesial surface of each root, samples were gained with a trephine mill. The inner diameter of the drill amounted to 3 mm. The dentine cylinders were embedded in acrylic resin (Palavit G, Kulzer, Wehrheim, Germany) in metal moulds with an inner diameter of 6 mm. The dentine surface was ground with abrasive paper (800, 1000, 1200, 2400, and 4000 grit; Water Proof Silicon Carbide Paper, Streuers, Erkrat, Germany). In this grinding step, the cementum was removed, which was additionally checked with a stereomicroscope (40x).

After sample preparation, samples were randomly allocated to ten groups (1–10) with 12 samples per group. Sample allocation and experimental procedure is shown in a flow chart (Figure 1).

2.2. Treatment of the Samples. The samples in group 1 remained untreated and served as a control group.

Samples of the groups 2–4 were treated with Seal&Protect (pH 2.5–3; DENTSPLY DeTrey GmbH, Konstanz, Germany), while the samples in groups 5–7 were treated with K-0184 (pH 2.5–3; experimental sealer; DENTSPLY DeTrey GmbH, Konstanz, Germany). The respective sealants were applied on the dentine surface and left undisturbed for 20 s. After these 20 s, the remaining solvent was removed with an air syringe, and the sealant was light-cured. A second layer of sealant was applied, the solvent evaporated with an air syringe and light-cured again. Samples in groups 8–10 were treated with Syntac Primer (pH: 1.6; Ivoclar Vivadent, Schaan, Liechtenstein) for 15 s (gently rub in), excess was dispersed and thoroughly air-dried. Syntac Adhesive (Ivoclar Vivadent, Schaan, Liechtenstein) was applied, left for 10 s, and again thoroughly dried with an air syringe. Finally, Heliobond (Ivoclar Vivadent, Schaan, Liechtenstein) was applied, blown to a thin layer and light-cured. The composition of the used materials is given in Table 1.

Light curing was performed with the VALO LED light-curing device (Ultradent Products, South Jordan, USA). In groups 2, 5, and 8 light curing was performed at standard mode (1000 mW/cm^2) for 10 s (=10 J/cm^2), in groups 3, 6, and 9 at high power mode (1400 mW/cm^2) for 7 s (=9.8 J/cm^2) and in groups 4, 7, and 10 at plasma-emulation mode (3200 mW/cm^2) for 3 s (=9.6 J/cm^2). The light curing units were checked for consistency prior to curing using a radiometer (Optilux Radiometer, SDS Kerr; Orange, CA, USA). Holding the samples with a forceps and resting the light output window on the forceps guaranteed a constant distance between light-curing tip and samples surface of 0.5 cm.

2.3. Analysis of Sealer Permeability and Stability. After one-week storage in water (37°C, distilled water), the first measurement was performed to evaluate baseline permeability of the surface sealants. For this, samples were stored in hydrochloric acid for 24 h (pH 2.3; 4.5 mmol/L). Each sample was stored in a separate Eppendorf Tube (Eppendorf International, Hamburg, Germany) with 1 mL of HCl under constant motion.

For testing permeability of the sealants, the amount of dentine calcium dissolved in the HCl was measured. For this determination, 500 μL of the HCl was mixed with the same amount of water and strontium chloride (0.25%). Strontium chloride was added to mask the phosphate dissolved in the acid that might otherwise falsify the following measurement of calcium by atomic absorption spectroscopy (2380 Atomic Absorption Spectrophotometer, Perkin-Elmer, Schwerzenbach, Switzerland). Measurement was performed at 422.7 nm.

To test stability of the surface sealants, the samples were subjected to the following thermomechanical loading: 5000 cycles of changing the surrounding water temperature every 120 s from 5°C to 50°C and 12000 brushing strokes (BS) with toothpaste slurry in an automatic brushing machine applying reciprocating linear motion to the toothbrushes (ParoM43, Esro AG, Thalwil, Zürich, Switzerland). The brushing machine was adjusted to a constant brushing

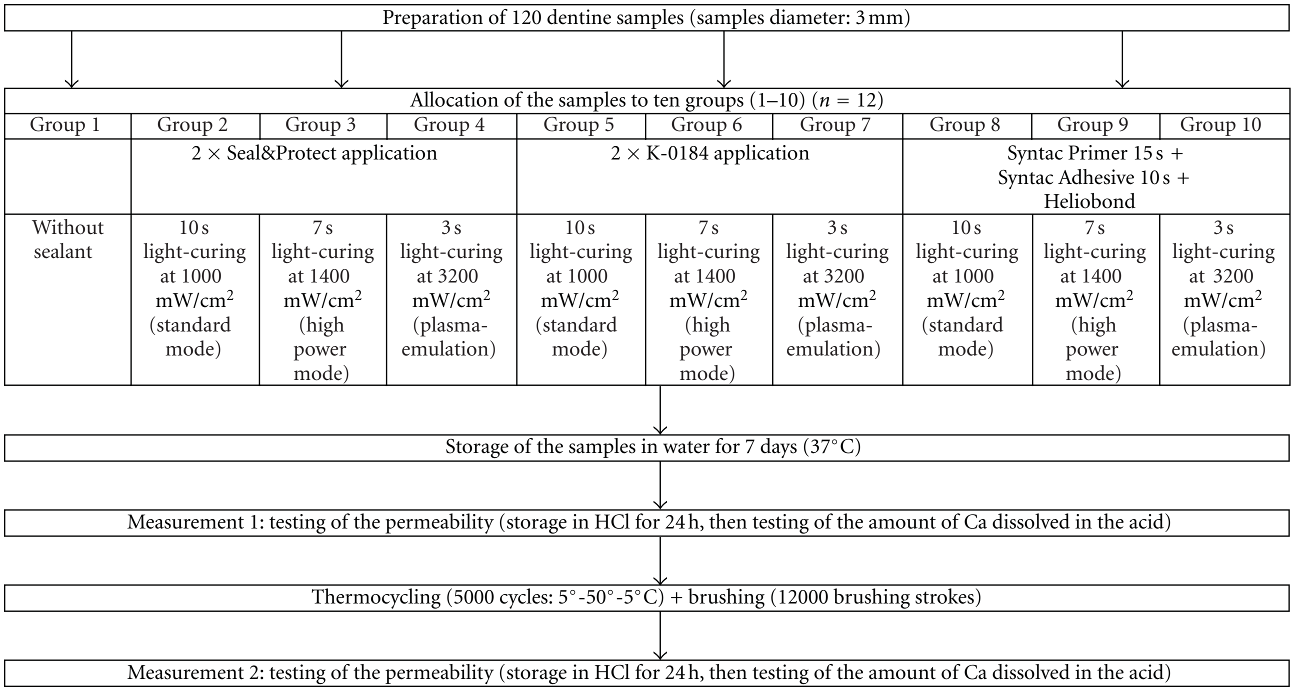

FIGURE 1: Sample allocation and experimental procedure.

TABLE 1: Composition of the used surface sealants and adhesive system (manufacturer's information).

Product	Composition
Seal&Protect (Dentsply Detrey, Konstanz, Germany)	Di- and trimethacrylate resins, PENTA (dipentaerythritol penta acrylate monophosphate), functionalised amorphous silica, photoinitiators, butylated hydroxytoluene, cetylamine hydrofluoride, triclosan, acetone
K-0184 (Dentsply Detrey, Konstanz, Germany)	Di- and trimethacrylate resins; PENTA (dipentaerythritol penta acrylate monophosphate), functionalised amorphous silica, photoinitiators, butylated hydroxytoluene, cetylamine hydrofluoride, acetone
Syntac Classic system (Ivoclar Vivadent, Schaan, Liechtenstein)	Syntac Primer: triethylene glycol dimethacrylate, polyethylene glycol dimethacrylate, maleic acid and acetone in an aqueous solution Syntac Adhesive: polyethylene glycol dimethacrylate and glutaraldehyde in an aqueous solution Heliobond: Bis-GMA, triethylene glycol dimethacrylate, stabilizers and catalysts

frequency of 120 strokes per minute and a constant brushing load of 2.5 N. The toothpaste slurry was prepared by mixing 300 mL artificial saliva (composition given by Klimek et al., 1982) [20] and 100 mL toothpaste (elmex, Gaba, Münchenstein, Switzerland; 1400 ppm, amine fluoride, RDA: 75). After each 500 brushing strokes, the slurry was renewed.

After this thermomechanical loading, the permeability of the sealant was again tested as described above (second measurement). Before storing the samples in the HCl, the samples were thoroughly cleaned with deionised water.

3. Statistical Methods

Data were coded in EXCEL and analyzed with SPSS Version 16.

Descriptive statistics such as mean and standard deviation of calcium released (SD) were computed for each group (1–10) at each measurement (measurement 1 and 2) and for the cumulative calcium release (measurement 1 plus 2) and was interpreted as the permeability of the materials.

Furthermore, the difference in the calcium release was calculated (calcium release in measurement 1; calcium release of the respective sample in measurement 2 = Δ calcium release). This difference was interpreted as stability of the surface sealants. Lower values represent a lower stability of the surface sealant with a higher susceptibility to thermomechanical and erosive wear.

The assumption of normal distribution of errors was checked, using Kolmogorov-Smirnov test.

Statistical analysis was performed using ANOVA followed by Scheffé post hoc tests in order to investigate the differences in the amount of calcium dissolved in the

Table 2: Sealer permeability.

Group	Material	Light-curing time (s)	Measurement 1	Measurement 2	Cumulative
1	Unsealed control		103.4 (30.3)	75.3 (29.9)	178.7 (50.6)
2		10	$^*2.9^a$ (2.2)	85.9^a (31.8)	$^*88.8^a$ (31.9)
3	S&P	7	$^*2.5^c$ (2.5)	77.5^b (29.7)	$^*80.1^b$ (28.9)
4		3	$^*2.6^e$ (2.1)	$^*105.1^c$ (20.2)	$^*107.8^c$ (20.9)
5		10	$^*6.1^a$ (7.6)	50.7^a (16.0)	$^*56.8^a$ (21.4)
6	K-0184	7	$^*5.7^c$ (4.4)	$^*42.8^b$ (15.3)	$^*48.4^b$ (16.1)
7		3	$^*2.9^e$ (1.7)	49.1^d (17.0)	$^*52.0^c$ (17.1)
8		10	$^*50.2^b$ (26.9)	48.2^a (28.7)	$^*98.3^a$ (49.5)
9	SCS	7	$^*47.1^d$ (27.9)	65.5^b (32.3)	$^*112.6^b$ (55.0)
10		3	$^*44.0^f$ (29.0)	58.7^d (28.0)	$^*102.7^c$ (53.1)

Calcium release μg ($\pm$SD) ($v = 500\,\mu$L) in the different groups (1–10) (S&P: Seal&Protect, K-0184: experimental sealer and SCS: syntac classic system).
Values that are significantly different to the respective untreated controls are marked with*.
Within the same measurement and same material, values for different light-curing durations, were not statistically significantly different.
Comparisons between values within the same measurement and same light-curing duration for different materials that are not significantly different are marked with same lower case letter.

experimental groups 2–10 and to compare the stability of the surface sealants (Δ calcium release). The calcium release in the treated groups (2–10) was compared with that of the unsealed control group (1) by ANOVA followed by Dunnett *t*-test.

Results of the statistical analysis with P values < 5% were interpreted as statistically significant.

4. Results

4.1. Sealer Permeability. Calcium release during the first and second measurements and the cumulative calcium release in the groups 1–10 are presented in Table 2.

At the first measurement, ANOVA revealed no significant influence of light exposure time on the calcium release ($P = 0.704$).

At the first measurement, the highest calcium release was observed for the untreated control group ($103.4 \pm 30.3\,\mu$g). The calcium release in the samples sealed with Seal&Protect (groups 2, 3, and 4) and K-0184 (groups 5, 6, and 7) were significantly lower compared to the calcium release of groups 8, 9, and 10 (Syntac Classic system) at the corresponding light exposure times ($P < 0.05$, resp.).

Also at the second measurement, no significant influence of the light-curing time on the calcium release could be observed (ANOVA; $P = 0.205$).

At this timepoint, calcium release in the sealed samples was either not significantly different (groups 2, 3, 5, 7–10) or significantly higher (group 4: Seal&Protect 3 s) compared to the unsealed control group ($75.3 \pm 29.9\,\mu$g). Only group 6 (K-0184 7 s) showed a significantly lower calcium release.

Moreover, the data showed that cumulative calcium release was not significantly influenced by the different light exposure times (ANOVA; $P = 0.660$).

The cumulative calcium release in all groups treated with surface sealants was significantly lower compared to the cumulative calcium release in the unsealed control group ($P < 0.05$, resp.).

4.2. Stability of Surface Sealants. The stability of the respective surface sealants applied with different light exposure times is presented in Figure 2.

For the stability of the surface sealants, a significant influence of the light exposure times could be observed (ANOVA; $P = 0.0404$). However, the respective post hoc tests showed no significant differences in the stability of the respective surface sealants when light cured for different durations ($P > 0.05$, resp.).

Within the 10 s light exposure time, the significantly highest stability was observed for the Syntac Classis system with no significant difference between Seal&Protect and K-0184. At 7 s light-curing duration, the stabilities of Seal&Protect and K-0184 were not significantly different ($P = 0.101$). Also the stabilities of the Syntac Classis system and K-0184 were not significantly different ($P = 0.932$). When light-cured for 3 s, the significantly lowest stability was observed for Seal&Protect.

5. Discussion

For the present study, samples were prepared from bovine dentine. Bovine teeth have the advantage that they are easy to obtain and that between two and six teeth can be harvested from one animal, while this number of teeth can rarely be gained from one human subject. Additionally, bovine teeth used for studies mostly stem from cattle raised in a comparable environment, with similar forage. Further, these teeth do not have a history of caries and/or fluoridation measures as many human teeth have, which might influence erosive demineralization or adhesion of applied surface sealants. Previous studies have proven bovine dentine to be a suitable alternative for human dentine in *in vitro* studies with regards to permeability characteristics [21] and adhesion tests [22]. In addition, human and bovine dentine does not perform differently under the same *in vitro* erosion/abrasion conditions [23]. Although it is favourable to use human dentine, it seems to be acceptable to substitute human with bovine dentine especially when the values of the respective

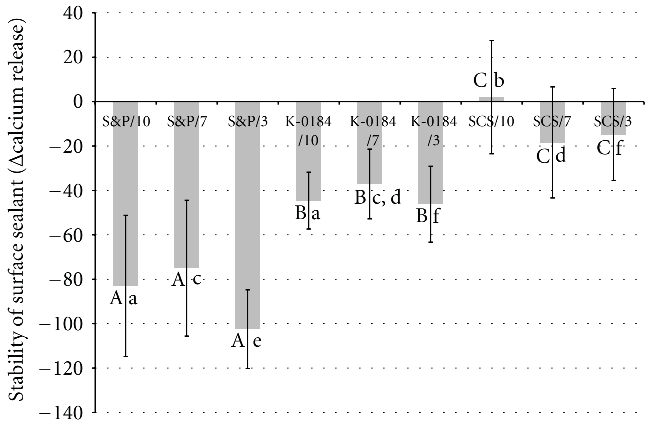

Figure 2: Stability (mean Δ calcium release (μg) ± SD) ($v = 500\,\mu$L) of the respective surface sealants cured with different light exposure times. (S&P = Seal&Protect, K-0184 = experimental sealer and SCS = Syntac Classis system; 10 = 10 s light-curing time, 7 = 7 s light-curing time, etc.) Lower values indicate lower stability of the surface sealants. Within the same material, values for different light-curing durations, which are not statistically significantly different, are marked with identical uppercase letters. Comparisons between values within the same light-curing duration that are not significantly different are marked with same lower case letters.

test groups are compared with each other and with the untreated controls of the same study (relative values).

A limitation of the present study might be that a smear layer was present on the dentine surfaces after the sample preparation. Under clinical conditions no such smear layer would be found. This smear layer might have an influence on the interaction of the sealants with the dentine during application, resulting in a poorer quality of the bonding of the sealants to the dentine. As the surface sealants used have an acidic pH (manufacturers information: Seal&Protect and K-0184: pH 2.5–3 and Syntac Primer: 1.6), it might be assumed that they are all able to remove or incorporate the minerals of the smear layer. Furthermore, it might be assumed that due to its lower pH, Syntac Primer is able to modify and/or remove the smear layer and to demineralise the dentine during bonding better than Seal&Protect and K-0184. However, as all sealants used provided a good protective effect, even under these disadvantageous conditions, it might be assumed that under clinical conditions at least the same protective effect and stability of the sealants might be observed. It has to be taken in consideration that the performance (antierosive effect) of the Syntac Classic system is not only determined by the pH of Syntac Primer but also by the performance of the later applied Syntac Adhesive and Heliobond.

Simulation of the erosive attack was performed with pure hydrochloric acid. Hydrochloric acid was selected for use, as it is the acidic compound found in stomach content [24]. However, beside hydrochloric acid, the gastric juice also contains various enzymes [24]. One such enzyme, pepsin, is a proteolytic enzyme well known for its ability to degrade collagen [25, 26]. A previous study has shown that the erosive mineral loss from dentine is higher, when the collagen matrix, exposed during the erosive attack, is removed [27]. Taking these findings into consideration, it is imaginable that an admixture of pepsin to the hydrochloric acid results in a higher erosive dentine loss. However, a study by Schlueter et al. (2007) [28] found no influence of pepsin admixture on hydrochloric-acid-induced dentine loss. Nevertheless, another study [29] by the same group showed an intensified dentine erosion progression *in vitro* when pepsin and trypsin were added to the hydrochloric acid. As the results of these studies are inconclusive, in the present study the simulation of the erosive attack was performed with pure hydrochloric acid, as performed in numerous other studies. In the present study, hydrochloric acid with a pH of 2.3 (titratble acidity: 0.05 mL of 0.05 M NaOH for 1 mL HCl) was used to simulate gastric juice. For gastric juice, Bartlett and Coward (2001) [30] found a mean pH of 2.92 (range 1.2–6.78) (in seven gastric acid samples) and mean titratable acidity of 0.68 mL of 0.05 M NaOH (range 0.03–1.64). However, it has to be taken in to consideration that Bartlett and Coward (2001) [30] found a broad range for both the pH and the titratable acidity of the gastric juice, which also covers the pH and titratable acidity of the hydrochloric acid solution used in the present study. Furthermore, we assume that using a higher pH, more like the one of the gastric juice found by Bartlett and Coward (2001) [30], might result in lower amounts of calcium dissolved in the same time periods but should not fundamentally change the findings of the present study.

The primary hypothesis of the present study, which is shortening the light-curing time while simultaneously increasing of the light intensity, results in a worse erosion-preventing efficacy of the surface sealants, has to be rejected. No significant influence of the light-curing duration on the permeability of the respective surface sealants was observed; neither at the initial measurement 1, final measurement 2, nor for the overall performance (cumulative calcium loss) of the surface sealants. It might be hypothesised that the shrinkage stress of the resin-based surfaces sealants, which might be influenced by the increasing light-curing intensity [31], had no negative effect on the permeability of the surface sealants used. This might be explained with the observation that during setting of resin-based materials, shrinkage stress can be compensated by flow of incompletely polymerised compounds [32].

Furthermore, it has to be taken in to consideration that the negative influence of high light intensities on shrinkage stress of resin-based dental materials was found in cavities simulating disadvantageous cavity configuration [18, 33]. In the present study, the material was applied in a flat layer on the dentine surface resulting in one bonded surface only, thus representing a favourable C-factor with low shrinkage stress during polymerisation [34].

The mechanical stability of resin-based materials is influenced by the degree of conversion [13]. Shortening the light-curing time without increasing the light intensity might reduce the degree of conversion of the surface sealants [12], which might then affect their stability. In the present study, no significant differences in the stability values of the respective surface sealants were observed when the surface sealants were light-cured for different durations but using similar energy densities. Nevertheless, it should be clarified in further studies if the polymerisation approaches used in the present study might have an influence on the material properties, such as biocompatibility and degree of conversion, and on temperature changes in the pulp chamber. However, a recent study [19] found the increase in temperature in the pulp chamber directly related to the light intensity and exposure time and recommend that curing devices with high power density ($>1200\,\mathrm{mW/cm^2}$) should only be activated for a short period of time ($<15\,\mathrm{s}$). As in the present study the light density over $1200\,\mathrm{mW/cm^2}$ was used for a maximum of 7 s, it might be assumed that this application mode does not have a negative influence on the pulp under clinical conditions.

The absolute stability values and the existing differences in the stability values of the different materials tested have to be interpreted with caution, due to the method of calculating the stability in the present study (calcium release in measurement 1; calcium release of the respective sample in measurement 2). In the Syntac Classic system group, for which slightly better stability values have been calculated, at the initial measurement (measurement 1), directly after application of the sealants without any loading, the permeability was between 8 and 17 times higher than that of the other sealants. However, at the second measurement, no difference in the permeability of the different surface sealants was observed. Thus, it has to be acknowledged that the superior stability of the Syntac Classic system was due to the fact that the initial permeability was already high, or in other words, that the antierosive effect was poor compared with the other surface sealants used. When looking at the values for the cumulative calcium loss, representing the performance of the products during the whole experimental procedure, it becomes obvious that the protective effect of the Syntac Classic system might be similar or even worse than that of the other sealants.

Beside the commercially available Seal&Protect, the experimental sealant K-0184 was also tested in the present study. The chemical composition of this sealant is similar to Seal&Protect, with the single difference that no triclosan is incorporated in the experimental sealant. As no significant difference in the cumulative calcium release and the stability values between Seal&Protect and the triclosan-free experimental sealant were observed, one might conclude that absence or presence of triclosan in the sealant used has no significant effect on its antierosive effect and stability.

The secondary hypothesis of the present study has to be partially rejected. In the present study the tested surfaces sealants showed a very good protective effect against erosive demineralisation during the first measurement (24 h erosion in hydrochloric acid). For the samples sealed with the tested surface sealants a reduction of the calcium release of 51% (Syntac Classic system light-cured for 10 s) up to 98% (Seal&Protect light-cured for 7 s) was observed. These findings are in accordance with the findings of a recent study [9], investigating the protective effect of surface sealants against erosive enamel wear caused by extrinsic and intrinsic acids under long-term exposition. In that study, a reduction of 81% of the calcium release during a 24 h storage in hydrochloric acid was found after application of Seal&Protect. Furthermore, Seal&Protect was able to provide a protective effect up to 4 days against erosive demineralisation caused by HCl.

At the final measurement (2) of the present study, after the thermomechanical loading, no further differences in the calcium release from the sealed samples, as compared to that of the unsealed control samples, were observed. Due to this finding, it might be concluded that the tested surface sealants are not able to maintain the initially observed antierosive protective effect during the thermomechanical loading. However it has to be taken in consideration that the mechanical loading used here, 12000 brushing strokes (BS), represents approximately 13 to 20 months *in vivo*, following the findings of a recent study by Wiegand and Attin (2011) [35]. They assumed 10–15 brushing strokes per tooth during a single tooth brushing session being adequate to simulate the clinical condition *in vitro*. Assuming tooth brushing twice a day, the 12000 BS of the present study equals 400 to 600 days under clinical conditions.

In 1996, Bartlett et al. [36] found a drop of the oral pH below 5.5 for 0.3% and below pH 6 for 4.4% of the total time during 24-hour pH telemetry in gastrooesophageal reflux patients. This corresponds to an erosion time between 4.3 and 60 min per day, respectively. Taking these findings into account, the here used 24 h erosive attack is equivalent to 24–330 days under clinical conditions. As the erosive attack has been performed twice, this results in a total simulation time of up to 660 days, similar to the duration simulated by the tooth brushing.

6. Conclusion

By the findings of the present study it can be concluded that

(1) shortening the light exposure times, while maintaining the energy density, has no negative influence on the erosion prevention potential and stability of the surface sealants used;

(2) initially (first 24 h erosion) all surface sealants show a good protective effect against erosive demineralisation, with a loss of this protective effect due to the thermomechanical loading used here;

(3) further studies are needed to evaluate how long the observed protective effect will last under more *in vivo*-like conditions.

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Research Article

Opinions on Dental Erosive Lesions, Knowledge of Diagnosis, and Treatment Strategies among Norwegian Dentists: A Questionnaire Survey

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This study aimed to investigate dentists' general experience, knowledge about diagnosis, and treatment of dental erosive wear in young adults. A questionnaire was sent to 1262 Norwegian public dental health-employed dentists. The response rate was 60%. Results indicated that most dentists recorded erosive wear, half of them used a specific scoring system, and half registered lesions at the tooth surface level. Lesions were reported most often on palatal surfaces of upper anterior teeth (79% of dentists), on occlusal surfaces of lower 1st molars (74%), and on upper 1st molars (32%). Half the dentists used clinical photographs for documentation and 60% made study models. While 40% reported more erosive lesions in males, 36% reported no gender differences. High intake of carbonated beverages and acidic juices were reported as the most common cause by 97% and 72% of the dentists, respectively. Only 21% of dentists recorded the patient's dietary history, and 73% never measured saliva secretion. The majority (78%) of the dentists treated patients with erosive wear themselves. In general, the survey suggests that the dentists are relatively up to date regarding the clinical recording, diagnosis, and treatment of dental erosive wear. However, dietary and salivary analyses were not given priority, and early, preventive treatment was lacking.

1. Introduction

The focus on dental erosive wear by both the public and dental health professionals appears to be increasing. This may be due to a decrease in caries levels in industrialized countries [1] and/or to high prevalence of erosion lesions documented in recent epidemiological studies [2–4]. The diagnosis and the potential treatment needs of patients with dental erosive lesions are considered to be a significant challenge to clinicians [5].

It is known that dental erosive wear is a multifactorial condition [6], and that it is important to evaluate the potential factors which could lead to identification of persons at risk. In order to assess and reveal possible predisposing factors, a record of patient's dietary intake should be registered as part of a comprehensive case history involving general health, habits, saliva flow rates and buffer capacity [5]. A study by Lussi and Schaffner showed that active lesions will progress when no adequate preventive measures are implemented [7]. It is therefore important to detect the condition as early as possible and dentists should be trained to register even the first signs of the condition. This becomes especially important when taking into account the trend that patients often do not seek treatment until the lesions have reached such an advanced stage that restorative therapy is needed [8]. Furthermore, the clinical appearance of the eroded tooth surfaces is the most essential sign since there are no diagnostic tools available for early, quantitative detection. The clinical examination of the teeth should be performed systematically using a simple and accurate scoring system [8].

The scientific literature shows that there has been an increasing focus on the aetiology, prevalence, and treatment options for dental erosive lesions. However, a survey among general dental practitioners from 2003 showed that a large proportion of dentists advised their patients about the erosive wear only occasionally or rarely [9]. Of the 1686 12 year olds included in that study, less than 10% could recall their dentist mentioning the condition. Recent studies have also identified that an inadequate level of information about dental erosive wear was given by dental practitioners to adolescents/adults in possible risk groups [10, 11]. Furthermore, another recent study showed that the knowledge and awareness about dental erosion among dental students, faculty members and patients in a Brazilian dental school were poor [12].

Therefore, the aim of the present study was to perform a survey on public dental practitioners asking them about their experiences, awareness, knowledge of diagnosis, and choice of treatment options for dental erosive wear in 18- to 30-year-old patients.

2. Material and Methods

A precoded questionnaire was sent electronically to all dentists ($n = 1262$) employed in the Public Dental Health Service (PDHS) in Norway in April 2011, using the Internet-based software program QuestBack. Information was collected on the respondents' sex, age, home county, and type of dental practice, and to which extent the respondents were involved in the diagnosis and treatment of dental erosive wear. The questionnaire had two parts. In one part the dentists were asked how they register and document dental erosive wear in their patients aged 18–30 years. They were also asked questions relating to their experience of erosive lesions, such as their estimation of the distribution and prevalence of erosive lesions among these patients. The dentists' opinions on probable causes related to erosive lesions were also recorded. The second part of the questionnaire involved a patient case, where the dentists were asked to record their choice of treatment including general patient advice and/or type of restoration. A brief patient history as well as colour clinical photographs of labial and palatal surfaces of upper anterior and molar teeth with differing severity of erosive lesions was provided (Figure 1). The questions are described below.

Patient Case. A 28-year-old woman who had an eating disorder with vomiting as a teenager, but is now healthy.

Question One. What type of advice would you give this patient (you can make more than one choice)? (1) Information about dietary and drinking habits; (2) information about brushing technique/habits; (3) recommend rinsing with fluoride; (4) recommend rinsing with chlorhexidine; (5) recommend fluoride tablets; (6) refer to specialist, faculty clinic, or other dentist; (7) recommend specific toothpaste or rinse.

Question Two. How would you treat the teeth in the maxillary anterior and posterior regions and the mandibular posterior teeth (you can make more than one choice)? (1) No treatment; (2) treat locally with fluoride solution (e.g., 2% NaF, Duraphat, Fluor Protector); (3) apply bonding material; (4) apply flow composite; (5) restore with glass ionomer cement; (6) restore with composite filling material; (7) restore with compomer; (8) restore with ceramic laminate/facet/inlay/onlay; (9) restore with crown.

The QuestBack program was configured to automatically send reminders to all participants who had not replied after 4, 7, and 12 weeks.

2.1. Statistical Analysis. The data was processed using SPSS version 18.0.3 (Statistical Package for the Social Sciences; SPSS Inc., Chicago, Ill., USA) and statistical evaluation was carried out by means of descriptive statistics. As the upper 1st molars were most commonly suggested for operative treatment by the dentists, multivariate logistic regression analyses were performed to test the association between operative treatment of these teeth (dependent variable) and the following independent variables: dentists' age, gender, practice location, and choice to refer. Unadjusted results were obtained by performing separate regression analyses for each selected variable. Adjusted results were obtained by including all the selected variables in one regression analysis. In the adjusted analysis the result for each variable was adjusted for all the other variables. Results were reported using odds ratio (OR) with 95% confidence interval (CI) and P value (P). The probability level for statistical significance was set at $\alpha = 0.05$.

2.2. Ethical Considerations. Participation was voluntary and no compensation was given to the respondents. Anonymity was ensured by QuestBack. The study was approved by the Norwegian Social Science Data Services.

3. Results

3.1. Details about the Respondents. A total of 1262 dentists were invited to participate in the study, and 783 dentists had responded after three reminders. Respondents who stated that they did not normally work with patients having dental erosive wear ($n = 78$) were excluded from the statistical analyses. A response rate of 60% was calculated according to Standard Definitions of the American Association for Public Opinion Research [13]. The respondents ranged in age from 25 to 72 years (mean 43.3 years, SD 13 years) and consisted of 65% females and 35% males.

3.2. Descriptive Analyses. Almost all respondents ($n = 695$, 98.6%) registered dental erosive lesions in their patient's charts. Of these, 50% used a specific scoring system: 31% used a two-graded scoring system (enamel dentine) and 14% used a more detailed system, while 5% did not report details on their scoring system. Erosive lesions were registered at the surface level by 58% of the respondents, at the tooth level by 15%, and at the individual level by 26%, while 1% did not answer the question. Documentation with clinical photographs was reported as being often performed by 9% of the dentists, sometimes by 51%, and never by 40%. The corresponding values for documentation with study

Figure 1: Clinical photographs of the patient case: A 28-year-old woman who had an eating disorder with vomiting as a teenager, but is now healthy. (a) palatal surfaces of the upper incisors, (b) occlusal surfaces of upper right 1st and 2nd molars, (c) occlusal surfaces of upper left 1st and 2nd molars, (d) occlusal surfaces of lower right 1st and 2nd molars, and (e) occlusal surfaces of lower left 1st and 2nd molars.

models were 5% (often), 60% (sometimes), and 35% (never). The dentists reported that the erosive lesions were most often located on the palatal surfaces in upper anterior teeth (79%), followed by first mandibular molars (74%) and first maxillary molars (32%). According to 36% of dentists there were no gender differences in the prevalence of erosive lesions. While 40% of the dentists reported that erosive lesions were more common in males than in females, only 4% of the dentists stated that more females than males had erosive lesions, and 20% were unsure.

Of the dentists with more than 10–15 years clinical experience ($n = 404$), the majority (81%) reported a higher prevalence of erosive lesions today compared with 10–15 years ago. Eight percent of the dentists did not have the impression of seeing more erosive lesions today, while 11% were unsure.

Half of the dentists (52%) did not think that young adults (aged 18–30 years) with dental erosive lesions had more caries than those without erosive lesions. Higher caries prevalence amongst patients with erosive lesions was estimated by 19% of dentists; 19% stated no differences and 10% were unsure.

Most of the dentists (77%) reported that they usually found a probable cause of the erosive lesions, 22% occasionally, and only 1% reported that they seldom found a probable cause. The most common causes given were consumption of carbonated beverages (97%), followed by acidic juices (72%), fruits (46%), sport drinks (24%), and acidic diet (20%). Reflux and eating disorders with vomiting were reported as more uncommon causes (8%) by the dentists.

When patients were registered as having erosive lesions, 21% of the dentists always recorded a dietary history. A quarter of the dentists (24%) reported that they often recorded a dietary history, 36% only occasionally, and 19% reported that they never recorded dietary history in patients registered with erosive lesions. The type of dietary

Table 1: The frequency (%) of dentists' general patient advice(s) in the patient case. *n*: number of dentists responding to each question.

Advice	%
Information about good dietary and drinking habits	88.2
Information about brushing technique/habits	58.4
Recommend rinsing with fluoride	87.1
Recommend rinsing with chlorhexidine	0.4
Recommend fluoride tablets	14.8
Refer to specialist, faculty clinic, or other dentist	9.0
Recommend specific toothpaste or rinse	11.2

questionnaire used was highly variable; 51 dentists (9%) used a precoded questionnaire, 31% asked the patient to record all they consumed during a certain period of time (amount, time of day, etc.), while the remaining 60% used different techniques, such as oral interviews and describing text in the dental chart.

More than half of the respondents (57%) estimated that their patients with erosive lesions had a normal amount of saliva, while 37% did not know. Only a small percentage of dentists estimated that their erosion patients had less than normal saliva flow (4%) and 2% thought they had more than normal amounts of saliva. Only two dentists (1%) reported that they always measured saliva secretion, 1% reported measuring saliva flow often, 25% only occasionally, while 73% reported that they never measured saliva secretion in patients with dental erosive lesions.

Most of the dentists (78%) treated their patients with dental erosive wear themselves, while 9% referred the patients to another dentist or specialist/university faculty clinic. The remaining 13% treated many patients themselves, but referred the more severe cases. The two latter categories were combined into one variable when performing multivariate analyses. Eight percent of all the male dentists would refer all patients with erosive lesions to another dentist or specialist/university faculty clinic, compared with 10% of the female dentists. The mean age was 44.6 years (males) compared with 43.3 years (females) among the dentists who treated their patients themselves. Significantly more dentists living in the eastern part of Norway (12%) referred patients compared with dentists living in the northern (7%) and western (7%) parts of Norway ($P = 0.03$).

3.3. Patient Case. A previously calibrated examiner scored the severity of the erosive lesions on the surface level based on the clinical photos [14] using a tested scoring system for erosive lesions (VEDE system) [14]. Enamel lesions (grade 2) were registered on central incisors and upper 2nd molars, while dentine lesions (grades 3, 4, and 5) were seen on lateral incisors, all 1st molars and lower 2nd molars, indicating a case with severe erosive lesions (Figure 1).

The results are presented in Tables 1 and 2. The majority of dentists (88.2%) would give the patient information about good dietary and drinking habits and recommend that the patient should rinse with fluoride (87.1% of the dentists). More than half the dentists (58.4%) would give information about tooth-brushing technique and/or brushing habits. Operative treatment was the most common choice of treatment for the upper 1st molars. Forty-four percent of the dentists chose to place a filling, and 18.8% chose prosthodontic treatment for these teeth. The lower 2nd molars were the most likely teeth to receive either no treatment other than advice (35.3%) or local treatment with fluoride solution/use of a bonding material (40.6%). These teeth were also least likely to be restored with a filling (20.1%). There were no obvious differences between the treatments chosen for the different upper incisor teeth, and most dentists would choose to restore them with a filling.

The results of the multivariate logistic regression analyses are shown in Table 3. Only the gender of the dentist remained significant in the analysis. An odds ratio of 1.5 indicated that for upper 1st molars, the female dentists suggested operative treatment significantly more often than the male dentists.

4. Discussion

Even though there are few comparable studies, this survey tends to suggest that the majority of dentists are aware of the problem of dental erosion and are relatively up to date regarding the clinical recording and diagnosis of dental erosive wear. The major points to note are that the documentation of erosive dental wear was not standardized, and little or no priority was given to dietary and salivary analyses. Despite this, and more encouragingly, the majority of dentists reported that they were confident of finding the cause of the erosive wear and also in treating their own patients that need reparative therapy due to dental erosive wear.

The questionnaire used in this study was aimed at dentists working in the Norwegian PDHS who were currently involved in the diagnosis and treatment of dental erosion in their clinical practice. In general, these dentists see a large proportion of adolescents and young adults, and based on recent prevalence studies this patient group may be considered as being at high risk for dental erosion [3, 4, 15, 16]. Importantly, responding dentists who reported that they did not normally work clinically with erosive dental wear were excluded from the study.

Measures were taken to ensure a high response rate in accordance with a systematic review of questionnaires [17]. The questionnaire was styled in a personal manner, and a realistic and illustrative case with background information was presented early in the questionnaire, as was proven successful in a previous questionnaire-study on dental caries [18]. In order to emphasize the importance of a reply, when sending reminders to the dentists, respondents were informed of the response rate so far for the questionnaire. Unfortunately, as this questionnaire was anonymous, no information could be retrieved about the nonresponders, and a meaningful nonresponse analysis could not be performed. Another limitation of this study is that such questionnaires are based on the respondents' ability to recall. Furthermore, given that the specificity and sensitivity of the dentists' diagnosis of erosive wear were not assessed, the accuracy of the data cannot be quality controlled.

Recent prevalence studies have shown that erosive tooth wear is a common problem in current dental practice and

Table 2: The frequency (%) of dentists' general choice of treatment and/or type of restorative material in the patient case. *n*: number of dentists responding to each question.

Treatment decision (%)	Incisors		1st molars		2nd molars	
	Centrals	Laterals	Lower	Upper	Lower	Upper
	$n = 705$	$n = 656$	$n = 681$	$n = 680$	$n = 648$	$n = 649$
No treatment	28.1	26.4	26.1	16.0	35.3	22.3
Treat locally with fluoride solution or bonding material	29.6	27.3	31.0	20.9	40.6	28.5
Restore with filling	34.9	38.7	34.0	44.3	20.1	36.1
Restore with ceramic laminate/facet/inlay/onlay	5.4	5.8	4.6	8.8	1.7	5.4
Restore with crown	2.0	1.8	4.3	10.0	2.3	7.7

Table 3: Associations between selected variables and operative restoration of upper 1st molars. Results are presented as odds ratios (OR) with 95% confidence interval (CI). Results significant at 5% level are marked in bold. ref: reference category.

Selected variables	Unadjusted			Adjusted		
	OR	95% CI	*P* value	OR	95% CI	*P* value
Age						
43–72 years (ref)						
25–42 years	1.1	0.8–1.5	0.71	1.2	0.9–1.7	0.31
Gender						
Male (ref)						
Female	**1.4**	**1.0–2.0**	**0.03**	**1.5**	**1.1–2.1**	**0.02**
Practice location						
East (ref)						
West	1.1	0.8–1.6	0.53	1.1	0.8–1.6	0.55
North	0.8	0.5–1.2	0.29	0.8	0.5–1.3	0.36
Refer cases						
No (ref)						
Yes	1.2	0.7–2.0	0.56	1.2	0.7–2.1	0.5

is a challenge for the practitioner [3, 4, 12, 15, 16]. In the present survey, the dentists were primarily asked to report on dental erosive wear on younger individuals. The majority of dentists participating in this study reported a higher prevalence of erosion lesions today, compared with 10–15 years ago. Even though lesion due to the combination of erosion and abrasion/attrition is a common phenomenon, one can expect that the presence of mainly erosive wear lesions exceeded the number of erosion/abrasion/attrition lesions in the age group included in this study [19].

To be able to record the presence and severity, as well as the progression of erosive lesions, use of a grading system is required [20]. In the present study, the majority of the dentists reported that they used a specific scoring system registered at the tooth surface level. In most cases this was the registration system included in the electronic patient journal system. Approximately one quarter of the dentists registered the erosions at the patient level only, which does not allow for specific followup of lesion progression. Regarding the oral distribution of the erosive lesions, the most commonly affected tooth surfaces were the palatal surfaces of the upper incisors and the occlusal surfaces of the lower 1st molars. The occlusal surfaces of upper 1st molars were also relatively frequently affected. These results are in accordance with many previous studies in adolescents [10, 21, 22].

Even though approximately one-third of dentists had the impression that there were no differences between the sexes, 40% reported more dental erosive wear in males. Several studies have reported a higher prevalence of dental erosion in male adolescents [2, 4, 15, 22]. In a recent study the consumption of soft drinks and other acidic beverages was found to be related to both age and gender [23]. Boys consumed significantly greater amounts than girls. One could expect that a higher intake of such beverages (often containing sugars), typically consumed between meals, could also lead to more dental caries. While half the respondents in the present study thought that patients with dental erosion did not have more dental caries, about one fifth of the dentists reported a higher incidence of caries in these patients, and about the same percentage reported that patients with dental erosion had the same amount of caries as patients without dental erosion. Results from other studies regarding presence of dental erosive wear and caries experience are also ambiguous. Some studies have demonstrated a significantly higher caries experience among children with high prevalence of dental erosion [24, 25], whereas others could not verify this association [26, 27].

In order to identify possible aetiological factors in patients presenting with dental erosion, a dietary history is extremely important, as behavioural factors such as eating and drinking habits play a key role in the pathogenesis [28]. Only half of the dentists in this study reported either occasionally or never, recording a dietary history in patients with dental erosion, and only a small proportion used a precoded questionnaire. This is a worrying finding, as the effective prevention of progression of erosive lesions often requires clear, individualized dietary advice.

Apart from behavioural factors, biological patient factors also play an important role in the pathogenesis of dental erosion [28]. It is well known that a high salivary flow rate favours the prevention or minimization of initial acid attack [29]. In the present study the majority of dentists reported that they did not measure saliva secretion in their dental erosion patients. This reflected the estimate by more than half the dentists that their patients had normal amounts of saliva. However, more than one-third of the dentists reported that they did not know about the saliva status of their dental erosion patients, possibly indicating a lack of knowledge about the importance of saliva in the aetiology of dental erosive wear. However, if the large majority of patients seen by these dentists are adolescents or young adults, the percentage of patients presenting with subnormal levels of saliva secretion is not expected to be very high.

In the present study, approximately two-thirds of the dentists reported that they usually found a probable cause of the dental erosion in their patients. Nearly all dentists reported that high consumption of carbonated soft drinks was the most likely cause. Acidic drinks are thought to be one of the most important factors leading to dental erosion, especially when taking into account that the consumption of such drinks has increased greatly over the last decades [30, 31]. Norway is no exception, with a yearly mean consumption of well over 100 liter per person, placing them in the top five among the European countries. Frequent intake of fruit and/or fruit juices was also commonly reported by the dentists to be causative factors in their patients. The risk of developing erosive tooth wear was found to be 37-times higher in persons with a greater than twice daily intake of citrus fruits than in persons eating fruit less often [32].

Dental personnel may in many cases be the first health workers to observe signs of eating disorders. In the present questionnaire dentists regarded reflux and eating disorders with vomiting as relatively uncommon causes of dental erosion in their patients. While no difference was reported in the prevalence of dental erosion between young Icelandic adults and patients with gastroesophageal reflux disease (GERD) [31], a more recent study found that patients with eating disorders (mean age 21 years) were at 8.5-times higher risk of having dental erosion [33].

Thorough documentation of the patient case is a standard part of following the progression of erosive lesions and treating patients with erosive tooth wear. For patients where the degree of damage suggests the need for extensive rehabilitation, insurance companies or social security systems usually expect solid documentation. In the present study, the majority of dentists only occasionally documented their patient cases by taking clinical photographs or making study models. Surprisingly, more than a third of the dentists never used this type of documentation. This may be related to the possible mild severity of the patient cases or the fact that they are not aware of the importance of documenting such wear.

A 3-year longitudinal study on the dynamics of tooth erosion in adolescents concluded that the condition progressed steadily in children with dental erosion [34]. At the same time, there are reports that according to patients, dentists do not appear to give enough information about this problem. In a recent study examining the status of the dentition in professional Norwegian wine tasters, seven of the nine wine tasters registered with dental erosive wear had not been informed by their dentist/dental hygienist about the presence of these lesions [11]. In another study examining dental erosion in a group of individuals training regularly at a training studio, 82% of the physically active young adults with erosive wear who recently had been to their dentist/dental hygienist claimed they had not been informed about the presence of these lesions [10]. Furthermore, in the study on dental students, faculty members, and patients in a Brazilian dental school, Hermont and coworkers [12] have recently shown that knowledge and awareness about dental erosion are poor, and they concluded that there was a need for better understanding and communication. Maybe it is still true that many dentists typically ignore or overlook the very early stages of erosive tooth wear, dismissing tooth surface loss as a normal and inevitable occurrence of daily living, and thus not worth spending time on any specific interventive activity, as was suggested a few years ago [5].

In the present survey most of the dentists reported that they perform the reparative treatment of their own patients with dental erosion, and only nine percent referred the patients to another dentist, a specialist or a university faculty clinic. There were no significant age or gender differences with regard to this aspect, an observation that was made in a similar clinical decision-making questionnaire among prosthodontists [35]. This indicates that most of the general dentists in this study feel competent in their technical skills. However, with respect to treatment options, significantly more of the female dentists who responded to the present questionnaire chose to restore the upper 1st molars. Regarding referral patterns, not surprisingly a greater proportion of dentists in the eastern, more populated part of Norway, where there are many specialists, referred their patients for restorative treatment, compared with those located in the northern and western parts of Norway, where there are few specialists.

The patient case included in the current study is not an uncommon example of a contemporary young adult. Prior registration of the erosive damage by one of the authors indicated that the patient had a combination of mild enamel and severe dentine lesions. The results of the questionnaire suggest that the responding dentists had few problems registering this difference. The 1st upper molars were regarded as the teeth with the greatest need for a restoration, probably because in this patient the palatal cusp was almost completely worn away. However, also the lateral incisors, upper 2nd molars, and central incisors were recommended to be filled by over a third of the dentists. Surprisingly, even though the lateral incisors and lower 1st molars had exposed dentine, more than a quarter of the dentists suggested that no restorative treatment was necessary. In contrast, about two-thirds of the dentists would restore enamel lesions on central incisors and 2nd molars using resin composites. This inconsistency could be a result of the difficulty for dentists in differentiating between more severe enamel lesions and early dentine lesions, as discussed in the literature [36]. Another consideration could be related to dentists' focus on the tooth level rather than considering the patient case as a whole.

In a similar questionnaire-based study [35], for a patient case involving palatal erosion in maxillary incisors without loss of the incisal edges, most specialists chose the prevention option that involved covering the worn surfaces with a dentine bonding agent and prescription of fluoride mouthwash. Restorative management in the form of direct composites was most commonly chosen by UK dentists while the non-UK prosthodontists were more divided, half of them choosing to leave the teeth untreated, about one-third choosing composites, and almost 10% choosing to crown the teeth. In a recent study performed on dentists in the Dental Practice-Based Research Network, direct resin-based composites were the most common type of restorations placed in noncarious tooth defects [37]. Although the patient group in that study was older than the average age of patients seen by the dentists in the present study, the choice of resin composites is a common factor.

Several authors have published strategies for the restorative treatment of patients with dental erosive tooth wear. Some clinicians have suggested that adequate function and aesthetics can be achieved when dental erosion lesions are restored using a combination of bonded ceramic overlays to reestablish vertical dimension and composite resin to restore the worn palatal and incisal surfaces of the upper anterior teeth [38]. Recently a new classification system (ACE classification: anterior clinical erosion), strictly related to the clinical observation of the status of eroded anterior maxillary teeth affected, was proposed [39]. Minimally invasive techniques with composite were recommended to restore the palatal surfaces, while the facial surfaces were recommended to be treated with ceramic veneers when necessary. The authors concluded that the question of whether it is preferable to start earlier with a lighter, less invasive rehabilitation or later with a highly aggressive but eventually more resistant one is still open for debate. In all these studies it would appear that direct composite restorations are considered to be the main approach in less severe cases.

5. Conclusion

Despite the limitations of gathering information using a survey of this type, the results tend to suggest that the responding dentists are relatively up to date regarding the clinical recording and diagnosis of dental erosive wear, although dietary and salivary analyses were not given priority. Furthermore, although documentation was not standardized, the majority of dentists reported that they felt confident of finding the cause of the erosive wear and of treating their patients themselves. As dental erosion appears to be increasing in prevalence, one would expect that newly trained dental health personnel will need to be even more aware of the necessity of careful and early diagnosis of this problem and that they will be even better equipped to face the future clinical challenges related to dental erosive wear.

Conflict of Interests

The authors report no conflict of interests. The authors alone are responsible for the content and writing of the paper.

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Research Article

A Nanomechanical Investigation of Three Putative Anti-Erosion Agents: Remineralisation and Protection against Demineralisation

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An increasing interest in dental erosion as a clinical and scientific phenomenon has led to concerted efforts to identify agents which might protect against erosion. In this study, nanoindentation was used to investigate inhibition of erosive enamel demineralisation over time scales with direct clinical relevance. Nanohardness of polished human enamel specimens ($n = 8$ per group) was measured at baseline (B), after demineralisation (D1: citric acid, 0.3% w/v, pH3.20, 20s), after treatment (T), and after a second demineralisation (D2: as above). Data were analysed using repeated measures ANOVA. All specimens exhibited a similar reduction in nanohardness B-D1 in the range 35.2–39.5%. The positive control solution (saturated hydroxyapatite solution) and 4500 mg/L fluoride as NaF significantly increased nanohardness D1-T by 19.9% and 24.1%, respectively, whereas 1400 mg/L fluoride as NaF, casein phosphopeptide-amorphous calcium phosphate mousse and negative control (deionised water) had no significant effect. Nanohardness at D2 was indistinguishable for all groups, with total reduction in nanohardness B-D2 of 31.6% (4500 mg/L fluoride), 35.2% (positive control), 39.9% (1400 mg/L fluoride), 42.4% (negative control), and 43.7% (CPP-ACP product). In summary, 4500 mg/L fluoride significantly increased the nanohardness of previously demineralised enamel and resulted in the smallest total reduction in nanohardness but there were few statistically significant differences among the groups.

1. Introduction

Dental erosion is widely acknowledged as a common problem among children, adolescents, and adults [1]. There is a great deal of interest in the development and evaluation of treatments which might reduce the severity of erosion. Of these, salts of fluoride have received perhaps the most attention, and there is convincing evidence that applications of fluoride, particularly at high concentrations, can provide protection against erosion [2]. A number of different fluoride salts have been investigated, including stannous (tin) fluoride [3, 4], amine fluoride [3], titanium fluoride [5, 6], zirconium, and hafnium fluoride [6], but sodium fluoride has been the subject of the most studies owing to its widespread use in commercially available oral care products.

Another agent which has received attention with regard to its ability to protect against erosion is a peptide of the bovine milk protein casein, casein phosphopeptide, in association with nanoparticles of calcium phosphate [7]. This is most commonly described in the literature and in marketing material as CPP-ACP (casein phosphopeptide-amorphous calcium phosphate). It has been incorporated, under the name Recaldent, into various products, one of the most successfully marketed being GC Tooth Mousse (Europe and Australia) or MI Paste (Japan, North and South America) (GC Corporation, Japan) [8].

Some investigations of agents which protect against erosion published in the research literature utilise prolonged exposures to the treatment agents. There are instances in which it could be argued that these are difficult to justify—it is hard to imagine the clinical relevance of a study where the tooth specimens are treated with a solution or paste for hours or even days before the erosive challenge is applied. Other studies utilise a cycling approach where the treatment and/or erosion/wear and/or saliva exposure are alternated with the intention of mimicking the changing oral environment over a period of several hours or days. Thus although in some cases the total exposure time to the treatment agent may be rather long, this is interspersed with acid and saliva exposures and thereby represents an accelerated aging model of erosion. In other cases the treatment may only be applied once and then a number of cycles of demineralisation and remineralisation are applied, in part to give sufficient erosive tooth loss to be detectable using laboratory techniques [9, 10]. As long as each exposure is of clinically relevant duration [11], these studies are much more justifiable and, while they may overestimate the amount of wear that would be seen clinically [12], are very useful in assessing the relative merits of protective strategies. There is also benefit in developing experimental models in which the treatment and erosion phases are very short, comparable to those seen in a single *in vivo* event, such as a single brushing of the teeth and/or a single intake of drink. Some recent examples of such studies can be found in references [13–15].

The aim of this study was to investigate three potentially erosion-preventive measures in a model which allows for single, clinically relevant exposure times. The experiment was designed such that we could assess both the effect of the treatments on the nanohardness of softened enamel (whether the treatment had caused any remineralisation) and whether the treatments affected the nanohardness of the same enamel after a subsequent, second erosive challenge (whether the treatment inhibited further demineralisation).

2. Material and Methods

2.1. Specimen Preparation. Forty-four human enamel specimens were prepared by sectioning healthy, sound enamel from the mesial, distal, buccal, and lingual surfaces of 22 human premolars and molars, which had previously been sterilised by immersion in 20000 ppm available chlorine for 24 h, followed by immersion in 4% formaldehyde for 7 days, and subsequently long-term storage in 70% ethanol. The specimens were randomly assigned to five groups of 8 specimens. Specimens measuring 1-2 mm wide (around the perimeter of the tooth) and 2-3 mm long (along the central axis of the tooth) were sectioned from the teeth using a water-cooled, diamond tipped annular saw (Microsilice 2; Metals Research, Royston, UK) and mounted in epoxy resin (Stycast; Hitek Electronic Materials, Scunthorpe, UK) using silicone molds. The natural surface of the enamel was lapped using silicon carbide paper of 120 and 1200 grit size under slow water flow for the minimum possible time needed to remove any thin layer of resin that had flowed over the enamel surface and to remove the very outer enamel to provide a flat working surface. The specimens were then ultrasonicated in industrial methylated spirit (IMS) for approximately 2 min at room temperature to remove polishing debris and polished using an aqueous slurry of 0.25 μm aluminium oxide to achieve a mirror finish and ultrasonicated again in IMS. All specimens were carefully inspected for lesions or damage before they were accepted for use in the study. Specimens were also assessed at D1 (see below) for their nanohardness and any outlying specimens, with nanohardness more than 1.5 standard deviations different from the mean, were eliminated and replaced with new specimens. This was done in order to exclude specimens that proved to be unusually susceptible, or resistant, to demineralisation and applied to 4 specimens (10%) in this study giving a final $n = 40$.

2.2. Nanoindentation. A nanoindentation system comprising a diamond-tipped Berkovich tip and vertical engagement with continual monitoring and control of vertical displacement was used (Triboscope; Hysitron Inc., Minneapolis, MN, USA) on an atomic force microscope (Nanoscope IIIa; Digital Instruments, Santa Barbara, CA, USA). Five widely spaced nanoindentations were performed on each specimen at each time point of the experiment and the mean from each specimen was used for statistical analysis. The AFM was used, with the Berkovich tip, to scan an area of $5 \times 5\,\mu$m prior to each indent to establish that the specimen was free from debris or microscopic scratches or cracks, and after indentation to check that the indent was equilateral and thus that the tip was engaged normal to the specimen surface. The nanoindentation data were analysed using Hysitron software using the Oliver & Pharr method to calculate nanohardness [16].

2.3. Study Regime. Specimens were treated as follows:

Baseline nanohardness (B) measured on polished, untreated specimens

D1: 1st demineralisation phase

D1 nanohardness measured

T: treatment phase with one of three test treatments or positive or negative control

T nanohardness measured

D2: 2nd demineralisation phase

D2 nanohardness measured.

2.4. Demineralisation. The demineralising solution was 0.3% w/v citric acid monohydrate (Fisher Scientific, Loughborough, UK) adjusted to pH 3.20 using KOH. The specimens were attached to a disc 30 mm in diameter which was mounted on a quantitative overhead stirrer (R50D; CAT, Staufen, Germany) in order to provide a standardised method for agitation [17]. The angular velocity of the stirrer was adjusted to give an equivalent linear velocity of the specimens in the solution of 0.25 m/s. Specimens were exposed to acid for 20 seconds at room temperature. After each acid exposure, specimens were rinsed by immersion in deionised water for 60 seconds.

2.5. Treatment. Three test treatments were investigated alongside positive and negative control solutions.

2.5.1. Test Treatments. Two sodium fluoride solutions, with fluoride concentrations of 1400 (pH 6.68) and 4500 (pH 6.74) mg/L (Fisher Scientific, Loughborough, UK) were used, and a CPP-ACP-containing mousse product, hereafter referred to as CPP-ACP product (GC Tooth Mousse, GC Corporation, Tokyo, Japan).

2.5.2. Control Solutions. The negative control was deionised water. The positive control was a solution saturated with respect to hydroxyapatite (HA), which was prepared by incrementally adding 0.1 g aliquots of HA powder (Sigma Aldrich, Gillingham, UK) to 1 L deionised water at 70°C under moderate stirring until no further hydroxyapatite dissolved. The solution was maintained at 70°C for 72 hours then left to cool slowly to room temperature and used immediately.

2.6. Exposure to Treatment Solutions. Specimens were attached to a disc to facilitate their handling. In the groups tested with fluoride or deionised water, specimens were immersed in the solution for 2 minutes without stirring. For the positive control, specimens were immersed without agitation for 60 minutes. The final group was covered with CPP-ACP product for 5 minutes according to the manufacturer's recommendation. After treatment, all specimens were rinsed with deionised water for 60 seconds.

2.7. Scanning Electron Microscopy. Scanning electron micrographs of enamel specimens at D2 were obtained using a Phenom scanning electron microscope (FEI, Netherlands) at nominal magnifications of 10000x and 20000x.

2.8. Statistical Analysis. Nanohardness data were analysed by repeated measures ANOVA using SPSS statistical software package for Windows, version 16.0 (IBM Corporation, New York, USA).

3. Results

The mean nanohardness of the specimens as a function of stage and treatment and standard deviations are given in Table 1. In the reporting and discussion of the results, data are expressed as percent change from baseline nanohardness in order to provide a simple comparison of the effects of the treatments by eliminating the need for the reader to continually refer back to the baseline nanohardness. However, raw data can be seen in Table 1 to allow a comparison of the actual nanohardness values.

All specimens displayed a statistically significant softening from B to D1, with reduction in nanohardness in the range 35.2–39.5%. All specimens showed a numerical increase in hardness from D1 to T, but this was only statistically significant for the positive control (19.9% increase in nanohardness) and the 4500 mg/L fluoride (24.1% increase in nanohardness). All specimens showed a numerical softening from T to D2, but this was only statistically significant for 1400 mg/L fluoride (22.2% decrease in nanohardness) and CPP-ACP mousse (24.8% decrease in nanohardness).

Table 1: Mean nanohardness and standard deviations (GPa) of human enamel specimens as a function of stage and treatment. B: at baseline, D1: after first demineralisation, T: after treatment, D2: after second demineralisation.

Treatment	Mean nanohardness (GPa) (Standard deviation)			
	B	D1	T	D2
Negative control	3.98 (0.37)	2.58 (0.32)	2.76 (0.35)	2.29 (0.25)
1400 mg/L F	4.25 (0.66)	2.82 (0.32)	3.28 (0.68)	2.55 (0.28)
4500 mg/L F	4.12 (0.52)	2.56 (0.27)	3.38 (0.54)	2.82 (0.49)
CPP-ACP product	4.30 (0.53)	2.78 (0.36)	3.22 (0.39)	2.42 (0.27)
Positive control	4.72 (0.46)	2.86 (0.29)	3.56 (0.50)	3.06 (0.42)

The overall reduction in hardness from B to D2 was CPP-ACP product (43.7%), negative control (42.4%), 1400 mg/L fluoride (39.9%), positive control (35.2%), and 4500 mg/L fluoride (31.6%). The statistical analysis indicated that there was a statistically significant difference between the positive and negative controls, but not between either control and the test solutions.

Scanning electron micrographs of a representative selection of the enamel samples are shown in Figures 1(a)–1(j). Areas of the specimens show the characteristic honeycomb pattern of etched enamel, interspersed with areas where the original polishing lines are apparent. This demonstrates that the etching was at an early stage without bulk tissue loss. No surface deposits were observed in any specimen group and all specimens from all treatment groups had a similar appearance.

4. Discussion

In this *in vitro* study we investigated three agents with respect to their capacity to protect human enamel against dietary acid-mediated demineralisation. The use of nanoindentation allowed us to employ exposure times for acid and treatment that are relevant to a single clinical application, as was recommended in a recent thorough review of laboratory erosion and abrasion models [12]. The study design allowed the investigation of whether the test agents increased the nanohardness of previously demineralised enamel, and also whether the test agents protected the enamel against subsequent demineralisation.

Nanohardness was used as the outcome measurement and is interpreted as an indication of the extent of demineralisation that had taken place. Nanoindentation is recognised as one of the most sensitive methods for investigating enamel demineralisation [10, 14]. It has been shown that enamel nanohardness reduces as a function of acid exposure time and thus can be used as an indication of the extent of erosionlike demineralisation that has taken place up to exposure times of around 5 minutes [14, 18]. A correlation between nanohardness and the mineral content of calcium and phosphate-like species PO_xH_y in demineralised human enamel has been previously demonstrated [19], and so

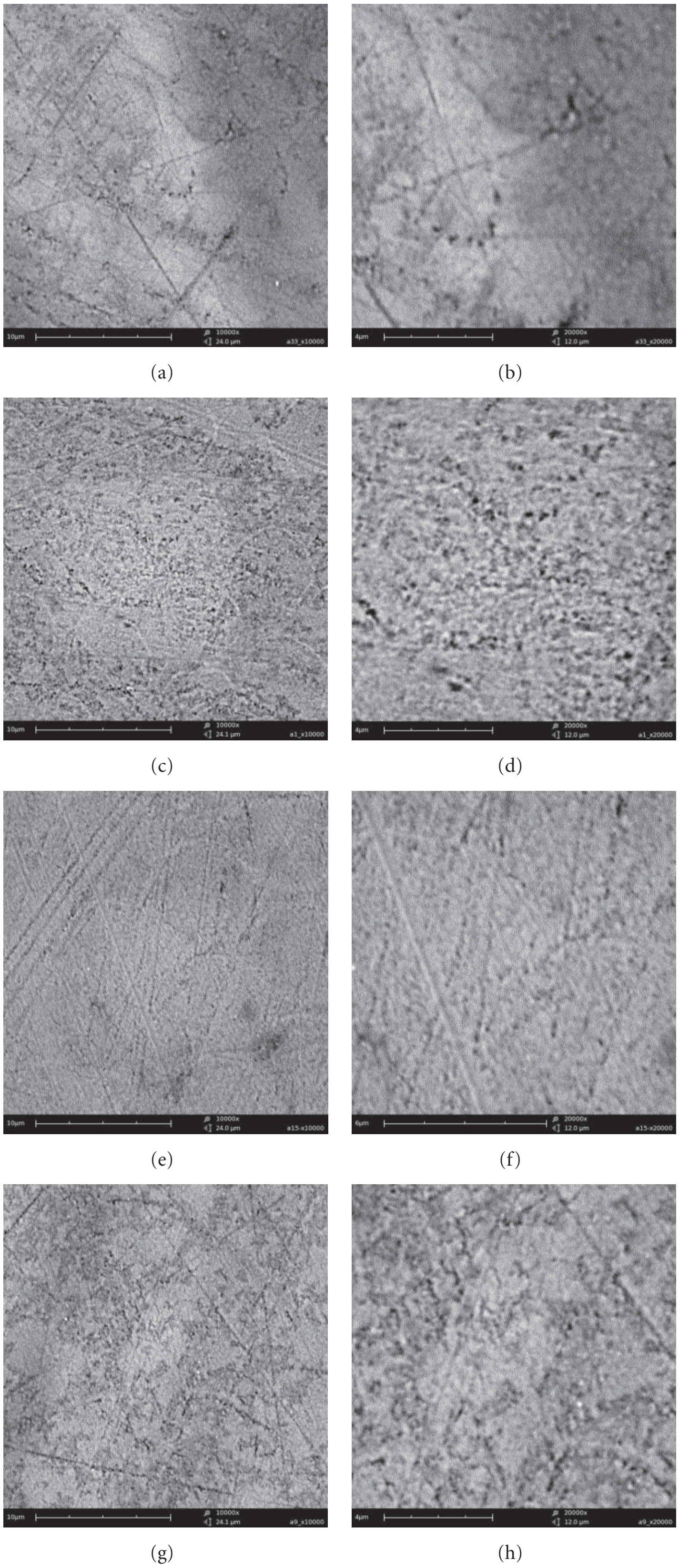
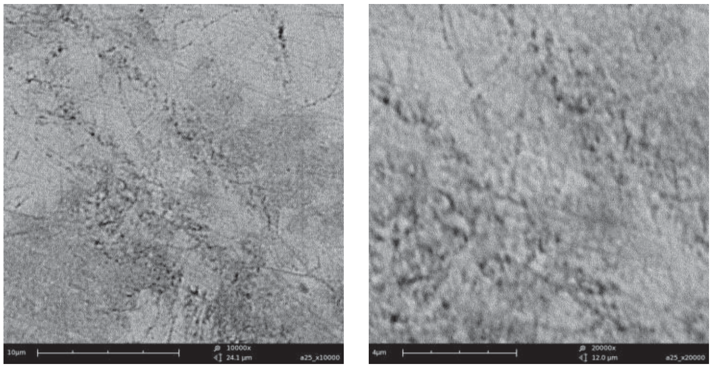


Figure 1: Continued.



(i) (j)

Figure 1: Scanning electron micrographs of enamel specimens after D2. (a) and (b) negative control; (c) and (d) 1400 mg/L F; (e) and (f) 4500 mg/L F; (g) and (h) CPP-ACP product; (j) positive control. Scale bars represent 10 μm ((a), (c), (e), (g), (i)) and 4 μm ((b), (d), (f), (h), (j)).

nanoindentation was selected for use in this study. This is because it offers the possibility to investigate enamel demineralisation and remineralisation at very early stages, using time scales of relevance to clinical exposure [20], where the bulk tissue is still in place, and where only localised mineral depletion within the enamel structure has occurred, as indicated in Figure 1 [18].

Experimental conditions for the treatment stage of the study were, likewise, designed to some extent to mimic the clinical situation. The fluoride solutions were applied for 2 minutes, since the recommendation for toothbrushing is typically to brush for 2 minutes, and the concentration of fluoride in saliva is quite rapidly depleted after the brushing ceases. That is not to say each tooth receives 2 minutes' brushing—the figure is more likely to be of the order of 5 seconds [12]—but there will be an elevated concentration of fluoride in the mouth throughout the brushing process. The fluoride concentrations were chosen as representative of mass market toothpastes (1400 mg/L) and comparable to prescription-only products (4500 mg/L). The CPP-ACP product was applied according to the manufacturer's guidelines, by smoothing onto the enamel surface and allowing it to remain undisturbed for 5 minutes. The positive control, saturated hydroxyapatite solution, was applied to the enamel specimens for 60 minutes to represent a period of inactivity during the day where the tooth surface is bathed in saliva.

The results indicate that 2 minutes' exposure to 4500 mg/L fluoride solution significantly increased the nanohardness of demineralised enamel, but 1400 mg/L fluoride solution did not. Furthermore, specimens treated with 4500 mg/L fluoride did not display significant softening when exposed to acid for a second time, whereas those treated with 1400 mg/L fluoride did soften significantly. Thus it appears that 4500 mg/L fluoride solution did, under these experimental conditions, provide both some rehardening of the enamel and some protection against subsequent demineralisation. We would interpret these results as signifying that the 4500 mg/L fluoride solution caused some precipitation of mineral within the softened surface enamel and that this resulted in both an increase in hardness and a reduced susceptibility to subsequent dissolution. This may suggest that the mineral deposited was fluorhydroxyapatite rather than calcium fluoride, and the moderately low concentration of fluoride and near-neutral pH of the treatment solution would support this inference [21]. The scanning electron micrographs in Figures 1(e) and 1(f) would also appear to offer support to this hypothesis, as they did not reveal any evidence of deposits *on* the surface, for instance of calcium fluoride. It should be noted, however, that the increase in enamel nanohardness afforded by treatment with 4500 mg/L fluoride was somewhat modest, and the resultant nanohardness after stage T was still some 18% lower than at baseline. The 1400 mg/L fluoride solution was either unable to produce such mineral deposits or those that were effected were insufficient to significantly increase nanohardness or reduce subsequent softening. A number of authors have sought to investigate the protective effects of fluoride compounds, and particularly sodium fluoride, against dental erosion [2]. A dose response effect showing increased protection at elevated sodium fluoride concentration has been observed both *in vitro* [13, 22–24] and *in situ* [25], although other studies have failed to reveal a dependence on fluoride concentration [26, 27].

Specimens that were demineralised and then treated with CPP-ACP product for 5 minutes, as recommended by the manufacturer, did not display a significant increase in nanohardness; neither did the treatment with CPP-ACP product provide any protection against subsequent demineralisation. There was no evidence of any of the product on the surface after D2 (Figures 1(g) and 1(h)). In a recent study, a significant increase in enamel microhardness was observed after treatment with the same CPP-ACP product [28], but the minimum exposure time investigated was 2 weeks. The contact time with the CPP-ACP product was, therefore, some 4000 times longer than in the present study. Another study using a 15-minute exposure to CPP-ACP demonstrated that enamel wear, as distinct from softening, was reduced by this treatment [29]. On the other hand, CPP-ACP product also failed to protect enamel against a subsequent erosive challenge in a model using 5 cycles of treatment, erosion, and remineralisation, although it was able to provide some protection when applied in conjunction with fluoride [30]. In a study where enamel specimens were eroded, placed in the mouth to facilitate remineralisation, treated with CPP-ACP product for 3 minutes applied either *ex vivo* or *in situ*, and finally replaced in the mouth for a further period of remineralisation, the CPP-ACP product did not confer any significant rehardening or protection of the enamel specimens [31]. It is plausible that putative protective effects of CPP-ACP are time dependent, with a significant effect only becoming apparent with long and/or repeated exposures.

5. Conclusion

Only two treatments significantly rehardened the softened enamel (from D1 to T): the positive control and 4500 mg/L fluoride (as sodium fluoride). The CPP-ACP product, 1400 mg/L fluoride and negative control solutions did not reharden the enamel. The total reduction in nanohardness (from B to D2) was 31.6% (4500mg/L fluoride), 35.2% (positive control), 39.9% (1400 mg/L fluoride), 42.4% (negative control), and 43.7% (CPP-ACP product), but over the entire time course the repeated measures ANOVA indicated that only the positive and negative controls differed to a statistically significant degree.

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