Original Paper

Caries Research

Caries Res 2005;39:371–379
DOI: 10.1159/000086843

Received: May 25, 2004
Accepted after revision: October 12, 2004

Effect of Titanium Tetrafluoride, Amine Fluoride and Fluoride Varnish on Enamel Erosion in vitro

A. Vieira J.L. Ruben M.C.D.N.J.M. Huysmans

Department of Dentistry and Oral Hygiene, Faculty of Medical Sciences, Groningen, The Netherlands

Key Words
Amine fluoride · Erosion · Fluoride varnish · Titanium tetrafluoride

Abstract
This study aimed at evaluating the effect of 1 and 4% titanium tetrafluoride (TiF_4) gels, amine fluoride (AmF) 1 and 0.25% and a fluoride varnish (FP) on the prevention of dental erosion. Two experimental groups served as controls, one with no pretreatment and another one pretreated with a fluoride-free varnish (FP-blanco). Dental erosion was modelled using bovine enamel samples submitted to alternate cycles of acid exposure in citric acid and remineralization in artificial saliva. Calcium loss of all samples involved in the study was quantified by atomic absorption spectroscopy and erosion depths were estimated. Two samples of each experimental group were also analyzed by white light confocal microscopy. The cumulative erosion depth (in μm) after 72 min was: TiF_4 gel 1% 8.29 ± 0.39; TiF_4 gel 4% 8.27 ± 0.55; AmF 1% 8.69 ± 0.66; AmF 0.25% 8.86 ± 0.33; FP 3.43 ± 1.07; FP-blanco 14.86 ± 1.59 and control 9.77 ± 0.49. A statistically significant protective effect ($p \leq 0.001$) was found only for the group pretreated with the fluoride varnish. Within the limitations of an in vitro study it may be concluded that topical applications of the fluoride varnish tested have a protective effect on the prevention of dental erosion.

Dental erosion is a pathologic, chronic, localized loss of dental hard tissue that is chemically etched away from the tooth surface by acid and/or chelation without bacterial involvement [Imfeld, 1996a]. Frequent consumption of acid-containing foods is an important etiologic factor of this condition, with fruit acids and phosphoric acid contained in fresh fruits, fruit juices and soft drinks being the most commonly consumed erosive acids [Zero, 1996]. Changes in lifestyle and dietary habits contributed to an increase of acid challenges to the dentition [ten Cate and Imfeld, 1996], making erosion a pathologic condition of special interest in contemporary dental research.

The pathology of dental erosion involves superficial demineralization of dental hard tissues [Meurman and ten Cate, 1996]. Since etching of enamel increases the surface-reactive area, and topically applied fluoride accumulates in demineralized lesions, the use of topical fluoride seems appropriate in the prevention of dental erosion [Imfeld, 1996b]. Although scientific evidence for the protective effect of fluoride on dental erosion is still lacking

KARGER

Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0008–6568/05/0395–0371$22.00/0

Accessible online at:
www.karger.com/cre

Ana Vieira
Department of Dentistry and Oral Hygiene
Faculty of Medical Sciences, University of Groningen
A. Deusinglaan 1, NL–9713 AV Groningen (The Netherlands)
Tel. +31 50 3633116, Fax +31 50 3632696, E-Mail a.vieira@med.rug.nl

[Meurman and ten Cate, 1996], some in vitro studies [Attin et al., 1998, 1999; van Rijkom et al., 2003] have reported a significant reduction of the erosion and abrasion of eroded enamel and dentin after application of topical fluoride agents. These results suggest the need for further research into the mechanism of action of fluoride products in the prevention of dental erosion. We hypothesized that the key for the possible success of some of these products in erosion prevention lies in the fact that they interact with tooth tissues, both chemically and physically. Therefore, in this study fluoride products were selected which are believed to combine those characteristics. Titanium tetrafluoride (TiF_4) has been shown to reduce artificial caries lesion formation and enamel solubility and enables high fluoride uptake [Büyükyilmaz et al., 1997a; Tezel et al., 2002]. At low pH Ti should have a tendency to bind with oxygen atoms in phosphate groups on the tooth surface (phosphate-bound oxygen) resulting in the production of a titanium dioxide glaze [Büyükyilmaz et al., 1997b]. The high fluoride uptake reduces the solubility of enamel and the titanium dioxide glaze protects the enamel from acid attacks [Gu et al., 1996]. van Rijkom et al. [2003] reported a significant erosion-inhibiting effect of TiF_4 4% compared to 1% neutral NaF gel. Amine fluorides (AmFs) act like surfactants, forming a homogeneous film on all oral surfaces [GABA, 2003]. The molecules adsorb readily to hydroxyapatite [Busscher et al., 1988] and their slightly acidic pH promotes the formation of calcium fluoride giving them caries-inhibiting properties [GABA, 2003]. Fluoride varnishes provide long contact periods between the dental tissues and the fluoride agent resulting in high fluoride uptake and the formation of calcium fluoride deposits that act as fluoride reservoirs [Arends and Schuthof, 1975; Grobler et al., 1983; de Bruyn, 1987; Petersson, 1993]. Besides its effect on de- and remineralization, we hypothesized that the films formed at the application of these products may act as a mechanical barrier against acid challenges.

The purpose of this study was to investigate in vitro the effect of the above-mentioned topical fluoride products in preventing dental erosion.

Material and Methods

Chemical Reagents

TiF_4 gels 1% (pH 2.5) and 4% (pH 3.2) were prepared from titanium (IV) fluoride (Aldrich Chemical Co., USA), carboxymethyl cellulose (akucell AC 2801, Enka-Akzo, Arnhem, The Netherlands) and distilled water. The AmF-containing agents used were: Elmex® fluid and Elmex medical (both from GABA BV). Elmex fluid is an aqueous solution containing 1% AmF in the form of Olaflur and Dectaflur. Elmex medical contains a total of 12,500 ppm F^-, 0.25% in the form of the AmFs Olaflur and Dectaflur, and 1% in the form of sodium fluoride. The varnishes used were Fluor Protector®, a polyurethane-based varnish containing fluoride (0.1%) in the form of difluoro silane and a fluoride-free varnish based on the composition of Fluor Protector (both from Vivadent, Liechtenstein). The composition and pH of the demineralization solution were based on data reported by Larsen and Nyvad [1999] for soft drinks. It consisted of 50 m*M* citric acid anhydrate, 0.4 m*M* $CaCl_2 \cdot 2\ H_2O$, 0.4 m*M* KH_2PO_4 and 1 m*M* NaN_3. The pH of the solution was adjusted to 3.0 with 1 *M* NaOH. For the Ca analysis a 0.11 wt% $LaCl_3$ solution was prepared from La_2O_3 and 0.6 *M* HCl. All reagents were obtained from Merck, Darmstadt, Germany. The enamel samples were remineralized with the commercially available artificial saliva Saliva Orthana® (Pharmachemie, Haarlem, The Netherlands), which according to the manufacturer consists of 3.5% mucin, 2% xylitol, 0.02% $CaCl_2$, 0.04% K_2HPO_4, preservatives and flavoring agents.

Sample Preparation

Fifteen bovine incisors were selected and enamel samples (3 × 3 mm) were prepared from the buccal surface of each tooth. Each sample was embedded in acrylic resin and ground flat on a rotating polishing machine (Phoenix Beta grinder/polisher, Buehler, Germany) under water cooling using Buehler SiC grinding paper (P1200). The samples were subsequently polished on the machine with diamond suspensions (6, 3, 1 and 0.1 μm) Buehler Metadi on a Buehler polishing cloth. After each polishing step the samples were rinsed for about 1 min under running tap water, sonicated for 5 min in water and rinsed again under running tap water for one more minute. The preparation resulted in the removal of about 100 μm enamel from the surface of the sample. The enamel surface area of each sample was measured using a stereomicroscope with a measuring grid. The samples were randomly divided into seven groups of six samples each.

Fluoride Pretreatment

Each of the experimental groups was pretreated with one of the topical fluoride agents as follows: TiF_4 1%, TiF_4 4%, Elmex fluid (AmF 1%), Elmex medical (AmF 0.25%) and Fluor Protector (FP). One group was pretreated with the fluoride-free varnish (FP-blanco) – control for the group pretreated with FP – and another group was not pretreated with any product (control). The TiF_4 gels were applied with cotton pellets by carefully dropwise alternately touching and awaiting the absorption into the tooth during 4 min. AmF 1% was applied with a cotton pellet and AmF 0.25% was applied with a microbrush, both for 4 min. FP and the FP-blanco were applied in a thin layer using a microbrush and air-dried. All groups were stored overnight in artificial saliva.

Erosive Demineralization

In order to mimic the conditions in the mouth during the consumption of erosive beverages, dental erosion was modelled by submitting the enamel samples to alternate cycles of demineralization within the demineralization solution under gentle agitation, and remineralization in artificial saliva. One complete cycle consisted of the following steps: (1) 4 min in 1 ml demineralization solution; (2) rinse with 4.4 ml distilled water; (3) 4 min in 1 ml demineralization solution; (4) rinse with 4.4 ml distilled water; (5) 4 min in 1 ml demineralization solution; (6) rinse with 4.4 ml distilled water; (7) remineralization in artificial saliva for 7 days. All groups were submitted to 6 cycles resulting in a total erosion time of 72 min.

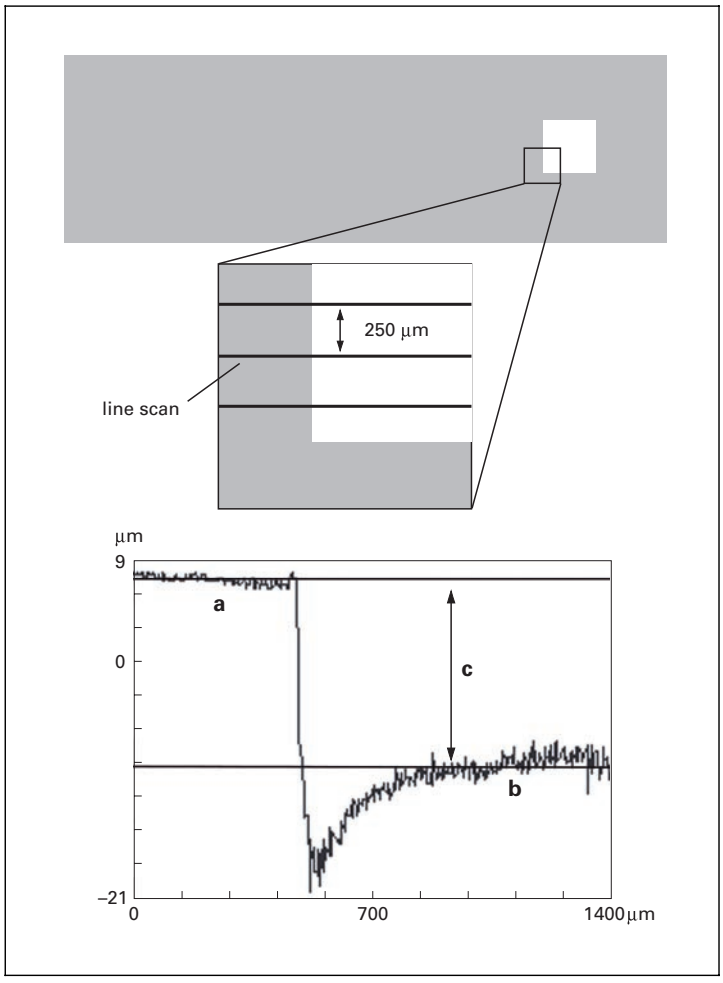

Fig. 1. Schematic drawing of the measurements performed with the CM. The grey area represents the acrylic resin (reference surface) and the white square the enamel surface. The profile tracing corresponds to one of the line scans. On each profile tracing two average lines were taken, one over the reference surface (a) and another over the eroded enamel surface (b). The distance between the two lines (c) was the erosion depth for the respective profile tracing.

Ca Analysis

The calcium loss following each acid exposure was determined by atomic absorption spectroscopy (AAS; AS 90, Perkin Elmer Analytical Instruments, Shelton, Conn., USA) [ten Cate, 1979].

The analysis was carried out in test tubes containing the collected demineralization solution (1 ml) combined with the distilled water (4.4 ml) used to rinse the sample. 0.6 ml 0.11 wt% $LaCl_3$ was added to the test tubes and the content was vortex agitated. The $LaCl_3$ solution was added to suppress phosphate interference with the calcium determination. A calibration curve was obtained with 1, 2.5, 5 and 10 mg/l standard Ca solutions with the same La concentration as in the solutions to be analyzed. The calcium loss from the enamel samples was determined by subtracting the calcium content of the demineralization solution – blanco solutions were analyzed – before the enamel exposure from the total calcium content of the solution. The lesion depth was calculated from the calcium loss, using the average calcium content per unit volume for bovine enamel and the exposed enamel area [ten Cate, 1979; Dijkman, 1982].

White Light Confocal Microscopy

Erosion depths were quantified with a white light confocal microscope (CM) from NanoFocus µSurf, Duisburg, Germany. The measurements were done on two samples of every experimental group. Baseline measurements were performed and after each demineralization cycle the samples were again analyzed. A 10× microscope objective with a basic field of 1,400 × 1,320 (µm × µm) and a vertical resolution of 50 nm was used. A scan was obtained from the left inferior corner of the sample and surrounding acrylic resin (reference surface). Using the equipment's software package three line scans were performed on each scan at intervals of 250 µm resulting in three profile tracings. On each profile tracing two average lines were taken, one over the reference surface and another over the eroded enamel surface. The distance between the two lines was the erosion depth for the respective profile tracing (fig. 1). The average of the erosion depths calculated from each of the three profile tracings was assumed to be the average erosion depth for the respective sample.

Statistical Analysis

Repeated measures analysis of variance with SPSS 11 for Windows 2000 with the General Linear Model (GLM) was used to analyze the data. The data were checked for normal distribution (QQ plots) and Levene's statistic was used to test the null hypothesis of equality of variance across groups. Multiple comparisons were performed with the Bonferroni procedure and the Dunnett t test (two-

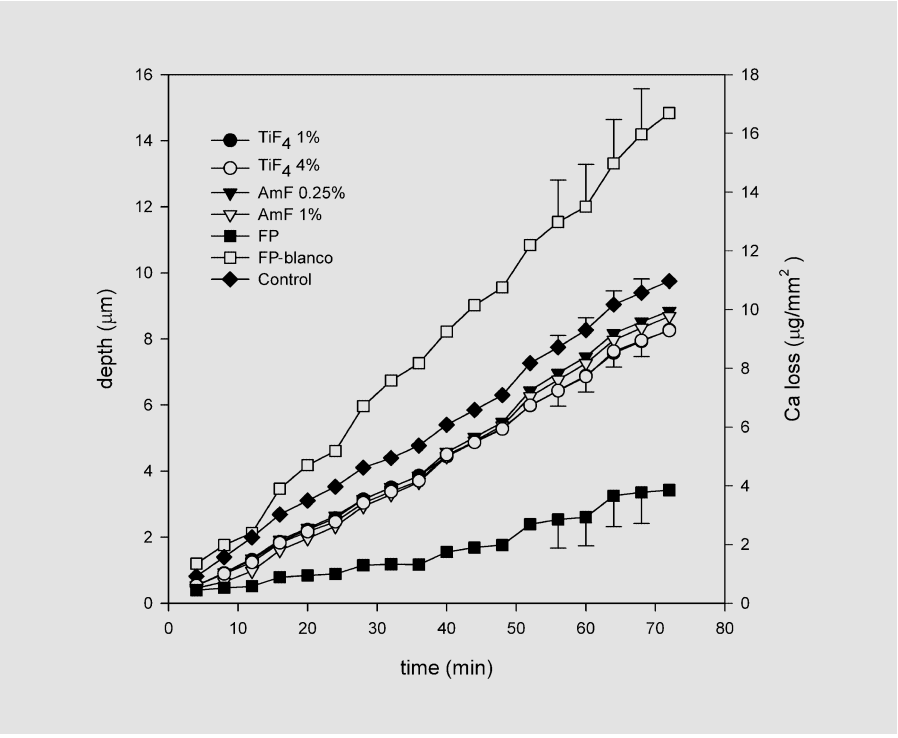

Fig. 2. Cumulative calcium loss and calculated erosion depth obtained by AAS.

sided). Regression analysis was used to evaluate erosion rates obtained with AAS and to compare the results obtained with AAS and CM. Since only two samples per group were analyzed with CM, the regression analysis included the results of the groups pretreated with TiF_4, AmF and control. The significance level for all tests was set at 0.05.

Results

In figure 2 the cumulative calcium loss and erosion depths calculated from it are plotted against erosion time. For all groups depth and time were linearly related, with the r^2 for the group pretreated with the fluoride varnish (0.950) slightly lower than those of the other groups (r^2 between 0.993 and 0.998). The fluoride varnish group showed a markedly lower erosion rate (slope 0.210) in comparison with the other fluoride-treated groups (slopes between 0.528 and 0.570). The group treated with the fluoride-free varnish showed the highest erosion rate (slope 0.921), followed by the control group (slope 0.590). The latter showed slightly higher erosion depths than the fluoride-treated groups. The cumulative erosion depth (in μm) after 72 min was: TiF_4 gel 1% 8.29 ± 0.39, TiF_4 gel 4% 8.27 ± 0.55, AmF 1% 8.69 ± 0.66, AmF 0.25% 8.86 ± 0.33, FP 3.43 ± 1.07, FP-blanco 14.86 ± 1.59 and control 9.77 ± 0.49. Multiple comparisons using both the Bonferroni procedure and the Dunnett t test yielded a statistically significant difference ($p \leq 0.001$) for both the group pretreated with FP and FP-blanco.

Figure 3 shows the mean calcium loss and erosion depths obtained with AAS at each acid exposure for the different groups. An oscillation of the depth values with time between each erosion cycle was observed for all groups with distinctly higher erosion depths observed at the first erosive challenge of each cycle (fig. 3a). The multivariate tests showed a significant interaction ($p \leq 0.001$) between the groups and the erosion time (cycles). A trend analysis of the erosion time showed that the interaction was particularly significant for the first ($p = 0.001$) and last ($p = 0.002$) cycles. Some degree of oscillation was still observed when the results were plotted without the first erosive challenge of each cycle (fig. 3b).

In figure 4 the cumulative erosion depths obtained with the CM are plotted against the time. Once again the erosion depths increased linearly with time, except for the fluoride varnish group that did not show any increase in material loss from the surface. The group pretreated with the fluoride-free varnish showed markedly less material loss from the surface than the groups pretreated with TiF_4 and AmF.

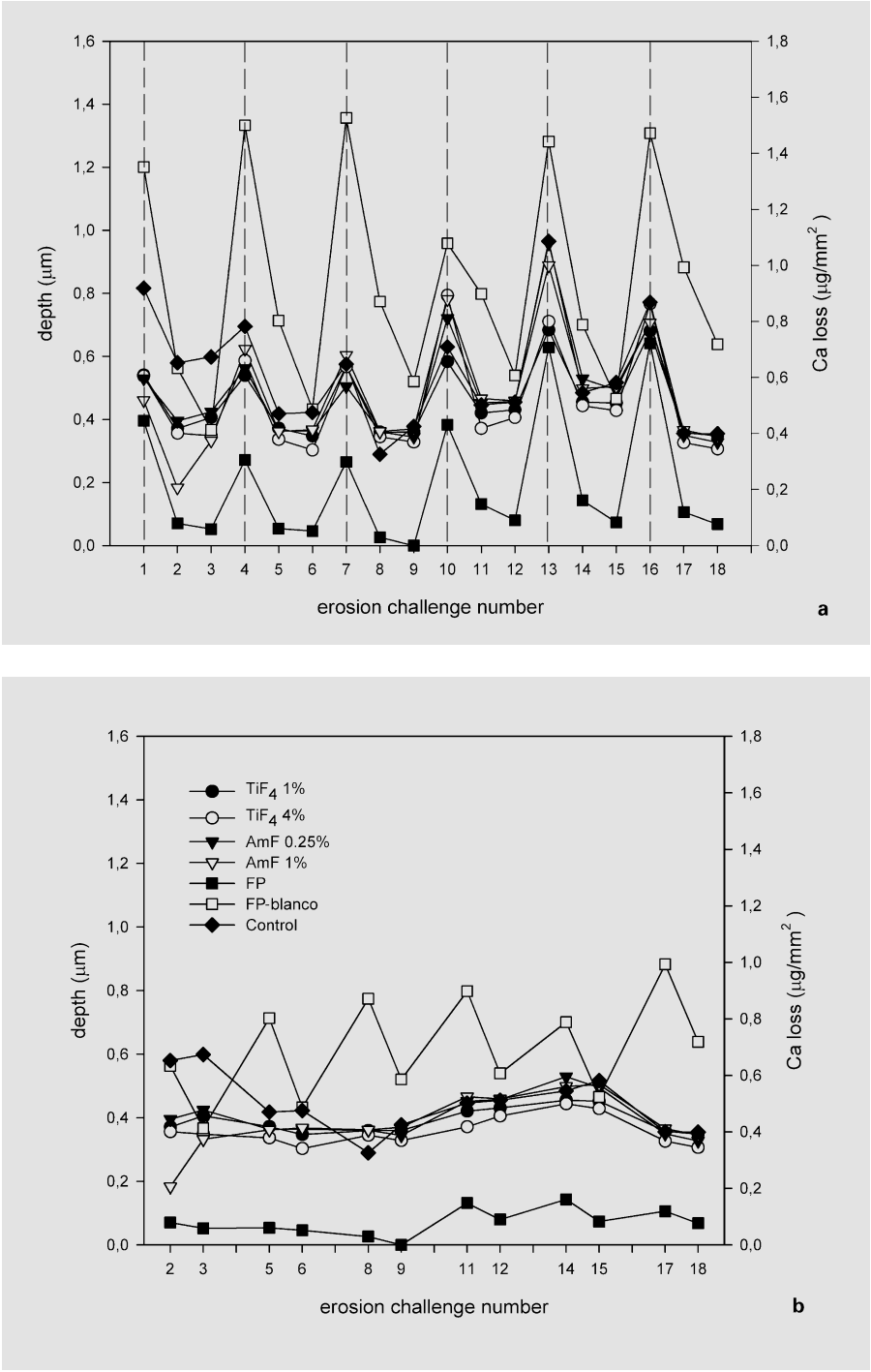

Fig. 3. Mean calcium loss and erosion depth obtained by AAS (**a**) at each acid exposure (**b**) without the first acid exposure of each erosion cycle. **a** The vertical dashed lines indicate the first acid exposure of each cycle.

In figure 5 the erosion depths obtained with the CM for the two samples of the control group and the groups pretreated with TiF_4 and AmF are plotted against the ones obtained with AAS for the same samples. The methods were highly linearly correlated ($r^2 = 0.946$); however, the results obtained with confocal microscopy were significantly higher than those obtained with AAS (slope 6.215, $p \leq 0.001$). Regression analysis yielded a Y-intercept of –1.043, which was not statistically different from zero ($p = 0.091$).

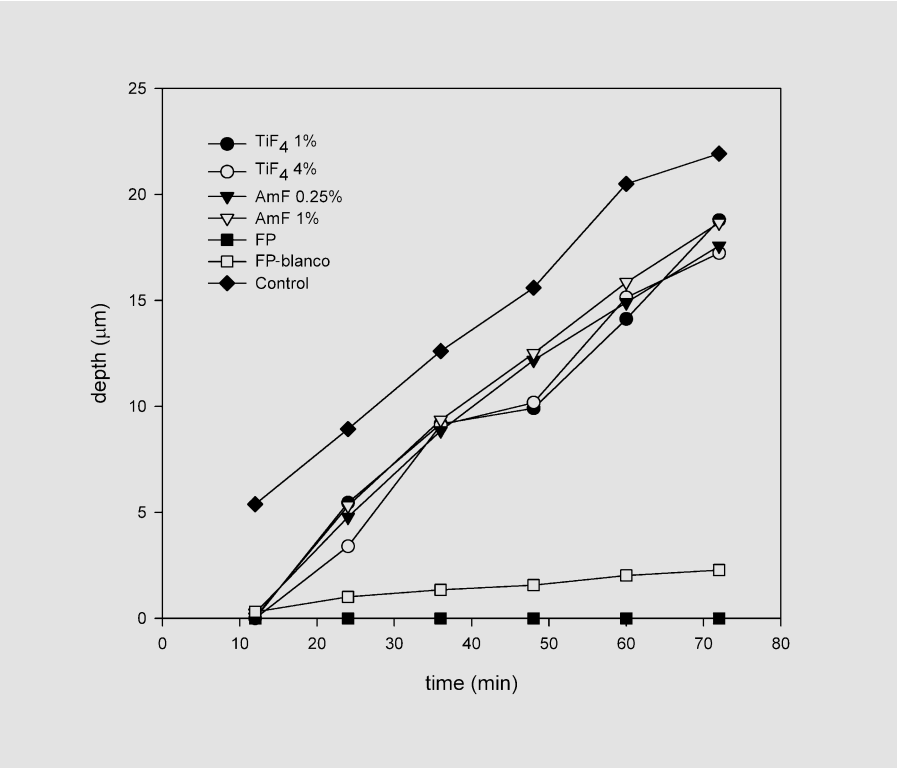

Fig. 4. Cumulative erosion depth obtained by confocal microscopy (n = 2 per group).

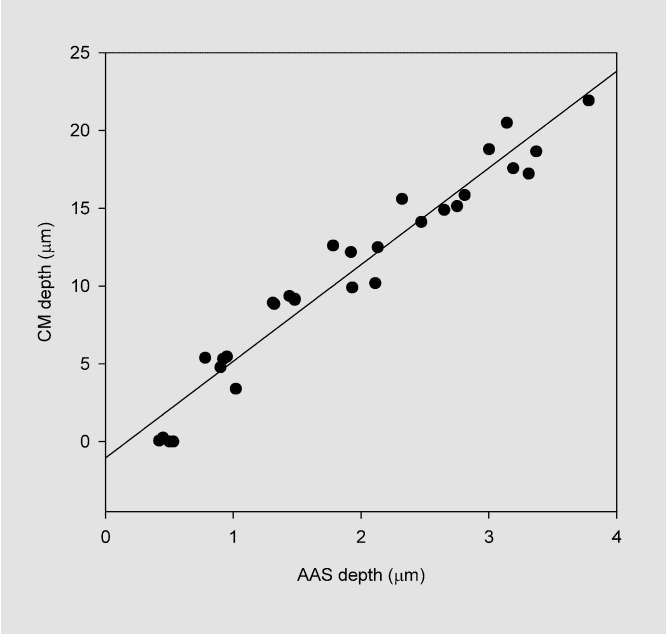

Fig. 5. Correlation between the values of cumulative erosion depth determined by CM and AAS, obtained for two samples of the control group and the groups pretreated with TiF_4 and AmF. The line indicates the least-squares regression (slope = 6.215, Y-intercept = –1.043, $r^2 = 0.946$).

Discussion

The methodology used in this in vitro study intended to reproduce, within the limitations of an in vitro study, the conditions in the mouth during the consumption of erosive beverages. The use of bovine incisors was found to be more convenient due to the larger availability, larger surface area and flatness of the labial surface of the specimens when compared to human enamel. Although bovine enamel has been widely used in dental research as a model for human enamel, morphological differences such as higher porosity [Featherstone and Mellberg, 1981] exist when compared to human enamel, which result in higher rates of lesion formation. This must be taken into account when interpreting the results. Some authors prefer long erosive periods [Jaeggi and Lussi, 1999; Ganss et al., 2000]. In the present study successive short contact periods with the erosive agent, under gentle agitation, were used in order to mimic the sipping of a beverage. Between the erosion cycles the samples were remineralized in artificial saliva. Artificial saliva has been previously used for this purpose and its efficacy has been proved [Attin et al., 2000]. Although the authors would have preferred shorter remineralization periods, a period of 1 week was chosen

due to operator logistics. One might suspect that under these conditions a more complete rehardening of the softened enamel layer has taken place. This may have had some influence on the higher calcium loss observed at the first acid exposure of each erosion cycle (see fig. 3a), though van Rijkom et al. [2003] also observed this phenomenon using much shorter remineralization periods. Therefore, it can be assumed that the effect of 1-week remineralization is in principle of minor importance.

It is well known that dental erosion is influenced by several factors, such as pH, the acid used, concentration, temperature, remineralization period and exposure time among several others [Attin et al., 2000; West et al., 2000]. West et al. [2000] found a 3-fold increase in enamel loss over the temperature range of 5–60°C. In figure 3b an oscillation of the erosion depths was observed between each erosion cycle. Since almost all variables except the room temperature were controlled, and approximately the same effect was observed for all experimental groups, the temperature may have played a role in the oscillation of the depth values. However, the oscillation was greater than would be expected from a variation of ±2°C as is reasonable in the lab.

Although all fluoride-treated groups showed lower erosion rates than the control groups, a statistically significant protective effect was only found for the group pretreated with fluoride varnish. This is not in agreement with van Rijkom et al. [2003], who reported for equivalent experimental conditions statistically significant differences between an experimental group pretreated with a 4% TiF_4 aqueous solution and a control group with no fluoride pretreatment. In order to prevent additional enamel loss with the application of the extremely acidic TiF_4 solutions (pH 1.5), in this study TiF_4 gels were used instead of an aqueous solution. Since the efficacy of the gels relative to the aqueous solutions was previously evaluated in a pilot study, where bovine enamel samples pretreated with 1% TiF_4 gel and solution showed comparable Ca loss after three erosion/remineralization cycles with a total of 30 min erosion, the differences found between the results of these two studies may be partly due to different application methods or differences between the statistics used. If we compare the estimated lesion depth for the 4% TiF_4 groups in the present study with those obtained by van Rijkom et al. [2003] after 28 min, the actual difference is small (2.7 vs. 2.9 μm) supporting the latter explanation.

Arends and Schuthof [1975] reported considerably higher amounts of fluoride permanently bound to dental enamel – up to a depth of 50 μm – after the application of fluoride varnish than those yielded with APF, AmF or NH_4F. They explained this by the longer effective time available for fluoride to react with hydroxyapatite in enamel provided by the varnish. Moreover, the varnish serves also as a fluoride reservoir since the silane fluoride is not only a source of fluoride ions but also reacts with water producing HF. This may explain the lower calcium loss for the group pretreated with fluoride varnish in the present experiments. Hunter et al. [2000] reported a linear pattern for enamel erosion with increasing etching time. In the present study a linear pattern was also found for all experimental groups, though the r^2 for the group pretreated with fluoride varnish was slightly lower than those of the other groups. In fact, the FP group showed several plateaus between the consecutive erosion cycles. This phenomenon is probably associated with the mechanical protection from the varnish, which prevents a more progressive calcium loss as was observed for the other fluoride products.

Confocal microscopy also revealed a linear pattern for the erosion depths with time except for the experimental group treated with FP. This group did not show any material loss from the surface through the erosion challenges. Since the varnish layer is not homogeneous, there may have been some places where the protective effect against the acid was diminished and calcium ions were still able to diffuse to the erosive medium. This would explain the calcium loss observed by AAS. This may also be a good explanation for the extremely high calcium loss observed for the group pretreated with the fluoride-free varnish. Presuming the varnish becomes porous after some erosion challenges, it would act as a reservoir for erosive medium underneath until the next remineralization period. This would provide longer contact periods between the medium and the enamel surfaces which, in the absence of fluoride, would result in a higher calcium loss than in the control samples or samples pretreated with other fluorides, where the medium can easily be washed away. With confocal microscopy the group pretreated with fluoride-free varnish showed less material loss from the surface than the control group and the groups pretreated with fluoride, with the exception of fluoride varnish. This emphasizes the mechanical protective effect of the varnish. The fact that some material loss was detected with the FP-blanco is possibly related to a slightly different composition of the varnish: the solvent used is not the same. This makes the fluoride-free varnish a less suitable control for the effect of the varnish.

The erosion depths obtained by confocal microscopy were significantly higher than those obtained by AAS.

One possible explanation for this discrepancy may be found in the differences between the principles by which both methods operate. With the AAS technique the calcium loss at each erosion challenge was measured, and the thickness of the etched layers was estimated according to the method reported by ten Cate [1979] and Dijkman [1982]. With the CM the reference and etched surfaces were scanned and the height difference was actually measured. A limitation of this technique was the relative small basic field used, which did not allow for the screening of the whole sample surface. In figure 1 an increase of height of the enamel surface towards the center of the sample is observed. Thus the measurements made on the periphery of the samples may have caused an overestimation of the erosion depths. Another possible explanation for the dissimilarity between the results obtained by both methods is the collapse of the softened layer created during the erosion challenges. Eisenburger et al. [2004] found in an SEM study a very delicate surface of softened enamel left after an erosion challenge of 2 h in 0.3% citric acid (pH 3.2). The acid damage extended to a depth of 9–12 μm below the surface. The layer was according to the authors extremely vulnerable to mechanical forces and drying led to its collapse. As in the present study the surface of the samples was wet but not immersed in water during the measurements with the CM, collapse of the softened layer may have taken place. This would result in higher erosion depths measured with the system. Furthermore, the accuracy of the CM in scanning the eroded enamel surface was evaluated in a pilot study where erosion depths of samples before and after Au-Pd (13-nm-thick layer) sputtering were compared. A difference of approximately 3 μm was found with smaller erosion depths after sputtering, indicating the possibility of some light penetration through the samples and consequently overestimation of the erosion depths. However, this cannot by itself explain the differences found. The Y-intercept of the least-squares regression between the erosion depths obtained by CM and the ones estimated from AAS (fig. 5) did not significantly differ from zero. This indicated that CM-measurable depth apparently did not proceed through the softened layer. The varnish-treated groups were not included in the analysis since their results were statistically different from the other groups. The accuracy of the erosion depth estimate from Ca loss (i.e. softening detection) can, however, also be questioned and therefore contribute to the discrepancies found between the two methods.

Within the limitations of an in vitro study it might be concluded that topical applications of fluoride varnish have a protective effect on the prevention of dental erosion. The differences found between the results obtained with AAS and white light confocal microscopy, for the measurement of the erosion depths, suggest the need of further research focusing on the investigation of techniques better suited for the quantification of dental erosion.

Acknowledgments

The authors gratefully acknowledge the company Ivoclar Vivadent, Schaan, Lichtenstein, for supplying the fluoride-free Fluor Protector. Dr. H. Breukelman and Marchien Velvis are thanked for their assistance with atomic absorption spectroscopy analysis. The authors also want to express their gratitude to Dr. V. Ocelik, Dr. N.J.M. Carvalho and Prof. J.Th. De Hosson of the Department of Applied Physics of the University of Groningen for their support with the confocal microscope. Dr. A.G. Dijkman is acknowledged for the helpful discussions.

References

Arends J, Schuthof J: Fluoride content in human enamel after fluoride application and washing – an in vitro study. Caries Res 1975;9:363–372.

Attin T, Buchalla W, Gollner M, Hellwig E: Use of variable remineralisation periods to improve the abrasion resistance of previously eroded enamel. Caries Res 2000;34:48–52.

Attin T, Deifuss H, Hellwig E: Influence of acidified fluoride gel on abrasion resistance of eroded enamel. Caries Res 1999;33:135–139.

Attin T, Zirkel C, Hellwig E: Brushing abrasion of eroded dentin after application of sodium fluoride solutions. Caries Res 1998;32:344–350.

de Bruyn H: Fluoride Varnishes and Enamel Caries; PhD thesis, Groningen, 1987.

Busscher HJ, Uyen HM, De Jong HP, Arends J, Kip GAM: Adsorption of amine fluorides on human enamel. J Dent 1988;16:166–171.

Büyükyilmaz T, Øgaard B, Duschner H, Ruben J, Arends J: The caries-preventive effect of titanium tetrafluoride on root surfaces in situ as evaluated by microradiography and confocal laser scanning microscopy. Adv Dent Res 1997a;11:448–452.

Büyükyilmaz T, Øgaard B, Rølla G: The resistance of titanium tetrafluoride-treated human enamel to strong hydrochloric acid. Eur J Oral Sci 1997b;105:473–477.

ten Cate B: Remineralisation of Enamel Lesions: A Study of the Physico-Chemical Mechanism; PhD thesis, Groningen, 1979.

ten Cate JM, Imfeld T: Etiology, Mechanisms and Implications of Dental Erosion. Workshop proceedings. Limelette, Belgium, September 28–29, 1995 (preface). Eur J Oral Sci 1996;104:149.

Dijkman T: Topical Fluoride Applications on Human Enamel – A Combined in vivo-in vitro Study; PhD thesis, Groningen, 1982.

Eisenburger M, Shellis RP, Addy M: Scanning electron micoscopy of softened enamel. Caries Res 2004;38:67–74.

Featherstone JDB, Mellberg JR: Relative rates of progress of artificial caries lesion in bovine, ovine and human enamel. Caries Res 1981;15:109–114.

GABA International: Active substances – amine fluoride 2003; available from URL: http://www.gaba.com/dental_profession/active_substances.html.

Ganss C, Klimek J, Schwarz N: A comparative profilometric in vitro study of the susceptibility of polished and natural human enamel and dentine surfaces to erosive demineralisation. Arch Oral Biol 2000;45:897–902.

Grobler SR, Øgaard B, Rølla G: Fluoride uptake and retention by sound enamel after in vivo Duraphat application. J Dent Assoc S Afr 1983;38:55–58.

Gu Z, Li J, Söremark R: Influence of tooth surface conditions on enamel fluoride uptake after topical application of TiF_4 in vitro. Acta Odontol Scand 1996;54:279–281.

Hunter ML, West NX, Hughes JA, Newcombe RG, Addy M: Relative susceptibility of deciduous and permanent dental hard tissues to erosion by a low pH fruit drink in vitro. J Dent 2000; 28:265–270.

Imfeld T: Dental erosion. Definition, classification and links. Eur J Oral Sci 1996a;104:151–155.

Imfeld T: Prevention of progression of dental erosion by professional and individual prophylactic measures. Eur J Oral Sci 1996b;104:215–220.

Jaeggi T, Lussi A: Toothbrush abrasion of erosively altered enamel after intraoral exposure to saliva: An in situ study. Caries Res 1999;33:455–461.

Larsen MJ, Nyvad B: Enamel erosion by some soft drinks and orange juices relative to their pH, buffering effect and content of calcium and phosphate. Caries Res 1999;33:81–87.

Meurman JH, ten Cate JM: Pathogenesis and modifying factors of dental erosion. Eur J Oral Sci 1996;104:199–206.

Petersson LG: Fluoride mouthrinses and fluoride varnishes. Caries Res 1993;27(suppl 1):35–42.

van Rijkom H, Ruben J, Vieira A, Huysmans MCDNJM, Truin G: Erosion-inhibiting effect of sodium fluoride and titanium tetrafluoride treatment in vitro. Eur J Oral Sci 2003;111: 253–257.

Tezel H, Ergücü Z, Önal B: Effects of topical fluoride agents on artificial enamel lesion formation in vitro. Quintessence Int 2002;33:347–352.

West NX, Hughes JA, Addy M: Erosion of dentine and enamel in vitro by dietary acids: The effect of temperature, acid character, concentration and exposure time. J Oral Rehabil 2000;27: 875–880.

Zero DT: Etiology of dental erosion – extrinsic factors. Eur J Oral Sci 1996;104:162–177.