Erosive enamel wear and the inhibiting effect of topical fluorides

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Dental erosion, the dissolution of hard tooth tissues due to acids of non-bacterial origin, can cause extreme tooth material loss especially if combined with mechanical factors in the mouth. The best approach to prevent the problem is to reduce the acid challenges in the mouth. This, however, poses a problem when dealing with erosion due to intrinsic factors as gastro-intestinal problems or low patient's compliance in erosion due to acidic diets. Therefore complementary preventive measures such as fluoride applications have been proposed. Commercially available fluoride products have been developed for the purpose of caries prevention and treatment, and fundamental differences exist between the caries and erosion processes. For this reason research should focus on understanding the process of erosive wear in order to choose the fluoride products best suited for its prevention.

Chapter 1 gives an overview of the processes involved in tooth wear. Erosion assumes a central role and is therefore discussed in more detail than the other wear processes. Preventive measures for erosive tooth wear are reviewed, with a particular interest in the use of fluoride and the considerations that need to be made when choosing a particular compound. The interaction between mechanical factors in the mouth, the eroded enamel and the fluoride products and, in particular the wear resistance of the products, seem important points to take into account when selecting fluoride products for wear prevention. However, little is known about the performance of the available topical fluoride products under wear conditions. Therefore, the aim of the research described in this thesis was to evaluate topical fluorides in their ability to prevent erosive wear and to understand their mechanism of action. Subsidiary aims were to evaluate the interaction between enamel erosive wear and mechanical factors in the mouth and the validation a new method for enamel wear quantification.

The pathology of enamel erosion involves superficial demineralisation causing the loss of the outermost enamel surface. In addition, diffusion of erosive agents into the enamel also causes subsurface demineralisation and the formation of a softened layer. Although it is known that the softened layer is very susceptible to disruption by mechanical factors such as brushing abrasion, tongue friction and attrition, the extent of damage caused by these factors had not been compared before. This is an important matter to be considered in the development of preventive measures for erosive wear prevention and was therefore investigated in the first part of this thesis (**Chapter 2**). Forty two bovine enamel samples were randomly divided into 7 groups (n = 6 per group) and submitted to 3 cycles of one of the following regimens: erosion and remineralisation (er/remin); toothbrush abrasion and remineralisation (abr/remin); erosion, toothbrush abrasion and remineralisation (er/abr/remin); attrition and remineralisation (at/remin); erosion, attrition and remineralisation (er/at/remin); simulated tongue friction and remineralisation (tg/remin); erosion, simulated tongue friction and remineralisation (er/tg/remin). Erosion took place in a demineralisation solution (50 mM citric acid, pH 3) for 10 min under controlled agitation (100 rpm). Brushing abrasion, tongue friction and attrition were simulated for 1 min using a home-made wear device. Remineralisation was carried out in artificial saliva for 2 h. Enamel loss was quantified using optical profilometry. One-way ANOVA indicated a significant difference between the amounts of enamel lost due to the different wear regimes ($p \leq 0.001$). Multiple comparisons with Bonferroni procedure showed that the wear depths found for the er/at/remin ($p \leq 0.001$) and er/tg/remin ($p \leq 0.001$) were significantly higher than the amount found for the er/remin group (4.4 ± 0.7 μm). This was not the case for er/abr/remin group ($p = 0.075$). The results suggested that the three forms of mechanical insults remove the softened layer formed by erosion to varying extent. This resulted in the hypothesis that a mineral gradient exists in the softened layer, with the outer part most demineralised and consequently the most fragile.

The results obtained in Chapter 2 confirm the fragility of the softened layer and raise important questions concerning the quantification of erosive wear such as: Are contact methods appropriate for erosion quantification? And, if the reliability of contact methods such as profilometry is influenced by possible damage of the softened layer by the stylus of the equipment which comes in contact with the sample during the scanning of the surface, is this dependent on the wear regimen applied? The answers to these questions are found in **Chapter 3**, where the performance of an optical profilometer and a contact profilometer in the quantification of enamel loss under 3 different wear regimens was evaluated. In this study eighteen bovine enamel samples were divided into 3 groups and submitted to 3 erosion/remineralisation, erosion/brushing abrasion/remineralisation or erosion/tongue friction/remineralisation cycles. Erosion was modelled by immersion of the samples in a demineralisation solution consisting of 50 mM citric acid (pH 3) for 10 min under controlled agitation (100 rpm). Brushing abrasion and tongue friction were simulated for 1 min using a home-made wear device. Remineralisation took place in artificial saliva for 2 h. Enamel loss was quantified using optical profilometry and contact profilometry and the methods were compared. One-way ANOVA showed no significant differences for the wear depths obtained with the two profilometers. The two methods were highly linearly correlated ($r = 0.95$). Least-squares linear regression analysis yielded a y-intercept of - 0.78 (95% C.I.: -2.08 to 0.52) and a slope of 1.14 (95% C.I.: 0.95 to 1.33). Although all samples showed scratches on the enamel surface after contact profilometry, the results indicated that the profilometer stylus penetrated only the very surface of the softened enamel. The scratch depths were inversely related to the wear depths what seems to confirm the previous hypothesis about the morphology of the softened layer. Nevertheless the use of non-contact methods for erosion quantification seems preferable and optical profilometry is considered a feasible method for that purpose.

The second part of this thesis focuses on the investigation of fluoride compounds capable of remineralising the softened layer and therefore limiting the extent of enamel loss. Based on the knowledge obtained in Chapters 2 and 3 on the disruption of the softened layer by mechanical factors, we selected fluoride products that may provide chemical and mechanical protection of the surface.

In **Chapter 4,** 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 was evaluated. Two experimental groups served as controls, one with no pretreatment, and another one pretreated with a fluoride-free varnish (FP-bl). Dental erosion was modelled using bovine enamel samples submitted to alternate cycles of acid exposure in citric acid and remineralisation 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 analysed by white light confocal microscopy. The cumulative erosion depth (in μm) after 72 min was: TiF_4 gel 1 % 8.29 $\pm$ 0.39; TiF_4 gel 4 % 8.27 $\pm$ 0.55; AmF 1% 8.69 $\pm$ 0.66; AmF 0.25% 8.86 $\pm$ 0.33; FP 3.43 $\pm$ 1.07; FP-bl 14.86 $\pm$ 1.59 and control 9.77 $\pm$ 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 was concluded that topical applications of the fluoride varnish tested have a protective effect on the prevention of dental erosion.

Since erosion is rarely the only factor contributing to dental wear, it is important to know whether the fluoride products tested in Chapter 4 can withstand the challenge of mechanical factors in the mouth. In **Chapter 5**, single professional applications of 4% titanium tetrafluoride (TiF_4), 1% amine fluoride (AmF) and 0.1% difluorosilane varnish (FV) were evaluated in the prevention of wear due to combined erosion and brushing abrasion. One hundred and eight bovine enamel samples were used. Control groups were not pretreated with any product (C), pretreated with a fluoride-free varnish (FV-bl) or pretreated with fluoride varnish and subsequently submitted to varnish removal (FV-r). Wear was modelled submitting the fluoride and control groups to 3 cycles of the following regimens: erosion/remineralisation (er/remin), abrasion/remineralisation (abr/remin) or erosion/abrasion/remineralisation (er/abr/remin). Erosion was simulated by immersion of the samples for 10 min in citric acid 50 mM (pH 3) under agitation (100 rpm). Abrasion was carried out for 1 min (200 strokes, load 150 g) in a wear device. Remineralisation (2 h artificial saliva) took place between the cycles. Two-way ANOVA showed that there was a significant interaction ($p \leq 0.001$) between the fluoride treatments and the wear regimens. Under er/remin a significant wear protective effect was found for the FV, FV-r and FV-bl groups. Abr/remin resulted in some enamel loss for the TiF_4 and AmF groups, however the amounts were not statistically significant ($p = 0.185$ and $p = 1.000$ respectively). Under er/abr/remin all products showed a significant protective effect except TiF_4. It was concluded that FV and AmF can protect bovine enamel against wear due to combined erosion and brushing abrasion *in vitro*.

A step further in the direction of a clinical application was taken with an *in situ* study where the fluoride product with the best *in vitro* performance was studied under more realistic conditions. In **Chapter 6,** a study on the effect of fluoride varnish (FV) in the prevention of wear due to erosion and combined erosion and toothbrush abrasion is reported. Eleven volunteers wore for 3 weeks, during working hours, appliances containing 2 control and 2 FV-treated human enamel samples. Erosion took place extraorally 3 times a day (5 min) in the soft drink Sprite under controlled agitation (100 rpm). At the end of each experimental day one control and one FV sample (C-er+abr and FV-er+abr) were brushed (5 s) with fluoridated dentrifice. The remaining control and FV sample (C-er and FV-er) were left unbrushed. Enamel volume loss was quantified by optical profilometry at day 5, 10 and 15. A statistically significant progression in enamel loss was found for the C-er, C-er+abr and FV-er+abr groups ($p < 0.001$, $p < 0.001$ and $p = 0.001$, respectively) but not for the FV-er group ($p = 0.053$). The values of cumulative normalised volume loss ($\times 10^8$ μm) at day 15 were: C-er 5.53 ± 2.14; C-er+abr 5.70 ± 2.07; FV-er 0.79 ± 0.67 and FV-er+abr 2.76 ± 1.35. The FV-er and FV-er+abr groups showed significant lower volume loss than the C-er group ($p < 0.001$ and $p = 0.005$, respectively) and the C-er+abr group ($p < 0.001$ and $p = 0.002$, respectively). The results indicated that fluoride varnish is effective in the reduction of erosive wear.

Although the *in vitro* and *in situ* studies provided much information on the performance of the tested fluoride products, their exact mechanism of action in erosive wear prevention remained to be explained. To clarify this matter a more fundamental approach was taken in the study described in **Chapter 7**, where the morphology of the fluoride-treated enamel surfaces was investigated with SEM. In this study thirty-six bovine enamel samples were randomly divided into 18 groups of two samples each. Three groups of samples (uneroded, 5 min erosion and 15 min erosion) were assigned to each of the controls (untreated samples (C) and samples pretreated with a fluoride-free varnish (FV-bl)) and to each of the following fluoride products: titanium tetrafluoride solution (TiF_4-s), titanium tetrafluoride gel (TiF_4-g), 1% amine fluoride (AmF) and fluoride varnish (FV). Erosion took place in a demineralisation solution consisting of citric acid 50 mM (pH 3.0) under controlled agitation (100 rpm). A thin section was prepared from each sample. The sections were cryo-fixated and subsequently fractured in liquid nitrogen at - 196°C. The surface and fractured surface were analysed with SEM. The micrographs of the eroded C samples showed the fragile appearance of the intraprismatic crystals. The fractured surfaces showed dissolution of the crystals at the prism boundaries up to approximately 3 and 5 μm beneath the surface after 5 and 15 min erosion, respectively. Application of TiF_4 resulted in the formation of a dense surface layer composed partly of sheet-like material and globular structures, which were still visible after erosion. The acidity of the products produced however demineralisation at prism junctions up to approximately 3 μm from the surface. AmF application resulted in a thin amorphous layer formed on the surface, which was almost completely removed after 5 min erosion. The application of FV resulted in the formation of a varnish layer approximately 2 μm thick on the surface of the samples, which became porous after erosion. The results helped to explain previous findings on the performance of TiF_4, AmF and FV under erosive conditions, however further physical and chemical analysis of the fluoride-treated surfaces before and after erosive wear seems necessary to further clarify the mechanism of action of the products.

In **Chapter 8** the critical points of the methodology and findings are discussed. It is pointed out that the *in vitro* and *in situ* models used to simulate the wear processes, although not able to reproduce exactly the phenomena that occur in the oral environment, were helpful for the evaluation of the extent of softened layer damage by the different mechanical factors and for the testing of the fluoride products under simulated wear conditions. The importance of choosing fluoride products that combine chemical and mechanical protective properties is stressed and it is suggested that the wear resistance of the products is the determinant factor in their performance. Finally some suggestions for future research are given. Concerning the fluoride varnish, it seems necessary to improve its mechanical resistance or alternatively consider a multilayer application of the product. The development of higher pH TiF_4 products is a necessary development in order to evaluate the true wear inhibition of this compound. With respect to the individual variation found in the *in situ* study reported in Chapter 6, it seems interesting to explore the inhibition potential of the salivary pellicle in the progression of erosion. Topics like pellicle modification and its interaction with the softened layer can provide valuable information in the development of evidence based approaches for erosive wear prevention.