Effect of ten selected compounds against acid-induced enamel softening and erosion

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ENAMEL DEMINERALISATION AND DENTAL EROSION - CAUSES AND PROPHYLAXIS

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SHORT TITLE

Toothpaste and dental erosion

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WHAT IS DENTAL EROSION?

Dental erosion is chemical wear of the hard tooth tissue without involvement of bacteria (1). Thus, dental erosion can develop on completely clean tooth surfaces and hence differ from caries, which occurs when bacteria producing acids degrade the hard tooth tissue under plaque covering tooth surfaces that are not clean. Contrary to caries, the progression of which to a level requiring treatment is usually measured in years, there is probably no lower limit for how fast dental erosion can develop (2). Overall, dental erosion belongs under wear of teeth and is hence closely linked with attrition and abrasion. Here attrition covers teeth wear which is caused by one tooth against another while abrasion covers teeth wear caused by other things than teeth, e.g. coarse foods or toothbrush. Dental erosion, attrition, and abrasion are all conditions that can compromise the teeth of the patient cosmetically and taking cosmetics into consideration in the following treatment can be of major importance to the patient. In addition to cosmetic considerations, tooth wear can also lead to an increased sensitivity towards cold and hot and in serious cases enamel fracture, altered occlusion, and pain. All tooth wear is irreversible and thus cannot be restored naturally to the starting point once it has occurred. Therefore there is good reason to routinely inform the patients about the risk of dental erosion and pay attention to beginning dental erosion even during standard dental check-ups.

WHAT IS ENAMEL DEMINERALISATION?

Enamel attrition and abrasion are wear conditions that have not been preceded by chemical changes in the tooth substance. On the contrary, dental erosion in the enamel will always be preceded by chemical changes in the tooth substance. These changes occur as a consequence of the acid influence on the outer micrometers of the enamel before the tooth substances is lost. The changes occur because the enamel is not dissolved instantaneously and uniformly across the surface but initially becomes porous as the prismatic structure is laid bare when some hydroxylapatite crystals are dissolved and others remain intact. The most obvious sign of this porosity is that the enamel becomes measurably softer (3). Usually, it takes a load of 350-400 kg for a diamond to make a mark of just a square millimetre in healthy enamel. Thus, when it comes to the hardness of healthy enamel it can match medium metal. But after acid influence the hardness can in some cases be more than halved as opposed to the starting point. This means that enamel exposed to acid to a much larger extent is exposed to attrition and abrasion and in case of continuous acid influence the enamel structure could be fully dissolved whereby dental erosion has occurred. Figure 1 shows that the softer the enamel becomes after having been exposed to acid influence, the more can subsequently be lost if the tooth surface is exposed to e.g. abrasive influences (4). In cases where the acid influence is moderate and the tooth surface not as such is exposed to attrition and abrasion, the enamel can be re-mineralised with minerals from saliva and thus regain its original hardness over a few hours. There is no actual term, nor diagnosis, for the condition where the enamel is softened after exposure to acid but where dental erosion has yet to occur. However, the concept of "enamel demineralisation" seems to cover these changes.

OCCURRENCE

Reports from several European countries show that there has been an increase in the occurrence of dental erosion over the last decades (5, 6). In Denmark there has also been found a substantial occurrence among children and young people (7) while dental erosion probably is less frequent among the adult and senior part of the population. The increase in dental erosion has happened simultaneously with a significant decline in the occurrence of caries over the developed part of the world (8). This has led to a generally increased focus on dental erosion, both among professionals and in the media. As enamel demineralisation are episodic microscopic changes in the outer micrometres of the enamel and thus difficult to measure in the patient it is not possible to do any studies of the occurrence among the population. But if you assume that dental erosion is an accumulated result of numerous instances of enamel demineralisation, you would also have to assume that enamel demineralisation occur more frequently among the current population than previously.

WHAT ARE THE MOST SIGNIFICANT CAUSES OF DENTAL EROSION AND ENAMEL DEMINERALISATION?

What the most significant causes of dental erosion are depend on the risk of getting dental erosion as a result of a given act at well as the frequency of this act among the population in general. For this reason, the most significant causes of dental erosion are not necessarily those that include the highest risk of dental erosion, but maybe to a higher degree those that are practiced most often. Table 1 shows a top 5 of the risk of developing dental erosion in relation to various factors. Here, it is relevant to distinguish between external factors vis-à-vis the patient ("extrinsic") and internal factors from the patient ("intrinsic"). The table shows that the greatest risk of dental erosion is linked to the consumption of citrus fruits several times daily. The next three factors are all related to the patient (intrinsic) with weekly vomiting and reflux disease as the greatest risk factors. Hyposalivation is also an important but somewhat smaller risk factor. Surprisingly, the consumption of sports drinks and soft drinks comes last in relation to the other factors examined.

Table 1. Factors that may be linked to dental erosion

Risk factors	Risk for dental erosion (odds)
Citrus fruits more than twice a day	37
Vomiting once a week or more	31
Reflux symptoms once a week or more	10
Hyposalivation (unstimulated saliva flow < 0.1 ml/min)	5
Soft drinks or sports drinks weekly*	4

* in case of sports drinks once a week or more and for soft drinks more than four to six times a week. Modified from Jarvinen et al. (9)

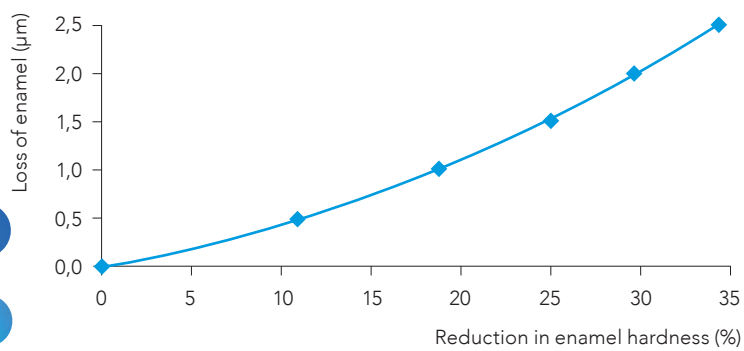

Figure 1. Loss of tooth substance after enamel demineralisation to the enamel.
[vertically] Loss of tooth enamel (µm), 0.0 – 0.5 – 1.0 – 1.5 – 2.0 – 2.5, [horizontally] Reduction of the enamel hardness (%). The figure is made based on the results in Attin et al. (4)

In actual numbers, the calculated risk means that there among those who frequently consumed soft drinks also were a number of people without dental erosion while among those who practised more severe risk factors, e.g. consumption of citrus fruits several times daily, only were very few without dental erosion. Even though the study was done 20 years ago it is a perfect example of how various behavioural and dietary factors can result in dental erosion. If you convert the figures to today it will probably be in the consumption of sports drinks and soft drinks that the greatest change in exposure has happened. Where 20 years ago it was less usual to consume sports drinks and soft drinks many times a week, it is now much more usual and especially among children and young people. It is very likely that this is the reason that the consumption of sports drinks and soft drinks very frequently can be linked to dental erosion (10). However, this should also be considered in relation to the fact that consumption of such drinks not necessarily will result in dental erosion for all who consume them. Here, the way they are consumed, the pH value of the soft drink, the bonding to the tooth surface as well as other factors play a significant role regarding the risk of dental erosion (11). In this connection, most epidemiological studies have determined that especially consumption of soft drinks with the lowest pH values are related to the development of dental erosion (12). A considerable effort against enamel demineralisation and dental erosion is thus to reduce the consumption of especially such soft drinks. In this context, you have to be aware of the fact that if one or more risk factors are present simultaneously, the risk for dental erosion is increased corresponding to the total effect of the factors.

It could be reduced saliva secretion as a result of dehydration at the same times as consuming sports drinks as seen in athletes (13) or reduced saliva secretion in combination with regular vomiting as seen in patients with eating disorders (14). If the source of enamel demineralisation is solid acidic foods, such as the citrus fruits in Table 1, there will be increased risk of interaction between attrition, abrasion and erosion. Here, the areas of the enamel that have been softened by enamel demineralisation will also be exposed to attrition and abrasion. Thus, the chemical effect in the form of demineralisation to the enamel combined with a mechanic action is the worst possible combination for loss of tooth substance. Thus, knowledge about the negative effect of combined risk factors, both biological as well as those related to foods, is vital if enamel demineralisation and dental erosion are to be avoided.

MODIFICATION OF ACIDIC SOFT DRINKS AND FOODS

Enamel demineralisation and dental erosion could easily be limited if the consumption of acidic soft drinks and other acidic foods was reduced. However, this is easier said than done, primarily because many people prefer the acidic taste. Thus, a lot of the drinks and foods we consume have a pH value that is under the neutral area, which is around pH 7, while almost none have a pH value that is higher than the neutral

area (15). It is because the sensory experience of acidic taste is perceived as a fresh taste. If you increase the pH value in acidic foods the taste will often suffer and become less fresh and thereby less appealing for the consumer. Because of this, many have tried to use tooth protecting minerals and proteins to modify acidic foods in order to make them less erosive with unchanged pH.

The foods where it has turned out to be the easiest to remove the erosive potential have been non-liquid foods that upon consumption have to be dissolved in saliva, e.g. acidic hard candy. In this case, adding moderate amounts of calcium and/or phosphate have made otherwise erosive products more or less non-erosive in healthy patients as well as in patients with reduced saliva secretion (16). It has been possible to reduce the erosive potential in drinks with a limited acidity. Thus, adding moderate amounts of calcium and phosphate to orange juice (from concentrate) basically remove the erosive potential in this drink. Unfortunately, the gap between juice with a pH value of about 4 to e.g. cola with a pH of about 2 corresponds to an increase in acid of more than 100 times. For this reason, it is not possible to remove the erosive potential from the most acidic soft drinks that unfortunately also in many cases are the most popular. But studies have shown that by adding a limited amount of calcium, phosphate, and other agents it might be possible to reduce the erosive potential of even the very acidic soft drinks (17).

EFFECTS OF ORAL HYGIENE ON ENAMEL DEMINERALISATION AND DENTAL EROSION

The primary effort to avoid caries, which is caused by bacterial plaque on the surface of the teeth, is still good oral hygiene in the form of tooth brushing. But tooth brushing may have the opposite effect on dental erosion. Thus, enamel demineralisation after consumption of something acidic may lead to five times more dental erosion/wear if softened enamel is brushed immediately after the enamel demineralisation has occurred. Figure 2 shows how many micrometres of enamel are lost when tooth brushing is done at various times after the occurrence of enamel demineralisation. If the enamel is brushed immediately after the enamel demineralisation has occurred, about 5 micrometres will be lost while only about 1 micrometre is lost if you wait about 3 hours. In the study, the loss of enamel after 3 hours in a saliva-like dissolution was almost the same as the acid influence only caused in the enamel (the dotted line in the figure).

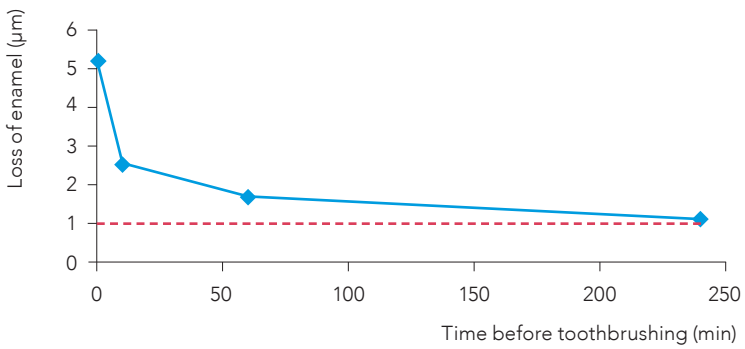

Figure 2. The effect of tooth brushing after demineralisation to the enamel [vertically] Loss of tooth enamel (µm), [horizontally] Time before tooth brushing after enamel demineralisation (min). The figure is made based on the results in Attin et al. (18)

In general, the loss of enamel can be described based on reduction of hardness, the greater the loss of hardness, the greater the loss of enamel in case of subsequent abrasion (4). Thus, most professionalsand toothpaste manufacturers generally recommend not to brush your teeth for a period of at least onehour after consumption of something acidic. Therefore, there is no doubt that tooth brushing should be performed at some point before consumption of acidic soft drinks and foods and not after consumption of such products if dental erosion/wear is to be avoided.

But the time of tooth brushing should probably not be right before the consumption of something acidic.Thus, it has also been established that completely clean tooth surfaces that are then subjected tosomething acidic develop many times more enamel demineralisation and dental erosion than not cleaned tooth surfaces with bacterial plaque (19). The reason is that bacterial plaque efficiently prevents the acid from diffusing to the tooth surface at the same time as dissolved calcium and phosphate most likely is retained close to the tooth whereas the enamel demineralisation more quickly is re-mineralised. In that perspective, you could say that as regards dental erosion and enamel demineralisation it is better not to brush your teeth that in fact brushing your teeth. But since brushing your teeth is the most important prevention of caries, lack of oral hygiene can obviously never become a strategy to avoid enamel demineralisation and dental erosion. It is also difficult to say how long before consumption of something sour it would be best not to brush you teeth. A study has recommended that wine tasters that are at risk of developing enamel demineralisation and dental erosion do not brush their teeth on the same day as they have planned a wine tasting (20). Such measures would obviously only be possible for very select groups as e.g. wine tasters and others who are exposed to singular but massive acid influence on their teeth. For all other "ordinary patients" the general recommendation has to be not to brush your teeth immediately before and as a minimum not for one hour after consumption of something acidic.

EFFECTS OF TOOTHPASTE ON ENAMEL DEMINERALISATION AND DENTAL EROSION

Besides the direct effect of tooth brushing before and after consumption of something acidic on enamel demineralisation and dental erosion, tooth brushing may through the agents in the toothpaste have an effect on these conditions. Here, the abrasive effect of the toothpaste is important in those cases where the teeth against recommendations are brushed after they have been exposed to enamel demineralisation. In this connection, there is a great difference between the various toothpastes' abrasive effect and knowledge about this could be beneficial to avoid this kind of damages (21). But also in those cases where the teeth are brushed before the enamel demineralisation have occurred, the active ingredients of the toothpaste may be of importance. Here, it is especially certain types of detergent that can dissolve and inhibit the forming of the protective layer of saliva proteins that usually is on the tooth surface and called the pellicle.

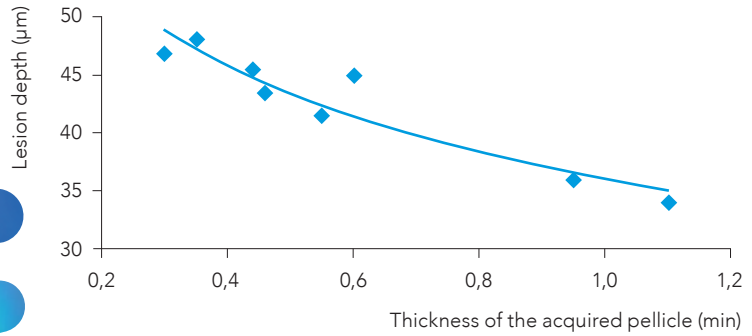

Figure 3. The effect of pellicle thickness on the lesion depth of the enamel [vertically] Lesion depth (µm), [horizontally] Pellicle thickness (µm), the figure is made based on the results in Amacchi et al. (23)

The pellicle actually works the same way as plaque by preventing acid from diffusing to the tooth surface and at the same time retain calcium and phosphate close to the tooth after enamel demineralisation. The protective layer of the pellicle is probably different individually and in some individuals this protection can result in halving the enamel demineralisation compared to a tooth surface with no pellicle (22). It is still unknown exactly why there are individual differences in the protective layer of the pellicle but functionally the varying protection is most likely based on differences in the thickness of the pellicle.

Thus, figure 3 shows that a thick pellicle provides better protection against acid influences than a thin pellicle. You therefore have to assume that rapid formation of a thick pellicle or a lack of dissolving the existing pellicle during and after tooth brushing will provide the teeth with better protection against enamel demineralisation. Unfortunately, detergents and especially negatively charged detergents, e.g. sodium lauryl sulfate (SLS), affect the bonding of the protective proteins on the tooth substance. Here, previous studies have shown an average reduction in bonding of protein to the tooth substance of more than 50% if the tooth substance prior to this had been exposed to SLS. The same study also showed a delayed pellicle formation after two hours in vivo on the tooth surface that had been in contact with SLS compared to tooth surfaces that had not been in contact with SLS (24). The effect most likely occurs because SLS changes the charge of the enamel surface from positive towards neutral so that the negatively charged saliva proteins to a lesser extent are attracted and bonded to the enamel. In addition to the fact that the protective effect from the pellicle varies from one person to the next, the pellicle's protective effect also varies depending on the type of erosive affect in question.

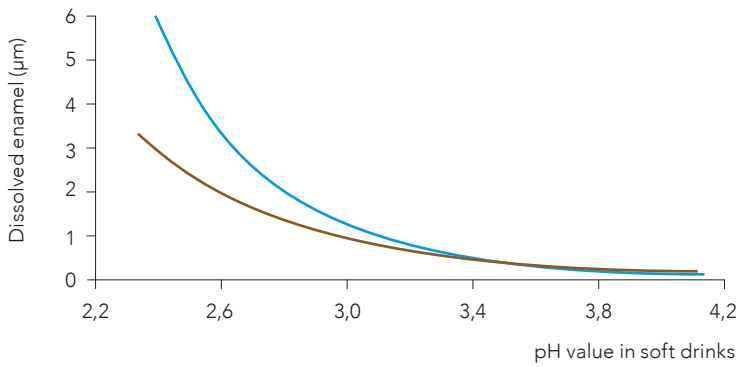

Figure 4. The effect of pH and pellicle on dissolution of tooth substance [vertically] Dissolved tooth substance (mg), [horizontally] pH value of soft drink, the figure is based on the results of Jensdottir et al. (25).

Protection from the pellicle is thus relatively bigger at very low pH values compared to slightly higher pH values. Figure 4 shows how dissolution of tooth substance becomes much less with pellicle (brown line) than without pellicle (blue line) at pH values below 3. This is interesting because it is exactly in this pH range that the most erosive soft drinks such as cola and sports drinks are to be found. Thus, you can imagine that the tooth surface will be even more exposed to enamel demineralisation if it just before exposure to e.g. cola has been cleaned with toothpaste containing SLS.

THE EFFECT OF FLUORIDE COMPOUNDS ON ENAMEL DEMINERALISATION AND DENTAL EROSION

It has been frequently discussed whether fluoride can prevent the development of dental erosion and it has often been put forward that fluoride does not provide any protection in this connection. But to discount an effect of fluoride on dental erosion and enamel demineralisation would not be correct. However, the effect of fluoride on these conditions is not the same as the effect of fluoride on caries. A significant part of the beneficial effect of the day-to-day exposure to fluoride toothpaste is thus a small increase of fluoride concentration in the oral cavity fluids by continuous daily use (26). Even though the increase is measured in the ppm range, it is still enough to result in a significant reduction in caries development because this process happens at relatively modest acid concentrations (27). But because enamel demineralisation and dental erosion occurs at acid concentrations that can be 1,000 times higher than in a caries attack, small changes in the fluoride concentrations in the oral cavity fluids has no effect on these conditions. In order for fluoride to have an effect on dental erosion and enamel demineralisation it would most likely require that fluoride in fair amounts are bonded to the tooth surface before the acid influence occurs. The prerequisite for deposit of fluoride compounds on the tooth surface is that the surface comes into contact with very high fluoride concentrations. In case of toothpaste that can happen at the highest concentration allowed close to 1,500 ppm and by avoiding sodium monoflourophosphate where fluoride is bonded to phosphate in the fluid phase of the toothpaste. It is most likely also important that the toothpaste does not contain foaming agents as SLS because this compound can inhibit the deposit of fluoride compounds on the tooth surface (28). In addition to the fluoride deposited from the toothpaste, deposits can also be achieved by professional fluoride treatment, e.g. with Duraphat varnish or the like. Regardless of how the deposit takes place, long-lasting protection is prerequisite on the fact that fluoride deposits are repeated frequently because the deposited fluoride, usually in the form of calcium fluoride, will be reduced/used every time the tooth surface is exposed to enamel demineralisation. Most likely, it will require almost daily exposure in order to achieve protection against regular consumption of soft drinks and sports drinks (29).

During the last decade, as a consequence of the increased occurrence of dental erosion there has also come more focus on other fluoride compounds than standard sodium fluoride and sodium monofluorophosphate. These are tin fluoride, zinc fluoride, titanium fluoride, and amine fluoride and several other compounds that all are tested in connection with studies of enamel demineralisation and dental erosion. One of the hypotheses in such studies is that the metal ion

of the fluoride compound, e.g. tin, is also bonded to the tooth surface where it can form a coating that will protect the tooth surface against acid in somewhat the same manner as plaque and pellicle. Arguments in favour of this hypothesis are that metal ions as tin with certainty are bonded to the tooth surface where tin in connection with long time use can result in a brownish discolouration. Also these types of fluoride compounds actually provide very adequate protection against enamel demineralisation and dental erosion (30). An argument against that this kind of fluoride compound provides special protection against enamel demineralisation is that ordinary sodium fluoride in some studies is shown to provide the same protective effect if applied at the same acidic pH values as the aforementioned fluoride compounds often are. If the latter is correct, part of the effect of these other fluoride compounds is maybe to a higher degree a result of increased deposits of calcium fluoride at low pH values. However, it is important to stress that there are still conflicting reports regarding the effect of various fluoride compounds on enamel demineralisation and dental erosion and that this area is thus still in development (31).

OTHER SUBSTANCES AND PRODUCTS AGAINST ENAMEL DEMINERALISATION AND DENTAL EROSION

In addition to calcium and phosphate for enrichment of certain beverages and foods and various fluoride compounds in oral hygiene products the increased focus on enamel demineralisation and dental erosion has also lead to development and tests of products with numerous other substances and compounds. Some of the most frequently used are different types of protein, predominantly milk protein, that like the pellicle also can bond to the tooth surface and provide a protective effect against dental erosion. Very few of these products have reached the Danish market. Of those on the market in other countries, some are used to re-mineralise already demineralised tooth surfaces (32) while other are used as a prophylactic before the tooth surface is exposed to enamel demineralisation (33). The complexity of the relationship between oral hygiene, enamel demineralisation, and other types of wear, however, make the application of specific products, of which some have to be applied before enamel demineralisation and others after, fairly complicated for the consumer. In a worst case scenario wrongful application could lead to exacerbation of the condition instead of improvement.

Recently protein compounds have also found their way into toothpaste. For these types of toothpaste, as well as all other types of toothpaste, it applies that the product should be used before the tooth surface is exposed to enamel demineralisation. With this approach it is possible to obtain extra protection against enamel demineralisation by adding protein to toothpaste rather than toothpaste without protein (34). The best results are achieved when the toothpaste at the same time contains no SLS as foaming agent and contains the maximum allowed concentration of fluoride in a compound where fluoride is not bonded to the liquid phase of the toothpaste. Tests have shown that this toothpaste composition, with or without protein, can protect the tooth surface against erosion-like changes from acid influence against, which other more traditional toothpastes provide limited protection against (35).

FACT BOX

- Dental erosion is chemical wear of the hard tooth tissue and enamel demineralisation is chemically induced softening of the enamel – both without the involvement of bacteria.
- The greatest risk of dental erosion comes with daily consumption of citrus fruits or in case of frequent vomiting.
- Acidic solid foods are generally more harmful than acidic beverages.
- Among soft drinks, consumption of beverages with the lowest pH value has been shown most frequently to result in dental erosion.
- In addition to the risk from other factors, patients with reduced saliva secretion have a further five time higher risk for developing dental erosion than healthy patients.
- Acidic beverages with a moderate pH value can be modified and thus have a less eroding potential.
- Acidic solid foods even with very low pH values can be modified and thus have a less eroding potential.
- Tooth brushing must not take place for at least one hour after consuming acidic foods as enamel demineralisation of enamel is easily worn off by the abrasion from toothbrush and toothpaste.
- Tooth brushing should maybe also be avoided immediately before consumption of acidic items in order not to remove the protective effect of the pellicle and other deposition on the teeth.
- To avoid enamel demineralisation and wear it is important to choose toothpaste with low abrasive effect, high free fluoride contents, and a mild foaming agent.
- New fluoride compounds have shown promise regarding protection of the enamel against enamel demineralisation and dental erosion – but this area is still in development.
- Other products than toothpaste that are specifically directed at enamel demineralisation and dental erosion have shown some effect, e.g. as increase re-mineralisation of demineralised enamel, but this area is also in development.

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PROTECTION AGAINST ACID-INDUCED ENAMEL SOFTENING BY FOUR COMMERCIAL TOOTHPASTES

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SHORT TITLE

Toothpaste and dental erosion

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ABSTRACT

Objective. To test whether or not and to what degree four commercially available toothpastes, all containing the same fluoride compound (NaF) at concentrations of 1400 or 1450 ppm, were able to prevent acid-induced enamel softening. Enamel softening was used as outcome measure because one of the toothpastes claimed to protect against this condition, another toothpaste claimed to protect against dental erosion, and two toothpastes did not claim any protecting effects against either of these conditions. Materials and methods. For the tests bovine enamel specimens, having a baseline Knoop surface microhardness (SMH) of 377±10 kp/mm2, were used. The experiment lasted 4 hours and could be divided into four 1-hour phases, one of toothpaste-remineralisation and three of demineralisation-remineralisation. Toothpaste slurries were made from toothpastes and deionised water (1:2 w/w) and demineralisation was obtained in a citric acid solution at pH 2.3. SMH measurements were performed after the toothpaste-remineralisation cycle, and after each of the three demineralisation-remineralisation cycles (AC1 to AC3). SMH data obtained after baseline were converted into relative hardness loss (HNL). Results. Regardless of what toothpaste slurry the specimens had been exposed to, all became gradually softer during acid-cycles AC1 to AC3 with increasing HNL values. However, at time point AC1 one toothpaste had significantly lower HNL compared to the other three toothpastes ($p<0.01$). This difference in HNL remained at AC2 ($p<0.01$) but disappeared at AC3. Conclusion. Although no product was able to prevent HNL caused by a highly acidic fluid, toothpastes differ in protection against this type of erosive challenge.

KEY WORDS

Tooth erosion, dental enamel, dentifrices, in vitro

INTRODUCTION

Dental erosion is a chemical wear of dental hard tissue caused by acids that are not produced by bacteria in the dental plaque (1). Most often exposure to soft drinks, sport drinks, fruit juices, and fresh fruits are the source of the acid causing dental erosion (2,3) and increased consumption of such foodstuffs has made dental erosion a growing problem in many countries (4,5). Like other wear processes, dental erosion is a progressive loss of tooth surface (6) that may expose the dentine if the acidic exposure is frequent and continues over a period of time. Therefore, considerable effort has been made to identify patients with high risk of developing dental erosion (7) and to chemically modify acidic foodstuffs and drinks (2,8,9). Recently, oral formulations and toothpastes with protecting effects against dental erosion have also been commercially introduced (10-12). However, especially the use of toothpaste against dental erosion demands a change of present paradigms. Thus, opposite to reducing dental caries, the mechanical action of tooth brushing does not prevent dental erosion. On the contrary, the impact of acids on enamel leads to a decrease of surface microhardness, making the surface highly susceptible to mechanical wear from brushing with abrasive toothpaste after intake of acid (13). Tooth brushing before an intake of acidic foods is also a problem because surfaces that are covered by a thick saliva protein pellicle (14) or dental plaque (15) are better protected against acid-induced softening than newly cleaned enamel surfaces.

With respect to dental erosion, one may say that not brushing ones teeth is better than brushing them. However, as the main preventive measure against dental caries, tooth-brushing cannot be avoided or realistically replaced by other measures. Therefore, the main role of toothpaste in relation to dental erosion should be to minimise the temporary, but necessary, vulnerability of a clean tooth surface to acid for period until the physiological protection is re-established. For this purpose, chemical interactions between toothpaste and enamel that persist after brushing, and influences the resistance of enamel to acid, are important. The present study aimed at testing the effect of such interactions in four toothpastes from the European market. All toothpastes contained the same fluoride compound (NaF) and nearly the same fluoride concentration (1400-1450 ppm), but differed with respect to detergent type and other additional ingredients added to increase the protection against acid-induced enamel softening and/or dental erosion.

MATERIAL AND METHODS

Toothpastes

The following four toothpastes were purchased from supermarkets in Denmark (1 and 4) and the Netherlands (2 and 3) and used for the experiments.

1. Aquafresh Fresh Mint Triple Protection (AF) from GlaxoSmithKline Consumer Healthcare A/S, Copenhagen, Denmark (batch 25091) with 1,400 ppm NaF

2. Colgate Total (CT) from Colgate-Palmolive, Weesp, The Netherlands (batch 8261PL1130) with 1,450 ppm NaF

3. Sensodyne Pronamel (SP) from GlaxoSmithKline Consumer Healthcare B.V., Zeist, The Netherlands (batch 386E P2) with 1,450 ppm NaF

4. Zendium Acid Defence (ZS) from Sara Lee H&BC, Den Haag, The Netherlands (batch 824101 125222) with 1,450 ppm NaF

Toothpastes 1 and 2 claimed no effect against acid-induced enamel softening or dental erosion whereas toothpastes 3 claimed a protective effect against dental erosion and toothpaste 4 against acid-induced enamel softening. Toothpastes 1, 2 and 3 are marketed in several European countries, whereas toothpaste 4 is marketed in Scandinavia, Iceland and the Netherlands only.

Specimen Preparation

Fully erupted permanent bovine incisors, which were extracted from mandibles obtained from young cattle at a local slaughterhouse, were used for the study. Only teeth exhibiting sound enamel by visually inspection were selected for the study. The teeth were mounted with the buccal enamel surface outwards in a block of epoxy resin (SpeciFix 20, Struers, Denmark), ground flat (TegraPol 11, TegraForce 1; Struers Denmark) with water-cooled SiC paper, at three different grits, i.e. 500, 1200 and 4000 (Struers, Denmark) until a surface of about 0.5 x 0.5 cm was exposed. This enamel surface was then polished for 3 minutes with a diamond paste (diamond polish 1 µm, Struers, Denmark). After polishing, the specimens were sonicated for 15 minutes in deionised water (Bransom 1510, USA) and the border of the exposed enamel surface was marked with nail varnish. Until use, the specimens were stored in a chamber with 100% humidity with thymol in the water vapour to prevent microbial growth and thus biofilm formation on their surfaces.

Toothpaste slurry and solutions for de- and remineralisation

The toothpaste slurry was prepared from a mixture of one of the four toothpastes and deionised water (1:2 w/w). The slurry was carefully obtained by mixing toothpaste and water with an Ultra-Turrax Ika T-25 (Germany). During the experiment each specimen was immersed for 2 minutes in 10 ml of freshly prepared toothpaste slurry. After immersion, the specimens were thoroughly rinsed for 30 seconds with running tap water and 15 seconds with deionised water.

The demineralisation solution was prepared from 0.05 M citric acid (Alfa Aeser, Germany) at pH 2.3. This low pH was chosen to mimic the pH of highly erosive soft drinks. Each tooth specimen was immersed for 2 minutes in 10 ml demineralisation solution. After immersion, the specimens were carefully rinsed with running tap water (from the Netherlands) for 30 seconds and with deionised water for 15 seconds. The demineralisation solution was used for maximally three demineralisation steps.

The remineralisation solution was prepared according to Exterkate et al. (16) and contained 20 mM acid free HEPES-buffer (Merck, Germany), 130 mM KCl (Merck, Germany), 1.5 mM $Ca(NO_3)_2$ (Alfa Aeser, Germany), 0.9 mM KH_2PO_4 (Merck, Germany), adjusted to pH of 7 with KOH (Merck, Germany). The solution was approximately seven times saturated with respect to hydroxyapatite by calculation (17) using 117.3 (pK) as solubility product for hydroxyapatite (18). In order to simulate the effect of human saliva the solution was refreshed daily and stirred gently by a rotating magnet at 37°C whenever it was in contact with the specimens.

Experimental protocol and procedure

Experimental conditions were developed to comply with general recommendations for use of toothpaste and to resemble an acid exposure from extrinsic acidic dietary sources. The protocol consisted of one 2-minute exposure to toothpaste followed by three 2-minutes exposures to the demineralising solution. Between toothpaste and acid exposures the specimens were kept in 15 ml of the remineralisation solution for 58 minutes. The experimental procedure lasted for 4 hours, divided into four 1-hour phases consisting of one toothpaste-remineralisation phase and three of demineralisation-remineralisation. Surface microhardness (SMH) measurements were performed before the experimental procedure started (baseline), after the toothpaste-remineralisation cycle (TP), and after each of the three demineralisation-remineralisation cycles (AC1 to AC3). For each experiment, five specimens were used per toothpaste and the combined 4-hour toothpaste-erosion cycle was repeated 3 times, requiring a total of 15 specimens per toothpaste.

Surface Microhardness Measurement

At baseline, TP, and AC1-AC3, SMH measurements were performed with a Knoop diamond indenter with a load of 50 grams, for 5 seconds using a Shimadzu HMV-2T hardness tester (Shimadzu, Japan). Each indentation was repeated five times per specimen with a distance interval of 20 µm between the centres of the short axis of the impressions. In this way the indentations were neighboured to the previously made indentations in the same area of the enamel. Baseline SMH was determined on a large number of specimens, of which 60 specimens with baseline values between 350 and 400 kg/mm^2 were selected. These specimens were divided in 12 groups of 5 teeth each and randomly assigning to each of the four toothpastes (three groups per toothpaste). SMH data at time points TP and AC1-AC3 were converted into relative hardness loss (HNL) and calculated according to:

$$HNL = \frac{[\,(SMH\ baseline - SMH\ measured)}{SMH\ baseline\,] \times 100}$$

Statistical analysis

Statistical analyses were performed with Excel and the R statistical software (www.r-project.org). In text and figures, results are given as mean±SD or as mean±SEM when indicated. The effects of experimental cycles (TP, AC1, AC2 and AC3) as well as differences among toothpastes on SMH in each cycle (Figure 1A) were tested by analysis of variance (ANOVA). Differences between toothpastes in each cycle were tested by Welch's t-test (Figure 1B). The level of significance was set at 1%, corresponding to the maximal Bonferroni correction (κ=5), in order to correct for repeated tests of statistical significance on related data.

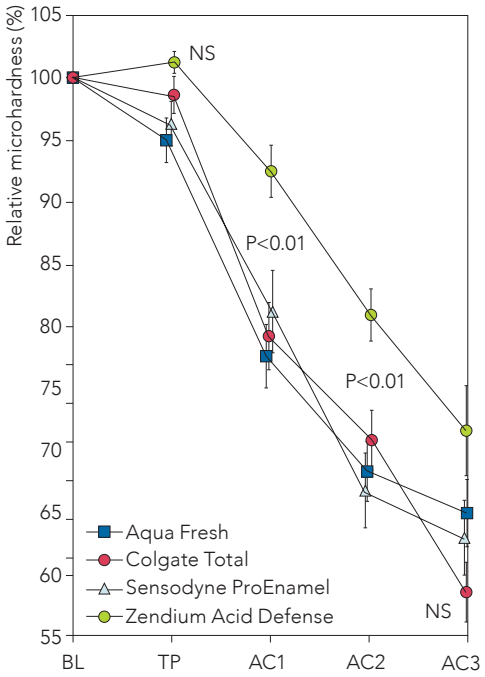
RESULTS

Figure 1A shows the relative microhardness (%), compared to the baseline microhardness (377±10 kg/mm^2), upon exposure to toothpaste and demineralisation solutions during the experimental procedure (BL, TP, AC1-AC3) for each of

the four toothpastes (AF, CT, SP, and ZS). Over the whole experimental procedures, SMH values were systematically and significantly influenced by the three consecutive cycles of demineralisation and remineralisation ($p<0.001$) and within the first two cycles SMH values were significantly influenced by the type of toothpaste that they had been exposed to at the beginning of the experiment ($p<0.01$). During the demineralisation-remineralisation cycles, enamel that had been in contact with toothpaste ZS was considerably harder than the other three toothpastes both after the first and second acid exposure ($p<0.01$). However, after the third exposure to acid (AC3) no statistical difference was obtained. At this time point, the average SMH value (mean of all toothpastes) was reduced to 65% of baseline (Figure 1A) and equal to a microhardness of 244±47 kg/mm^2.

Figure 1B shows the loss of surface microhardness (HNL) from the enamel surfaces during the experiment, which was determined after toothpaste-remineralisation cycle and after each of the three acid cycles. After the toothpaste-remineralisation cycle HNL did not differ between enamel surfaces that had been in contact with toothpastes AF, CT, and SP. However, at AC1 and AC2 enamel surfaces that had been in contact with toothpaste ZS showed significantly less loss of microhardness compared with the other toothpastes. During the whole experiment, this difference was most noticeable when ZD is compared with toothpastes AF and CT, which showed a significantly higher HNL at three time points each (AC1-AC3) although in a different manner. Differences in HNL between toothpastes SP and ZS were only significant at two time points (AC1 and AC2).

FIGUR A



FIGUR B

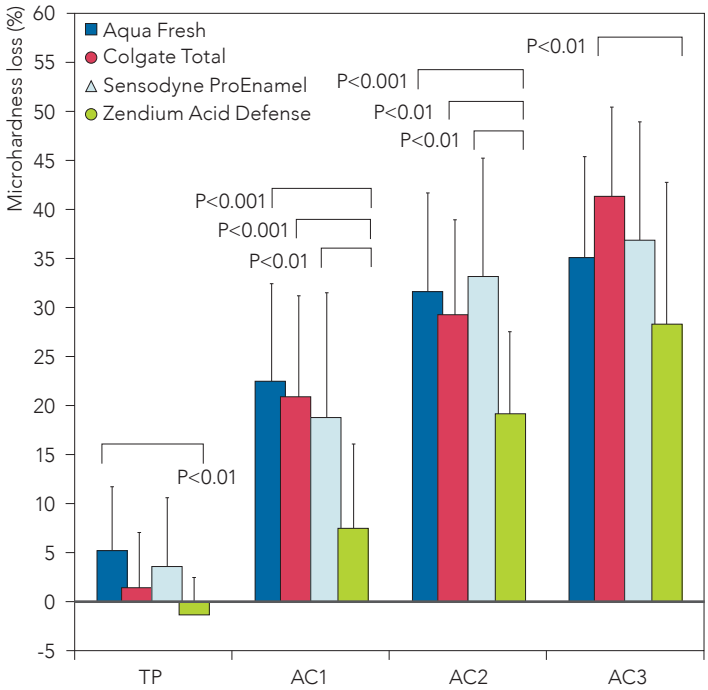


FIGURE LEGEND

Figure 1A shows the percent (mean±SEM) surface microhardness of baseline (i.e. 377±10 kg/mm^2), of bovine enamel specimens before and after exposure to four different toothpastes (AF, CT, SD, and ZS) followed by three exposures to citric acid with pH 2.3. P-values show the difference among the four toothpastes at the five time points obtained by analyses of variance (ANOVA) and NS denotes non-significant. Figure 1B shows the loss of surface microhardness (HNL) of enamel surfaces after exposure to toothpaste and acidic solution (mean±SD). The bars above columns indicate the level of statistical significance for difference between two toothpastes determined by t-tests. In total 15 enamel specimens were used per toothpaste with five indentations per specimen per time point (i.e. baseline, TP1 and AC1 to AC3).

DISCUSSION

With the present in vitro setup, four commercial toothpastes were shown to differ in protection against a strong erosive challenge having a pH similar to Cola drinks. Among the limited number of previous reports about this topic, most have also been based on in vitro experiments, few on in situ experiments, and none on in vivo experiments. Among these studies, considerable variations can be found in the number of toothpaste treatments ranging from one toothpaste application (19) to cyclic models with toothpaste treatments in the morning and in the evening (20,21) or three times per day (22,23). Depending on the study setup, the evaluation of the effect of the toothpaste has been carried out after one experimental cycle (19), after 5 days (22,23), 14 days (20,21) or even 20 days (24). Regardless of the number of toothpaste treatments it is, however, crucial for the outcome of the study that the toothpaste is applied to the tooth surface before the acid. Thus, this sequence is in accordance with general recommendations and toothpaste product manuals. In the present study, the toothpaste was applied before the acid and the experiment was terminated at the same day after the third demineralisation-remineralisation cycle (AC3) without any additional exposure to toothpaste. At this point, i.e. after AC3, the enamel surfaces had become so soft (65% of baseline) that tooth brushing in vivo would have been unadvisable due to the increased susceptible to mechanical wear brushing the softened enamel surface with toothpaste. Thereby, the present study focused only on the immediate effects of toothpaste exposure followed by deand remineralisation, and not on prolonged cumulative effects.

Since no general protocol is accepted for testing the effect of toothpaste on dental erosion, exposure time between toothpaste and enamel has also varied considerably among studies. Exposure times of 1 minute (25), 2 minutes (11,20,21), 3 minutes (19) and up to 5 minutes (22,23) have all been described in literature. Also exposure to the acid challenge has varied considerably from 30 seconds (20,21), 3 minutes (19), 5 minutes (23), 10 minutes (11,22), and up to 20 and 30 min (25). With respect to the present study, we believe that the most important factor in relation to exposure times is that the exposure to toothpaste is within the range of what can be expected in the oral cavity and that the exposure to acid is shorter or similar to that of toothpaste. Equally important is the pH of the acidic challenge, which should be comparable to soft drinks and foods that are known to cause dental erosion in vivo. Here, previously reported acid challenges seem to fall in two groups, one around pH 2.3 (20-23) and another group around pH 4.0 (11,19,25). The present study used an exposure time to toothpaste of 2 minutes, according to the general recommendations for tooth brushing, and an equally long exposure time to acid. However, from a physiological point of view the exposure time to acid in vivo would most likely be shorter than the exposure to toothpaste, because sour taste is the strongest stimulant of saliva secretion (26) resulting in rapid elimination from the oral cavity. It may therefore be debatable that either the exposure to toothpaste could be longer than the 2 min used in this study or that the exposure to acid could be shorter than the 2 min used in this study. With respect to the pH value of the demineralisation solution the present study is comparable to past studies using the low pH value of 2.3. This pH value is close to the most acidic soft drinks on the international market and probably resembles a worst-case scenario in vivo. To further mimic in vivo conditions, the remineralising solution in the present study had a saturation level with respect to hydroxyapatite that is comparable to average unstimulated human saliva (i.e. approximately seven times saturated).

Using this setup, toothpaste ZS was able to protect the tooth surfaces better than the three other toothpastes during the first two acid challenges, while differences in protection tended to disappear at the third acid challenge. Because all toothpastes had the same fluoride compound and nearly the same fluoride concentration the differences must be due to other ingredients in the toothpastes. ZS and SP, which overall was second best, are different from AF and CT in terms of the type of detergent used in the toothpaste. Where AF and CT have the strong anionic detergent sodium lauryl sulphate (SLS), SP has a zwitterionic detergent (i.e. Cocamidopropyl betaine) and ZS a non-ionic detergent (i.e. steareth-30). It has been shown previously, that the presence of SLS near enamel surfaces can inhibit the deposition of calcium fluoride normally obtained by high fluoride levels (27) and this inhibition most likely occurs due to calcium binding by the negative lauryl sulphate. In this way, the acid protective calcium fluoride deposition may have been higher in ZS and SP compared to the other toothpastes, even though the available fluoride concentrations exceeded hundreds of ppm in all toothpaste slurries. With respect to wear processes in general, Moore and Addy (28) showed that also mechanical wear of teeth is increased in the presence of anionic detergents compared to both zwitterionic and non-ionic detergents. Thus, it is possible that strong anionic detergents like SLS have a negative impact on both mechanical as well as chemical wear of tooth surfaces.

A second explanation for the differences is that ZS contains different proteins, among others, milk proteins. As many different proteins, for example salivary proteins and milk proteins, can protect enamel surfaces against erosion like demineralisation by the formation of a proteinaceous layer on the tooth surface (29-31), we speculate that the higher protection from ZS also was due to the formation of such a proteinaceous layer on the enamel surfaces in this study. This positive effect of the proteins is supported by the fact that the difference in protection between ZS and the other toothpastes was most pronounced during the first two acid challenges. Thus, most likely the proteinaceous layer was removed from the enamel surface during the three acid challenges. With respect to ZS and SP our results are in part comparable to Lussi et al. (19), who showed that enamel surfaces exposed to ZS and SP did not develop a significant decrease in microhardness when subjected to an acidic challenge, whereas enamel exposed to three other commercial available toothpastes did. However, in the study by Lussi et al. (19) no significant differences could be obtained between the levels of protection offered by the different toothpastes. We speculate that this difference could be due to various testing conditions and in this case especially the pH of the demineralisation solution. In the present study pH 2.3 was used for demineralisation compared to pH 4.0 in the study by Lussi et al. (19). This difference in pH values could be important because the protective effect of experimentally developed pellicles has been shown to be higher at pH values between 2 and 3 compared to pH values around 4 (32). Therefore, a protection from a proteinaceous layer developed after exposure to ZS could have been higher at the low pH conditions in the present study compared to conditions at higher pH.

In spite of the differences obtained, the present setup did not allow for an estimation of the relative contribution from the different toothpaste components; i.e. detergents, proteins and other ingredients, to the combined protection of enamel.

CONCLUSION

In conclusion this study has shown that commercially available toothpastes differ in protection against a highly acidic worst-case scenario erosive challenge. Furthermore, no product was able entirely to prevent loss of enamel surface microhardness caused by the present erosive challenge.

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EFFECT OF TEN SELECTED NATURAL COMPOUNDS AGAINST ACID-INDUCED ENAMEL SOFTENING

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KEY WORDS

Dental erosion, erosion prevention, colostrum, Neem oil, saliva proteins

ABSTRACT

Dental erosion is increasing in most of the developed world. Its main causes are increased consumption of acidic soft drinks and an altered dental disease pattern, which make conditions other than caries more visible than just decades ago. Furthermore, increased oral hygiene, which prevents dental caries, may have an undesirable role in making enamel more vulnerable to dietary acids. It seems clear that the problem is greatest among adolescence and less among elderly. Dietary advices on reducing the intake of acidic drinks and avoiding tooth brushing immediately after intake of such beverages will not solve the problem alone. Therefore, research about protective agents against enamel erosion is mandatory. Consequently, the purpose of this study was to investigate protective effects of ten compounds against acid-induced enamel softening. All compounds were tested as extracts from their natural form. According to our findings, focus shall be given to colostrum proteins and Neem oil as promising protective agents.

WHAT THIS PAPER ADDS

- A comprehensive screening of a variety of compounds and their ability to protect enamel against acid-induced softening
- A finding that colostrum proteins and an indication that Neem oil, mucin and casein may be candidates for protecting enamel against acid-induced softening

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INTRODUCTION

Dental erosion is the chemical wear of tooth substance without the presence of bacterial acids in dental biofilm [Eccles, 1979] and this condition seems to increase in most developed countries [Bardow et al., 2014]. Apart from seriously ill patients, which are not the focus of the present paper, a key aspect seems to be a high consumption of acidic soft drinks and foodstuffs [Jensdottir et al., 2004; Johansson, 1997]. Nonetheless, dental erosion may also become more apparent due to a considerable decrease in caries activity and cavity formation [Petersen, 2003]. Thus, with the exception of seriously ill patients, caries seems to be under control at this point in time in many countries [Bardow and Vissink, 2015]. Decreased caries activity, especially among teenagers, may contribute to higher visibility of other more or less subtle conditions like erosive wear or erosion in the enamel than in previous decades [Lussi and Ganss, 2014] when much higher caries activity, decay and cavity formation were part of everyday practice. In addition, it is not unlikely that antagonistic effects between dental caries and erosion may exist, so that dental erosion is most likely to develop under conditions that are nonfavorable to dental caries and vice versa [Honorio et al., 2008]. Throughout this paper, dental erosion is used synonymously with acid-induced softening, although the latter condition is reversible compared to dental erosion that is irreversible.

Regardless of the causes of dental erosion, increased awareness of the disease has introduced a broad range of products that are marketed with proclaimed preventive or therapeutic effects against this disease [Honorio et al., 2008; Buzalaf et al., 2014; Souza et al., 2014]. The present paper focuses on testing some of these compounds, as well as new compounds that may be potential alternatives for future dental products aiming at preventing dental erosion and/or acid induced softening of the enamel. In this context, bovine colostrum was chosen because previous research [Bardow et al., 2004] has shown considerable effects of colostrum proteins in protecting enamel against acidinduced dissolution (Figure 1, modified from Bardow et al., 2004). Accordingly, the aim of this study was to identify novel or infrequently tested compounds for prevention of dental erosion. Our hypothesis was that some of these compounds could be superior to other known agents in protecting against dental erosion. As a screening of potential compounds, nearly no compounds available in whole commercial formulas for retail, e.g. dentifrices or mouthwashes, were included. Only extracts of compounds in their natural form were tested in the present study, instead of being incorporated in any commercial embodiment. For comparison,

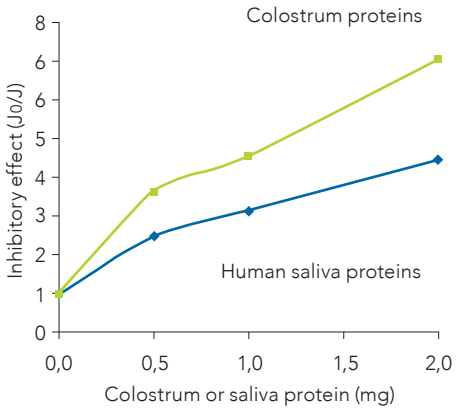

Figure 1

the protective effect of all tested compounds was related to the protective effect of human whole saliva proteins, which should be considered the natural physiological background in the oral cavity. Since the human saliva and the acquired pellicle cannot alone completely prevent dental erosion [Bruvo et al., 2009], the investigation of compounds that could be added to dentifrices, mouthwashes and perhaps the eroding substance itself are of interest.

MATERIALS AND METHODS

Specimen preparation

Twenty-five crowns from central bovine incisors (36-month-old cattle from the same stable) were cleaned with a toothbrush in tap water (0.46 ppm fluoride and 105 mg/L calcium) following extraction. The crowns were then divided from the root and sectioned in buccal and lingual blocks using a water-cooled diamond saw (Struers, Copenhagen, Denmark). All buccal blocks (minimum surface area 2 cm^2) were sectioned once again resulting in 50 enamel specimens. All specimens were then embedded into epoxy resin within a circular silicone form (diameter 6.0 cm and height 1.5 cm). After hardening of the epoxy, all specimens were ground until an enamel window of 1 cm^2 was visible. Hereafter, specimens were wet polished until plano-parallel using a water-cooled polishing machine rotating in different aspects with waterproof silicon carbide grinding paper (Struers, Copenhagen, Denmark) having FEPA grit numbers of 1,000, 2,400 and 4,000 corresponding to particle sizes of 18.3, 8.4 and 5.0 µm, respectively. All specimens were then subjected to surface microhardness testing by the Vickers method, using an indentation force of 100 grams and with 3 indentations 20-50 µm apart. Only samples with a surface microhardness between 355 to 375 kg/mm^2 (n=11) were selected for the study. For optimal precision, the hardness tester was calibrated using 3 certified (Struers, Copenhagen, Denmark) metal blocks (hardness values from 150 to 400 kg/mm^2) and all determinations on enamel were corrected accordingly using non-linear mathematical extrapolation. In between tests, all specimens were stored at 4°C in Millipore water saturated with respect to hydroxyapatite (100% saturation) to avoid demineralization or remineralization of the freshly polished surfaces.

Test solutions for protection of enamel

Ten test solutions were prepared for coating of the enamel against a standardized acid challenge. For comparison, human whole saliva proteins collected from >100 healthy young individuals, with the pooled saliva subsequently dialyzed and lyophilized, were used [Bardow et al., 2000]. The concentration used for coating with salivary proteins was 4 mg/mL corresponding to an optimal human saliva protein composition [Bardow and Vissink, 2015]. The lyophilized proteins were resuspended in Millipore water and brought into solution by gentle stirring of a rotating magnet at 5°C for 24 hours. After testing the saliva proteins, ten different compounds were brought into solution by cutting or mixing 10 grams of each compound and then transferring one at a time to 500 mL of Millipore water (2% in total w/w). The solutions were prepared using a commercial blender (Phillips, Amsterdam, Holland). The test compounds in question were in alphabetical order:

(1) Albumin that is a relatively large protein of which the most common is serum albumin. Albumins consist of proteins that are water-soluble and soluble in concentrated salt solutions. Albumin is unique from other blood proteins in that it is non-glycosylated. In this study, analytical grade bovine serum albumin was used.

(2) Colostrum is the milk produced by the mammalian glands in late pregnancy. Colostrum is high in protein and contains a variety of proteins. Colostrum is claimed effective in the treatment or prevention of a variety of oral illnesses and conditions and therefore is commercially used in dentifrice and other oral hygiene products. Because colostrum proteins have the ability to bind strongly to enamel and most likely create some kind of a pellicle [Bardow et al., 2004], bovine colostrum has also been used in oral care products against dental erosion.

(3) Calcium fluoride is a compound with the formula CaF_2 that also can occur as mineral fluorite. Due to the formation of hydrogen fluoride on enamel surfaces, we hypothesized that this compound also could have anti erosive effects.

(4) Casein is a phosphoprotein commonly found in mammalian milk, making up to 80% of the proteins in bovine milk and comprises a variety of different chemical variants. Casein-derived compounds are used in tooth remineralization products to stabilize amorphous calcium phosphate (ACP) and release the ACP onto tooth surfaces, where it has a well-known effect to facilitate remineralization and thus "prevent and repair" erosive damages originating from exposure to food borne acids [Reynolds, 2009].

(5) Guarana is a plant native to the Amazon rainforest. Guarana is known for its fruit and in Brazil several soft drinks from guarana extract are available for retail. Guarana has been claimed to have antioxidant and antimicrobial activity in sugar rich soft drink [Majhenic et al., 2007] but no effects against dental erosion.

(6) Hydroxyapatite is the main inorganic component of dental enamel. The hydroxyapatite unit cell (least countable ionic entity in integers) has the formula $Ca_{10}(PO_4)_6(OH)_2$. It is a heavily soluble salt with a solubility product of $10^{-117.3}$ M^{18}. Hydroxyapatite is normally not used in dentifrice because it binds fluoride ions and thus decreases the effect of the toothpaste. However, in recent years, several derivatives of hydroxyapatite have been developed for dentifrices, including Novamin™ and nano-hydroxyapatite. Some of these have been tested against dental erosion, although with varying results. In this experiment only pure hydroxyapatite crystals (Ca/P ratio = 1.66) were used [Christoffersen, 1981].

(7) Liquorice is a root with a somewhat sweet flavor that can be extracted from the Glycyrrhizaglabra plant. The plant is native to southern Europe, India, and parts of Asia. It has demonstrated antiviral, antimicrobial and anti-inflammatory effects. Liquorice has also demonstrated efficacy in treating dental caries [Messier et al., 2012], but has not been tested for anti-erosive effects.

(8) Mucins are a family of high molecular weight, heavily glycosylated proteins. Mucins have considerable water-holding capacity, which is important in maintaining mucosal barriers. Mucins have been shown to have anti-caries effects and also a considerable ability to protect dental enamel from erosion [Kielbassa et al., 2005]. In the present study analytical grade porcine stomach mucins were used.

(9) Neem oil is extracted from the Neem tree, native to the Indian subcontinent. Neem oil is composed mainly of triglycerides and contains many triterpenoid compounds. Azadirachtin and nimbin have been credited with antiseptic, antifungal, antiseptic, antipyretic, antihistamine and anti-caries effects, but never anti-erosion effects.

(10) Salvadora Persica is often called Miswak and is used as tooth cleaning sticks in the Middle East and some Asian and African cultures. Miswak is known for its antimicrobial effects on the oral tissues and teeth [Akhtar et al., 2011; Chaurasia et al., 2013] and also antioxidant activities [Ibrahim et al., 2015]. Miswak has, however, never been tested for any possible anti-erosion effects.

Coating of specimens and erosive challenge

For each experiment, eleven enamel blocks were submersed in 2% solutions of the compounds in question, except for the solution containing human saliva proteins that was prepared as a 4 mg/mL solution. The test solutions were adjusted to neutral pH by HCl or NaCl. The specimens were mounted within a bowl attached to a shaker moving slowly in a rocking fashion. The shaker was adjusted to a gentle rocking movement, so that the solution in question slowly rinsed back and forth over the specimens' surfaces. With this set-up, specimens were either fully submersed or out of the solution numerous times during the coating procedure that lasted 24 hours. This set-up was constructed to simulate the effect of saliva secretion to the mouth and subsequent swallowing. For the erosive challenge, the specimens were transferred from the coating solution into 2 liters of an acidic solution made of Millipore water containing 2% tartaric acid. The acidic solution was buffered with 1 mmol/L $CaHPO_4$ to promote softening of the enamel instead of direct erosive wear (i.e. surface loss), and had a final pH of 2.3. The specimens were bathed in the acidic solution, which was agitated using a stirrer with a rotating magnet, in gentle pace at room temperature for 4 minutes. Following the erosive challenge, specimens were gently air dried prior to microhardness measurements.

Determination of protective effects of the tested substances

Protective effects of the tested substances against enamel softening were determined according to microhardness measurements before and after exposure to the acidic solution. The initial microhardness of the selected freshly polished enamel pieces was on average 365 kg/mm^2. In contrast, the microhardness of enamel coated only with Millipore water and subsequently exposed to the eroding solution was on average 140 kg/mm^2. This gave rise to a difference in microhardness of 225 kg/mm^2. Using this number, the protective effect of all test compounds after acid exposure could be calculated. Thus, a drop in microhardness of 225 kg/mm^2 equaled 0% protection, whereas no drop in microhardness equaled 100% protection. Within this range, the protective effects of all tested compounds were calculated and given in percent. During the experiments, each enamel specimen served as its own control, according to the calculations described, in order to avoid differences among specimens in response to the erosive challenge. For all comparisons with the test compounds, the protective effect of human salivary proteins (28 ± 8%) was used as control because this is the natural background within the oral cavity and what can be expected without the use of anti-erosion products.

Statistical analyses and assessment of compounds

Statistical analyses were done with Excel and SPSS™. Among the eleven enamel specimens used in each experiment, the specimen with lowest microhardness was discarded so that each solution was tested on the ten best enamel specimens in total. This procedure was performed to avoid bias from misleading determinations and thereby give optimal chances for all solutions and compounds. Because the Shapiro-Wilk test revealed that the data were not normally distributed ($p<0.05$), a non-parametric approach was adopted in all analyses and during the comparisons that are shown in Figure 2 and Table 1. By this approach, one-to-one comparisons were performed by the Wilcoxon signed rank test for paired analysis. This was used to compare the protective effect of the investigated substances against the physiological salivary background (the percent protective effect of human salivary proteins). The level of significance was set at 0.05 for all comparisons. For selected compounds, a speculative approximation of the results was estimated by amplifying the sample size to n=60, while maintaining the same differences, as this would show tentative results with a theoretical larger sample size.

RESULTS

Figure 2 shows the protective effect of the tested compounds (in % compared to no protection i.e. Millipore water). Colostrum proteins, saliva proteins and Neem oil had the best protective effect among the investigated compounds. However, it is important to emphasize that considerable differences were obtained among the various compounds. Thus, the protective effect ranged from nearly zero to a considerable protection of approximately 50%. Some of the compounds had a protective effect that, although inferior, was still close to that provided by human salivary proteins (Figure 2). These included proteins such as mucin and casein. Among the inorganic compounds, only hydroxyapatite had a reasonable protective effect against acid induced enamel softening.

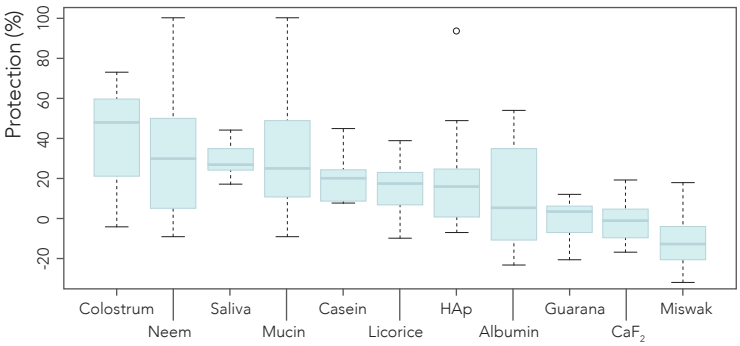

Figure 2. Boxplot of the percent protective effect of the various compounds tested. The protective effect was defined as the gain in reducing hardness loss compared to exposure to Millipore water only. HAp denotes hydroxyapatite and CaF2 calcium fluoride.

Comparing the results from Figure 2 with whole saliva proteins, a pattern became apparent in descending order (Table 1). Colostrum was better in protecting the enamel compared to all other compounds tested, however with no significant difference when compared to saliva. Thus, in this context, the following results should only be seen as a pilot study for screening of potential candidates against

enamel softening. Especially due to the manpower required to conduct such a comprehensive study, it seems most interesting to test as many compounds as possible and then focus on the most promising ones. The main result was that colostrum proteins and Neem oil were statistically noninferior, although with a somewhat better effect than human saliva (Table 1 and Figure 2). Mucin, casein, albumin and hydroxyapatite demonstrated slightly lower protection, although statistically non-inferior to than of human saliva. All other investigated compounds showed clear statistical inferiority in comparison to the protective effects of human saliva proteins on enamel.

TABLE 1

No.	Solutions	P-value	Result
1	Colostrum vs. saliva	0.426 / 0.046*	Non-inferiority / Superiority*
2	Neem vs. saliva	0.575	Non-inferiority
3	Mucin vs. saliva	0.938	Non-inferiority
4	Casein vs. saliva	0.207	Non-inferiority
5	Licorice vs. saliva	0.014	Inferiority
6	Hydroxyapatite vs. saliva	0.441	Non-inferiority
7	Albumin vs. saliva	0.383	Non-inferiority
8	Guarana vs. saliva	0.009	Inferiority
9	Calcium fluoride vs. saliva	0.009	Inferiority
10	Miswak vs. saliva	0.004	Inferiority

* Denotes an estimated amplified sample size of n=60 compared to n=10 as in the present study. Only significant numbers relating to superiority are shown.

DISCUSSION

The awareness of dental erosion has obviously increased in recent years, which has lead major companies to develop products that may prevent or repair erosive wear. It is important to state that the oral disease pattern today is completely different from decades ago, when massive decay and caries were the main problems to solve [Petersen, 2003]. In this perspective, the introduction of fluoride dentifrices clearly decreased caries, although nothing indicates that fluoride dentifrices can solve the erosion problem. Thus, with proper caries control in place requiring meticulous oral hygiene, dental erosion is clearly becoming a prominent problem that in the worst case scenario may be antagonistically related to caries [Honorio et al., 2008]. This means that treating caries successfully could have a negative effect on dental erosion. It is therefore beneficial to reconsider part of the toothpaste embodiment so that toothpastes will benefit both diseases simultaneously instead of focusing on dental caries only.

Substances that may help prevent dental erosion should at minimum not promote caries. In terms of medians, colostrum proteins and Neem oil fulfilled this prerequisite to varying degrees (Figure 2 and Table 1). Despite the apparent protective effect of colostrum, the lack of statistical significance in Table 1 could be due to the relatively small sample size. Only 10 specimens were chosen for this screening study. However, by projecting an amplified sample size to 60 specimens, while maintaining the same differences, statistical significance may be expected (p=0.046) for the colostrum proteins, and thus superiority in comparison to human saliva proteins. This finding is not surprising as a similar study performed more than ten years ago showed clear protective effects of colostrum when compared to human saliva proteins [Bardow et al., 2004], findings which were subsequently patented [EP1568356-B1].

However, it was surprising that Neem oil also had a positive effect. Since to the best of our knowledge the effect of this oil against dental erosion has not been investigated, Neem oil could be an interesting and potentially new candidate for the prevention of dental erosion. Nonetheless, it is likely that other and perhaps more effective compounds against dental erosion still can and will be unveiled. However, this requires clear information about not brushing the teeth immediately after intake of acidic foodstuffs, due to enamel softening and thus increased risk of abrasion [Wiegand, 2008]. In a different perspective it could also be speculated that mixtures of some of the compounds tested in the present paper could be interesting. Such mixtures could perhaps contain colostrum, Neem oil, mucin and casein.

CONSLUSIONS AND FUTURE SPECTIVES

Organic substances such as proteins and oils seem interesting for future research on the prevention of dental erosion. Our recommendation at this point in time is to screen many more compounds, as there are numerous potential candidates that have not been tested yet. Such investigations should help preventing dental erosion.

ACKNOWLEGDEMENTS

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CONFLICT OF INTEREST

Author Allan Bardow is the co-inventor on a patent claiming anti erosive effects of colostrum proteins in a toothpaste embodiment (EP1568356-B1). The remaining authors declare no conflicts of interest in the present compounds tested for this study.

AUTHOR CONTRIBUTIONS

Al-Kahwa and Al-Qazaz were responsible for selecting the compounds to be tested. They also carried out the laboratory analysis by themselves after instruction in the use of the equipment by Allan Bardow, who also assisted with microhardness testing, statistical analyses and writing the paper. Ana Benetti assisted with epoxy casting, polishing of the enamel and day to day microhardness analyses. All authors were involved in finalizing the manuscript and approved to submit the manuscript for publication in its present form.

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