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Fluoride application before early and advanced erosion

S O'Toole *et al.*

Efficacy of sodium and stannous fluoride mouthrinses when used before single and multiple erosive challenges.

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ABSTRACT

Background: Application of fluoride mouthrinse before an acidic challenge may decrease enamel erosion. This paper compares the efficacy of stannous (SnF_2) and sodium (NaF) fluoride when facing single and multiple erosive cycles *in vitro*.

Methods: Human enamel samples (N = 60) were randomly assigned to groups testing SnF_2 and NaF mouthrinses (225 p.p.m.) and a water control. Samples were allocated into subgroups testing one or five erosive cycles. Samples were immersed in test solution for 1 min prior to citric acid immersion (0.3%, pH 3.2, 10 min), and the cycle repeated either one or five times. Analysis was done using profilometry and microhardness change.

Results: After one cycle, SnF_2 resulted in least step height followed by NaF and water (1.3 µm (0.63), 2.3 µm (0.39), 4.3 µm (0.41) respectively; $P < 0.0001$). After five cycles SnF_2 continued to reduce step height but pre-application of NaF was no different to water (4.6 µm (0.7), 10.5 µm (1.1) and 11.1 µm (0.38) respectively; $P < 0.0001$). There were no statistical differences in microhardness change between fluorides. After one erosive cycle, fluoride application resulted in statistically softer enamel compared with water.

Conclusions: Both SnF_2 and NaF reduced erosion after one cycle. After five cycles, SnF_2 continued to offer protection whereas NaF was statistically comparable with water. Softening of enamel may not imply less erosion has occurred.

Keywords: Enamel erosion, fluoride, prevention, tooth wear, tooth demineralization.

Abbreviations and acronyms: Stannous Fluoride = SnF_2, Sodium Fluoride = NaF.

INTRODUCTION

Dental erosion is a condition of growing concern in the dental community. Increasingly, preventive treatments are being aimed at surface protection rather than attempting remineralization of erosive lesions.[1] The optimal use of fluoride in the prevention of enamel erosion is unclear. Some authors report that the established caries model of fluoride application, whereby fluoride is applied frequently and at higher concentrations, is of limited use in dental erosion.[2,3] Other authors report that fluoride has a protective effect depending on the fluoride concentration or the form of delivery.[4–6]

Previous studies have demonstrated the effectiveness of stannous fluoride in reducing enamel erosion.[7–9] It is thought that both the stannous ion and fluoride ion have a role in protecting enamel during an erosive challenge.[10] Interestingly, the divalent stannous ion has been shown to be stable and effective when present at low pH.[7] Below a pH of 4, stannous fluoride can release ions to re-establish saturation of mineral content in the oral environment with regards to enamel structure.[7,11] The stannous ion (Sn^{2+}) can replace the calcium ion (Ca^{2+}) in hydroxyapatite.[7,11] Acidulated sodium fluoride has been shown to work by a similar mechanism whereby the fluoride becomes incorporated into the hydroxyapatite structure forming fluoroapatite. Fluoroapatite has been shown to be more stable and acid resistant than hydroxyapatite.[12] Above a pH of 4, stannous fluoride can undergo oxidation and hydrolysis reactions forming inactive precipitates such as stannous fluorophosphate, calcium stannous fluoride and stannous hydroxyphosphate.[13] These are less soluble than calcium fluoride and may act as a physical barrier preventing contact of the acid with the enamel.[13] *In vitro* studies have suggested that the stannous ion does not need to be present in high doses in order to achieve an anti-erosive effect.[14]

Although multiple studies have compared stannous fluoride with sodium fluoride, few studies have contrasted them under differing erosive conditions. The fluorides may be more or less effective depending on the level of the erosive challenge they must protect against. The purpose of this research was to investigate the effectiveness of low-concentration stannous fluoride and sodium fluoride when facing differing levels of erosive challenges. The protective effect of a single application and erosive challenge was compared with the protective effect observed after multiple applications and erosive challenges. The null hypothesis was that there will be no difference between stannous fluoride and sodium fluoride mouthrinses of equal fluoride concentration after both one and five erosive cycles.

METHODS

Previously extracted, caries-free human molars (N = 60) were collected (Research Ethics Committee reference 12/LO/1836) and stored in sodium hypochlorite solution for 3 days. Buccal surfaces were sectioned using a circular saw (Buehler Isomet 1000 precision saw with an Extex diamond wafering blade) at a speed of 300 rpm and force of 150 *g*. These were mounted in acrylic resin (Oracryl; Bracon, East Sussex, UK) and polished in a grinder-polisher (Metaserv 3000 variable speed grinder-polisher; Buehler, Coventry, UK) at 200 rpm using Federation of European Producers of Abrasives standard silicon carbide sandpaper disks of grit 80, 180, 600, 1200, 2400 and 4000. This resulted in a smooth, highly polished surface. Samples were then placed in distilled water in an ultrasonic bath (GP-70; Nusonics, Lakewood, CA, USA) for 15 min to remove debris and dried for 12 h at room temperature. Two pieces of adhesive tape were placed 1 mm apart. This allowed a 1 mm × 3 mm exposure area and two unexposed reference areas on either side.

The 60 samples were randomly divided into two groups. Half of the samples were subjected to one treatment cycle (N = 30) and the other half were subjected to five treatment cycles (N = 30). Both groups tested a stannous fluoride mouthrinse (Periomed, alcohol-free, stannous fluoride 0.63% w/w, fluoride 0.12% w/w; 3M ESPE, St Paul, MI, USA) (N = 10), a sodium fluoride mouthrinse (Fluoriguard, alcohol-free, sodium fluoride 0.05% w/w 225 p.p.m.; Colgate, Surrey, UK, (pH 6)) (N = 10) and a distilled water control (N = 10). Periomed was diluted to ensure a uniform solution containing 225 p.p.m. of the fluoride ion and 956 p.p.m. stannous ion (187.5 mL in 1000 mL of distilled water) and stirred for 5 min using a magnetic stirrer. The final pH of the solution was 3.8. Fluoriguard was supplied at a fluoride concentration of 225 p.p.m. (pH 6) and required no preparation.

Within each group, samples were immersed in 80 mL of fluoride solution or distilled water for 1 min using an orbital shaker (Stuart Orbital Shaker SS1; Bibby Scientific, Staffordshire, UK, set at 62.5 rpm). To simulate the manufacturer's instructions, a wait period of 30 min was performed, whereby samples were placed in a distilled water bath (100 mL) for 30 min. Following treatment, the samples were immersed in 80 mL 0.3% citric acid, pH 3.2 for 10 min under agitation (Stuart Orbital Shaker SS1; Bibby Scientific, set at 62.5 rpm). After the erosive challenge, samples were rinsed using the orbital shaker in 100 mL of distilled water for 2 min. For the one-cycle group, samples were allowed to dry and were analysed. For the five-cycle group, the complete cycle, including erosion and fluoride application was performed five times before they were allowed to dry and analysed. The experiment was carried out at 22 ± 1°C (Fig. 1).

Samples were allowed to air-dry at room temperature for 12 h, the tape was carefully removed and then scanned using a confocal non-contacting red light laser profilometer (XYRIS 2000; Taicaan, Southampton, UK) with a spot size of 2 μm and resolution of 0.01 μm. Using the integrated microscope video camera to ensure equal widths of reference and eroded areas were captured, 500 data points were scanned in a raster pattern. Data were analysed using Boddies version 1.92 (Taicaan, Southampton, UK). The step height between the reference area and the eroded area was calculated in µm.

Surface microhardness was measured using a Knoop hardness tester (Duramin-1/-2; Struers, Catcliffe, UK). For each sample, three indentations (10 s at 981 mN loading) were made 100 µm apart in both the eroded area and the reference area. The Knoop hardness number (KHN) was calculated for each of the indents using the supplied Duramin program. This software automatically calculates the hardness of the surface based upon the length of the indent and force applied. The mean reference area value was then subtracted from the mean eroded value to obtain the change in microhardness in KHN.

Gpower version 3.1.5 (Heinrich Heine, Düsseldorf, Germany) was used to perform a power calculation based on ANOVA and comparing the mean step height loss and microhardness change between different groups. A sample size of 54 yielded 80% power at 5% level and would give an effect size of 0.31 using two-tailed test within groups.

Statistical analysis was performed on SPSS version 22.0 software (IBM, Armonk, NY, USA). The data were checked for normality using normality plots and Shapiro–Wilk tests. Data were normally distributed and thus presented as means and standard deviations (SD). A two-way ANOVA was used to assess for group differences. A post-hoc Bonferroni test was then applied to assess for treatment comparisons. The level of significance was set at $P \leq 0.05$.

RESULTS

Step height

The step height results obtained are represented in Fig. 2. After one cycle, the mean (SD) step heights for stannous fluoride, sodium fluoride and distilled water were 1.3 µm (0.63), 2.3 µm (0.39) and 4.3 µm (0.41), respectively. Stannous fluoride application resulted in statistically significant less step height compared with both sodium fluoride and the water control ($P < 0.0001$). Sodium fluoride also reduced step height compared with water ($P < 0.0001$). After five cycles, all groups displayed statistically increased step height as expected ($P < 0.0001$). The mean (SD) step heights for stannous fluoride, sodium fluoride and distilled water were 4.6 µm (0.7) and 10.5 µm (1.1) and 11.1 µm (0.38) of step height, respectively. Stannous fluoride again reduced step height compared with sodium fluoride and water ($P < 0.0001$). However, there was no statistical difference in step height between sodium fluoride and water after five cycles (Fig. 2).

Microhardness

The microhardness results obtained are represented in Fig. 3. A greater microhardness change means the resulting enamel was softer whereas a low microhardness change is indicative of a harder remaining structure. After one cycle the mean (SD) microhardness changes for stannous fluoride, sodium fluoride and distilled water were 152.8 KHN (22.9), 133.3 KHN (27.6) and 91.4 KHN (27.7), respectively. Stannous fluoride was statistically softer than both sodium fluoride and the water control ($P = 0.019$). There was no statistical difference between sodium fluoride and water. After five cycles, the mean microhardness change (SD) for stannous fluoride, sodium fluoride and distilled water were 78.3 KHN (16.57) and 81.4 KHN (28.61) and 114.9 KHN (11.9), respectively. The water control group was softer than both stannous fluoride ($P = 0.003$) and sodium fluoride ($P = 0.002$) after five cycles. When comparing one cycle to five cycles, both stannous fluoride and sodium fluoride were statistically softer after one cycle compared with five cycles ($P = 0.001$ and $P = 0.003$, respectively). This contrasted with water application where the enamel was harder after one cycle compared with five erosive cycles ($P = 0.009$).

DISCUSSION

The unique finding in this study is that sodium fluoride reduced step height after one application and erosive challenge. However, after five applications and erosive challenges, it was not statistically different to the water control. In contrast, stannous fluoride reduced step height after both a single erosive cycle and five erosive cycles. As statistical differences were observed between stannous and sodium fluoride after both a single erosive challenge and five erosive challenges, the null hypothesis was rejected.

While other studies have shown high-concentration fluoride gels[15] and other metal ions[16] to have different protective effects, this is the first study observing differences in the surface protection offered by different low-concentration fluorides after single and multiple erosive cycles. One study investigated a stannous fluoride product after multiple applications. As the number of applications increased they observed a thickening stannous layer. However, they did not perform any erosive cycling and did not compare the product with any other dentrifices.[17] Another *in situ* trial compared stannous and sodium fluoride dentifrices after 5 days (20 erosive challenges) and 15 days (60 erosive challenges).[9] Although the authors also observed less step height with stannous fluoride, they did not compare the dentifrices to a water control. We do not know if sodium fluoride offered improved protection over water at advancing levels of erosion using their experimental model.

Other studies have also found sodium fluoride to show no additional protective effect over water when assessing advanced erosion. One study investigated the effect of a pretreatment of stannous fluoride and sodium fluoride on enamel to a short hydrochloric acid exposure (4 min 0.01 M HCL), using atomic absorption spectroscopy to detect mineral loss. Both SnF_2 and NaF reduced calcium loss over a water control. However, stannous fluoride prevented calcium release to a greater extent (water treatment resulted in 1.6 absorbance units of calcium release compared with 0.3 for SnF_2 and 1.1 for NaF, significance levels $P < 0.0001$ and $P < 0.05$, respectively).[18] When exposed to a longer erosive challenge

(30 min 0.01 M HCl), SnF_2 was still able to reduce calcium loss ($P < 0.001$) whereas for NaF there was no difference to the water control.[18] Wiegand *et al.* found that step height with NaF was also no different to a fluoride-free placebo after a 3-day *in vitro* cycling regime of application and erosion.[19] Hystad *et al.* found both high and low concentrations of NaF resulted in similar levels of step height to water in an *in situ* study simulating the severe erosive challenge of gastro-oesophageal reflux.[20] NaF appears to offer more protection *in situ* where the interaction with saliva may aid in preventing enamel erosion.[21,22]

Our second interesting finding was that the lower step heights observed after one cycle when fluorides were applied were associated with enamel softening compared with the water control. This interesting observation could mean that in the early stages of erosion a softer, affected layer of enamel is retained with potential for remineralization. After five cycles, however, microhardness results reversed. Water application resulted in statistically softer enamel, whilst fluoride application resulted in enamel hardening. These results could be explained by a plateauing effect of enamel hardness which has been observed in studies after prolonged erosive challenges.[8,23] The authors hypothesized that a hardness equilibrium is established as softened surface layers are removed by a continuing erosive challenge. This exposes harder enamel underneath.[8,23] Microhardness analysis can determine the degree to which the acid has affected the top surface of enamel (initial softening of a newly exposed layer or deeper softening of an exposed layer), but is not always an indicator of how much erosion has taken place. Rakhmatullina *et al.* observed a rapid linear loss of enamel hardness after 12–16 min of erosion with 0.65% citric acid at a pH of 3.6. After a certain point, the relationship between erosion and microhardness was not linear and hardness measurements stabilised.[8] Reviews of laboratory-based methods for testing erosion have suggested that microhardness testing is useful when assessing initial softening but is of limited use when assessing advanced erosion.[24,25] Microhardness data at advanced stages of erosion may be assumed to be a qualitative method of analysis.

This study used profilometry and microhardness testing to measure the erosion that occurred. There is conflict in ideal experimental conditions between profilometry and microhardness. The primary measure of outcome for this experiment was profilometry. Profilometry is the gold standard method for measuring step height formation in *in vitro* erosion studies[26]. Our profilometer has a resolution of 0.01 µm and repeatability of 0.2 µm. The use of adhesive tape to provide reference areas for profilometric measurements is well-established in the *in vitro* assessment of profilometric measurements.[27] However, sufficient erosion must occur in order to assess profilometric tissue loss. After a certain level of erosion, microhardness readings become less accurate for reasons outlined above and results should be interpreted with care. The qualitative microhardness results obtained from this study may provide further insight into the erosion process but need to be reinforced by additional experiments.

This is the first study comparing the effect of fluorides after one erosive cycle to five erosive cycles and gives a better understanding into the mechanism of action of the fluorides. This study adds to the increasing evidence that stannous fluoride can be recommended to patients at risk from repeated or severe erosive challenges. The remineralizing properties of sodium fluoride may be more useful in early stages of erosion.

CONCLUSIONS

Both stannous and sodium fluoride reduced step height after one erosive cycle. After five cycles, application of stannous fluoride continued to reduce step height but sodium fluoride resulted in the same step height as the water control. Softening of enamel structure may not imply less erosion has occurred.Taking into consideration the limitations of *in vitro* research, stannous fluoride mouthrinses may have an inhibitory effect on enamel erosion in patients anticipating multiple erosive challenges whereas sodium fluoride may be of use in early erosion.

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REFERENCES

1 Magalhães AC, Wiegand A, Rios D, Buzalaf MA, Lussi A. Fluoride in Dental Erosion. In: *Monograph Oral Science*. 2011, pp 158–170.

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

3 Larsen MJ, Richards a. Fluoride is unable to reduce dental erosion from soft drinks. *Caries Res* 2002;36:75–80.

4 Mohammed A, Dusara K. What is the role of topical fluoride application in preventing dental erosion? *Evid Based Dent* 2013;14:59–62.

5 Austin RS, Stenhagen KR, Hove LH, Tveit AB, Moazzez R, Bartlett DW. The effect of single-application fluoride treatment on simulated gastric erosion and erosion-abrasion of enamel in vitro. *Int J Prosthodont* 2014; 27:425–426.

6 Ganss C, Klimek J, Brune V, Schürmann A. Effects of two fluoridation measures on erosion progression in human enamel and dentine in situ. *Caries Res* 2004;38:561–566.

7 Schlueter N, Hardt M, Lussi A, Engelmann F, Klimek J, Ganss C. Tin-containing fluoride solutions as anti-erosive agents in enamel: an in vitro tin-uptake, tissue-loss, and scanning electron micrograph study. *Eur J Oral Sci* 2009;117:427–434.

8 Rakhmatullina E, Beyeler B, Lussi A. Inhibition of enamel erosion by stannous fluoride containing rinsing solutions. *Schweiz Monatsschr Zahnmed* 2013;123:296–302.

9 Hooper S, Seong J, Macdonald E, *et al.* A randomised in situ trial, measuring the anti-erosive properties of a stannous-containing sodium fluoride dentifrice compared with a sodium fluoride/potassium nitrate dentifrice. *Int Dent J* 2014;64:35–42.

10 Ganss C, Schlueter N, Hardt M, Schattenberg P, Klimek J. Effect of fluoride compounds on enamel erosion in vitro: a comparison of amine, sodium and stannous fluoride. *Caries Res* 2008;42:2–7.

11 Babcock FD, King JC, Jordan TH. The reaction of stannous fluoride and hydroxyapatite. *J Dent Res* 1978;57:933–938.

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

13 Schlueter N, Klimek J, Ganss C. Effect of stannous and fluoride concentration in a mouth rinse on erosive tissue loss in enamel in vitro. *Arch Oral Biol* 2009;54:432–436.

14 Schlueter N, Klimek J, Ganss C. In vitro efficacy of experimental tin- and fluoride-containing mouth rinses as anti-erosive agents in enamel. *J Dent* 2009;37:944–948.

15 Sar Sancakli H, Austin R, Al-Saqabi F, Moazzez R, Bartlett DW. The influence of varnish and high fluoride on erosion and abrasion in a laboratory investigation. *Aust Dent J* 2015;60:38–42.

16 Bueno MG, Marsicano J a, Sales-Peres SHC. Preventive effect of iron gel with or without fluoride on bovine enamel erosion in vitro. *Aust Dent J* 2010;55:177–180.

17 Khambe D, Eversole SL, Mills T, Faller R V. Protective effects of SnF 2 – Part II . Deposition and retention on pellicle-coated enamel. *Int Dent J* 2014;64:11–15.

18 Willumsen T, Ogaard B, Hansen B, Rolla G. Effects from pretreatment of stannous fluoride versus sodium fluoride on enamel exposed to 0.1 M or 0.01 M hydrochloric acid. *Acta Odontol Scand* 2004;62:278–281.

19 Wiegand A, Bichsel D, Magalhães AC, Becker K, Attin T. Effect of sodium, amine

and stannous fluoride at the same concentration and different pH on in vitro erosion. *J Dent* 2009;37:591–595.

20 Hystad Hove L, Stenhagen KR, Mulic A, Holme B, Tveit AB. May caries-preventive fluoride regimes have an effect on dental erosive wear? An in situ study. *Acta Odontol Scand* 2014;73:1–7.

21 Ganss C, Neutard L, von Hinckeldey J, Klimek J, Schlueter N. Efficacy of a tin/fluoride rinse: a randomized in situ trial on erosion. *J Dent Res* 2010;89:1214–1218.

22 Schlueter N, Klimek J, Ganss C. Efficacy of an experimental tin-F-containing solution in erosive tissue loss in enamel and dentine in situ. *Caries Res* 2009;43:415–421.

23 Venasakulchai A, Williams N a, Gracia LH, Rees GD. A comparative evaluation of fluoridated and non-fluoridated mouthrinses using a 5-day cycling enamel erosion model. *J Dent* 2010;38:21–29.

24 Hara a T, Zero DT. Analysis of the erosive potential of calcium-containing acidic beverages. *Eur J Oral Sci* 2008;116:60–65.

25 Stenhagen KR, Hove LH, Holme B, Taxt-Lamolle S, Tveit a B. Comparing different methods to assess erosive lesion depths and progression in vitro. *Caries Res* 2010;44:555–561.

26 Paepegaey A-M, Barker ML, Bartlett DW, *et al.* Measuring enamel erosion: a comparative study of contact profilometry, non-contact profilometry and confocal laser scanning microscopy. *Dent Mater* 2013;29:1265–1272.

27 Barbour ME, Rees JS. The laboratory assessment of enamel erosion: a review. *J Dent* 2004;32:591–602.

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Fig. 1. Simplified experiment process.

Fig. 2 Step height comparing stannous fluoride (SnF_2) and sodium fluoride (NaF) after one and five experimental cycles.

Fig. 3 Microhardness change comparing stannous fluoride (SnF_2) and sodium fluoride (NaF) after one and five experimental cycles.