



## Sequence of stannous and sodium fluoride solutions to prevent enamel erosion



Saoirse O'Toole<sup>a,\*</sup>, Miten Mistry<sup>b</sup>, Mahdi Mutahar<sup>c</sup>, Rebecca Moazzez<sup>d</sup>, David Bartlett<sup>e</sup>

<sup>a</sup> King's College London Dental Institute, London, England, United Kingdom

<sup>b</sup> King's College London Dental Institute, London, England, United Kingdom

<sup>c</sup> King's College London Dental Institute, London, England, United Kingdom

<sup>d</sup> Dental Institute Oral Clinical Research Unit, King's College London Dental Institute, London, England, United Kingdom

<sup>e</sup> Prosthodontics, King's College London Dental Institute, London, England, United Kingdom

### ARTICLE INFO

#### Article history:

Received 8 June 2015

Received in revised form 28 September 2015

Accepted 4 October 2015

#### Keywords:

Erosion  
Prevention  
Saliva  
Fluoride  
Enamel  
Tooth wear

### ABSTRACT

**Objectives:** Investigate the timing of stannous (SnF<sub>2</sub>) and sodium fluoride (NaF) application with and without salivary pellicle to prevent enamel erosion.

**Methods:** Human buccal molar enamel samples ( $n = 120$ , REC ref 12/LO/1836) were randomly assigned to three groups testing SnF<sub>2</sub> and NaF basic fluoride formulation and commercial mouthrinses with and without the presence of human saliva. Samples were randomly allocated to 2 subgroups: immersion in either fluoride for 1 min either before or after citric acid immersion (0.3%, pH 3.2, 10 min), and the cycle repeated 5 times. For human saliva group, samples were immersed in 80 ml of natural saliva for 24 h prior to the experiment. Analysis was done using non-contacting profilometry and microhardness change. Data were not normal and were log transformed. A linear model tested statistical differences between the groups.

**Results:** SnF<sub>2</sub> application before erosion statistically reduced step height compared to application after erosion for all groups (solutions: 6.5  $\mu\text{m}$  ( $\pm 1.2$ ), 7.5  $\mu\text{m}$  ( $\pm 0.8$ );  $p = 0.01$ , mouthrinses: 3.2  $\mu\text{m}$  ( $\pm 0.6$ ), 4.2  $\mu\text{m}$  ( $\pm 0.7$ );  $p < 0.0001$ , mouthrinses with saliva: 2.5  $\mu\text{m}$  ( $\pm 0.4$ ), 3.1  $\mu\text{m}$  ( $\pm 0.6$ );  $p = 0.002$ , before and after respectively). In contrast, application of NaF before erosion increased step height compared to application after, but this was only statistically significant for the saliva group (before: 5.6  $\mu\text{m}$  ( $\pm 0.3$ ) and after: 4.9  $\mu\text{m}$  ( $\pm 0.3$ );  $p = 0.023$ ). Presence of saliva increased microhardness change ( $p < 0.0001$ ). Within this group, greatest microhardness change was observed when SnF<sub>2</sub> was applied before erosion and when NaF was applied after erosion (SnF<sub>2</sub>: 156.6KHN ( $\pm 32.8$ ), 123KHN ( $\pm 20.1$ );  $p = 0.02$ . NaF: 119.5KHN ( $\pm 33.5$ ), 218KHN ( $\pm 24.9$ ), before, and after respectively).

**Conclusion:** SnF<sub>2</sub> reduced step height formation overall when compared to NaF, but particularly when applied before citric acid immersion. In contrast, NaF reduced step height when applied after citric acid immersion, but only in the presence of saliva.

**Clinical significance:** Stannous fluoride can be recommended over sodium fluoride to patients at risk of dental erosion and is optimally applied before erosion occurs. If sodium fluoride is to be used in the presence of saliva it is optimally applied after erosion has occurred.

© 2015 Elsevier Ltd. All rights reserved.

## 1. Introduction

Tooth wear is a multifactorial condition consisting of erosion, abrasion and attrition and is common to many European adults [1]. Dental erosion is a condition of growing concern in the dental community and there is debate over the optimal timing of oral

hygiene procedures in relation to an erosive challenge. After an erosive challenge, the softened enamel may be more susceptible to mechanical abrasion, such as, toothbrushing [2]. Based on previous laboratory and clinical studies some authors have recommended not to brush for at least one hour after an erosive challenge [3–5]. However more recently, other authors have demonstrated that eroded enamel showed no increased abrasion resistance even after a 2–4 hour remineralisation period [6,7]. Fluoride, applied as a mouthrinse either before [8] or after [9] an erosive challenge has been shown to protect enamel without an abrasive element.

\* Corresponding author at: Floor 25, Guy's Tower, King's College London, London Bridge, SE1 9RT, England, United Kingdom.  
E-mail address: [saoirse.otoole@kcl.ac.uk](mailto:saoirse.otoole@kcl.ac.uk) (S. O'Toole).

The two interpretations on the role of fluoride in erosion are surface protection or remineralisation of erosive lesions [10]. Two theories on surface protection are the presence of fluoride deposits on the dental surfaces and incorporation of the fluoride ion into the hydroxyapatite structure [11]. The concept of remineralisation in erosion is not universally accepted and is partly based on the caries process where the lost surface minerals are replaced by the fluoride ions [12].

The stannous ion shows promising results in the prevention of dental erosion, either combined with fluoride or in the form of other stannous salts [13]. Interestingly, there are indications that deposits of the stannous ion are more stable on dental surfaces than sodium fluoride deposits when facing an erosive challenge [14].

Both stannous and sodium fluorides have shown to be protective against an erosive challenge albeit under different conditions [9,15]. The properties of different fluoride compounds indicate they may react differently depending on the condition of the enamel and the environment (neutral or acidic) into which it is placed.

In vivo, tooth surfaces are covered with an acquired salivary pellicle which helps to protect enamel from tooth erosion [16]. The pellicle acts as a diffusion barrier aiding the protection against demineralisation [17]. Due to its high protein and mineral content saliva can increase mineralisation of demineralised enamel if the matrix is still intact [18]. Salivary pellicle can also alter the efficacy of products making them more effective [19,20].

In vitro studies provide the opportunity for highly controlled conditions to study individual risk factors or novel compounds on erosion to better understand their role. The aim of this study was to investigate the timing of application of fluoride in relation to the erosive challenge. The second aim was to investigate under laboratory conditions the application of sodium and stannous fluoride as a pure solution, a commercial mouth rinse or in the presence of a salivary pellicle. The first null hypotheses proposed that altering the timing of application of different fluorides to enamel would not affect enamel erosion. The second null hypothesis proposed that enamel erosion is not influenced by sodium and stannous fluoride applied as a solution or as a commercial mouthrinse with and without the presence of saliva.

## 2. Materials and methods

Enamel from previously extracted, caries free teeth were sectioned, using a circular saw (Isomet 1000 with an Extex diamond wafering blade; Buehler, Coventry, UK) at a speed of 300 rpm with a force of 150 g, from the buccal surfaces of molar teeth to produce 120 sound enamel specimens. The sectioned enamel specimens were placed into a custom-made silicone mould (specimen size  $8 \times 21.5 \times 24$  mm) and embedded in cold cure acrylic resin (Oracryl; Bracon, East Sussex, UK). Specimens were then polished (Metaserv 3000 variable speed grinder-polisher; Buehler, Coventry, UK) using the Federation of European Producers of Abrasives (FEPA) standard silicon carbide sandpaper, starting at 80 grit, followed by the 180, 600, 1200, 2400 and 4000 grit. Following polishing, specimens were immersed in 80 ml of deionised water and ultrasonicated (GP-70; Nusonics, Lakewood, US) at 60 Hz for 15 min, after which they were rinsed and allowed to dry. Adhesive tape was placed on the enamel surface to create a window approximately  $1 \text{ mm} \times 3 \text{ mm}$  wide for two reference areas. Specimens were stored in dry conditions prior to the erosive cycling except for the saliva experiment.

Citric acid (99%; Sigma Aldrich, Haverhill, UK) at 0.3% adjusted to pH 3.2 with sodium hydroxide was used as the erosive solution. Sodium fluoride (99%; Alfa Aesar, Lancashire, UK) and stannous fluoride (99%; Sigma-Aldrich, Haverhill UK) solutions were diluted with deionised water to create 225 ppm concentration of fluoride at pH 6 and 4 respectively. Commercial sodium and stannous fluoride mouth rinses were used at a 225 ppm concentration (Fluoriguard, alcohol free, sodium fluoride 0.05% w/w 225 ppm; Colgate, Surrey, UK, (pH 6) and Periomed alcohol free, stannous fluoride 0.63% w/w, fluoride 0.12% w/w; 3 M ESPE, Minnesota, US, diluted in deionised water to produce a 225 ppm fluoride concentration solution (pH 3.8)). Acid and fluoride solutions were freshly made each day. Stimulated human saliva was collected from healthy volunteers and was obtained after an absence of food or drink for 1 h prior to donation. Volunteers were asked to chew flavourless paraffin wax for 5 min while the saliva was collected in a 20 ml polypropylene tube. The samples were immediately frozen at  $-80^\circ\text{C}$  within 15 min of collection. Prior to use in the experimental cycling, the saliva was fully defrosted at room temperature and then pooled.

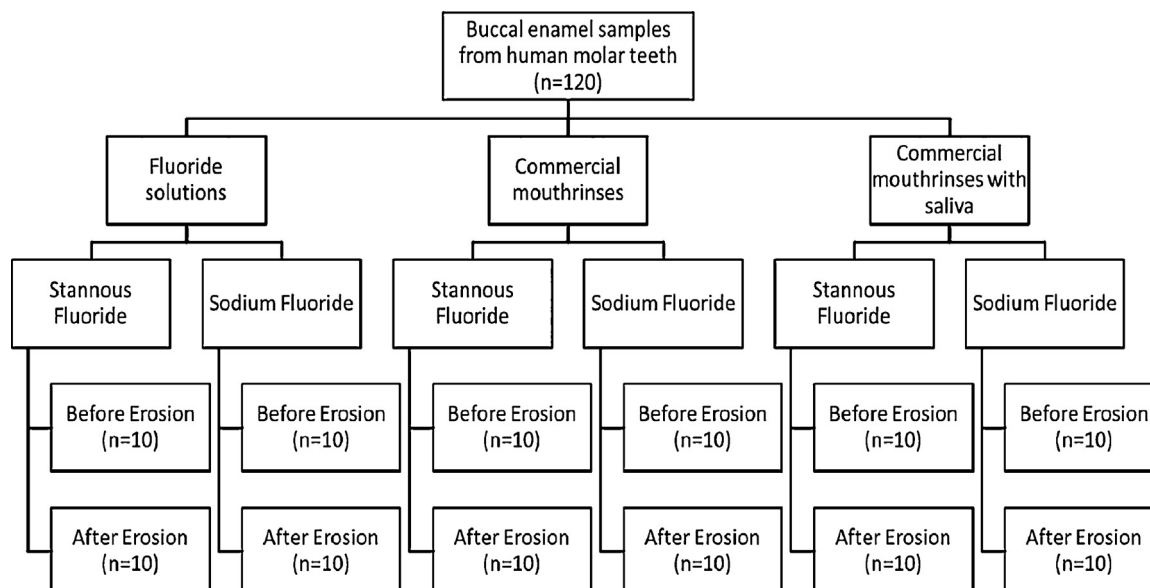


Fig. 1. Random allocation of samples.

The enamel specimens ( $n = 120$ ) were randomly divided into three groups for allocation to the pure solutions ( $n = 40$ ), commercial mouthrinses ( $n = 40$ ) or commercial mouthrinses in saliva ( $n = 40$ ) groups. Within each of the three groups, the specimens were further divided into two groups for immersion in sodium fluoride ( $n = 20$ ) or stannous fluoride ( $n = 20$ ). Furthermore, each group were then again divided into those when the fluoride was applied prior to the citric acid ( $n = 10$ ) and those that were applied afterwards ( $n = 10$ ) as shown in Fig. 1.

One cycle consisted of immersing the specimens in 80 ml of citric acid, agitating with an orbital shaker (Stuart Orbital Shaker SS1; Bibby Scientific Limited, Staffordshire, UK) at 60 rpm for 10 min, following which the specimens were rinsed in 100 ml of deionised water, again, under agitation with an orbital shaker set at 60 rpm for a final 2 min. The specimens were then placed in 80 ml of the respective fluoride solution and agitated with the orbital shaker at 60 rpm for 1 min. Specimens were rinsed and placed in 100 ml of deionised water and left, unstirred, for 30 min. This cycle was carried out five times. Where the fluoride was to be applied first, the enamel specimens were immersed in fluoride solution, using the same procedure described above and thereafter immersed in acid and the process repeated another four times.

To assess the impact of saliva, the specimens in the saliva group ( $n = 40$ ) were placed in 80 ml of natural saliva, un-agitated for 24 h and stored overnight. The specimens were then rinsed prior to acid cycling and the fluoride was applied either before or after the acid immersion as previously described. For this group, samples were placed in 100 ml of natural saliva for 30 min instead of deionised water between erosive challenges.

Specimens were air-dried for 24 h after which the tape was removed and profilometric measurement and microhardness data were obtained. Profiles were measured using a white light non-contacting laser profilometer with a spotsize of  $7 \mu\text{m}$  and a resolution of  $0.01 \mu\text{m}$  (XYRIS 2000; Taicaan, Southampton, UK) with a single line mid-point step height calculated with Boddies software (Taicaan, Southampton, UK). Knoop microhardness

(Duramin-1/-2; Struers, Catcliffe, UK) was performed at a press load of 981.2 mN and a press time of 10 s. Each specimen had 3 indentations taken  $100 \mu\text{m}$  apart on the worn area and on the reference area. Knoop microhardness was calculated manually using the Duramin software (Struers, Catcliffe, UK) and the Knoop microhardness change (KHC) calculated by subtracting the average of the worn and reference areas for each specimen. There were no missing values in the data set. Data were analysed using SPSS version 22 software (IBM SPSS Statistics for Windows, Version 22.0; IBM Corp, New York, US). Data was checked for normality using Shapiro-Wilks test and visually using histograms, Q-Q plots and box plots. Both the step height and microhardness data were right-skewed. Original data were transformed by  $\log_{10}$  to fulfill the normality assumption. Further analysis were carried out on the transformed data. A linear model was used to test the effects of the solution used, fluoride used and the timing of application. If the interaction effect was significant, then further post hoc analysis were carried out to test which combination order and material was statistically significant. A power calculation based on ANOVA and comparing the mean step height loss between different groups, solutions and orders showed that a sample size of 118 yielding 80% power at 5% level would give an effect size of 0.31 using two tailed test using Gpower ver 3.1.5.

### 3. Results

Figs. 2 and 3 show the mean step height formation and mean Knoop microhardness change with standard deviations for the fluoride solutions, commercial mouthrinses and commercial mouthrinses in saliva experiments respectively.

#### 3.1. Step height data

For all groups, stannous fluoride produced statistically lower step heights than sodium fluoride ( $p < 0.0001$ ) independent of order of application or the type of solution being used.

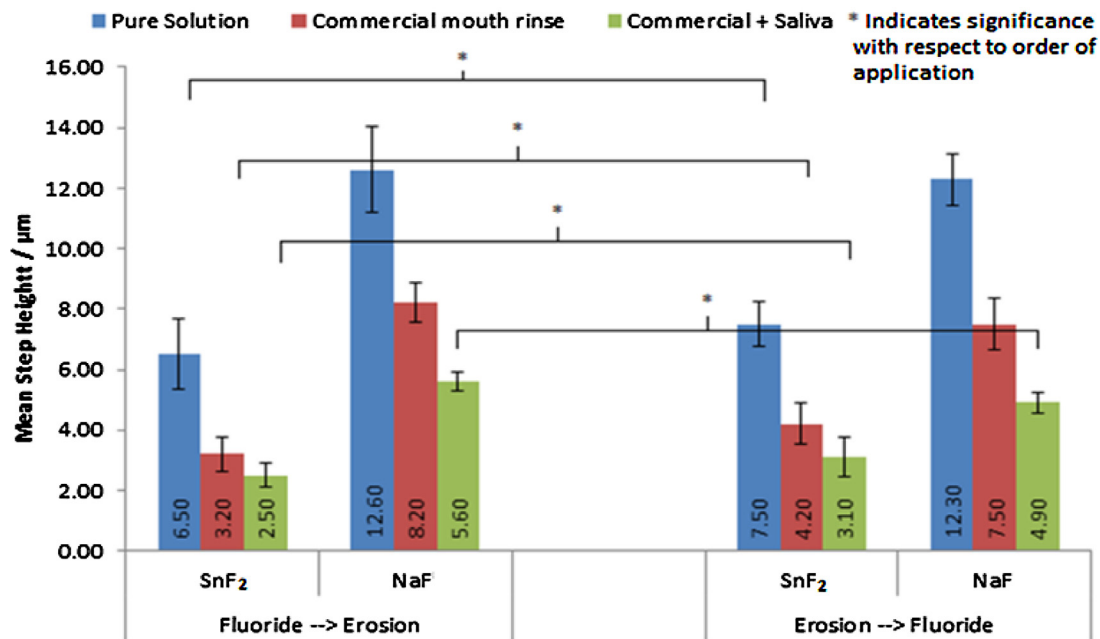


Fig. 2. Mean step height ( $\mu\text{m}$ ) with standard deviations.

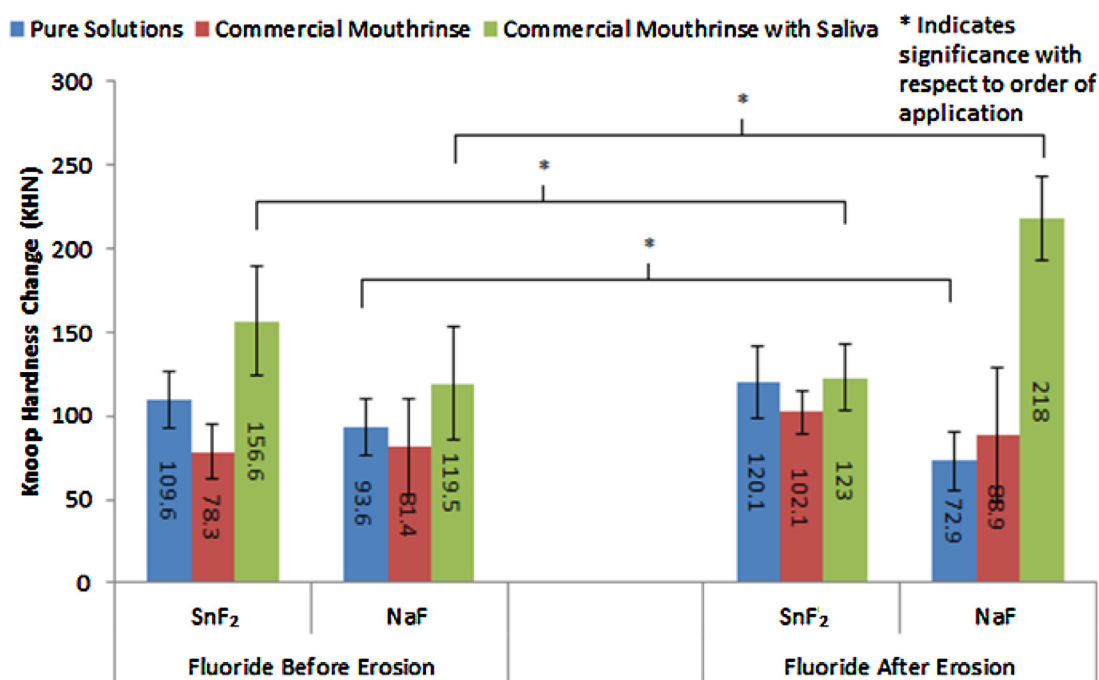


Fig. 3. Knoop microhardness change KHN with standard deviations.

Pure fluoride solutions produced the greatest mean step heights. Commercial mouthrinses statistically reduced mean step height formation ( $p < 0.0001$ ) and the addition of saliva to the commercial mouthrinses further statistically reduced step height formation ( $p < 0.0001$ ).

For all groups, stannous fluoride produced statistically lower step heights when applied before citric acid immersion compared to after (pure fluoride solutions ( $p = 0.01$ ), commercial mouthrinses with and without saliva ( $p = 0.002$  and  $p < 0.0001$  respectively)). In contrast, sodium fluoride produced lower step heights when applied after citric acid immersion although this was only significant for commercial mouthrinses in saliva ( $p = 0.023$ ).

### 3.2. Microhardness data

Within the pure solution group, the only statistical difference observed was a decreased microhardness change when sodium fluoride was applied before citric acid immersion compared to after ( $p = 0.006$ ). There were no statistical differences with respect to microhardness change in the commercial mouthrinse group. When saliva was added to the commercial mouthrinses an overall increase in microhardness change was observed ( $p < 0.0001$ ). Within this group, a statistically greater microhardness change was observed when stannous fluoride was applied before citric acid immersion ( $p = 0.02$ ) and when sodium fluoride was applied after citric acid immersion ( $p < 0.0001$ ).

Microhardness data were statistically different for all stannous fluoride solutions ( $p < 0.05$ ). For sodium fluoride solutions, microhardness changes were statistically different only when saliva was present ( $p < 0.0001$ ).

## 4. Discussion

The timing of application of fluoride had a significant effect on step height formation and microhardness for both stannous and sodium fluoride and therefore the first null hypothesis was rejected. The second null hypothesis was also rejected as significant

differences were found in the step height and microhardness change when the fluorides were applied as pure solutions and commercial mouthrinses with and without the presence of saliva.

Overall, stannous fluoride produced statistically lower mean step heights compared to sodium fluoride and this finding supports the work of other authors [21–25]. Stannous fluoride resulted in statistically reduced step height formation when applied before the erosive challenge for all groups. To the author's knowledge, this is the first study to investigate the timing of stannous fluoride application in relation to an erosive challenge. SnF<sub>2</sub> deposits have been reported to be more acid resistant than CaF<sub>2</sub> deposits [14] which may explain the improved surface protection when applied before an erosive challenge. The stannous ion also has the same valency as the calcium ion and, when incorporated into demineralised enamel, has been shown to be less acid-soluble [36]. Stannous fluoride formulations are acidic as stannous fluoride is not stable at neutral solutions [26]. This mildly acidic formulation may allow for incorporation of stannous and fluoride ions into the hydroxyapatite structure before an erosive challenge.

Sodium fluoride resulted in less step height formation when applied after an erosive challenge compared to application before, however this was only significant when it was applied in the presence of saliva ( $p = 0.023$ ). Sodium fluoride is well established in the literature as an effective remineralisation agent for caries [27] and has also been shown to be effective when remineralising erosive lesions both in vitro [9] and in situ [15]. The fluoride remineralisation process observed in caries is different to erosion as the lesions are diffuse and open to the oral environment. Any remineralisation that can occur is restricted to demineralised enamel layer [12]. This data would suggest that sodium fluoride has a role in the protection against dental erosion, particularly when applied after an erosive challenge. Saliva enhanced the action of sodium fluoride and this result supports data reported in other in situ studies [20,28]. Under these laboratory conditions, stannous fluoride still protected against erosion is the absence of saliva.



The primary measurement for the outcome of this experiment was non-contacting laser profilometry which is an internationally accepted reliable method of surface profile loss due to erosion [29–31]. We performed an erosive challenge on the enamel specimens in order to obtain a measurable and reliable step height and to simulate severe erosive conditions to test the fluorides. Consequently, at this level of erosion, microhardness readings are not as reliable. There will always be a conflict between accurate profilometry and microhardness. However, under the conditions of this model, surface softening was detected by the microhardness measurement and supports the concept that the erosive process involves surface softening with tissue loss [25,32]. The microhardness data in this experiment is indicative of surface change rather than a measurement for the amount of enamel lost [33,34].

The second unique finding in this study is that the introduction of saliva created, a statistically significant greater microhardness change which was associated with the statistically lowest step height for each fluoride compound. This means that although there was reduced profilometric loss the remaining structure was softer. This interesting finding that lower step height formation may leave behind a softer surface is worth investigating further. This softened structure could be an advantage implying the presence of an intact enamel matrix which may have improved potential for remineralisation. Conversely, it has been shown that softened enamel is more susceptible to degradation from mechanical wear processes [6,35] and this softened structure could result in further enamel loss. Our samples were rinsed after each treatment so observation of the long term effect of the combination of fluorides and saliva was outside the remit of this experiment. Further investigation and clinical studies are needed to answer this question.

The data suggests that stannous fluoride is more protective in the prevention of demineralisation. It also suggests that sodium fluoride is optimally applied when attempting to remineralise an eroded lesion. This might explain to some degree the conflicting data on the ideal timing for application of fluoride. Some authors have applied it before the erosive challenge and found little to no effect [12,26]. Whereas other authors have applied sodium fluoride after erosion and found a protective effect [15,38]. These data suggest, albeit in a laboratory investigation, that different fluoride compounds work in different ways and are ideally applied at different times in relation to the acid challenge.

## 5. Conclusions

The results of this study, bearing in mind the limitations of in vitro research, suggest that sodium and stannous fluoride react differently depending on when they are applied. In this in vitro experiment it was observed that stannous fluoride was optimally applied before the erosive challenge whereas sodium fluoride after. Clinically, a different approach may be needed as to how toothpastes are used by patients with erosion. The additional components in the commercial mouth rinses appear to increase the efficacy of the fluorides and this should be taken into consideration when interpreting the results of in vitro studies. The presence of the salivary pellicle added further protection and this was particularly relevant for sodium fluoride. This work does show differences between different fluoride compounds and application time and can provide the bases for in situ studies to further understand this complex problem.

## Conflicts of interest

There are no conflicts of interests.

## Acknowledgement

Research funded by King's College London, UK.

## References

- [1] D.W. Bartlett, A. Lussi, N.X. West, P. Bouchard, M. Sanz, D. Bourgeois, Prevalence of tooth wear on buccal and lingual surfaces and possible risk factors in young European adults, *J. Dent.* 41 (2013) 1007–1013.
- [2] A. Wiegand, S. Egert, T. Attin, Toothbrushing before or after an acidic challenge to minimize tooth wear? An in situ/ex vivo study, *Am. J. Dent.* 21 (2008) 13–16.
- [3] A. Lussi, B. Megert, D. Eggenberger, T. Jaeggi, Impact of different toothpastes on the prevention of erosion, *Caries Res.* 42 (2008) 62–67.
- [4] J.A. Hughes, N.X. West, M. Addy, The protective effect of fluoride treatments against enamel erosion in vitro, *J. Oral Rehabil.* 31 (2004) 357–363.
- [5] S. Ponduri, E. Macdonald, M. Addy, A study in vitro of the combined effects of soft drinks and tooth brushing with fluoride toothpaste on the wear of dentine, *Int. J. Dent. Hyg.* 3 (2005) 7–12.
- [6] A. Lussi, J. Lussi, T.S. Carvalho, B. Cvikl, Toothbrushing after an erosive attack: will waiting avoid tooth wear? *Eur. J. Oral Sci.* 122 (2014) 353–359.
- [7] C. Ganss, N. Schlueter, D. Friedrich, J. Klimek, Efficacy of waiting periods and topical fluoride treatment on toothbrush abrasion of eroded enamel in situ, *Caries Res.* 41 (2007) 146–151.
- [8] E. Rakhmatullina, B. Beyeler, A. Lussi, Inhibition of enamel erosion by stannous fluoride containing rinsing solutions, *Schweizer Monatsschrift fur Zahnmedizin* 123 (2013) 296–302.
- [9] C. Ganss, N. Schlueter, M. Hardt, P. Schattenberg, J. Klimek, Effect of fluoride compounds on enamel erosion in vitro: a comparison of amine, sodium and stannous fluoride, *Caries Res.* 42 (2008) 2–7.
- [10] A.C. Magalhães, A. Wiegand, D. Rios, M.A. Buzalaf, A. Lussi, Fluoride in dental erosion, *Monogr. Oral Sci.* (2011) 158–170.
- [11] A. Lussi, T. Saads, The future of fluorides and other protective agents in erosion prevention, *Caries Res.* 49 (2015) 18–29.
- [12] L. Hystad Hove, K. Refsholt Stenhagen, A. Mulic, B. Holme, A.B. Tveit, May caries-preventive fluoride regimes have an effect on dental erosive wear? An in situ study, *Acta Odontol. Scand.* (2014) 1–7.
- [13] N. Schlueter, L. Neutard, J. von Hinckeldey, J. Klimek, C. Ganss, Tin and fluoride as anti-erosive agents in enamel and dentine in vitro, *Acta Odontol. Scand.* 68 (2010) 180–184.
- [14] D. Khambe, S.L. Eversole, T. Mills, R.V. Faller, Protective effects of SnF<sub>2</sub>—Part II. Deposition and retention on pellicle-coated enamel, *Int. Dent. J.* 64 (2014) 11–15.
- [15] M.S. Mathews, B.T. Amaechi, K. Ramalingam, R.A. Ccahuana-Vasquez, I.P. Chedjieu, A.C. Mackey, et al., In situ remineralisation of eroded enamel lesions by NaF rinses, *Arch. Oral Biol.* 57 (2012) 525–530.
- [16] W.L. Siqueira, W. Custodio, E.E. McDonald, New insights into the composition and functions of the acquired enamel pellicle, *J. Dent. Res.* 91 (2012) 1110–1118.
- [17] W.L. Siqueira, H.C. Margolis, E.J. Helmerhorst, F.M. Mendes, F.G. Oppenheim, Evidence of intact histatins in the in vivo acquired enamel pellicle, *J. Dent. Res.* 89 (2010) 626–630.
- [18] B.T. Amaechi, S.M. Higham, In vitro remineralisation of eroded enamel lesions by saliva, *J. Dent.* 29 (2001) 371–376.
- [19] A.T. Hara, F. Lippert, D.T. Zero, Interplay between experimental dental pellicles and stannous-containing toothpaste on dental erosion-abrasion, *Caries Res.* 47 (2013) 325–329.
- [20] C. Ganss, N. Schlueter, J. Klimek, Retention of KOH-soluble fluoride on enamel and dentine under erosive conditions—A comparison of in vitro and in situ results, *Arch. Oral Biol.* 52 (2007) 9–14.
- [21] M.C. Huysmans, D.H. Jager, J.L. Ruben, D.E. Unk, C.P. Klijn, A.M. Vieira, Reduction of erosive wear in situ by stannous fluoride-containing toothpaste, *Caries Res.* 45 (2011) 518–523.
- [22] R.V. Faller, S.L. Eversole, K. Saunders-Burkhardt, Protective benefits of a stabilised stannous-containing fluoride dentifrice against erosive acid damage, *Int. Dent. J.* 64 (Suppl. 1) (2014) 29–34.
- [23] S. Hooper, J. Seong, E. Macdonald, N. Claydon, N. Hellin, M.L. Barker, 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.* 64 (Suppl 1) (2014) 35–42.
- [24] L.H. Hove, A. Mulic, A.B. Tveit, K.R. Stenhagen, A.B. Skaare, I. Espelid, Registration of dental erosive wear on study models and intra-oral photographs, *Eur. Arch. Paediatr. Dent.* 14 (2013) 29–34.
- [25] A. Venasakulchai, N.A. Williams, L.H. Gracia, G.D. Rees, A comparative evaluation of fluoridated and non-fluoridated mouthrinses using a 5-day cycling enamel erosion model, *J. Dent.* 38 (2010) 21–29.
- [26] A. Wiegand, D. Bichsel, A.C. Magalhães, K. Becker, T. Attin, Effect of sodium, amine and stannous fluoride at the same concentration and different pH on in vitro erosion, *J. Dent.* 37 (2009) 591–595.
- [27] V.C.C. Marinho, J.P.T. Higgins, A. Sheiham, S. Logan, Combinations of topical fluoride (toothpastes, mouthrinses, gels, varnishes) versus single topical fluoride for preventing dental caries in children and adolescents, *Cochrane Database Syst. Rev.* (2004) CD002781.
- [28] N. Schlueter, J. Klimek, C. Ganss, Efficacy of an experimental tin-F-containing solution in erosive tissue loss in enamel and dentine in situ, *Caries Res.* 43 (2009) 415–421.

- [29] A.-M. Paepegaey, M.L. Barker, D.W. Bartlett, M. Mistry, N.X. West, N. Hellin, et al., Measuring enamel erosion: a comparative study of contact profilometry, non-contact profilometry and confocal laser scanning microscopy, *Dent. Mater.* 29 (2013) 1265–1272.
- [30] M.E. Barbour, J.S. Rees, The laboratory assessment of enamel erosion: a review, *J. Dent.* 32 (2004) 591–602.
- [31] N. Schlueter, A.T. Hara, R.P. Shellis, C. Ganss, Methods for the measurement and characterization of erosion in enamel and dentine, *Caries Res.* 45 (Suppl. 1) (2011) 13–23.
- [32] Hara, T. a, D.T. Zero, Analysis of the erosive potential of calcium-containing acidic beverages, *Eur. J. Oral Sci.* 116 (2008) 60–65.
- [33] M. Hannig, M. Balz, Influence of in vivo formed salivary pellicle on enamel erosion, *Caries Res.* 33 (1999) 372–379.
- [34] Z. Cheaib, A. Lussi, Impact of acquired enamel pellicle modification on initial dental erosion, *Caries Res.* 45 (2011) 107–112.
- [35] A. Wiegand, J.P.M. Burkhard, F. Eggmann, T. Attin, Brushing force of manual and sonic toothbrushes affects dental hard tissue abrasion, *Clin. Oral Invest.* 17 (2013) 815–822.
- [36] C. Ganss, M. Hardt, A. Lussi, A.-K. Cocks, J. Klimek, N. Schlueter, Mechanism of action of tin-containing fluoride solutions as anti-erosive agents in dentine—an in vitro tin-uptake, tissue loss, and scanning electron microscopy study, *Eur. J. Oral Sci.* 118 (2010) 376–384.
- [38] L.P. Comar, M.F. Gomes, N. Ito, P.A. Salomão, L.T. Grizzo, A.C. Magalhães, Effect of NaF, SnF(2), and TiF(4) toothpastes on bovine enamel and dentin erosion-abrasion in vitro, *Int. J. Dent.* 2012 (2012) 134350.