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Abstract: The aim of this study was to measure loss of dentine produced by soft drinks alone and combined with tooth brushing with and without toothpastes. Groups of flat human dentine specimens were exposed for 10 min and then 30 min to orange juice (OJ), carbonated cola (CC) or modified blackcurrant (MB) drinks alone or after the exposures brushed with a fluoride toothpaste for 10 s. Further groups were exposed to OJ as before but brushed with water or non-fluoride toothpaste or placed in slurries of fluoride paste. Five cycles of each regimen were carried out. Tissue loss was determined by profilometry. Water immersion/brushing and brushing controls were included. OJ and CC produced similar erosion and significantly more than MB. Compared with drinks alone, dentine loss was reduced by fluoride toothpaste brushing but increased by water and non-fluoride toothpaste brushing. Fluoride toothpaste slurry had no significant effect on soft drink erosion. Very little abrasion with brushing alone was recorded over the time frame of these experiments. It is concluded that fluoride toothpaste could provide protection, albeit small, against erosion. The data again support the concept of brushing before meals.

Key words: tooth wear; dentine; erosion; abrasion; fluoride; toothpaste

Introduction

Reviews on tooth wear consistently reach similar conclusions (1–5) namely: enamel and, to a lesser extent, dentine are both hard tissues which are quite resistant to physiological levels of

attrition and abrasion respectively; both tissues have a much, reduced resistance to chemical–physical wear or acid corrosion: in dental terminology referred to as erosion; erosion of enamel and dentine reduces considerably their normal resistance to physical wear, particularly by abrasion, by a surface-softening process. Research on softening of dental hard tissues by acids has been conducted over several decades and has focused mainly on enamel (6–8) but with some data for dentine (9, 10). Using various measurement techniques and methodologies researchers report that enamel softening plateaus at around 5 μm presumably when acid penetration and surface dissolution of enamel reaches equilibrium (6–8). This softened zone appears visibly very brittle (11) and can be removed easily by a range of physical agents including ultrasonication and tooth brushing with and without toothpaste (7, 8, 12). Indeed, there is evidence that the softened zone can be removed by the action of the tongue (13). Remineralization of softened enamel is possible but studies in vitro suggest this may take several hours (14). Dentine appears more susceptible to erosion than enamel at least from studies *in situ* (15). Softening also occurs in dentine but from studies *in vitro* the depth is less than with enamel (10). Whether rehardening of softened dentine occurs and within clinically relevant time periods, is unclear and has been debated (16). A study *in vitro* was unable to remineralize dentine softened by acid after 24 h in artificial saliva (10), although a study *in situ* suggested some rehardening of dentine occurred (9).

Taking into account the aforementioned observations, it is not difficult to imagine a bad case scenario for the progress of wear, in dental hard tissues, initiated by dietary erosion. The intake of soft drinks in many countries including the UK is high and frequent during any one day (17, 18). The resultant chronic, irreversible loss of tissue and surface softening without sufficient intervening time for rehardening will mean loss of the softened zone by the various physical impacts teeth incur during the waking hours (4, 5). The additive, even synergistic interplay between erosion and abrasion by tooth brushes, alone or with toothpaste, is well established from studies *in vitro* (7, 12, 19) and, more recently, *in situ* (20). Most interest has centred on enamel with less work carried out on dentine. Also protocols *in vitro* often have not been pragmatic in design. The aim of the present study was to compare the wear of dentine exposed to two conventional acidic drinks and one modified drink with and without brushing with standard fluoride toothpaste. The model attempted to simulate twice daily tooth brushing and four times daily intake of each soft drink. Appropriate controls to determine the effects of brushing and fluoride toothpaste were applied to one of the conventional acidic drink/brushing regimens.

Method and materials

Flat human dentine specimens were prepared from human third molar teeth extracted from individuals aged 18–35 years. After removing any soft tissue remnants the teeth were sterilized in 20 000 ppm hypochlorite for 24 h and stored in isotonic saline at 4°C until required. The method of preparation has been described in detail in several prior publications (10, 15, 21). Essentially pieces of dentine were embedded in resin and polished to 1200 grit to have an acceptance profile of ±0.3 μm measured on a contacting profilometer. The area of exposed dentine in the resin was then taped to leave a 2-mm wide window of dentine. Groups of six specimens were allocated to each regimen. The soft drinks were:

1 orange juice (OJ) (J. Sainsbury PLC, London, UK), pH 3.8;

2 carbonated cola (CC) (Coca-Cola Enterprises Ltd, Uxbridge, UK), pH 2.8;

3 modified blackcurrent (MB) (Ribena Toothkind; GlaxoSmithKline, Weybridge, UK), pH 3.8.

Tap water was used as the placebo control, pH 7.0.

The pHs of the study liquids were determined using a pH meter and electrode.

A total of 10 treatment regimens were performed as follows:

Regimens 1–3: Groups of specimens were placed into 300 ml volumes of the three drinks or water at 35°C for 10 min then removed, rinsed with water and returned to the respective liquid for 30 min. Taping was removed and tissue loss recorded with the profilometer across two zones from one tape boundary to the other. Specimens were then re-taped and the above cycles repeated four more times.

Regimens 4–6: Groups of specimens were placed into the same liquids as above together with a fourth group placed in water. After the 10- and 30-min immersions specimens were brushed for 10 s with a standard brush (Oral B 35, Oral B, London, UK) and 3 gm in 10 ml water slurry fluoride toothpaste (Colgate Regular, Colgate Palmolive, London, UK), in a reciprocal action brushing machine, with a 200-g head load at a speed of 50 cycles per minute. This constituted one cycle, which was repeated four more times with profilometric measurements taken after each cycle.

Regimen 7: Specimens were immersed in OJ as for regimens 4–6 but post-immersion brushings were with non-fluoride toothpaste (The Boots Company PLC, Nottingham, UK). Profilometry was as before. A total of five cycles were performed.

Regimen 8: Specimens were immersed in OJ as for regimens 4–6 but post-immersion brushings were with water. Profilometry was as before. A total of five cycles were performed.

Regimen 9: Specimens were immersed in OJ as for regimens 1–3 with post-immersion exposures to the slurry of fluoride toothpaste for 10 s. Profilometry was as before. A total of five cycles were performed.

Regimen 10: Specimens were immersed in water for the same time periods as OJ in regimens 4–6 and then brushed with the slurry of fluoride toothpaste for 10 s. Profilometry was as before. A total of five cycles were performed.

Regimen 11: Specimens were brushed with the fluoride toothpaste slurry for 100 s. Profilometry was only at the 100 s time point.

In all experiments the immersion liquids were stirred at constant speed (270 rpm) with an overhead propeller stirrer (Lab-Egg; Ika-Werke GmbH & Co., Stanfan, Germany). A placebo control of immersion in water was not employed as previous data drawn from *in vitro* and *in situ* studies revealed no detectable changes in specimens.

Statistical methods

Averages of the two measurements across each specimen at each cycle were calculated and used to calculate the means and standard deviations for each treatment group at each cycle. The mean dentine loss per treatment regimen at cycle 5 was used as the outcome measure for statistical analysis. In view of the large number of possible paired comparisons, those of most interest were chosen *a priori* but, based on the assumptions that water immersion with toothpaste brushing and toothpaste brushing would have very limited effects and data would be presented but not used in analyses.

Analysis of variance was used to determine the significance of differences between immersion only in the three drinks, between immersion in the three drinks followed by brushing and between the four orange juice regimens. If significant unpaired t-tests were used to determine the significance of differences between OJ and MB with and without brushing with toothpaste and between the remaining 6 possible pairs of regimens of orange juice. Additionally each drink was compared with and without brushing.

Results

Unless otherwise stated reference to toothpaste applies to the fluoride product. The mean and standard deviation of dentine loss in microns for regimens 1–10 involving immersion in a liquid with and without brushing at each cycle are shown in Table 1 together with the data at 100 s for brushing with toothpaste only (regimen 11). For all regimens 1–11, with the exception of water immersion and toothpaste brushing, dentine loss increased with each treatment cycle. With the exception of MB immersion without brushing, the incremental loss after the first cycle was always the greatest. Increments after the first cycle varied in magnitude for each regimen and at best the data approximated to a linear pattern. As predicted, water soaking followed by brushing with toothpaste and brushing with toothpaste alone had negligible effects although individual measurements revealed that extremely small amounts of abrasion had occurred to specimens.

Immersion in MB resulted in less erosion than was the case with OJ or CC. OJ and CC were similar in both with and without brushing regimens. Individual analyses across with brushing and without brushing protocols revealed significant differences ($P \ll 0.001$). The planned paired comparison of MB with OJ revealed significantly less erosion with MB both with and without brushing ($P \ll 0.001$). For the respective drinks erosion alone was significantly greater than erosion with toothpaste brushing (OJ, $P < 0.05$, CC, $P < 0.001$, MB, $P < 0.001$). Analysis of variance for the OJ

Table 1. **The mean (standard deviation) loss of dentine in microns by soft drink erosion without and with brushing over five cycles**

	Cycle				
Regimen	1	2	3	4	5
1. OJ	8.98 (0.98)	12.88 (1.09)	17.68 (1.26)	20.22 (2.50)	24.40 (2.82)
2. CC	8.83 (0.80	14.51 (1.69)	18.86 (1.24)	21.44 (1.99)	25.01 (1.15)
3. MB	2.85 (1.10)	6.60 (0.85)	9.63 (0.84)	11.99 (0.49)	16.63 (1.06)
4. OJ FTP.B	7.58 (0.44)	11.96 (0.30)	16.74 (1.12)	19.40 (0.82)	21.45 (2.33)
5. CC FTP.B	7.00 (0.68)	10.92 (0.59)	16.81 (1.26)	19.74 (1.12)	20.63 (1.61)
6. MB FTP.B	4.21 (0.76)	6.03 (0.20)	8.58 (0.80)	10.53 (1.06)	12.33 (1.14)
7. OJ NFTP.B	9.38 (1.49)	14.45 (2.10)	17.83 (2.08)	21.58 (3.31)	26.70 (3.24)
8. OJ W.B	9.76 (0.76)	14.70 (1.59)	19.88 (2.11)	23.42 (1.67)	27.72 (1.52)
9. OJ FTP.S	8.04 (1.42)	14.39 (2.07)	17.73 (2.59)	20.32 (2.77)	24.25 (2.68)
10. W FTP.B	0.26 (0.12)	0.17 (0.09)	0.28 (0.11)	0.24 (0.12)	0.33 (0.13)
11. FTP.B					0.09 (0.21)

FTP, fluoride tooth paste; NFTP, non-fluoride tooth paste; B, brush; W, water.

regimens was significant ($P < 0.01$). OJ brushed with water produced the most tissue loss and significantly more than with OJ alone ($P < 0.05$), OJ with toothpaste brushing ($P < 0.01$) and OJ with toothpaste slurry ($P < 0.05$). OJ alone was not significantly different from OJ with the toothpaste slurry but OJ with toothpaste brushing was significantly less erosive than OJ with toothpaste slurry ($P < 0.05$). OJ with brushing with the non-fluoride toothpaste was similar to brushing with water after OJ and not significantly different ($P > 0.05$). Brushing with the non-fluoride paste after OJ was greater than OJ alone but the difference did not reach significance. Brushing with the non-fluoride paste after OJ produced significantly more tissue loss than OJ followed by brushing with the fluoride paste ($P < 0.01$).

Discussion

The design of this study attempted to simulate *in vitro* commonly practiced oral hygiene habits alongside the intake of soft drinks and determine their effects on dentine. The protocol would more relate to adults than adolescents and in whom there has been exposed cervical dentine. Thus, tooth brushing with toothpaste is recommended as twice daily, which advice is often followed and often is the case (22–24). Brushing times have more recently been recommended to be 2 min, although the majority of studies suggest 1 min is more the actual norm (3). Based on these two recommendations and observations from studies on tooth brushing (25, 26), it was estimated that any one, tooth surface would be unlikely to receive more than 10 s brushing in any one cycle. Data also suggest that 1 l of soft drink consumption in any one day is common, particularly in the young (17, 18). It would not seem unreasonable to suggest that such a volume may be imbibed as four separate drinks taking 10 min to consume. Indeed, this has been the protocol for a number of studies *in situ* on soft drink erosion (27–30). The model therefore simulated a soft drink at breakfast (10 min immersion) followed by tooth brushing, then three further drinks during the day (30 min immersion) followed by the evening tooth brushing. The cycles were repeated to represent effects on dentine of 5 days of such a regimen. The appropriate controls for individual variables became obvious and 10 regimens were considered necessary. An intervening remineralizing period using artificial saliva was not used as a study *in vitro* from this group revealed no rehardening of dentine even after 24 h immersion (10). As with most studies *in vitro*, the model is a worst-case scenario, particularly for erosion and abrasion and compared with studies *in situ* the present model has exaggerated the effect (29). However, this is irrelevant because, using highly standardized conditions, it was the differences between the regimens, which were of interest.

As expected in the brushing with toothpaste alone or after water immersion very little dentine was removed. These regimens were only included as controls for the model. Toothpaste abrasivity has been studied over decades and by a variety of methods. Indeed there are International Standards Organisation (ISO) and British Standards Institute (BSI) standards for this (4). Unlike ISO methods for toothpaste abrasion, the time frame of the present study was too short to draw meaningful clinical extrapolations of the data. Reviews on the subject however conclude that toothpastes conforming to ISO or BSI standards in normal use should not cause clinically significant dentine wear in a lifetime (4, 5). As seen here erosion appears by far the greater threat to dentine than abrasion. Thus all three drinks produced enormous tissue loss relative to that caused by toothpaste abrasion alone. The drink modified by the addition of calcium, MB, produced approximately one third less erosion than the other two drinks. Most research with MB has been concerned with studies *in situ* and *in vitro* on enamel (28, 29). Proportionately, there was very much reduced erosion of enamel by MB compared with conventional soft drinks, indeed some data showed little difference from water controls (30). Presumably this may reflect the calculated much lower solubility product of dentine compared with enamel (31) apparently raising the critical dissolution pH from 5.5 for enamel to 6.7 for dentine. Certainly, studies *in vitro* and *in situ* reveal that dentine is much more soluble than enamel in acidic solutions (15, 16).

Experiments which have investigated the loss of enamel when combining erosion with some form of physical impact, such as tooth brushing with or without toothpaste, or ultrasonication, report at least additive if not synergistic effects (7, 8, 12, 19). Less data are available for dentine, although additive and synergistic effects are reported from studies *in vitro* and *in situ* (10, 19, 20). The mechanism for increased enamel and dentine loss is thought to be removal of the erosion softened tissue zone by abrasion. One might expect the combined actions of erosion and abrasion to be greater on enamel because the softening depth appears larger and more brittle than for dentine (8, 10, 12). In studies on this subject, including the present investigation, there is the potential for a confounding factor in the model, namely the presence of fluoride. Although not great, it does appear that fluoride can afford protection to enamel and dentine against erosion (32–35). The data reported here support this concept with less erosion seen when specimens were brushed with toothpaste after the immersion in the drinks than after immersion alone. Further

support was provided from the OJ erosion followed by water or non-fluoride toothpaste brushing regimens where dentine loss was greater than OJ alone, which in turn was greater than OJ with fluoride toothpaste brushing. Presumably, the fluoride was taken up onto the dentine as calcium fluoride or fluoroapatite so reducing the acid solubility (36). An alternative explanation could be that other ingredients in the paste, notably abrasives adhered to the dentine surface and or plugged the tubules, thereby producing a protective layer. Certainly artificial silica in non-ionic detergent based toothpastes do, according to studies *in vitro* and *in situ*, firmly adhere to dentine and occlude tubules (37, 38). In the present study this explanation is unlikely as the toothpaste did not have a silica abrasive non-ionic detergent formula and was shown in a study *in situ* to produce little visible topographical effects on dentine (38). Also, again, the non-fluoride paste produced similar results to water brushings after OJ immersion. There does appear however, to be one anomaly in the data set namely that OJ alone and OJ with exposure to the toothpaste slurry produced similar dentine loss. One might have expected to observe at least similar protection from the topically applied slurry as the brushed slurry. A possible explanation could be the accumulation of collagen matrix on the dentine surface as erosion progressed. This layer could then reduce or prevent fluoride uptake onto the intact dentine below. Brushing with toothpaste would remove the acid insoluble collagen matrix to bring the fluoride in contact with the dentine surface. Such an explanation was propounded for the apparent difference in the rate of dentine erosion *in vitro* compared with *in situ*: the collagen matrix would not be expected to accumulate *in situ* because of the action of the tongue (15, 21).

To conclude, with the caution that must be afforded to extrapolating all data generated *in vitro* to clinical meaning, this study indicates as to how susceptible dentine is to erosion by soft drinks. Tooth brushing with toothpaste rather than accelerating dentine loss appeared to afford protection, albeit relatively small, against erosion. This protection appeared to come from the contained fluoride in the paste. Brushing before meals for the preventive effects of fluoride would seem not only logical but also, based on this and other studies, biological.

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