

## Studies on dental erosion: An *in vivo-in vitro* model of endogenous dental erosion – its application to testing protection by fluoride gel application

L Jones,\* D Lekkas,\* D Hunt,\* J McIntyre,\* W Rafir\*

### Abstract

**Background:** The objective in this study was to develop an *in vivo-in vitro* model of endogenous erosion, with a view to exploring the potential for some degree of its control by the use of topical fluoride gel application to teeth.

**Methods:** Six volunteers each wore a small clasp retained palatal acrylic appliance to which six sterilized enamel tiles were bonded. Three tiles on each appliance were coated extra-orally with either 1.23 per cent acidulated phosphate fluoride (APF) or 2.2 per cent sodium fluoride (NaF) neutral gel for four minutes prior to multiple periods of exposure to the simulated gastric acid, cumulating in 16, 36, 80 and 150 minutes of exposure. Impressions of the enamel tiles prior to and following acid exposure permitted dies to be prepared. These were sectioned through the exposed areas and examined under a stereomicroscope to assess maximum depths of erosion.

**Results:** The depth of erosive demineralization of enamel was found to be greatly reduced with increased frequency of APF gel application. The reduction in enamel loss was less following topical application of NaF gel.

**Conclusions:** It was concluded that fluoride gels significantly reduced enamel erosion using this *in vivo-in vitro* model and therefore, if prescribed appropriately, should help reduce tooth tissue loss from endogenous erosion.

**Key words:** Gastric acid, demineralization, tooth erosion, fluoride.

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This may occur with conditions such as gastro-oesophageal reflux,<sup>1</sup> duodenal ulcers, anorexia nervosa or bulimia syndrome,<sup>2</sup> or as a result of prolonged severe nausea during pregnancy.

A review of the dental literature suggests that more research is needed to find measures which effectively inhibit the damage to teeth from erosion.<sup>3,4</sup> Such measures include the use of antacids,<sup>5</sup> fluorides<sup>6</sup> and oral hygiene practices. Even though fluoride has been successfully used to inhibit dental caries, its availability through dietary fluoride supplementation programmes or through regular use of fluoridated dentifrices does not appear to result in teeth being more resistant to erosion. Teo *et al.*<sup>7</sup> found, in a comprehensive study, that systemic fluoride use during childhood provided little protection against erosion lesions, even though it inhibited caries development.

However, the more concentrated topical fluorides may provide some protection against erosion. Fung<sup>8</sup> used an *in vitro* erosion model to simulate endogenous erosion of human enamel. She demonstrated that erosion could be significantly reduced by a repeating cycle of a four minute application of 1.23 per cent acidulated phosphate fluoride (APF) and sodium fluoride (NaF) gel prior to every two, four or six, two minute exposures to 0.06mol/L HCl. The HCl solution contained 2.2mmol/L CaHPO<sub>4</sub> to simulate concentrations of calcium and phosphate normally present in saliva. Even though this result provided promise that these fluoride gels may provide a degree of protection clinically, results from *in vitro* simulations and animal studies need to be confirmed by *in vivo* investigations as they either exclude the protective effects of saliva, dental pellicle and dental plaque or involve a non-human oral environment. Saliva in particular has been shown to play a critical role in protecting teeth against carious demineralization.<sup>9</sup> Bevenius and L'Estrange<sup>10</sup> proposed that salivary parameters should also be assessed in determining susceptibility to dental erosion. Khan *et al.*<sup>11</sup> found that salivary factors influenced the degree of erosion in specific locations within the mouth.

### INTRODUCTION

Dental erosion is becoming an increasing problem in dentistry and can be attributed to either exogenous or endogenous factors. Endogenous dental erosion is associated with frequent exposure of teeth to gastric juice, containing hydrochloric acid (HCl) at pH 1-1.5.

\*Dental School, The University of Adelaide, South Australia.

In developing a model of endogenous dental erosion which most closely simulates the conditions encountered in the human mouth, it is necessary to have these intra-oral protective factors present and actively interacting with the experimental process as closely as possible, yet without the risks of the erosive agents damaging the subjects' teeth. This can most realistically be achieved by the use of a test system in which the enamel surfaces undergo a period of time intra-orally in order to permit formation of a layer of pellicle glycoprotein, and possibly some plaque, prior to acidic challenge. Such *in situ* models have been widely used in caries research with beneficial results.<sup>12</sup> The essential requirement is that no ill effects result to the subject from the test agent. Thus substances potentially damaging to the teeth are applied to the teeth during the *in vitro* component of the test, and their effects are monitored at this stage. These substances are then thoroughly removed before the test enamel again enters the mouth to resume the *in vivo* phase of the study.

Lekkas *et al.*<sup>13</sup> developed such an *in vivo-in vitro* model of dental erosion, and found that the resultant enamel erosion lesions had many characteristics which were similar to those observed clinically. However, they found that the rate of erosion varied significantly between enamel tiles taken from different teeth, presumably because of differing degrees of enamel maturation present. They also found limitations in adequately quantitating the extent of erosive damage.

The objective of this study was to further develop this *in vivo-in vitro* model to simulate the effect of endogenous erosion on tooth enamel, and to use this model to investigate to what extent fluoride gels can inhibit erosive demineralization.

## MATERIALS AND METHODS

### Subjects

Ethical approval for the study was provided by the Committee for the Ethics of Human Experimentation, University of Adelaide. Six staff and students of the Adelaide Dental Hospital/University of Adelaide volunteered as subjects. Informed consent was gained prior to the study. Each subject was required to be caries free, have a normal salivary flow rate and show no clinical signs of erosion before acceptance as a subject. A small clasp retained palatal acrylic appliance was constructed for each subject, and they were required to wear it during working hours each day for a period of three weeks.

### Enamel tile preparation

Ten intact human molar crowns, which had been stored at 4°C in deionized distilled water (DDW) with 0.1 per cent thymol added, were used for enamel tile preparation. Each crown had been examined microscopically to ensure minimal surface defects. Six square tiles of enamel with a minimum size of 3mm square were cut from each molar and stored under

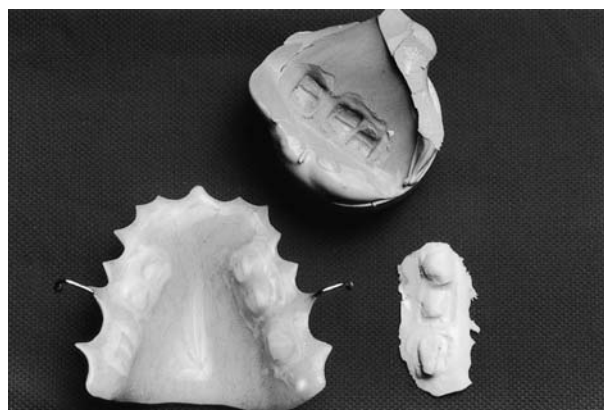


Fig 1. Appliance with six tiles attached, an impression, and a die prepared from the impression.

moist conditions in separate containers. All tiles to be used in the study were sterilized using ethylene oxide at the Central Sterilization Department of the Royal Adelaide Hospital. The packets of tiles were thoroughly evacuated of sterilizing agent and exposed to air in an aseptic environment for a week before use.

Six enamel tiles from the same molar tooth were then embedded into the palatal surface of each appliance, three on either side, using composite resin as the bonding agent (Fig 1). The embedded enamel tiles were checked with the stereomicroscope to ensure surface contamination with resin had not occurred. Each enamel tile had a 1mm wide strip of unfilled resin placed across the middle of the exposed area to protect a central band of enamel thus providing a reference zone of uneroded enamel for measurement and comparison with adjacent eroded areas.

### Simulation of gastric secretions

In previous *in vitro* and *in vivo-in vitro* studies,<sup>8,13</sup> a 0.06mol/L HCl solution with 2.2mmol/L CaHPO<sub>4</sub> produced tooth enamel erosion within a two week period which closely resembled the clinical appearance of gastric acid erosion. As well, the pH (1.4) was similar to that of gastric secretions. Five litres of this concentration of HCl together with CaHPO<sub>4</sub>, was prepared and used to simulate the effect of gastric juice on enamel.

### Experimental method

#### Overall organization

Subjects were given a non-fluoridated toothpaste (Blackmores Herbal and Mineral Toothpaste, Blackmores Laboratories Ltd, Balgowlah, NSW, Australia) to use with their normal toothbrushing routine and asked to refrain from drinking tea for two days prior to and daily during the study. Two named plastic denture containers were reserved for each subject. One was used to store the appliances in DDW when not being worn, and the other formed a receptacle for holding the appliance during the periods of acid exposure. The acid test solution was placed in

shallow plastic containers and the appliance positioned to ensure that the enamel tile surfaces were submerged. Normal clinical protocol for the protection of volunteers from cross-infection was observed, i.e., wearing gloves, use of named clean containers, use of fresh acid test solutions, and washing with DDW, etc.

The experiment was conducted over a period of approximately three weeks. Initially subjects wore appliances for two days to develop a layer of pellicle over the surface of the enamel slabs. Subsequently plates were worn for a minimum of 60 minutes at the beginning of each day in order to re-establish the pellicle layer. Erosive demineralization was performed three times a day, during a period of one hour from 10am, 1 and 3pm. The subjects organized their tea and lunch periods and thus removed the appliances around these times. Following exposure to acid, the whole appliance was thoroughly rinsed before being replaced in the mouth until the next test period. Every evening plates were removed, and all surfaces except the enamel tiles were cleaned with a non-fluoride tooth paste using a new tooth brush, following which they were stored in individual containers at 4°C. A daily log sheet was kept for each subject including starting times, times removed and re-inserted and finishing times, to ensure each plate received a similar period of exposure to acid, and relatively similar period of time intra-orally.

#### **Summary of the protocol for topical fluoride placement, erosive acid challenge and assessment of enamel loss**

Day 1 and 2: subjects to wear the appliances for five hours each day.

Day 3: subjects to commence wearing the appliance at 9am.

Subjects to attend between 10 and 11am to leave their appliances for 45 minutes. The appliances to be treated as follows: subjects 1-3: APF 1.23 per cent gel applied to the test enamel tiles; subjects 4-6 to have NaF 2.2 per cent gel applied:

- (i) subjects 1 and 4: gel applied before every two minute exposure to acid
- (ii) subjects 2 and 5: gel applied before every two, two minute exposures to acid
- (iii) subjects 3 and 6: gel applied before every four, two minute exposures to acid.

On category (i) subjects' appliances, the gel was washed from the enamel after four minutes with a strong jet of DDW, the slates dried and then all six slates were submerged in erosive acid for two minutes. The tiles were washed thoroughly with DDW and then dried. This procedure was repeated a further three times, taking approximately 40 minutes before the plate was washed and delivered back to the subject to wear. At 1pm, the second test run was carried out. The third was carried out commencing at 3pm. For subjects in category (ii), two repeats of the two, two minute acid exposure with prior gel application before each were

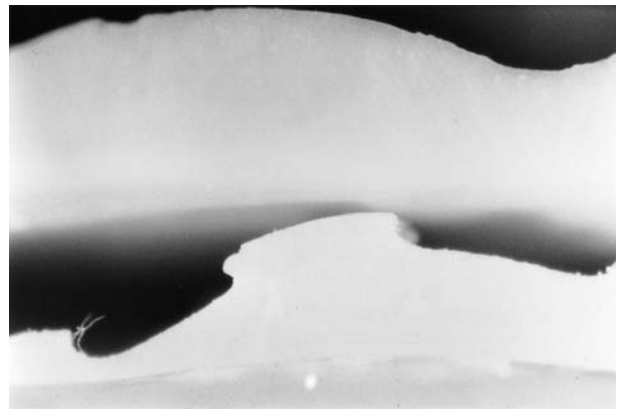


Fig 2. Two sections through dies, demonstrating the depth of erosion in an unprotected enamel tile (lower sample), and in one protected with APF gel (upper sample) prior to every two minutes of acid exposure, after 150 minutes of accumulated exposure.

carried out. Only one cycle of gel application and erosive challenge were required for samples from category (iii) subjects. The purpose of these repetitions of the demineralization procedure at each session was to ensure all subjects slates experienced similar cumulative periods of acid exposure as the experiment proceeded.

A strict plan of action had been previously prepared, with numerous helpers available to deliver plates, work timers, wash, dry and keep the experimental area clean. The experiment was terminated after each subject's plate had been subjected to 75 two minute acid exposures, i.e., 150 cumulative minutes total.

#### **Monitoring erosion lesion progression**

In order to obtain progressive quantitative data on erosive loss of enamel, it was decided to take impressions of the enamel tiles using polyvinyl siloxane impression materials (Extrude Light Body Impression Material, Kerr Manufacturing Coy, Romulus, Mississippi, USA) before the experiment started, and after 16, 36, 80 and 150 minutes of acid exposure. Impressions were then poured with Epoxy Die Material (Epoxy Die Material, Ivoclar-AG, Schaan, Liechtenstein) and marked clearly. At the end of the experiment, these dies were hemi-sectioned through the centre of the lesion, perpendicularly to the line of protected enamel, and examined under a stereomicroscope. The maximum depth of erosion of both the protected and unprotected tiles was determined with a graduated  $\mu\text{m}$  scale. This had previously been calibrated against a standard scale on the same microscope. The monitoring was carried out at the end of each test day. Figure 1, as well as showing an appliance with enamel tiles in place, shows an impression and the die which was prepared from it.

#### **RESULTS**

Figure 2 shows samples of two sections of dies superimposed, the lower one from the unprotected tiles, the upper one from the APF protected tiles, all from the same subject.

**Table 1. Mean depth of erosive demineralization of enamel between tiles ( $\mu$ ) with and without a prior application of acidulated phosphate fluoride (APF) gel, after varying times of exposure to the erosive acid solution (SD)**

Time (min)	APF before two minute acid	APF before four minute acid	APF before eight minute acid	No gel
0	0	0	0	0
16	0	0	31 (4)	33 (9)
36	12 (3)	50 (7)	62 (7)	101 (13)
80	13 (7)	101 (8)	119 (9)	166 (19)
150	31 (5)	125 (10)	214 (13)	265 (17)

**Table 2. Mean depth of erosive demineralization of enamel between tiles ( $\mu$ ) with and without a prior application of sodium fluoride (NaF) gel, after varying times of exposure to the erosive acid solution (SD)**

Time (min)	NaF before two minute acid	NaF before four minute acid	NaF before eight minute acid	No gel
0	0	0	0	0
16	0	21 (2)	26 (3)	33 (4)
36	63 (7)	75 (4)	87 (7)	101 (9)
80	83 (9)	125 (7)	145 (9)	166 (14)
150	156 (4)	193 (11)	219 (13)	265 (16)

The mean depths of enamel erosion from the three unprotected and the three protected enamel tiles used in each experiment are presented in Table 1 and 2, and Fig 3 and 4. Using this experimental design, the depth of demineralization of enamel was found to be greatly reduced with increased frequency of topical APF application. When APF was applied for four minutes before each two minutes of acid exposure, depth of surface enamel loss after 150 minutes of acid exposure was reduced by approximately 88 per cent compared with that in the control tiles. Depth of loss of surface was reduced to nearly half that of the control when APF was applied prior to every four accumulated minutes of acid exposure. The reduction was much less when applied prior to an accumulation of eight minutes of erosive demineralization.

Topical application of 2 per cent NaF gel was also found to decrease the depth of demineralization of enamel, though to a lesser extent than that observed when 1.23 per cent APF gel was used. When NaF was applied for four minutes prior to each two minutes of acid exposure, approximately 40 per cent reduction of depth of surface loss was seen after 150 minutes in comparison with the control.

## DISCUSSION

The objective of this project was to use an *in vivo-in vitro* model system to investigate the effectiveness of topical fluoride application in controlling simulated endogenous erosion. The results indicate that one method of controlling endogenous dental erosion may be by applying 1.23 per cent APF gel for four minutes prior to any anticipated episode of gastric acid contact.

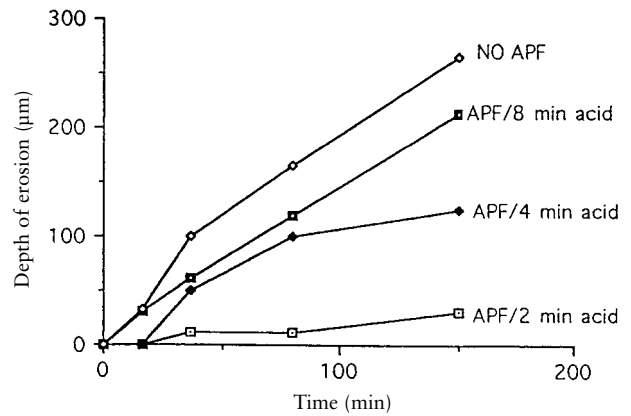


Fig 3. Mean depth of erosion between tiles ( $\mu$ ) with time from enamel samples coated with APF gel prior to every two, four and eight minutes of acid exposure.

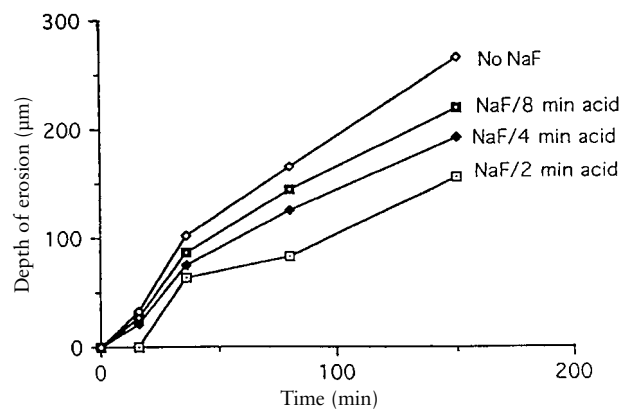


Fig 4. Mean depth of erosion between tiles ( $\mu$ ) with time from enamel samples coated with NaF gel prior to every two, four and eight minutes of acid exposure.

If glass ionomer cements or ceramic restorations are present, neutral gel should be recommended for such frequent use, as acidulated gels etch such restorations if used often.<sup>14</sup>

Patients, referred to a tooth wear clinic at the Adelaide Dental Hospital, who suffer from many forms of endogenous erosion, generally report that they have no warning as to precisely when an erosive attack is likely to occur. These episodes are usually more frequent over a period of a few weeks at a time. In such cases, it might still be useful for patients to self apply the appropriate gel to teeth every evening before bed, and/or every morning on rising during the weeks when bouts of erosion occur. However, with some forms of gastric reflux, regurgitation or frequent emesis associated with prescribed drugs, chronic alcoholism, eating disorders or from nausea during pregnancy, the pattern of acid reflux, regurgitation or emesis is more predictable, and protective gels may be able to be placed at a time just before the acid attack. Yet in many such cases this is practicably not possible, and again regular nightly application of gel to teeth during periods of frequent erosive attacks may be the most realistic approach to providing some protection against severe demineralization.



These patients also report that the episodes of acid reflux, regurgitation or emesis last only a short time, though the sour taste can linger for some time unless the mouth is well rinsed. The choice in this investigation of increments of two minute acid exposure was intended to simulate the acidic conditions to which teeth are exposed in such erosion episodes.

Some clinicians have questioned the wisdom of using acidulated gels to protect against acidic erosion. Acidulated fluorides have been formulated to ensure that the concentration of fluoride ion present is sufficient to protect the tooth against any resultant mineral loss.<sup>15</sup> The acidulated forms of fluoride are used to enhance the uptake of fluoride into the tooth surface, and the lowered pH associated with the gel is present for only a short time before being neutralized by salivary buffering. Fluoride gels should be most effective if used some time prior to a gastric acid attack, and are most often applied late in the evening. Thus the acidity temporarily generated by their placement in the mouth usually occurs at times far removed from when the endogenous erosion attacks occur. Hence there should be no overlap of acidity from both factors.

It is unlikely that fluoride gels will be able to control prolonged episodes of endogenous erosion totally. Most forms of erosion subject teeth to pH levels well below 4.5 for varying intervals, during which time even fluorapatite and fluoride enriched apatite will dissolve. Consequently, the potential for topical fluoride to inhibit endogenous erosion will be less than for the control of dental caries, where the pH is unlikely to be so low. In the more severe cases of endogenous erosion, medical help should be sought, and sealants and protective restorations may be necessary to protect the tooth surface.

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*Address for correspondence/reprints:*

Dr John McIntyre  
Dental School  
The University of Adelaide  
Adelaide, South Australia 5005  
Email: john.mcintyre@adelaide.edu.au