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REVIEW ARTICLE

# The laboratory assessment of enamel erosion: a review

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## KEYWORDS

Tooth surface loss;  
Erosion; Erosive  
potential; Tooth wear

**Summary Objectives.** To review the various methods and techniques available to assess enamel erosion in vitro.

**Data.** Peer reviewed scientific articles.

**Sources.** Medline and Web of Science searches and manual searching.

**Study selection.** Laboratory based assessments only included.

**Conclusions.** A number of macroscopic and microscopic techniques have been used to assess enamel erosion in vitro and in situ. This review examines techniques which are either well established or comparatively novel techniques that are being explored for their potential.

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## Introduction

Dental erosion may be defined as an irreversible loss of dental hard tissues due to a chemical process without the involvement of microorganisms.<sup>1,2</sup> This process may be caused by either extrinsic or intrinsic agents. Extrinsic agents include acidic substances, beverages, foods, medication and environmental exposure to acidic agents.<sup>3-5</sup> Intrinsic causes of erosion include recurrent vomiting as part of the eating disorders anorexia or bulimia

nervosa or due to the regurgitation of the gastric contents.<sup>6,7</sup>

Various surveys have shown that there is a high prevalence of erosion within the UK population<sup>8,9</sup> and the prevalence is likely to increase as an ageing population retains their teeth for longer. Anecdotally, one of the major causes of erosion is the consumption of acidic drinks and researchers are keen to quantify the amount of erosion that various beverages may cause as this information is particularly useful for counselling patients. It also helps provide further insights into the chemical process of erosion, and ways in which erosion can be modified, reduced or prevented.

Many techniques have been used to investigate the loss of tooth substance during erosion. The list

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discussed in this review is not exhaustive, but is composed of those techniques which are either well established or are comparatively novel techniques that are being explored for their potential.

## Surface hardness and nanoindentation techniques

Indentation techniques have been used extensively to investigate enamel erosion by measuring the hardness of the enamel surface. Initially, the erosive process involves enamel dissolution, which is associated with a softening of the surface due to weakening of the enamel structure. Thus, hardness measurements are a simple method of observing the early stages of enamel erosion. The popularity of this technique is likely to be associated with the low initial start up cost and the ease with which data can be acquired.

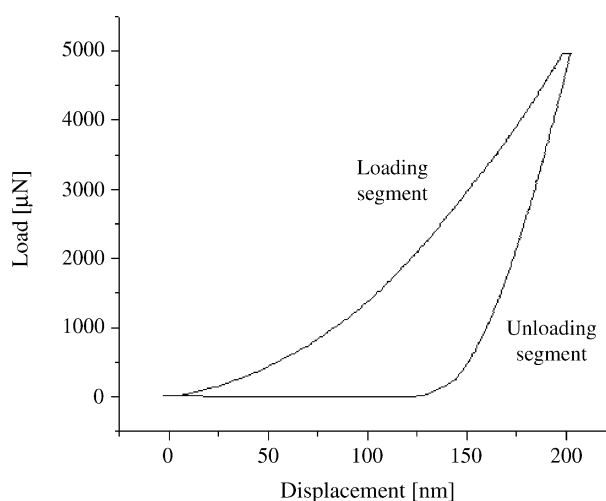
Two techniques are commonly used to measure enamel hardness. Surface hardness, or microindentation, is the more established and traditional method, whereas nanoindentation (also known as ultra-microindentation) is emerging as a new technique that is readily applicable to enamel erosion. The basic technique of both microindentation and nanoindentation is a diamond tip of known dimensions, which is pressed onto a surface with a given load and duration. The microindentation technique yields data in arbitrary units, usually Knoop hardness number (KHN) or Vickers hardness number (VHN), and nanoindentation yields hardness and reduced elastic modulus in the SI unit of Pascals ( $\text{Nm}^{-2}$ ).

One main difference between micro- and nanoindentation is, as suggested by the names, the scale of the indentation. Microindentations in sound enamel have typical indentation depths of micrometres or tens of micrometres,<sup>10</sup> whereas, nanoindentations in sound enamel have sub-micrometre indentation depths, typically around 200 nm.<sup>11</sup> The hardness measured by indentation is affected not only by the immediately surrounding material, but also by the material at a distance of approximately 10 times the dimensions of the indentation.<sup>12</sup> Thus for typical indentations, microhardness is a function of the mechanical properties of the material at some tens to hundreds of micrometres from the enamel surface, whereas nanoindentation is affected by the material to a depth of a few micrometres. Considering that the thickness of the softened region of enamel during dissolution is estimated to be 2–5  $\mu\text{m}$ ,<sup>13,14</sup> it is clear that microindentation is affected by not only the softened layer but also the intact, underlying

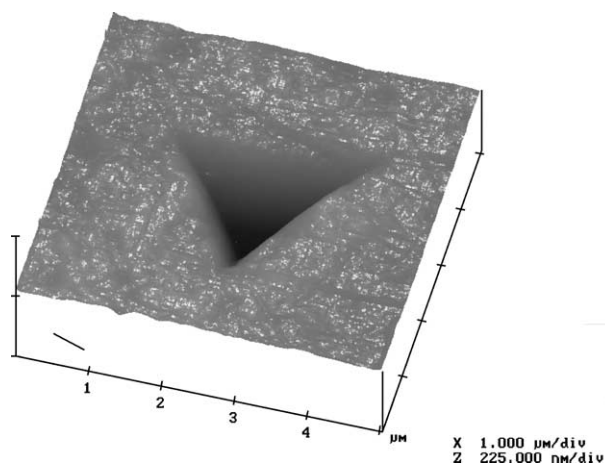
enamel. Nanoindentation can therefore be expected to be more sensitive to the early stages of enamel dissolution when the thickness of the softened layer may be smaller.

There are two further major differences between microindentation and nanoindentation with relevance to enamel erosion studies. These involve capabilities often incorporated into nanoindentation apparatus which are not usually used with microindentation, although this is not due to any conceptual or fundamental limitations of microindentation and rather to manufacturing practice.

Microindentation hardness is determined by measuring the dimensions of the indent left in the enamel surface. As such only plastic (permanent) deformation is investigated and there is no information regarding the elastic response and recovery of the surface. In typical nanoindentation apparatus, the displacement of the tip as a function of the applied load is continually monitored during indentation, and as such the plastic and elastic deformation of the surface can be determined. An example of such load-displacement data for a sound, polished enamel surface is shown in Fig. 1. In practical terms, both the hardness (plastic, permanent deformation) and the elastic modulus (or Young's modulus) may be readily calculated from nanoindentation load-displacement data. Elastic modulus data may be useful in studies of erosion, since it has been shown in studies of thin films<sup>12,15</sup> and of enamel erosion<sup>16</sup> to be more sensitive than hardness to the presence of underlying hard material (intact enamel in the case of erosion).



**Figure 1** Load-displacement data for a typical nanoindentation in sound, polished human enamel taken from an erupted permanent molar. There is a holding segment between loading and unloading. The difference between the loading and unloading segments indicates plastic deformation and elastic recovery.



**Figure 2** A nanoindentation in a sound, polished enamel surface taken from an unerupted third molar. The image was obtained by scanning the surface using the nanoindentation tip, and measures  $5\ \mu\text{m} \times 5\ \mu\text{m}$ .

In some nanoindentation apparatus, the tip can be scanned across the substrate surface line by line, building up an image of the area in contact with the tip. An example of such an image of sound enamel, taken after indentation, is shown in Fig. 2. This is particularly useful when analysing inhomogeneous substrates as different regions of the surface can be identified and indented, and also in rough substrates as locally flat areas can be selected for indentation. After indentation, the indent can be examined for cracks and other artefacts. Since dissolved enamel surfaces are both inhomogeneous and rough, nanoindentation imaging is beneficial when applied to enamel dissolution studies.

Nanoindentation is a comparatively new technique and there are only a few reports of its use to investigate enamel erosion. In the first such report, and the only of in situ erosion, enamel samples were placed in an intra-oral appliance in the mouth of a volunteer who drank one of three drinks.<sup>11</sup> Nanoindentation demonstrated clear differences between the mechanical properties of the enamel samples as a function of the different drinks, with orange juice resulting in significantly more softening than mineral water or a blackcurrant drink. A related study in which in vitro erosion by soft drinks was investigated using 'ultra-microindentation' apparatus also indicated different degrees of softening by different drinks.<sup>17</sup> In a preliminary study, another group observed changes in the mechanical properties of enamel after storage in deionised water and saline solution, and advised caution in the choice of storage medium for tooth specimens.<sup>18</sup> More recently, a series of investigations of in vitro enamel erosion has previously demonstrated unknown features of the dependence of enamel

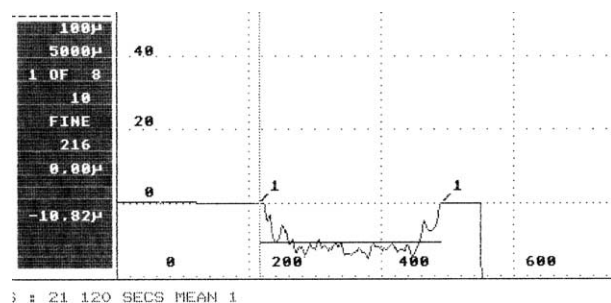
erosion on the calcium and phosphate concentrations, degree of saturation and pH of acidic solutions.<sup>16,19-21</sup> These studies used exposure times of 30 s-10 min, which is comparable to the clearance times of acids in the mouth.<sup>22</sup> This demonstrates that nanoindentation is extremely sensitive to changes in the mechanical properties of the enamel surface and is likely to find increasing application in enamel erosion research.

Microindentation has also been used to compare enamel erosion by different solutions. Statistically significant changes due to erosion by cola drinks<sup>23,24</sup>, fruit juices,<sup>25,26</sup> sport drinks,<sup>26</sup> red wine,<sup>27</sup> bleaching agents,<sup>28</sup> citric acid solution<sup>29</sup> and acidic lozenges<sup>30</sup> have been reported. Softening of enamel samples followed by rehardening has been observed after exposure to saliva and soft cheeses<sup>31</sup> and milk.<sup>32</sup> In some studies, microhardness changes after short exposure times were observed, for example, five minutes of exposure to a cola drink.<sup>23,24</sup> In these studies, agitation of the cola was shown to increase the degree of erosion, and the presence of an early salivary pellicle was shown to reduce erosion. Other studies using microindentation have also indicated that salivary pellicle reduces the degree of softening caused by erosion.<sup>33,34</sup>

One advantage of microindentation is that it has lower start-up costs than nanoindentation, and is faster and simpler to perform. A drawback of both techniques is that the enamel surface must be polished to provide a flat substrate prior to erosion. The outer layer of the enamel, which contains much greater concentrations of fluoride and lower concentrations of magnesium and carbonate than enamel, is removed. Since the solubility of inner enamel is known to be higher than that of surface enamel, erosion proceeds more quickly in polished samples.<sup>35</sup>

## Profilometry

Profilometry, also known as surfometry, has been extensively used to characterise enamel loss caused by erosion. The device uses a small metal stylus with a diameter of  $20\ \mu\text{m}$  that scans across the enamel sample at a rate of around 10 mm/min. To assess the effect of an erosive agent part of the sample, surface is protected with adhesive tape, nail varnish or similar. The unprotected surface is exposed to the erosive agents, thus providing a direct comparison between treated and protected areas. The sample surface is scanned before and after erosion, and the amount of material loss can be measured from the trace produced, as shown



**Figure 3** A profilometry trace obtained by scanning across an eroded pit in an enamel surface taken from an erupted permanent molar. The scale is in  $\mu\text{m}$ .

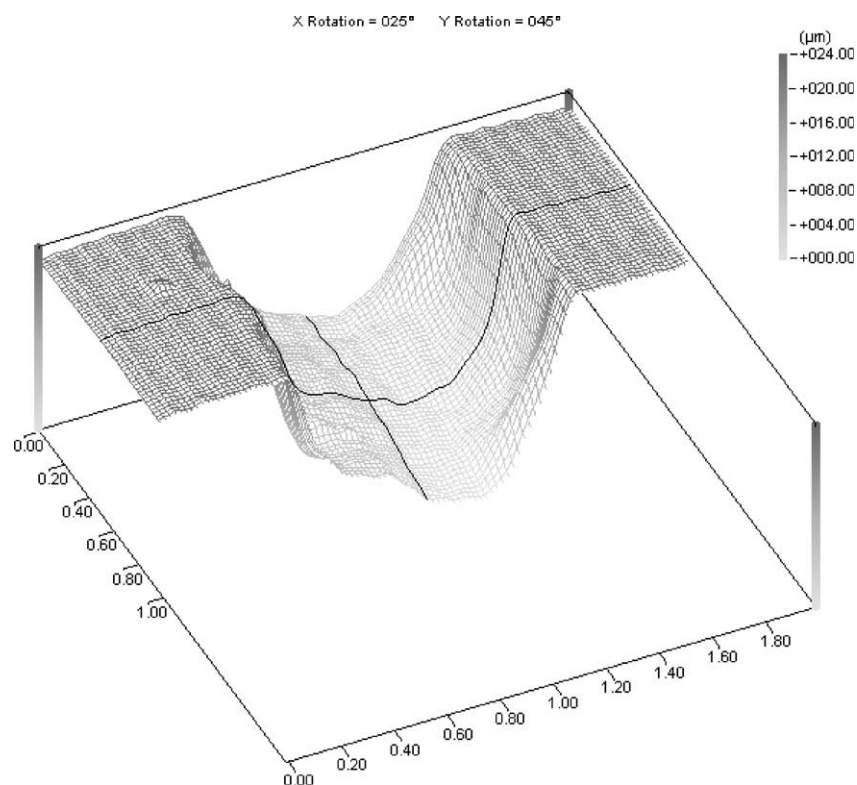
in Fig. 3. Alternatively, a cast may be made of the eroded enamel surface and the profilometer used to measure the profile of the cast.<sup>36</sup>

More recently, laser profilometry has been used to assess erosion. In this technique, the traditional contact stylus is replaced with a laser, and interferometry is used to build up a map of the surface. Such a map of an eroded enamel surface is shown in Fig. 4. One main advantage of this technique is that it can provide data for the volume of enamel loss as well as vertical loss.<sup>37</sup> Furthermore, since a laser beam is used to probe the surface rather than a stylus, there is no direct physical contact between the probe and the surface

and no damage occurs to the surface by scratching of the soft, eroded surface. In addition, many devices have interchangeable scanning heads that allow the specimen size to be varied from a small enamel sample of  $1\text{ mm}^2$  to a study model.

Profilometry is a simple and fast assessment to carry out over a relatively large area of enamel. However, as with indentation techniques, the enamel sample has to be ground flat prior to use. Since material loss is assessed rather than surface softening, profilometry is used to investigate more advanced stages of erosion than indentation techniques. The optical properties of the substrate are also important as this can limit the use of this technique.<sup>38</sup>

Profilometry has been used to assess the erosive potential of various products in vitro including fruit cordials,<sup>39,40</sup> carbonated drinks,<sup>41</sup> alcopops,<sup>42,43</sup> white wines,<sup>44</sup> ciders,<sup>45</sup> herbal teas,<sup>46</sup> mouthrinses<sup>47</sup> and various acid solutions.<sup>48</sup> The technique has also been adapted for use in clinical trials.<sup>25</sup> The enamel specimen is incorporated in an upper removable appliance, similar to an orthodontic appliance, and is worn during the trial period. At the end of each day, the enamel specimen can be easily removed and scanned in the profilometer. Using this technique, it has been



**Figure 4** A three-dimensional laser profilometry image of an eroded enamel surface taken from an erupted permanent molar. The areas at the left and right hand sides were protected using adhesive tape, and the area in the centre was exposed to an erosive solution for 30 min. The scale is in  $\mu\text{m}$ .

shown that the ranking of erosive drinks in vitro and in situ remains the same.<sup>25</sup> However, the amount of erosion produced in the mouth is approximately 10 times smaller than that found in the laboratory. This is probably due to the protective and reparative effects of pellicle and saliva.<sup>25</sup>

Recently, a new experimental protocol has been developed that measures both the material loss during erosion and the thickness of the softened layer. Ultrasonication has been shown to remove the softened enamel layer observed during erosion.<sup>14</sup> This has been exploited in a series of papers<sup>14,49-51</sup> in which profilometry was used to measure erosion depths, then the same samples were placed in an ultrasonic bath, and profilometry was used a second time to measure the erosion depth after the softened layer of enamel was removed. This has provided an estimate of the thickness of the softened layer of approximately 2-5 µm.

## Microradiography

Microradiography is a technique in which a beam of X-rays is incident on an enamel section or block, and a photographic plate<sup>10</sup> or a photon counter<sup>52</sup> is used to record the penetrating radiation. The degree of blackening of the film or the photon density, together with a calibration sample, provides a map of the mineral density of the enamel.<sup>10</sup> There are several reviews in the literature on the use of microradiography in dental research, and the technique is usually divided into one of three 'generations' of microradiography. These are: transverse microradiography, which is used in the majority of published studies; longitudinal microradiography, used for thick sections of teeth; and wavelength-independent microradiography, which is used to quantify mineral content in whole teeth.<sup>53</sup>

Although, microradiography has been predominantly used in the investigation of caries, it has also been used to study erosion in vitro. Erosive lesions after 24 h exposure to orange juice have been observed, with statistically significantly different degrees of erosion in different brands of juice.<sup>53</sup> Another study indicated the importance of treatment between erosive challenges, by investigating erosion in orange juice alternated with storage in either water or artificial saliva.<sup>55</sup> Microradiography results demonstrated a significant difference between the erosion depth of samples stored in water and artificial saliva. In one unusual and interesting study, microradiography was used to demonstrate different rates of erosive lesion

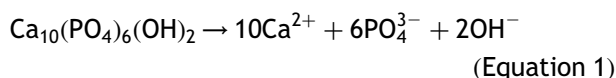
progression perpendicular and parallel to the natural tooth surface, with the rate of acid attack shown to be 14% higher perpendicular than parallel to the natural surface.<sup>56</sup>

Microradiography has the potential to observe both material loss, as with profilometry, and softening, as with indentation techniques. One study has demonstrated a strong correlation between microradiography and profilometry of erosive lesions.<sup>57</sup> Another pair of studies compared enamel dissolution using microradiography and hydroxyapatite dissolution using chemical analysis in different acid solutions,<sup>58,59</sup> indicating that there may be a good correlation between these two techniques.

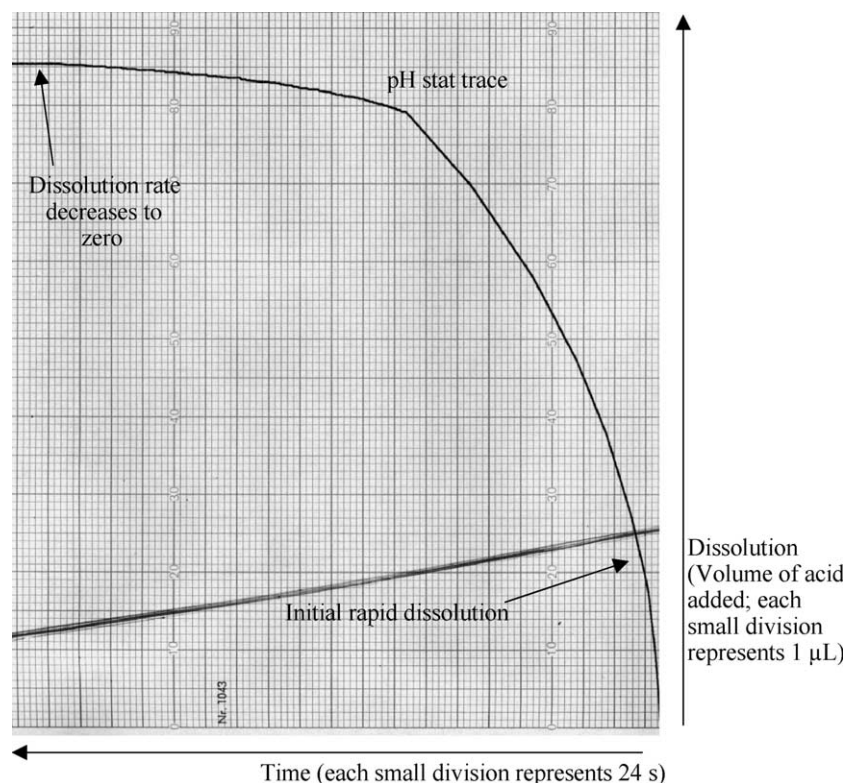
## Chemical analysis

Chemical analysis has been used extensively to investigate the kinetics and thermodynamics of enamel and hydroxyapatite dissolution by measuring the concentrations of calcium and phosphate released into the dissolving solution, as well as the pH and uptake or release of minor constituents of enamel such as fluoride or magnesium.<sup>10,58-66</sup> Calcium analysis is usually performed using atomic absorption spectroscopy (AAS) and phosphate concentration is usually determined using spectrophotometry of a coloured phosphate complex.<sup>67</sup>

The pH stat is a technique which is frequently applied to studies of chemically pure hydroxyapatite dissolution, but also has great potential for enamel erosion studies. It is a chemical analysis device, which makes use of the fact that, on dissolution, hydroxyapatite and enamel release hydroxyl (OH<sup>-</sup>) ions (Eq. (1)).



Thus, by measuring the change in pH of a solution, it is possible to determine the rate of release of OH<sup>-</sup> ions, and from this the rate of hydroxyapatite or enamel dissolution can be calculated. The pH stat includes a feedback loop, which acts to hold the pH at a stationary value. For example, enamel dissolution may be investigated in an acidic solution at, say, pH 3.3. When the enamel sample is immersed in the acid, it starts to dissolve, releasing OH<sup>-</sup> ions which neutralise H<sup>+</sup> ions and cause the pH to increase. The pH stat equipment automatically adds acid to compensate for this neutralisation of H<sup>+</sup> ions and maintain the pH at the initial value of 3.3. By measuring the rate at which it is necessary to add acid to maintain a constant pH, it is possible to calculate the dissolution rate of



**Figure 5** An example of data obtained using a pH stat to investigate dissolution of hydroxyapatite powder in an acidic solution. The x-axis represents time (moving from right to left), with five small boxes (one large box) indicating 2 min. The y-axis represents the total amount of acid added, with five small boxes (one large box) indicating 5  $\mu\text{L}$ . The rate is initially rapid, indicated by a steep gradient. As the dissolution progresses the rate decreases, eventually reaching zero when all the hydroxyapatite powder has dissolved.

the enamel at a chosen pH. An example of such pH stat data for hydroxyapatite dissolution is shown in Fig. 5. Although, this technique has predominantly been applied to hydroxyapatite dissolution studies, it is readily applicable to the investigation of real-time enamel erosion.<sup>60</sup>

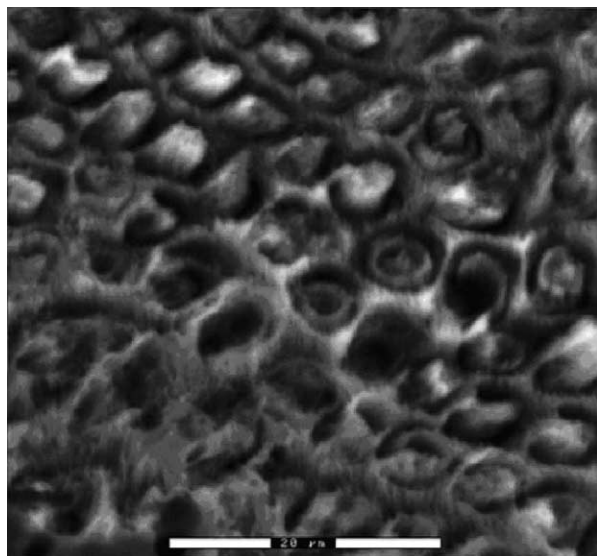
These chemical analysis techniques are well established, sensitive and accurate but they provide information only on the net concentrations of ions released. Additional techniques are necessary to visualise the crystal surfaces and deposition or nucleation of new material, and only in vitro processes can be investigated.<sup>10</sup> However, chemical techniques do have the advantage that erosion of natural enamel surfaces can be investigated, since no polishing is required. In addition, this method of investigation is one of the few with which genuinely real-time data can be obtained.

### Microscopy techniques

Scanning electron microscopy (SEM) is a well-established technique in virtually all areas of

modern science. Its main use in the investigation of enamel erosion is as an imaging tool, and many researchers have produced excellent, high-resolution images of eroded enamel surfaces.<sup>35,68-70</sup> In this sense, SEM is usually used as a supporting technique in an investigation. It may also be used to help identify other phases of calcium phosphate such as brushite ( $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ) and monetite ( $\text{CaHPO}_4$ ) which form during enamel dissolution under some conditions.<sup>63</sup>

The environmental scanning electron microscope (ESEM) is a modification of the SEM.<sup>71,72</sup> The main differences between ESEM and conventional SEM are that the sample can be examined without coating with metal or carbon, in low vacuum, and in wet conditions. The resolution and magnification afforded by ESEM are comparable to those of SEM.<sup>73</sup> An ESEM image of a polished, erupted molar surface exposed to citric acid for 10 min is shown in Fig. 6. To date, there are very few published reports of the application of ESEM to enamel erosion research; one showing typical images of etched premolar enamel,<sup>74</sup> and another showing a sequence of images of the progression of erosion in premolar enamel after 30, 60, and 90 s.<sup>75</sup>



**Figure 6** An environmental scanning electron micrograph of a polished, permanent erupted molar surface after exposure to citric acid (pH 3.3) for 10 min. The prismatic structure of the enamel is clearly visible, and different etching patterns can be seen in different regions of the image. Protruding prisms can be seen in the top left of the image, protruding prism boundaries in the middle right and aprismatic enamel can be seen in the bottom left. The scale bar represents 20  $\mu\text{m}$ .

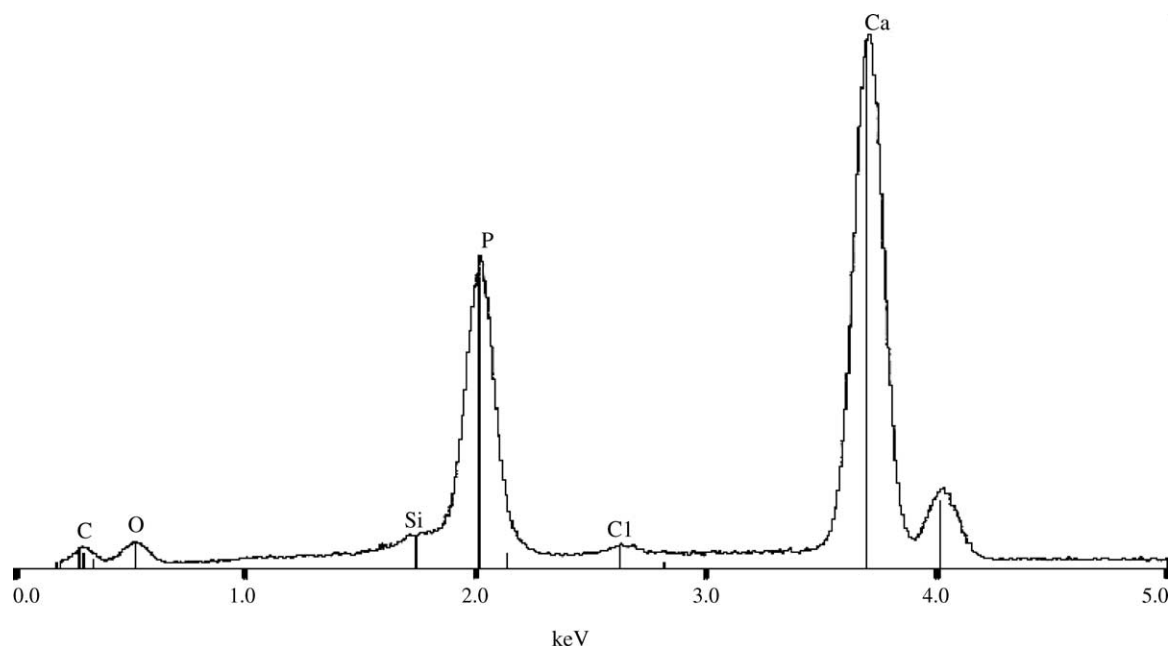
Energy dispersive X-ray spectroscopy (EDX, also known as EDS) is a form of microanalysis that may be incorporated into SEM or ESEM. The interaction of the electron beam with the sample surface causes X-rays to be emitted by the atoms and ions in

the top few micrometres of the sample surface. An electron is ejected from an inner shell of an atom, and when an outer shell electron takes the place of the missing electron, energy is emitted in the form of X-ray radiation. The X-rays are analysed and provide information on the elemental distribution in the surface. An example of an EDX spectrum of a sound, polished enamel surface is shown in Fig. 7. Peaks corresponding to calcium, phosphorus, oxygen, carbon and chlorine can be seen, in addition to a small peak for silicon, which is thought to be contamination due to lubrication with silicon grease.

The application of EDX to enamel erosion is limited to two preliminary studies with some relevance to erosion processes, in which the effects of fluoride<sup>76</sup> and peroxide<sup>77</sup> on the chemical composition of enamel surfaces were investigated. Since ions with a concentration of 1 wt. % can be reliably detected,<sup>78</sup> it is conceivable that minor ion concentrations of sound and eroded enamel might be compared.

### Atomic force microscopy (AFM)

The atomic force microscope is one of the family of scanning probe microscopes (SPMs) first developed in the 1980s<sup>79</sup> and has become an invaluable tool in biological and biomaterials research. In AFM, the sample surface is probed using a sharp tip attached



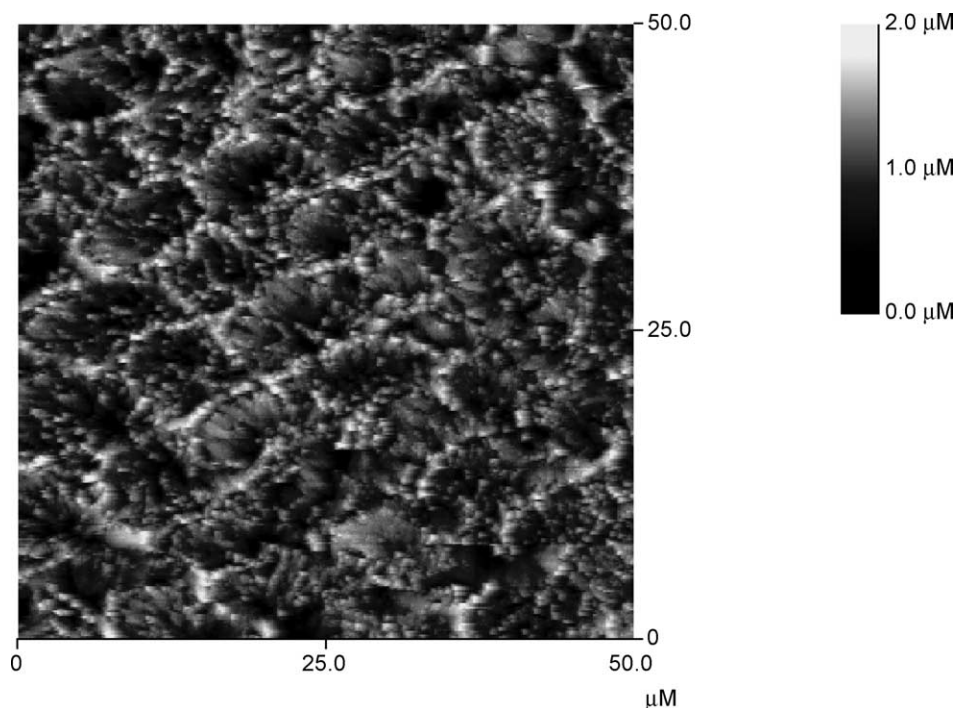
**Figure 7** An energy dispersive X-ray (EDX) spectrum of a polished erupted molar surface. Major peaks can be seen indicating calcium and phosphorus, and smaller peaks indicating oxygen, carbon, silicon and chlorine. The silicon is thought to be due to contamination by silicon grease used during sample preparation.

to a flexible cantilever. The tip or sample is moved such that the tip moves back and forth across the sample and tracks the surface features. A diode laser beam is reflected from the back of the cantilever and is incident on a four-segment photodiode. As the tip moves, the deflection of the cantilever is indicated by the position of the laser on the photodiode, and a map is built up of the surface of the specimen. An example of such an AFM image of eroded enamel is shown in Fig. 8.

There are a number of different modes of AFM, the most commonly used being contact mode (CMAFM) and tapping mode (TMAFM). In contact mode, the tip is moved laterally in constant contact with the surface, and is deflected by surface features much in the same way as with profilometry. The main difference between the techniques is the resolution of AFM, which is orders of magnitude greater than with profilometry, and the imaging capability, which is not usually incorporated in profilometry equipment. In tapping mode, the tip is held at a distance from the surface and is driven to oscillate close to its resonant frequency. The tip gently taps on the surface and the mean position of the laser beam on the photodiode is used to generate an image of the surface. The intermittent tip-sample contact and the lack of shear forces on the surface result in less damage to fragile surface features than with contact mode.

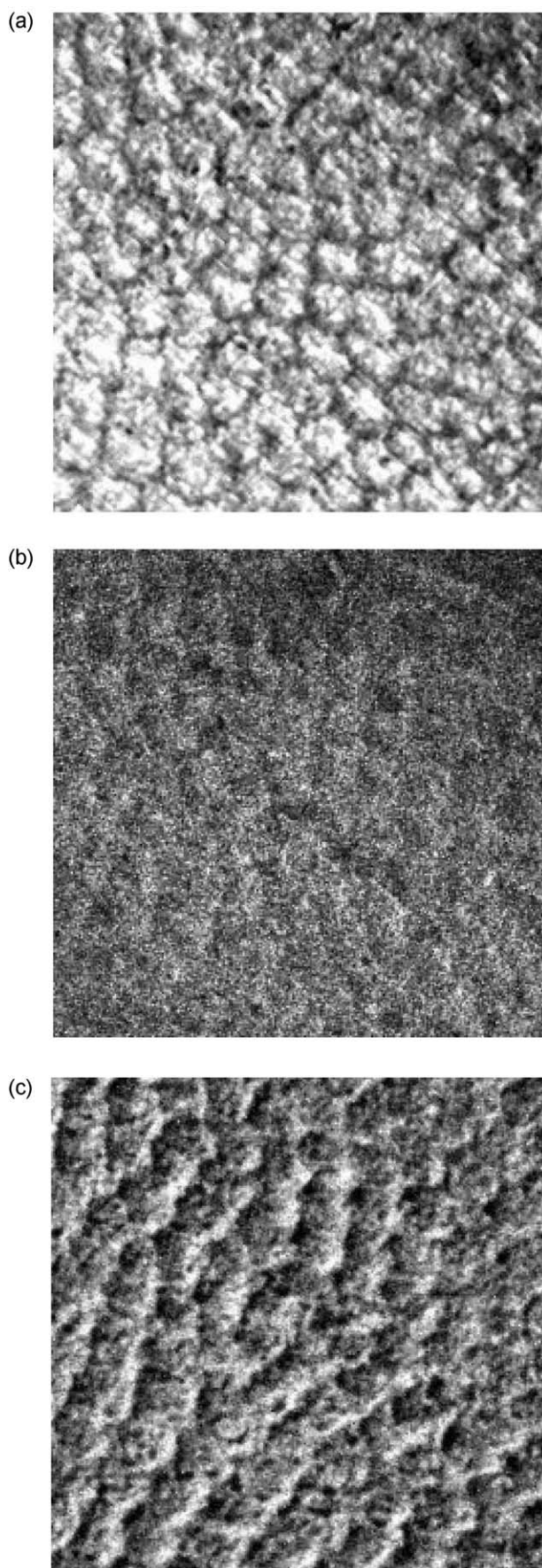
One main advantage of AFM over techniques such as SEM and scanning tunnelling microscopy is that of sample preparation. AFM can be used equally well on conducting and insulating surfaces and can be performed in ambient conditions, in air or liquids as well as in a vacuum. Thus, fragile samples are not damaged by harsh sample preparation techniques such as coating, dehydration and exposure to vacuum and artefacts associated with such techniques are avoided. Furthermore, the same sample can be imaged in AFM in real time. A further advantage is its extremely accurate, quantitative nature. However, the scan size is limited to  $<0.5 \times 0.5 \text{ mm}^2$  and it can take around 60 min to scan this area. Time limitations must therefore be taken into account, and several areas must be scanned to ensure that the images are representative of the whole enamel area being tested.

There are a few reports of the use of AFM to investigate enamel erosion.<sup>80-83</sup> In one study, a series of images of enamel surfaces was obtained, and between taking each image a drop of soft drink was placed on the enamel, without removing the sample from the microscope.<sup>82</sup> By doing this, it was possible to observe exactly the same area of the enamel after consecutive exposures to different soft drinks. Inhomogeneous erosion patterns were observed, and image processing allowed these authors to calculate the amount of enamel lost



**Figure 8** A tapping mode atomic force microscopy (AFM) image of a polished, unerupted third molar surface, exposed to citric acid solution (pH 3.3) for 10 min.





from the surface with a sensitivity of  $\pm 50$  nm. In other studies, a more qualitative approach has been used, comparing the appearance of enamel surfaces after exposure to different erosive soft drinks.<sup>80,81,83</sup>

### Secondary ion mass spectroscopy (SIMS)

SIMS is a form of mass spectroscopy in which a beam of ions is incident on a surface, causing the ejection of secondary ions which are spectroscopically analysed. SIMS is extremely sensitive and can detect ions at the sample surface with concentrations of parts per billion.<sup>84</sup>

There are three main measurements that can be made using SIMS. First, the concentrations of positive or negative ions can be compared, with reference to the sensitivity factors for each ion. SIMS is, however, only partially quantitative and actual concentrations cannot be accurately measured. Second, since SIMS is a destructive process, a depth profile of ions can be observed as the surface is sputtered by the incident ion beam. Third, a surface map can be obtained, showing the distribution of different species in the surface.

SIMS has mostly been used in dental research to determine the relative concentrations of major and trace elements in dental tissues.<sup>85-87</sup> Although problems with surface charging are usually encountered, there has been one published report of maps of ions in enamel surfaces, showing localisation of  $\text{CN}^-$  ions at prism boundaries.<sup>88</sup> This was thought to be due to the elevated levels of organic material found in the prism boundaries as compared with the prisms.

Although, SIMS has not been described in the literature as a technique to examine erosion, the potential for monitoring trace elements in the process is obvious. A series of previously unpublished SIMS images of eroded enamel is shown in Fig. 9. Fig. 9a shows a topographic image of the enamel surface, which displays the characteristic honeycomb erosion structure. Lighter regions in the image indicate higher areas in the surface, and darker regions indicate lower areas. Fig. 9b shows an elemental map of exactly the same area, but this time the shades of grey represent different concentrations of calcium in the surface. It can be seen

**Figure 9** Secondary ion mass spectroscopy images of a polished permanent erupted molar enamel surface. The enamel has been exposed to citric acid (pH 3.3) for 10 min. Each image measures  $75 \mu\text{m} \times 75 \mu\text{m}$ , (a) shows the surface topography, (b) shows a calcium map and (c) shows a magnesium map.

that there are higher concentrations of calcium in the prism cores than at the prism boundaries, which is likely to be due to the higher mineral density in these regions. Fig. 9c shows a magnesium map of the same area, and it can be seen that there appear to be slightly higher concentrations of magnesium at the prism boundaries than in the prism cores. Although, it has been tentatively suggested that the magnesium in enamel is associated with the organic material, this has not been directly observed before. It should be noted, however, that these figures represent preliminary investigations and a thorough study has not yet been conducted.

### Quantitative light-induced fluorescence

Quantitative light-induced fluorescence (QLF) is a technique that was developed for the detection of early demineralisation found in enamel caries. Essentially, the technique measures a loss in auto-fluorescence of enamel in the presence of demineralisation.<sup>89</sup> However, the exact mechanisms by which QLF measures erosion is unclear, but is probably a combination of mineral loss detection from the softened layer below the crater that is inevitably formed as erosion proceeds.

Due to its narrow focal depth, images are of a constant size and positioning and angulation artefacts are avoided. The fluorescence on which the system is based also creates a 10-fold increase in contrast between sound and eroded enamel.<sup>90</sup> Furthermore, the technique requires no tooth preparation and could potentially be used both in vitro and clinically to provide real time data.

### Conclusions

Several techniques have been discussed with respect to their application to enamel erosion studies. The most established of these are micro-indentation, profilometry, microradiography, chemical analysis and SEM. Profilometry and microradiography are readily applicable to enamel erosion at more advanced stages, but to investigate earlier stages of erosion, it is preferable to use more sensitive techniques such as microindentation. More sensitive still is nanoindentation, which is likely to find increasing application in the field. Chemical analysis has the notable benefit that it is applicable from the earliest to the most advanced stages, but has the drawback that it is usually only an indirect measurement of erosion. SEM is rarely used on its own as it is difficult to quantify

the degree of erosion; however, it is invaluable as a supporting technique, particularly when using techniques which do not allow for direct observation of the surface, such as microindentation. In this respect, the quantitative nature of AFM imaging is likely to promote greater use of this technique in erosion studies. QLF and SIMS have only recently been applied to enamel erosion investigations, but may find increasing application in the future.

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