

Methods for the Measurement and Characterization of Erosion in Enamel and Dentine

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Key Words

Abrasion · Assessment methods · Dentine · Enamel · Erosion

Abstract

The advantages, limitations and potential applications of available methods for studying erosion of enamel and dentine are reviewed. Special emphasis is placed on the influence of histological differences between the dental hard tissue and the stage of the erosive lesion. No method is suitable for all stages of the lesion. Factors determining the applicability of the methods are: surface condition of the specimen, type of the experimental model, nature of the lesion, need for longitudinal measurements and type of outcome. The most suitable and most widely used methods are: chemical analyses of mineral release and enamel surface hardness for early erosion, and surface profilometry and microradiography for advanced erosion. Morphological changes in eroded dental tissue have usually been characterised by scanning electron microscopy. Novel methods have also been used, but little is known of their potential and limitations. Therefore, there is a need for their further development, evaluation, consolidation and, in particular, validation.

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Dental erosion involves histological changes in dental hard tissue. In early stages, the mechanical and physical properties of the tooth are modified, as minerals are released to the erosive acid. Enamel and dentine are affected differently [Lussi et al., 2011]. Because of the very high mineral content, erosion of enamel is initially manifested as partial demineralisation at the surface leading to softening and increased roughness. Dentine contains less mineral and more organic material, and shows mineral loss initially at the border between the peri- and intertubular dentine. Subsequently, there is loss of peritubular dentine, enlargement of the tubules and finally demineralisation of the intertubular dentine with exposure of the organic matrix [Selvig, 1968; Meurman et al., 1991; Kinney et al., 1995]. In more advanced stages, continuing exposure to acids and to mechanical and chemical challenges leads to surface loss, either through dissolution or abrasion of the fragile softened enamel surface or through enzymatic or mechanical degradation of the exposed dentine matrix.

The choice of the method for evaluating erosion depends primarily on the stage of the lesion, the expected changes in the structure of the erosive lesion during the study and on the tissue of interest. The exposed organic matrix of eroded dentine is prone to desiccation and

shrinkage, which interferes with several measuring methods or creates artefacts. Some methods are indirect, e.g. the release of calcium or phosphate, but most methods directly evaluate erosive changes in morphology, composition or physical properties.

Methods for assessing enamel erosion have been reviewed previously [Grenby, 1996; Azzopardi et al., 2000; Barbour and Rees, 2004; Attin, 2006; Field et al., 2010], but reviews on the evaluation of dentine are lacking. Methods suitable to assess enamel are not necessarily applicable to dentine due to histological differences. In this paper, we describe the potential applications and limitations of the existing methods regarding the type of surface required (natural or flattened), the nature of the study (erosion only or erosion and abrasion), the type of experimental model (in vitro, in situ and clinical), the need for repeated measurements over time (using destructive or non-destructive methods), and the need for qualitative or quantitative data.

General Approach

A literature search, described and tabulated in the online supplementary material (for all online suppl. material, see www.karger.com/doi/10.1159/000326819), showed that profilometry was the most commonly applied quantitative method to determine both dentine and enamel in in vitro, in situ and also in clinical models, followed by quantitative methods evaluating surface hardness (enamel) and microradiography (dentine). Scanning electron microscopy (SEM) was the most commonly used method for qualitative study of erosion in both tissues.

Quantitative Methods

Chemical Analysis of Dissolved Minerals

Quantification of calcium and phosphate release by an acid solution is a well-established method for assessing erosion [Grenby, 1996]. Calcium analysis using an ion-selective electrode [Hara and Zero, 2008] may be subject to error due to complexation by certain acids [Attin et al., 2005a]. Atomic absorption spectrophotometry is a reliable and sensitive method for calcium analysis [Willis, 1961; Trudeau and Freier, 1967], and interference by other solutes, such as phosphate, is easily avoided. It can be used to quantify erosion of both enamel and dentine [Grenby et al., 1990; Hara and Zero, 2008]. Calcium and

phosphate can also be analysed colorimetrically [Attin et al., 2005]. These methods allow analysis of very small volumes of demineralisation solution (10 μ l) and of very small concentrations: 12.4 μ mol/l for calcium [Attin et al., 2005a] and, depending on the acid used, between 1.9 and 9.0 μ mol/l for phosphate [Attin et al., 2005b]. These methods can be employed to measure both tissues. Preparation of specimen surfaces is not required. If prepared specimens are used, it should be considered that insufficient removal of the smear layer can cause an artefactual increase in mineral concentration.

Analysis of mineral release has been used both in vitro and in situ using extra-oral erosive challenge. It has also been used in vivo [Young et al., 2006] and is suitable for longitudinal measurements. One limitation is that the erosive challenge cannot take place in the presence of saliva, which would interfere with the analysis. Further, this method does not provide information about possible mineral gain, or about physical and morphological changes.

Surface Hardness

Softening, i.e. loss of hardness, is measured by the resistance of a substrate to the penetration of an indenter. Microhardness is measured with either a Knoop or a Vickers diamond indenter, which are rhomboidal and tetra-pyramidal, respectively. The Knoop or Vickers hardness numbers are calculated from the length of the indentation and the applied load. The costs of microhardness analysis are relatively low, and it is a simple way to obtain accurate information about early erosion. This may help explain the high popularity of this method [Barbour and Rees, 2004].

For the most accurate assessment of enamel hardness, flattened polished surfaces are necessary and the test surface must be positioned perpendicularly to the long axis of the indenter. These requirements limit the accuracy of hardness measurements on natural tooth surfaces. However, a few studies have explored the possibility of in vivo hardness measurements [Caldwell et al., 1957; Fosse et al., 1986]. Fosse [1986] developed an instrument for in vivo models, but only two studies seem to have used it [Albrecht et al., 1991; Takats et al., 1991]. Therefore, while surface hardness measurements are in principle applicable in vivo as well as in vitro and in situ, this application remains to be tested in further studies.

The Knoop diamond penetrates sound enamel by about 1.5 μ m, while that of Vickers would penetrate about 5 μ m given the usual loads [Featherstone, 1992] of 50 and 200 g, respectively. Therefore, it can be speculated

that Knoop hardness is more sensitive to changes in the most superficial layer of an erosive lesion.

Changes in surface hardness of enamel can be observed even after a few minutes of exposure to an erosive agent [Hara et al., 2006; Hara and Zero, 2008]. A clear limitation is that in highly eroded dental substrates the indentation boundaries are not clearly defined, so that measurements are either inaccurate or impossible. The decrease in surface of advanced erosive lesions cannot be quantified by hardness measurements of the remaining surface. Another limitation is that, when material is deposited on the surface, e.g. by application of certain fluorides, surface hardness measurements may not be representative.

Correctly performed enamel hardness measurements have proved to be robust, with a great amount of accumulated experience in the literature. Indentations placed in enamel are stable and not vulnerable to time-dependent changes in their morphology, since enamel shows low elasticity and in principle no retraction [Herkstroter et al., 1989]. In demineralised dentine, the indentations can be reduced in length by about 30% within 24 h after indentation (500-gram load for 10 s) [Herkstroter et al., 1989], mainly through retraction of exposed matrix after compression and shrinkage due to desiccation. This means that surface hardness measurements are not appropriate for the assessment of dentine erosion.

Nano-indentation uses the same principle as micro-hardness indentation but at a smaller scale. It uses a trigonal pyramidal Berkovich diamond indenter that creates an indentation usually maximally 1 μm in length under loads of 0.25–50 mN [Kinney et al., 1996; Mahoney et al., 2003]. This indenter applies an increasing load to the test substrate to a preset value, and then the load is decreased until partial or complete relaxation of the test substrate occurs. The load/displacement recording potentially allows calculation of mechanical properties: Young's modulus, hardness, fracture toughness, time-dependent creep, and plastic and elastic energy. Erosion studies have focused on hardness (plastic deformation) analysis. Nano-indenters penetrate less deeply than microhardness indenters (150–500 nm) [Finke et al., 2000], allowing better sensitivity for differentiating small changes on the surface. Nano-indentation can detect enamel softening after only 30 s of exposure to citric acid (19.1 mM, pH 3.3) [Barbour et al., 2005], an exposure time similar to the duration of a pH fall at tooth surfaces during ingestion of acidic beverages in vivo. Therefore, this technique is sufficiently sensitive for the study of initial erosion.

Because of their deeper penetration, micro-indenters are thought to be influenced not only by the displaced area but also by its surroundings, which may involve sound enamel in shallow lesions. With nano-indentation, there is the possibility of calculating the Young modulus (elastic deformation). This seems also to be a relevant parameter for the characterization of very shallow erosive lesions, considering that it would not be influenced by the underlying intact enamel [Barbour and Rees, 2004; Attin, 2006]. Therefore, the elastic modulus is sometimes considered a better parameter to assess the effect of an acid impact [Barbour et al., 2003].

Flattened and polished surfaces seem to be required for adequate measurements, although it has been speculated that, because of the small magnitude of the measurement, the natural curvature of the tooth may not be a problem for nano-indentation [Attin, 2006].

Nano-indentation of dentine is often combined with atomic force microscopy (AFM) for visual control, because nano-indenters are small enough to be placed on peri- or intertubular dentine or even in a dentine tubule, and these all have distinct properties [Kinney et al., 1996; Angker et al., 2003].

Hardness indentations can also be used to quantify the amount of surface loss of enamel, either by placing indentations of different depths (using different loads) on the surface with subsequent verification of their presence [Schweizer-Hirt et al., 1978] or by placing indentations and measuring their lengths before and after any experimental treatment. This approach is useful for testing abrasion procedures in erosion-abrasion [Jaeggi and Lussi, 1999; Wiegand et al., 2007] and abrasion [Joiner et al., 2004] studies, but it is questionable when erosion is performed after the indentation, since erosion affects both indented and non-indented areas [Attin, 2006].

Surface Profilometry

Surface profilometry quantifies the loss of dental tissue in relation to a non-treated reference area. It also provides information on surface roughness – most commonly R_a and R_q [Field et al., 2010]. However, roughness measurements seem to be useful only for early stages of erosion.

In profilometry, the surface of a specimen is scanned to generate a two- or three-dimensional profile, using either a contact or a non-contact measuring device. In contact profilometry, the surface is scanned with a stylus with a diamond or steel tip. Non-contact profilometry uses a probe of laser light (white or blue light). The vertical range for white-light non-contact profilometry varies from 300

μm to 10 mm, depending on the sensor selected. This gives good flexibility for analysing very deep erosion pits and even curved natural surfaces. However, for maximum sensitivity and accuracy, flattened specimens are required. With such specimens, erosive lesions around 0.5 μm deep can be consistently detected and measured with non-contact profilometry [Hara and Zero, 2008]. It is common practice to check the flatness of polished specimens before using them in experiments. For an experienced technician, the overall reproducibility (within-sample standard deviation) of the white-light profilometer, including errors in measurement, repositioning and depth calculation, was $\pm 0.06 \mu\text{m}$ for enamel and $\pm 0.09 \mu\text{m}$ for dentine for erosion depths of 0.5–3.5 μm for enamel and 4.0–10.0 μm for dentine [Steiner-Oliveira et al., 2010]. When examiners with different experience levels were included, the values increased to $\pm 0.18 \mu\text{m}$ for enamel and $\pm 0.21 \mu\text{m}$ for dentine [Hara et al., unpubl. data].

Profilometry can also be used to measure erosion depth on natural surfaces [Ganss et al., 2000], but this has rarely been done in vitro, because the accuracy is less than for flattened surfaces. However, the capability for studying natural surfaces makes profilometry potentially applicable to studies of erosion in vivo. For this purpose, high-quality replicas are used and a method for superimposing the surface profiles at different times is required. Ground marks [Lambrechts et al., 1989] or acid-resistant markers bonded to tooth surfaces [Bartlett et al., 1997; Schlueter et al., 2005] have been used to provide fixed reference points, resulting in a detection limit of approximately 15–20 μm . Chadwick et al. [1997] developed a surface-mapping instrument, which employs a computer-controlled probe to map electroconductive replicas of tooth surfaces. Mitchell et al. [2003] estimated that this method had an overall precision of about 15 μm and hence a detection limit of about 50 μm .

With profilometry, erosive, abrasive and combined erosive-abrasive tissue loss can be measured, but not the depth of softening on enamel surfaces. In an attempt to overcome this limitation, Eisenburger et al. [2000] recorded surface profiles of acid-treated specimens before and after ultrasonication in saline solution. The ultrasonication was intended to remove softened enamel, so any difference between the two profiles should give the depth of the softened layer. However, SEM shows that the softened layer is incompletely removed from the surface by ultrasonication [Eisenburger et al., 2004].

A disadvantage of contact profilometry is that the stylus penetrates the eroded surface, which is either partially demineralised (enamel) [Ren et al., 2009] or complete-

ly demineralised (dentine) [Ganss et al., 2009b]. In enamel, this can cause damage to the surface and can lead to an overestimate of early erosion depth.

In dentine, the performance of profilometry depends on the target criterion, which can be the level or loss of organic material from the surface or the loss of mineral. The measurement of the level of the organic matrix in relation to a non-eroded reference can be of interest, for example, in evaluating organic matrix degradation by enzymes. In this case, moisture control is essential, because dentine, especially when demineralised, is prone to shrinkage, particularly if measurement takes some time. Since most non-contact devices do not allow measurements in water, it is recommended to keep the specimens wet until measurements are performed to avoid any dimensional change [Ganss et al., 2007; Attin et al., 2009]. If moisture is thoroughly controlled, little or no step height will be measured between a reference and an experimental area [Ganss et al., 2009b] (fig. 1a). If moisture control is inadequate, the organic matrix shrinks to some extent and, although a step height can be measured (fig. 1a), it reflects neither the surface level of the wet organic matrix nor the loss of mineral at the demineralisation front (fig. 1b). The same ambiguity is encountered with contact profilometry because, while the stylus penetrates the matrix, the tip normally does not reach the demineralisation front [Ganss et al., 2009b].

To avoid these problems, the organic matrix should be removed before profilometric measurement of demineralisation depth. This can be achieved by treatment with proteolytic agents. Sodium hypochlorite removes not only the totally but also the partially demineralised part of the matrix [Ahmed et al., 2008], which would cause demineralisation depth to be overestimated. Non-specific proteolytic enzymes like pepsin or trypsin do not remove organic structures completely [Schlueter et al., 2007a, 2010], so demineralisation depth could be underestimated. However, collagenase (e.g. from *Clostridium histolyticum*) removes the totally demineralised matrix [Klont and ten Cate, 1991; Ganss et al., 2007, 2009], while leaving the partially demineralised dentine [Ahmed et al., 2008; Ganss et al., 2009a]. Sometimes very small remnants of organic matrix can persist on the surface of collagenase-treated specimens. Therefore, measurement with contact profilometry is recommended, as it is less sensitive to such remnants and shows a higher concordance with calcium analysis [Ganss et al., 2009b]. Of course, removal of the organic matrix turns non-destructive profilometry into a destructive method, and this is a shortcoming of this procedure.

Microradiography

Microradiography quantifies the mineral content of dental hard tissue by measuring the attenuation of X-rays transmitted through a section by comparison with a reference aluminium step wedge. The intensity of the emergent beam is recorded on photographic emulsion or with a photon counter [Arends and ten Bosch, 1992; Anderson et al., 1998]. In transversal microradiography (TMR), the X-ray beam is perpendicular to the direction of lesion progress [de Josselin de Jong et al., 1987a], while in longitudinal microradiography (LMR) the beam is approximately parallel with this direction [de Josselin de Jong et al., 1987b]. These methods use X-rays at a specific wavelength. Wavelength-independent microradiography [Herkstroter et al., 1990] uses higher-energy, polychromatic X-rays. This allows the study of thicker specimens and thus, in principle, longitudinal non-destructive measurements of lesion progress.

LMR has been used to study erosion, abrasion and erosion-abrasion of both enamel and dentine *in vitro* and *in situ* [Herkstroter et al., 1991; Ganss et al., 2004a; Schlueter et al., 2007b; Ganss et al., 2008, 2009b; Schlueter et al., 2010]. LMR provides information about changes in the total mineral content of plano-parallel specimens, expressed as the equivalent thickness of hydroxyapatite loss, but not about the mineral content/depth profile of a lesion. It was originally developed for quantifying de- and remineralisation of enamel and was optimised for sections approximately 400 μm thick [de Josselin de Jong et al., 1987b, 1988]. With LMR, advanced enamel erosion (mineral loss $>20 \mu\text{m}$) can be reliably measured [Ganss et al., 2005]. For dentine, the thickness of the specimens must be increased to about 800 μm to obtain an equivalent thickness of mineral similar to that in a 400- μm slice of enamel. The lower limit of detection is also raised to about 50 μm ($\pm 25 \mu\text{m}$ loss of mineral) [Ganss et al., 2009b]. Results for dentine are more variable than for enamel [Ganss et al., 2009a]. On the other hand, results for dentine erosion are not influenced by the exposed matrix, because its X-ray absorption is negligible, so this does not have to be removed before measurement. Sample preparation and measurement are time consuming but LMR is not destructive, which enables serial measurements and should be considered for monitoring progression of advanced erosion, for which it gives similar results to profilometry [Ganss et al., 2007].

The classical TMR method widely employed in caries research requires plano-parallel, thin sections (50–200 μm thick) and is therefore destructive, but it provides information on the distribution of mineral with depth, in-

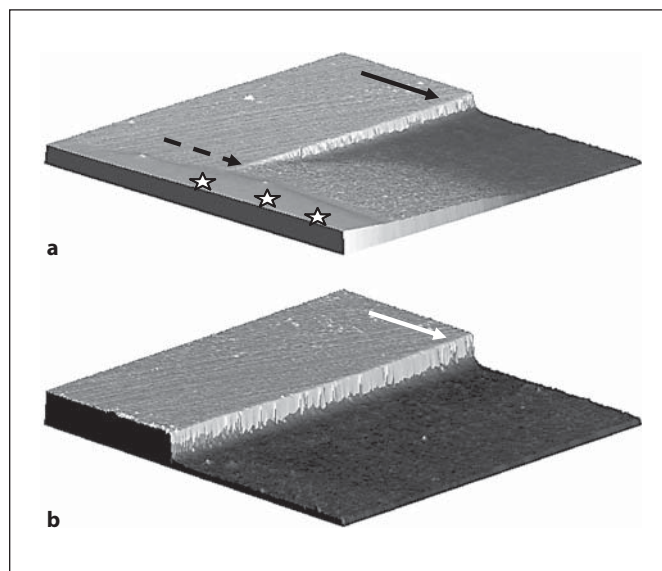


Fig. 1. Representative 3D scan (MicroProf, FRT GmbH, Bergisch-Gladbach, Germany) of an eroded dentine specimen (120 min, 0.05 M citric acid, pH 2.3). The scanning time was 5 min (scanned area $2 \times 2 \text{ mm}$, resolution 300×300 points). The specimen was wetted until scanning. Directly prior to scanning, the water was removed with a piece of tissue. **a** The area marked by white stars is due to water residues on the surface where the scan was started. The shrinkage of the organic matrix during scanning time is clearly visible, expressed in an increase in step height between the reference and the experimental area (step height at the region marked by the black dashed arrow was $0.7 \mu\text{m}$ and by the black solid arrow it was $31.1 \mu\text{m}$). However, the step height does not reflect the total loss of mineral. **b** Same specimen after removal of the organic matrix with collagenase solution. The step height distinctly increased due to the removal of the organic matrix (step height at the region marked by the white solid arrow: $50.6 \mu\text{m}$). No shrinkage occurs during the scanning procedure, indicating that the organic matrix was removed.

tegrated mineral loss and lesion depth [Arends and ten Bosch, 1992]. Since thin sections are used, this method is more sensitive to small changes than LMR. Larsen and Nyvad [1999] used classical TMR to measure erosion depth on natural surfaces. The classical method has been modified for the study of erosion [Hall et al., 1997; Amaechi et al., 1998]. In one adaptation [Amaechi et al., 1998], the most important change is a modification of the analysis software. It is possible to quantify the extent of mineral loss or gain, and the erosion depth of an eroded area by extrapolating the level of a reference area across the experimental area [Amaechi et al., 1999b]. In the modification by Hall et al. [1997, 1999], a strip extending across

the enamel and dentine on 100- to 150- μm -thick longitudinal sections of a tooth is exposed to acid, and mineral loss is determined from profiles across the eroded strip. The method is not destructive, but handling thin sections through consecutive measurements without breakage appears to be difficult.

Both TMR methods can be used for in vitro and in situ studies of both enamel and dentine [Hall et al., 1997; Amaechi et al., 1998, 1999a; Hall et al., 1999; Hara et al., 2005, 2008]. The degree of de- and remineralisation [Hara et al., 2008] can be quantified. Measurements in dentine are not influenced by the organic matrix. However, specimen preparation is demanding and time consuming, and the exposure time to X-rays can be quite long (15–65 min) [Amaechi et al., 1998; Hara et al., 2005, 2008]. Nevertheless, overall, transversal microradiography is a method providing many possibilities for evaluating erosive demineralisation [Barbour and Rees, 2004; Attin, 2006].

Other Methods

Other methods can be used to determine erosion progression in enamel and dentine. Some are at a more experimental stage and are not at all established or are semi-quantitative rather than quantitative. Of these newer methods, *quantitative light-induced fluorescence* (QLF) and *optical coherence tomography* (OCT) are non-invasive optical techniques which are potentially useful for clinical study of erosion and are discussed in detail by Huysmans et al. [2011].

QLF is based on the fact that the fluorescence of a tooth surface, which emanates from the dentine, is reduced by mineral loss in the enamel. The demineralisation increases porosity and this in turn causes scattering of incident light. On tooth surfaces irradiated with blue-green light, demineralised areas thus appear darker and can be quantified [de Josselin et al., 1995; Pretty et al., 2003b]. In vitro tests of its suitability for enamel erosion research [Pretty et al., 2003a, 2004; Ablal et al., 2009; Nakata et al., 2009] have given variable results. The applicability of QLF to determine dentine erosion has not been investigated up to now. However, an in vitro study suggests that QLF in combination with fluorescein can be used to detect carious demineralisation on root surfaces [Pretty et al., 2003b], and this approach may also be useful for erosion.

OCT is a high-resolution interferometric technique that generates subsurface images of enamel samples [Wilder-Smith et al., 2009] using near-infrared light. The images provide information about enamel thickness and

about porosity, which is related to the degree of mineral loss. However, in vivo accessibility and positioning of the probe are problematic.

Ultrasound has been suggested as a tool for the measurement of dental enamel thickness [Bozkurt et al., 2005] and may potentially be suitable for studying dental erosion [Huysmans and Thijssen, 2000]. Its non-destructive nature allows repeated measurements and its simplicity advocates its clinical use. Unfortunately, the detection limit of approximately 300 μm and the variation observed in settings simulating clinical conditions, which is only partially explained by poor probe tip positioning and poor repeatability, limit its use in erosion studies [Louwerse et al., 2004].

The novel methods for the assessment of erosion, such as QLF, OCT and ultrasound, have not yet been fully validated, and this should be done prior to their routine application, e.g. in in vivo studies. Further studies of correlations with established methods are required to corroborate that the methods measure what they purport to measure and to test their accuracy, reliability and reproducibility. As QLF is strongly influenced by enamel porosity, tests should focus on measurements of the extent of partial mineral loss, i.e. softening (nano-/microhardness and TMR), but under experimental conditions including varying amounts of tissue loss (measured by profilometry). A similar combined approach would be appropriate for OCT as this is claimed to detect both softening and tissue loss.

In the *iodide permeability test*, enamel samples are soaked in potassium iodide for a few minutes, and the amount of iodide recovered provides information on the pore volume of the test sample, and hence the level of demineralisation [Bakhos and Brudevold, 1982; Zero et al., 1990]. It is suitable only for in vitro study of erosion. This low-cost technique can be used for rapid screening of the erosive potential on enamel [Attin, 2006], but not on dentine.

Qualitative and Semiquantitative Methods

The structural changes due to different challenges (e.g. acids or anti-erosive treatment) of dental hard tissue can be studied by qualitative methods, mostly microscopy techniques, which can be used either alone or combined with quantitative measurements.

Transmitted light microscopy of thin ground sections allows the depth of erosion in both enamel and dentine, and the thickness of the demineralised matrix overlying

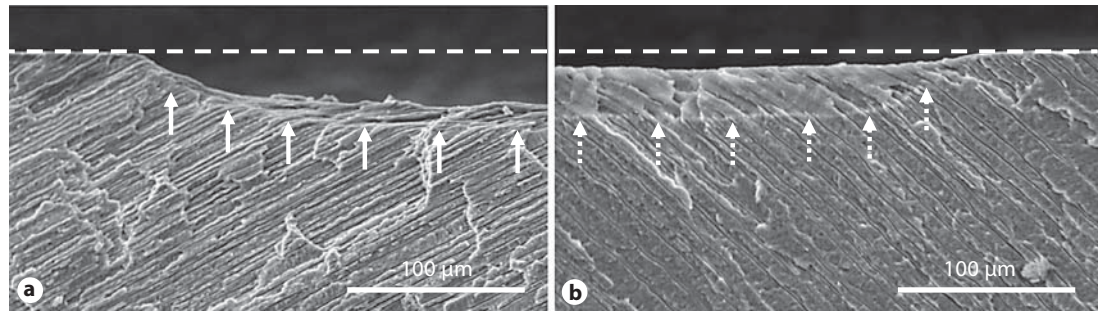


Fig. 2. Cross section of dentine specimens after immersion in citric acid (0.05 M; pH 2.3) for 10 days, 6 times per day, 5 min each. The level of the reference area is marked by dashed lines. **a** Specimen dried under ambient conditions. The organic matrix has collapsed but is still visible as a layer on the surface of the specimen. A step between the reference area and the experimental area can

be found, but this does not reflect the level of the demineralisation front (white arrows). **b** Critical point-dried specimen. The demineralised organic matrix is visible as a broad zone persisting on the surface, which is clearly demarcated against the mineralised dentine (dashed white arrows). The surface level of the demineralised tissue almost reaches the surface of the reference area.

eroded dentine to be visualised and quantified [Kleter et al., 1994; Ganss et al., 2004b]. For enamel, *polarised-light microscopy* provides much less information on erosive lesions than on caries lesions, but in eroded dentine, it discriminates between partly and fully demineralised tissue [White et al., 2001; Saunders and McIntyre, 2005].

Confocal laser scanning microscopy (CLSM) uses monochromatic laser light to collect images from specific focal planes. Images from a sequence of focal planes can be combined by computer software to generate 2D optical sections perpendicular to the focal plane or 3D images. Conclusions about changes in mineral content and morphology due to demineralisation can be drawn from the changes in reflection and scattering of light [Zentner and Duschner, 1996]. The advantages of CLSM are the high resolution (less than 300 nm in the x and y directions and 20 nm in the z direction) and fast recording of the surface topography. CLSM is mostly used to obtain qualitative information, but it has also been used to quantify erosive tissue loss [Heurich et al., 2010] and softening depth. While CLSM has been used to visualise the demineralisation of carious dentine [Banerjee and Boyde, 1998; de Carvalho et al., 2008], its use for studying dentine erosion has yet to be elucidated.

Transmission electron microscopy has been used to study the ultrastructure of dentine erosion by acetic acid [Selvig, 1968], but otherwise it has mainly been used in erosion research to investigate the impact of acids on the salivary pellicle [Hannig and Balz, 1999, 2001].

SEM, in contrast, has proven to be essential for studying ultrastructural changes associated with erosion in both enamel and dentine. Conventional SEM uses a high

vacuum, which causes drying artefacts, and the specimens must be coated with metal (e.g. gold) or carbon to avoid charging. Therefore, the effects of treatments cannot be studied by conventional SEM, as serial measurements are not possible. This limitation can be partly overcome using environmental SEM, which uses a lower vacuum and allows observation under humid conditions, so specimens need not be dried or coated. The resolution is lower than that of conventional SEM, but environmental SEM might prove useful in future studies, especially where repeated examination is needed.

For the examination of acid-treated enamel surfaces, air-drying is adequate for detecting the presence of changes (increased roughness) and for studying etching patterns, but for closer study, special steps are necessary to avoid re-precipitation artefacts and to prevent collapse of the fragile, partly demineralised surface [Eisenburger et al., 2004]. The surfaces of eroded dentine specimens have been studied after air-drying [Meurman et al., 1991], but it is preferable to use more careful preparation techniques to avoid collapse of the demineralised organic matrix at the surface. Although no technique can totally prevent shrinkage of demineralised dentine [Carvalho et al., 1996], freeze-drying or critical-point drying can minimise shrinkage artefacts (fig. 2).

To study subsurface effects of erosion, it is necessary to prepare internal surfaces by fracturing or sectioning. In the study of enamel erosion, fractured surfaces are highly irregular and of limited value. However, the pattern of mineral loss can be visualised by backscattered or secondary electron imaging on highly polished sections [Shellis et al., 2005; Schlueter et al., 2009], and subsurface

porosity can be visualised by replica techniques [Eisenburger et al., 2004]. In the study of dentine erosion, the junction between demineralised and intact tissue can be visualised on fractured surfaces or polished sections and thus the linear or areal extent of structural changes can be measured [Schlueter et al., 2010]. Differences in the angulation of specimens in SEM influence such measurements; therefore careful specimen alignment is essential.

Scanning probe microscopies, such as *AFM* and *scanning tunnelling microscopy*, can reach a resolution at a molecular or atomic level. A major advantage of AFM is that it can be performed under ambient conditions (in air or liquids) as well as under vacuum, so artefacts can be minimised or avoided and serial measurements are possible. Using AFM, demineralisation can be investigated in detail in both enamel [Finke et al., 2000] and dentine [Habelitz et al., 2002; Ma et al., 2009], and demineralised dentine can be visualised at a level at which single collagen fibres can be recognised [Habelitz et al., 2002]. In tapping mode, AFM can also measure height differences at atomic level, thus it is suitable for quantifying early surface changes. As already mentioned above, AFM is often used to identify the sites of nano-indentations. Because of its high resolution, a disadvantage of AFM is that it is very time consuming. Scanning a field of 0.5×0.5 mm takes approximately 60 min, and it is necessary to scan various areas on the surface in order to find a representative one [Barbour and Rees, 2004].

SEM can be coupled with *energy-dispersive X-ray spectroscopy*, which provides information about the composition of a specimen from the characteristic X-rays emitted under electron bombardment. Energy-dispersive X-ray spectroscopy can thus be used to determine quantitative changes in elemental composition on both eroded surfaces and cross sections. It can also be applied to detect the deposition of active agents from therapeutic treatments at the tooth surface [Schlueter et al., 2009; Wiegang et al., 2009; Ganss et al., 2010] and beneath the sur-

face from concentration profiles of the element in cross sections [Schlueter et al., 2009; Ganss et al., 2010].

Secondary ion mass spectroscopy allows semiquantitative analysis at the part-per-billion level of the elemental, isotopic or molecular composition of specimen surfaces, although with some surface damage [Barbour and Rees, 2004; Attin, 2006]. Secondary ion mass spectroscopy has been employed to study enamel erosion [Barbour and Rees, 2004] and fluoride uptake by early erosive lesions [Fowler et al., 2009], but it has not been applied to dentine erosion to our knowledge.

Conclusions

Although all existing methods have limitations, in combination they can adequately fulfil most of the needs of research into dental erosion. For early erosion, chemical analyses of mineral release and enamel surface hardness have been used. Advanced erosion has been mostly analyzed by surface profilometry and microradiography. Morphological changes due to erosion have commonly been studied by SEM.

The applications and limitations of each method have been determined mostly by experience; therefore, traditional methods are well understood and have been validated. However, there is a need for further development, evaluation and, in particular, validation of novel methods, since such methods have sometimes been used without knowing what they actually measure. Validation of these methods could improve the study of erosion at all stages, not only in vitro or in situ, but also in clinical settings.

Disclosure Statement

The authors declare that they have no conflict of interest in relation to this paper.

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