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Review Article

In Vitro Techniques of Dental Erosion Characterisation; A Brief Review

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Abstract

Aim and Objective: This review aims to explore different procedures and techniques used in dental research for assessment of dental erosion *in vitro*.

Data: Peer reviewed scientific articles.

Sources: Medline, Web of Science and Google Scholar searches and manual searching.

Study selection: *In vitro* or laboratory based techniques were included only.

Conclusions: In this review several macroscopic and microscopic techniques have been gathered to measure dental erosive tooth wear both in laboratory research and *in situ*. This review presents conventional research techniques which are either standard or relatively innovative practices that are being explored for their potential.

Keywords: *Dental Erosion; Dentine; Enamel*

Introduction

Dental hard tissues undergo histological changes as a result of erosion. Because of the histological differences between dentine and enamel, these two tissues respond differently to erosion [1]. When assessing dental erosion, the researchers should bear in mind that the choice of the technique depends mainly on the stage of erosion, the expected modifications in the structure of the eroded tissue, and the tissue studied. According to a previous report [2], profilometry was the most common quantitative technique to assess dentine and enamel *in vitro, in situ*, and in clinical studies. The next commonly applied technique was quantitative evaluation of surface hardness (enamel) and microradiography (dentine). Regarding qualitative studies, the authors reported scanning electron microscopy (SEM) to be the most frequent technique used for qualitative assessment of erosion in dentine and enamel. This paper has been designed to produce a detailed outlook for assessment and characterization of dental erosion.

Chemical analysis of dissolved materials

Chemical analysis has been employed to investigate erosion by measuring the concentrations of calcium and phosphate ions that are released into the dissolving solution. Similarly, the pH and uptake or release of other constituents, including fluoride and magnesium ions have been measured [3]. Calcium analysis is performed using an ion-selective electrode [4]. However, the shortcoming of this technique involves errors caused by complexation of some acids [5]. To overcome such errors, the atomic absorption spectrometry has been suggested. It has proven to be a reliable and sensitive method for analysing calcium [2], as interference by other solutes are circumvented. Another advantage of the atomic absorption spectrometry is the possibility of using it to quantify erosion in both dentine and enamel [4].

In an *in vivo* study, Young., *et al.* [6] used extra-oral erosive challenge to analyse mineral release in healthy human teeth. However, this technique is limited in that the presence of saliva precludes erosive challenge. In addition, with this method, it is impossible to determine possible mineral gain or the occurrence of physical and morphological modifications.

Surface hardness

Surface Microhardness is measured with the use of a Knoop or Vickers diamond indenter, which determines the resistance of a substance to the indenter. The use of surface Microhardness is more appropriate during the early stages of erosion [2]. It also has the added advantage of obtaining accurate information about erosion in its early stages and of being relatively inexpensive. It is probably for these reasons that this method is commonly used in dentistry [7].

The depth of penetration by the Knoop and Vickers diamond indenters vary, with the Vickers diamond indenter having a greater penetration, estimated at 5 µm as compared with 1.5 µm for the Knoop diamond indenter [8]. These differences arise due to the varying loads that are impressed by the indenters on dental tissue, typically 50 and 200g for the Knoop and Vickers diamond indenter, respectively. Hence, it can be implied that sensitivity is higher with the Knoop indenter for changes that involve the most superficial layers of eroded tooth.

Surface hardness tests have the advantage of detecting modifications in the surface hardness of enamel within a few minutes of exposure to an erosive substance [4]. Nevertheless, these tests cannot clearly delineate the indentation boundaries in advanced erosion. Furthermore, when certain substances such as fluorides are deposited on tooth surface, these tests may not accurately determine surface hardness. This is because the application of fluorides causes the formation of precipitates on dental surface, making it difficult for indenters to penetrate the surface of the test material [2].

Further research shows that surface hardness tests may not be appropriate for assessing dentine erosion. Herkströter., *et al.* [9] demonstrated that demineralized dentine sustained changes in length (of approximately 30% reduction) within 2 hours after indentation. The authors attributed this change to retraction of the exposed matrix after compression and shrinkage, which was caused by desiccation.

Nano indenters have also been successfully used in assessing enamel erosion [10]. Nano indentation is a newer technique that uses the same principle as microhardness indentation although at a smaller scale. This technique usually gives results as hardness and reduced elastic modulus in Pascal's (Nm-2). While microhardness techniques usually yield indentation depths of micrometres or tens of micrometres in healthy enamel, Nano indentation yields depths in sub-micrometres [11]. Nano indenters are better in that they penetrate dental tissue less deeply than microhardness indenters [12] and hence, they can better detect minimal changes on tooth surface. They are limited because they have to be combined with atomic force microscopy (AFM) to assess dentine erosion [13].

Surface profilometry

Surface roughness is generally measured by using a profilometer, which can be contact (uses a diamond tip) or optical (uses a light beam). In contact profilometry, a diamond tip of fixed radius 1.5 - 2.5 mm is usually used [14]; however, the shape of the tip can vary [15]. While chisel-point (0.25 µm x 2.5 µm) tips may be used for detecting bumps in a surface, conical tip are almost exclusively used for measuring surface micro roughness, with the load ranging from 0.05 to 100 mg [16]. An analogue/digital signal is generated by the vertical movements of the stylus, as it is dragged across the surface of the material. A recording speed of approximately 1 mm/s is usually maintained in order to minimize the effect of external vibrations and electrical interferences on the accuracy of the lateral resolution [16]. The vertical resolution can be as low as 0.1 nm for smooth surfaces or as high as 1 nm for rough surfaces. However, because the stylus is practically in constant contact with the surface that is being measured, there is a risk of the diamond tip causing damage to the specimen [7].

Several drawbacks of stylus profilometers can be overcome by laser profilometers, as they do not directly touch the surface of the specimen. In this technique, a light spot is directed at the surface of the specimen, typically below 100 mm in diameter. Surface topography can be profiles either by measuring the deflection of the laser beam, or (with white light) by utilizing the confocal principle [17]. A main limitation of laser profilometry is that the results can be affected by colour and transparency [16]. It is usually necessary to record a polyvinyl siloxane impression of the sample, which is then scanned by the laser profilometer to overcome translucencies on the surface.

The surface colour of the specimen, however, affects the laser profile. In studies using laser profilometers at wavelengths of 785 nm, it was demonstrated that specimens with darker colours had a higher roughness. Further, the authors De Long., *et al.* [18] showed if an impression material absorbed colour at a wavelength similar to that of the laser, then the surface will not be scanned.

Other parameters are also used to measure tooth surface changes (Table 1). These variants usually measure the average distance between the highest peaks and valleys of the profile and can also truncate some outlying peaks and valleys, depending on the engineering system employed for measurement [16]. Although these systems measure the effects of surface change in the best possible way, it is very challenging for inexpert or unqualified personnel to interpret the tabular form of the parameters.

Parameter	Description
R_a	Arithmetic average of all deviations of the profile from the centreline
R_q	Geometric average of all deviations of the profile from the centreline
R_z	Mean of five roughness depths of five successive sample lengths of the profile
R_{max}	Largest of the five roughness depths
R_p	Height of the highest point above the centreline within the length of the profile
R_v	Depth of the lowest point below the centreline within the length of the profile
R_{pm}	Mean value of Rp in five consecutive sample lengths
R_t	Vertical height between the highest and lowest points of the profile within the evaluation length
R_{tm}	Mean value or Rmax in five consecutive sampling lengths
R_{3z}	Similar to Rz except the individual roughness depth is the depth from the highest peak to the third lowest valley within the sample length

Table 1: *Common amplitude parameters for surface measurement [adapted from Field., et al. (2010) (19)].*

Microradiography

In this technique, X-ray beams are directed toward an enamel section, and a photographic plate [11] is used to record the penetrating radiation. The mineral content of enamel is thus quantified by measuring the attenuation of X-rays transmitted through a section of the tissue by comparing it with a reference value. There are three generations of micro radiography [7]. These include: longitudinal micrography, which is used to assess erosion, abrasion, and erosion-abrasion in dentine and enamel *in vitro* and *in situ* [20]; transverse micrography, which is widely used in assessing carious lesions but has been modified for studying erosion [21]; and wavelength-dependent micrography, which is used to measure mineral content in whole teeth [22].

Other methods

Other methods have been used by researchers to assess erosion in dentine and enamel. These include quantitative light-induced fluorescence and optical coherence tomography, which are non-invasive techniques. Schlueter., *et al.* and Bozkurt., *et al.* [2,23] suggested the use of ultrasound for assessing enamel thickness, while Huysmans and Thijssen [24] suggested that it may be a potential tool for studying dental erosion. However, the above-mentioned techniques are novel and their use has not yet been validated in clinical practice [2].

The iodide permeability test has also been used in assessing erosion. According to Attin [25], this technique is cheap and it can be used for the rapid detection of erosion in enamel but not dentine.

Qualitative and semi-quantitative methods

Changes to dental tissue that are brought about by erosive agents can be studied by qualitative methods. In most cases, microscopy, either alone or in combination with quantitative techniques can be employed to study erosion in dentine or enamel.

Transmitted light microscopy

In transmitted light microscopy, erosive lesion of dentine and enamel can be visualized and quantified [26]. Saunders and McIntyre [27] found that polarized-light microscopy detected changes on erosive enamel to a lesser extent than on carious enamel. Conversely, it could accurately differentiate between partly and completely demineralized tissues in the case of eroded dentine.

Confocal laser scanning microscopy

This technique is mostly employed to obtain qualitative data by using monochromatic laser light. It has been used to quantify loss of dental tissue brought about by erosion and softening depth [2]. Although it is used in quantifying demineralisation in carious dentine, its use in dentine erosion has not yet been determined [2].

Transmission electron microscopy

Researchers have used transmission electron microscopy to assess the impact of acids on the salivary pellicle [28]. It has also been used *in vivo* to study changes caused by early microbial colonization of human enamel [29].

Scanning electron microscopy

Scanning electron microscopy (SEM) is used to qualitatively estimate surface alterations after erosive attacks. It has also been used to evaluate the efficacy of salivary acquired pellicle to protect underlying enamel surface from acidic attacks [28] to show superficially deposited precipitates that resulted from mineral dissolution with acids [30]. This test can be performed on polished and unpolished surfaces after gold-sputtering, and the severity of surface alteration can be graded by using individually adopted scales.

In enamel, acid attacks due to immersion of specimens in erosive solutions cause the surface to have an etching pattern, and enamel prisms are exposed to an extent that depends on the severity of the erosive challenge. In dentine, treatment with an acidic solution may cause the dentin tubules to open [31]. Common scanning electron microscopes could cause the specimens to lose moisture due to necessary preparation of the specimens for the SEM investigation. The loss of moisture may cause additional changes in the eroded surface. To prevent collapse of the fragile eroded enamel surface structure, freeze drying of samples was suggested [32]. Precipitates formed by dissolved enamel mineral may block the enamel surface so that the eroded enamel prism structure might not be seen with SEM. Eisenburger., *et al.* [32] recommended that before removing samples from the acidic bath, the acid should be neutralized to decrease the risk of art factual re-precipitation. The delicate surface should be impregnated with methacrylate or dentin adhesives to permit the production of resin replicas [31]. After the enamel samples has been completely dissolved with hydrogen chloride, the resin replicas could then be examined with SEM, providing insight into structural surface and subsurface alterations.

Although SEM has been demonstrated to be an efficient tool for studying the ultra-structural changes associated with erosion in enamel and dentine [33,34], the investigation is limited because it causes drying artefacts, and specimens must be coated with metal or carbon to prevent charging. Serial measurements are not possible with conventional SEM and hence, the effects of treatments cannot be studied using this test. Environmental SEM partially overcomes this limitation, as it permits the observation of specimens under humid conditions [2]. Thus, specimens do not have to be dried or coated. While the resolution is lower than that of conventional SEM, environmental SEM is potentially useful for future investigation, particularly in cases where specimens have to be examined several times [2].

Scanning probe microscopies

Some examples of scanning probe microscopies include atomic force microscopy (AFM) and scanning tunnelling microscopy, which can give resolutions at a molecular or atomic level [2]. Atomic force microscopy is advantageous in that artefacts can be decreased or circumvented and demineralisation can be assessed in both enamel [12] and dentine [35].

Energy-dispersive X-ray spectroscopy

When used in combination with environmental SEM, energy-dispersive X-ray spectroscopy can provide data about the constitution of a specimen from the nature of the X-rays that are given off after bombardment [2].

Secondary ion mass spectroscopy

This is a semi-quantitative technique which has been used to study enamel erosion [7]. However, there are no data in dental research that describe the use of this method in assessing dentine erosion.

Conclusions

A number of techniques have been discussed in this review for the analysis and characterisation of dental erosion from numerous scientific studies. The paramount recognised techniques to study dental erosion according to literature are microradiography, profilometry, micro indentation, chemical analysis and SEM. For the investigation of initial stages of dental erosion, it is desirable to use extra sensitive techniques such as microindentation although a further sensitive and advanced technique of analysis using nanoindentation is likely to find increasing application in the field of scientific research. Profilometry and microradiography are readily applicable to dental

Erosion at more advanced stages. Literature indicates another technique named as Chemical analysis which has the distinguished advantage for the study of dental erosion from the initial to extreme advanced stages, but it also has a disadvantage that it is generally an indirect method of dental erosion measurement. SEM is also occasionally used in such studies but due to its difficulty in quantifying the degree of erosion it is instrumental mostly as a supporting technique only with techniques which do not allow direct observation of the eroded surface, such as microindentation. In this case, the quantifiable nature of AFM imaging promotes greater use of this technique in erosion studies. Recently SIMS, OCT and QLF have been introduced for the investigations of dental erosion, these may attract researchers in the future.

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