Real-time measurement of *in vitro* enamel demineralization in the vicinity of the restoration–tooth interface

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An X-ray attenuation method using photon counting (scanning microradiography) is presented for the real-time study of *in vitro* demineralization of dental tissues in the vicinity of the restoration–tooth interface. By repeated measurement of mineral content profiles during the course of demineralization, the pattern of lesion development and the rate of mineral loss can be studied. The method is illustrated by comparison of enamel demineralization near a polyacid-modified composite resin restoration, near a bis-GMA/TEGMA composite resin restoration, and in an unrestored control. The method has potential for study of the influence of restorative materials on susceptibility of tooth tissue to demineralization.

1. Introduction

The replacement of dental restorations is commonly needed for treatment of secondary caries at the interface with dental tissues. An important approach to reduction of secondary caries is the development of fluoride-releasing restorative materials [1]. The use of model systems, *in vitro* and *in situ*, for comparison of the demineralization of tissues surrounding these materials and non-fluoridated analogs has been reviewed [2, 3]. The distribution of mineral loss has been investigated by microhardness measurements [4], polarized light microscopy [5–7], and contact microradiography [8, 9]. For examination of tissue deep to the tooth surface, microhardness measurements and polarized light microscopy give information about one time in the course of demineralization, at the point when the section is cut. Microradiographic techniques have the advantage that repeated measurements of the distribution of mineral in a section can be made during the course of de- or remineralization. In such experiments, a section cut from a sound tooth is coated in acid-resistant varnish leaving a window on the natural surface exposed to the de- or remineralizing solution.

In scanning microradiography (SMR) (Fig. 1) [10, 11], a section can be kept in an aqueous medium throughout the experiment, so that dehydration and shrinkage of the tissue are avoided. For *in vitro* studies, de- and remineralizing solutions can be pumped through the environmental cell in which the section is mounted. For measurement of mineral distribution, the section is stepped in one or two dimensions across a fine X-ray beam (10–20 μm) and the transmitted photons counted for a fixed time at each point. Photon counting has the advantage over film emulsion (used in contact microradiography) of having a wider dynamic range, greater sensitivity to small intensity changes and potentially greater accuracy [11]. The section can also be accurately repositioned ($\pm$ 5 μm) so that repeated measurements of the same point or area can be made during the course of an experiment.

For investigation of the restoration–tooth interface, the use of a thick section ($\sim$ 500 μm) provides a relatively rigid base for the restoration, so that risk of disruption of the interface is reduced. For SMR, the target can be chosen so that the X-ray energy is appropriate for measurement of a thick section.

The purpose of this work was to demonstrate the use of SMR for the study of *in vitro* demineralization in relation to the restoration–tooth interface, during acid attack. Two commercially available materials, a polyacid-modified composite resin and a bis-GMA/TEGMA composite resin, were used as illustrative examples.

2. Materials and methods

2.1. Preparation of tooth specimens and restoration application

An erupted, caries-free, lower third human molar, which had been stored in 70% ethanol/30% water for 3 years since extraction, was immersed in distilled water for one day. A $\sim$ 4 mm thick transverse slice was cut (Microslice 2, Malvern Instruments, UK) from the crown and divided mesiodistally to produce two blocks. These were immersed in distilled water for a further 2 days. A restoration (about 3 mm × 2 mm × 2 mm) was placed on the coronal surface of each block so

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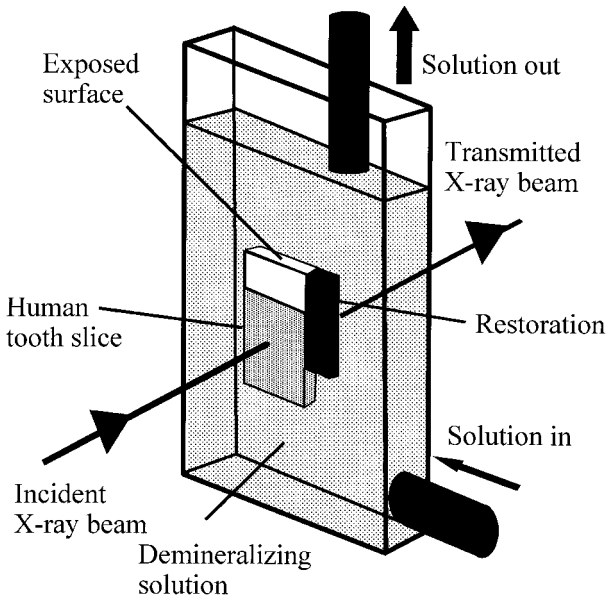

Figure 1 Schematic of SMR cell.

as to cover the enamel–dentine junction and extend to the natural surface (Fig. 2). A polyacid-modified composite system (Compoglass, Vivadent) was used for one restoration and a bis-GMA/TEGMA composite resin restorative system (made by another manufacturer) for the other. The pre-treatment of the tissue surface and placement of the restoration was in accordance with the respective manufacturer's instructions. Each block was coated with three layers of acid-resistant nail varnish (Rimmel, London, UK), leaving exposed the natural surface (enamel and restoration), and stored separately in 2.5 ml distilled water at 35 °C for 1 week. From each block, a $\sim 500\,\mu m$ thick buccolingual section was cut perpendicular to the restoration–tooth interface through the restoration and tooth (Fig. 2). The cut surfaces were coated with three layers of acid-resistant nail varnish so as to leave a window straddling the restoration–enamel interface on the natural surface. An additional control section, cut outside the restoration and parallel to the test sections, was varnished to leave a window on the natural enamel surface. The three sections were mounted in individual environmental cells which were then filled with deionized water.

2.2. SMR system and demineralizing procedure

A detailed description of SMR with multiple specimen capability has been given previously [10]. In this experiment, a Hilger and Watts Y33 microfocus X-ray generator ($100\,\mu m \times 100\,\mu m$ foreshortened source, Ag target, 35 kV, 2 mA tube current) was used. The aperture comprised two perpendicular tantalum crossed slits ($15\,\mu m$ gap), positioned 30 mm from the source, to give a $15 \times 15\,\mu m^2$ X-ray beam. X-ray photons were counted by a high purity germanium solid state detector. Standard readings were repeated every ~ 20 mins so that compensation could be made for any long term drift in the system. Taking the mass attenuation coefficient for enamel mineral at 22.1 keV as $4.69\,g^{-1}\,cm^2$ [11], the projected mass of enamel mineral (or, for the restoration, the equivalent projected mass of mineral) was determined for each measurement.

The sections were mounted so that they could be

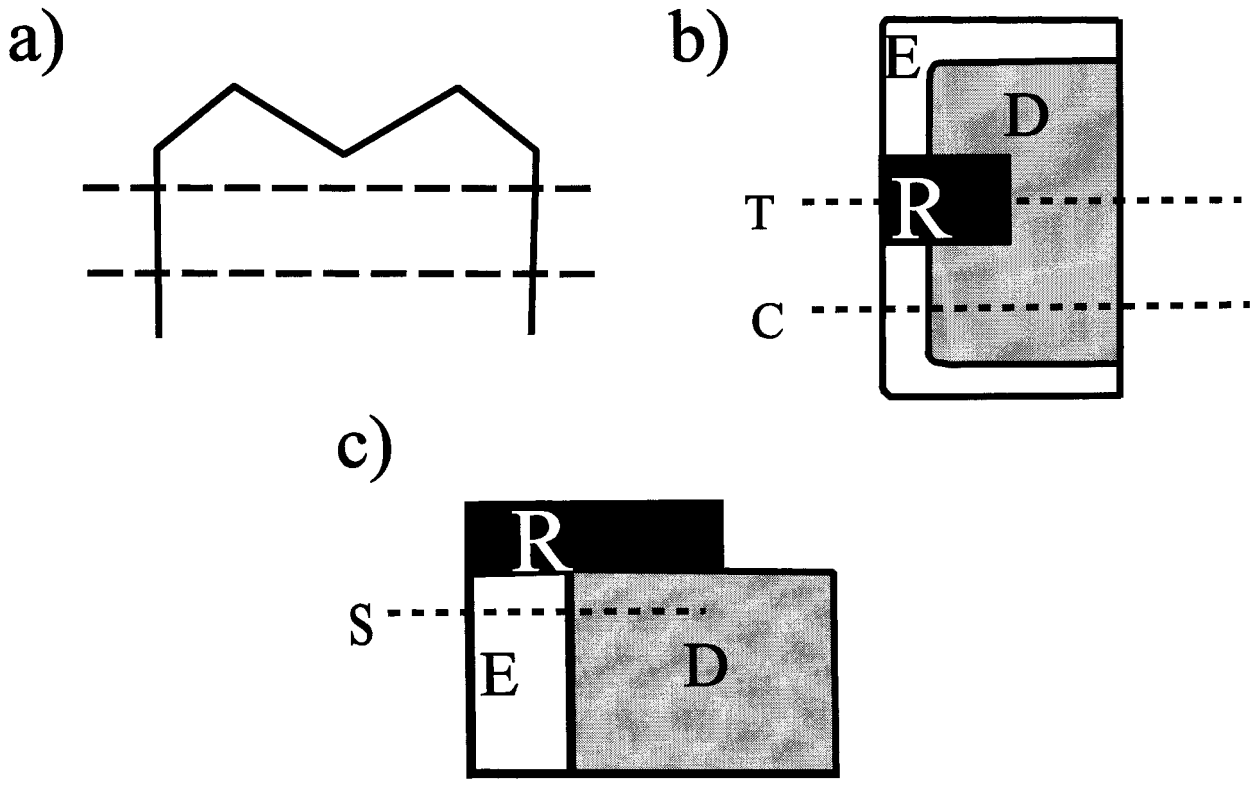

Figure 2 Schematic of section preparation. Restoration (R), enamel (E) and dentine (D). (a) Transverse cuts through tooth crown. (b) Tooth block with restoration (from above) showing position of test (T) and control (C) sections. (c) Test section with a scan line (S).

scanned over 2.5 mm parallel to the restoration-tooth interface in the test sections and the equivalent surface in the control (Fig. 2).

Before the start of demineralization, an area was scanned with 50 scans parallel to the interface at 80 μm separation (50 × 50 μm steps, 2 s counting time) in each test section. The scan line closest to the restoration and entirely in tooth tissue, was identified. (This line was subsequently used as the second of the lines scanned during demineralization: see below.) A comparable area of enamel in the control section was also scanned, but with 70 μm steps.

A demineralizing solution (100 mmol l^{-1} acetic acid, buffered to pH 4.5 with NaOH) was then continuously pumped through the three environmental cells in parallel. The scanning sequence for the sections was: test 1 (five scans of 100 × 10 μm), test 2 (as test 1), control section (five scans of 100 × 50 μm), test 1 (five scans of 50 × 50 μm), test 2 (as test 1), control (five scans of 50 × 70 μm), test 1 (five scans of 100 × 10 μm) . . . , etc. All scans started near the natural surface and were separated by 800 μm. For the test samples, the first scan was in the restoration, the second was that previously identified as being outside the restoration but < 160 μm from the interface throughout its length. The counting time was 10 s at all points and the scanning sequence cycled every 10.9 h.

After 2 weeks, when substantial demineralization had occurred in all of the sections, the solution in each cell was replaced by water and the initial area scans repeated. In view of the enhanced mineral loss observed for the composite resin test section (see Results), an additional area scan of this interfacial region was made to examine for gap formation (10 μm line separation).

The mineral loss after each scan was calculated by integrating the projected mass along each scan, subtracting from the original value and plotting as a function of time.

3. Results

In the area scans, the regions of restorative material, enamel and dentine were clearly discriminated, with both restorative materials having higher X-ray attenuation than enamel. The natural gradient in mineral content of enamel with depth from the surface was also observed.

The scans made during demineralization showed the development of the lesions with time. In the control section, all scans showed the occurrence of slow demineralization, with maintenance of a mineralized surface layer (Fig. 3b). A similar pattern of lesion development was found in both test sections along the lines most distant (> 2 mm) from the restoration–tooth interface.

In the vicinity of the interfaces, lesion development differed. At < 160 μm from the interface with the polyacid-modified composite resin, the surface layer was more pronounced (Fig. 3a), but at < 160 μm from the composite resin restoration, the surface layer was less pronounced and there was more rapid progression of the demineralizing front (Fig. 3c). The corresponding rates of integrated mineral loss increased in the order: polyacid-modified composite resin test < control

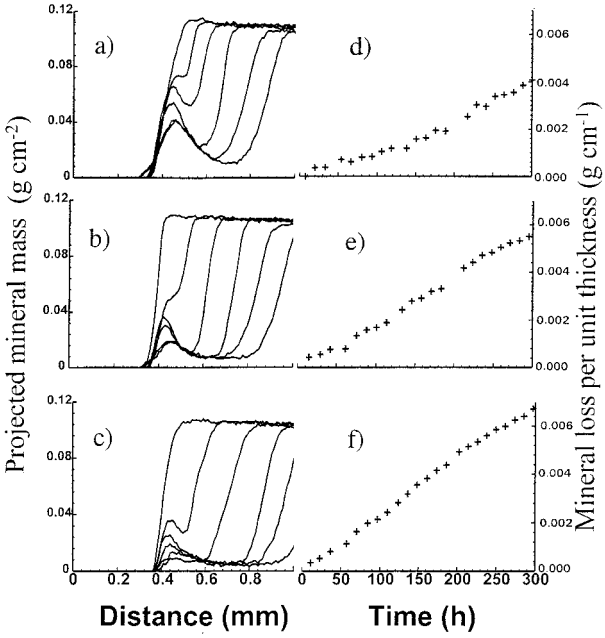

Figure 3 (a, b, c) Profiles of projected enamel mineral mass (at 10 μm steps) at 10.9 h intervals during demineralization. (d, e, f) Change in integrated mineral loss with time. (a, d) <160 μm from interface with polyacid-modified composite resin; (b, e) Control; (c, f) <160 μm from interface with bis-GMA/TEGMA composite resin.

< composite resin test section (Fig. 3d,e,f). For test and control sections the initial rate of mineral loss (to about 70 h) was slower than that observed later.

With increasing distance from the interface, the patterns of mineral loss moved towards that observed at > 2 mm for both test sections.

The final area scans at 80 μm intervals between lines confirmed that the final patterns of demineralization seen in the line scans were representative of the regions studied. The additional scan of the composite resin test section (10 μm intervals between lines) showed no evidence of a gap in the interfacial region.

4. Discussion

The SMR results show the real-time development of *in vitro* enamel demineralization in the vicinity of the restoration–tooth interface for two materials. The repeated measurements of the distribution of mineral during demineralization allow both the rate and pattern of mineral loss to be studied (Fig. 3).

The slow initial rate of mineral loss from both test and control sections appeared to be associated with the phase of demineralization during which the surface layer was established (Fig. 3). The mechanisms of subsurface demineralization are imperfectly understood [12], but the availability of fluoride affects formation of the surface layer [13].

The reduced demineralization of enamel in proximity to the polyacid-modified composite resin (Fig. 3a,d), relative to the control enamel (Fig. 3b,e), cannot be attributed to the accumulation of fluoride in the solution during demineralization because the solution was constantly refreshed. However the variation in mineral loss with distance from the restoration might be explained by release of fluoride during the preliminary storage, diffusion in the static water and uptake by

enamel. This could be tested by comparison of specimens stored in static and flowing water.

The reason for the enhanced mineral loss associated with the composite resin restoration (Fig. 3c,f) is uncertain, since there was no radiographic evidence of an interfacial gap after demineralization. Moreover the slow initial demineralization that was observed would be inconsistent with leakage at the restoration–enamel interface. However, we note that it has been suggested [4] that a similar finding of relatively increased demineralization at 0.2 mm from bis-GMA resin restorations in an *in vitro* study might be explained by local demineralization from acid residues in bis-GMA resin.

Although *in vitro* models do not replicate the complex conditions in the mouth, they provide a powerful approach to understanding fundamental mechanisms because individual variables can be rigorously controlled. For example, SMR systems allow the use of customized de- and remineralization regimes by switching the solution pumped over the section. Although the realtime measurement of mineral content is limited to *in vitro* studies, SMR can also be used for measurement of sections from *in situ* studies, following each episode of exposure in the mouth.

A further possibility is to use the recently developed [14] parallel integrating SMR for direct measurement of changes in integrated mineral loss with time. In this, the attenuation of a 10–20 μm X-ray beam *normal* to the natural surface and parallel to the restoration–tooth interface would be measured with time. This beam would pass through enamel and dentine (vertical for the specimen shown in Fig. 1), and could be repeatedly scanned over a line normal to the restoration-tooth interface, thus giving information about the integrated mineral loss with time as a function of distance from the interface. The advantages of using parallel integrating SMR are that more precise measurements of integrated mineral loss would be obtained and that there would be no need to cut the restored specimen, thus obviating the possibility of sectioning damaging the adhesion at the interface. A disadvantage would be that profile information would not be obtained.

5. Conclusions

A scanning microradiographic (SMR) method is presented for the real-time study of *in vitro* demineralization of dental tissue in the vicinity of the restoration-tooth interface, during acid attack. By repeated measurement of mineral content profiles during the course of demineralization, the pattern of lesion development and the rate of mineral loss can be studied.

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