Short communication

A minimally destructive technique for removing the smear layer from dentine surfaces

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ABSTRACT

Objectives: To develop a minimally destructive technique for removing the smear layer produced by cutting and polishing specimens of dentine prepared for use in experimental studies, e.g. on occlusion of dentinal tubules by oral health products. The aim was to avoid the damage caused by conventional techniques utilising short exposures to solutions with very low pH.

Methods: Two acetate buffers, pH 5.5, containing different concentrations of calcium and phosphate, with log(ion activity product with respect to hydroxyapatite) (pIHA) of 55 or 56, were tested on slices of dentine using scanning electron microscopy (SEM).

Results: A solution which, from previous work, was slightly undersaturated with respect to dentine mineral, with a pIHA of 56, was found to remove smear layers produced by cutting and/or polishing after 15 min. However, to reliably remove debris occluding the tubules an exposure time of 2 h, followed by brief ultrasonication, was necessary. After 2 h treatment with this buffer, only a small amount of demineralization of the surface was detectable by SEM, while calcium and phosphorus were detectable by X-ray dispersive spectroscopy.

Conclusion: It is possible to remove smear layers, and to open dentinal tubules, by a reasonably short exposure to an acidic buffer which is undersaturated with respect to dentine mineral.

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1. Introduction

The permeability of dentine is believed to be an important factor in hypersensitivity, since it is generally accepted that fluid movement within tubules, caused by mechanical, thermal or osmotic stimuli, is thought to be responsible for the stimulation of intrapulpal nerve endings. Dentine becomes permeable when the outer ends of the tubules are opened, following exposure of the dentine surface through gingival recession and subsequent wear processes. In experimental studies related to dentine hypersensitivity, test specimens often consist of slices of dentine cut transverse to the tubules. In such specimens, the cut surfaces are covered by a smear layer – a tenacious layer of debris produced by the cutting processes which is not removed completely by mechanical polishing. To study, for instance, permeability, it is necessary to remove the smear layer. This is conventionally achieved by treating the specimens with acidic solutions. However, because the treatment tends to be relatively aggressive, it can lead to significant demineralization of the
underlying dentine surface, resulting in formation of a layer of demineralized collagenous matrix at the surface and removal of peritubular dentine. When the aim of a study is to determine the extent to which tubules are occluded by oral health products designed to reduce hypersensitivity, the increase in apparent tubule diameter caused by loss of peritubular dentine is a potential source of error in quantitative determinations of the extent of occlusion.

The aim of the present study was to develop a technique for removing the smear layer with minimal damage to the underlying dentine. The approach was based on previous semi-quantitative estimates of tooth tissue solubility.

2. Materials and methods

All the reagents were purchased from AWV (Poole, Dorset, U.K.) or from Sigma–Aldrich (Poole, Dorset, U.K.).

Dentine specimens were prepared from the crowns of extracted molars and premolars and stored in 70% ethanol, sometimes after prior treatment with sodium dichloroisocyanurate solution containing 10,000 mg/L available chlorine (HazTabs; Guest Medical, Edenbridge, Kent, U.K.) for 24 h. Appropriate ethical permission to use extracted teeth was obtained from North Somerset and South Bristol Research Ethics Committee. Serial transverse slices, approximately 0.5 mm thick, were cut through the tooth crowns using a diamond-coated annular saw blade (Microslice; Metals Research Ltd., Royston, Herts, U.K.). Slices containing dentine, and located occlusal to the pulp horns were selected for study, as these contained large areas in which the dentinal tubules were oriented perpendicular to the section surface. In preliminary experiments, slices were used without further treatment and the object of study was the thick smear layer left after sectioning. In subsequent experiments, the slices were polished with a slurry of 1200 grade silicon carbide powder in water.

Two test solutions were prepared. Both were based on 100 mmol/L acetate buffer, pH 5.5, to which were added calcium and phosphate in equimolar concentrations to produce defined values of the $\log(\text{ion activity product, } I)$ ($p_I$) with respect to hydroxyapatite ($p_I^{HA}$). In initial studies the concentrations of calcium and phosphate were 6.55 mmol/L and 4.875 mmol/L respectively in the solutions with $p_I^{HA} = 55$ and $p_I = 56$, and were added in the form of calcium carbonate and phosphoric acid. In later experiments, CaCl$_2$·2H$_2$O and KH$_2$PO$_4$ were employed and the concentrations adjusted to 6.67 and 4.95 mmol/L respectively. Ion activity products were calculated using an ion speciation programme. For comparison, the effects of treatment with 6% citric acid solution for 15 s and 30 s were examined.

In the experiments, one half of each approximately circular slice was coated with clear nail varnish. The slice was then immersed in the test solution for the required time and then washed in deionised water and dried. In some experiments, the slices were ultrasonicated after the initial exposure whilst still in the test solution (Pul 55; Kerry Ultrasonics, Hitchin, U.K.: 100 W power output, frequency 38 kHz). Half of the exposed area was then coated with nail varnish and the slice was placed in domestic bleach (Domestos; Unilever, Port Sunlight, U.K.: approximately 5% sodium hypochlorite) overnight and then finally washed in deionised water. The slices were allowed to air-dry and the nail varnish removed by several rinses with acetone. After allowing the specimens to air-dry, they were fixed to aluminium stubs with styrene cement, sputter-coated with gold/palladium and examined by scanning electron microscopy (SEM). Figs. 1–5 were obtained using a Phenom scanning electron microscope (FEI Europe, Eindhoven, The Netherlands) (5 kV thermionic source; backscattered electron detector). Some silicon carbide-polished specimens were examined after treatment with the $p_I^{HA}$ 56 solution, but without subsequent ultrasonication, using a JSM 5600 scan-
ning electron microscope (Jeol Ltd., Akishima, Tokyo, Japan) in the secondary emission mode. EDX spectra of these specimens were obtained with an EDX attachment running Oxford Link ISIS-300 software for energy dispersive X-ray spectroscopy.

3. Results

3.1. Unpolished specimens

Specimens that had been cut with the diamond saw but not polished showed a thick smear layer (Fig. 1).

The pHHA 55 solution had no visible effect on this smear layer after 10 min, whilst after 2 h and 24 h there was partial loss of the smear layer but no open tubules were visible. The pHHA 56 solution likewise had no visible effect after 10 min but after treatment for 2 h or more the smear layer was removed and all exposed tubules were at least partly open, but material was visible inside the openings of many tubules after this exposure period. In unpolished specimens that had been treated with the pHHA 56 solution for 2 h and then ultrasonicated in the solution during the last 1 min of the exposure, the tubules were consistently open and free of occluding material (Figs. 1 and 2). In such specimens, peritubular dentine could be distinguished at the tubule openings (Fig. 2). Small cracks were seen on the surface, mainly in the peritubular dentine. After treatment with NaOCl the peritubular dentine appeared as more clearly-defined ring-like structures, slightly elevated above the intertubular dentine (Fig. 3). Cracks were present in the peritubular dentine in NaOCl-treated regions. The openings of the tubules in the NaOCl-treated regions usually appeared funnel-shaped (Fig. 3). These morphological changes were associated with a very small loss in height of the NaOCl-treated area of the surface (Fig. 1).

In specimens treated with 6% citric acid for 15 s or 30 s all tubules were fully opened, with no signs of peritubular dentine. The tubule diameters seemed greater than after treatment with the pHHA 56 solution (Fig. 4). After treatment with NaOCl, the tubule openings were widened even further and peritubular dentine, when visible, was located beneath the intertubular dentine surface (Fig. 5).

3.2. Polished specimens

In specimens which had been polished, the tubules were not fully open after 30 min or 60 min treatment with pHHA 56 solution but were consistently fully opened after 2 h treatment. Polished specimens that had been subject to this process without ultrasonication retained debris in some tubule openings (Fig. 6). EDX spectra acquired from surfaces of specimens that had been polished and then treated with pHHA 56 solution showed distinct peaks for Ca and P (Fig. 7).
4. Discussion

Whilst exposure to the pH$_{55}$ solution resulted in partial loss of the smeared dentine after prolonged exposure, the underlying dentine surface showed no visible loss of mineral. This supports the conclusion$^3$ that solutions with this pH$_{55}$ are close to saturation with dentine mineral and the smear layer might have been removed by dispersion of the constituent particles into the solution rather than by dissolution. In contrast, a solution with a pH$_{56}$ of 56 is undersaturated with respect to dentine mineral, since not only was the smear layer completely removed after 2 h but the underlying surface showed changes due to partial demineralization. This is consistent with previous data.$^3$

The cracks seen on dentine surfaces treated with pH$_{56}$ solution are most likely due to shrinkage caused by air-drying of the demineralized matrix layer and partly-dissolved peritubular dentine. In the NaOCl-treated regions, cracks were confined to the peritubular dentine, suggesting that treatment with the solution had partly demineralized the tissue beneath the surface, like the softening seen in dental erosion. The funnel-shaped openings of the tubules in the NaOCl-treated areas suggested that the peritubular dentine had been dissolved preferentially at the luminal surface.

Dentine surfaces treated with the pH$_{56}$ solution, whilst no longer being covered by a smear layer, would have different chemical and physical properties to intact dentine, because they would be covered by a thin film of demineralized collagen. Removal of this layer by NaOCl probably does not restore the surface properties, because there is a chance that this agent can extract collagen from the superficial dentine. If a particular experiment requires both removal of the smear layer and relatively unaltered surface properties, one approach would be to remove the demineralized matrix layer by digestion with proteolytic enzymes.$^5$

References
