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# Effects of sodium hypochlorite solution on root dentine composition

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Sodium hypochlorite (NaOCl) solution, $\leq 5\%$ w/v available chlorine (abbreviated subsequently to %), is widely used as an irrigant in root canal treatment of teeth, so its effects on dentine are of clinical importance. The effects of $\sim 0.5\%$, 3% and 5% NaOCl solution on the composition of root dentine were studied at ambient temperature. For dentine powder treated for 30 min, depletion of the organic phase was confirmed by infrared spectroscopy. Apatite lattice parameters showed no significant change, but NaCl was also detected by X-ray powder diffraction. The low solubility of apatite mineral in the NaOCl solutions was demonstrated by the constant weight of bulk enamel specimens immersed for seven days. The stability of the mineral phase was confirmed by scanning microradiography (SMR), an X-ray attenuation method employing photon counting. Repeated SMR measurements of the local mineral content of bulk samples of root dentine and a synthetic hydroxyapatite aggregate during exposure to pumped NaOCl solutions for 100 h showed no mineral loss. As predicted from apatite chemistry, reaction of NaOCl with the mineral phase can be excluded as a primary factor in changes in mechanical properties of treated dentine. Effects of retention of NaCl on endodontic sealants requires further investigation.

## 1. Introduction

Root canal treatment (RCT) is a common dental procedure, usually provided with the aim of retaining teeth in which the pulp has become irreversibly infected or necrotic. The procedure includes the removal of pulp tissue and heavily infected dentine surrounding it. This is usually effected by mechanical debridement in the presence of an irrigating solution. A common clinical problem affecting root canal treated teeth is fracture that can necessitate extraction. Factors that may predispose to fracture have been identified as changes in the mechanical properties of dentine due to the action of irrigants, medicaments or root canal filling materials [1–4], as well as loss of structural integrity due to caries or access cavity preparation [5, 6] and increased loading resulting from reduction in proprioception [7, 8]. This work concerns the effect of NaOCl irrigating solutions on the composition of human dentine, which is a composite comprising $\sim 70$ wt % apatite mineral, $\sim 20$ wt % organic material (mostly collagen), and $\sim 10$ wt % water.

NaOCl solution, in the concentration range 0.5% to $\sim 5\%$, is a recommended root canal irrigant [9] that degrades necrotic tissue [10] and has broad spectrum antimicrobial activity [11, 12]. Dentine surrounding the canal is softened [13]. Further, dentine bars exposed *in vitro* to 5.25% NaOCl solution for 30 min had a lower elastic modulus and flexural strength than controls [3, 4].

NaOCl is widely used as a deproteinating agent in preparation of calcific biominerals for scanning electron microscopy [14], but less is known about any effects of NaOCl on the mineral components. The use of 2.5% NaOCl solution at 6 °C in deproteination of apatitic biominerals, from explanted heart valve bioprostheses (bovine and porcine), bovine bone and human aortic calcified deposits, resulted in materials with higher Ca/P ratio and greater crystallinity than the corresponding untreated deposits [15]. In a detailed study [16] of the effect of 10% NaOCl solution on bovine dentine powder (30 min treatment at ambient temperature), an increase in crystallinity of the mineral apatite phase and the formation of calcite ($CaCO_3$) was found by X-ray powder diffraction.

The purpose of this work was to investigate the effects on composition of human dentine of NaOCl solutions at concentrations relevant to root canal treatment ($\sim 0.5\%$, 3%, 5%).

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## 2. Materials and methods

### 2.1. Preparation of solutions and specimens

Concentrated ($\sim 12\%$) NaOCl solution (BDH Laboratory Supplies, Poole, Dorset) was diluted to produce 0.5%, 3% and 5% solutions whose concentrations were confirmed by iodometric titration [17]. Samples of enamel and root dentine were prepared from clinically sound single-rooted human premolars (stored in 4 wt % formal-saline for two weeks since extraction). The tissues were cut under water to avoid local heating. Sections were prepared by cutting (Microslice II, Malvern Instruments, Devon, UK) tooth blocks, then removing dentine or cementum with a diamond-coated dental bur. Root dentine powder was ground from sections using a dental bur, the slurry filtered, dried at 37 °C, and sieved (100 mesh, giving sample particle size = 150 μm). Sections of synthetic hydroxyapatite (HAp) aggregate were cut from pellets made by isostatic compression [18] of HAp powder (prepared by dropwise addition of ammonium orthophosphate solution to calcium nitrate solution at 70 °C and pH 11). Further details of preparation are given below for each experiment.

### 2.2. Fourier transform infrared (FTIR) spectroscopy

Specimens (30 mg) of dentine powder were stirred in 90 ml NaOCl solution (0%, 0.5%, 3% or 5%) for 30 min, filtered (Whatman No. 1 paper, UK), washed (50 ml deionized water for 1 min) and dried at 37 °C for 30 min. FTIR spectra of samples (2 mg), dispersed in a KBr matrix (300 mg), were recorded using a Perkin Elmer 1600 series FTIR spectrometer.

### 2.3. X-ray powder diffraction (XRD)

Specimens (5 mg) of dentine powder were stirred in 90 ml NaOCl solution (0% or 5%) for 30 min or 24 h, filtered, then dried at 37 °C. XRD patterns (Cu$K\alpha_1$ radiation, $\lambda = 1.5406$ Å) for $\sim 2$ mg samples were collected using an INEL CPS-120 position sensitive detector and analyzed by Rietveld refinement using the program GSAS [19]. Further details of the powder data collection and Rietveld refinement have been given [20].

### 2.4. Weight changes of specimens exposed to NaOCl solutions

Five specimens (typically 100 mg) each of enamel (bisected enamel cap), dentine (complete root), and apatite aggregate ($\sim 5 \times 5 \times 3$ mm block) were weighed ($\pm 0.5$ mg) after immersion (0, 30, 90, 180 min, 1, 5, 7 days) in 2 ml NaOCl solution (0%, 0.5%, 3%, 5%) that was replaced after each weighing. Before weighing, each specimen was washed in 50 ml distilled water for 1 min, blotted on absorbent paper, then left to dry in air for 30 min (the time determined in a preliminary study for specimens to dry to constant weight after immersion in water for a day).

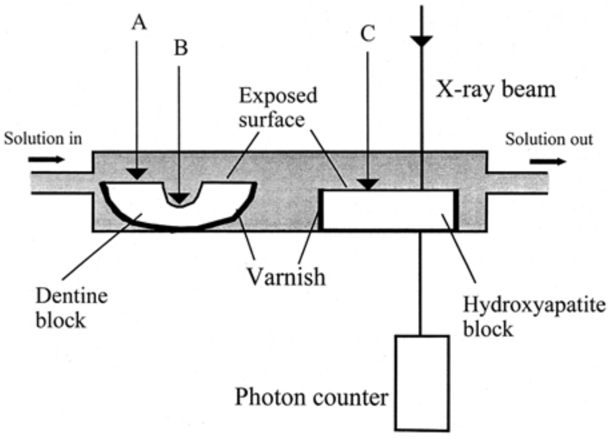

*Figure 1* Schematic of cell for SMR showing direction of X-ray beam path for measurement of projected mineral mass through: A, cut surface of root dentine; B, root canal dentine; C, HAp block.

### 2.5. Scanning microradiography (SMR)

SMR is an X-ray attenuation method using photon counting for repeated real-time measurements of projected mineral mass, at a resolution of 15 μm, of specimens (section or block) immersed in an aqueous medium. SMR with multiple specimen capability has been described [21]. In this work measurements were made with Ag$K\alpha$ (22.1 keV) radiation. (For whole dentine, $\sim 97\%$ of X-ray attenuation at 22.1 keV is due to mineral.)

Tooth roots bisected longitudinally were cut transversely to provide dentine blocks. The outer root surface was coated with nail varnish, leaving exposed the surface of the root canal and the adjacent cut face of dentine. Blocks of the HAp aggregate ($\sim 3$ mm thick) were similarly varnished so that one cut face was left exposed. One dentine and one HAp block were mounted in each of four SMR cells (Fig. 1), which were then filled with deionized water. An initial SMR area scan of each block was used both to confirm absence of prior damage and to select tracks for monitoring the projected mineral mass.

NaOCl solution (0.5%, 3% or 5%) or deionized water (control) was then pumped through each cell at 0.42 ml min$^{-1}$ and recirculated via a 1 L reservoir. SMR was used to measure the projected mineral mass at points along the two selected scan lines for each block. Measurements (10 s count time) were made for 11 points along each scan line in the dentine blocks (5 through the root canal surface, 6 through the cut face) with 300 μm separation and at 11 points along each scan line in the HAp blocks with 600 μm separation. The time between successive measurements at each point was 1.8 h. The projected mineral mass was calculated from the numbers of incident and transmitted protons assuming a mass attenuation coefficient for dental mineral of 4.69 g$^{-1}$ cm$^2$ at 22.1 keV [22]. The projected mineral mass was plotted as a function of time for each measurement point in the dentine blocks and HAp blocks.

## 3. Results

### 3.1. FTIR spectroscopy

By comparison with the spectrum of untreated dentine (Fig. 2), the reduction in intensity of the C-H stretching

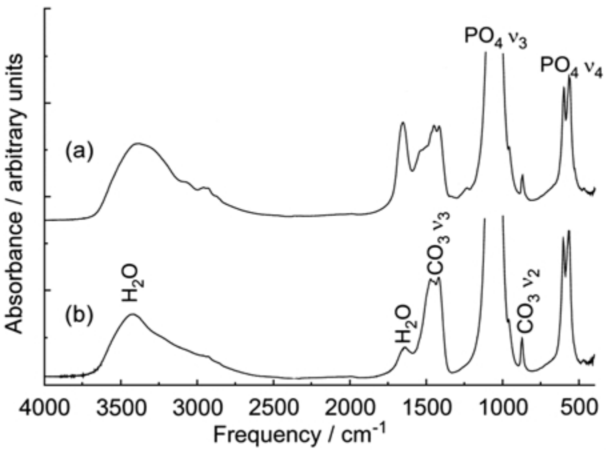

*Figure 2* FTIR spectra of root dentine powder following 30 min immersion in (a) water (b) 0.5% NaOCl solution.

bands (2960–2850 cm$^{-1}$) and amide bands (about 1655, 1525, 1228 cm$^{-1}$) characteristic of demineralized bone protein (mostly collagen) [23] demonstrated a reduction in the organic phase of dentine by NaOCl treatment. The spectrum of the treated powder exhibited bands characteristic of a carbonate-containing apatite with predominant carbonate substitution in phosphate ion sites. In contrast to enamel, the 1545 cm$^{-1}$ ($v_3$) carbonate band, that is characteristic of carbonate substitution in hydroxyl sites, was not observed.

### 3.2. XRD

The XRD patterns revealed no significant difference in the lattice parameters of the mineral apatite phase for NaOCl-treated and control dentine powder (Table I). However, NaCl was detected as a substantial second phase in treated dentine powder (33 wt % NaCl in powder treated with 5% NaOCl for 24 h). There was no evidence for the formation of calcite ( $\geq 1$ wt %).

### 3.3. Weight changes

The weight loss from bulk dentine specimens was initially rapid, with greater loss from treatment with 3% or 5% than with 0.5% NaOCl solution (Fig. 3), but there was little further loss between 1 and 7 days. In contrast to dentine ( $\sim 6$ wt % loss at 24 h in 5% NaOCl solution), enamel showed no change in weight (within the precision of the method, $\pm$ 0.5 mg). HAp aggregate specimens exhibited variable gains in weight that showed no relation to NaOCl concentration or duration of treatment.

TABLE I Lattice parameters and NaCl content of NaOCl-treated dentine and control

| Dentine treatment regime | *a*/Å | *b*/Å | Wt % NaCl |
|---|---|---|---|
| Deionized water for 30 mins | 9.449(6) | 6.915(4) | 0 |
| 5% NaOCl for 30 mins | 9.438(7) | 6.907(7) | 25 |
| 5% NaOCl for 24 h | 9.437(9) | 6.900(11) | 33 |

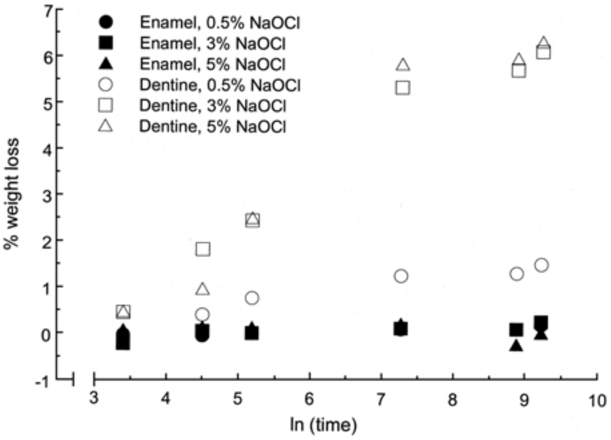

*Figure 3* Mean weight changes in dentine and enamel blocks with time (minutes) of exposure to NaOCl solutions.

### 3.4. SMR

Fig. 4 shows typical results of projected mineral mass as a function of time of exposure to NaOCl solution for (a) a point in cut surface root dentine, (b) a point in root canal dentine and (c) a point in HAp. No long-term change was observed in the integrated X-ray mineral mass for HAp aggregate or dentine at any of the 176 sites monitored over 100 h during exposure to NaOCl solution or deionised water. Apparent short-term fluctuations in the integrated X-ray mineral mass (seen in Fig. 4) had greater magnitude and higher frequency with increasing NaOCl concentration. These apparent fluctuations are attributable to the effect of gas bubbles (principally $O_2$ from the decomposition of NaOCl) in the X-ray beampath. Error originating from counting statistics, $\sim 0.000634$ g cm$^{-2}$, was negligible in relation to the magnitude of the integrated mineral mass of the specimens (about 0.15–0.4 g cm$^{-2}$).

## 4. Discussion

FTIR spectra showed that there was substantial loss of the organic phase from dentine powder exposed to 0.5% NaOCl for 30 min. Detection of changes in dentine mineral with NaOCl treatment was precluded by the overlap of organic phase bands with apatite $v_3$ carbonate bands. However, the spectra demonstrated that the

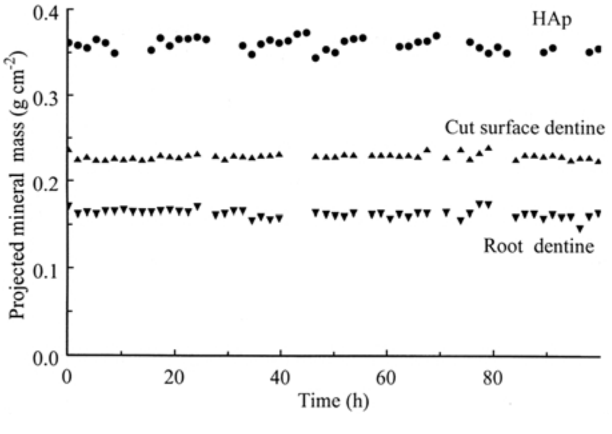

*Figure 4* Typical SMR time sequences of projected mineral mass of blocks of root dentine, enamel and HAp exposed to 3% NaOCl solution.

structure of NaOCl-treated dentine mineral differed from that of enamel apatite in having a smaller fraction of carbonate ions (if any) in hydroxyl ion sites.

There was no evidence, from measurements of lattice parameters, that there was any change in the atomic-scale structure of the apatite mineral in dentine following treatment with 5% NaOCl solution. However this does not exclude the possibility of a change in overall composition, reported [16] for bovine dentine after 10% NaOCl treatment. The apatite crystallites are sufficiently small that adsorbed species could make a significant contribution to the overall composition.

For bovine dentine powder, variation between samples in amounts of calcite formed and magnesium ions removed with 10% NaOCl treatment has been attributed to variation in chemical composition [16]. The absence (at $\geq 1$ wt %) of calcite in human dentine powder treated with 5% NaOCl in this study may indicate species differences in dentine composition, which should be further investigated if bovine dentine is to be used as an *in vitro* model for human dentine.

NaCl is present in commercially available NaOCl solutions manufactured by the hydrolysis of brine and is also a decomposition product of NaOCl. Further, in the context of SEM sample preparation, Boyde [14] has commented that ''careful and extensive washing with distilled water is required to remove residual NaCl'' from mineralized tissues treated with NaOCl. Thus the presence of NaCl in the treated dentine powder in this study was unsurprising. Its absence in previous work [16] may illustrate the effect of post-treatment washing.

The negligible weight change of enamel specimens with NaOCl treatment was consistent with the very low solubility expected for apatite mineral in an alkaline solution and the very low ( $\sim 1.5$ wt %) organic content of enamel. The erratic gains in weight, relative to baseline, for synthetic HAp are attributable to variable retention of NaCl and water in these very porous specimens. Thus the maximum observed weight loss ( $\sim 6$ wt %) from blocks of dentine (organic phase $\sim 20$ wt %) is almost certainly an underestimate of the loss of tissue mass. It is likely to reflect both the incomplete removal of NaCl by washing and the retention of increasing amounts of water as the porosity of the dentine increased with degradation of the organic phase. Crystals of NaCl have been observed, by SEM, in main and lateral canals and in dentinal tubules following alternating canal irrigation with NaOCl and hydrogen peroxide solutions [24]. Since NaCl is bactericidal through removal of water from bacterial cells [24], its retention has potential clinical benefit. However the desirability of NaCl retention also requires knowledge of possible reactions between NaCl and root canal sealants, which do not appear to have been investigated.

The long-term constancy of integrated X-ray mineral mass for HAp aggregate and for dentine exposed at the root canal surface and at a cut face indicated that there was no mineral loss in this system at concentrations of NaOCl $\leq 5\%$. This observation was consistent with the low solubility of apatite in alkaline solutions. However the remarkable retention of mineral, despite substantial degradation of the collagenous phase of dentine, may also have been dependent on the lack of physical disturbance of the specimens in the SMR cells. Complete retention of mineral cannot be assumed to occur with use of NaOCl solution for root canal irrigation, in which mechanical disturbance may result in some loss of apatite crystallites from the degraded dentine.

Although reaction of NaOCl with the mineral phase can be excluded as a primary factor in change in mechanical properties for treated dentine, degradation of the organic phase will necessarily result in changed properties of this composite material.

## Conclusions

NaOCl (0.5%, 3%, 5%) solution degrades the organic phase of dentine, but no mineral loss was observed in the mechanically undisturbed system studied by SMR. Further, no evidence was found for a change in apatite crystal structure of dentine mineral following NaOCl treatment. Reported changes in the mechanical properties of treated dentine are, therefore, attributed primarily to structural changes in this composite material resulting from degradation of the organic phase.

Investigation of the effects of retained NaCl on endodontic sealants is required, in order to inform the decision whether canal irrigation with water is indicated following use of NaOCl solution.

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