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Effect of sodium hypochlorite on human root dentine – mechanical, chemical and structural evaluation

M. Marending[1], H. U. Luder[2], T. J. Brunner[3], S. Knecht[4], W. J. Stark[3] & M. Zehnder[1]

[1]Division of Endodontology, Department of Preventive Dentistry, Periodontology, and Cariology, University of Zürich Center of Dental Medicine, Zürich, Switzerland; [2]Institute of Oral Biology, Section of Orofacial Structures and Development, University of Zürich Center of Dental Medicine, Zürich, Switzerland; [3]Institute for Chemical and Bioengineering, Department of Chemistry and Applied Biosciences; and [4]Institute for Biomechanics, Department of Mechanical and Process Engineering, Swiss Federal Institute of Technology (ETH), Zürich, Switzerland

Abstract

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Aim To investigate the mechanical, chemical and structural alterations of human root dentine following exposure to ascending sodium hypochlorite concentrations.

Methodology Three-point bending tests were carried out on standardized root dentine bars ($n = 8$ per group, sectioned from sound extracted human third molar teeth) to evaluate their flexural strength and modulus of elasticity after immersion in 5 mL of water (control), 1% NaOCl, 5% NaOCl or 9% NaOCl at 37 °C for 1 h. Additional dentine specimens were studied using microelemental analysis, light microscopy following bulk staining with basic fuchsin, and scanning electron microscopy (SEM). Numerical data were compared using one-way ANOVA. Bonferroni's correction was applied for multiple testing.

Results Immersion in 1% NaOCl did not cause a significant drop in elastic modulus or flexural strength values in comparison to water, whilst immersion in 5% and 9% hypochlorite reduced these values by half ($P < 0.05$). Both, carbon and nitrogen contents of the specimens were significantly ($P < 0.05$) reduced by 5% and 9% NaOCl, whilst 1% NaOCl had no such effect. Exposure to 5% NaOCl rendered the superficial 80–100 μm of the intertubular dentine permeable to basic fuchsin. Three-dimensional SEM reconstructions of partly demineralized specimens showed NaOCl concentration-dependent matrix deterioration. Backscattered electron micrographs revealed that hypochlorite at any of the tested concentrations left the inorganic dentine components intact.

Conclusions The current data link the concentration-dependent hypochlorite effect on the mechanical dentine properties with the dissolution of organic dentine components.

Keywords: biomechanics, dentine, sodium hypochlorite.

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Introduction

The use of antiseptic irrigating solutions is an important part of chemomechanical root canal preparation. It enhances elimination of microbiota and facilitates removal of necrotic tissue and dentine debris from the root canal system. Based on its unique capacity to dissolve necrotic tissue remnants, sodium hypochlorite (NaOCl) remains the most widely recommended irrigating solution in endodontics (Zehnder 2006). Concentrations ranging from 0.5% to 5.25% have been advocated in the past, with no consensus on the ideal concentration. To maximize the tissue-dissolving and antimicrobial

Correspondence: Dr Matthias Zehnder, Department of Preventive Dentistry, Periodontology, and Cariology, University of Zürich Center for Dental Medicine, Plattenstrasse 11, CH 8032 Zürich, Switzerland (Tel.: +41 44 632 8610; fax: +41 44 634 4308; e-mail: matthias.zehnder@zzmk.unizh.ch).

effects, more and more dentists seem to use hypochlorite solutions at the higher end of the concentration range. Apparently, some clinicians have used solutions at a concentration of up to 10% hypochlorite to irrigate root canals (Matsumoto *et al.* 1987). However, not only the tissue dissolving and antimicrobial hypochlorite effects are concentration-dependent (Baumgartner & Cuenin 1992), but also the caustic potential of this irrigant (Hidalgo *et al.* 2002). This is because hypochlorite is a nonspecific oxidizing agent (Dychdala 1991). Thus, in addition to its application as root canal irrigant, NaOCl is commonly used to deproteinize hard tissues for biomedical applications (Johnson *et al.* 2000).

One NaOCl side effect has received relatively little attention in the endodontic literature: the impact on the dentine matrix (Oyarzun *et al.* 2002). Dentine is composed of approximately 22% organic material by weight. Most of this consists of type I collagen, which contributes considerably to the mechanical properties of dentine (Currey *et al.* 1994). Sodium hypochlorite is known to fragment long peptide chains and to chlorinate protein terminal groups; the resulting *N*-chloramines are broken down into other species (Stoward 1975, Davies *et al.* 1993). Consequently, hypochlorite solutions may affect mechanical dentine properties via the degradation of organic dentine components. A study on bovine dentine suggested that, within the time frame of a root canal treatment, concentrated hypochlorite solutions cause untoward effects on dentine biomechanics (Slutzky-Goldberg *et al.* 2004). A 2-h exposure of dentine to NaOCl solutions of more than 3% (w/v) significantly decreases the elastic modulus and flexural strength of human dentine compared to physiological saline (Grigoratos *et al.* 2001, Sim *et al.* 2001). However, contrasting results have also been published (Machnick *et al.* 2003).

It was the aim of the current study to characterize further the impact of hypochlorite concentration on root dentine under controlled laboratory conditions.

Materials and methods

Preparation of dentine specimens

In adapting the method described by Grigoratos *et al.* (2001), human root dentine bars were prepared as follows: intact human maxillary third molars with fully formed roots were selected from the department's collection of extracted teeth. Eight teeth (32 dentine bars, $n = 8$ per group) were used for the three-point bending test and subsequent microelemental analysis. Nine more teeth (three teeth per experiment, 36 dentine bars in total, $n = 3$ per group) were used for backscattered electron imagery, light microscopy and three-dimensional (3D) reconstructions of scanning electron micrographs (SEM; see below). Teeth had been stored in 0.1% thymol solution immediately after extraction for up to 1 year. The absence of caries and cracks was verified under a stereo dissecting microscope (Leica Wild M3Z, Wild, Heerbrugg, Switzerland) with an inbuilt light source (intralux 4000, SOWODENT, Birmensdorf, Switzerland). Teeth were mounted on stubs and longitudinally sectioned using a saw microtome (Leica SP 1600, Leica Microsystems, Glattbrugg, Switzerland) with a diamond-coated internal-hole blade under continuous water flow. In the first step, longitudinal tooth slices of 1.2 mm thickness were obtained. These slices were then clamped in a custom-made small vice to remove the outer dentine and cementum with a second rectangular cut. The third cut was made parallel to the second at a distance of 0.8 mm towards the root canal, yielding a plane-parallel dentine bar of 0.8×1.2 mm, that was finally cut to a length of 10 mm using a diamond saw in a handpiece. Based on an inspection for cracks and irregularities under the dissecting microscope, four perfect bars per tooth were selected and stored in sterile 0.9% saline solution until further use.

NaOCl exposure

Eight teeth (32 dentine bars) were used for this part of the study. The four dentine bars per tooth were randomly assigned to be immersed in one of four solutions: ultrapure water (control), 1% (w/v) NaOCl, 5% NaOCl and 9% NaOCl. The ultrapure water was prepared from deionized water (Ministil P-21, Christ, Aesch, Switzerland) in a Milli-Q Plus system (Millipore Inc., Billerica, MA, USA); it had a resistance of 18.2 MΩ cm^{-1}. Hypochlorite solutions were prepared from a 9.2% stock solution. The concentrations of all NaOCl solutions were verified by iodometric titration. The bars were individually soaked in 5 mL of the respective solutions in polypropylene containers, which were constantly agitated in a shaking water bath (LAUDA A120S, Lauda-Königshofen, Germany) at 37 °C for 1 h. Subsequently, specimens were thoroughly rinsed with ultrapure water. Immediately thereafter, the bars were subjected to a three-point bending test.

Three-point bending test

Three-point bending tests were performed using an ElectroForce 3200 apparatus (Bose, EnduraTEC Systems Group, Minnetonka, MN, USA) directly after treatment with test and control solutions. The dentine bars were kept moist with physiological saline solution during all manipulations. Before they were transferred to the testing apparatus, their width and depth were measured using a sliding calliper. Specimens were then placed with the greater bearing surface on the support span (i.e. with the tubules parallel to the cross-head). The cross-head speed of the testing machine was set to 0.5 mm min^{-1} and the bars were tested until failure. The Young's modulus E and flexural bend strength (FBS) were determined according to the American Society for Testing and Materials (1989). The Young's modulus was calculated from the slope m of the load-displacement curves within the linear elastic region using the formula

$$E = \frac{L^3 m}{4bd^3} \quad (1)$$

with the support span width L, the width b and the depth d of the sample.

The FBS was calculated according to the formula

$$\mathrm{FBS} = \frac{3PL}{2bd^2} \quad (2)$$

with P representing the load at fracture (Grigoratos *et al.* 2001).

Microelemental analysis

Fractured dentine bars from the biomechanical tests were used for this part of the study ($n = 8$ per group). Specimens were dried overnight at 110 °C in a vacuum oven (Salis, Aarburg, Switzerland). Subsequently, the dentine bars were ground and homogenized using a metal mortar and pestle. The C and N contents in wt% of 2 mg dentine powder per specimen were determined in duplicate using a CHN-900 apparatus (LECO, St Joseph, MI, USA).

Light microscopy

Specimens ($n = 3$ per group, four bars per tooth) were treated with test and control solutions as described above. Subsequently, they were washed in ultrapure water and then immersed for 2 h at room temperature in half-strength Karnovsky's fixative (2.5% glutaraldehyde and 2% paraformaldehyde in 0.02 mol L^{-1} sodium cacodylate buffer, pH 7.4) containing 1% tannic acid to stabilize the collagen. Following a rinse in 0.185 mol L^{-1} Na-cacodylate buffer (pH 7.2), they were dehydrated in an ascending ethanol series. The ethanol solutions up to 96% contained 1% of basic fuchsin, whilst the 100% ethanol solution was pure. Specimens were embedded Technovit 7200 VLC (Heraeus Kulzer, Wehrheim, Germany) and polymerized for 4 h under white and blue light. Ground sections of about 50 μm thickness were prepared using a cutting/grinding system (EXAKT, Norderstedt, Germany) and examined without further staining in a Leitz Dialux 20 light microscope (Leica Microsystems) at magnifications up to 25×. Digital micrographs were made at a resolution of 1950 × 1525 pixels using a ProgRes C14 camera (Jenoptik, Jena, Germany).

To reduce *bias*, the researcher viewing the microscopy pictures was blinded to the experimental protocol.

Scanning electron microscopy

Dentine bars ($n = 6$ per group, four bars per tooth, treated with hypochlorite or ultrapure water as described above) were processed through half Karnovsky's/1% tannic acid fixative as described above. Specimens destined for examination in secondary emission mode ($n = 3$) were subsequently decalcified in 17% EDTA for 2 h at room temperature, resulting in the dissolution of inorganic dentine components from the superficial 100 μm (Kawasaki *et al.* 2000). Following a rinse in 0.185 mol L^{-1} Na-cacodylate buffer (pH 7.4), they were dehydrated in ascending grades of acetone and dried using the critical point method in an CPD 030 device (BAL-TEC, Balzers, FL, USA). Dry specimens were glued to SEM stubs and sputter-coated with platinum in an MED 010 apparatus (BAL-TEC). They were examined in a Tescan VEGA TS 5316 XM scanning electron microscope (Brno, Czech Republic) at an accelerating voltage of 20 kV and 10–20 mm working distance. Digital images were taken at a resolution of 2048 × 1536 pixels.

For 3D representations of the dentine bar faces, stereopairs of micrographs were captured at eucentric tilting angles of +10° and −10°. Surfaces and selected profiles were then reconstructed at a resolution of 1024 × 768 pixels using the program MEX 4.1 (Alicona Imaging, Grambach, Austria).

To reveal possible changes in the inorganic dentine components, backscattered electron imagery was chosen. Dentine bars destined for examination in backscattered emission mode ($n = 3$) were embedded

in Technovit 7200 VLC as described for the light microscopic preparations, except that no basic fuchsin was added to the alcohol during dehydration. The cutting faces of the polymerized blocks were polished with silicon carbide paper down to grit 4000, followed by a polishing cloth with diamond paste of 3, 1 and 0.5 μm grain size. The polished block faces were then coated with carbon at a thickness of about 10–15 nm using a MED020/EVM030 electron beam evaporator (BAL-TEC) and examined with an annular monocrystal scintillation type (YAG) backscatter detector contained in the Tescan scanning electron microscope. Digital images were taken at 20 kV, about 20 mm working distance, and a resolution of 2048 × 1536 pixels.

Data analysis

Numerical data pertaining to the mechanical properties of the dentine bars and their elemental composition were compared between groups using one-way analysis of variance (ANOVA) followed by Bonferroni's adjustment for multiple testing.

Results

Immersion of root dentine bars in 5 mL of 1% NaOCl at 37 °C for 1 h did not cause a significant drop in their elastic modulus or flexural strength compared to the corresponding values obtained with specimens immersed in water. In contrast, 5% and 9% hypochlorite reduced elastic modulus and flexural strength values by half (Fig. 1, $P < 0.05$).

Both, carbon and nitrogen content of the specimens were significantly ($P < 0.05$) reduced by 5% and 9% NaOCl, whilst 1% NaOCl did not significantly reduce these values against the water control (Table 1). This indicated a concentration-dependent effect of hypochlorite on organic dentine components.

Backscattered electron micrographs of the ground sections revealed that hypochlorite at any of the concentrations tested left the inorganic dentine components intact (Fig. 2a–c). Ground sections of basic fuchsin-stained specimens showed that in the dentine bars previously immersed in water, dye penetration occurred only through the tubules (Fig. 2d). In the specimens exposed to 1% (Fig. 2e) and 5% NaOCl (Fig. 2f), the intertubular dentine was apparently also stained. The depth of the zone penetrated by the dye was markedly greater at the higher compared with the lower hypochlorite concentration (Fig. 2f).

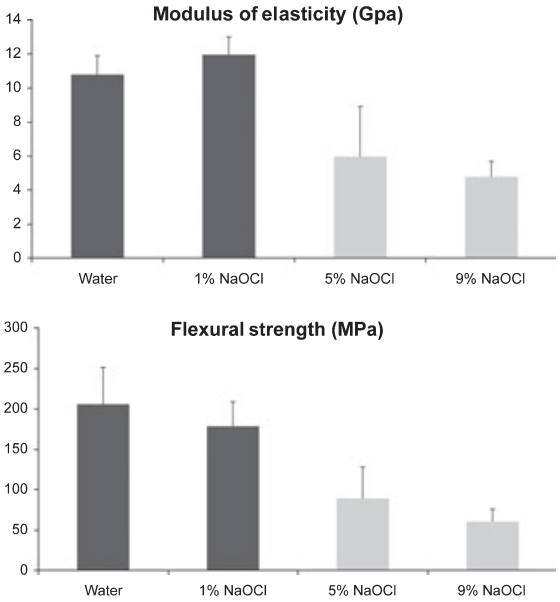

Figure 1 Mechanical parameters of the standardized dentine bars after immersion in 5 mL of test or control solutions at 37 °C for 1 h. Columns indicate mean values, bars standard deviations. Columns in the same shade represent data sets that did not significantly differ from each other at the 5% level (ANOVA).

Table 1 Carbon and nitrogen content (in wt% mean ± SD, $n = 8$) of root dentine specimens immersed in different concentrations of sodium hypochlorite

Element	Water	1% NaOCl	5% NaOCl	9% NaOCl
C	11.3 ± 0.3^{A}	10.9 ± 0.3^{A}	9.4 ± 1.4^{B}	9.0 ± 0.3^{B}
N	3.6 ± 0.2^{a}	3.3 ± 0.1^{a}	2.7 ± 0.4^{b}	2.6 ± 0.1^{b}

Identical superscript letters indicate that there was no significant difference at the 5% level between data sets (ANOVA, Bonferroni).

In a further experiment, dentine specimens were treated with the different hypochlorite concentrations or water and then demineralized in EDTA for 2 h to uncover the superficial dentine matrix. Three-dimensional surface reconstructions based on SEM scans differed markedly (Fig. 3). Whereas some detachment of the superficial matrix was visible after exposure to 1% hypochlorite (Fig. 3b), treatment with 5% NaOCl (Fig. 3c) resulted in readily apparent deterioration of the superficial dentine characterized by craters up to 10 μm in depth.

Discussion

The current study showed a clear concentration-dependent effect of NaOCl solutions on mechanical

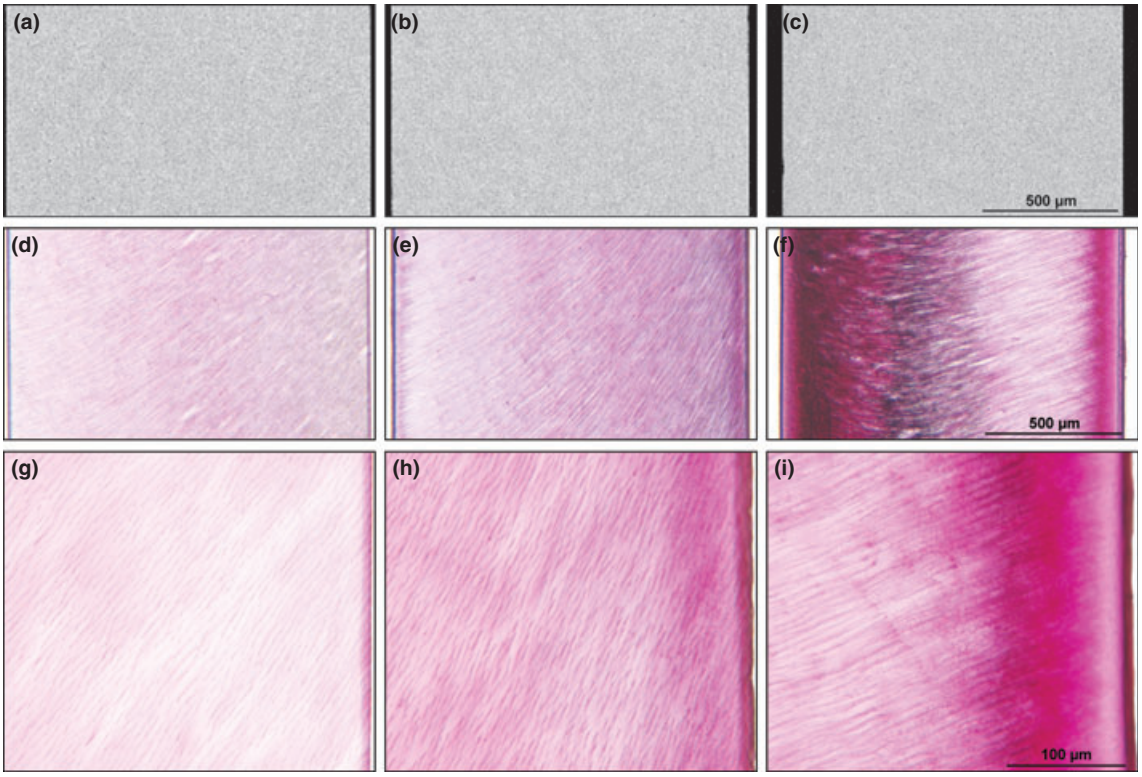

Figure 2 Typical scanning electron micrographs obtained in backscattered emission mode of specimens immersed in water (a), 1% (b) and 5% (c) sodium hypochlorite for 1 h at 37 °C. Note the homogenous appearance of the specimens in the backscattered electron micrographs, indicating a homogeneous distribution of inorganic dentine components regardless of the hypochlorite concentration. Overviews (d–f) and details (g–i) of dentine bars, which had been immersed in water (d and g), 1% NaOCl (e and h) or 5% NaOCl (f and i) at 37 °C for 1 h.

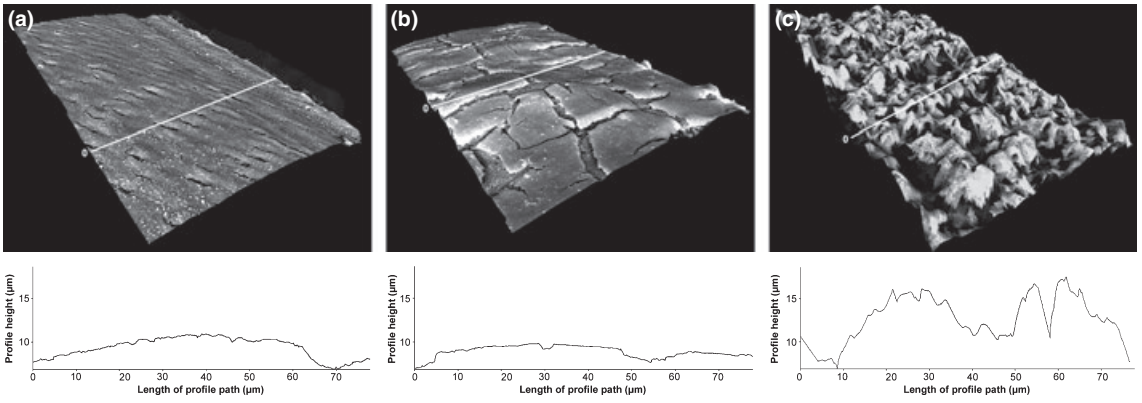

Figure 3 Three-dimensional surface reconstructions and profiles of dentine specimens after immersion in water (a), 1% (b) and 5% (c) NaOCl for 1 h at 37 °C. After exposure to these solutions, the specimens were immersed in EDTA for 1 h to expose the superficial dentine matrix.

dentine properties resulting from the disintegration of the organic dentine matrix.

In the current study, standardized human root dentine bars were immersed in hypochlorite solutions of different concentration for 1 h. Hypochlorite-induced mechanical alterations were measured applying a three-point bending test. Furthermore, dentine specimens were analysed for their organic content via

elemental C and N analysis. Light microscopy of specimens stained *en bloc* with basic fuchsin revealed spatial information regarding the alterations in dentine permeability resulting from the destruction of organic moieties entailed by hypochlorite. Alterations of the organic and inorganic matrix at the hypochlorite-exposed surfaces were studied by SEM. The mechanical part of this investigation was a repetition of a published study (Grigoratos *et al.* 2001) with different hypochlorite concentrations and volumes, whilst elemental analysis, bulk staining with basic fuchsin, and 3D surface reconstructions of human root dentine after hypochlorite exposure offered some new information.

Standardized bars were used to study hypochlorite effects on root dentine. This approach does not allow direct clinical conclusions. On the other hand, variance in the outcome variables was reduced, and the studied effects could be observed in a laboratory setting. Variability in dentine microanatomy (Vasiliadis *et al.* 1983, Kinney *et al.* 2005) was controlled by preparing four specimens per tooth, one per experimental group. This compensated for the relatively small number of specimens used in the current investigation. Furthermore, the rectangular cross-section of the specimens allowed their standardized positioning for the mechanical tests. There is a strong dependence of the elastic dentine properties and fracture behaviour on the orientation of the mineralized collagen fibrils (Arola & Reprogel 2006). It was found that the fracture toughness of dentine was lower where cracking occurred perpendicular to the dentinal tubules, when compared with crack paths in the plane of the tubules (Nalla *et al.* 2003).

The elastic modulus or flexural strength values of root dentine after exposure to hypochlorite reported here confirm published results (Grigoratos *et al.* 2001). However, in the previous study, longer incubation times (2 h instead of 1 h used in the current investigation) and higher irrigant volumes (50 mL instead of 5 mL) were used. One hour of exposure to 5 mL of the test and control irrigants was chosen. These lower irrigant volumes and exposition times were used here to compensate for the fact that dentine in rectangular bars is exposed from four sides, which is different from the situation in the root canal. Nevertheless, the current results are comparable to the ones published by Grigoratos *et al.* (2001). Immersion in 5% NaOCl for 2 h (instead of 1 h) under the current conditions resulted in spontaneous, macroscopically visible fracture lines in the dentine bars (Fig. 4). Such cracks on the dentine surface after exposure to 5% NaOCl have also been reported in the literature (Lee *et al.* 2004). Furthermore, the present results are in line with the reported concentration-dependent effect of hypochlorite on root dentine microhardness (Slutzky-Goldberg *et al.* 2004). In contrast to the current study and the investigation by Grigoratos *et al.* (2001), the work group of Machnick *et al.* (2003) found no significant effect of hypochlorite concentration on root dentine flexural strength or modulus of elasticity. They immersed rectangular dentine bars of 1 × 1 mm diameter in ascending concentrations of 30 mL of hypochlorite or chelating solutions for 2 h. Based on the similarity of theirs and the current methodology, differences in outcome variables cannot be explained. However, as indicated above, there is a strong evidence that hypochlorite does weaken dentine. Consequently, a systematic error in the investigation by Machnick *et al.* (2003) cannot be excluded.

Figure 4 Macrophotograph depicting a dentin bar that was immersed in water (a) and a counterpart that was immersed in 5% NaOCl (b) for 2 h. Not after 1 h (cf. Results section), but after 2 h immersion in 5% NaOCl (b), spontaneous cracking of the dentine surface could be observed macroscopically ($n = 3$ per group).

Immersion in basic fuchsin of the dentine bars treated with hypochlorite was performed to assess the permeability of the altered dentine. Bulk staining with basic fuchsin has long been used to detect microdamage in the bone, because this alcohol-soluble dye lodges in cracks and voids and is not washed out by cutting and grinding with water cooling (Lee *et al.* 2003). The method thus allows to differentiate damage due to the applied treatment from artefactual cracks resulting from specimen preparation. The findings indicate that exposure to 1% and particularly

5% hypochlorite solution entails markedly enhanced dentine permeability.

Taken together, the results from microelemental analysis, SEM and light microscopy reported here show that hypochlorite solutions dissolve organic dentine components, whilst the inorganic dentine moieties are left intact. The finding that hypochlorite does not alter the mineral content is in line with the previous studies (Di Renzo *et al.* 2001, Dogan & Calt 2001, Driscoll *et al.* 2002). However, contrasting results regarding the hypochlorite effects on the organic dentine components have been published. These can be explained by differing experimental conditions and analytical methods. In line with the current observations, an immunohistochemical evaluation of the effects of NaOCl on dentine type I collagen showed a 7 μm surface zone without immunoreactivity after 2 min exposure to 5.25% hypochlorite (Oyarzun *et al.* 2002). On the other hand, Di Renzo *et al.* (2001), using 12% NaOCl exposure up to 48 h and Fourier transform infrared spectroscopy (FT-IRS) to visualize treatment-induced chemical modifications of human dentine surfaces did not find any significant alterations in organic moieties, unless the specimen was previously demineralized. This can be explained by the fact that collagen fibrils in dentine are embedded in hydroxyapatite crystals (Butler 1992, Hoshi *et al.* 2001), and consequently, may not only be protected from chemical attacks, but also shielded from infrared light. Thus, even if hypochlorite *does* alter the superficial organic matrix, this may not be identified using spectroscopic methods.

As a consequence of the nonspecific action of NaOCl on organic moieties, properties of hypochlorite such as the tissue-dissolving and antibacterial effects, which are desired in endodontics, appear to be mutually interlinked with the untoward effects. In this context, it appears important to notice that the mineral phase in dentine has a protective effect on collagen and, hence, that demineralizing agents used for the removal of the smear layer can expedite the destructive hypochlorite effect (Di Renzo *et al.* 2001, Oyarzun *et al.* 2002).

Future research should focus on the ideal mode of hypochlorite delivery into the root canal to obtain maximum disinfection and canal cleanliness without hampering mechanical dentine properties.

Conclusions

Under the conditions of the current study, the following observations were made:

1. sodium hypochlorite caused a concentration-dependent reduction of elastic modulus and flexural strength in human root dentine bars;
2. similar to the effects on mechanical properties, the reduction of C and N in the specimens was a function of hypochlorite concentration;
3. sodium hypochlorite made altered intertubular dentine permeable to basic fuchsin dye, although no effect of hypochlorite on inorganic dentine components could be detected in backscattered SEMs and
4. as visualized on SEM 3D reconstructions of the exposed dentine surface after demineralization, 5% NaOCl severely altered the peripheral dentine matrix.

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