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Evaluation of the surface free energy on root canal dentine walls treated with chelating agents and NaOCl

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Abstract

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Aim To evaluate *ex vivo* the effects of combined and single use of EDTA, RC-Prep and NaOCl on the surface free energy of canal wall dentine using the captive bubble technique.

Methodology Eighteen extracted human pre-molar teeth were sectioned at the crown and the apical third, the remaining mid-root portion were bisected longitudinally. Thereafter, the root halves were embedded in resin blocks that exposed the dentine surface of the canal wall. The specimens were randomly assigned to six experimental groups ($n = 6$) after polishing. The root dentine surfaces of the first two groups were treated with 17% EDTA or RC-Prep followed by 2.5% NaOCl irrigation. Groups 3, 4 and 5 were treated with either 17% EDTA, RC-Prep or 2.5% NaOCl alone. Control specimens were irrigated with saline solution. The surface free energies of experimental groups were calculated by measuring air and octane contact angles on the canal wall dentine. Statistical analysis was performed using the Mann–Whitney U and Bonferroni post-tests at $P = 0.05$.

Results Compared with the control group; combined and single use of EDTA, RC-Prep and NaOCl irrigation significantly decreased the surface free energy of canal wall dentine surfaces ($P < 0.05$). Among all groups tested, the use of NaOCl as a final flush following RC-Prep treatment yielded increased wettability. Nevertheless, this value remained lower than that of the control group.

Conclusion Use of chelating agents alone or in combination with NaOCl decreased the wettability of root canal wall dentine.

Keywords: chelating agents, dentine, sodium hypochlorite, surface free energy.

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Introduction

Chelating agents remove the smear layer from the root canal and potentially allow better dentinal tubule penetration of root canal sealers as well as demineralizing and softening dentine (Şen *et al.* 1995, Hülsmann *et al.* 2003). In order to obtain the maximum effect during and after instrumentation, it is necessary to use chelating agents in conjunction with a tissue solvent (Yamada *et al.* 1983, Baumgartner & Mader 1987). An effective method to remove the organic and inorganic remnants is to irrigate the root canal with EDTA followed by NaOCl (Yamada *et al.* 1983, Gulabivala *et al.* 2005). It has been reported that dentine surface treatment with different agents may cause alterations in the chemical and structural composition of human dentine, which in turn may change its permeability and solubility characteristics (Pashley 1992, Van Meerbeek *et al.* 1992, Dogan & Calt 2001). These alterations may effect the adhesion of materials to dentine surfaces (Erickson 1992),

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resistance to stress (Ruse & Smith 1991, Driscoll *et al.* 2002) and also bacterial attachment to the dentine surface (Yang & Bae 2002). In the context of adhesion, the adhesive material must come into intimate contact with the substrate to facilitate molecular attraction and allow either chemical adhesion or penetration for micromechanical surface attachment. The adhesion processes are mainly influenced by the relative surface free energy (wetting ability) of the solid surface (Erickson 1992, Eick *et al.* 1997, Al-Omari *et al.* 2001). The surface free energy is a measure of the surfaces' reactivity or adhesiveness to its environment. This phenomenon occurs as a result of inter-atomic attraction. Measurement of surface free energy can be expressed in terms of the angle formed between a drop of liquid and the plane surface of the solid on which it rests. This is termed 'contact angle' (θ) and it has an inverse relationship with surface free energy (wettability), i.e. the lower the contact angle the greater the surface free energy and, hence, the greater the adhesion (Miloseviç 1992, Kane *et al.* 1993).

It has been reported that EDTA significantly decreases the wettability of dentine surface, while NaOCl does not cause any change (Attal *et al.* 1994, Tanı *et al.* 1996). However, combined uses of EDTA and NaOCl is generally suggested in endodontic practice so as to remove the smear layer effectively during root canal treatment (Yamada *et al.* 1983, Gulabivala *et al.* 2005).

The aim of this study was, therefore, to evaluate the effect of combined and single use of EDTA, RC-Prep and NaOCl on surface free energy of root dentine using the captive bubble technique.

Materials and methods

Sample preparation

The study was carried out using 18 sound human premolar teeth freshly extracted for orthodontic purposes. The teeth were immediately placed in saline after debridement of surrounding soft tissue and debris. The crowns and apical thirds of the teeth were removed, and the remaining roots bisected longitudinally using a low-speed rotary diamond disk at low speed under coolant water. The 36 root halves were embedded in resin blocks that exposed the canal wall dentine surface. The specimens were randomly assigned to six experimental groups ($n = 6$) and smoothed under distilled water with polishing disks (Soflex; 3M ESPE, St Paul, MN, USA). Thereafter, the samples were rinsed with distilled water and dried at 37 °C for 30 min.

Sample treatment

The samples were randomly divided into the following treatment groups:

Group 1: The samples were immersed in 10 mL 17% EDTA (Merck Co., Darmstadt, Germany) for 15 min followed by irrigation with 10 mL, 2.5% NaOCl (Sultan Chemists, Inc., Englewood, NJ, USA).

Group 2: The samples were treated with RC-Prep (Premier Dental Products, Norristown, PA, USA) for 15 min followed by irrigation with 10 mL, 2.5% NaOCl.

Group 3: The samples were immersed in 10 mL 17% EDTA for 15 min and thereafter rinsed with 10 mL saline solution.

Group 4: The samples were treated with RC-Prep for 15 min followed by rinsing with 10 mL saline solution.

Group 5: The samples were irrigated with 10 mL, 2.5% NaOCl.

Group 6 (Control): The samples were irrigated with 10 mL saline solution.

Contact angle measurement

Underwater contact angles of air and octane were measured on dentine surfaces using goniometry (Olympus, VS-IV, Tokyo, Japan) with the captive bubble technique (Andrade *et al.* 1979a). This technique allows contact-angle measurement on surfaces immersed in a liquid (Fig. 1). After completion of the treatments the dentine specimen was mounted horizontally to the microscopic lamel which was completely immersed into the glass cuvette. The cuvette was filled with double-distilled water. While the dentine surface was equilibrating in the cuvette, the goniometer was aligned and focused on the dentine-water interface. At this point, a microsyringe (Hamilton Gastight Syringe-Model 1701ASRN; Sigma, St Louis, MO, USA) containing either room air or pure *n*-octane was lowered into the water. A bubble volume of approximately 5 μL was released from syringe tip by positioning it underneath the dentine surface and allowing it to rise to the dentine/water interface. All measurements were carried out by one calibrated operator. For each droplet, three measurements were performed on the same dentine surface. Determination of the contact angles for the control and experimental groups were made on

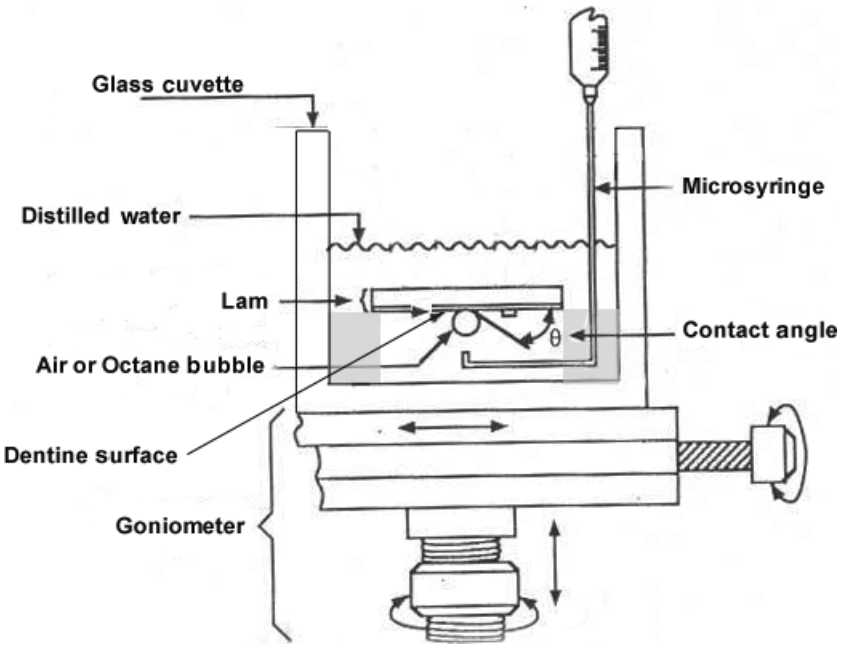

Figure 1 Schematic diagram showing the contact angle measurement apparatus.

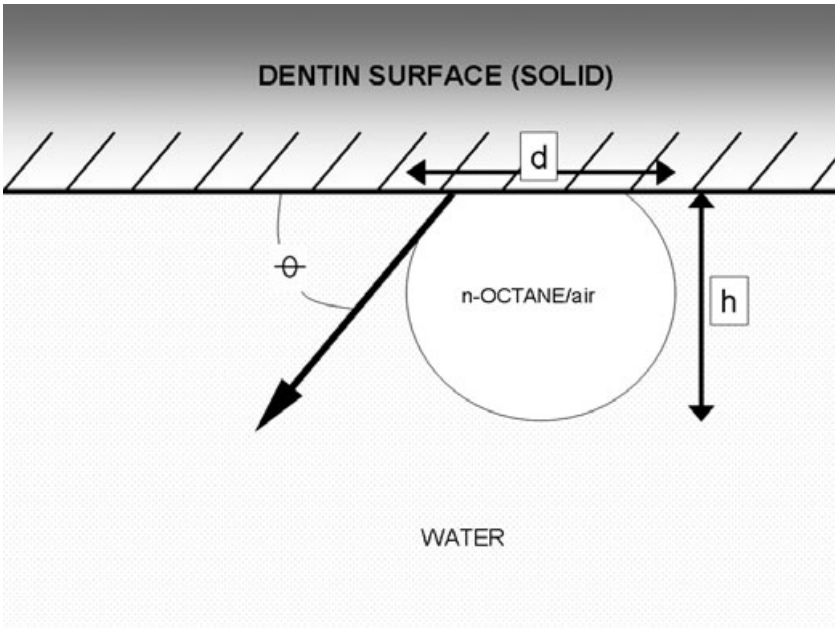

Figure 2 Captive bubble technique showing the three phase equilibrium between dentin surface, water and *n*-octane/air $\{\theta \leq 90°, \theta_{octane/air} = \cos^{-1}[(2h/d) - 1]\}$.

the film negatives at ×10 magnification on an enlarger. The height (h) and diameter (d) of bubbles were then measured and two contact angles (θ air or octane) were determined (Fig. 2).

Determination of surface free energies of samples

Air bubbles and *n*-octane droplets at the sample-water interface can be used to derive the surface free energy

components, i.e. polar component (γ_{sv}^{p}), and dispersive component (γ_{sv}^{d}), and surface free energies (γ_{sv}) of fully hydrated samples. In the present study, surface free energies (γ_{sv}) and its polar and dispersive components (γ_{sv}^{p}) and (γ_{sv}^{d}), respectively, were calculated according to the harmonic mean equation (eqn 1) developed by Andrade *et al.* (1979b). This method utilises Young's equation (eqns 2 and 3) for non-deformable solids.

$$\gamma_{12} = \gamma_1 + \gamma_2 - 4\left(\frac{\gamma_1^{d}\gamma_2^{d}}{\gamma_1^{d} + \gamma_2^{d}}\right) - \left(\frac{\gamma_1^{p}\gamma_2^{p}}{\gamma_1^{p} + \gamma_2^{p}}\right), \quad (1)$$

where the subscripts d and p refer to the dispersed and polar components, respectively, γ refers to the interface tension, and the subscripts 1 and 2 refer to the two phases of interest.

$$\text{For air: } \gamma_{sv} = \gamma_s - \pi_e = \gamma_{sw} + \gamma_{wv}\cos\theta_{air}, \quad (2)$$

$$\text{for octane: } \gamma_{sv} = \gamma_s - \pi_e = \gamma_{sL} + \gamma_{wL}\cos\theta_{L}, \quad (3)$$

where subscript L refers to water-immiscible liquid (here *n*-octane), subscript v refers to water vapour and π_e is the water vapour equilibrium spreading pressure. The subscripts s and w refers to solid phase (here root dentine) and water, respectively.

Equation 1 can be written for air–water–solid and octane–water–solid triple phases, separately. Combining these equations with eqns 2 and 3 (γ_{sv}^{p}) and (γ_{sv}^{d}) were calculated. For octane, $\gamma_{LV}^{p} = 0$ and $\gamma_{LV}^{d} = \gamma_{WV}^{d}$ and the spreading pressure for water vapour on octane is assumed zero. For air, $\gamma_{LV}^{d} = \gamma_{LV}^{p} = 0$. The self-consistent values for the surface and interfacial tensions at 25 °C are (in ergs cm^{-2}): $\gamma_{wv} = 72.1$; $\gamma_{ov} = 21.6$; $\gamma_{wv}^{d} = 21.6$; $\gamma_{ow} = 50.5$ (16). Then surface free energy of root dentin was calculated by using the following equation (eqn 4).

$$\gamma_{sv} = \gamma_{sv}^{p} + \gamma_{sv}^{d} \quad (4)$$

The differences between the means of each group were analysed statistically using the Mann–Whitney *U* and Bonferroni tests ($P < 0.05$).

Table 1 Air and octane contact angle values of root dentine treated with chelating agents and NaOCl (mean ± SD)

Groups	θ_{air} (degree)	θ_{octane} (degree)
Control	31.7 ± 1.9	38.2 ± 1.9
EDTA	44.1 ± 3.2	45.0 ± 1.4
EDTA + NaOCl	47.5 ± 3.2	43.4 ± 3.1
RC-Prep	49.3 ± 0.8	42.4 ± 4.7
RC-Prep + NaOCl	40.7 ± 2.2	43.7 ± 3.0
NaOCl	46.7 ± 1.5	42.2 ± 3.1

Results

Tables 1 and 2 depict the contact angle and surface free energy values (dispersive and polar forces) of dentine surfaces treated with EDTA, RC-Prep and NaOCl. The statistical analyse between the experimental and control groups are summarized in Table 3. When compared with the control group; the θ_{air} degrees of all treated surfaces increased significantly, whereas the θ_{octane} degrees increased significantly only for EDTA-treated surfaces ($P < 0.05$). The surface free energy of all treated surfaces and its dispersive components except for RC-Prep + NaOCl decreased significantly ($P < 0.05$; Table 3). However, its polar components decreased significantly only for EDTA treatment ($P < 0.05$; Table 3). There was a significant difference in θ_{air} and surface free energy of RC-Prep treated dentine surfaces when compared to the RC-Prep + NaOCl-treated group ($P < 0.05$).

Discussion

The results of this study show that single or combined use of EDTA, RC-Prep and NaOCl increased the contact angles and decreased significantly the surface free energy of root dentine. This result suggests that the agents mentioned above lead to a decrease in the wettability of dentine surface, thereby interfering with the adhesion mechanism. It has been reported that wetting of dentine surfaces decreases after application of EDTA (Attal *et al.* 1994, Tanı *et al.* 1996). The present results corroborate with those findings. This may be because the weak demineralization created by EDTA compounds creates a relatively smooth surface of the organic dentine structure, which does not offer an increased area for adhesion (Saleh *et al.* 2002). In this study, NaOCl alone decreased the wettability of dentine surfaces in contrast to previous reports (Attal *et al.* 1994). This may be due to the differences in experimental procedures. Wettability of a surface has been shown to be dependent on the chemical composition of the solid surface (Rosales *et al.* 1999). It has been reported that the organic element of dentine is decreased significantly by soaking in NaOCl (Driscoll *et al.* 2002). Moreover, NaOCl was found to have similar surface tension levels to EDTA which is important for the wetting capacity of a material (Tasman *et al.* 2000) and it may be possible to obtain a similar degree of wetting on the dentine surface.

Groups	γ_{sv}^{p}	γ_{sv}^{d}	γ_{sv}
Control	40.74 ± 0.86	21.69 ± 1.71	62.43 ± 1.34
EDTA	37.61 ± 0.67	16.77 ± 2.09	54.37 ± 2.09
EDTA + NaOCl	38.35 ± 1.47	13.84 ± 2.35	52.20 ± 1.98
RC-Prep	38.77 ± 2.20	12.66 ± 2.30	51.43 ± 0.30
RC-Prep + NaOCl	38.20 ± 1.38	18.59 ± 2.51	56.79 ± 1.43
NaOCl	39.34 ± 0.90	13.41 ± 0.44	52.75 ± 1.02

Table 2 Surface free energy parameters of root dentin treated with chelating agents and NaOCl (erg cm^{-2}; mean ± SD)

Table 3 Statistical comparison of surface free energy parameters among experimental groups

	Groups						
	EDTA + NaOCl ⇓ Control	RC-Prep + NaOCl ⇓ Control	EDTA ⇓ Control	RC-Prep ⇓ Control	NaOCl ⇓ Control	EDTA + NaOCl ⇓ EDTA	RC-Prep + NaOCl ⇓ RC-Prep
γ_{sv}	<0.05	<0.05	<0.05	<0.05	<0.05	NS	<0.05
γ_{sv}^{p}	NS	NS	<0.05	NS	NS	NS	NS
γ_{sv}^{d}	<0.05	NS	<0.05	<0.05	<0.05	NS	NS

NS, nonsignificant.

Interpretation of the contact angle can only be achieved if the following assumptions are valid: the solid surface is clean, rigid, nondeformable and highly smooth (Miloseviç 1992). Contact angle can be measured using sessile drop or captive bubble techniques. In this study, the captive bubble method was selected. The advantage of this method is that the solid surface is kept in a fully hydrated state. Therefore, it is possible to study in an environment similar to the conditions in the mouth.

The phenomenon of adhesion is to obtain optimum spreading and adhesion on a high-energy surface and to keep the interface as free as possible from low energy organic films. The decrease in free surface energy decreases the spreading and thus reduces the adhesion of a material to the solid surface (Baier 1992). Free surface energy (γ_{sv}) possesses two components; one (γ_{sv}^{d}) is nonpolar and refers to the apolar Lifshitz-Van der Waals forces often called 'dispersive forces' and gives information mainly on hydrophobic interaction. The other (γ_{sv}^{p}) involves the polar interaction often called 'nondispersive forces' and refers to hydrophilic interactions (Armengol *et al.* 2003). In this study, the polar component (γ_{sv}^{p}) of the surface free energy in treated dentine remained approximately constant in all experimental groups, whereas the dispersion component (γ_{sv}^{d}) of the surface free energy decreased significantly. As regards the hydrophobic and hydrophilic considerations, all the surface treatment groups reduced their hydrophobic character when compared with the control group with smear layer.

In the present study, the use of NaOCl as a final flush following RC-Prep treatment increased the surface free energy, although the results were still lower than those in the control group. This difference may have been caused either by the presence of urea peroxide in RC-Prep, which may oxidize collagen and/or the dentine matrix, or by the polyethylene glycol vehicle (Carbowax) that provides lubricant properties to RC-Prep (Morris *et al.* 2001). Prati *et al.* (1999) demonstrated that the diameter and size of open dentinal tubules increased after NaOCl treatment of demineralized dentine. Since the micromechanical retention increased, it may have altered the adhesion mechanism. Micromechanical bonding requires the presence of irregularities on the surface of the dentine matrix into which the material can penetrate (Saleh *et al.* 2002).

It has been reported that irrigation of the root canal system with water or saline does not alter the mechanical and chemical properties of root canal dentine (Sim *et al.* 2001, Driscoll *et al.* 2002). In the present study, smear layer was intentionally retained in the control group before irrigating with saline solution, and the surface free energy levels were found to be the highest in this group. The morphology and thickness of the smear layer may have a prominent role in determining wettability. Smear layer tends to act as a physical barrier to the diffusion of oral fluids and restorative materials. If the smear layer occludes the dentinal tubules, the permeability of dentine would be reduced (Pashley 1992). The high surface free energy of the ground dentin surface can be explained by

microcapillary penetration into the smear layer. However, this high surface free energy does not create high bond strength, since smear layer disturbs the bonding between dentine and the resin materials, and consequently reduces adhesion (Tanı *et al.* 1996).

Various agents have been incorporated into EDTA solutions to increase their antibacterial properties and reduce the surface tension of solution in an attempt to facilitate wetting and increase the ability of the chelators to penetrate the dentine (Tasman *et al.* 2000, Hülsmann *et al.* 2003). Further studies are needed to evaluate the wettability characteristics of the low surface tensioned EDTA compounds and how this relationship would affect the adhesion mechanism of dentine.

Conclusions

This study investigated the wettability of root dentine surface by calculating their surface free energy after applying different treatments. The wettability of a surface depends on its both components (dispersive and nondispersive) of surface free energy and is measured by the contact angle between surface of the solid and the polar and nonpolar liquids. Both chelating agents and NaOCl decreased the wettability of dentine surface when compared with the control group with smear layer. However, the use of NaOCl as a final flush following chelating agents did not ideally wet the root canal dentine.

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