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Effects of sodium hypochlorite and ethylenediaminetetraacetic acid on rotary nickel–titanium instruments evaluated using atomic force microscopy

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Abstract

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Aim To use atomic force microscopy (AFM) to evaluate the effects of sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA) on the surface characteristics of ProTaper rotary nickel–titanium instruments.

Methodology A total of twenty ProTaper (Dentsply Maillefer, Ballaigues, Switzerland) instruments (S1, S2, F1, F2) were divided into five groups: no immersion, immersion in 5.25% NaOCl for 5 or 10 min and immersion in 17% EDTA for 5 or 10 min. Twenty surface areas along 3- mm sections at the tip of the files (perfect squares of 1×1 μm) were analysed by AFM operating in contact mode under ambient conditions. Three-dimensional images (400×400 lines) were processed using Gwyddion software, and the roughness average (Ra) and the root mean square value (RMS) of the scanned surface profiles were recorded. Data were analysed by means of ANOVA and paired samples *t*-test.

Results Three-dimensional AFM images of the surface of ProTaper instruments, including new and those immersed in NaOCl and EDTA solutions, revealed topographic irregularities at the nanometric scale. RMS and Ra values of instruments treated with NaOCl and EDTA solutions were statistically higher than that of the new ones ($P < 0.05$).

Conclusions Atomic force microscopy three-dimensional images and roughness values indicated that short-term contact between NaOCl and EDTA endodontic irrigants and ProTaper instruments caused alterations in the surface of instruments.

Keywords: atomic force microscopy, EDTA, NaOCl, NiTi.

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Introduction

In recent years, rotary nickel–titanium (NiTi) instruments have become popular because of their superior elasticity and resistance to torsional fracture compared to stainless steel hand files (Walia *et al.* 1988). Despite the many advantages of NiTi instruments, unexpected fractures can occur during clinical use (Alpati *et al.* 2005, Troian *et al.* 2006) and there are different opinions about how many times rotary NiTi instruments should be used. The manufacturer, several government agencies around the world and many clinicians recommend single patient use of a set of rotary files (Sonntag & Peters 2007). Similar suggestions were proposed by Arens *et al.* (2003) to reduce fracture frequency, but other studies have reported that NiTi rotary instruments may be used up to ten times in simulated canals, or to prepare four molar teeth without fracture (Yared *et al.* 1999, Peters & Barbakow

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2002). However, because individual root canals are not anatomically the same, as well as other factors, it is difficult to give general recommendations on the appropriate number of uses for NiTi rotary instruments.

Cleaning and sterilization procedures plus contact between the NiTi instruments and the irrigating solutions, including sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA), can enhance their corrosion and deterioration which could, in turn, influence their mechanical properties and lead to undesirable fracture (Busslinger *et al.* 1998, Haikel *et al.* 1998, O'Hoy *et al.* 2003, Darabara *et al.* 2004, Berutti *et al.* 2006, Peters *et al.* 2007, Bonaccorso *et al.* 2008, Mohammadi 2008). Moreover, several studies suggested that the presence of defects on the surface of NiTi instruments (Sattapan *et al.* 2000, Tripi *et al.* 2001) and the deterioration of the files might explain the increase in fracture rates even under simple clinical conditions that should present no risk of breakage (Pruett *et al.* 1997, Gambarini 2001).

To date, the surfaces of NiTi rotary instruments have been analysed using a variety of different techniques. Scanning Electron Microscopy (SEM) has been widely used to evaluate the topographical characteristics of different kinds of materials (Cazaux 2005) and has been accepted as a suitable method for the evaluation of NiTi instrument surfaces (Tripi *et al.* 2001, Martins *et al.* 2002, Alpati *et al.* 2003, 2005). Recently, atomic force microscopy (AFM) has become increasingly popular for imaging the three-dimensional surfaces and interfaces of biomaterials (Siedlecki & Marchant 1998, Jandt *et al.* 2000). In addition, AFM has also been recommended as a valuable research tool for investigating the topography of various endodontic instruments (Valois *et al.* 2005, 2008, Inan *et al.* 2007, Topuz *et al.* 2008). AFM is part of the scanning probe microscopy family and is able to reconstruct three-dimensional surface topography images in real time. The sample surface is probed with a sharp tip attached to a flexible cantilever that deflects in the z-direction as a result of the surface topography during tip scanning over the sample surface. A photodiode detects the deflection of the cantilever through a laser beam focused on, and reflected from, the rear of the cantilever. Moreover, AFM records the data of samples in digital form as sets of x, y and z values. These sets can be analysed with dedicated digital software to give all the data pertaining to the examined surface in quantitative form by using vertical topographic parameters (Siedlecki & Marchant 1998, Jandt *et al.* 2000). With these parameters, it is possible to evaluate the vertical amplitude of a surface topography.

Because AFM has recently been successfully used to investigate the surface characteristics of new and used ProTaper NiTi instruments (Inan *et al.* 2007), the purpose of the present study was to evaluate the effects of NaOCl and EDTA irrigating solutions on the nanostructure surface of ProTaper instruments using AFM analysis. In particular, roughness average (Ra) and root mean square (RMS) values of instrument surfaces were analysed to quantify the effects of endodontic irrigants tested.

Materials and methods

A total of twenty ProTaper NiTi rotary (Dentsply Maillefer, Ballaigues, Switzerland) instruments were analysed. The instruments were divided into five groups; in each group, S1, S2, F1 and F2 instruments were tested. One group was set as the control (no treatment), and the other four groups were immersed in 5.25% NaOCl or 17% EDTA solutions for 5 or 10 min, respectively.

The files were attached to a metal holder using a rapid-drying cyanoacrylate glue. Each sample was placed on the AFM (Assing, Italy), and then 20 areas of the surface were analysed on a 3-mm section at the tip of the files. The AFM images of the file samples were recorded using the contact mode operation under ambient conditions (Fig. 1).

Atomic force microscopy probes (curvature radius < 10 nm) mounted on cantilevers (250 μm), with a spring constant of 0.1 N m^{-1}, were used. Scanned areas were perfect squares (1 × 1 μm). Three-dimensional images (400 × 400 lines) were processed using Gwyddion software 2.19 (http://www.gwyddion.net). The Ra and the RMS of the scanned surface profiles were recorded. These parameters indicated changes in vertical surface topography, and an increase in Ra and RMS values meant alterations of NiTi instruments surface caused by the irrigants.

Statistical analysis of the data was performed by means of an analysis of variance (ANOVA) followed by a Tukey's test for multiple comparisons. Moreover, differences of Ra and RMS values between new instruments and immersed in irrigating solutions were analysed statistically by the paired samples t-test. The level of significance was set at $P < 0.05$.

Results

Three-dimensional AFM images of the surfaces of ProTaper instruments (S1, S2, F1 and F2), including

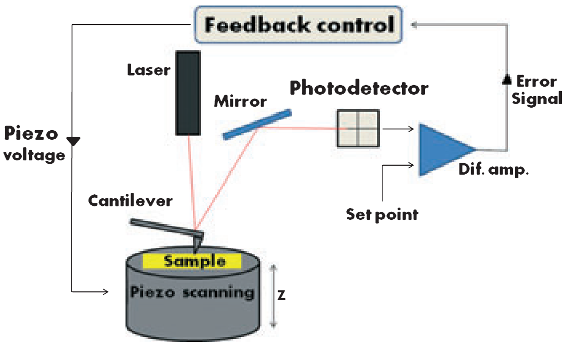

Figure 1 Schematic representation of the operating principle of an atomic force microscope.

new and those immersed in NaOCl and EDTA solutions, showed topographic irregularities at the nanometric scale (Figs 2 and 3). The control group images of S1 and S2 files were different compared to F1 and F2 control instruments (Figs 2 and 3), whilst all treated ProTaper images revealed an increase in roughness compared to controls (Figs 2 and 3). To investigate the quantitative differences in topography resulting from immersion in canal irrigants, vertical topographic parameters (Ra and RMS) were evaluated. Ra and RMS values of S1 and S2 files were significantly lower compared to F1 and F2 files ($P < 0.001$, ANOVA). The immersion of ProTaper instruments in NaOCl and EDTA solutions for 5 and 10 min demonstrated a significant increase ($P < 0.05$, paired samples *t*-test) in the mean values of Ra and RMS compared to controls (Table 1). In particular, F1 and F2 had the highest overall values of Ra and RMS, whilst the largest increase was observed for S2 instruments immersed in NaOCl and EDTA solution for 10 min compared to control and the other experimental groups (Table 1). The increase in Ra and RMS values was time and irrigating solution dependent ($P < 0.001$, ANOVA).

Discussion

Sodium hypochlorite is an effective antimicrobial with tissue-dissolving capabilities that has also been recommended as a cleaning agent for endodontic instruments (Spencer *et al.* 2007). EDTA was introduced into endodontics as a chelating and lubricating agent used for narrow or calcified canals but has little or no antibacterial activity (Calt & Serper 2002, Zehnder *et al.* 2005, Dotto *et al.* 2007, Putzer *et al.* 2008). In the present study, topographic surface changes in ProTaper instruments immersed in NaOCl and EDTA solution were evaluated using AFM. Both irrigants caused significant deterioration of instrument surfaces resulting in an increase in RMS and Ra values compared to new instruments. It has been shown that immersion in 5.25% NaOCl for 5 min caused localized surface pitting and cracks that modifies the integrity and resistance to fracture of NiTi instruments (Topuz *et al.* 2008). It has also been reported that 5% NaOCl solution induced significant corrosion after immersing NiTi instruments for 30 and 60 min (Busslinger *et al.* 1998). Moreover, energy dispersive X-ray spectroscopy analysis (EDX) revealed that 5.25% NaOCl affected the chemical composition of the surface after 1–4 h and the exposed bulk of instruments through machining marks and fabrication microcracks (Darabara *et al.* 2004). The present findings on NaOCl are in line with the latter studies, whereas they contradict other recent investigations which reported no effects caused by NaOCl and EDTA on NiTi instruments with different surface treatments (Darabara *et al.* 2004, Bonaccorso *et al.* 2008, Cavalleri *et al.* 2009). Darabara *et al.* (2004) used the cyclic potentiodynamic polarization method to evaluate the pitting and crevice corrosion characteristics of NiTi instruments exposed to 17% EDTA and 5.25% NaOCl solutions, demonstrating high corrosion resistance of the instruments to both irrigating substances. Bonaccorso *et al.* (2008) confirmed that long-term EDTA contact did not alter the surface structure of files. However, the data from the latter studies were based on different contact times between files and irrigants and on SEM analysis instead of AFM, which might explain the differences between the results.

To date, SEM has been widely used to evaluate the surface characteristics of different NiTi instruments (Cazaux 2005). Recently, AFM analysis was introduced to provide qualitative and quantitative information on the topography of various materials, including endodontic files (Valois *et al.* 2005, 2008, Inan *et al.* 2007, Topuz *et al.* 2008). SEM and AFM are both able to resolve structure down to the nanometer scale, but the image formation mechanisms are quite different, resulting in disparate types of information. SEM uses an electron beam operated under vacuum to produce a two-dimensional 'photographic' image of the samples, but cannot directly provide any quantitative data (Cazaux 2005). In contrast, AFM reconstructs a three-dimensional image of the surface topography in real time. These data sets can be analysed with dedicated digital software to provide all the data pertaining to the examined surface in a quantitative

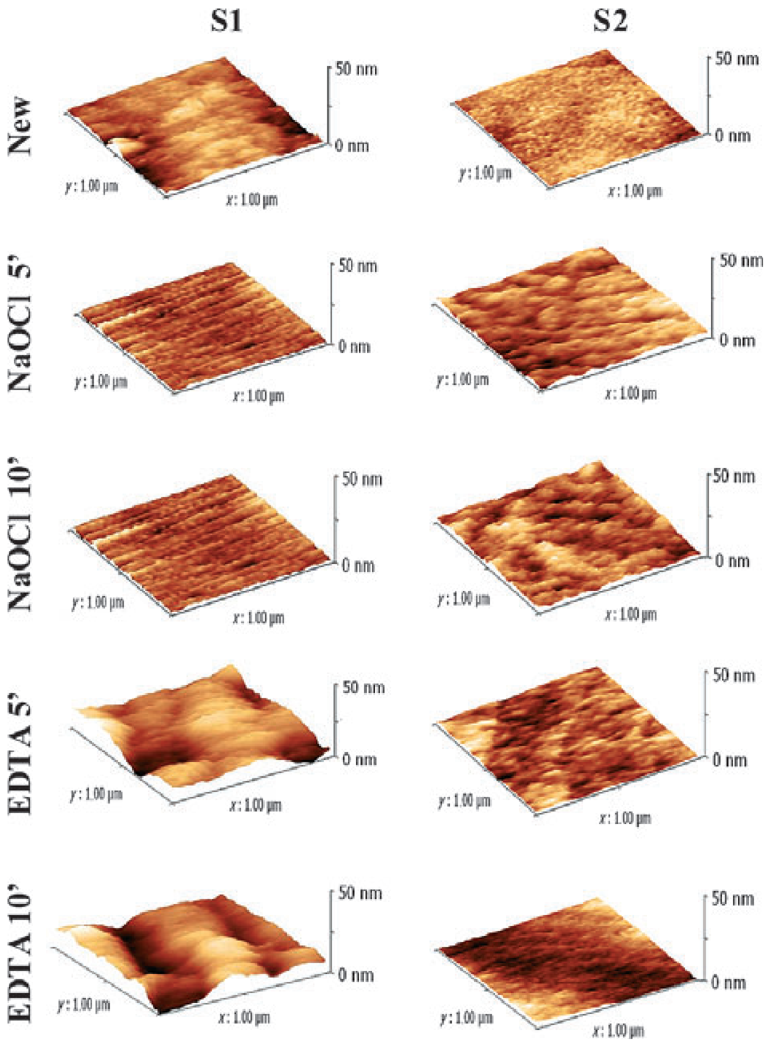

Figure 2 Three-dimensional atomic force microscopy images of new and immersed ProTaper S1 and S2 instruments.

form. Of additional significance, AFM provides appropriate topographic contrast with greater detail, direct measurements in all three dimensions with a vertical resolution of ~0.1 nm and views of surface features in a broad range of conditions (Siedlecki & Marchant 1998, Jandt *et al.* 2000).

Recently, AFM has been recommended for the study of surface characteristics of rotary NiTi instruments (Valois *et al.* 2005, 2008, Inan *et al.* 2007, Topuz *et al.* 2008). The three-dimensional images and roughness values of rotary instruments were in agreement with previous AFM studies (Valois *et al.* 2005, 2008, Inan *et al.* 2007, Topuz *et al.* 2008). The data revealed lower Ra and RMS values for S compared to F instruments, which is likely to be related to differences arising from the manufacturing process (Martins *et al.* 2002, Alpati *et al.* 2005). Moreover, different instrument tapers might influence their topography and, in turn, the effect of irrigate solutions on deterioration (Inan *et al.* 2007, Topuz *et al.* 2008). Furthermore, the present study demonstrated a clear effect of NaOCl on the surface of ProTaper instruments and provided original and new information on the deterioration caused by short-term EDTA contact. The data are in line with previous AFM studies that evaluated the effect of NaOCl on RaCe rotary instruments (Topuz *et al.* 2008) and on ProTaper instruments after clinical use (Inan *et al.* 2007). However, as no AFM data was reported previously regarding the influence of EDTA, the present study appears to be the first that provided evidence that EDTA could alter the surface of NiTi endodontic instruments. Interestingly, the RMS and Ra values resulting from the AFM analysis suggest that EDTA alters the ProTaper surface more than NaOCl.

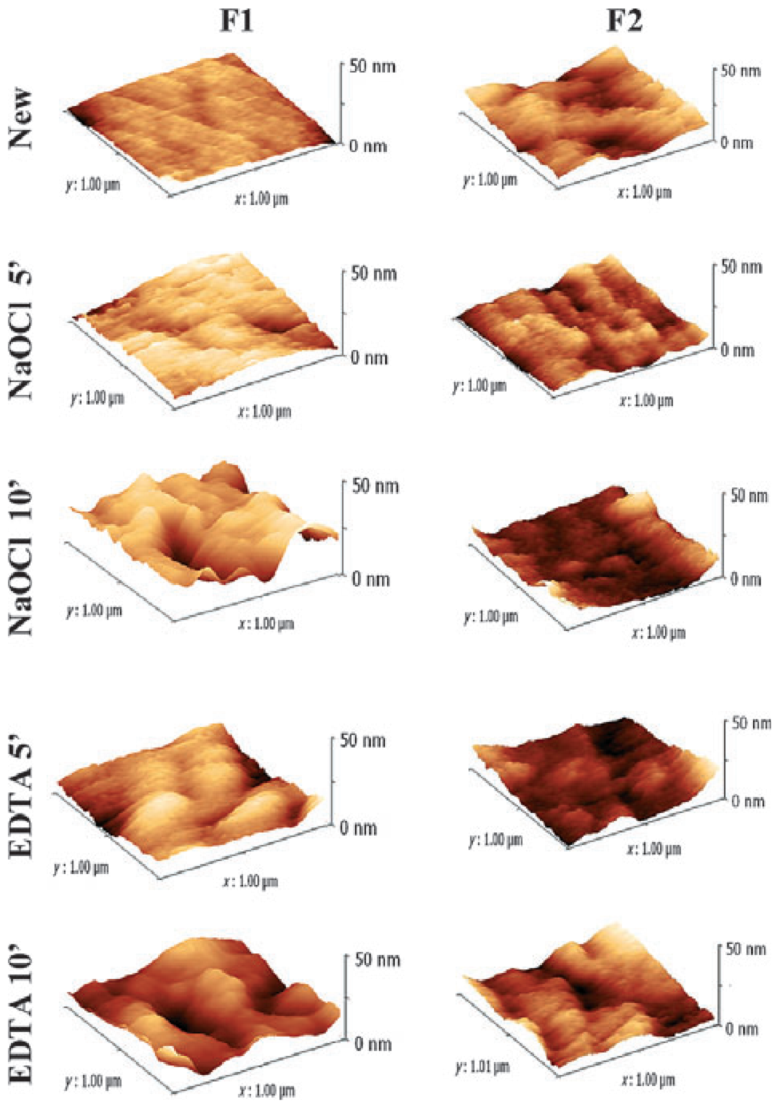

Figure 3 Three-dimensional atomic force microscopy images of new and immersed ProTaper F1and F2 instruments.

Table 1 Mean ± standard deviation of Ra and RMS values (nm) in the experimental groups

	Ra				RMS			
Treatments	S1	S2	F1	F2	S1	S2	F1	F2
New	0.77 ± 0.21	0.50 ± 0.13	2.36 ± 1.02	2.3 ± 0.69	1.09 ± 0.36	0.65 ± 0.17	3.09 ± 1.47	2.88 ± 0.72
NaOCl 5′	1.20 ± 0.79*	0.73 ± 0.22**	3.27 ± 1.34*	3.3 ± 0.87*	1.71 ± 0.98*	0.95 ± 0.32**	4.33 ± 1.90*	4.10 ± 1.13*
NaOCl 10′	2.46 ± 0.70***	1.19 ± 0.67**	4.26 ± 1.04**	4.18 ± 1.37**	3.28 ± 1.17***	1.60 ± 0.82***	4.85 ± 1.70*	5.10 ± 2.22*
EDTA 5′	1.86 ± 0.67***	3.87 ± 0.30***	5.10 ± 1.07***	3.82 ± 0.61***	2.45 ± 0.80***	4.96 ± 1.20***	5.52 ± 1.16***	4.79 ± 0.74***
EDTA 10′	4.02 ± 1.17***	4.56 ± 1.28***	5.07 ± 0.91***	5.18 ± 1.67***	5.09 ± 1.47***	6.15 ± 0.90***	7.16 ± 1.18***	7.00 ± 3.14**

*($P < 0.05$), **($P < 0.01$) and ***($P < 0.001$) indicates statistically significant differences between files new and immersed in irrigating solutions (paired samples *t*-test).
EDTA, ethylenediaminetetraacetic acid; NaOCl, sodium hypochlorite; Ra, roughness average; RMS, root mean square value.

These differences can likely be attributed to the lower pH of EDTA compared with NaOCl (Bayramoğlu *et al.* 2000).

In summary, short-term contact between NaOCl and EDTA solutions and ProTaper instruments increased their RMS and Ra values indicating deterioration on the surface. The results suggest that AFM is a suitable method for quantifying and evaluating surface of endodontic instruments and the effects of irrigates. However, further studies are needed to compare the effect of different endodontic irrigants on various NiTi rotary instrument surfaces during clinical performance.

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