The effect of irrigation solutions and sterilization process on the surface properties of ProTaper Next and TruNatomy rotary files: An atomic force microscopy study

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Abstract

Aim: This study sought to evaluate the effects of different irrigation solutions and sterilization processes on the surface properties of ProTaper Next (PTN; Dentsply-Maillefer, Ballaigues, Switzerland) and TruNatomy (TN; Dentsply Sirona, Ballaigues, Switzerland) nickel-titanium rotary files.
Methodology: 30 ProTaper Next and 30 TruNatomy files were used. Both types of files were divided into five groups. The files in three groups were dipped into 5.25% NaOCI (CanalPro, Coltene/Whaledent, Altstätten, Switzerland), BioAKT (New Tech Solutions s.r.l., Brescia, Italy), and 18% etidronic acid (Dual Rinse HEDP, Medcem, Weinfelden, Switzerland), respectively, for 5 minutes, while the files in the fourth group were exposed to five autoclave cycles. The files in the fifth (control) group did not receive any treatment. Scans were performed from five different points using an atomic force microscope (AFM). The assumption of the conformity of the quantitative variables to a normal distribution was evaluated using the Kolmogorov-Smirnov test. A one-way analysis of variance was used to compare the quantitative measures between more than two independent groups, while the post-hoc least significant difference test was used for the pairwise comparisons ($p<0.05$).
Results: AFM images of the PTN and TN files in all the groups revealed surface irregularities. The root mean square (RMS) and depth values of the files dipped in NaOCI, BioAKT, and Etidronic acid were statistically higher than those of the control group ($p<0.05$). Moreover, the RMS and depth values were statistically higher in the files that had been subjected to five autoclave cycles when compared with the control group ($p<0.05$). The RMS values and depth values among the groups were found to be compatible.
Conclusion: All the applications increased the surface roughness of the PTN and TN files. The Etidronic acid and sterilization applications were found to increase the RMS and depth values the most when compared with the control group.

Keywords: Etidronic acid, BioAKT, surface analysis, atomic force microscopy, autoclave sterilization, heat treatment

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Introduction

Root canal treatment is mainly intended to ensure the mechanical and chemical disinfection of irritants from the root canal system as well as the hermetic filling and obturation of the conically shaped root canal in order to prevent reinfection (1). Biomechanical preparation, which represents a key stage of endodontic treatment, is defined as a procedure involving the removal of tissue residues, bacteria, and bacterial outputs from the pulp chamber and root canal, followed by the cleaning, shaping, and preparation of the apical region (2).

Sodium hypochlorite (NaOCI) is known to be effective against spores and viruses, in addition to having a broad antibacterial spectrum. Moreover, NaOCI, which is also an organic solvent, exhibits a higher capability to dissolve necrotic tissues when compared with living tissues (3). Due to these valuable characteristics, liquid NaOCI has been used as an irrigation solution in root canal treatment since the 1920s.

Recently, etidronic acid (HEDP) has been proposed as a weak alkaline chelating agent with a pH of around 10.7 and can be mixed with NaOCl without compromising the antimicrobial/antibiofilm and tissue dissolving properties of the latter. The mixture of NaOCl and HEDP has the ability to reduce smear layer formation and reducing the accumulation of hard tissue debris during root canal instrumentation to an extent similar to the traditional use of NaOCl followed by EDTA (4). As an inorganic solvent, HEDP has also been recommended as an alternative to ethylenediaminetetraacetic acid (EDTA) or citric acid (CA). In a randomized clinical study evaluating the antimicrobial efficacy of a combined NaOCl/HEDP solution, it was reported that pure NaOCl made 40% of the canals culture-negative, the mixture with HEDP resulted in 50% root canals bacteria-free, but this difference was not statistically significant (5). Furthermore, HEDP (1- hydroxyethane 1, 1-diphosphonic acid) is a biocompatible chelator that can be used in combination with sodium hypochlorite without the short-term loss of the desired properties of either compound (6).

HEDP exhibits proteolytic and antimicrobial properties. Yet, it is widely thought that NaOCI is the only chelator that can be used without restricting the antimicrobial effects and tissue-dissolving efficiency (3). The recommended concentrations of HEDP for use in root canal irrigation are 9% and 18% (7). Most previous studies on a combined HEDP/NaOCl irrigation have used 9% HEDP in 2.5% NaOCl; this was because the researchers of these studies combined an 18% HEDP solution with a 5% NaOCl (7). To the best of our knowledge, no prior study has investigated the effect of HEDP on the surface roughness of nickel-titanium rotary file systems.

BioAKT is recognized as an innovative root canal irrigation solution. It contains 4.846% CA, 0.003% silver ions, unknown detergents, and water (8). It effectively kills microorganisms by impairing the membrane integrity of the microbial cells with its silver ions. BioAKT also removes the smear layer with CA through its activity in the silver citrate complex (9, 10). Again, to the best of our knowledge, no prior study has examined the effect of BioAKT on the surface roughness of nickel-titanium rotary file systems.

Nickel-titanium (Ni-Ti) files prevent apical transportation, preserve the original form of the root canal better than classic files, and are more flexible than classic files (11). However, Ni-Ti files are also associated with the disadvantages of being expensive and having a tendency to break unexpectedly (12). In fact, the unexpected breaking of Ni-Ti files represents one of the most common complications during root canal treatment (13). Chemical mechanisms such as disinfection and sterilization applied to an Ni-Ti file prior to or during instrumentation, as well as the interaction between the utilized irrigation solution and the file, can cause abrasion and/or the deterioration of the file, which may lead to premature breakage (14). Currently, there is no consensus as to how many times Ni-Ti rotary instruments can be used (15).

ProTaper Next (PTN) is multifile rotary system with an off-centered rectangular cross section that is multitapered over its length and made of heat-treated M-Wire Ni-Ti alloy which works in a continuous asymmetrical rotational motion. In addition, its offset design facilitates the removal of debris and fillers from the canal and also increases the flexibility of PTN files throughout the active portion of the process (16). PTN is available in 5 sizes: X1 (17.04), X2 (25.06) X3 (30.07), X4 (40.06), and X5 (50.06).

Recently, a novel heat-treated NiTi instrument, TruNatomy (TN) was introduced that manufactured from a unique thin Ni-Ti wire with a 0.8-mm maximum flute diameter instead of 1.2 mm, which is commonly used in other conventional Ni-Ti files. The manufacturer claims that this difference serves to preserve the tooth structure and canal geometry, especially in a severely curved canal, due to the regressive slim taper, reduced shape memory, and special heat treatment of the Ni-Ti alloy. It has an off-centered parallelogram cross section to preserve the radicular dentin during mechanical preparation and provides space for additional debris removal (17). It consists of 5 specific instruments (Orifice Modifier, Glider and three shaping files) which work in continuous rotation at 500 rpm with 1.5 N.cm torque.

Atomic force microscopy (AFM) is a visualization technique with high atomic resolution that enables the microscopic visualization of the surfaces of samples (18). As AFM can obtain both qualitative and quantitative information regarding surface properties and three-dimensional (3D) images, it has become very popular for use in examining the surface properties of materials. Moreover, it is easy to use and can provide detailed surface information at even the molecular level (19).

The present study sought to examine the effects of various irrigation solutions and sterilization procedures on the surface properties of PTN and TN root canal files. The null hypothesis was that the utilized treatments do not affect the surface properties of the files.

Materials and Methods

Evaluating the files for control purposes

In this study, a total of 30 PTN and 30 TN files were used. Both types of files were divided into five groups, each of which contained six files (Table 1). The PTN (X2 #25/0.06) and TN (prime #26/0.04) files selected for the groups were all newly unpacked rotary instrument files. Although new files were used, they were still examined under a stereomicroscope to identify any possible deformation that might have occurred during the production phase. All files suspected to be deformed were excluded from the study.

Dipping the files in irrigation solutions

Three groups of files were dynamically dipped into NaOCl (5.25%), HEDP (18%), and BioAKT solutions, respectively, for 5 minutes in a rotating state. The files were then washed with distilled water and dried. Subsequently, they were cut with a carbon separating disc with a 5 mm distance from the apical regions for the purpose of examination. The samples were placed in an AFM device for analysis.

Applying sterilization treatment to the files

For the sterilization treatment, six each of the newly unpacked PTN and TN files were used. More specifically, the files were repacked and the sterilization process was repeated five times in an NC 23B autoclave (Nüve, Ankara, Turkey) at 134°C for 20 minutes. The apical 5 mm parts of the files were cut using a carbon separating disc and placed in an AFM device for analysis.

AFM surface evaluation

All the file samples were placed on an AFM device (XE-100, PSIA Corp., Korea) with a 5 µm x 5 µm area, a scanning speed of 1.0 Hz, and a resolution of 256 pixels. Each sample was scanned at five different points using silicon nitride tips compatible with the tapping (non-contact) mode. During the AFM examinations, the average roughness of each sample was calculated using the XEI analysis program. Moreover, 3D analysis images, depth data, and root mean square (RMS) data were obtained for the purpose of numerical comparison.

Statistical analysis

The raw RMS data regarding the surface roughness and the raw depth data regarding the maximum depth obtained in the study were transferred to statistical data analysis software. More specifically, the statistical analysis of all the gathered data was conducted using SPSS Version 22 software (IBM SPSS Inc., Armonk, NY, USA). The assumption of the normal distribution of the quantitative variables was evaluated using the Kolmogorov-Smirnov test. All descriptive statistics were presented as the mean ± standard deviation. A one-way analysis of variance (ANOVA) was used to compare the quantitative measurements among more than two independent groups, while the post-hoc least significant difference (LSD) test was used to perform the pairwise comparisons. The level of significance was assumed to be $p<0.05$.

Table 1. Categorization of the files.

	Protaper next	TruNatomy
Group 1: Control	No treatment applied	No treatment applied
Group 2: %5.25 NaOCl	Dipped for 5 minutes	Dipped for 5 minutes
Group 3: %18 Etidronic acid	Dipped for 5 minutes	Dipped for 5 minutes
Group 4: BioAKT	Dipped for 5 minutes	Dipped for 5 minutes
Group 5: Sterilization	Autoclaved for 5 times	Autoclaved for 5 times

Results

In this study, a statistically significant difference was found between the PTN file and the TN file groups in terms of the mean RMS values ($p<0.05$). All the treatment groups exhibited increased surface roughness when compared with the control group. The mean RMS and standard deviation values according to the in-group applications are presented in Table 2. The sterilization and HEDP treatments increased the RMS values of both the PTN and the TN files the most.

Furthermore, a statistically significant difference was found between the PTN file, and the TN file groups in terms of the mean depth values ($p<0.05$). All the treatment groups exhibited increased depth values when compared with the control group. The mean depth and standard deviation values according to the in-group applications are shown in Table 3. The sterilization and HEDP treatments increased the depth values of both the PTN and the TN files the most.

In addition, 3D AFM topographic surface images of files in the PTN and TN groups are shown in Figure 1 and Figure 2, respectively.

Table 2. Mean RMS and standard deviation values as per the in-group applications

	Protaper Next	TruNatomy
Control	32.74 ± 6,76 (nm)	36,63 ± 6,77 (nm)
%5.25 NaOCl	38.78 ± 4,69 (nm)[a]	46,66 ± 5,92 (nm)[a]
%18 Etidronic acid	44.12 ± 5,43 (nm)[b]	52,48 ± 9,93 (nm)[b]
BioAKT	39.22 ± 5.91 (nm)[a]	44,88 ± 8,82 (nm)[a]
Sterilization	46.27 ± 6,95 (nm)[b]	55,18 ± 6,96 (nm)[b]
p	<0,05	<0,05

* p<0.05 statistically significant
* One-way ANOVA
* Within the same measurement category, values with the same capital letter are not statistically significant by Tukey's method for post-hoc analysis.

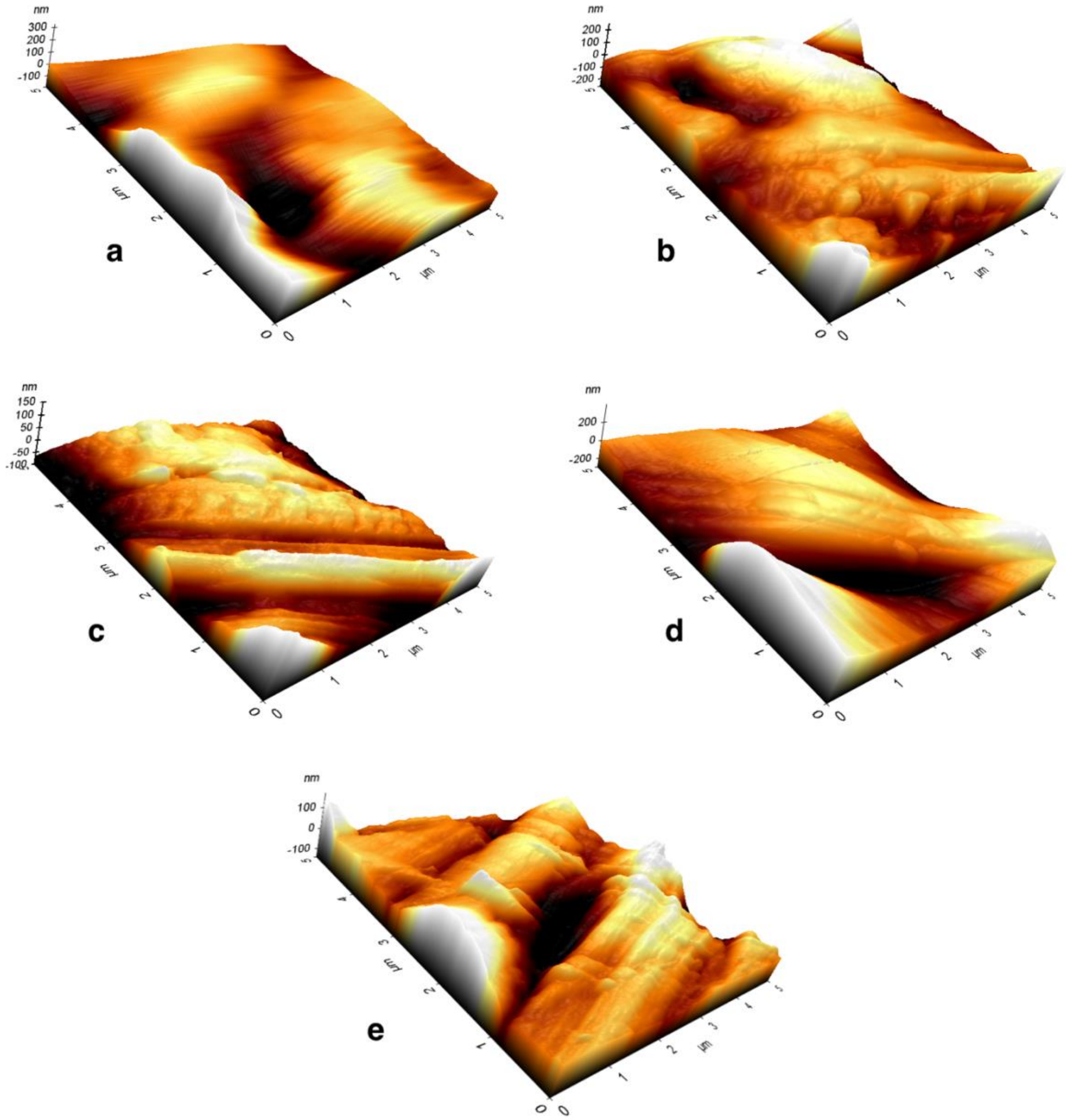

Figure 1. 3-D AFM topographic surface images for ProTaper Next groups **a)** Control, **b)** NaOCl, **c)** Etidronic acid, **d)** BioAKT, **e)** Sterilization

Table 3. Mean “Depth” and standard deviation values as per the in-group applications

	Protaper Next	TruNatomy
Control	303.15 ± 48,73 (nm)	327,83 ± 67,77 (nm)
%5.25 NaOCl	361.87 ± 71,12 (nm)[a]	397,58 ± 62,41 (nm)[a]
%18 Etidronic acid	430.14 ± 65,82 (nm)[b]	432,42 ± 74,03 (nm)[b]
BioAKT	363.84 ± 47,88 (nm)[a]	388,44 ± 62,82 (nm)[a]
Sterilization	465.16 ± 74,01 (nm)[b]	448,83 ± 58,53 (nm)[b]
p	<0,05	<0,05

*p<0.05 statistically significant
*One-way ANOVA
*Within the same measurement category, values with the same capital letter are not statistically significant by Tukey's method for post-hoc analysis.

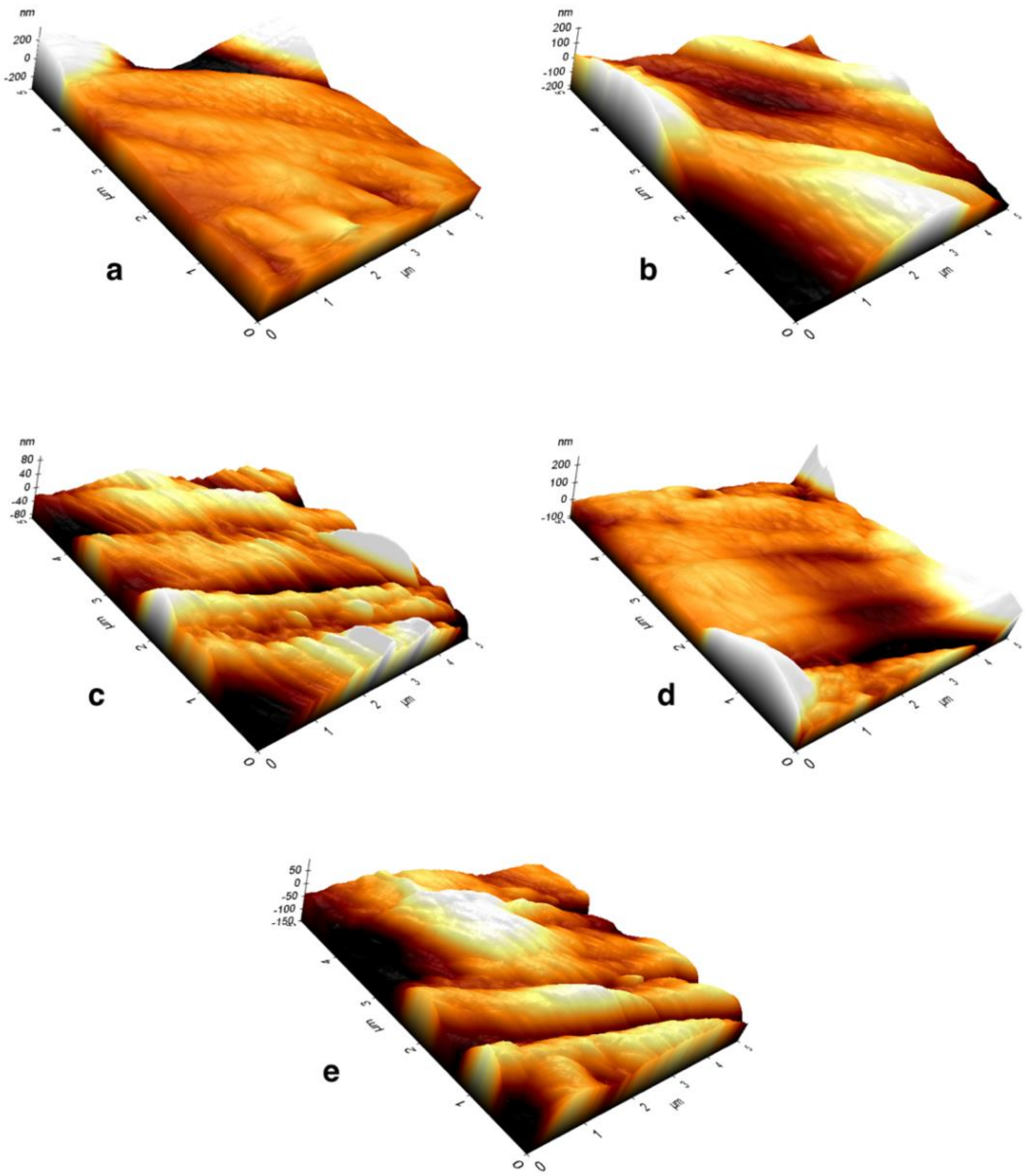

Figure 2. 3-D AFM topographic surface images for TruNatomy groups **a)** Control, **b)** NaOCl, **c)** Etidronic acid, **d)** BioAKT, **e)** Sterilization

Discussion

Mechanical preparation and irrigation allow for both microorganisms and necrotic tissues within the root canal to be removed (20). Moreover, biomechanical preparation is recognized as a key stage of endodontic treatment (2). Despite the many advantages offered by Ni-Ti files, unexpected breakage is a common complication during root canal treatment, and instrument fragments stemming from breakages may limit the success of root canal treatment and result in tooth loss (21).

In addition to the physical effects of using Ni-Ti rotary files in relation to root canal preparation, the files also come into contact with different irrigation solutions and need to be sterilized in an autoclave prior to being used again. Chemical mechanisms such as exposure to high temperatures during the sterilization process and interactions with different irrigation solutions may cause the deformation, abrasion, or deterioration of the files (14). Previous studies concerning this matter have made use of different visualization methods.

Techniques such as stereomicroscopy, scanning electron microscopy (SEM), and AFM can be used to examine the surface properties of materials. For instance, SEM uses a vacuum-operated electron beam to obtain two-dimensional "photographic" views of samples, although it cannot directly provide any quantitative data (22). In the case of AFM, both qualitative and quantitative information can be obtained regarding surface features, as can a 3D image. AFM is simple to use and able to provide detailed surface information at even the molecular level (19). As a consequence, many studies have examined the surface topography of files by means of AFM (23, 24). The present study used AFM to evaluate the surface properties of Ni-Ti files.

Martins et al. identified the presence of debris, metallic glow, and deep scraping marks formed during the production phase of new files (25), while Pirani et al. reported that new Ni-Ti files exhibited surface defects (26). The findings of this study are compatible with the findings of Martins et al. and Pirani et al. The AFN examination of the untreated PTN and TN files (i.e., the control groups) revealed that they also exhibited surface roughness. This finding is consistent with the findings of other studies reporting surface irregularities to occur during the production of Ni-Ti files (15, 24).

Furthermore, Martins et al. reported that after dipping Ni-Ti files in 5.25% NaOCl solution for 24 hours, no change was observed in the surface properties of the tested files when examined using SEM (25). However, Peters et al. showed that the use of 5.25% NaOCl reduced the cyclic fatigue resistance of both ProFile and RaCe Ni-Ti files (27). This difference in findings may stem from the difference in operating standards. Berutti et al. reported that galvanic corrosion occurred in Ni-Ti files upon contact with the NaOCl solution, causing pits and cracks that changed the integrity of the instrument's surface and paved the way for reduced cyclic fatigue fracture resistance (28). Fayyad and Mahran showed that the application of NaOCl at a concentration of 5.25% for 5 minutes to a TF file increased its surface roughness (29). This study revealed that after the application of 5.25% NaOCI, the increase in the surface roughness was statistically significant ($p<0.05$). This finding is consistent with the findings of other studies showing that NaOCI application increases the surface roughness of Ni-Ti files (30). The recommended concentrations of HEDP for use in root canal irrigation are 9% and 18%. HEDP is a weak chelator and in one study in which 9% and 18% concentrations were used to remove the smear layer, the 18% concentration was found to give better results (32), which is why the higher concentration was used in the present study.

Berutti et al. reported that surface roughness served to reduce cyclic fatigue resistance (28). Erik and Özyürek found that when used together with HEDP at body temperature, NaOCl solution significantly reduced the cyclic fatigue resistance of the tested HyFlex EDM, Reciproc Blue, and WaveOne Gold files, although neither HEDP nor NaOCI affected the cyclic fatigue resistance when used on their own (33). Erik and Özyürek also considered that corrosion might have contributed to the decrease in the cyclic fatigue resistance of the files. In the present study, the surface roughness of the PTN and TN files significantly increased as a result of the dynamic immersion in HEDP ($p<0.01$). Thus, the findings of this study support those of the study by Erik and Özyürek (33). No prior study has examined the effect of HEDP on the surface roughness of Ni-Ti rotary files.

Moreover, in this study, BioAKT was found to significantly increase the surface roughness of both the PTN and the TN files ($p<0.05$). Again, no prior study has investigated the effect of BioAKT on the surface roughness of Ni-Ti rotary files.

Infection control is highly important in relation to clinical practices. Dentistry patients are considered to be at high risk based on their potential for catching and spreading contagious disease. Indeed, there is a constant risk of cross-contamination among patients, dentists, and dental assistants during the course of dental practices (34). There is currently no consensus as to the effect of sterilization procedures applied to the surface of Ni-Ti files (19).

Razavian et al. reported that multiple rounds of autoclave sterilization increased the surface roughness of the tested files, and this roughness was correlated with the number of autoclave cycles (35). In a study involving Greater Taper and ProFile Ni-Ti files, Valois et al. found that more than one autoclave cycle increased the surface irregularities of the rotating Ni-Ti files (25). Nair et al. reported that after multiple autoclave cycles performed on ProTaper and Mtwo files, the surface roughness of both types of files significantly increased (36). The present study found that the surface roughness of the PTN and TN files significantly increased following the application of the sterilization process for 20 minutes at 134°C five times ($p<0.01$). This finding is consistent with the findings of other studies revealing that autoclave sterilization increases the surface roughness of Ni-Ti files (35, 36).

Conclusions

Within the limits of this study, it can be concluded that the application of NaOCl, HEDP, BioAKT, and sterilization increased the surface roughness of the PTN and TN files. Thus, the study's null hypothesis can be rejected. The RMS and depth values were compatible with each other among the groups. Moreover, the HEDP and sterilization groups showed the greatest increases in terms of the RMS and depth values when compared with the control group. Further studies are required to compare the effects of HEDP and BioAKT on the surface roughness of Ni-Ti files.

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