

Research Article

The Impact of Multiple Autoclave Cycles on the Surface Roughness of Thermally Treated Nickel-Titanium Endodontic Files

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In this study, we evaluate the surface roughness (SR) of three thermally treated nickel-titanium rotary instruments (i.e., Tru-Natomy [TN], ProTaper Gold [PTG], and EdgeTaper Platinum [ETP]) after impact testing with multiple autoclave sterilization cycles using scanning electron microscopy (SEM). Overall, 60 thermally treated files were sourced (i.e., 20 TN, 20 ETP, and 20 PTG files), and each group was divided into four equal subgroups of five files ($n = 5$). An unsterilized group was assigned as the pre-autoclaving group (Cycle 0), and the other three groups underwent various autoclave sterilization cycles (Cycles 1, 5, and 10). The roughness average (Ra), root mean square (Rq), and average maximum peak-to-valley height (Rz) values were evaluated using SEM and ImageJ software. The Shapiro–Wilk test, one-way analysis of variance, and post-hoc Tukey test were used to analyze the data. The statistical significance level was set at $p < 0.05$. Before autoclaving, all instruments showed debris and SR but with no statistically significant differences existing between the groups. SR values decreased after one autoclaving cycle for all instruments. After five autoclave cycles, the Rz value of ETP was statistically higher compared with PTG and TN. After 10 autoclave cycles, the Ra and Rq values of TN were statistically higher compared with ETP and PTG. These results suggest that multiple autoclaving sterilization cycles influence the SR of TN, PTG, and ETP, with ETP showing higher levels of surface irregularities than PTG and TN.

1. Introduction

The innovation of nickel-titanium (NiTi) rotary files has enriched the field of endodontics. Various manufacturing strategies have been developed to optimize mechanical properties and reduce the incidence of file fractures [1, 2].

NiTi files are often reused after autoclave sterilization due to their high cost [3, 4], which is a critical step in infection control to prevent cross-infection between endodontic treatments [5]. Additionally, many dental clinicians

may use predetermined sets of NiTi files; some files go unused in a single visit but will nevertheless require autoclaving before the next visit [6].

The thermal treatment employed in manufacturing NiTi files enhances their mechanical properties [7]. However, the mechanical properties may be negatively or positively affected by heat generated during the sterilization process [8–10]. The effect of repeated sterilization is reflected by an increase in the surface roughness (SR) of NiTi rotary files [3, 11, 12] and a decrease in their cutting efficiency, which

can generally be observed after five or 10 repeated autoclaving cycles [13–15]. The surface changes may lead to unfavorable file separation during root-canal preparation, which may jeopardize the treatment outcome [3].

Recently, TruNatomy (TN; Dentsply Sirona, Baillagues, Switzerland) has been introduced with special heat treatment. To manufacture TN, a thin 0.8 mm wire with a common file diameter (1.2 mm) is utilized. TN files are less prone to separation, as they are extremely flexible and resistant to cyclic fatigue [16, 17].

ProTaper Gold (PTG; Dentsply Maillefer, Baillagues, Switzerland) is the successor of the ProTaper Universal system (Dentsply Maillefer, Baillagues, Switzerland). PTG files have the same sequence and design as ProTaper Universal, but they are thermally treated and possess controlled memory properties [18]. The mechanical properties of PTG demonstrated superior results compared with ProTaper Universal, which could have implications for clinical use [18–21].

Comparable to other systems on the market, a number of NiTi systems have recently been launched with similar designs and preparation procedures. Among these is EdgeTaper Platinum (ETP; Albuquerque, NM, US). According to the manufacturer, ETP instruments have a similar sequence and design to PTG instruments [22], but it has been proved that they are actually different in terms of design and surface treatment [23]. Unlike TN and PTG, ETP instruments are not provided in pre-sterile packs [24].

A thorough literature review found no studies on the impact of repeated autoclave sterilization on the SR of TN, PTG, and ETP. Accordingly, the purpose of this study is to evaluate the impact of multiple autoclave sterilization cycles on the SR of three thermally treated NiTi rotary instruments (i.e., TN, PTG, and ETP) using scanning electron microscopy (SEM). The hypothesis is that multiple autoclaving sterilization cycles influence the SR of TN, PTG, and ETP and that there are significant differences among them.

2. Materials and Methods

The Institutional Review Board of Princess Nourah Bint Abdulrahman University in Riyadh, Saudi Arabia, authorized this randomized controlled *in vitro* study (approval no. 21–0281).

This investigation employed 60 NiTi files: 20 TN (26/0.04), 20 PTG F2 (25/0.08), and 20 ETP F2 (25/0.06). The files were divided into four equal subgroups ($n=5$). An unsterilized group was assigned as a pre-autoclaving group (Cycle 0). The other three groups underwent various amounts of autoclave sterilization cycles (Cycles 1, 5, and 10). All files were removed from their packaging, and a code was blindly drawn for each file corresponding to the number of autoclaving cycles they were subjected to. At each cycle, a steam sterilizer (Steris Amsco Century Prevac Steam Sterilizerv 148H, OH, US) was used to sterilize the files for four minutes at 132°C under a pressure of 29 psi before being dried for five minutes.



FIGURE 1: Laboratory setup for SEM images.

2.1. Scanning Electron Microscopy with ImageJ Analysis. SEM (JSM-IT500HR, JEOL, Japan) was used to measure the SR 3 mm from the tip of the file at magnifications of $\times 450$ and $\times 1,000$. A high-brightness electron gun generated high-resolution pictures with an accelerating voltage of 10.0 kV. All files were fixed in a predetermined position (defined by a marked rubber stopper) to standardize the imaging zone (Figure 1).

For image analysis, the roughness-calculation plugin was utilized in the open-source image-processing software ImageJ (LOCI, University of Wisconsin, Version 5.2). NiTi rotary files were evaluated and compared using roughness average (Ra), root mean square (Rq), and average maximum peak-to-valley height (Rz). An increase in these values indicates a change in the vertical surface topology of NiTi files.

2.2. Statistical Analysis. SR was analyzed using SPSS (IBM, version 22). The Shapiro–Wilks test was used to assess data normality after descriptive analysis. Significant variations in SR values across groups were determined using one-way analysis of variance. A Tukey post-hoc test was used when significant differences were revealed in SR values. Statistical significance was established at a confidence level of 95%, and statistical significance was defined as $p < 0.05$.

3. Results

Table 1 shows the mean and standard deviation of SR values for the experimental groups.

At Cycle 0 (pre-autoclaving), SEM showed debris and SR (Figure 2) on all surfaces.

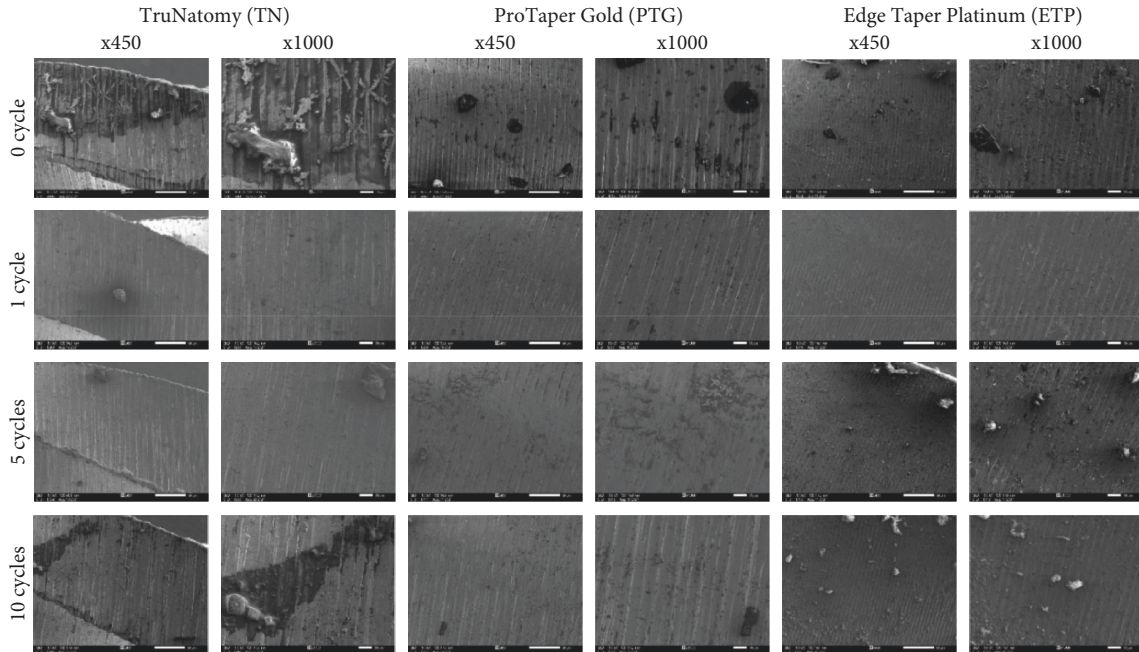
Following Cycle 1, there was a statistically significant decrease in all SR parameters for all groups ($p < 0.0001$). The Ra and Rq values of TN increased after Cycles 5 and 10 but were significantly lower compared with Cycle 0 ($p < 0.05$). For PTG, the SR values were essentially constant for all autoclaving cycles; the Rz value increased minimally after Cycle 10 but remained significantly lower than Cycle 0 ($p < 0.05$). For ETP, after Cycle 5, the Rz value significantly increased compared with Cycle 1 ($p < 0.01$). In comparing the groups, there are no statistically significant differences in the SR values before autoclaving or after one autoclaving cycle, as shown in Table 2.

After Cycle 5, the Rz value of ETP was statistically higher compared with PTG and TN ($p < 0.05$). After Cycle 10, the Ra and Rq values of TN were statistically higher compared

TABLE 1: Mean and standard deviations of SR parameters before and after different autoclaving cycles.

SR parameters		TN	PTG	ETP
NiTi	Cycle	Mean \pm SD	Mean \pm SD	Mean \pm SD
Ra	0	17.00 \pm 4.70 ^a	16.52 \pm 2.84 ^a	15.99 \pm 5.14 ^a
	1	9.68 \pm 1.80 ^b	8.33 \pm 1.66 ^b	8.16 \pm 0.88 ^b
	5	11.36 \pm 2.80 ^b	8.80 \pm 1.65 ^b	11.66 \pm 1.47 ^{a,b}
	10	12.92 \pm 1.12 ^{a,b}	8.23 \pm 0.83 ^b	10.19 \pm 1.54 ^b
<i>p</i> value		0.008*	0.0001*	0.004*
Rq	0	22.51 \pm 6.057 ^a	22.46 \pm 4.13 ^a	21.04 \pm 6.50 ^a
	1	13.13 \pm 2.28 ^b	11.44 \pm 2.25 ^b	11.28 \pm 1.05 ^b
	5	15.62 \pm 3.53 ^b	11.96 \pm 2.15 ^b	16.21 \pm 2.05 ^{a,b}
	10	17.84 \pm 1.38 ^{a,b}	11.51 \pm 1.04 ^b	13.81 \pm 1.85 ^b
<i>p</i> value		0.008*	0.0001*	0.003*
Rz	0	51.84 \pm 21.20 ^a	64.85 \pm 12.34 ^a	63.88 \pm 19.32 ^a
	1	39.81 \pm 8.54 ^b	35.70 \pm 12.00 ^b	43.89 \pm 8.85 ^b
	5	45.28 \pm 9.80 ^{a,b}	35.99 \pm 6.95 ^b	82.08 \pm 22.22 ^{a,c}
	10	68.26 \pm 7.25 ^{a,c}	38.60 \pm 7.38 ^b	55.69 \pm 3.05 ^{a,b}
<i>p</i> value		0.009*	0.004*	0.003*

a, b, and c indicate cycles with the same letters have no significant differences. * indicates statistically significant difference ≤ 0.05 .

FIGURE 2: SEM images of TN, PTG, and ETP at magnifications of $\times 450$ and $\times 1,000$ for Cycles 0, 1, 5, and 10.

with ETP and PTG ($p < 0.05$), but the Rz value of PTG was statistically lower compared with ETP and TN ($p < 0.05$). By comparing the SR values recorded under SEM magnifications of $\times 450$ and $\times 1,000$, it is evident that the Ra and Rq values for the latter are slightly lower compared with the former, but the difference is not statistically significant. However, for SEM magnification of $\times 1,000$, the Rz values are significantly higher compared with SEM magnification of $\times 450$ ($p < 0.05$) (Table 3).

4. Discussion

The surface characteristics of NiTi instruments should be considered when assessing instrument quality, as various

conditions may affect corrosion resistance [13]. NiTi file corrosion occurs by the selective removal of nickel from the surface when the instrument is exposed to sodium hypochlorite during root-canal preparation or the disinfection process. Alteration on the instrument surface produces micropitting and cracks, which increase the likelihood of fracture [25]. Additionally, NiTi files are machined instruments rather than twisted, and they have recently been subjected to thermal treatment to improve their mechanical properties. This complex manufacturing process produces pits, groves, and cracks on the surface of NiTi instruments [26], which increases the friction between the instruments and the canal wall and subsequently deteriorates the cutting angle, cutting efficiency, strength, and fatigue resistance.

TABLE 2: Comparison of SR parameters between the instruments (means \pm standard deviation).

Cycle	0	1	5	10
Ra				
TN	17.00 \pm 4.7 ^a	9.68 \pm 1.80 ^a	11.36 \pm 2.80 ^a	12.92 \pm 1.12 ^a
PTG	16.52 \pm 2.84 ^a	8.33 \pm 1.66 ^a	8.80 \pm 1.65 ^a	8.23 \pm 0.83 ^b
ETP	15.99 \pm 5.14 ^a	8.16 \pm 0.88 ^a	11.66 \pm 1.47 ^a	10.19 \pm 1.54 ^b
Rq				
TN	22.51 \pm 6.057 ^a	13.13 \pm 2.28 ^a	15.62 \pm 3.53 ^a	17.84 \pm 1.38 ^a
PTG	22.46 \pm 4.13 ^a	11.44 \pm 2.25 ^a	11.96 \pm 2.15 ^a	11.51 \pm 1.04 ^b
ETP	21.04 \pm 6.50 ^a	11.28 \pm 1.05 ^a	16.21 \pm 2.05 ^a	13.81 \pm 1.85 ^b
Rz				
TN	51.84 \pm 21.20 ^a	39.81 \pm 8.54 ^a	45.28 \pm 9.81 ^a	68.26 \pm 7.25 ^a
PTG	64.85 \pm 12.34 ^a	35.70 \pm 12.00 ^a	35.99 \pm 6.95 ^a	38.60 \pm 7.38 ^b
ETP	63.88 \pm 19.32 ^a	43.89 \pm 8.85 ^a	82.09 \pm 22.22 ^b	55.69 \pm 3.05 ^a

a, b, and c indicate groups with the same letters have no significant differences. 0 cycle is the pre-autoclaving cycle.

TABLE 3: SR parameters recorded under $\times 450$ and $\times 1,000$ magnification ($*p < 0.05$).

SR parameter	SEM magnification	Mean	Std. deviation	Sig.
Rq	$\times 450$	15.7372	5.1478	0.54
	$\times 1,000$	14.3978	5.2471	
Ra	$\times 450$	11.5681	3.9665	0.57
	$\times 1,000$	10.4723	4.0930	
Rz	$\times 450$	53.1684	18.1849	0.04*
	$\times 1,000$	73.8068	25.0038	

Thus, high SR values can be understood as stress-concentration points, which lead to crack initiation and propagation during clinical use and finally to instrument separation [27–29].

To analyze the SR of NiTi files, a variety of techniques have been used [30–35]. Atomic force microscopy (AFM) and SEM are the most commonly used methods to evaluate the surface characteristics of endodontic files. AFM has the advantage that both quantitative and qualitative data can be obtained [36, 37], but it is time consuming and the repeated measurement of small scanned areas is not easy [33]. SEM has the disadvantage that only qualitative evaluations can be obtained, as the two-dimensional images created by SEM cannot be processed for quantitative surface data [38]. However, a number of researchers have shown that data obtained via field-emission SEM (FE-SEM) is reliable and accurate, and, therefore, SEM and ImageJ software were used to measure SR values and recreate three-dimensional images [34, 39–41]. In the present study, the SR of three thermally treated NiTi instruments (i.e., TN, PTG, and ETP) is evaluated using SEM aided by ImageJ software after multiple autoclaving sterilization cycles.

The results suggest that all files had surface irregularities before autoclave sterilization (Cycle 0) with no statistically significant differences among the groups. This is consistent with previous studies that proved the presence of irregularities and debris on the surface of new unutilized endodontic files [11, 15, 42]. These studies attribute the presence of such SR to the complex manufacturing process of NiTi files and their packing. Therefore, all files must be checked carefully before being used (even new ones) to avoid

unwanted instrument separation during root-canal preparation.

After Cycle 1, the SR parameters of all NiTi groups reduced significantly, which means that surface irregularities were barely registered on the surface. These results are consistent with a previous study [34], who used FE-SEM combined with ImageJ software to assess the SR changes of thermally treated NiTi instruments before and after simulated root-canal preparation. They observed that the surface parameters of Wave One Gold (Dentsply Sirona, Baillagues, Switzerland) and Reciproc Blue (Dentsply Sirona, Baillagues, Switzerland) decreased after root-canal preparation. Previous studies suggest that SR values increase after Cycle 1, which is inconsistent with the present study [3, 11, 12, 15, 42, 43]. These studies used different methods to assess instrument SR, and therefore, we cannot directly compare the findings of the present study.

In the present study, TN registered the highest Rz value after Cycle 10, but statistically significant differences were not observed compared with Cycle 0. Previous report [44] showed that TN has significantly higher SR values compared with XP-endo Shaper (FKG Dentaire SA, La Chaux-de-Fonds, Switzerland) when exposed to different irrigants.

Regarding ETP, the highest Rz values were obtained after Cycle 5 ($p < 0.05$), which is consistent with previous studies [3, 12, 14, 28], who concluded that the SR of NiTi files increases after multiple autoclave cycles. These results should be considered when reusing the files.

Concerning PTG, it is interesting to note that the SR values did not increase until after Cycle 10, where a minor increase in the Rz value can be observed. However, this value

is still significantly lower compared with Cycle 0. The difference in the results of PTG and ETP can be attributed to the difference in surface treatment, which has been proven earlier [23].

Our results clearly show that multiple autoclave cycles enhance the surface topography of PTG and TN, as the SR decreased. Therefore, it would be helpful to sterilize the new files before using them to remove debris and reduce SR and thereby enhance the mechanical properties during clinical use. Although it was reported that multiple autoclave cycles have no impact on the cyclic fatigue resistance of thermally treated instruments [45], some researchers suggest that cyclic fatigue resistance of thermally treated instruments may be enhanced after many autoclave cycles [46, 47]. Again, it is unfair to directly compare the present study with previous reports due to inconsistent methods.

With respect to SEM, high magnifications ($\times 1,000$) show significantly higher Rz values compared with low magnifications ($\times 450$). This result is consistent with a study [11], which proved the accuracy of high magnification in detecting instrument SR.

It may be argued that PTG and NT manufacturers recommend that these instruments be discarded after single-use, negating the need for their autoclave sterilization. However, this single-use policy has been put into question, with nothing to prove that autoclave-resistant prion-related infections may be transmitted by dental instruments. Not to mention it is having an increasing impact on the environment, especially with endodontic instruments being the second largest contributor to greenhouse gas emissions, compared to other root-canal procedure materials [48]. It appears to simply translate into more profit for the manufacturer [49] and an increase in the cost of root-canal treatment [4].

The instruments in this study have different surface colors, which suggest different metallurgical manufacturing methods [50]. The complex heating-cooling treatment results in a unique titanium-oxide surface layer [23]. This suggests that a possible explanation for the current findings is the restructuring of surface oxides under the heat and pressure of autoclave sterilization [51].

This study is limited in so far as it does not reflect the many complicated factors that instruments are subjected to in clinical settings. For instance, the effect of irrigating solutions and dynamic forces that instruments undergo between autoclaving cycles were not simulated.

In summary, multiple autoclave sterilization cycles influenced the SR of the three investigated thermally treated NiTi groups; significant differences were observed between each group. High-magnification SEM along with ImageJ software can be used to accurately detect slight changes in the surface topography of NiTi instruments.

5. Conclusions

Multiple autoclaving sterilization cycles influenced the SR of thermally treated NiTi files, i.e., TN, PTG, and ETP. ETP showed higher levels of SR than TN and PTG after five autoclave cycles. Accordingly, to avoid file separation and

compromised endodontic outcomes, repeated use of ETP should be avoided in clinical endodontic treatments.

Data Availability

The datasets generated and/or analyzed during the current study are available as a supplementary file.

Conflicts of Interest

The authors declare no conflicts of interest.

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Supplementary Materials

See Figures S1 in the Supplementary Material for whole dataset. (*Supplementary Materials*)

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