

Original Article

Microstructural, microchemical, and mechanical changes associated with the clinical reuse of two nickel–titanium endodontic instruments

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ABSTRACT

Background: Nickel–titanium (NiTi) instruments have represented a great technological development that enabled endodontists conforming irregular-shaped root canals. Notwithstanding, the repeated use of these instruments may lead to the fracture without any prior visible warning signs. This study aimed to evaluate how multiple clinical instrumentation/sterilization cycles of two NiTi mechanized instruments can affect their microstructural, microchemical, and mechanical characteristics.

Materials and Methods: In this observational descriptive study, a total of 140 NiTi instruments, 70 ProTaper Gold® (PTG) and 70 WaveOne Gold® (WOG) were analyzed. For each brand system, instruments were evaluated in the as-received condition ($n = 10$) and after one ($n = 20$), two ($n = 20$), and three ($n = 20$) instrumentation/sterilization cycles. Intraoperative instrumentation parameters were recorded for all used instruments. Afterward, the files were examined using scanning electron microscopy and energy-dispersive X-ray microanalysis. All of the instruments were tensile-fatigue tested until rupture in order to calculate the mechanical tensile strength and the maximum elongation percentage for the samples. Statistical analysis was completed using Chi-square, Kruskal–Wallis H -, or Mann–Whitney U -tests with a statistical significance set at $P < 0.05$.

Results: Significant increasing changes in surface topography ($P < 0.05$, Chi-square test) and chemical composition ($P < 0.05$, Kruskal–Wallis H -test) in both brand systems through instrumentation/sterilization cycles were detected. In addition, values of mechanical tensile strength and maximum elongation percentage increased significantly through instrumentation/sterilization cycles in the PTG group, whereas only the median values of mechanical tensile strength increased significantly in the WOG group (all $P < 0.01$, Kruskal–Wallis H -test).

Conclusion: Although multiple instrumentation/sterilization cycles may render NiTi instruments more flexible and fatigue resistant, the significant changes detected in their surface topography and chemical composition should preclude their repeated clinical use in the routine endodontic practice as prevention for breakage.

Key Words: Electron probe microanalysis, endodontics, instrumentation, scanning electron microscopy, tensile strength

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INTRODUCTION

There is the general perception that nickel–titanium (NiTi) instruments show a high fracture risk mainly due to situations such as torsional stress and cyclic fatigue during instrumentation.^[1-4] The first situation occurs when the tip of the instrument binds but the shank of the file continues to rotate,^[3] whereas the second is caused by repeated tensile-compressive stress in a curved canal, although generally the fracture of the instrument is due to the combination of both situations.^[4] Nevertheless, several factors may influence the resistance to cyclic fatigue fracture of NiTi mechanized instruments, including the root canal curvature, diameter and design of the instruments, operational speed, torque range, movement kinematics, metal surface treatments, irrigation solutions, and the sterilization process.^[2,5]

Whereas it has been demonstrated that multiple autoclave sterilization cycles can modify the surface topography and chemical composition of NiTi instruments,^[2] a corrosive phenomenon may also occur triggered by contact between metals with different electrochemical activities in the presence of sodium hypochlorite (NaOCl) solution, which may alter the structural integrity of the surface of a NiTi instrument and thus, in critical fatigue conditions, predispose it to fracture.^[5] It is well known that NiTi instruments come into contact with NaOCl during disinfection or when the solution is present in the pulp chamber and root canal system during instrumentation.^[4,5] Usually, the corrosion patterns involving selective removal of nickel from the surface can create micropitting that weakens the structure of the instrument.^[6,7] In turn, these microstructural defects can lead to areas of stress collection and crack formation, weakening the structure of the instrument.^[7,8] Despite the former, some authors^[9] have found that cyclic fatigue resistance may be not adversely affected by NaOCl immersion and/or autoclave sterilization.

In order to prevent the fracture of the instruments inside the root canal and the risk of cross-contamination, manufacturers recommend discarding them after certain number of uses. Nevertheless, there is no agreement or evidence about the exact number of uses to which an instrument can be submitted with safety before failure. Moreover, although some researchers suggest that NiTi instruments could be reused in up to

10 canals^[10] or more,^[11-13] others^[14] have demonstrated that new instruments also can fracture during their first use. Considering not only that the repeated use may lead to undesired deterioration of NiTi instruments, but also that the analysis of chemical, mechanical, and physical characteristics of the instruments used for root canal preparation should be based on reliable results of studies performed under conditions reflecting the clinical setting,^[15] this study aimed to evaluate how multiple clinical instrumentation/sterilization cycles of two NiTi mechanized instruments can affect their microstructural, microchemical, and mechanical characteristics.

MATERIALS AND METHODS

Study design

This experimental study was approved by the Institutional Ethics Committee for Human Studies of the University of Antioquia (Medellín, Colombia) and carried out at the Faculty of Dentistry following the ethical guidelines of the Helsinki Declaration. Two brands of NiTi instruments with different cross-sectional geometries were tested: ProTaper Gold® (PTG) F2 #25/0.08 taper (Dentsply Maillefer®, Ballaigues, Switzerland) with a convex triangle cross-section and WaveOne Gold® (WOG) Primary #25/0.07 taper (Dentsply Maillefer®) of parallelogram-shaped cross-section. The sets of instruments were obtained from those files routinely used during root canal treatments performed on patients in the postgraduate endodontic clinics between January 2017 and April 2019. Informed consent was obtained from all patients before starting root canal treatment. The sample size calculation for each group was based on a previous study regarding the assessment of the resistance to fatigue failure of five different NiTi rotary files.^[16] It was increased by 33% to maintain the estimates at an optimal level of precision against the effect of size reduction due to dropouts. Hence, the final sample size for different assessments was set to 140 files to determine significant differences in outcomes at the 95% confidence level, with an alpha value = 0.05 and 80% power. Based on this sample size, four groups for each brand system were established as follows: a control group (Group I), constituted by 10 new unused instruments of each brand, and Groups II, III, and IV containing 20 instruments of each brand which underwent one, two, and three instrumentation/sterilization cycles, respectively.

Root canal preparation

Preoperative cone-beam computed tomography (CBCT) images of each tooth were obtained using a 3D Accuitomo 80[®] CBCT scanner (J. Morita[®] Manufacturing Corp., Kyoto, Japan) for assessment of the maximum root curvature angle following previously described methods.^[17] Endodontic procedures were performed by senior endodontic postgraduate students under infiltrative and/or regional anesthesia and intraoperative isolation with rubber dam. Briefly, the access cavities were prepared, the orifices were located, and the canals were explored with sizes 10 stainless steel K-files (Dentsply Maillefer). The cleaning and shaping of the canals were completed in accordance with the crown-down technique recommended by the manufacturer, and canal irrigation was performed with 5.25% NaOCl solution after the usage of each file, as well as with 17% ethylenediaminetetraacetic acid (EDTA) and distilled water as final irrigants. Except in the control group, PTG files were rotated in continuous motion with a 16:1 reduction handpiece, at 300 rpm and torque of 3.1 Ncm, and WOG files were used in reciprocating motion, at 300 rpm and torque of 2.5 Ncm using an electric motor (X-Smart IQ[®]; Dentsply Maillefer, Ballaigues, Switzerland). The instrument sequence for PTG instruments included the shaping of the coronal two-thirds (to resistance) with S1 and S2 instruments, followed by working length (WL) determination using Root ZX Mini-Morita apex locator (J Morita[®], Kyoto, Japan). Thereafter, the instrumentation of the apical one-third (to WL) with F1 and F2 instruments was performed. For WOG files, instrument sequence included the shaping of the coronal two-thirds with the primary file, WL determination as described, and apical one-third instrumentation performed with the same primary file. After each use of rotary instrument, recapitulations using a patency file (size 10 K-file) were performed. For each instrument, the total instrumentation time, the time with a torque between 50% and 75%, and the time with a torque >75% were recorded. After use, the instruments were washed, ultrasonically cleaned with an enzymatic agent (Alkazyme[®], Alkapharm UK, Penkridge, Staffordshire, UK) and sterilized at 121°C and 20 PSI for 60 min in autoclave.

Scanning electron microscopy and energy-dispersive X-ray microanalysis

The files were mounted on aluminum stubs using graphite tape and examined uncoated using a JEOL

JSM-6490 LV[®] (JEOL Ltd, Tokyo, Japan) scanning electron microscopy (SEM) under an acceleration voltage of 20 kV and high vacuum conditions (10⁻³ torr). The secondary electron images were acquired from tip (D0) and 5 mm beyond tip (D5) of each instrument. Topographic surface analysis included the recording of presence/absence of unwinding, tip flattening, micropitting, and irregularities of cutting edges. Furthermore, corrosion areas were identified on the metal surface by the presence of electron-dense spots^[2] and categorized arbitrarily as absent versus present. Likewise, microcrack formation was recorded as absent and initials/propagated. Subsequently, energy-dispersive X-ray (EDX) microanalysis was performed to evaluate the changes in the elemental composition and corrosion products generated through the instrumentation/sterilization cycles of the instruments at selected areas using an EDX microprobe (INCAPentaFETx3, Oxford Instruments PLC, Oxford, UK) coupled to the SEM.

Mechanical tests

Tensile tests were performed on a universal testing machine (3345, Instron[®], Norwood, Ma, USA) with a load cell capacity of 5000 N (Instron[®]). The initial distance between the upper and lower clamps was set to 11 mm in all the files, and tension force was applied with a 5-mm/min crosshead speed until file fracture occurred. In order to obtain a better grip, the samples were prepared by removing the attachment section that fits the handpiece, and the lower clamp held the apical portion of the active area of the files (~6 mm), whereas the upper clamp held the shaft area. Stress-strain graphics were used to calculate both mechanical tensile strength (i.e., the maximum stress the specimen can withstand before rupture) and the maximum elongation percentage (i.e., percentage of plastic strain at breakage) for each instrument.

Statistical methods and data analysis

Data collected were evaluated using the statistical package IBM[®] SPSS[®] 25.0 (Chicago, Ill, USA). All parameters were tested for normal distribution using the Shapiro–Wilk test. Because the results for each group and subgroup did not follow a normal distribution, the variables were analyzed using nonparametric methods. In the bivariate analyses, categorical variables were analyzed by Pearson's Chi-square probability test, and continuous variables were assessed using the Kruskal–Wallis *H*-test, followed by *post hoc* Bonferroni/Dunn multiple-comparison test. Furthermore, Mann–

Whitney *U*-test was used for pairwise comparisons, and Spearman's rank correlation coefficient[®] was used to describe the relationship between pairs of quantitative variables. All analyses were two-sided, and statistical significance was assumed at $P < 0.05$.

RESULTS

Clinical instrumentation data analysis

The summary statistics of intraoperative instrumentation parameters for each system according to the instrumentation/sterilization cycles are presented in Table 1. As evident from this table, while no significant differences ($P > 0.05$, Kruskal–Wallis *H*-test) in relation to the maximum root curvature angle were observed between instrumentation/sterilization subgroups for PTG instruments, median values of the total instrumentation time, time with a torque between 50% and 75%, and time with a torque >75% showed significant upward trends ($P < 0.05$) through instrumentation/sterilization cycles. In contrast, only the median values of the time with a torque between 50% and 75% and the maximum root curvature angle increased significantly in the WOG group ($P < 0.05$), whereas the total instrumentation time and the time with a torque >75% were statistically similar between

the instrumentation/sterilization subgroups ($P > 0.05$). In addition, the total instrumentation time was significantly greater ($P < 0.01$, Mann–Whitney *U*-test) in the three instrumentation/sterilization subgroups for WOG instruments as compared with those recorded for the PTG group. Furthermore, although the time with a torque between 50% and 75% was usually lower in the WOG group in comparison with the data recorded for the PTG group, the difference was only significant between the subgroups of three cycles of instrumentation/sterilization ($P < 0.001$). Likewise, the time with a torque >75% was significantly lesser in the subgroups of two and three instrumentation/sterilization cycles, whereas the maximum root curvature angle was significantly lesser in the subgroups of one and two cycles of WOG instruments in comparison with the PTG group (All $P < 0.05$). In addition to the former, Spearman correlation analysis showed evidence of positive correlations between the maximum root curvature angle and the data related to total instrumentation time, time with a torque between 50% and 75%, and the time with a torque >75% ($r > 0.390$, $P < 0.01$, data not shown) in the PTG group. Alternatively, these positive correlations were only noted regarding total instrumentation time and the time with a torque

Table 1: Between-group comparisons of intraoperative instrumentation parameters according to instrumentation/sterilization cycles and brand systems

Intraoperative variables ^a	Brand systems ^b		
	PTG (n=60)	WOG (n=60)	<i>P</i> ^d
Total instrumentation time (Sec)			
One cycle	155.0 (9.0-778.0)	635.5 (42.0-2815.0)	<0.001
Two cycles	392.5 (27.0-647.0)*	860.5 (39.0-1853.0)	0.002
Three cycles	450.5 (203.0-939.0)*	883.5 (396.0-2914.0)	<0.001
<i>P</i> ^e	0.001	0.203	
Time with a torque between 50 to 75% (Sec)			
One cycle	8.1 (0.0-23.3)	0.4 (0.0-47.2)	0.068
Two cycles	24.2 (0.0-122.0)*	18.1 (0.0-193.4)*	0.841
Three cycles	89.3 (8.1-291.1)*,†	23.7 (0.0-152.3)*	<0.001
<i>P</i> ^e	<0.001	0.001	
Time with a torque>75% (Sec)			
One cycle	0.0 (0.0-5.4)	0.0 (0.0-0.8)	0.529
Two cycles	1.6 (0.0-34.9)*	0.0 (0.0-18.6)	0.026
Three cycles	11.6 (0.0-131.2)*,†	0.0 (0.0-39.6)	<0.001
<i>P</i> ^e	<0.001	0.385	
Maximum root curvature angle (degrees)			
One cycle	32.5 (8.2-59.6)	20.2 (0.0-42.9)	0.013
Two cycles	37.8 (0.0-69.6)	25.5 (0.0-70.7)	0.048
Three cycles	36.4 (17.3-85.7)	32.7 (3.3-64.2)*	0.267
<i>P</i> ^e	0.432	0.023	

^aExcluding control groups, ^bValues are given as median (range) of each measurement, ^cKruskal–Wallis *H* test, ^dTwo-sided Mann–Whitney *U*-test, ^eStatistically significant difference ($P < 0.01$, *post hoc* Bonferroni/Dunn multiple-comparison test) as compared with one-cycle subgroup, ^fStatistically significant difference ($P < 0.01$, *post hoc* Bonferroni/Dunn multiple-comparison test) as compared with two-cycle subgroup. PTG: ProTaper Gold[®]; WOG: WaveOne Gold[®]

between 50% and 75% in the WOG group ($r > 0.369$, $P < 0.01$, data not shown). It is important to emphasize that under these experimental conditions, none of the instruments fractured during root canal preparations.

Microstructural findings

Overall, surface analysis of unused PTG and WOG instruments confirmed the presence of a regular surface texture with milling marks along the surface, mainly perpendicular to the long axis of the files [Figure 1a-d]. These machining marks did not present changes and were shallower in the WOG group when compared with those observed in PTG files. Table 2 shows the details of the different instrument defects observed under SEM conditions. Although no macroscopic defects were found in any of the samples, several limited microscopic defects were observed after initial instrumentation/sterilization cycle. Overall, both in PTG and WOG instruments, unwinding, tip flattening, microcracks, irregularities of cutting edges, and corrosion areas were absent before use. As can be seen from this table, while PTG files did not show unwinding under different instrumentation conditions, in the WOG groups, unwinding increased throughout the three cycles of instrumentation/sterilization. The beginning point of unwinding of these instruments arisen mostly at, or very close to, the tip of the

instrument [Figure 2a]. However, the observed differences among the instrumentation/sterilization

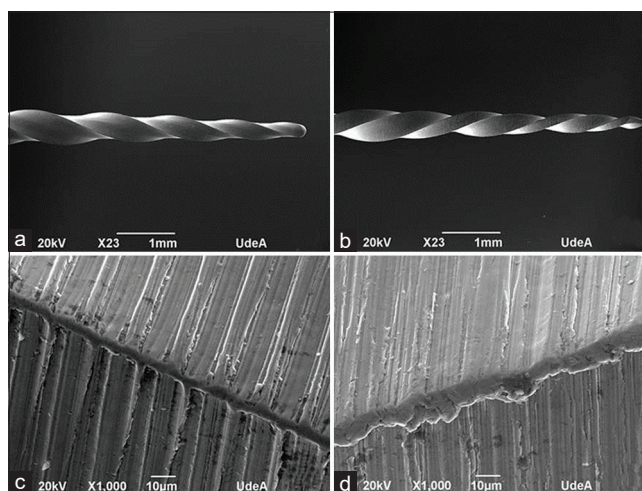


Figure 1: Unused (a) PTG and (b) WOG instruments ($\times 23$ magnification) showing a regular surface texture with milling marks that run perpendicular to the long axis of the files. At higher magnification ($\times 1000$), it can be noted that (c) PTG instruments possess sharp cutting edges and noncutting tips, whereas (d) WOG files show a morphology of cutting edges that appear rounded with a wrapped portion similar to a “wave curl” and have a tapering tip with a terminal rounded portion. Note that machining marks are shallower in WOG instruments when compared with those observed in PTG files. PTG: ProTaper Gold®; WOG: WaveOne Gold®.

Table 2: Comparison of microstructural features according to instrumentation/sterilization cycles and brand systems

Microstructural findings	Brand systems/experimental groups ^a									
	PTG				<i>P</i> ^b	WOG				<i>P</i> ^b
	Control (<i>n</i> =10)	One cycle (<i>n</i> =20)	Two cycles (<i>n</i> =20)	Three cycles (<i>n</i> =20)		Control (<i>n</i> =10)	One cycle (<i>n</i> =20)	Two cycles (<i>n</i> =20)	Three cycles (<i>n</i> =20)	
Unwinding										
Absent	10 (14.2)	20 (28.6)	20 (28.6)	20 (28.6)	-	10 (17.2)	17 (29.3)	16 (27.6)	15 (25.9)	0.374
Present	-	-	-	-			3 (25.0)	4 (33.3)	5 (41.7)	
Tip flattening										
Absent	10 (16.4)	18 (31.1)	14 (23.0)	18 (29.5)	0.068	10 (16.4)	17 (27.9)	18 (29.5)	16 (26.2)	0.455
Present	-	1 (10.0)	6 (60.0)	3 (30.0)			3 (33.3)	2 (22.2)	4 (44.4)	
Microcracks										
Absent	10 (34.5)	8 (27.6)	6 (20.7)	5 (17.2)	0.001	10 (30.3)	7 (21.2)	13 (39.4)	3 (9.1)	<0.001
Initials/propagated	-	12 (29.3)	14 (34.1)	15 (36.6)			13 (35.1)	7 (18.9)	17 (45.9)	
Micropitting										
Absent	9 (15.8)	17 (29.8)	14 (24.6)	17 (29.8)	0.466	10 (17.5)	15 (26.3)	17 (29.8)	15 (26.3)	0.315
Present	1 (7.7)	3 (23.1)	6 (46.2)	3 (23.1)			5 (36.5)	3 (23.1)	5 (36.5)	
Irregularities of cutting edges										
Absent	10 (18.5)	16 (29.6)	16 (29.6)	12 (22.2)	0.090	10 (22.2)	13 (28.9)	13 (28.9)	9 (20.0)	0.032
Present	-	4 (25.0)	4 (25.0)	8 (50.0)			7 (28.0)	7 (28.0)	11 (44.0)	
Corrosion areas										
Absent	10 (20.8)	13 (27.1)	11 (22.9)	14 (29.2)	0.092	10 (30.3)	6 (18.2)	8 (24.2)	9 (27.3)	0.003
Present	-	7 (31.8)	9 (40.9)	6 (27.3)			14 (37.8)	12 (32.4)	11 (29.7)	

^aValues are given as *n* (%) of instruments within each parameter according to the instrumentation/sterilization groups, ^bTwo-sided Pearson's Chi-square test. PTG: ProTaper Gold®; WOG: WaveOne Gold®

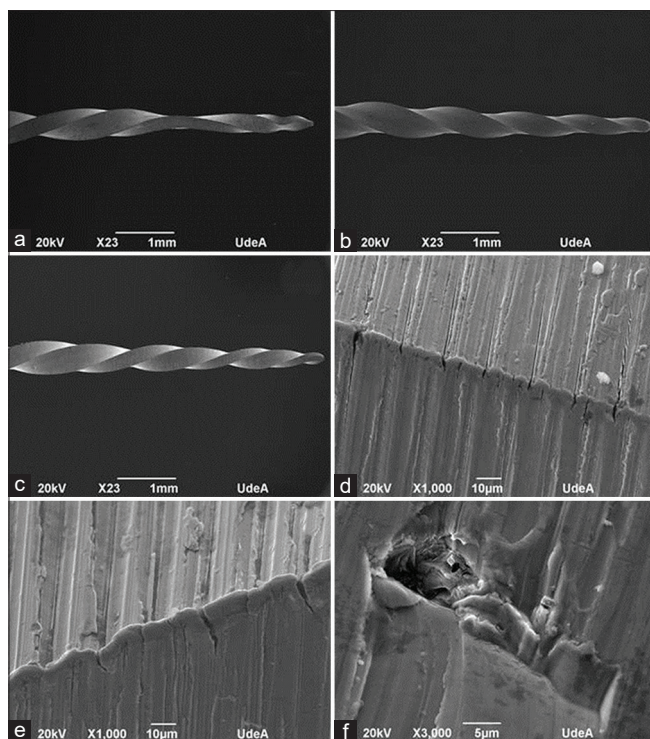


Figure 2: Micrographs of PTG/WOG files showing surface defects: (a) WOG file showing unwinding after two instrumentation/sterilization cycles. (b) PTG file showing tip flattening after two instrumentation/sterilization cycles. (c) The tip of a WOG file resulted worn out after three instrumentation/sterilization cycles. (d) Crack lines running perpendicular to the axis of a PTG file after three instrumentation/sterilization cycles. (e) Cracks of cutting edge of a WOG file after three instrumentation/sterilization cycles. (f) Unused PTG file showing a surface micropit adjacent to the cutting edge. PTG: ProTaper Gold®; WOG: WaveOne Gold®.

cycles were not statistically significant ($P > 0.05$, Chi-square test). After use, although the incidence of tip flattening was higher in PTG instruments (10 files) in comparison with WOG instruments (9 files), no statistical differences regarding the brand system nor instrumentation/sterilization cycles could be detected [Figure 2b and c, all $P > 0.05$]. Furthermore, as a result, in both brands of instruments, a significantly increasing trend of microcrack formation ($P < 0.01$) perpendicular to the axis of the instrument through the instrumentation/sterilization cycles was observed [Figure 2d and e], although the number of microfractured instruments was statistically similar among the two brand systems ($P = 0.496$, data not shown). In turn, the incidence of micropitting was statistically similar between the two brand systems with no significant differences according to the instrumentation/sterilization cycles ($P > 0.05$). It was remarkable that one unused PTG file showed

evidence of micropitting and microstructural alteration of the area around the cavity [Figure 2f]. SEM analysis also demonstrated wear, blunting, and disruption of the cutting edges in the instruments after use [Figure 3a and b]. These irregularities of cutting edges showed an increasing trend through the instrumentation/sterilization cycles, being significantly higher ($P < 0.05$) in WOG instruments which underwent three instrumentation/sterilization cycles. Even so, no statistical differences regarding the brand systems or instrumentation/sterilization cycles in PTG files could be detected (all $P > 0.05$). In the same line, signs of corrosion were identified on the instrument surfaces with increasing instrumentation/sterilization cycles [Figure 3c and d], being significantly higher ($P < 0.05$) in WOG instruments which underwent one instrumentation/sterilization cycle, although no significant differences were noticeable between instrumentation/sterilization cycles of PTG files.

It was noteworthy that, regardless of instrumentation/sterilization cycles, those unwound instruments were used in teeth showing significantly greater root curvature angle ($P = 0.016$, Mann–Whitney U -test) and underwent significantly higher total instrumentation time ($P = 0.002$, data not shown). Similarly, while all instrumentation parameters were significantly greater in those files exhibiting microcrack formation (all $P < 0.05$, data not shown), only the total instrumentation time was significantly higher in those instruments showing irregularities of cutting edges and/or corrosion areas ($P < 0.05$). Conversely, there were no statistical differences in the instrumentation parameters regarding tip flattening and micropitting ($P < 0.05$).

Microchemical findings

EDX microanalysis revealed the composition of the samples in weight percentage of elements detected [Table 3]. As can be seen, this assessment confirmed that both brand systems were composed mainly of nickel (Ni) and titanium (Ti). As expected, control PTG and WOG instruments contained mainly Ni and Ti, with only small traces of oxygen (O). It was noteworthy that, in both brands tested, the weight percentage of Ni and SecSec showed a significant downward trend according the instrumentation/sterilization cycles (all $P < 0.001$, Kruskal–Wallis H /Bonferroni/Dunn multiple-comparison test). Whereas Ni content was significantly higher in the control and three instrumentation/sterilization cycle

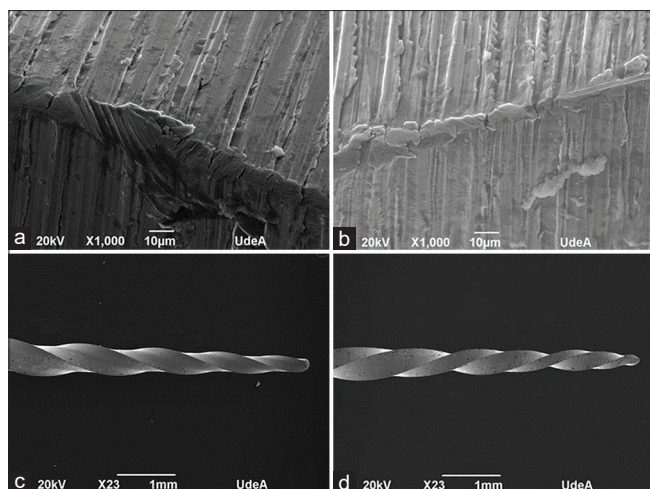


Figure 3: High-magnification scanning electron microscopy images showing topographical changes of PTG and WOG instruments after use. (a) Blunt cutting edge in a PTG instrument after one instrumentation/sterilization cycle. (b) Cutting edge disruption in a WOG file after three instrumentation/sterilization cycles. (c) Presence of signs of corrosion on the surface of a PTG file after one instrumentation/sterilization cycle. (d) WOG instrument showing corrosion spots along the flat surfaces after three instrumentation/sterilization cycles. PTG: ProTaper Gold®; WOG: WaveOne Gold®.

groups of PTG files when compared with those of WOG instruments ($P < 0.05$, Mann–Whitney U -test), no significant differences in Ti content between the two brands through the instrumentation/sterilization cycles could be noted (all $P > 0.05$). On the contrary, although the weight percentage of oxygen and carbon increased significantly through the instrumentation/sterilization cycles in each brand system ($P < 0.001$, Kruskal–Wallis H /Bonferroni/Dunn multiple-comparison test), except for oxygen composition which was significantly higher in WOG files which underwent three instrumentation/sterilization cycles ($P < 0.05$, Mann–Whitney U -test), there was no significant difference between the brand systems in terms of the oxygen and carbon composition ($P > 0.05$) before or after use. In addition to the former, the EDX analysis also pointed out the presence of calcium, aluminum, phosphorus, silicon, sulfur, iron, and sodium on the surface of the instruments, without intra- or intergroup significant differences (All $P > 0.05$, Kruskal–Wallis H - and Mann–Whitney U -tests). Figure 4 shows a representative EDX spectrum, with characteristic peaks of Ni and Ti on the noncorroded areas, while in the zones where localized attacks took place, the corrosion products were identified as oxides of Ni and Ti together with calcium carbonate. These corrosion

products were present in all instruments after clinical use and were absent in the control groups ($P < 0.001$, Chi-square test).

Mechanical characteristics

Table 4 depicts the results of mechanical tests for each system according the instrumentation/sterilization cycles. Overall, median values of mechanical tensile strength and maximum elongation percentage increased significantly through instrumentation/sterilization cycles in the PTG group, whereas only the median values of mechanical tensile strength increased significantly in the WOG group (all $P < 0.01$, Kruskal–Wallis H -test). Furthermore, it was noticeable that the results of the two mechanical tests were significantly greater ($P < 0.01$, Mann–Whitney U -test) in the three instrumentation/sterilization subgroups for PTG instruments when compared with those recorded for the WOG group. Typical tensile stress-strain curves used for mechanical tensile tests are presented in Figure 5a-d. Moreover, irrespective of instrumentation/sterilization cycles, no significant differences were observed in median values of mechanical tensile strength and maximum elongation percentage regarding the different subgroups of instrument defects detected under SEM conditions (all $P > 0.05$, Mann–Whitney U -test, data not shown).

Alternatively, whereas Spearman correlation analysis [Table 5] showed significant, albeit moderate, positive correlations between mechanical tensile strength and the data related to total instrumentation time, time with a torque $>75\%$, and maximum root curvature angle (all $P < 0.05$) in the PTG group, these positive correlations were only noted regarding the time with a torque between 50% and 75% and the time with a torque $>75\%$ in the WOG group. On the contrary, no significant correlations could be detected between the maximum elongation percentage and intraoperative instrumentation parameters in any of the brand systems (all $P > 0.05$).

DISCUSSION

During root canal treatment, NiTi instruments are used under rotational or reciprocating speeds ranging from 300 to 500 rpm, applied by an endodontic electric motor operating with torques varying from 2.5 to 5 Ncm in a handpiece of 16:1 reduction.^[18,19] Although the preparation time usually ranges from 12 to 40 min,^[19] it may vary according to the type of instrument used and specific anatomical

Table 3: Bivariate comparisons of elemental composition of the samples analyzed throughout the instrumentation/sterilization cycles

Elemental composition (weight percentage) and instrumentation/sterilization cycle subgroups	Brand systems ^a		P ^c
	PTG	WOG	
Niquel (Ni)			
Control group	54.6 (50.3-54.8)	54.2 (50.2-54.6)	0.011
One cycle	46.6 (21.8-52.7)*	45.9 (13.1-52.5)*	0.516
Two cycles	44.1 (32.5-47.0)*	41.5 (32.1-52.1)*	0.066
Three cycles	42.5 (36.2-45.5)*,†	37.4 (24.6-45.4)*,†	0.002
P ^b	<0.001	<0.001	
Titanium (Ti)			
Control group	45.0 (41.4-45.4)	45.1 (41.5-45.8)	0.705
One cycle	39.2 (19.6-43.1)*	36.3 (11.3-43.2)*	0.172
Two cycles	36.3 (26.7-38.7)*	35.7 (28.3-43.6)*	0.620
Three cycles	34.9 (30.5-37.2)*,†	31.4 (22.5-39.1)*	0.060
P ^b	<0.001	<0.001	
Oxygen (O)			
Control group	0.0 (0.0-7.4)	0.0 (0.0-7.8)	0.315
One cycle	7.2 (3.6-17.0)*	8.9 (0.0-25.9)*	0.072
Two cycles	8.1 (0.0-20.9)*	9.6 (0.0-14.5)*	0.904
Three cycles	8.6 (6.1-15.4)*	13.5 (4.8-19.3)*	0.021
P ^b	<0.001	<0.001	
Carbon (C)			
Control group	0.0 (0.0-0.0)	0.0 (0.0-0.0)	1.000
One cycle	5.6 (3.2-54.5)*	7.4 (0.0-25.5)*	0.495
Two cycles	11.8 (5.1-21.4)*	10.9 (0.0-27.5)*	0.947
Three cycles	12.7 (7.8-22.5)*	15.3 (6.8-33.3)*,‡	0.327
P ^b	<0.001	<0.001	

^aValues are given as median (range) of each measurement, ^bKruskal-Wallis *H* test, ^cTwo-sided Mann-Whitney *U*-test. *Statistically significant difference ($P < 0.01$, *post hoc* Bonferroni/Dunn multiple-comparison test) as compared with control subgroup, †Statistically significant difference ($P < 0.05$, *post hoc* Bonferroni/Dunn multiple-comparison test) as compared with one-cycle subgroup, ‡Statistically significant difference ($P < 0.01$, *post hoc* Bonferroni/Dunn multiple-comparison test) as compared with one-cycle subgroup. Ni: Nickel; Ti: Titanium; PTG: ProTaper Gold[®]; WOG: WaveOne Gold[®]

Table 4: Comparison of data resulting from mechanical tests according to instrumentation/sterilization cycles and brand systems

Mechanical parameters and instrumentation/sterilization cycle subgroups	Brand systems ^a		P ^c
	PTG	WOG	
Mechanical tensile strength (Mpa)^b			
Control group	2080.9 (1094.4-2914.8)	1593.6 (1365.6-2128.3)	0.063
One cycle	2901.7 (1459.0-3705.6)*	2186.2 (1013.2-4233.6)	<0.001
Two cycles	3430.7 (1985.6-7673.2)*,†	2728.8 (2083.2-5864.6)*,†	<0.001
Three cycles	2904.4 (2126.4-4023.4)*	2629.5 (977.9-3596.8)*	0.001
P ^d	<0.001	<0.001	
Maximum elongation (%)^e			
Control group	14.5 (12.9-16.2)	14.8 (13.6-16.5)	0.684
One cycle	16.0 (13.4-18.8)*	14.8 (12.0-16.0)	<0.001
Two cycles	16.0 (15.2-17.2)*	13.9 (12.0-16.0)	<0.001
Three cycles	15.9 (13.0-16.0)*	14.4 (4.8-16.0)	<0.001
P ^d	0.002	0.103	

^aValues are given as median (range) of each measurement, ^bData based on maximum charge divided by cross-sectional area, ^cData based on the maximum elongation of the instrument length divided by the original instrument length, ^dKruskal-Wallis *H* test, ^eTwo-sided Mann-Whitney *U* test, *Statistically significant difference ($P < 0.05$, *post hoc* Bonferroni/Dunn multiple-comparison test) as compared with control subgroup, †Statistically significant difference ($P < 0.05$, *post hoc* Bonferroni/Dunn multiple-comparison test) as compared with one-cycle subgroup. PTG: ProTaper Gold[®]; WOG: WaveOne Gold[®]

characteristics of the root canals instrumented such as curvature, length, and width.^[1] The current study represents a comprehensive evaluation of the cyclic fatigue resistance that incorporates different torque

time values applied, root curvature angles, and repeated clinical use, as factors that might be involved in the eventual breakage of NiTi engine-driven instruments. The results showed several differences

Table 5: Statistical correlation analysis between the results of mechanical tests and instrumentation parameters irrespective of instrumentation/sterilization cycles

Intraoperative instrumentation parameters	Mechanical test							
	PTG				WOG			
	Mechanical tensile strength (Mpa)		Maximum elongation (%)		Mechanical tensile strength (Mpa)		Maximum elongation (%)	
	r^a	P	r^a	P	r^a	P	r^a	P
Total instrumentation time (Sec)	0.313	0.008	0.089	0.117	0.144	0.235	0.159	0.188
Time with a torque between 50% and 75% (sec)	0.228	0.058	0.047	0.697	0.300	0.012	0.222	0.065
Time with a torque >75% (Sec)	0.255	0.033	0.010	0.935	0.256	0.033	0.051	0.677
Maximum root curvature angle (degrees)	0.328	0.006	0.107	0.379	0.127	0.296	0.149	0.219

^a r =Spearman's rank correlation coefficient. PTG: ProTaper Gold®; WOG: WaveOne Gold®

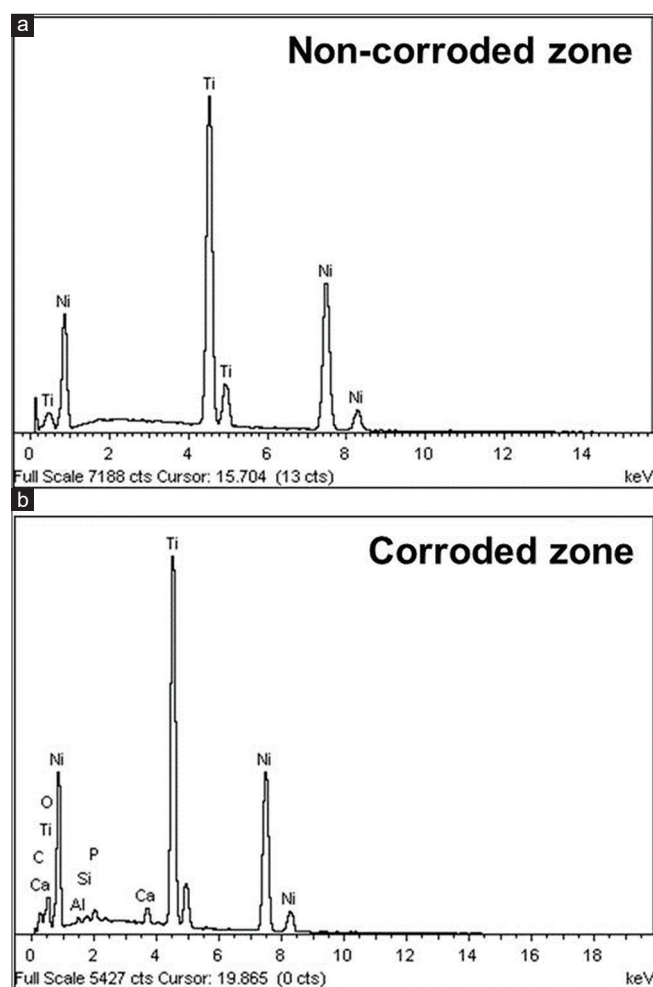


Figure 4: Representative energy-dispersive X-ray spectrum of the surfaces of (a) new WOG and (b) used PTG files. The presence of NiTi on the noncorroded areas is clearly seen; while in the corroded zones an increase of oxygen and traces of other elements were detected. PTG: ProTaper Gold®; WOG: WaveOne Gold®.

in intraoperative instrumentation parameters for each system according to the instrumentation/sterilization cycles. It is important to point out that these differences were related to variations inherent

not only to the types of instrumentation systems (i.e., sequential multiple file vs. single file) but also the movement kinematics (continuous vs. reciprocating motion) and degree of root curvature.

Although several studies have demonstrated that the repeated clinical use of NiTi instruments may cause a considerable amount of surface defects, such as unwinding, tip flattening, micropitting, microcracks, irregularities of cutting edges, and corrosion areas,^[18,20,21] other studies have also found defects in new instruments.^[21,22] In the present study, whereas only one unused PTG file showed evidence of micropitting and microstructural alteration of the area around the cavity, in both brand systems, several reused instruments presented surface defects, thus confirming that the number of instrumentation/sterilization cycles may alter the surface topography of the instruments. In this line, the occurrence of unwinding of WOG instruments, but not in PTG ones, was not only due to that WOG technique is a single-file concept^[23] but also to a longer preparation time in canals with abrupt apical curves. Given that the reciprocating motion of WOG instruments relieves the stress on the file by special counterclockwise and clockwise movements, thus preventing the taper-lock phenomenon,^[24] it would be possible to state that this movement kinematics has an anti-breakage control feature that allows the instrument to unwind so as to prevent the risk of fracture.^[25] In addition to the former, SEM analysis of reused instruments revealed how the friction forces generated by the interaction of the file with the root canal wall can lead to damage of the tip and cutting edge of the instruments, the latter being significantly more frequent in WOG files which underwent three instrumentation/sterilization cycles. Since there were no statistical differences in the instrumentation parameters regarding tip flattening, its clinical effects on the canal centering ability of

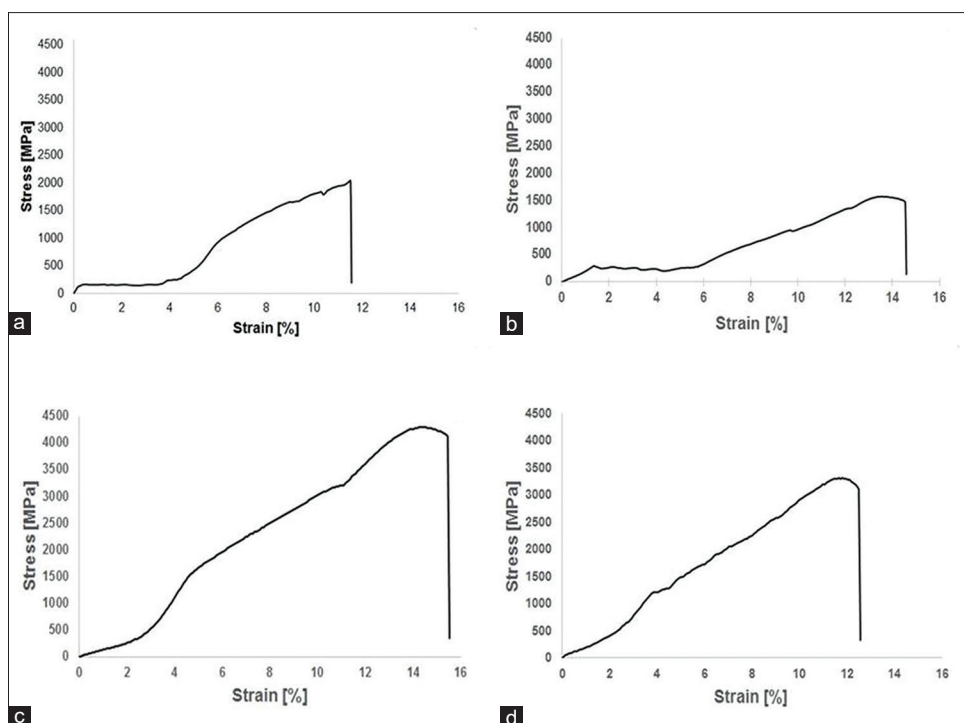


Figure 5: Representative stress-strain curves of the instruments obtained in mechanical tensile tests. The lines show the stress produced by increasing tensile strain. As can be seen, the curves continue upward up to the final fracture. Note that stress-strain values were lower in the new instruments and higher after clinical use. (a) Unused PTG instrument. (b) Unused WOG instrument. (c) PTG instrument underwent two instrumentation/sterilization cycles. (d) WOG instrument underwent two instrumentation/sterilization cycles. PTG: ProTaper Gold®; WOG: WaveOne Gold®.

the file are difficult to evaluate. Even so, it has been suggested that the wear of the tip could be related to the high apical force applied during instrumentation.^[26] On the contrast, as the total instrumentation time was significantly higher in those instruments showing irregularities of cutting edges, it may be possible to speculate that the presence of these defects can yield a decrease in the cutting efficiency of these files.^[27]

On the other hand, many studies have demonstrated a relationship between the increase in surface microcracks, micropitting, and corrosion areas^[18,22,26,27] and the fracture of NiTi rotary files, especially during use in curved root canals.^[28] In the present study, although none of the instruments fractured during root canal preparations, SEM analysis showed a significantly increasing trend of microcrack formation through the instrumentation/sterilization cycles, being statistically similar among the two brand systems. Considering that all instrumentation parameters were significantly greater in files showing microcracks, and that such surface defects appeared since the initial instrumentation/sterilization cycles, it is quite possible that microcracks may nucleate, grow, and propagate slowly through the instruments, until the sudden, final

fracture occurs.^[20] Interestingly, SEM evaluations also showed signs of micropitting and corrosion areas on the surface of the two brand systems. Although in the current study there were no statistical differences in the instrumentation parameters regarding micropitting, it has been acknowledged that these microcavitated areas may present a concentration of corrosion and possibly become sites susceptible to instrument breakage.^[8,29] Likewise, given that the present results showed that the total instrumentation time was significantly higher in those instruments showing corrosion areas, it is probable that they have been produced by the contact of instruments with NaOCl solutions during instrumentation.^[4,5] This phenomenon may be attributed to galvanic corrosion, also known as dissimilar metal corrosion, i.e., corrosion induced when two (or more) dissimilar metals are coupled in a corrosive electrolyte^[5] and it has been recognized that the electron-dense appearance of these deposits is caused by their much lower mean atomic number, compared with the NiTi alloy.^[28] Subsequently, these localized corrosion areas could further expand and catalyze the dissolution of metals weakening the structure of the instruments with the repeated clinical use.^[6,7]

The results of the EDX analysis showed that the instruments tested were manufactured by NiTi alloys with elemental composition ranging from 50.2 to 54.8 wt% in Ni content and from 41.4 to 45.8 wt% in Ti content. These values are close to those reported previously^[2,30,31] and are within the nominal composition range, as specified by the American Society for Testing and Materials for wrought NiTi alloys used in medical devices and surgical implants.^[32] After use, EDX analysis indicated a chemical alteration of the surface in both brand systems characterized by a significant decrease in Ni and Ti with a significant increase in oxygen and carbon through the instrumentation/sterilization cycles. Other elements including calcium, aluminum, phosphorus, silicon, sulfur, iron, and sodium were also detected on the surface of the reused instruments, possibly associated with the presence of dentin and/or restorations debris lodged along machining grooves.^[28,33] In addition to the former, the EDX spectra also revealed the presence of Ni and Ti on the noncorroded areas, while in the zones where localized attacks took place, the corrosion products were identified as oxides of Ni and Ti together with calcium carbonate probably coming from the tap water employed in disinfectant solutions.^[5]

It was acknowledged that any change in the fatigue strength of a material is a consequence of both the residual stresses and the increased hardness due to work hardening.^[34,35] Because plastic deformation is necessary for producing residual stresses, the material which has deformed plastically will be work hardened.^[35,36] In this way, both work hardening and work softening may be observed on the NiTi shape memory alloy during the cyclic fatigue process.^[37] In consonance with the aforementioned, the present study showed a significant increase in the mechanical tensile strength of the two brand systems through instrumentation/sterilization cycles. Moreover, it was also evident that for the PTG instruments, the maximum elongation percentage increased with respect to the new unused files, whereas remained statistically similar in the WOG files through instrumentation/sterilization cycles. Although in the current research, Vickers microhardness measurements were not carried out, it has been previously accepted that this test is in agreement with mechanical tensile strength results.^[19] Consequently, it would be possible to state that, in this research, the effect of work hardening became pronounced with the

increase of instrumentation/sterilization cycles and was related to the amount of its plastic deformation, a process also known as dislocation density because of work hardening.^[34,38] These findings are in line with those previously described,^[34] which demonstrated that the effect of work hardening and the intrinsic NiTi intermetallic microstructure can cause irregular and unexpected changes in the hardness of the metal. Other authors, however, have found no work-hardening effect associated with increasing reuse of NiTi rotary files.^[23,35] Possible reasons for these conflicting findings are different experimental settings and different NiTi instruments.^[35] Taking into consideration that the temperature used in sterilization may not be high enough to cause significant changes in the NiTi alloy structure,^[39,40] and that in this research, the required changes to increase the fatigue resistance of NiTi instruments would be correlated to the total instrumentation time, time with a torque >75%, and maximum root curvature angle, it might be possible to state that consecutive cycles of instrumentation/sterilization may give rise to cumulative effects, leading to increase the hardness in rotary NiTi endodontic instruments after sterilization, as previously observed.^[40,41]

From the metallurgical point of view, flexibility is considered as one of the foremost mechanical properties of NiTi rotary instruments, and the selection of one instrument often hinges on this issue.^[42] Attempts to enhance the surface of NiTi instruments and thereby to increase surface hardness, flexibility, and resistance to cyclic fatigue have resulted in a variety of strategies^[36] among which thermomechanical process applied during manufacturing is of great relevance. In this sense, two of the most recent alloys that have been developed are CM-wire (PTG instruments) and M-wire (WOG instruments). These instruments contain a mixture of austenite and martensite, whereas others consist mainly of austenite,^[43] and are able to undergo a displacive, nondiffusive transformation of the lattice structure into a martensitic phase when suitably stressed.^[44] Instruments in the martensite phase can easily be deformed, yet they will recover their shape on heating above the transformation temperatures.^[45] The explanation for this may be that heating transforms the metal temporarily into the austenitic phase and makes it superelastic, which makes it possible for the file to regain its original shape before cooling down again. In addition, the martensitic form of NiTi has a remarkable fatigue resistance.^[45] The results of the two mechanical

tests presented herein were significantly greater in the three instrumentation/sterilization subgroups of PTG instruments when compared with those recorded for the WOG group, thus suggesting that PTG instruments resist a much higher maximum strain before fracture and confirming the superior flexibility of CM-wire instruments compared with M-wire technology.^[46-48]

CONCLUSION

It can be concluded that although multiple instrumentation/sterilization cycles may render, these NiTi instruments more flexible and fatigue resistant, the significant changes detected in their surface topography and chemical composition preclude the repeated clinical use in the routinely endodontic practice as prevention for breakage.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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