Cyclic Fatigue Resistance of Heat-treated Nickel-titanium Instruments after Immersion in Sodium Hypochlorite and/or Sterilization

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Abstract

Introduction: The purpose of this study was to assess the effects of sodium hypochlorite (NaOCl) immersion and sterilization on the cyclic fatigue resistance of heat-treated nickel-titanium (NiTi) rotary instruments. **Methods:** Two hundred ten new 25/.06 Twisted Files (TFs; SybronEndo, Orange, CA) and Hyflex CM (Coltene Whaledent, Cuyahoga Falls, OH) files were divided into 7 groups (n = 15) for each brand. Group 1 (control group) included new instruments that were not immersed in NaOCl or subjected to autoclave sterilization. Groups 2 and 3 were composed of instruments dynamically immersed for 3 minutes in 5% NaOCl solution 1 and 3 times, respectively. Groups 4 and 5 consisted of instruments only autoclaved 1 and 3 times, respectively. Groups 6 and 7 recruited instruments that received a cycle of both immersion in NaOCl and sterilization 1 and 3 times, respectively. Instruments were subsequently subjected to a fatigue test. The surface morphology of fractured instruments was studied by field-emission scanning electron microscopy and x-ray energy-dispersive spectrometric (EDS) analyses. The means and standard deviations of the number of cycles to failure (NCF) were calculated and statistically analyzed using 2-way analysis of variance ($P < .05$). **Results:** Comparison among groups indicated no significant difference of NCF ($P > .05$) except for the groups of TFs sterilized 3 times without and with immersion in NaOCl ($P < .05$). HyFlex CM files exhibited higher cyclic fatigue resistance than TFs when files were sterilized 3 times, independently from immersion in NaOCl ($P < .05$). EDS analysis showed the presence of an oxide-rich layer on the Hyflex CM files' external surface. No morphologic or chemical differences were found between files of the same brand subjected to different treatments. **Conclusions:** Repeated cycles of sterilization did not influence the cyclic fatigue of NiTi files except for TFs, which showed a significant decrease of flexural resistance after 3 cycles of sterilization. Immersion in NaOCl did not reduce significantly the cyclic fatigue resistance of all heat-treated NiTi files tested. *(J Endod 2018;■:1–6)*

Key Words

Autoclave sterilization, controlled memory wire, cyclic fatigue, heat-treated files, sodium hypochlorite, R-phase

Significance

Immersion in sodium hypochlorite as well as autoclave sterilization could influence the cyclic fatigue resistance of NiTi instruments. It is important to know because these procedures influence the flexural fatigue of heat-treated NiTi files.

Root canal instruments manufactured from nickel-titanium (NiTi) alloy were introduced in 1988 to overcome the rigidity of stainless steel material (1, 2). However, there is a general perception that NiTi instruments have a high risk of fracture during their use (3), and manufacturers have continued their efforts to improve the properties and fracture resistance of NiTi instruments. Separation of NiTi files can be caused by torsional or flexural fatigue (cyclic fatigue) (4–6).

Many factors can influence the fatigue of NiTi instruments such as the type of kinematics (7) as well as raw materials and manufacturing processes (8). Resistance to cyclic fatigue of NiTi rotary instruments can be increased by improvements in the manufacturing process or the use of heat-treated alloys with superior mechanical properties (9).

The Twisted File (TF; SybronEndo, Orange, CA) is a NiTi rotary system manufactured with R-phase alloy using a twisting method. Its manufacturing process involves the transformation of a basic austenite NiTi wire into the R (premartensitic)-phase by a process of heating and cooling. After the twisted shape is achieved, a series of heating and cooling is said to convert the twisted R-phase wire back to the austenite crystalline structure, which becomes superelastic while stressed (10). This treatment would impart an increased cyclic fatigue resistance to the files (11).

Another development in the fabrication of endodontic NiTi instruments was the introduction of controlled memory wire (CM Wire; DS Dental, Johnson City, TN) as Hy-

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flex CM (Coltene Whaledent, Cuyahoga Falls, OH) files. These instruments have high resistance to cyclic fatigue and do not rebound to their original shape because of their alloy and specific manufacturing process (11, 12).

Because postmachining thermal treatment influences the properties of heat-treated alloys, the heat generated by sterilization procedures could influence the mechanical properties of endodontic instruments (9, 13). Sterilization of NiTi files must be ensured before clinical use except for the presterilized ones. Repetitive use of files under clinical conditions requires autoclave sterilization after every use. Also, prearranged sets of selected files may not be used during the same appointment. As a result, the unused rotary files are also subjected to multiple autoclave cycles (13, 14). Researchers reported that the additional "heat treatment" during autoclave sterilization might improve the flexibility of files, and the sterilization of files by using dry hot air and an autoclave would have a positive effect on the cyclic fatigue resistance (13, 14).

Although no effect of autoclave sterilization on Lightspeed NiTi rotary instruments (Discus Dental, Culver City, CA) has been reported, ProFile NiTi rotary files (Dentsply Maillefer, Ballaigues, Switzerland) have shown a higher mean cycle to failure when exposed to both dry heat and autoclave sterilization (14, 15). Similar findings of autoclave conditions either improving or degrading both the performance and physical properties of various marketed rotary NiTi systems have been reported (9, 14–17).

One additional factor potentially limiting the resistance of NiTi files to fatigue fracture is corrosion, which may occur in the presence of sodium hypochlorite (NaOCl) solution (18). NaOCl is used as an irrigant, and it selectively removes nickel from the instrument surface and causes micropitting (19), negatively affecting the physical and mechanical properties of NiTi files (20).

Even if the effects of NaOCl contact or autoclave sterilization on the cyclic fatigue of NiTi instruments have been separately investigated (14–18, 21), few studies analyzed the influence of both autoclave sterilization and NaOCl immersion on the cyclic fatigue of NiTi endodontic files (9, 22, 23). Moreover, no studies have investigated the influence of autoclave sterilization and immersion in NaOCl of instruments made by different heat-treated alloys such as the TF and Hyflex CM. Thus, the purpose of this study was to investigate the effect of both NaOCl contact and repeated autoclave cycles on the cyclic fatigue resistance of R-phase and CM Wire files.

Materials and Methods

Sample size estimation was calculated a priori with G*Power 3.1.9.2 software (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany). A total of 210 new 25/.06 TFs and Hyflex CM files were selected for the test. All instruments had been previously inspected using a measuring stereomicroscope (SZR-10; Optika, Ponteranica, Bergamo, Italy) for any signs of visible deformation. None were discarded.

Instruments all from the same production lot were randomly assigned into 7 groups (n = 15) for each brand. Group 1 (the control group) included new instruments that were not immersed in NaOCl or subjected to autoclave sterilization. Groups 2 and 3 were composed of instruments dynamically immersed for 3 minutes in 5% NaOCl solution (Niclor; OGNA Laboratory, Milan, Italy) at 37°C 1 and 3 times, respectively. All files were placed in small separate glass containers with the amount of NaOCl solution necessary to contact 16 mm of the instrument's length; dynamic immersion was allowed, activating the endodontic instruments with a 6:1 reduction handpiece (Sirona Dental Systems GmbH, Bensheim, Germany) powered by the VDW Silver Reciproc motor (VDW, Munich, Germany) at 500 rpm. Immediately after removal from the solutions, all files were rinsed with bidistilled water to neutralize the effect of NaOCl, dried, registered with an identification number, and stored in glass vials. Groups 4 and 5 consisted of instruments only autoclaved 1 and 3 times, respectively. The remaining groups (groups 6 and 7) recruited instruments that received a cycle of both immersion in NaOCl (as described previously) and sterilization 1 and 3 times, respectively.

The sterilized instruments were subjected to 1 or 3 cycles of autoclave sterilization (Sterilix Vacuum Plus; Reverberi, Barco, Italy), and each cycle was performed at a temperature of 134°C for 17 minutes (9). Each file was placed in a separate endodontic sponge and packaged singularly for sterilization in pouches. Instruments that underwent multiple autoclave cycles were allowed to cool to room temperature after sterilization. Sponges were removed from sterilization packaging and repackaged singularly in pouches before the subsequent autoclave cycles. In groups 4 and 5, no additional cleaning or surface treatment procedures were performed on the files before, during, or after sterilization. In groups 6 and 7, immersion in NaOCl was performed before autoclaving.

Instruments of all groups of each brand were then subjected to cyclic fatigue testing using an artificial stainless steel canal. It consists of a 36.8 mm × 25.4 mm × 9.5 mm metal block with a suitable artificial canal with a 60° angle of curvature and a 5-mm radius of curvature to the center of the 1.5-mm-wide canal. The radius was measured to the central axis of the curvature according to the method of Schneider (24). The center of the curvature was 5 mm from the tip of the instrument. The apparatus enabled the instrument to rotate freely within a stainless steel artificial canal at a constant pressure (7, 24, 25). The apparatus was connected to the same 6:1 reduction handpiece and motor used for dynamic immersion.

Cyclic fatigue tests were performed by rotating the instruments in continuous rotation at 500 rpm (as suggested by the manufacturers). Torque was set at the maximum level. The canals were covered with glass to prevent the instruments from slipping out (26). To reduce friction between the instrument and the metal canal walls, a special high-flow synthetic oil designed for lubrication of mechanical parts (Super Oil; Singer Co Ltd, Elizabethport, NJ) was applied.

All instruments were rotated until fracture occurred; timing was stopped as fracture was detected visually and/or audibly. To obviate human error, video recording was performed simultaneously, and the recordings were observed to cross-check the time of file separation.

The number of cycles to failure (NCF) for each instrument was calculated by multiplying the number of rotations per the effective seconds of continuous rotation required for fracture. The length of the fractured file tip was measured by using a digital microcaliper (Mitutoyo Italiana srl, Lainate, Milan, Italy).

Data were subjected to the Shapiro-Wilk test to characterize their normality and statistically analyzed using 2-way analyses of variance and the Tukey multiple comparison post hoc test to assess significant differences among groups ($P < .05$).

The broken fragments were analyzed using a field-emission scanning electron microscope (Nova NanoSEM 450; FEI, Eindhoven, the Netherlands) using 600× magnification to investigate the morphology of the fracture surface and 500× for the lateral views. This magnification was chosen to disclose the main feature morphology while maintaining a large field of view.

The chemical composition of the files' surface and eventual debris identified by imaging was obtained by a field-emission scanning electron microscope equipped with a Si-drift detector for energy-dispersive X-ray spectrometry (Quantax-200; Bruker, Berlin, Germany). Energy-dispersive X-ray spectrometric (EDS) spectra were collected from the middle region of each fractured specimen on 2000× magnified images.

Results

The means and standard deviations of NCF after the cyclic fatigue test for all groups of investigated instruments are presented in Table 1. The inferential analysis revealed statistically significant differences among the different groups considering the type of instrument as the independent variable (2-way ANOVA, $P < .05$; interaction <.05). Moreover, there were statistically significant differences between the instruments tested considering the type of treatment as the independent variable (2-way ANOVA, $P < .05$; interaction <.05).

Three minutes of immersion in 5% NaOCl both for 1 or 3 times did not exert any significant overall effect on file performance for any of the instrument systems tested ($P > .05$). TFs autoclaved 3 times had significantly lower resistance to cyclic fatigue than new ones, independent of immersion in NaOCl ($P < .05$). Instead, the cyclic fatigue of TF was not adversely affected by the sterilization procedure for 1 time, with or without contact with NaOCl ($P > .05$). On the other hand, no cyclic fatigue difference was found between the control and all test groups of Hyflex CM ($P > .05$).

Post hoc analysis revealed a significantly higher cyclic fatigue resistance of HyFlex CM files than TFs in groups 5 and 7 (files sterilized 3 times without and with immersion in NaOCl, respectively) ($P < .01$). The lengths of the fractured file fragments were not statistically different among the tested instruments (mean = 5.1 mm) ($P > .05$) (Table 1).

Field-emission scanning electron microscopic analysis of the fractured surfaces showed the typical features of cyclic fatigue failure for both instruments. The crack initiation areas and the overload fast fracture zone caused by cyclic fatigue are identifiable in the fracture surface of all instruments (Fig. 1*A–F*). HyFlex CM files showed surface grooves approximately orthogonal to their longitudinal axes (Fig. 2*A* and *B*), whereas surfaces grooves approximately parallel to the longitudinal axes were found on TFs (Fig. 2*D* and *E*).

EDS analysis indicated that both tested instruments (Hyflex CM and TF) had near equiatomic NiTi compositions (Fig. 2*C* [Sp. 3] and *F* [Sp. 3]). Debris were visible on the file surfaces of Hyflex CM (Fig. 2*C* [Sp. 1]) and TFs (Fig. 2*F*[Sp. 1]). Moreover, EDS analysis showed the presence of an oxide-rich layer on the Hyflex CM files' external surface (Fig. 2C [Sp. 3]), whereas the underlying substrate consisted of the NiTi alloy with only a tiny amount of oxygen (Fig. 2*C* [Sp. 2]).

Discussion

NiTi instruments are frequently reused for multiple cases, leading to an accumulation of cyclic fatigue. Material fatigue appears to be an important reason for the separation of rotary instruments during clinical use (27, 28).

TABLE 1. Means and Standard Deviations (SDs) of Number of Cycles to Failure (NCF) for Hyflex CM and Twisted File after Immersion in Sodium Hypochlorite and/or Autoclave Sterilization

Instrument		Hyflex CM		Twisted File	
Group	*n*	NCF	SD	NCF	SD
1	15	710.5^{a1}	71.2	705.8^{a1}	75.8
2	15	792.6^{a1}	55.8	674.4^{a1}	77.6
3	15	686.4^{a1}	63.5	661.4^{a1}	81.5
4	15	750.0^{a1}	51.5	638.9^{a1}	70.2
5	15	797.5^{a1}	65.0	484.9^{b2}	74.4
6	15	681.4^{a1}	42.6	641.9^{a1}	83.7
7	15	732.2^{a1}	74.5	548.8^{b2}	68.2

The same letters show differences not statistically significant ($P > .05$) in comparison with different groups of the same instrument; the same number show differences not statistically significant ($P > .05$) in comparison with the same group of different instruments.

The choice to reuse NiTi files for more than 1 patient makes the sterilization process necessary in order to prevent cross infections (29). Several studies had evaluated the effects of sterilization on the mechanical properties of files manufactured with the conventional NiTi alloy (14, 15), sometimes resulting in conflicting results. Although some authors reported no effect of autoclave sterilization on the fatigue resistance of NiTi files (9, 15), others have shown a higher (14, 16) or lower (17) mean NCF when exposed to both dry heat and autoclave sterilization. In addition, few reports investigated the influence of the autoclave sterilization procedure on the cyclic fatigue of heat-treated NiTi instruments (9, 16, 17, 30), and only 1 also involved immersion in NaOCl (9).

Thus, in the present study, we enquired into the effect of both NaOCl contact and autoclave sterilization on the cyclic fatigue resistance of 2 heat-treated NiTi files. The present investigation was based on rotary endodontic instruments that had not been used clinically in order to avoid instrument exposure to the uncontrolled stresses of routine clinical conditions, as in previous reports (14, 15). In order to get closer to clinical conditions, the protocol of this study provides dynamic immersion in 5% NaOCl solution at 37°C for 3 minutes only of the working part (16 mm) (21). Only 16 mm from the tip of instruments was immersed in solution to avoid galvanic corrosion phenomena (31). To simulate a realistic number of times that files can be reused, 1 or 3 cycles of sterilization and/or immersion in NaOCl were chosen (32).

An artificial canal with a 60° angle of curvature and a 5-mm radius of curvature was chosen because most of the previous studies on cyclic fatigue were based on these parameters (13, 21, 26). Each cycle of autoclave sterilization was performed at a temperature of 134°C for 17 minutes. These temperatures and sterilization times ensure a better simulation of clinical conditions, maintaining a correct protocol for an efficient disinfection (33). Under the present conditions, a statistically significant decrease in TF cycles to failure after autoclaving for 3 times was observed.

The current results suggest any enthalpy generated during autoclave processing does not provide enough energy to enable a heat treatment effect that could cause a crystalline phase change. It has been reported that a temperature of 170°C is required to initiate reordering and 430°C–440°C to obtain maximum fatigue resistance (15). Furthermore, multiple autoclave cycles have been reported to increase the depth of NiTi file surface irregularities, causing fatigue propagation (34, 35).

Even if the present report is in agreement with a previous study (17) in which autoclaving procedures significantly reduced the NCF of 25/.06 TF instruments, it is in contrast with another one in which repeated cycles of autoclave sterilization did not affect the mechanical properties of NiTi endodontic instruments except for the K3 XF prototype rotary instruments (SybronEndo, Orange, CA), which showed a significant increase in their cyclic fatigue resistance (16). These different findings could be caused by the different instruments tested as well as the sterilization protocol followed (16). Only 1 study analyzed the effect of NaOCl immersion and autoclave sterilization on the cyclic fatigue of heat-treated NiTi alloys (9), excluding any influence on cyclic fatigue resistance when TFs are subjected to 5 repeated cycles of NaOCl and autoclave sterilization. The different results with our study could be explained by the different cyclic fatigue test performed as well as the other experimental conditions (NaOCl concentration and time of immersion used) (9).

Regarding Hyflex CM, autoclave sterilization conditions did not significantly affect its cyclic fatigue behavior. In addition, HyFlex CM files exhibited higher cyclic fatigue resistance than TFs, only in files sterilized 3 times without or with immersion in NaOCl. These results could be attributed to the different postmachining thermomechanical treatment that the NiTi alloy is subjected to during the fabrication of Hyflex CM

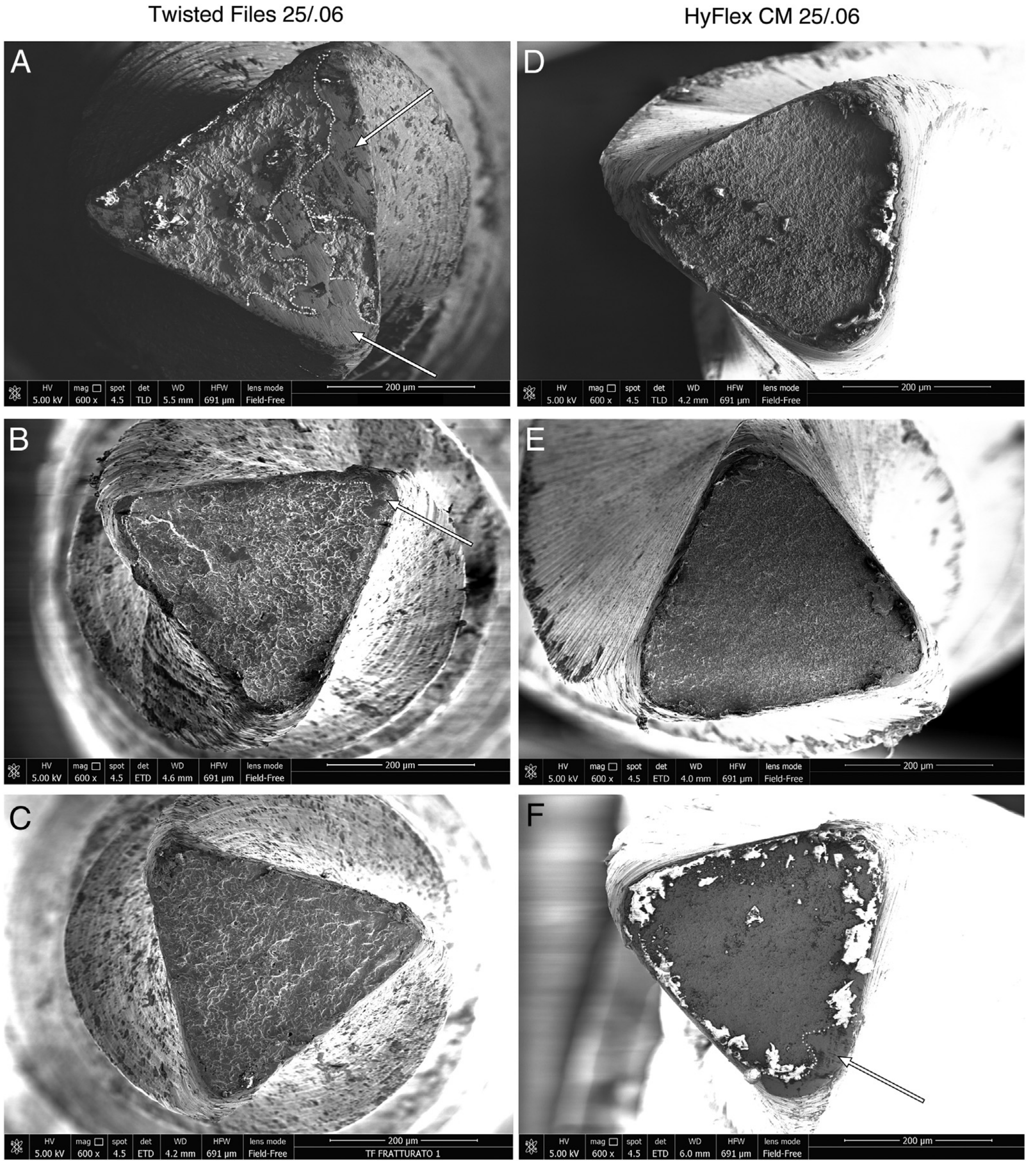

Figure 1. Field-emission scanning electron microscopic analysis performed on fractured specimens (axial views) at 600× of (*A–C*) TFs and (*D–F*) Hyflex CM files. Fatigue fracture surface of TFs and Hyflex CM files: (*A* and *D*, respectively) new (ie, not treated), (*B* and *E*, respectively) dynamically immersed for 3 minutes in 5% NaOCl 3 times, and (*C* and *F*, respectively) dynamically immersed in 5% NaOCl and sterilized 3 times. Similar fracture patterns (composed of distinguishable brittle and plastic areas) are visible on the same surface plane. In some samples (*A*, *B*, and *F*), the visible parts of brittle fracture are wider (*white dotted lines* and *arrows*).

and TF instruments. According to the manufacturer, TFs were developed by transforming a raw NiTi wire in the austenite phase into the R-phase through a thermal process (10) within a very narrow temperature range (28). On the other hand, CM Wire is an NiTi alloy manufactured using a special thermomechanical process that controls the memory of the material (11, 12), allowing an increased cyclic fatigue resistance (36). NiTi is a ''sensitive'' alloy to both thermal and mechanical stresses. Patented processes are highly influenced by temperature and time intervals (16). Any further machining process will affect transitional temperatures and the percentage of phases of the alloy (11). This can explain why the performance of NiTi rotary instruments can be influenced by the quality of the manufacturing processes and different thermal treatments.

A recent study (30) reported that HyFlex CM files showed an increase in cyclic fatigue resistance after autoclave sterilization, whereas no difference was observed for TFs. In addition, it was reported that no statistically significant difference existed between the 2 instruments in cyclic fatigue resistance. The different results with our study may be explained by the different instrument size, sterilization conditions, and methodology used (30).

EDS analysis of the Hyflex CM and TF surfaces after immersion in NaOCl and sterilization for 3 times revealed a higher amount of oxygen and a lesser amount of nickel on the Hyflex CM surface than on the TF surface (Fig. 2*C* [Sp. 3] and *F* [Sp. 3]). This confirms the existence of an oxide coating on shape memory files including Hyflex CM as previously reported (37). An increase of the electrode potential is commonly observed in the presence of oxide layers in several alloys in corrosive environments like NaOCl (37). Therefore, an increment of the corrosion resistance is expected in this condition for CM Wire files compared with conventional, M-Wire, or R-phase files because of the oxide layer that reduces the amount of nickel on the instrument surface by covering it (37).

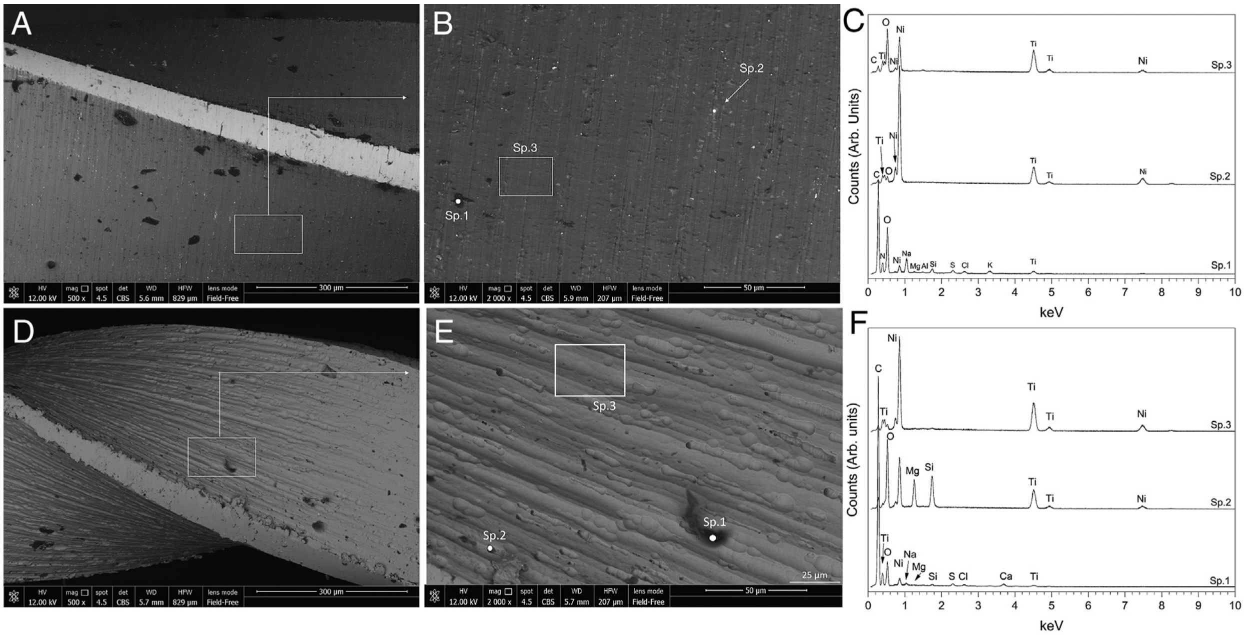

Figure 2. Field-emission scanning electron microscopic micrographs at 500× of the middle region of an (*A*) HyFlex CM 25/.06 and (*D*) TF 25/.06 dynamically immersed in 5% NaOCl and sterilized 3 times in the lateral view. The *white rectangular area with the connected arrow* represents the middle region at higher magnification (2000×) used for EDS analysis of (*B*) Hyflex CM and (*E*) TFs. EDS spectra acquired in the *rectangular marked area* (Sp. 3) and in 2 different points (Sp.1 and Sp.2) on the (*C*) HyFlex CM and (*F*) TF surface.

Under our testing conditions, dynamic immersion in NaOCl did not significantly reduce the cyclic fatigue resistance of the NiTi files examined, as previously reported (21, 38). These findings may be caused by the type of analysis conducted. The cyclic fatigue device generates the maximum stress at the center of the simulated curve (about 5 mm from the tip), so, if a corrosive zone was present at that level, the instrument could break early. However, if the corrosive attack hits the instrument in a different area, the resistance to cyclic fatigue of the instrument will probably not be reduced (21).

Within the limitations of this study, it can be concluded that repeated cycles of sterilization did not influence the cyclic fatigue of NiTi endodontic instruments except for TFs, which showed a significant decrease of flexural resistance after 3 cycles of sterilization. Immersion in NaOCl did not significantly reduce the cyclic fatigue resistance of either NiTi file tested.

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The authors deny any conflicts of interest related to this study.

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