

## ORIGINAL ARTICLE/ARTICOLO ORIGINALE

# Ni-Ti alloy remnants after root canal preparation with Ni-Ti engine-driven files: a preliminary report

**KEYWORDS**

Root Canal,  
Ni-Ti Instruments, Scanning  
Electron Microscope,  
EDX Analysis

**PAROLE CHIAVE**

Canale radicolare,  
strumenti Ni-Ti, microscopio  
elettronico a scansione,  
analisi EDX

**Stefan Vasile  
Stefanescu<sup>1\*</sup>,  
Mugur George Popescu<sup>2</sup>,  
Lucian Barbu Tudoran<sup>3</sup>,  
Haider Al-Saffar<sup>4</sup>**

<sup>1</sup>My Dentist, Europa House,  
Stoneclough Rd.,  
Manchester M26 1GG,  
United Kingdom

<sup>2</sup>V. Goldis University of West,  
86 L. Rebreanu str, Arad,  
Romania

<sup>3</sup>Babes-Bolyai University,  
1 M. Kogalniceanu str.,  
Cluj-Napoca, Romania

<sup>4</sup>My Dentist, Europa House,  
Stoneclough Rd.,  
Manchester M26 1GG,  
United Kingdom

Received 2019, February 19

Accepted 2019, October 14

*Residui di lega di Ni-Ti dopo la preparazione del canale radicolare con file rotanti in Ni-Ti: uno studio preliminare*

**Abstract**

**Aim:** Aim of the present study was to assess any signs of Ni-Ti alloy remnants in the root canal after preparation and investigate if this aspect may be influenced by the steam sterilization of the files.

**Materials and Methods:** 20 extracted upper first premolars with complete root formation and apical foramina and three kits of BioRace instruments (FKG, Le Chaux-of-Fonds, Switzerland) were used in the present study. The first BioRace kit was used to prepare 10 teeth (20 canals), without sterilization between the root canal preparations, while the second sample group was subjected to steam sterilization after each tooth (two canals) prepared. The third kit of instruments was used as a control group. Files were then analyzed by means of scanning electron microscope (SEM) using magnifications between 500 and 4.000x. Images were taken on sections D1-D8 of each file. Files weight was measured using a precision weight scale with readability of 0,001g to quantify the possible alloy loss and file degradation occurred during their use. Teeth were then sectioned on their length and submitted to EDX analysis in search of possible alloy particles within the root canals, using magnifications between 30 and 300x. In the same manner, the irrigant used during the root shaping was collected and analyzed. Differences in weight were statistically analysed using the independent sample Student t-test ( $p < 0.05$ ).

**Results:** No file separation was registered in both groups analyzed. Weight measurements and SEM micrographs showed signs of higher degradation for samples from group 2. EDX analysis showed some Ni-Ti particles in roots prepared in group 2.

**Conclusions:** Metal strips, weight loss and micro-fractures appeared on the files subjected to steam sterilization between the uses. Ni-Ti particles detached from files, during their intra-canal use were found on the root canal walls.

**Obiettivi:** il presente studio ha lo scopo di valutare eventuali segni di residui di lega di Ni-Ti nel canale radicolare dopo la preparazione e indagare se questo aspetto può essere influenzato dalla sterilizzazione dei file.

**Metodologia:** 20 primi premolari superiori estratti con completa formazione delle radici e forame apicale e tre kit di strumenti BioRace (FKG, Le Chaux-of-Fonds, Svizzera) sono stati utilizzati in questo studio. Il primo kit di BioRace è stato utilizzato per preparare 10 denti (20 canali), senza sterilizzazione tra gli utilizzi, mentre il secondo gruppo di campioni è stato sottoposto a sterilizzazione a vapore dopo ciascun dente (due canali) preparato. Il terzo kit di strumenti è stato usato come gruppo di controllo. I file sono stati quindi analizzati mediante microscopio elettronico a scansione (SEM) usando ingrandimenti tra 500 e 4.000x. Le immagini sono state scattate sulle sezioni D1-D8 di ciascun file. Il peso dei file è stato misurato utilizzando una bilancia con precisione di 0,001 g per quantificare la possibile perdita di lega e il degrado dei file durante l'utilizzo. I denti sono stati quindi sezionati sulla loro lunghezza e sottoposti all'analisi EDX alla ricerca di possibili particelle di lega all'interno dei canali radicolari, utilizzando ingrandimenti tra 30 e 300x. Allo stesso modo, l'irrigante utilizzato durante la modellatura della radice è stato raccolto e analizzato. I dati sulla differenza di peso sono stati analizzati statisticamente con test di Student ( $p < 0.05$ ).

**Risultati:** nessuna frattura è stata registrata negli strumenti di entrambi i gruppi analizzati. Le misurazioni del peso e le micrografie SEM hanno mostrato segni di degradazione più elevata per i campioni del gruppo 2. L'analisi EDX ha mostrato alcune particelle di Ni-Ti nelle radici preparate del gruppo 2.

**Conclusioni:** strisce metalliche, perdita di peso e micro-fratture sono apparsi sui file sottoposti a sterilizzazione a vapore tra gli usi. Le particelle di Ni-Ti staccate dai file, durante il loro uso intra-canale, sono state trovate sulle pareti del canale radicolare.

**Corresponding author**

Stefan Vasile Stefanescu | 193A Warrington rd, Whiston L35 5AF, Merseyside, UK | Phone: +44-7712702307 | email: amedeo\_1@yahoo.com

Peer review under responsibility of Società Italiana di Endodonzia

10.32067/GIE.2019.33.02.05

Società Italiana di Endodonzia. Production and hosting by Ariesdue. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).



## Introduction

Endodontic treatments involve accessing a sterile or non-sterile pulp chamber and root canal content, following an inflammation of the pulp or a bacterial colonization of the pulp chamber and root canals, as a septic complication of decay. For this purpose stainless-steel instruments were developed in the last century serving for the different purposes during different procedures having the unique goal to clean and seal the root canal respecting the biologic and mechanical principles stated by Schilder (1). Over the last quarter of the century, the place of the stainless-steel instruments were taken slowly and surely by nickel-titanium files, becoming an instrument *sine qua non* for root canal treatments, even if steel-instruments are still used, mainly at the beginning of the root canal treatment. Nickel-Titanium (Ni-Ti) files are increasingly used by dentists for their properties that overlap those of stainless-steel instruments in facilitating the cleaning and shaping of root canals, during the chemical and mechanical treatment of all kind of pulp diseases. It is well known the fragility and the rigidity of the steel instruments compared with the Ni-Ti ones. These new instruments not only increased the speed of the treatment but also managed to assure a proper shape of the canal preparation and, subsequently, of the canal filling (2). These files proved to be twice as flexible in bending and torsion, having superior resistance to torsional fracturing when compared to similar stainless-steel instruments (2). The use of nickel-titanium (Ni-Ti) rotary instruments has increased the effectiveness and efficiency of root canal preparation. Studies made on extracted teeth proved that there are less debris and smear layer after preparation with nickel-titanium rotary files than after preparation with hand instruments (3, 4, 5, 6, 7).

During the time many techniques were tried to further enhance instrument res-

istance to cyclic fatigue and cutting efficiency, resulting in a variety of strategies including heat-treatment and electro-polishing (8). Being so useful, reliable, resistant and ultimately, not cheap, the initially single-use instruments got to be reused by a wide number of practitioners for at least five times, with great success, being assured by the manufacturers that both files and the tooth are safe. There are reports that experimented to reuse the same file on 12 (9) or 24 (10) extracted roots having medium curvature. Legislation worldwide is not prohibiting the reuse nor imposing the single-use, with very few exceptions. However, reusing the endodontic files raises many debates and questions. First of all there is the issue of cross-infection then the debate on sterilization and its effect on the Ni-Ti alloy itself and not ultimately the file mechanical resistance threshold. According to Walia (11), a material will fail by torsion when the ultimate shear strength is exceeded. Others stated that being a ductile material, a Ni-Ti instrument would deform plastically and will fracture if its ultimate strength is exceeded (12, 13, 14, 15) or if a fracture line has extended to such an extent that the remaining intact cross-section of file is unable to bear the functional load (16), thus complicating the whole treatment. While stainless-steel instruments separate due to excessive torque, in case of the Ni-Ti files, the combined stress exerted by torsional and cyclic fatigue is to be blamed for the separation that occurs (16, 17, 18). Since Buehler (19) discovered the equiatomic Ni-Ti alloy and Walia (11) first reports on the use of Ni-Ti in endodontics, a large number of studies were made to assess the effect of steam or dry sterilization on Ni-Ti files. The idea that sterilization might work as a heat-treatment, inducing a “regeneration” of the used file served as ground for file reuse. Although a sterilization cycle might be assumed as a thermal treatment that should enhance the endurance of Ni-Ti files, this might be valid only in case of dry sterilization to some extent, considering that thermal treatment means heating up the



alloy to approximately 550 °C (20). However, for the best practice in decontamination and cross-infection control, vacuum steam autoclaving is recommended, not dry heat. Several studies were made to assess the effect of sterilization process on the fatigue life of Ni-Ti rotary files, some of them with no significant effects (22). Others have found a better fatigue life after five cycles of dry-heat sterilization at 180 °C (21). But there are also reports where the sterilization proved to affect the Ni-Ti files by decreasing the cutting efficiency, increasing the depth of surface irregularities and surface roughness and crack initiation and propagation (23, 24, 25).

Many studies concluded that instrument usage can significantly influence the potential for fracture (10, 26, 27, 28). Although new instruments can fracture at their first canal use, those that are used for three or more canals may have a higher susceptibility for fracture. Even if fracture incidence is relatively low in clinical practice, the fracture rates in reused files are very high and it can occur with any Ni-Ti rotary instrument (29). Some reports found that the incidence of separation of unused rotary Ni-Ti files is about 1% (30, 31, 32, 33, 34, 35) while distortion was noticed to occur in up to 60% of the instruments used (36), but it is estimated that the incidence of instrument fracture in instruments used multiple times varies between 0.39% and 21% (12, 14, 15, 29, 37, 38, 39). It has been also reported that 55.7% of these fractures are due to torsional mechanism and the rest of 44.3%, to cyclic fatigue (12).

Endodontic irrigants are substances used, both as liquids as well as gels, to eliminate microorganisms, smear layer and pulp tissue from the endodontic system, due to the anatomic complexity. For this purpose sodium hypochlorite (NaOCl) and Ethylenediaminetetraacetic acid (EDTA) are widely used, for its antibacterial and protheolytic effect, as well as for its chelating properties. It was shown that during extended periods of time in NaOCl solutions, corrosion

may be enhanced (38) or minimized depending on the pH of the environment (41). Though the impact of NaOCl did not show any difference in the cutting efficiency or resistance to fracture of Ni-Ti instruments (42, 43) it did result in a reduced resistance to cyclic fatigue (44, 45) and the presence of corrosion (21). There are also studies that showed that a sterilization cycle along with immersion in NaOCl did not result any substantial instrument changes (46). Since no studies are present on Ni-Ti files degradation, aim of the present study was to evaluate the degradation of Ni-Ti rotary files, after root canal preparation, by measuring their weight and by means of scanning electron microscope (SEM) evaluation and energy dispersive X-ray spectroscopy (EDX) examination of the prepared canal.

## Materials and Methods

20 extracted upper first bicuspid with complete root formation and two separate canals were used in present study. After the access cavity was prepared, the canals were negotiated to the working length with stainless-steel hand K-files up to size 15, confirming the apical patency. Standardized pre-operative radiographs were taken in both projections with the 15 K-file inserted into the canal, using a radiographic paralleling device attached to a Minray (Soredex, Milwaukee, United States) 70 kV, 8 mA X-ray generator. Teeth were included in a silicon-based material (Zetaplus, Zhermack, Badia Polesine, Italy) in order to maintain position, the X-ray tube was aligned perpendicular to the root canal, with an object to film distance of 3 mm and the exposure time was set at 0,2 seconds. Digora Optime UV intraoral imaging plate system (Soredex, Milwaukee, United States) was used to capture the images. The degree of the canal curvature was determined using the Digora DfW2.6 and Scanora (Soredex, Milwaukee, United States) image processing software. Schneider's method (47) was used to determine the canal



curvature drawing two lines, one parallel to the long axis of the root canal and the second one crossing the foramen and intersecting the first line when it began to leave the long axis of the canal. Only canals categorized as moderately curved (10-20°) were selected for the present study. Since upper bicuspid usually has two root canals, both needed to have a curvature of 10-200, thus every tooth had two xrays done, with both canals having a size 15 K-file inserted one at a time. Teeth exclusion criteria were also: teeth with open apices, single root canal, previous restorative and endodontic treatment, developmental defects, abnormalities in root canal shape, root resorption, calcified canals. The 20 teeth were then divided in two groups of 10.

Three kits of BioRace instruments (FKG, Le Chaux-of-Fonds, Switzerland) consisting of six files each (BR0 tip size 25/.08 taper, BR1 15/.05, BR2 24/.04, BR3 25/.06, BR4 35/.04, BR5 40/.04) were used in present study. The six instruments of the first BioRace kit sample (group 1) were used in sequence without sterilization between their use in the preparation of 20 root canals (10 teeth), while the second kit sample of six instruments (group 2) was used in 20 root canals (10 teeth) and subjected to steam sterilization (Alphaclave23, HMCE, Taillis-France) for 70 minutes, after each two root canals prepared, a total of 10 cycles of sterilization. The third kit of six new instruments was used as a control group.

Teeth were decoronated at the cemento-enamel junction (CEJ) to avoid coronal interferences during root canal preparation, using a diamond disc (Bessqual, Meta Dental Corp, Glendale, USA). The BioRace engine-driven endodontic files were used to prepare the root canals using the crown-down preparation technique by the same experienced operator, using an electric NSK NLXplus motor (NSK Europe, Maidenhead, UK) having a 20:1 reduction hand-piece NSK Ti-Max X-SG20L, at 300 rpm and torque set at 1 N/cm as recommended by the manufacturer.

Each root canal was irrigated copiously

with 2 ml of both 5,25% NaOCl (Cerkamed PPH, Stalowa Wola, Poland) and 17% EDTA (ethylene-diamine-tetra-acetic acid) (Meta-Biomed Co Ltd., Chungbuk, Korea), after each instrument was used. Irrigation was carried out above a Berzelius glass, one for each sample group. Collected liquid was analyzed by means of Energy-dispersive X-ray spectroscopy (EDS, EDX or XEDS). After the liquid was analyzed, it was filtered using a filter paper and sediment left to dry at room temperature and then analyzed by means of Energy-dispersive X-ray spectroscopy. Broken files were registered and the instrument and the tooth involved were excluded from further examination and substituted.

After root canal instrumentation the files were cleaned in ultrasonic bath (Hygea2850VM, Ultrawave Ltd., Cardiff, U.K.) in alcohol, for eight minutes, then all the instruments, including the control group, were subjected to weight measurement using a precision weight scale (Partner AS.3Y, Partner SRL, Bucharest, Romania), with readability of 0,001 g. Each file of the sample kit tested was subjected to three consecutive measurements and the mean value was calculated to evaluate differences among new files and the instruments used with and without sterilization.

Then, roots were decalcified in 10% EDTA aqueous solution for 30 days, sectioned along their long axis and prepared for SEM analysis. SEM analysis was performed on each Ni-Ti file used and on the prepared roots, using a High-Resolution Scanning Electron Microscope (Hitachi SU8230, Chiyoda, Japan) equipped with a cold field emission gun. For morphological analysis the samples were deposited on aluminum stubs and sputter-coated with 10 nm gold on a Q150T ES Quorum. Loss of integrity, surface modifications, crack line propagation and deformations of the instruments were analyzed by means of SEM, using magnifications between 500 and 4.000x.

Energy-dispersive X-ray spectroscopy (EDX, EDS or XEDS) was conducted us-

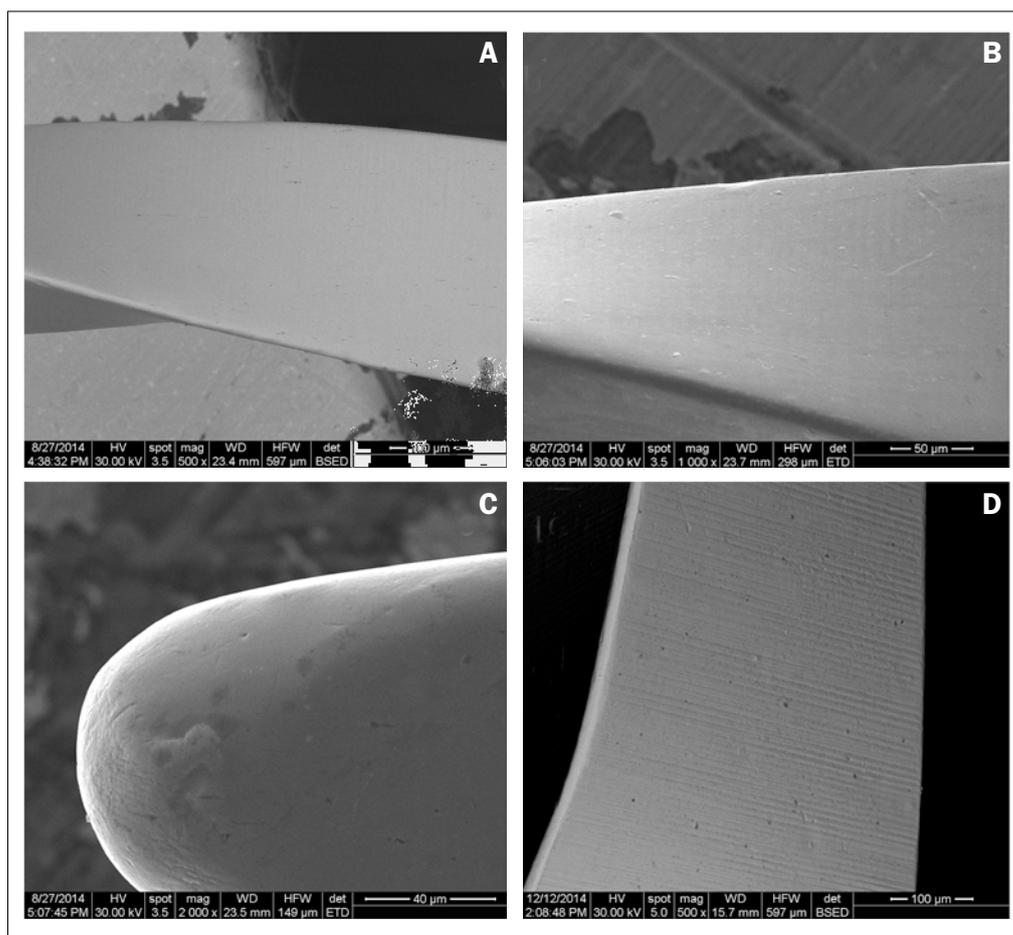
ing an EDX System (X-Max N80TLE Silicon Drift Detector, Oxford Instruments, Abingdon, Oxfordshire, UK) to identify microscopic alloy fragments inside the roots or in the irrigating agent. Roots were analyzed in search of Ni-Ti alloy fragments on the inner surface of the root canals using magnifications between 30 and 300x to identify their existence in the roots prepared, to identify the group in which this phenomenon happened more and the moment of degradation to better understand if the phenomenon happened in all the roots prepared, or only by the end of instruments life. Differences between the weight measurements at the baseline and after treatment for both groups were tested for distribution using the Kolmogorov-Smirnoff test. They had normal (gaussian) distribution, which allowed using the independent sample Student t-test to compare the data ( $p < 0.05$ ).

## Results

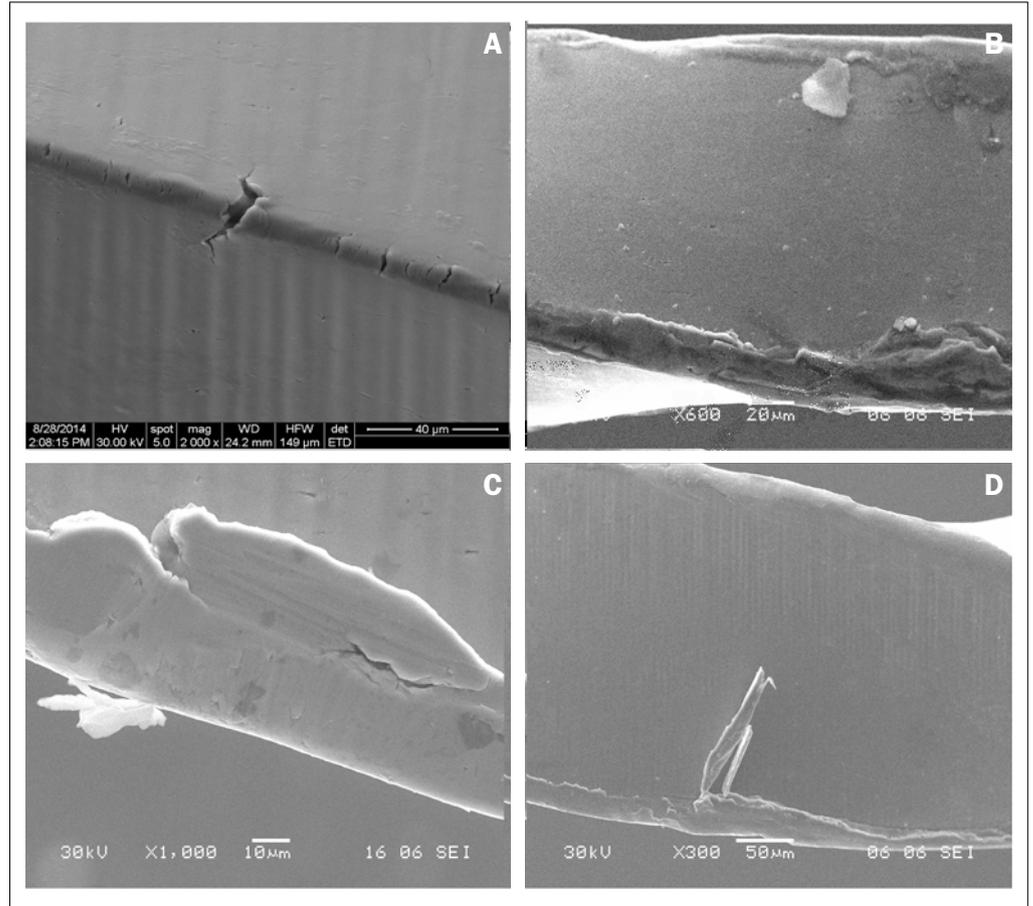
No file separation was registered in both groups analyzed. Weight measurements of the different instruments are shown in Table 1 and there were no statistical differences between two groups ( $p = 0.9140$ ). In fact, a minimum weight loss was registered in both groups of instruments used, with respect to the control group (0,1-0,2 mg for instruments from group 1 and 0,1-0,5 mg for instruments from group 2), except for BR5 instruments, which recorded a higher weight loss (3 mg and 2,5 mg for samples from group 1 and 2 respectively).

SEM microphotographs of the tested instruments showed that the files from group 2 presented higher degradation with respect to files from group 1. At SEM analysis, instruments from the control group did not report any sign of degradation (fig. 1), while slight structure disruptions, cutting edge disruption, metal strips and

**Figure 1**  
Surface of new files:  
BR0 (A), BR1 (B), BR2 (C)  
and BR3 (D).



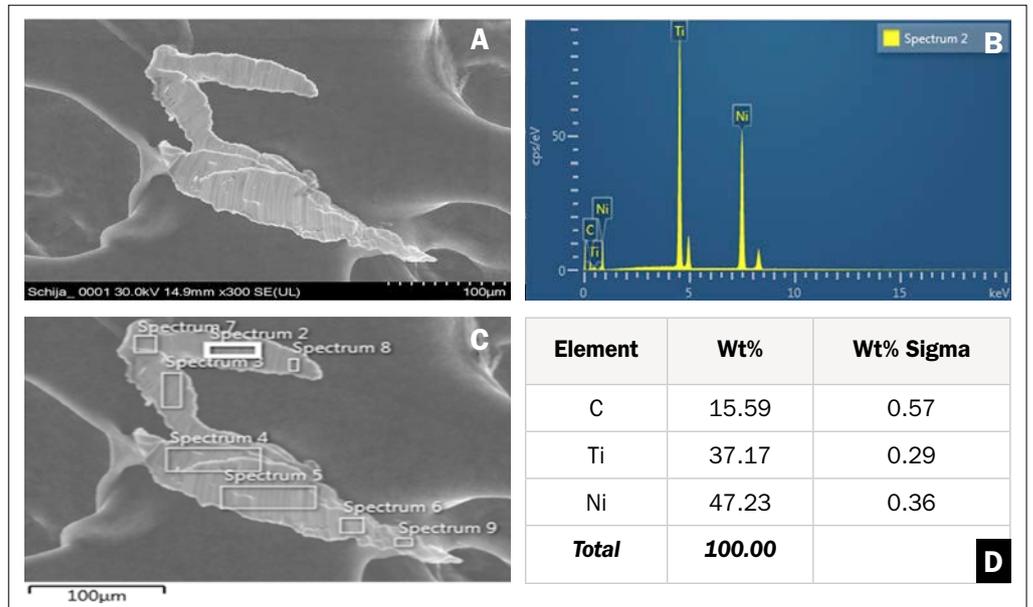
**Figure 2**  
Cutting edge disruption, metal strips and heavy surface degradation on BR3 file from group 1 (A); same file from sample group 2 (B); BR5 file from sample group 1 (C); same file from sample group 2 (D).



heavy surface degradation (fig. 2) maybe seen in both groups analyzed. EDX analysis revealed the presence of metal strips in the irrigating sample col-

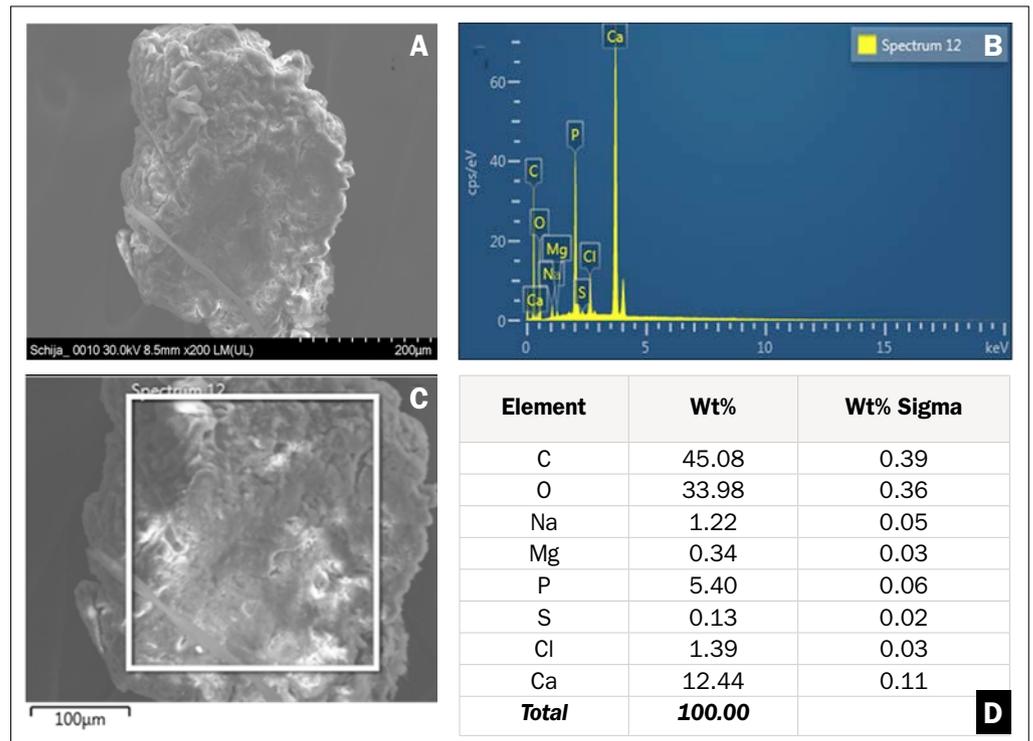
lected from group 2 (fig. 3), while only organic and inorganic debris could be found in the irrigating sample collected from group 1 (fig. 4). SEM analysis revealed

**Figure 3**  
EDX analysis results on the alloy fragment found in a tooth sample from group 2. (A) Alloy fragment collected in the rising agent; (B) Elements weight ratio in metal fragment; (C) EDX sampling on Ni-Ti fragment; (D) Elements atomic ratio in metal fragment.



**Figure 4**

EDX analysis on the debris structure from a sample of group 1. (A) Calcific debris collected in the rinsing agent; (B) Elements weight ratio in debris fragment; (C) EDX sampling of debris; (D) Elements atomic ratio in debris fragment.



that the last four roots prepared with files from group 2 were richer in particles than other roots prepared before in the same group, as well as then all the roots prepared with files from group 1.

Small scattered Ni-Ti particles may be seen on EDX layered images on the internal walls of the root canals in two samples from group 2 (fig. 5A, C) compared to the unlayered SEM image of the same section of the canals (fig. 5B). Elements atomic ratio of the analysed canal segment is

displayed in fig. 5D, where Nickel and Titanium elements are clearly identified by the EDX technique.

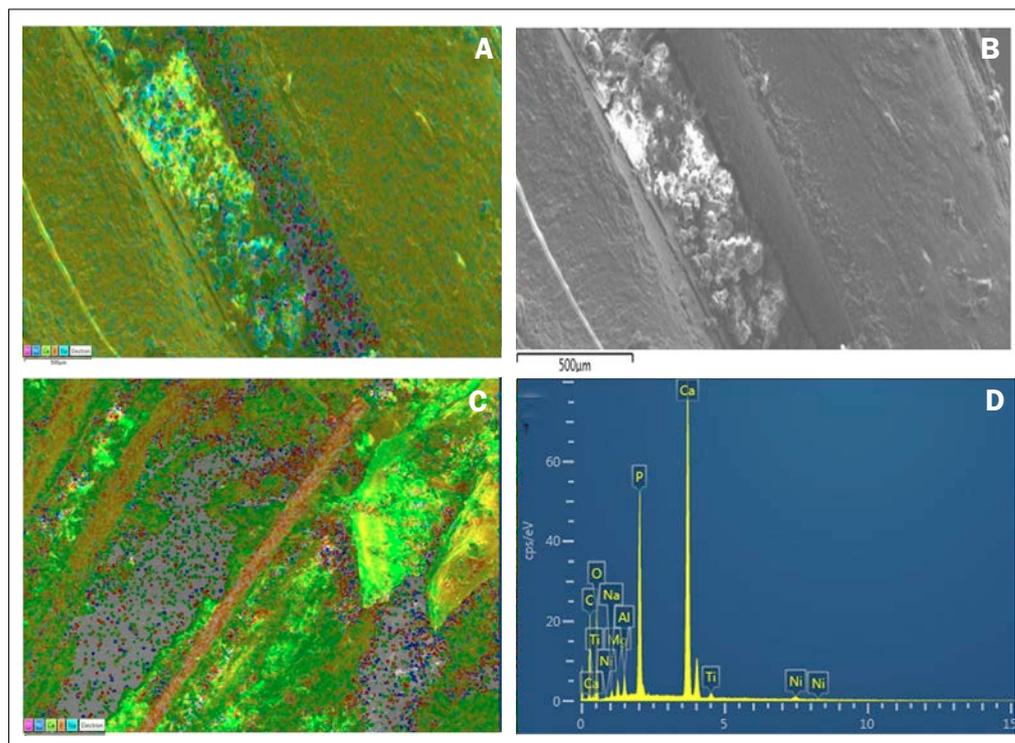
## Discussion

Ni-Ti rotary files have two mechanisms of fracture: shear (torsional) and accumulation of metal fatigue (12, 14, 15). Metal, being a ductile material, would deform plastically before a fracture occurs, if its yield stress is exceeded (13, 14, 15). Studies have clas-

**Table 1. Mean weight (in mg) of the different files analyzed in the three different groups.**

BioRace	Control group (mg)	Group 1 (mg)	Group 2 (mg)
BR0	435,7	435,6	435,4
BR1	436,8	436,7	436,5
BR2	436,2	436,0	435,7
BR3	456,3	456,1	455,9
BR4	430,9	430,8	430,8
BR5	445,1	442,1	442,6

**Figure 5**  
**(A)** EDX layered image of root canal section with multiple Ni-Ti particles; **(B)** SEM image on the apical third of the root, without EDS layering; **(C)** EDX layered image of root canal section with multiple Ni-Ti particles; **(D)** Elements atomic ratio of root canal section presented in sections **(A)** and **(B)**.



sified the mechanism of instrument breakage into “torsional” when macroscopic, plastic deformation is present (13, 14, 15), even if such plastic deformations may not always appear when the instrument undergoes shear failure (49, 50). On the other hand, a detailed examination of the fracture surface of an instrument that fails purely due to fatigue would reveal the presence of one or more crack origins, an area of steady fatigue-crack growth adjacent to each crack origin, an area of rapid crack growth manifested as microscopic dimples of irregular size and shape and, occasionally, a shear lip where the rapid crack-growth region meets the periphery of the cross-section (10). Corrosion pits (the result of corrosive attacks on the material) may be found in specimens fatigued in hypochlorite solution (10, 28, 33). The fatigue-crack origins are usually situated at or near the cutting edge (in cross-section) or on some flaws of the surface such as machining grooves, or subsurface defects, such as microscopic fatigue striations (9). These inner structure changes may lead also to surface defects, besides those inflicted mechanically and through chemical corrosion (10, 21, 40, 42, 43, 44, 45).

As it may be assumed that structure disintegration leads to weight loss that is measurable with precise equipment, the present study focused on weight loss assessment and identification of the sample group that suffered the most for this phenomenon. The purpose of this study was to assess the degradation of the Ni-Ti files by measuring them before and after use. The present study found Ni-Ti fragments in the collected and filtered rinsing agent and smaller fragments on the inner surface of the dentinal root canal walls of the prepared teeth. The alloy fragments collected in the irrigating solutions were probably detached from the cutting edge of the files, having comparable sizes with similar fragments found still attached on the cutting surface of files from the same group in the SEM micrographs. Smaller sized particles having similar or smaller size than the dentinal tubules were trapped in the tubules as it can be noticed in the EDX images. EDX analysis made possible through different staining to identify the alloy particles and to create an idea about their presence and location. Energy-dispersive X-ray spectroscopy (EDS, EDX, or XEDS) is an analytical technique used for the elemental analysis



or chemical characterization of a sample. Its characterization capabilities are due to the fundamental principle that each element has a unique atomic structure allowing a unique set of peaks on its X-ray emission spectrum. To stimulate the emission of characteristic X-rays from a specimen, a high-energy beam of charged particles such as electrons or protons or a beam of X-rays is focused into the sample being studied. At rest, an atom within the sample contains ground state (or unexcited) electrons in discrete energy levels or electron shells bound to the nucleus. The incident beam may excite an electron in an inner shell, ejecting it from the shell while creating an electron-hole where the electron was. An electron from an outer, higher-energy shell then fills the hole and the difference in energy between the higher-energy shell and the lower energy shell may be released in the form of an X-ray. The number and energy of the X-rays emitted from a specimen can be measured by an energy-dispersive spectrometer. As the energies of the X-rays are characteristic of the difference in energy between the two shells and of the atomic structure of the emitting element, EDS allows the elemental composition of the specimen to be measured (51).

Instruments from the kit used in group 1 and not autoclaved presented distortion and bluntness rather than disruption of the cutting edge, while instrument samples from the kit used in group 2 and submitted to sequential autoclave sterilization presented metal chips and cutting edge disruption. Thus, files from group 2 seemed to be more affected structurally, by weight calculation, SEM images and EDX measurements. Given the fact that instruments from both groups were used in similar clinical conditions, on a similar number of root canals, with a similar degree of curvature, these differences may be due to environmental conditions and the inner transformation that alloy suffered during steam sterilization. A limitation of the present study is given by the small sample size and by the fact that samples from group 1 were tested in a clinically non-reproducible condition, meaning the absence of sterilization. Previous studies reported that multiple

cycles of autoclave sterilization significantly decrease the cyclic fatigue resistance of the Twisted File (52), decreased instruments cutting efficiency, increased the depth of surface irregularities and surface roughness and evidenced crack initiation and propagation (22, 23, 24). In another study (10), steam sterilization affected the inner structure of nitinol, so that files with increased taper seemed to be more fragile than files with a smaller taper from the same file kit, due to decreased flexibility. However, there are studies that showed no influence of autoclave on cyclic fatigue resistance of the files, while others (53) identified the same type of increased resistance to cyclic fatigue, although the thermal applications did not alter instrument surface morphology, but resulted in significant changes in the instrument bulk with the appearance of an R-phase and improved fatigue resistance.

In the present study, instruments were used to prepare 10 roots and 20 root canals because the findings of a previous study reported that files subjected to intermediate steam sterilization separated preparing the 12th root canal (10) while in another study (9), where no sterilization was used, the same instruments used in the previous study were managed to prepare 24 root canals. It may be assumed that this damage happened when a certain load was applied after a certain number of rotations, being torque and fatigue dependent (14, 29, 47, 48).

Another aspect that needs to be addressed is the aspect of cross-infection. It has been a long time argued about the chances of prion cross-infection by means of dental instruments. Those who argued in favor of possible transmission based their theory on prion's alleged transmission from the neural tissues to the tissues located in the mouth, due to prion accumulation in the perioral ganglia system of patients with this disease (54). The assumptions followed the logical conclusion that since dental pulp originates from the neural crest, theoretically, the dental pulp of people suffering from any type of Creutzfeldt-Jakob Disease could be infectious. There were reported cases of infected laboratory animals that



developed certain contagiousness in the oral tissues (55, 56). It was stressed that even if the oral tissues are of merely not detectable contagiousness and the presence of prion protein in these tissues has not been confirmed in humans, we cannot rule out the nosocomial transmission of prions during dental treatments (57). It was reported that classic sterilization methods like steam sterilization or by ethylene gas are ineffective against prion agents (58, 59). Therefore instruments that can be reused have to pass through strict decontamination protocols before cleaning and then to be subjected to steam sterilization at 134 °C for 10-18 minutes in a vacuum environment. However, in a later report, dentists are requested to use endodontic files as single-use instruments regardless of the case (60).

On the other hand, as there is no instrument that prevents apical extrusion (61), the present study reports on the possibility for apical extrusion of Ni-Ti particles along with dentin debris, even if the periodontal space is a closed system, being different with the open system used during this study. It is therefore assumable that such metal particles may migrate through the foramens being able to create an allergic reaction (59).

In endodontics the potential allergens are haptens which can turn in full antigens in certain conditions. New researches showed that Type I reactions can be triggered by haptenic substances like metal ions (60), with the possible creation of an immediate or delayed dermal or mucosal reaction (62). If the individual is initially sensitized the reaction can have anaphylactic symptoms due to cross-reaction with barrier equipment (rubber dam, gloves etc.) which also contains allergens (59). These reactions can be the result of previous sensitization. It is known that dermal exposure to metallic allergens is more likely to trigger an allergic reaction than mucosal exposure, therefore allergic reactions in endodontics is extremely low if we take in consideration only the type 1 reactions. But if we take into account the side effects of allergic reactions which are almost neglected as symptoms, like delayed apical healing, persisting discomfort after root filling, post

operatorial urticaria, or allergy to Nickel, their number may increase considerably. There are cases of allergic reactions due to cross-reactions between natural rubber latex and gutta-percha using the chloroform technique (63, 64) or between NRL and antibodies to avocado or bananas. Braun et al. (65) presented arguments in favor of possible sensitization to formaldehyde after several root canal treatments. A reaction between the metal ions and the sealer haptens combined with the residual pulp tissue proteins can form allergens that trigger systemic or local reactions (59). Although it is difficult to prove the link between a failed root canal treatment and a suspected local reaction to an allergen, there are cases described in the literature (63, 64) where the symptoms (urticaria, persisting discomfort) required the removal of the root filling after which the symptoms disappeared.

## Conclusions

Although no file separated in any of the groups, metal strips, weight loss and micro-fractures appeared more frequently on the files subjected to steam sterilization between the uses. Ni-Ti particles detached from the files during their intra-canal use were found in the irrigating solutions and on the root canal walls. Single-use of Ni-Ti endodontic engine-driven files is always advisable in order to reduce instruments wear due to their excessive use and reduce the chances of cross-infection and unwanted reactions.

## Clinical Relevance

The results of this study should warn the dentist about the risks of multiple-use of endodontic files encouraging them to consider Ni-Ti files as single-use equipment.

## Conflict of Interest

The authors deny any conflict of interest related to this study. There were no other contributors to the article. The article was funded from authors' self sources.



## References

1. Schilder H, Cleaning and shaping the root canal. *Dental Clinics of North America*, 18 (1974), 269-296.
2. Hulsmann M, Peters OA, Dummer PMH. Mechanical preparation of root canals: shaping goals, techniques and means. *Endodontic Topics*, 10 (2005), pp. 30-76.
3. Orstavik D. Time-course and risk analysis of the development and healing of chronic apical periodontitis in man. *Int Endod J*, 29 (1996), pp. 150-5.
4. De Paula Silva FWG, Hassan B, Bezerra da Silva LA, Leonardo MR, Wu M-K. Outcome of root canal treatment in dogs determined by periapical radiography and cone-beam computed tomography scans. *J Endod*, 35 (2009), pp. 723-6.
5. Salehrabi R, Rotstein I. Endodontic Treatment Outcomes in a Large Patient Population in the USA: An Epidemiological Study. *J Endod*, 30 (2004), pp. 846-50.
6. Dammaschke T, Steven D, Kaup M, Ott KH. Long-term survival of root-canal-treated teeth: a retrospective study over 10 years. *J Endod*, 29 (2003) pp. 638-43.
7. Stoll R, Betke K, Stachniss V. The influence of different factors on the survival of root canal fillings: a 10-year retrospective study. *J Endod*, 31 (2005) pp. 783-90.
8. Bürklein S, Schäfer E. Critical evaluation of root canal transportation by instrumentation. *Endodontic Topics* 29 (2013), pp. 110-124.
9. Stefanescu T, Antoniac IV, Popovici RA, Galuscan A, Tirca T (2016) Ni-Ti rotary instrument fracture analysis after clinical use. Structure changes in used instruments. *EEMJ*, 15(2016), pp. 981-88.
10. Stefanescu T, Galuscan A, Tudoran LB, Monea MD, Jumanca D. Environmental effect on Ni-Ti rotary files: Steam sterilization & corrosion. A SEM study. *Rev. Chim*, 67(2016), pp. 2114-18.
11. Walia HM, Brantley WA, Gerstein H. An initial investigation of the bending and torsional properties of Nitinol root canal files. *J Endod*, 14(1988), 346-51.
12. Sattapan B, Palamara JE, Messer HH. Torque during canal instrumentation using rotary nickel-titanium files. *J Endod*, 26(2000), 156-60.
13. Sattapan B, Nervo GJ, Palamara JE, Messer HH. Defects in rotary nickel-titanium files after clinical use. *J Endod* 26(2000), 161-5.
14. Parashos P, Gordon I, Messer HH. Factors influencing defects of rotary nickel-titanium endodontic instruments after clinical use. *J Endod*, 30(2004), pp. 722-5.
15. Peng B, Shen Y, Cheung GS, Xia TJ. Defects in ProTaper S1 instruments after clinical use: longitudinal examination. *Int Endod J*, 38(2005), pp. 550-7
16. Cheung GSP. Instrument fracture: mechanisms, removal of fragments, and clinical outcomes. *Endodontic Topics*, 16(2009), 1-26.
17. Dalton BC, Ørstavik D, Phillips C, Pettiette M, Trope M. Bacterial reduction with nickel-titanium rotary instrumentation. *J Endod* 24(1998), 763-767.
18. Gulabivala K., Stock C.J.R. Root canal system preparation. In: Stock CJR, Gulabivala K, Walker RT, eds. *Endodontics*, 3rd Edn. Edinburgh (2004): Mosby, 135-172.
19. Buehler WJ, Gilfrich JW, Wiley RC. Effects of Low-Temperature Phase Changes on the Mechanical Properties of Alloys Near Composition TiNi. *Journal of Applied Physics* 34 (5) (1963): 1475-1477-
20. Liu Y, Xie ZL, Van Humbeeck J, Dalaey L. Effect of texture orientation on the martensite deformation of NiTi shape memory alloy sheet. *Acta Materialia* 47(1999): 645-660.
21. Sonntag D, Peters OA. Effect of prion decontamination protocols on nickel-titanium rotary surfaces. *J Endod* 33(2007), 442-6.
22. Mize SB, Clement DJ, Pruett JP, Carnes DL Jr. Effect of sterilization on cyclic fatigue of rotary nickel-titanium endodontic instruments. *J Endod* 24(1998), 843-7.
23. Alexandrou GB, Chrissafis K, Vasiliadis LP, Pavlidou E, Polychroniadis EK. SEM observations and differential scanning calorimetric studies of new and sterilized nickel-titanium rotary endodontic instruments. *J Endod* 32(2006), 675-9.
24. Valois CR, Silva LP, Azevedo RB. Multiple autoclave cycles affect the surface of rotary nickel-titanium files: an atomic force microscopy study. *J Endod* 34(2008), 859-62.
25. Viana AC, Gonzalez BM, Bueno VT, Bahia MG. Influence of sterilization on mechanical properties and fatigue resistance of nickel-titanium rotary endodontic instruments. *Int Endod J* 39(2006): 709-715.
26. Gambarini G. Cyclic fatigue of ProFile rotary instruments after prolonged clinical use. *Int Endod J* 34(2001), 386-9.
27. Yared GM. In vitro study of the torsional properties of new and used ProFile nickel-titanium rotary files. *J Endod* 30(2004), 410-2.
28. Yared GM, Kulkarni GK. An in vitro study of the torsional properties of new and used rotary nickel-titanium files in plastic blocks. *Oral Surg Oral Med Oral Path* 96(2003), 466-71.
29. Di Fiore PM, Genov KA, Komaroff E, Li Y, Lin L. Nickel-Titanium rotary instrument fracture: a clinical practice assessment. *Int Endod J*, 39(2006), 700-708.
30. Ankrum MT, Hartwell GR, Truitt JE. K3 Endo, ProTaper, and ProFile systems: breakage and distortion in severely curved roots of molars. *J Endod*, 30(2004), 234-237.
31. Paqué F, Musch U, Hülsmann M, Comparison of root canal preparation using RaCe and ProTaper rotary NiTi instruments. *Int Endod J*, 38(2005), 8-16.
32. Rangel S, Cremonese R, Bryant S, Dummer P. Shaping ability of RaCe rotary nickel-titanium instruments in simulated root canal. *J Endod*, 31(2005), 460463.
33. Schäfer E, Schlingemann R. Efficiency of rotary nickel-titanium K3 instruments compared with stainless-steel hand K-Flexofile. Part 2. Cleaning effectiveness and instrumentation results in severely curved root canals of extracted teeth. *Int Endod J*, 36 (2003), 208-217.
34. Schäfer E., Vlassis M. Comparative investigation of two rotary nickel-titanium instruments: ProTaper versus RaCe. Part 2. Cleaning effectiveness and instrumentation results in severely curved root canals

- of extracted teeth. *Int Endod J*, 37(2004), 239-248.
35. Yoshimine Y, Ono M, Akamine A. The shaping effects of three nickel-titanium rotary instruments in simulated S-shaped canals. *J Endod*, 31(2005), 373-375.
  36. Stewart JT, Lafkowitz S, Appelbaum K, Hartwell G. Distortion and breakage of Liberator, EndoSequence, and ProFile systems in severely curved roots of molars. *J Endod*, 36(2010), 729-731.
  37. Alapati SB, Brantley WA, Svec TA, Powers JM, Nusstein JM, Daehn GS. SEM observations of nickel-titanium rotary endodontic instruments that fractured during clinical use. *J Endod*, 31 (2005), pp. 40-3.
  38. Shen Y, Cheung GS, Bian Z, Peng B. Comparison of defects in ProFile and ProTaper systems after clinical use. *J Endod*, 32(2006), pp.61-5.
  39. Shen Y, Cheung GS, Peng B, Haapasalo M. Defects in nickel-titanium instruments after clinical use. Part 2: fractographic analysis of fractured surface in a cohort study. *J Endod*, 35(2009), pp.133-6.
  40. O'Hoy PY, Messer HH, Palamara JE. The effect of cleaning procedures on fracture properties and corrosion of NiTi files. *Int Endod J* 36(2003), 724-32.
  41. Novoa XR, Martin-Biedma B, Varela-Patino P. The corrosion of nickel-titanium rotary endodontic instruments in sodium hypochlorite. *Int Endod J* 40(2007), 36-44.
  42. Ormiga Galvao Barbosa F, Antonio da Cunha Ponciano Gomes J, Pimenta de Araujo MC. Influence of sodium hypochlorite on mechanical properties of K3 nickel-titanium rotary instruments. *J Endod* 33(2007), 982-5.
  43. Cavalleri G, Cantatore G, Costa A, Grillenzoni M, Comin Chiaramonti L, Gerosa R. The corrosive effects of sodium hypochlorite on nickel-titanium endodontic instruments: assessment by digital scanning microscope. *Minerva Stomatologica* 58(2009), 225-31.
  44. Berutti E, Angelini E, Rigolone M, Migliaretti G, Pasqualini D. Influence of sodium hypochlorite on fracture properties and corrosion of ProTaper Rotary instruments. *Int Endod J* 39(2006), 693-9.
  45. Peters OA, Roehlike JO, Baumann MA. Effect of immersion in sodium hypochlorite on torque and fatigue resistance of nickel-titanium instruments. *J Endod* 33(2007), 589-93.
  46. Haikel Y, Serfaty R, Wilson P, Speisser JM, Allemann C. Mechanical properties of nickel-titanium endodontic instruments and the effect of sodium hypochlorite treatment. *J Endod*, 24(1998): 731-735.
  47. Schneider SS. A comparison of canal preparations in straight and curved root canals. *Oral Surg Oral Med Oral Pathol*, 32 (1971), pp. 271-5.
  48. Lopes HP, Ferreira AA, Elias CN, Moreira EJ, de Oliveira JC, Siqueira JF Jr. Influence of rotational speed on the cyclic fatigue of rotary nickel-titanium endodontic instruments. *JOE*, 35 (2009), pp. 1013-6.
  49. Cheung GSP, Peng B, Bian Z, Shen Y, Darvell BW. Defects in ProTaper S1 instruments after clinical use: fractographic examination. *IEJ*, 38 (2005), pp. 802-809.
  50. Wei X, Ling J, Jiang J, Huang X, Liu L. Modes of failure of ProTaper nickel-titanium rotary instruments after clinical use. *JOE*, 33 (2007), pp. 276-279.
  51. Wikipedia; [https://en.wikipedia.org/wiki/Energy-dispersive\\_X-ray\\_spectroscopy](https://en.wikipedia.org/wiki/Energy-dispersive_X-ray_spectroscopy)
  52. Hilfer PB, Bergeron BE, Mayerchak MJ, Roberts HW, Jeansonne BG. Multiple autoclave cycle effects on cyclic fatigue of nickel-titanium rotary files produced by new manufacturing methods. *JOE*, 37 (2011), pp.72-4.
  53. Condorelli GG, Bonaccorso A, Smecca E, Schäfer E, Cantatore G, Tripi TR. Improvement of the fatigue resistance of NiTi endodontic files by surface and bulk modifications. *IEJ* 43(2010), 866-73.
  54. Guiryo DC, Shankar SK, Gibbs J, Messenheimer JA, Das S, Gajdusek DC. Neuronal degeneration and neurofilament accumulation in the trigeminal ganglia in Creutzfeldt-Jakob disease. *Annals of Neurology*, 25(1989), 102-6.
  55. Advisory Committee on Dangerous Pathogens. Spongiform Encephalopathy Advisory Committee. Transmissible spongiform encephalopathy agents: Safe working and the prevention of infection. London: The Stationery Office (1998), 1-54.
  56. Flechsig E, Hegyi I, Enari M, Schwarz P, Collinge J, Weissmann C. Transmission of scrapie by steelsurface-bound prions. *Journal of Molecular Medicine*, 7(2001), 679-84.
  57. Bali Z, Bali RK, Nagrath S. Prion diseases: risks, characteristics, and infection control considerations in dentistry. *Journal of Investigative and Clinical Dentistry*, 2(2011), 236-240.
  58. Brown SA, Merritt K. Use of containment pans and lids for autoclaving caustic solutions. *American Journal of Infection Control*, 31(2003), 257.
  59. Lodi G, Porter SR, Scully C. Hepatitis C virus infection: review and implications for the dentist. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology*, 86(1998), 8-22.
  60. Department of Health, UK. Commissioning and Systems Management. Health Technical Memorandum 01-05: Decontamination in primary care dental practices. London, UK: Department of Health (2009).
  61. Ingle JI, Bakland LK, Peters DL, Buchanan LS, Mullaney TP. Endodontic cavity preparation. In: Ingle JI, Bakland LK, eds. *Endodontics*. 4th Edn. Baltimore: Williams & Wilkins, 1994.
  62. Hensten A, Jacobsen N. Allergic reactions in endodontic practice. *Endodontic Topics*, 12(2005), 44-51.
  63. Hostynek JJ. Aspects of nickel allergy: epidemiology, etiology, immune reactions, prevention, and therapy. In: Hostynek JJ, Maibach HI, eds. *Nickel and the Skin. Absorption, Immunology, Epidemiology and Metallurgy* (Chapter 1). Boca Raton: CRC Press, 2002: 1-38.
  64. Kelly KJ, Banerjee B. Natural rubber latex allergy. In: Grammer LC, Greenberger PA, eds. *Petterson's Allergic Diseases* (Chapter 31), 6th edn. Philadelphia: Lippincott Williams & Wilkins, 2002: 653-671.
  65. Gazelius B, Olgart L, Wrangsjö K. Unexpected symptoms to root filling with gutta-percha: a case report. *Int Endod J* 19(1986), 202-204.
  66. Boxer MB, Grammer LC, Orfan N. Gutta-percha allergy in a health care worker with latex allergy. *J Allergy Clin Immunol* 93(1994), 943-944.
  67. Braun JJ, Zana H, Purohit J, Valfrey A, Scherer J, Haikel Y, de Blay F, Pauli G. Anaphylactic reactions to formaldehyde in root canal sealant after endodontic treatment: four cases of anaphylactic shock and three of generalized urticaria. *Allergy* 58(2003), 1210-1215.