EVALUATION OF THE TITANIUM OXIDE LAYER (TIO2) OF HYFLEX CM AND EDM FILES BY MEANS OF DETECTION OF RETRODISPERSED ELECTRONS (EDX)

INTRODUCTION

Unprecedented advances over the last decades in endodontic technology and materials have implied significant changes in terms of endodontic prognosis. One of the main developments has been observed with the appearance of the nickel titanium (NiTi) endodontic instruments. since 1990s, NiTi rotary instruments have undergone revolutionary changes in terms of design, shape and surface treatment, what translates into greater flexibility in comparison with previous stainless steel (SS) instruments [1]; allowing biomechanical preparation techniques to address root canals of greater anatomical complexity with fewer procedural errors and reducing the time of endodontic treatment [2, 3].

Original Niti endodontic instruments were built from a conventional superelastic alloy, composed of 56% nickel and 44% titanium. The resultant combination is a 1:1 atomic ratio (equiatomic) of the major components, where nickel is a ductile, malleable and corrosion resistant metal; and titanium has a low elastic modulus, which improves flexibility and confers the characteristic of returning to its original shape after bending [1, 4, 5].However, In spite of the advanced flexibility of NiTi alloy compared with stainless steel, fracture of NiTi endodontic instruments remains a problem in clinical practice.

Seeking to increase the resistance to fracture, the Niti alloy has been subjected to different thermal treatments, leading to the formation of a very thin layer of titanium oxide (TiO_2), formed by a reaction between oxygen of the atmosphere and titanium of the alloy surface: as a result of an oxidation process [6], making it permeable to oxygen ,thus conferring more resistance to corrosion; and producing a better arrangement of the crystal structure, thereby leading to increased flexibility, an improved fatigue resistance or plastic behaviour [7,8].

Instruments coated with an oxide layer are 300% to 800% more resistant to fracture at room temperature compared to conventional super elastic NiTi. Fragmentation and micro cracks in the surface oxide layer can be a contributing factor to this greater resistance to fracture, since part of the instrument's life is affected by the deterioration of this layer, however, nucleation and slow propagation cracking occurs only later in the NiTi substrate [9,10].

Pedulla, et al. demonstrated by X-ray energy dispersion spectrometry (EDS) technique how HyFlex CM instruments after immersion in NaOCl and their respective sterilization , a greater amount of oxygen and a Less nickel on its surface thus confirming the existence of an oxide layer in shape memory instruments, which increases corrosion resistance [11].

On the other hand, Pereira et. al, reported that after clinical use a decrease in the titanium oxide layer is observed, and in turn the possibility of a galvanic interaction between the surfaces covered with dentinal slit and the exposed surface of the instrument generating more severe corrosion processes that lead to the fracture [10].

In view of the foregoing, the main objective of this study was to assess of the effect of repeated biomechanical preparations and sterilization cycles on the composition and behaviour of the titanium oxide layer of the HyFlex CM and EDM instruments.

MATERIALS AND METHODS

A total of 18 human third molars extracted and selected according to the following inclusion criteria which were evaluated clinically and under a periapical radiography : no signs of tooth decay, fractures or cracks; Permeability of root canals; absence of internal or external resorption , fully close apices and root canals with severe curvatures of 30-40 ° [12]. Teeth were placed in chloramine T for 48 hours and then in distilled water until the cameral opening were performed.

The cameral opening of each tooth was carried out with a round carbide and endo-zeta burs; the working length was determined with periapical radiographs and manual stainless steel files 06, 08 and 10 to keep the duct permeable and subsequently the teeth were mounted in an acrylic cube. The HyFlex CM and EDM instruments were used according to the manufacturer's recommendations [13]. Briefly, for the preparation of root canals, all files were activated with a VDW Silver Reciproc engine set at 500 rpm and 2.5 Ncm, and under a slight apical pressure and brushing movement. The following sequences were defined:

Hyflex CM: 25/08, in two thirds of the working length, 04/20 to the working length; and size 25/04 to work length.

HyFlex EDM: 25 / .12 size two thirds of the working length; size 10/05 to work length, and size 25/~.

Endodontic Irrigation was carried out with 1 ml 5.25% NaOCl between each instrument, followed by an sterilization cycle as described by Pirani et al [14], files were left in a container with enzymatic soap for 15 minutes, which was prepared in 25 gr in 5 litters of water, before a mechanical washing , finally the files were passed through the Digital Ultrasonic Cleaner Blazer (Sonic GT) for 15 minutes and then autoclaved at 134 ° C, 30 pounds for 1 hour [14].

4 sets of instruments were used and asigned into four groups, as follows: Hyflex CM; Group 1: Instruments 3,5 and 6; Group 2: 4, 7 and 8; Group 3: 1, 2 and 11; Group 4: 9, 10 and 12. HyFlex EDM: Group 1: Instruments 1,2 and 9; Group 2: 3, 4 and 10; Group 3: 5, 6 and 11; Group 4: 7, 8 and 12; . Mesial root canal were prepared with a deferent system (HyFlex CM HyFlex EDM).

During the second preparation an EDM 25/~ instrument was fractured, therefore a CM 25/04 was also ruled out. The chemical composition of the file´s surface was identified by field - emission scanning electron microscope (TESCAN LYRA3, EDX). All the endodontic files were evaluated before use and after every cycle of miomechanical preparation and sterilization (a total of 4EDX analysis). Each instrument was analyzed at 3 - 6 mm length under the microscope, taking into account the following parameters: EDX spectrum, 20X magnification, 10.00 mm working distance , the acceleration voltage was set at 10Kw and the speed electron beam 5 m/sg.

STATISTIC ANALYSIS

A descriptive statistical analysis of variance and comparison graphs with Excel and SPSS 20 software respectively were performed. The Kolmogorov-Smirnov test was applied to determine the standard normal distribution of the evaluated variables(weight percentage (Wt%) of Nickel, Titanium, Oxygen and Carbon), which are the elements that represent more than 98% Wt% on the surface of both types of instruments, finding that the KS test statistic ($p > 0.05$) accepts the normality hypothesis. The remaining 2% of the Wt% of the composition is given by silicon, phosphorus, calcium related dentin residues; and chlorine and sodium related to contact with sodium hypochlorite during the biomechanical preparation process [15].

Means and standard deviation of WT before and after of each chemical element for the two types of instruments were expressed in the different preparations and lengths. Comparisons of both instruments were performed with the Tukey B mean comparison test.

RESULTS

From every analysis with the EDX technique of the HyFlex CM and EDM instruments (both at 3 mm and 6 mm in length) before use and after every biomechanical preparations and sterilization cycle , tables and graphs were obtained defining the approximate Wt% ; in which the surface composition of both types of instruments was evidenced. No significant differences between the Results obtained at different lengths were observed.

When new instruments were analysed before use, it was observed that CM files had a lower Wt of Ni 25.21%, compared with the Wt of titanium 35.41Wt%; while EDM files were composed by a 40.52% WT of Ni and the Wt of Ti was 39.03% which means that the chemical composition of EDM files tends to be more equiatomic on its surface. Also, higher percentage of oxygen is observed in CM 31.03Wt% compared to that of EDM 16.38Wt%.

Table 1: Weight percentage of Nickel, Titanium, Oxygen and Carbon for new Hyflex CM and EDM files at different lengths.

file/length	Ni	Ti	O	C
CM - 3mm	25,26	36,88	32,53	4,56
CM - 6mm	25,15	33,93	29,52	5,57
EDM - 3mm	39,30	38,16	16,43	5,42
EDM - 6mm	41,75	39,89	16,32	1,96

After the first biomechanical preparation and the respective sterilization protocol, it was observed that in the HyFlex CM the percentage of Nickel (13.91 Wt%) on its surface decreases significantly while the behaviour of the Ti is relatively stable (41.37Wt%) while oxygen (39.98Wt%) increases slightly. On the other hand, the behavior of the three elements (Ni: 39.49Wt%, Ti: 40.16Wt% and O: 16.52Wt %) on EDM files is more stable when compared with the first analysis.

Table 2: Weight percentage of Nickel, Titanium, Oxygen and Carbon for Hyflex CM and EDM files in the first preparation at the different lengths.

file/lengths	Ni	Ti	O	C
CM - 3mm	16,38	39,95	38,32	4,35
CM - 6mm	11,43	42,80	41,64	3,64
EDM - 3mm	40,11	40,49	16,57	2,58
EDM - 6mm	38,86	39,84	16,47	4,41

After the second preparation and the respective sterilization protocol, it is observed on the surface of the HyFlex CM that the Ni (14.91 Wt.%) is stabilized, the Ti (41.52Wt%) increases slightly and the O (37.27Wt %) decreases aslightly . In EDM, the Wt% of Ni (37.02 Wt%) and Ti (36.80Wt%) decrease and the O (19.08Wt%) increases slightly.

Table 3: Percentage of the weight of Nickel, Titanium, Oxygen and Carbon for Hyflex CM and EDM files in the second preparation at the different lengths

file/length	Ni	Ti	O	C
CM - 3mm	16,04	41,31	35,63	5,36
CM - 6mm	13,78	41,72	38,91	4,74
EDM- 3mm	35,80	36,18	19,20	5,32
EDM- 6mm	38,23	37,42	18,95	3,76

At the fourth analysis (after the third preparation), an increase in Ni (20.39Wt%) and Ti (48.21Wt%) is observed on the surface of the HyFlex CM, while O (28.89Wt%) decreases; likewise EDM files showed the same increase behaviour Ni:(36.96Wt%), Ti: (38.45Wt%) and O: 18.43Wt% more stable.

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Table 4: Percentage of the weight of Nickel, Titanium, Oxygen and Carbon for Hyflex CM and EDM files in the third preparation at the different lengths.

file/lengths	Ni	Ti	O	C
CM- 3mm	21,67	47,46	28,30	2,55
CM - 6mm	19,10	48,96	29,48	2,18
EDM - 3mm	39,46	39,21	17,04	3,69
EDM - 6mm	34,46	37,70	19,81	6,51

DISCUSSION

The risk of a torsional and flexural fracture during biomechanical preparation remains one of the concerns regarding NiTi endodontic instruments. For this reason, improvements in NiTi metallurgy, such as heat treatment (thermal processing) is one of the fundamental approaches to adjust the transition temperatures of NiTi alloys and thus optimize the microstructure of the alloys [16].

HyFlex instruments (HyFlex; Coltene Whaledent, Cuyahoga Falls, OH) are made from control memory wire (CM), composed by titanium (46%) and nickel (52%), (Zinelis et al 2010) [17]. A reduction in nickel percentage with regard to conventional Niti files gives HyFlex instruments greater flexibility and resistance to cyclic fatigue [17]. According to Thompson et. al (2013), the decrease in Nickel concentration generates a softer metal [18]. HyFlex instruments are subjected to a thermomechanical process which controls the memory of the alloy, making it extremely flexible but without the shape memory of conventional superelastic NiTi instruments since the austenite / martensite transition temperature changes to approximately 50 ° C. which generates an increase in the final austenitic temperature and the martensitic microstructure remains stable at body temperature [14,19], therefore the instrument would contain a significant proportion of martensitic alloy (Shen et al. 2011) [20]. Esta bibliografia cual es Instruments in the martensitic phase can be easily deformed and will regain their shape when heated beyond the transformation temperature [16, 21].

Currently, two types of HyFlex instruments (CM and EDM) are available in the market ,the HyFlex CM instruments, in addition to above described characteristic, own a surface without irregularities, while HyFlex EDM, have a rough and crater-like irregular surface, which is produced by a manufacturing process named electrical discharge machining, which consists of generating high-frequency electric discharges between an electrode and the workpiece within a dielectric fluid. Electric sparks cause local melting and partial evaporation of small portions of material that are removed from this local area and have a crater-like surface finish (Theisen & Schuermann 2004) [22].

Pirani et al. (2016) reported that the wire of HyFlex EDM files is made up of 55% nickel, 45% titanium and 10-20% oxygen [14]. Additionally, EDM files are composed of a longer R and martensite phases with peculiar structural properties, such as increasing phase transformation temperatures and greater hardness compared to HyFlex CM [19].

The analysis performed with EDX, evaluated the behaviour of the chemical elements on the surface of the HyFlex CM and EDM [23] which differs in percentage weight of the substrate material by which these instruments are made of [14].

EDX analysis of this study shows that CM files, at the first analysis (new instruments), are composed by lower percentages of nickel 25.21 Wt%, titanium 35.41Wt%, and oxygen 31.03 Wt%. While EDMs are composed of 40.52 Wt% nickel, 39.03 Wt% titanium, and 16.38 Wt% oxygen , what represents a more equiatomic composition , by which it would suggest that EDM files possess greater hardness with a low percentage of nickel compared with the percentage of titanium. Comparing the percentages of oxygen, almost twice as much is observed in the CM, which verifies the finding of Iijima, et al, regarding the composition and oxygen permeability of the titanium oxide layer [7].

In the analysis after the first and second preparation a very similar behavior was observed in the CM, which consists of a downward trend in nickel (13.91 Wt% and 14.91Wt%), the light titanium increased (41, 37Wt% and 40.16Wt%) and the same as oxygen (39.98 and 37.27Wt%) similar to that reported by Pedulla et al. (2018) in that after the HyFlex CM instruments are immersed in sodium hypochlorite and sterilized, they present more oxygen content and less nickel on their surface [11]; and from other studies (Mize et al. 1998, Alexandrou et al. 2006, Valois et al. 2008), which explain why instruments that underwent major sterilization cycles revealed changes in the chemical composition , as a result of higher amounts of titanium oxide on the surfaces of sterilized instruments [24,25,26]. On the other hand EDM files show a more stable behaviour of its components, showing no abrupt changes in any of the observed elements.

EDX analysis after the third preparation, reflect that CM files show a slight rise in nickel 20.39Wt% and

48.21Wt% of titanium and oxygen 28.89Wt% which represents a significant decrease since it is less than value obtained at first analysis, results that coincide with those reported by Pereira et. al, in that the titanium oxide layer is worn away by friction during root canal instrumentation, with more nickel in its content, taking into account that the thicker the layers formed, the greater the tendency to crack formation [10]. EDM instruments maintain this behaviour with a tendency to an equiatomic and stable composition (nickel 36.96Wt%, titanium 38.45% and oxygen 18.43Wt%), which identifies that with the use of the instruments the reaction between the oxygen of the Atmosphere and titanium of the alloy surface, as a result of an oxidation process when subjected to different temperature changes during heat treatment is different [5].

Results from this study are in agreement with those from Seago et al. (2015), in that HyFlex CM files can be reused, but after repeated clinical uses and sterilizations, there is a change in the austenite finishing temperature, generating a change in the proportions of austenite and martensite phases NiTi microstructural, which could affects the mechanical properties of these files [27]. On the other hand, Iacono et al. (2016) conclude that HyFlex EDM files after several uses do not modify their properties [18] and Fayyad, et. al., refers to the fact that a stable oxide layer on the surface of NiTi instruments benefits corrosion resistance through a "passivation phenomenon" and oxidation, thus increasing fracture resistance [8].

Bonaccorso et al (2008), mentions that the surface characteristics seems to be an important factor affecting the long-term corrosion resistance of NiTi files, because different surface compositions are observed within the machining marks and micro cracks in instruments Race electropoliched and not electropoliched. This suggests that the difference in the manufacture of the instruments can justify the difference in the behavior of the elements on the surface of the instruments [15].

CONCLUSIONS

HyFlex CM files have greater instability of the components on their surface, especially nickel, while in EDM, greater stability of their components on the surface is observed.

The titanium oxide layer is modified with the reuse of the instruments and can affect the hardness, flexibility and clinical resistance especially in the Hyflex CM.

The different phase composition, the improved hardness and the manufacturing process can affect the mechanical behavior of the instruments.

RECOMMENDATIONS

It is recommended for future research to verify the structure of the HyFlex CM and EDM instruments before and after use and thus be able to relate it to the behavior of the oxide layer and that of the cracks within the NiTi alloy substrate and how this It affects cyclic fatigue for these instruments.

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