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Abstract
Aim: The current study was conducted to evaluate the effect of autoclave cycles on surface characteristics of Nickel-Titanium Rotary Files by scanning electron microscopy (SEM).

Materials and Method: In this experimental study, 3 groups of rotary file systems
- Hyflex EDM
- Protaper Next
- One Shape

were used. The surface characteristics of the files were examined (without autoclave, 1 autoclave cycle, 3 autoclave cycles) by SEM under 1000X magnification. Data were analysed using the SPSS software and the multifactorial repeated measures ANOVA.

Result: New files had debris and pitting on their surfaces. When the autoclave cycles were increased, the mean of surface roughness also increased showing more surface roughness.

Conclusion: Sterilization by autoclave increased the surface roughness of the files but not significantly.

Introduction
Nickel-titanium instruments have become the mainstay of endodontics because of their super elasticity, excellent shape memory, wider elastic limits and superior resistance to bending and torsional failure compared to stainless steel instruments. These instruments are classified as "critical items" and require a strict sterilization protocol using autoclave, glass bead sterilizer, sodium hypochlorite, laser, chemical sterilization or dry heat sterilization when reused¹. Most endodontic instruments as supplied from the manufacturer are not sterile and have been found to have metallic spurs and debris on their surfaces². The manufacturing process produces milling marks and metal debris, and dentine fragments appear to adhere to deposits of carbon and sulphur resulting from the decomposition and oxidation of the lubricating oil used during machining³. There is uncertainty regarding the potential changes to the physical and mechanical properties of NiTi instruments that are subject to repeated sterilization cycles under autoclaves or dry heat sterilizers⁴. Some authors reported that heat sterilization increases number of rotations-to-breakage of NiTi instruments⁵,⁶, while others showed that had no consistent or significant effect⁷,⁸. Some researchers found that heat sterilization leads to reduction of cutting efficiency of the NiTi files⁹,¹⁰. The sterilization process and material fatigue due to the repeated usage are two important factors affecting files microstructure. The purpose of this study was to study the effect of autoclave on the surface characteristics of "as-received" and multipe autoclaved instruments using SEM.

II. Materials and Methodology
Patients aged 18-45 yrs were randomly selected having irreversible pulpitis or peri-apical pathology in maxillary I molar with maximum 30° curvature of canal. The 3 groups of rotary file systems
- Hyflex EDM
- Protaper Next
- One Shape

were divided in 2 sub-groups:
- i. Unsterilized Controlled group ("as-received" condition of file)
- ii. Sterilized Experimental group (files subject to 3 cycles of sterilization)

3 commercially available file systems of length 25mm were selected and the last file used was examined. 5 files (from the same lot of the batch) of size 25/06 taper from each of the 3 groups were taken. However, a control file for each group were not subjected to use or sterilization. The remaining 4 files were used to carry the
endodontic treatment in 3 cases each and were autoclaved after each case. SEM (Scanning Electron Microscopy) study of the CONTROL GROUP i.e. unsterilized files in the as-received condition will be done. The Remaining Groups of files will be used and autoclaved for first cycle(H1,P1,V1), and used for next case and sterilized for second cycle(H2,P2,V2), and used again for the next case and autoclaved for third cycle(H3,P3,V3). Each file group will be packaged for sterilization separately and each autoclave cycle will be performed for 15 mins at a temperature of 132°C and 15 lbs pressure (UNIQUE CLAVE C-79B). SEM of files will be done after cycle 1 and 3. Samples were allowed to cool to room temperature for at least 30 mins. between cycles.

III. Results

The surface roughness scores were summarized as Mean ± SD (standard deviation). The surface roughness scores were compared by two way (groups and cycles) analysis of variance (ANOVA) and the significance of mean difference within (inter) and between (intra) the groups was done by Tukey’s HSD (honestly significant difference) post hoc test after ascertaining normality by Shapiro-Wilk’s test and homogeneity of variance by Levene’s test. The outcome measure of the study was surface roughness scores assessed on SEM. The objective of the study was to compare the surface roughness score between groups (Hyflex Edm, One Shape And Protaper Next) And Cycles (Cycles 0, Cycles 1 And Cycles 3).

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of sterilization cycles</th>
<th>CYCLE 0</th>
<th>CYCLE 1</th>
<th>CYCLE 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>HYFLEX EDM</td>
<td>2.00 ± 0.71</td>
<td>2.40 ± 0.55</td>
<td>2.80 ± 0.84</td>
<td></td>
</tr>
<tr>
<td>ONE SHAPE</td>
<td>1.40 ± 0.55</td>
<td>1.60 ± 0.55</td>
<td>2.20 ± 0.45</td>
<td></td>
</tr>
<tr>
<td>PROTAPER NEXT</td>
<td>1.20 ± 0.45</td>
<td>1.60 ± 0.55</td>
<td>1.60 ± 0.55</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Surface roughness scores (Mean ± SD) of three groups and three cycles

![Surface roughness score](image)

Fig. 1. For each group, mean surface roughness scores between the cycles.

<table>
<thead>
<tr>
<th>Comparisons</th>
<th>Hyflex Edm</th>
<th>One Shape</th>
<th>Protaper Next</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYCLES 0 Vs. CYCLES 1</td>
<td>0.974</td>
<td>1.000</td>
<td>0.974</td>
</tr>
<tr>
<td>CYCLES 0 Vs. CYCLES 3</td>
<td>0.455</td>
<td>0.455</td>
<td>0.974</td>
</tr>
<tr>
<td>CYCLES 1 Vs. CYCLES 3</td>
<td>0.974</td>
<td>0.790</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Table 2: For each group, comparison (p value) of mean difference in surface roughness between the cycles by Tukey test
A comparative evaluation of the surface characteristics of three types of NiTi rotary files.

For each group, comparing the mean surface roughness scores between the cycles, Tukey test showed similar (p>0.05) surface roughness scores among the cycles in all groups i.e. did not differ significantly (Table 2 and Fig. 1). Similarly, for each cycle, comparing the mean surface roughness scores between the groups, Tukey test showed similar (p>0.05) surface roughness scores among the groups at all cycles i.e. also did not differ significantly (Table 3 and Fig. 3).

IV. Discussion

The present study evaluated the effect of multiple cycles of sterilization procedures, and root canal preparations on nickel-titanium rotary files from three different manufacturers, and determined whether the resistance to surface alterations was under the influence of these factors. Differences in physical properties or surface topography between endodontic instruments might be related to different metal alloys or their design features. Moreover, it has been observed that properties and performance of NiTi rotary instruments might be affected by sterilization procedures.

Several different techniques were used to analyse surface topographic proprieties and chemical composition by means of SEM, AFM and EDS. The SEM has been the gold standard to evaluate the surface characteristics of NiTi instruments because SEM produces a high-resolution two-dimensional image of the sample. Magnification is an indispensable aid in visualizing the features leading to failure process as well as gaining insight into the mechanism of failure which is not possible with the naked eye. Hence in the present study the files were viewed under the Scanning Electron Microscope at 1000X. SEM was used because it is the gold standard for correct topographic analysis of file surface.

The analysis of this study was first performed on as-received instruments. SEM showed surface roughness and irregularities caused by manufacturing process. Hyflex EDM micrographs of the new disclosed a nonuniform structure where pits, pores and voids caused the peculiar aspect of a rough-spark-machined crater-like irregular surface. These results confirmed the data from previous studies, which demonstrated the presence of surface irregularities, machining marks, metal flashes and irregular cutting edges as characteristic features of the manufacturing process in new NiTi instruments. Heat treatment (thermal processing) is one of the most fundamental reasons for the morphometric variations amongst instruments of the same batch and toward adjusting the transition temperatures of NiTi alloys and affecting the fatigue and torsional resistance of NiTi endodontic files.

Examination of all samples subjected to repeated sterilization cycle revealed an increase of surface alterations compared with those that were not sterilized. SEM analysis demonstrated minor irregular cutting edge in Hyflex EDM compared with ProTaper Next and One Shape. Surface changes observed after a repeated number of autoclave procedures probably might be due to a cumulative effect on structure and chemical composition of rotary NiTi file surfaces. In contrast Eggert et al supported that these surface irregularities are insignificant. Some techniques have been suggested to improve surface microhardness and corrosion resistance involving ion implantation, electropolishing etc. Protaper Next surfaces did not dramatically change after 3 autoclave cycles compared with as-received instruments, whilst others showed some increase in roughness in the cutting edge areas. All rotary instruments examined in this study presented debris on their surface.

The manufacturer of Hyflex EDM reports that the shape and strength of files with straightened spirals can be restored during autoclaving. This means that the file appears to regain its shape after sterilization and reuse. In particular, superelasticity and shape memory are strongly affected by heat treatment as part of the manufacturing processes. The M-Wire technology of ProTaper Next allows the NiTi instruments more flexibility and resistance to cyclic fatigue compared with non-M-Wire NiTi instruments. Differential scanning calorimetric analyses found that at 37°C conventional superelastic NiTi wire has the austenite structure, whereas M-Wire is a mixture of nearly equal amounts of R-phase and austenite.

The composition of the NiTi alloy, especially the nickel content, has a great influence on the transformation temperatures. It has been reported that the phase transformation temperature shifts 12°C toward a lower temperature when the nickel atom content of a Ni-rich Ni-Ti alloy increases by 0.1% . CM wire possesses a relatively high As and Af compared with regular SE wire. The CM wires had no SE at room temperature or at 37°C, whereas they exhibited SE when heated to 60°C. The CM wires exhibited different phase transformation behavior and mechanical properties compared with SE wires, attributing to the special heat treatment history of CM wires. ing temperature (Af) of austenite transformation of the raw CM wires were much higher than those of the SE wires, and the enthalpy changes during the heating and cooling processes for the CM wires were also larger than for SE wires. Thermomechanical treatment during manufacturing of endodontic instruments is believed to have a great influence on the thermal behavior of NiTi instruments.
Surface structure and alloy of NiTi instruments are fundamental characteristics when re-using endodontic instruments subjected to repeated cycles of autoclave sterilization. The surface roughness increased with an increase in the number of autoclave cycles but was not as significant. Files removed from the manufacturer’s packaging may have minimal or no bacterial contamination but sterility of the files cannot be guaranteed. The findings of this study showed that new files have debris and roughness before sterilization by autoclave particularly in Hyflex EDM. This was due to the method of machining process employed.

References