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Evaluation of Residual Debris and Smear layer After Root Canal Preparation by Three Different Methods: A Scanning Electron Microscopy Study

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Introduction: This study investigated the amount of debris and smear layer remaining followed chemomechanical preparation using three systems: ProTaper Universal, reciprocating SafeSider, and hand K-Flexofiles with scanning electron microscope (SEM). **Materials and Methods:** Sixty-five mandibular molars with mesiobuccal canal curvature (25 to 40°) were extracted and divided into one control group (n=5), and three experimental groups (n=20) according to the preparation method; K-Flexofile, ProTaper Universal and SafeSider instruments. All canals were irrigated with 3 ml of 5.25% sodium hypochlorite solution and 3 mL of 17% EDTA. Subsequently, the canals were irrigated with 5 ml of normal saline. Then the teeth were examined under the scanning electron microscope (SEM). Kruskal-Wallis, Dunn-Q Bonferroni, and Friedman tests were used for statistical analysis of results. **Results:** To assess the accumulation of debris, statistically significant differences were observed only in the coronal area among ProTaper Universal, SafeSider, K-Flexofile, and the control group. (P=0.029). To evaluate the residual smear layer amount, statistically significant differences were observed only in the coronal and middle areas, following the preparation of the canals using ProTaper Universal, SafeSider, and hand K-Flexofiles and control groups (P=0.019). **Conclusions:** Based on the present *in vitro* study, we can declare that the canals were utterly cleaned of debris and smear layer in none of the groups. Manual Flexofile and ProTaper Universal groups result in cleaner canal walls than reciprocal SafeSider, in the coronal and middle thirds.

Keywords: ProTaper; Root Canal Preparation; SafeSider; Smear Layer; Scanning Electron Microscopy

Introduction

The favorable outcome of root canal treatment depends on proper cleaning and shaping and optimal disinfection of the canals. During root canal cleaning and shaping, dentin mineral debris adheres to the canal walls and the pulp's organic component. The smear layer, alongside bacteria and their by-products, is composed and compressed into dentinal tubules, which have a thickness of about 1-5 microns [1]. Debris is often infectious and prevents microorganisms' removal from the root canal [2].

On the other hand, smear layer removal leads to a better adaptation of filling material with canal walls. It also helps establish a better seal along the canal and microleakage reduction. The smear layer prevents intracanal medicaments from penetrating the dentinal tubules. The smear layer and debris left in the canal can also contain microorganisms. Microorganisms infiltration into dentinal tubules can have adverse effects on the outcomes of endodontic treatments; thus, its elimination is necessary [3, 4].

To solve the problems of traditional method, rotary systems with nickel-titanium (NiTi) files were introduced to the market [5]. Rotary instruments have high elasticity and high resistance to cyclic fatigue. They are more prone to fracture than manual methods, however they have much less mistakes during root canal preparation. ProTaper Universal, a cross-section of a convex triangle with a variety of tapers, is one of these systems. In the shaping and finishing files, the taper, respectively, rises and decreases toward the coronal. This tapered design reduces file

fatigue, increases flexibility and simultaneously removes dentin [6].

Today, there are various methods for cleaning and shaping. According to several studies, the instruments with different cross-sections leave various amounts of debris and smear layers in the canal [7]. These results are contradictory in the studies concerning the cleaning ability of hand instruments and rotary systems [8]. According to a study by Burklein *et al.*, the amount of residual smear layer with a ProTaper file (triangular cross-section, three cutting edges and small flutes) was slightly higher than the Reciproc instrument (S-shape cross-section with a sharp cutting edge) [9].

We used SafeSider and K-Flexofile instruments. SafeSider system consists of a series of files with non-circular cross-sections with a flat lateral surface that believed their flat side surface facilitates debris removal. With 16 flutes, these files have reduced resistance and engagement with root canal walls, which lowers the risk of file breakage (14). K-Flexofiles are triangular-sectioned flexible hand devices made of stainless steel. Because there is no cutting edge on the files in this system, ledge development is less likely. A hand tool preparation, however, results in a longer treatment session and an irregular root canal design (15-17) .Since the elimination of residual debris and smear layer is essential and challenging, it is worth minimizing the creation of smear layer by proper rotary systems. Thus, this study aimed to compare the amount of debris and smear layer left on dental canal walls following chemo-mechanical preparation using three systems: ProTaper Universal, Reciprocating SafeSider, and hand K-Flexofile with scanning electron microscopic (SEM) assistance.

Materials and Methods

After approval by Ethics & Research Committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.RIDS.REC.1395.277), 65 mandibular first and second mandibular molar teeth (the same number of each tooth in each group), with 25 to 40° curvature of mesiobuccal canals according to the Schneider method [10], were selected from about 1000 human adult teeth extracted for periodontal or prosthetic reasons. Teeth with previous endodontic treatment, immature roots, external root resorption, calcified canals, and caries extended to the root were excluded. The teeth were swamped in a 5.25% sodium hypochlorite solution (Morvabon, Iran) for 24 h and then transferred to a normal saline solution and used within one week. Access cavity was prepared with diamond bur (Brasseler USA, Savannah, GA, USA), and a size 08 K-file (Dentsply Maillefer, Ballaignes, Switzerland) was used passively to confirm root canal anatomy. The inclusion criteria were separate patent canals confirmed by radiographs from mesial and distal sides [11, 12]. Then, the teeth were randomly numbered (according to the random numbering table) and divided into equal numbers in study groups. The teeth were decoronated at the cementoenamel junction (CEJ), and the study included mesial root canals with 15 mm length [13]. Patency was established using #10 K-file (Dentsply Maillefer), and working length was determined with K-file #15 (Dentsply Maillefer) up to the apical foramen. A plastic tube was fixed on the coronal part of specimens as an irrigant reservoir.

The canal preparation methods in all three groups were according to the manufacturer's recommendation, and the master apical file was considered #25 in all systems.

In group one, the mesiobuccal canals were instrumented by hand K-Flexofiles (Dentsply Maillefer, Ballaigues, Switzerland) and the passive step-back method [14]. In the second group, the canals were prepared using ProTaper Universal system (Dentsply Maillefer, Ballaigues, Switzerland) and rotary electromotor (NSK, Nakanishi Inc., Tokyo, Japan) up to the F2 file. In this group, first, the length of the canal was measured with file #15 (Dentsply Maillefer, Ballaigues, Switzerland), and then the S series of files were used to shape the coronal and middle parts, and the F series to finish the apical third [(S1-Sx) - (S1-S2-F1-F2)] [15]. Finally, in the third group, the canals were prepared with the SafeSider system (Essential Dental Systems, South Hackensack, NJ, USA) and with reciprocating movement by Endo-Express handpiece (Essential Dental Systems) according to the manufacturer's recommendation. In this order, after preparing the access cavity and checking the canal path with hand file number 08 (Dentsply Maillefer, Ballaigues, Switzerland), canals were prepared up to #25 stainless-steel file (2%) by reciprocal movement (30 degrees clockwise and 60 degrees counter clockwise) using the handpiece. A peeso provided by the factory (Pleezer) was used for coronal enlargement. Apical preparation was done with NiTi file #25 (6%), and then #25 (8%) was used 2 mm shorter than the working length [13].

According to the recommendation of da Silva Beraldo *et al.* [16], the type of irrigant and the syringe were considered the same for all groups. The root canals were rinsed with 3 mL of 5.25% NaOCl solution (Morvabon, Iran) and 3 mL of 17% ethylenediaminetetraacetic acid (EDTA) (Morvabon, Iran), using a 30-gauge syringe passively, between two files each. Finally, the canals were irrigated with 5 ml of normal saline after the last file [17]. After drying with a paper point, a cotton pellet was placed on the orifice of the canals. The control group consisted of five teeth

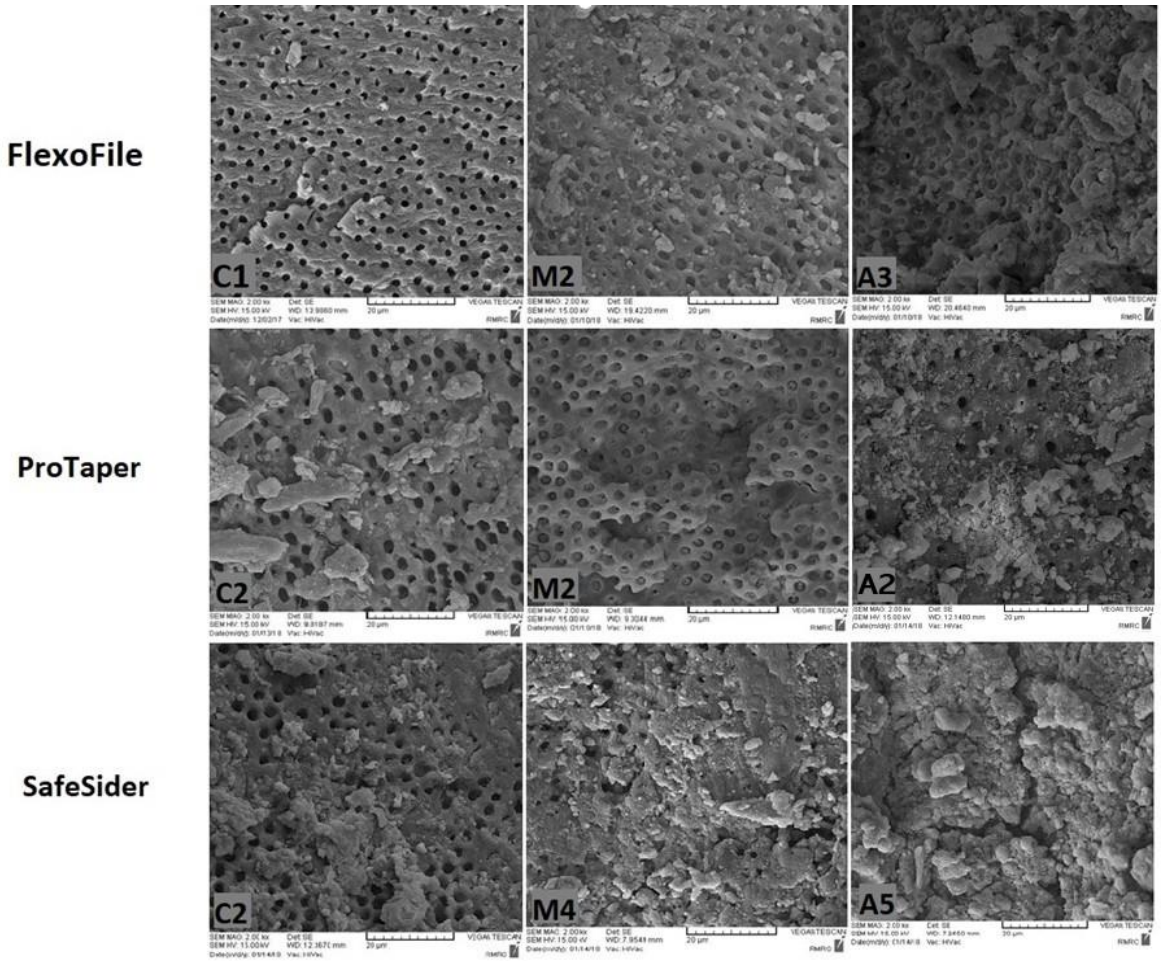

Figure 1. SEM photomicrographs of smear layer (2000×) of different experimental groups at coronal (C), middle (M), apical (A) thirds. Score 1 to 5

with no preparation. Root apices of all teeth were sealed and isolated by a small piece of carbowax (DOW Chemical Co, Midland, MI, USA) to create a closed irrigation system.

After preparing the samples, a shallow guide groove was created on the mesiobuccal roots from the mesial and distal side by a handpiece (NSK, Mio, Japan) and diamond disk (Jota, Ruthi, Switzerland) with water spray and the aid of an operating microscope (Carl Zeiss Meditec AG, Oberkochen, Germany). These grooves were made very carefully so that the depth of these grooves did not reach the canals. Using a chisel and mallet in the longitudinal axis of the roots, the roots were then split into buccal and lingual halves. Broken teeth were removed and fresh specimens were used in their stead. Then, the specimens were first dried and vacuumed in high vacuum operation mode, then covered with a gold sheet (30-nm) and observed under SEM (VEGA\\TESCAN-LMU, Czech Republic) with secondary electron emission mode, and 15 kV accelerated voltage) [18]. Images were taken randomly from the coronal, middle, and apical thirds (three images from each section with 200× magnification (for debris evaluation) and six images with 2000× magnification (for the smear layer evaluation).

SEM operator was blinded to the type of instrumentation systems, and random selection of imaging sites was considered adequate by the operator's performance monitoring. Separate blindness calibration was done for two trained observers (endodontists) using specified photographs and five score index for debris and smear layer according to the Hulsmann method [19] with the following criteria:

For the remaining debris:

1. The walls are clean, with no debris.
2. Small accumulations of debris are observed in canal walls.
3. Less than 50% of the canal walls have debris.
4. More than 50% of canal walls contain debris.
5. The walls are entirely or almost covered with debris.

For the smear layer:

1. There is no smear layer, and the tubules are open.

2. Some tubules are open.
3. There is a uniform smear layer, and the dentin tubules are not open.
4. The walls are entirely covered with a smear layer, and there is no open tubules.
5. Observation of thick and non-uniform smear layer.

SPSS software was used to examine the data (SPSS version 20; SPSS, Chicago, LL, USA). For intergroup comparison, the Kruskal-Wallis test was used. We used the Dunn-Q Bonferroni test for paired comparisons. After preparation, the Friedman test was used to analyze the debris and smear layer values in the coronal, middle, and apical regions for each group. The agreement amongst endodontists in assessing debris and smear layer was evaluated using the Kappa agreement coefficient analysis. The type one error in this study was considered 0.05, so *P*-value<0.05 was considered statistically significant.

Results

The kappa values for inter-observer agreement for evaluating debris and smear layer were 0.84 and 0.94, respectively.

We observed different amounts of debris and smear layer in all three parts of the prepared canals. Figures 1 to 3 show SEM micrographs of different groups. To assess the residual debris, statistically significant differences were observed only in the coronal area of the prepared canals among ProTaper Universal, SafeSider, K-Flexofile and the control group (Kruskal-Wallis test, *P*=0.029) (Table 1). The ProTaper system remained less debris than the control group (*P*=0.028). There was no statistically significant difference in remaining debris between the three experimental groups in coronal, middle, and apical areas (Kruskal-Wallis test, *P*=0.32, *P*=0.94, *P*=0.38).

Only the coronal and middle regions of the root canals between the experimental groups and the control group showed statistically significant variations in the residual smear layer score (Kruskal-Wallis test, coronal *P*=0.019, middle *P*=0.021) (Table 2).In the coronal part, ProTaper and K-Flexofile had less residual smear layer than the control group (*P*=0.042 and *P*=0.015). In the middle segment, ProTaper and K-Flexofile had less residual smear layer than the control group (*P*=0.036 and *P*=0.013). There was no statistically significant difference in remaining smear layer between the three experimental groups in coronal, middle, and apical areas (Kruskal-Wallis test, *P*=0.31, *P*=0.57, *P*=0.28, respectively).

Friedman test showed a significant difference in remaining smear layer between three zones of root canals after preparation with K-Flexofiles (*P*=0.041). Apical regions had a residual smear layer that was more obvious than coronal or intermediate regions. Following K-Flexofile preparation alone, there was a statistically significant difference in residual debris across various root canal thirds (*P*=0.034). As a result, there is much more debris in the apical third than in the coronal.

Table 1. Frequency distribution of remaining debris in different groups of files based on Hulsmann scoring system and 200× magnification

Variable	Score	ProTaper	SafeSider	FlexoFile	Control	*P*-value
Coronal Debris	1	2(%10)	0	1(%5)		0.029
	2	3(%15)	2(%10)	7(%35)		
	3	7 (%35)	4 (%20)	3(%15)		
	4	5(%25)	11 (%55)	3(%15)	1(%20)	
	5	3(%15)	3(%15)	6(%30)	4(%80)	
Middle Debris	1	3(%15)	0	0		0.23
	2	2(%10)	2(%10)	5(%25)		
	3	3(%15)	5(%25)	5(%25)		
	4	7(%35)	10(%50)	4(%20)	2(%40)	
	5	5(%25)	3(%15)	6(%30)	3(%60)	
Apical Debris	1	2(%10)	0	0		0.36
	2	2(%10)	1(%5)	2(%10)		
	3	3(%15)	4(%20)	1(%5)		
	4	6(%30)	7(%35)	7(%35)	2(%40)	
	5	7(%35)	8(%40)	10(%50)	3(%60)	
P-value		0.38	0.14	0.34		

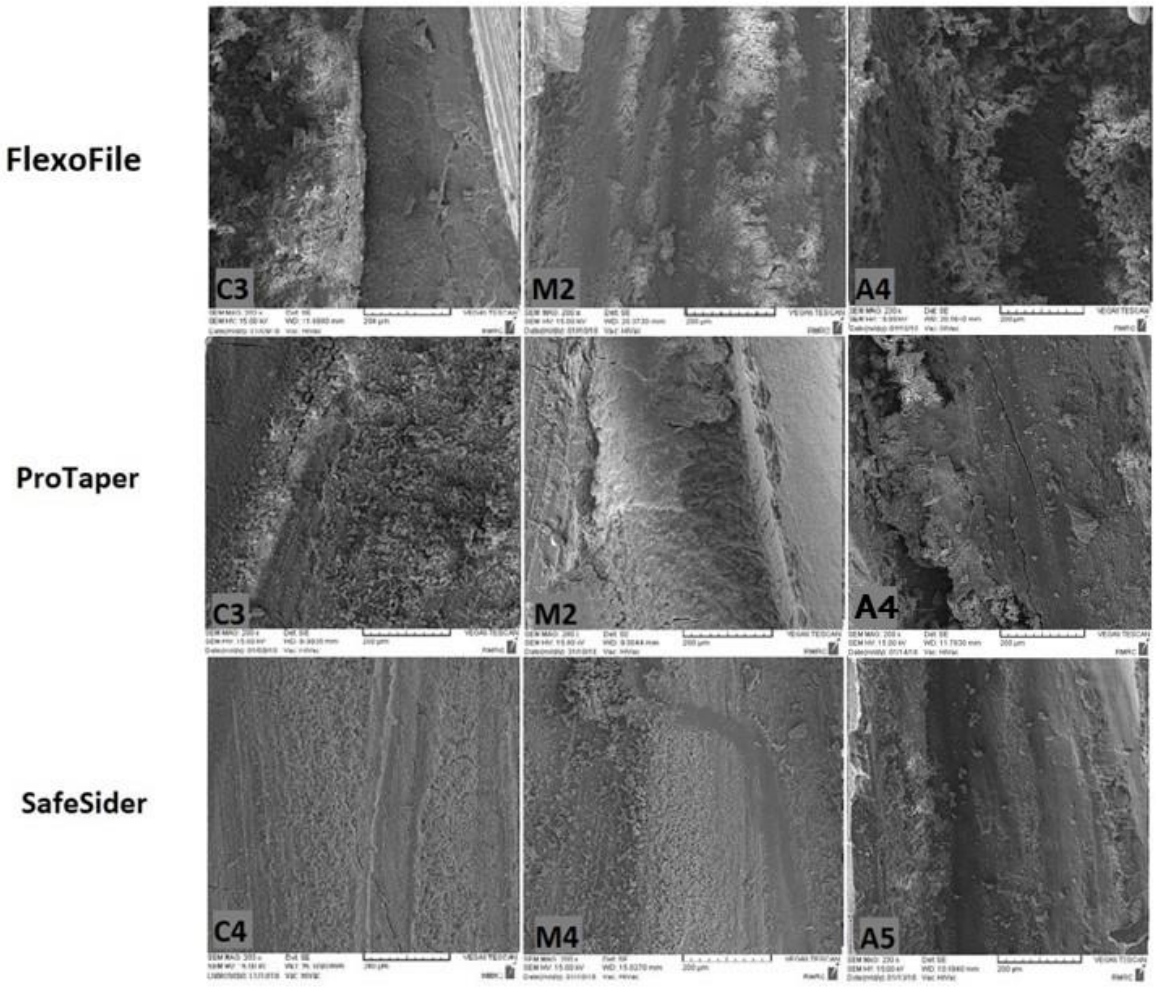

Figure 2. SEM photomicrographs of debris (x200) of different experimental groups at coronal (C), middle (M), apical (A) thirds. Score 1 to 5

Discussion

There are various techniques for observing and evaluating of dentinal tubules and remaining smear layer after canal preparation. One of these methods is scanning electron microscopy (SEM). The challenge of using different magnifications of SEM has been expressed in previous studies, showing that it may affect the results of scoring systems. Conventional SEM studies on the remaining smear layer have severe disadvantages and undergo a high risk of bias. While using this method, it is impossible to examine the same root canal's area before and after intervention. Instruments leave a substantial section of the root canal wall undisturbed [27], and no smear layer forms on these regions.

Therefore, it is hard to prove beyond a reasonable doubt that the smear layer was at a specific location and was eliminated by a specific action since the preceding state is unknown. Besides, only a very small part of root canal is evaluated; this area may not represent the complete root canal. The SEM operator bias towards selecting relatively cleaner regions was documented [20]. Further problems may arise during the drying and coating of the specimens, which can introduce several artifacts. Previous studies have used a range of 45-2500 times magnification [21, 22]. While analyzing the smear layer and dentinal tubules needs higher magnifications, debris is readily defined at low magnifications. On the other hand, the higher the magnification, the smaller the area will inevitably be examined [23]. We used a magnification of 200-2000 to evaluate debris and smear layer. The limitations of SEM were extensively discussed in the literature[24] and cannot be solved simply by calculating the number of open tubules using the software.

There are several systems for scoring the remaining debris and smear layer. The Hulsman five-point numerical scoring system, a reliable system, was used in this study [25].

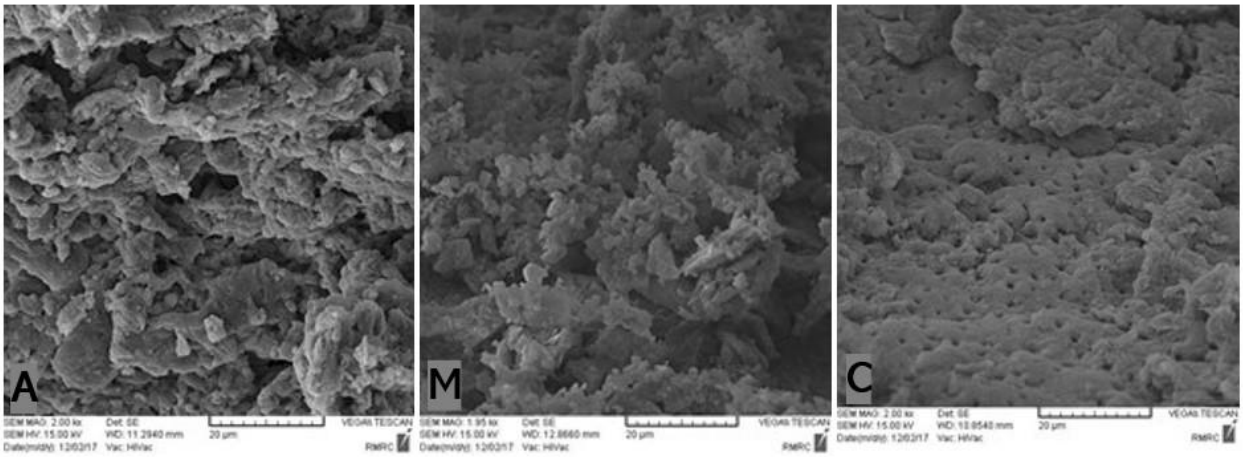

Figure 3. SEM photomicrographs of control groups at coronal (C), middle (M), and apical (A) thirds

The quality of preparation and cleaning of canal walls contributes to the success of root canal treatment [26]. The efficiency of canal cleaning was evaluated based on residual debris and smear. The debris consists of dentin chips and vital or necrotic pulpal tissue attached to the canal walls (4). The smear layer, which is the product of the canal shaping process, has a crystalline structure composed of inorganic and organic residues, microorganisms and their by-products. It has a 1-2 microns thickness and non-uniformly covers the canal walls [27]. Although there are conflicting regards on the removal or preservation of this layer, the studies show that it is an infectious layer that can protect microorganisms against canal disinfectant. The combination of these factors jeopardizes the establishment of a complete seal, as the smear layer prevents the sealer from penetrating the dentinal tubules that eventually leads to microleakage [28].

In addition to using different irrigants to remove the smear layer and debris, it is also essential to pay attention to the instruments used. For example, the use of a sharp instrument has a significant effect on the production of the smear layer. The type of canal preparation also affects the amount of this layer. Due to longer contact time with the canal walls, rotary instruments produce more smear layer than standard manual methods [29].

In this study, we tried to perform standardization among all three groups. First, the specimens were decoronated, and canals with 15 mm working length entered the study. All canals had a curvature of 25-40 degrees. In all three groups, we had equalized the irrigation method, in which it was performed entirely passively with a 30-gauge syringe, allowing better penetration of the needle into the greater curvature of the canal [30].

Table 2. Frequency distribution of remaining smear layer in different groups of files based on the Hulsmann scoring system and 2000× magnification

Variable	Score	ProTaper	SafeSider	FlexoFile	Control	*P*-value
Coronal Smear	1	3(%15)	0	2(%10)		0.019
	2	6(%30)	6(%30)	9(%45)		
	3	5(%25)	6(%30)	4(%20)		
	4	1(%5)	4(%20)	2(%10)		
	5	5(%25)	4(%20)	3(%15)	5(%100)	
Middle Smear	1	3(%15)	2(%10)	4(%20)		0.021
	2	4(%20)	4(%20)	6(%30)		
	3	7(%35)	6(%30)	4(%20)		
	4	3(%15)	5(%25)	3(%15)		
	5	3(%15)	3(%15)	3(%15)	5(%100)	
Apical Smear	1	1(%5)	0	1(%5)		0.095
	2	5(%25)	5(%25)	4(%20)		
	3	4(%20)	4(%20)	2(%10)		
	4	6(%30)	2(%10)	3(%15)		
	5	4(%20)	9(%45)	10(%50)	5(%100)	
P-value		0.27	0.075	0.041		

None of the three systems used were able to leave canals free of debris and smear layer. Previous similar studies have presented the same result, and so far, none of the filing systems has resulted in thoroughly cleaned canals [31]. It could be stated that there were no significant differences in residual debris and smear layer in the coronal, middle, and apical areas of the canal between hand K-Flexofile, ProTaper Universal rotary, and SafeSider reciprocal systems. However, in the coronal and middle areas, the hand K-Flexofile and ProTaper Universal remained less smear and debris than SafeSider. While in the apical part, ProTaper Universal was superior.

Shetty *et al.* [32] reported less residual smear layer in single-canal teeth prepared by rotary ProTaper than hand ProTaper and K-File. However, K-Flexofiles showed a better cleaning ability in the middle and coronal thirds than the ProTaper rotary system, which could be related to the differences in diameter and curvature of canals. In a review, Elnagar *et al.* [33] reported NiTi K-file and the Revo-S rotary system had significantly overall better performance, despite the lack of complete cleaning in all areas. Our study contradicted this result, which may be attributed to the difference among file systems, asymmetrical cross-sections, and snake-like movement in the Revo-S system.

Intragroup analysis revealed that Flexofiles had better clearance in coronal and middle parts than the apical third. However, the difference between residual smear and debris after preparation with ProTaper Universal rotary and SafeSider reciprocal systems was insignificant.

Elnagar *et al.* [33] reported better cleaning in the coronal and middle areas for the manual system (Ni-Ti K-flex file). They stated that there were no discernible differences among the three zones of the rotating system (Revo-S). This discovery matched the outcome of our research. In the intragroup comparison of three file systems (ProTaper Universal, K3, and Mtwo), which was consistent with the current investigation, Raut *et al.* [34] revealed no significant variation in various parts of the canal. Hoppe *et al.* reported no significant difference in the amount of debris and residual smear layer amongst one thirds of the canals in the ProTaper and reciprocating WaveOne file systems [35].

Considering the results of previous studies are consistent with the present study, it can be concluded that manual systems have better cleaning performance in coronal and middle areas. These findings may be attributed to the better accessibility to the coronal parts of the canal and the clinician's force to clean the canal. As the canal is wider in the coronal and middle areas, it is easier to apply force for cleaning, while in motor systems, uniform force is applied along the entire length of the canal.

It is impossible to say that one system is completely better than another without taking into account all the various variables, such as file type, movement type, preparation system, amount and type of irrigant, root canal length, and apical diameter that influence the amount of removal or residual debris and smear layer [9]. So, it seems that more studies should be designed considering more variables.

One of the study's limitations is the apparent differences between laboratory and clinical conditions and eliminating some of the problems in the clinic, such as calcification.

Conclusion

The results of present *in vitro* study, which was designed to compare the residual debris and smear layer following preparation with Flexofile, ProTaper Universal rotary, and SafeSider reciprocal systems, showed that in none of the groups, the canals were utterly cleaned of debris and smear layer. However, Flexofile and ProTaper Universal groups outperformed reciprocal SafeSider in the coronal and middle thirds. The ProTaper Universal system performed superior than the other two in the apical area. This distinction, nevertheless, was not statistically significant. Overall, every group outperformed the favorable control group.

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Conflict of Interest: 'None declared'.

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