

Effect of different separated nickel–titanium rotary instruments on apical microleakage: An *in vitro* study

Roohollah Sharifi, Ehsan Heidarzadi, Ehsan Azizi¹, Abbasali Khademi², Saber Khazaei, Pedram Iranmanesh²

Department of Endodontics, School of Dentistry, Kermanshah University of Medical Sciences, ¹Department of Microbiology, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, ²Department of Endodontics, Dental Research Center, Dental Research Institute, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran

Abstract

Introduction: Endodontic instrument separation is one of the iatrogenic errors which may affect the outcome of treatment. The present *in vitro* study was aimed to investigate the effect of different cross-sectional designs of separated nickel–titanium (Ni-Ti) rotary files on apical microleakage.

Materials and Methods: A total of 48 mandibular premolar roots were selected. The samples were randomly assigned to four experimental groups including Mtwo ($n = 10$), HERO Shaper ($n = 10$), RaCe ($n = 10$), and FlexMaster ($n = 10$) as well as positive ($n = 4$) and negative ($n = 4$) control groups. Ni-Ti rotary instruments with the same size and taper (0.06, #30) were separated in the apical region after complete cleaning and shaping, and the remaining root canal was obturated with gutta-percha and AH-26 sealer. The bacterial microleakage of each group was assessed through *Enterococcus faecalis* for 60 days. Survival analysis was done by SPSS 22.

Results: There were no statistically significant differences between the studied groups in terms of the time of microleakage ($P = 0.955$).

Conclusion: The separated Ni-Ti rotary instruments with different cross-sectional designs had no impact on apical microleakage.

Keywords: Dental instruments, dental leakage, *Enterococcus faecalis*, nickel–titanium, root canal therapy

Address for correspondence: Dr. Pedram Iranmanesh, Department of Endodontics, Dental Research Center, Dental Research Institute, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran.

E-mail: pedram.iranmanesh@yahoo.com

Submission: 26-02-21 **Acceptance:** 03-06-21 **Web Publication:** 08-01-22

INTRODUCTION

Endodontic instrument separation is one of the iatrogenic errors which may affect the treatment outcome through impairing the cleaning and shaping of the root canal.^[1] The frequency of instrument separation ranges from 1.83% to 8.2%.^[2] Insufficient access cavity preparation, inadequate knowledge of clinician, industrial defects, and physical

properties of the instrument may cause torsional failure or cyclic fatigue which leads to instrument separation.^[3,4] Besides, the influence of the cross-sectional design in enhancing the cyclic fatigue resistance on nickel–titanium (Ni-Ti) rotary instruments has been investigated previously.^[5]

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How to cite this article: Sharifi R, Heidarzadi E, Azizi E, Khademi A, Khazaei S, Iranmanesh P. Effect of different separated nickel–titanium rotary instruments on apical microleakage: An *in vitro* study. Saudi Endod J 2022;12:61-6.

Access this article online

Quick Response Code:



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DOI:

10.4103/sej.sej_42_21

Several techniques and methods have been evaluated when clinicians encounter a file separation. Bypassing is a simple, effective, and safe treatment path since it avoids the removal of an excessive quantity of root dentin.^[6] The other methods for removing the separated file include retrieval kits, ultrasonic, and recent minimally invasive gentle wave system.^[7-9] Dissolution of the separated segment has also been proposed in experimental studies.^[10] Clinicians might consider surgical management for this mishap.^[11] However, in some cases, the separated segment cannot be retrieved, and the patient should be monitored.

Several Ni-Ti rotary files with various designs are available in the market. For instance, RaCe file has a triangular cross-section, Mtwo has an S-form with two cutting edges, FlexMaster has a triangular with convex sides, and HERO Shaper file has a triangular cross-section with a positive rake angle.^[12,13] It is assumed that, besides root canal morphology, rotation speed of files, cycles of autoclave, frequency of usage, and the cross-sectional design of files may influence the separation rate.^[12,13]

The effect of separated instrument on microleakage has been evaluated in several studies; however, the consistent outcome has not been reported. The obturation techniques, microleakage assessment, and the designs of Ni-Ti rotary instrument attributed to the debate.^[12,14,15] Owing to different cross-section designs of Ni-Ti rotary instruments, which may impact the microleakage rate, the aim of the present study was to investigate the association between different cross-sectional designs of Ni-Ti rotary file and apical microleakage.

MATERIALS AND METHODS

The present study was approved by the regional bioethics committee affiliated to Kermanshah University of Medical Sciences, Iran #94261.

This experimental study was conducted on 48 human mandibular premolars which were extracted due to periodontal disease and collected from the dental clinics of Kermanshah city, Iran. According to the 95% confidence interval and the Type I error ($\alpha = 0.05$), the $Z = 1.96$. Considering $1 - P = 0.5$ and the value of $d = 0.3$, at least ten samples were obtained in each interval group. The characteristics of the selected samples included healthy teeth without root canal treatment and caries lower than cemento-enamel junction (CEJ), with mild-to-moderate root curvature ($<20^\circ$), no canal calcification and root resorption, and teeth with no root fracture or crack. A periodontal curette was used to remove the debris from

the tooth surface before the procedure. The crowns of the samples were cut perpendicular to the longitudinal axis by a fissure diamond bur (Tizkavan, Tehran, Iran) so that the roots with the same length (12 mm) were obtained.

A #15 K-file (MANI, Tochigi, Japan) was used for navigation. The working length was considered 1 mm shorter than the anatomic apex. The coronal part of all canals was flared by #1, #2, and #3 Gates Glidden burs (Dentsply/Maillefer, Ballaigues, Switzerland), respectively. The apical region of all canals was prepared up to #30 K-file (MANI, Tochigi, Japan) using the step-back technique. For irrigation and smear layer removal, we used 2 mL 2.5% sodium hypochlorite (Cerkamed, Stalowa Wola, Poland), 2 mL 17% ethylenediaminetetraacetic acid (Vericom CO. LTD, Gyeonggi-do, South Korea), and normal saline (Daropakhsh, Tehran, Iran) using a 27G syringe (Vista Dental Products, Racine, WI).^[16] The roots were randomly assigned to four experimental groups ($n = 40$), one positive control group ($n = 4$), and one negative control group ($n = 4$). After preparation of canals, the rotary files Mtwo (VDW, Munich, Germany), HERO Shaper (Micro-Mega, Besancon, France), RaCe (FKG Dentaire, La-Chaux-de-Fonds, Switzerland), and FlexMaster (VDW, Munich, Germany), with the same size and taper (0.06, #30 and 300 rpm), were inserted using an electric motor (VDW. SILVER, Munich, Germany) until separation occurred at apical one-third and 0–1 mm from the anatomic apex, which was confirmed by radiographic images. All experimental groups were obturated with gutta-percha cone (Dentsply Tulsa Dental Products, Tulsa, OK) and AH-26 sealer (Dentsply-Detrey, Konstanz, Germany) using lateral compaction technique. The outer surface of the samples was coated with a layer of nail polish up to 2 mm from the apex to prevent the penetration of bacteria. The control groups underwent the same preparation procedure, in which the negative control group was filled with adhesive wax and the positive control group remained unfilled after root canal preparation.

The double-room technique was used for bacterial microleakage assessment. The 3 mm micropipettes were cut 1.5 mm from their ends, and the samples were placed in the end side of cap. The space between the tooth and micropipette was sealed with adhesive wax. The micropipettes with samples were exposed to 12 h ethylene oxide for sterilization. Then, 10 mL of tryptic soy broth (TSB) (Merch, Darmstadt, Germany) was added to the glass test tubes. All test tubes containing TSB were sterilized in autoclave and incubated at 37°C to ensure sterilization. Apparent lack of turbidity in media was indicative of proper sterilization. The microtubes

containing the samples were placed in test tubes under a sterile hood and adjacent to the flame so that the root apex of the sample was in the culture medium. This set was isolated with Parafilm (Supa Co., Tehran, Iran) and was placed in an incubator at 37°C for 3 days. Lack of turbidity was also checked to confirm sterilization.

Using a sterile micropipette, 0.1 ml *Enterococcus faecalis* (ATCC.29212) (each milliliter containing 1.5×10^8 bacteria) was added to the test tubes in contact to the coronal part of samples every 5 days for 60 days. Then, Parafilm for sealing and 50 mL of the test tube was used after each addition of bacteria. The samples were incubated at 37°C, and culture media at lower part of chamber were checked for turbidity every day. Any observed turbidity was considered microbial contamination. The turbid media were cultured in the bile esculin agar to confirm the presence of *Enterococcus faecalis*.^[17]

Kaplan–Meier survival analysis was conducted by SPSS-22 (IBM Corp, Armonk, NY, USA) using the log-rank test to measure the trend of microleakage over 60 days. $P < 0.05$ was considered statistically significant.

RESULTS

The positive control group had bacterial microleakage on the 1st day after culture. However, no microleakage was reported for the negative control group after 60 days indicating aseptic conditions.

In the Mtwo group, one sample had microleakage on day 52, with an incidence rate of 0.00166. In the HERO Shaper group, two samples were found to have microleakage in days 52 and 57, with an incidence rate of 0.00335. As for

the RaCe and FlexMaster groups, one sample showed microleakage on the 2nd day, with an incidence rate of 0.00181 [Figure 1]. The results of the log-rank test showed no significant difference among groups in terms of microleakage time ($P = 0.955$, $\chi^2 = 0.67$).

DISCUSSION

Increased use of Ni-Ti rotary files for root canal treatment in recent years and their separation in the curved canals, particularly at apical one-third, have turned the separation of files into a major challenge,^[13] which may affect the prognosis of treatment. Despite new and developed technologies for the extraction of separated files, it is still a complicated and occasionally impossible procedure.

The effect of file separation on the microleakage is a controversial issue in experimental studies. Although several studies have shown the negative effect of rotary file separation,^[14,18-20] others have indicated no influence of separated rotary files on the leakage.^[15,21,22] Notably, one study showed a lower leakage when a file was separated.^[12] The present study also indicated no impact of rotary file separation on the bacterial microleakage. It should be noted that the methodology was not homogeneous in the abovementioned studies; for instance, the preparation method, separated rotary file, obturation technique, and the microleakage method were not similar [Table 1].

Glucose penetration,^[12] fluid transport test,^[18] dye extraction method,^[19] and bacterial penetration^[15] were applied to measure apical microleakage. In the present study, the double-room technique was utilized owing to advantages such as similarity to clinical conditions for the use of the bacterial flora of oral cavity, the samples' remaining healthy

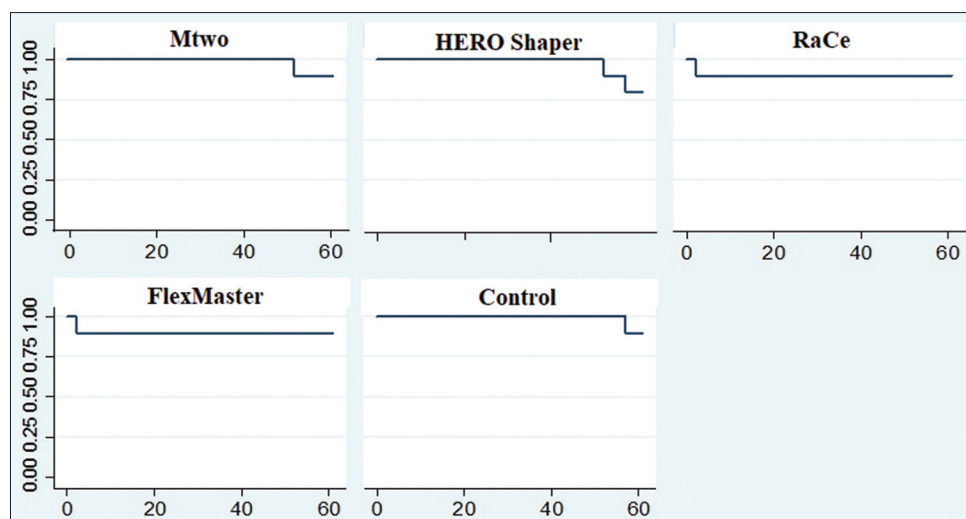


Figure 1: Kaplan–Meier plot of microleakage for different studied groups

Table 1: Characteristics of similar studies

Study	Sample size	Root canal preparation/ master apical file	Separated file	Obturation technique	Microleakage technique	Outcome of file separation on leakage	Outcome of obturation on leakage
Saunders et al., 2004 ^[15]	26 human mandibular premolars	ProFile 04, #40	ProFile 04, #40	Lateral compaction: gutta-percha + Roth 811	Bacterial penetration (<i>S. sanguis</i>), 90 days	No difference	n/a
Mohammadi and Khademi, 2006 ^[21]	26 human mandibular premolars	-	K3 #40	Lateral compaction: gutta-percha + AH plus	Bacterial penetration (<i>E. faecalis</i>)	No difference	n/a
Altundasar et al., 2008 ^[18]	80 human mandibular premolars	ProTaper F3	ProTaper F3	Thermafil + AH plus	Modified fluid transport test, 1 min	Leakage was higher in ProFile group but was less in ProTaper groups	Thermafil showed less leakage
Taneja et al., 2012 ^[12]	40 human mandibular premolars	ProFile 0.06, #30	ProFile 0.06, #30	Lateral compaction: ProTaper or gutta-percha cones + AH plus	Modified glucose penetration	Leakage was significantly lower	Thermafil showed less leakage
Hegde et al., 2013 ^[19]	62 mandibular premolars	ProTaperF2	ProTaperF2	Thermafil + AH plus	Dye extraction	Leakage was higher (K3 showed higher leakage)	Obtura II showed less leakage
Moren et al., 2013 ^[14]	30 human single rooted	RaCe. 04, # 25	RaCe. 04, # 25	Lateral compaction: ProTaper or gutta-percha cones + AH plus	Dye penetration technique	Leakage was higher	McSpadden thermo-mechanical and Schilder's vertical compaction techniques showed less leakage
Ok et al., 2015 ^[20]	36 human single-rooted anterior teeth	RaCe. 06, #25	RaCe. 06, #25	Lateral compaction: gutta-percha + AH plus	Dye extraction method/methylene blue solution	Leakage was higher	n/a
Mohammadi et al., 2015 ^[21]	84 human maxillary premolars	ProTaperF2	ProTaperF2	Schilder's vertical compaction	Dye penetration technique	No difference	No difference
<i>E. faecalis</i> : <i>Enterococcus faecalis</i> , <i>S. sanguis</i> : <i>Streptococcus sanguis</i> , n/a: Not applicable							

and the possibility of repetition if required, and lack of interference in the experimenter's interpretation.^[17] Further, similar to other studies,^[21,22] *Enterococcus faecalis* was used for bacterial penetration. *Enterococcus faecalis* is resistant to the mechanical methods of canal cleaning, can survive alone in the root canal, and cause failure of root canal treatment.^[23] Nevertheless, a certain number of bacterial in oral cavity cause pathogenesis.

The canal preparation comprising the final size and taper rate of instrumentation can affect the leakage.^[15] Although four rotary systems with different designs were used in the present study, the same final size and taper rate (0.06%, #30) were considered for all separated files. Moreover, the final preparation size and taper rate were homogenous for the separated files. Except for one study,^[20] the size and taper of the separated file or canal preparation were analogous among the studied groups. Notwithstanding, various rotary files with different designs have been separated in experimental studies, most of which have been ProTaper (particularly F3) with a convex triangular cross-sectional design.^[24,25] It has been reported that F3 files have the highest incidence rate of separation among all ProTaper sequence files.^[26] A ProFile with a concave triangular (triple-U) and radial land,^[24,25] K3 with three radial lands and a relatively bulky triangular cross-sectional area,^[19,25] Revo-S with an asymmetrical triple-helical cross-section,^[27] and RaCe with nonradial lands and a triangle cross-section have also been utilized in experimental studies.^[19] Producing a smear layer during the instrumentation is assumed to be a reason for the higher leakage of cases with a separated file since the smear layer may act as a barrier between the dentinal wall and filling material, thereby jeopardizing the sealing. It has been reported that rotary files with a radial land design tend to burnish the cut dentin, while nonradial land files with positive cutting angles tend to cut and eradicate the dentin debris; hence, radial landed files show higher leakage.^[19] Notably, the present study revealed that different cross-sectional designs had no impact on the bacterial microleakage; however, all files had a nonradial land and cross-sectional design.

The obturation technique may also affect the leakage in case of file separation. While most studies^[12,18,19] have reported a lower leakage for Thermafil gutta-percha carrier or Obtura II injectable gutta-percha than for the lateral compaction technique, one study has shown the superiority of McSpadden thermomechanical and schilders vertical compaction over Thermafil in the presence of a separated file.^[14] Although there is a controversial outcome about the sealing and quality of Thermafil and vertical

compaction,^[28-30] the superiority of Thermafil over lateral compaction has been attributed to the minimum volume and thickness of the sealer.^[31,32] Notably, the taper of the separated file can affect the amount of sealer entering the dentinal tubules.^[33] Nevertheless, an appropriate obturation technique should be applied in the case of separated instruments as a normal situation.

The limitation of the present study should be noted; sterile canals and an appropriate obturation technique were used; hence, the outcome may differ in clinical settings. Yet, there are still few clinical studies in this regard. A meta-analysis^[34] which pooled the outcomes of two case-control studies (with 199 cases) and a case-control study^[35] (with 8460 cases) indicated that file separation had no adverse effects on the outcome of endodontic treatment. Hence, more clinical studies are recommended. Finally, clinicians should enhance their knowledge about the causes, prevention, and management of file separation.

CONCLUSION

The present *in vitro* study indicated that the separated Ni-Ti rotary instruments with different cross-sections were not associated with bacterial microleakage.

Financial support and sponsorship

This study was financially supported by the Vice Chancellery for Research and Technology, Kermanshah University of Medical Sciences, Kermanshah, Iran (#94261).

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Gambarini G. Cyclic fatigue of ProFile rotary instruments after prolonged clinical use. *Int Endod J* 2001;34:386-9.
2. Vouzara T, Mel C, Lyroutdia K. Separated instrument in endodontics: Frequency, treatment and prognosis. *Balk J Dent Med* 2018;22:123-32.
3. McGuigan MB, Louca C, Duncan HF. Endodontic instrument fracture: Causes and prevention. *Br Dent J* 2013;214:341-8.
4. Gambarini G, Cicconetti A, Di Nardo D, Miccoli G, Zanza A, Testarelli L, et al. Influence of different heat treatments on torsional and cyclic fatigue resistance of nickel-titanium rotary files: A comparative study. *Appl Sci* 2020;10:5604.
5. Di Nardo D, Gambarini G, Seracchiani M, Mazzoni A, Zanza A, Giudice A, et al. Influence of different cross-section on cyclic fatigue resistance of two nickel-titanium rotary instruments with same heat treatment: An in vitro study. *Saudi Endod J* 2020;10:221-5.
6. Parveen S, Hossain M, Uddin MF. Management of broken instrument by file bypass technique. *Bangabandhu Sheikh Mujib Med Univ J* 2017;10:41.
7. Cruz A, Mercado-Soto CG, Ceja I, Gascón LG, Cholico P, Palafox-Sánchez CA. Removal of an instrument fractured by ultrasound and the instrument removal system under visual magnification. *J Contemp Dent Pract* 2015;16:238-42.

8. Ebihara A, Takashina M, Anjo T, Takeda A, Suda H. Removal of root canal obstructions using pulsed Nd: YAG laser. *Int Congr Ser* 2003;1248C: 257-9.
9. Wohlgemuth P, Cuocolo D, Vandrangi P, Sigurdsson A. Effectiveness of the GentleWave system in removing separated instruments. *J Endod* 2015;41:1895-8.
10. Ormiga F, da Cunha Ponciano Gomes JA, de Araújo MC. Dissolution of nickel-titanium endodontic files via an electrochemical process: A new concept for future retrieval of fractured files in root canals. *J Endod* 2010;36:717-20.
11. Satheesh SL, Jain S, Bhuyan AC, Devi LS. Surgical management of a separated endodontic instrument using second generation platelet concentrate and hydroxyapatite. *J Clin Diagn Res* 2017;11:D01-3.
12. Taneja S, Chadha R, Gupta R, Gupta A. Comparative evaluation of sealing properties of different obturation systems placed over apically fractured rotary NiTi files. *J Conserv Dent* 2012;15:36-40.
13. Di Fiore PM, Genov KA, Komaroff E, Li Y, Lin L. Nickel-titanium rotary instrument fracture: A clinical practice assessment. *Int Endod J* 2006;39:700-8.
14. Moreno GV, Argüello RG, Pérez TH. Assessment of apical sealing of three warm obturation techniques in the presence of fractured NiTi rotary instruments. *Rev Odont Mex* 2013;17:20-5.
15. Saunders JL, Eleazer PD, Zhang P, Michalek S. Effect of a separated instrument on bacterial penetration of obturated root canals. *J Endod* 2004;30:177-9.
16. Alamoudi RA. The smear layer in endodontic: To keep or remove-an updated overview. *Saudi Endod J* 2019;9:71-81.
17. Shantiaee Y, Maziar F, Dianat O, Mahjour F. Comparing microleakage in root canals obturated with nanosilver coated gutta-percha to standard gutta-percha by two different methods. *Iran Endod J* 2011;6:140-5.
18. Altundasar E, Sahin C, Ozcelik B, Cehreli ZC. Sealing properties of different obturation systems applied over apically fractured rotary nickel-titanium files. *J Endod* 2008;34:194-7.
19. Rangappa KG, Hegde J, Chikkamallaiiah C, Rashmi K. Comparative evaluation of the sealing ability of different obturation systems used over apically separated rotary nickel-titanium files: An *in vitro* study. *J Conserv Dent* 2013;16:408-12.
20. Ok E, Ertaş H, İysal B. Effect of separated rotary instruments on the the apical microleakage of root canal fillings. *J Dent Fac Atatürk Uni* 2015;24:246-51.
21. Mohammadi Z, Mashouf RY, Jafarzadeh H, Heydari A, Shalavi S. Effect of apically separated rotary instruments on bacterial leakage of obturated root canals using resilon. *N Y State Dent J* 2015;81:42-6.
22. Mohammadi Z, Khademi AA. Effect of a separated rotary instrument on bacterial penetration of obturated root canals. *J Clin Dent* 2006;17:131-3.
23. Alghamdi F, Shakir M. The influence of *Enterococcus faecalis* as a dental root canal pathogen on endodontic treatment: A systematic review. *Cureus* 2020;12:e7257.
24. Berutti E, Chiandussi G, Gaviglio I, Ibba A. Comparative analysis of torsional and bending stresses in two mathematical models of nickel-titanium rotary instruments: ProTaper versus ProFile. *J Endod* 2003;29:15-9.
25. Hwang SY, Oh SR, Lee Y, Lim SM, Kum KY. Effect of cross-sectional area of 6 nickel-titanium rotary instruments on the fatigue fracture under cyclic flexural stress: A fractographic analysis. *J Korean Acad Conserv Dent* 2009;34:424.
26. Wu J, Lei G, Yan M, Yu Y, Yu J, Zhang G. Instrument separation analysis of multi-used ProTaper Universal rotary system during root canal therapy. *J Endod* 2011;37:758-63.
27. Sekar V, Kumar R, Nandini S, Ballal S, Velmurugan N. Assessment of the role of cross section on fatigue resistance of rotary files when used in reciprocation. *Eur J Dent* 2016;10:541-5.
28. Pommel L, Camps J. *In vitro* apical leakage of system B compared with other filling techniques. *J Endod* 2001;27:449-51.
29. Haikel Y, Freymann M, Fanti V, Claisse A, Poumier F, Watson M. Apical microleakage of radiolabeled lysozyme over time in three techniques of root canal obturation. *J Endod* 2000;26:148-52.
30. Bhambhani SM, Sprechman K. Microleakage comparison of thermafil versus vertical condensation using two different sealers. *Oral Surg Oral Med Oral Pathol* 1994;78:105-8.
31. Weis MV, Parashos P, Messer HH. Effect of obturation technique on sealer cement thickness and dentinal tubule penetration. *Int Endod J* 2004;37:653-63.
32. Gulsahi K, Cehreli ZC, Kuraner T, Dagli FT. Sealer area associated with cold lateral condensation of gutta-percha and warm coated carrier filling systems in canals prepared with various rotary NiTi systems. *Int Endod J* 2007;40:275-81.
33. Eymirli A, Uzunoğlu Özyürek E, Serper A. Sealer penetration: Effect of separated file's cross-section, taper and motion characteristics. *Clin Oral Investig* 2021;25:1077-84.
34. Panitvisai P, Parunnit P, Sathorn C, Messer HH. Impact of a retained instrument on treatment outcome: A systematic review and meta-analysis. *J Endod* 2010;36:775-80.
35. Spili P, Parashos P, Messer HH. The impact of instrument fracture on outcome of endodontic treatment. *J Endod* 2005;31:845-50.