Effect of Sterilization and Irrigation on Surface Roughness of Ni-Ti Rotary Instruments: An In-Vitro Study

Dr. Jhankriti Babbar[1], Dr. Akanksha Bhatt[2], Dr. Vishesh Gupta[3*], Prof.(Dr.) Praveen Singh Samant[4]

[1]*Junior Resident, Department of Conservative Dentistry & Endodontics, Babu Banarasi Das College of Dental Sciences, BBD University, Lucknow, Uttar Pradesh, India*

[2]Assistant Professor, Department of Conservative Dentistry & Endodontics, Babu Banarasi Das College of Dental Sciences, BBD University, Lucknow, Uttar Pradesh, India

[3*]Professor & Head, Department of Conservative Dentistry & Endodontics, Babu Banarasi Das College of Dental Sciences, BBD University, Lucknow, Uttar Pradesh, India

[4] Consultant Endodontist, Lucknow,Uttar Pradesh, India

***Corresponding Author:**

Dr. Vishesh Gupta

Professor & Head, Department of Conservative Dentistry & Endodontics, Babu Banarasi Das College of Dental Sciences, BBD University, Lucknow, Uttar Pradesh, India

Email ID: drvisheshgupta@gmail.com

ABSTRACT

The cornerstone of contemporary root canal therapy lies in the chemo-mechanical preparation of the root canals. This involves both shaping and the application of intracanal irrigants within the canals to ensure optimal disinfection of the system.[1] Effective sterilization and disinfection are essential for controlling infections within dental settings, helping to minimize healthcare-associated infections and enhance patient safety.[2]

This study aimed to assess the individual change along with a comparative evaluation between the surface roughness of TruNatomy and RaceEvo Ni-Ti endodontic instruments after sterilization by autoclave, dynamic immersion in 3% Sodium Hypochlorite (NaOCl) followed by 17% Ethylenediaminetetraacetic acid (EDTA) or 17% EDTA followed by 2% Chlorhexidine (CHX) used as endodontic irrigants. Evaluation of surface roughness was done by using Scanning Electron Microscope.

Statistical analysis performed using the *SPSS* statistical software *23.0 Version* concluded that autoclaving and dynamic immersion in 3% NaOCl, 17% EDTA and 2% CHX irrigants cumulatively increased surface roughness of Ni-Ti rotary files. RaceEvo file system consistently showed higher surface roughness in comparison to TruNatomy file system across multiple treatment cycles. Autoclaving showed the least increase in surface roughness. Autoclaving followed by dynamic immersion in 3% NaOCl and 17% EDTA showed highest surface roughness alterations in both the file systems.

KEYWORDS

Autoclaving; Endodontic irrigants; Ni-Ti rotary files; Rotary files; Surface roughness

INTRODUCTION

Endodontic therapy holds eradication of infection by eliminating harmful microorganisms as the primary objective.[3] Numerous strategies exist to achieve the objective of clearing the canal contents and eradicating infection.[4]

The TruNatomy system developed by Dentsply Sirona is constructed from a heat-treated NiTi alloy including three categories of shaping instruments differentiated by size. This file has an off-centered parallelogram cross-section and a slip design that facilitates additional space for debris removal hence maintaining the integrity of radicular dentin and effectively navigate curved canals.[5] The RACE EVO from FKG Dentaire SA is an electropolished file system that has a triangular cross-section, alternating cutting edges, and a rounded tip. It has improved cyclic fatigue resistance making it more flexible with better cutting efficiency while exerting less stress on dentin and minimizing the screwing effect.[6] The surface of these files is blue in color indicating the existence of a titanium oxide layer, which contributes to its superior resistance.[7]

Relying solely on mechanical preparation, even with highly flexible instruments, does not suffice for achieving a disinfected root canal system. Hence, chemical irrigation should consistently accompany mechanical preparation. Sodium hypochlorite, CHX, and EDTA are among the frequently utilized irrigants in endodontic treatments.[8]

Sodium hypochlorite is used in concentrations from 0.5% to 8% and serves as a potent antimicrobial agent. It effectively breaks down pulp and organic material within dentin. Research by Vianna et al. indicated that 0.5% NaOCl could eradicate C. albicans when used for about 30 minutes, while a 5.25% solution could do the same in just 15 seconds. In contrast to NaOCl, CHX completely lacks the ability to dissolve tissue. When applied in concentrations between 0.2% and 2%, CHX effectively eradicated E. faecalis cells in 30 seconds or less across all tested concentrations. EDTA serves as a potent chelating agent within the root canal system. Typically, EDTA is utilized at a concentration of 17%, and it can remove smear layers from the root canal walls in less than one minute of direct contact. The synergistic use of these selected irrigation solutions significantly enhances treatment success rates.[1]

The sterilization of both medical and dental instruments is crucial to avert cross-contamination and uphold patient safety. Conventional techniques such as steam sterilization are still widely employed due to their effectiveness in eradicating microorganisms, while alternative methods like low-temperature chemical sterilization and radiation techniques are suitable for heat-sensitive materials.[9]
A primary concern regarding the clinical application of NiTi files is their unexpected fractures resulting from flexural fatigue and torsion. When stress is exerted on the instrument, microstructural alterations take place, leading to phase changes. Both stress and heat exposure can modify NiTi alloys mechanically.[10,11]
Serene et.al. suggested that heat applied while sterilization processes may restore the integrity of previously utilized NiTi files at a molecular level, thus enhancing their fracture resistance. Such behavior has been observed in modern martensitic instruments.[3]
Consequently, this study seeks to evaluate the comparative effects of sterilization methods and endodontic irrigants on the surface roughness of innovative nickel-titanium rotary instruments.

METHOD

This study was conducted at Babu Banarasi Das College of Dental Sciences, Lucknow in collaboration with Birbal Sahani Institute of Palaeobotany, Lucknow, Uttar Pradesh

A total sample size of 30 Ni Ti rotary files were used after careful assessment under a Dental Operating Microscope for any gross impurities or surface defects.
INCLUSION CRITERIA- Files with no defects or impurities.
EXCLUSION CRITERIA- Files with defects or impurities.

All rotary file samples were autoclaved 10 times before and after each instrumentation cycle. Autoclaving cycle was set at 121ºC at 15 psi for 15 mins. Eppendorf tubes with a capacity of 0.5 mL were taken and filled at full capacity with respective experimental irrigant solution for each group. The endomotor was set at recommended speed and torque for the respective rotary file systems and immersed dynamically into the irrigant solutions for 30 seconds each.

GROUP I: 15 TruNatomy files were subdivided into 3 subgroups with 5 samples each and subjected to further treatment.
Subgroup A: A total of 5 samples of TruNatomy files were autoclaved 10 times according to protocol.
Subgroup B: A total of 5 samples of TruNatomy files were autoclaved, then dynamically immersed in 3% NaOCl followed by 17% EDTA. This cycle repeated 10 times and samples were evaluated by SEM analysis.
Subgroup C: A total of 5 samples of TruNatomy files were autoclaved, then dynamically immersed in 17% EDTA followed by 3% CHX. This cycle was repeated 10 times and samples were evaluated by SEM analysis.
GROUP II: 15 RaceEvo files were subdivided into 3 subgroups with 5 samples each and subjected to further treatment.
Subgroup A: A total of 5 samples of RaceEvo files were autoclaved 10 times according to protocol.
Subgroup B: A total of 5 samples of RaceEvo files were autoclaved, then dynamically immersed in 3% NaOCl followed by 17% EDTA. This cycle was repeated 10 times and samples were evaluated by SEM analysis.
Subgroup C: A total of 5 samples of RaceEvo files were autoclaved, then dynamically immersed in 17% EDTA followed by 3% CHX. This cycle was repeated 10 times and samples were evaluated by SEM analysis.

Samples were mounted on a stub prior to SEM assessment and surface roughness of respective files was performed at 3mm from the tip of the files. SEM images of all samples were evaluated using ImageJ software for mean surface roughness values and a surface plot was obtained.

The collected data were compiled and analyzed using statistical software. Descriptive statistics were expressed as mean ± standard deviation for continuous variables (Ra and Rq). Intragroup comparisons across treatment cycles (1st, 3rd, and 10th cycles) within each subgroup were performed using repeated measures Analysis of Variance (ANOVA) to assess changes over time.
When statistically significant differences were detected, post-hoc pairwise comparisons were conducted to identify specific cycle-wise differences. Intergroup comparisons between Group I and Group II at corresponding treatment cycles were performed using independent samples t-tests. Subgroup comparisons within each group at the 10th cycle were analyzed using one-way ANOVA.
A p-value of less than 0.05 was considered statistically significant, and values less than 0.001 were considered highly statistically significant. Results are presented in tabular form to facilitate clear comparison and interpretation.
Table 1 presents the mean and standard deviation of surface roughness (Ra) values across the 1st, 3rd, and 10th treatment cycles in Group I and Group II for all subgroups. In both groups, a progressive increase in Ra values was observed with advancing treatment cycles across all subgroups. Subgroup B in both groups demonstrated the highest mean Ra values at each cycle, particularly at the 10th cycle, indicating a greater cumulative effect of repeated treatments. Subgroup A consistently showed lower Ra values compared to subgroups B and C. Overall, Group II exhibited comparatively higher Ra values than Group I during the initial cycles; however, differences narrowed by the 10th cycle.

TABLE 1: Descriptive Statistics of the Outcome Variable (Ra) at Different Treatment Cycles

Group	Subgroup	n	1st Cycle (Mean ± SD)	3rd Cycle (Mean ± SD)	10th Cycle (Mean ± SD)
Group I	A	5	14.07 ± 0.87	18.09 ± 2.03	27.05 ± 3.81
	B	5	15.16 ± 2.99	30.10 ± 3.36	37.48 ± 4.09
	C	5	19.69 ± 4.08	23.91 ± 2.16	30.07 ± 3.06
Group II	A	5	18.93 ± 3.37	23.44 ± 3.34	25.16 ± 3.52
	B	5	22.95 ± 3.41	31.00 ± 4.60	42.31 ± 6.10
	C	5	20.40 ± 2.82	24.11 ± 4.13	28.86 ± 7.24

TABLE 2: Intergroup Comparison Between Group I and Group II at Different Cycles

Subgroup	Cycle	Group I (Mean ± SD)	Group II (Mean ± SD)	p-value
A	1st	14.07 ± 0.87	18.93 ± 3.37	p = 0.021
	3rd	18.09 ± 2.03	23.44 ± 3.34	p = 0.029
	10th	27.05 ± 3.81	25.16 ± 3.52	p = 0.368
B	1st	15.16 ± 2.99	22.95 ± 3.41	p = 0.004
	3rd	30.10 ± 3.36	31.00 ± 4.60	p = 0.706
	10th	37.48 ± 4.09	42.31 ± 6.10	p = 0.135
C	1st	19.69 ± 4.08	20.40 ± 2.82	p = 0.683
	3rd	23.91 ± 2.16	24.11 ± 4.13	p = 0.928
	10th	30.07 ± 3.06	28.86 ± 7.24	p = 0.690

Table 2 presents Intergroup comparison of Ra between Group I and Group II revealing statistically significant differences between Group I and Group II in subgroups A and B during the 1st cycle (p = 0.021 and p = 0.004, respectively) and in subgroup A during the 3rd cycle (p = 0.029). However, no statistically significant differences were observed between the two groups at the 10th cycle for any subgroup. This indicates that although initial differences existed, prolonged treatment cycles resulted in comparable surface roughness outcomes between the groups.

DISCUSSION

In this study, both the RaceEvo and TruNatomy file systems showed a progressive increase in Ra values with advancing treatment cycles across all subgroups. Cumulative effect of Subgroup B (autoclaving followed by dynamic irrigation in 3% NaOCl and 17% EDTA) in both file systems demonstrated the highest mean Ra values at each cycle, particularly at the 10th cycle, indicating a greater cumulative effect of repeated treatments. Subgroup A (Autoclaving) consistently showed lower Ra values compared to subgroups B and C (autoclaving followed by dynamic irrigation in 17% EDTA and 2% CHX). Overall, Group II (RaceEvo) exhibited comparatively higher Ra values than Group I (TruNatomy) during the initial cycles; however, differences narrowed by the 10th cycle.

This result was in alignment with a study done by Özcan T. et.al. where they compared the electrochemical corrosion rates and areas of corrosion of ProTaper Universal Ni-Ti rotary files when immersed in 5% NaOCl, 15% EDTA, 2% CHX and 2.5% NaOCl solutions and concluded that denser and increased areas of surface roughness were observed in all the samples.[12] Jose J. et.al. also concluded in their study that XP-endo Shaper and TN files upon exposure to 5.25% NaOCl and 17% EDTA at 10 minutes time showed significantly higher surface roughness in both file systems with 17% EDTA causing significantly more surface irregularities when compared to 5.25% NaOCl.[13]

Intergroup comparison revealed statistically significant differences between Group I and Group II in subgroups A and B during the 1st cycle ($p = 0.021$ and $p = 0.004$, respectively) and in subgroup A during the 3rd cycle ($p = 0.029$). However, no statistically significant differences were observed between the two groups at the 10th cycle for any subgroup. This indicates that although initial differences existed, prolonged treatment cycles resulted in comparable surface roughness outcomes between the groups. This is in alignment with the study by Özdemir O. et.al. where the HyFlex CM, The ProTaper Next, TF Adaptive, and 2Shape presented similar surface responses after five cycles of autoclave sterilization with HyFlex CM files demonstrating superior surface properties after several cycles of sterilization.[14]

Therefore, it can be concluded from this study that autoclaving and dynamic immersion in 3% NaOCl, 17% EDTA and 2% CHX irrigants has a cumulative effect over subsequent cycles and causes an increased surface roughness in both RaceEvo and TruNatomy file systems. The maximum increase in surface roughness was observed in RaceEvo file system upon autoclaving followed by dynamic immersion in 3% NaOCl followed by 17 % EDTA whereas autoclaving showed least surface roughness changes throughout.

Heat treatments modify the phase-transformation temperature of NiTi instruments to control the instrument's phase composition at its operating temperature and improve its mechanical and metallurgical properties.[15] To decrease the stress accumulation during bending of rotary files in curved root canals, the rotary NiTi instruments should be designed with rectangle cross-section configuration, low pitch, reduced taper, and with centered cross section.[16] The present study demonstrated significantly greater surface alteration of RaceEvo instruments compared with TruNatomy following immersion in endodontic irrigants.

This difference may be attributed to variations in proprietary manufacturing processes, alloy metallurgy, and surface finishing rather than heat treatment alone. RaceEvo files have alternating cutting edges, triangular cross-section and sharp cutting flutes.[6] These features may create localized stress concentration and expose more active surface area. TruNatomy files are off-centred, have a parallelogram cross-section and a slimmer core which may distribute stresses more evenly and reduce localized corrosive attack.[5]

Surface machining defects act as preferential sites for corrosion initiation, and exposure to sodium hypochlorite and EDTA may enlarge these defects through oxidation and chelation, resulting in increased surface roughness. Furthermore, the stability of the protective titanium oxide layer, baseline surface quality, and electrochemical behaviour of the alloy are likely to influence the degree of corrosion.[17]

CONCLUSION

Within the constraints of this study, it can be concluded that autoclaving and dynamic immersion in 3% NaOCl, 17% EDTA and 2% CHX irrigants has a cumulative effect over subsequent cycles and causes significant increase in surface roughness of Ni-Ti rotary files. RaceEvo file system consistently showed higher surface roughness when compared to TruNatomy file system across multiple treatment cycles. Autoclaving showed the least increase in surface roughness followed. Autoclaving followed by dynamic immersion in 3% NaOCl and 17% EDTA showed maximum increase in surface roughness in both the file systems.

REFERENCES

1. Tanalp J, Güngör T. Apical extrusion of debris: a literature review of an inherent occurrence during root canal treatment. International endodontic journal. 2014 Mar;47(3):211-21.
2. Patiño-Marín N, Villa García LD, Aguirre López EC, Medina-Solís CE, Martínez Zumarán A, Martínez Rider R, Márquez Preciado R, Rosales García P, Salas Orozco MF. Sterilization and Disinfection: Ensuring Infection Control in Dental Practices. Cureus. 2025 Feb 15;17(2):e79041. doi: 10.7759/cureus.79041. PMID: 40099062; PMCID: PMC11912515.

3. Fabricius L, Dahlén G, Sundqvist G, Happonen RP, Möller ÅJ. Influence of residual bacteria on periapical tissue healing after chemomechanical treatment and root filling of experimentally infected monkey teeth. European journal of oral sciences. 2006 Aug;114(4):278-85.
4. Berman LH, Hargreaves KM, Rotstein I. Cohen's Pathways of the Pulp. Elsevier Health Sciences; 2020 Sep 8.
5. Jaggi, Prakriti; Mulay, Sanjyot; Tandale, Anita Sanap; Jadhao, Renuka; Aras, Sanket; Patil, Rutuja. Comparative Evaluation of Shaping Ability of TruNatomy Rotary Endodontic File versus Different Rotary Endodontic File Systems. Journal of the International Clinical Dental Research Organization 15(1):p 13-22, Jan–Jun 2023. | DOI: 10.4103/jicdro.jicdro_16_23
6. Almohareb R.A., Barakat R., et al. Effect of autoclaving cycles on the cyclic fatigue resistance of race and RACE EVO nickel-titanium endodontic rotary files: an in vitro study. Metals. 2021;11(12):1947.
7. Allahem Z, Bendahmash M, Almeaither R, Alfawaz H, Alqedairi A. Evaluation of cyclic fatigue behavior of RACE EVO rotary Files: In-vitro comparative study. Saudi Dent J. 2024 Apr;36(4):656-660. doi: 10.1016/j.sdentj.2024.03.009. Epub 2024 Mar 18. PMID: 38690374; PMCID: PMC11056425.
8. Rotstein I, Ingle JI, editors. Ingle's endodontics. PMPH USA; 2019 Jun 1.
9. McDaniel J, Canares G. Infection Control in the Dental Office in the Era of COVID-19. Cham: Springer; 2024. Maintenance and sterilization of dental handpieces; pp. 183–194. [Google Scholar]
10. Cheung, G.S.; Oh, S.H.; Ha, J.H.; Kim, S.K.; Park, S.H.; Kim, H.C. Effect of torsional loading of nickel-titanium instruments on cyclic fatigue resistance. *J. Endod.* 2013, *39*, 1593–1597. [Google Scholar] [CrossRef] [PubMed]
11. El Abed, R.; Al Raeesi, D.; Alshehhi, A.; Alkhatib, Z.; Khamis, A.H.; Jamal, M.; Kim, H.-C. Effect from Autoclave Sterilization and Usage on the Fracture Resistance of Heat-Treated Nickel–Titanium Rotary Files. *Materials* 2023, *16*, 2261. https://doi.org/10.3390/ma16062261
12. Özcan T, Sonat B, Öztan MD, Basmaci F, Aksoy U. The Determination of the Corrosion Rates of Rotary Ni-Ti Instruments in Various Irrigation Solutions. Cyprus Journal of Medical Sciences. 2023 May 9.
13. Jose J, Khandelwal A, Siddique R. Qualitative assessment of the surface topographic changes of XP-endo Shaper and TruNatomy files after exposure to sodium hypochlorite and ethylenediaminetetraacetic acid. European Endodontic Journal. 2021 Jun 3;6(2):197.
14. Özdemir O, Koçak S, Koçak MM, Sağlam BC. Effects of repeated sterilization cycles on the surface alterations of ProTaper Next, TF Adaptive, HyFlex CM, and 2Shape instruments. Journal of Dental Research, Dental Clinics, Dental Prospects. 2021 May 5;15(2):76.
15. Basturk FB, Özyürek T, Uslu G, Gündoğar M. Mechanical properties of the new generation RACE EVO and R-Motion nickel–titanium instruments. Materials. 2022 May 6;15(9):3330.
16. Galal M, Hamdy TM. Evaluation of stress distribution in nickel-titanium rotary instruments with different geometrical designs subjected to bending and torsional load: a finite element study. Bulletin of the National Research Centre. 2020 Jul 22;44(1):121.
17. Prasad PS, Sam JE, Kumar A. The effect of 5% sodium hypochlorite, 17% EDTA and triphala on two different rotary Ni-Ti instruments: An AFM and EDS analysis. Journal of Conservative Dentistry and Endodontics. 2014 Sep 1;17(5):462-6.