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Stereomicroscopic evaluation of dentinal microcrack formation induced by rotary file systems: An *in vitro* study

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Abstract:
Root canal instrumentation should preserve radicular dentin to maintain fracture resistance, yet different rotary systems may produce dentinal defects during shaping. Therefore, it is of interest to compare the incidence and types of defects caused by ProTaper Next and OneShape in single-rooted mandibular premolars. Forty-five extracted premolars were divided into control, ProTaper Next and OneShape groups and then sectioned at 2, 4 and 6 mm from the apex for stereomicroscopic evaluation. Both rotary systems produced significantly more defects than the control, but no significant difference was found between ProTaper Next and OneShape in defect incidence or distribution. Thus, data shows that continuous rotary instrumentation with either system increases dentinal microcracks compared with uninstrumented teeth.

Keywords: Stereomicroscopic**,** rotary file, root canal, obturation, endodontics

Background:
Root canal instrumentation aims to clean and shape the canal system while preserving the maximum radicular dentin to maintain long-term fracture resistance of endodontically treated teeth **[1]**. However, excessive dentin removal and the stress generated during mechanical preparation can initiate dentinal microcracks and craze lines that may propagate under functional loads, eventually leading to catastrophic vertical root fracture, one of the most frustrating causes of tooth loss after root canal treatment **[2]**. With the evolution of nickel-titanium (NiTi) rotary systems, clinicians now choose between traditional multifile sequences and newer single-file techniques, each with improved safety and efficiency **[3]**. ProTaper Next (Dentsply Maillefer) is a widely used multi-file system manufactured with M-Wire technology and features an off-centered rectangular cross-section with a variable taper. These design characteristics reduce the taper lock and screw-in forces but still require the sequential use of multiple instruments **[3, 4]**. In contrast, OneShape (Micro-Mega) is a single-file system in continuous rotation with a constant 0.06 taper and variable cross-sectional geometry along its working length, designed to minimize the number of instruments in contact with dentin, thereby theoretically lowering the risk of defect formation **[5]**. Tufenkci *et al.* **[6]** compared the ProTaper Next and OneShape systems using micro-computed tomography and found that the OneShape system led to significantly less transportation in the apical region. In contrast, Zhu *et al.* **[7]** reported no significant differences between the two file systems in terms of root canal transportation. Therefore, it of interest to evaluate and compare the incidence and types of dentinal defects (complete fractures, partial cracks and craze lines) produced by the ProTaper Next and OneShape rotary systems when used in continuous rotation motion in single-rooted mandibular premolars, using stereomicroscopic analysis of root sections at 2, 4 and 6 mm from the apex.

Materials and Methods:
Study design and setting:
This *in vitro* comparative experimental study was conducted in the Department of Conservative Dentistry and Endodontics, over duration of six months. Because the study utilized only anonymized human teeth extracted for orthodontic or therapeutic reasons, with no linkable patient data, the Institutional Ethics Committee granted full ethical clearance (DDC/ACAD/ETHICAL/2016/1330) and waived the requirement for additional written informed consent, specifically for this study. All teeth were collected with written informed consent from the patients at the time of extraction, permitting their use for research and educational purposes. This study was conducted in strict accordance with the Declaration of Helsinki (2013 revision).

Sample size calculation:
The sample size was determined using the G*Power 3.0.10 software (Franz Faul, Universität Kiel, Germany) to ensure adequate statistical power. Based on the power analysis with F-test analysis of variance (ANOVA) a priori analysis, a minimum of 45 samples for three groups were obtained with an effect size of 0.47 **[8]**, 80% power of study and 5% alpha error. Thus, 15 teeth were included in each group, resulting in 45 teeth.

Sample selection and preparation:
45 single-rooted mandibular premolar teeth extracted for orthodontic reasons from patients aged 18-30 years with healthy periodontal conditions were collected from the Department of Oral and Maxillofacial Surgery. This selection criterion was chosen to standardize the samples and minimize pre-existing dentinal defects, as orthodontically extracted teeth are typically intact and free from caries, restorations and traumatic forces that could induce cracks. The inclusion criteria were teeth with single, straight roots (curvature ≤10° as assessed by Schneider's method); single canals confirmed radiographically, non-carious surfaces and completely formed apices. Exclusion criteria included fractured teeth, root resorption, severe anatomic variations, or calcified canals. All extracted teeth were initially cleaned to remove visible blood and debris using ultrasonic scaling and stored in distilled water at 37^{0}C for 24 h to maintain hydration. To ensure standardization and the absence of pre-existing cracks at the beginning of the study, each tooth was meticulously examined under a stereomicroscope (Lawrence and Mayo, Mumbai, India) at 20 x magnifications for any external craze lines, microcracks, or defects on the root surface. Additionally, periapical radiographs were obtained to confirm single canals and rule out internal anomalies. Teeth showing signs of pre-existing defects were discarded and replaced. After inspection, the teeth were measured with a standardized root length of 15 mm using a digital Vernier caliper (Mitutoyo Corporation, Kawasaki, Japan) and decoronated at this length using a diamond disc (DFS Diamon GmbH, Riedenburg, Germany) mounted on a low-speed micromotor handpiece (NSK Ltd., Kanuma, Japan) under constant water irrigation to prevent overheating and dehydration-induced cracks.

Randomization and blinding:
To minimize bias, 45 teeth were randomly allocated into three groups (n = 15 each) using a computer-generated randomization sequence (https://www.random.org/). Allocation was performed by an independent investigator who was blinded to the instrumentation protocols. Group 1 served as the control (uninstrumented), Group 2 was instrumented with ProTaper Next (Dentsply Maillefer, Ballaigues, Switzerland) and Group 3 was instrumented with OneShape (Micro-Mega, Besançon, France). The study followed a triple-blind design: participants (not applicable *ex vivo*), outcome assessors (endodontists evaluating sections) and the statistician were blinded to the group assignments. The group identities were masked using coded labels throughout the procedure, sectioning and analysis.

Biomechanical preparation:
All instrumentation procedures were performed by a single experienced endodontist to ensure consistency. Canals in the experimental groups (Groups 2 and 3) were irrigated with 0.5 mL of 3% sodium hypochlorite (Hypo 3%, Prevest DenPro Ltd., Jammu, India) for 1 minute, followed by 1 mL of saline (0.9% sodium chloride, Fresenius Kabi AG, Bad Homburg, Germany), after each file change. The roots were kept moist in saline to prevent dehydration. No instrumentation was performed in Group 1 (control). In Group 2, ProTaper Next was used according to the manufacturer's instructions in the continuous rotation mode. Initial negotiations were performed with#10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) of 21 mm length. Working length was established by advancing the file until it was visible at the apical foramen, subtracting 1 mm and confirming it radiographically. Instrumentation was performed with #15 and #20 K-files to working length, followed by X1 (17/0.04) and X2 (25/0.06) files in a brushing motion with ethylenediaminetetraacetic acid (EDTA) gel (Glyde File Prep, Dentsply Maillefer, Ballaigues, Switzerland) at 300 rpm and 2 Ncm torque using an endodontic motor (X-Smart Plus, Dentsply Maillefer, Ballaigues, Switzerland). For Group 3, OneShape was employed per the manufacturer's guidelines for continuous rotation. Working length determination mirrored that of Group 2. After initial patency with a #20 K-file (Micro-Mega, Besançon, France), the OneShape file (25/0.06) was used in a pecking motion to working length with EDTA gel (MD-ChelCream, Meta Biomed Co. Ltd., Cheongju, South Korea) at 350 rpm and 2.5 Ncm torque on the same endodontic motor.

Sectioning and microscopic observations:
After instrumentation, roots were marked at 2 mm, 4 mm and 6 mm from the apex using a digital Vernier caliper. Horizontal sectioning was performed under continuous saline irrigation using a diamond disc (DFS Diamon GmbH, Riedenburg, Germany) on a low-speed handpiece to obtain three sections per root. The sections were stabilized in a custom acrylic jig (4x2 cm) and mounted on modeling wax for evaluation. Observations were conducted under a stereomicroscope (Lawrence & Mayo, Mumbai, India) at 20x magnification.

Defects were categorized as follows **[9, 10]**:

[1] Score 0 = No defect (dentin devoid of lines/cracks),
[2] Score 1 = Defects (line from the canal wall into dentin with or without reaching the outer surface), or
[3] Score 2 = Craze lines (other lines not extending to the outer surface).

Images were then captured for documentation **(Figure 1)**. The scoring criteria employed in this study necessitate proper citation when used for research purpose.

Statistical analysis:
Scoring data for dentinal cracks were recorded in a Microsoft Excel spreadsheet and subsequently analyzed using Statistical Package for Social Sciences (SPSS) version 26.0 (IBM Corp., Armonk, New York, USA). The normality of the outcome data was assessed using the Shapiro-Wilk test, which indicated a non-normal distribution. The scoring data are presented as frequency and percentage, along with median values. Consequently, the non-parametric Friedman test (intragroup) and Kruskal-Wallis test (intergroup) were used to compare dentinal crack scores at varying root lengths. The association between dentinal cracks and the method of canal preparation was evaluated using the

Fisher exact test. A p-value less than 0.05 were considered statistically significant for all analysis.

Results:

The Kruskal-Wallis test revealed statistically significant differences in dentinal crack scores between groups at all root levels (2 mm, 4 mm and 6 mm), with a p-value of 0.001 at each depth. Both rotary instrumentation groups, ProTaper Next and One Shape, exhibited a median crack score of 2, whereas the control group had a median score of 0. The mean ranks were substantially higher in both groups than in the control across all measured levels. However, no significant differences were observed between the two rotary systems. This implies that rotary instrumentation significantly increases the incidence of dentinal cracks compared with unprepared teeth, but the choice between the ProTaper Next and OneShape systems does not appear to influence crack formation **(Table 1)**. The post-hoc analysis revealed highly significant differences in dentinal crack scores at all root depths when comparing the control group to both rotary instrumentation groups, with a p-value of 0.001. However, no statistically significant difference was found between the ProTaper Next and OneShape systems at any measured level (2, 4, or 6 mm), with p-values ranging from 0.842 to 0.864. This result suggests that rotary instrumentation inherently increases the risk of dentinal cracks compared to unprepared teeth **(Table 2)**. Intragroup analysis using the Friedman test revealed no statistically significant differences in dentinal crack scores at different root depths for either rotary instrumentation system. For the ProTaper Next group, the median score remained constant at 2 across all measured levels (2, 4 and 6 mm), with a p-value of 0.861. Similarly, the OneShape group showed a consistent median score of 2 at each depth, yielding a p-value of 0.951 **(Table 3)**. Fisher's exact test revealed a statistically significant association between the method of canal preparation and the formation of dentinal defects at all three root levels (2 mm: p = 0.001; 4 mm: p = 0.001; 6 mm: p = 0.001). In the control group (group 1), 100% of the specimens exhibited no defects at any depth. In contrast, in both rotary instrumentation groups, a high prevalence of defects (primarily cracks with some craze lines) was observed. The proportion of defects in the rotary groups was consistently high and increased slightly with depth, whereas craze lines also became more frequent at 6 mm. This suggests that rotary instrumentation techniques are strongly and significantly associated with the induction of dentinal cracks and craze lines, whereas the unprepared controls remained unaffected **(Table 4)**.

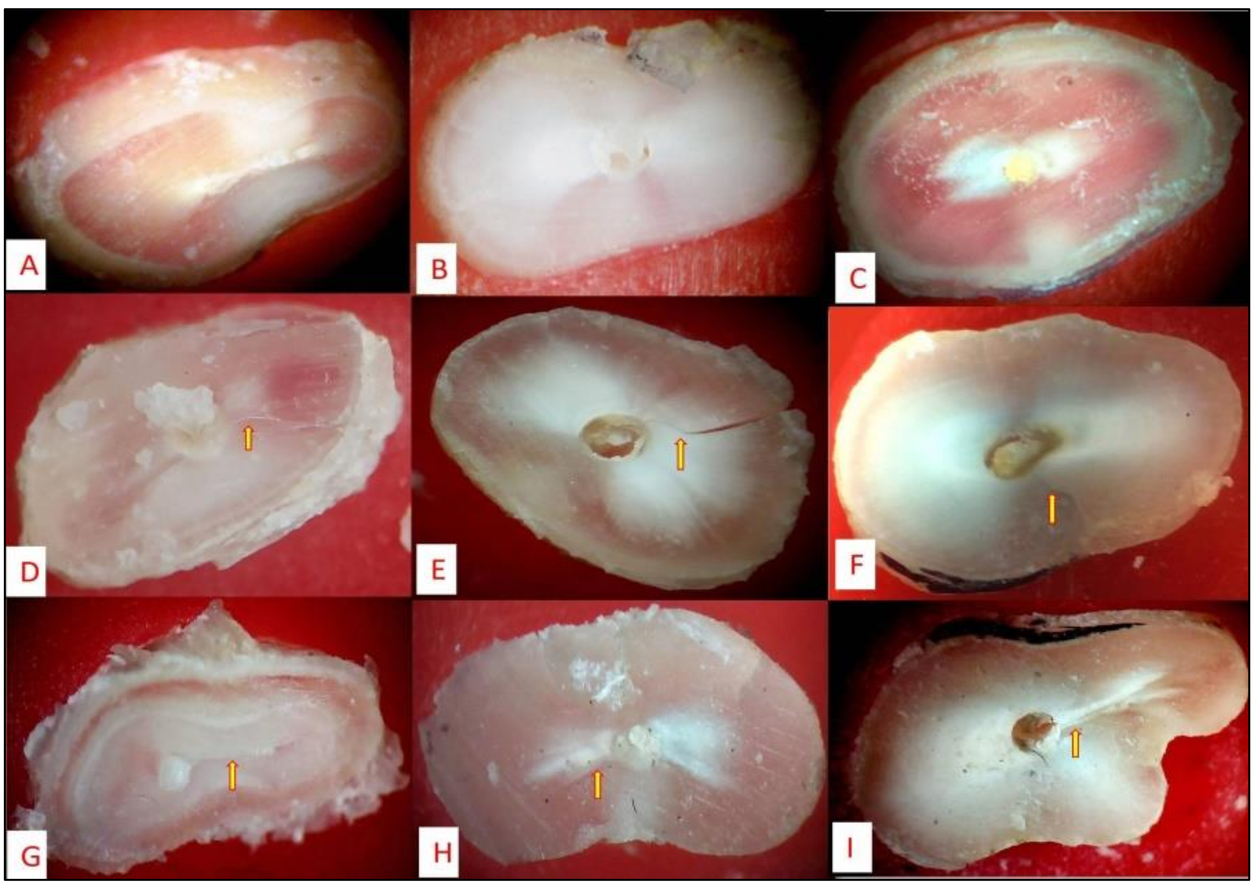

Figure 1: Stereomicroscopic assessment (at 20x) of dentinal defects at 2 mm, 4 mm and 6 mm from the apex in the control, ProTaper Next and OneShape groups (A) Control group showing no dentinal defects at 2-mm section, (B) Control group showing no dentinal defects at 4-mm section, (C) Control group showing no dentinal defects at 6-mm section, (D) ProTaper Next group showing dentinal

defects at 2-mm section, (E) ProTaper Next group showing dentinal defects at 4-mm section, (F) ProTaper Next group showing dentinal defects at 6-mm section, (G) OneShape group showing dentinal defects at 2-mm section, (H) OneShape group showing dentinal defects at 4-mm section, (I) OneShape group showing dentinal defects at 6-mm section.s

Table 1: Intergroup comparison of dentinal crack scores at different root levels using the Kruskal–Wallis test

Root lengths	Groups	n	Median	Mean Rank	Kruskal–Wallis χ^2 stats	p-value
At 2 mm	Control	15	0	12.5	18.94	0.001*
	ProTaper Next	15	2	28.67		
	One Shape	15	2	27.83		
At 4 mm	Control	15	0	12	19.97	0.001*
	ProTaper Next	15	2	28.13		
	One Shape	15	2	28.87		
At 6 mm	Control	15	0	9.5	29.09	0.001*
	ProTaper Next	15	2	30.13		
	One Shape	15	2	29.37		

*Data are expressed as median and mean rank; *p = 0.001 indicates statistically significant difference among groups (Kruskal–Wallis test).*

Table 2: Post-hoc pairwise comparison between groups at different root levels using Dunn–Bonferroni test

Pairwise comparison	2 mm		4 mm		6 mm	
	Test Statistic	p-value	Test Statistic	p-value	Test Statistic	p-value
Control - ProTaper Next	-16.17	0.001*	-16.13	0.001*	-20.63	0.001*
Control - OneShape	-15.33	0.001*	-16.87	0.001*	-19.87	0.001*
ProTaper Next - OneShape	0.83	0.842	-0.73	0.864	0.77	0.860

**p = 0.001 indicates statistically significant difference between groups (Dunn–Bonferroni post-hoc test).*

Table 3: Intragroup comparison of dentinal crack scores at different root levels using the Friedman test

Groups	Root length	n	Median	Mean Rank	Friedman χ^2 stats	p-value
ProTaper Next	At 2mm	15	2	2	0.3	0.861
	At 4 mm	15	2	1.9		
	At 6 mm	15	2	2.1		
OneShape	At 2mm	15	2	1.97	0.1	0.951
	At 4 mm	15	2	1.97		
	At 6 mm	15	2	2.07		

Data are expressed as median and mean rank. p > 0.05 indicates no statistically significant difference within the group (Friedman test).

Table 4: Association between canal preparation technique and dentinal crack formation at different root levels

Root lengths	Groups	No defect	Defect	Craze lines	Chi stats	p-value
At 2 mm	Control group	15 (100%)	0 (0.0%)	0 (0.0%)	21.25	0.001*
	ProTaper Next group	4 (26.7%)	10 (66.6%)	1 (6.7%)		
	OneShape group	5 (33.5%)	10 (66.6%)	0 (0.0%)		
At 4 mm	Control group	15 (100.0%)	0 (0.0%)	0(0.0%)	22.1	0.001*
	ProTaper Next group	4 (26.7%)	9 (60.0%)	2 (13.3%)		
	OneShape group	4 (26.7%)	10 (66.6%)	1 (6.7%)		
At 6 mm	Control group	15 (100.0%)	0 (0.0%)	0(0.0%)	34.13	0.001*
	ProTaper Next group	1 (6.7%)	11 (73.3%)	3 (20.0%)		
	OneShape group	2 (13.3%)	11 (73.3%)	2 (13.3%)		

*Data are presented as number (percentage); *p = 0.001 indicates statistically significant association using the Fisher exact test.*

Discussion:

The results of this study showed that both the ProTaper Next multi-file system and the OneShape single-file system significantly increased the incidence of dentinal defects, including partial cracks, complete fractures and craze lines, in mandibular premolars compared to uninstrumented controls. However, no statistically significant differences were observed between the two systems in terms of defect formation at any of the evaluated apical levels (2 mm, 4 mm and 6 mm). These findings support the rejection of the null hypothesis only in part, as instrumentation-induced defects, but the specific system, multi-file versus single file, did not differentially influence outcomes. The consistent median crack score of 2 across both experimental groups and all root depths underscores the inherent stress imposed by rotary instrumentation on radicular dentin, irrespective of the file number or design nuances. This aligns with substantial evidence from prior research, indicating that NiTi rotary instrumentation predisposes roots to microcracks by generating torsional and cyclic fatigue stresses during canal shaping. For instance, Bilaiya *et al.* **[11]** reported a higher prevalence of dentinal cracks in roots prepared with rotary systems compared to controls, which was attributed to the mechanical contact and frictional forces between files and dentin walls. Similarly, Oak *et al.* **[12]** found that ProTaper Next and HyFlex instruments induced cracks in 50-60% of specimens, although fewer than traditional ProTaper Universal systems, highlighting the role of advanced metallurgy, such as M-Wire in ProTaper Next, which enhances flexibility and reduces stress concentration. These findings are consistent with a study conducted by Gupta *et al.* **[13]**, which reported no significant differences in dentinal defect formation among rotary and reciprocating systems despite minor variations in fracture patterns. Similar observations were reported by another study **[14]**, in which all instrumented groups exhibited dentinal defects while the unprepared control group remained unaffected; although WaveOne and HyFlex EDM produced significantly more defects than some other systems, no significant differences were observed between several contemporary NiTi file systems **[15]**. Collectively, these findings support the present results, suggesting that canal instrumentation itself is the principal factor contributing to dentinal damage, whereas the influence of the specific file system appears to be comparatively limited. In our study, the elevated defect rates in both groups corroborated these observations, suggesting that heat-treated NiTi alloys cannot fully mitigate dentin weakening. Regarding the lack of difference between ProTaper Next and One Shape, our results are consistent with studies showing comparable defect induction between multi file and single-file systems when used in continuous rotation. Delavan *et al.* **[16]** evaluated OneShape alongside other systems using stereomicroscopy and found that OneShape system produced 10% cracks at 2 mm, 5% at 4 mm and 35% at 6 mm. similar findings were obtained in our study. More dentinal cracks at the 6 mm apical region can be attributed to the reduced dentinal thickness and greater stress concentration in the apical third during biomechanical preparation. The tapered design and aggressive cutting action of rotary instruments generate higher radial forces in the apical area, making it more susceptible to microcrack initiation and propagation. Additionally, the limited canal diameter apically restricts stress dissipation, leading to greater structural damage compared to the coronal levels, which is supported by the significantly higher defect prevalence observed at 6 mm in the experimental groups. Eliasz *et al.* **[17]** found that ProTaper Next produced fewer cracks than One Shape, possibly due to ProTaper Next's progressive taper reducing apical stress, whereas One Shape's single-file approach might concentrate forces. In contrast, Frater *et al.* **[18]** found no significant disparity in dentinal defects between rotary systems, irrespective of rotary motion. This may be explained by the shared design features: both ProTaper Next and OneShape systems employ 0.06 taper and asymmetric cross-sections (off-centered rectangular in ProTaper Next and variable in One Shape), which minimize screw-in effects but still exert similar hoop stresses on dentin. Methodological differences, such as varying tooth types (molars versus premolars) or evaluation techniques (micro-computed tomography versus sectioning), could account for these discrepancies. Our use of mandibular premolars, with their thin dentin walls, may amplify the uniform defect susceptibility and override system-specific advantages observed elsewhere. The absence of intragroup variations in crack scores across root depths further supports a homogeneous distribution of defects. In our protocol, standardized working lengths, EDTA lubrication and controlled torque (2-2.5 Ncm) likely distributed stresses evenly, preventing depth-specific escalation. This uniformity implies that defects arise from cumulative instrumentation rather than localized apical preparation, as both systems achieve similar final apical sizes. These findings contribute to the ongoing debate on single-file versus multifile efficacy. While single-file systems, such as One Shape, promise efficiency by reducing procedural steps and cross-contamination risks **[19]**, our data suggest that they do not inherently spare dentin integrity compared to sequenced multi-file systems, such as ProTaper Next. Clinically, these results underscore the need for caution when instrumenting teeth prone to vertical root fractures such as mandibular premolars. Although *in vitro* defects may not directly translate to clinical failures, they represent potential initiation sites for crack propagation under occlusal loads, potentially compromising long-term prognosis. Clinicians might prioritize systems based on efficiency (favoring OneShape for time savings) or centering ability (favoring ProTaper Next per some transportation studies); however, they should incorporate adjunctive measures such as passive ultrasonic irrigation or bioceramic sealers to enhance outcomes. Limitations include the *in vitro* nature, which excludes periodontal ligament simulation and functional loading, potentially overestimating defects, sectioning artifacts from diamond discs, though minimized by irrigation and reliance on stereomicroscopy, which is destructive and less sensitive than non-invasive micro-computed tomography for detecting subclinical microcracks. Future studies should employ longitudinal micro-CT assessments and incorporate fatigue testing to better simulate the clinical scenarios.

Conclusion:

Rotary instrumentation significantly increased dentinal defects irrespective of the file system used. ProTaper Next and OneShape showed comparable performance with no significant difference in defect formation. Preservation of root dentin should remain a key consideration during biomechanical preparation.

Advancement to knowledge:

This study provides comparative evidence that both ProTaper Next and OneShape rotary systems produce a similar incidence and distribution of dentinal microcracks at standardized apical levels (2, 4 and 6 mm) in mandibular premolars. The findings suggest that the rotary instrumentation process itself, rather than the choice of these two continuous-rotation file systems, is the primary contributor to dentinal defect formation, thereby informing evidence-based file selection in endodontic practice.

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