

Evaluation of surface wear in rotary and reciprocating nickel-titanium instruments after use in curved root canals

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Doctoral thesis / Disertacija

2023

Degree Grantor / Ustanova koja je dodijelila akademski / stručni stupanj: **University of Zagreb, School of Dental Medicine / Sveučilište u Zagrebu, Stomatološki fakultet**

Permanent link / Trajna poveznica: <https://um.nsk.hr/um:nbn:hr:127:833816>

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School of Dental Medicine
University of Zagreb

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DISSERTATION

Zagreb, 2022



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Supervisor: professor Ivica Anić, PhD

Zagreb, 2022



Sveučilište u Zagrebu

Stomatološki fakultet

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**Evaluacija površinskoga trošenja
rotacijskih i recipročnih nikal-titanskih
instrumenata nakon uporabe u
zavijenim korijenskim kanalima**

DOKTORSKI RAD

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Zagreb, 2022.

The dissertation was completed at the Department of Endodontics and Restorative Dentistry at the School of Dental Medicine, University of Zagreb and Vinča Institute of Nuclear Sciences, Belgrade.

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1. _____
2. _____
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Committee for the doctoral dissertation defense:

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Dissertation defense date: _____

This dissertation contains: 83 pages
5 tables
25 figures
1 CD

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Acknowledgments

I thank my mentor, Professor Ivica Anić, for sharing his knowledge about scientific research, academic writing, and research ethics, and his love for endodontics. I thank Professor Ivona Bago for her great help with the publishing and reviewing of the papers. I thank Dubravka Milovanović and Jelena Savović from the Vinča Institute of Nuclear Sciences without whom optical profilometry would be impossible. I thank my parents for their constant support and for teaching me about the importance of academic education and advancement. I thank my brother for considering me his role model.

I dedicate my dissertation to Dela, Adem and Bedžo.

Without you, I wouldn't be the person I am today.

ABSTRACT

EVALUATION OF SURFACE WEAR IN ROTARY AND RECIPROCATING NICKEL-TITANIUM INSTRUMENTS AFTER USE IN CURVED ROOT CANALS

The aim of the study was the evaluation of surface roughness in reciprocating and rotary single-file nickel-titanium (NiTi) instruments, after two uses in curved root canals. A total of 40 new NiTi instruments were divided into 4 groups of 10 instruments, according to their manufacturer: Reciproc Blue, WaveOne Gold, XP-endo Shaper and TruNatomy. Instruments in every group were then randomly divided into 2 subgroups: a) instruments for use in artificial curved canals and b) instruments for use in human curved canals. Artificial blocks containing a simulated curved canal (length 19 mm; angle of curvature of 40°) were used (*Diadent* Group International, Korea). In the human teeth subgroup, roots of molar teeth with a single root canal and 25° – 40° curvature (Schneider) were selected. Each instrument was used twice in two different root canals. Instrumentation was performed according to the manufacturer's instructions by the same researcher using the same irrigation protocol, at body temperature (37°C). 3D noncontact optical profilometry (NewView™ 7100; Zygo Corporation, Middlefield, CT, USA) was used for surface roughness analysis, before and after the first and second usage. After conducting two instrumentation procedures, tested instruments were submitted to cyclic fatigue test, to determine their time to fracture (TTF). The data were statistically analyzed using one-way ANOVA with Bonferroni posthoc tests using MedCalc® (MedCalc Software Ltd, Ostend, Belgium) software. The Mann-Whitney U test was used to compare cyclic fatigue between the tested instruments. The statistical significance level was set at 5%. In the artificial canals, the XPS instruments showed significantly higher roughness values compared to TruNatomy and reciprocating instruments, at every stage, especially after the second use ($p < .008$). In extracted teeth, the novel rotary instruments showed no significant difference in roughness compared to the reciprocating instruments, irrespective of the evaluation stage ($p = 1.0$). Contrary, in both subgroups, reciprocating instruments had significantly higher CF resistance compared to rotary instruments ($p < .05$). The novel rotary and reciprocating systems showed similar surface wear, after two uses in curved root canals. Reciprocating instruments exhibited superior cyclic fatigue resistance compared to novel rotary instruments. Optical profilometry enabled precise, accurate, and repeatable measurements of surface roughness, and could be considered an adequate method in these types of studies.

Keywords: instrument wear, NiTi instruments, cyclic fatigue, optical profilometry

SAŽETAK

EVALUACIJA POVRŠINSKOGA TROŠENJA ROTACIJSKIH I RECIPROČNIH NIKAL-TITANSKIH INSTRUMENATA NAKON UPORABE U ZAVIJENIM KORIJENSKIM KANALIMA

Svrha istraživanja jest analiza trošenja rotacijskih i recipročnih single file nikal-titanskih (Ni-Ti) instrumentacijskih sustava nakon dviju uporaba u zavijenim korijenskim kanalima. Ukupno 40 novih Ni-Ti instrumenata podijeljeno je u 4 skupine (n=10), prema proizvođaču: Reciproc Blue, WaveOne Gold, XP-endo Shaper i TruNatomy. Instrumenti su potom nasumično podijeljeni u dvije podskupine (n=5): a) za primjenu u umjetnim zavijenim kanalima, n=40 i b) instrumenti za primjenu u humanim zavijenim kanalima, n=40. U prvoj podskupini korišteni su simulirani korijenski kanali, duljine od 19 mm i zavijenosti 40° (*Diadent Group International, Korea*). Umjetni kanali odabrani su zbog standardizirane duljine i zakrivljenosti. Podskupinu humanih kanala sačinjavali su korijeni molara s jednim korijenskim kanalom, zavijenosti od 25° do 40° prema Schneideru. Humani kanali odabrani su tako da budu što sličniji umjetnim, prema stupnju zakrivljenosti, a s ciljem usporedbe rezultata i imitiranjem kliničkih uvjeta. Svaki instrument korišten je dva puta, za instrumentaciju dvaju različitih korijenskih kanala. Isti istraživač, s iskustvom u strojnoj endodonciji napravio je instrumentaciju, prema uputama proizvođača za svaki instrumentacijski sustav, na tjelesnoj temperaturi (37°C). Tijekom instrumentacije korišteno je ukupno 5 mL 2.5% natrijevog hipoklorita za ispiranje u svakom korijenskom kanalu. Metodom 3D beskontaktna optičke profilometrije (*NewView™ 7100; Zygo Corporation, Middlefield, CT, USA*) analizirana su tri parametra površinske hrapavosti, 3 mm udaljeno od vrha instrumenta. Profilometrijska analiza napravljena je tri puta za svaku skupinu: prije, nakon prve i nakon druge uporabe instrumenta. Nakon dviju uporaba instrumenti su podvrgnuti testu cikličkog zamora, kao pokazatelja otpornosti na lom. U cikličkom testu svaki instrument korišten je u umjetnom kanalu zavijenosti 60°, tijekom kojeg je mjereno vrijeme potrebno da dođe do loma instrumenta. Anova test i Bonferroni post-hoc testovi korišteni su za analizu podataka dobivenih optičkom profilometrijom, a Mann-Whitney U test za analizu i usporedbu rezultata testa cikličkog zamora, uz razinu statističke značajnosti od 5%, u svim testovima, osim Mann-Whitney U testa u HK skupini, gdje je razina značajnosti bila 1%.

U podskupini umjetnih zavijenih kanala, XP-endo Shaper pokazao je značajno veće trošenje u odnosu na sve ostale skupine, pri svakom mjerenju, a osobito nakon druge primjene ($p < .008$). U podskupini humanih zavijenih kanala, vrijednosti hrapavosti povećale su se nakon primjene u svim skupinama, ali razlike između skupina nisu bile statistički značajne ($p = 1.0$). U testu cikličkog zamora, rezultati su bili u korelaciji u objema podskupinama. Recipročni instrumenti pokazali su značajno veću otpornost na lom u odnosu na rotacijske instrumente nakon dviju primjena u zavijenim kanalima ($p < .05$). Recipro Blue je imao najduže vrijeme potrebno da dođe do loma instrumenta, a Xp-endo Shaper najkraće.

U ovom istraživanju, recipročni i rotacijski instrumenti imali su slično i minimalno površinsko trošenje nakon dvostruke uporabe u zavijenim korijenskim kanalima. Recipročni instrumenti pokazali su značajno veću otpornost na ciklički zamor u odnosu na rotacijske ($p < 0.05$). Optička profilometrija omogućila je precizno, ponovljivo i objektivno mjerenje parametara površinskog trošenja, te se može smatrati pouzdanom metodom za ovu vrstu istraživanja.

Uz ograničenja ovog tipa istraživanja, a obzirom na klinički značaj, moglo bi se zaključiti da su i recipročni i novi rotacijski single-file instrumentacijski sustavi, jednako sigurni za primjenu u dva zavijena korijenska kanala, bez straha od loma instrumenta.

Ključne riječi: površinsko trošenje, nikal-titanski instrumenti, ciklički zamor, optička profilometrija

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List of abbreviations:

AFM	Atomic force microscopy
AB	Acrylic blocks
ANOVA	Analysis of variance
CCW	Counterclockwise
CW	Clockwise
ET	Extracted teeth
NaOCl	Sodium hypochlorite
Ni-Ti	Nickel – Titan
RB	Reciproc Blue
SEM	Scanning electron microscopy
SS	Stainless steel
SR	Surface roughness
TRN	TruNatomy
WOG	WaveOne Gold
XPS	XP-endo Shaper
WL	Working length

1. INTRODUCTION

The main goal of root canal treatment is the elimination of infection (infected pulp tissue, bacterial biofilm, debris) from the root canal system. This is achieved by mechanical instrumentation and chemical irrigation. Manual and engine-driven endodontic instruments are used for the mechanical shaping of the root canal, while chemical irrigation ensures disinfection and dissolution of the pulpal tissue. Criteria for successful root canal instrumentation include: shaping a continuously tapered root canal, maintaining the original shape of the canal, maintaining the position of the apical foramen, smooth canal walls, and making the apical stop (1).

Currently, there are many instrumentation systems on the market. They differ in diameter, cross-section, conicity, number of cutting surfaces, length, number of instruments in the sequence, and movement kinematics. The development of new instruments nowadays is a fast and market-driven process. Clinical procedures and ideal clinical characteristics are still being refined as new instruments continue to be introduced to the market.

1.1. History and development of endodontic instruments

In 1838, Edward Maynard created the first endodontic instrument made from a spring of a clock and used it for cleaning and widening the root canal. The first endodontic instruments were made of carbon steel, whose hardness, greater than dentin, made them very rigid. They were also highly corrosive, which negatively affected their resistance to fracture (2). The replacement of carbon steel with stainless steel instruments solved the corrosion problem. These stainless steel manual instruments have a colored plastic handle and a 0.02 mm taper increase in the active part. However, lack of flexibility remained a concern, which made the instrumentation in curved canals difficult, producing iatrogenic errors and changes in the root canal anatomy.

The introduction of the NiTi alloy into dentistry in 1963 presented a revolution in the development of endodontic instruments. This alloy, characterized by great flexibility, shape memory, and super-elasticity, enabled further development of engine-driven instruments (3).

The first reference to rotary instrumentation was made by Oltramare in 1892 (4), who used needles that could be attached and rotated in a dental handpiece. Consequently, after the first endodontic rotary handpiece was introduced and together with the appearance of the NiTi

alloy in endodontics, engine-driven endodontic instruments have started to develop. That resulted in numerous instrumentation systems currently available on the market that are chronologically divided into five generations, based on time of appearance (5).

1.1.1. First-generation endodontic instruments

The first commercially available rotary files appeared on the market in the mid-1990s. These rotary files had passive cutting radial lands and fixed tapers of 4% and 6%. The most important NiTi rotary instruments in this category are LightSpeed (Kerr, USA), Profile (Maillefer, Ballaigues, Switzerland), Quantec (SybronEndo, USA), and GT system (Dentsply, Tulsa). The passive cutting blade and the same conicity along the working part were the main features of this generation of NiTi rotary instruments. Furthermore, this generation's deficiency was the large number of files required to achieve optimal shaping (6).

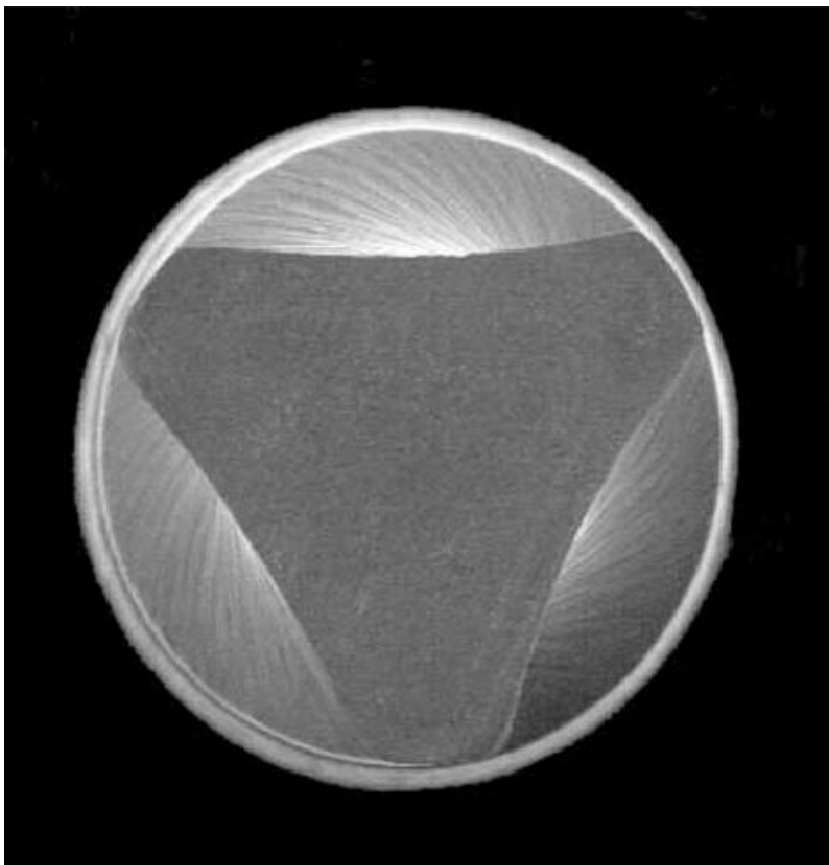


Figure 1. Cross-section of Profile. Courtesy of Kim et al. (7).

1.1.2. Second-generation endodontic instruments

NiTi rotary files of the second generation were released onto the market in 2001. Compared to the previous generation, these instruments needed fewer instruments for complete cleaning and shaping because they had active cutting edges and greater cutting efficiency. ProTaper Universal (Dentsply Maillefer, Switzerland), K3 (SybronEndo, USA), M-two (VDW, Germany), Hero Shaper (Micro-Mega, France), I Race, and I Race Plus are among the instruments from this generation (FKG Dentaire, Switzerland).

ProTaper instruments have variable conicity along the same instrument. The system consists of 6 instruments: three shaping and three finishing files. During the manufacturing process, BioRaCe instruments (FKG Dentaire, Switzerland) undergo an electropolishing procedure to reduce the number of surface defects and enhance the mechanical properties (8). The surface properties of the NiTi instrument contribute to fatigue resistance because most fatigue failures stem from the surface, especially in the presence of surface defects (9). As a result, manufacturers began to focus on other methods to increase the instruments' resistance to fracture during this time period. Attempts to improve the surface of NiTi instruments, cyclic fatigue resistance, and cutting efficiency have resulted in a variety of strategies, including ion implantation (10) and electropolishing (11-13).

Instrument conicity varies from one ProTaper to the next. This system consists of six instruments: three for shaping and three for finishing. In order to decrease the number of surface defects and improve the mechanical properties, BioRaCe instruments (FKG Dentaire, Switzerland) are electropolished throughout the production process (8). Since most fatigue failures originate at the surface, especially when surface defects are present, the surface characteristics of the NiTi instrument are important factors that influence fatigue resistance (9). Because of this, at this time, manufacturers started concentrating on other strategies to improve the instruments' resistance to fracture. Ion implantation (10) and electropolishing are two techniques that have been used to improve the surface of NiTi instruments, resistance to cyclic fatigue, and cutting efficiency (11-13).

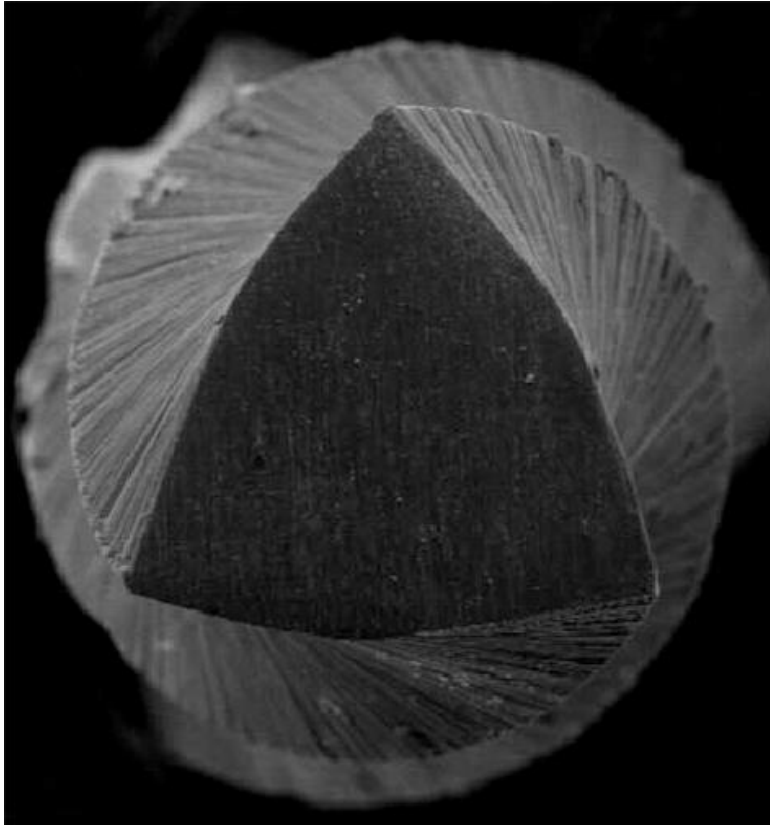


Figure 2. Cross-section of ProTaper. Courtesy of Kim et al. (7).

1.1.3. Third-generation endodontic instruments

Improvements in NiTi metallurgy marked the third generation of endodontic instruments. The use of heat treatment (thermal processing) in manufacturing procedures was the key to adjusting the transition temperatures of NiTi alloys and affecting the fatigue resistance of NiTi endodontic files (14). M-wire technology, R-phase technology, Controlled Memory Wire, and Electrical Discharge Machining were notable changes in NiTi metallurgy.

M-wire (SportsWire, Langley, OK) was introduced in 2007. It is created by subjecting NiTi wire to several heat treatments. Dentsply's ProFile GT Series X, ProFile Vortex, and Vortex Blue are M-wire instruments.

In 2010, a unique NiTi alloy with increased flexibility called CM Wire (DS Dental, Johnson City, TN) was introduced. The memory of the material can be controlled during the special thermomechanical process, making instruments made from CM Wire exceptionally flexible, but with less shape memory than conventional NiTi files.

The first fluted NiTi file made via plastic deformation - a method similar to twisting used to make the majority of stainless-steel K-files and reamers - was introduced by SybronEndo in 2008.

1.1.4. Fourth-generation endodontic instruments

Since 1958, a reciprocating movement, defined as any repetitive back-and-forth motion, has been used to drive stainless-steel files. In 2008, Dr. Ghassan Yared described the unequal clockwise and counterclockwise motion that could make optimal shaping of any root canal possible, using just one 25/0.08 ProTaper instrument (15). Since then, the new reciprocation technique has sparked a lot of interest.

Three years after, in 2011, the first reciprocating single-file shaping techniques were introduced: WaveOne (Dentsply Tulsa Dental Specialties, USA/Dentsply Maillefer, Switzerland) and Reciproc (VDW, Germany). Both files are made from M-wire. The use of a single instrument for complete cleaning and shaping of the root canal, so-called “single-file technique“, was a big success derived from the reciprocating philosophy.

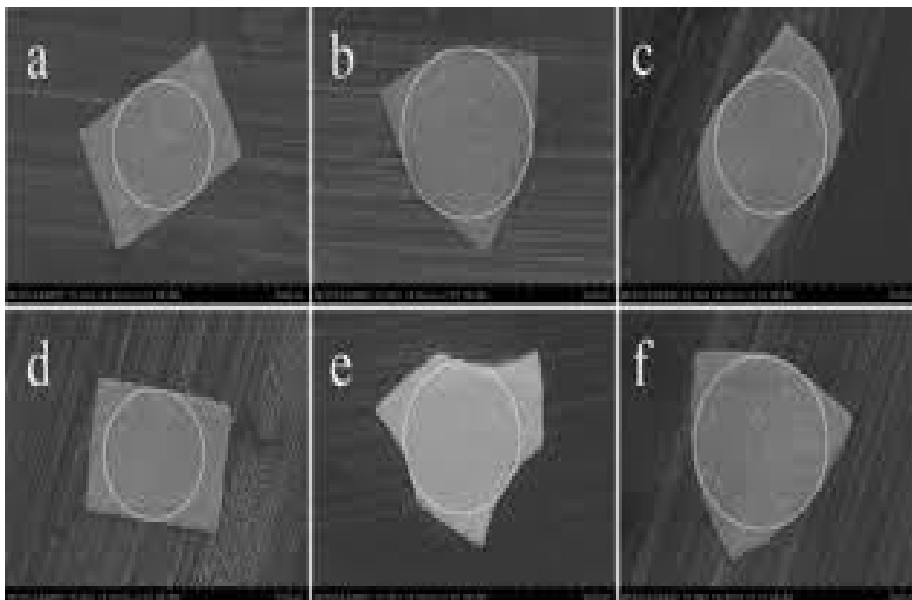


Figure 3. Cross-sectional configurations for the six files (110×): WaveOne Gold, ProTaper Gold, Reciproc Blue, ProTaper Next, WaveOne, and ProTaper. Courtesy of Hou et al. (16).

1.1.5. Fifth-generation endodontic instruments

The fifth generation of endodontic instruments has been designed so that the center of rotation is offset, compared to the previously used centered mass of rotation. This design provides less engagement between the instrument and dentin (14). Reduced engagement protects the instrument from undesirable screw-in effect, limits the torque, and increases resistance to torsional fatigue.

Revo-S (Micro-Mega, France), One Shape (Micro-Mega, France), and ProTaper Next (Dentsply Maillefer, Switzerland/Dentsply Tulsa Dental Specialties, USA) are commercial examples of instrument brands that offer variations of this technology. The ProTaper Next instruments have three notable design features: multiple tapers on a single instrument, M-wire technology, and an offset design. The “single-file“ NiTi instruments used in continuous rotation are One Shape, TruNatomy, and Xp-endo Shaper.

1.2. Mechanical properties of the nickel-titanium alloy

The introduction of the Nickel-Titanium (NiTi) alloy into fundamentally altered root canal instrumentation can be considered a technological revolution that marked the beginning of 'modern endodontics' (17).

The era of “modern endodontics” is connected to the publication of the article titled "An initial investigation of the bending and torsional properties of Nitinol root canal files", in which the NiTi alloy was proposed as a material for the manufacturing of endodontic instruments for the first time. (18). This article highlighted the limitations of stainless-steel (SS) hand instruments. These limitations are particularly seen in curved root canals, where due to the stiffness of SS, ledge formation, root perforation or transportation, and separation of the instrument are more likely to occur (19-21).

The NiTi alloy was developed by the Naval Ordnance Laboratory (White Oak, MD, USA) (22). The name Nitinol is an acronym for nickel (Ni), titanium (Ti), and Naval Ordnance Laboratory (nol). Andreasen and Hilleman (23) used this alloy for the first time in dentistry in 1971 for the manufacture of orthodontic wires. In particular, in endodontics, the fabrication of

endodontic instruments from the NiTi alloy was conceived in 1975. Walia, Brantley, and Gerstein later introduced the first NiTi endodontic instruments made of orthodontic wire in 1988 (18).

The elastic behavior of a material is defined by Hooke's Law, which states that deformation made in a material is directly proportional to the deforming force or load. When the applied force exceeds a certain point, called yield strength, the deformation is irreversible, and it is called plastic deformation. Most metal alloys exhibit an elastic range of 0.1 or 0.2%. The NiTi alloy, on the other hand, can be deformed up to 8% beyond the yield strength point, without any plastic deformation (24). This property is known as superflexibility or pseudo-elasticity and is defined as the material's ability to recover its original shape after deformation.

The NiTi alloy used in endodontic instruments contains approximately 56 wt% nickel and 44 wt% titanium, resulting in a nearly equiatomic ratio (24). This equiatomic NiTi alloy has the ability to alter its crystallographic structure when exposed to different temperatures or stresses, which significantly influences its mechanical properties (25).

There are three different temperature-dependent crystallographic structures of the NiTi alloy:

1. Austenitic NiTi (austenite) has a body-centered cubic crystal structure, stable at higher temperatures and lower stresses. The alloy in this phase is hard and has super-elastic properties (26). Austenitic instruments exhibit high torque values at fracture. As a result, these files are suitable for shaping straight or slightly curved root canals.
2. Martensitic NiTi (martensite) has a stable monoclinic structure at higher temperatures and stresses. When in the martensitic phase, the NiTi alloy is soft and ductile, easily deformed, and has shape memory (26). Martensitic instruments are more flexible and have greater cyclic fatigue resistance. Failure caused by cyclic fatigue more likely occurs in severely curved root canals. That is why martensitic instruments should be used to treat curved root canals with greater safety. Furthermore, martensitic instruments are prebendable, due to shape memory, which may be very useful in treating complex root canal anatomies.
3. The R-phase is an intermediate phase with a rhombohedral structure that is formed during the transformation from martensite to austenite upon heating and during the reverse transformation from austenite to martensite upon cooling (27). The formation of the R-phase is favored by the presence of dislocations and precipitates in the NiTi

alloy (28). A substantial density of dislocations is expected in NiTi orthodontic wires and endodontic instruments because the alloy experiences considerable permanent deformation during the manufacturing processes (27).

1.1.6. Martensitic transformation

Austenite to martensite transition, or martensitic transformation, can be induced by stress or temperature.

If a NiTi orthodontic wire or endodontic instrument is exposed to sufficiently low temperatures, it will consist entirely of martensite. Contrary, upon heating, martensite will start transforming to R-phase at the R_s temperature, and this transformation will be finished at the R_f temperature. With further heating, R-phase starts transforming to austenite at the A_s temperature, and the transformation is finished at the A_f temperature. Alternatively, if a NiTi orthodontic wire or endodontic instrument is heated above the A_f temperature, it will be converted entirely to austenite. This transformation process is reversible. Thus, upon cooling to a sufficiently lower temperature, the alloy starts transforming the opposite way, from austenite to R-phase and eventually to martensite at the M_f temperature. These transformation processes are summarized in Figure 4.

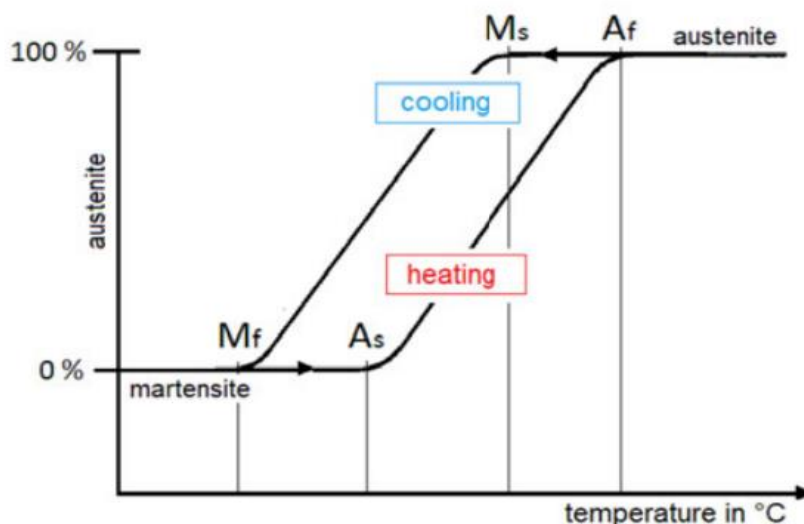


Figure 4. Temperature hysteresis diagram of the NiTi alloy. (M_s) martensite start temperature, (M_f) martensite finish temperature, (A_s) austenite start temperature, and (A_f) austenite finish temperature. Courtesy of Zupanc et al. (29).

Therefore, the most important tool for manufacturers to alter the phase composition and consequently mechanical properties of the NiTi alloy is a change in the transformation temperature. This can be achieved through thermal and mechanical treatment or chemical composition variation (30).

The other factor which can induce martensitic transformation is stress. This is known as stress-induced martensite (SIM) transformation. For example, SIM transformation can be induced by the insertion of the instrument into a curved root canal. As the alloy is not stable at the body temperature, unloading the instrument or withdrawing the instrument out of a curved root canal causes retransformation to the austenite phase (29).

The two unique features that occur as a result of the martensitic transformation in the NiTi alloy are shape memory and super-elasticity (24).

1.1.7. Super-elasticity

The super-elastic behavior of NiTi wires means that they have the ability to return to their original shape upon unloading, removing the force that caused the deformation (24). In order to utilize this property of the NiTi alloy, endodontic instruments should mainly consist of austenite (26). Austenite possesses super-elastic properties and consequently, they tend to spring back to their original shape, after deformation. As previously mentioned, the super-elasticity of nickel-titanium allows the range of 8% elastic deformation, compared to the maximum of 1% in other alloys, such as stainless steel. Although some other alloys have been found to have even better super-elastic properties, they could not overcome the superiority of NiTi in terms of its great biocompatibility and resistance to corrosion (24).

1.1.8. Shape memory

The shape memory is a property of an alloy that can be deformed when cold but returns to its pre-deformed ("remembered") shape when heated. It occurs through forming strong, energetic electron bonds that pull back displaced atoms to their previous positions. Martensitic instruments show a shape memory effect when heated. The instruments made of martensitic alloy are more flexible, with increased cyclic fatigue resistance, compared to austenitic alloy.

1.1.9. Advantages of using NiTi engine-driven instruments

The introduction of NiTi into endodontics has resulted in undeniable benefits, which can be summarized in three points (31, 32).

- Root canal treatment time is reduced

The instrumentation technique with manual SS files needs a greater number of instruments, resulting in a longer treatment time. On the other hand, the increased cutting efficiency of NiTi rotary instruments combined with the use of increased taper allows clinicians to significantly reduce treatment time (17).

- Instrumentation procedure simplification

In general, NiTi instruments have significantly simplified the instrumentation technique when compared to traditional instrumentation using SS files. Because of the improved mechanical properties, it is possible to shape the root canal while maintaining its original trajectory and anatomy (33).

- Improved predictability and efficacy of endodontic treatments

The super-elasticity of the NiTi alloy allows the use of endodontic instruments with a larger taper with a lower risk of fracture, thus improving the root canal shaping and filling process (34).

1.3. Thermomechanical treatment of the Ni-Ti alloy

At the beginning of 2000, many studies found that heat-induced changes were effective in increasing the flexibility of NiTi endodontic instruments (35-38). Since then, heat treatment is used in manufacturing procedures to alter the properties of NiTi endodontic instruments. This proprietary thermomechanical process is very sophisticated and integrates hardening and heat treatment.

The conventional NiTi alloy has the austenitic configuration in the root canal because martensite is unstable at body temperature. However, different thermal or mechanical treatments can change the transformation temperature in such a way that endodontic instruments could be martensitic under clinical conditions. Precisely that was the main

objective of different thermomechanical treatments, which resulted in a variety of types of the NiTi alloy and, consequently, a great number of engine-driven instruments (Table 1).

Table 1. Overview of NiTi alloys used for the manufacture of endodontic instruments
Courtesy of Zanza et al. (39).

Alloy	Crystallographic Phase	NiTi System
<i>Conventional NiTi alloy</i>	Austenitic	Mtwo OneShape ProFile ProTaper Universal RaCe, BioRaCe, iRace F360, F6 Skytaper EdgeTaper
<i>R-phase</i>	Austenitic	Twisted File Twisted File Adaptive K3XF (not twisted)
<i>M-Wire</i>	Austenitic with small amounts of R-phase and martensite	ProFile Vortex ProFile GT Series X ProTaper Next Reciproc WaveOne
<i>CM Wire</i>	Martensitic with varying amounts of austenite and R-phase	Hyflex CM TYPHOON Infinite Flex NiTi Files V-Taper 2H Hyflex EDM
<i>Gold heat-treated</i>	Martensitic with varying amounts of austenite and R-phase	ProTaper Gold WaveOne Gold
<i>Blue heat-treated</i>	Martensitic with varying amounts of austenite and R-phase	ProFile Vortex Blue Reciproc Blue Rotate
<i>MaxWire</i>	Martensitic (20 °C), austenitic (35 °C)	XP-endo Finisher XP-endo Shaper
<i>T-Wire</i>	Martensitic with varying	2Shape

	amounts of austenite and R-phase	
<i>c-Wire</i>	Martensitic with varying amounts of austenite and R-phase	OneCurve RECI One
<i>FireWire</i>	Martensitic with varying amounts of austenite and R-phase	EdgeOne Fire EdgeSequel Sapphire EdgeTaper Platinum EdgeFile X7
<i>AF-R Wire</i>	Martensitic with varying amounts of austenite and R-phase	AF-One
<i>FKG heat treatment</i>	Phase transition ranging from 32 °C to 35 °C (between martensite and austenite)	R-Motion file System Race Evo

1.4. Rotary instrumentation systems

1.4.1. Rotational movement

a) Centric rotary motion

Rotary instrumentation was first mentioned by Oltramare in 1892. (4). He described the usage of fine needles with a rectangular cross-section attached to a dental handpiece for root canal instrumentation. In 1899, Rollins (40) developed the first endodontic handpiece for root canal instrumentation. After the introduction of NiTi endodontic hand instruments by Walia et al. (18), many rotary systems have been introduced into the market.

Centric rotary motion, described in the late 1980s, is performed by electric motors and reduction contra-angle handpieces driving NiTi files in full rotational motion of 360° within the root canal.

b) Eccentric rotary motion

Eccentric rotary motion means that the instrument rotates eccentrically or asymmetrically because of the non-centered axis of rotation. This motion is achieved by the specific design of an instrument, with an off-centered cross-section. The first available systems using this asymmetrical motion were Revo-S (MicroMega, France), followed by ProTaper Next (Dentsply, Tulsa), OneShape (MicroMega, France), TRUShape (Dentsply, Tulsa), and more recently XP-endo Shaper (FKG, Switzerland). ProTaper Next system has an asymmetrical rectangular cross-section. TRUShape system (Dentsply Sirona) instruments are heat-treated, with an S-shaped long axis, a triangular cross-section, and variable taper. Thus, these systems are recommended for canals with irregular anatomies because they can achieve more conservative preparation and also have a greater contact of the instruments with the canal walls (41).

1.4.2. Xp-endo Shaper

In 2015, a special NiTi alloy known as MaxWire (Martensite-Austenite Electropolishing-Flex, FKG) was developed for the manufacture of instruments in the XP-endo family. In 2019, this company introduced the latest expansions to this system, so-called XP-endo solutions, which comprise 2 different files: XP-endo Shaper and XP-endo Finisher. These, so-called “3D“ instruments, expand at body temperature to achieve safe and easy management of root canal treatment while preserving dentine. These instruments are used in eccentric rotary motion at higher speed and lower torque.

The XP-endo Shaper is a snake-like instrument used to shape root canals. When expanded at temperatures of 35°C or higher, it takes a semicircular shape. It is in the martensitic phase at room temperature. When introduced into the canal, at body temperature, it changes shape due to the austenitic phase's molecular memory. It has a Booster tip, which gives it a distinct geometry, and six sharp edges.

According to the manufacturer, it can start shaping at ISO diameter 15 and achieve ISO diameter 30, but also increase the taper from .01 to at least .04. It allows a canal shaping of a minimum of 30/.04 with only one instrument (42).

In contrast to traditional rotary files that change root canal shape into their shape, these instruments adapt to the root canal anatomy. Thus, XP-endo Shaper adapts to the morphology

of the root canal, expanding or contracting along the working length. This implies that the final preparation size depends on the root canal morphology and not *vice versa* (43).

The XP-endo Finisher is available in two sizes with zero taper: ISO 25 and ISO 30. It is intended to provide additional cleaning after chemo-mechanical preparation by touching hard-to-reach areas of the root canal. At the same time, it preserves dentin and the root canal anatomy. It has been reported that in combination with chemical irrigation, the XP-endo Finisher promotes significant bacterial reduction and biofilm removal from the main canal and dentin tubules (42).

1.4.3. *TruNatomy*

TruNatomy (TRN; Dentsply Sirona, Maillefer, Ballaigues, Switzerland) is, together with the ProTaper Ultimate, the newest released rotary file system on the market. The system consists of an orifice modifier, TruNatomy Glider, and three shaping files. The TRN shaping files are provided in three different sizes: Small, Prime, and Medium. TRN Small prepares the root canal to a diameter of 0.20 mm with a taper of .04 over the first apical millimeters. TRN Prime prepares the root canal to a diameter of 0.26 mm with a taper of .04 over the first apical millimeters. TRN Medium prepares the root canal to a diameter of 0.36 mm with a taper of .03.

The name “TruNatomy“ is an abbreviation of the words true natural anatomy, thus emphasizing the aim of preserving the structural dentine and ensuring the instrument adapts to the canal, not *vice versa*.

These instruments are manufactured from 0.8 mm NiTi wire instead of 1.2 mm NiTi wire, which is used to manufacture most other instruments. According to the manufacturer, this provides slim shaping of the root canal and provides more space for debridement. The manufacturing process results in a file that has a colored appearance. Due to this proprietary processing and special NiTi heat-treated wire, they have greater flexibility, which allows the file to be pre-bent when needed. Thanks to its superior canal-centering ability, the instrument can adapt to the canal. However, the manufacturer did not disclose any details about the type of NiTi wire used (44). The TRN instruments have an off-centered parallelogram cross-section design and operate in rotational motion at a higher speed (500 rpm) and with less torque (1.5 Ncm). All the TruNatomy files are intended for “single use only“ (on one patient

during a single procedure) and support at least 4 canals, 35° curved (i.e. Schneider technique). TRN instruments preserve the structural dentine and tooth integrity due to instrument geometry, regressive tapers, and slim design, along with the heat-treatment of the NiTi alloy (45).

1.5. Reciprocating instrumentation systems

1.5.1. Reciprocating movement

a) Rotational reciprocating motion

Since the Giromatic system (MicroMega, France) introduced reciprocation in 1964, various endodontic reciprocating handpieces have been manufactured (46). These systems used 90° clockwise (CW) and counterclockwise (CCW) motion angles. The Giromatic system fell out of use over time because it produced more procedural errors than hand files. Current examples of reciprocating handpieces that use small and equal 30° angles of CW and CCW rotation include the M4 (SybronEndo, Orange, CA, USA), Endo-Eze (Ultradent Products Inc., South Jordan, UT, USA), and Endo-Express SafeSider (Essential Dental Systems, South Hackensack, NJ, USA). Using small stainless-steel hand files, these handpieces allow the formation of an endodontic glide path (47). With the introduction of the NiTi alloy, endodontic torque control motors, and a balanced-force technique (unequal CW and CCW motions), reciprocating motion, regained popularity (46).

b) Single file reciprocation

Yared first proposed single-file reciprocation in 2008 (REF). In his experiment, he used a single F2 ProTaper instrument in reciprocating motion with different counterclockwise (CCW) and clockwise (CW) angles, allowing the instrument to advance with little apical pressure. This landmark study represented a revolution in the instrument's kinematics, as it stated that tapered root canal shaping can be achieved using just one instrument (15).

Consequently, new reciprocating instruments for 'single-instrument shaping' were developed: Reciproc (VDW) and WaveOne (Dentsply Sirona) systems, made of the M-Wire alloy. To avoid the screw-in effect, these two instrument systems use centric reciprocating motion. The

counterclockwise rotation (Reciproc 150°, Wave One 170°) serves to cut dentin, and the clockwise (Reciproc 30°, WaveOne 50°) rotation to clear the debris. To complete a full 360° rotation, these instruments require five rotation circles. The angles were calculated to be less than the degree required for instrument fracture if it binds to dentin. Because the instruments have a CCW cutting direction, they can cut if the CCW movement is greater than the CW movement. With the exception of these reciprocating instruments, all the other instruments are designed to cut in the clockwise direction. When compared to continuous rotary motion, reciprocating motion reduces tensile and compressive stress in the flexed region of the instrument, providing greater resistance to cyclic fatigue (48, 49).

Plotino et al. (50), used a total of 1,696 Reciproc instruments in clinical conditions and evaluated fracture and deformation rates. They discovered a very low incidence of both fracture, 0.47%, and deformation, 0.35% (50). Despite the risk of fracture, it is relatively common to reuse reciprocating instruments in clinical practice. Bueno et al. (51), in their *in vivo* study, used Reciproc R25 and WaveOne Primary instruments in three posterior teeth (a total of 358 endodontic treatments). They found no signs of deformation in any of the instruments, and only three fractured during use, indicating a low incidence of fracture when using reciprocating motion. Previous studies indicate that it provides excellent *in vitro* and *in vivo* results, including mechanical properties, root canal disinfection, root canal shaping, and postoperative pain (51-53).

1.5.2. Reciproc Blue

In 2011, the first blue-colored instrument, ProFile Vortex Blue, was introduced by Dentsply Tulsa Dental (Tulsa, OK, USA). It is a consequence of a special heat treatment that results in a visible titanium-oxide layer on the surface. This proprietary process is used for manufacturing two gold and two blue instrumentation systems. Two of them are used in a reciprocating motion (Reciproc Blue, VDW, and WaveOne Gold), while the other two are used in rotary motion (ProFile Vortex Blue; ProTaper Gold).

Surface-treated instruments exhibit a controlled memory effect, which means the instrument can be bent before inserting it into the root canal. They contain a greater amount of stable martensite than M-wire, with increased softness and ductility. Blue and Gold instruments

were found to have greater flexibility and fatigue resistance than conventional NiTi and M-wire instruments. They are claimed to exhibit a well-centered shaping, even in severely curved root canals (54).

The Reciproc blue CM (VDW GmbH, Munich, Germany) NiTi instruments are an upgrade of the original Reciproc system. The system consists of three files: R25 for narrow canals, R40 for medium canals, and R50 for wide canals. R25 shapes the root canal to a diameter of 0.25 mm with a taper of .08 over the first apical millimeters. R40 shapes the root canal to a diameter of 0.40 mm with a taper of .06 over the first apical millimeters. R50 shapes the root canal to a diameter of 0.50 mm with a taper of .05 over the first apical millimeters. The instruments have specific s-shaped cross-sections and variable tapers. The file's tip is non-cutting for a gentle treatment near the apex (55).

Reciproc Blue has the same geometry, size, and design as standard Reciproc files. Reciproc blue has lower microhardness but similar surface properties, as well as increased cyclic fatigue resistance and flexibility (56).

1.5.3. WaveOne Gold

The WaveOne Gold (Dentsply, Tulsa) NiTi instruments are improved versions of the original WaveOne system. The system consists of four shaping files: Small - 20/.07, Primary - 25/.07 (recommended for most root canals), Medium - 35/.06, and Large - 45/.05. Small has a diameter of 0.20 mm and .07 taper over the first apical millimeters. Primary has a diameter of 0.25 mm and .07 taper over the first apical millimeters. Medium has a diameter of 0.35 mm with .06 taper over the first apical millimeters. Large has a diameter of 0.45 mm with .05 taper over the first apical millimeters (57). The Wave One instruments have varying cross-sections along their active part. The taper is constant for the first three millimeters and then decreases.

This system has been upgraded by Wave One Gold, which uses the Gold thermal process and has a specific gold appearance. It has four cutting edges, of which only two are in constant contact with dentin, which keeps the instrument centered in the root canal. This design ensures a very low screw-in effect and enough space for debris removal. Wave One Gold has been found to have a greater resistance to torsional fatigue when compared to Reciproc and

TF Adaptive files (58, 59). The WaveOne Gold instrument's tip is tapered and semi-active. These design properties enable a smooth reciprocating movement, increase safety, and provide better cutting efficiency (38).

The variable tip diameters allow the clinician to use this system in a variety of root canal anatomies, while the reduced taper ensures a more conservative preparation with greater dentin preservation.

1.6. Failure of endodontic instruments

Despite the undeniable advantages of NiTi files over stainless steel ones, fracture of NiTi instruments inside root canals can occur even without visible macroscopic deformation, which may be correlated with a negative treatment prognosis (60, 61). Fracture of NiTi instruments occurs due to torsional fatigue, cyclic fatigue, or a combination of both (62). In curved root canals, the risk of fracture is mainly related to cyclic and in narrow root canals, mainly to torsional fatigue (63).

1.6.1. Cyclic fatigue (CF)

Metal fatigue is the reason for fracture caused by fatigue through flexure. Clinically, this often happens in curved canals (64). When the instrument rotates inside the curvature, part of the instrument on the outer side of the curve is in tension, while the part of the instrument on the inner side of the curve is in compression. This repeated tension-compression cycle, caused by rotation within the curved canal, increases cyclic fatigue of the instrument over time and may be an important factor in instrument fracture (62).

Irregularities on the instrument's surface may lead to fracture due to cyclic fatigue. Surface defects may propagate when exposed to tension/compression forces inside the curvature, therefore increasing the risk of separation (65). Therefore, greater flexibility and smaller taper of the instrument increase resistance to cyclic fatigue. Contrary, instruments with larger sizes and tapers have to be used with caution in curved canals to avoid described fractures due to cyclic fatigue (66).

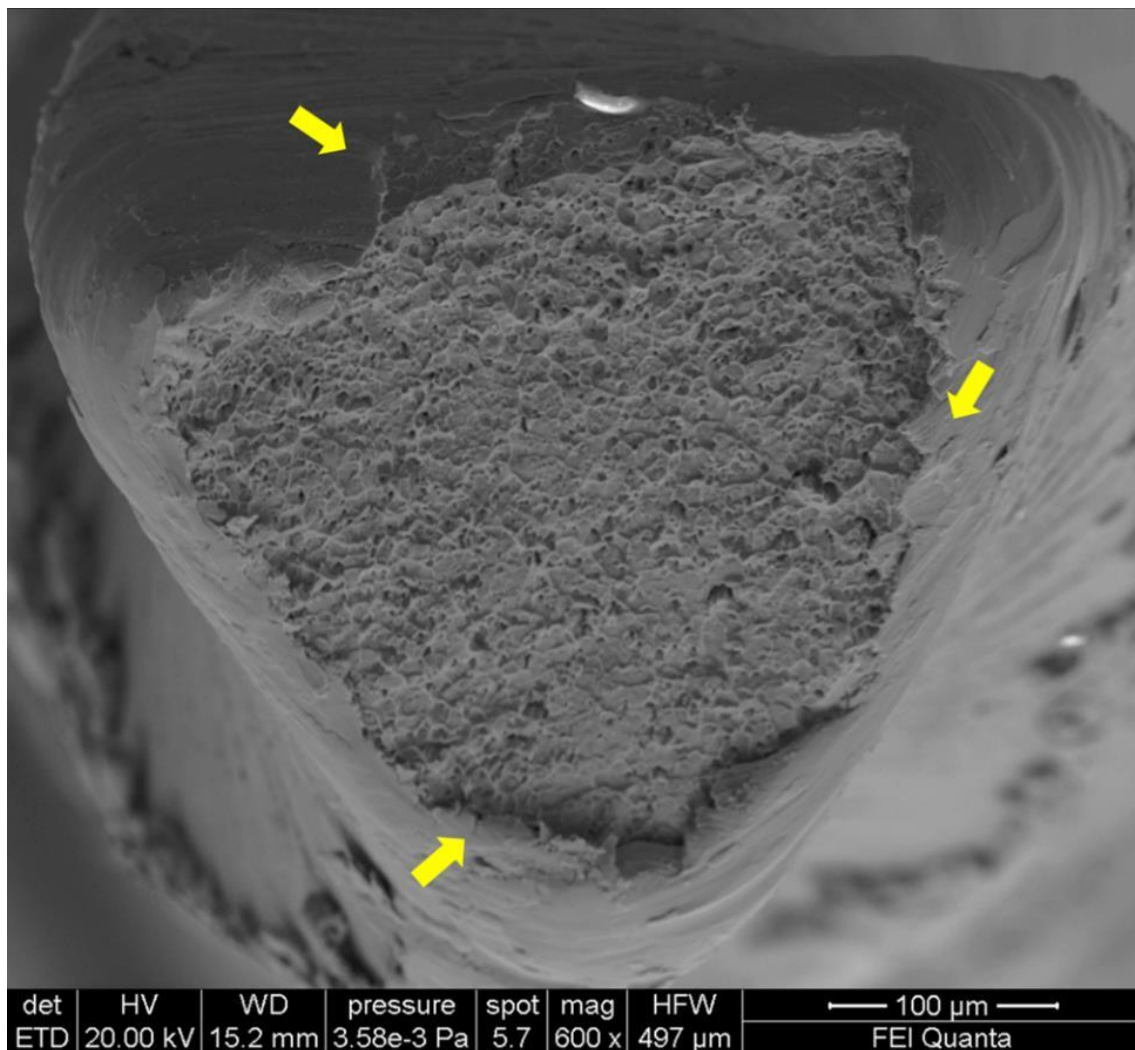


Figure 5. SEM image of the fractured surface of F2 EdgeTaper Platinum (Albuquerque, NM, USA) in a transversal view at $\times 600$ magnification after cyclic fatigue testing. A typical feature of ductile fracture, which originates from the external part of the instrument with visible cracks (yellow arrows). Courtesy of Zanza et al. (39).

1.6.2. Torsional fatigue (TF)

The torsional fracture occurs when the tip of an instrument binds in a canal and the remaining portion continues to rotate. When the elastic limit (yield strength) of the metal is exceeded by the torque exerted by the handpiece, fracture of the tip occurs (67). Instrument fractures due to TF often carry specific signs of plastic deformation or distortion (68). Torsional stress is generated by the twisting of the instrument around its longitudinal axis at one end, while the other end is fixed. Clinically, this occurs more often in narrow and constricted canals, when the instruments are subjected to high torsional loads (69). TF is characterized by a maximum torsional load and angle of rotation. This last property reveals the twisting limit of the instrument before separation (70). Hence, greater cross-section, bigger taper, or stiffer instruments have greater resistance to TF.

According to Sattapan et al. (71), failure due to TF occurred in 55.7% of the instruments tested after clinical use. On the other hand, Wei et al. (72) analyzed 100 instruments that fractured during clinical use and found that 91% of the separations were due to cyclic fatigue. Torsional fatigue occurred in just 3% of the cases and, the combination of both in 6% of the cases.

Therefore, the selection of the proper instrument for the proper case is paramount in avoiding instrument failure. Instruments with greater flexibility are preferred for instrumentation of curved canals and stiffer instruments can be suitable for narrow canals.

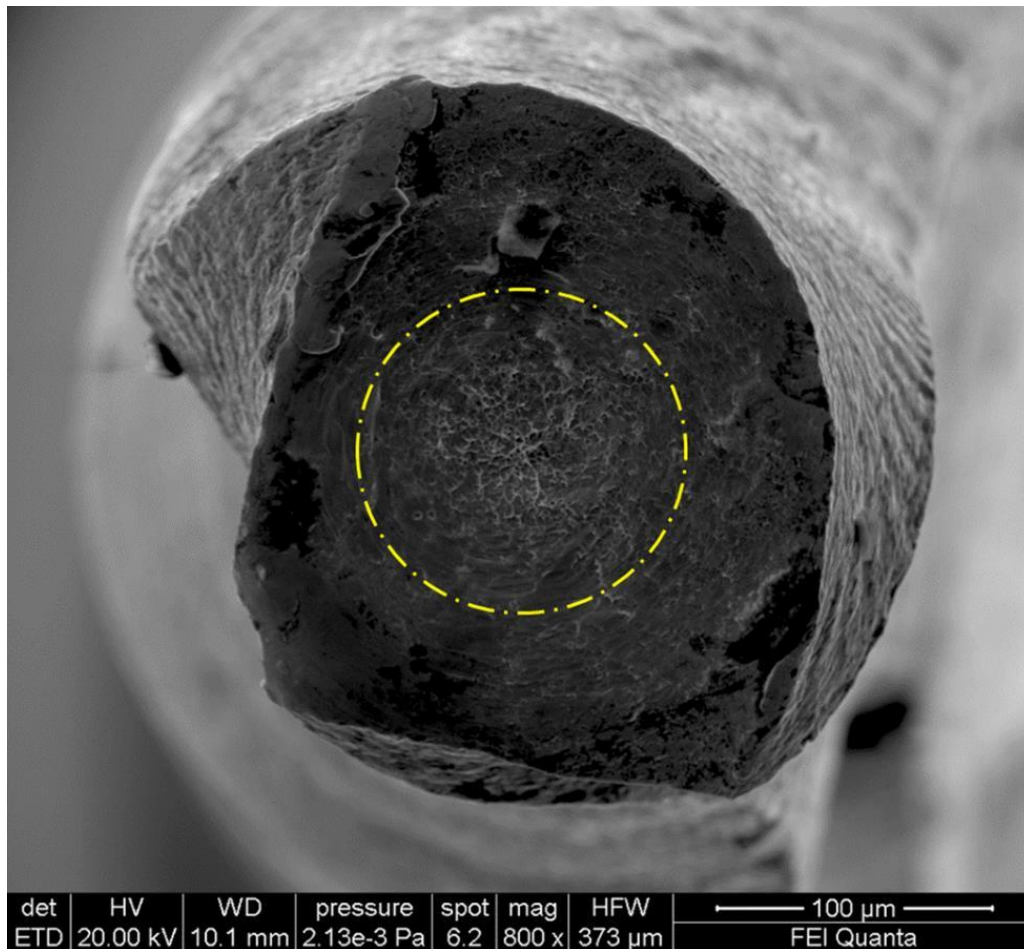


Figure 6. SEM image of the fractured surface of an F-One #20 after torsional testing. The typical features of excessive torsional load, showing concentric circular abrasion marks. Courtesy of Zanza et al. (39).

1.7. The surface wear of NiTi instruments

The very first study about surface wear of NiTi instruments was published in 1997. The study compared rotary NiTi with stainless steel instruments after repetitive usage in curved canals (73). The surface characteristics of NiTi instruments have mainly been evaluated using scanning electron microscopy (SEM) or atomic force microscopy (AFM).

1.7.1. Scanning electron microscopy

A scanning electron microscope (SEM) is an electron microscope that produces two-dimensional images of a sample, by scanning its surface with a focused beam of electrons. The electrons interact with atoms in the sample and produce a signal which contains information about the surface topography and composition of the sample. In the SEM mode, secondary electrons that are emitted by atoms excited by the electron beam are detected using a secondary electron detector. The number of secondary electrons that can be detected, and consequently the signal intensity, depends on sample topography. SEM can achieve a resolution higher than 1 nanometer.

In conventional SEM, specimens are observed in high vacuum, low vacuum, or wet conditions, under variable pressure or environmental SEM, and at a wide range of cryogenic or elevated temperatures using specialized instruments. This microscope has a wide field of view and can produce accurate representations of the sample's three-dimensional shape (74).

Scanning electron microscopy (SEM) has been widely used for qualitative analysis of surface topography (75), although it is known that two-dimensional images generated by SEM cannot provide quantitative surface data (76). Furthermore, specimen preparation and vacuum-induced changes may be time-consuming and may cause morphological inaccuracy of the sample (77).

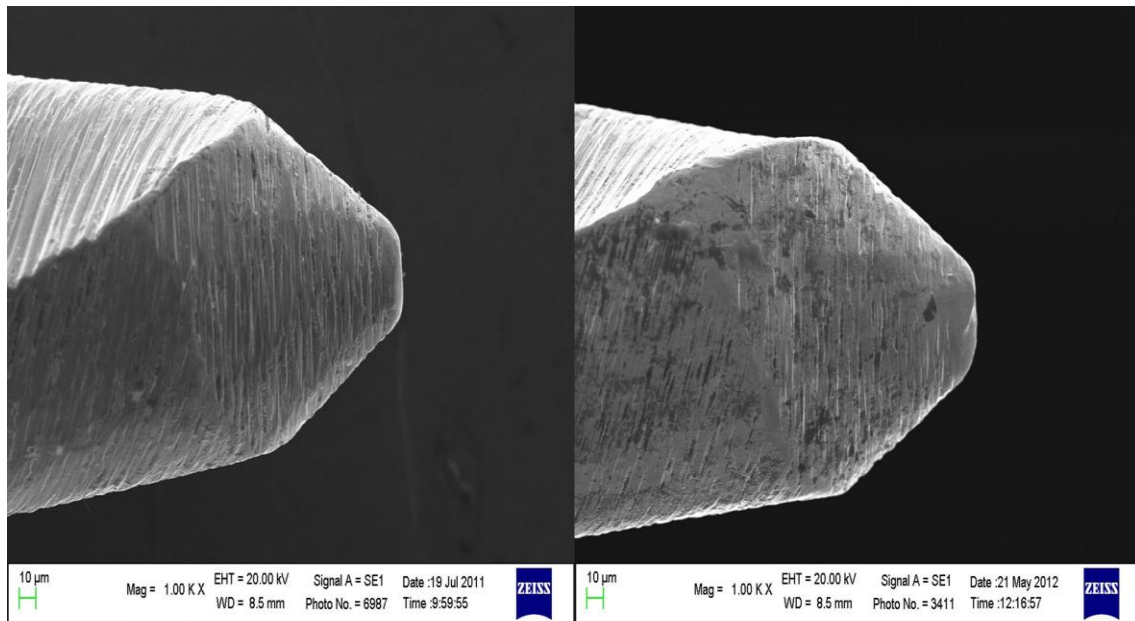


Figure 7. Unused Reciproc R25 instrument and Reciproc R25 instrument after third use (tip portion), 1,000x. Surface wear is noticeable, modifying the instrument outline in the tip portion. Courtesy of Piriani et al. (78).

1.7.2. Atomic force microscopy

An atomic force microscope (AFM) is a scanning probe microscope that analyzes the sample's surface by "touching" the sample with a mechanical probe. The sample is mounted on the sample stage and the interaction between the probe and sample causes the changes in the motion of the probe. The detector records and measures the deflection and motion of the probe. The value of deflection is most commonly used to quantify the surface topography of the sample. Detected signals can be used to form a three-dimensional image of a sample's surface at a high resolution.

When compared to SEM, the size of the scanned image is a disadvantage of AFM. The SEM can scan an area sized in millimeters, whereas the AFM can scan a maximum area of 150×150 micrometers. AFM has a longer scanning time and requires several minutes for a typical scan. AFM images can be also affected by the irregularity of the specimen, which may require software enhancement and filtering. Such filtering could "flatten out" or change real surface topography (79).

AFM has been proposed to three-dimensionally assess the instrument surfaces by the analysis of nanoscale high-resolution images (80). This method can quantitatively evaluate changes in the surface topography of NiTi instruments (80,81). As the scanning probe can cause deformations on the surface of the sample, the sample needs to be flat and rigid in order to achieve accurate atomic resolution (82). However, the surface of endodontic instruments is curved and angled, which makes this method not an ideal choice for their surface assessment. Moreover, as very small areas of the surface can be scanned, the representativeness of the measurements is reduced, preventing repeatable evaluation of similar areas at different times (82, 83).

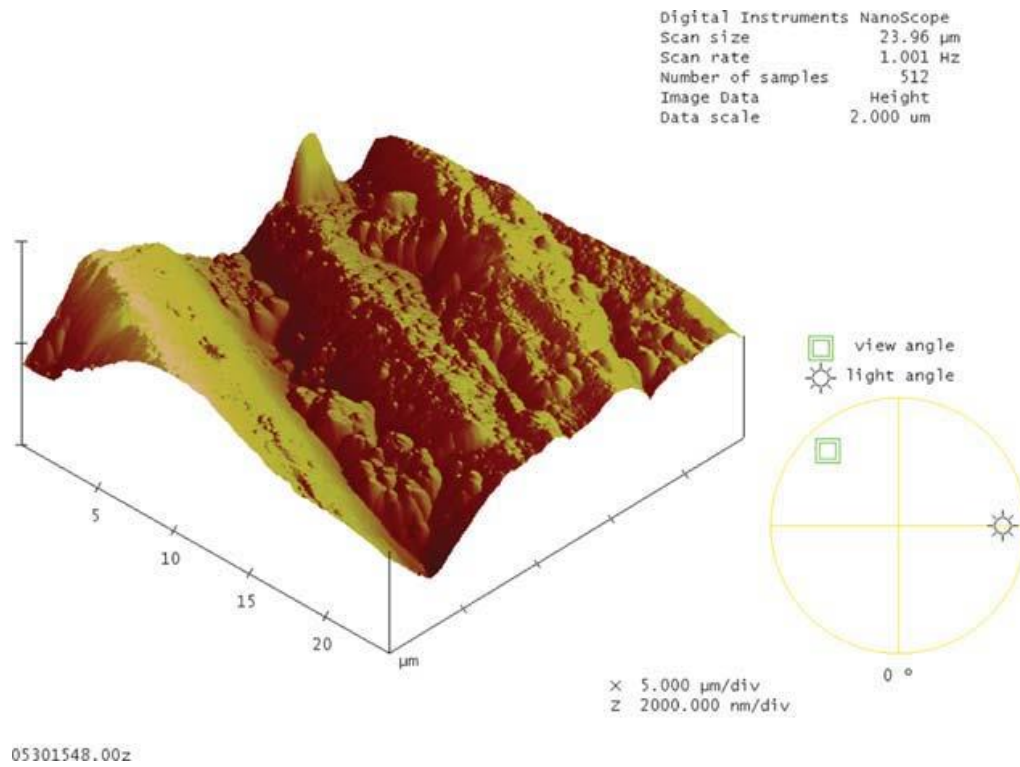


Figure 8. Atomic force micrograph of the cervical region of a new ProTaper rotary file. Courtesy of Yamazaki et al. (84).

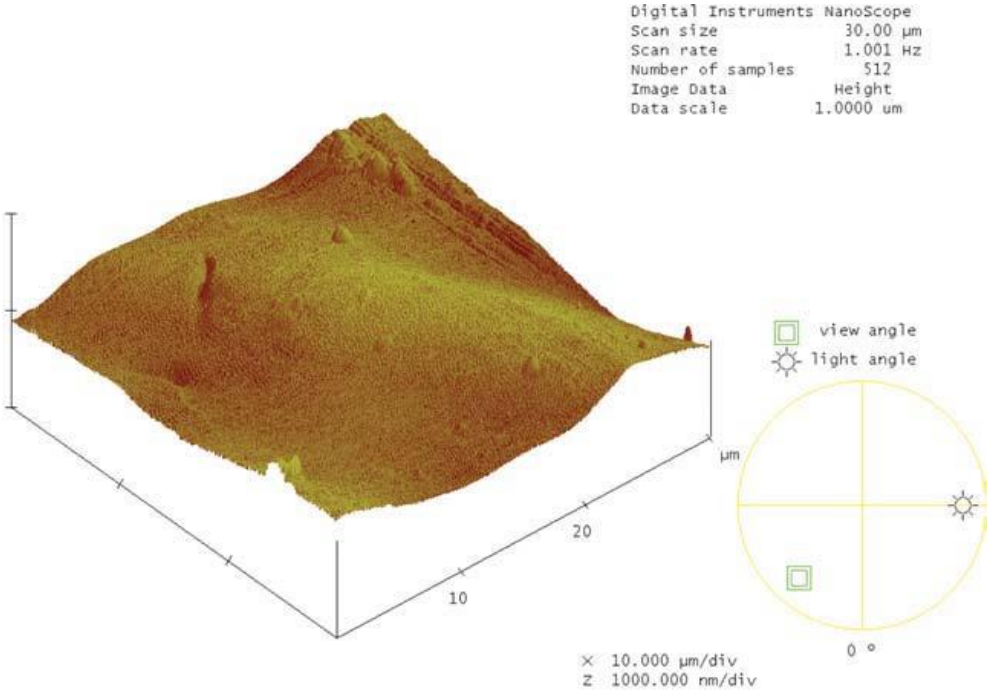


Figure 9. Atomic force micrograph of the cervical region of a ProTaper rotary file after being used 12 times—the third dimension. Courtesy of Yamazaki et al. (84).

1.7.3. Optical profilometry

White-light interferometry is a proven method for noncontact surface analysis that is approved in ISO/DIS 25178 Part 604 (International Organization for Standardization). It can be applied to a wide range of surfaces. The technique enables fast measurements without any specific sample preparation. Optical profilers measure and quantify surface roughness, step heights, and surface topography. Every profilometer has two components: a surface detector and a sample stage. The sample stage holds and orientates the sample. The detector detects and measures surface irregularities.

There are two types of optical profilometers: contact and noncontact.

Contact stylus profilometers use a diamond probe to “touch“ the surface. The probe moves across the sample and detects the height of its surface irregularities. The result is a two-dimensional roughness measurement.

Noncontact optical profilometry uses light instead of a probe to detect surface irregularities. The light source of the microscope is split into two paths. One path directs the light onto the sample’s surface, the other path directs the light to a reference mirror. Reflections from the two surfaces are recombined and projected to the detector. This interference of two light beams contains information about the surface topography and results in a three-dimensional surface roughness measurement (85).

1.7.3.1. Advantages and disadvantages of optical profilometry

A great advantage of optical profilometry is three-dimensional measurement, resulting in greater roughness details. Optical profilometry has been a well-established technique for more than 20 years. The profilers give ultra-precise 3-dimensional analyses of any surface and rapidly measure heights from 0.1 nm to 1.0 mm, with a vertical resolution of 0.1 nm (87). They are stable solid-state instruments that need just a periodic calibration using specific specimens. Compared to a contact stylus, optical profilometers can scan a greater field of the surface at very high resolutions.

Optical profilers also have some limitations. They have large and delicate lenses and are sensitive to vibration. The sample must be very clean for proper focus. Although profilers can scan a surface relatively fast, the instrument setup takes more time. Another sensitive point of optical profilometry is the adequate direction of the light. The orientation of the light and the distance between the objective and the sample are critical for successful optical measurement. In order to distinguish micro surface irregularities, the distance between the measured surface and the objective has to be around 1 millimeter, which may be a limitation in some samples. Furthermore, as the direction of the light is essential for accurate measurements, angled surfaces can cause unprecise and inaccurate measurements (85).

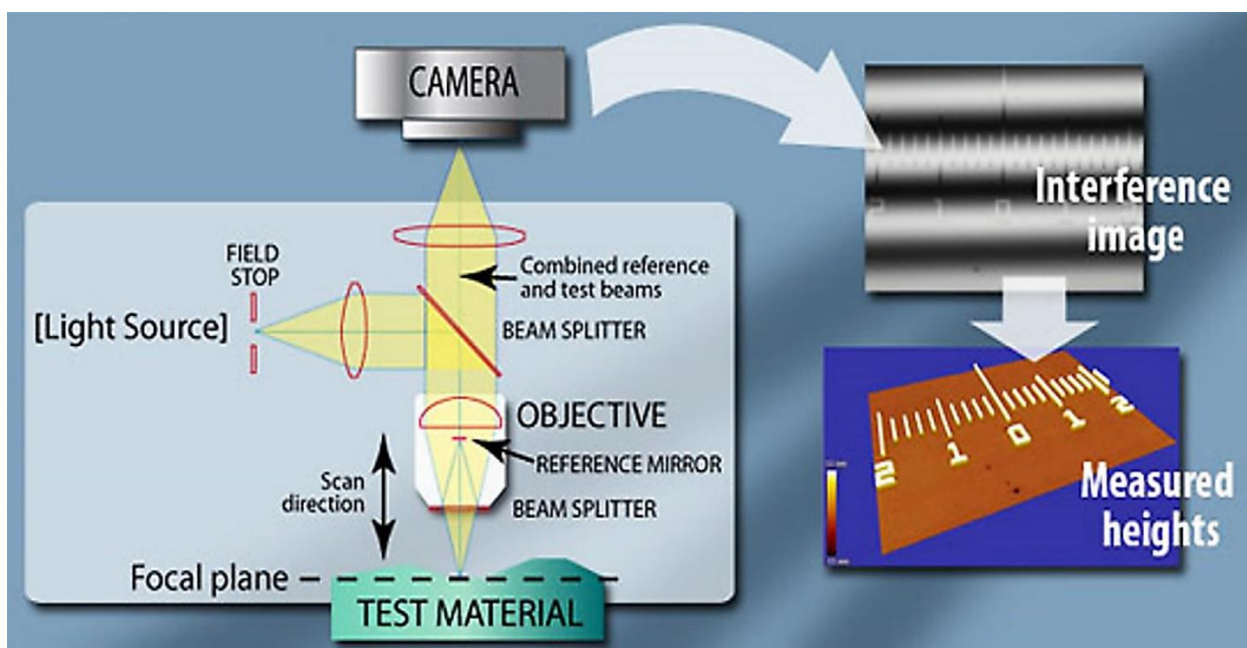


Figure 10. Schematic of an optical interferometer. Courtesy of Zygo® Corporation (86).

2. AIMS AND OBJECTIVES

Primary aims:

1. Qualitative and quantitative surface topography comparison of reciprocating (Reciproc Blue, WaveOne Gold) and rotary (XP-endo Shaper, TruNatomy) instrumentation systems after use in curved root canals.
2. Evaluation of cyclic fatigue resistance of reciprocating (Reciproc Blue, WaveOne Gold) and rotary (XP-endo Shaper, TruNatomy) instrumentation systems after use in curved root canals.

Secondary aims:

1. Evaluation of 3D optical profilometry as a method for surface topography analysis of NiTi instruments before and after use, and comparison of the accuracy of the technique between acrylic blocks and human teeth.
2. Comparison of surface topography in XP-endo Shaper, TruNatomy, Reciproc Blue, and WaveOne Gold instruments after use in acrylic resin blocks and extracted human teeth.

Null hypothesis:

1. There is no significant difference in surface topography between reciprocating and rotary instrumentation systems after use in acrylic blocks and extracted human teeth.
2. There is no significant difference in cyclic fatigue resistance of reciprocating (Reciproc Blue, WaveOne Gold) and rotary (XP-endo Shaper, TruNatomy) instrumentation systems.

Working hypothesis:

1. There will be a significant difference in surface topography between reciprocating and rotary instrumentation systems after use in acrylic blocks and extracted human teeth.
2. There will be a significant difference between reciprocating and rotary instrumentation systems in resistance to cyclic fatigue.

3. MATERIALS AND METHODS

This study was approved by the Ethical Committee, School of Dental Medicine, University of Zagreb (No: 05-PA-15-12/2017).

A total of 40 new NiTi instruments were selected and divided into 2 groups according to their use in

1. Acrylic blocks (AB), n= 20
2. Extracted human teeth (ET), n=20

Instruments in each group (n=20) were further randomly divided into 4 subgroups according to their specific manufacturer:

1. Reciproc Blue, RB (VDW, Germany), n=10
2. WaveOne Gold, WG (Dentsply Maillefer, Ballaigues, Switzerland), n=10
3. XP-endo Shaper, XPS (FKG Dentaire, La Chaux-de-Fonds, Switzerland), n=10
4. TruNatomy, TRN (Dentsply Maillefer, Ballaigues, Switzerland), n=10

3.1. Sample selection

The surface of all instruments was examined under an optical microscope H600 (Helmut Hund, Wetzler, Germany) at 20x magnification to confirm the absence of any visible manufacturing defects. The criterium for sample selection was: new instruments from the package with the absence of any irregularities visible at 20x magnification.

3.2. Root canals selection

a) Curved root canals in extracted human teeth (n=40)

From a pool of extracted human molar teeth, extracted due to orthodontic or periodontic indications, mostly third molars, 40 roots with one single canal and curvatures between 25° and 40° were selected. The presence of canal curvature was measured in both directions according to the method of Schneider (88). Teeth with previous endodontic treatment,

intracanal calcifications, root caries, and external or internal resorption were excluded. The teeth were stored in 0.1% thymol solution before use.



Figure 11. Selected and extracted molar teeth and new instruments for instrumentation.

b) Curved root canals in acrylic blocks (n=40)

A total of 40 clear resin blocks (*Diadent* Group International, Seoul, Korea) containing a simulated root canal were used. The length of simulated root canals is standardized at 19 mm, with a 40° angle of curvature according to Schneider's method (88).



Figure 12. Acrylic blocks and new instruments for instrumentation.

3.3. Root canal instrumentation



Figure 13. Artificial resin block with a simulated curved root canal.

One operator, experienced in engine-driven root canal treatment, performed all endodontic instrumentation procedures. Instrumentation was performed in the water bath at 37°C to simulate clinical conditions.

In extracted human teeth access cavity was made using a water-cooled diamond fissure bur No. 016 (Komet, Rock Hill, SC, USA). The tooth cusps were shortened to standardize the working length at 19 mm. Canal patency was confirmed by the insertion of a 10 K-file (Dentsply-Sirona Endodontics, Ballaigues, Switzerland) through the apical foramen. Teeth with apical foramen diameter smaller than size 10 hand K-file and teeth in which a 10 K-file was loose at apical foramen were not included. In the artificial root canals, patency was confirmed by the insertion of a 10 K-file through the apical foramen.

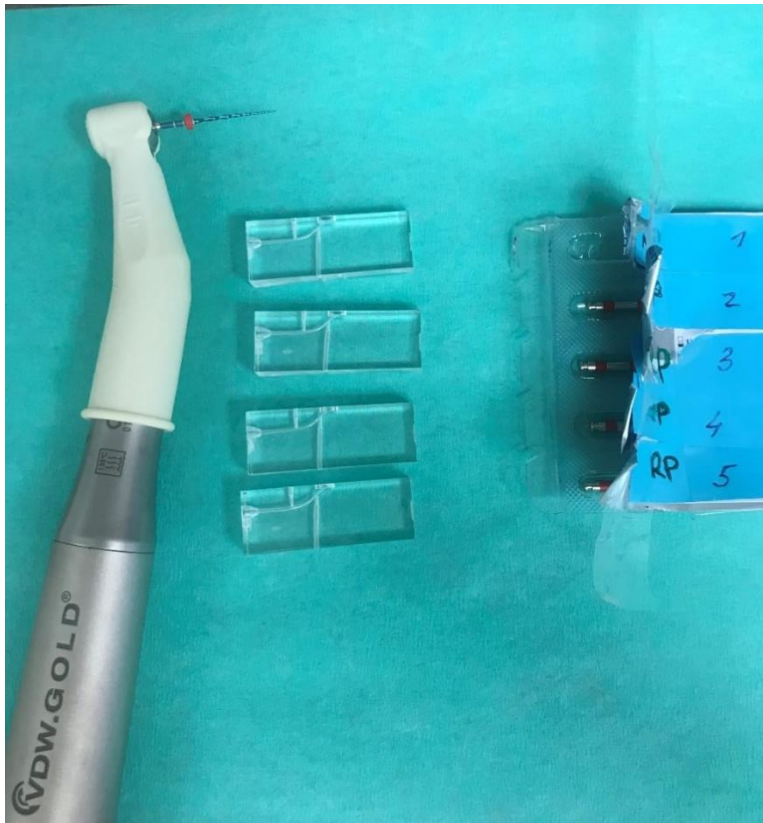


Figure 14. Handpiece with artificial resin block and instruments for the second usage.

Subgroup I: Reciproc Blue system (n=10)

a) instrumentation in extracted teeth (n=5)

Initially, a glide path was created using an ISO 10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) to reach the working length (WL). The preparation was performed with the Reciproc Blue R25 file (tip size 25; variable taper; VDW) using the VDW Gold motor (Dentsply Maillefer, Ballaigues, Switzerland) set at reciprocation RECIPROC ALL mode. In-and-out pecking motion, 3 mm amplitude, with gentle apical pressure, was applied with a brushing action against the lateral walls. After three pecks, the instrument was removed from the canal and cleaned with sterile gauze. The irrigation is made using a total of 5 mL of 2.5% sodium hypochlorite. The whole procedure was repeated until the instrument reached working length (WL), mostly three times. Each instrument was used twice to instrument two root canals.

b) instrumentation in acrylic blocks (n=5)

The same procedure was made in 10 artificial canals. Each instrument was used twice in two different blocks.



Figure 15. Reciproc Blue instrument.

During instrumentation, a total of 5 mL of 2.5% sodium hypochlorite (NaOCl) was used for each canal irrigation using a 30G needle (BD Microlance, Becton Dickinson, Madrid, Spain). The instruments were cleaned from debris remnants with gauze moistened in the same solution.

Subgroup II: Wave One Gold system (n=10)

a) instrumentation in extracted teeth (n=5)

The Wave One Gold primary file (tip size 25 and variable taper WG/Dentsply Maillefer, Ballaigues, Switzerland) was used with the VDW Gold motor set at reciprocation WAVEONE ALL mode. In-and-out pecking motion, the amplitude of approximately 3 mm, with gentle apical pressure, was applied with a brushing action against the lateral walls. After three pecks, the instrument was removed from the canal and cleaned with sterile gauze, and the canal was irrigated with a total of 5 mL of 2.5% NaOCl. This procedure was repeated until the instrument reached WL. Each instrument was used twice to instrument two root canals.

b) instrumentation in resin blocks (n=5)

The same procedure was made in 10 artificial canals. Each instrument was used twice in two different blocks.

During instrumentation, a total of 5 mL of 2.5% NaOCl was used for each canal irrigation using a 30G needle (BD Microlance, Becton Dickinson, Madrid, Spain). The instruments were cleaned from debris remnants with gauze moistened in the same solution.



Figure 16. WaveOne Gold Primary instrument.

Subgroup III. XP-endo Shaper (n=10)

a) instrumentation in extracted teeth (n=5)

The glide path was created using ISO 10 hand file. According to the manufacturer, XP-endo Shaper can start shaping at ISO diameter 15 and achieve ISO diameter 30, but also increase the taper from .01 to at least .04. It allows a canal shaping of a minimum of 30/.04. In the presence of NaOCl, the instrument was inserted into the canal until resistance, retracted till the tip is loose, then the motor was started with the rotational speed of 800 rpm and torque of 1 Ncm. Long gentle strokes were used to progress down the WL. If WL was not reached in 5 strokes, the canal is irrigated and recapitulated. Pecking motion was not used at any time

during preparation. Once WL was reached, the canal was irrigated as described below, and additional 15 long gentle strokes were applied to WL. Each instrument was used twice to instrument two root canals.

b) instrumentation in acrylic blocks (n=5)

The same procedure was followed in 10 artificial canals. Each instrument was used twice in two different blocks.

During instrumentation, a total of 5 mL of 2.5% NaOCl was used for each canal irrigation using a 30G needle (BD Microlance, Becton Dickinson, Madrid, Spain). The instruments were cleaned from debris remnants with gauze moistened in the same solution.

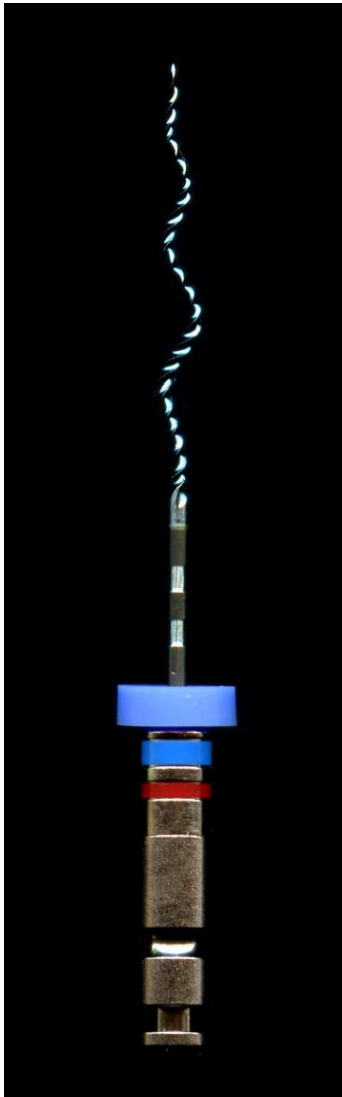


Figure 17. XP-Endo Shaper (FKG) instrument.

Subgroup IV. TruNatomy (n=10)

a) instrumentation in extracted teeth (n=5)

The working length is initially determined using ISO 10 hand file. WL and glide path were confirmed using the TruNatomy glider. TruNatomy Prime (TRN) instrument has a tip size of 0.26 mm with a taper of .04 over the first apical millimeters. According to the manufacturer, rotational speed was 500 rpm and torque 1.5 Ncm. After irrigation with NaOCl, the TRN instrument is used in presence of NaOCl with 2-3 gentle in and out motions (approx. amplitude 2–5 mm). Irrigation and preparation were done until the WL is reached. After reaching the WL, the Prime file was removed to avoid over-enlarging the apical foramen. Each TruNatomy Prime instrument was used twice to instrument two root canals.

b) instrumentation in acrylic blocks (n=5)

The same procedure was made in 10 artificial canals. Each instrument was used twice in two different blocks.

During instrumentation, a total of 5 mL of 2.5% NaOCl was used for each canal irrigation using a 30G needle (BD Microlance, Becton Dickinson, Madrid, Spain). The instruments were cleaned from debris remnants with gauze moistened in the same solution.



Figure 18. TruNaromy Prime instrument.

3.4. Three-dimensional optical profilometry analysis

All tested instruments were analyzed three times: before any instrumentation (T0), after the first (T1), and after the second (T2) instrumentation cycle using a noncontact three-dimensional optical surface profilometer (NewView™ 7100; Zygo Corporation, Middlefield, CT, USA).

A standardized procedure for specimen analysis was conducted according to the method proposed by Ferreira et al. (83). Marks on the shafts of the files were made with ISO 014 diamond round bur (Komet, Rock Hill, SC, USA), as described by Uslu et al. (89). Those marks served as reference points to ensure that the same areas on the surfaces of the files were analyzed before and after the root canal preparation. The motorized table was moved transversely forward until the profilometer reached the tip of the instrument, and then moved 3 mm back again, to obtain the reading areas of the flute regions of the instrument. The scanning areas were located 3 mm coronal from the tip of the instrument.

All tested instruments were analyzed three times: before any instrumentation (T0), after the first (T1), and after the second (T2) instrumentation cycle. The surface of the endodontic instruments was measured and analyzed using a non-contact 3D optical surface profilometer (NewView™ 7100; Zygo Corporation, Middlefield, CT, USA). All the measurements were conducted on areas of $211 \times 211 \mu\text{m}$. Three amplitude parameters were evaluated in the quantitative analysis: the average roughness over the measurement field (Ra), root mean square roughness (Rq), and peak to valley height (Rz). All the analyses were provided by Mx™ software (Zygo Corporation, Middlefield, CT, USA).

The 3D optical profiler used in this study has a scan range from $<1 \text{ nm}$ up to $20000 \mu\text{m}$, independent of surface texture and magnification, with high vertical optical resolution (0.01 nm) and $0.4\text{-}0.6 \mu\text{m}$ lateral resolution. Repeatability of 0.02 nm is an important requirement because it enables reliable analyses at different times.



Figure 19. 3D non-contact optical profiler.

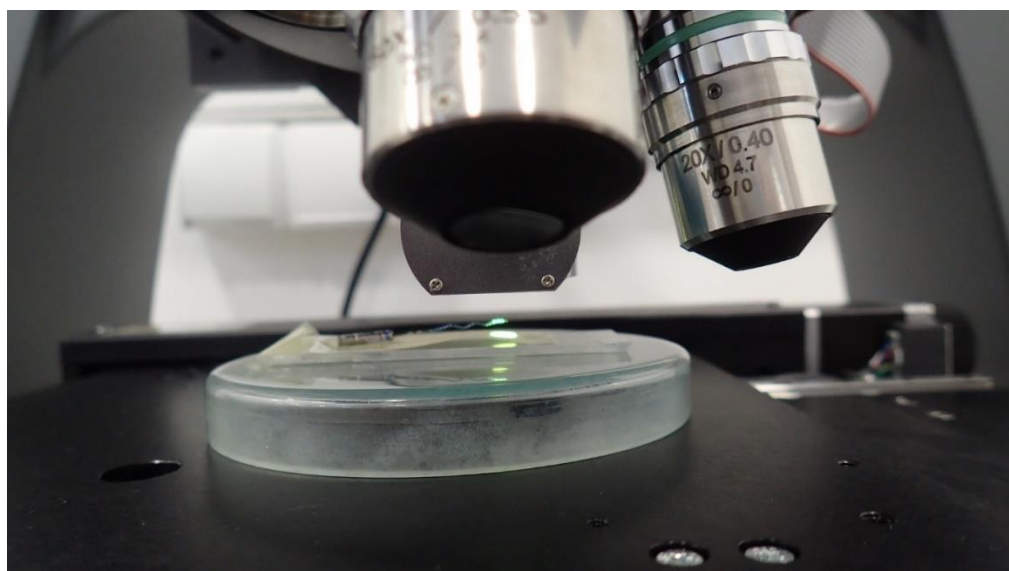


Figure 20. Positioning the tip of the instrument for measuring.

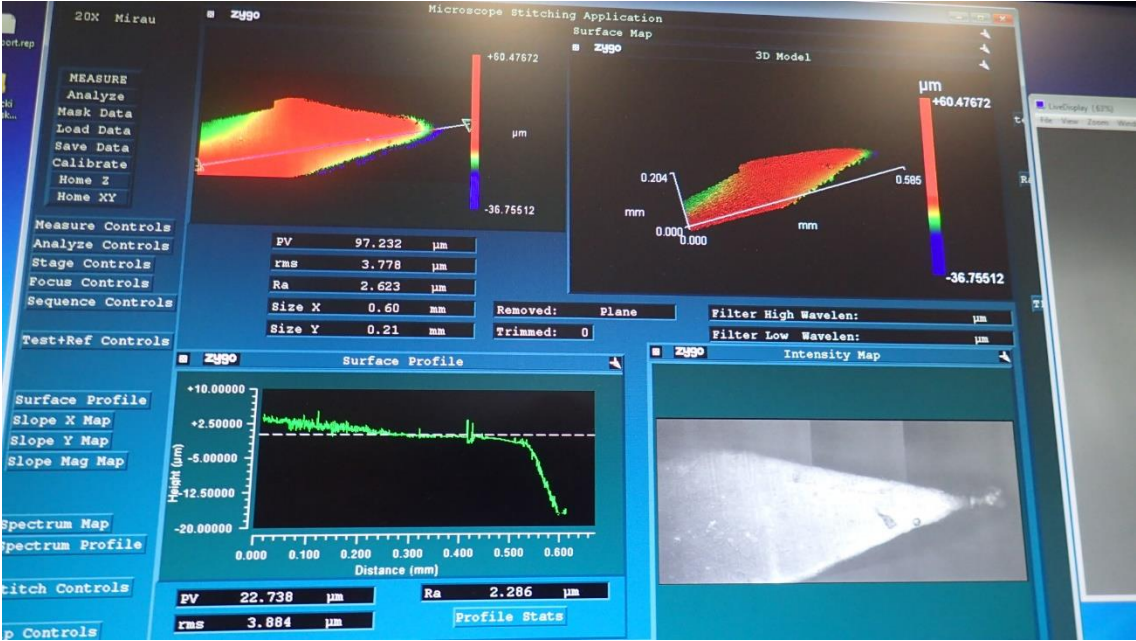


Figure 21. Surface parameters analysis and quantification by Mx™ software.

3.5. Cyclic fatigue test

After conducting two instrumentation procedures, the same 40 instruments were submitted to a cyclic fatigue test. The artificial canals for testing are made of stainless steel and manufactured to reproduce the size and taper of the instruments, with a 60° angle of curvature and a 5 mm radius of curvature, and the center of the curvature located 5 mm from the apical portion of the canal (90). The files were inserted into the artificial canal and operated in 35 °C water using a torque-controlled endodontic motor (VDW Gold; VDW Munich, Germany) following the instructions for use given by the manufacturers: XP-endo shaper at 800 rpm and 1.0 Ncm torque, TruNatomy at 500 rpm and 1.5 N.cm torque, and Reciproc Blue and WaveOne Gold in the proprietary 'Reciproc ALL' mode that is pre-programmed by the manufacturer in the endodontic motor without disclosing speed, torque, and angles of reciprocation (91). The time to fracture (TTF) was recorded in seconds for each instrument using a digital chronometer. A digital microcaliper was used to measure the length of each fractured fragment (FL). The mean FL was recorded to evaluate the correct positioning of the instrument inside the canal curvature.

3.6. Statistical analysis

Data distribution was checked with the Kolmogorov-Smirnov test, and according to test findings, the appropriate parametric test was used in the analysis of the tested instrument brands at the different evaluation time points, considering the Ra, Rz, and Rq parameters (repeated measures ANOVA) and between instruments regarding T1 – T0, T2 – T0, and T2 – T1 changes (one-way ANOVA) with appropriate post-hoc tests (Bonferroni).

For cyclic fatigue difference between the tested instrument brands, Mann-Whitney U test was used due to the non-parametric distribution of the time to failure variable. All p values below 0.05 are considered significant. Statistical analysis was performed with MedCalc® Statistical Software version 20.007 (MedCalc Software Ltd, Ostend, Belgium; <https://www.medcalc.org>; 2021).

4. RESULTS

In the acrylic blocks (AB) subgroup, the surface analysis was made after instruments were used in simulated curved canals in acrylic blocks. The data obtained, considering three roughness parameters in three evaluation stages, are displayed in Table 2. XPS instruments, showed the highest values of roughness, both before and after their use. Roughness parameters significantly changed after the first (Ra, Rq) and second use (Ra, Rq, Rz). For other instruments in this subgroup, the difference was not statistically significant ($p > .05$).

The intergroup comparison data, displayed in Table 3., showed that XPS had significantly higher values for all roughness parameters after the second use, compared to the TRN ($p < .007$; $p < .001$; $p < .006$). The two reciprocating systems (RB and WG) showed no significant differences. The TRN group showed minimal changes in roughness values, without significant differences compared to reciprocating files, irrespective of the evaluation stage ($p > .05$)

In the extracted teeth (ET) subgroup, the surface analysis, for all tested instrument brands, was made after their use in extracted human curved root canals. Although the surface roughness values changed with usage in all groups, the differences were not significant ($p > .05$). The comparison of surface roughness parameters at different time points and intergroup data, are displayed in Tables 4. and 5.

The qualitative comparison of 3D surface images, obtained by profilometry, before and after instrumentation is displayed in Figure 24. for the AB subgroup and Figure 25. for the ET subgroup.

Table 2. SR parameters comparison in three evaluation stages after use in acrylic blocks.

Instrument	Parameter	T0 (mean ± SD)	T1 (mean ± SD)	T2 (mean ± SD)	p value
RB n=5	Ra	0.44 ± 0.09	0.54 ± 0.14	0.51 ± 0.18	0.518
	Rz	11.70 ± 3.01	14.08 ± 1.48	9.80 ± 3.04	0.069
	Rq	0.71 ± 0.18	0.95 ± 0.23	0.76 ± 0.28	0.275
WG n=5	Ra	0.38 ± 0.18	0.46 ± 0.06	0.41 ± 0.08	0.565
	Rz	11.06 ± 5.64	12.89 ± 2.00	8.53 ± 4.25	0.300
	Rq	0.61 ± 0.30	0.77 ± 0.07	0.65 ± 0.17	0.457
XPS n=5	Ra	0.19 ± 0.04 (a)	0.34 ± 0.09 (b)	0.35 ± 0.08 (b)	0.008
	Rz	2.39 ± 0.60 (a)	6.29 ± 2.98	6.60 ± 3.28 (b)	0.043
	Rq	0.30 ± 0.06 (a)	0.58 ± 0.15 (b)	0.61 ± 0.13 (b)	0.003
TRN n=5	Ra	0.41 ± 0.01	0.41 ± 0.04	0.39 ± 0.06	0.796
	Rz	10.72 ± 2.02	13.00 ± 3.24	9.34 ± 1.93	0.100
	Rq	0.62 ± 0.03	0.71 ± 0.09	0.61 ± 0.12	0.168
*Different lower-case letters in parentheses indicate statistically significant differences on Bonferroni post-hoc test ($p < 0.05$). Significant values are marked in bold letters.					

Table 3. SR parameters comparison, considering the T1 – T0, T2 – T0, and T2 – T1 changes presented in percentages after use in acrylic blocks.

Instrument	Parameter	RP n=5 (mean ± SD)	WG n=5 (mean ± SD)	XPS n=5 (mean ± SD)	TRN n=5 (mean ± SD)	p value
T1-T0 change (%)	Ra	26.55 ± 35.16	32.71 ± 31.74	82.49 ± 56.91 (b)	0.34 ± 8.93 (a)	0.022*
	Rz	24.56 ± 22.15 (a)	43.58 ± 80.19 (a)	156.22 ± 72.27 (b)	23.08 ± 30.51 (a)	0.005*
	Rq	40.12 ± 46.14	42.81 ± 47.71	97.03 ± 64.34	15.10 ± 11.31	0.077
T2-T0 change (%)	Ra	18.15 ± 41.52	17.59 ± 28.00	93.23 ± 60.36 (b)	-3.66 ± 13.22 (a)	0.007*
	Rz	-12.44 ± 32.78 (a)	-8.76 ± 55.50 (a)	160.43 ± 83.55 (b)	-8.31 ± 35.34 (a)	<0.001*
	Rq	10.95 ± 41.68 (a)	15.59 ± 35.26 (a)	112.62 ± 75.68 (b)	-0.24 ± 22.68 (a)	0.006*
T2-T1 change (%)	Ra	-6.00 ± 23.99	-10.70 ± 10.11	7.42 ± 22.89	-3.75 ± 11.93	0.473
	Rz	-29.96 ± 21.30	-34.95 ± 33.18	3.45 ± 29.61	-24.60 ± 24.09	0.161
	Rq	-18.77 ± 24.40	-15.85 ± 18.87	9.31 ± 25.97	-12.91 ± 19.42	0.220
Different lower-case letters in parentheses indicate statistically significant differences on Bonferroni post-hoc test ($p < 0.05$). Significant values are marked in bold.						

Table 4. SR parameters comparison in three evaluation stages after use in extracted human teeth.

Instrument	Parameter	T0 (mean ± SD)	T1 (mean ± SD)	T2 (mean ± SD)	p value
XPS n=5	Ra	0.25 ± 0.25	0.16 ± 0.08	0.24 ± 0.09	0.631
	Rz	3.87 ± 2.78	2.59 ± 1.75	5.93 ± 2.70	0.138
	Rq	0.42 ± 0.39	0.26 ± 0.13	0.41 ± 0.18	0.551
TRN n=5	Ra	0.40 ± 0.06	0.42 ± 0.09	0.42 ± 0.14	0.975
	Rz	8.08 ± 1.79	6.69 ± 1.20	10.05 ± 2.78	0.067
	Rq	0.61 ± 0.11	0.60 ± 0.11	0.64 ± 0.26	0.915
RP n=5	Ra	0.37 ± 0.12	0.45 ± 0.12	0.45 ± 0.13	0.517
	Rz	12.52 ± 4.72	11.46 ± 4.17	13.35 ± 4.01	0.790
	Rq	0.65 ± 0.23	0.79 ± 0.27	0.83 ± 0.30	0.539
WO n=5	Ra	0.40 ± 0.08	0.43 ± 0.13	0.66 ± 0.51	0.380
	Rz	11.56 ± 4.76	7.99 ± 1.71	12.33 ± 5.76	0.292
	Rq	0.63 ± 0.13	0.66 ± 0.23	0.91 ± 0.70	0.557

Table 5. SR parameters comparison, considering the T1 – T0, T2 – T0, and T2 – T1 changes presented in percentages after use in extracted human teeth.

Instrument	Parameter	XPS n=5 (mean ± SD)	TRN n=5 (mean ± SD)	RP n=5 (mean ± SD)	WO n=5 (mean ± SD)	p value
T1-T0 change (%)	Ra	27.51 ± 19.65	3.44 ± 18.76	30.41 ± 46.07	15.70 ± 57.19	0,927
	Rz	4.73 ± 86.36	-11.48 ± 36.57	5.41 ± 49.90	-17.35 ± 45.61	0,662
	Rq	18.15 ± 97.08	-0.83 ± 19.95	32.59 ± 51.01	12.13 ± 62.19	0,200
T2-T0 change (%)	Ra	97,13 ± 182,05	3,34 ± 28,67	32,52 ± 63,54	88,20 ± 191,50	0,896
	Rz	198,54 ± 366,56	28,02 ± 38,70	24,81 ± 66,73	20,74 ± 78,94	0,404
	Rq	108,94 ± 222,00	4,17 ± 30,35	42,85 ± 78,61	61,14 ± 161,48	0,076
T2-T1 change (%)	Ra	58,06 ± 65,34	0,08 ± 22,02	2,20 ± 24,09	39,41 ± 63,55	0,872
	Rz	171,89 ± 158,69	50,22 ± 30,91	27,49 ± 47,69	52,50 ± 49,01	0,715
	Rq	71,79 ± 74,03	5,54 ± 26,11	9,91 ± 33,83	26,27 ± 47,40	0,167

Cyclic fatigue differences between the tested instrument groups are shown in Figure 1 (acrylic blocks) and Figure 2 (extracted teeth).

The intergroup comparison of the CF resistance of the instruments used in the two curved root canals showed that RB had the highest CF resistance, followed by WG, TRN, and XPS ($p < .05$). The same results were obtained in the second subgroup after use in extracted teeth, but with a higher level of significance of 0.01.

The reciprocating instruments had a significantly higher CF resistance than the rotary instruments. The difference between rotary instruments TRN and XPS was not statistically significant. No significant difference was also observed in the length of the fractured fragments, which confirms the accurate positioning of instruments inside the testing device ($p > .05$).

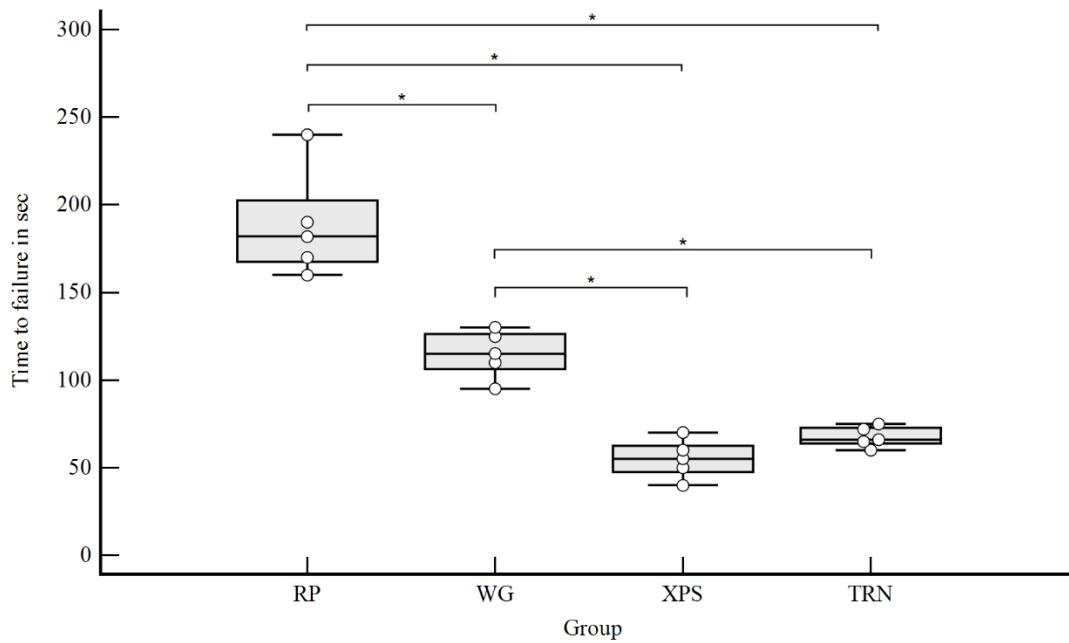


Figure 22. Cyclic fatigue difference between the tested instrument groups after use in acrylic blocks; p values < 0.05 were marked with *.

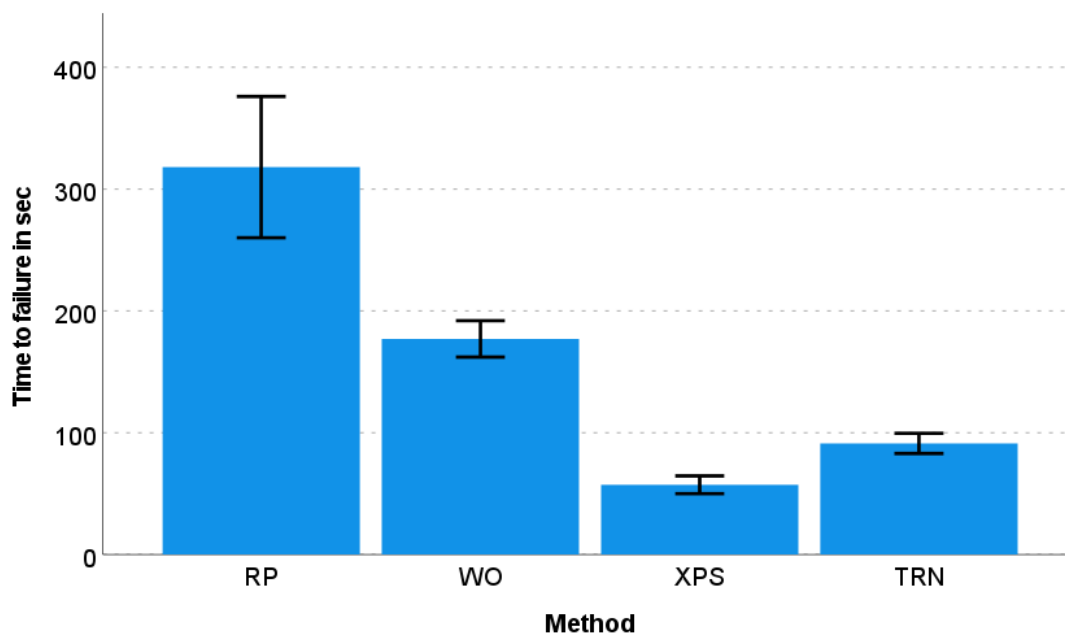


Figure 23. Cyclic fatigue difference between the tested instrument groups after use in extracted human teeth (mean ± 95% CI).

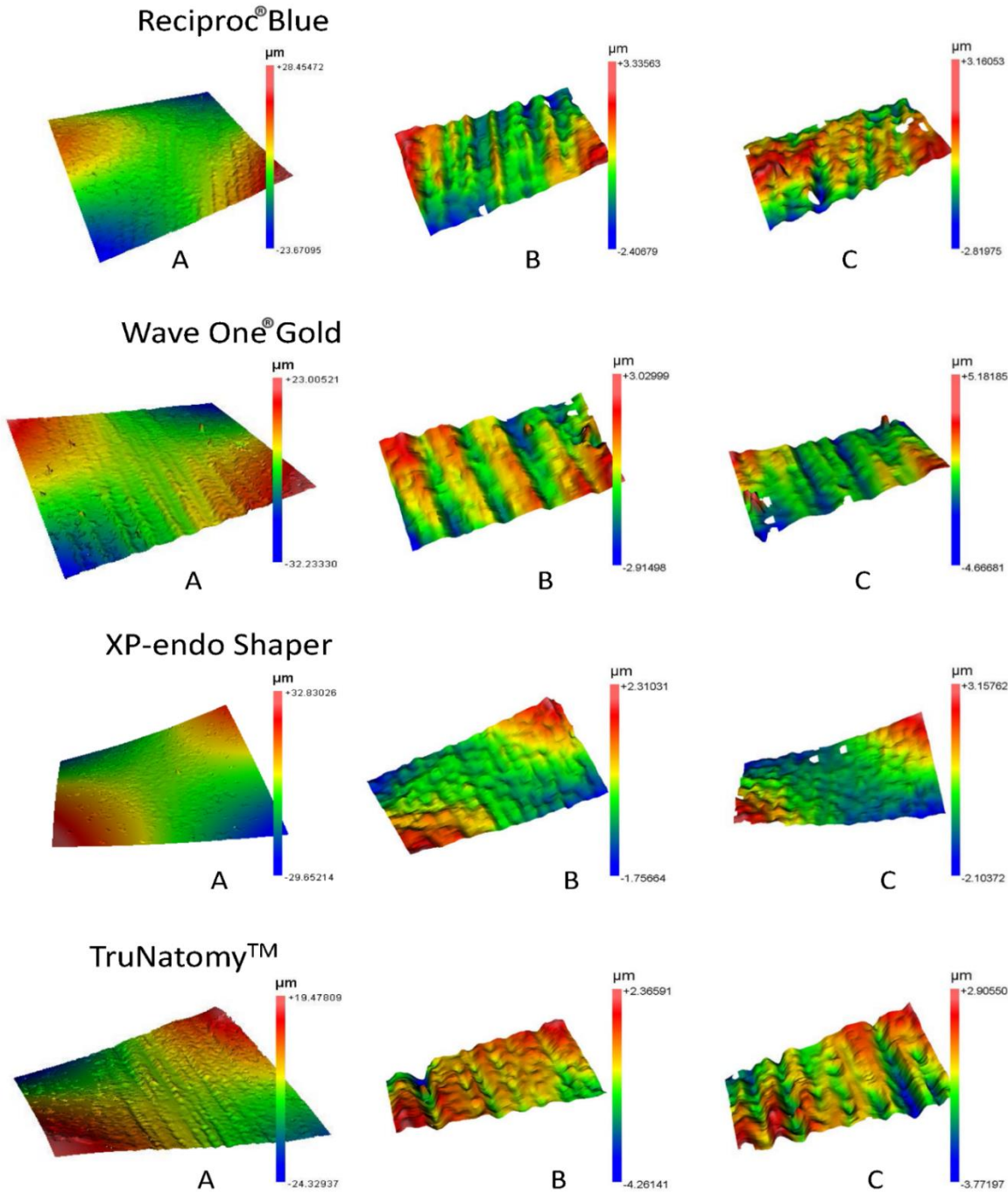


Figure 24. Surface morphology obtained by profilometry before (a), after the 1st use (b), and after the 2nd use (c) for each instrument group: Reciproc Blue (RC), WaveOne Gold (WG), TruNatomy (TRN), and Xp-Endo Shaper (XPS).

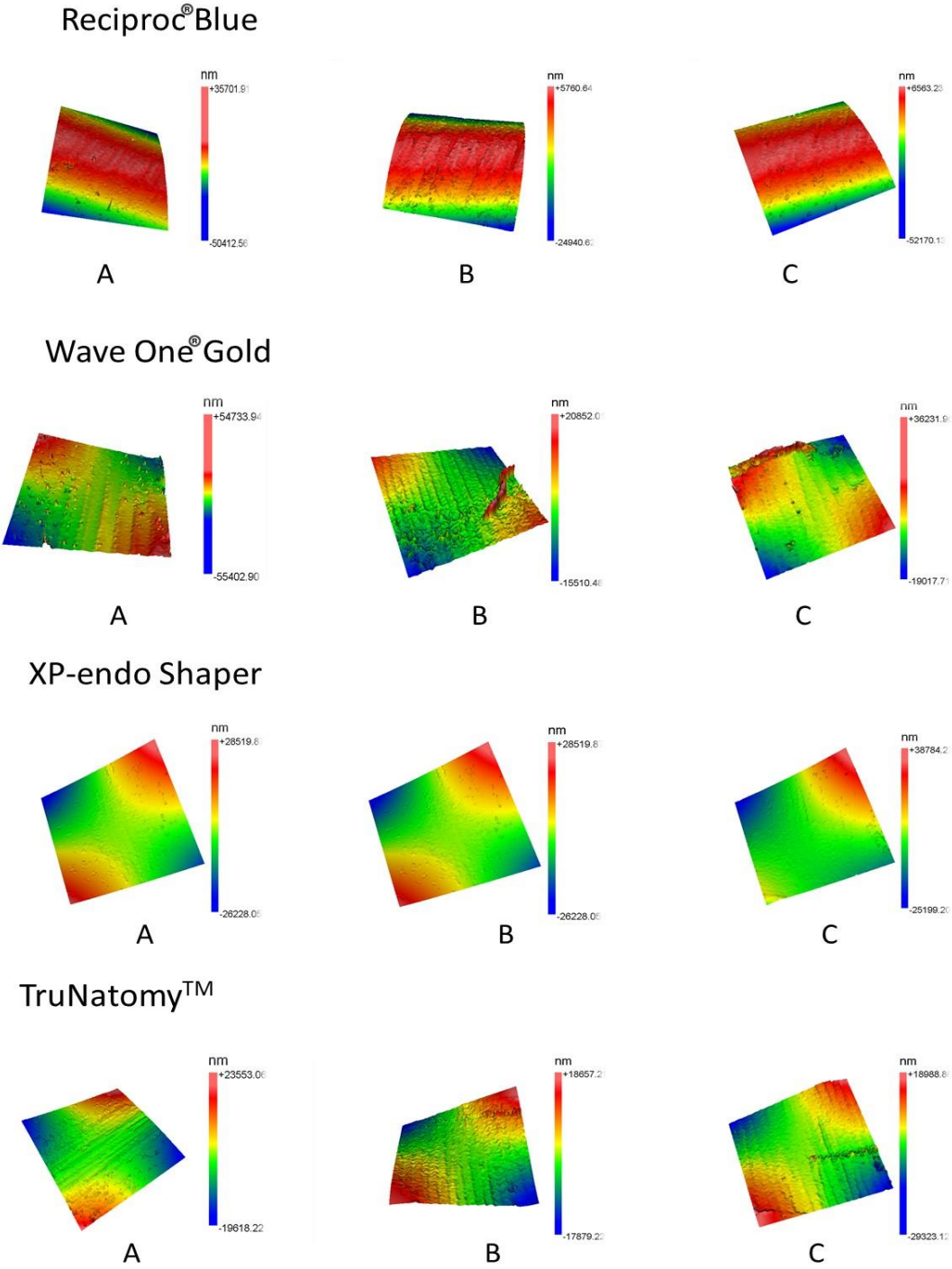


Figure 25. Surface morphology obtained by profilometry before (a), after the 1st use (b), and after the 2nd use (c) for each instrument group: Reciprocity Blue (RC), WaveOne Gold (WG), TruNatomy (TRN), and Xp-Endo Shaper (XPS).

5. DISCUSSION

This study compared the surface roughness (SR) before and after instrumentation to investigate the effects of endodontic instrumentation on different NiTi instruments. Endodontic instrumentation was carried out in extracted human teeth (ET) with curved root canals and in acrylic blocks (AB) with a simulated curved root canal (curvature: 20-40°). The presence of defects formed during the manufacturing process can make NiTi rotary instruments prone to spontaneous fracture (92, 93). The qualitative analysis of intact instruments showed minor surface defects even before their use, which is attributed to the machining and grinding of NiTi instruments. Furthermore, the surface properties of the alloy are significantly affected by heat treatment. Silva et al. (94) compared surface roughness and torsional resistance of identical heat-treated and non-heat-treated NiTi instruments. Surface roughness analysis revealed significant differences between heat-treated and non-heat-treated instruments in their study, with groove depth being lower on the surface of heat-treated ProDesign R files compared to non-heat-treated ProDesign R files. These findings demonstrated that thermal treatment has a direct impact on the surface properties of the instruments. Thermally treated NiTi alloys have an optimized microstructure, which may result in a reduction of the instrument's surface roughness (95, 96). As a result, reducing surface roughness can postpone the onset of instrument failure caused by rapid crack growth (97).

The cyclic loading and unloading during instrumentation result in repeated transitions between austenite and martensite, thereby, altering the mechanical properties of the instrument. (98). The stresses caused by mechanical instrumentation may increase the SR, friction, and incidence of instrument failure (87). Therefore, understanding the effects of usage on the surface properties of NiTi instruments may provide useful information about their clinical safety (90).

In this study, noncontact 3D optical profilometry was used to analyze the surface topography. Different microscopic techniques have been used to examine the surface topography of used NiTi files (99, 100). Scanning electron microscopy (SEM) provides 2-D images for subjective qualitative analysis by an observer (76). On the other hand, atomic force microscopy (AFM) requires a flat surface of the sample and examines very small areas; however, the curved or uneven surface of NiTi files makes AFM analysis very difficult. Ferreira et al. (83) first suggested the use of optical profilometry as a better alternative to AFM and SEM for analyzing the surface properties of NiTi files as it could examine larger areas and give

quantitative repeatable results (101, 102). For this reason, non-contact 3-D optical profilometry was used to quantitatively measure the usage-induced surface wear of novel rotary instruments in the present study.

The first study in dentistry using optical profilometry was published in 2001 to compare different methods of polishing composite restorations (103). Recently, Ferreira et al. (83) introduced noncontact 3D optical profilometry as a new and accurate method for the assessment of the surface topography of NiTi instruments. The technique is reported to provide accurate reproduction of constructed three-dimensional images, permitting full characterization and quantification of surface topographical features such as roughness and wear on the same surface area at different time points.

Thus, the ability to quantitatively evaluate surface topography is the primary benefit of 3D optical profilometry. In contrast to the other techniques, this technique does not require sample preparation for the analysis of any type of surface, with high precision and independent of magnification (82). This methodology, described by Ferreira et al. (83), enables repeatable, reliable, and accurate analysis of similar areas of the instrument surface at different time points. The ability to analyze areas from 0.1mm to 200 mm in three dimensions, including sharp-angled surfaces, enables complete characterization of the surface of endodontic instruments. The noncontact 3D optical profilometry is claimed to be accurate and reliable for qualitative and quantitative evaluation of the surface topography of endodontic instruments during manufacturing and after repeated use. The technique is a useful tool for future research on this subject (83).

In this study, the overall results from the AB group showed that TruNatomy instruments had minimal surface roughness similar to that of reciprocating files. There was no significant difference in roughness compared to reciprocating files. Contrarily, the XPS instruments showed significantly higher values of roughness compared to TruNatomy at every stage, especially after the second use ($p < .008$). This study was the first that evaluated the surface characteristics of XPS and TRN. TRN instruments are manufactured from thinner NiTi wire (0.8 instead of 1.2 mm) compared to other NiTi files. Thin wire combined with special heat treatment may have caused less friction during instrumentation in resin blocks and subsequently lower roughness values.

The TRN instruments, manufactured from thinner NiTi wire (0.8 mm), probably caused less friction during instrumentation and subsequent lower roughness values. The special

thermomechanical processing of the NiTi instruments and heat treatment reduce the internal stresses and surface defects, resulting from the grinding process, and might play a role in improving the surface properties (104).

The XPS instrument showed the greatest surface wear at every evaluation stage, especially after the second use. The greater surface wear in XPS could be attributed to its high rotational speed with increased time of instrument contact with the canal walls. Additionally, instrumentation with XPS results in more contact with the intracanal surface (105, 106), which could support the idea of greater surface wear due to increased contact with the canal walls of XPS.

RB and WG showed similar roughness values before and after two uses in curved canals. In contrast, AlRahabi and Atta (101) observed that WaveOne and WaveOne Gold instruments had higher roughness values than Reciproc and Reciproc blue instruments after four uses. This difference between the results of other studies could be explained by instrument usage in resin blocks in this study compared to their usage in extracted human teeth in the study by AlRahabi (101). Similar to our findings, Ferreira et al. (107) found similar surface roughness between Reciproc and Wave One instruments before and after two uses in resin blocks.

Previous studies evaluated surface topography either on acrylic resin blocks or extracted teeth, using different methods, thus providing hardly comparable results (60,78, 101). The main advantage of resin blocks is the ability to provide standardized root canal diameter, length, and curvature (108). On the other hand, studies on extracted human teeth should provide more reliable results with a better simulation of clinical conditions. Therefore, in the present study, the same instrumentation procedures are conducted on both human teeth and acrylic blocks, with similar curvature, in order to distinguish a better methodological approach in these types of studies.

Acrylic blocks (AB) with simulated canals were used in the past to assess cutting efficiency and other mechanical properties of endodontic instruments (107,109-112). They have uniform composition and homogeneous hardness, which could be advantageous in comparison with human or bovine dentin. However, this material does not reflect the clinical conditions of use and has been used to make comparisons (113). As previously reported, the reliability of canals in AB is an acceptable experimental model because simulated root canals provide standardization of root canal diameter, length, and curvature in terms of angle and radius (108, 114).

The recent studies (107, 109) on surface roughness, using 3D noncontact optical profilometry, were also conducted in simulated root canals in AB. In this study, the same protocol was used, but in order to evaluate the methodology, the same instrumentation procedures were conducted in acrylic blocks and selected extracted human teeth with a similar angle of curvature (20-40°).

Interestingly, the results between the two subgroups were not in line. As mentioned above, when used in AB, instruments showed significant changes in SR. On the contrary, when used in human teeth, there was no significant difference in SR between the groups, irrespective of the evaluation stage ($p = 1.0$). Different results between two subgroups can be explained by two factors: dentine microstructure and instrument design. Dentine is a dynamic tissue that undergoes a gradual transition in translucency with age that results in the filling of the dentin tubules with minerals, a process regarded as sclerosis (115). Dentin sclerosis occurs first and is most severe in tissue near the root apex (116). Additionally, surface roughness was measured exactly there, 3 mm from the tip of the instrument. That may suggest that the thinnest part of the instrument was in contact with the parts of the dentine where the sclerosis is most severe. In contrast, acrylic blocks have constant hardness, which is not changed along the simulated canal. On the other hand, the specific design of the XPS instrument, with the tip that has 6 sharp edges, may explain its different behavior in contact with dentin and acrylic walls. Although it has been claimed that acrylic resin has a lower hardness than dentine (117), the exact specifications of plastic block's hardness from different manufacturers are not clearly specified.

These results may suggest questionable reliability of results from the studies conducted in AB. An objection to the use of acrylic blocks is also the relative softness of the clear casting resin compared with root dentine (117 Lim). Polymethylmethacrylate (PMMA) in acrylic blocks presents Knoop hardness values between 18 and 20, whereas human radicular dentin shows values around 45 Knoop hardness values (118,119). It seems that Knoop hardness is not the only factor influencing the differences in results. Young's elastic modulus is significantly different between dentine (highly mineralized 40–42 GPa, whereas weakly mineralized 17 GP) and PMMA (2.9 GPa). Consequently, the friction between NiTi and acrylic surfaces may be greater, causing higher SR values.

Interestingly, although profilometry analysis revealed a change in instrument roughness after their use, this difference was significant only in the AB subgroup for XPS. The values of SR parameters mostly increased after the first and decreased after the second use in all groups. In

the HT subgroup, SR values were dominantly decreased after the first and increased after the second use (Rz, Rq), but the difference was not statistically significant. However, Ra values slightly, but not significantly, increased with usage in all instrument groups. These observations are partly in accordance with the results published by Moreira et al. (120), who concluded that the clinical use of Reciproc decreased surface roughness. In some previous studies using optical profilometry, the roughness values of reciprocating instruments increased after their use (90, 101, 109).

Various factors influence the surface roughness of NiTi instruments during root canal instrumentation such as mechanical or torsional stress produced by friction, and chemical corrosion caused by root canal irrigators. Also, root canal enlargement prior to NiTi instrumentation reduces torsional stress and the associated instrument wear (121).

In the present study, in order to simulate the clinical conditions, a glide path was created by a stainless steel K-file (size#10) to reduce torsional stress on NiTi instruments. In addition, constant irrigation by sodium hypochlorite was performed.

Irrigation solutions affect the surface and increase the SR of NiTi instruments (122). Fayyad and Mahran (123) investigated the effects of immersing NiTi in various irrigating solutions using AFM. Shahi et al. (124), Stokes et al. (125), and Casella et al. (126) investigated the effects of hypochlorite on instrument surfaces using electrochemical systems. They found that immersion in sodium hypochlorite at concentrations of 5.25 percent at 37°C does not decrease corrosion resistance, despite the fact that both SEM and AFM reveal effects on the investigated surface. Contrarily, corrosive effects will be visible not only microscopically but also macroscopically if the exposure times are greater than 12 to 24 hours (127).

A recent study by Zafar (128) investigated the surface roughness of ProTaper Next, ProTaper Gold, and WaveOne Gold before and after four instrumentation cycles. The results revealed significant differences for all roughness parameters, both before and after instrumentation, with WOG exhibiting the highest values. In their study, instruments were cleaned using an ultrasonic cleaner and sterilized (134°C for 4 min; pressure: 30 psi) prior to optical profilometry analysis.

Surface alterations are caused by both irrigants and autoclave sterilization according to scientific literature. The formation of surface irregularities or micro-pitting on the surface of instruments is an unfavorable effect of autoclaving and dry heat sterilization (129). There are contradictory findings concerning the various effects of sterilization procedures. Sterilization

and disinfection procedures, according to Dioguardi et al. (127), cause changes in physical and mechanical properties, but whether the effects are significant or insignificant for clinical use is not consistent across studies. In general, changes can be summarized as a reduction in cutting efficacy (statistically significant after five autoclaving cycles), and a corrosive effect after the use of NaOCl (when the exposure time is greater than 12 hours) (127).

Those findings and effects of sterilization can explain the differences between our results and results from Zafar (128). Contrarily, the corrosive effect of NaOCl can be considered irrelevant, in both studies, because the exposure time does not exceed 12 hours if the instruments are used twice (in our study) or four times (in the study by Zafar).

Although many similar studies on this topic have evaluated SR after four uses of the instruments, in the present study, each instrument was used twice in two separate root canals. Moderately curved canals were selected (20-40° angle of curvature), in order to simulate clinical conditions. All evaluated instruments are single-use instruments, which are not supposed to be sterilized or used in more than four root canals. In clinical conditions, having more than two curved canals in the same patient is extremely rare. Therefore, evaluation of surface wear after two uses may be considered relevant for clinical practice.

It has been claimed that increased surface roughness shortens the fatigue life of endodontic files (87). For this reason, to evaluate the possible correlation between SR and resistance to fracture, all instruments in this study were subjected to a CF test after their two uses. The results in both subgroups showed that the reciprocating instruments had a significantly higher CF resistance than the rotary instruments. These results are in accordance with previous studies (110-112, 130) that suggest that reciprocating motion improves the CF resistance of instruments, compared to continuous rotation, independent of other variables such as the speed of rotation, the angle of curvature, and NiTi instrument design.

In contrast, Gomes et al. (131) conducted a systematic review of the literature to find evidence on the clinical incidence of fracture of NiTi files used in rotary or reciprocating motions because the majority of the evidence indicating a higher fracture resistance of reciprocating instruments is based on laboratory studies, with results that are hardly comparable with clinical conditions. They emphasized that the impact of reciprocation may be less significant than other confounding factors. When the number of uses, for example, was considered, significant differences were discovered. A higher number of uses was found to be associated

with a higher risk of fracture. According to their findings, the overall clinical incidence of fracture of engine-driven NiTi files was 2.27 percent, with the occurrence of fracture decreasing over time. According to this review, the type of kinematics may not be a deciding factor in reducing the incidence of fracture of NiTi instruments. Other factors, such as the single use of the NiTi instruments and the operator's experience were found to be stronger predictors of instrument fracture (131).

RB showed the highest CF resistance followed by WG, XPS, and TRN. Similar to our findings, Duque et al. (132) observed that RB instruments presented a higher CF resistance than WG instruments after three uses in extracted mandibular premolars. Although both instruments have special surface treatment, the S-shaped cross-section of RB and smaller metal mass volume than the parallelogram cross-section of WG may increase the flexibility and lead to greater CF resistance (133).

In this study, XPS had the lowest CF resistance compared to the RB and WG instruments; however, there was no difference compared to the TRN. As this is the first study to evaluate the CF resistance of used XPS and TRN instruments, the results cannot be compared with those of previous studies. Azim et al. (134) observed superior CF resistance of XPS compared to WG, Hyflex EDM, and F2 ProTaper Universal. The different results between these studies could be attributed to the methodology, in which the CF of the instruments used was evaluated, compared to the non-used instruments evaluated in their study. Additionally, the angle of curvature of the artificial canal in our study was 60° compared to 90° in their study. In general, present results have not revealed any correlation between SR values and CF resistance, which may suggest questionable reliability of cyclic fatigue as a predictor of instrument safety in clinical use. Greater significance in the incidence of fracture may have the number of uses, operator's experience, or sterilization procedures.

Regarding clinical relevance, the overall result, which shows non-significant SR change after two uses of the instruments, suggests that reciprocating, as well as novel rotary instruments, can be safely used in more than one moderately curved root canal, without risk of fracture. On the contrary, surface changes are not correlated with resistance to fracture due to cyclic fatigue.

6. CONCLUSIONS

Primary conclusions

1. In this study, reciprocating instruments showed surface wear similar to a novel rotary instrument, after two uses in curved root canals. XP-endo Shaper exhibited significantly higher surface wear after use in acrylic blocks. Contrarily, differences were not significant after use in extracted human teeth.

2. The reciprocating instruments exhibited significantly longer time to failure, and consequently higher resistance to cyclic fatigue, compared to novel rotary instruments.

Reciproc blue instruments had the highest resistance to fracture, followed by WaveOne Gold, TruNatomy, and Xp-endo Shaper.

Secondary conclusions

1. 3D optical profilometry enabled repeatable and precise measurement of surface roughness and can be suggested as an accurate method for evaluation of surface properties of NiTi instruments.

2. Different results between instruments used in acrylic blocks and extracted teeth suggest that acrylic blocks are not an adequate substitute for the evaluation of the mechanical properties of NiTi instruments.

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8. BIOGRAPHY

Merima Balić was born on December 23, in Tuzla, Bosnia and Herzegovina. In 2010, she graduated from High School “Meša Selimović” and Music High School (class: piano) in Tuzla, in both schools with an excellent average grade of 5.0.

Upon graduation from Music High School in 2010, in the Hotel Tuzla, at the age of 18, she had a solo piano concert. From the age of seven, she was also a professional swimmer and a member of Bosnia and Herzegovina's National Swimming Team. She is a five-time national champion and record holder, and the owner of more than 150 international and national medals.

In 2010, she moved to Zagreb, where she started studies at the School of Dental Medicine. During her studies, she received the “Deans award” for the best academic success in 2011/2012. She received three “Award scholarships for excellence in studies” at the University of Zagreb. She participated in two European Dental Student exchange programs: in Belgrade (2013) and Istanbul (2014). Merima graduated on June 30, 2016, and was the first graduate of her generation, with an average grade of 4.9/5.0. She is currently in the last year of her Ph.D. studies at the University of Zagreb. During her studies, she published several scientific papers in domestic and international dental journals.

After graduation, she started working as a general dentist in “Štimac centar” in Zagreb, where she gained her first clinical experience. Two years later, in 2019, she moved to Budapest and started working as a prosthodontist in the international dental clinic “Madenta”. She is currently a self-employed dentist, working as a prosthodontist in two private clinics.

She fluently speaks English and German and has a good comprehension of Spanish, Turkish, and Hungarian language. Her native language is Bosnian/Croatian. Now, she plays music and does sports as hobbies.

Scientific publications:

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- Balić, Merima

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