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Is minimally invasive endodontics the next step? – Comparative study of two single-file rotary systems

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Is minimally invasive endodontics the next step? – Comparative study of two single-file rotary systems

Doctoral degree thesis submitted to the Faculty of Dental Medicine, University of Porto

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Resumo

Introdução: Este estudo teve como objetivo investigar e comparar sistemas de limas endodônticas relativamente à preservação da anatomia radicular interna, a avaliação da superfície dos instrumentos, a limpeza do sistema radicular e remoção da *smear layer* no terço apical assim como o grau de preservação de dentina peri-cervical recorrendo a um modelo experimental em dentes humanos. Os sistemas avaliados foram a TruNatomy (TN), a Protaper Ultimate (PU) e WaveOne Gold (WOG).

Métodos: Os sistemas de limas endodônticas avaliados foram utilizados de acordo com as recomendações de utilização do fabricante, utilizando-se um protocolo de irrigação que incluiu NaOCl a 1.1% e EDTA a 17%, com uma agulha de irrigação de diâmetro 27G.

Para o estudo da preservação da dentina peri-cervical e transporte canalar foram selecionados dentes monoradiculares maxilares e mandibulares e raízes mesio vestibulares de primeiros e segundos molares de ambas as arcadas. Para ambas investigações foi utilizada a microtomografia computorizada. Adicionalmente foram selecionados dentes pré-molares de ambas as arcadas para avaliar o grau de limpeza e quantificação da *smear layer* no terço apical com recurso a microscopia electrónica de varrimento.

As amostras foram digitalizadas com recurso a microtomografia computorizada antes e após a preparação químico-mecânica,de forma a avaliar o transporte canalar a 3,5 e 7 mm do ápex. Para a avaliação da preservação da dentina peri-cervical foram selecionados três pontos de interesse – a junção cemento-esmalte, uma localização 1 mm acima e abaixo deste ponto de referência.

Para a análise da *smear layer*, as porções radiculares dos dentes pré-molares foram secionadas longitudinalmente em duas partes. Nas imagens obtidas por microscopia eletrónica de varrimento, foi aplicado um sistema numérico para quantificar o grau de limpeza e remoção da *smear layer*.

A morfologia dos instrumentos foi avaliada antes e após uma utilização, recorrendo a microscopia eletrónica de varrimento.

Resultados: TN e PU apresentaram menos redução de volume canalar após instrumentação, em comparação com a variação de volume verificada com WOG ($p < 0.05$). Verificou-se uma redução de dentina peri-cervical em todos os grupos após a instrumentação, tendo os

sistemas TN e PU preservado de forma similar a dentina peri-cervical, e de forma superior à verificada com o sistema WOG ($p < 0.05$).
Em termos do transporte canalar e habilidade da lima permanecer no centro do canal radicular, verificou-se que todos os sistemas obtiveram resultados similares. Todos se mantiveram centrados e respeitaram a anatomia original, com desvios mínimos.

As imagens obtidas com a microscopia electrónica de varrimento revelaram uma remoção incompleta da *smear layer*, com os túbulos dentinários parcialmente abertos. Não se verificou uma diferença estatisticamente significativa entre os sistemas as avaliados.
A avaliação da superfície das limas revelou estrias nas limas antes da sua utilização, provavelmente como consequência do processo de fabrico, assim como, outras alterações - crateras, cavitações e irregularidades. Estas últimas três alterações aumentaram em quantidade nas limas observadas após uma utilização.

Conclusão: Os sistemas TN e a PU promoveram uma maior preservação da dentina peri-cervical e radicular. Nenhum, dos sistemas foi capaz de remover por completo a *smear layer* na região apical. Todos os sistemas permaneceram centrados no canal e demonstraram um desvio mínimo da sua anatomia original do canal.

Palavras-chave: Microtomografia computorizada, Dentina peri-cervical, Microscopia eletrónica de varredura, Smear layer, Transporte canalar, Distorção de limas, TruNatomy, WaveOne Gold, Protaper Ultimate

Abstract

Background: This study aimed to investigate and compare the shaping ability and file morphology, the degree of smear layer removal, and the degree of peri-cervical preservation of different endodontic file systems, namely TruNatomy (TN), Protaper Ultimate (PU), WaveOne Gold (WOG), within an experimental study with human teeth.

Methods: The endodontic file systems were used in accordance with manufacturer's instructions, and the irrigation protocol included using 1.1% NaOCl and 17% EDTA with a 27-gauge needle.
Maxillary and mandibular human single-rooted teeth and mesiobuccal canals of maxillary and mandibular first and second molars were selected for the peri-cervical dentin analysis and canal transportation assessment using microtomography.
Upon preparation with the distinct systems, maxillary and mandibular premolars were selected to assess smear layer removal in the apical third, using scanning electron microscopy. The samples were scanned before and after preparation with microtomography to evaluate root canal transportation at 3-, 5- and 7-mm levels from the root apex and peri-cervical dentin preservation at three selected locations – the cementoenamel junction and 1 mm above and 1 mm below this landmark. File morphology was compared before and after single-use upon scanning electron microscopy examination.

To study smear layer removal, premolar teeth roots were split into two portions. The canal surface was evaluated with scanning electron microscopy and micrographs were taken and assessed at x2000 magnification for cleanliness using a numerical scoring system.

Results: TN and PU presented the lowest canal volume reduction after instrumentation, with values significantly lower than WOG ($p<0.05$). Pericervical dentin was reduced in all groups upon instrumentation, with TN and PU evidencing similar levels of dentin preservation, significantly higher than those attained with WOG ($p<0.05$).
Similar findings were attained for the shaping ability comparison between all systems. All systems performed equally in terms of canal transportation and centering ability.

SEM-acquired imaging revealed incomplete smear layer removal and partially opened dentinal tubules in the apical third. No differences were observed between the systems.

Files imaging demonstrates machining grooves present before instrumentation and surface alterations such as grooves, cavitations and irregularities. These surface alterations increased after single-use instrumentation.

Conclusions: TN and PU showed the highest preservation of peri-cervical and root dentin. None of the assessed systems completely removed the smear layer in the apical region. All systems shape the root system maintaining files centered with minimal deviation of original canal anatomy.

Keywords: Micro-computed tomography, Peri-cervical dentin, Scanning Electron Microscopy, Smear layer, Canal transportation, File distortion, TruNatomy, WaveOne Gold, Protaper Ultimate

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Research papers published or in preparation for publishing derived from the doctorate research project

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II. Comparison of the canal transportation and centering ability of TruNatomy, WaveOne Gold and Protaper Ultimate – an ex vivo study in human molar teeth
   - Manuscript in the late stages for Journal submission

Abbreviations

ANOVA	One-way analysis of variance
Ba	Barium
CA	Centering ability
CEJ	Cementoenamel junction
CT	Canal transportation
EDS	X-ray Spectroscopy
EDTA	Ethylenediamine tetraacetic acid
ES	Endodontic Specialist
F1-F2-F3	Finishing files for Protaper System
FMDUP	Faculty of Dental Medicine of Porto University
GDP	General Dental Practitioner
HSD	Honest Significant Difference
Hv	High-voltage
Kv	Source voltage
Micro-CT	Microcomputer tomography
M-wire	Martensite wire
ms	Exposure measured in milliseconds
NaOCl	Sodium hypochlorite
Ni-Ti	Nickel-Titanium
NPI	Negative Pressure Irrigation
PCD	Peri-cervical dentin
PU	Protaper Ultimate
PUI	Passive Ultrassonic Irrigation
S	Sulphur
Se	Secondary electrons
SEM	Scanning Electron Microscopy
SPSS	Statistical Package for the Social Sciences
TN	TruNatomy

µA	Source current measured in microampere
µm	Image pixel size measured in micrometre
WD	Working distance
WOG	WaveOne Gold

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Chapter I|

1.1. | Introduction

Endodontics is a field of Dentistry which focuses on the assessment and management of the dental pulp and periradicular tissues. In other words, it encompasses the prevention, diagnosis and treatment of pathologies related to the dental pulp and surrounding tissues. [1]

For both the General Dental Practitioner (GDP) and the Endodontic Specialist (ES), selecting the appropriate options when considering root canal therapy can be a strenuous and impractical process, given the wide range of available approaches. In addition, the current literature has yet to provide a clear decision-making pathway for the clinician. This is observed due to the conflicting results from different studies, with the release of new research, systems and materials further accentuating this complexity. [2,3] Of additional relevance, it is regarded that despite all the technological advances, the success rate has not changed significantly for orthograde root canal treatments, with a rise from 82% to approximately 90% in recent publications. [5,6,7]

Most recently, there has been a paradigm shift in the assessment of root canal treatments. The concept now used for the outcome assessment focuses on survival rather than success, thus allowing inter-group comparisons with other treatment modalities such as dental implants. [7,8] Success and survival are two distinct endodontic outcomes. Success is defined as an outcome of an asymptomatic root-treated tooth with signs of apical periodontitis resolution and healing; while on the other hand, survival is defined as the outcome of a root-treated tooth with persistent signs of apical periodontitis lesions that have either reduced or remained unchanged in size. [9,10] Root canal successful treatment seems to mainly depend on reducing and eliminating microorganisms and preventing contamination of the root canal system by bacteria. [11]

With the advent of Ni-Ti endodontic file systems, root canal preparation has improved, allowing for a more efficient process and more time dedicated to the subsequent disinfection. However, a crucial question that new modern century endodontics faces is whether the emphasis should be placed on the chemical aspect of the chemical-mechanical preparation, considering that a significant technical improvement has been achieved through rotary Ni-Ti file systems. [12,13,14]

The issues with disinfection do not rely solely on the choice of the irrigant used but also on complementary methods of supporting/improving it, such as using systems like Passive

Ultrasonic Irrigation (PUI) or negative pressure irrigation (NPI), that are aimed to improve the disinfection outcomes. (15,16,17)

Another aspect to consider is the final taper of the root canal after instrumentation. Not all clinical scenarios allow standard tapers, such as 6%, to be used, otherwise increasing the risk of strip perforation. Current data suggest that smaller tapers may be preferred to minimize microleakage and improve long-term prognosis. (18,19,20)

In the same way, some literature favours larger apical diameters and larger tapered preparations, as stated previously. The ideal size and shape for the root system after preparation, which would enable optimal debridement, still needs to be determined. Some authors advocate large preparation sizes as this would allow for enhanced fluid movement and contact in the apical third. (21,22,23) Nonetheless, the satisfactory preparation of the root canal system does not rely on taper alone, but results from a combination of factors - some of which are shown in Figure 1.1. – the significance/impact of each has yet to be fully understood.

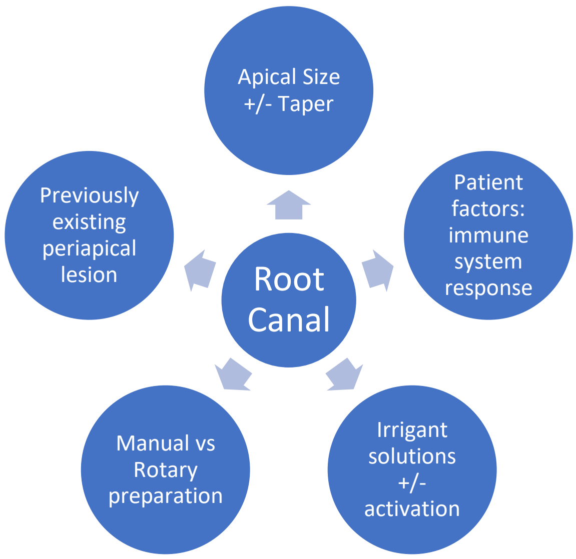


Figure 1.1 – Graphic representation of elements contributing to the outcome of an endodontic root canal preparation.(24)

Some of the systems available in the market include endodontic files with tapers above 6%. (25,26) Others promote a sizeable apical preparation whilst having a decreasing taper in the middle or coronal portions of the root canal. (27) The question arises if a reduced taper file system adequately shapes the root canal without inducing iatrogenic errors such as canal transportation whilst preserving peri-cervical dentin (PCD), thus increasing the long-term prognosis for treated teeth. Furthermore, curiosity and debate arise about whether the reduced taper impacts the apical third's irrigation and smear layer removal. [27,28]

The reduced taper and peri-cervical preservation fall within the concept of minimally invasive dentistry. When applied to endodontic therapy in dentistry, this has been envisioned as a system that still operates in the root canal, removing enough tooth tissue to allow penetration of irrigants and obturation to the working length whilst ensuring minimal dentin removal and maximum preservation of the root canal anatomy. In ideal circumstances, this would also entail the complete smear layer removal. Notwithstanding, the validation of these attributes, as well as the long-term impact on the root canal success, is also a topic of recent debate. [29]

1.2. | Background

1.2.1. | Areas of focus

1.2.1.1. | Canal transportation and centering

Centering ability (CA) is defined as the minimal or non-existent deviation of a root canal from its original curvature, ensuring that the endodontic instrument remains centred. [30] Canal transportation (CT) is one of the iatrogenic errors that can cause deviation from the original canal anatomy. [31]

Research demonstrates that rotary and reciprocating instruments have been shown to provide better centering ability than hand instrumentation. [32,33] However, research results are conflicting regarding differences between rotary and reciprocating systems. While some studies demonstrate a statistically significant difference concerning CA and CT, others do not. [34,35] [36,37]

CT can be classified as either internal or external. Internal transportation occurs when there is an elliptic preparation whilst the file is within the confines of the root canal; external transportation takes place if transportation occurs when the instrument is outside the confines of the root canal. [38]

This change in root canal anatomy can usually be seen where the canal's curvature rendered the file tip uncontrollable or where rigid instrumentation (with stainless steel files or large-size files) is performed in curved canals. [39,40]

The aetiology of CT is less dependent on the type of alloy used but more related to the inherent tendency of any root canal instrument to straighten itself inside the root canal. [31,40]

This iatrogenic error can impact the outcome and long-term prognosis of a root-treated tooth. [31] This reduces the ability of the clinician to properly remove the biofilm and smear layer, thus reducing the chances for success. [41] As such, it is essential to understand the mechanic-chemical principles of root canal treatment, acknowledge the properties of the endodontic file systems, and be mindful of the potential adverse effects these can cause during the procedures. Likewise, the clinician must have a thorough knowledge of which systems are more suitable for specific clinical scenarios, thus minimising the risk of such iatrogenic errors.

1.2.1.2. | Smear layer in the apical third

The smear layer, or microcrystalline debris, is formed whenever dentin surfaces are cut with hand or rotary instruments. It is composed of microscopic mineral crystals and an organic matrix. [40] The smear layer exhibits characteristics such as microbial penetration, occlusion of the dentin tubules within the root surface, and slowly dissolves over months to years.

There is debate as to if it should be left *in situ* or removed, as some argue that it could affect the quality of the seal between the dentin tubules and the endodontic cement, whilst others support the view that removing the smear layer increases the permeability of the dentin tubules potentially leading to bacterial infiltration. [40,42] Another consideration is that the smear layer may allow bacteria to persist or proliferate after shaping and cleaning procedures. [38]

The current view in Endodontics is that the smear layer should be removed as it enhances the efficiency of irrigant penetration. [43] This is typically achieved by using chelating agents in the irrigation protocol. [44,45] Some of these agents used in the irrigation of the root canal systems are citric acid, EDTA (ethylenediaminetetraacetic acid) and maleic acid, used at different concentrations, respectively, 6%, 17% and 7%. [46] These chelating agents show great capacity for removing the smear layer produced during instrumentation. [44,45] However, their effectiveness is seen in the coronal and middle thirds of the root canal. To date, no technique or irrigation solution has been able to completely remove the smear layer in the apical region. [47,46] Additional methods such as ultrasonic and manual pumping complement the traditional irrigation technique and improve smear layer removal. [48]

1.2.1.3. | Peri-cervical dentin preservation in root canal therapy

Endodontic treatment is performed to preserve a functional dentition in the long term. [49] Conversely, endodontically-treated teeth are considered to have lower survival rates when compared to non-root canal-treated counterparts. [50] However, this discrepancy has been a reason for debate, because the reasons for failure are often attributed to prosthodontic factors rather than issues stemming from the endodontic procedures. [51]

Many views and hypotheses have been proposed and investigated, such as changes in dentin properties, extensive loss of tooth structure, including marginal ridges before instrumentation, and/or excessive removal of coronal and cervical dentin during access and preparation of the canals. [49,52,53] Based on the current evidence available, tooth structure loss plays a significant role in the tooth's long-term survival. [51]

One of the critical areas of the tooth structure is the PCD. It is located approximately 4 mm above and 4 mm below the crestal bone level and is an area of force concentration, thus playing an essential role in a tooth's resistance to fracture. [49,51]

When endodontic treatment is carried out, coronal flaring is advocated to allow straight-line access to deeper portions of the root canal system. In addition, some instruments of endodontic engine-driven systems have larger tapers – 6% or above compared, in contrast to the standard 2% taper of hand files. [51] As a result, large areas of peri-cervical dentin are removed in the mechanical-chemical preparation phase, ultimately compromising the long-term survival of these teeth.

A treatment idealised to preserve the dentin and the tooth's function originates a wide range of challenges and factors affecting the initially planned outcome. One must be aware that it is not solely the PCD that is being removed, as the coronal tooth structure such as the pulp chamber roof and, in cases of previous pathology, the marginal ridges and large portions of the occlusal surface of a tooth. It could present an even more significant challenge in smaller-diameter teeth, such as anterior teeth and premolars.

With the advent of new rotary systems and the combination of concepts such as minimally invasive endodontics, manufacturers have created endodontic file systems designed to provide adequate canal space preparation and conservation of the peri-cervical dentin, thus ensuring a better long-term prognosis for the teeth. Such is the case for TruNatomy (TN) and Protaper Ultimate (PU), with a smaller flute diameter compared to most systems on the market.

1.2.1.4. | Endodontic file alterations following instrumentation

The root canal treatment sequence has largely remained consistent. One must shape and disinfect, and then obturate the canal space. How this is achieved can vary from clinician to clinician. The current trend is the increased use of rotary systems, as these allow for shaping to be performed in less time than manual instrumentation.

Accordingly, rotary file development is an area of dentistry with significant innovations. Historically shaping was performed using stainless steel manual files, and later with nickel-titanium (Ni-Ti) alloy instruments. However, these files and techniques presented shortcomings, such as rigidity and extended procedure times. Iatrogenic errors were also more frequent with hand instrumentation. (54)

With the advent of rotary instrumentation in the 1990's, Ni-Ti endodontic file systems became the norm in endodontic practice. (55,56) Current Ni-Ti alloys used in dentistry typically comprise 55% Ni and 45% Ti as the standard composition. (57) These files offer advantages like the ability of surface treatment, optimized alloy composition, and increased elasticity. The defenders of the rotary Ni-Ti systems used this argument to shift perspectives. They claimed rotary Ni-Ti systems were safer, and those iatrogenic mishaps could be easily prevented due to file flexibility. (58)

Despite these modifications intended to reduce fracture risk and deformation, the use of Ni-Ti alloys lead to the development of potential iatrogenic changes during canal preparation. The risks, if used incorrectly, are well-documented and described in the literature. (59) As the files are used and endure stress, it is possible to observe upon closer inspection, distortion and curving, and even unwinding of the flutes. (60,56)

The two primary reasons for the failure of an endodontic rotary instrument are excessive torsional and/or flexural load that generates stresses that exceed the elastic deformation capacity of the instrument, causing it to first deform plastically, and eventually fracture. There could also be a combination of the two. These would be considered permanent changes; however, before becoming permanent, the files can undergo temporary alterations that revert to normal once the stress is reduced or removed. (61,62,63,64)

Regarding movement kinematics, reciprocating motion (as exemplified in systems like Wave One), appears to generate less stress and/or expose the instrument to less prolonged stress during clinical use. (65)

1.2.2. Engine-driven systems assessed

Engine-driven endodontic systems have grown exponentially due to their cost-effectiveness and ease of use. They have become indispensable and are a routine element in root canal therapy. (55, 66)

The first Ni-Ti file became commercially available in the 1990s and have constantly been subject to improvements with a focus on different aspects such as taper, manufacturing treatment, Ni-Ti wire material, and motion mode. (55,67) These rotary files have evolved through several generations. The first generation focused on the geometric design of the files. The second generation, which included files like Protaper Universal, introduced modifications such as surface modifications. The third generation saw the introduction of materials in the martensite phase, such as the M-wire (martensite wire), emphasizing material improvement. The fourth file generation focused on improving the motion modes and reducing the risk of torsional fracture. At this time, other file motions, such as reciprocation, were developed, alongside the introduction of single file systems (such as WaveOne). These files provided efficient cutting, reduced operating time, and cost control. (68,69) The fifth generation of endodontic files includes files released from 2010 and onwards. (55) These more recently developed systems have been created in line with current treatment philosophies, such as minimally invasive endodontics, thus having reduced tapers, smaller flute sizes, and extremely flexible alloys, allowing them to preserve the original root system anatomy. (57)
Notable examples of such engine-driven systems of relevance are TN, WOG and PU.

1.2.2.1. | TruNatomy

The TN system encompasses various components, including an orifice modifier file (20.08), a glide path instrument (17.02v) and four shaping files – Prime (26.04v), Small (20.04v), Medium (36.03v) and Large (46.02v). It comes with a recommended irrigation needle which is flexible, thin, and long, allowing the irrigant to be delivered in the more apical portions of the root system. The package also includes file-specific obturation gutta-percha points and paper points.

This rotary system comprises standard Ni-Ti wires but incorporates special modifications such as micro-milling and post-machining heat treatment. It also features a patented geometric form: combining a parallelogram and a reduced flute diameter of 0.8mm. The glider file has centred parallelogram cross-sections, whilst the shaping files have an off-centred parallelogram section. All files have a variable taper.

It is essential to know that both the glider file and the shaping files have a reduced taper – 2% and 4% and the same diameter at the coronal third, thus effectively preserving peri-cervical dentin. However, the orifice modifier has an 8% taper, which could pose a contradiction to its acclaimed feature of dentin preservation. Its potential is clear to what concerns root dentin, but it might have similar results to the other endodontic file systems in the market, given that the orifice taper is 8%. [70] Thus, the tooth's long-term prognosis may be similar, if there is evidence that the PCD was not preserved. TN has shown excellent results in terms of cyclic fatigue resistance in both single and double-curvature canals, showing less canal transportation compared to WOG and Protaper Gold. [71]

1.2.2.2. | WaveOne Gold

The WOG system is presented as a single-file reciprocating system, aiming to simply the endodontic treatment. According to manufacturers, in cases where a glide path cannot be established, or the canals are tight, the system provides instruments to create a glide path. The WOG system encompasses a glider file (015), and four shaping file sizes – Small (020), Primary (025), Medium (035) and Large (045). It includes the same irrigation needle offered for the TN system in its packaging, as well as file-specific obturation gutta-percha points, GutaCore for WOG and paper points.

All files possess variable taper. For the small and primary files, the first 3 mm have a continuous taper of 7%; for the medium file, the first 3 mm have a continuous taper of 6%; and for the large file, the first 3 mm have a continuous taper of 5%. Beyond the 3 mm mark, the taper progressively decreases, imparting greater flexibility to the file and preserving more dentin in the body of the prepared canal. (72,73)

This rotary system is made of Ni-Ti metal, which features improved attributes such as reduced memory. In addition, it undergoes modifications such as post-manufacturing heat treatment, also known as gold heat treatment, further enhancing its mechanical properties and durability. The geometric cross-section is off-centred, following a parallelogram shape.

This system represents an improvement from its predecessor, WaveOne, offering a balance of simplicity and safety. As a result, it is a widely used system in general and endodontic practice. (74)

1.2.2.3. | Protaper Ultimate

The PU system offers an array of innovative features for endodontic procedures. It encompasses an orifice opener SX (020.003v) and a slider file (016.002v), which corresponds to the roles of the WOG glider and the TN glide path instruments. Preceding the use of the three main finish files, F1 (020.007v), F2 (025.008v), and F3 (030.009v), it offers a shaping file called shaper (020.004v). All files possess multiple tapers and are wider at the apical region for enhanced apical cleaning while remaining conservative in the coronal portions. This system also includes a flexible irrigating needle, as mentioned for the previous rotary systems. The system also includes file-specific obturation gutta-percha points (Conform Fit) and paper points.

This rotary system is made of Ni-Ti and introduces the concept of Deep Shape. It intends to address the challenge of cleaning the apical portion effectively. As such, it was designed to have an increased apical taper, leading to optimised hydraulics of the disinfection fluid and better evacuation of the debris.

The PU files have parallelogram cross-sections. The Slider file receives a pre-thermal treatment (M- wire technology) while the Shaper and Finisher files receive a post-grinding heat treatment – Gold heat treatment. Conferring the files with reduced memory and increased flexibility. (75)

All systems are designed to increase efficiency during shaping and for chemical disinfection to be carried out successfully. Moreover, focus has been established on designing some files to increase the effectiveness of smear layer removal in the apical third, contributing to the overall success of root canal procedures.

Currently, there is a lack of similar independent studies where TN, PU and WOG have been assessed regarding PCD preservation, shaping ability and smear layer removal, among other important features and properties which are per the norm assessed when a new endodontic system is added onto the market and is ready to be used in clinical practice.

From a literature search, it is possible to identify articles of interest comparing two systems here referenced and assessed such as a comparison between TN and WOG or the comparison of TN, WOG and other versions of Protaper. [76,77,78]

The focus of the current research is of importance seeing the practical and clinical implications that the data acquired provided.

1.2.3. | Study objectives

The main objective of this work was to compare TN, PU and WOG endodontic file systems and assess them regarding canal transportation, cleaning effectiveness in the apical third, and how these instruments fare regarding peri-cervical dentin preservation in an *ex vivo* research study with human teeth. As the alloys have been processed using modern techniques, another objective was to assess each file for visual changes following instrumentation.
To summarise, the null hypothesis is that there were no statistically significant differences between the file systems in all parameters assessed.

CHAPTER II|

2.1. | Materials and Methods

The approach to our analysis within each subgroup was characterised by a commitment to reproducibility and skilful execution. The objective was to provide new knowledge and high-quality evidence for the field of endodontics so that can translate into better patient care.

Micro-CT, a cutting -edge imaging technique, was used to identify root canal variations in the apical and peri-cervical regions, as it offers enhanced resolution and detailed information about minor anatomical structures. It also offers a non-destructive, three-dimensional replication of the root canal system that can be repeated multiple times. [79]

Scanning electron microscopy (SEM) is the most commonly used methodology for assessing cleanliness effectiveness and file deformation visualisation and was adopted for the method of this study. [80,81]

Extracted human teeth were used to emulate the clinical setting as closely as possible, enhancing the reliability and relevance of the attained data and results. [82]

In this context, is important to highlight that crestal bone levels are challenging to determine once the teeth have been extracted. For standardisation, the cementoenamel junction (CEJ) was selected as the reference point for this analysis. This is reproducible and has been previously advocated. [83]

The endodontic files for the different groups - WOG, PU and TN were used to instrument root canal systems in extracted human teeth.

The different rotary instrument systems were new and used per the manufacturer's recommendations.

It is essential to highlight that all manufacturers advocate for single-use. There is no mention about the number of canals the files can be used before it is recommended they are discarded.

The only system that provides more precise and relevant information is the TN files.

These are intended for Single Use only (on one patient during a single procedure). The TN file's mechanical characteristics support at least four canals, 35° curved (i.e. Schneider technique). [83] The clinical applications are that the molar tooth, which contains in average 4 canals, can have a single-use file perform the shaping and debridement. [85]

2.1.1. | Research design

The research final design includes a comparative analysis of three endodontic mechanically driven systems (TN, PU and WOG) mentioned in human extracted teeth.

The use of natural human teeth aims at modifying the experiment conditions so that they more closely resemble the naturally occurring conditions such as those seen *in vivo*.
As such, this study is labelled as an *ex vivo* comparison which refers to an investigation carried outside of a living organism. [86]

Due to the lack of clinical data this first stage of basic research is required before clinical case reports can be offered with a comprehensive understanding of all file features and overall performance in these and other parameters of relevance. [87]
Preclinical or laboratory-based research can offer important data before clinical studies and investigations can be safely planned and executed. [87] There is however the need to ensure the findings are reliable and can be replicated. This study followed previously published research methodologies and techniques thus offering comparable results that can be reproduced. [88]
The current analysis offers a technical and experimental testing which compared files qualities and effects under controlled conditions by using random allocation of interventions to comparison groups - human extracted teeth. [89]

As the research design included the use of organic human sample materials, it required the need for ethical approval.

2.1.2. | Samples selection and initial preparation

The study received the ethical approval from the FMDUP ethics committee (Reference number 18/2021), and consent forms to be used for specimen collection were appropriately obtained. Written consent was obtained for all the samples collected.

For the Micro-CT analysis, the research sample included forty-five maxillary and mandibular single-root teeth (incisors and canines) and twenty-seven curved mesiobuccal canals of extracted human mandibular and maxillary first and second molars. The latter sample had at least one curved and operable mesiobuccal canal. Additionally, eighteen extracted human maxillary and mandible premolars were selected for the SEM investigation.

Permanent teeth with completely formed apices and with a patent canal with curvatures between 10° and 40°, as measured according to the criteria described by Schneider, were included in the canal transportation analysis. [90] The teeth with calcified, non-patent canals, teeth with resorptive defects, broken apices, vertical root fracture and teeth with curvatures less than 10° and more than 40° were excluded from this analysis.

The exclusion criteria for the SEM and smear layer analysis were: teeth with incomplete apices, teeth with previous root canal treatments, teeth with vertical root fracture, broken apices, resorption – internal and/or external, deep caries or restorations affecting the CEJ (internally).

The teeth were disinfected initially using buffered formalin 10% (APC Pure, Cheshire, United Kingdom) for a minimum period of 1 week. After this initial step, the samples were submerged in saline when not handled or prepared.

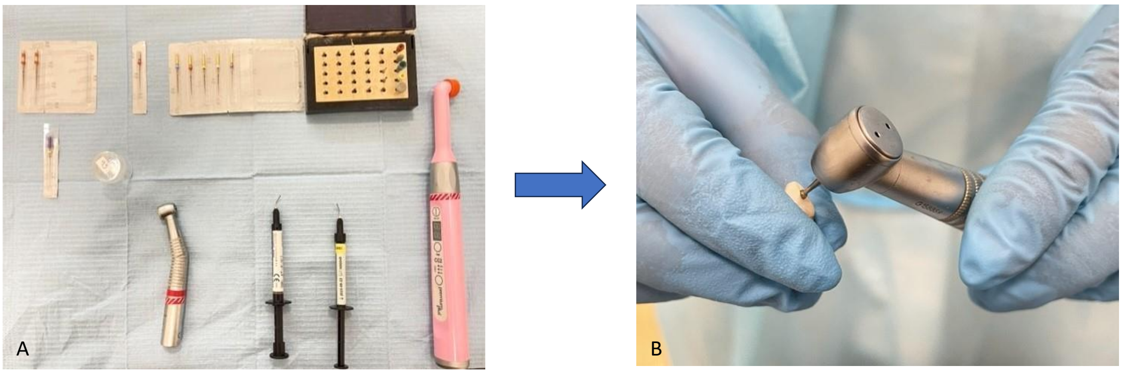
Each specimen was subjected to an access opening, which was skilfully performed using a medium grit medium-sized round diamond bur (126210, Dentaleader, Lisbon) and a fast handpiece with water cooling. The Endo Z bur was used to refine the access cavity. The initial file used in all selected teeth was a K10 (Dentsply Tulsa Dental, Charlotte, USA), which was gently passed through the foramen to minimise canal lumen changes. The working length was established after the K10 file reached the foramen, with a subsequent subtraction of 1 mm from the measurement. This adjustment was made since the file tip was positioned at the apex foramen, thus providing the working length for each specimen. A single operator with postgraduate training in endodontics executed all preparations, including access, irrigation, shaping and temporary restoration.

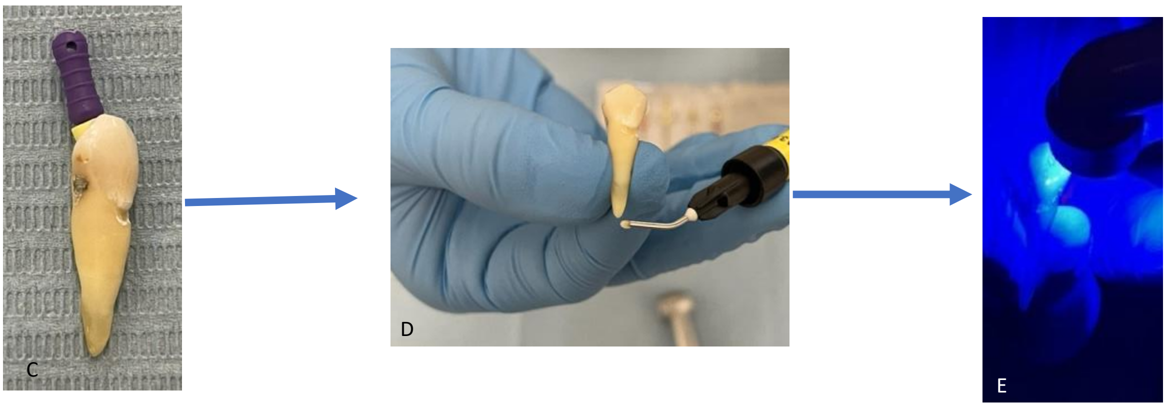
2.1.3. | Shaping protocol

The canals were prepared with WOG, TN or PU files according to the manufacturer's protocols, including orifice openers if included in the manufacturer's recommendations. The samples were all instrumented with rotatory and reciprocating instruments with a 0.25-0.26 tip to maintain standardisation. The shaping sequence is summarised in Table 2.1 and Figure 2.1.

Table 2.1 – Shaping sequence summary for all three file systems.

PU	WOG	TN
Glide path was established by using a K10 2% file and the slider file. Sequential enlargement was carried out until the WL was reached in a wet canal, with F2 being the last file used. Small pecking movements of 2-3 mm were applied. Frequent recapitulation and irrigation were accomplished.	Glide path established with a K10 ISO 2% file and WOG Proglider 0.16 2% ISO. The WOG primary file was used in short increments 2-3 mm with irrigation and recapitulation between each shaping attempt. Once the WL was reached, shaping was concluded and the irrigation protocol was carried out.	Glide path was established after the use of the orifice modifier with a K10 2% and TN glider files. The files were advanced in the presence of an irrigant solution, in a pecking motion of 2-3 mm. Irrigation and recapitulation were repeated as necessary. The last file used was TN Prime.
Irrigation protocol: A 27-gauge needle, placed 3 mm short of the working length and employing a 1.1% sodium hypochlorite solution. The subsequent sections will provide a more comprehensive description of the irrigation protocol where needed.		


A
B


C
D
E

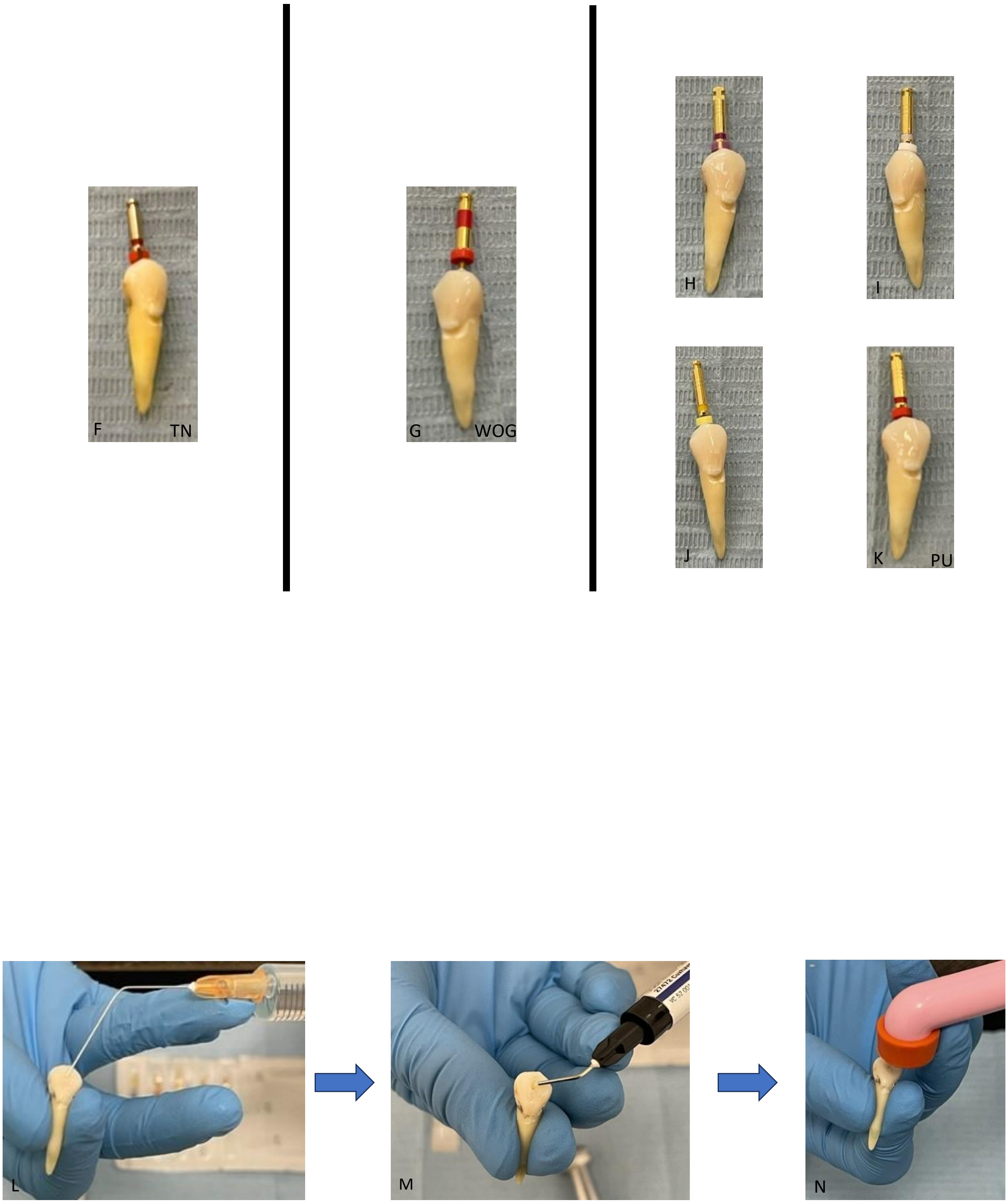


Figure 2.1 – Representative image sequence showing the steps of tooth preparation and shaping

2.2. | Analyses

2.2.1. | Endodontic files morphology and structure – scanning electron microscopy (SEM)

All the files (control and used files study group) were observed under SEM (FEI Quanta 400FEG scanning electron microscope, Hillsboro, USA) and photomicrographs were taken at 100x, 500x,1000x and 2500x magnification with the following settings:

- High Voltage (HV): 15.00kV
- Working distance (WD):15.2 mm
- Secondary electrons (SE) mode

An evaluation of the morphological changes was carried out, including the number and the type of defect for a magnification of 2000x.

The surface defects recorded were:

a. Irregularities - broad areas of rough, non-smooth surfaces and not including grooves and cavitations;
b. Grooves – extended, narrow cut or depression into the metallic surface of the file – cavitations may or may not be present;
c. Cavitations - localised round or spherical spaces which may or may not be associated with grooves.

The literature search presented several articles with morphological assessment methods; however, their description needed more information on how each defect was classified. Such analysis presents limitations such as difficulty in characterising findings, the subjective nature of the observation and operator bias. [91] Thus, the qualitative analysis here determines the form and the type of defects it would incorporate and is specific about the characteristics of each defect. [92,93,94]

2.2.2. | Peri-cervical dentin and volume wear assessment - microcomputed tomography (micro-CT)

The peri-cervical dentin was analysed through micro-CT scans. The PCD thickness was calculated as the shortest distance from the canal outline to the closest adjacent root surface, which was measured on four surfaces, i.e., facial, lingual, mesial, and distal, for all the groups in the two obtained scans (pre- and post-preparation) using axial cuts. [95] Accordingly, a micro-CT scan was performed before and after preparation using a BRUKER- Skyscan1276 (Bruker Corporation, Kontich, Belgium) equipment, with the following settings:

- Source voltage (kV) = 100
- Source Current (uA) = 200
- Image pixel size (um) = 20.014000
- Exposure(ms) = 432
- Scan performed with 360 rotation

The scanned datasets were reconstructed using the software NRecon version 1.7.4.2 with the following settings:

- Pixel size (um) = 20.01400
- Smoothing = 0
- Ring Artifact Correction = 8
- Beam Hardening Correction (%) = 6
- Minimum for CS to Image Conversion = 0.000000
- Maximum for CS to Image Conversion =0 .040000

A volumetric representation of the datasets was prepared with DataViewer software version 1.5.6.3. To assess volume changes and the remaining dentin thickness, measurements were taken at 1 mm above, 1 mm below, and at the CEJ – a reference point chosen for standardisation – and the volume changes following root canal preparation were recorded by measuring the distance from the edge of the canal lumen to the tooth's margin, (Figure 2.2.). The volume variation was calculated using volume extraction modules and with the assistance of a computer-assisted software program – CT Analyser software (Bruker, version 1.17.7.2) following the guidelines from Bruker (MN110), wherein root canal volumes were calculated both before and after preparation, and the differences were subtracted for volume change quantification. Representative three-dimensional images were captured using CTVox software (Bruker, version 3.3.0).

An assessment of the volume (mm^3) of the entire canal length was carried out. The same software and parameters were used by deducting the scores of the prepped canal spaces from the data obtained from the unprepped, original canals.

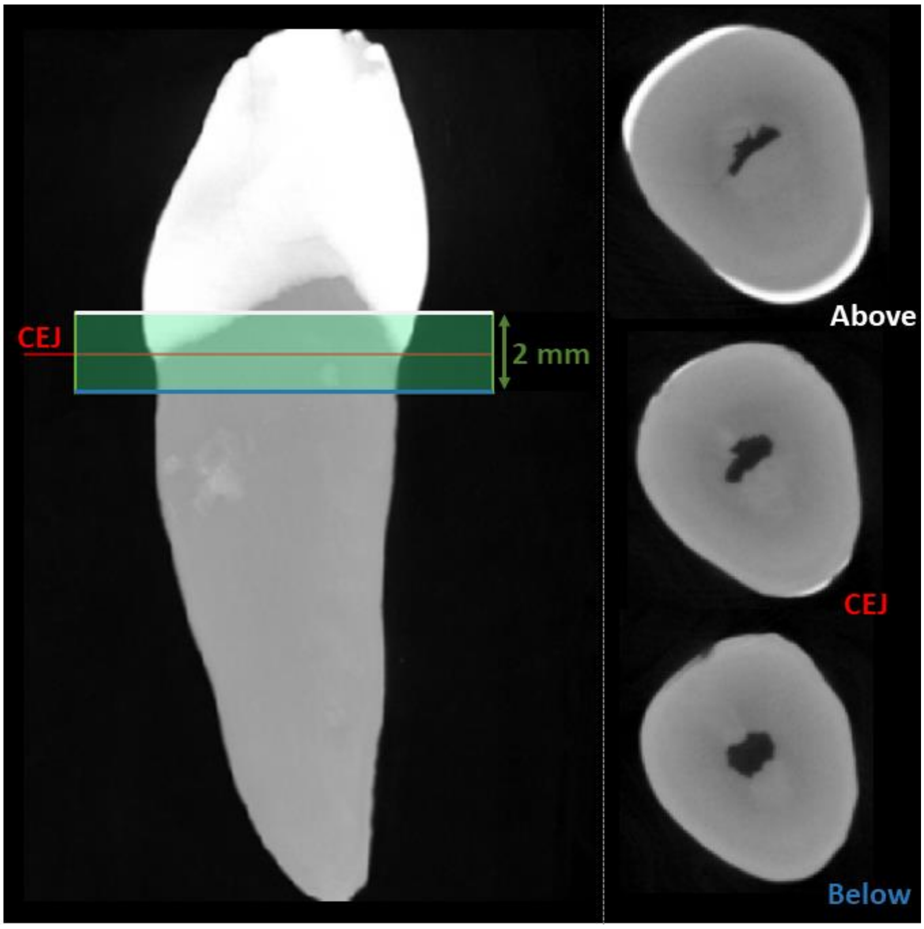


Figure 2.2 – Representative microtomographic image highlighting the CEJ, and regions for analysis, from a sagittal and an axial view

2.2.3. | Smear layer evaluation - scanning electron microscopy (SEM)

For this evaluation, eighteen extracted human maxillary and mandible premolars were selected.

The sub-analysis followed the sample preparation previously mentioned, differing on the following described procedures. Root ends were sealed with flowable composite A3.5 Synergy D6 flow (Coltene, USA) to prevent irrigants from escaping through the apex, simulating *in vivo* closed apex conditions. Irrigation was performed with a 27-gauge needle using 1.1 % sodium hypochlorite. The needle was inserted 3 mm short of the working length. The final irrigation protocol for this sample group was 5ml EDTA for 1 min, followed by 5ml 1.1% hypochlorite for 1 min. The prepared canals were dried using paper points specific to each endodontic file system. The canal orifices were sealed using a cotton pellet and glass ionomer (Ionoseal, Voco,Germany) to prevent the entry of debris into the canal system during tooth sectioning. The samples were sectioned with a 4.2 mm straight osteotome (Hufriedy, Germany) splinting samples into two halves. Specimens were dehydrated in a graded series of ethanol solutions (50%-100%) and then left to dry in the open air for 24 hours. The split halves were secured with SEM specimen stubs and coated with a 30 nanometers-thin gold-palladium (Au-Pd) coating for SEM analysis (FEI Quanta 400FEG ESEM/EDAX Genesis X4M scanning electron microscope, Hillsboro, USA). SEM photomicrographs were taken at different magnifications.

The most representative micrographs at 1 mm from the apex were taken and assessed for smear layer content and dentin tubules patency using a numeric scoring system extracted from a previously published report Table 2.2. [96]:

Table 2.2 – Smear layer scoring system

Scores	Criteria
Score I	No smear layer and dentin tubules open.
Score 2	Small amounts of scattered smear layer and dentinal tubules open.
Score 3	Thin smear layer and dentinal tubules partially open.
Score 4	Partial covering with a thick smear layer.
Score 5	Total covering with a thick smear layer.

The images were assessed by 2 researchers and in the case of divergent score, the lowest mark given was selected.

2.2.4. | Canal transportation and centering ability – micro-CT

After establishing the initial access and patency, the teeth were radiographed with a K10 file *in situ* to measure root canal curvatures according to Schneider's method. [97]
The method consists on drawing two distinct lines on the radiograph: the first line is drawn parallel to the long axis of the canal, providing a reference for its orientation; and the second line, starting from the apical foramen, intersects the first line at a point where the canal begins to deviate from the long axis of the tooth. This intersection point marks the precise location where the curvature of the root canal begins. Angulation was herein calculated.

For evaluation, a micro-CT scan was performed before and after preparation using a micro-CT BRUKER – Skyscan1276 (Bruker Corporation, Kontich, Belgium) with the following parameters:

- Source voltage (kV) = 100
- Source Current (uA) = 200
- Image pixel size (um) = 20.014
- Exposure(ms) = 432
- Scan performed with 360^0 rotation
- An aluminium-copper filter was attached

The images were reconstructed with the NRecon software version 1.7.4.2. (Bruker Corporation, Kontich, Blegium).

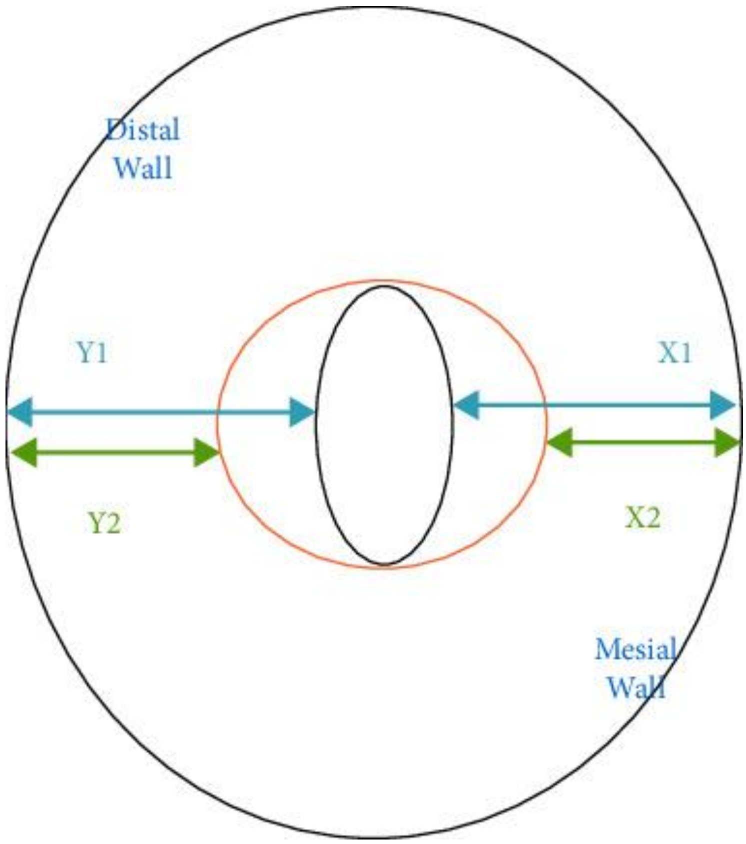
After image reconstruction, the root canal systems were binarized, and their volumes were calculated allowing a comparative analysis. Three-dimensional images were captured using CTVox software (Bruker, version 3.3.0).

Three zones were investigated:

1. Coronal (7 mm from the apical foramen)
2. Middle (5 mm from the apical foramen)
3. Apical (3 mm from the apical foramen), according to a previously established methodology [98]

For a detailed analysis, 1mm thick cross-sectional slices, perpendicular to the root canal's long axis, were collected from the acquired dataset. [99] In assessing the extent and direction of the canal transportation, these were determined by measuring the shortest distance from the edge of the un-instrumented canal to the edge of the outermost boundary of the tooth in both the mesial and distal directions, and then compared with those taken from the instrumented images. [99] A visual representation is seen in figure 2.3.

This was calculated using the following formula: $(Y1–Y2)−(X1–X2)$, wherein Y1 represents the shortest distance between the canal's distal wall and the peripheral edge of the root before instrumentation; Y2 denotes the shortest distance between the canal's distal wall and the peripheral edge of the root after instrumentation; X1 stands for the shortest distance between the canal's mesial wall and the mesial periphery of the root before instrumentation; and X2 signifies the shortest distance between the canal's mesial walls and the mesial periphery of the root, after instrumentation. [100]



A result of 0 from the canal transportation formula indicates that no canal transportation occurred during the instrumentation process.

.

Figure 2.3 – Schematic representation highlighting the formula used for the canal transportation scoring.

The mean centering ratio measures the ability off the instrument to maintain its central alignment in the canal. This ratio can be calculated for each section using the following equation (Equation1):

$$\frac{(X1 - X2)}{(Y1 - Y2)}$$

The numerator for the centering ratio formula is determined as the smallest of the two numbers, between (XI - X2) or (Y1 - Y2). [98] Using this formula, a result of 1 for the centering ratio would indicate perfect centering. [98]

2.3. | Statistical analysis

Analysis of variance (ANOVA) was used to find the significance of study parameters between three or more groups, followed by post hoc Tukey HSD analysis of the ANOVA values that were statistically significant. The level of significance was fixed at $p = 0.05$ and any values ≤0.05 were considered statistically significant.

The software SPSS (Statistical Package for the Social Sciences, 28.0, IBM, USA) was used for calculations.

CHAPTER III|

3.1. | Results

3.1. | Endodontic files morphological analysis

The SEM images acquired for the three endodontic instruments studied show different surface characteristics, prior and upon canal preparation. Apart from the machining grooves which are expected as part of the manufacturing process other surface alterations could be seen. Data represent the number and type of defects before and after a single use.

Table 3.1. – Scores of the qualitative analysis of the SEM images acquired of the endodontic files and their irregularities and deformations after instrumentation

Groups	Irregular	Grooves	Microcavities
WOG	2-2	13-18	10-7
TN	5-3	15 -19	6-3
PU	3-3	14-14	9-7

The visual assessment shows a build-up of organic and non-organic matter before and after use. In some systems, like PU, there is evidence of composition changes in the file's surface. Spectrum analysis was carried out and is available below.

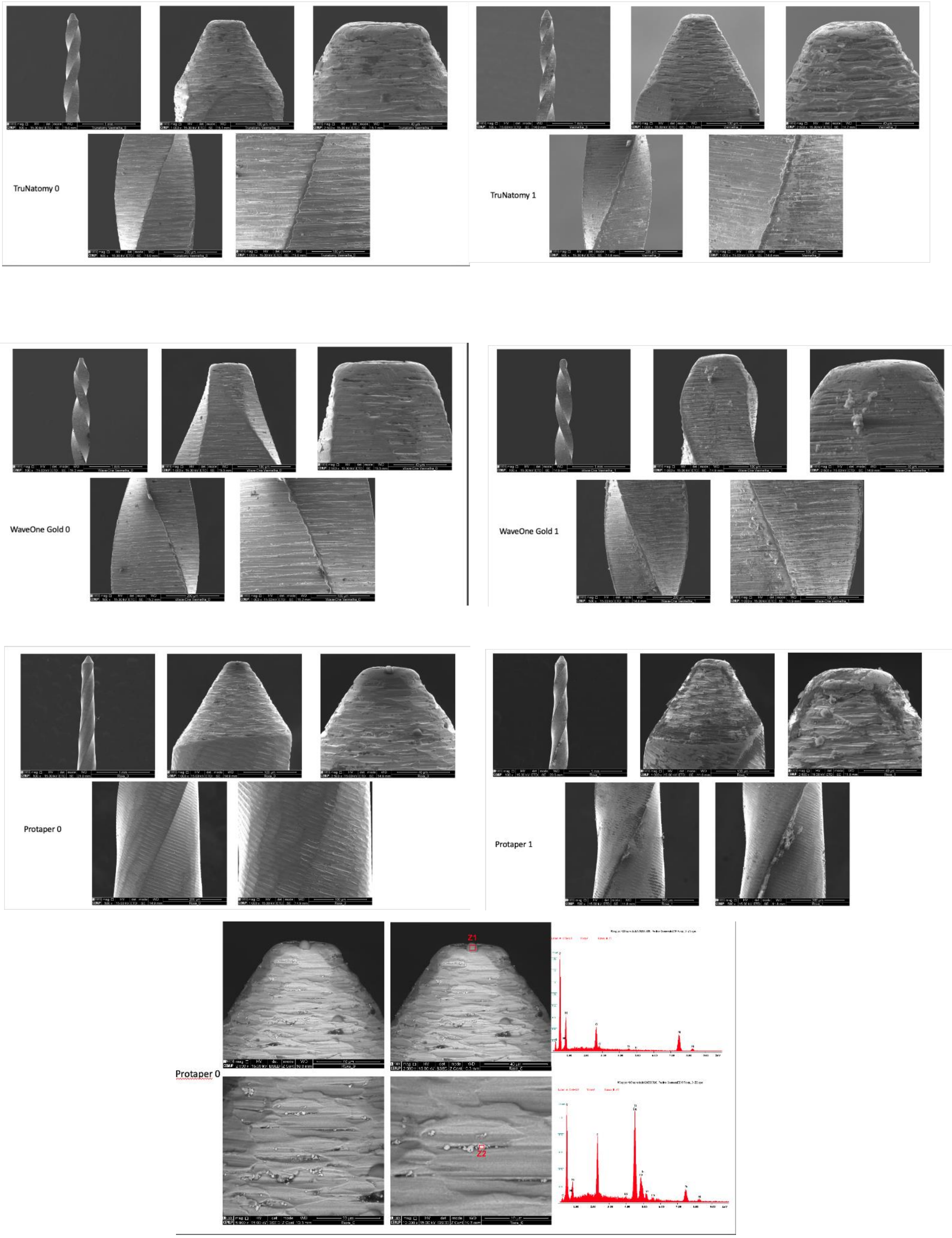


Figure 3.1. – SEM micrographs before (0) and after one use (1) for all three file systems and spectral analysis (bottom right) on particles identified on PU files prior to its use.

3.1.2. | <u>Peri-cervical dentin analysis</u>

The remaining dentin thickness following endodontic preparation at different levels (CEJ, as well as 1 mm below and above this reference) demonstrates a constant tendency, showing increased dentin thickness with TN, compared to PU and WOG, with the latter evidencing the lowest score. The table below (Table 3.2.) shows the relevant data for the three regions assessed.

Table 3.2 – Quantitative data regarding remaining dentin thickness upon WOG, TN and PU preparation, at the CEJ and 1 mm above and below the region of interest.

Average/ Standard Deviation	***CEJ***	***1 mm above CEJ***	***1mm below CEJ***
TruNatomy	1.8876/0.4223	1.814/0.5420	1.831/0.0622
Protaper Ultimate	1.4765/0.3741	1.369/0.4345	1.462/0.2047
WaveOne Gold	1.2128/0.5724	1.163/0.6279	1.136/0.4265

Representative microtomographic bidimensional images and measurements are presented before and upon instrumentation (Figure 3.2.A). Comparatively, the dentinal thickness reduction at the CEJ was 0.1243 ± 0.04 mm, 0.2531 ± 0.15 mm and 0.5784 ± 0.17 mm for TN, PU, and WOG, respectively. At a coronal position from the CEJ (+ 1 mm), dentin reduction levels were 0.1805 ± 0.07 mm, 0.3762 ± 0.11 mm and 0.6507 ± 0.14 mm, and at an apical position from the CEJ (- 1 mm), the variations were of 0.0725 ± 0.03 mm, 0.2177 ± 0.10 mm and 0.5584 ± 0.12 mm for TN, PU and WOG, respectively. For the three assessed locations, TN values were found to be significantly lower than those of WOG (Figure 3.2.B). In addition, when the full ROI was considered, TN levels were found to be significantly inferior to those of WOG and PU (Figure 3.2.C).

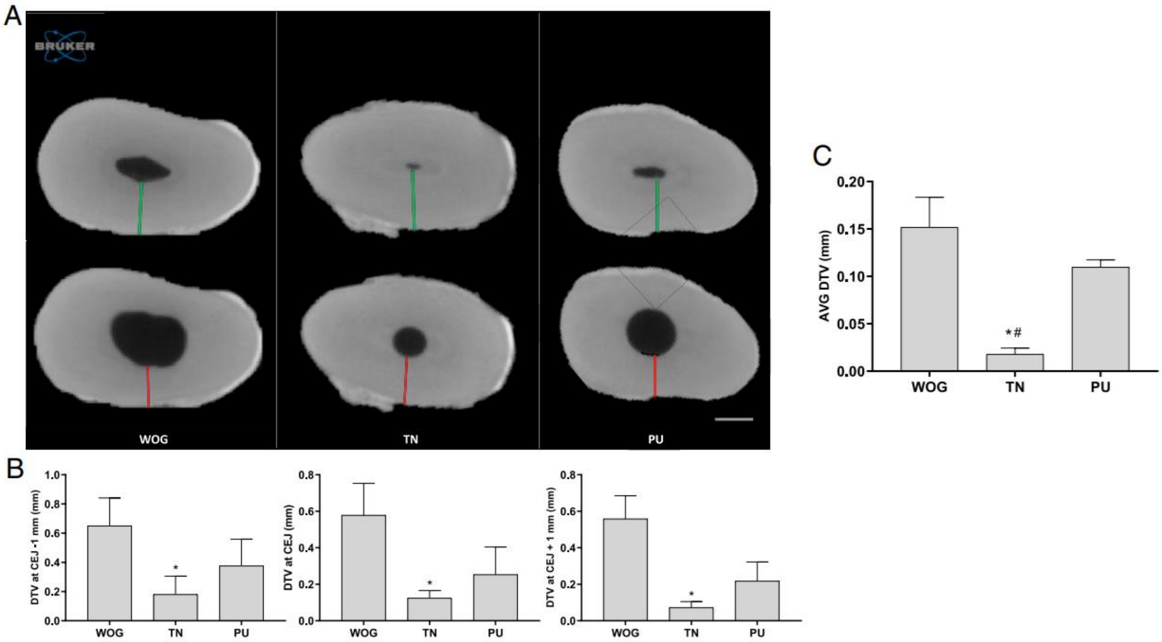


Figure 3.2. - (A) Representative bidimensional microtomographic sections of the dentin thickness analysis, before (top) and upon (bottom) instrumentation with the different systems, scale bar corresponds to 1 mm. (B) Assessment of the linear variation of the dentin thickness at the selected references bordering the CEJ. (C) Assessment of the dentin thickness variation at the selected region of interest, delimited at 1 mm coronal and 1 mm apical of the CEJ. *Significantly different from WOG ($p<0.05$); #Significantly different from PU ($p<0.05$). CEJ, cementoenamel junction; DTV, dentin thickness value; PU, Protaper Ultimate; TN, TruNatomy; WOG, WaveOne Gold.

In addition, dentin thickness tends to show the highest scores at the level of the CEJ, followed by the values at 1 mm below the CEJ and lastly, at 1 mm above the CEJ.

3.1.3. | Volume wear analysis

Upon instrumentation, the volume variation of the canal was found to be dissimilar among the assayed systems (Figure 3.3). Representative microtomographic three-dimensional reconstructions (Figure 3.3 A) show the original canal trajectory (in green) and shown the removed volumes upon instrumentation (in red). These results are suggestive of an increased volume variation attained with WOG when compared to the other systems. Quantitative assessment (Figure 3.3 B) validated these findings by showing that TN presented the lowest volume variation value (0.66 ± 0.26 mm3), followed by PU presenting an intermediate volume variation (1.91 ± 0.91 mm3), and WOG demonstrating the highest levels (3.59 ± 1.58 mm3).

In addition, WOG levels were further found to be significantly higher than those of TN and PU. The figure below (Figure 3.4) presents the volume wear for both analysis subtypes: Total and root volume wear.

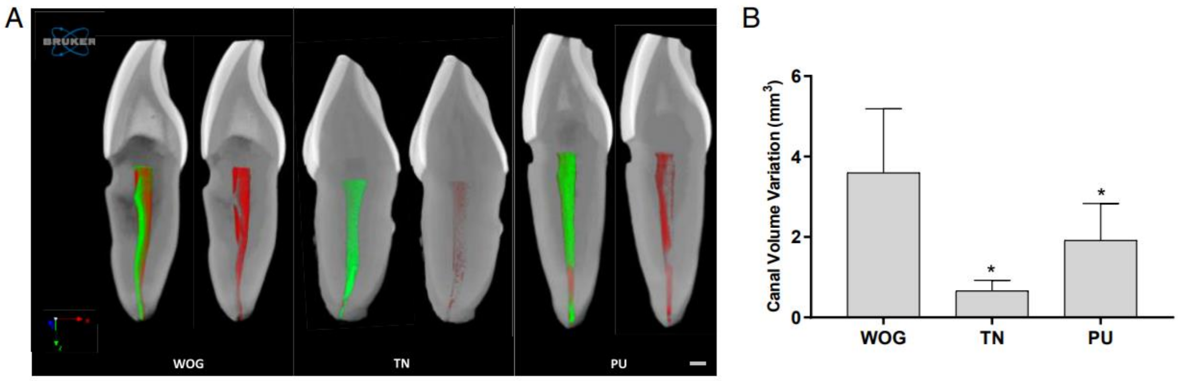


Figure 3.3.- (A) Representative microtomographic images of the canals, prior (in green) and upon (in red) instrumentation, with the different systems, scale bar corresponds to 1 mm. (B) Quantitative assessment of the canal volume variation upon instrumentation with the different systems. *Significantly different from WOG ($p<0.05$). PU, Protaper Ultimate; TN, TruNatomy; WOG, WaveOne Gold.

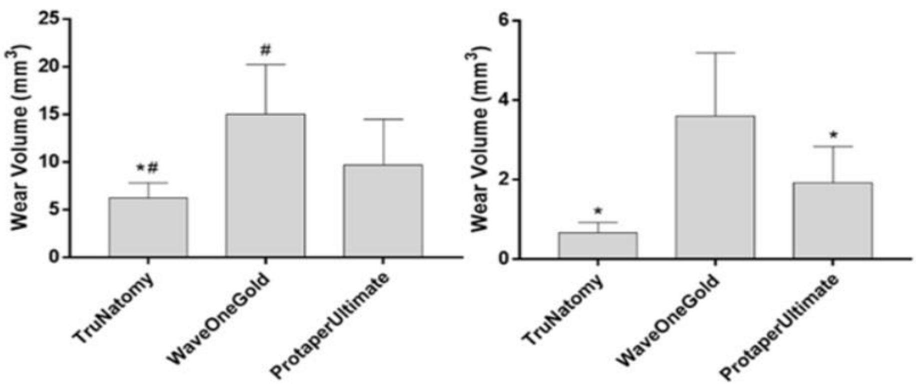
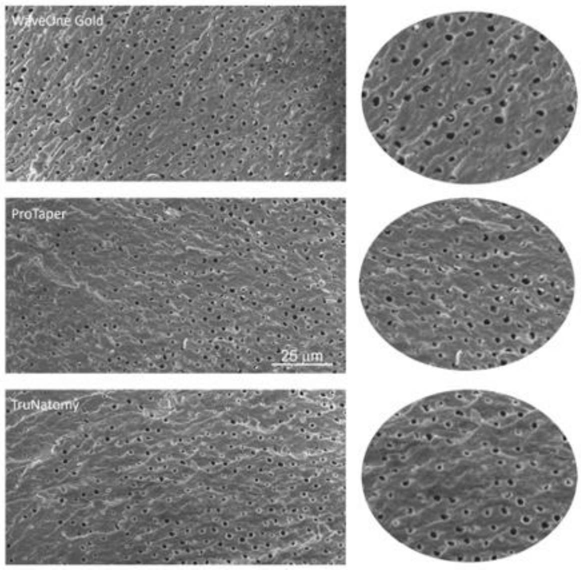


Figure 3.4.– Reduction in volume due to instrumentation. The first graph englobes the entire tooth (including the coronal aperture), while the analysis of the second graph englobes the CEJ until the apical region (does not include the coronal aperture). * Different from WaveOne Gold; # Different from Protaper Ultimate. ($p<0.05$)

3.1.4. | Smear layer

The SEM imaging of the canal wall at the apical location (Figure 3.5) revealed the presence of scattered remnants of the smear layer, and the dentinal tubules were opened or partially opened in all the assayed samples (Figure 3.5 A). Semi-quantitative scores revealed similar values, within the 2-3 range, evidencing no significant differences among experimental groups (Figure 3.5 B).

A



B

System	Mean	Median	SD	SE
WaveOne Gold	1.9	2	0.567	0.179
Protaper	2.8	2.5	0.918	0.290
TruNatomy	2.3	2	1.059	0.334

Figure 3.5 –(A)- Representative SEM images of the instrumented canal at 1 mm from the apex. (B) – Scores and descriptive statistics of the analysis of the SEM images.

3.1.5. | Canal Transportation and centering ability

Regarding the assessed parameters, results show no statistically significant difference between the three file systems assessed.

At 3 mm from the apex, the results were 0.0012 (0.074), 0.032 (0.096) and -0.0038 (0.083) for TN, PU and WOG respectively.

For centering ability at 3 mm from the apex, the results were 1.799 (2.460), 1.820 (1.927) and 1.180 (0.79), in the same order described in the previous paragraph.

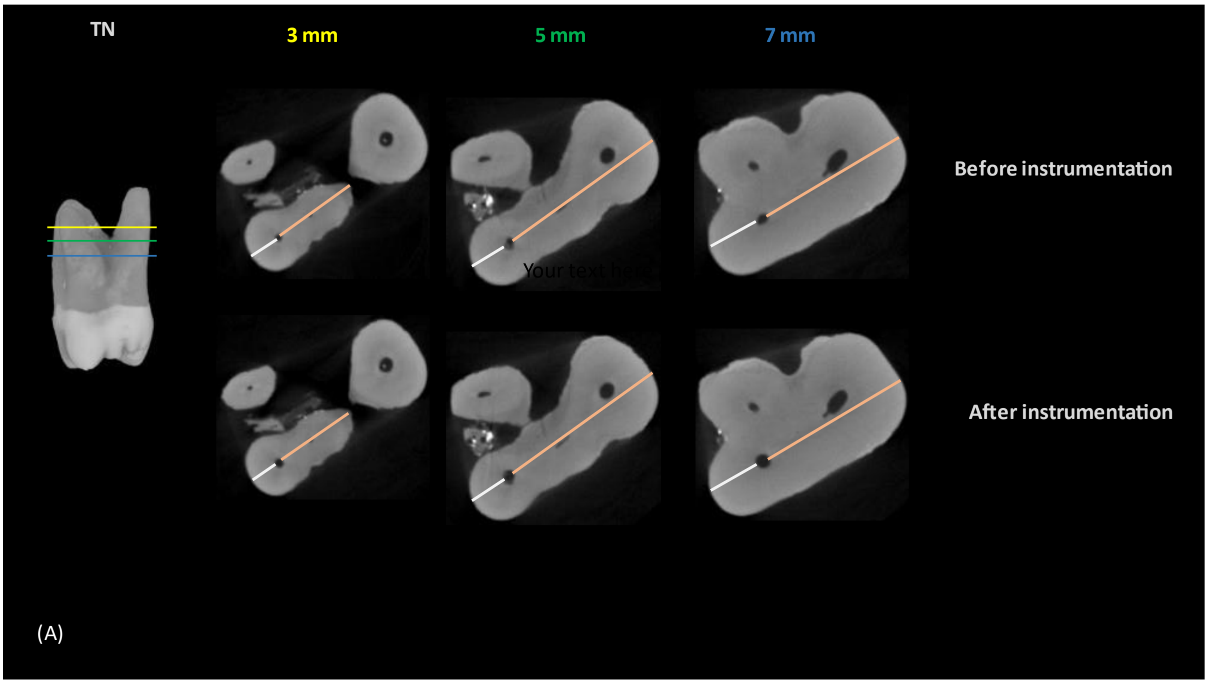
Similar results were seen at all other areas assessed – at 5mm and 7mm from the apex, for canal transportation and centering ability. Included below are the reported quantitative data and images obtained before and after preparation of the root canal system.

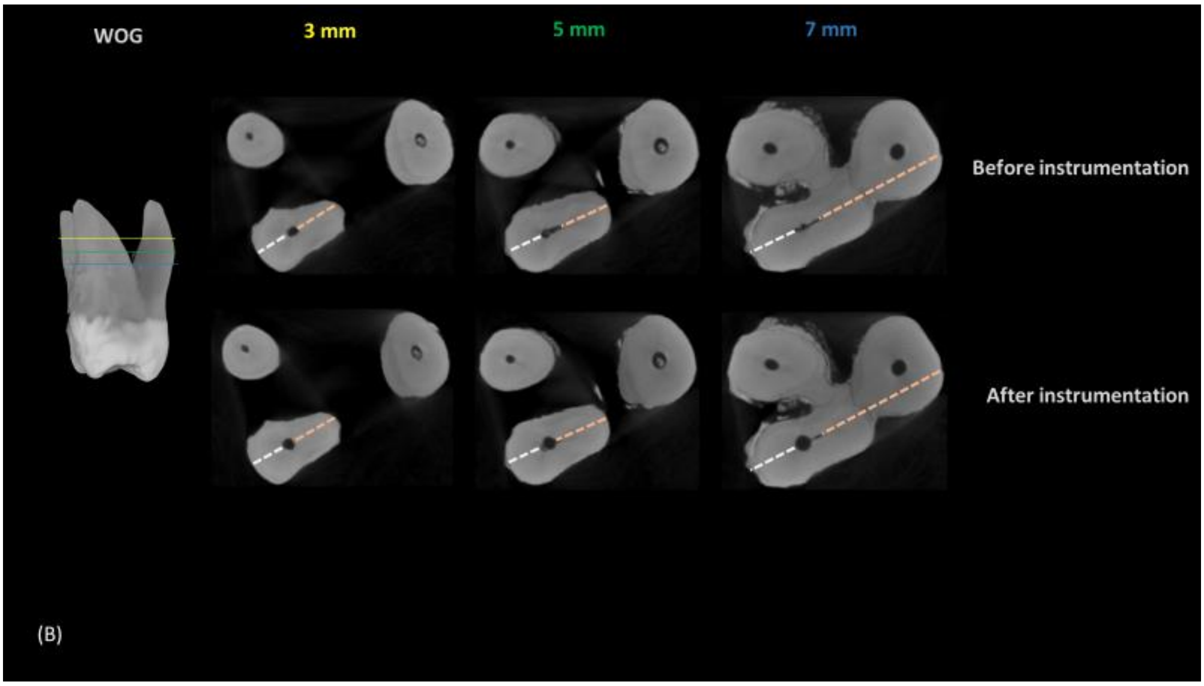
Table 3.3 - Quantitative data regarding canal transportation and centering ability at three selected reference points away from the apex, for the assayed endodontic systems.

Transportation	**3 mm**			**5 mm**			**7 mm**		
	AVG	MED	SD	AVG	MED	SD	AVG	MED	SD
TruNatomy	0.001222	-0.001	0.07439	0.042222	0.031	0.063352	-0.06578	-0.049	0.070253
WaveOneGold	-0.00389	-0.026	0.083156	-0.04478	0.02	0.157583	-0.14856	-0.142	0.104948
ProtaperUltimate	0.032333	-0.001	0.096651	-0.10022	-0.066	0.098195	-0.07067	0.008	0.168251
Centering	**3 mm**			**5 mm**			**7 mm**		
	AVG	MED	SD	AVG	MED	SD	AVG	MED	SD
TruNatomy	1.799946	0.992754	2.460403	2.874739	1.490822	3.052619	0.556685	0.379679	0.369894
WaveOneGold	1.180981	0.813853	0.792116	1.515336	1.150376	1.420676	0.365306	0.358974	0.261845
ProtaperUltimate	1.820588	0.979167	1.927636	0.520793	0.481132	0.39187	1.031299	1.163265	0.743774

Transportation					
Tukey's multiple comparisons test	Mean Diff,	95,00% CI (	Significant?	Summary	Adjusted P Value
3 mm					
TruNatomy vs. WaveOneGold	0.005112	-0,2195 to (	No	ns	0.9981
TruNatomy vs. ProtaperUltimate	-0.03111	-0,2557 to (	No	ns	0.9337
WaveOneGold vs. ProtaperUltimate	-0.03622	-0,2608 to (	No	ns	0.9113
5 mm					
TruNatomy vs. WaveOneGold	0.087	-0,1376 to (	No	ns	0.5931
TruNatomy vs. ProtaperUltimate	0.1424	-0,08214 to	No	ns	0.2636
WaveOneGold vs. ProtaperUltimate	0.05544	-0,1691 to (	No	ns	0.8057
7 mm					
TruNatomy vs. WaveOneGold	0.08278	-0,1418 to (	No	ns	0.6224
TruNatomy vs. ProtaperUltimate	0.00489	-0,2197 to (	No	ns	0.9983
WaveOneGold vs. ProtaperUltimate	-0.07789	-0,3025 to (	No	ns	0.6563

Centering					
Tukey's multiple comparisons test	Mean Diff,	95,00% CI (	Significant?	Summary	Adjusted P Value
3 mm					
TruNatomy vs. WaveOneGold	0.619	-2,687 to 3,	No	ns	0.8825
TruNatomy vs. ProtaperUltimate	-0.02064	-3,326 to 3,	No	ns	0.9999
WaveOneGold vs. ProtaperUltimate	-0.6396	-3,945 to 2,	No	ns	0.8751
5 mm					
TruNatomy vs. WaveOneGold	1.359	-1,946 to 4,	No	ns	0.5563
TruNatomy vs. ProtaperUltimate	2.354	-0,9515 to 5	No	ns	0.1922
WaveOneGold vs. ProtaperUltimate	0.9945	-2,311 to 4,	No	ns	0.7269
7 mm					
TruNatomy vs. WaveOneGold	0.1914	-3,114 to 3,	No	ns	0.988
TruNatomy vs. ProtaperUltimate	-0.4746	-3,78 to 2,8	No	ns	0.9289
WaveOneGold vs. ProtaperUltimate	-0.666	-3,971 to 2,	No	ns	0.8654


TN
3 mm
5 mm
7 mm
Before instrumentation
After instrumentation
(A)


WOG
3 mm
5 mm
7 mm
Before instrumentation
After instrumentation
(B)

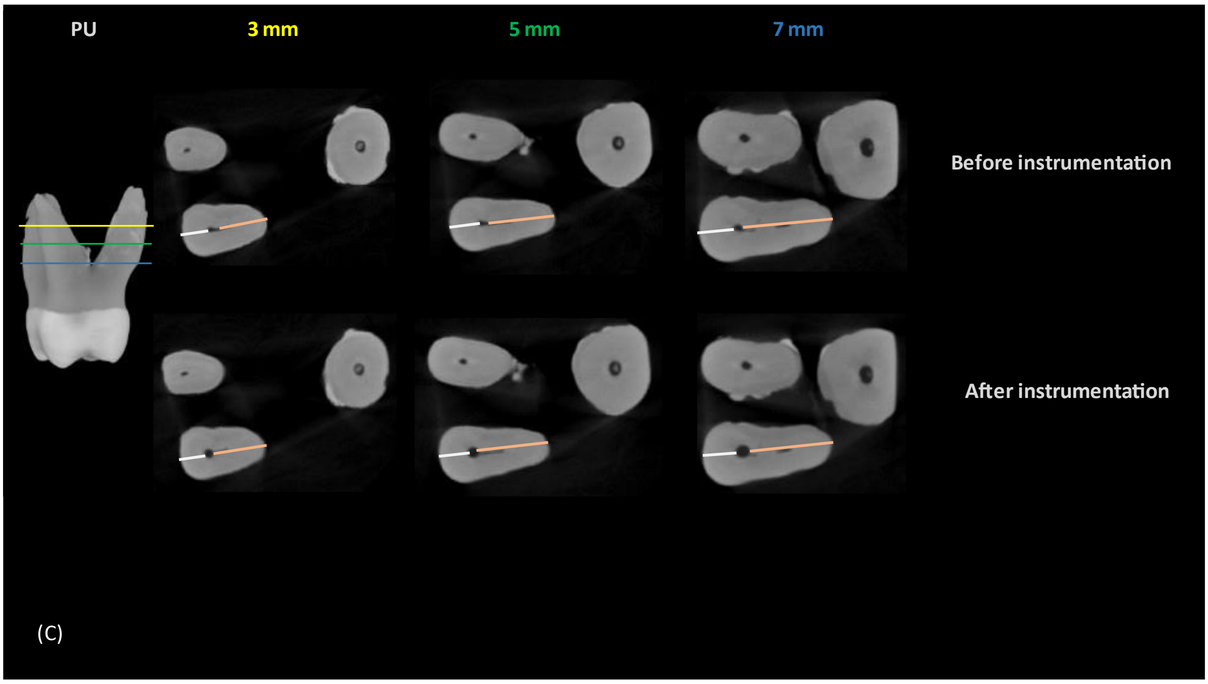


Figure 3.6 – (A,B,C) Representative microtomographic images of the canal transportation methodology used to assess TN, PU and WOG at 3,5 and 7 mm from the apex.

CHAPTER IV|

4 | Discussion

This research project undertook a comprehensive comparison between reciprocating and rotary systems with different tapers, evaluating key properties such as morphological changes of the endodontic files, peri-cervical dentin preservation, smear layer removal in the apical third, canal transportation and file centring ability.

These elements play a crucial role in the success of endodontic treatment, impacting the long-term prognosis and tooth survival of treated teeth. [101] However, such notions and dogmas long established in the endodontic field may be slowly changing. A recent publication proposes a dynamic nature and interpretation of these key factors to what concerns the success and tooth retention over the years. Both variables, tooth retention and case success, are known to reducing probabilities over time. Furthermore, the percentage of cases deemed successful is similar to previous findings. [102]

This poses an excellent point for reflection and consideration as to why despite technological developments there has been no noticeable change in the percentages of survival and/or success. As the masticatory system and each individual tooth presents multiple factors that are inter-related, the same way oral health affects systemic health and vice-versa, the same extrapolation can be considered in endodontic medicine, in particular the importance of the elements studied in this research and overall impact in the endodontic success of the case and tooth survival.

The same study however mentions that a small proportion of the variables were significantly associated with root canal treatment failure and tooth extraction, most of which were pathological conditions – the presence of apical radiolucency and the inference contamination of the root canal and patient characteristics such as the tooth in which the intervention was carried out - rather than technical variables. [102]

According to the authors, there seems to be no differences regarding tooth survival according to type and location. This is a contradictory view to other similar publications. [103]

It has been shown that incisors have a lower success rate despite being seen as easy and cases of low complexity. Molars are more complex per norm but possess higher success rates when it comes to endodontic success. If tooth survival is considered, then molar teeth come higher in terms of reduced function and early extraction need.

When it comes to the oral cavity, molars receive higher masticatory loads and are more susceptible to cracks. Thus, low root wear and reduction of peri-cervical dentin thickness can affect the prognosis, and can be seen as secondary factors that compound and could greatly influence the outcomes considered of relevance in Endodontics.

The authors also state that complications can precipitate tooth extraction such as with perforations. Thus, the use of files that are conservative and follow the original canal anatomy can lead to improved outcomes. [(102)]

The primary goal in root canal therapy remains the prevention or treatment of apical pathology due to intra-radicular biofilm and its propagation in the periapical tissues, as previously mentioned. [(101)] Despite advances in metallurgy and root canal treatment supplemental materials and equipment, complete removal of colonies and bioproducts, such as toxins, cannot be achieved. [(104,105)] This is not to say and dismiss that the success of root canal therapy has improved over the years. With the continuous advancements in this field, root canal outcomes have taken steps towards the gold standard of achieving 100% success rate. This progress is expected to continue even more so as more research and technology are integrated in the field of endodontics.

As endodontic systems follow modern treatment philosophies, it has been proposed that a reduced taper root canal preparation may impede the total root system debridement, as the smear layer is not easily removed, particularly in the apical third. [(106)] This creates a dissociation between balancing the organic elements of health vs disease in endodontics and its bio-structural and technical aspects, such as preserving the original canal anatomy and the dentin at the cervical level.

Some recently published literature assessing WOG and TN concerning access cavity preparation and peri-cervical data provide valuable support for continuing in this direction. [(82)]

The results from this research show that TN presented the lowest volume variation, followed by PU, and last, WOG, which presented a significantly higher volume variation than the former. A similar trend was attained for the dentin preservation assessment at the three distinct levels bordering the CEJ. TN presented with a significantly reduced value when compared with PU and WOG when the full region of interest was disclosed.

Although no comparative data was available for the three systems, previous studies comparing TN and WOG showed TN's superior shaping ability, and increased capacity to preserve the original canal shape whilst having limited canal transportation and maintaining a high centering ability. [(76, 98)]

TN presents excellent potential, particularly in cases of complex coronal fractures affecting CEJ, deep caries or previous restorations with associated periapical pathology as it would allow deep debridement in the apical segment (with the use of complementary techniques) whilst applying the concept of Minimally Invasive Endodontic Dentistry by keeping as much dentin cervically as clinically possible due to the regressive taper it possesses.

Notably, no differences were found between the three systems regarding smear layer cleanliness. This indicates that conventional irrigation systems such as needle and tip in reduced tapers appear to compare to other systems of greater preparation taper. The data shows that the irrigant can reach the apical third and remove the smear layer even in a 4% taper such as the one for TN. However, similarly to previous studies, the smear layer was not completely removed. (107,57)

The concept of deep shape did not show significant advantages in this comparison. This may be due to the reduced irrigant force and flow, as the apical third presents a wider preparation taper. Further investigation, including the use of additional means of smear layer removal such as ultrasonics, is essential to assess these new file's performance in terms of smear layer removal.

All systems provided a centered canal preparation with preservation of the initial anatomy without changes considered statistically significant.

All systems use modified Ni-Ti files known for their flexibility and adaptation to the root system with a tendency to remain centered. (108,109)

The glide path, established initially with a K10 file, may have positively affected the results because it facilitates instrumentation with engine-driven files and reduces procedural errors. The fact that the root system for all samples was also patent as part of the selection criteria, significantly homogenizes the outcome seeing as these are standard cases of low-level complexity. Results will invariably change with more challenging conditions such as root calcifications. (110)

The results contradict recent studies regarding the centering ability and canal transportation of rotary and reciprocation endodontic file systems, which state there are differences between rotary and reciprocation shaping movements. (111)

However, other studies support the findings from our analysis which showed no difference between rotary and reciprocating systems. (36, 112)

Previous studies show TN performs better than other compared groups such as Protaper Gold having removed less structure in simulated S-shaped canals. (76)

The results of this research are derived from natural human teeth which was selected to mimic the in vivo conditions. Results may vary if the sample size increases, resulting in a more robust analysis.

The research also produced data regarding the surface changes of these endodontic file systems. Previous studies demonstrate that surface changes are expected when endodontic files are used in root canal preparations. [94,113] Data suggests a tendency for groove defects to increase whilst irregularities and cavitations appear to reduce.

After single use, the elementary composition of PU changed. Energy-dispersive X-ray spectroscopy (EDS) was carried out, and new elements not seen initially were reported, such as Sulphur (S) and Barium (Ba).

It is known that file composition can vary following instrumentation due to exposure to the irrigating agents; however, these elements are present before use. Carbon contamination can be caused by manufacturing processing by using vacuum induction. [114,115]

It may be possible that the manufacturing process of PU may allow for S and Ba contamination. The fact that this is detected following single-use instrumentation may give an idea of the processing stage at which the contamination happens. What impact this composition change has on the Protaper system is yet unknown. A more comprehensive analysis incorporating clinical simulation may offer additional data.

Further studies are warranted with a larger sample for analysis.

CHAPTER V|

5 | Conclusions

Within the limitations of this study, it was observed that TN and PU exhibit superior performance compared to WOG in of preserving peri-cervical dentin and reducing total canal volume.

TN, PU and WOG, demonstrated comparable shaping abilities, TN showed better conservation of the canal anatomy. All the file systems were able to clean and shape moderately curved canals with minimal apical transportation.

All systems exhibited a similar cleaning capacity, smear layer removal and capability to keep the dentin tubules open. Notably, all files displayed machining grooves and other surface defects even before use, and the number of grooves increased after a single use.

CHAPTER VI|

6 | References

1. San Chong B, editor. Harty's Endodontics in Clinical Practice E-Book. Elsevier Health Sciences; 2016 Jul 28.

2. Mustafa M, Attur K, Bagda KK, Singh S, Oak A, Kathiria N. An Appraisal on Newer Endodontic File Systems: A Narrative Review. J Contemp Dent Pract. 2022 Sep 1;23(9):944-952. doi: 10.5005/jp-journals-10024-3398. PMID: 37283003.

3. Dablanca-Blanco AB, Castelo-Baz P, Miguéns-Vila R, Álvarez-Novoa P, Martín-Biedma B. Endodontic Rotary Files, What Should an Endodontist Know? Medicina (Kaunas). 2022 May 27;58(6):719. doi: 10.3390/medicina58060719. PMID: 35743982; PMCID: PMC9230915.

4. Burns LE, Kim J, Wu Y, Alzwaideh R, McGowan R, Sigurdsson A. Outcomes of primary root canal therapy: An updated systematic review of longitudinal clinical studies published between 2003 and 2020. Int Endod J. 2022 Jul;55(7):714-731. doi: 10.1111/iej.13736. Epub 2022 May 4. PMID: 35334111; PMCID: PMC9322405.

5. Elemam RF, Pretty I. Comparison of the success rate of endodontic treatment and implant treatment. ISRN Dent. 2011;2011:640509. doi: 10.5402/2011/640509. Epub 2011 Jun 15. PMID: 21991484; PMCID: PMC3168915.

6. Moazami F, Sahebi S, Sobhnamayan F, Alipour A. Success rate of nonsurgical endodontic treatment of nonvital teeth with variable periradicular lesions. Iran Endod J. 2011 Summer;6(3):119-24. Epub 2011 Aug 15. PMID: 23130063; PMCID: PMC3487520.

7. López-Valverde I, Vignoletti F, Vignoletti G, Martin C, Sanz M. Long-term tooth survival and success following primary root canal treatment: a 5- to 37-year retrospective observation. Clin Oral Investig. 2023 Jun;27(6):3233-3244. doi: 10.1007/s00784-023-04938-y. Epub 2023 Mar 18. PMID: 36933044; PMCID: PMC10264502.

8. Jazea AAA, Alshammari AG, Alotaibi MM, Alshammeri N, Alotaibi MM, Abdullah A, Alemzi F, Almanea E. Tooth survival following non-surgical root canal treatment: a review. International Journal of Medicine in Developing Countries. 2021; 5(2):738-742. doi: 10.24911/IJMDC.51-1606741962.

9. Setzer FC, Kim S. Comparison of long-term survival of implants and endodontically treated teeth. J Dent Res. 2014 Jan;93(1):19-26. doi: 10.1177/0022034513504782. Epub 2013 Sep 24. PMID: 24065635; PMCID: PMC3872851.

10. de Kuijper MCFM, Meisberger EW, Rijpkema AG, Fong CT, De Beus JHW, Özcan M, Cune MS, Gresnigt MMM. Survival of molar teeth in need of complex endodontic treatment: Influence of the endodontic treatment and quality of the restoration. J Dent. 2021 May;108:103611. doi: 10.1016/j.jdent.2021.103611. Epub 2021 Feb 19. PMID: 33617944.

11. Alsinaidi YA, Almotairi TA, Alyami IM. Factors Affecting Root Canal Treatment Outcomes: A Systematic Review. Saudi J Oral Dent Res. 2022 Nov;7(11):270-5. doi: 10.36348/sjodr.2022.v07i11.001.

12. Arias A, Peters OA. Present status and future directions: Canal shaping. Int Endod J. 2022 May;55 Suppl 3(Suppl 3):637-655. doi: 10.1111/iej.13698. Epub 2022 Feb 16. PMID: 35118683; PMCID: PMC9303733.

13. Abu-Tahun I, Al-Rabab'ah MA, Hammad M, Khraisat A. Technical quality of root canal treatment of posterior teeth after rotary or hand preparation by fifth year undergraduate students, The University of Jordan. Aust Endod J. 2014 Dec;40(3):123-30. doi: 10.1111/aej.12069. Epub 2014 Sep 4. PMID: 25197021.

14. Locke M, Thomas MB, Dummer PM. A survey of adoption of endodontic nickel-titanium rotary instrumentation part 1: general dental practitioners in Wales. Br Dent J. 2013 Feb;214(3):E6. doi: 10.1038/sj.bdj.2013.108. PMID: 23392051.

15. Gyulbenkiyan E, Gusiyska A. Impact of the passive/active ultrasonic activation on the endodontic irrigants effectiveness - a review. *J of IMAB.* 2023 Jan-Mar;29(1):4826-4831. doi: 10.5272/jimab.2022281.4826.

16. Miguéns-Vila R, Martín-Biedma B, Aboy-Pazos S, Uroz-Torres D, Álvarez-Nóvoa P, Dablanca-Blanco AB, Varela-Aneiros I, Castelo-Baz P. Effectiveness of Different Irrigant Activation Systems on Smear Layer Removal: A Scanning Electron Microscopic Study. J Clin Med. 2022 Feb 15;11(4):1003. doi: 10.3390/jcm11041003. PMID: 35207275; PMCID: PMC8879012.

17. Machado R, da Silva I, Comparin D, de Mattos BAM, Alberton LR, da Silva Neto UX. Smear layer removal by passive ultrasonic irrigation and 2 new mechanical methods for activation of the chelating solution. Restor Dent Endod. 2021 Jan 26;46(1):e11. doi: 10.5395/rde.2021.46.e11. PMID: 33680900; PMCID: PMC7906843.

18. Zogheib C, Sfeir G, Plotino G, Deus G, Daou M, Khalil I. Impact of Minimal Root Canal Taper on the Fracture Resistance of Endodontically Treated Bicuspids. J Int Soc Prev Community Dent. 2018 Mar-Apr;8(2):179-183. doi: 10.4103/jispcd.JISPCD_88_18. Epub 2018 Apr 24. PMID: 29780745; PMCID: PMC5946528.

19. Tabrizizadeh M, Kazemipoor M, Hekmati-Moghadam SH, Hakimian R. Impact of root canal preparation size and taper on coronal-apical micro-leakage using glucose penetration method. J Clin Exp Dent. 2014 Oct 1;6(4):e344-9. doi: 10.4317/jced.51452. PMID: 25593654; PMCID: PMC4282899.

20. Mohammadzadeh Akhlaghi N, Rahimifard N, Moshari A, Vatanpour M, Darmiani S. The Effect of Size and Taper of Apical Preparation in Reducing Intra-Canal Bacteria: A Quantitative SEM Study. Iran Endod J. 2014 Winter;9(1):61-5. Epub 2013 Dec 24. PMID: 24396378; PMCID: PMC3881304.

21. Brunson M, Heilborn C, Johnson DJ, Cohenca N. Effect of apical preparation size and preparation taper on irrigant volume delivered by using negative pressure irrigation system. J Endod. 2010 Apr;36(4):721-4. doi: 10.1016/j.joen.2009.11.028. Epub 2010 Feb 6. PMID: 20307751.

22. A, S., P, S., Vijay R, S. A., & S, A. Effect of Apical Preparation Size and Taper on Irrigant Penetration in Apical Third of Root Canal using Two Different Endodontic Needles: An In Vivo Study. Journal of Dentistry and Oral Biology. 2017 Sep 2(13).

23. Boutsioukis C, Gogos C, Verhaagen B, Versluis M, Kastrinakis E, Van der Sluis LW. The effect of root canal taper on the irrigant flow: evaluation using an unsteady Computational Fluid Dynamics model. Int Endod J. 2010 Oct;43(10):909-16. doi: 10.1111/j.1365-2591.2010.01767.x. Epub 2010 Jul 5. PMID: 20618877.

24. Marending M, Peters OA, Zehnder M. Factors affecting the outcome of orthograde root canal therapy in a general dentistry hospital practice. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2005 Jan;99(1):119-24. doi: 10.1016/j.tripleo.2004.06.065. PMID: 15599359Mirmohammadi H, Sitarz M, Shemesh H. Intra-manufacture Diameter Variability of Rotary Files and Their Corresponding Gutta-Percha Cones Using Laser Scan Micrometre. Iran Endod J. 2018 Spring;13(2):159-162. doi: 10.22037/iej.v13i2.14710. PMID: 29707008; PMCID: PMC5911287.

25. Reddy PJ, Kumar VS, Aravind K, Kumar HT, Vishal M B, Vizaikumar VN, Das R, Vamsilatha K. Canal shaping with one shape file and twisted files: a comparative study. J Clin Diagn Res. 2014 Dec;8(12):ZF01-3. doi: 10.7860/JCDR/2014/11126.5333. Epub 2014 Dec 5. PMID: 25654059; PMCID: PMC4316365.

26. Butcher S, Mansour A, Ibrahim M. Influence of Apical Preparation Size on Effective Conventional Irrigation in the Apical Third: A Scanning Electron Microscopic Study. Eur Endod J. 2019 Feb 6;4(1):9-14. doi: 10.14744/eej.2018.06078. PMID: 32161880; PMCID: PMC7006559.

27. Usta SN, Solana C, Ruiz-Linares M, Baca P, Ferrer-Luque CM, Cabeo M, Arias-Moliz MT. Effectiveness of conservative instrumentation in root canal disinfection. Clin Oral Investig. 2023 Jun;27(6):3181-3188. doi: 10.1007/s00784-023-04929-z. Epub 2023 Mar 3. PMID: 36867258; PMCID: PMC10264279.

28. Puleio F, Lo Giudice G, Militi A, Bellezza U, Lo Giudice R. Does Low-Taper Root Canal Shaping Decrease the Risk of Root Fracture? A Systematic Review. Dent J (Basel). 2022 Jun 1;10(6):94. doi: 10.3390/dj10060094. PMID: 35735636; PMCID: PMC9222076.

29. Neelakantan P, Vishwanath V, Taschieri S, Corbella S. Present status and future directions: Minimally invasive root canal preparation and periradicular surgery. Int Endod J. 2022 Oct;55 Suppl 4:845-871. doi: 10.1111/iej.13750. Epub 2022 Apr 29. PMID: 35426157.

30. Palanisamy V, Rao A, Shenoy R, Mahabala KY, Srikrishna SB. Comparison of centering ability of three different endodontic instruments using cone-beam computed tomography: An in vitro study. World. 2019 Jul;10(4):307. doi:10.5005/jp-journals-10015-1654.

31. van der Vyver PJ, Vorster M, Paleker F, de Wet FA. Errors in root canal preparation: a review of the literature and clinical case reports. S. Afr. dent. j. [Internet]. 2019 June [cited 2023 Nov 18] ; 74(5): 246-254. Available from: http://www.scielo.org.za/scielo.php?script=sci_arttext&pid=S0011-85162019000500008&lng=en. http://dx.doi.org/10.17159/2519-0105/2019/v74no5a6.

32. Gergi R, Rjeily JA, Sader J, Naaman A. Comparison of canal transportation and centering ability of twisted files, Pathfile-ProTaper system, and stainless steel hand K-files by using computed tomography. J Endod. 2010 May;36(5):904-7. doi: 10.1016/j.joen.2009.12.038. Epub 2010 Mar 7. PMID: 20416443.

33. Rejula F, Christalin R, Ahmed W, Dinakaran S, Gopinathan AS, Babu A. Measure and compare the Degree of Root Canal Transportation and Canal-centering ability of Twisted, ProTaper, and Conventional Stainless Steel K Files using Spiral Computed Tomography: An in vitro Study. J Contemp Dent Pract. 2017 Jun 1;18(6):463-469. doi: 10.5005/jp-journals-10024-2066. PMID: 28621275.

34. Shi L, Yang Y, Wan J, Xie W, Yang R, Yao Y. Shaping ability of rotary and reciprocating single-file systems in combination with and without different glide path techniques in simulated curved canals. J Dent Sci. 2022 Oct;17(4):1520-1527. doi: 10.1016/j.jds.2022.04.029. Epub 2022 May 12. PMID: 36299306; PMCID: PMC9588838.

35. Sunildath MS, Mathew J, George L, Vineet RV, Thomas P, John D. Canal transportation and centering ability of root canals prepared using rotary and reciprocating systems with and without PathFiles in cone-beam computed tomography-based three-dimensional molar prototypes. J Conserv Dent. 2021 May-Jun;24(3):246-251. doi: 10.4103/jcd.jcd_200_21. Epub 2021 Dec 8. PMID: 35035149; PMCID: PMC8717843.

36. Mittal A, Dadu S, Singh NS, Singh S, Gupta B, Abraham A, Yendrembam B, Kumari S. Comparative Assessment of Canal Transportation and Centering Ability of Reciproc and One Shape File Systems Using CBCT-An In Vitro Study. J Clin Diagn Res. 2017 Apr;11(4):ZC31-ZC34. doi: 10.7860/JCDR/2017/24257.9660. Epub 2017 Apr 1. PMID: 28571257; PMCID: PMC5449913.

37. Barbosa-Ribeiro M, Albergaria SJ, Malvar MD, Crusoé-Rebello IM, Gomes BP, Carvalho FB. Canal transportation and centering ability of curved root canals prepared using rotary and reciprocating systems. Brazilian Journal of Oral Sciences. 2015 Jul;14:214-8. doi:10.1590/1677-3225V14N3A08.

38. Chandra S. Grossman's endodontic practice. Wolters kluwer india Pvt Ltd; 2014.

39. Eliasz W, Kubiak K, Poncyljusz W, Surdacka A. Root Canal Transportation after Root Canal Preparation with ProTaper Next, WaveOne Gold, and Twisted Files. J Clin Med. 2020 Nov 14;9(11):3661. doi: 10.3390/jcm9113661. PMID: 33202555; PMCID: PMC7696375.

40. Rotstein I, Ingle JI, editors. Ingle's endodontics. PMPH USA; 2019 Jun 1.

41. Chaniotis A, Ordinola-Zapata R. Present status and future directions: Management of curved and calcified root canals. Int Endod J. 2022 May;55 Suppl 3:656-684. doi: 10.1111/iej.13685. Epub 2022 Feb 27. PMID: 35106792.

42. Alamoudi Ruua A. The smear layer in endodontic. to keep or remove - an updated overview. Saudi Endodontic Journal. 2019 May-Aug 9(2):71-81. *doi:* 10.4103/sej.sej_95_18.

43. Dewi A, Upara C, Chaiariyakul D, Louwakul P. Smear Layer Removal from Root Canal Dentin and Antimicrobial Effect of Citric Acid-modified Chlorhexidine. Eur Endod J. 2020 Dec;5(3):257-263. doi: 10.14744/eej.2020.38258. PMID: 33353912; PMCID: PMC7881384.

44. Hülsmann M, Heckendorff M, Lennon A. Chelating agents in root canal treatment: mode of action and indications for their use. Int Endod J. 2003 Dec;36(12):810-30. doi: 10.1111/j.1365-2591.2003.00754.x. PMID: 14641420.

45. Poggio C, Dagna A, Chiesa M, Beltrami R, Bianchi S. Cleaning Effectiveness of Three NiTi Rotary Instruments: A Focus on Biomaterial Properties. J Funct Biomater. 2015 Feb 16;6(1):66-76. doi: 10.3390/jfb6010066. PMID: 25692796; PMCID: PMC4384101.

46. Doumani M, Habib A, Doumani A, Seirawan MK, Sadeka MA, Alnofiai SR. A review: the applications of EDTA in endodontics (Part I). IOSR Journal of Dental and Medical Sciences. 2017;16(9):83-5.

47. Murugesan K, Vishwanath S, Kadandale S, Thanikachalam Y, Parthasarathy R, Ilango S. Comparative Evaluation of Smear Layer Removal in Apical Third Using Four Different Irrigants With Ultrasonic Agitation: An In Vitro Scanning Electron Microscopy (SEM) Analysis. Cureus. 2022 Mar 14;14(3):e23142. doi: 10.7759/cureus.23142. PMID: 35444899; PMCID: PMC9009992.

48. Darcey J, Jawad S, Taylor C, Roudsari RV, Hunter M. Modern Endodontic Principles Part 4: Irrigation. Dent Update. 2016 Jan-Feb;43(1):20-2, 25-6, 28-30 passim. doi: 10.12968/denu.2016.43.1.20. PMID: 27024899.

49. Gluskin AH, Peters CI, Peters OA. Minimally invasive endodontics: challenging prevailing paradigms. Br Dent J. 2014 Mar;216(6):347-53. doi: 10.1038/sj.bdj.2014.201. PMID: 24651341.

50. Geramy A, Eghbal MJ, Ehsani S. Stress distribution changes after root canal therapy in canine model: a finite element study. Iran Endod J. 2008 Fall;3(4):113-8. Epub 2008 Oct 1. PMID: 24082903; PMCID: PMC3782244.

51. Acharya N, Hasan MR, Kafle D, Chakradhar A, Saito T. Effect of Hand and Rotary Instruments on the Fracture Resistance of Teeth: An In Vitro Study. Dent J (Basel). 2020 Apr 29;8(2):38. doi: 10.3390/dj8020038. PMID: 32365583; PMCID: PMC7345062.

52. Maqbool M, Noorani TY, Asif JA, Makandar SD, Jamayet NB. Controversies in endodontic access cavity design: A literature review. Dental Update. 2020 Oct 2;47(9):747-54. Doi: 10.12968/denu.2020.47.9.747.

53. Kishen A. Mechanisms and risk factors for fracture predilection in endodontically treated teeth. Endodontic topics. 2006 Mar;13(1):57-83.

54. Yared G. Canal preparation with nickel-titanium or stainless steel instruments without the risk of instrument fracture: preliminary observations. Restor Dent Endod. 2015 Feb;40(1):85-90. doi: 10.5395/rde.2015.40.1.85. Epub 2014 Nov 13. PMID: 25671218; PMCID: PMC4320282.

55. Liang Y, Yue L. Evolution and development: engine-driven endodontic rotary nickel-titanium instruments. Int J Oral Sci. 2022 Feb 18;14(1):12. doi: 10.1038/s41368-021-00154-0. PMID: 35181648; PMCID: PMC8857196.

56. Yılmaz A, Gökyay SS, Dağlaroğlu R, Küçükay IK. Evaluation of deformation and fracture rates for nickel-titanium rotary instruments according to the frequency of clinical use. Eur Oral Res. 2018 May;52(2):89-93. doi: 10.26650/eor.2018.461. Epub 2018 May 1. PMID: 30775708; PMCID: PMC6365127.

57. Martina S, Pisano M, Amato A, Abdellatif D, Iandolo A. Modern rotary files in minimally invasive endodontics: a case report. Front Biosci (Elite Ed). 2021 Dec 20;13(2):299-304. doi: 10.52586/E886. PMID: 34937316.

58. Namazikhah MS, Mokhlis HR, Alasmakh K. Comparison between a hand stainless-steel K file and a rotary NiTi 0.04 taper. J Calif Dent Assoc. 2000 Jun;28(6):421-6. PMID: 11324127.

59. Cheung GS, Peng B, Bian Z, Shen Y, Darvell BW. Defects in ProTaper S1 instruments after clinical use: fractographic examination. Int Endod J. 2005 Nov;38(11):802-9. doi: 10.1111/j.1365-2591.2005.01020.x. PMID: 16218972.

60. Özyürek T, Demiryürek EÖ. Surface deformation of several nickel-titanium rotary instruments after removal of the root canal filling materials: a scanning electron microscope evaluation. Odontology. 2020 Apr;108(2):213-221. doi: 10.1007/s10266-019-00454-2. Epub 2019 Sep 17. PMID: 31531770.

61. Nešković J, Damjanov M, Živković S, Grga Đ, Koruga Đ, Kojić D. Deformations of the manual endodontic instruments during root canal instrumentation. Stomatološki glasnik Srbije. 2010;57(1):21-9. doi:10.2298/SGS1001021N.

62. Gomes MS, Vieira RM, Böttcher DE, Plotino G, Celeste RK, Rossi-Fedele G. Clinical fracture incidence of rotary and reciprocating NiTi files: A systematic review and meta-regression. Aust Endod J. 2021 Aug;47(2):372-385. doi: 10.1111/aej.12484. Epub 2021 Jan 7. PMID: 33410578.

63. Inan U, Gonulol N. Deformation and fracture of Mtwo rotary nickel-titanium instruments after clinical use. J Endod. 2009 Oct;35(10):1396-9. doi: 10.1016/j.joen.2009.06.014. Epub 2009 Aug 15. PMID: 19801238.

64. Mohamed RH, Abdelrahman AM, Sharaf AA. Evaluation of rotary file system (Kedo-S-Square) in root canal preparation of primary anterior teeth using cone beam computed

tomography (CBCT)-in vitro study. BMC Oral Health. 2022 Jan 18;22(1):13. doi: 10.1186/s12903-021-02021-0. PMID: 35042489; PMCID: PMC8764846.

65. da Frota MF, Espir CG, Berbert FL, Marques AA, Sponchiado-Junior EC, Tanomaru-Filho M, Garcia LF, Bonetti-Filho I. Comparison of cyclic fatigue and torsional resistance in reciprocating single-file systems and continuous rotary instrumentation systems. J Oral Sci. 2014 Dec;56(4):269-75. doi: 10.2334/josnusd.56.269. PMID: 25500924.

66. Bansode, P.V., Wavdhane, M.B., Pathak, S.D., Khedgikar, S., & Rana, H.B. Evolution of Rotary NI-TI File Systems: A Literature Review. Indian journal of applied research. 2016 Jan; 6(12):91-94.

67. Ounsi HF, Nassif W, Grandini S, Salameh Z, Neelakantan P, Anil S. Evolution of Nickel-titanium Alloys in Endodontics. J Contemp Dent Pract. 2017 Nov 1;18(11):1090-1096. doi: 10.5005/jp-journals-10024-2181. PMID: 29109327.

68. Bartols A, Laux G, Walther W. Multiple-file vs. single-file endodontics in dental practice: a study in routine care. PeerJ. 2016 Dec 7;4:e2765. doi: 10.7717/peerj.2765. PMID: 27957398; PMCID: PMC5147020.

69. Alsilani R, Jadu F, Bogari DF, Jan AM, Alhazzazi TY. Single file reciprocating systems: A systematic review and meta-analysis of the literature: Comparison of reciproc and WaveOne. J Int Soc Prev Community Dent. 2016 Sep-Oct;6(5):402-409. doi: 10.4103/2231-0762.192945. Epub 2016 Oct 24. PMID: 27891305; PMCID: PMC5109853.

70. Silva EJNL, Lima CO, Barbosa AFA, Lopes RT, Sassone LM, Versiani MA. The Impact of TruNatomy and ProTaper Gold Instruments on the Preservation of the Periradicular Dentin and on the Enlargement of the Apical Canal of Mandibular Molars. J Endod. 2022 May;48(5):650-658. doi: 10.1016/j.joen.2022.02.003. Epub 2022 Feb 15. PMID: 35181453.

71. DENTSPLY Sirona. Scientific Manual TruNatomy®. 2022. Available from: https://www.dentsplysirona.com/content/dam/master/product-procedure-brand-categories/endodontics/product-categories/full-solutions/trunatomy-solution/scientific-support-clinical-education/END-Scientific-Information-TruNatomy-Scientific-Manual-US.pdf.

72. Van der Vyver PJ, Vorster M. WaveOne® Gold reciprocating instruments: clinical application in the private practice: Part 1. Available from: https://repository.up.ac.za/handle/2263/66162.

73. Van der Vyver PJ, Vorster M. WaveOne®Gold reciprocating instruments: clinical application in the private practice: Part 2. Available from: http://www.moderndentistrymedia.com/aug_sep2017/van-der-vyver-part2.pdf

74. DENTSPLY Sirona. WaveOne® Gold. 2020. Available from: https://www.dentsplysirona.com/content/dam/master/regions-countries/north-america/product-procedure-brand/endodontics/brands/waveone-gold/end-brochure-waveone-gold-reciprocating-files-en.pdf.

75. DENTSPLY Sirona. Protaper UltimateTM. 2020. Available from: https://www.dentsplysirona.com/content/dam/master/product-procedure-brand-categories/endodontics/product-categories/full-solutions/protaper-ultimate-solution/scientific-support-clinical-education/END-scientific-information-ProTaper-Ultimate-FactFile.pdf

76. Eren İ, Sezer B. Comparison of the Effects of WaveOne Gold, TruNatomy, and Conventional Hand Files on Remaining Dentin Volume in the Coronal Part of the Root and Preparation Efficiency in Mandibular Molars. J Endod. 2023 Aug;49(8):1027-1034. doi: 10.1016/j.joen.2023.05.020. Epub 2023 Jun 2. PMID: 37269976.

77. Kim H, Jeon SJ, Seo MS. Comparison of the canal transportation of ProTaper GOLD, WaveOne GOLD, and TruNatomy in simulated double-curved canals. BMC Oral Health. 2021 Oct 16;21(1):533. doi: 10.1186/s12903-021-01854-z. PMID: 34656099; PMCID: PMC8520641.

78. García G, Alfie D, Goldberg F. Comparative study of the shaping ability obtained with TruNatomy, Wave One Gold and Protaper Gold systems in simulated curved canals. Endodoncía (Madrid). 2019;37(3):23-30. ID: ibc-189959.

79. Acar B, Kamburoğlu K, Tatar İ, Arıkan V, Çelik HH, Yüksel S, Özen T. Comparison of micro-computerized tomography and cone-beam computerized tomography in the detection of accessory canals in primary molars. Imaging Sci Dent. 2015 Dec;45(4):205-11. doi: 10.5624/isd.2015.45.4.205. Epub 2015 Dec 17. PMID: 26730367; PMCID: PMC4697004.

80. Ashraf H, Zargar N, Zandi B, Azizi A, Amiri M. The Evaluation of Debris and Smear Layer Generated by Three Rotary Instruments Neo NiTi, 2Shape and Revo_S: An Ex-vivo Scanning Electron Microscopic Study. Iran Endod J. 2023;18(2):96-103. doi: 10.22037/iej.v18i2.39966. PMID: 37152856; PMCID: PMC10155103.

81. Mankeliya S, Singhal RK, Gupta A, Jaiswal N, Pathak VK, Kushwah A. A Comparative Evaluation of Smear Layer Removal by Using Four Different Irrigation Solutions like Root Canal Irrigants: An *In Vitro* SEM Study. J Contemp Dent Pract. 2021 May 1;22(5):527-531. PMID: 34318772.

82. Hülsmann M. A critical appraisal of research methods and experimental models for studies on root canal preparation. Int Endod J. 2022 Mar;55 Suppl 1:95-118. doi: 10.1111/iej.13665. Epub 2021 Dec 17. PMID: 34800299.

83. Vorster M, van der Vyver PJ, Markou G. The Effect of Different Access Cavity Designs in Combination with WaveOne Gold and TruNatomy Instrumentation on Remaining Dentin Thickness and Volume. J Endod. 2023 Jan;49(1):83-88. doi: 10.1016/j.joen.2022.11.002. Epub 2022 Nov 12. PMID: 36375650.

84. DENTSPLY Sirona. MAILLEFER TruNatomy™. 2018. Available from: https://www.dentsplysirona.com/content/dam/flagship/en/explore/endodontics/dfu/trunatomy/TRUNATOMY%20ROW_DFU_1218_WEB_DSE_EN.pdf.

85. Kashyap RR, Beedubail SP, Kini R, Rao PK. Assessment of the number of root canals in the maxillary and mandibular molars: A radiographic study using cone beam computed tomography. J Conserv Dent. 2017 Sep-Oct;20(5):288-291. doi: 10.4103/JCD.JCD_73_17. PMID: 29386772; PMCID: PMC5767819.

86. Westreich D, Pence BW, Turner AN. In populo. Epidemiology. 2010 Jan;21(1):152-3. doi: 10.1097/EDE.0b013e3181c1e92a. PMID: 20010219; PMCID: PMC2882154.

87. Nagendrababu V, Murray PE, Ordinola-Zapata R, Peters OA, Rôças IN, Siqueira JF Jr, Priya E, Jayaraman J, Pulikkotil SJ, Dummer PMH. A protocol for developing reporting guidelines for laboratory studies in Endodontology. Int Endod J. 2019 Aug;52(8):1090-1095. doi: 10.1111/iej.13123. Epub 2019 Apr 25. PMID: 30908638.

88. Voehringer, P., Nicholson, J.R. (2019). Minimum Information in In Vivo Research. In: Bespalov, A., Michel, M., Steckler, T. (eds) Good Research Practice in Non-Clinical Pharmacology and Biomedicine. Handbook of Experimental Pharmacology, vol 257. Springer, Cham. https://doi.org/10.1007/164_2019_285.

89. Bhide A, Shah PS, Acharya G. A simplified guide to randomized controlled trials. Acta Obstet Gynecol Scand. 2018 Apr;97(4):380-387. doi: 10.1111/aogs.13309. Epub 2018 Feb 27. PMID: 29377058.

90. Shivashankar MB, Niranjan NT, Jayasheel A, Kenchanagoudra MG. Computed Tomography Evaluation of Canal Transportation and Volumetric Changes in Root Canal Dentin of Curved Canals Using Mtwo, ProTaper and ProTaper Next Rotary System-An In-vitro Study. J Clin Diagn Res. 2016 Nov;10(11):ZC10-ZC14. doi: 10.7860/JCDR/2016/20373.8788. Epub 2016 Nov 1. PMID: 28050495; PMCID: PMC5198448.

91. Anderson C. Presenting and evaluating qualitative research. Am J Pharm Educ. 2010 Oct 11;74(8):141. doi: 10.5688/aj7408141. PMID: 21179252; PMCID: PMC2987281.

92. Javed F, Motiwala MA, Khan FR, Ghafoor R. Comparison of surface defects in Protaper Next and Hyflex EDM files after single clinical use: A stereoscopic evaluation. J Pak Med Assoc. 2022 Jan;72(1):37-41. doi: 10.47391/JPMA.20-1056. PMID: 35099435.

93. Howait M. Reciproc Endodontic File Surface Defects After Single Use: An SEM Analysis. J Int Soc Prev Community Dent. 2021 Jan 30;11(1):98-103. doi: 10.4103/jispcd.JISPCD_412_20. PMID: 33688479; PMCID: PMC7934823.

94. Bhatia S, Nagendrababu V, Peters OA, Fawzy A, Daood U. Evaluation of usage-induced degradation of different endodontic file systems. Sci Rep. 2021 Apr 27;11(1):9027. doi: 10.1038/s41598-021-88570-4. Erratum in: Sci Rep. 2021 Aug 2;11(1):16015. PMID: 33907252; PMCID: PMC8079699.

95. Zinge PR, Patil J. Comparative evaluation of effect of rotary and reciprocating single-file systems on pericervical dentin: A cone-beam computed tomography study. J Conserv Dent. 2017 Nov-Dec;20(6):424-428. doi: 10.4103/JCD.JCD_201_17. PMID: 29430095; PMCID: PMC5799989.

96. Butcher S, Mansour A, Ibrahim M. Influence of Apical Preparation Size on Effective Conventional Irrigation in the Apical Third: A Scanning Electron Microscopic Study. Eur Endod J. 2019 Feb 6;4(1):9-14. doi: 10.14744/eej.2018.06078. PMID: 32161880; PMCID: PMC7006559.

97. Malur MH, Chandra A. Curvature height and distance of MB canal of mandibular molar with Schneider angle and its comparison with canal access angle. J Oral Biol Craniofac Res. 2018 Sep-Dec;8(3):212-216. doi: 10.1016/j.jobcr.2017.07.002. Epub 2017 Jul 17. PMID: 30191111; PMCID: PMC6107922.

98. Kabil E, Katić M, Anić I, Bago I. Micro-computed Evaluation of Canal Transportation and Centering Ability of 5 Rotary and Reciprocating Systems with Different Metallurgical Properties and Surface Treatments in Curved Root Canals. J Endod. 2021 Mar;47(3):477-484. doi: 10.1016/j.joen.2020.11.003. Epub 2020 Nov 18. PMID: 33217469.

99. Gambill JM, Alder M, del Rio CE. Comparison of nickel-titanium and stainless steel hand-file instrumentation using computed tomography. J Endod. 1996 Jul;22(7):369-75. doi: 10.1016/S0099-2399(96)80221-4. PMID: 8935064.

100. Kuzekanani M, Sadeghi F, Hatami N, Rad M, Darijani M, Walsh LJ. Comparison of Canal Transportation, Separation Rate, and Preparation Time between One Shape and Neoniti (Neolix): An In Vitro CBCT Study. Int J Dent. 2021 Sep 7;2021:6457071. doi: 10.1155/2021/6457071. PMID: 34539788; PMCID: PMC8443393.

101. Gulabivala K, Ng YL. Factors that affect the outcomes of root canal treatment and retreatment-A reframing of the principles. Int Endod J. 2023 Mar;56 Suppl 2:82-115. doi: 10.1111/iej.13897. Epub 2023 Feb 10. PMID: 36710532.

102. Van Nieuwenhuysen JP, D'Hoore W, Leprince JG. What ultimately matters in root canal treatment success and tooth preservation: A 25-year cohort study. Int Endod J. 2023 May;56(5):544-557. doi: 10.1111/iej.13895. Epub 2023 Feb 8. PMID: 36683563.

103. Fransson H, Dawson V. Tooth survival after endodontic treatment. Int Endod J. 2023 Mar;56 Suppl 2:140-153. doi: 10.1111/iej.13835. Epub 2022 Oct 2. PMID: 36149887.

104. Kaushal R, Bansal R, Malhan S. A comparative evaluation of smear layer removal by using ethylenediamine tetraacetic acid, citric acid, and maleic acid as root canal irrigants: An *in vitro* scanning electron microscopic study. J Conserv Dent. 2020 Jan-Feb;23(1):71-78. doi: 10.4103/JCD.JCD_43_20. Epub 2020 Oct 10. PMID: 33223646; PMCID: PMC7657419.

105. AAE. Root Canal Irrigants and Disinfectants. AAE. 2011. 2023.

106. Plotino G, Özyürek T, Grande NM, Gündoğar M. Influence of size and taper of basic root canal preparation on root canal cleanliness: a scanning electron microscopy study. Int Endod J. 2019 Mar;52(3):343-351. doi: 10.1111/iej.13002. Epub 2018 Sep 14. PMID: 30129186.

107. Waleed D, Selivany BJ, Mohammad JR. Debridement Ability of TruNatomy, S-One Plus, and Other Single File Systems. Open Access Maced J Med Sci [Internet]. 2022 Jan. 27 [cited

2023 Nov. 16];10(D):91-7. Available from: https://oamjms.eu/index.php/mjms/article/view/8398.

108.de Albuquerque MS, Nascimento AS, Gialain IO, de Lima EA, Nery JA, de Souza Araujo PR, de Menezes RF, Kato AS, Braz R. Canal Transportation, Centering Ability, and Dentin Removal after Instrumentation: A Micro-CT Evaluation. J Contemp Dent Pract. 2019 Jul 1;20(7):806-811. PMID: 31597800.

109.Kandaswamy D, Venkateshbabu N, Porkodi I, Pradeep G. Canal-centering ability: An endodontic challenge. J Conserv Dent. 2009 Jan;12(1):3-9. doi: 10.4103/0972-0707.53334. PMID: 20379433; PMCID: PMC2848810.

110.Liu JY, Zhou ZX, Tseng WJ, Karabucak B. Comparison of canal transportation and centering ability of manual K-files and reciprocating files in glide path preparation: a micro-computed tomography study of constricted canals. BMC Oral Health. 2021 Feb 23;21(1):83. doi: 10.1186/s12903-021-01440-3. PMID: 33622296; PMCID: PMC7903633.

111.Mann NS, Rana M, Sarika DA, Batra D. Comparison of the rotary and reciprocating file systems using the CBCT: an original study. Journal of Positive School Psychology. 2022 Jun 1:10967-73.

112.Saber SE, Nagy MM, Schäfer E. Comparative evaluation of the shaping ability of WaveOne, Reciproc and OneShape single-file systems in severely curved root canals of extracted teeth. Int Endod J. 2015 Jan;48(1):109-14. doi: 10.1111/iej.12289. Epub 2014 Apr 18. PMID: 24673737.

113.Menecy, A., Mokhless, N., Hanafy, S. Evaluation of Surface Characteristic Changes of WaveOne Gold and WaveOne Single Reciprocating Files Using Scanning Electron Microscopy: An in-vitro Study. *Journal of Dental Materials and Techniques*, 2018; 7(1): 25-32. doi: 10.22038/jdmt.2017.10004.

114.Jose J, Khandelwal A, Siddique R. Qualitative Assessment of the Surface Topographic Changes of XP-endo Shaper and TruNatomy files after exposure to Sodium Hypochlorite and Ethylenediaminetetraacetic Acid. Eur Endod J. 2021 Aug;6(2):197-204. doi: 10.14744/eej.2021.10437. PMID: 34650015; PMCID: PMC8461496.

115.Hamdy TM, Galal M, Ismail AG, Abdelraouf RM. Evaluation of Flexibility, Microstructure and Elemental Analysis of Some Contemporary Nickel-Titanium Rotary Instruments. Open Access Maced J Med Sci. 2019 Oct 13;7(21):3647-3654. doi: 10.3889/oamjms.2019.811. PMID: 32010393; PMCID: PMC6986506.

Appendices

Appendix I – Template of Informed Consent for specimen collection

Rua Dr. Manuel Pereira da Silva
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Tel. 220 901 100

Informação aos Participantes

Título do estudo: Endodontia Minimamente Invasiva – Será o próximo passo? – Estudo comparativo de dois sistemas de instrumentação de lima única.

Investigador Responsável: Dr. Gilberto Miguel Serôdio Ribeiro

Orientadores: Prof. Doutora Cláudia Rodrigues (FMDUP) e Prof. Doutor Pedro de Sousa Gomes (FMDUP)

Contacto e-mail: gilberto.ribeiro@nhs.net

Contacto telefónico: 00447478480

Este documento pretende fornecer informação sobre o estudo para o qual foi convidado a participar e que está a ser conduzido pelo Dr. Gilberto Ribeiro, no âmbito do seu projeto de Doutoramento na FMDUP, de modo a que possa decidir de forma informada e consciente sobre a sua participação.
Adicionalmente, poderá fazer todas as perguntas que entender necessárias, de forma a compreender os aspetos do estudo que estiverem menos claros. Se aceitar participar, guarde este documento para consulta futura.

Descrição do estudo
Este é um estudo de investigação que decorrerá na FMDUP. As amostras serão recolhidas na Clínica Pedagógica da FMDUP e CC Dental Clinic.
O principal objetivo deste estudo visa avaliar, *in vitro*, a eficácia na preparação canal de dois sistemas de lima única com diferentes características - TruNatomy e Wave One Gold.
Este estudo foi sujeito a apreciação e encontra-se aprovado pela Comissão de Ética da FMDUP, garantindo a proteção dos direitos, da segurança e do bem-estar de todos os participantes.

Em que consistirá a sua participação
O estudo não envolve a realização de qualquer questionário, exame clínico, intervenção clínica, tratamento, ou qualquer forma de intervenção.
Se aceitar participar no estudo pedimos-lhe que consinta na doação do(s) dente(s) extraídos, para a realização da investigação acima descrita. O(s) dente(s) não será(ão) utilizado(s) para qualquer outro estudo, ou outros objetivos, e não será utilizado para qualquer procedimento que permita a identificação ou qualquer forma de análise genética.

Que riscos ou benefícios podem resultar da sua participação
Não se identificam riscos para o participante com a realização deste estudo.

Como potenciais benefícios identifica-se a possibilidade contribuir para a realização de um estudo experimental *in vitro* que visa uma avaliação comparativa de novos sistemas de limas endodônticas, contribuindo para o melhor conhecimento da utilização destes dispositivos médicos.

De que forma serão tratados os seus dados
Neste estudo não serão recolhidos dados pessoais.

Que direitos é que poderá exercer enquanto titular dos dados

Tem o direito de retirar o consentimento e desistir de participar no estudo, sem qualquer consequência para si, sem precisar de explicar as razões, sem qualquer penalização e sem comprometer a sua relação com o investigador responsável.
Contudo, o exercício deste direito só poderá ser garantido até à doação do(s) dente(s), uma vez que após esse momento não será possível a identificação individual do(s) dente(s).
Para exercer estes direitos deverá contactar o investigador responsável.

Como poderá obter mais informações

Se tiver questões sobre este estudo deve contactar o investigador responsável:
Dr. Gilberto Miguel Serôdio Ribeiro
Faculdade de Medicina Dentária da Universidade do Porto
Rua Dr. Manuel Pereira da Silva, 4200-393 Porto
gilberto.ribeiro@nhs.net

Para qualquer questão relativa aos seus direitos como participante neste estudo, deve contactar o Presidente da Comissão de Ética da FMDUP, para cetica@fmd.up.pt

Quaisquer dúvidas relacionadas especificamente com o tratamento dos seus dados poderão também ser endereçadas à Encarregada da Proteção de Dados da Universidade do Porto, através do e-mail dpo@reit.up.pt.

Rua Dr. Manuel Pereira da Silva
4200-393 Porto
Tel. 220 901 100

Consentimento

Eu, abaixo assinado, ____ (nome completo), compreendi a informação que me foi fornecida, por escrito e verbalmente, sobre o estudo "Endodontia Minimamente Invasiva – Será o próximo passo? – Estudo comparativo de dois sistemas de instrumentação de lima única", que está a ser conduzido no âmbito da Tese de Doutoramento em Medicina Dentária da Faculdade de Medicina Dentária da Universidade do Porto e para o qual é pedida a minha participação.

Sei que poderei desistir do estudo a qualquer momento, sem ter que dar justificações e sem que isso possa trazer-me quaisquer consequências.

Nestas circunstâncias,

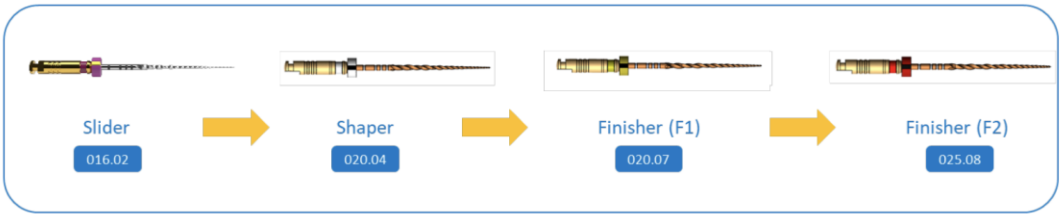
☐ Aceito livremente participar neste estudo, tal como me foi apresentado pelo Investigador Responsável.

Data ___ / ___ / ______

Participante: ____

Investigador: ____

Appendix II – File systems visual aid



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(n.d.) https://www.dentsplysirona.com/content/dam/master/product-procedure-brand-categories/endodontics/product-categories/full-solutions/trunatomy-solution/scientific-support-clinical-education/END-Scientific-Information-TruNatomy-Scientific-Manual-US.pdf

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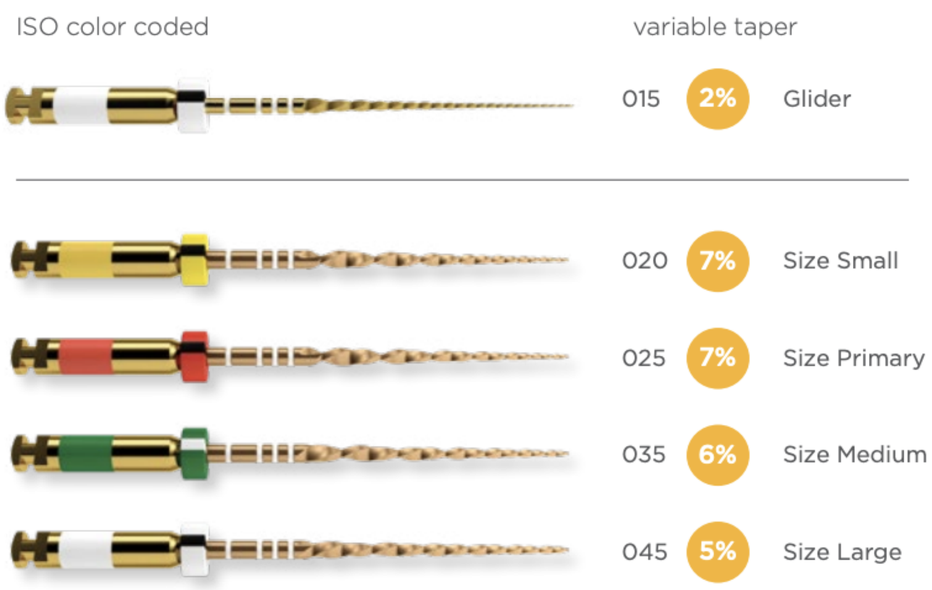

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Appendix III – Statistical data table for Volume and Root wear following shaping using 3 endodontic systems

Tukey's multiple comparisons test	n	Mean Diff,	95,00% CI of diff,	Adjusted P Value		
Total Wear Volume (mm^3)						
TruNatomy vs. WaveOneGold	15	-8.806	-10,15 to -7,464	<0,0001		
TruNatomy vs. ProtaperUltimate	15	-3.47	-4,812 to -2,128	<0,0001		
WaveOneGold vs. ProtaperUltimate	15	5.336	3,994 to 6,678	<0,0001		
System	TruNatomy		WaveOneGold		ProtaperUltimate	
AVG / SD	6.19	1.601853	14.99567	5.217102	9.66	4.831439

Tukey's multiple comparisons test	n	Mean Diff,	95,00% CI of diff,	Adjusted P Value		
Root Wear Volume (mm^3)						
TruNatomy vs. WaveOneGold	15	-2.936	-4,278 to -1,594	<0,0001		
TruNatomy vs. ProtaperUltimate	15	-1.257	-2,599 to 0,08539	0.0719		
WaveOneGold vs. ProtaperUltimate	15	1.679	0,3373 to 3,021	0.0096		
System	TruNatomy		WaveOneGold		ProtaperUltimate	
AVG / SD	0.6608	0.262399	3.596667	1.589627	1.917417	0.914552

Appendix IV – Statistical data regarding smear layer content and grading in the apical third

	Pictures analyzed (A - J)													
System	A	B	C	D	E	F	G	H	I	J	Mean	Median	SD	SE
WaveOne Gold	1	2	2	2	3	1	2	2	2	2	1,9	2	0,567646	0,179505
Protaper	4	3	4	4	3	2	2	2	2	2	2,8	2,5	0,918937	0,290593
TruNatomy	4	4	2	3	2	2	1	2	2	1	2,3	2	1,05935	0,334996

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value	
TruNatomy vs. WaveOneGold	0,4	-0,5679 to 1,368	No	ns	0,5680	A-B
TruNatomy vs. ProtaperUltimate	-0,5	-1,468 to 0,4679	No	ns	0,4179	A-C
WaveOneGold vs. ProtaperUltimate	-0,9	-1,868 to 0,06786	No	ns	0,0721	B-C

Appendix V - Statistical data regarding Dentin thickness reduction and remaining dentin thickness at the three selected reference points selected

Tukey's multiple comparisons test	n	Mean Diff,	95,00% CI of diff,	Adjusted P Value		
Dentin Thickness Reduction (mm) - 1 mm above CEJ						
TruNatomy vs. WaveOneGold	15	-0.4702	-0,8456 to -0,09469	0.0095		
TruNatomy vs. ProtaperUltimate	15	-0.1957	-1,007 to 0,6155	0.8377		
WaveOneGold vs. ProtaperUltimate	15	0.2745	-0,5366 to 1,086	0.706		
System	TruNatomy		WaveOneGold		ProtaperUltimate	
AVG / SD	0.1806	0.075156	0.65075	0.140327	0.37625	0.112745

Tukey's multiple comparisons test	n	Mean Diff,	95,00% CI of diff,	Adjusted P Value		
Dentin Thickness Reduction (mm) - CEJ						
TruNatomy vs. WaveOneGold	15	-0.4541	-0,778 to -0,1302	0.003		
TruNatomy vs. ProtaperUltimate	15	-0.1288	-0,8285 to 0,5708	0.9021		
WaveOneGold vs. ProtaperUltimate	15	0.3253	-0,3744 to 1,025	0.5194		
System	TruNatomy		WaveOneGold		ProtaperUltimate	
AVG / SD	0.1243	0.041122	0.578417	0.174308	0.253167	0.15115

Tukey's multiple comparisons test	n	Mean Diff,	95,00% CI of diff,	Adjusted P Value		
Dentin Thickness Reduction (mm) - 1 mm bellow CEJ						
TruNatomy vs. WaveOneGold	15	-0.4859	-0,8439 to -0,1279	0.0043		
TruNatomy vs. ProtaperUltimate	15	-0.1453	-0,807 to 0,5165	0.8639		
WaveOneGold vs. ProtaperUltimate	15	0.3407	-0,3211 to 1,002	0.4483		
System	TruNatomy		WaveOneGold		ProtaperUltimate	
AVG / SD	0.0725	0.032247	0.558417	0.126518	0.21775	0.104722

Tukey's multiple comparisons test	n	Mean Diff,	95,00% CI of diff,	Adjusted P Value		
Remaining Dentin Thickness (mm) - 1 mm above CEJ						
TruNatomy vs. WaveOneGold	15	0.6507	-0,2141 to 1,515	0.1815		
TruNatomy vs. ProtaperUltimate	15	0.4441	-0,4206 to 1,309	0.45		
WaveOneGold vs. ProtaperUltimate	15	-0.2066	-1,071 to 0,6581	0.8409		
System	TruNatomy		WaveOneGold		ProtaperUltimate	
AVG / SD	1.8137	0.542082	1.163	0.62796	1.369583	0.434541

Tukey's multiple comparisons test	n	Mean Diff,	95,00% CI of diff,	Adjusted P Value		
Remaining Dentin Thickness (mm) - CEJ						
TruNatomy vs. WaveOneGold	15	0.6748	-0,1899 to 1,54	0.1597		
TruNatomy vs. ProtaperUltimate	15	0.4111	-0,4537 to 1,276	0.5043		
WaveOneGold vs. ProtaperUltimate	15	-0.2638	-1,128 to 0,601	0.754		
System	TruNatomy		WaveOneGold		ProtaperUltimate	
AVG / SD	1.8876	0.422371	1.212833	0.572445	1.476583	0.374117

Tukey's multiple comparisons test	n	Mean Diff,	95,00% CI of diff,	Adjusted P Value		
Remaining Dentin Thickness (mm) - 1 mm bellow CEJ						
TruNatomy vs. WaveOneGold	15	0.694	-0,1707 to 1,559	0.1438		
TruNatomy vs. ProtaperUltimate	15	0.3689	-0,4958 to 1,234	0.5759		
WaveOneGold vs. ProtaperUltimate	15	-0.3251	-1,19 to 0,5396	0.6514		
System	TruNatomy		WaveOneGold		ProtaperUltimate	
AVG / SD	1.8309	0.062247	1.136917	0.426518	1.462	0.204722

Tukey's multiple comparisons test	n	Mean Diff,	95,00% CI of diff,	Adjusted P Value		
Average Reduction of Dentin Thickness (2 mm around CEJ, mm)						
TruNatomy vs. WaveOneGold	15	-0.1338	-0,2133 to -0,05434	0.0002		
TruNatomy vs. ProtaperUltimate	15	-0.09184	-0,1713 to -0,01234	0.0186		
WaveOneGold vs. ProtaperUltimate	15	0.042	-0,0375 to 0,1215	0.4303		
System	TruNatomy		WaveOneGold		ProtaperUltimate	
AVG / SD	0.018	0.00646585	0.151833333	0.031526215	0.10983333	0.00754034

Tukey's multiple comparisons test	n	Mean Diff,	95,00% CI of diff,	Adjusted P Value		
Average of Remaining Dentin Thickness (2 mm around CEJ, mm)						
TruNatomy vs. WaveOneGold	15	0.1554	-0,3578 to 0,6686	0.7575		
TruNatomy vs. ProtaperUltimate	15	0.1114	-0,4018 to 0,6246	0.8669		
WaveOneGold vs. ProtaperUltimate	15	-0.044	-0,5572 to 0,4692	0.978		
System	TruNatomy		WaveOneGold		ProtaperUltimate	
AVG / SD	1.8777	0.0858914	1.72225	0.180089435	1.76625	0.10205981

Appendix VI - Published manuscript - Comparative evaluation of the canal shaping ability, pericervical dentin preservation and smear layer removal of TruNatomy, WaveOne Gold and Protaper Ultimate – an ex vivo study in human teeth

Comparative evaluation of the canal shaping ability, pericervical dentin preservation and smear layer removal of TruNatomy, WaveOne Gold and Protaper Ultimate – an *ex vivo* study in human teeth

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ABSTRACT

Introduction: Innovative file systems have been recently introduced, claiming improved effectiveness and superior ability to preserve the tooth structure, still allowing an efficient preparation and disinfection up to the apical region. Regardless, few data are available on the comparative effectiveness of the most recently developed systems. Thus, this ex vivo study aimed to comparatively evaluate, for the first time, the functionality of WaveOne Gold (WOG), TruNatomy (TN), and Protaper Ultimate (PU) file systems regarding canal shaping, dentin preservation, and smear layer removal ability.

Methods: Human maxillary incisors were randomly divided for instrumentation with one of the assayed systems. Canal shaping ability and pericervical dentin preservation were characterized through microtomographic evaluation and morphometric assessment (n=15). Smear layer removal ability was evaluated by scanning electron microscopy (n=6).

Results: TN and PU presented the lowest canal volume variation upon instrumentation, found to be significantly lower than that attained with WOG ($p<0.05$). Pericervical dentin was reduced in all groups upon instrumentation, with TN evidencing the highest preservation, quantitatively similar to PU, and significantly higher than that attained with WOG ($p<0.05$). SEM imaging revealed the presence of scattered remnants of the smear layer and partially opened dentinal tubules at the apical portion, with no significant differences between systems.

Conclusions: TN and PU allowed for the highest tissue preservation, reporting the lowest volume variation and the highest preservation of the pericervical dentin. None of the assessed systems provided a complete removal of the smear layer in the apical region.

Keywords: TruNatomy; WaveOne Gold; Protaper Ultimate; Micro-computed tomography; Pericervical dentin preservation; Smear layer removal.

INTRODUCTION

Endodontic treatment poses many challenges when all the steps required to ensure a successful clinical outcome are considered. Current therapeutic concepts for root canal treatment (RCT) rely on the preservation of the maximum tooth structure, by maintaining the original canal shape during instrumentation, and the total obturation of the canal system (1). In this frame, nickel-titanium instruments have been developed for effective root canal preparation, outperforming traditional files, and minimizing the risk of preparation-related accidents, given their increased flexibility (2). Moreover, efforts to maintain the original structure of the canal anatomy and to preserve the remaining dentin, particularly the pericervical dentin, a region acknowledged to directly impact the tooth's fracture resistance and long-term prognosis, have been developed (3). This realization has led to the development of innovative file systems whose aim is to preserve tooth structure. This is accomplished by a reduction of the shaft diameter and taper while still allowing an efficient preparation and disinfection up to the apical region (4). Such systems embrace those that have been on the market for a longer time such as WaveOne Gold (WOG), the more recent TruNatomy (TN), and the latest system ProTaper Ultimate (PU).

WOG is a reciprocating single-file system with a parallelogram-shaped cross section with two cutting edges alternated with one cutting edge from an off centered cross section. It has a reverse helix structure, a semiactive guiding tip, and a maximum flute diameter of 1.2 mm. It has also a fixed taper from D1-D3, and a decreasing one from D4-D16, which appears to result in improved effectiveness and flexibility compared to the WaveOne system (5, 6). Instruments are available at different sizes, with distinct tip size and taper, as follows: #20/0.07, #25/0.07, #35/0.06 and #45/0.05. TN is a newer generation system that is constructed with an off-centered rectangular section, a regressive taper, and a smaller flute diameter of 0.8 mm, when compared to WOG. Taper ranges from 0.02 in larger instruments to 0.04 in smaller ones. As a result of its metallurgic processing the TN system shows greater flexibility as a result of the application of heat in the post-manufacturing process (7, 8). PU is the most recent of the systems considered in this study which is a multi-file system that demonstrates different crystallographic arrangements which delivers instruments with complementary mechanical outcomes (9). It also presents a parallelogram cross section with distinctive acute angles, a partially off centered and a maximal flute diameter of 1.0 mm, thus conservatively approaching the dentin removal in critical areas such as the CEJ. It differs from WOG and TN by providing wider apical preparation sizes – with Finisher files presenting increased tip sizes and tapers: #20/0.07, #25/0.08 and #30/0.09), which produces a more efficient debridement in the apical third (9, 10). As a newly marketed system, little data is available on PU functionality and effectiveness, with no previous comparative assessment of these three systems being previously published, to the best of the authors' knowledge.

This ex vivo study with human teeth aims to compare the effectiveness of the systems by assessing the canal shaping, volume variation, and pericervical dentin preservation through microtomography and morphometric quantitative evaluation, further evaluating the smear layer removal capability through scanning electron microscopy. The null hypothesis is that there is no difference between the endodontic file systems regarding instrumentation effectiveness and smear layer removal.

MATERIALS AND METHODS

Sample selection

The Ethics Committee for Health of the Faculty of Dental Medicine, University of Porto approved the research protocol, with the reference number 18/2021. The sample size was calculated using G*Power 3.1 software (Heinrich Heine Universität, Düsseldorf, Germany), using 12 specimens per group, and the alpha error set at 0.05 and the power set to 95%. From the initial selection of extracted human teeth, 15 maxillary incisors per group were selected for the microtomographic analysis, while 6 maxillary incisors per group were selected for the qualitative smear layer assessment using SEM. Included teeth met the following criteria: unrestored or moderately restored teeth without previous root canal treatment; a restoration or carious lesion must have not reached the critical area, 4 mm below or above the CEJ. In addition, teeth with endodontic treatment, root fractures, broken apices, internal resorption defects, immature apices, and vertical or horizontal root fractures were excluded. Samples were distributed randomly within the three test groups.

Instrumentation

A single operator with postgraduate training in endodontics performed all the procedures in accordance with the manufacturers' recommendations. An access opening was made on each specimen using a medium grit medium-sized round diamond bur (126210, Dentaleader, Lisbon, Portugal) with a handpiece using continuous water spray. An Endo Z bur (671796, Mailleffer, USA) was used to refine the access cavity, and a size 10 hand K-type file (Dentsply Tulsa Dental, Charlotte, USA) was used to define the working length (WL). Canals were prepared with WOG, TN, or PU files according to the manufacturer's recommendations including the use of orifice openers.

Following to the manufacturer's recommendations, the TN system was used in continuous rotation at 500 rpm and 1.5 Ncm, WOG was used in a reciprocating motion with a 350 rpm rotation at 170° counterclockwise and 50° clockwise, and PU was used in continuous rotation at 400 rpm and 4 Ncm. All systems were powered by the endodontic micromotor control unit WaveOneTM (DENTSPLY Maillefer, Tulsa, OK, USA). The teeth were instrumented in short amplitude movements until the working length (WL) was reached. Patency was maintained with a K10 file. Canal irrigation was conducted with 3 mL of 1.1% NaOCl using a 27-gauge needle placed 3 mm short of the working length for a total volume of 9 mL and short amplitude vertical movements were used.

Microtomographic evaluation

Microtomographic scans were conducted before and after instrumentation with the microtomographic equipment Skyscan1276 and software both from the Bruker Corporation, Kontich, Belgium. The following parameters were used: image pixel size 20.014 □m, 100 kV, 200 uA, 360o rotation around the vertical axis. Images were reconstructed using the NRecon software version 1.7.4.2., with the following parameters: 432 ms exposure time, rotation set at 0.040 with a framing average of 4, CS to image conversion values of 0.0 to 0.04, ring artifact correction of 8, beam hardening correction of 6%, and smoothing of 0 were used. An aluminum/copper filter was attached. Reconstructed 3D datasets of pre- and post-instrumentation were three-dimensionally registered, oriented, and aligned in the same multidimensional space, using DataViewer software version 1.5.6.3. Volume analysis: the tissue removed through instrumentation was analyzed using the CTAnalyser software version 1.17.7.2, which follows the guidelines from Bruker (MN110), in a region of interest (ROI) defined between the cementoenamel junction (CEJ) and the apex. Dentin thickness analysis: the reduction of the dentin thickness and the average reduction of dentin thickness were measured from datasets obtained before and after instrumentation. Evaluation was conducted at defined levels, as well as at a ROI defined between the levels 1 mm in the coronal direction and 1 mm in the apical direction from the CEJ, selected as the reference point for standardization. The defined regions are represented on Supplementary material 1. Representative three-dimensional images were captured using CTVox software (Bruker, version 3.3.0).

Smear layer removal analysis

For the SEM imaging, teeth were sectioned after the canals were obturated, and selected surfaces were coated by a thin film of Au/Pd (SPI Module Spitter Coater) and imaged in a Quanta 400 FEG ESEM/EDAX Genesis X4M) microscope, using a voltage of 10 kV. Images at 1 mm from the apical region were used to assess the presence or absence of the smear layer using a semi-quantitative score: (1) no smear layer and dentin tubules open; (2) small amounts of scattered smear layer and dentinal tubules open; (3) thin smear layer and dentinal tubules partially open; (4) partial covering with a thick smear layer; (5) total covering with a smear layer, as previously reported (11). Images were randomized and evaluated blinded.

Statistical analysis

The data was examined using the one-way ANOVA for intragroup comparison and Post Hoc Tukey HDS for intergroup examination. The level of significance was set at $p < 0.05$. Statistical software (Statistical Package for the Social Sciences, 28.0, IBM, USA) was used for all analyses.

RESULTS

Upon instrumentation, the volume variation of the canal was found to be dissimilar among the assayed systems (Figure 1). Representative microtomographic three-dimensional reconstructions (Figure 1A) show the original canal trajectory (in green) and shown the removed volumes upon instrumentation (in red). These results are suggestive of an increased volume variation attained with

WOG when compared to the other systems. Quantitative assessment (Figure 1B) validated these findings by showing that TN presented the lowest volume variation value (0.66 ± 0.26 mm3), followed by PU presenting an intermediate volume variation (1.91 ± 0.91 mm3), and WOG demonstrating the highest levels (3.59 ± 1.58 mm3). Whether no significant differences were attained between TN and PU, WOG levels were found to be significantly higher than those of the other systems ($p<0.05$).

Pericervical dentin assessment (detailed in Supplemental figure 1) was found to be reduced upon instrumentation within all three systems, with significant differences between the systems when assayed (Figure 2). Representative microtomographic bidimensional images and measurements are presented before and upon instrumentation (Figure 2A). Comparatively, the dentinal thickness reduction at the CEJ was 0.1243 ± 0.04 mm, 0.5784 ± 0.17 mm, and 0.2531 ± 0.15 mm for TN, WOG, and PU, respectively. At a coronal position from the CEJ (+ 1 mm), dentin reduction levels were 0.1805 ± 0.07 mm, 0.6507 ± 0.14 mm, and 0.3762 ± 0.11 mm, and at an apical position from the CEJ (- 1 mm), the variations were of 0.0725 ± 0.03 mm, 0.5584 ± 0.12 mm, and 0.2177 ± 0.10 mm for TN, WOG, and PU, respectively. For the three assessed locations, TN values were found to be significantly lower than those of WOG ($p<0.05$), while no significant differences to PU were disclosed (Figure 2B). In addition, when the full ROI was considered, TN levels were found to be significantly lower than those of WOG and PU ($p< 0.05$), with no significant differences between the latter (Figure 2C).

The SEM imaging of the canal wall at the apical location (Figure 3) revealed the presence of scattered remnants of the smear layer, and the dentinal tubules were opened or partially opened in all of the assayed samples (Figure 3A). Semi-quantitative scores revealed similar values, within the 2-3 range (Figure 3B), evidencing no significant differences among experimental groups (Figure 3C).

DISCUSSION

Treatment approaches based on minimally invasive endodontics focus on radicular structure and pericervical dentin preservation in an effort to increase the fracture resistance and overall mechanical properties of teeth treated endodontically. This has led to the development of endodontic systems whose features promote maximum tooth preservation such as the reciprocating WOG, and the more recent continuous rotation systems TN and PU. To the best of the authors' knowledge, these systems have not been previously comparatively evaluated, with only very few reports on PU assessment. Thus, this ex vivo study evaluated comparatively the effectiveness of WOG, TN, and PU, in extracted human anterior teeth with attention to canal shaping, volume wear, pericervical dentin preservation, and smear layer removal in the apical third. Upon assessment, the null hypothesis was rejected as significant differences were found among the three systems within the evaluated parameters.

After instrumentation TN presented the lowest volume variation, followed by PU, and lastly, WOG which presented a significantly higher volume variation than the former. A similar trend was attained for the dentin preservation assessment at the three distinct levels bordering the CEJ. TN presented with a significantly reduced value when compared to WOG and PU, when the full ROI was disclosed. Whether no comparative data is available for the 3 systems, previous studies comparing TN and WOG showed an increased shaping ability of TN with an increased capacity to preserve the original canal shape, while limiting canal transportation (12, 13), and maintaining a high centering ability (8). The full off-centered cross-sectional design of the TN system seems to allow the shaping of a larger canal surface when compared to concentric instruments with the same cross-sectional area. This would further endorse a more favorable stress distribution during instrumentation (14). This approach allows the use of smaller diameter instruments, known to improve canal shaping and pericervical dentin preservation (15). Accordingly, TN presented the lowest diameter (0.8 mm), followed by PU (1.0 mm) and lastly, WOG (1.2 mm), which correlates with the overall outcomes regarding canal volume variation and pericervical dentin preservation. These results affirm the major influence of this parameter on the reported data. Of additional relevance, the distinct taper values, among systems and within the multi-file system, may further influence the overall outcomes. Conflicting literature reports exist, with some studies indicating the absence (16, 17) or presence (18) of significant variances when comparing different tapers in terms of their impact on root canal preparation and dentin removal effectiveness.

Regarding the evaluation of the smear layer content, no significant differences were verified between the distinct systems. Attained data is in line with previous reports evidencing similar range scores for WOG (19, 20) and the absence of significant differences between WOG and TN (21). Whether no data for PU is available, comparatively, no significant differences were found between WOG and TN, indicating their comparable effectiveness in removing the smear layer. However, it is important to note that neither system was able to achieve complete removal of the smear layer. Consequently, the integration of complementary methods, such as ultrasonic irrigation, is necessary and holds the potential to enhance cleaning outcomes.

Overall, this study was the first to compare the WOG, TN, and PU systems, demonstrating the capability of TN and PU to outperform WOG in both canal volume variation and pericervical dentin preservation. Consequently, the null hypothesis is rejected. However, it is imperative to acknowledge the study's limitations, chiefly its ex vivo nature and focus on maxillary anterior teeth with straight, patent canals. Future investigations should broaden their scope to include multi-rooted teeth and explore various irrigation techniques to enhance canal debridement. Additionally, further validation through clinical studies is imperative to substantiate these findings.

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Declaration of interest: none.

FIGURES

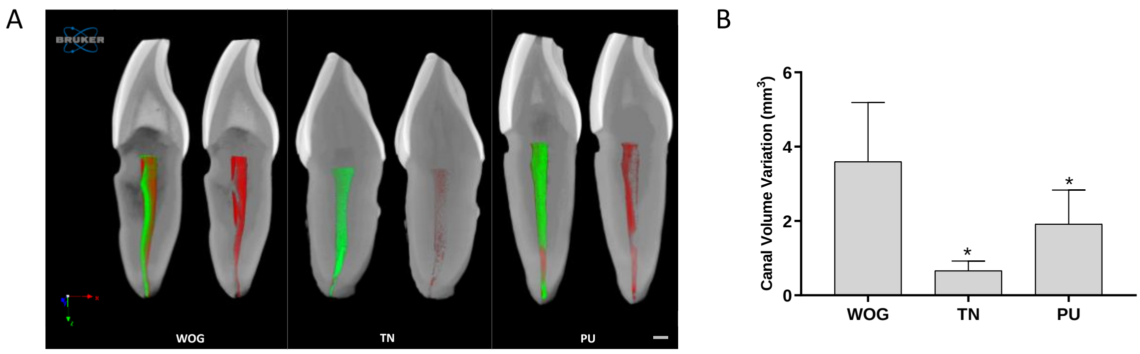


Figure 1 – A – Representative microtomographic images of the canals, prior (in green) and upon (in red) instrumentation, with the different systems, scale bar corresponds to 1mm. B – Quantitative assessment of the canal volume variation upon instrumentation with the different systems. * - significantly different from WOG (p<0.05).

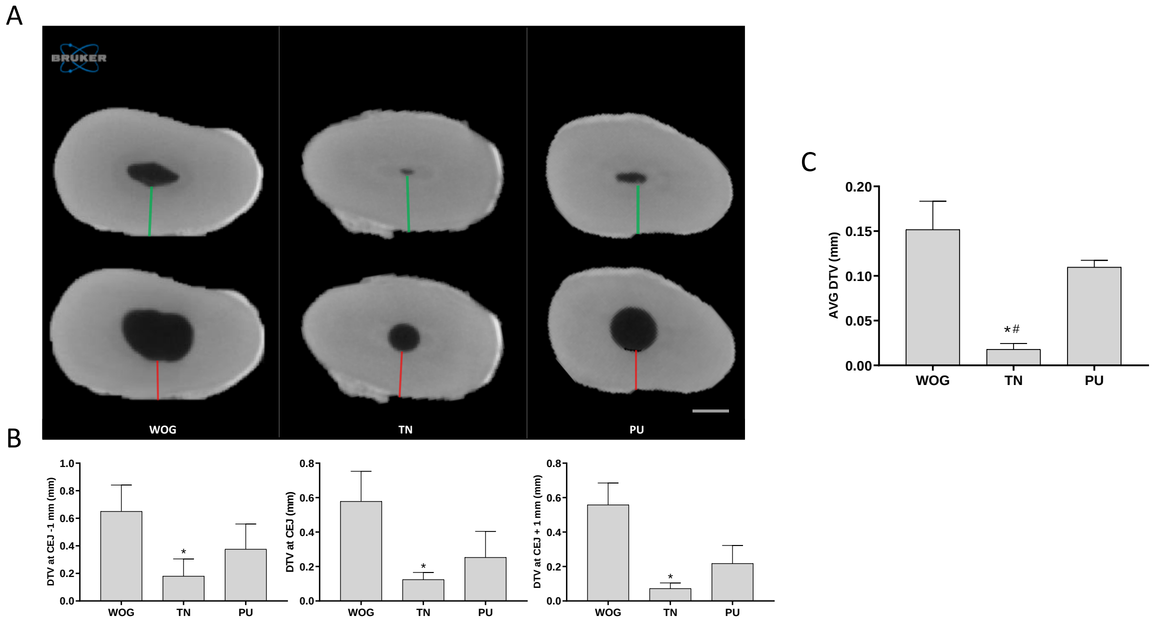
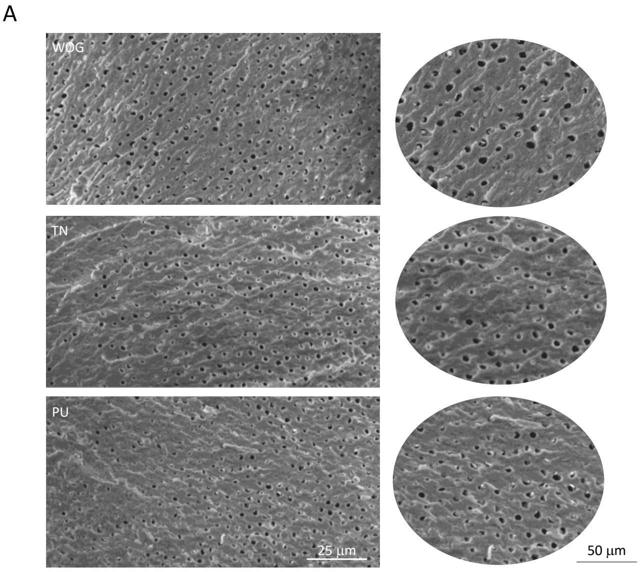


Figure 2 – A - Representative bidimensional microtomographic sections of the dentin thickness analysis, prior (top) and upon (bottom) instrumentation with the different systems, scale bar corresponds to 1mm. B – Assessment of the linear variation of the dentin thickness at the selected references bordering the cementoenamel junction. C – Assessment of the dentin thickness variation at the selected ROI, delimited at 1 mm coronal and 1 mm apical of the CEJ. * - significantly different from WOG ($p<0.05$); # - significantly different from PU ($p< 0.05$).



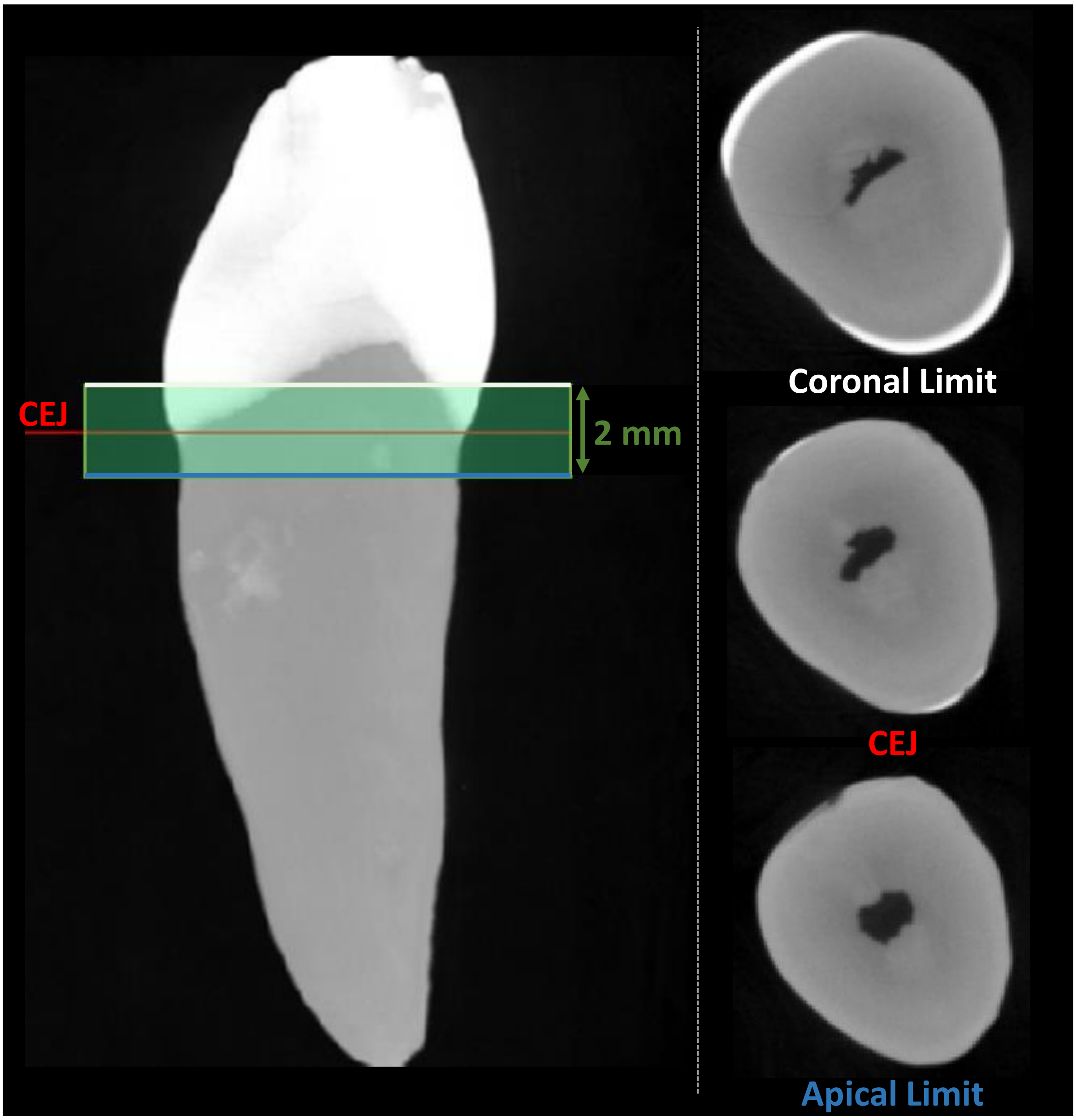
B

	Mean	Median	SD	SE
WOG	1.9	2	0.567	0.179
TN	2.3	2.5	0.918	0.290
PU	2.8	2	1.059	0.334

C

	Mean Diff.	95.00% CI of diff.	p value
TN vs WOG	0.4	-0.5679 to 1.368	0.5680
TN vs PU	-0.5	-1.468 to 0.4679	0.4179
WOG vs PU	-0.9	-1.868 to 0.06786	0.0721

Figure 3 – Representative SEM images of the canal wall at 1 mm from the apex, upon instrumentation with the three different systems. B – Scores and descriptive statistics of the semi-quantitative analysis of the SEM images. C – Statistical analysis and inter-group comparison of the attained scores.



Supplementary Figure 1 – Microtomographic image of the region of interest for the assessment of pericervical dentin upon instrumentation with the different systems. The red line defines the cementoenamel junction (CEJ), while the white line defines the region of analysis 1 mm in the coronal direction, and the blue line defines the region of analysis 1 mm in the apical direction.

REFERENCES

1. Colombo M, Bassi C, Beltrami R, Vigorelli P, Spinelli A, Cavada A, et al. Radiographic technical quality of root canal treatment performed by a new rotary single-file system. Annali di Stomatologia 2017;8(1).

2. Pedullà E, Kharouf N, Caruso S, al e. Torsional, Static, and Dynamic Cyclic Fatigue Resistance of Reciprocating and Continuous Rotating Nickel-Titanium Instruments. J Endod 2022;48:1421-1427.

3. Brasil S, Marceliano-Alves M, Marques M, al e. Canal Transportation, Unprepared Areas, and Dentin Removal after Preparation with BT-RaCe and ProTaper Next Systems. J Endod 2017;43:1683-1687.

4. Tabassum S, Zafar K, Umer F. Nickel-titanium rotary file systems: What's new? Europ Endod J 2019;4:111.

5. Adıgüzel M, Capar I. Comparison of cyclic fatigue resistance of WaveOne and WaveOne Gold small, primary, and large instruments. J Endod 2017;43:623-627.

6. Prados-Privado M, Rojo R, Ivorra C, Prados-Frutos J. Finite element analysis comparing WaveOne, WaveOne Gold, Reciproc and Reciproc Blue responses with bending and torsion tests. J Mechanic Beahv Biomed MAt 2019;90:165-172.

7. Elnaghy A, Elsaka S, Mandorah A. In vitro comparison of cyclic fatigue resistance of TruNatomy in single and double curvature canals compared with different nickel-titanium rotary instruments. BMC Oral Health 2020;20:38.

8. Kabil E, Katić M, Anić I, al. e. Micro-computed Evaluation of Canal Transportation and Centering Ability of 5 Rotary and Reciprocating Systems with Different Metallurgical Properties and Surface Treatments in Curved Root Canals. J Endod 2021;47:477-484.

9. Martins J, Silva E, Marques D, Ajuz N, Rito Pereira M, Pereira da Costa R, et al. Characterization of the file-specific heat-treated ProTaper Ultimate rotary system. Int Endod J 2023;56:530-542.

10. Mustafa M, Attur K, Bagda K, Singh S, Oak A, Kathiria N. An Appraisal on Newer Endodontic File Systems: A Narrative Review. J Contemp Dent Pract 2022;23:944-952.

11. Butcher S. Influence of apical preparation size on effective conventional irrigation in the apical third: a scanning electron microscopic study. Europ Endod J 2018;4:9-14.

12. Shaheen N, Elhelbawy N. Shaping ability and buckling resistance of TruNatomy, WaveOne gold, and XP-Endo shaper single-file systems. Contemp Clinical Dent 2022;13:261.

13. Kim H, Jeon S, Seo M. Comparison of the canal transportation of ProTaper GOLD, WaveOne GOLD, and TruNatomy in simulated double-curved canals. BMC Oral Health 2021;21:533.

14. Martins S, Garcia P, Viana A, Buono V, Santos L. Off-centered geometry and influence on NiTi endodontic file performance evaluated by finite element analysis. J Mat Eng Performance 2020;29:2095-2102.

15. Aazzouzi-Raiss K, Ramírez-Muñoz A, Vieira G, Aranguren J, Pérez A. Effects of conservative access and apical enlargement on shaping and dentin preservation with traditional and modern instruments: A micro-computed tomographic study. J Endod 2023;in press.

16. Seema T, Ahammed H, Parul S, Cheranjeevi J. Comparative evaluation of dentin removal and taper of root canal preparation of hand K file, ProTaper rotary file, and Kedo S rotary file in primary molars using cone-beam computed tomography. Int J Clin Peditric Dent 2020;13:332.

17. Elkholy M, Nawar N, Ha W, Saber S, Kim H. Impact of canal taper and access cavity design on the life span of an endodontically treated mandibular molar: a finite element analysis. J Endod 2021;47:1472-1480.

18. Lima C, Barbosa A, Ferreira C, Augusto C, Sassone L, Lopes R, et al. The impact of minimally invasive root canal preparation strategies on the ability to shape root canals of mandibular molars. Int Endod J 2020;53:1680-1688.

19. Feghali M, Jabbour E, Koyess E, Sabbagh J. Scanning electron microscopy evaluation of debris and smear layer generated by two instruments used in reciprocating motion WaveOne Gold® and Reciproc Blue®. Australian Endod J 2019;45:388-393.

20. Liu Y, Chen M, Tang W, al. e. Comparison of five single-file systems in the preparation of severely curved root canals: an ex vivo study. BMC Oral Health 22:649.

21. Waleed D, Selivany B, Mohammad J. Debridement Ability of TruNatomy, S-One Plus, and Other Single File Systems. Open Access Macedonian Journal of Medical Sciences 2022;10:91-97.