THE EFFICACY OF MECHANICAL ROTARY, CHEMICAL SOLVENT AND LASER-ACTIVATED IRRIGATION TO REMOVE INTRACANAL OBTURATION MATERIAL WITH VARIOUS TYPE OF SEALER IN RETREATMENT ENDODONTIC

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by

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LIST OF ABBREVIATIONS

AMDI	Advanced Medical and Dental Institute.
KSA	Kingdom of Saudi Arabia
KSU	King Saud University
LAI	Laser-Activated Irrigation
PTUR	Protaper Universal Rotary
RCFM	Root canal filling material
RCT	Root Canal Therapy
RFM	Residual filling materials
CBCT	Cone Beam Computed Tomography

LIST OF APPENDICES

KEUPAYAAN ALAT MEKANIKAL BERPUTAR, PELARUT KIMIA DAN PENGAKTIFAN LASER LARUTAN DALAM MENGHILANGKAN MATERIAL TAMPALAN SALUR AKAR GIGI DAN PELBAGAI JENIS BAHAN PENGAP DI DALAM RAWATAN ULANGAN ENDODONTIK

ABSTRAK

Kajian ini bertujuan untuk menilai keberkesanan pelbagai protokol rawatan semula endodontik dalam menghapuskan bahan pengisian saluran akar daripada premolar manusia yang diekstrak dan untuk menilai pengaruh jenis bahan pengap terhadap prestasi penyingkiran. Tiga puluh enam spesimen gigi berakar tunggal telah diperuntukkan kepada empat kumpulan berdasarkan kaedah rawatan semula: Kumpulan 1 - ProTaper Universal Retreatment (PTUR), Kumpulan 2 - PTUR + kloroform, Kumpulan 3 - PTUR + pengairan diaktifkan laser (PDL), dan Kumpulan 4 - PTUR + kloroform + PDL. Setiap kumpulan dibahagikan lagi mengikut jenis bahan pengap: AH Plus, MTA Fillapex dan CeraSeal (n = 3 setiap subkumpulan). Patensi apikal dinilai selepas rawatan semula. Bahan obturasi yang tertinggal dianalisis menggunakan tomografi terkomputer mikro (mikro-CT) untuk mengukur isipadu dan stereomikroskopi digunakan untuk memeriksa sisa pada dinding saluran. Analisis statistik termasuk ujian Chi-Square untuk patensi apikal dan ANOVA dua hala dengan ujian post hoc Tukey untuk pengukuran kuantitatif. Tiada perbezaan ketara dalam patensi apikal diperhatikan dalam kalangan kumpulan ($p = 1.000$). Kumpulan 4 mempamerkan tahap bahan tertahan yang paling rendah ($p = 0.0037$), ini menunjukkan kecekapan yang dipertingkatkan. Di antara bahan pengap, MTA Fillapex dikaitkan dengan jumlah sisa yang lebih tinggi berbanding dengan AH Plus dan CeraSeal. Sebagai kesimpulan, penggunaan gabungan PTUR, kloroform, dan PDL

terbukti lebih berkesan dalam membersihkan saluran akar tanpa menjejaskan patensi apikal. Penemuan ini menyokong manfaat klinikal untuk mengintegrasikan kedua-dua teknik pelarut dan bantuan laser semasa prosedur rawatan semula.

THE EFFICACY OF MECHANICAL ROTARY, CHEMICAL SOLVENT AND LASER-ACTIVATED IRRIGATION TO REMOVE INTRACANAL OBTURATION MATERIAL WITH VARIOUS TYPE OF SEALER IN RETREATMENT ENDODONTIC

ABSTRACT

This study aimed to evaluate the efficacy of different retreatment protocols in eliminating root canal filling materials (RCFM) from extracted human premolars and to assess the influence of sealer type on removal performance. Thirty-six single-rooted specimens were allocated into four groups based on the retreatment method: Group 1 – ProTaper Universal Retreatment (PTUR), Group 2 – PTUR + chloroform, Group 3 – PTUR + laser-activated irrigation (LAI), and Group 4 – PTUR + chloroform + LAI. Each group was further subdivided according to sealer type: AH Plus, MTA Fillapex, and CeraSeal (n = 3 per subgroup). Apical patency was assessed post-retreatment. The remaining obturation material was analyzed using micro-computed tomography (micro-CT) to quantify its volume and stereomicroscopy to examine remnants on the canal walls. Statistical analyses included the Chi-Square test for apical patency and a two-way ANOVA with Tukey's post hoc test for quantitative measurements. No significant differences in apical patency were observed among the groups ($p = 1.000$). Group 4 exhibited the lowest levels of retained material ($p = 0.0037$), suggesting enhanced efficiency. Among the sealers, MTA Fillapex was associated with a higher amount of remaining debris compared to AH Plus and CeraSeal. In conclusion, the combined use of PTUR, chloroform, and LAI proved more effective in debriding root canals without adversely affecting apical patency. These findings support the clinical

benefit of integrating both solvent and laser-assisted techniques during retreatment procedures.

CHAPTER 1

INTRODUCTION

1.1 Research background

Root canal retreatment's primary goal is to stop the spread of infection by removing the filling material, debris, and microorganisms that cause apical periodontitis (More et al., 2022). It can be accomplished using chemical solvents and mechanical tools to dissolve the Gutta-Percha (GP). This includes hand files, rotary, reciprocating, or ultrasonic tools to remove the intracanal filling material (Nguyen et al., 2019). Furthermore, few studies have discovered the utilisation of Laser-Activated Irrigation (LAI) to enhance the removal of root canal fillings (De Meyer et al., 2017).

Although the filling material has been entirely removed from the dentinal tubules, multiple in vitro studies have demonstrated the remnants of Root Canal Filling Material (RCFM) on the canal walls, provided that the dentinal tubules may be deemed as a reservoir (Tomer et al., 2021). Endodontic retreatment, as opposed to using hand files, using mechanical devices, was faster in eliminating endodontic material, which is in agreement with most of the earlier published investigations. Simultaneously, chloroform can chemically soften the GP, which is intertwined with the canal anatomy (such as lateral canals, isthmuses, and irregularities) that instruments cannot reach (İriboz et al., 2019).

1.2 Problem statement

Many studies have reported the difficulties of completely removing calcium-based sealers during retreatment endodontic procedures (Kim et al., 2015; Uzunoglu et al., 2015). Although various techniques have been proposed during the retreatment procedure, there is still no best way to conclude thus far. Many of the study's findings varied, and the researchers disagreed on the most efficient technique to use during root canal retreatment. Furthermore, numerous investigations have revealed that, regardless of the retreatment technique employed, there is still a sizable amount of debris on the root canal walls (Bernardes et al., 2016). LAI refers to a laser-activated irrigant (Er:YAG, Er, Cr:YSGG) in the root canal system, using low-energy lasers (20 mJ) with ultra-short pulses (50 μs) to generate cavitations and shock waves, known as Photon-Initiated Photoacoustic Streaming (PIPS) (Peters, Ove et al., 2011). Notably, the effectiveness of the LAI in eliminating the smear layer, debris, and biofilm has been thoroughly examined (Ordinola-Zapata et al., 2014). However, only a few studies have been identified that utilize LAI in root canal retreatment, with two specifically assessing the retreatment of epoxy resin-based sealer (Jiang et al., 2016; Keleş et al., 2015), as well as another two studies on bioceramic sealer (Suk et al., 2017; Yang et al., 2021). After using LAI in all groups, the authors observed a substantial decrease in the filling remnants (Jiang et al., 2016; Keleş et al., 2015; Suk et al., 2017; Yang et al., 2021).

1.3 Justification of the Study

Despite the existence of several retreatment techniques, there is still no universally accepted method for the complete and predictable removal of RCFMs. Most existing studies either focus on a single retreatment approach or evaluate limited sealer types, leading to inconsistent and inconclusive findings.

This study is necessary since it investigates a combined retreatment protocol that integrates mechanical instrumentation (Protaper Universal Rotary (PTUR)), chemical dissolution (chloroform), and advanced irrigation (LAI). This combination has not been thoroughly explored, particularly with different sealer types. Thus, by comparing these protocols across AH Plus, MTA Fillapex, and CeraSeal, the study aims to identify a more effective, clinically relevant method that could improve the success rate of retreatment procedures. Therefore, this research fills a critical gap in endodontic literature and supports evidence-based decision-making in clinical practice.

1.4 Research question (RQs)

RQ1. Is apical patency difficult to achieve in retreatment endodontics?

RQ2. Does LAI improve the elimination of the filling material of root canals in retreatment endodontics?

RQ3. What does the internal canal wall look like after the retreatment procedure?

1.5 Hypothesis

Null hypothesis: There is no noticeable difference among the retreatment techniques tested in this study. Alternative hypothesis: The combination of PTUR, chloroform, and the LAI technique may significantly remove the intracanal obturation materials in the endodontic retreatment procedure.

1.6 Objective

1.6.1 General objectives

To assess the effectiveness of mechanical rotary (PTUR) supplemented with chemical solvent (chloroform) and LAI in removing intracanal obturation material in extracted human permanent premolar teeth obturated using various types of sealers during retreatment endodontic.

1.6.2 Specific objectives

i. To investigate the achievement of apical patency following the use of PTUR supplemented with chloroform and LAI in root canal retreatment procedure on tooth obturated, using various types of sealers: AH Plus, MTA Fillapex, and CeraSeal bioceramic sealer.

ii. To assess the residual volume of RCFM through Micro-Computed Tomography (Micro-CT) assessment following root canal retreatment using PTUR supplemented with chloroform and LAI on tooth obturated using various types of sealers: AH Plus, MTA Fillapex, and CeraSeal bioceramic sealer.

iii. To assess the remnant of RCFM on the canal wall of extracted human permanent premolar teeth under a stereomicroscope after retreatment, endodontic procedures using PTUR supplemented with chloroform and LAI on tooth obturated with various types of sealers: AH Plus, MTA Fillapex, and CeraSeal bioceramic sealer.

1.7 Conceptual framework

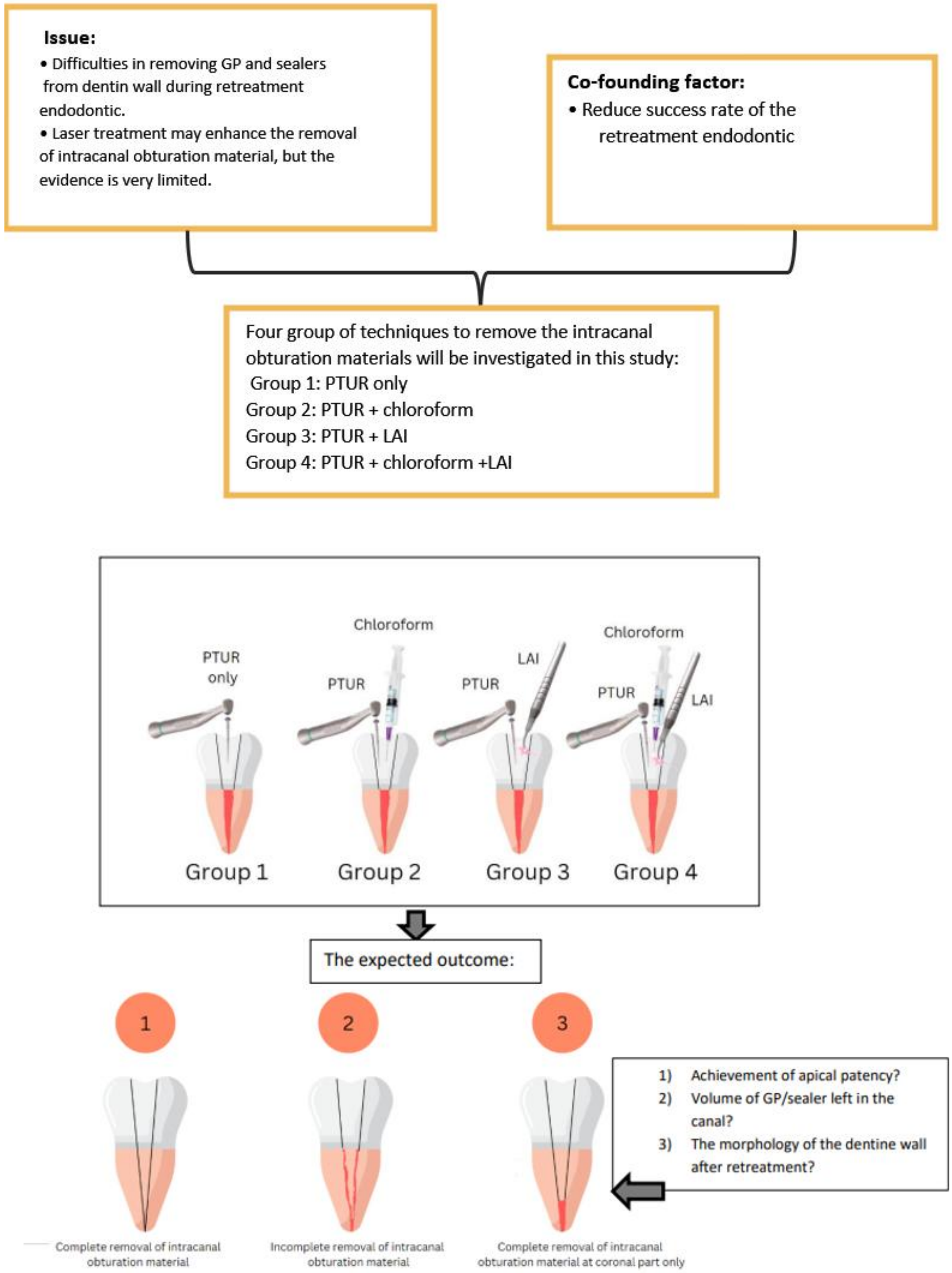

Figure 1.1 Conceptual framework

CHAPTER 2

LITERATURE REVIEW

2.1 Indication of endodontic retreatment

The endodontic retreatment aims to eliminate residual pathogens from the root canal system; however, achieving this goal remains clinically challenging. Although cleaning and shaping are fundamental steps, their effectiveness is limited by the complexity of root canal anatomy. Many published studies emphasize the importance of debridement, yet they often overlook the variations in canal morphology that can compromise outcomes. Research has shown that even single-rooted teeth may possess two or more canals, and these canals can exhibit irregular or unpredictable configurations. Despite the development of advanced mechanical and chemical techniques, none can consistently achieve complete disinfection, especially in canals with severe curvature or narrow diameters. Thus, while the reported success rate of Root Canal Therapy (RCT) is approximately 74%, this figure may overestimate the effectiveness in more anatomically complex cases.

RCT is a reliable and effective therapy. Nevertheless, some patients have post-treatment infections **(Figure 2-1)**. Here, removing the filling material in the retreatment procedure is significantly different from the first RCT treatment. Note that the filling material may contain some necrotic tissue or bacterial material **(Figure 2-1)**, which could result in chronic inflammation and pain. Thus, the initial retreatment stage must ensure the complete removal of all dental materials from the root canal system. Nevertheless, removing Gutta-Percha (GP) and sealant filling materials is complex due to their entrapment within the root canal's irregularities (Gulabivala & Ng, 2023).

Figure 2.1 Root Canal Retreatment procedure ("Root canal retreatment procedure," n.d.)

2.2 Evaluation of Physical Properties and Bioactivity of Common Root Canal Sealers

The physical properties of root canal sealers, such as flow, alkalinity, solubility, and radiopacity, are key factors influencing their clinical effectiveness and long-term durability. However, many comparative studies report these characteristics without critically analyzing their impact on clinical outcomes. A recent study evaluated three commonly used sealers: AH Plus, MTA Fillapex, and CeraSeal. AH Plus had the highest flow, potentially improving its adaptability within the canal system. However, high flow alone does not ensure clinical superiority, especially when other properties may be compromised. CeraSeal exhibited the highest alkalinity after 72 hours, a characteristic linked to antimicrobial potential and bioactivity, but its significantly higher solubility raises concerns about dimensional stability and sealing ability. Although this solubility may suggest bioactivity, it could also result in material degradation and microleakage. The study did not assess these properties under dynamic clinical conditions. Thus, while the results are promising, further well-designed in vitro and in vivo studies are needed to confirm their clinical relevance and long-term performance (Choudhary et al., n.d.).

AH Plus, an epoxy resin-based sealer, has been widely utilized in endodontics due to its excellent sealing ability, dimensional stability, and low solubility (Ashraf et al., 2020).

MTA Fillapex is a calcium silicate-based sealer widely recognized for its biocompatibility and favorable physical properties. In a study comparing MTA Fillapex with the epoxy resin-based AH Plus, MTA Fillapex exhibited significantly lower values for flow, working time, setting time, solubility, and water absorption. This indicates excellent handling characteristics and dimensional stability (Vitti et al., 2013). Additionally, another investigation compared MTA Fillapex with nano-MTA Plus, focusing on sealing ability, adaptability, and antibacterial effect. Although both sealers demonstrated comparable sealing ability and antibacterial activity, MTA Fillapex demonstrated significantly superior adaptability to canal walls compared to nano-MTA Plus. This may enhance its clinical performance during root canal obturation (Fahmy et al., 2024). Together, these findings support MTA Fillapex as a sealer that combines favorable physical behavior with reliable sealing and biological compatibility.

CeraSeal is a bioceramic sealer composed of calcium silicate and has been increasingly proposed as an alternative to conventional epoxy resin-based sealers. Several studies have highlighted its improved penetration into dentinal tubules, which may enhance the bonding interface between the sealer and the canal walls, potentially leading to better sealing and improved treatment outcomes. Its reported biocompatibility is another advantage, as it supports safe interaction with periapical tissues. Additionally, CeraSeal has been found to be adaptable to various obturation techniques, including both cold lateral compaction and warm vertical compaction, offering clinicians greater flexibility during treatment.

However, while in vitro findings suggest favorable sealing ability and reduced microleakage, these results must be interpreted with caution. Most available studies are laboratory-based and may not fully capture the clinical challenges associated with anatomical complexity, intracanal moisture, or long-term performance. Therefore, although CeraSeal presents promising characteristics, more comprehensive in vivo research is required to establish its superiority and predictability under real clinical conditions (Omaia et al., 2023).

2.3 Importance of apical patency in endodontic treatments

Procedure errors such as apical transportation, ledge formation, and perforation may arise when residual debris from both soft and hard dental tissues obstructs the apical region of the root canal. This debris can also harbor microorganisms, contributing to or exacerbating periradicular pathologies. To mitigate these complications, maintaining apical patency has been advocated. The most commonly adopted approach involves the use of a "patency file" during canal instrumentation. This technique employs a fine, flexible K-file that is gently extended slightly beyond the apical foramen without enlarging it, thereby reducing the likelihood of procedural mishaps. Furthermore, preserving apical patency has been associated with improved irrigation dynamics, better tactile feedback, and a lower risk of working length loss, all of which contribute to more predictable endodontic outcomes (Mounce, 2015).

2.4 Techniques used to remove the intracanal obturation materials

Endodontic retreatment involves removing the existing obturation material, which is essential since any remaining germs, as well as necrotic tissue in the original filling, may lead to retreatment failure and potential disease (Tandon et al., 2022).

In the past, research has proven that using a solvent associated with other GP retrieval techniques can help remove GP from the canal wall. However, no technique has been discovered to be completely effective (Das et al., 2017).

Stainless steel hand files, ultrasonic tips, and nickel-titanium rotary instruments are the techniques that have been utilized in removing Root Canal Filling Materials (RCFMs). Removing filling material with rotary instruments is generally effective and safe and has a high success rate (Nguyen et al., 2019) **(Figure 2-2)**.

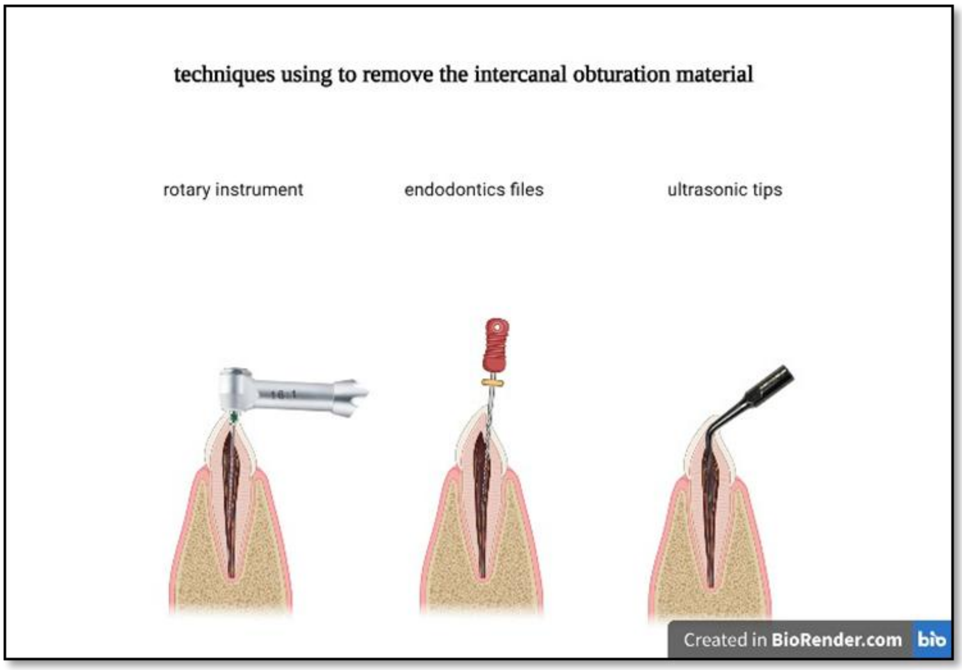

Figure 2.2 Rotary instruments, hand files, and ultrasonic tips

2.5 Challenges and complications in endodontic retreatment

The factors affecting periapical status (whether healing or maintaining periapical health) following root canal treatment can be classified into three main categories:

1. **Patient and Tooth Factors:** These include gender, age, overall immune or health status, tooth anatomy, and pre-operative pulpal and periapical conditions.These variables are often inherent and may influence healing outcomes regardless of treatment quality.

2. **Treatment Factors:** This category encompasses aspects related to the procedure itself, including tooth management and isolation, operator characteristics, access to the canal system, canal shaping and enlargement, control of working length throughout the process, medication, irrigation, culture test outcomes, and obturation. While many of these aspects are controllable by the clinician, the literature shows some inconsistency in determining their individual impact on long-term success.

3. **Postoperative Restorative Factors:** Post-treatment factors such as the quality of the remaining tooth structure, restoration type and timing, marginal adaptation, and occlusal dynamics can affect treatment outcomes. However, evidence varies regarding their individual impact, possibly due to differences in study design, case selection, and outcome assessment across published research. Patient and tooth-related factors generally exert the strongest influence on periapical status, while most treatment factors have moderate effects. Notable exceptions include the apical extent and quality of root filling, as well as the standard of restorative care, which show substantial impact on healing (Gulabivala & Ng, 2023).

2.6 Evaluation of various root canal re-treatment methods

According to the study, Two-Dimensional (2D) radiographs did not accurately depict the canal's true cleanliness or the retreatment efficacy. The effectiveness of the retreatment was evaluated using Three-Dimensional (3D) CBCT, which is more accessible to researchers than Micro-Computed Tomography (Micro-CT) (Pawar et al., 2016).

Radiographs, as well as digital images of vertically sectioned teeth, offer 2D information about a 3D structure, making it impossible to obtain precise measurements of the total canal area. Furthermore, a significant limitation is the subjective assessment of the residual material among different observers. Consequently, numerous recent studies have employed Micro-CT as a non-invasive and reproducible assessment technique, allowing for quantitative measurement of remaining debris with minimal operator bias. In addition, a stereomicroscope can accurately depict the thickness and depth of sealer penetration into dentinal tubules (Kakoura & Pantelidou, 2018).

2.7 Efficacy of rotary instruments in endodontic retreatment

The primary goal of retreatment is to clean and reshape the root canal by eliminating the existing root-filling material. Rotary instruments or hand instruments can be used to accomplish this.

The Protaper Universal Retreatment System consists of instruments designed for shaping, finishing, and retreatment, specifically engineered to remove filling materials from root canals. These instruments have varying tapers and tip diameters: D1, D2, and D3 measure 30, 25, and 20 in diameter, respectively. Their lengths are 16 mm for D1, 18 mm for D2, and 22 mm for D3. All instruments feature a convex triangular cross-section, with D1 having an active working tip to assist in penetrating filling materials (Tomer et al., 2021).

The Protaper retreatment files were used following the manufacturer's instructions, employing a crown-down technique with a brushing motion at a constant speed of 500 rpm. D1 (size 30; 9% taper) was applied to the coronal third, D2 (size 25; 8% taper) to the middle third, and D3 (size 20; 7% taper) to the apical third (Das et al., 2017). The effectiveness of utilizing the Protaper Universal Rotary (PTUR) for removing GP in the canal has been demonstrated in numerous prior studies (Al-Dahman & Al-Omari, 2021). However, there is not a single study that successfully removes the material completely.

2.8 Gutta-Percha solvent in endodontic retreatment

Most of the removal techniques for GP involve hand files, Gates Glidden drills, or rotary files, with or without solvents. Historically, eucalyptol, turpentine oil, and chloroform were commonly used. Chloroform has often been considered the gold standard due to its ability to dissolve GP rapidly. It was later found to be toxic and possibly carcinogenic. In 1976, the US FDA restricted its use in medications and cosmetics based on safety concerns. Although alternatives have been studied, none match its effectiveness (Maria et al., 2021). Moreover, it is proven to be an advantage when mechanical techniques fail to retrieve GP (Jain et al., 2015), and it may also result in shorter retreatment periods (Parvin et al., 2022). Still, its clinical use remains controversial, reflecting the trade-off between effectiveness and safety in endodontic retreatment.

2.9 Laser in dentistry

Light Amplification by Stimulated Emission of Radiation is the abbreviation for the word (Laser). The care and well-being of patients are greatly impacted by laser contributions to medicine and dentistry. Based on the active medium that is stimulated, dental lasers are categorized. The active media can be a gas, liquid, solid, or semiconductor. Excitation mechanisms that inject energy into the active medium through one or more fundamental optical, electrical, or chemical techniques may excite the active medium (Fenelon et al., 2022). Additionally, they can also be classified according to their wavelengths and the risks linked to laser application or based on how effectively hard and soft tissue lasers can be utilized on various types of tissue (Hedge et al., 2018). Despite these established classifications, clinical outcomes may vary depending on the procedure and laser type used.

2.10 Lasers technology for activated irrigation in endodontics

Weichman and Johnson were the first to introduce a laser in endodontic procedures in 1971. They utilized a high-powered Carbon Dioxide (CO_2) laser to seal the apical foramen in vitro. Since then, numerous studies have been published on the use of lasers in endodontics. However, the advent of innovative delivery technologies, including flexible and thin fibers alongside specialized endodontic tips, during the late 1990s marked the inception of practical laser use in endodontics. Currently, laser technology can be utilized in various endodontic procedures, including cleaning and disinfecting the root canal system, pulp capping or pulpotomy, obturation, endodontic retreatment, and apical surgery (Jurič & Anić, 2014).

The recommended laser wavelengths for cleaning and disinfecting the root canal system include erbium: yttrium aluminum garnet (Er:YAG) at 2,940 nm, erbium, chromium: yttrium scandium gallium garnet (Er,Cr:YSGG) at 2,780 nm, neodymium: yttrium aluminum garnet (Nd:YAG) at 1,064 nm, and diode lasers within the range of 635 to 980 nm. These specific wavelengths are selected based on their absorption by biological substances and chromophores present in root canals. This includes water, apatite minerals, and various pigmented substances like microorganisms. Notably, the interaction of these lasers' wavelengths with these materials determines their physical effects during root canal treatments (Jurič & Anić, 2014).

The Laser-Activated Irrigation (LAI) mechanism depends on cavitation bubble generation caused by the irrigant absorbing the laser energy. Here, LAI, having a pulsed Er:YAG laser, is an additional means of activating irrigants. Pulsed erbium lasers create optical cavitation within the irrigant, causing vapor bubbles to form and collapse near the fiber tip. In contrast, smaller secondary bubbles penetrate deeper into the canal and experience acoustic streaming. Note that these processes happen at a very high speed (within the microsecond region), causing the liquid to move rapidly throughout the canal. This confirms that LAI could 99.5% lower the bacterial load in infected root canals, and the laboratory setting cannot detect any bacteria after LAI (Liapis et al., 2021).

Multiple laser systems can access areas inaccessible via traditional means (such as bacteria residing deeply within fins, isthmuses, lateral canals, and dentinal tubules). Apart from that, among the recent advancements in laser technology, the diode laser is compelling due to its compact delivery tip sizes, cost-effectiveness, adaptable power output options, and user-friendly operational modes like continuous wave, pulsed power, and gated mode (Mathew et al., 2014). Several studies have utilized LAI to assist in removing RCMFs. Nonetheless, none have succeeded in completely eliminating the material adhering to the dentinal wall (Keleş et al., 2015; Suk et al., 2017; Yang et al., 2021).

2.11 Micro-CT technology in endodontics

Technological advancements during the 20th century facilitated the successful use of different techniques to illustrate the structure of human teeth. These techniques encompass digital radiography, resin injection, radiopaque contrast agents in radiographic methods, 3D wax modeling, and scanning electron microscopy (Perrini & Versiani, 2019). These techniques have revealed considerable potential in endodontic studies, yielding results that have profoundly influenced dental education and clinical practices (Versiani et al., 2013) devised a technique for computer-aided imaging in endodontic research, which included capturing six radiographs per tooth from specified angles and introducing a contrast medium into the root canals of extracted teeth. By combining these six images, a 3D model of the canals was generated. The root canal volumes and diameters were subsequently measured with computerized video image processing software, utilizing the integrated data (Blasković-Subat et al., 1995). Bjørndal et al. (1999) pioneered the use of Micro-CT technology for conducting quantitative analysis of the anatomy of root canals (Bjørndal et al., 1999). This advanced, noninvasive, and nondestructive technology, through the reconstruction of digital cross-sections of teeth into 3D models, facilitates the detailed examination of the root canal system. Moreover, examining the system's impact on different dental treatment and retreatment approaches is crucial. In addition, the generated 3D models enable the creation of digital versions of the specimens, allowing for measurements and modifications to visualize their internal as well as external structures. In essence, Micro-CT is currently deemed the most precise and vital tool for examining root canal anatomy (Peters et al., 2000; Versiani et al., 2012).

However, its high cost, limited accessibility in clinical settings, and time-intensive data processing continue to pose challenges to its routine use in everyday dental practice.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Research design

This is a cross-sectional laboratory study involving extracted permanent human premolar teeth, whereby the pulp in the canal was removed and filled up with Gutta-Percha (GP)/sealer. Radiographs were taken to ensure proper obturation, and the teeth underwent GP/sealer removal utilising the Protaper Universal Rotary Instrument (PTUR) and supplemented with a combination of chloroform and Laser-Activated Irrigation (LAI).

A cross-sectional laboratory design was selected to accurately compare retreatment protocols under standardized conditions. Using extracted human premolars enabled precise evaluation of residual filling material and apical patency through Micro-Computed Tomography (Micro-CT) and stereomicroscopy, minimizing variability and enhancing the reliability of the findings. This design enabled an accurate assessment of treatment efficacy that would be difficult to achieve in a clinical setting.

3.2 Study area

The study was performed at the dental clinic and Biomaterial lab, CRL 5, Advanced Dental and Medical Institute (AMDI), USM, Bertam Campus.

3.3 Study population

1. Reference population: Patients attending the AMDI dental clinic.
2. Sample population: Patients who attended the AMDI dental clinic requiring tooth extraction as part of orthodontic treatment, or periodontal disease.

3.4 The subject criteria

Inclusion criteria:

1. Subjects aged more than 12 years old have premolar teeth that are indicated for extraction due to periodontal disease or for orthodontic treatment.
2. Healthy subjects without a bleeding problem that prevents tooth extraction.
3. Healthy subjects who did not take any medication that predisposed to bleeding tendency, e.g., aspirin or warfarin.
4. Subjects who do not have any hearing problems so that they can understand the procedures involved and the study protocol.

Exclusion criteria:

1. Subjects with bleeding problems that prevent tooth extraction.
2. Subjects with an underlying medical problem such as hypertension or taking medication that predisposes to bleeding tendency, e.g., aspirin or warfarin.
3. Subjects who have hearing problems or physical disability that may interfere with the procedure involved and the study protocol.

3.5 Tooth criteria

We follow the recommendation of sample criteria from a similar study by Oltra et al. (2017) and Yang et al. (2021). The inclusion as well as exclusion criteria with respect to the tooth sample are as follows:

Inclusion criteria:

Due to orthodontic treatment or periodontal disease, freshly extracted human single-rooted permanent premolars will be collected from subjects undergoing tooth extractions at the AMDI dental clinic.

Exclusion criteria:

1. Multi-rooted/multi-canal permanent premolar teeth will be excluded from this study.
2. Premolar teeth with past root canal treatment, open apices, and root resorption will be excluded from this study.
3. Premolar teeth with root curvature of more than 20 (degree of curvature measured using DBSWIN, imaging software, USA).
4. The root stump of a permanent premolar tooth will be excluded from this study.

3.6 Sample size estimation

For specific objective i: To investigate the achievement of apical patency after root canal retreatment using PTUR instruments supplemented with chloroform and LAI.

The sample size for specific objective one was calculated using G*power software version 3.1.9.4. The selected test was the t-test, specifically the Wilcoxon-Mann-Whitney test (two groups), based on proportions p1 = 0.6 and p2 = 0.9 (Nouri et al., 2021), a margin of error of 0.05, and a power of 0.8. Meanwhile, the estimated sample size for objective one is 79 subjects, accounting for a 10% dropout rate (approximately seven subjects).

Figure 3.1 Sample size calculation

For specific objective ii: To assess the residual quantity of root canal filling through Micro-CT evaluation following the retreatment of the root canal utilizing PTUR and enhanced with chloroform and LAI.

The sample size for specific objective two was calculated using G*power software version 3.1.9.4. The selected test was the t-test, specifically the Wilcoxon-Mann-Whitney test (two groups), based on an effect size of 0.8 (Yang et al., 2021), a margin of error of 0.05, as well as a power of 0.8. Meanwhile, the estimated sample size is 59 subjects, including a 10% dropout rate (approximately five subjects).

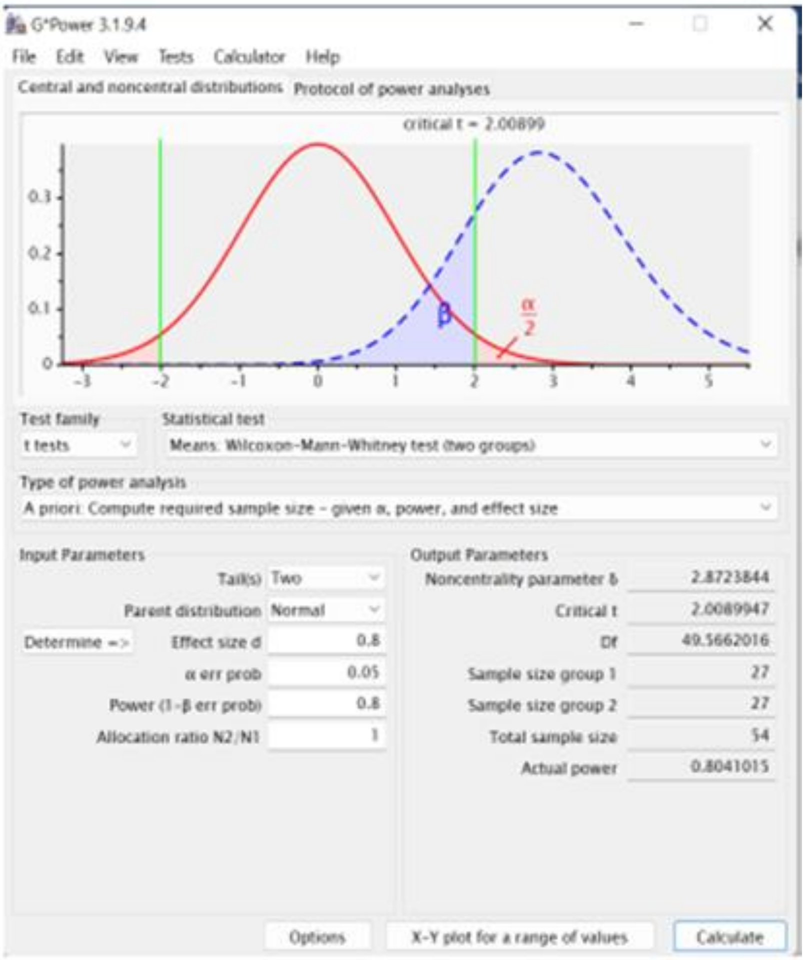

Figure 3.2 Sample size calculation

For specific objective iii: To analyze the remnants of endodontic filling material on the canal wall of extracted human permanent premolar teeth under a stereomicroscope after retreatment endodontic using PTUR instruments, chloroform, and LAI.

The sample size for specific objective three was calculated utilising G*power software version 3.1.9.4.

The selected test was a t-test for the difference from a constant (one-sample case), based on an effect size of 0.5 (Nguyen et al., 2019), a margin of error of 0.05, and a power of 0.8. The estimated sample size for objective three is 37 subjects, including a 10% dropout rate (approximately three subjects).

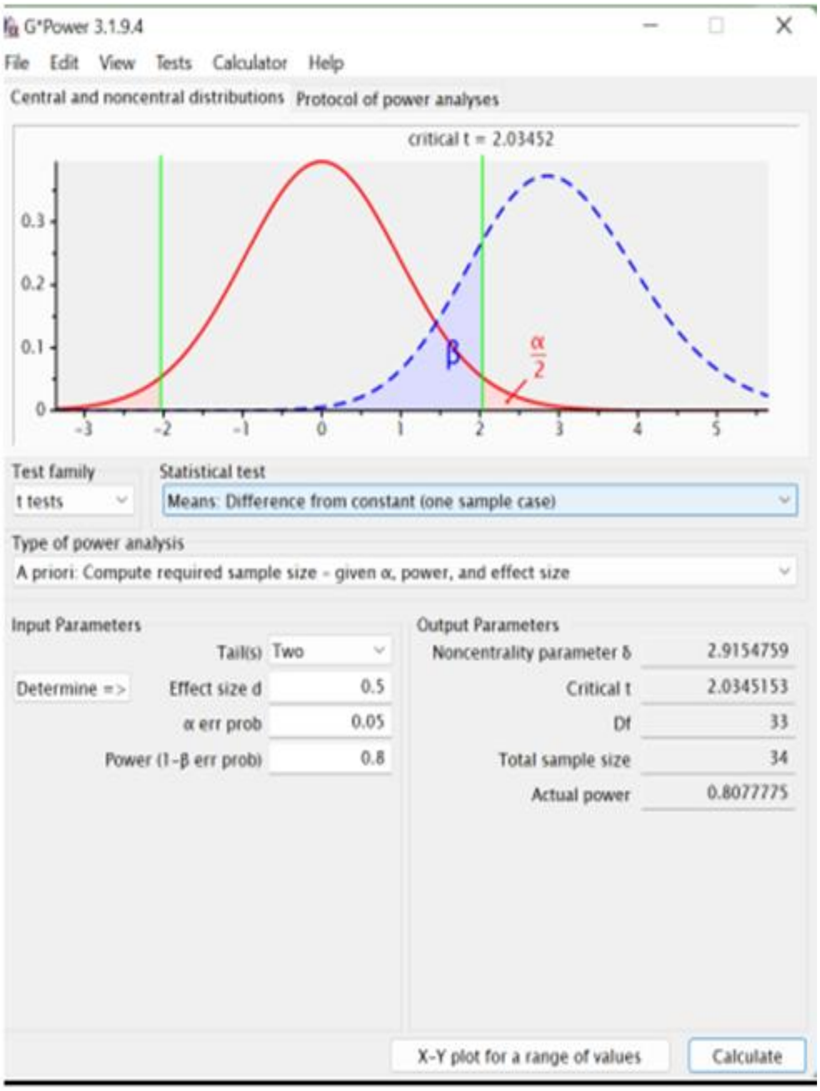

Figure 3.3 Sample size calculation

According to the sample size calculation from all objectives, this study selected the sample size from the specific objective three, around 37 teeth were required for this study. Since we only have four groups (Group 1: PTUR, Group 2: PTUR + chloroform, Group 3: PTUR + LAI as well as Group 4: PTUR + chloroform + LAI) with three different sealers will be used (refer to Table 3-1), it is decided to only use nine teeth per group with three replicates for each treatment as provided in Table 3.1 below. Thus, the total extracted tooth samples required was 36 teeth.

Conducting a separate power analysis for each objective ensures that the study has sufficient statistical power to produce reliable results, even when using the same sample. In addition, this approach follows standard research practices, ensuring the results are dependable and can be applied to relevant populations.

Table 3.1 Sample size calculation

GROUP	**TREATMENT**	**SUBGROUP A GP/AH Plus**	**SUBGROUP B GP/MTA Fillapex**	**SUBGROUP C GP/ CeraSeal**
1	Protaper Universal Rotary Instrument (PTUR)	3 samples	3 samples	3 samples
2	Protaper Universal Rotary Instrument (PTUR) + chloroform	3 samples	3 samples	3 samples
3	Protaper Universal Rotary Instrument (PTUR) + Laser-Activated Irrigation (LAI)	3 samples	3 samples	3 samples
4	Protaper Universal Rotary Instrument (PTUR) + chloroform + Laser-Activated Irrigation (LAI)	3 samples	3 samples	3 samples