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SELF-ADJUSTING FILE (SAF) COMPARED TO NEWLY DESIGNED ROTARY AND RECIPROCATING FILES: COMPOSITION AND MICROSTRUCTURE, MICROBIAL BIOFILM REMOVAL CAPABILITY AND SHAPING EFFECTIVENESS.

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Preface

The selection of the correct shaping technique in clinical endodontics has always been one of the greatest challenges. Since the introduction of Ni-Ti files the choice has been greater and the options bigger. Even though research is mainly focused on durability and safety of the instruments, the most important task, their effectiveness is not that clear and so intensively tested. Obviously the biggest problem in endodontics is the endodontic space which is variable and most of the times unique. Lately there has been a greater awareness in the way Ni-Ti files work in the endodontic space and how they manage to clean different anatomic sites such long oval canals. This is the reason of this doctoral thesis, testing and understanding better different instruments with different designs and different mentalities and their effectiveness in such difficult anatomic site.

Undoubtful such task required the support and guidance of many colleagues to whom I would like to express my sincere and absolute gratitude. Prof. Cerutti who provided me the opportunity to work with him in his facilities in University of Brescia, and his guidance as a reviewer of my doctoral thesis. Prof. Krejci who provided me access to his laboratory in University of Geneva and also for his guidance during microscopy studies. All the three members of the supporting committee, Prof. Panopoulos, Prof. Zinelis and Prof. Cerutti for the correct supervision and review of this doctoral thesis.

Contents

CHAPTER 1- GENERAL PART

1. Introduction
2. Cleaning the root canal system

- Microbial causes of pulpitis and apical periodontitis
- Overview of ways of infection control
- Purpose of endodontic treatment
- Instrumentation of the root canal
- Results of manual instrumentation on root canal bacteria
- Rotary instrumentation

3. The NiTi Instruments

- Metallurgy of NiTi alloys
- Ability to shape the root canal
- Ability to clean the root canal
- Working time
- Instrument fracture
- Impact of specific instrument design characteristics
- The Self Adjusting File
- The WaveOne File
- The BT Race files

4. Methods of study of microbial flora of root canal system

- Microscope
- Microbial cultivation
- Immunological methods
- Molecular genetic methods

5. Root canal cleanliness - Smear layer

- Clinical implications
- Influencing factors
- Irrigants, chelating agents
- Irrigation devices and techniques

CHAPTER 2- EXPERIMENTAL PART

6. Materials and Methods:
7. Results
8. Discussion
9. Conclusions

CHAPTER .1

1.Introduction

The goal of endodontic therapy is the removal of all tissue, (either vital or necrotic), microorganisms, and microbial byproducts from the root canal system but effective debridement of all areas of the root canal system may sometimes be extremely difficult. The intricate nature of canal anatomy, consisting of root irregularities, isthmuses, and anastomoses, can lead to residual tissue and debris after chemo-mechanical instrumentation.[1-9] Removal of debris and microorganisms is further facilitated by the flushing action of irrigation solution.[1-8, 10-12]

Research conducted in vivo has failed to demonstrate total elimination of the microbial population after traditional instrumentation and irrigation procedures in infected canals.[13-19] Dalton et al.[13] showed that only 28 percent of all canals could be rendered bacteria-free after rotary instrumentation and irrigation with sterile saline. After Nickel-Titanium (Ni-Ti) rotary instrumentation and irrigation with 1.25-percent sodium hypochlorite, Shuping et al.[15] were only able to achieve negative cultures in 62 percent of teeth. These percentages can be attributed to the complexity of canal anatomy.

Therefore, improving the antibacterial efficacy of our current endodontic instrumentation techniques and procedures is essential.

With the advent of Ni-Ti technology, the shaping of the root canal system can be accomplished easier, faster and more predictable than with the stainless-steel (SS) files. Iatrogenic errors during canal debridement, such as ledges, transportations or loss of patency are less probable with the use of Ni-Ti rotary files than with conventional techniques.

Nevertheless, Ni-Ti files are not devoid of disadvantages. Fracture is common and it mainly unexpected[20-21]. But the main problem which causes bigger concerns is their lack of ability to remove dentine from the entire surface of the root canal walls. Several studies have shown that hand and rotary instrumentation techniques tend to produce round preparations [22-23], leaving areas of the canal wall uninstrumented, especially in oval canals [24-27]. These uninstrumented areas can be as extended as to 45-79% of the total circumference of the root canal wall mostly in long oval canals[28-30] . Inevitably, these regions cannot be mechanically cleaned and since sodium hypochlorite is mostly transported to the anatomy through shaping files, the disinfection of these territories is expected to be more difficult and problematic. A possible solution to the problem of effective debridement and disinfection of the root canal system is through the use of anatomically designed Ni-Ti files which would be able to adapt in the treating anatomy. The Self Adjusting File (SAF) which was introduced in the later years, has been the first anatomical file, claiming that it adapts in the anatomy and cleans better the endodontic space. There are different opinions in the literature about

the effectiveness of this file to clean successfully the root canal space in comparison to traditional round shaped files. Some claim that the SAF is more effective (31-33) and mostly effective in oval canals and others that still round files clean better.

The purpose of this study is to test the SAF in comparison to newly introduced round files about their ability to clean and disinfect the endodontic space in long oval canals. It is important to clarify the effectiveness of the SAF file and decide wether an anatomically designed non round file would be more effective in cleaning the root canal space which is highly irregular and not round.

2. Cleaning the root canal system

Microbial causes of pulpitis and apical periodontitis

Pulpal inflammation or pulpitis (ie irritation or even necrosis of the pulp) may be caused by various factors, both chemical and physical. The most common cause, however is the inflow of bacteria and/or their products in the pulp through a deep caries lesion or a leaking fillin. In this case, the inflammatory reaction in the pulp starts long before bacteria invade the pulp tissue. The first stage of an inflammatory reaction is caused by bacterial antigens interacting with the immune system[34-36.] At this stage and to the extent the carious lesion has not yet entered the pulp, the pulpal inflammation is likely to be reversed. If, however, the carious lesion reaches the pulp and breach the hard tissue barrier bacteria can invade the pulp. Nevertheless even in this case the infection may still be rather superficial and the largest part of the pulp tissue remains vital and bacteria free.
. Apical periodontitis is an inflammatory process in the periradicular tissues caused by microorganisms in the necrotic root canal [37-39]. Therefore the elimination of the microorganisms in the is essential for the treatment of apical periodontitis. Various studies have demonstrated that the prognosis of apical periodontitis following root canal treatment is poorer if viable micro-organisms exist in the canal at the time of the root filling [40-42]. However, other studies have failed to indicate meaningful differences in healing between teeth filled after obtaining positive or negative cultures from the root canal[43], and between treatments finished in one or two appointments[43,44]. Nevertheless, there is a common agreement that successful elimination of the causative agents in the root canal system is the key to health[45].

Overview of ways of infection control

Elimination of endodontic infection cannot be affected through the same ways as in other parts of the human body. Whereas in most parts of the human body, elimination of possible infections is carried out solely by the host defense system, sometimes enhanced by a systemic antibiotic therapy, in endodontic infections a

combination of several host and treatment factors must be used.
With the most necessary of such factors being the host defense system, instrumentation and irrigation are the most important ways to treat it. The systemic antibiotic therapy only occasionally and under certain circumstances can be helpful. Also locally used intracanal medicaments between appointments, root canal filling, and coronal restoration are important factors elimination and maintenance of the good health of the endodontic space [46].

Purpose of endodontic treatment

In the vast majority of teeth in need of root canal treatment, the purpose is to prevent or, if possible, treat apical periodontitis[47], or more specifically, to prevent or treat a microbial infection in the root canal system. It goes without saying clear that in some cases, (.e.g resorptions and endodontic complications) there can be various intermediate goals, but even then the final success depends on whether the infection is controlled successfully.

Instrumentation of the root canal

The goal of instrumentation and irrigation is from a technical perspective the removal of all necrotic and vital organic tissue along with some hard tissue from the root canal system and the shaping of the canal system in a way that permits easy debridement and controlloed placement of locally used medicaments yielding a permanent root filling of high quality. From a microbiological perspective, the goal of instrumentation and irrigation is the removal and/or elimination of all microorganisms existing in the root canal system and neutralization of any antigenic/ biological potential of the microbial components remaining in the canal.

If the complete eradication of root canal microorganisms could be achieved at the first appointment, most treatments could be finished in one visit, provided that there would be enough time to do so. In cases where this cannot be achieved, instrumentation and irrigation aims to create optimal conditions for the placement of an antibacterial interappointment dressing to enhance disinfection of the canal.

Results of manual instrumentation on root canal bacteria

Mechanical instrumentation is the main method for bacterial reduction and/or elimination in the infected root canal. In a paper written by Bystro¨m & Sundqvist[48] the reduction of bacterial counts cultured from infected canals, was measured by instrumentation with hand SS instruments under irrigation with physiological saline solution. Fifteen root canals with necrotic pulps and periapical lesions were instrumented at five sequential appointments. The canals were left empty with no intraradicular medicament. A substantial

reduction in bacterial population was evidenced, (100–1000-fold), but bacteriafree root canals were limited to seven out of 15. Also Ørstavik et al.[49] reported the limited efficiency of manual mechanical preparation. In a comparison performed by Cvek et al[50] of the antibacterial effect of different irrigation solutions in permanent necrotic maxillary incisors with immature apices with those with mature roots. Three groups (34, 46, and 28 teeth) were compared, where mechanical cleansing was accompanied by irrigation with sterile saline and 0.5% or 5.0% sodium hypochlorite (NaOCl) solutions respectively. The antibacterial effect of sterile saline was very low and limited to the teeth with mature root. Contrary NaOCl was more effective in decreasing bacterial counts as compared with saline irrigation. It is worthwhile to be mentionted that no statistical difference was found in the antibacterial effect between 0.5% and 5.0% NaOCl solutions.

Both techniques obtained similar reduction in bacterial population while enlarging progressively the root canl with only 28% of bacteria free teeth. A poor antibacterial effect of instrumentation combined with saline irrigation was also proven by Siqueira et al. [51] in a group of teeth enlarged manually with Ni-Ti flex K-files to apical size #40. Interestinlgy, increasing the size of apical preparation from #30 to #40 resulted in a significant reduction in the numbers of cultivable bacteria. In a study by Pataky et al.[52], the antimicrobial efficacy of various root canal hand preparation techniques and instruments was compared in 40 human first maxillary premolars. Teeth after sterilization and infection with E. faecalis for 24 h., were instrumented using saline irrigation. Before and after the root canal preparation samples were taken for culture. All types of preparation obtained considerable reduction in bacterial counts; but none of the abive reached sterilization [52]

The above studies taken together show that mechanical preparation using hand instruments and irrigation with saline cannot predictably eliminate the bacteria existing in the infected root canals. Taking into consideration the current knowledge on the frequency of invasion of bacteria into dentinal tubules and the lateral canals from the main root canal, the complicated anatomy of the root canal system, the physical restrictions of metal instruments (SS or Ni-Ti), and the minimal antibacterial activity of saline, it would be quite surprising if these studies showed high numbers of sterile root canals. Furthermore as regards the limitations of sampling from the root canal, it is likely that the true frequency of canals with viable microorganisms is higher than reported. Therefore, the focus of interest in relation to the antibacterial efficiency of instrumentation and irrigation has been concentrated on the use of irrigating solutions with strong anti-bacterial activity as an essential supplement to mechanical preparation.

Rotary instrumentation

Following the development of rotary Ni-Ti instruments for root canal preparation taken place the last ten years, there has been an increasing shift

from manual to rotary, engine- driven preparation. Despite the fact that manual instrumentation is still the most popular approach for preparing the root canals, many specialists and a constantly growing number of general practitioners are using rotary NiTi instruments.

Besides the fact that the main incentive for a practitioner to start using rotary NiTi instruments have been in most cases, the desire to complete the canal preparation in a shorter time, rotary preparation with the use of such instruments could offer other advantages including the quality of the apical preparation. Nevertheless, not in all cases, where the various aspects of preparation have been compared[53] the rotary instruments have been found to be superior to hand instruments. Ahlquist et al.[54] and Scha¨fer & Lohmann[55] demonstrated that hand instrumentation preparation resulted to cleaner canals compared to rotary instruments. On the other hand, however, rotary NiTi instruments seem to maintain the original canal curvature better than hand SS instruments, especially in the apical part of the root canal[56]. Sonntag et al.[57] compared the quality of root canal shaping performed by dental students using both manual and rotary NiTi files. The preparations were performed in standardized simulated canals. Zips and elbows occurred fairly less frequently with rotary than with manual preparation. Moreover, the correct preparation length was accomplished in significantly more cases with rotary instruments. Instrument fractures were documented in 1.3% of the cases with both rotary and manual preparation. The time required for the preparation was also significantly longer with the manual preparation than with the rotary one [57]. Although it is difficult to be proven through research, it is fairly clear that a symmetrical preparation following the original path of the root canal facilitates the elimination of intracanal infection [58,59]). On the other hand, another study, [Deplazes et al. [53]] showed no significant differences in displacement of canal centers or between the mean cross-sectional areas of the instrumented root canals between the Lightspeed and NiTi and K-file groups. Dalton et al. [60] in their study compared the capability of SS K-type files and NiTi rotary instruments to remove bacteria from infected root canals using saline as an irrigating solution. The canals were sampled for microbes before, during, and after instrumentation. In the above-mentioned study, almost 1/3 of the canals were rendered bacteria free, and no significant difference was identified between canals instrumented with hand files and rotary instruments. It is interesting the fact that with larger apical preparation, a significant decrease in bacterial counts was accomplished. Coldero et al [61] studied the effect of the size of apical preparation on the number of residual bacteria in the root canal. This study reached the conclusion that additional apical enlargement to size #35 did not reduce further the number of surviving bacteria. However, in light of our knowledge about the natural size of the apical root canal, there is the possibility, the sizes #25/#35 to be too small to show differences in bacterial elimination. In fact, the study performed by Rollison et al.[62] demonstrated that apical enlargement from size #35 to size #50

resulted in a greater reduction of bacteria in the root canal. This study also showed the difficulty in achieving a sterile root canal. Contradictory to these results, Card et al.[63] showed sterility in a majority of root canals instrumented by rotary NiTi instruments using large apical sizes and irrigation with 1% NaOCl. The instrumentation and bacterial sampling were executed in two stages: the first instrumentation utilized 1% NaOCl and 0.04 taper ProFile rotary files. Canals in cuspids and bicuspids were instrumented to size #8 and the molar canals to size #7. After bacteriological sampling, the canals were instrumented in the apical third by LightSpeed files and 1% NaOCl irrigation and were sampled again. Molar canals were instrumented to size #60 and cuspid/bicuspid to size #80. There was no growth detected from any of the cuspid/bicuspid canals (11 teeth), and 81.5% of the molar canals after the first instrumentation. In the molars, the proportion of bacteria-free canals increased to 89% after the second instrumentation. The interesting fact was that in molars with no visible anastomoses between their roots reached sterilisation in 93% after the first instrumentation. The undoubtedly higher grade of difficulty in eliminating bacteria from molar canals than from premolars and canines [63] may be partially due to a greater variation in morphology in molar canals than in other teeth: (i) in many cases molar roots have two (or even more) canals in one root, which canals often communicate with each other through a complex network of anastomoses, (ii) the cross-section of most molar canals is oval, with long and narrow extensions at one end of the canal, and (iii) most molar canals are curved, some of them severely, which makes their instrumentation a challenge. Peters et al.[64] studied the rotary preparation of root canals of maxillary first molars through the comparison of the effects of four techniques for preparation on canal volume and surface area using three-dimensionally reconstructed root canals in extracted teeth. Micro CT data were used to describe morphometric parameters in relation to the above preparation techniques. Specimens were scanned prior to and following the preparation of the canals using K-type hand files and three rotary instruments[64]. The canals that had been prepared were significantly more rounded and with greater diameters. But were also straighter than unprepared specimens, and with all instrumentation techniques at least 35% of the surface area of the dentine surface was left untouched. While there were great differences between the three canal types reviewed, very few differences were identified among the four types of instruments.

2.The Ni-Ti Instruments

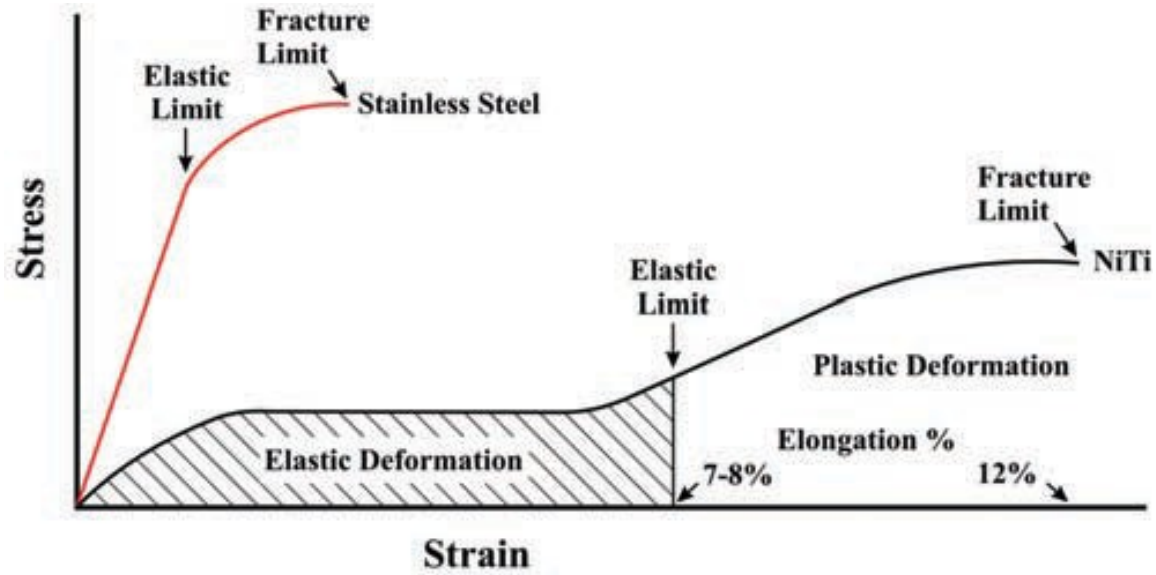
After the introduction of rotary NiTi instruments back in 1988[65,] there has been a growing shift from manual to rotary engine-driven preparation. A survey of Australian dentists[66] showed that even though hand instrumentation was still the most popular method for root canals preparation, the majority of endodontists

(64%) and a growing number of general practitioners were using rotary NiTi instruments. The numbers of general practitioner and endodontists who have adopted this new technology has exceeded the critical level required (10–20 %) in order to ensure that the rate of rotary NiTi adoption is self-sustaining.[66,67]

Metallurgy of NiTi alloys

The NiTi alloy which is used to manufacture endodontic instruments consists of approximately 56 % (wt) nickel and 44 % (wt) titanium and is broadly known as 55-

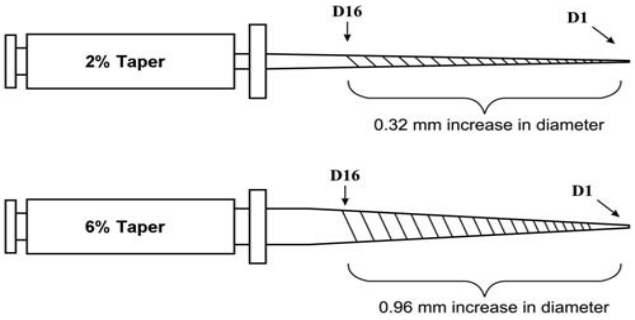
Nitinol.[68] The superelasticity of NiTi instruments is due to a stress-induced phase transformation in the crystalline structure of the material. The austensitic phase is transformed into the martensitic phase on stressing, and when the NiTi instrument is in this form can be bent by using light force only[69] After the release of stresses, the metal returns to the austensitic phase and the file regains its original shape. The superelasticity of NiTi allows deformation of as much as 8 % strain to be fully recoverable, **compared to** a maximum of less than 1 % with using alloys such as stainless steel[68].



= stress at which fracture occurs; elongation % refers to the deformation that results from application of a tensile stress, calculated as (change in length/ original length) x 100%.

The improved flexibility and unique features of NiTi alloy grant an advantage when preparing curved canals and has made possible the creation of engineer instruments with greater tapers (4-12 per cent), which allow better control of

root canal shape [69]. The outcome is a predictably machined tapered preparation that facilitates both the cleaning of the canal and its obturation.



The diameter of the 2% taper instrument increases 0.02mm for every millimetre of length from D1 to D16 on ISO or standard taper. The diameter of the greater tapered instrument increases 0.06mm (6% taper) for every millimetre of length from D1 to D16.

Ability to shape the root canal

NiTi rotary files have become a backbone in clinical endodontics due to their ability to shape root canals with less procedural difficulties. Many studies on extracted human teeth have demonstrated that rotary NiTi instruments maintain the original canal curvature better than stainless steel hand instruments, especially in the apical region of the root canal[70-76].Esposito and Cunningham[77] showed that NiTi files has been significantly more efficient than stainless steel hand files in maintaining the original canal path in cases when the apical preparation was enlarged beyond ISO size 30.Generally, in vitro studies indicate that NiTi instruments produce fairly less straightening and better centred preparations than SS hand files, this way reducing the potential for iatrogenic errors.

Although the use of rotary NiTi instrumentation offers significant shaping advantages there is very limited direct evidence from clinical follow-up studies on the infuence of improved canal shapes on healing results. Petiette et al.[78] prepared 40 teeth with both NiTi hand files or SS K-files and noticed that NiTi instrumentation was better at maintaining the original canal shape. At the recall of the two groups a year after completion of the endodontic treatment, the authors identified a rather higher healing rate (assessed by change in densitometric ratio) for teeth which had been prepared with NiTi files thus concluding that instrumentation with NiTi files led to a better prognosis compared to SS files due to the better maintenance of original canal shape and access to apical anatomy. Nevertheless there is no evidence for rotary instrumentation with NiTi.

Ability to clean the root canal

Studies on the cleaning ability of endodontic instruments have reviewed their ability to remove debris from root canals. Tan and Messer[79] noticed that instrumentation to larger file sizes using rotary NiTi instruments resulted to significantly cleaner canals in the apical 3mm compared to hand instrumentation. However, none of the above techniques was compeletely effective in cleaning the apical canal space. After instrumenting curved root canals of extracted human teeth with either rotary NiTi or SS hand files, Schäfer et al.[76,78] found uninstrumented areas with remaining debris in all areas of the canals regardless of the preparation technique used. In addition it was identified that cleaness decreased from the coronal to the apical part of the root canal. Peters et al[80] used micro-CT data to analyse preparation of root canals of maxillary first molars after instrumentation using K-type hand files and three rotary NiTi file systems and identified that all instrumentation techniques left 35 % or more of the canal's surface of the touth untouched, with little differences between the four instrument types. These findings point out the limited ability of endodontic instruments to clean effectively the root canal and emphasize the significance of antibacterial irrigation for the enhanced disinfection of root canals.

Working time

Although some comparative studies have indicated that rotary NiTi preparations required shorter working times compared with manual instrumentation,[75,77,79] other studies have not shown any difference.[76,78] However working time depends more on the operator factors and preparation technique rather than the instruments used.

Instrument fracture

Each endodontic instrument is likely to break within the canal in case of improper application. Although it is a common understanding among dental practitioners that rotary NiTi instruments break more frequently than SS hand files, current clinical evidence does not support such view[81]. A research in the relevant literature shows that the mean clinical fracture frequency of rotary Ni-Ti instruments is approximately 1.0 % with a range of 0.4–3.7 %[81,] while the mean prevalence of retained fractured endodontic hand instruments (mainly SS files) is approximately 1.6 % with a range of 0.7–7.4 %[81.]

Safe clinical usage of Ni-Ti instruments requires an understanding of basic fracture mechanisms and their relationhip with canal anatomy. Sattapan et al.[79] found two types, of fracture for rotary Ni-Ti instruments namely torsional fracture and flexural fracture. Torsional fracture occurs when the tip or any part of the instrument locks into the canal while rotary motion continues. The elastic limit of the metal is exceeded, and the instrument shows plastic deformation

(unwinding, reverse winding) followed by fracture. Torsional fracture may typically occur if excessive apical force is placed on the instrument and is more likely to occur with smaller size files.[79] Flexural fracture is caused by work hardening and metal fatigue. It occurs at the point of maximum flexure when the instrument is freely rotating in a curved canal, and may start from defects in the instrument surface that occur after cyclic fatigue[82]. Flexural fractures showed a sharp break without any accompanying defect, and it was found that they happen more frequently with larger file sizes, indicating that larger instruments have fewer cycles to failure.[80] In order to avoid flexural fracture, the authors of the research propose to discard the instruments after substantial use. Increased severity of angle and radius of root canal curvature around of which the instrument rotates, reduces instrument lifespans[83].

In light of the finding that rotary Ni-Ti files may be subject to fracture due to fatigue without any prior evidence of plastic deformation, single-use of these instruments has been supported by certain researchers [84], and currently there is no agreement on the recommended number of uses for these instruments. Parashos et al.[85] examined discarded rotary Ni-Ti instruments from 14 endodontists and identified parameters that may affect deficiency after clinical use. The aforementioned study did not propose the routine single use of instruments as to prevent fracture on the basis that, instrument separation is a multifactorial problem. The operator of the instruments himself (meaning his clinical skill or a decision to use instruments a specified number of times) was found to be the most important factor of defect rate.

However the absence or presence of plastic deformation is not a proof of fatigue fracture as other conditions such as high strain rates can eliminate the plastic deformation of ductile metals as well. According to Cheung[85] the only way to acquire real information about the fracture mechanism is through fractographic analysis, fact which only few studies have used, and none of them have demonstrated characteristic patterns of fracture mechanism. In general fatigue loading causes numerous cracks that evolve simultaneously, but only one provokes the separation of the file while the rest remain as secondary cracks. From the clinical point of view the fracture mechanism of single overloading fits better with the real clinical conditions since as it was mentioned before the fracture is independent of times used as it can occur at any time during operation while it also explains the fact that even brand new instruments have 0.9% incidence of separation[81]. This approach also explains the general outcome of many studies evidencing that file separation is more dependent to the way the files are used, rather than by the number of uses[82,83]. Given that the failure mechanism is not associated with cumulative damage but with a sudden overloading, probably when the tip of the instrument is locked in a constricted region of the root canal, the aim of research to determine a safe number of uses of Ni-Ti files is a rather questionable value while the training of operators for a proper use of those instruments seems more substantial.

Impact of specific instrument design characteristics

In latest years various different rotary NiTi systems have been introduced into endodontic practice. The specific design features of each system (such as cross-sectional geometry, tip design, and taper) vary and influence the flexibility, cutting efficiency and torsional resistance of the instrument. In any case it is difficult to predict the impact that the specific instrument design characteristics will have on clinical outcomes[94]. It is recommended to use instruments with safety tips rather than those with cutting tips such as Quantec SC which have been reported to result in a high rate of procedural errors including root perforation, zipping and ledging[95,96]. There is some evidence that NiTi instruments with active cutting blades (such as ProTaper, FlexMaster, RaCe, Mtwo) achieve better canal cleanliness than instruments with radial lands (e.g. ProFile). Comparisons of instruments with and without radial lands on the basis of SEM-evaluation of root canal walls for residual debris have shown that radial lands tend to burnish the cut dentine into the root canal wall, whereas instruments with positive cutting angles seem to cut and remove the dentine chips[97,98]. In vitro studies have showed that actively cutting cross-sections do not affect centering of the canal preparation.[98,99] However, instruments with active cutting blades must be used with caution in the apical region as over-instrumentation with such instruments can possibly creates an apical zip.[100] Certain studies have indicated that instrument shaft design does not significantly modify canal shapes of similar apical sizes[101], whereas other studies have suggested that a thin and flexible shaft will permit larger apical sizes with less aberrations[102].

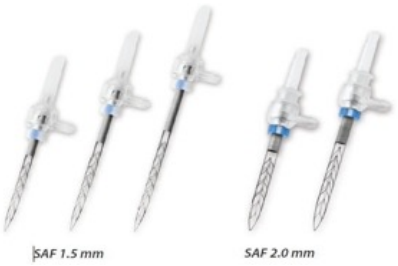
The Self Adjusting File

The self-adjusting file (SAF) (IMG. 1) (ReDedent-Nova, Ra'anana, Israel) is a recently developed system which mirrors a new concept in cleaning and shaping of root canals compared to other NiTi rotary instruments.

The SAF is a hollow file designed as a compressible, thin-walled pointed cylinder with diameter either 1.5 or 2.0 mm, composed of 120-mm-thick Ni-Ti lattice and with an asymmetrical tip.

The 1.5-mm. file is claimed to be compressed to the extent of being inserted into any canal previously prepared or negotiated with a #20 K-file[103]. The 2.0-mm file will be compressed into a canal that was prepared with a #30 K-file. The file will then attempt to regain its original dimensions, thus applying a constant delicate pressure on the canal walls[103] When inserted into a root canal, the SAF is automatically adjusted to the canal's shape, both longitudinally (as will be the case with any nickel titanium file) and along the cross-section. In a round canal, it is foressen to maintain a round cross-section, while in an oval or flat canal it is foreseen to maintain a flat or oval cross-section, providing a three-dimensional adaptation. The wall thickness of the lattice comprising the file is 100 mm,

therefore when fully compressed mesio-distally, the file may spread bucco-lingually up until 2.4 mm[104]
The surface of the lattice threads is lightly abrasive and remains a very close contact along the entire circumference and length of the canal. This fact, combined with the vibrating movement of the handpiece, allows it to remove dentin with a back and-forth grinding motion[104]



IMG. 1: The self adjusting file (SAF) in the two different diameters (1.5 & 2 mm.)

The SAF is operated through a special handpiece head, RDT3 (ReDent Nova, Raanana, Israel) (IMG.2) designed to be used with various available handpieces such as KaVo GENTLE power. The RDT3 head turns the rotation of the motor into an in-and-out vibrating motion of the SAF file. It is operated at 5000 rpm and generates 5000 vibrations per minute with amplitude of 0.4 mm. The RDT3 includes also a special clutch element that allows it to slowly rotate counterclockwise when not engaged in the canal and to stop the rotation immediately when the file enters the canal and engages its walls. Therefore, the file never rotates when it is in contact with the canal walls, but rotates slowly during every outbound motion of the operator. This way, the SAF works as a file with only the vibratory mode, but enters the canal in a different circular position in every inbound motion of the operator[104]

IMG. 2: The Vatea pump and the RTD handpiece

The hollow design of the SAF is used in order to provide a continuous flow of fresh sodium hypochlorite, or any other irrigant, throughout the file operation [103,104.] The SAF file is equipped with a rotating hub with an attached silicone tube The other end of the tube is attached to the special VATEA peristaltic pump (ReDent-Nova, Raanana, Israel). (IMG.2) The pump is operated through a rechargeable battery and has a container that contains 500 ml of irrigant. A control panel allows the operator to set the flow rate between 1 and 10 ml / min and indicate the time elapsed.

The pump and all its connectors are compatible with any irrigating solution, including full-strength NaOCl or EDTA solution [105]. The pump is activated by a foot pedal or a handswitch, with an on/off action.

The SAF removes dentin with a filing motion in a manner that resembles the way that sandpaper is used. When using sandpaper, a rough surface is applied with light pressure and with repeated back and forth motion, which allows the material to be removed in a uniform way. The surface of the SAF is delicately rough with 3 μm peak to bottom dimensions This rough surface exists on each thin element of the NiTi lattice. The compression of the file causes circumferential pressure on the canal walls. By using the vibrating motion of the file, dentin is gradually removed, thus creating a presumably smooth surface [103]

The pressure reaches its greatest point when the file is inserted into the root canal and declines with the gradual enlargement of the canal [103] This change in pressure in tum results in the decrease in a similar manner or the amount of dentin removed.

The SAF file manufacturers support that it maintain the original shape of the canal and extracts a uniform dentin layer from the entire perimeter of the root canal [102,104]. Thus, a round canal will be enlarged as a round canal, whereas a flat-oval canal will be enlarged as a flat-oval canal.

The SAF is inserted into the canal while in vibration mode and is carefully pushed in until it reaches the predetermined working length. Then it is operated with in-and-out manual motion of 3 to 5 mm and continuous irrigation using two cycles of 2 minutes each for a total of 4 minutes per canal. The above procedure removes a uniform dentin layer 60 to 75-mm thick from the canal perimeter [104]. A single SAF file is used all through the procedure, starting as a compressed file which is gradually enlarged in size during dentin removal with close adaptation to the canal walls [104]

One of the most crucial steps when working with the SAF system is the initial preparation of a glide path that will permit free insertion of a #20 K-file to its working length which in turn will permit the insertion of the SAF to the full length of the prepared canal[102].

Instrumentation protocols should be carefully chosen after taking into account the degree of difficulty expected in the specific canal, based on the first instrument to bind (FITB) in the apical part of the canal.

It is proposed to use this step to classify the following levels of difficulty of an individual root canal:

1. easy canals: canals that allow a #20 (or larger) file to be inserted to a working length with no prior instrumentation, (FITB = #20)
2. medium canals: canals that allow only #15 file to be inserted to a working length, (FITB = #15)and
3. difficult canals: canals that allow only a #10 file (or smaller) to be inserted to a working length. (FITB = #10)

As already mentioned, SAF has the exceptional feature of allowing constant irrigation all through the preparation procedure. In cases where EDTA is to be used for smear layer removal,[104] suggested the following protocol: the SAF will be operated in two cycles of 2 minutes each for a total of 4 minutes. During the first minute of each cycle, NaOCl will be used as irrigant, and whereas during the second minute EDTA will be used as irrigant. Finally the canal will be flushed with NaOCl.

The WaveOne File

The WaveOne NiTi single file system has been fairly recently introduced by Dentsply Mailefer (Ballaigues, Switzerland), consists of 3 single-use files available in lengths of 21, 25 and 31 mm and is designed to be used with a dedicated reciprocating motion motor. The WaveOne Small file is used in fine canals. The tip size is ISO 21 with a continuous taper of 6%.

The WaveOne Primary file is used in the majority of canals. The tip size is ISO 25 with an apical taper of 8% that is being reduced towards the coronal end.

The WaveOne Large file is used in large canals. The tip size is ISO 40 with an apical taper of 8% that is being reduced towards the coronal end. (IMG.3)

As already mentioned, the Small 21/06 file has a fixed taper of 6% over its active portion. The Primary 25/08 and the large 40/08 WaveOne files, though, have fixed tapers of 8% from D1-D3, while from D4-D16, they have a unique progressively decreasing percentage tapered design. This design allegedly helps to improve flexibility and preserve remaining dentin in the coronal two-thirds of the finished preparation.

IMG. 3:The Wave One files.

Another unique feature of design of the WaveOne files is that they have a reverse helix and 2 distinct cross-sections along the length of their active portions. From D1-D8, the WaveOne files have a modified convex triangular cross-section, while, these files have a convex triangular cross-section from D9-D16. The design of the two WaveOne cross-sections is further improved by a changing pitch and helical angle along their active portions. The WaveOne files have noncutting modified guiding tips, so as to enable the files to safely progress through canals. Particularly, WaveOne Primary file (#25.08) has a constantly decreasing taper from its tip to its shaft (0.8, 0.65, 0.6, and 0.55) and is defined by different cross-sectional designs over the entire length of the working part. In the tip region, the cross-section presents radial lands, while in the middle part of the working length and near the shaft, the cross-sectional design is converted from a modified triangular convex cross-section with radial lands to a neutral rake angle with a triangular convex cross section similar to the ProTaper F2 file near the shaft.
The WaveOne files are constructed with M-Wire (Dentsply Tulsa Dental, Tulsa, UK) NiTi alloy, which is produced through an innovative thermal treatment process [107]. Studies have indicated that M-Wire technology improves the resistance to cyclic fatigue by almost 400% compared to 25/04 NiTi files (which are commercially available). Higher flexibility, less debris removal and the ability to maintain the shape of the canal are some of the other advantages of this newly developped supermetal files [65,76]
The WaveOne motor is operated with a 6:1 reducing handpiece and the pre-programmed motor is set for the angles of reciprocation and speed for WaveOne instruments. (IMG.4)

IMG. 4 The Wave One micro-motor
The reciprocation working motion includes a clockwise (CW) and a counterclockwise (CCW) motionThe blades of the WaveOne files have a left-handed angulation, thus they cut in the counterclockwise direction. A large rotating angle in the cutting direction (CCW) determines the instrument advances in the canal and engages dentine to cut it, whereas a smaller angle in the opposite direction (CW) allows the file to be immediately disengaged and safely progress along the canal path, whilst reducing the screwing effect and file breakage. In principle, the WaveOne system utilizes an engaging angle that is 5 times the disengaging angle in a way that after three engaging/disengaging cutting cycles, the file will have rotated 360° or turned one CCW circle [107]). Since the

counterclockwise angle is larger than the clockwise one, it is supported that the instrument constantly progresses towards the apex of the root canal. (IMG.5)

IMG. 5: The reciprocating motion .

The reciprocating motion purports to diminish the risk of instrument fracture caused by torsional stress. In particular the angle of CCW rotation is designed to be smaller than the elastic limit of the instrument. Despite the fact that the instrument performs one rotation of 360° in several reciprocating movements, accumulation of metal fatigue is still a concern. A recent study showed that Reciproc (VDW, Munich, Germany) instruments are related with significantly higher cyclic fatigue resistance than WaveOne instruments [108]

According to the protocol of use introduced by Webber et al, information from a preoperative radiograph (size and length of canal, number of canals, degree and severity of curvature), but primarily the first hand file into the canal are the main factors taken into account for the selection of the applicable WaveOne file as follows:

- WaveOne Small file should be used, if a file size 10 K-file is very resistant to movement,
- WaveOne Primary file should be used, if size 10-K file moves to length easily, is loose or very loose; and
- WaveOne Large file should be used, if a size 20-K file or larger goes to length[47]

With an estimated working length a #10 file is inserted into the orifice to determine whether the file will move towards the terminus of the canal easily. A loose #10 file ensures that sufficient existing space is available in order to immediately initiate mechanical shaping procedures utilizing the Primary 25/08 WaveOne file. However, in longer, narrower, and more curved canals, the #10 file cannot be initially and safely worked with to length. In these cases, [106] does not suggest the use of size 06 and/or 08 hand files in an effort to immediately reach the terminus of the canal, but rather simply work the size 10 hand file, within any region of the canal, until it is completely loose.

After a glide path has been established, shaping can commence, starting with the Primary 25/08 WaveOne file. The file should be directed apically with gentle pressure. After every few millimeters of advancement, or if the Primary 25/08 WaveOne file will not easily progress, file should be removed for cleaning and inspection of its flutes. As with any mechanical shaping file, it is advised to irrigate, recapitulate with a 10 file and then re-irrigate. Recapitulating with the 10 file moves debris into solution, confirms the glide path, and makes it easier for the same 25/08 WaveOne file to continue shaping procedures.

When the canal is secured, the Primary 25/08 WaveOne file can generally be carried to the full working length in one or more passes. When this Primary file will not readily advance in a secured canal, then the Small 21/06 WaveOne file may be utilized. This file will typically reach the desired working length in one or more passes. The Small 21/06 file may be the only shaping file taken to the full working length, especially in more apically or abruptly curved canals. However, with the anatomy in mind, to encourage 3D disinfection and filling root canal systems, more shape may be indicated. In these instances, the 25/08 file will generally advance through any region of a canal where the shape has been previously expanded utilizing the Small 21/06 bridge file.

Once the Primary 25/08 WaveOne file readily moves to the working length, it is removed. The finished shape is confirmed when the apical flutes of this file are loaded with dentin. Alternatively, the size of the foramen can be gauged with a size 25/02 hand file.

When the size 25 hand file is snug at length, the shape is done. If the size 25/02 hand file is loose at length, it simply means the foramen is larger than 0.25 mm.

In this instance, foramen can be gauged with a size 30/02 hand file. If the size 30 hand file is snug at length, the shape is done. If the size 30 hand file is loose at length, then the Large 40/08 WaveOne file should be used to optimally prepare and finish these larger canals. Upon reaching the working length, the 40/08 WaveOne file is removed for inspection of its apical cutting flutes. If the flutes are loaded with dentine mud, there is visual confirmation that this file has cut its shape in the apical one-third. Alternatively, the terminal size of the preparation can be gauged using a size 40/02 hand file. When the size 40/02 hand file is snug at length, the shape is done and the forarnen is confirmed to be 0.40 mm. When the 40/02 hand file is loose at length, it simply means the forarnen is larger than 0.40 mm. In these instances, other methods may be utilized to finish these larger, typically less curved, and more straightforward canals[45]

The BT Race files

The RaCe system was manufactured in 1999 by PKG. RaCe is an acronym for "reamer with alternating cutting edges" this file has flutes and reverse flutes alternating with straight areas; this design aims at reducing the tendency to thread the file into the root canal. Cross sections are triangular or square for .02 instruments with 10 size #15 and #20 tips. The tips are round and non cutting.

RaCe instruments have been marketed in various packages to address small and large canals.

The BT Race system was recently presented and differs from standard RaCe instruments regarding taper, instrumentation sequence, tip. It is claimed by the manufacturer that sufficient apical preparation sizes can be obtained by using BT Race with a decreased number of instruments.

The new BT-Race have a “Booster Tip” (BT) patented by FKG which increases the the files efficiency. The sequence and Booster Tip allows the practitioner to achieve adequate apical preparation sizes in all types of canals with unparalleled ease with only 3 les. Further exclusive advantages include:

Sterile

Files are sterile and packed in individual cells. Thus the instruments are ready to use and files that are not used stay sterile.

Associated costs are reduced (such as storage and handling)

Instrument use is guaranteed to be totally hygienic

Single-use instruments

Patient cross-contamination is prevented

Few instruments, the practitioner follows a simplified workflow, which gains time and benefits the patient too.

The instruments are subject to less stress, reducing risk of breakage

Cleaning, autoclaving and maintenance of instruments are things of the past. The single use set up reduces time of handling the files used and cost involved

Adhere to biological standards

The efficiency of the files, the clean cut of dentine at 800 rpm and a sequence design that removes small parts of the canal wall ensure easy progression and minimise the risk of micro-cracks both coronally and in the apical parts Minimal weakening of the coronal part and the root of the tooth thanks to the low taper (final preparation of 35/.04)

The design of the Booster Tip (BT) and the safety tip ensure the canal anatomy is respected

Biological preparation to guarantee a sufficient cleaning of the apical third Outstanding removal of debris.

The BT-Race Sequence BT-Race : 3 sterile single-use instruments

The BT-Race Sequence ensures a minimal biological apical preparation of ISO 35/.04:

BT1, 10/.06 : is used for canal exploration, the creation of a glide path and conservative enlargement of the coronal third. Small apical diameter and large taper clears the coronal part of the canal.

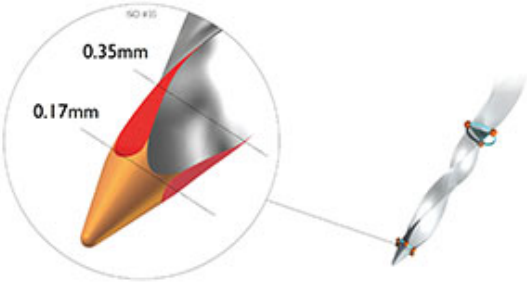
BT2, 35 : preparation of the apical third. Patented file with BT tip; in spite of ISO 35 diameter, file remains flexible thanks to the non taper design; easy and efficient penetration is accomplished thanks to the BT tip.

BT3, 35/.04 : final shaping for the most common canals. All the advantages of the Race design plus the BT tip allow this 35/04 file to effortlessly join the coronal and

apical preparations created by the BT1 and BT2. Thus stress on the file and dentine is minimized. (IMG.6)

IMG.6: The BT-Race files.

The BT tip specifics
(Booster Tip and Biological Treatment)
Removes an increased amount of material with each cut and enables thus a faster progression through the canal, while respecting its anatomy and shape Has 6 cutting edges at the tip, for increased cutting efficiency
Its revolutionary shape enables the use of fewer instruments per treatment. (IMG. 7)



IMG.7: The BT (booster) tip.

The specifics of BT2
Clears the apical canal to size #35 ensuring that files that follow are not blocked and are not stressed
No taper, the file remains very flexible and can therefore operate in any type of curvature
Thanks to the BT tip and safety tip, apical progression is efficient following the use of BT1
Breaking point located 16 mm from the tip to avoid all risk of breaks at the tip
Protocol BT-Race

For most cases
Speed : 800 rpm (600-1000 rpm)
1. After the coronal access is attained, the working length should be obtained with small hand files (ISO 06, 08, 10 or 15) depending the constriction of each canal.
2. A glide path should be performed with small stainless steel or NiTi files up to ISO 15 before using BT-Race sequence.
3. Files of the entire sequence should be used to full WL before changing to the next file in the sequence. Per file, total working time in one canal should not exceed 10 seconds.
4. Use the BT1 with a long and gentle pecking motion (3-4 back and forth strokes). If BT1 does not reach WL, clean the instrument, irrigate and repeat until the WL is achieved.
5. Recapitulate with K-File ISO 15 to keep the glide path open, irrigate.
6. Use BT2 up to WL in the same manner as BT1.
7. Recapitulate with K-File ISO 15, irrigate.
8. Use BT3 up to WL in the same way as BT1.

4.Methods of study of microbial flora of root canal system

The importance of studying the microbial flora associated with dental tissue colonization has been perceived since the early ages. Anthony Van Leeuwenhoek was the first to spot microbes in specimens of teeth with a microscope in 1684 [109.] In 1890, Miller observed microorganisms that he attempted to associate with dental pulp pathology and hence with endodontic aetiology [110]

The documentation of the above correlation came from Kakehashi in 1965, which demonstrated that pulp necrosis and its progression to inflammation of peri-crocental tissues was possible only when revealing the pulp in the oral environment of normal mice but not the corresponding mice that had not come not in contact with microorganisms[111]. Sundqvist also concluded 11 years later after extensive microbiological studies on dead dental pulp [112].

Based on the above, the identification of microorganisms involved in endodontic infections was necessary in order to study this microbial flora (detection and identification of microbial strains) and to develop effective protocols for the chemical and mechanical processing of infected root canals.

Methods that can be used to study and identify microbial flora in infected root canals are divided into two major categories. The first concerns those that are dependent on culture-dependent methods, while the latter is related to new modern methods based on molecular analysis of microbial load and are not dependent on culture-independent methods.

The most important of these methods are the following:.

Microscope

Microscopy is a method of observing, under high magnification, the morphological characteristics of the microbes found in the sample. It also provides important information about topographical distribution and organization. Microscopic examination provides quick and relatively low cost information, but usually the morphological characteristics observed are not sufficient to classify the microorganism at the level of the species [113,114]

Direct microscopy is possible with a variety of methods, depending on the question being asked. The microscopic examination based on the staining of the samples to be observed is used for the initial morphological classification of the microorganisms detected (e.g., Gram stain). This means that in addition to the shape of microbes, what happens when a pigment comes into contact with microorganisms is also considered. The differentiation of microbes in Gram (+) and Gram (-) is of great importance both in terms of the level of infection and their potential sensitivity and resistance to antibiotics [115,116].

Dark-field microscopy makes it possible to determine the mobility of microorganisms and can provide information on the presence of spirochetes in the observed sample. Samples are usually observed under magnification of the order of 400x or 1000x placed on a glass plate in water and covered with a special thin transparent protective film [117,118].

Finally, both the Scanning Electron Microscope (SEM) and the Transmission Electron Microscope (TEM) have been widely used in the past [114-115,119,120] as well as after biofilms [121-125].

The scanning electron microscope enables the morphological characteristics of the surface being scanned (e.g., a dentin surface colonized by microorganisms) to be observed under great magnification and at the same time high resolution. The passing electron microscope also provides the ability to record and visualize the internal structure of the observed surfaces (viamins, dentin colonies colonized by microorganisms)

Microscopy as a method of detecting and studying microorganisms generally has low sensitivity and specificity. Low sensitivity is justified by the fact that a sufficient range of microbial load is required to detect microscopic microbial cells (approximately 104-105 cells / ml of sample). The low specificity of the method is that the classification of microorganisms on the basis of their morphological characteristics is extremely difficult to impossible[126].

Its role, in contemporary endodontic literature, is now mostly auxiliary and to a lesser extent diagnosis for two main reasons. The first is that microscopic findings on the morphology of microbes can lead to erroneous conclusions due to the diversity of microorganisms. The second is that the interpretation of the results is consistently subjected to the subjective perception of the researcher [125].

Microbial cultivation

For over a century, microbial cultivation with the help of artificial nutrients has been and continues to be the basic diagnostic tool for many infectious diseases. Cultivation of microbes found in infected root canals has been widely used in the past and continues to be used even today at a particularly improved level with regard to sample sampling and transport and growth conditions of microbes [115,127-131].

Moller in 1966 was the one who initially demonstrated the importance of adequate antisepsis measures and the necessary conditions for taking and transferring the microbiological sample to the laboratory[132]. Through the relevant study, it has clearly described how to avoid potential contamination of the specimen, correct sampling and VMG III of the sample proposed for use at that time. Much later, the same medium was modified by taking its final form into VMGA III [134/133].

But the fact that gave a tremendous boost to the efforts to study and identify the microbial flora found in infected root canals was the development of anaerobic microbial culture techniques.

Anaerobic microbial cultivation techniques were originally introduced in the late 1960s and early 1970s. Sundqvist in 1976 clearly described the diversity of the microbial root canal ecosystem by combining the use of anaerobic cultivation techniques with the most accurate determination of the phenotype of microbes isolated [132]. Until today, cultivation methods are considered by many to be the gold standard of microorganism design and identification techniques, despite the rapid development and improvement of molecular methods in the last decade [135].

Taking the microbiological sample in any case is required to be done under severe antiseptic conditions. This is done in order to avoid false positives and consequently results. In particular, the teeth are initially isolated using a rubber sealer. Tooth decay or blockages are removed by using high and low-speed sterile balloons and simultaneous steaming of sterile saline. Dental floss tightly surrounds the neck of the tooth and the field (tooth mill, cavity, grapple, and rubber in the tooth area) is first decontaminated using 3% or 30% H_2O_2 until no spontaneous reaction occurs (bubbling). Then, all surfaces are disinfected with thorough cleaning using a sterile cotton ball impregnated with 2.5% NaOCl solution or 10% iodine tincture. Upon completion of the opening of the myrrh chamber (using resterilized high and low-speed clams and simultaneous sprinkling of normal serum), a new decontamination of the field, including the myrrh chamber, is followed in the same manner as mentioned above. Neutralization of 2.5% NaOCl solution or 10% iodine tincture is done using 5% sodium thiosulphate solution and then a microbiological sample is obtained by means of a sterile paper cone from the tooth surfaces. These are the control samples from each tooth with a prerequisite to be negative to take into account the results of microbiological specimens in the study [133,135,136].

For each tooth, three microbiological samples are usually taken using three sterile paper cones. Initially a knife K No 15 with a cut handle is inserted into the root tube about 1 mm from the working length (based on an edge finder and intermediate radiograph) with a slight tapping motion. The paper cones (No. 15) are placed at the same length for at least one minute (1) so that they are apparently impregnated by the fluids of the root canal. If the root canal is dry, then a minimum amount of sterile saline is introduced into it. It is worth noting that the last paper cone is the most important one, as it absorbs liquids from the most distal areas of the root of the root canal third root [133,135,136].

The paper cones collected together with the cut rine are sterilized and placed in an Eppendorf test tube (cryotubes) containing the VGMA III transport medium. This medium serves to maintain the vitality of microorganisms while being bacteriostatically preventing the proliferation of microorganisms in the sample before it reaches the laboratory

At the same time, VGMA III has the ability to inactivate antiseptic agents that are often used to disinfect root canals and which remain on the paper cones after taking the sample. Finally, it contains specific factors (cysteine) that do not allow its oxidation, as well as a redox index to control the above reaction. VGMA III medium is not commercially available. Its composition has been reported in detail by Dahlen et al. in 1993 [134].

The manipulations in the laboratory for the cultivation of the sample have three main purposes: First, to show if living microorganisms are found in the sample, secondly to recognize and classify these microorganisms at the species or genus level and thirdly, to ensure as much as possible a semi-quantitative sample of the microbial population found in the sample.

Thioglycolate, trypticase broth, HCMG Sula can be used initially to control the growth of micro-organisms. They also have the advantage that their use does not require further equipment for anaerobic incubation since the test tubes are oxygen-free. Thus, if the tubes are prepared under anaerobic conditions, hermetically sealed and the sample placed in the lower part of the tube, the culture medium ensures satisfactory anaerobic conditions even for the most oxygen sensitive microbial species.

An important end in this phase is to ensure a sufficient incubation time for the growth of micro-organisms likely to be in adverse environmental conditions. Usually 5 to 6 days are initially required to control growth while the incubation is continued for at least another 14 days. Solid culture media (Brucella blood agar) come to complement the use of liquid media in order to further assist in detecting the diversity of microbial flora and to quantify as much as possible each colony forming unit (CFU) [135]

The main advantage of microbial cultivation is mainly its wide range, which makes it possible to detect a relatively large number of microorganisms found in the clinical specimen. The physiology of growing microorganisms and their possible sensitivity to various antimicrobial agents are also studied based on the culture,

indirectly determining their endurance and their ability to cause disease in particularly adverse conditions [137].

At the same time, however, microbial culture is characterized by significant constraints that often make it difficult and problematic. High cost is initially a major disadvantage, as it usually takes a long time (several days to weeks) to cultivate and identify many anaerobic microbial strains. The moderate sensitivity of the cultivation methods was, in fact, the one that led to the rapid development of molecular analysis of microbial flora of root canals.

At the same time, however, microbial culture is characterized by significant constraints that often make it difficult and problematic.

This level of sensitivity is explained by the fact that the method of detecting anaerobic microbial strains which until now seems to be impossible to cultivate with the usual nutrients is explained by the inability. Finally, the specialization depends heavily on the experience of the individual researcher and on his ability to strictly apply the protocol of the method [138,139].

The inability to cultivate many micro-organisms is due to many reasons:

a. The lack or absence of specific nutrients or growth factors for use in artificial cultivation.

b. The toxicity of the culture medium itself, which is often an inhibitory factor for the proliferation and growth of microbes.

c. The production of substances from various microbes that acts inhibitory to the growth of other target micro-organisms likely to be found in the sample.

d. Metabolic dependence of various microbial strains from other germs for growth.

e. Cutting the intercommunication systems of the microbes during their possible separation on the cultivation medium which often results in the inhibition of the growth of many of them.

f. Bacterial inactivity or bacterial dormancy, which is a state of low metabolic activity in which microorganisms enter in situations where environmental conditions are unfavorable to them. In this condition, dormant bacterial cells can not grow naturally or form colonies unless they undergo a resuscitation phase [138-140].

The inability to identify micro-organisms previously cultivated has also been an additional limitation of the cultivation methods. The traditional way of identification based on the phenotype is always difficult and time-consuming. Also, the same process involves a significant degree of subjective perception of the individual researcher. Finally, a significant difficulty during phenotypic tests is related to the divergent and convergent genetic variation of genetics (Genetic divergence-convergence).

Deviating is the genetic progression that occurs for strains of the same species, which while genetically considered the same (same genotype), differ

phenotypically. Conversely, the genetic evolution of strains of different species (different genotype) is considered to be convergent, but they have similar phenotypic behavior. In both cases, the diagnosis based on phenotypic characters of bacterial strains is likely to lead to false conclusions [141].

Immunological methods

In these techniques, antibodies bind to cell antigens (immunohistochemistry) and can be visualized by binding of a fluorescent dye (immunofluorescence) to these bound antibodies. The fluorescent dyes commonly used are fluorescein, rhodamine and less phycoerythrin. Each of the fluorescent dyes absorbs light at a certain wavelength and emits light at a certain longer wavelength. Emitted light is generally observed with a fluorescence microscope [142,143].

Staining for immunofluorescence may be direct or indirect. In direct staining (direct immunofluorescence), the specific antibody (primary) is directly conjugated to the fluorescent dye. In contrast, in indirect staining (indirect immunofluorescence), the primary antibody is labeled and detected by means of a second antibody labeled with the fluorescent substance. Indirect staining has two advantages over direct staining. First, the primary antibody, which is not always readily available, does not need to be labeled so as to avoid any loss of labeling

Secondly, the indirect method increases the sensitivity of the technique because multiple fluorescent reagents can be blocked in the secondary antibody [142-144].

Detection of microorganisms by means of indirect immunofluorescence has occurred on several occasions in the past [145-148] but the development of molecular methods in recent years has led to their non-use for this purpose.

In the ELISA, the amount of the antigen-antibody complex is determined by the addition of a suitable substrate, the reaction product of which, by enzyme aid, produces a colored product or light. Measurement of light intensity by special photometers gives information on the number of labeled complexes and, by extension, the amount of antigen or antibody in the sample. The quantification is done by means of a standard reference curve.Enzymes commonly used in ELISA are alkaline phosphatase and peroxidase. Reinforcement of the signal gives the alkaline phosphatase reaction when NADP + is used as the substrate for NAD production. ELISA is a highly sensitive technique particularly useful for both clinical and laboratory microbiology, which combines simple organology, low cost and low risk of the tracers used [149,150].

Immunological methods of microorganism detection have several advantages which are [143]:

1. The short time (a few hours) usually required for the above procedure.
2. Detection of dead microorganisms.
3. The low cost.

However, there are no disadvantages, which are mainly:

1. The ability to detect only the target micro-organisms in the sample.

2. Their low relative sensitivity (104 cells).
3. Their dependency on the type of antibodies used each time [151,152].

Molecular genetic methods

The molecular methods used in Clinical Microbiology can be categorized into two main groups. Firstly, in techniques based on DNA hybridization techniques and secondly in Polymerase Chain Reaction (PCR) techniques.

Methods of hybridizing DNA fragments are based on the use of DNA probes. The probes are short, labeled, single-stranded DNA fragments, designed to hybridize to specific parts of the bacterial DNA. It is worth noting that the PCR method is characterized by a higher sensitivity but at the same time a lower specificity than the method of DNA probes [153-154].

The PCR reaction is a method of proliferating DNA fragments in vitro. This method involves knowledge of the nucleotide sequence of the target DNA as well as the synthesis of complementary monoclonal sequences which hybridize due to complementarity and are used as primers for its proliferation [155-157].

Socransky et al. were the first to introduce the Checkerboard technique by which hybridization of a large number of microbial DNA samples can be accomplished by means of specific probes (single stranded sequences) on a specific suspension membrane [153].

The technique is based on the following two conditions: The first is that the genetic material of the microorganism being detected consists of DNA sequences which are completely specific to the microorganism. The second concerns the fact that the specific sequences have been isolated and cloned before the method is applied. Isolation of the specific sequences of the microbial DNA has taken place with the help of the DNA-ligase enzyme while the cloning is done by means of a plasmid implanted in a carrier microorganism which is then allowed to multiply. Next, the labeling of these specific parts of the genetic material with dioxygenin follows, and the DNA probes are ready to apply the method. Next, the labeling of these specific parts of the genetic material with dioxygenin follows, and the DNA probes are ready to apply the method.

The microbial DNA resulting from the clinical specimens, after specific processing to become single-stranded, is applied in rows over a nylon membrane or nitrocellulose membrane. After applying the samples of the genetic material to the membrane, it is placed in a special device (Minislot device) with the rows of membrane samples perpendicular to the corresponding rows of the device.

Then, the surface of the membrane is processed with all of the pre-selected detectors. If the detector detects a complementary sequence on the membrane, its hybridization will take place and will be immobilized on its surface. This is revealed by the presence of a fluorescent spot along the membrane rows. The intensity of the spot is strictly proportional to the amount of DNA of the target microbial species from the clinical specimen. Subsequently, with a series of rinses

of the membrane surface under stringent stringency conditions, unhybridized probes are removed, taking the final result of the technique [153].

As can be seen from the above, the method can detect microorganisms for which a detector alone has been predicted [154,158]. This is the main reason why it is not a method of selecting the study and identification of microbial flora of root canals. A disadvantage of the method is also the rare cases of cross-hybridization that can occur during the process.

A modified version of the Checkerboard technique is the Reverse-Capture Checkerboard method[49]. In this technique, the probe is one that is immobilized along the membrane and not the portion of the genetic material resulting from the sample. In the process, more than 30 oligonucleotide probes are applied to the membrane by targeting regions of the 16S rRNA segment of the microorganisms to be detected. Each probe is attached to the membrane by ultraviolet radiation or heat, making it available for hybridization after the start of the procedure.

At the same time, by PCR, the 16S rRNA fragment of the resulting genetic material of the microorganisms is multiplied by specific dioxygenin-labeled primers. Then, the PCR reaction products are hybridized onto a Miniblotter device where the membrane with the probes is placed.

The optical effect of hybridization is detected by fluorescence procedures. The Reverse-Capture Checkerboard technique has a higher specificity than the classic Checkerboard technique, mainly due to the different use of the detectors by this method. In addition, it is possible for the detectors to be designed so that they can detect unknown microbial strains, which can not happen with the classical technique [160-162.]

The technique was developed for the purpose of in situ detection and identification of microorganisms found in a clinical specimen [163,164]. The principle is based on the detection of nucleotide sequences by a fluorescent labeled probe which hybridizes only with the complementary target sequence within the microbial cell. The technique also gives information on the morphology, number, organization and distribution of detectable microorganisms in the sample [165,166].

An important advantage of fluorescent in situ hybridization is the possibility of detection of microorganisms whose cultivation has not yet been achieved[163].

Ribosomal RNA (rRNA) is the main target of fluorescent in situ hybridization. This is because it can be found in all living micro-organisms. Based on this, regions of nucleotide sequences that are unique to specific microbial groups from the sex level up to specific microbial strains can be identified [164].

In most applications, the technique aims at detecting a portion of 16S rRNA. The probes used are mainly of 15 to 30 nucleotides and which are joined at their 5-terminus with a fluorescent dye. This may usually be fluorescein, tetramethylhodamine or Texas red pigment [164,165].

A standard protocol of the art includes four steps. Initially, sample fixation takes place and the appropriate probes are then placed to detect the target sequences.

Following is the leaching of the sample in order to remove the unbound probes and finally the detection of the labeled cells using a conventional scanning microscope [167].

The use of DNA microarray technology was first described in 1995[168.] Analyzes of DNA microarrays allow the detection of nucleotide sequences, with particular impetus in many scientific fields such as Microbiology, Cellular Physiology, Cancer Biology and Pharmacology [169].

A DNA microarray is essentially a set of typically thousands of defined DNA molecules, fixed to locations with specific coordinates on a glass surface, called spots. By convention, the fixed DNA molecules are called probes and the target target [169,170].

The principle of the method is based on the labeling of transcripts isolated from biological samples and their hybridization to the DNA microarray probes in order to determine the amount or relative level of expression. A common protocol involves isolating the total RNA from the biological sample which is then reverse transcribed to form complementary DNA (cDNA).

In-vitro transcription of the cDNA is then performed to incorporate modified nucleotides to label with fluorescent molecules. The labeled target hybridizes to the microarray DNA slide for several hours to achieve hybridization of the target sample molecules to the probes. Through the hybridization process and intense flushing of the plate to remove the excess of the target molecules, the microarray is scanned to determine the hybridization levels of the DNA probe, which respectively indicate the levels of gene expression in the test sample [171,172].

The DNA microarrays may also be used to enhance detection of the polymerase chain reaction products. After the reaction, as described below, the microarrays help to identify the products by hybridizing them. In this way and using wide range primers a single PCR can simultaneously detect hundreds of bacterial species [173].

The polymerase chain reaction technique was introduced by Kary Mullis in 1983, and since then, its effect on research mainly on Medical Science has been particularly important. The polymerase chain reaction (PCR) is the technique by which in vitro molecular cloning of selected bacterial DNA sequences is achieved by means of the heat-resistant DNA polymerase.

This reaction allows the rapid and selective replication of DNA sequences in many millions of copies and relies on the repetition of a thermal cycle of reactions each consisting of three steps [156,157].

In the first step, denaturation, the double-stranded target DNA to be propagated is incubated at a temperature of 94-96 oC, resulting in its denaturation and the formation of two single strands (strands). These clones are ready to be hybridized with oligonucleotide primers (primers).

In the second step, called the annealing step, the reaction temperature of the mixture falls between 50-65 ° C, so that the primers can be recombined into the complementary target sequences of the single stranded DNA. The hybridized

primers will then function as a substrate for the Taq DNA polymerase which is a thermosetting enzyme.
Finally, in the third step called the extension phase at a temperature of 72° C, each chain of the initially double-stranded molecule is replicated by means of DNA polymerase. Essentially what happens in this phase is the elongation of the primers. As the reaction occurs, the total number of double-stranded DNA molecules increases exponentially [155].
The paper cones harvested together with the cut rhin are aseptically transferred and placed in an Eppendorf test tube (cryotubes) containing 1 ml of TE buffer solution (10 mM Tris-HCl, 0.1 μM EDTA, pH: 7.6) for preservation of the samples. They are immediately placed in a freezer at a temperature of -20 ° C. Each test tube shows the name of the patient and the identification code with which it has been incorporated into the study.
Double PCR was described by Mullis and Faloona (1987) and is characterized by two consecutive PCR reactions with about 25 rounds for each. The first PCR uses a pair of primers (outer) that enclose the broadest region of the sequence to be multiplied. The first proliferation product is then transferred to a second test tube and the second PCR reaction is performed with one or more primer pair (s) specific to the internal sequence multiplied by the first primer pair [174.]
The internal PCR method was developed to increase the sensitivity of the reaction and to minimize possible non-specific products, which is achieved by the use of the second most specific primers [175]. However, after the end of the first cycles, dissolution of the mixture should be followed to reduce the concentration of the external primers and then add the pair of internal primers. However, this process involves the risk of contamination of the sample.
This is a major disadvantage of the method which in recent years has been attempted to overcome in a number of ways, one of which is the implementation of the double protocol of one tube. Based on this protocol, the pair of second most specific primers is placed into the first reaction tube but separated from the reaction products by a thick layer of oil. At the end of the first reaction, the mixture is centrifuged to mix with the pair of second more specific primers in order to carry out the second reaction [155,176].
Multiple PCR provides the ability to simultaneously multiply many different DNA fragments. A separate primer pair is designed for each segment. The pairs are pooled and then added to the mixture. In this way, multiplex PCR allows many microbial species simultaneously [67,68]. In the application of the method, however, special caution is needed in the selection of the primers to achieve the same degree of temperature at the same time and not to complement each other [69,70].
Reverse transcription is a technique in which initially total RNA is transcribed in vitro into a single-stranded complementary DNA (cDNA) with the help of the enzyme reverse transcriptase. Subsequently, with the addition of the appropriate primers and DNA polymerase, the remaining steps of conventional PCR follow to

multiply the genetic material in order to construct the second strand of the cDNA [181].

In the case where the material used is the mRNA having a poly (A) sequence at its 3 'end, then a small sequence of deoxyribonucleotides consisting of about 20 thymidine monophosphates is usually used as the initiator for the reaction. As the primers, random hexanucleotide sequences or other specific nucleotide sequences may also be used depending on the type of mRNA.

The PCR reaction using reverse transcriptase essentially detects the presence of RNA of the microorganisms in the clinical specimen. This demonstrates the vitality of the microbes found in the sample as RNA is an extremely sensitive molecule that is almost immediately destroyed in the case of necrosis of microorganisms [162].

This method can be used to determine whether two or more strains of the same species are epidemiologically related [182-184]. According to this, the primers are arbitrarily chosen without prior knowledge of the sequence to be multiplied, which is considered the greatest advantage of the technique.The method was based on the observations of Welsh and McClelland that when a randomly selected primer under low stringency conditions is used in a PCR reaction with a genomic DNA matrix of a microorganism, amplicon (the product of the reaction) is produced and the result is repeatable and characteristic of that micro-organism. The explanation of this phenomenon lies in the fact that by lowering the annealing temperature, a primer is expected to cling to the DNA even if there is a miss-matching in the bases [182,185].

Cloning can significantly help to clarify whether different strains of a microbial species are related to specific signs or symptoms of a disease. Accordingly, the origin of microorganisms found in root canals can be detected by comparing the results of the method with those from other areas of the mouth or even distant areas of the body that have shown signs and symptoms of microbial infection [186].

The results of conventional PCR are mainly of a qualitative nature. An exception to this rule is real-time PCR, which is characterized by continuous quantitative measurement of products throughout the reaction [187,188].

There are many different real-time PCR approaches that use different detection techniques. The three main techniques used are:

- the classic TaqMan system [189],
- SYBR-Green I dye [190]
- molecular beacons (molecular beacons) [191],

In the first description of real-time PCR, hydrolysis probes were used. This technique is based on the pairing of two important processes. First, the construction of double-tagged oligonucleotide probes, called hydrolysis probes or TaqMan, which emit fluorescence upon breakage based on the principle of fluorescence energy transfer by resonance. Secondly, the discovery that Taq DNA polymerase has a 5 '→ 3' exonuclease activity and can be used to degrade the fluorescently labeled probe. It is the most specific PCR detection technique in real time.

The oligonucleotide probe (TaqMan) used in the method is not extended from its 3 'end and is labeled with a reporter fluorescent reporter and a quencher fluorescence group. Based on its design, it hybridizes to the target sequence between the primers during the hybridization and reaction elongation phases. In its free intact form, no fluorescence emission is detected because the fluorescence from the reference dye is absorbed by the absorption dye. However, upon hybridization of the probe to one of the strands of the target molecule, the probe is cleaved due to the 5 '→ 3' exonuclease activity of Taq polymerase.
In this way, the reference dye and absorption dye are separated and the fluorescence of the reference dye is not transferred to the absorption dye resulting in the detection of the reference fluorescence emission. This process occurs in each cycle and does not affect the exponential accumulation of the reaction product. The increase in fluorescence is measured in each cycle and is directly proportional to the product produced [189,192].
The technique that uses the SYBR-Green dye that interferes with DNA is more simple to apply [193-195]. This pigment is incorporated into the small groove of double-stranded DNA, which greatly increases its fluorescence. During the course of the reaction, the amount of double-stranded target DNA exponentially increases while increasing the amount of SYBR-Green dye incorporated into the newly formed DNA and hence increasing the fluorescence emitted. In each cycle, the emitted fluorescence gradually increases during the reaction elongation phase while it is small or absent completely during the denaturation phase.
The measurement of the fluorescence emitted allows quantitative determination of the PCR products during the exponential phase of the reaction. The main advantage of the method compared to the use of fluorescence probes is that it can be used with any pair of primers and at any target. A major disadvantage is its low specificity compared to other techniques due to the risk of proliferation of non-specific products or primer dimers [196].
Finally, the third technique uses the so-called molecular beacons or markers. Molecular beacons are detectors that spontaneously adopt a stem-and-loop structure [197]. They are double labeled with a fluorescent group bound at one end of the molecule and an absorption group at the other end. When the detector has the "stalk and loop" structure, no fluorescence is detected because the fluorescent and absorbing groups are at a short distance, with the result that the photons emitted by the fluorescent group are fully absorbed.
When the probe hybridizes to a complementary target sequence, changing its configuration leads to the formation of a linear structure whenever the fluorescent group is removed from the absorption group resulting in fluorescence [198].
The broad range PCR allows for detection of the entire microbial population installed in a particular environment (eg, root canals). In particular, the oligonucleotide primers used in the broad-band PCR reaction are designed to hybridize to evolutionarily conserved genes (universal primers), sequences

common to the genome of different species of microorganisms. Larger distances between primer pairs result in lower sensitivity, but this often provides more information on the nucleotide sequences contained in this region [199].
In the wide-band PCR reaction, the amplification of the 16S rDNA fragment of the microbial DNA mixture from root canal samples is usually performed using primers for the 16S rDNA of all bacterial species (universal primers). The nucleotide sequences of the primers as described by Nübel et al., Commonly used are [200]:

Forward primer: 5′-AAC GCG AAG AAC CTT AC-3′
Reverse primer: 5′-CGG TGT GTA CAA GAC CC -3′

At the 5-end of the Forward primer is added a nucleotide sequence 40b rich in guanine (G) and cytosine (C) nucleotides with sequence
5-CGC CCG CCG CGC GCG GCG GGG GGG GGG GCA CGG GGG G -3 with the result that the first primer eventually takes the following form:

Forward primer: 5′-CGC CCG CCG CGC GCG GCG GGC GGG
GCG GGG GCA CGG GGG GAA CGC GAA GAA CCT TAC-3′

Reverse primer: 5′-CGG TGT GTA CAA GAC CC-3′.

The addition of the GC-rich loop to the first primer is made in order for the DNA not to completely denature during its movement on the polyacrylamide gel during electrophoresis[201] on a gel with a denaturing agent gradient, a technique to be described subsequently.
In cases of polymicrobial infections, the direct determination of the nucleotide sequence of the products obtained is not possible due to the fact that they are a heterogeneous mixture of many different microbial species. Thus, after the end of the reaction, the products are cloned into a vector (plasmid vector, multi-copy plasmid) of known sequence by means of which a library of separate fragments of 16S rRNA is generated from the sample.
In fact, the cloning process is done in order to distinguish the different sequences between them so that their complete determination is subsequently followed [202-204]. Once this process has taken place, the identified sequences are submitted for identification on specific web site addresses (eg www.ncbi.nlm.nih.gov National Center for Biotechnology Information or www.rpd.cme.msu.edu. Ribosomal Database Project II, GenBank).
DGGE is a molecular molecular fingerprinting approach that allows separation of the polymerase chain reaction products. The method was first described by

Fischer & Lerman[205] and its principle is based on the electrophoretic mobility of double-stranded DNA segments which is significantly reduced when they are partially denatured.
The DGGE method, compared to conventional horizontal agarose gel electrophoresis, enables separation of DNA fragments not only based on size but also on the basis of their differentiated denaturation characteristics, significantly increasing the separation potential. During DGGE electrophoresis, as negatively charged double stranded DNA fragments (PCR reaction products) proceed under the action of the electric field along the polyacrylamide gel, they encounter an ever increasing concentration of the chemical denaturing agent.
Different DNA sequences (e.g., DNA sequences of different bacteria) begin to denature at different concentrations of the denaturing agent. From the start of denaturation of a DNA sequence onwards, the sequence movement in the gel decreases significantly resulting in the formation of characteristic zones [206].
The method typically uses a 6% polyacrylamide gel containing a continuously increasing concentration of a mixture of two chemical denaturing agents, urea and formamide along its direction and in the direction of electrophoresis. At the top of the gel, the concentration of denaturing agents is about 30%, while at the bottom it reaches 70%. As a whole (100%), the mixture of denaturing agents contains 7mol / l of urea and 40% (v / v) formamide [206].

Each band created on the polyacrylamide gel during electrophoresis is extracted with a micropipette and stored at 4 ° C for 24h. After this time, the sequencing of the nucleotide sequence for each zone is followed. Thus, the sequence of each zone is compared with known nucleotide sequences of microorganisms stored in the GenBank database on the Internet for the identification of microbes in the samples.[207-209]
It is the second largest molecular imaging technique that can be used both to document genetic differences between different microbial strains and to analyze and identify the microbial population in the sample. The method essentially calculates the number and size of fragments produced by microbial DNA digestion using a restriction enzyme (endonuclease)[208].
Initially, the PCR reaction, according to what has been reported, takes place using broad-spectrum primers which have been labeled with a fluorescent dye such as 4,7,2,7-tetrachloro-6-carboxyfluorescein[209]. The reaction products are then separated by restriction enzymes, thereby creating different fragment sizes [210].
The length of these final fragments is calculated using automated systems, curves characteristic of each microorganism are obtained and then compared to the corresponding known fragments based on information stored in an Internet database [211,212]. Of great importance for the correct application of the method is the choice of restriction enzymes to be used. This is required to be based on two important criteria.

First, the final fragments must be suitable for size and frequency analysis (best results are obtained with fragments of 1,000 to 1,500 bp) and secondly, fragments of these sizes should not be too much to avoid overlapping zones that may be reduce the discretion of the method. Based on the above, this molecular imaging technique finds important applications in microbiology because the DNA of the microorganisms differs significantly in G / guanine + C (cytosine) content by 25-75% and therefore the number of fragments produced by the help of restriction endonucleases usually varies considerably.

5.Root canal cleanliness - Smear layer

Several studies have indicated that current methods of cleaning and shaping root canals create dentine debris as well as a smear layer that covers the instrumented wall areas [212-215] whuich contains organic and inorganic substances, (which include odontoblastic processes), microorganisms, and necrotic materials. This layer itself has two separate layers: 1) a superficial layer on the surface of the canal wall, of approximately 1 to 2 μm thick, and a deeper layer-smear plugs packed into the dentinal tubules to a depth from 6 and up to 40 μm^2. The contents of the smear layer can be forced into dentinal tubules to several different distances[212].

The compaction of smear layer into dentinal tubules iscause by:

- The linear movement of instruments: the up-and-down filling movement of instruments cuts dentin and dentinal tubules vertically, pushing debris into the tubules. The significance of this mechanism is largely depended on the orientation of the dentinal tubules. Dentinal tubules in the root run a relatively straight course between the pulp and the periphery [216].
- The rotation of instruments: while instruments are rotating (reaming), the centrifuge can cause dentin debris to escape the file flutes and push them into the dentinal tubules.
- The capillary effect: capillary action as a result of adhesive forces between the dentinal tubules and the smear material.[212]

Clinical implications

The effect of the smear layer on bacteria population has not yet been clearly determined. Its role as a physical barrier to bacteria and bacterial byproducts has been supported by many studies [217] which indicated that dentinal plugs stopped bacterial invasion into dentinal tubules. Similarly, Michelich[218] and Diamond and Carrell[7] stated that when the smear layer is present, bacteria could not penetrate into dentine.Furthermore, Drake [220] showed that the removal of the smear layer opened the tubules, allowing bacteria to colonize in the tubules in a much higher degree (IO-fold) compared with roots with an intact smear layer. It has been shown that smear layer removal facilitates passive bacteria penetration[212-223]. It seems that this bacterial invasion depends on the number and type of bacteria as well as on time[223,224].

On the other hand, it has been evidenced that present bacteria in the smear layer and in the dentinal tubules, can survive, multiply[13] and grow[224,226,227]. Bacteroides gingivalis and Treponema denticola[229] seemed to release proteolytic enzymes degradating the smear layer, creating a gap between the obturation and the canal wall,a fact which permits leakage of other bacterial species into dentinal tubules and the periradicular tissues. This degradation of the smear layer has been suggested as a possible cause of the failure of glass-ionomer retrograde fillings [230]

Intracanal medicaments

The presence of smear layer in the root canal space decreases the effect of intracanal irrigants and medicaments into the tubules [232-234]. Orstavik and Haapasalo [223] in an in vitro study contaminated for 7 days bovine incisors with E.faecalis and Demonstrated that, removing the smear layer, liquid camphorated monochlorophenol rapidly disinfected the dentinal tubules, while calcium hydroxide was ineffective. The same authors [221], showed the importance of removal of the smear layer and the presence of patent dental tubules in decreasing the time required to achieve the disinfecting effect of intracanal medications. They concluded that the presence of smear layer delayed, but did not completely negate the effect of intracanal medicaments.

Microleakage

In addition The presence of the smear layer also affect the adaptation of root filling sealers and materials to the canal walls. A number of studies [236-238] have indicated that smear layer removal means better bond of the obturation materials to root canal surface. Specifically, on one hand Oksan [239] observed that no tubular penetration occurred when smear layer was present in the root canal space. whereas On the other hand upon removal of the smear layer the penetration ranged from 40 to 60 μm. Kouvas et al [240] and more recently Kokkas et al [241] found that the presence of smear layer obstructed the penetration of several sealers (Sealapex, Roth 811, and CRCS) into dentinal tubules. They also found that smear layer constitutes an important part of the sealer penetration into the dentinal tubules, as well as in the potential clinical implications.

The view is that maximum sealer penetration into dentinal tubules, after removing the smear layer, results in the reduction of mikroleakage [244]. Nevertheless, several studies, based on various sealers and obturation techniques, did not identifiedany significant effect on root canals mikroleakage aftersmear layer removal[245-248]. Such conflicting conclusions is, due to differences in the way smear layer is produced, the method used for obturation and sealer as well as types of bacteria used in vitro. In the matter of fact, mikroleakadge of root canals is a complex phenomenon with many variables such as root canal anatomy, instrumentation size and filling technique, irrigants, sealer properties and the infectious state of the canal playing an important role and this is why there is no convincing evidence in the literature proving a direct correlation between filling materials penetration into dentinal tubules and mikroleakages.

Influencing factors

Dentine structure - dentinal tubules

Main aspects of the root dentine are the dentinal tubules, which are different from crown tubules. In the root, dentinal tubules extend from the pulp-predentin junction to the intermediate dentine just inside the cementum - dentin junction. They ran a relatively straight course between the pulp and the periphery in contrast to the typical S-shaped contours of the tubules in the tooth crown [249]. Their diameter ranges from 1 to 3 μm, at the pulp-predentin junction and is as small as 0.4 mm at the cementum - dentin junction [250]. The number of dentinal tubules per square millimeter (density) varies from 4900 to 90000 [251]. This density increases in an external to internal direction from the root surface, from 10000/ rnrn^2 at the outer root surface to 580000/mm^2 at the inner root wall. Similarly, it increases in an apical to coronal direction of the root. Mjor et al[249], in their study of the structure of apical root dentine, recorded the density of tubules being 14000/rnrn^2 neat the pulp, 8100/rnrn^2 in the middle and 2500/mm^2 in peripheral root dentin. These densities are much lower than those in root, not apical, dentin (ranging from 40000 to 8100/rnrn^2). It is important to note that the aforementioned study also noted the presence of irregular secondary dentin as well as areas completely devoid of dentinal tubules (fibrodentin).

Instruments - techniques

Root canal cleanliness has been studied for nearly 5 decades[252]. For example, the step-back technique was found to be superior in debridement compared to the standardized serial filing and reaming techniques[41]. Later on, it was shown that the balanced-forced technique produces a cleaner apical portion than the step-back and crown-down pressureless techniques [254]. Siqueira et al[43] found no significant differences when comparing the cleaning efficacy of five different instrumentation techniques (step-back-SS, step-back-NiTi, ultrasonic, balanced-force, canal master U technique) at the apical third of root canals.

However, it was after the advent of rotary NiTi systems[256] and files that studies on debridement quality became popular and numerous.

A great number of different NiTi systems has been designed and introduced over the last 20 years with various design features such as cutting angle, number of blades, tip design, taper and cross-section, which in tum influence instruments' cutting efficacy and, consequently, the type and amount of debris created inside the root canal.

Irrigants, chelating agents

Studies have demonstrated that mechanical preparation alone, either with stainless steel[231] or with nickel-titanium instruments [257] can significantly reduce bacterial load but not sufficiently disinfect root canal. Bystrom and Sundqvist[231] found a 10^2 - 10^3 fold reduction in bacterial population (pre-instrumentation mean population: 4 x 10^5 when they mechanically prepared root canals using saline as irrigation solution. Bacteria were eliminated from the root canals of eight teeth

during the treatment, while in 7/17 teeth bacteria persisted despite treatment on five successive occasions. Dalton et al [257] compared intracanal bacterial reduction on teeth instrumented with 0.04 tapered nickel-titanium (NiTi) rotary instrumentation, compared to stainless-steel K-file step-back technique (both utilizing sterile saline irrigation). They concluded that there was no detectable difference in colony-forming unit count between two techniques and that neither technique was able to produce bacteria free canals in more than 24% of specimens. Thus, the use of intracanal irrigants is obligatory in order to eradicate microorganisms from root canals.

However, the use of these intracanal chemical agents has much more effects than the already mentioned bacteria reduction. It is well documented that they also effect the mechanical [258,259] and chemical [260,261] properties of dentine. And, more importantly when discussing root canal cleanliness, they have an effect on the instrumented as well as the uninstrumented surface and smear layer.

The ideal irrigant should be able to disinfect and penetrate dentin and its tubules, offer long-term antibacterial effect, dissolve necrotic tissue, demonstrate good surface wetting, remove the smear layer and be biologically compatible. In addition, it should have no adverse effects on dentin or the sealing ability of filling materials. Furthermore, it should be relatively inexpensive, convenient to apply and cause no tooth discoloration [262-263].

Following there is a brief description of the most commonly used irrigants and their properties, emphasizing on their impact on dentine surface and smear layer.

Sodium Hypochlorite (NaOCl)

Sodium hypochlorite (NaOCl) was introduced as an endodontic irrigating solution since 1919 (Coolidge 1919) and is the most commonly used root canal irrigant that has been used in various concentrations (ranging from 0.5% to 5.25%).

It's antibacterial properties have been demonstrated in numerous studies and it is effective against endodontic microorganisms, including those difficult to eradicate from root canals, such as Enterococcus, Actinomyces and Candida organisms [264-270].

Free chlorine in NaOCl dissolves vital and necrotic tissue by breaking down proteins into amino acids, while the mineral component is left relatively intact [250]. It is known to reduce the modulus of elasticity and the flexural strength of dentine [,258,259].

Temperature rise of a 5% NaOCl solution from 21° to 50°C resulted in a thinner, made of finer, less well-organized particles smear layer[269]. However, higher concentrations of NaOCl don't seem to have an impact on flushing out loose debris from the canals. Baumgartner et al [270] found similar results for NaOCl solutions 1%, 2.5% and 5.25% on instrumented and uninstrumented surfaces in the middle third of root canals.

Chlorhexidine (CHX)

Chlorhexidine is a broad-spectrum antimicrobial agent effective against gram negative and gram-positive bacteria, which was first suggested for use in

Endodontics in 1959 [271]. Unlike NaOCl, it cannot dissolve organic substances and necrotic tissues present in the root canal system. In addition, like NaOCl, it is ineffective in killing all bacteria and removing the smear layer [272,273].
A comparison of the cleaning effects of 2% chlorhexidine and NaOCI gave similar residual debris scores in the cervical third of roots with both agents, although smear layer removal was poor [62].

Hygrogen Peroxide (H202)

Hydrogen peroxide (H_20_2) has been used as an endodontic irrigant for years, mainly in concentrations ranging between 3% and 5%. It is active against bacteria, viruses, and yeasts. The tissue-dissolving capacity of H202 is clearly lower than that of NaOCI[275]. When used in combination with NaOCI, bubbling will occur as a result of nascent oxygen being released through the chemical reaction between these two liquids. Studies have shown that the combined use of NaOCI and H_20_2 results in a weakened cleaning effect[270]. Hydrogen Peroxide is generally no longer recommended as a routine irrigant.

Chelator solutions

Unfortunately, no irrigating solution is capable of acting simultaneously on the organic and inorganic elements of the smear layer. Thus, chelating agents, such as EDTA, citric acid and MTAD, are used for the removal of the inorganic portion of the smear layer. Chelators act through the creation of a stable calcium complex with dentin mud, smear layers, or calcific deposits along the canal walls. Removal of smear layer facilitates access of solutions and may help prevent apical blockage and aid disinfection [280]. Effectiveness of these agents depends on the length of the canal, the depth of penetration of the material, application time, hardness of dentin, pH and concentration of the material to obtain maximum effect [281].

Ethylenediamine Tetra-Acetic Acid (EDTA)

Ethylenediamine Tetra-Acetic Acid (EDTA) was the first chelating agent described by Nygaard - Ostby in 1957 for use in Endodontics. It is a specific chelating agent for the calcium ion, and therefore for the dentin. Dentin is a molecular complex which counts with calcium ions in its composition. The chelating agent is applied over dentin; this facilitates dentin disintegration for the EDTA [282]. Calcium binding results in the release of protons, and EDTA loses its efficiency in an acidic environment. Thus the action of EDTA is thought to be self-limiting [283]. EDTA used for one minute inside the root canal is effective to remove dentinal debris[283]. Nevertheless, a 10 minute application will erode dentin around and inside the canals. This erosion is due to an excessive opening of the tubules, and a broadening of the tubule diameter. For the aforementioned reasons, use of EDTA for periods longer that 1 minute is not recommended [284]. EDTA had a significantly better antimicrobial effect than saline solution; however it exerts its stronger effect when used synergistically alternating with NaOCl, although no disinfecting effect on colonized dentin could be demonstrated [285]. Moreover, chemical

analyses indicated that chlorine, the active agent in NaOCl, becomes inactivated by EDTA[74]. EDTA does not dissolve organic matter [234,287].

Concluding, an EDTA solution should be preferably used at the end of root canal preparation in order to remove the smear layer[233]. Many authors indicate canals should be irrigated at the end of instrumentation with the sequential use of EDTA and NaOCl [234,287,288]. Irrigation with 17% EDTA for one minute followed by a final rinse with NaOCl is the most commonly recommended method for smear layer removal [289].

Citric acid

The use of 10% citric acid as final irrigation has shown good results in smear layer removal [290]. In vitro studies have shown their cytotoxicity, and 10% citric acid has proven to be more biocompatible than 17% EDTA-T and 17% EDTA [291,292]. Sceiza et al evaluated the inflammatory response of 17% EDTA, 17% EDTA-T, and 10% citric acid in bony defect created in rat jaws and they concluded that 10% citric acid showed less aggressive in inflammatory response [292]. The use of 25% citric acid was found to be ineffective in eradication of biofilms of Efaecalis after 1, 5, and 10 min of exposure [293].

MTAD

MTAD is relatively new irrigating solution supposedly capable of both removing the smear layer and disinfecting the root canal system[294]. It is a mixture of 3% doxycycline hyclate, 4.25% citric acid, and 0.5% polysorbate-80 (Tween 80) detergent[294]. Compared to EDTA 17% it has shown better results in removing the smear layer from the apical, but not the cervical and middle root canal third [294]. It exhibits superior cleaning action when used in conjunction with NaOCl and the erosive effects of this combination are less than those of EDTA and NaOCl [294].

Irrigation devices and techniques

In a continuous effort to overcome the well documented limitations of current chemomechanical preparation of root canals and to augment the antibacterial and cleaning effect of the irrigants, several methods and devices have been proposed, tested and used.

Passive ultrasonic irrigation (PUI)

The first use of ultrasonics in endodontics was reported by Richman (1957). There are two types of ultrasonic irrigation: one where irrigation is combined with simultaneous ultrasonic instrumentation (UI) and another without simultaneous instrumentation, called passive ultrasonic irrigation (PUI) [297,298]. PUI has been shown to be more effective in removing simulated pulp tissue from the root canal system or smear layer from the root canal wall than UL This can be explained by a reduction of acoustic streaming and cavitation [298], although several studies have shown that acoustic streaming should be regarded the main mode of action of ultrasonics [299].

Passive ultrasonic activation was first described by Weller et al [297], and relies on the transmission of acoustic energy from an oscillating file or smooth wire to an

irrigant in the root canal. The energy is transmitted by means of ultrasonic waves and can induce acoustic streaming and cavitation of the irrigant. After the root canal has been shaped to the master apical file (irrespective of the preparation technique used), a small file or smooth wire (for example, size #15) is introduced in the center of the root canal, as far as the apical region. The root canal is then filled with an irrigant solution, and the ultrasonically oscillating file activates the irrigant.

Several studies however, are yet to deliver a conclusive evidence-based answer for the effectiveness of this method in smear layer removal. Cameron et al[300,301] reported the complete removal of the smear layer when combining 3% NaOCl with PUI for 3 and 5 minutes. Likewise, Alacam [302] and Huque et al [303] could completely remove the smear layer using 5% NaOCl with 3 min of PUI and 12% NaOCl with 20s of PUI respectively. Also, a 5% NaOCl during 3 min PUI removed smear layer more effectively than 0.5% NaOCl from the middle and apical third of the canals[91]. It is obvious that there is a great variation on the concentration of the NaOCl as well as the time of activation of the irrigant among the studies, which certainly does not contribute in reaching more definitive conclusions.

Ciucchi et al [304] and Abbott et al [305] both reported that the use of ultrasounds did not enhance smear layer removal either with EDTA or combination of NaOCl and EDTA as irrigants. It is also well documented that PUI with water used as irrigant is not effective in removing the smear layer [303,306], a fact that is attributed to the difference in physical properties between water and NaOCL.

EndoActivator

The EndoActivator System (Dentsply Tulsa Dental Specialties) is comprised of a cordless, contra-angled, sonic handpiece and the EndoActivator tips. Its 3-speed sonic motor switch provides options of 10000, 6000 and 2000 cycles per minute (cpm). The tips are made from a medical-grade polymer and are 22 mm long. There are small, medium and large tips (yellow, red, and blue color-coded) that correspond to file sizes 20/02, 25/04, and 30/06, respectively.

In a recent study by Mancini et al.[307], the EndoActivator was more effective in removing the smear layer than PUI at the 3, 5 and 8 mm from the apex when tested in single-rooted mandibular premolars. Kanter et al[308] also found that the EndoActivator produced better results on canal cleanliness than ultrasonic and syringe irrigation. Rodig et al [309] evaluated the cleaning efficacy of various techniques in curved root canals. They concluded that activation of NaOCl and EDTA did not enhance debris removal but did result in better smear layer removal only the coronal region. EndoActivator was significantly more effective than ultrasonic agitation and CanalBrush.

On the other hand a study by Klyn et al [310] showed no statistical difference in canal and isthmus cleanliness when EndoActivator, F file, ultrasonic agitation and syringe irrigation were compared in mandibular molars.

EndoVac

The EndoVac system (Discus Dental, Culver City, California) is an irrigation system consisting of a deliver / evacuation tip attached to a syringe of irrigant and a high-speed suction source of the dental unit. As the cannulas are placed in the canal, negative pressure pulls irrigant from a fresh supply in the chamber and down into the canal to the tip of the cannula and then out through the suction hose [311]. The dimensions of the needle are size #55 with a 2% taper. It is assumed that apical extrusion of the irrigant will probably be reduced, since the canal is irrigated with negative (as opposed to positive) pressure [311].

Early studies showed significantly better debridement for the EndoVac compared to needle irrigation, at 1 mm from the working length[311,312]. Yoo et al [313] also reported favorable results for the EndoVac in cleaning debris from canal and isthmus of mandibular molars compared to syringe irrigation and ultrasonic agitation. Likewise, Howard et al.[314] found that EndoVac, PiezoFlow and Max-i-Probe with similar volumes significantly brush-covered NaviTip FX needle, manual dynamic irrigation, PUI, and Endovac), none of the methods completely removed debris and smear layer but ultrasound and EndoVac removed more debris than manual techniques. Finally, a study by Saini et al [315,316] also found better cleaning efficacy for the EndoVac, compared to Max-i-Probe and NaviTip, both at 3.5 and 1.5 mm levels of single-rooted teeth.

Self-Adjusting File (SAF)

The SAF is a recently introduced endodontic file which is discussed in detail in Chapter 3. However, it can also be considered as an irrigation device since the file is hollow, which allows for continuous irrigation. The irrigant is delivered through a free-rotating hub to which a silicone tube is attached. Either a special irrigation unit (VATEA, ReDent, Ra'anana, Israel) or any physio-dispenser-type unit may be used to deliver a constant flow of irrigant at 5 ml/min. This maintains a continuous flow of fresh, fully active irrigant, facilitating an outflow of tissue debris and dentin powder that is generated by the file use. No positive pressure is thought to be created in the canal during this continuous irrigation procedure. The open metal lattice allows the irrigant to escape freely, presumably minimizing the risk of irrigant transportation beyond the apical foramen [317].

Lasers

The first use of lasers in endodontics has been reported by Weichman and Johnson [318]. Some investigators suggested the use of lasers to vaporize tissue in the main canal, remove the smear layer and eliminate the residual tissue in the apical portion of root canals[319]. However, the efficacy of lasers greatly depends on factors such as wavelength, power level, duration of exposure, the absorption of light in the tissue, the geometry of the root canal and the tip-to-target distance [320-322]. Although, for some lasers the removal of debris and smear layer has been reported [323], the main difficulty continues to be access to small canal spaces with the relatively large probes emitting the light straight ahead [324]. There is promising evidence on smear layer removal by the use of Photon-induced photo-acoustic streaming phenomenon [325].

Ozone (HealOzone)

Ozone, a naturally occurring compound consisting of three oxygen atoms, has been shown to be a powerful and reliable antimicrobial agent against bacteria, fungi, protozoa and viruses [326]. A special device for intracanal application of ozone named HealOzone (KaVo Dental GmbH, Biberach/Riss, Germany) is marketed for use in Endodontics. However, in vitro studies on its effectiveness produced controversial results except that it is ineffective in removing the smear layer efficiently, unless combined with NaOCl or EDTA [327-330].

CHAPTER 2- EXPERIMENTAL PART

5.Materials and Methods:

Aim

The aim of this study is to analyse composition of the SAF file and all the other NiTi files tested, through scanning electron microscopy with x-ray energy-dispersive spectrometric analysis (EDX), compare the ability of these files to remove bacterial biofilm from oval anatomies, and finally evaluate, through scanning electron microscopy, how all these different shaping techniques affect the root canal walls. (Tab.1)

Null hypothesis :

All files share equal efficacy on cleaning and disinfection of long oval canals.

Tab.1 : Ni-Ti files tested during this investigation

Brand name/ taper	**Movement**	**Manufacturer**	**ISO num. /taper**	**Lot num.**
Self Adjusting File/	Pecking motion	ReDent-Nova, Ra'anana, Israel	Hollow	
Waveone/ Variable .08-.04	Reciprocating	Dentsply, Maillefer, Ballaigues, Switzerland	Variable	1095671
BT-Race /0-.06	Rotating	FKG Denatire, LaCaux De Fonts, Switzerland	Variable	AK22

Experiment 1:Composition and microstructure study

For this investigation three files from each NiTi group were used. For the WO group, three WaveOne (40/.08) were tested, for the BTR group three BT4 (40/0.04) and for the SAF group three SAF files. The instruments were placed on stage and Seconday Electron (SE) images and Baclscattered Electron (BE) images were acquired with a scanning electron microscope (Quanta 200, FEI[331], Hillsboro, Oregon, USA) operating at high vacuum chamber conditions (4.9 X 10−6 Torr pressure), 20 kV accelerating voltage, 98μA beam current and 200X nominal magnification. The elemental composition of each sample was determined by X-ray energy dispersive spectroscopy (EDX). Three spectra were collected from the surface of each file employing an X Flash 6|10 Silicon Drift Detector (Bruker, Berlin, Germany) under the aforementioned conditions and spot analysis mode. The quantification of EDX spectra was carried out by the dedicated software (ESPRIT version 1.9, Bruker) operating in a standardless mode with ZAF (atomic number, absorbance, fluorescence) correction factors.[331].

Experiment 2:Microbial biofilm removal capability

One hundred single-rooted and single-canaled extracted teeth were used. All teeth were initially examined radiographically in both directions (buccal-lingual and mesio-distal) for the presence of caries and to confirm that they exhibited an oval-shaped ratio (bl-md >2.5:1, 5 mm from the apex).

Standard access cavities were prepared in all teeth, and the root canals were enlarged up to an apical diameter of K-file size 25 with hand files under continuous irrigation with isotonic saline. The smear layer was removed by irrigation with 5 ml of 17% EDTA (pH 7.8) (Ultradent, South Jordan, UT) for 3 min followed by 5 ml of 2.5% NaOCl. Irrigation was performed with a 30G NaviTip needle (Ultradent, South Jordan, UT) placed as apically as possible to ensure that the irrigants reached the entire length of the canal. Residual NaOCl was inactivated with 10 ml of 10% $Na_2O_3S_2$, which was then rinsed with 5 ml of distilled water. Teeth were immersed in trypticase soy broth (TSB), ultrasonicated for 1 min to release entrapped air and allow penetration of the culture media into root irregularities and then sterilized in an autoclave for 20 min at 121 °C [332-334]

An E. faecalis strain (ATCC 29212) was used to infect the root canals (16). A suspension was prepared by adding 1 ml of a pure E. faecalis culture grown in TSB for 24 hours to 5 ml of fresh TSB. One millilitre of this suspension was used to inoculate each of the flasks where teeth were immersed to become infected. E. faecalis was allowed to grow for 30 days at 37 °C under gentle shaking. The culture media was replenished every week. After the incubation period, the incubation media excess was removed from the external root surface with sterile gauze.

Teeth were randomly divided into five groups, each comprised of 25 teeth (n=25). Each tooth apex was sealed with epoxy resin to create a vapour lock effect and to prevent apical bacterial leakage[335](IMG. 8). The working length was determined

with a size 20 K-file. At this point and before the canal preparation, initial bacteriological samples were taken, as described later. The teeth were then treated as follows (IMG: 9,10,11):

Group 1: The SAF was introduced into the canal until resistance was felt and activated. Shaping proceeded with a pecking motion in an apical direction until a working length was reached. The file had a working frequency of 5,000 movements per min and an amplitude of 0.4 mm. The system employed constant NaOCl irrigation. A total of 15 ml of 2.5% NaOCl was used for each tooth. This was the standard amount of irrigant used for all teeth of all groups during shaping. After shaping, the canals were rinsed with 5 ml of 17% EDTA, followed by 5 ml of 2.5% NaOCl. Inactivation of sodium hypochlorite was performed by adding 5 ml of 10% $Na_2O_3S_2$, which was then rinsed with distilled water. The average total time that the NaOCl remained in the canal was 4.9 min (range 4.4-6 min). The working length in this group varied from 21 to 24 mm with an average of 21.4 mm.

Group 2: The teeth of this group were shaped with the BT system. First, the BT1 file (10/0.06) was introduced into the canal and, using a pecking motion, reached the working length. Then, the BT2 (35/0.0), BT3 (35/0.04) and BT4 (40/0.04) files were used in the same manner. During shaping, 15 ml of 2.5% NaOCl was used, 3.75 ml after each file. After shaping, each canal was rinsed with 5 ml of 17% EDTA, followed by 5 ml of 2.5% NaOCl, which was inactivated by 5 ml of 10% $Na_2O_3S_2$ and then washed with distilled water. The average total time that the NaOCl remained in the canal was 6.7 min. (range 5.5-8.2 min.). The working length in this group varied from 20 to 25 mm with an average of 22.3 mm.

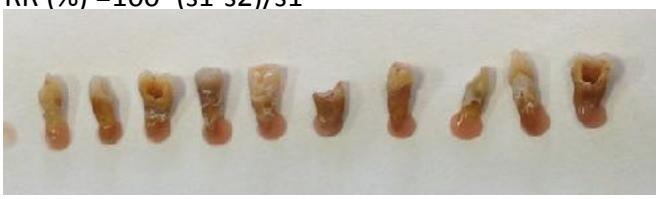
Group 3: a WaveOne file (40/.08) was used to shape the canal in a reciprocating motion. After the file encountered resistance, it was removed, cleaned of debris, and re-introduced until it reached the working length. During and after shaping, the volume of irrigant and the irrigation protocol were the same as those utilised in the the rest of the groups, as well as the protocol for inactivation of the irrigating solutions. The average time NaOCl remained in the canal was 5 min (range: 3.5-8.3 min). The working length in this group ranged from 21 to 24 mm, with an average of 22.5 mm. Regarding the speed and torque selection, all rotary files were used according to the manufacturer's recommendations.

Group 4: Teeth were shaped with stainless steel hand K-files (FKG Dentaire, LaSaux-de-Fonds, Switzerland; 0.02) with the step back technique. The final apical size was 40/0.02. During shaping, 15 ml of 2.5% NaOCl was used. Smear layer removal and inactivation of irrigants were performed as in Groups 1, 2 and 3. The average time that NaOCl remained in the canal was 12 min (range 8.9-14.2 min.). The working length in this group varied from 21 to 25 mm, with an average of 21.2 mm.

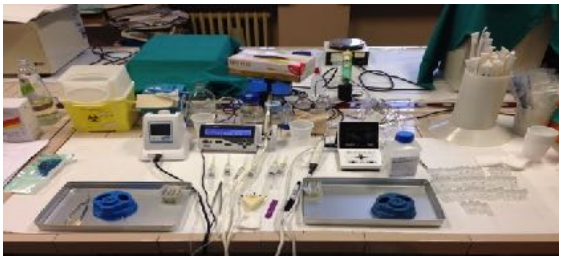
Sampling Procedures:

Two samples were collected from each tooth: one before shaping (s1) and one after chemomechanical preparation (s2).

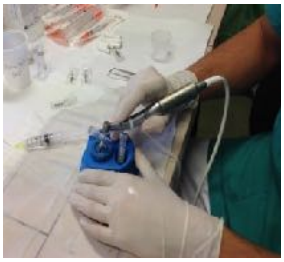
The first sample (s1) was collected after working length determination. The root canal was filled with sterile saline solution, and 3-5 sterile paper points (diameters 30.02 and 20.02) were consecutively placed to the working length and remained in the canal for 1 min each. The paper points were immediately transferred to tubes containing 1 ml of sterile saline solution and processed immediately.
The second sample (s2) was collected after inactivating NaOCl in the root canal by flooding the canal with sodium thiosulfate for 5 min and then washing with saline solution (10 ml). Then, a sterile precurved stainless steel hand K-file (#20) was used to dislocate debris from the buccal and lingual canal surface by a pulling motion. All of the content of the root canal was absorbed by sterile paper points, each remaining in the canal for 1 min. Sampling continued until the canal was completely dry. The paper points were transferred to tubes containing 1 ml of sterile saline solution and processed immediately[332]
All samples were vortexed for one min, followed by 10-fold serial dilutions in saline. Then, 100 ml aliquots were plated onto blood agar and incubated at 37 °C for 48 hours. The number colony forming units (CFUs) grown were counted and then transformed into actual counts based on the known dilution factors. The number of CFUs (quantitative) per sample was evaluated. The relative percentage reduction (RR) from s1 to s2 was calculated according to the formula:
RR (%) =100*(s1-s2)/s1



IMG. 8: Contaminated teeth with the apical block of epoxy resin



IMG. 9: Laboratory setting



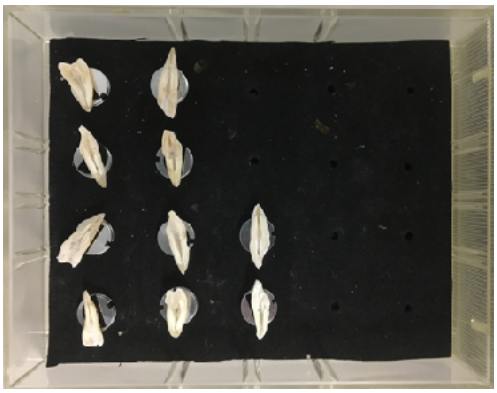
IMG. 10: During chemomechanical preparation IMG. 11: During sampling

Experiment 3:Root canal surface cleanliness evaluation

The aim of this part of the study was to evaluate the shaping efficacy and morphology of root canal after root canal preparation with the files tested. For this evaluation, twenty teeth were used, five teeth for each group (n=5).

After endodontic treatment following the shaping and irrigation protocols described in the microbiological evaluation all teeth were grooved longitudinally along the buccal and lingual surfaces with a carborundum disc at medium speed, with care not to penetrate into the canal. Teeth were then cleaned and dried, immersing them in to ascending concentrations of alcohol (50%-75%-85%-100%) each for 30 min and then left in the silicate dehydrator for one night (IMG.12), before being split with a micro-blade and mallet (IMG.13). High magnification optical microscope (keyennce digital microscope VHX-500, Osaka, Japan)(IMG.14) and scanning electron microscope (Zeiss Sigma 300VP, Oberkochen, Germany) (IMG.15) were utilised for the evaluation of the half with the most visible part of the apex present.

IMG. 12: Teeth after dehydration and ready for optical observation



IMG. 13: Teeth after dehydration and ready for optical observation

Microscopic evaluation

SEM microscopic evaluation was performed in all samples in 200x for debris and 1000x for smear layer. The SEM operator did this under X50 magnification. Magnification was then increased to 200x and 1000x and the area of the canal was photographed and used for scoring. Each of these sections was examined using the Zeiss High Vacuum Scanning Electron Microscope (sigma VP300). To ensure standardization of the area examined for each sample, the central beam of the SEM was directed to the center of each third of the canal space being analysed. This distance was 3 mm (location A), 6 mm (location B), and 9 mm (location C) from the apex. In order to confirm findings from SEM microscopy, a mapping of each root canal was performed through optical microscopy in 200x magnification using the optical digital microscope. This microscope has the ability to acquire in focus photos in high magnifications of the whole extent of the surface of the root and compose them in a single image. In that way the evaluation was performed in the whole extend of the root canal instead of selected spots. For the best evaluation from the examiners the photos where then divided in three different areas, apical middle and coronal third, calculating 3,6 and 9 mm from the apex. To ensure intra-examiner consistency, all specimens were evaluated twice by each examiner.

The recordings of the groups were statistically analysed in order to explore potential differences between techniques but also between examiners and root specimens. Afterwards, Presence of debris was defined as the presence of particles or chips of any structure on the surface of the root canal [336]

Smear layer was defined as a surface film of debris retained on dentine after instrumentation with either rotary instruments or endodontic files, consisting of dentine particles, remnants of vital or necrotic pulp tissue, bacterial components and retained irrigant. [336]

The amount of debris present were graded between 1 and 4, as follows: [337]

Score 1: clumps of debris covering less than 25% of the canal wall area Score 2: clumps of debris covering 25 to 50% of the canal wall area

Score 3: clumps of debris covering 50% to 75% of the canal wall area

Score 4: clumps of debris covering more than 75% of the canal wall area

The amount of smear layer present were graded between 1 and 4, as follows: [337]

Score 1, little or no smear layer; covering less than 25% of the specimen; tubules visible and patent.

Score 2, little to moderate or patchy amounts of smear layer; covering between 25 and 50% of the specimen; many tubules visible and patent.

Score 3, moderate amounts of scattered or aggregated smear layer; covering between 50% and 75% of the specimen; minimal to no tubule visibility or patency.

Score 4, heavy smear layering covering over 75% of the specimen; no tubule orifices visible or patent.

IMG. 14: The optical microscope

IMG. 15: The SEM microscope

Statistical analysis:

*For the microbiological study, c*ontinuous variables are presented with median and interquartile range (IQR). Quantitative variables are presented with absolute and relative frequencies. For the comparison of S1 and S2 between the four study groups the non parametric Mann-Whitney test was computed. Bonferroni correction was used in order to control for type I error in case of multiple testing. Wilcoxon signed test was computed to compare S1 and S2 measurements. Differences in changes of CFU between the four study groups were evaluated using repeated measurements analysis of variance (ANOVA). CFU was log-transformed for the analysis of variance due to its skewed distribution. All p values reported are two-tailed. Statistical significance was set at 0.05 and analyses were conducted using SPSS statistical software (version 22.0).

*For the evaluation of cleanliness, k*appa values were calculated to assess the agreement between two measurements of the two examiners and between the two examiners; a maximum value of 1, corresponds to perfect agreement, values ≥ 0.75 are considered as excellent agreement, and values >0.4 indicate acceptable reliability. Chi-square tests were used for the comparison of proportions. For the comparisons of the results between the four methods multivariable ordinal logistic regression models was used. Odds Ratios reported by these models correspond to the probabilities of higher scores which mean worse outcomes. Odds ratios with

their 95% confidence intervals were computed from the results of the ordinal logistic regression analyses. P values reported are two-tailed. Statistical significance was set at 0.05 and analysis was conducted using SPSS, version 22.00.

6.Results

Experiment 1:Composition and microstructure study

Representative EDX spectra with the characteristic peaks of Ni and Ti is illustrated in Fig. 1,2 and 3 for WaveOne, BtRace and SAF respectively. Images of the files testes are demonstrated (IMG. 16-21). The results of the elemental composition obtained from the EDX analysis are listed in Table 2. Wave One demonstrated higher content in Ni than the other two files tested.

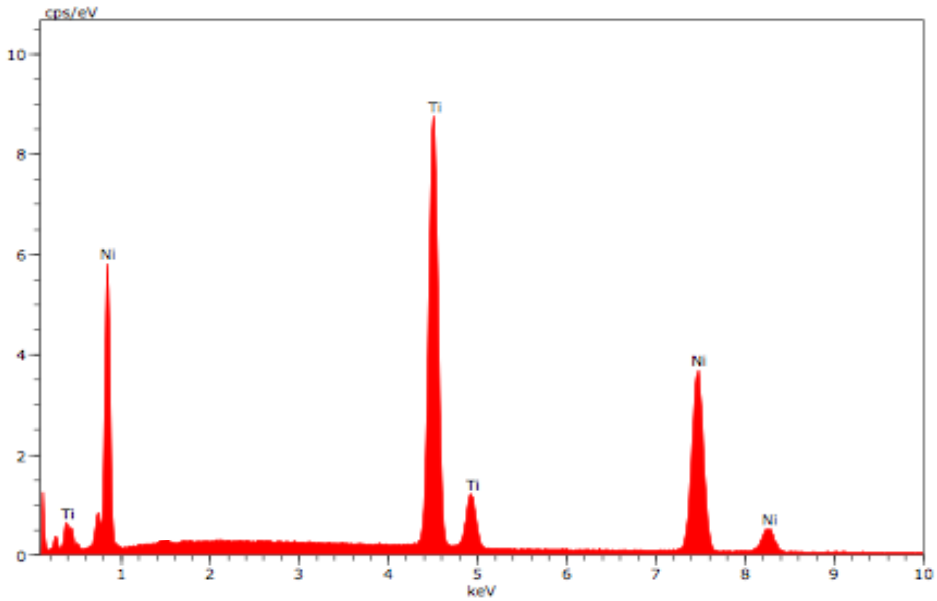
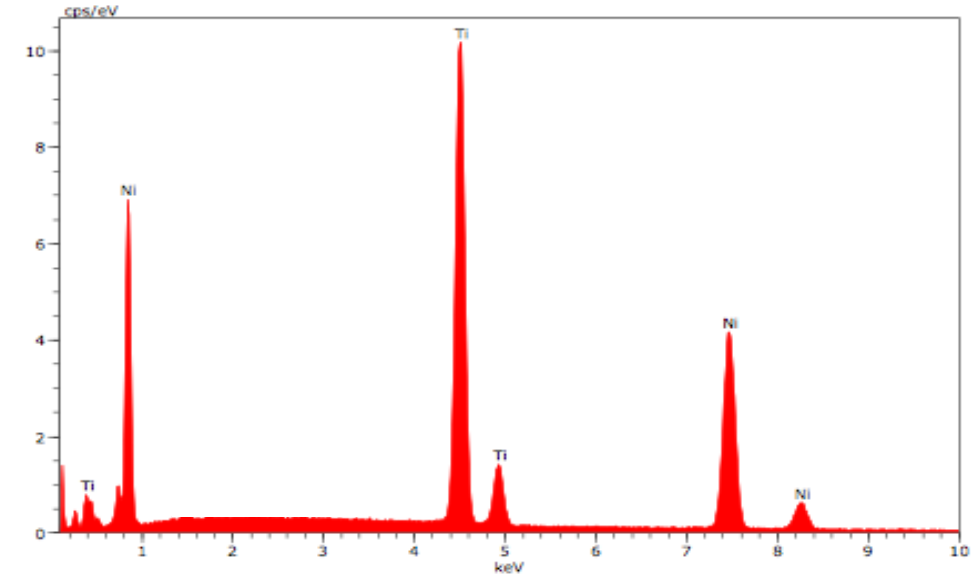


FIG. 1: Representative Wave One spectrum



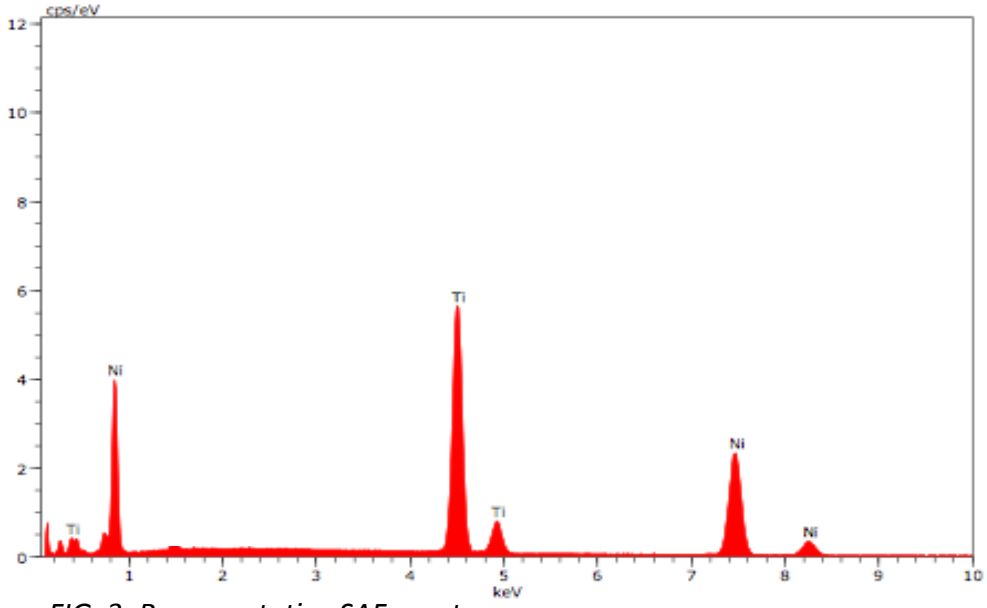
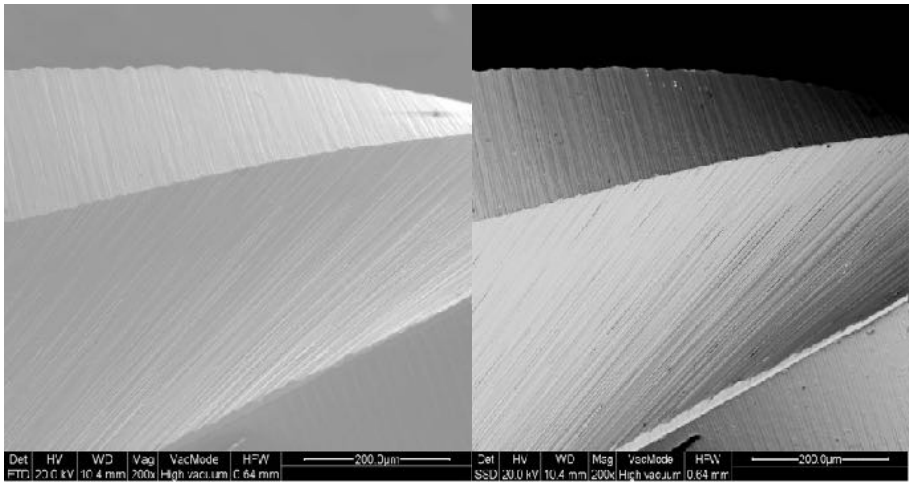
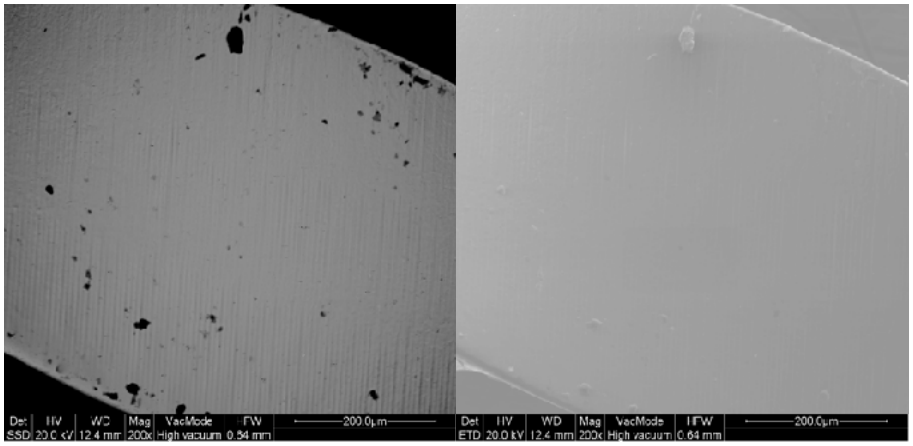


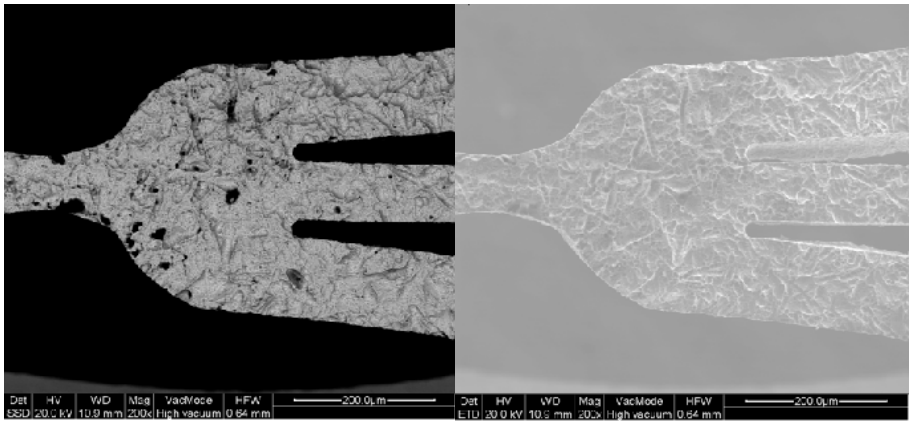
FIG. 3: Representative SAF spectrum



IMG. 16-17: Wave One under SEM-EDX investigation



IMG. 18-19: BT-Race under SEM-EDX investigation



IMG. 20-21: SAF under SEM-EDX investigation

Tab.2: The quantitative results (% wt) for the products tested, according to EDX analysis. The results are sorted in an increasing Ni content

	Ni(%wt)	Ti(%wt)	SD
Wave One	52,14	46,79	
BT Race	51,99	47,12	
SAF	51,49	47,31	

Experiment 2:Microbial biofilm removal capability

The median S1 and S2 are shown in table 3. CFU median values at baseline were similar between the four groups. As far as it concerns S2 samples, CFU was significantly greater in Manual group as compared with WO, BT Race and SAF groups, while it was significantly lower in BT Race group as compared with all other study groups. (Tab.3)

Intragroup analyses evaluating the CFU reduction from S1 to S2 indicated a significant decrease in all study groups ($p<0.001$). The reduction from S1 to S2 for all groups is shown in Table 4.(Tab.4)

The mean reduction was 93.20% in the Manual group, 97.26% in the SAF group, 99.95% in the WO group and 99.96% in the BT Race group. The median reduction is shown in table 2. The overall reduction of CFU from S1 to S2 measurements was significantly different between the four groups as defined from repeated measurements ANOVA ($p<0.001$)-figure 1. Specifically, the reduction of CFU was lower in the Manual group as compared with the other groups, it was lower in the SAF and WO groups as compared with BT Race group ($p<0.05$). (FIG.4)

Tab.3: CFU values from the first and second sample for all study groups

	S1		S2		
	Median	IQR	Median	IQR	P**
Manual[a]	$6.53x10^5$	$4.15x10^5$-$1.33x10^6$	$9.73x10^3$	$4.93x10^3$-$2.87x10^4$	<0.001
SAF[b]	$5.60x10^5$	$3.48x10^5$-$1.49x10^6$	$7.82x10^2$	$1.74\ x10^2$-$1.60x10^3$	<0.001
WO[c]	$5.65x10^5$	$4.02x10^5$-$8.75x10^5$	$4.5x10^2$	$1.2x10^2$-$1.92x10^3$	<0.001
BT Race[d]	$1.13x10^6$	$4.30x10^5$-$2.35x10^6$	$1.45x10^2$	$1.50x10^1$-$4.55x10^2$	<0.001

P* (a vs. b)	0.938	<0.001
P* (a vs. c)	0.823	<0.001
P* (a vs. d)	0.332	<0.001
P* (b vs. c)	0.954	0.455
P* (b vs. d)	0.347	0.002
P* (c vs. d)	0.342	0.030

*p_value for group effect;** p-value for time effect

Tab.4 : % reduction from S1 to S2 measurements for all study groups

% Reduction from S1 to S2		
Group	Median	IQR
Manual	97.84	96.81-99.49
SAF	99.92	99.42-99.98
WO	99.95	99.96-100.00
BT Race	99.98	99.96-100.00

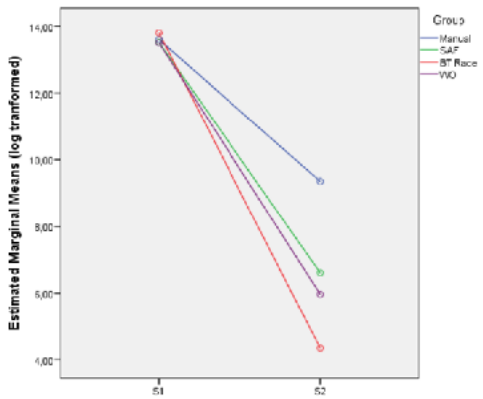


FIG.4: Marginal means of log transformed S1 and S2 values for the four study groups

Experiment 3: Root canal surface cleanliness evaluation:

Results for debris:

The average score of the two examiners for the four groups are shown at the following table, for each method separately:(Tab5)

Tab.5 : Average score of the two examiners for the four groups tested

Average score of the two examiners	Method															
	Optical								SEM							
	Group								Group							
	BTR		MANUAL		SAF		WO		BTR		MANUAL		SAF		WO	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
1	12	40.0	0	0.0	3	10.0	9	30.0	12	40.0	2	6.7	3	10.0	9	30.0
2	10	33.3	6	20.0	10	33.3	12	40.0	11	36.7	4	13.3	10	33.3	12	40.0
3	6	20.0	11	36.7	6	20.0	6	20.0	4	13.3	11	36.7	5	16.7	5	16.7
4	2	6.7	13	43.3	11	36.7	3	10.0	3	10.0	13	43.3	12	40.0	4	13.3

In optical method: There was a significant difference in the score between the four groups ($p<0.001$). Specifically, differences were found between groups BTR and SAF ($p=0.002$), where BTR method obtained cleaner specimens. The comparison between Manual and BTR ($p<0.001$) was also significant with BTR obtaining also cleaner specimens. Significant wasn't also the comparison between WO and Manual, where WO resulted better ($p<0.001$). Between WO and SAF the difference was not found significant ($p=0.008$).

In SEM method: There was a significant difference in the score between the four groups ($p<0.001$). Specifically, BTR achieved cleaner surfaces compared to SAF and Manual method ($p<0.001$). Significant was also the difference between WO and

Manual ($p<0.001$) with WO resulting cleaner root surfaces. Between WO and SAF the difference wasn't not significant($p=0.010$).

Neither in the optical method, nor in the SEM method the comparison between BTR and WO was found significant.

The average score of the two examiners for the four groups separately for locations A (apical), B (medium) and C (coronal), are shown table 6, for each method separately (Tab6):

Tab. 6: Average score of the two examiners for each location A,B&C

Location	Average score	Method															
		Optical								SEM							
		Group								Group							
		BTR		MAN.		SAF		WO		BTR		MAN.		SAF		WO	
		N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
C (9mm)	1	5	50	0	0	2	20	4	40	5	50	1	10.0	2	20	4	40
	2	4	40	4	40	5	50	3	30	4	40	3	30.0	5	50	3	30
	3	1	10	3	30	2	20	2	20	1	10	3	30.0	1	10	2	20
	4	0	0	3	30	1	10	1	10	0	0	3	30.0	2	20	1	10
B (6mm)	1	5	50	0	0	1	10	1	10	5	50	1	10.0	1	10	1	10
	2	3	30	2	20	4	40	6	60	3	30	1	10.0	4	40	6	60
	3	2	20	3	30	3	30	3	30	2	20	3	30.0	3	30	2	20
	4	0	0	5	50	2	20	0	0	0	0.0	5	50.0	2	20	1	10
A (3mm)	1	2	20	0	0	0	0	4	40	2	20	0	0.0	0	0	4	40
	2	3	30	0	0	1	10	3	30	4	40	0	0.0	1	10	3	30
	3	3	30	5	50	1	10	1	10	1	10	5	50	1	10	1	10
	4	2	20	5	50	8	80	2	20	3	30	5	50	8	80	2	20

Tab. 7: Results from ordinal logistic regression analysis for both methods combined:

	OR (95% CI)	P
Group		
SAF vs. Manual	0.56 (0.29-1.08)	0.085
WO vs. Manual	0.14 (0.07-0.27)	<0.001
BTR vs. Manual	0.09 (0.04-0.19)	<0.001
SAF vs. WO	4.13 (2.08-8.22)	<0.001
SAF vs. BTR	6.16 (3.07-12.34)	<0.001
WO vs. BTR	1.49 (0.76-2.93)	0.250
LOCATION		
B vs.C	1.82 (1.03-3.23)	0.039
A vs. C	4.62 (2.51-8.50)	<0.001
METHOD		
SEM vs Optimal	0.99 (0.62 – 1.60)	0.984

In the intergroup comparison WO and BTR groups were significantly better compared to Manual and SAF group. The comparison between the first two groups was did not result significant. (Tab.7)

The location with the cleaner surfaces was location C (9mm from the apex), and the worst was location A (3mm from the apex). The difference between them was significant.

In the comparison between the two investigation methods (optical microscope vs. SEM), no significance was found (p>.05).

Tab. 8: Results from ordinal logistic regression analysis for optical microscope method:

	OR (95% CI)	P
Group		
SAF vs. Manual	0.51 (0.20-1.30)	0.159
WO vs. Manual	0.12 (0.04-0.32)	<0.001
BTR vs. Manual	0.08 (0.03-0.22)	<0.001
SAF vs. WO	4.36 (1.64-11.62)	<0.001
SAF vs. BTR	6.36 (2.37-17.09)	0.003
WO vs. BTR	1.46 (0.56-3.82)	0.443
LOCATION		
B vs. C	1.88 (0.84-4.24)	0.126
A vs. C	5.03 (2.10-12.04)	<0.001

In the intergroup comparison WO group achieved significantly cleaner surfaces compared to the Manual method and also to the SAF group. Also the BTR group resulted significantly more effective in obtaining cleaner surfaces, compared to the Manual group. (Tab.8)

Overall the WO group achieved the better scores under this investigating method.

In the location comparison, the location A (3mm from the apex) resulted the most difficult to clean since it obtained the lowest score in all four groups.

Tab. 9: Results from ordinal logistic regression for SEM method:

	OR (95% CI)	P
Group		
SAF vs. Manual	0.61 (0.24-1.56)	0.306
WO vs. Manual	0.16 (0.06-0.42)	<0.001
BTR vs. Manual	0.10 (0.04-0.28)	<0.001
SAF vs. WO	3.93 (1.50-10.33)	0.005
SAF vs. BTR	5.97 (2.24-15.92)	<0.001
WO vs. BTR	1.52 (0.58-3.94)	0.391
LOCATION		
B vs.C	1.77 (0.79-3.97)	0.165
A vs. C	4.28 (1.82-10.07)	0.001

With the SEM microscope investigation, the group WO obtained cleaner surfaces compared to the Manual group. Significantly cleaner surfaces obtained also the BTR group compared to the Manual group. Even though the SAF group scored lower in comparison with the WO and BTR group, non of these differences resulted significant.

The cleaner are in all four groups tested was Location C (9mm from the apex), and compared to location A, the difference was significant.

The percent of agreement was 82.5% for both methods and the level of agreement between two measurements of the same examiner was excellent and significant ($p < 0.001$) as defined from the weighted Kappa coefficient that was equal to 0.86.

The percent of agreement was 84.2% for optical method and the level of agreement between two measurements of the same examiner was excellent and significant ($p < 0.001$) as defined from the weighted Kappa coefficient that was equal to 0.87.

The percent of agreement was 80.3% for SEM method and the level of agreement between two measurements of the same examiner was excellent and significant (p <0.001) as defined from the weighted Kappa coefficient that was equal to 0.85.

The percent of agreement was 76.7% for both methods and for each method separately and the level of agreement between two measurements of the same examiner was excellent and significant (p <0.001) as defined from the weighted Kappa coefficient that was equal to 0.79. (Tab.9)

An average score of the two measurements of each examiner was calculated. In cases where the average of the examiners' scores was not an integer the result was rounded to the nearest integer. (Tab. 10)

Tab. 10: Comparison of the average scores of the two examiners for both methods combined and for each one separately:

	Both methods				Method							
					Optical				SEM			
	Average score of examiner 2				Average score of examiner 2				Average score of examiner 2			
	1	2	3	4	1	2	3	4	1	2	3	4
Average score of of examiner 1	N	N	N	N	N	N	N	N	N	N	N	N
1	50	39	1	0	24	20	0	0	26	19	1	0
2	4	31	16	0	3	15	8	0	1	16	8	0
3	0	5	33	14	0	3	18	8	0	2	15	6
4	0	0	8	39	0	0	4	17	0	0	4	22

The percent of agreement was 63.8% for both methods and the level of agreement between the two examiners was acceptable and significant (p <0.001) as defined from the weighted Kappa coefficient that was equal to 0.71.

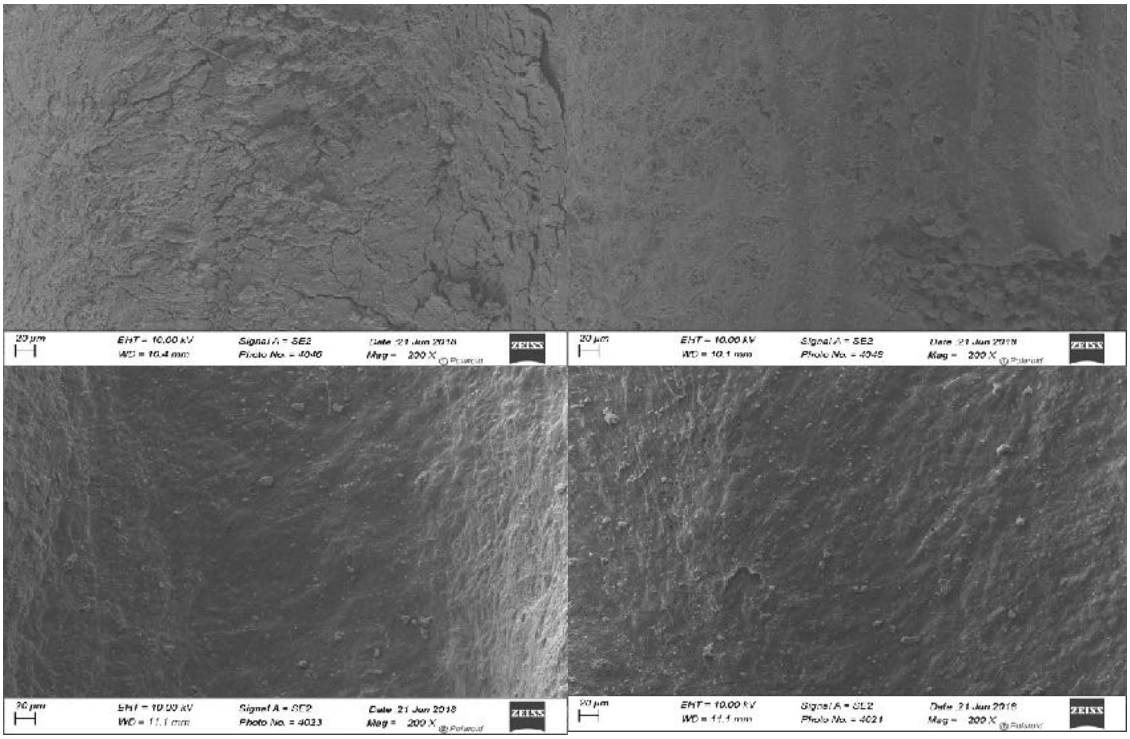
The percent of agreement was 61.7% for optical method and the level of agreement between the two examiners was acceptable and significant (p <0.001) as defined from the weighted Kappa coefficient that was equal to 0.69.

The percent of agreement was 65.8% for SEM method and the level of agreement between the two examiners was acceptable and significant (p <0.001) as defined from the weighted Kappa coefficient that was equal to 0.73.

Tab. 11: An average score of the two examiners for both methods combined and for each one separately

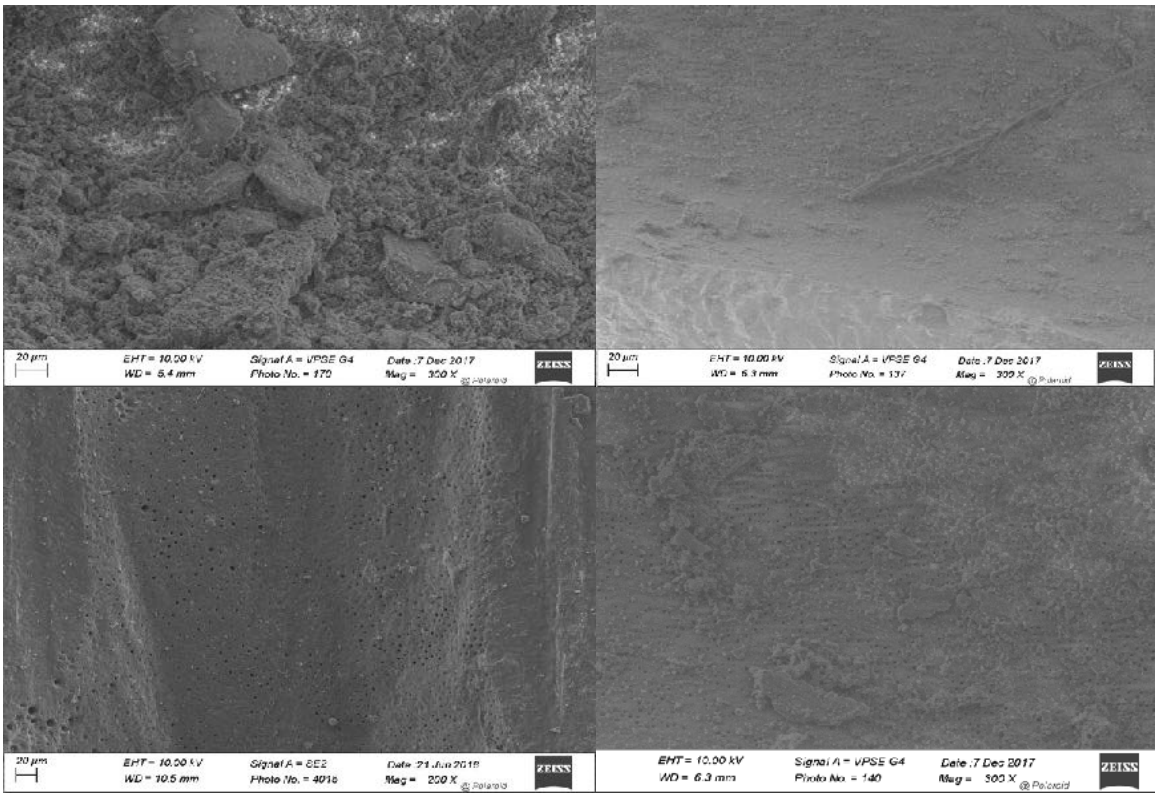
Average score of the two examiners	Both methods		Method			
			Optical		SEM	
	N	%	N	%	N	%
1	50	20.8	24	20.0	26	21.7
2	75	31.3	38	31.7	37	30.8
3	54	22.5	29	24.2	25	20.8
4	61	25.4	29	24.2	32	26.7

In Images 22-40 (IMG. 22-40) are shown characteristic tooth samples evaluated under SEM (200x) and optical microscope of all four groups which obtained all four scores (4-1).



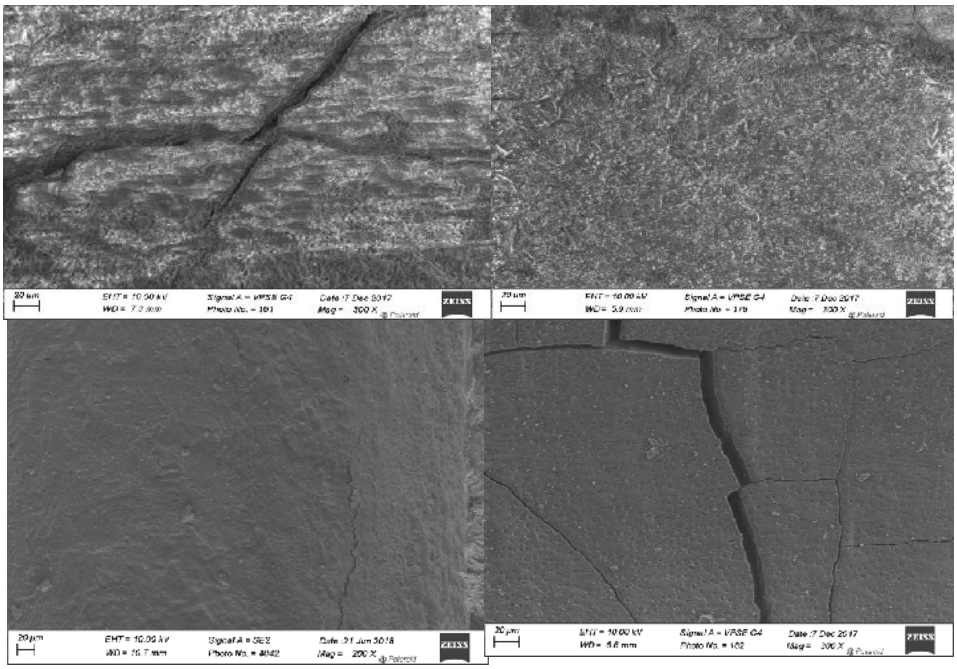
IMG. 22-25: Characteristic images from teeth treated with WO evaluated under SEM for debris (200x) with score 4 -1 (clockwise)

IMG. 26: Tooth treated with WO evaluated under evaluated under optical microscope for debris



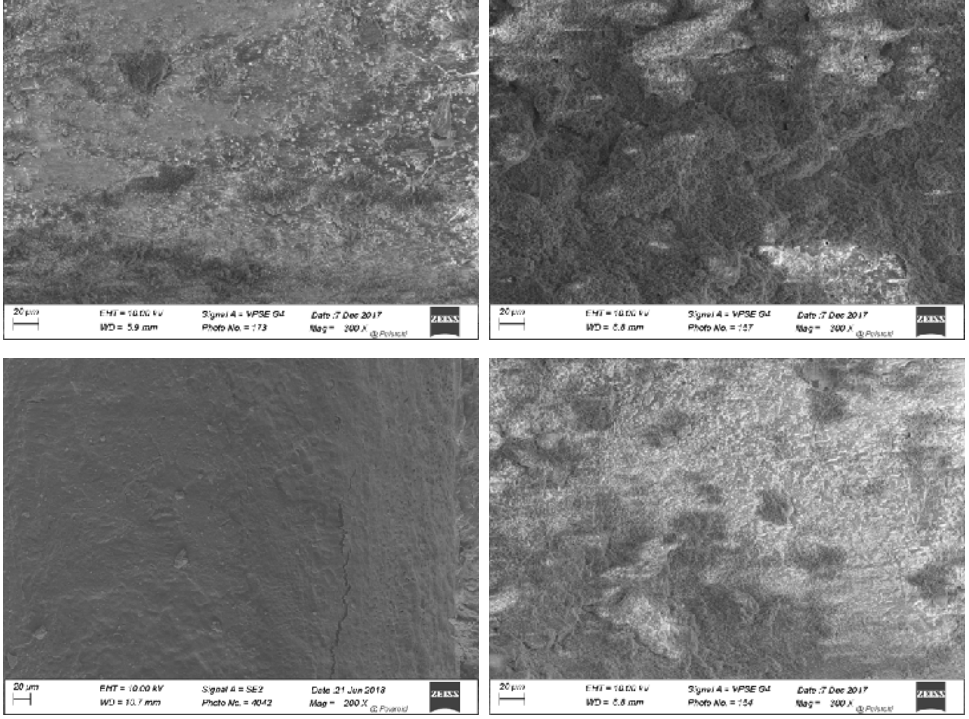
IMG. 27-30: Characteristic images from teeth treated with BTR evaluated under SEM for debris (200x) with score 4 -1 (clockwise)

IMG. 31: Tooth treated with BTR evaluated under evaluated under optical microscope for debris



IMG. 32-35: Characteristic images from teeth treated with SAF evaluated under SEM for debris (200x) with score 4 -1 (clockwise)

IMG. 36: Tooth treated with SAF evaluated under evaluated under optical microscope for debris



IMG. 37-40: Characteristic images from teeth treated with MAN evaluated under SEM for debris (200x) with score 4 -1 (clockwise)

IMG. 40: Tooth treated with MAN evaluated under evaluated under optical microscope for debris

Results for Smear Layer:

In Images 41-56 (IMG. 41-56) are shown characteristic tooth samples evaluated under SEM microscope (1000x) of all four groups which obtained all four scores (4-1).

Tab. 12: The average score of the two examiners for the four groups:

		Group							
		Manual		SAF		WO		BTR	
		N	%	N	%	N	%	N	%
Average score of the two examiners	1	2	6,7	3	10	9	30	12	40
	2	4	13,3	10	33,3	12	40	11	36,7
	3	11	36,7	6	20	5	16,7	4	13,3
	4	13	43,3	11	36,7	4	13,3	3	10

There was a significant difference in the score between the four groups ($p<0.001$). Specifically, differences were found between groups Manual and WO ($p=0.001$), Manual and BTR ($p<0.001$) and between SAF and BTR ($p=0.015$).

Tab.13: The average score of the two examiners for the four groups separately for locations A (apex), B (medium) and C (coronal):

Location			Group							
			Manual		SAF		WO		BTR	
			N	%	N	%	N	%	N	%
C (9mm)	Av.Score	1	1	10	2	20	4	40	5	50
		2	3	30	5	50	3	30	4	40
		3	3	30	1	10	2	20	1	10
		4	3	30	2	20	1	10	0	0
B (6mm)	Av.Score	1	1	10	1	10	1	10	5	50
		2	1	10	4	40	6	60	3	30
		3	3	30	3	30	2	20	2	20
		4	5	50	2	20	1	10	0	0
A (3mm)	Av.Score	1	0	0	0	0	4	40	2	20
		2	0	0	1	10	3	30	4	40
		3	5	50	2	20	1	10	1	10
		4	5	50	7	70	2	20	3	30

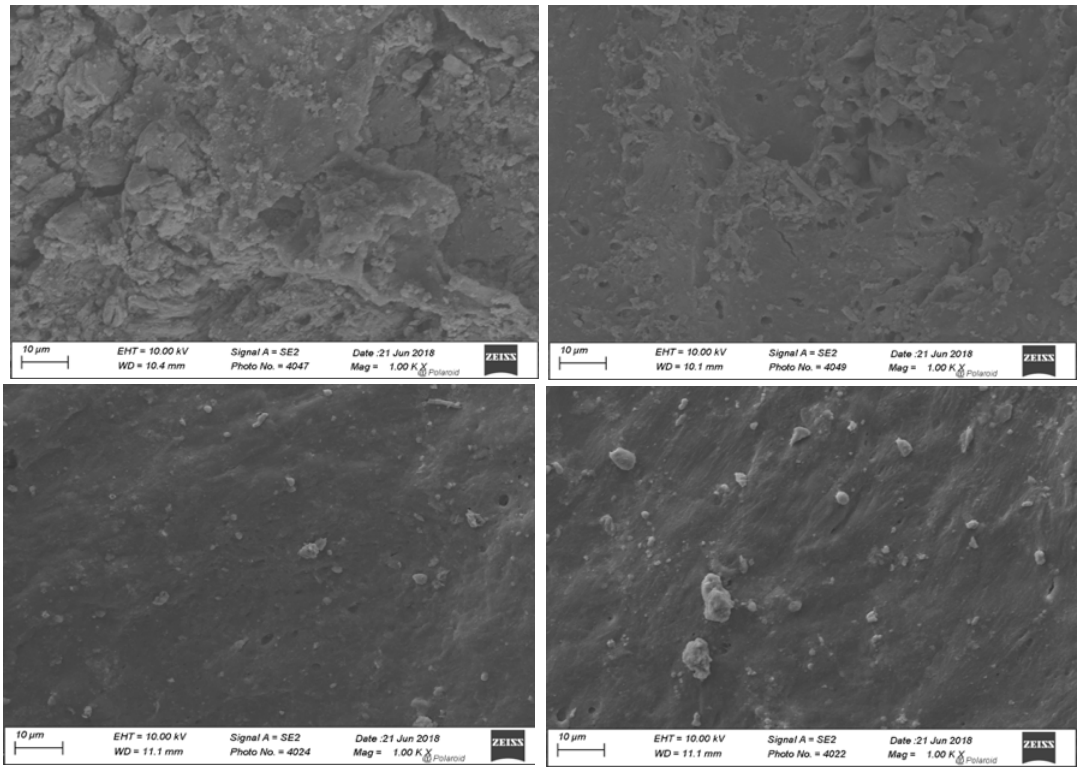
Tab.14: Results from ordinal logistic regression analysis

	OR (95% CI)	P
Group		
SAF vs. Manual	0.56(0.22-1.41)	217
WO vs. Manual	0.15(0.06-0.41)	<0.001
BTR vs. Manual	0.10(0.04-0.27)	<0.001
SAF vs. WO	3.65(1.40-9.53)	8
SAF vs. BTR	5.54 (2.09-14.68)	1
WO vs. BTR	1.51(0.58-3.93)	393
LOCATION		
B vs. C	1.78 (0.79-3.99)	161
A vs. C	4.04(1.72-9.45)	1

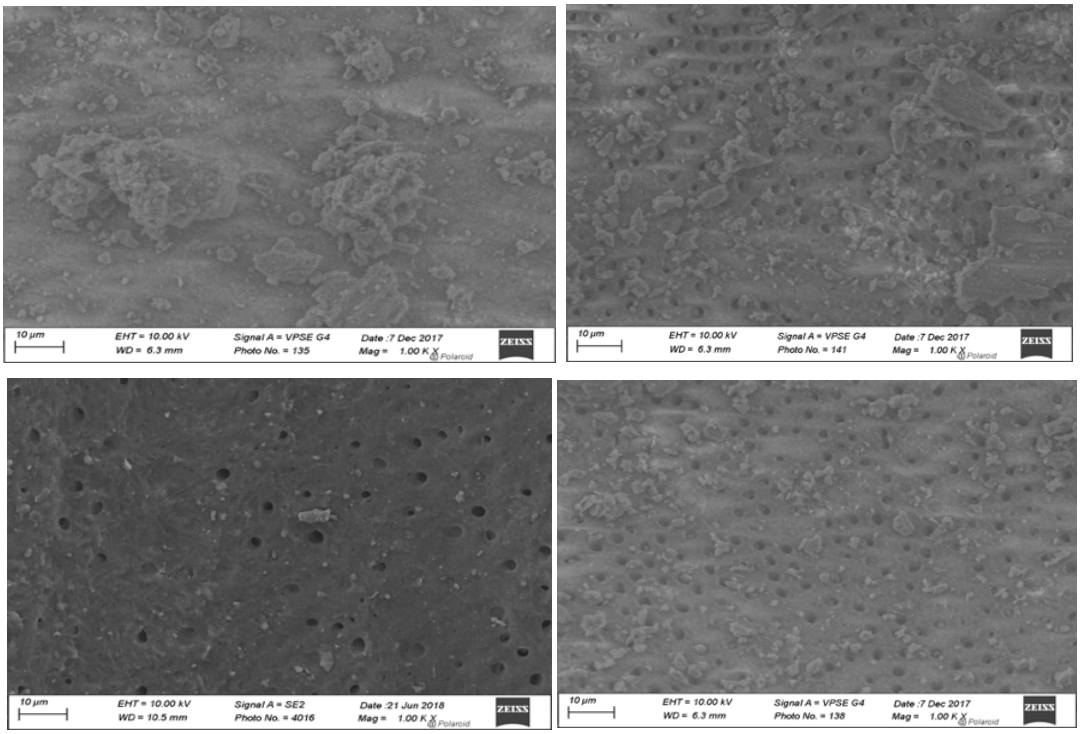
In the intergroup comparison, the WO group achieved significantly better results in clean root canal areas compared to Manual group. The same result was achieved from the BTR group compared to the Manual group. In the direct comparison between SAF group and WO, and between SAF and BTR, the SAF group resulted significantly less clean surfaces. No significance was found between WO and BTR group, but the WO group was slightly better in cleaning the apical third (location A) and the BTR group performed better in the rest two thirds (locations B and C).

All four groups performed worse in location A (3mm from the apex) compared to location C (9mm from the apex) and to location B (6mm from the apex). Non of these comparisons were significant.

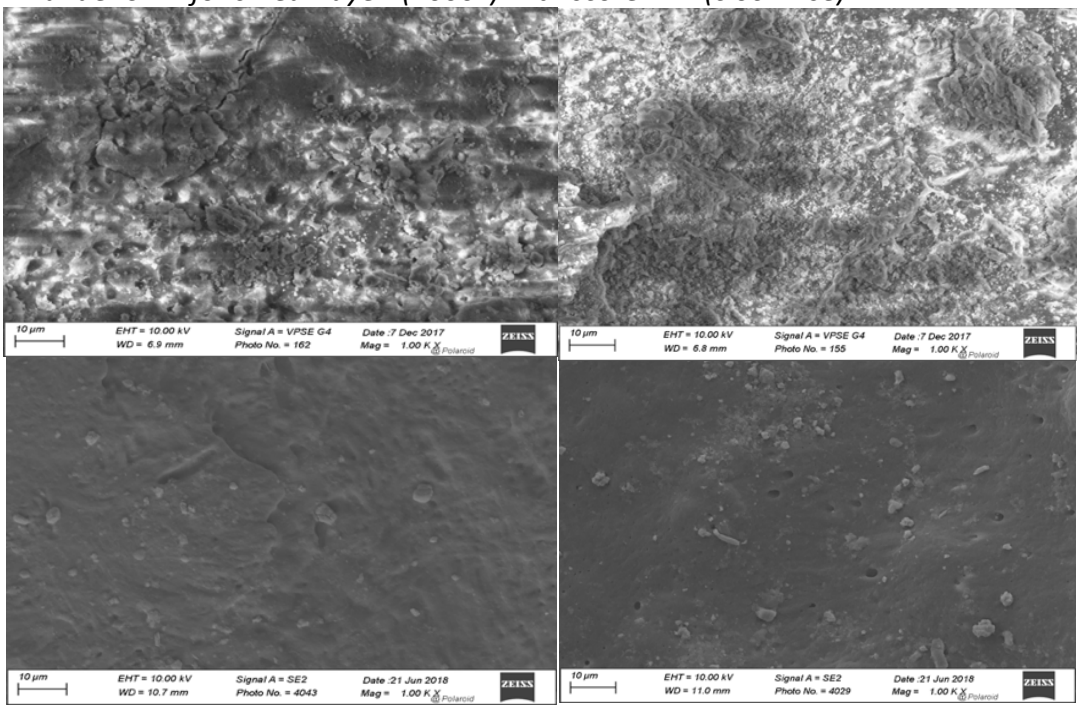
The percent agreement was 93.1% and the level of agreement between two measurements of the same examiner was excellent and significant ($p < 0.001$) as defined from the weighted Kappa coefficient that was equal to 0.84.



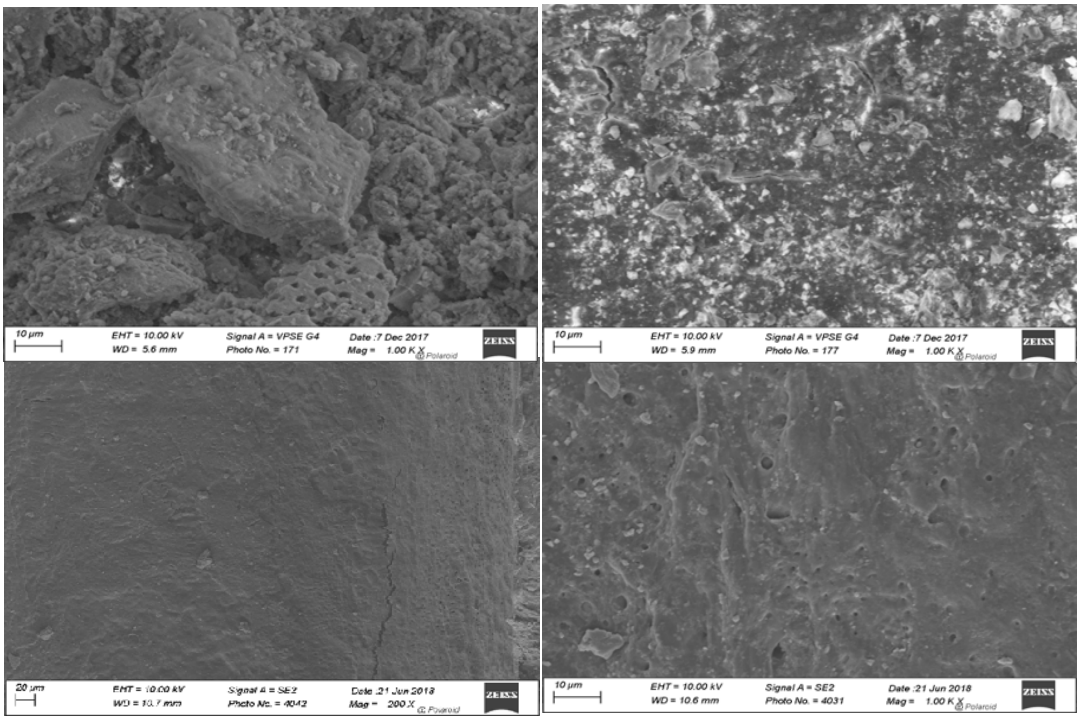
IMG. 41-44: Characteristic images from teeth treated with WO evaluated under SEM for smear layer (1000x) with score 4 -1 (clockwise)



IMG. 45-48: Characteristic images from teeth treated with BTR evaluated under SEM for smear layer (1000x) with score 4 -1 (clockwise)



IMG. 49-52: Characteristic images from teeth treated with SAF evaluated under SEM for smear layer (1000x) with score 4 -1 (clockwise)



IMG. 53-56: Characteristic images from teeth treated with MAN evaluated under SEM for smear layer (1000x) with score 4 -1 (clockwise)

7.Discussion

In this study we evaluated the effectiveness of four different shaping files in disinfecting and cleaning the root canal in long oval canals. The null hypothesis of this study was that all files share equal efficacy on cleaning and disinfection of long oval canals. As resulted from our investigation manual files performed worse compared to all three Ni-Ti systems in all tests performed.In addition the SAF obtained significantly worse results compared to WO and BTR files tested. This fact rejects the null hypothesis that all files are equally efficient in long oval canals.

It is known that both canines and premolars with one root canal present an oval diameter, especially at the coronal and middle level[338]. Moreover, 2/3 of upper and 1/3 of lower single rooted premolars present a long oval root canal anatomy at the 5mm from the apex level[2]. That means that the long diameter (bucco-lingual) is at least 2 times greater than the short diameter (mesio-distal) of the canal. Considering the difficulties in cleaning this type of canals, especially their buccal and lingual extensions[339] the selected teeth presented a suitable choice for examining the cleaning efficacy of the relatively new file systems tested.

The results of the EDX analysis showed that the instruments tested were manufactured by Ni-Ti alloys with elemental composition ranging from 51.49 to 52.4%wt in Ni content, values close to those (56 wt%Ni, 44 wt% Ti) reported previously [340,341]. These values are not within the nominal composition range specified in the ASTM standards for wrought Ni-Ti alloys used in medical devices and surgical implants[9]. Nevertheless, such compositional deviations, probably attributed to raw material variations during manufacturing, do not seem to affect the mechanical properties of the Ni-Ti instruments, as the thermomechanical history has a much more crucial effect on the final mechanical strength [343-345].

From the SEM analysis during EDX analysis the SAF instrument presented with an etched surface which is already mentioned by the manufacturer and described before [346]. The manufacturer claims that this surface treatment is meant to increase effectiveness of the file during chemomechanical preparation. In any case as far as the composition is concerned this surface treatment seems not to affect the composition of the file.

Regarding the effectiveness of the files tested in cleaning the root canal space of long oval canals from viable Enterococcus faecalis, the tested files showed statistically significant differences in the reduction of CFUs. All four files succeeded in significantly reducing the intracanal bacterial population. This is in line with several previous reports on the antibacterial efficacy of chemomechanical procedures [347-349]

According to Ricucci and Siqueira Jr,[350] most cases of periradicular infections are caused by intraradicular bacterial biofilms. They found biofilm structures in 80% and 74% cases of primary and secondary infections, respectively. In the present work, a biofilm was formed after 30 days of constant contamination of a root canal in order to simulate clinical conditions. This period has been shown to be sufficient to promote E. faecalis biofilm formation inside root canals[351], infact the root canals in the initial samples (S1) showed a high bacterial count, confirming the validity of the method.

A microbiological culture method was employed to assess the presence of viable microorganisms, which were sampled from the root canal lumen before and after chemomechanical preparation, using sterile paper points. Microbial populations were reduced by more than 97% after manual instrumentation and by 99% with the rest 3 Ni-Ti preparation techniques tested.

The intergroup analysis revealed a significantly greater ability of the rotary, reciprocating instrument and the SAF to remove bacteria than the manual technique, which is consistent with previous studies [354,355]. In comparison to manual files, the designs of both the round files and the SAF permit more effective opening of the space of the root canal [356], more effective transport of sodium hypochlorite to the apex and allow continuous irrigation in the case of the SAF [356], which provides a very logical explanation for our results.

In contrast there are studies that find manual instrumentation either more or equal effective to rotary files in cleaning the root canal system [357-361],a fact which is not in agreement to our results. However, all these studies were conducted in different anatomical sites (either round canals or small oval canals), and the rotary files used were different than the files tested in this experiment. In addition, none of these experiments were performed in long oval canals.

In the comparison of the SAF system to the other two NiTi shaping systems, the SAF proved less effective in disinfecting the root canal, these findings are compareable to the ones found in a direct comparison of BioRaCe to the SAF [362].

The results with round files (BTR and WO) could be surprising since many authors have underlined the difficulty of the files to prepare all root canal walls, especially in oval-shaped canals, where recesses are commonly left untouched [363-369]. In our study all teeth prepared with round files were shaped until diameter 0.040, which is a rather large preparation and is known to improve antibacterial effectiveness during chemomechanical preparation [370]

The evaluation method of CFU reduction used in this study is a not as sophisticated as oother microbiological techniques, but it is also a reliable method to conduct such comparisons [371-373]. Although PCR is considered a more advanced technique [370], it has been demonstrated that when studying bacteriological cleaning and the effectiveness of endodontic files, culture evaluation is as reliable as PCR [42].

The method used to obtain the post instrumentation sample (S2) was a modified method described by Alves et al. [374]. The modification consisted of scraping the narrow extremities of the oval canal with a sterile K-file to disengage residual bacteria attached to the walls, which otherwise would not have been counted.

Another finding was that instrumentation with the SAF left significantly more CFUs inside the root canals than the BT method. It has been previously found that rotary instrumentation was more effective, both microbiologically and through SEM observation, for cleaning oval canals than the SAF [375]. In contrast there is evidence suggesting that the SAF is more effective in eliminating bacteria from oval root canals than other rotary systems [43]. However, this finding was based on more negative cultures obtained by the SAF in S2 and not by actual differences in reducing CFU percentages.

Finally, the root canal cleanliness was evaluated through optical and SEM microscopy for the debris and SEM microscopy for smear layer. Debris and smear layer have been used as criteria to evaluate the cleaning efficiency of the different instruments because debris comprises dentine chips, residual vital or necrotic pulp tissue attached to the root canal wall that is considered

to be infected in many cases [376]. The smear layer is a thin surface film (1–2 μm) consisting mostly of inorganic material [377] that is produced when a canal is instrumented [378].Hence, no smear layer is found on areas that are not instrumented [379]. Although the influence of smear layer on the outcome of the endodontic treatment is controversial [380], it is recommended to remove the smear layer because of its potential deleterious effects [381,391]. This could be achieved using chelating agents [47,49,53,54]

In this last study the cleaning efficiency was examined on the basis of a numerical evaluation scheme for debris and smear layer, by means of an SEM-evaluation of the coronal, the middle and the apical parts of the canals [377,385].

With all four systems, partially un-instrumented areas with remaining debris were found in all canal sections. This finding has also been described by others[377,386-390] and is consistent with other investigations using microcomputer tomography assessment of canal shapes. [391-394] Additionally, the present results confirm previous observations that cleanliness decreased from the coronal to the apical part of the root canal [391,392,395-399].

In the direct comparison of the two techniques (SEM vs optical) for the debris evaluation, no significance was evidenced. This finding can be used in future investigations since optical microscopy is easier to perform and less expensive, in addition through optical observation we are able to evaluate the whole extent of the root and not only specific spots which excludes possible biases. Unfortunately this was not feasible in higher magnifications since optical microscopy lack of depth of fieldd in high magnifications and mapping the root canal under x1000 magnifications was practically impossible.

In our study NaOCl and EDTA were used as irrigants. It has been shown that the use of these two irrigant solutions produces a synergistic effect that results in effective removal of the smear layer[400]. In the present study, it was decided to ensure equal volumes of both irrigants after instrumentation, (10 mL final flushing of NaOCl and 10 mL of 17% EDTA), in an attempt to simulate clinical conditions.

Findings from the SEM evaluation showed a significant difference between manual preparation an all the other techniques, a fact which was confirmed also by another study[396]. On the other hand many previous papers have found no difference between manual and NiTi files [411,412,413] whereas some authors claimed that manual files performed better comparing to rotary files[414,415,416], however none of these studies were performed in long oval canals.

In the direct comparison of the four shaping techniques the WO group achieved the best results. In both comparisons to manual and SAF groups the difference was significant in all investigations. The only non-significant comparison found was between WO and BTR group.

In previous publications direct comparisons of the files tested in our research were found only two studies between WO and SAF. The two studies comparing SAF to WaveOne used either horizontal sections in the mesial root of mandibular molars [417] or a micro-computed tomography analysis [418]. Up to date, there are 8 studies evaluating the cleaning efficacy of the single file systems examined in our study in terms of debris and smear layer removal; five for the SAF (but only one on oval canals and one on curved canals) and three for the WaveOne (none on oval canals).

The results of our study find differences to other studies comparing the SAF to round files [419-421]. This is mostly because of the anatomical site where the studies were conducted, most of this studies used mandibular incisors that are not as oval as premolars and canines, and also the final preparation apical of these studies was never at least 40.04, which as we have mentioned before in the microbiology experiment is a fundamental point of the effectiveness of round NiTi files[378].

In the only study in oval canals [424], it was compared the cleaning efficacy of the SAF to the ProTaper rotary file system, in terms of both microbiological and SEM evaluation. The results found were similar to our findings with the rotary file obtaining cleaner surfaces comparing to SAF both in debris and smear layer evaluation.

After having considered each experiment separately and evaluated our findings we could reach to an overall evaluation of the four systems tested and their clinical efficiency regarding both their ability to reduce biofilm and clean the root canal walls in long oval canals.

The first observation which is in agreement to many other authors is the superiority of NiTi files to manual instrumentation. Apart from the design of the files that make them more efficient in cleaning and in concequence disinfecting the root canal space, the fact of the grater taper of NiTi files provide a greater possibility to be more effective[,361,362,424,426,428].

In the comparison between NiTi files and the SAF, both round shaping systems achieved better results, facts that is in agreement with some authors[378,416] but not to other authors[424-426], who conducted similar experiments but not in long oval canals. The two most important points to define the superiority of the round files in comparison to the SAF was the selection of the large apical preparation[378,416] (at least 40.04) and the choice of the same amount sodium hypoclorite.

In the direct comparison of the the two round shaping systems, even though the differences were significant the significance was by little. In fact the rotary system obtained better disinfection but the reciprocating better debridement.

Admittingly, none of these techniques was clearly superior to the other.

Nevertheless having considered all these data, and understanding the limitation of the studies conducted in vitro it is important to realise that

there is need of more clinical studies in order to define the most effective protocol for successful chemomechanical preparation, especially in complex anatomies such as long oval canals.

8.Conclusions

The purpose of this thesis was to evaluate three innovative shaping systems and their effectiveness in long oval canals. The Self Adjusting File which is the first anatomical file, the WaveOne which is a single reciprocating file, and the BT Race, a three file system with particular design were investigated in their metallurgical composition, in their effectiveness in disinfecting and cleaning in vitro long oval canals.

Within the limitations of in vitro studies we were able to draw some useful conclusions.

All tested techniques achieved better results compared to manual instrumentation.

The SAF technique which was supposed to be more effective in long oval canals did not achieve the best results, contrary both round shaping systems performed better in disinfecting the root canal and also cleaning the root canal.

The WO system obtained the cleaner root canal surfaces but did not disinfect in the best way.

The BT Race system was more effective in cleaning the root canal from E. Faecalis, but produced more smear layer compared to the reciprocating system.

It is obvious that no technique was able to achieve perfect results neither in disinfection nor in root canal cleanliness and none of the files tested was able to obtain a perfect cleaning and disinfection in long oval canals.

It is imperative to realize that in order to obtain the best disinfection and cleanliness in root canal treatments shaping the root canal is not sufficient. In order to standardize the most effective way of chemomecanical preparation more clinical research is needed combining shaping and disinfection methods.

Finally after this investigation we can conclude that:

- Not all shaping instruments are able to obtain the same cleanliness of the root canal space.
- Manual shaping through stainless steel files resulted to be inferior to Ni-Ti intruments in cleaning long oval canals
- Even though anatomical instruments is a very interesting concept there is need of more evolution in order to make them more effective in cleaning three-dimensionally the endodontic space of long oval canals

Ελληνική Περίληψη :
ΕΙΣΑΓΩΓΗ

Η παρούσα διδακτορική διατριβή έχει ως σκοπό να μελετήσει τη βασική σύσταση, την αποτελεσματικότητα απομάκρυνσης μολυσματικού βιολογικού υλικού, όπως και την δυνατότητα καθαριότητας του ριζικού σωλήνα, από τέσσερα διαφορετικά συστήματα χημικομηχανικής επεξεργασίας. Τα συστήματα που μελετήθηκαν είναι: το Self Adjusting File (SAF) ένα ανατομικό εργαλείο που προσαρμόζεται στην ανατομία με σκοπό να έχει όσο μεγαλύτερη επαφή με τα τοιχώματα του ρίσκου σωλήνα , το WaveOne (WO) ένα εργαλείο παλίνδρομης κίνησης στο ριζικό σωλήνα, όπως επίσης και το σύστημα BT Race (BTR), σύστημα τριών εργαλείων με την ιδιαιτερότητα μιας ρίνης με αρνητική κωνικότητα και ευρύ άκρο (35.00), τέλος η τεχνική χειρός με ατσάλινες ρίνες.

ΥΛΙΚΑ ΚΑΙ ΜΕΘΟΔΟΙ

Μέρος 1: Μελέτη σύνθεσης και μικροδομής
Για την έρευνα αυτή χρησιμοποιήθηκαν τρεις ρίνες από κάθε ομάδα NiTi. Για την ομάδα WO, ελέγχθηκαν τρεις ρίνες (40 / .08), για την ομάδα BTR τρεις ρίνες (40 / 0.04) και για την ομάδα SAF τρία εργαλεία SAF. Μετά την προετοιμασία δειγμάτων, από την κεντρική περιοχή συλλέχθηκε ένα φάσμα EDX στο οποίο έγινε ποσοτική ανάλυση από το λογισμικό Genesis (έκδοση 5.2, EDAX). Τα αποτελέσματα της στοιχειακής σύνθεσης αναλύθηκαν μέσω περιγραφικής ανάλυσης. Οι ποσότητες των Ni, Ti ποσοτικοποιήθηκαν (%) ανά ρίνη.

Μέρος 2: Μικροβιακή απομάκρυνση βιοϋμενίου
Εκατό μονόριζα εξαγμένα δόντια ωοειδούς μορφής (παρειογλωσσικά-εγγυςαπω> 2,5: 1, 5 mm από το ακρορίζιο) χρησιμοποιήθηκαν. Μετά την αρχική εξέταση ακτινογραφικά, προετοιμάστηκαν (διάνοιξη, διαβατότητα και αποστείρωση) και έγινε μόλυνση των ριζικών σωλήνων με E. Faecalis (ATCC 29212) για 30 ημέρες στους 37 ° C υπό ήπια ανακίνηση.
Τα δόντια χωρίστηκαν τυχαία σε τέσσερις ομάδες, (n = 25). Η ακροριζική τους έξοδος σφραγίστηκε με εποξική ρητίνη για να αποφευχθεί διαρροή βακτηριδίων ακροριζικά. Σε αυτό το σημείο και πριν από την χημικομηχανική επεξεργασία των δοντιών, ελήφθησαν αρχικά βακτηριολογικά δείγματα, όπως περιγράφεται αργότερα. Τα δόντια στη συνέχεια διαμορφωθήκαν ανάλογα με την ομάδα τους:
Ομάδα 1: με εργαλείο SAF
Ομάδα 2: με BTRace
Ομάδα 3: με WaveOne

Ομάδα 4: με ρίνες χειρός.
Σε όλες τις ομάδες η τελική ακροριζική παρασκευή ήταν 40. Το ομοιογενές πρωτόκολλο διακλυσμών προέβλεπε συνολικά 15 ml 2,5% NaOCl για κάθε δόντι. Αυτή ήταν η κανονική ποσότητα διακλυσμών που χρησιμοποιήθηκε για όλα τα δόντια όλων των ομάδων κατά τη διάρκεια της διαμόρφωσης. Μετά τη διαμόρφωση, οι σωλήνες ξεπλύθηκαν με 5 ml 17% EDTA, ακολουθούμενα από 5 ml 2,5% NaOCl. Η αδρανοποίηση υποχλωριώδους νατρίου πραγματοποιήθηκε με προσθήκη 5 ml 10% Na2O3S2, το οποίο κατόπιν ξεπλύθηκε με αποσταγμένο νερό.
Διαδικασίες δειγματοληψίας:
Δύο δείγματα συλλέχθηκαν από κάθε δόντι: ένα πριν τη διαμόρφωση (s1) και ένα μετά από χημικόμηχανική επεξεργασία (s2).
Το πρώτο δείγμα (s1) συλλέχθηκε μετά τον προσδιορισμό του μήκους εργασίας.
Το δεύτερο δείγμα (s2) συλλέχθηκε μετά το πέρας της χημικομηχανικής επεξεργασίας και την αδρανοποίηση του NaOCl στο ριζικό σωλήνα πληρώνοντάς τον με θειοθειικό νάτριο για 5 λεπτά και στη συνέχεια έκπλυση με φυσιολογικό ορό (10 ml). Στη συνέχεια, χρησιμοποιήθηκε μια αποστειρωμένη ρίνη χειρός (# 20) με πρόκαμψη για να εκτοπίσει τα υπολείμματα από την παρειακή και γλωσσική επιφάνεια του σωλήνα με κίνηση έλξης. Το σύνολο του περιεχομένου του ριζικού σωλήνα απορροφήθηκε από αποστειρωμένου κώνους χαρτιού, ο καθένας από τα οποίους παρέμεινε για 1 λεπτό.
Όλα τα δείγματα στροβιλίστηκαν για ένα λεπτό, και αφού έγινε η διαδικασία αραίωσης και επώασης προέκυψαν οι μικροβιακές μονάδες. Οι αριθμοί μονάδων που σχηματίζουν αποικίες (CFUs) αναπτύχθηκαν και στη συνέχεια μετατράπηκαν σε πραγματικές μετρήσεις με βάση τους γνωστούς παράγοντες αραίωσης. Προσδιορίστηκε ο αριθμός των CFU (ποσοτικά) ανά δείγμα. Η σχετική ποσοστιαία μείωση (RR) από s1 σε s2 υπολογίστηκε σύμφωνα με τον τύπο:
RR (%) = 100 * (s1-s2) / s1

Μέρος 3: Αξιολόγηση της επιφάνειας του ριζικού σωλήνα
Για αυτή την αξιολόγηση, χρησιμοποιήθηκαν είκοσι δόντια, πέντε δόντια από κάθε ομάδα (n = 5). Μετά την προετοιμασία των δειγμάτων (επιμήκης διαχωρισμός και αφυδάτωση) τα δόντια παρατηρήθηκαν στο οπτικό μικροσκόπιο υψηλής μεγέθυνσης και στο ηλεκτρονικό μικροσκόπιο σάρωσης για την αξιολόγηση της καθαρότητας του ριζικού σωλήνα. Από κάθε δόντι αξιολογήθηκε το τμήμα με πιο εμφανές ακροριζικό τμήμα.
ΜΙΚΡΟΣΚΟΠΙΚΗ ΑΞΙΟΛΟΓΗΣΗ
Διεξήχθη μικροσκοπική αξιολόγηση σε SEM, σε όλα τα δείγματα σε μεγεθύνσεις 200x για αξιολόγηση παρουσίας θραυσμάτων και 1000x για την παρουσία επιχρίσματος σε απόσταση 3 mm (θέση A), 6 mm (θέση B)

και 9 mm (θέση C) από το ακρορίζιο. Παραλληλα για την μελετη καθαροτητας από θραυσματα εγινε και πραγματοποιήθηκε χαρτογράφηση κάθε ριζικού σωλήνα μέσω οπτικής μικροσκοπίας σε 200x μεγεθύνσεις χρησιμοποιώντας το οπτικό ψηφιακό μικροσκόπιο VHX-500. Προκειμένου να διασφαλιστεί η συνοχή μεταξύ των εξεταστών, όλα τα δείγματα αξιολογήθηκαν δύο φορές από κάθε εξεταστή.
Οι καταγραφές των ομάδων αναλύθηκαν στατιστικά προκειμένου να διερευνηθούν πιθανές διαφορές μεταξύ των τεχνικών αλλά και μεταξύ των εξεταστών και των δειγμάτων ριζών. Η ποσότητα των υφιστάμενων θραυσμάτων και επιχρίσματος βαθμολογήθηκε μεταξύ 1 και 4 ως εξής:
Βαθμός 1: συστάδες συντριμμιών που καλύπτουν λιγότερο από το 25% της περιοχής του τοιχώματος του διαύλου Βαθμολογία 2: συστάδες συντριμμιών που καλύπτουν το 25 έως 50% της περιοχής του τοιχώματος του καναλιού
Βαθμός 3: συστάδες συντριμμιών που καλύπτουν το 50% έως 75% της περιοχής του τοιχώματος του καναλιού
Βαθμός 4: συστάδες συντριμμιών που καλύπτουν περισσότερο από το 75% της περιοχής του τοιχώματος του καναλιού

ΑΠΟΤΕΛΕΣΜΑΤΑ

Μέρος 1: Μελέτη σύνθεσης και μικροδομής
Τα αποτελέσματα της στοιχειακής σύνθεσης που λαμβάνεται από την ανάλυση EDX παρατίθενται στον Πίνακα 1. Η ομάδα WO έδειξε υψηλότερη περιεκτικότητα σε Ni από τις άλλες δυο ομάδες που αναλύθηκαν ενώ η ομάδα BTR έδειξε τη χαμηλότερη περιεκτικότητα Ti.

Πίνακας 1. Τα ποσοτικά αποτελέσματα (% wt) για τα προϊόντα που ελέγχθηκαν, σύμφωνα με την ανάλυση EDX. Τα αποτελέσματα ταξινομούνται σε ένα αυξανόμενο περιεχόμενο Ni

	Ni(%wt)	Ti(%wt)	SD
WO (Wave One)	52,14	46,79	
BTR (BT Race)	51,99	47,12	
SAF	51,49	47,31	

Μέρος 2ο:

Οι μέσες τιμές CFU κατά την έναρξη (s1) ήταν παρόμοιες μεταξύ των τεσσάρων ομάδων και δεν παρουσιάζουν στατιστικά σημαντική διαφορά μετάξι τους. Όσον αφορά τα δείγματα (s2), οι CFU ήταν σημαντικά

περισσότερες στην ομάδα MAN (manual) συγκριτικά με τις ομάδες WO, BTR και SAF, ενώ ήταν στατιστικά σημαντικά χαμηλότερες στην ομάδα BTR συγκριτικά με όλες τις άλλες ομάδες μελέτης.
Οι αναλύσεις εντός ομάδας που αξιολογούν τη μείωση των CFU από τα s1 σε s2 έδειξαν σημαντική μείωση σε όλες τις ομάδες μελέτης (p <0.001). Η μείωση από s1 σε s2 για όλες τις ομάδες παρουσιάζεται στον πίνακα.
Η μέση μείωση ήταν 93.20% στην ομάδα MAN, 97.26% στην ομάδα SAF, 99.95% στην ομάδα WO και 99.96% στην ομάδα BTR. Η μέση μείωση παρουσιάζεται στον πίνακα 2. Η συνολική μείωση των CFU από τις μετρήσεις s1 σε s2 ήταν σημαντικά διαφορετική μεταξύ των τεσσάρων ομάδων όπως ορίστηκε από τις επαναλαμβανόμενες μετρήσεις ANOVA (p <0.001).
Συγκεκριμένα, η μείωση των CFU ήταν μικρότερη στην ομάδα MAN σε σύγκριση με τις άλλες ομάδες,
Ενώ στις ομάδες ομάδες SAF και WO η μείωση των CFU ήταν σημαντικά λιγότερη σε σύγκριση με την ομάδα BT Race (p <0,05).

Μέρος 3ο: Εκτίμηση της καθαρότητας της επιφάνειας του ριζικού σωλήνα:

Αποτελέσματα για συντρίμμια:

Στο οπτικό μικροσκόπιο:
Υπήρξε σημαντική διαφορά στην βαθμολογία μεταξύ των τεσσάρων ομάδων (p <0,001). Συγκεκριμένα, βρέθηκαν διαφορές μεταξύ των ομάδων BTR και SAF (p = 0.002), όπου η μέθοδος BTR απέκτησε καθαρότερα δείγματα. Η σύγκριση μεταξύ της ομάδας MAN και της BTR (p <0,001) ήταν επίσης στατιστικά σημαντική με την BTR να έχει καθαρότερα δείγματα. Σημαντική ήταν επίσης η σύγκριση μεταξύ WO και Manual, όπου η WO είχε καλύτερη επίδοση (p <0,001). Μεταξύ της WO και της SAF η διαφορά δεν βρέθηκε σημαντική (p = 0,008).
Στο μικροσκόπιο SEM:
Υπήρξε σημαντική διαφορά στην βαθμολογία μεταξύ των τεσσάρων ομάδων (p <0,001). Συγκεκριμένα, η BTR πέτυχε καθαρότερες επιφάνειες σε σύγκριση με τη μέθοδο SAF και MAN (p <0,001). Σημαντική ήταν επίσης η διαφορά μεταξύ WO και MAN (p <0.001) με την WO που παράγει καθαρότερες επιφάνειες ριζών. Μεταξύ της WO και της SAF η διαφορά δεν ήταν σημαντική (p = 0,010).
Ούτε στην οπτική μέθοδο ούτε στη μέθοδο SEM η σύγκριση μεταξύ BTR και WO βρέθηκε στατιστικά σημαντική.

Στη σύγκριση μεταξύ ομάδων οι ομάδες WO και BTR ήταν σημαντικά καλύτερες σε σύγκριση με τις ομάδες MAN και SAF. Η σύγκριση μεταξύ των δύο πρώτων ομάδων δεν ήταν στατιστικά σημαντική διαφορά.
Η περιοχή C (9mm από το ακρορίζιο) ήταν η θέση με τις καθαρότερες επιφάνειες σε όλες τις ομάδες, και η χειρότερη ήταν η θέση A (3mm από το ακρορίζιο). Η διαφορά μεταξύ τους ήταν στατιστικά σημαντική ($p < 0.001$).
Στη σύγκριση μεταξύ των δύο μεθόδων έρευνας (οπτικό μικροσκόπιο έναντι SEM), δεν βρέθηκε καμία σημαντικότητα ($p > .05$).

Αποτελέσματα για το επίχρισμα :

Υπήρξε σημαντική διαφορά στην βαθμολογία μεταξύ των τεσσάρων ομάδων ($p < 0.001$). Συγκεκριμένα, βρέθηκαν διαφορές μεταξύ των ομάδων MAN και WO ($p = 0.001$), MAN και BTR ($p < 0.001$) και μεταξύ SAF και BTR ($p = 0.015$).

Στη σύγκριση μεταξύ ομάδων, η ομάδα WO πέτυχε σημαντικά καλύτερα αποτελέσματα σε περιοχές καθαρού ριζικού σωλήνα σε σύγκριση με την ομάδα MAN. Το ίδιο αποτέλεσμα επιτεύχθηκε από την ομάδα BTR σε σύγκριση με την ομάδα MAN. Στην άμεση σύγκριση μεταξύ SAF ομάδας και WO και μεταξύ SAF και BTR, η ομάδα SAF είχε σημαντικά λιγότερες καθαρές επιφάνειες. Δεν βρέθηκε καμία σπουδαιότητα μεταξύ των ομάδων WO και BTR, αλλά η ομάδα WO ήταν ελαφρώς καλύτερη στον καθαρισμό της ακροριζικού τριτημόριου (θέση A) και η ομάδα BTR παρουσίασε καλύτερα στα υπόλοιπα δύο τριτημόρια (θέσεις Β και Γ).
Και οι τέσσερις ομάδες παρουσίασαν χειρότερη θέση A (3mm από το ακρορίζιο) σε σύγκριση με τη θέση C (9mm από το ακρορίζιο) και τη θέση B (6mm από το ακρορίζιο). Καμιά σύγκριση δεν ήταν σημαντική.

Μετάξι των εξεταστών, η ποσοστιαία συμφωνία ήταν 93,1% και το επίπεδο συμφωνίας μεταξύ δύο μετρήσεων του ίδιου εξεταστή ήταν εξαιρετική και στατιστικά σημαντική ($p < 0,001$) όπως ορίζεται από τον σταθμισμένο συντελεστή Kappa που ήταν ίσο με 0,84.

ΣΥΜΠΕΡΑΣΜΑΤΑ

Σκοπός αυτής της εργασίας ήταν να αξιολογήσει τρία καινοτόμα συστήματα διαμόρφωσης και την αποτελεσματικότητά τους σε μακρύς ωοειδείς ριζικούς σωλήνες.
Εντός των περιορισμών των in vitro μελετών είχαμε τη δυνατότητα να καταλήξουμε σε κάποια χρήσιμα συμπεράσματα.

Όλες οι δοκιμασμένες τεχνικές πέτυχαν καλύτερα αποτελέσματα σε σύγκριση με τη χημικομηχανική επεξεργασία με το χέρι.
Η τεχνική SAF που υποτίθεται ότι είναι πιο αποτελεσματική στους μακρύς ωοειδείς ριζικούς σωλήνες δεν πέτυχε τα καλύτερα αποτελέσματα, αντίθετα και τα δύο στρογγυλά συστήματα διαμόρφωσης έχουν καλύτερη απόδοση στην απολύμανση του ριζικού σωλήνα και επίσης στον καθαρισμό του ριζικού σωλήνα.
Είναι προφανές ότι καμία τεχνική δεν μπόρεσε να επιτύχει τέλεια αποτελέσματα ούτε στην απολύμανση ούτε στην καθαριότητα των ριζικών σωλήνων.
Είναι επιτακτικό να συνειδητοποιήσουμε ότι για να επιτευχθεί η καλύτερη απολύμανση και καθαριότητα δεν αρκεί η μηχανική διαμόρφωση. Προκειμένου να τυποποιηθεί ο αποτελεσματικότερος τρόπος χημικομηχανικης επεξεργασίας απαιτείται περισσότερη κλινική έρευνα συνδυάζοντας μεθόδους σχηματισμού και ενεργοποίησης.

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