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COMPARATIVE STUDY ON CLEANING EFFICACY OF TWO SINGLE FILE SYSTEMS IN OVAL CANALS: WAVEONE AND SELF-ADJUSTING FILE

ALEXANDROS PROUNTZOS

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Επιβλέπων Καθηγητής για την εκπόνηση της Μεταπτυχιακής Διπλωματικής Εργασίας: Κερεζούδης Νικόλαος, Επίκουρος Καθηγητής Ενδοδοντίας

Τριμελής Επιτροπή για την αξιολόγηση της Μεταπτυχιακής Διπλωματικής Εργασίας:

1. Πανόπουλος Παναγιώτης, Αναπληρωτής Καθηγητής Ενδοδοντίας

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Στους γονείς μου

Πρόλογος

Η παρούσα διπλωματική εργασία σηματοδοτεί την ολοκλήρωση των μεταπτυχιακών μου σπουδών στην Ενδοδοντία. Μου δίνεται έτσι η αφορμή να αναφερθώ σε ανθρώπους που με βοήθησαν σημαντικά, έχοντας επίγνωση πως κάποιες ευχαριστίες σε ένα χαρτί, τυπικές ή μη, δε μπορούν να αντικατοπτρίσουν σχέσεις και συναισθήματα που δημιουργήθηκαν σε όλο αυτό το χρονικό διάστημα.

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"But I'll know my song well before I start singing…"

Bob Dylan - "A *Hard Rain's* A-*Gonna Fall*"

CONTENTS

Introduction

The quest of chemomechanical preparation in the context of endodontic therapy is to:

a) shape the root canal to an adequate cavity to enable irrigation and obturation

b) clean the canal system mostly by promoting access for disinfecting solutions (Stewart 1955).

A variety of endodontic instruments and irrigant solutions have been used in order to achieve these goals. However, these instruments and irrigants produce and leave dentine debris and a smear layer as a result of their action on root canal walls (Torabinejad et al 2002). Research has shown that a significant amount of debris remains in the root canal space and smear layer covers the root canal walls, after completion of chemomechanical preparation. This debris may be compacted along the surface of canal walls, increasing the risk of bacteria 'contamination' or extrusion to the periapical tissues in infected cases (Bowman et al 2002, Fairbourn et al 1987, Myers and Montgomery 1991, Lambrianidis et al 2001, Tinaz et al 2005, Huang et al 2007, Leonardi et al 2007, Kustarci et al 2008a, b, Logani et al 2008, Uezu et al 2010). Additionally, it reduces the adaptation of sealer and gutta-percha on the canal walls and inhibits the sealer penetration into the dentinal tubules (Bowman et al 2002).

If tissue remnants and debris are not removed, the subsequent stage of root canal obturation may also be jeopardized, leading to a potential failure of treatment (Wu et al 2001). Therefore, several researchers have studied the cleaning efficacy of different techniques, systems and protocols, i.e. the amount of debris and/or smear layer that remains inside root canal walls at the end of chemomechanical preparation.

The present thesis consists of two parts:

- The general part, after a brief overview on the history of root canal preparation and its objectives, emphasizes on root canal cleanliness and its clinical implications. The factors that influence the production of debris and smear layer are also discussed in this part. Finally, oval root canals and the challenges derived from their special anatomy are discussed.

- The experimental part is a comparative study on the cleaning efficacy of two recently introduced, NiTi rotary single-file systems, the WaveOne and the Self-Adjusting file, in terms of debris and smear layer removal under optical microscopy observation.

GENERAL PART

CHAPTER 1

1.1 Root canal preparation

1.1.1 History

Pierre Fauchard, "the father of modern dentistry", in his famous book "Le chirurgien dentist" (1728), describes instruments for trepanation of teeth, cauterization of dental pulps and preparation of root canals**.** However, neither in this book nor in any literature of that time a systematic description of preparation of the root canal system is to be found. Indeed, in a survey for endodontic instrumentation up to 1800, Lilley concluded that "…only primitive hand instruments and excavators, some iron cauter instruments and only very few thin and flexible instruments for endodontic treatment has been available" (Lilley 1976).

The first ever endodontic hand instruments are credited to Edward Maynard, who used round wires (in the beginning watch springs, later piano wires) to create small needles for extirpation of pulp tissue (Grossman 1976, Bellizzi and Cruse 1980). In 1852 Arthur used small files to enlarge root canals (Bellizzi and Cruse 1980, Anthony and Grossman 1945, Curson 1965, Grossman 1987). During the middle of the 19th century broaches were recommended for the preparation of root canals: "The best method of forming these canals is with a three- or four- sided broach, tapering to a sharp point, and its inclination corresponding as far as possible, with that of the fang. This instrument is employed to enlarge the canal, and give it a regular shape"

(Hülsmann et al 2005). In 1885 the Gates Glidden drill and in 1915 the K-file were introduced.

Endodontic instruments were now available, lacking one important characteristic though: standardization. No number was descriptive of the instrument, other than to designate the succession of width. Each manufacturing company had its own control of widths and lengths, so that an instrument with a specific number made by one company did not necessarily correspond to the same-numbered instrument of another manufacturer. More importantly, there was no system for determining instrument taper. Interestingly, this very important development, although proposed by Trebiitsch in 1929 and Ingle in 1955 (Ingle 1955) was long overlooked until 1975 when ISO specifications for endodontic instruments were published (ISO/DIS 3630/1, 1975)

The first_description of rotary devices seems to have been by Oltramare in 1982 (Hülsmann et al 2005). He used fine needles with a rectangular cross-section, which were mounted into a dental handpiece. These needles were introduced passively into the canal up to working length and then the rotation started. In 1989 W. Rollins developed the first endodontic handpiece for automated root canal preparation. Specially designed needles were mounted into the handpiece with a 360° rotation and rotational speed up to 100 r.p.m. to avoid fractures (Hülsmann et al 2005). A great number of rotary systems were developed and introduced in the following years. In 1928 the 'Cursor filing contra-angle' was developed by the Austrian company W&H (Burmoos, Austria). This handpiece created a combined rotational and vertical motion of the file. In 1958 the Racer-handpiece (W&H), working in a vertical motion and in 1964 the Giromatic (MicroMega, Besancon, France), with a reciprocal 90° motion, were introduced. Several endodontic handpieces like Endolift (Kerr, Karlsruhe, Germany) with a combined vertical and 90° rotational motion and similar devices were marketed during this period. All these conventional handpieces had one main characteristic in common: their instruments, which were made of stainless steel, worked mainly in a rigid up and down motion with no or limited rotation (Hülsmann et al 2005).These were followed by the advent of modified endodontic handpieces. The Canal Finder (S.E.T., *Gröbenzell, Germany)* was the first endodontic handpiece that featured a partially flexible motion (Levy 1984). The vertical motion of the file had amplitude depending on the rotary speed and the resistance the file met into the root canal. When resistance increased, it changed into a 90° rotational motion, in an attempt to make the root canal anatomy, or at least the root canal diameter, one main influencing factor of the instruments behavior. More handpieces with modified working motions followed such as the Excalibur handpiece (W&H) with laterally oscillating instruments and the Endoplaner (Microna, Spreitenbach, Switzerland) with an upward filing motion (Hülsmann et al 2005).

A new aid in the quest of root canal preparation was the introduction of ultrasound in endodontics (Richman 1957, Martin and Cunningham 1985). The first ultrasonic device was marketed in 1980, while the first sonic device in 1984 (Hulsmann 2000). Attempts to use laser_devices can be traced since 1971 (Weichman and Johnson 1971).

Lussi et al (1995) introduced a novel approach to removing canal contents and accomplishing disinfection, the non-instrumental technique (NIT). This technique did not involve the use of any files, but consisted of a vacuum pump, a hose and a special valve cemented into the access cavity. Others attempts like the use of electro-physical devices such as ionophoresis in several versions and electrosurgical devices (Endox, Lysis, Munich, Germany) have also been described.

The most influential and promising development though was the introduction of endodontic instruments made from nickel-titanium (NiTi) (Walia et al 1988). Based on their unique properties, both hand but mainly rotary NiTi instruments, offered new perspectives for root canal preparation.

1.1.2 Goal and objectives

Root canal preparation, also termed as instrumentation, enlargement and shaping and cleaning, is an integral part of a successful endodontic treatment. Its main purpose is the prevention of periradicular disease and/or promotion of healing if disease already exists (Orstavik 2003).

Schilder (1974) in his classic work described the biologic and design objectives of a root canal preparation:

Biologic objectives:

- Instrumentation should be confined inside the root canal
- All tissue should be thoroughly removed from the root canal space
- Any forcing of necrotic debris beyond the apical foramen should be avoided
- Preparation must create sufficient space for intra-canal medicaments to reach full root canal length

Design objectives:

- The root canal preparation should flow with the shape of the original canal
- The apical foramen should remain in its original position
- The apical opening should be kept as small as practical
- Preparation should have a continuously tapering funnel from the apex to the access cavity
- Cross-sectional diameter should be narrower at every point apically

In order to achieve the aforementioned objectives of an ideal root canal preparation, a variety of techniques, instruments and systems have been proposed and used.

1.2 Root canal cleanliness - Smear layer

1.2.1 Definition - Structure

A number of studies have shown that current methods of cleaning and shaping root canals produce dentine debris and a smear layer that covers the instrumented wall areas (McComb 1975, Mader 1984, Cengiz 1990). This smear layer contains organic and inorganic substances, which include odontoblastic processes, microorganisms, and necrotic materials (Pashley 1992). The layer itself can be described as having two separate layers: a superficial layer on the surface of the canal wall, approximately 1 to 2 μm in thickness, and a deeper layer-smear plugs packed into the dentinal tubules to a depth from 6 and up to 40 μm (Mader 1984). The contents of the smear layer can be forced into dentinal tubules to varying distances (Cengiz 1990).

Compaction of smear layer into dentinal tubules is a result of:

a) 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 (Mjor 1996).

b) The rotation of instruments: while rotating instruments (reaming), the centrifuge can cause dentin debris to escape the file flutes and push them into the dentinal tubules.

c) The capillary effect: capillary action as a result of adhesive forces between the dentinal tubules and the smear material. (Cengiz 1990)

1.2.2 Clinical implications

- **Bacteria**

The effect of the smear layer on bacteria population has not yet been clearly defined. Its role as a physical barrier to bacteria and bacterial byproducts has been supported by many studies. Vojinovic et al (1973) showed that dentinal plugs stopped bacterial invasion into dentinal tubules. Likewise, Michelich et al (1980) and Diamond and Carrell (1984) stated that, in the presence of smear layer, bacteria could not penetrate into dentine. Moreover, Drake et al (1994) showed that removal of the smear layer opened the tubules, allowing bacteria to colonize in the tubules to a much higher degree (10-fold) compared with roots with an intact smear layer. It has been shown that smear layer removal facilitates passive bacteria penetration (Haapasalo and Orstavik 1987, Safavi 1989, Orstavik and Haapasalo 1990). It seems that this bacterial invasion depends on the number and type of bacteria as well as on time (Meyron and Brook 1990, Orstavik and Haapasalo 1990).

On the other hand, studies have shown that bacteria can remain in the smear layer (Baker et al 1983) and in the dentinal tubules, where they may survive, multiply (Brannstrom and Nyborg 1973) and grow into dentinal tubules (Olgart et al 1974, Meryon et al 1986, Meryon and Brook 1990). Williams and Goldman (1985) showed that smear layer delayed the penetration of Proteus vulgaris, but was not a complete barrier. Meryon et al (1986) found that Pseudomonas aeruginosa penetrated even thicker dentine slices, by removing the smear layer itself and by opening the orifices of dentinal tubules after possible collagenase production. Meyron and Brook (1990) observed that A.viscosus, Corynebacterium spp. and S.sanguis digested the smear layer and facilitated their penetration. It has been shown that certain bacteria, e.g., Bacteroides gingivalis and Treponema denticola (Uitto et al 1988) release proteolytic enzymes which cause degradation of the smear layer, resulting in a gap between the filling material and the canal wall, permitting the leakage of other bacterial species and their byproducts along the canal walls into dentinal tubules and the periradicular tissues. This degradation of the smear layer has been proposed as a possible cause in the failure of glass-ionomer retrograde fillings (Pitt Ford and Roberts 1990).

- **Intracanal medicaments**

Since it is well documented that bacteria can penetrate into dentinal tubules (Brännström 1984, Bystrom and Sundqvist 1985), many authors supported the idea that the presence of smear layer inhibits the antimicrobial effect of intracanal irrigants and medicaments into the tubules (Wayman et al 1979, Yamada et al 1983, Baumgartner and Mader 1987). Orstavik and Haapasalo (1987) in an in vitro study inoculated bovine incisors with E.faecalis for 7 days. They found that, with the removal of the smear layer, liquid camphorated monochlorophenol rapidly disinfected the dentinal tubules, while calcium hydroxide was ineffective. A subsequent study of the same authors (Haapasalo and Orstavik 1990), showed the importance of removal of the smear layer and the presence of patent dental tubules for decreasing the time necessary 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. Byström and Sundqvist (1985), have also shown that the presence of smear layer can inhibit or significantly delay the penetration, and therefore antimicrobial action, of intracanal irrigants and medicaments into dentinal tubules.

- **Microleakage**

Another aspect of the presence of the smear layer concerns its effect on the adaptation of root filling materials and sealers onto canal walls. Several studies (Abramovich 1976, Tidmarsh 1978, White 1984) have shown that smear layer removal resulted in better adhesion of obturation materials to root canal walls. In particular, Oksan (1993) found that the smear layer obstructed the penetration of filling materials into dentinal tubules, since no tubular penetration was observed in the presence of smear layer. On the contrary, when smear layer was removed penetration ranged from 40 to 60 μm. Kouvas et al (1998) also found that the presence of smear layer obstructed the penetration of several sealers (Sealapex, Roth 811, and CRCS) into dentinal tubules. More recently, Kokkas et al (2004) found similar results when examining the penetration depth of three different sealers (AH Plus, Apexit, and Roth 811) with lateral condensation. They also concluded that smear layer plays an important role in sealer penetration into the dentinal tubules, as well as in the potential clinical implications. Other investigators assessed the penetration depth of different sealers, including Tubliseal, AH26, Sealapex, Rosin, Roth's 811, and CRCS, into the dentinal tubules. They found the penetration to be 10 to 80 μm after removal of the smear layer, whereas no penetration was observed with the smear layer intact (Gutierrez et al 1990, Palleres et al 1995, Cengoglu et al 1993).

The idea is that maximum sealer penetration into dentinal tubules, after removing the smear layer, results in the reduction of mikroleakage (White 1984). However it should be noted, that a number of studies, using various sealers and obturation techniques, did not find any significant effect on root canals mikroleakage following smear layer removal (Evans and Simon 1986, Chailertvanitkul et al 1996, Saunders and Saunders 1994, Madison 1984). These conflicting results probably occur due to differences in the way smear layer is produced, method of obturation and sealer used as well as types of bacteria used in vitro. Indeed, mikroleakage of root canals is a complex phenomenon in which many variables such as root canal anatomy, instrumentation size and filling technique, irrigants, sealer properties and the infectious state of the canal play an important role. Consequently, there is no convincing evidence in the literature that a direct correlation between filling materials penetration into dentinal tubules and mikroleakage occurs.

1.3 Influencing factors

1.3.1 Dentine structure – dentinal tubules

Root canal cleanliness is the result of the interaction of files, irrigants, etc. on the inner structure of the root. Main aspects of the root dentine are the dentinal tubules, which have different characteristics 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 (Mjor 1996). Their diameter ranges from 1 to 3 μm, at the pulp – predentin junction and is as small as 0.4 μm at the cementum - dentin junction (Pashley 1989). The number of dentinal tubules per square millimeter (density) varies from 4900 to 90000 (Sen 1995). This density increases in an external to internal direction from the root surface, from 10000/mm^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 (2001), in their study of the structure of apical root dentine, recorded the density of tubules being 14000/mm^2 near the pulp, 8100/mm^2 in the middle and 2500/mm² in peripheral root dentin. These densities are much lower than those in root, not apical, dentin (ranging from 40000 to 8100/mm²). More importantly, they also noted the presence of irregular secondary dentin as well as areas completely devoid of dentinal tubules (fibrodentin).

1.3.2 Instruments - techniques

Root canal cleanliness has been studied for nearly 5 decades (McComb 1975, 1976). For example, the step-back technique was found to be superior in debridement compared to the standardized serial filing and reaming techniques (Walton 1976). Later on, it was shown that the balanced-forced technique produces a cleaner apical portion than the step-back and crown-down pressureless techniques (Wu and Wesselink 1995). Siqueira et al (1997) 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 (Walia 1988) 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, conicity and cross-section, which in turn influence instruments' cutting efficacy and, consequently, the type and amount of debris created inside the root canal.

What follows is a brief description of the most popular instruments/systems available nowadays for root canal instrumentation and their main properties, emphasizing on their cleaning ability in terms of research results.

ROTATIONAL

The majority of systems use files in a classic rotary way. These are:

- **LightSpeed and LightSpeed LSX**

The LightSpeed was introduced in the early 1990s by Senia and Wildey as an instrument with a long, thin non cutting shaft and short anterior cutting part. Cross sections of LightSpeed LS1 cutting parts show three round excavations, the U-shape design common to many earlier NiTi instruments, whereas the LSX is shaped like a flat chisel in cross section. A full set consists of 25 LightSpeed LS1 instruments in sizes #20 to #100, including half sizes (e.g., 22.5, 27.5); LSX does not have half sizes, and a set includes sizes #20 to #80.

Versumer et al (2002) reported best results for Lightspeed, in terms of debris and smear layer removal, but not statistically different from Profile .04. On the other hand, Bechelli et al (1999) reported a homogenous smear layer after Lightspeed preparation. Also, in a study by Rodig et al (2002) on oval distal root canals of mandibular molars, Lightspeed instruments left buccal and lingual extensions unprepared, leaving smear layer and debris. Moreover, in another study the cleaning ability of Lightspeed was judged unsatisfactory (Hulsmann et al 2003).

- **ProFile**

The Profile system (Dentsply, Tulsa Dental) was introduced in 1994 by Ben Johnson. ProFile instruments have increased tapers compared with conventional hand instruments. Cross sections of a ProFile instrument show a U-shape design with radial lands and a parallel central core. Lateral views show a 20-degree helix angle, a constant pitch, and bullet shaped noncutting tips. Together with a neutral or slightly negative rake angle, this configuration results in a reaming action on dentin rather than cutting. Also, debris is supposedly transported coronally and effectively removed from the root canals.

Roggendorf et al (1999) found superior cleaning ability for the ProFile compared to Lightspeed, Quantec and hand instrumentation. Moreover, in a study by Brkanic et al (2010) the least amount of debris and smear layer has been found in canals shaped with ProFile instruments compared to ProTaper, GT, K-3, FlexMaster, hand ProTaper and hand GT. However, in a study by Rodig et al on oval distal root canals of mandibular molars, Profile .04 instruments left buccal and lingual extensions unprepared, leaving smear layer and debris on canal walls (Rodig et al 2002).

- **GT and GTX Files**

The GT (Greater Taper) was introduced by Buchanan in 1994. They were hand instruments that came in four tapers (.06, .08, .10, and .12), and the maximum diameter of the working part was 1 mm. This decreased the length of the cutting flutes and increased the taper. The instruments had a variable pitch and an increasing number of flutes in progression to the tip; the apical instrument diameter was 0.2 mm. Instrument tips were non-cutting and rounded.

The GTX instrument incorporates the same design principles, as in GT, but is made of M-Wire and has a different approach emphasizing more in the use of the #20.06 rotary. The GTX set currently includes tip sizes 20, 30, and 40, in tapers ranging from .04 to .010.

Rodig et al (2007) found that GT gave better debridement results at the coronal part of mandibular molars than ProTaper 0.4, but for the middle and apical thirds the results did not differ significantly. However, Liu et al (2006) found better results from ProTaper than GT, in terms of debris removal, in all thirds of the root of maxillary and mandibular molars. Celic et al (2013) found that GT series X had similar cleaning effectiveness with more traditional NiTi files. Finally, Arvaniti and Khabbaz (2011) concluded that canal preparation with GT files and tapers 0.4, 0.6, 0.8 did not affect canal cleanliness.

- **HERO 642, Hero Shaper**

Hero Shaper *(Micro-Mega, Besançon, France)*, priorly named HERO 642 with little differences, is a system designed with positive rake angles. Tapers of .02, .04, and .06 are available in sizes ranging from #20 to #45. The instruments are relatively flexible but maintain an even distribution of force into the cutting areas. HERO instruments have a progressive flute pitch and a noncutting passive tip. Cross sections of HERO instruments show geometries similar to those of an H-file without radial lands.

Hulsmann et al (2001) found that HERO 642 achieved better results than Quantec in debris removal, while results for smear layer removal were similar. In another study by Hulsmann et al. HERO 642 failed to remove debris and smear layer in the majority of cases (Hulsmann et al 2003).

- **ProTaper and ProTaper Next**

The ProTaper system was designed by Ruddle, West and Machtou and consisted of six instruments: three shaping (SX, S1, S2) and three finishing instruments (F1, F2, F3), later added also two larger finishing files (F4, F5) and a retreatment kit. The novelty of ProTaper files was the introduction of varying tapers along the instruments’ long axes. Taper increases coronally for the shaping files, and decreases for the finishing files. The convex triangular cross section of ProTaper instruments reduces the contact areas between the file and the dentin.

Recently, ProTaper Next files (PTN) *(Dentsply Maillefer)* have been introduced. PTN files feature an off-centered rectangular cross-section design that further enhances canal shaping efficiency as claimed by the manufacturer. PTN files are available in five sizes: X1 (tip size 17 with a taper of .04), X2 (tip size 25 with a taper of .06), X3 (tip size 30 with a taper of .07), X4 (tip size 40 with a taper of .06) and X5 (tip size 50 with a taper of .06).

Yang et al (2008) found that canals prepared with ProTaper instruments showed smaller amounts of debris and smear layer remaining in the apical region compared to Hero Shaper. However, Paque at al. in their study concluded that preparation using ProTaper did not obtain an acceptable cleanliness of root canal (Paque et al 2005).

- **Quantec**

Quantec files (SybronEndo, Orange, CA) are available in tapers of 0.12, 0.10, 0.08, 0.06, 0.05, 0.04, 0.03, and 0.02 mm/mm. All have a D0 diameter of 0.25mm. Quantec files have a reduced radial land that minimizes surface tension, contact area, and stress on the instrument. Quantec files vary in taper and rate taper along their blanks and are available in non-cutting (LX) or safe-cutting (SC) tip.

Bertrand et al (1999) reported that Quantec instruments were clearly superior to hand instrumentation in the middle and apical third with the best results for the coronal third of the root canal. Medioni et al (1999) reported superior ability of Quantec when compared to HERO 642, ProFile and hand instrumentation. On the other hand, Kocjis et al (1998) found no difference between Quantec and manual preparation using K-files. Also, in a study by Hulsmann et al Quantec SC failed to remove debris and smear layer in the majority of cases (Hulsmann et al 2003).

- **K3**

The K3 system *(SybroEndo)* is the current concept of a series of systems designed by McSpadden (earlier: Quantec 2000, Quantec SC and Quantec LX). The overall design of the K3 is similar to that of the ProFile and the HERO in that it includes instruments with .02, .04, and .06 tapers. The most obvious difference between the Quantec and K3 models is the K3's unique cross sectional design with a slightly positive rake angle for greater cutting efficiency, wide radial lands, and a peripheral blade relief for reduced friction. Unlike the Quantec, a two flute file, the K3 features a third radial land to help prevent threading-in. The K3 has a variable pitch and variable core diameter in its lateral aspect, which provides more apical strength.

Following preparation with K3, Schafer & Lohmann (2002) and Schafer & Schlingermann (2003) found significantly more debris and smear layer than after manual preparation with K-Flexofiles, although these differences were not significant for the middle and apical thirds of the root canals.

- **FlexMaster**

The FlexMaster file system features .02, .04, and .06 tapers. The cross sections have a triangular shape, with sharp cutting edges and no radial lands.

Zand et al (2007) found that FlexMaster left significantly less smear layer in the apical third of the root canal compared to Ni-Ti flex K-files. On the other hand, in a study by Hulsmann et al. FlexMaster failed to remove debris and smear layer in the majority of cases (Hulsmann et al 2003). Following preparation with FlexMAster Schafer and Lohmann (2002) and Schafer and Schlingermann (2003) found significantly more debris and smear layer than after manual preparation with K-Flexofiles, although these differences were not significant for the middle and apical thirds of the root canals.

- **RaCe, BioRaCe**

The RaCe system was manufactured in 1999 by FKG. 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 size #15 and #20 tips. The tips are round and noncutting. RaCe instruments have been marketed in various packages to address small and large canals.

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

RaCe preformed better when compared with ProTaper (Schafer and Vlassis 2004). Zand et al (2007) found that RaCe left significantly less smear layer in the apical third of the root canal compared to Ni-Ti flex K-files. On the other hand, Paque at al in their study found that preparation using RaCe did not obtain an acceptable cleanliness of root canal (Paque et al 2005).

- **Mtwo**

Mtwo *(VDW, Munich, Germany)* instruments have an S-shaped cross-sectional design and a noncutting safety tip. These instruments are characterized by a negative rake angle *(Malagnino 2006)* with two cutting edges and an increasing pitch length (blade camber) from the tip to the shaft. This design is alleged to have two functions: (i) to eliminate threading and binding in continuous rotation, and (ii) to reduce the transportation of debris towards the apex. The basic series of Mtwo instruments comprises eight instruments with tapers ranging between 4% and 7% and sizes from 10 to 40.

A SEM study by Lichota et al. found that Mtwo instruments successfully removed debris, but left canal walls covered with smear layer (Lichota et al 2008). Poggio et al (2014) reported significantly lower smear layer scores for the Mtwo compared with Reciproc, in the middle and apical thirds. However, Burklein et al (2012) found Mtwo and Reciproc to be better at debris removal than ProTaper and WaveOne in the apical third of canals.

- **Twisted File**

The Twisted File was presented by SybroEndo in 2008 and it's the first NiTi file manufactured by plastic deformation. This unique production process is believed to result in superior physical properties. Twisted File is available in 5 tapers and various tip sizes: 25/40/50/.04, 25/30/35/.06, 25/.08, 25/.10, 25/.12 in 23 and 27 mm lengths.

Celic et al (2013) found that Twisted file presented similar cleaning effectiveness with more traditional NiTi files such as RaCe, Mtwo and ProTaper Universal

- **Self-Adjusting file (SAF)**

  The SAF is discussed in detail in **Chapter 2.**

<u>RECIPROCATION</u>

Researchers continued to look for innovative methods of root canal instrumentation in order to achieve better results. Thus, in 2008, G. Yared published an article stating that it is possible to completely shape a canal using only one instrument (Yared 2008). This new preparation technique, based on the reciprocating movement of this instrument, used a single instrument – F2 ProTaper –and was coined the single-file F2 ProTaper technique (Yared 2008). The single-file F2 ProTaper technique was the F2 used in conjunction with a 16:1 reduction ratio contra-angle connected to an ATR Vision *(ATR, Pistoia, Italy)* motor which allowed the reciprocating movement.

In general, reciprocating root canal preparation is considered an evolution of the balanced force technique (Roane 1985). Indeed, a mechanical reciprocating movement mimics manual movement and reduces the various risks associated with continuously rotating a file through canal curvatures (Rudlle 2012). The life of an instrument used with a reciprocating movement is increased of about 35% compared to the same instrument used with a continuous rotating movement (Valera-Patino et al 2010). This is because reciprocating movement prevents the “taper lock”, so reducing fractures due to torque forces (You S-Y et al 2010). Moreover, reciprocating movement promoted an extended cyclic fatigue life compared to a continuous rotating movement (De-Deus et al. 2010).

Reciprocation working motion consists of a counterclockwise (cutting direction) and a clockwise motion (release of the instrument); the angle of the counterclockwise cutting direction is greater than the angle of the reverse direction. Due to this fact, the instruments are claimed to continuously progress towards the apical part of the root canal. The angles of reciprocation are specific to the design of the particular instruments and programmed in an electronic monitor.

Two new NiTi systems, Reciproc *(VDW, Munich, Germany)* and WaveOne *(Dentsply Mailefer, Ballaigues, Switzerland)*, as well as a stainless-steel system, Endo-Eze Anatomic Endodontic Technology *(Ultradent Products Inc., South Jordan, UT, USA)* were recently introduced and are specifically designed for use in reciprocation.

- **Reciproc**

Reciproc *(VDW, Munich, Germany)* files are made of M-Wire NiTi alloy and are used in a reciprocal motion that requires special automated devices. The Reciproc clinical sequence, as suggested by the manufacturer, is a single instrument technique using one of three files: 25/.08, 40/.06 and 50/.05. However, the mentioned tapers describe only the first 3 apical millimeters which are followed by a decreasing taper until the shaft. The entire working part of the instrument has an S-shaped cross section.

Burklein et al (2012) reported better canal cleanliness for the Reciproc at the apical part, compared with ProTaper and WaveOne. More recently, Poggio et al (2014) compared the cleaning efficacy of the Reciproc with the Mtwo, and their results showed that Reciproc presented significantly higher smear layer scores in the middle and apical third of the canal.

- **WaveOne**

  The WaveOne system is discussed in detail in **Chapter 3.**

- **Endo-Eze (AET)**

  In 2004, Endo-Eze Anatomic Endodontic Technology (AET) was introduced as a minimally invasive endodontic preparation system *(Ultradent Products Inc., South Jordan, UT, USA).* The basic system has seven (stainless-steel) instruments: three shaping files with tip diameters of 0.10 - 0.13 mm and tapers 2.5, 4.5 and 6.5% respectively, and four apical files with tip diameters of 0.15 - 0.30 mm and tapers 2.0% (sizes 15, 20 and 25) and 2.5% (size 30), respectively.

  Endo-Eze files are used in a special reciprocating/oscillating handpiece for instrumentation of the coronal part of the root canal about 3 mm short of the apex. The apical files are hand files with shortened cutting flutes to cut only in the apical region of the canal in a clockwise turn and pull motion.

  Zmener et al (2005) found better instrumentation scores in oval canals prepared with AET compared to ProFile and K-Flexofiles.

1.3.3 Irrigants, chelating agents

Studies have demonstrated that mechanical preparation alone, either with stainless steel (Byström and Sundqvist 1981) or with nickel-titanium instruments (Dalton et al 1998) can significantly reduce bacterial load but not sufficiently disinfect root canal. Byström and Sundqvist (1981) found a $10^2 – 10^3$ fold reduction in bacterial population (pre-instrumentation mean population: 4×10^5) 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 (1998) 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 (Sim et al 2001, Grigoratos et al 2001) and chemical (Driscoll et al 2000, Reddington et al 2003) 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 (Gulabivala et al 2005, Zehnder 2006).

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%).

Its 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 (Bergenholtz 2004, Haapasalo 1983, Hancock 2001, Nair 1990, Waltimo 2000).

Free chlorine in NaOCl dissolves vital and necrotic tissue by breaking down proteins into amino acids (Johnson 2000), while the mineral component is left relatively intact (Driscoll et al 2002, Reddington et al 2003). It is known to reduce the modulus of elasticity and the flexural strength of dentine (Sim 2001, Grigoratos 2001).

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 (Berutti et al 2006). However, higher concentrations of NaOCl don’t seem to have an impact on flushing out loose debris from the canals. Baumgartner et al (1992) 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 (Cawson and Curson 1959). 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 (Estrela 2008, Shabahang 2008).

A comparison of the cleaning effects of 2% chlorhexidine and NaOCl gave similar residual debris scores in the cervical third of roots with both agents, although smear layer removal was poor (Yamashita et al 2003).

- **Hygrogen Peroxide (H_2O_2)**

Hydrogen peroxide (H_2O_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 H_2O_2 is clearly lower than that of NaOCl (Schafer 2007). When used in combination with NaOCl, 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 NaOCl and H_2O_2 results in a weakened cleaning effect (Baumgartner 1987).

Hydrogen Peroxide is generally no longer recommended as a routine irrigant.

- **Iodine Potassium Iodide (IKI)**

IKI, used as a solution of 2% iodine in 4% potassium iodide, has been proposed and is used as an endodontic disinfectant because of its antibacterial properties and low cytotoxicity (Spandberg 1973). However, it may act as a severe allergen (Popescu et al 1984) and also stains dentin (Schafer 2007).

Iodine acts as an oxidizing agent by reacting with free sulfhydryl groups of bacterial enzymes, cleaving disulfide bonds. IKI was found to be effective against E.Faecalis and Candida (Haapasalo and Ørstavik 1987, Safavi et al 1990). A study by Siren et al (2004) has shown that while calcium hydroxide was unable to kill E. faecalis in infected bovine dentine blocks, calcium hydroxide combined with IKI or CHX effectively disinfected the dentine.

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 (Torabinejad 2002). 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 (Dogan 2001).

- **Ethylenediamine Tetra-Acetic Acid (EDTA)**

Ethylenediamine Tetra-Acetic Acid (EDTA) was the first chelating agent described by Nygaard - Östby 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 (Tronstad 1993). 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 (Seidberg 1974). EDTA used for one minute inside the root canal is effective to remove dentinal debris (Seidberg 1974). 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 (Calt 2002). 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 (Heling 1998). Moreover, chemical analyses indicated that chlorine, the active agent in NaOCl, becomes inactivated by EDTA (Grawehr 2003). EDTA does not dissolve organic matter (Goldman et al 1981, Baumgartner and Mader 1987).

Concluding, an EDTA solution should be preferably used at the end of root canal preparation in order to remove the smear layer (Yamada 1983). Many authors indicate canals should be irrigated at the end of instrumentation with the sequential use of EDTA and NaOCl (Goldman et al 1982, Baumgartner and Mader 1987, Abbott et al 1991). Irrigation with 17% EDTA for one minute followed by a final rinse with

NaOCl is the most commonly recommended method for smear layer removal (Johnson 2009).

- **Citric acid**

The use of 10% citric acid as final irrigation has shown good results in smear layer removal (Smith and Weyman 1986). *In vitro* studies have shown their cytotoxicity, and 10% citric acid has proven to be more biocompatible than 17% EDTA-T and 17% EDTA (Sceiza et al 2001, Malheiros et al 2005). 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 (Sceiza et al 2010). The use of 25% citric acid was found to be ineffective in eradication of biofilms of *E faecalis* after 1, 5, and 10 min of exposure (Arias-Moliz et al 2009).

- **MTAD**

MTAD is relatively new irrigating solution supposedly capable of both removing the smear layer and disinfecting the root canal system (Torabinejad 2003). It is a mixture of 3% doxycycline hyclate, 4.25% citric acid, and 0.5% polysorbate-80 (Tween 80) detergent (Torabinejad 2003). 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 (Torabinejad 2003). 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 (Torabinejad 2003).

- **Tetraclean**

Tetraclean *(Ogna Laboratori Farmaceutici, Muggio, Italy)* is a new product very similar to MTAD. Their differences lie in the concentration of antibiotics (doxycycline 150 mg/5 ml for MTAD and 50 mg/5 ml for Tetraclean) and the kind of detergent (Tween 80 for MTAD, polypropylene glycol for Tetraclean). Doxycycline hydrochloride has shown ability to remove the smear layer in the middle and apical thirds of canals, probably due to its acid pH of 2 (Barkhordar et al 1997). Currently, there is no data on its interaction with NaOCl regarding smear layer removal.

1.3.4 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) (Weller 1980, Ahmad 1987). 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 UI. This can be explained by a reduction of acoustic streaming and cavitation (Ahmad 1987), although several studies have shown that acoustic streaming should be regarded the main mode of action of ultrasonics (Briseno 1993, Walmsley 1987, Walmsley 1989, Lumley 1991).

Passive ultrasonic activation was first described by Weller et al (1980), 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 (1983, 1987b) reported the complete removal of the smear layer when combining 3% NaOCl with PUI for 3 and 5 minutes. Likewise, Alacam (1987) and Huque et al (1998) 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 (Turkun and Gengiz 1997). 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 (1989) and Abbott et al (1991) 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 (Cameron 1983, 1987b, Heard &

Walton 1997, Turkun and Cengiz 1997, Huque et al 1998), 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 (2013), 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 (2011) also found that the EndoActivator produced better results on canal cleanliness than ultrasonic and syringe irrigation. Rodig et al (2010) 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 (2010) 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 (Nielsen 2007). 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 (Nielsen and Baumgartner 2007).

Early studies showed significantly better debridement for the EndoVac compared to needle irrigation, at 1 mm from the working length (Nielsen and Baumgartner 2007, Siu and Baumgartner 2010). Yoo et al (2013) 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 (2011) found that EndoVac, PiezoFlow and Max-i-Probe with similar volumes significantly improved canal and isthmus cleanliness, but there was no statistical difference between groups. In a study comparing debris and smear layer removal using five different irrigation techniques (NaviTip needle-no activation, active scrubbing with 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 (2013) 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.

- **Safety-Irrigator**

The Safety-Irrigator is an irrigation / evacuation system that apically delivers the irrigant under positive pressure through a thin needle containing a lateral opening, and evacuates the solution through a large needle at the root canal orifice. In a recent study, Safety-Irrigator produced better results in debris removal than syringe irrigation, but was less effective than continuous ultrasonic irrigation (CUI) and manual dynamic activation (MDA) with a tapered cone (Jiang 2012).

- **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 (Metzger et al 2010).

- **Lasers**

The first use of lasers in endodontics has been reported by Weichman and Johnson (1971). 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 (Torabinejad 2002). 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 (Dederich 1984, Onal 1993, Moshonov 1995). Although, for some lasers the removal of debris and smear layer has been reported (Koba 1999), the main difficulty continues to be access to small canal spaces with the relatively large probes emitting the light straight ahead (Kimura 2000). There is promising evidence on smear layer removal by the use of Photon-induced photo-acoustic streaming phenomenon (Zhu et al. 2013)

- **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 (Arita 2005, Kim 1999). 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 (Nagayoshi 2004, Muller, 2007, Estrela 2007, Hems 2005, Kustarci 2009, Huth 2009).

- **Non-instrumental technique (NIT)**

The NIT technique was developed by Lussi et al (1993) and uses a vacuum pump and an electrically driven piston, generating alternating pressure and bubbles in the irrigation solution, inside the root canal. This was expected to enhance the ability of sodium hypochlorite to dissolve organic pulp tissue. Although in vitro results seemed promising (Lussi 1993, Lussi 1995, Lussi 1999), an in vivo study showed minimal removal of debris from the root canal walls as well as intra-operative problems (Attin et al 2002). The NIT system is presently not marketed and cannot be regarded as an alternative to root canal instrumentation.

1.4 Oval canals

Several anatomical and histological studies have demonstrated the complexity of the anatomy of the root canal system, including wide variations in the number, length, curvature and diameter of root canals; the complexity of the apical anatomy with accessory canals and ramifications; communications between the canal space and the lateral periodontium and the furcation area; the anatomy of the peripheral root dentine (Vertucci 2005, Cunningham 1992). This complex anatomy is regarded as one of the major challenges in root canal preparation.

A funnel-shaped canal with a circular base is not the common configuration in root canal anatomy (Schilder 1974). Recently, cross-sectional root canal configurations have been classified as round, oval, long oval, flattened or irregular (Wu et al. 2000). Metrically, Wu et al (2000) defined “oval” as having a maximum diameter (bucco-lingual) of up to 2 times greater than the minimum (mesio-distal) diameter and “long oval” as having a maximum diameter of 2 to 4 times greater than the minimum diameter.

A high prevalence of oval and long oval canals even in the apical root canal portion has been reported (Mauger et al 1999, Gani and Visvisian 1999, Wu et al 2000). As reported by Mauger et al. (1999), after resecting the apices of mandibular incisors at a 20-degree facial bevel, the average faciolingual diameter of canals was 0.75 mm at 3 mm from the apex. They found that oval canals occupied 42% and long oval canals 40% of the investigated teeth. Gani and Visvisian (1999) investigated the apical canal diameter in the first upper molar. Cross sections of most mesiobuccal canals were found to be oval. In an investigation of 180 teeth of all groups Wu et al (2001) detected oval canals in 25% of all sections investigated. In some groups of teeth such as mandibular incisors and maxillary second premolars, the prevalence is greater than 50% and in distal roots of mandibular molars, the prevalence is 25 to 30%. The investigators concluded that “in approximately one fourth of the apical canals, the long canal diameter is equal to or larger than 2 times the short canal diameter; this discrepancy may complicate the root canal cleaning, shaping and filling procedures”.

A major aim in the preparation of infected root canals is to remove the inner layer of dentin (Wu et al 2001). However in many cases bacteria have penetrated deeply into the dentine (Armitage et al 1983, Ando and Hoshino 1990, Peters et al 2001), making it difficult to completely remove them from the dentinal tubules using instruments. This aim is particularly hard to achieve when preparing long oval root canals. Furthermore, after preparation, unistrumented recesses may be left in many oval canals, irrespective of the instrumentation technique, thus leaving debris and unprepared root canal surfaces behind (Wu et al 2001, Barbizam et al 2002, Grande et al 2007, Rodig et al 2002, Weiger et al 2002, Wu et al 2003).

Various techniques have been recommended for the preparation of oval root canals (Wu 2003). Difficult areas for instrumentation are the buccal and lingual extensions of these canals (Wu 2000). Using a larger file in long oval canals in order to include the entire oval canal in the preparation can result in perforation of the mesial or distal wall (Wu and Wesselink 2001). The most common technique, using hand instruments, is the circumferential technique with K-type or Hedstrom files. However, several studies have shown that circumferential filing is not capable of containing the entire canal wall (Reynolds et al 1987, Zuolo et al 1992, Siqueira et al 1997, Evans et al 2001).

Another technique proposed is the balanced force technique (Roane et al 1985). It has been found, however, that in two-thirds of oval canals use of the balanced force method left a portion of the root canal wall uninstrumented (Wu and Wesselink 2001).

In 2001, Peters et al investigated the effects of four NiTi techniques, namely K-files, Lightspeed instruments, ProFile.04 and GT rotary instruments, on root canal geometry. They came to the conclusion that all instrumentation techniques left 35% or more of the canals' surface area unchanged (Peters et al 2001).

Weiger et al (2002) in a laboratory research on extracted teeth has assessed ability of rotary NiTi instruments to prepare oval root canals which was not superior compared with hand instrument techniques. They also showed that when any amount of preparation was included between 44% and 68% of the canal surface was unprepared in oval canals.

El Ayouti et al (2008) though, have shown that the use of rotary instruments with taper larger than 4% was more efficient than hand files in preparing oval root canals. Unprepared canal perimeter with rotary instruments ranged from 25 to 35%.

Rodig et al (2002) examined the quality of preparation of oval distal root canals in mandibular molars using three NiTi instruments: LIghtspeed, ProFile .04 and Quantec SC. They concluded that all three did not achieve a controlled preparation of the buccal and lingual extensions of oval canals. Buccal and lingual extensions remained unprepared, leaving smear layer and debris. Accordingly, Zmener et al (2005) studied in vitro the cleanliness of root canal walls in oval-shaped root canals following three techniques: Anatomic Endodontic Technology (AET), ProFile and K-Flexofiles. They found that complete cleanliness was not achieved by any of the techniques and instruments investigated.

To summarize, root canal disinfection is critical for endodontic treatment success (Haapasalo 2005); eradication of microorganisms occurs as a combination of mechanical preparation (Bystrom et al 1981) and irrigation (Estrela et al 2008). Irrigation alone is not always effective, and mechanical action of instruments on canal walls, including removal of infected dentin is desirable. Both the mechanical effect of disinfection and irrigation efficacy depends on canal enlargement. Mechanical disinfection can also be related to the removal of a layer of infected dentin (Siqueira et al 1997). It has been shown that bacteria may penetrate dentinal tubules to depths of 200 μm and more (Love and Jenkinson 2002). However, when material removal of 200 μm was required in the study mentioned by El Ayouti et al (2008), less than 20% of the canal surface was counted as prepared. Moreover, Wu et al. in their work found that, regardless of the technique used, the instruments did not succeed in contacting 40% or more of oval root canal walls (Wu et al 2003). More recently, Paque et al (2010) concluded that more than 50% of canal surface has undergone less than 34 μm of dentin removal in long-oval canals. Based on the aforementioned results and the fact that bacterial persistence in the root canals in areas not affected by treatment seems to be the major cause of post-treatment apical periodontitis (Ricucci et al 2009), one may conclude that preparation of oval canals remains a problem to be solved.

CHAPTER 2

The self-adjusting file (SAF) *(ReDedent-Nova, Ra'anana, Israel)* is a newly developed system that represents a new concept in cleaning and shaping of canals compared to other NiTi rotary instruments.

2.1 Design of the file

The SAF is a hollow file designed as a compressible, thin-walled pointed cylinder either 1.5 or 2.0 mm in diameter, with an asymmetrical tip and composed of 120-mm-thick nickel-titanium lattice **(Figure 1).**

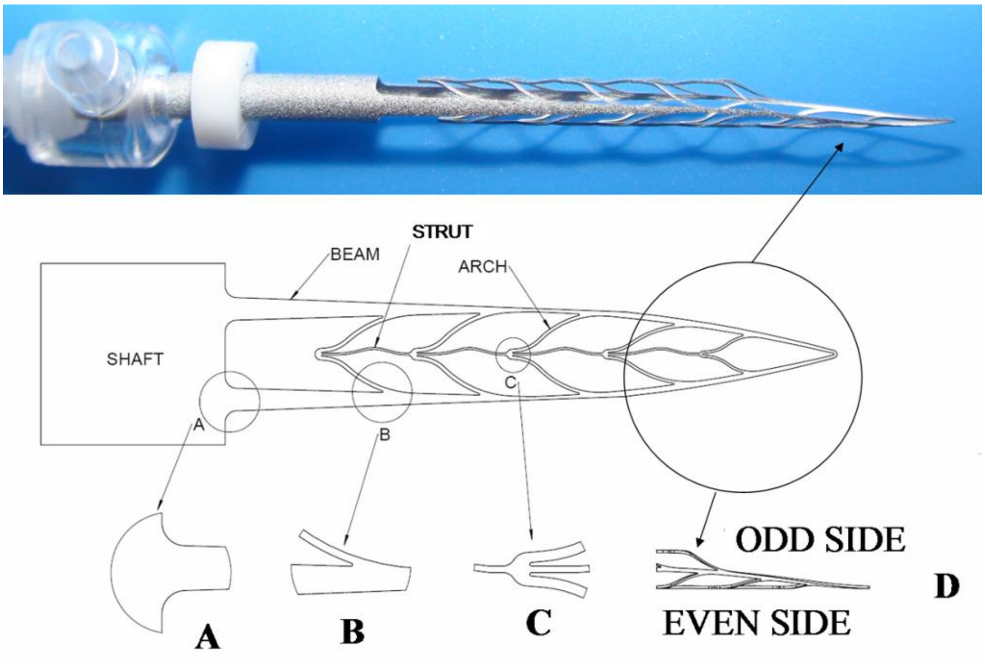


Figure 1. SAF file: Solid arrow shows beam, hollow arrows show arches and dashed arrows show struts. Figure 1b shows the connecting points of the components: (A) beam to the shaft, (B) arch to the beam and (C) strut to the arch.

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 (Hof et al 2010). The 2.0-mm file will compress 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 (Hof et al 2010). When inserted into a root canal, it adapts itself to the canal's shape, both longitudinally (as will any nickel titanium file) and along the cross-section. In a round canal, it is expected to attain a round cross-section, whereas in an oval or flat canal it is expected to attain a flat or oval cross-section, providing a three-dimensional adaptation. The wall thickness of the lattice making the file is ~100 μm, and thus when fully compressed mesio-distally, the file may spread bucco-lingually as far as 2.4 mm (De-Deus et al 2011).

The surface of the lattice threads is lightly abrasive and remains with intimate 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 (Hof et al 2010).

<u>The RDT3 Handpiece Head</u>

The SAF is operated with a special handpiece head, RDT3 *(ReDent Nova, Raanana, Israel)* that is designed to be used with a variety of available handpieces such as KaVo GENTLEpower or similar **(Figure 2).** 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 min with amplitude of 0.4 mm. The RDT3 also contains 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 is inserted into the canal and engages its walls. Thus, the file never rotates when 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 (Metzger 2011).

Figure 2. The RDT3 Handpiece Head

The VATEA Pump

The hollow design of the SAF is used to provide a continuous flow of fresh sodium hypochlorite, or any other irrigant, throughout the file operation (Hof et al 2010, Metzger et al 2010). The SAF file is equipped with a rotating hub, to which a silicone tube is attached. The other end of the tube is attached to the special VATEA peristaltic pump *(ReDent-Nova, Raanana, Israel)* **(Figure 3).** The pump is operated by a rechargeable battery and has a container that holds 500 ml of irrigant. A control panel allows the operator to set the flow rate between 1 and 10 ml / min and provides indications of the time elapsed.

The pump is activated by a foot pedal or a handswitch, with an on/off action. The pump and all its connectors are compatible with any irrigating solution, including full-strength sodium hypochlorite or EDTA solution (Metzger 2011).

Figure 3. The VATEA Pump

2.2 Mode of action

The SAF removes dentin with a filing motion in a manner similar to using sandpaper. When using sandpaper, a rough surface is applied with light pressure and with repeated motion back and forth, which allows the even removal of material. The surface of the SAF is delicately rough with 3 μm peak - to - bottom dimensions **(Figure 1).** This rough surface is present on every thin element of the NiTi lattice. The compression of the file generates circumferential pressure on the canal walls. With the vibrating motion of the file, dentin is gradually removed, resulting in a presumably smooth surface (Hof et al 2010).

The pressure is greatest when the file is inserted into the root canal and declines with the gradual enlargement of the canal (Hof et al 2010). This change in pressure in turn affects the amount of dentin removed, which declines in a similar manner ***(Figure 4).***

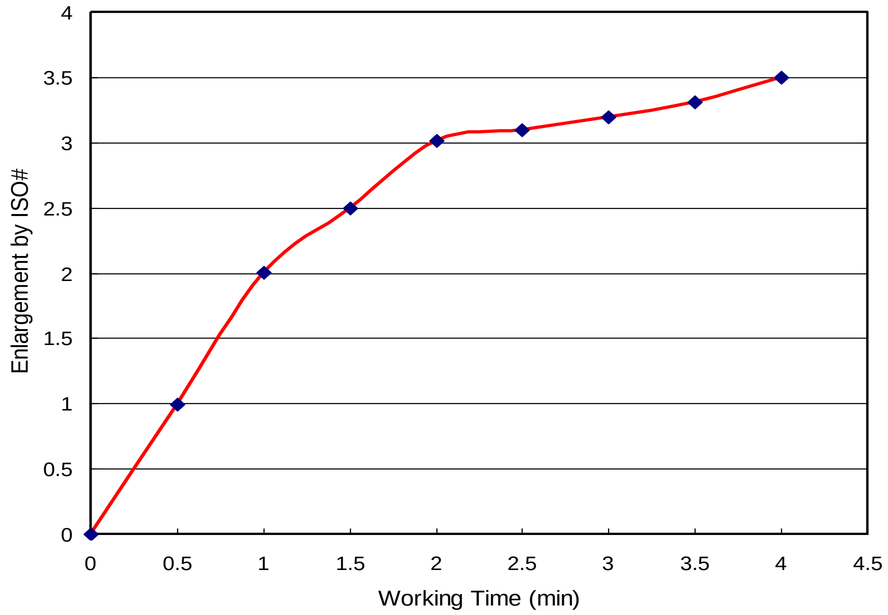


Figure 4. SAF mode of operation over time

The SAF file manufacturers claim that it preserves the original shape of the canal and removes a uniform dentin layer from the entire circumference of the root canal (Hof et al 2010, Metzger et al 2010)**.** Thus, a round canal will be enlarged as a round canal, while a flat-oval canal will be enlarged as a flat-oval canal.

The SAF is inserted into the canal while vibrating and is delicately pushed in until it reaches the predetermined working length. It is then operated with in-and-out manual motion of 3 to 5 mm and with continuous irrigation using two cycles of 2 minutes each for a total of 4 minutes per canal. This procedure will remove a uniform dentin layer 60- to 75-mm thick from the canal circumference (Metzger et al 2010). A single SAF file is used throughout the procedure, starting as a compressed file that gradually enlarges in size during dentin removal with close adaptation to the canal walls (Metzger et al 2010).

2.3 Protocol

According to Solomonov (2011) one of the most important steps when working with the SAF system is the initial preparation of a glide path that will allow free insertion of a #20 K-file to its working length. This, in turn, will allow insertion of the SAF to the full length of the prepared canal (Hof et al 2010).

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

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

Instrumentation protocols should be chosen according to the degree of difficulty expected in a given canal, based in the first instrument to bind (FITB) in the apical part of the canal.

Easy Canals (FITB = #20)

In easy canals, the SAF file is used for 4 minutes with continuous sodium hypochlorite irrigation at a flow rate of 4 ml / min. A short in – and - out pecking motion is used continuously to prevent binding of the file in the canal and to allow the file to change its position in the canal during every outbound stage of the pecking motion. The endpoint of the procedure is the completion of 4 minutes of continuous operation.

Medium Canals **(FITB = #15)**

In medium cases, either a Profile 20.04 (Dentsply-Tulsa, Tulsa, OK) or Mtwo 15.05 (VDW, Munich, Germany) rotary file or similar instruments may be used to establish the glide path required by the SAF file followed by a #20 NiTi K-file. The SAF system is then used as described for the easy canals. For mandibular molars, there is no need to remove the bulk of dentin in the coronal mesial part of mesial canals, as is commonly needed with rotary instruments. The SAF file can easily negotiate a double curve with no risk of file separation or transportation of the canal.

Difficult Canals **(FITB = #10)**

In difficult canals, one of the following glide-path establishment protocols may be applied using special path-establishment instruments from either the Mtwo system, PathFile (Dentsply-Maillefer, Ballegues, Switzerland), or EndoWave MGP (Morita, Tokyo, Japan). The purpose is to establish a glide path that will allow a #20 hand file to reach its working length effectively, safely and with minimal canal transportation.

Such protocols may include the following: (1) Mtwo 10.04 / Mtwo 15.05 / 20 NiTi K file, (2) PathFile 13.02 / 16.02 / 19.02 / 20 NiTi K file, and (3) EndoWave MGP 10.02/ 15.02/ 20.02/ 20 NiTi K-file.

Other similar instruments may be used to gain the same goal: a canal that allows a free passage of a # 20 K-file to the working length (Solomonov 2011).

Irrigation protocol

As mentioned before, SAF has the unique property of allowing continuous irrigation throughout the preparation procedure. When EDTA is to be used for smear layer removal, Metzger et al (2010) proposed the following protocol: the SAF is to be operated in two cycles of 2 minutes each for a total of 4 minutes. During the first minute of each cycle, NaOCl is used as irrigant, whereas EDTA is used during the second minute. At the end, the canal is flushed with NaOCl.

CHAPTER 3

The WaveOne NiTi single file system has been recently introduced by Dentsply Mailefer *(Ballaigues, Switzerland).* This system is designed to be used with a dedicated reciprocating motion motor. It consists of 3 single-use files available in lengths of 21, 25 and 31 mm:

- 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 reduces 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 reduces towards the coronal end **(Figure 1).**

Figure 1. The WaveOne files

3.1 Design of the file

As 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, whereas from D4-D16, they have a unique progressively decreasing percentage tapered design. This design supposedly serves to improve flexibility and conserve remaining dentin in the coronal two-thirds of the finished preparation.

Another unique design feature of the WaveOne files is 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, whereas from D9-D16, these files have a convex triangular cross-section **(Figure 2).** The design of the two WaveOne cross-sections is further enhanced by a changing pitch and helical angle along their active portions. The WaveOne files have noncutting modified guiding tips, in order to enable the files to safely progress through canals.

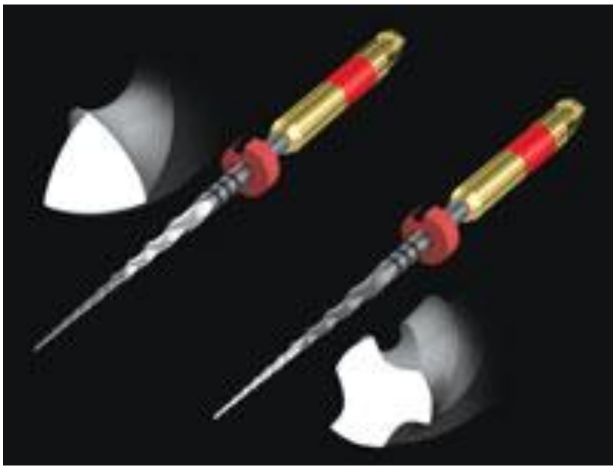


Figure 2. Cross-section in the middle and apical part

Specifically, WaveOne Primary file (#25.08) has a continuously decreasing taper from its tip to its shaft (0.8, 0.65, 0.6, and 0.55) and is characterized 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 changes from a modified triangular convex cross-section with radial lands to a neutral rake angle with a triangular convex cross-section analogue to the ProTaper F2 file near the shaft **(Figure 3).**

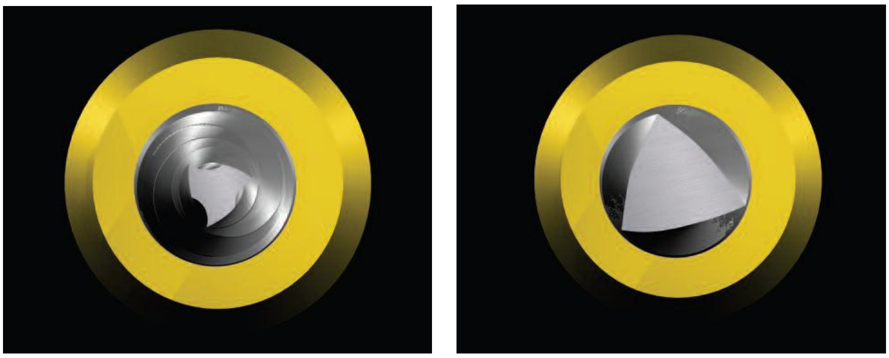


Figure 3. Modified triangular (apical) and triangular (middle) convex cross-section

The WaveOne files are manufactured with M-Wire *(Dentsply Tulsa Dental, Tulsa, OK)* NiTi alloy, which is produced with an innovative thermal treatment process (Gambarini et al 2011). Studies have shown that M-Wire technology significantly improves the resistance to cyclic fatigue by almost 400% compared to commercially available 25/04 NiTi files (Johnson et al 2008). Greater flexibility, less debris extrusion and maintaining canal shape are some of the other advantages of this new supermetal files (Walia et al 1998, Reddy et al 1998, Petiette et al 2001).

The WaveOne motor **(Figure 4)** 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.

Figure 4. The WaveOne motor

3.2 Reciprocation movement – mode of action

The reciprocation working motion consists of a counterclockwise (CCW) and a clockwise motion (CW). The WaveOne files have a left-handed angulation of the blades, which means they cut in the counterclockwise direction (CCW). 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 effect of a screwing effect and file breakage. In principle, the WaveOne system utilizes an engaging angle that is 5 times the disengaging angle **(Figure 5)** in a way that after three engaging/disengaging cutting cycles, the file will have rotated 360° or turned one CCW circle (Ruddle 2012). Due to the fact that the counterclockwise angle is greater than the clockwise one, it is claimed that the instrument continuously progresses towards the apex of the root canal (Yared 2008).

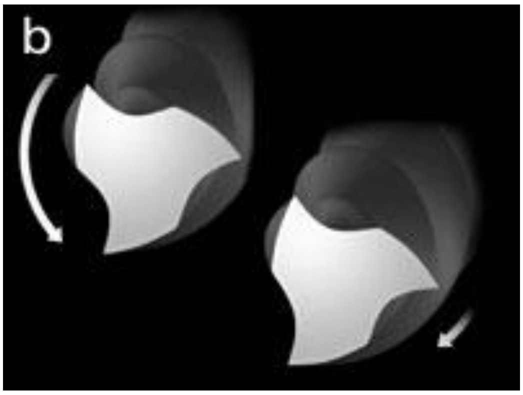


Figure 5. Reciprocation movement

The reciprocating movement aims to minimize the risk of instrument fracture caused by torsional stress: the angle of CCW rotation is designed to be smaller than the elastic limit of the instrument. Despite the mentioned fact that the instrument completes one rotation of 360° in several reciprocating movements, accumulation of metal fatigue remains a concern. A recent study demonstrated that Reciproc *(VDW, Munich, Germany)* instruments were associated with a significantly higher cyclic fatigue resistance than WaveOne instruments (Plotino et al 2012).

3.3 Protocol of use

File Selection

According to the protocol introduced by Webber et al, information from a preoperative radiograph (size and length of canal, number of canals, degree and severity of curvature), but mainly the first hand file into the canal will aid in the selection of the WaveOne file:

1. If a file size 10 K-file is very resistant to movement, WaveOne Small file should be used.
2. If size 10-K file moves to length easily, is loose or very loose, WaveOne Primary file is to be used.
3. If a size 20-K file or larger goes to length, WaveOne Large file should be used.

(Webber et al 2011)

Shaping Technique

With an estimated working length a #10 file is inserted into the orifice to determine if the file will easily move toward the terminus of the canal. A loose #10 file confirms sufficient existing space is available 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 to length. In these cases, Ruddle (2012) 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 foramen is confirmed to be 0.40 mm. When the 40/02 hand file is loose at length, it simply means the foramen 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 (Ruddle 2012).

EXPERIMENTAL PART

An ideal root canal preparation would eliminate all bacteria, their toxins, pulp remnants and debris, thus leaving a perfectly smooth and clean inner surface of dentin. However, despite advances and innovations concerning instruments, techniques and protocols of instrumentation, this is still not the case. In fact, due to the complexity of root canal systems and our inability to instrument all aspects of canal wall surfaces, it is still not possible to completely remove or destruct all bacteria (Bystrom and Sundqvist 1981, 1985). This seems to be even truer, in oval-shaped root canals (Rodig et al 2002, Weiger et al 2002, Paque et al 2010).

Therefore, research concerning “root canal cleanliness” is an ongoing field, and is divided into two distinct categories of studies. The first assesses the quantity and quality of bacteria inside the root canal before and after chemomechanical preparation, based on microbiological techniques (Baumgartner 2004, Bystrom and Sundqvist 1981, 1985). The second is about what “kind of surface” is left behind after instrumentation, concerning pulp remnants, bacteria and debris compacted along the root canal wall (Hulsmann et al 1997). The experimental part of the present thesis belongs in the second category, where the quality of root canal cleaning is evaluated via debris and smear layer removal.

Specifically, its purpose is to examine the cleaning efficacy of the WaveOne primary file (25/08, 25 mm long) and compare it with the SAF file (1.5 mm diameter, 25 mm long) in terms of debris and smear layer removal in oval canals under optical microscopy.

Materials and Method

Sample size calculation

Sample size was initially decided based on previous studies of similar nature (Foschi et al 2004, Metzger et al 2010, Kaya et al 2011, Alves et al 2011). A formal, a priori sample size calculation was not possible since there are no previously published results on the specific comparison.

However, we performed a detailed simulation-based post-hoc power analysis to determine the minimum effect (Odds Ratio for WaveOne vs. SAF) that a study with the same design and sample size would be able to detect at an alpha level of 0.05 while retaining a power of 0.80. Score distribution and differences across examiners, magnification rates and tertiles along with the intra-class correlation of results obtained from the same sample were assumed the same as the ones observed in the current study.

Tooth selection and Specimen Preparation

The protocol was approved by the ethics committee of the Dental School of the University of Athens, and a signed informed consent/assent was obtained from all patients.

Twenty four single rooted, intact permanent human premolars were selected from a random collection of teeth that were extracted for orthodontic or prosthetic reasons. Tissue remnants and external deposits were removed from the teeth by placing them in 2.5% NaOCl solution for 5 minutes, during which the remains were removed with curettes. Specimens were checked using a stereomicroscope to ensure the presence of one main apical foramen and mature apices. All teeth were checked for single-canal anatomy and for the presence of an oval canal using mesio-distal and bucco-lingual digital radiographs (Wu et al 2000). Only teeth with a curvature of less than 10° were used (Schneider 1971). Teeth that did not fulfill all inclusion criteria were excluded. All selected specimens were stored in a 0,1% thymol solution until use.

Group organization

The first stratification of the specimens for the organization of the groups was performed by pair-matching individual teeth based on the similarity of their physical appearance (total length, length of the root, bucco-lingual and mesio-distal dimensions at the cemento-enamel junction). Following, matching was verified by comparing the digital radiographs of both planes, detecting one not calcified, oval canal of same size (excluding long-oval ones) to ensure the creation of homogenous and standardized groups. The age of the teeth was also taken into account given that it affects dentins characteristics and the number of dentinal tubules; age frame 20-35 years (Carrigan et al 1984).

Group A was subjected to chemo-mechanical preparation with the SAF system, whereas Group B was processed using the WaveOne system.

Root canal preparation

<u>Group A - SAF:</u>

Endodontic access cavities were prepared with a #330 bur at first and an Endo Z bur *(Dentsply Maillefer, Ballaigues, Switzerland)* for completion in a high-speed handpiece. Pulpal remnants were extirpated using a broach, with care taken not to push the broach through the apical foramen.

Working length (WL) was determined by measuring the length of a #10 K file that emerged at the apical foramen, minus 1 mm. The tip of the protruding file was observed with the aid of a dental operating microscope (magnification x12.8, *Global Protege plus, Global Surgical Corporation, St. Louis, MA, USA)*.Teeth were initially instrumented using a Gates Glidden bur #3 to a level 2-3 mm apical to the cemento-enamel junction.

Thereafter, irrigation with 2 ml of 2.4% NaOCl solution with a 29g needle (NaviTip 29g; *Ultradent, South Jordan, UT, USA)* took place, and surgical suction was placed close to the access cavity to aspirate the excess irrigation solution. The needle was placed in the coronal third of the canal, and the flow rate was manually controlled at 4 mL/min to achieve maximum effectiveness (Park *et al* 2013). The same flow rate and setup were applied in all of the manually delivered irrigation procedures in this work.

K files #15/0.2 and #20/0.2 *(Dentsply-Maillefer, Ballaigues, Switzerland)* were then used to create the glide path. Only root canals with an initial apical gauging diameter of size 20.02 or less were used. In this phase, 4 teeth needed to be replaced (two from each group), as the #20 file was protruding through the apical foramen. They were replaced by ones that did not significantly differ in size, length or in other aspect (tolerance was set at 0.5mm) and the initial apical gauge was No#20 or less, to ensure the homogenous of the created groups.

A SAF file *(ReDent- Nova*, diameter of 1.5 mm, length of 25 mm) was then used, following a modification of the manufacturer's protocol (Tay *et al* 2010). The SAF file was operated using an RDT handpiece-head with the motor rotating at 5000 rpm, which resulted in 5000 in – and - out vibrations per minute. Continuous simultaneous irrigation was applied using the VATEA irrigation pump (ReDent-Nova), which was connected to the file by a polyethylene tube. Irrigation was applied at 5 mL/min and alternated every minute between 2.4% NaOCl and 17% EDTA until 4 minutes were completed. To allow this mode of irrigation, two VATEA pumps were used; one with NaOCl solution and one with EDTA solution, as described by Metzger et al. (Metzger *et al* 2010). Surgical suction was used to aspirate the excess irrigation solutions.

Each canal was prepared using a new SAF instrument. Final irrigation was performed in all specimens with 10 mL of 2.4% NaOCl, delivered with a syringe and needle that was placed into the canal as deep as possible without resistance, although not deeper than the predetermined WL minus 2 mm.

Recapitulation with a #10 K-file was performed 0.5 mm beyond the WL after every minute (for a total of 4 minutes) to ensure patency (Brady et al 1985) , avoid apical plugging (Holland et al 1980, Souza 2006) and prevent the vapor lock effect at the apical third (Tay et al 2010).

All procedures were done by a single operator.

Group B - WaveOne:

The teeth were initially handled and instrumented as in the SAF group, until the #20.02 glide path was established.

The WaveOne system *(Dentsply-Maillefer)* was then used for the chemomechanical preparation of the canals. The WaveOne files were operated with a reciprocating motion generated by the WaveOne electric motor *(Dentsply-Maillefer, Ballaigues, Switzerland),* which was operated with a 6:1 reduction handpiece *(Sirona, Bensheim, Germany).* The pre-programmed motor was set for the angles of reciprocation and speed designed for the WaveOne instruments. The counterclockwise (CCW) movement was greater than the clockwise (CW) movement and advanced the instrument by engaging and cutting the dentin. CW movement disengaged the instrument from the dentin before it locked into the canal. Three reciprocating cycles completed one complete reverse rotation, whereupon the instrument was gradually advanced into the canal with little apical pressure required (Webber et al 2011).

WaveOne primary files *(Dentsply-Maillefer, Ballaigues, Switzerland)* with a size of 25 and a taper of 0.08 (red colored) were used in a reciprocating and slow in and-out pecking motion, according to the manufacturer's instructions. After three slow in-and-out movements (pecks), 2 mL of 2.4% NaOCl was used as an irrigant. The 29g irrigation needle was placed into the canal as deep as possible without resistance, although not deeper than the predetermined WL minus 2 mm. The flutes of the instrument were cleaned by wiping with sterile gauze after the first three pecks as suggested by the manufacturer (Webber et al 2011).

The reciprocating file was then re-inserted for another set of three pecks and 2 mL NaOCl irrigation, as many times necessary, until the WL was reached and the mechanical preparation of the canal was completed.

During and until the completion of the WaveOne file operation, care was taken to ensure that a total volume of 10 mL of 2.4% NaOCl was used to irrigate each specimen's canal. Subsequently, a flushing with 10 mL of 17% EDTA solution that was activated with an Endo-Activator (15/.02 Yellow tip, *Dentsply-Maillefer, Ballaigues, Switzerland)* for one minute was performed. The EndoActivator tip was placed 2mm less than the working length, according to the manufacturers' protocol.

The final flushing was performed using 10 mL of 2.4% NaOCl solution that was activated again for 30 seconds, according to the manufacturers' protocol (Ruddle 2012).

The design of the study ensured that equal volumes of each irrigant were used for both groups: a total of 22 mL 2.4% NaOCl (2 mL during glide path preparation, 10 ml during instrumentation, 10 mL final flushing) and 10 mL of 17% EDTA solutions.

Each irrigation step was followed by recapitulation to ensure patency (Brady et al. 1985). A #10 K-file was passed 0.5 mm beyond the WL to prevent apical plugging (Holland et al. 1980, Souza 2006) and to prevent a vapor lock effect that could otherwise prevent effective irrigation at the apical third of the canal (Tay et al. 2010).

All procedures were done by a single operator.

Assessment of preparation

Following, two shallow longitudinal grooves were cut in each root in a buccolingual direction, with care not to penetrate into the canal. The sample was then immersed in liquid nitrogen and split longitudinally with a mallet and chisel, resulting in a mesial and distal half of the root canal (Zmener et al 2005). For each specimen, the half with the more intact canal and visible apex was conserved. During this process, two specimens from each group were discarded because roots were split inconveniently.

Specimens were then coded and blindly examined by one examiner under an optical microscope *(Nikon, Eclipse ME600 – camera Nikon FDX-35).*

Using an optical microscope instead of a scanning electron microscope (SEM) offers some important advantages (which will be described in the **Discussion** part of this thesis), but has one main disadvantage: as magnification increases the depth of field decreases. As a result and since the inside of a root canal is not a flat but rather a curved surface, only a part of the image taken is in focus.

To resolve this problem, special software called Helicon Focus *(Helicon Soft Ltd)* was used. Helicon Focus is a program for focus stacking. Focus stacking is a post-processing technique that can extend the depth of field beyond what is available in one shot. Thus, for each specimen, and each specific area of assessment, a number of shots were taken. For each shot, microscope lens were adjusted to focus on a slightly different part of the canal wall, under a slight linear movement from the nearest to the farthest part of the area. Then the software could merge all shots into one sharp image, by blending all the sharp areas together. This way, more "focused" images were produced by stacking a number of photos ranging from 10 to 70 or more, depending on the characteristics (depth of field, curvature) of each specimen.

Serial photo-micrographs were taken of the canal walls at x200 and x500 magnification at the cervical, middle and apical levels (9, 6, and 3 mm from the apex respectively). These serial photographs were then placed adjacent to each other, forming a continuous horizontal examination strip for each of the three levels of observation (Zmener et al 2005).

The resulting pictures were evaluated by 3 examiners for the presence of debris (x200) and smear layer (x500).

Presence of **debris** was defined as the presence of particles or chips of any structure on the surface of the root canal (Wu et al 2001).

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 (American Association of Endodontists' *Glossary of Endodontic Terms*, 2012).

The amounts of **debris** present at **200x** magnification were graded between 1 and 5, as follows:

Score 1: no debris or only isolated small particles present

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

Score 3: clumps of debris covering 25 to 50% of the canal wall area

Score 4: clumps of debris covering 50% to 75% of the canal wall area

Score 5: clumps of debris covering more than 75% of the canal wall area

The amounts of **smear layer** present at **500x** magnification were also graded between 1 and 5, as follows:

Score 1: no smear layer present, all dentinal tubules patent

Score 2: less than 25% of canal wall area covered by thin smear layer, dentinal tubule openings visible

Score 3: up to 50% of the area covered with smear layer, patchy distribution of smear layer

Score 4: a thin homogeneous smear layer visible

Score 5: a thick inhomogeneous smear layer completely covering the canal walls

(Peters et al 2000)

To ensure intraexaminer consistency, the first 8 specimens were evaluated twice by each examiner.

The recordings of the two groups were statistically analyzed in order to explore potential differences between techniques but also between examiners, root tertiles and magnification rates.

Statistical analysis

Data are initially described through two-way contingency tables showing absolute and relative % frequencies **(Tables 1, 2, 3, 4, 5).** Wherever appropriate **(Tables 2, 3, 4, 5)**, the non-parametric coefficient Kendall's τ is given as a measure of the strength of the association between group and Debris or Smear Layer scores (p-values in these cases are given only indicatively as formal confirmatory procedures are being used at the final stage of the analysis).

Data are also presented graphically through bar-charts showing the distribution of scores by group **(Charts 1-6, 7-12).** Results of the exploratory/descriptive analyses are given for each examiner separately but also through the average score of the three examiners. In cases where the average of the examiners' scores was not an integer the result was rounded to the nearest integer.

The agreement between the examiners was also assessed through two-way contingency tables accompanied by the overall percentage of agreement and the weighted version of the coefficient of agreement κ **(Table 6).**

Formal analysis of all results was performed through multivariable ordinal logistic regression models **(Tables 7, 8).** Standard Errors produced by these models were adjusted for the clustering of the results within teeth. Examiner effects were also considered as fixed effects in the multivariable models. Odds Ratios reported by these models correspond to the probabilities of higher scores. For example the Odds Ratio of 3.88 for WaveOne vs. SAF indicates that higher scores are almost 4-times more likely in the WaveOne group compared to the SAF group.

Interactions between group and tertile or magnification were also tested within the same framework of ordinal logistic regression in order to detect whether the group effect was consistent across tertiles and magnification rates.

Although the group by tertile interaction was not strictly statistical significant, a similar subgroup analysis for results from different tertiles is also presented **(Table 8).**

All analyses have been performed with Stata 11 *(Stata Corp., TX USA)*; p-values less than 0.05 are considered statistically significant.

RESULTS

Table 1. Overall results by group (from all 3 examiners)

	Group SAF N (%)	WaveOne N (%)	Overall N (%)
Tertile			
Cervical	60 (33.3)	60 (33.3)	120 (33.3)
Middle	60 (33.3)	60 (33.3)	120 (33.3)
Apical	60 (33.3)	60 (33.3)	120 (33.3)
Magnification			
x200 (Debris)	90 (50.0)	90 (50.0)	180 (50.0)
x500 (Smear layer)	90 (50.0)	90 (50.0)	180 (50.0)
Examiner			
1	60 (33.3)	60 (33.3)	120 (33.3)
2	60 (33.3)	60 (33.3)	120 (33.3)
3	60 (33.3)	60 (33.3)	120 (33.3)
Debris or Smear layer score			
1	116 (64.4)	68 (37.8)	184 (51.1)
2	60 (33.3)	84 (46.7)	144 (40.0)
3	4 (2.2)	22 (12.2)	26 (7.2)
.	0 (0.0)	6 (3.3)	6 (1.7)
Total	180 (100.0)	180 (100.0)	360 (100.0)

Four teeth (two for each group) were damaged during splitting procedures.

Completely cleaned root canals were not found after instrumentation with any of the two systems at any magnification (x200, x500).

Although a 5-score scale was used, all scores ranged between 1 and 3 (for both groups and magnifications). This means that in general root canals showed relatively minimal amounts of remaining debris and smear layer for both groups **(Figures 1-6).**

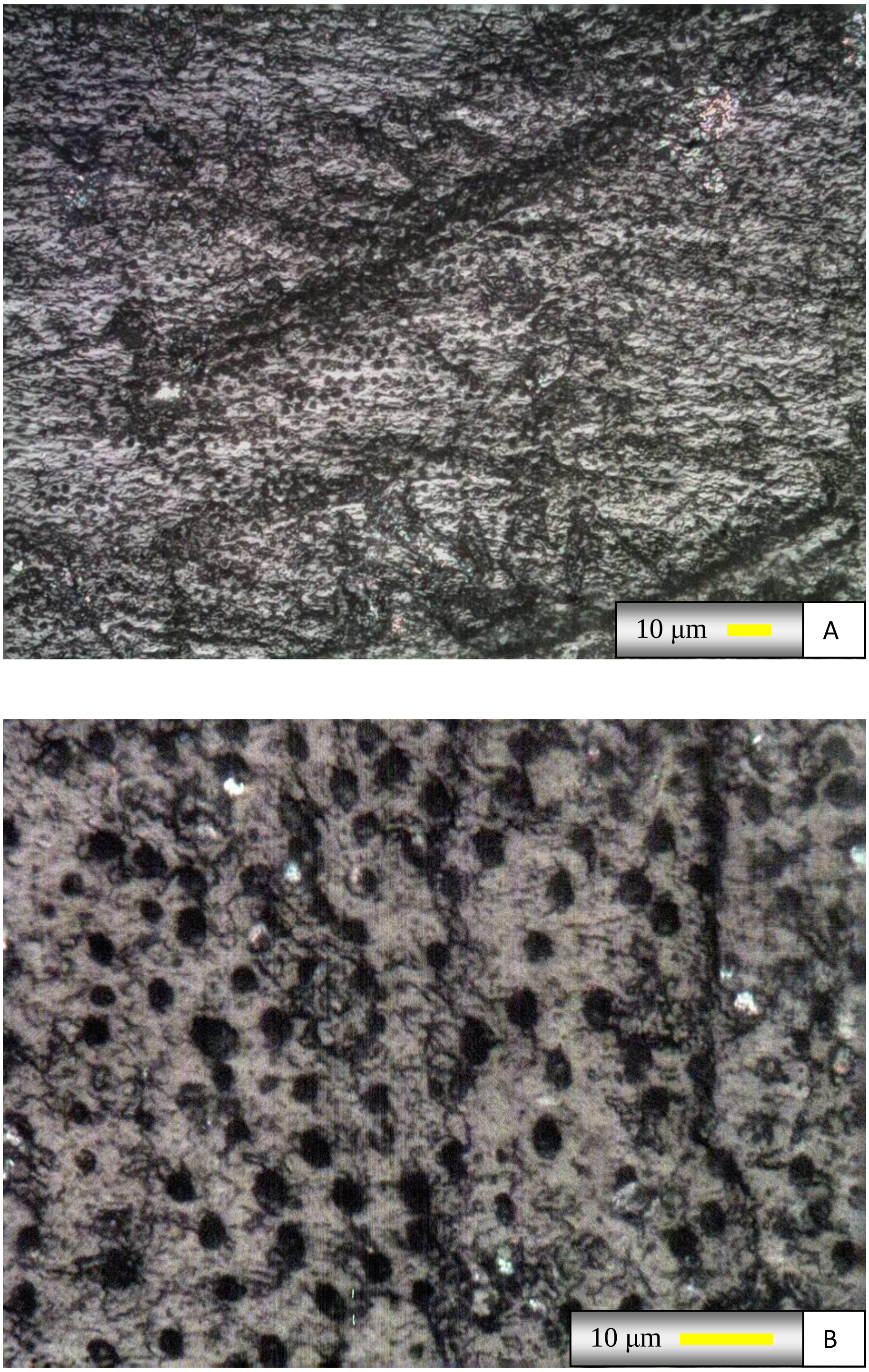


Figure 1. Canal wall after preparation with the **SAF** representative of a **Score 1** at A. 200x and B. 500x magnification

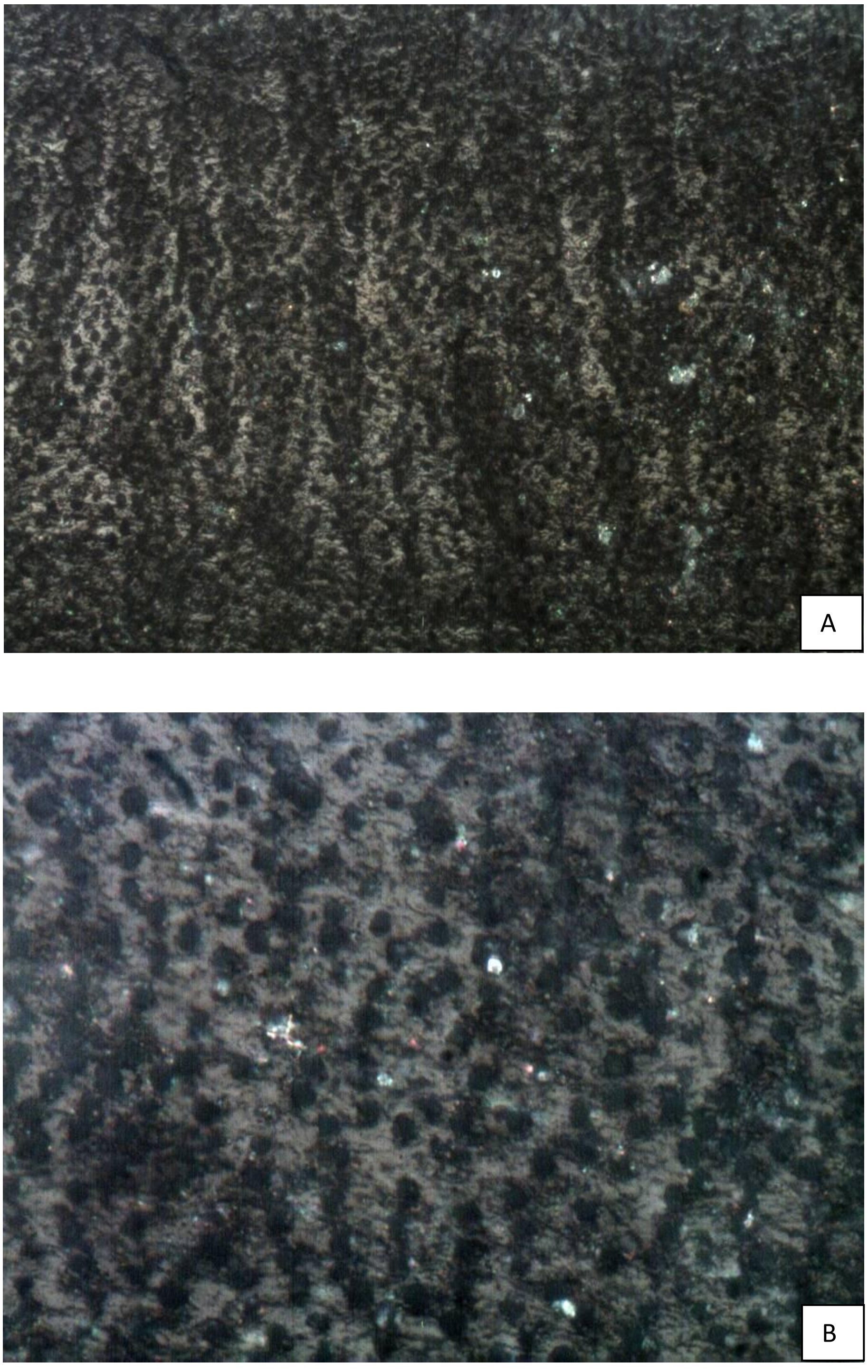


Figure 2. Canal wall after preparation with the **WaveOne** representative of a **Score 1** at A. 200x and B. 500x magnification

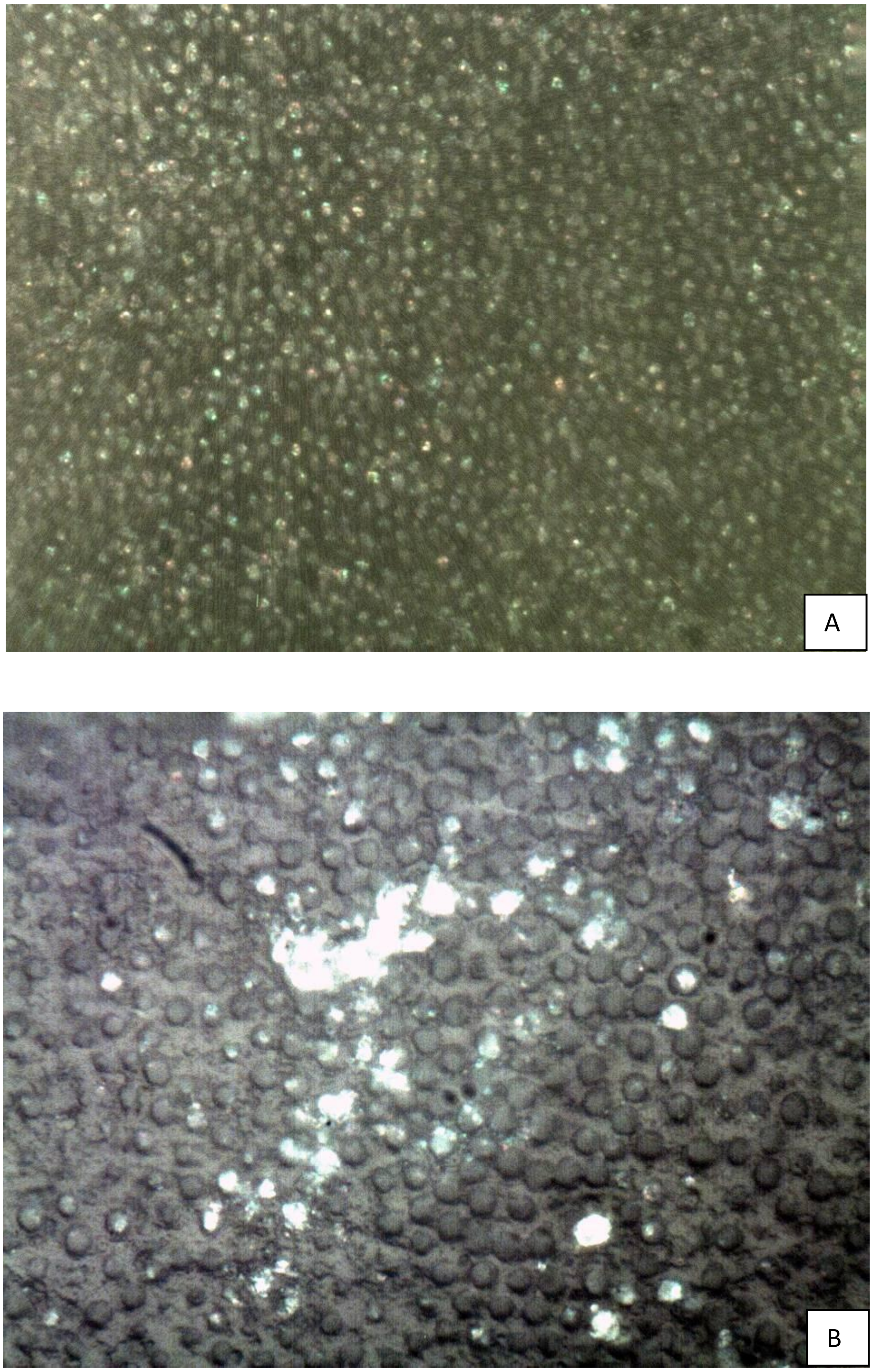


Figure 3. Canal wall after preparation with the **SAF** representative of a **Score 2** at A. 200x and B. 500x magnification

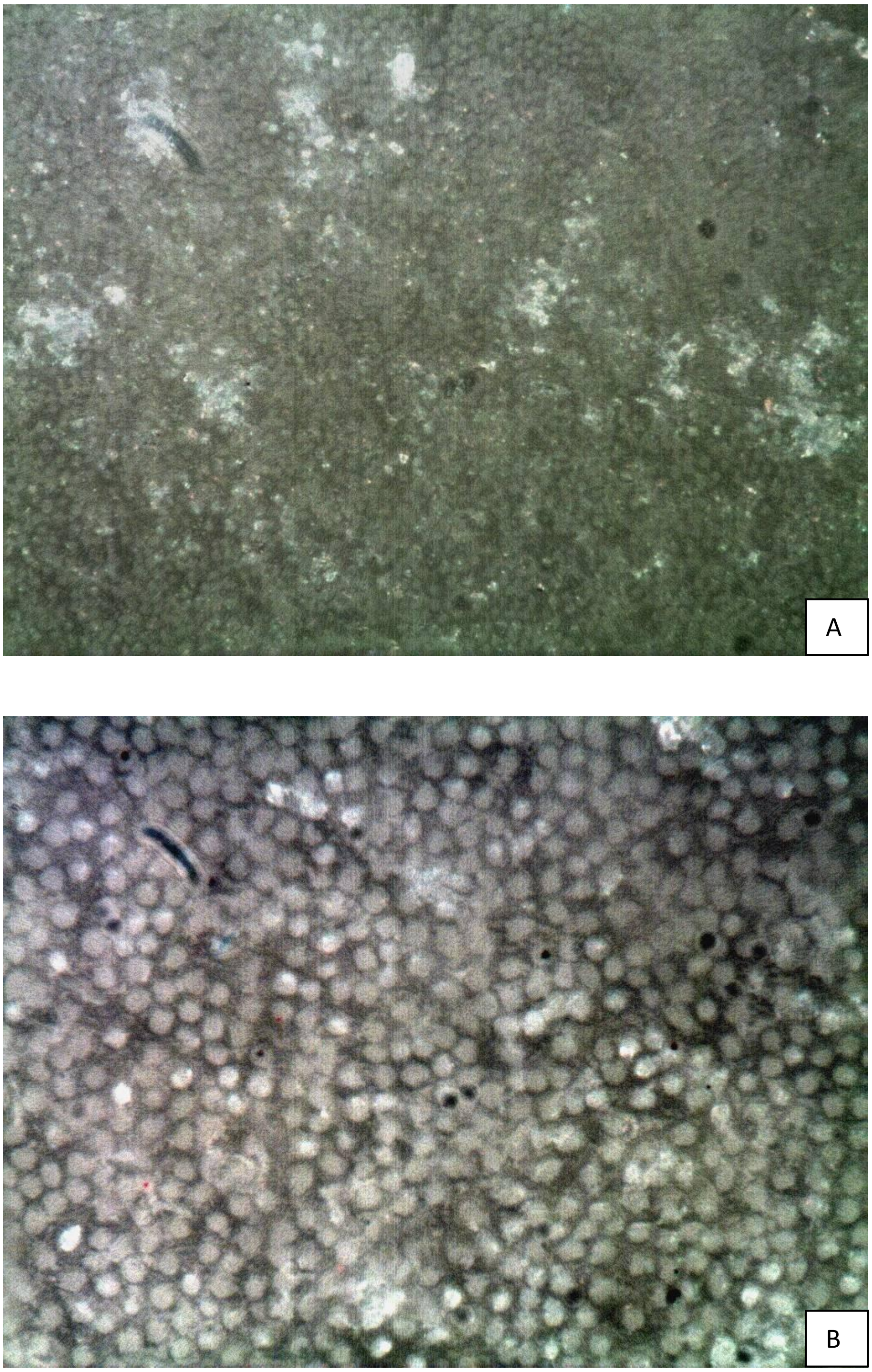


Figure 4. Canal wall after preparation with the **WaveOne** representative of a **Score 2** at A. 200x and B. 500x magnification

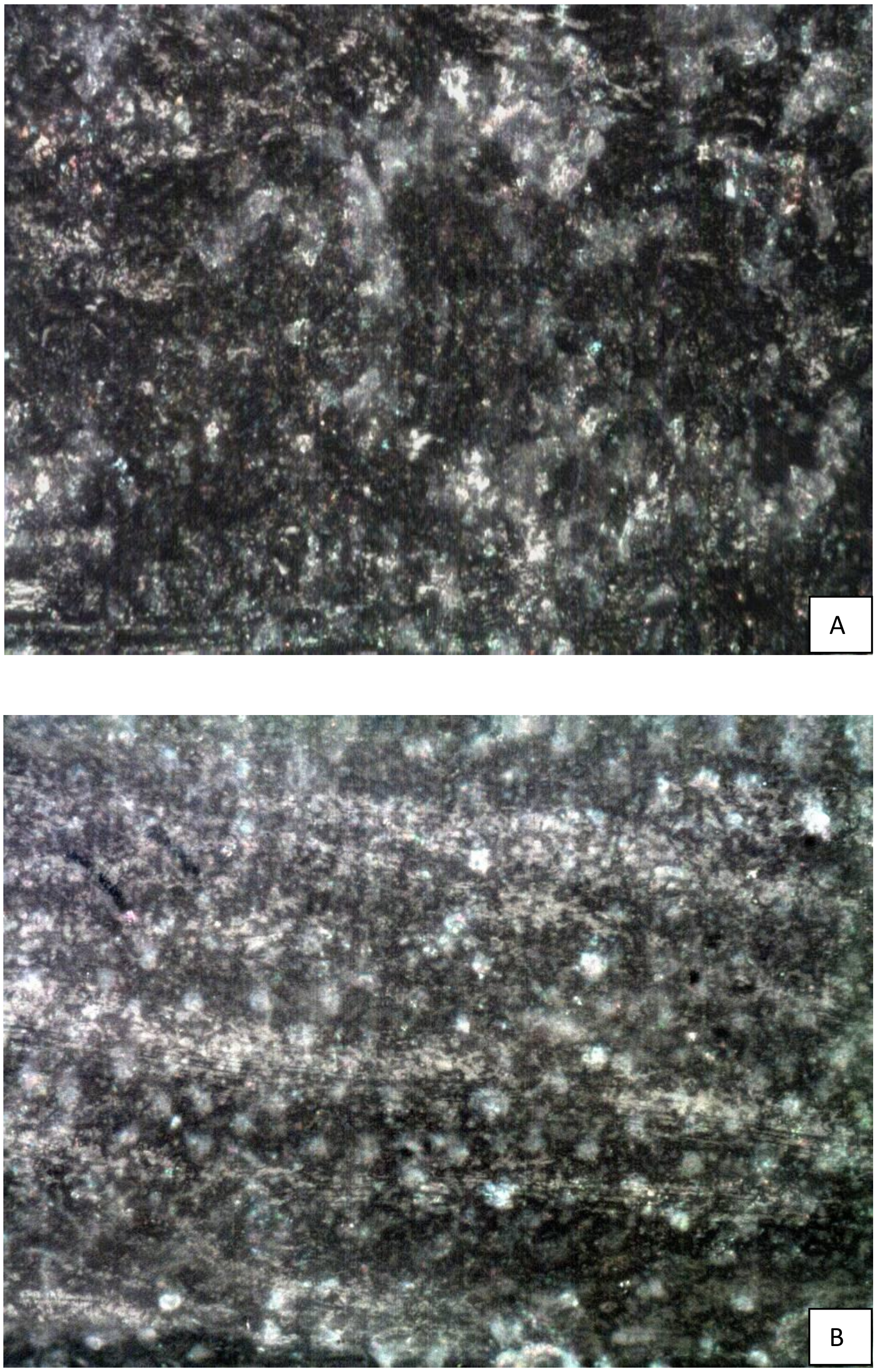


Figure 5. Canal wall after preparation with the **SAF** representative of a **Score 3** at A. 200x and B. 500x magnification

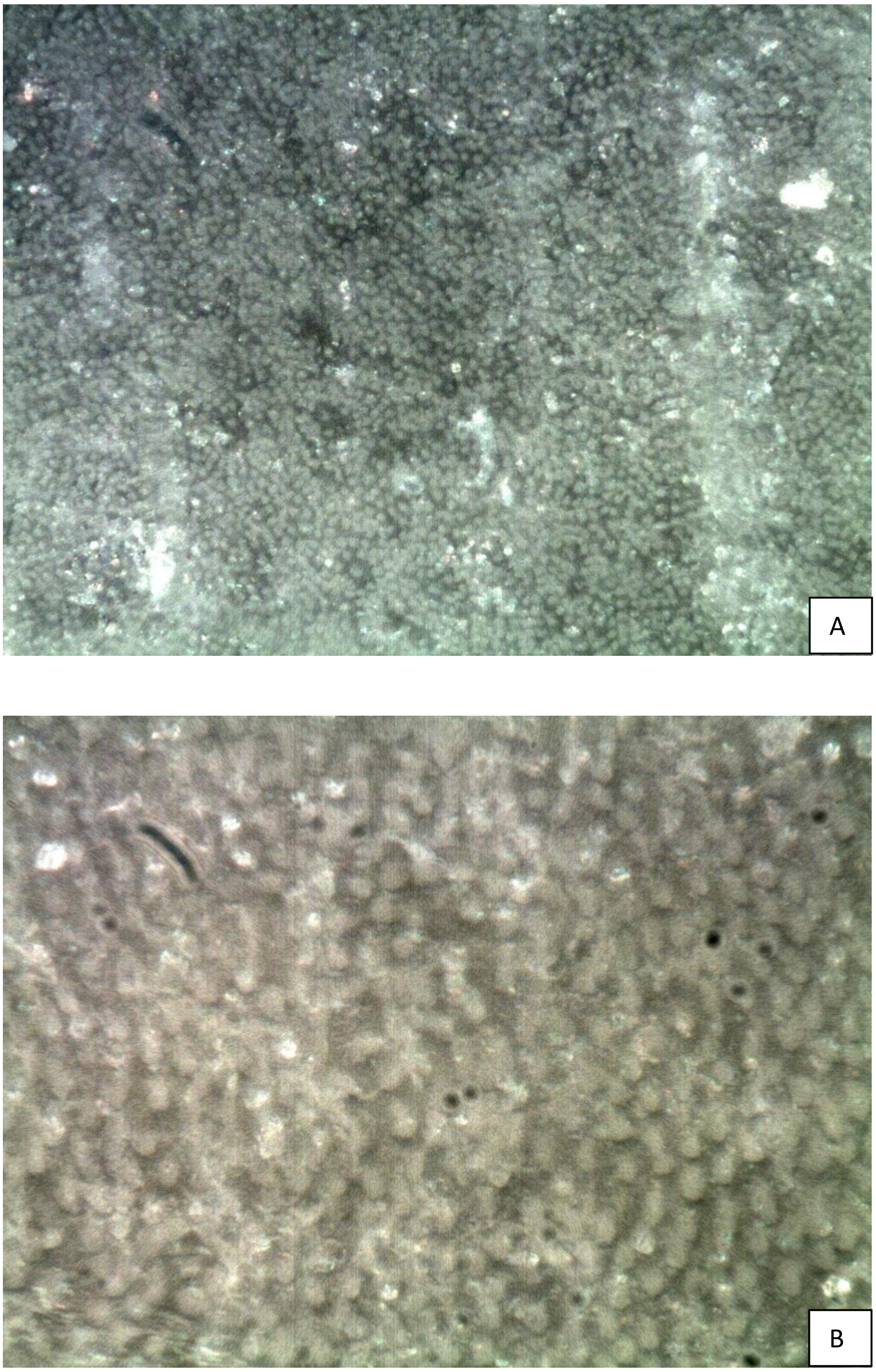


Figure 6. Canal wall after preparation with the **WaveOne** representative of a **Score 3** at A. 200x and B. 500x magnification

Table 2. Results by group - Examiner #1

	Group SAF N (%)	WaveOne N (%)	Overall N (%)	p (Kendall's τ)
Debris score - Cervical 3rd				0.014 (0.500)
1	10 (100.0)	5 (50.0)	15 (75.0)	
2	0 (0.0)	4 (40.0)	4 (20.0)	
3	0 (0.0)	1 (10.0)	1 (5.0)	
Smear layer score - Cervical 3rd				0.026 (0.550)
1	7 (70.0)	3 (30.0)	10 (50.0)	
2	3 (30.0)	2 (20.0)	5 (25.0)	
3	0 (0.0)	5 (50.0)	5 (25.0)	
Debris score - Middle 3rd				0.681 (0.105)
1	7 (70.0)	6 (60.0)	13 (65.0)	
2	3 (30.0)	4 (40.0)	7 (35.0)	
Smear layer score - Middle 3rd				0.024 (0.550)
1	3 (30.0)	1 (10.0)	4 (20.0)	
2	7 (70.0)	4 (40.0)	11 (55.0)	
3	0 (0.0)	5 (50.0)	5 (25.0)	
Debris score - Apical 3rd				0.288 (0.263)
1	8 (80.0)	5 (50.0)	13 (65.0)	
2	2 (20.0)	4 (40.0)	6 (30.0)	
.	0 (0.0)	1 (10.0)	1 (5.0)	
Smear layer score - Apical 3rd				0.101 (0.399)
1	8 (80.0)	3 (30.0)	11 (55.0)	
2	1 (10.0)	6 (60.0)	7 (35.0)	
3	1 (10.0)	0 (0.0)	1 (5.0)	
.	0 (0.0)	1 (10.0)	1 (5.0)	

Table 3. Results by group - Examiner #2

	Group SAF N (%)	WaveOne N (%)	Overall N (%)	p (Kendall's τ)
Debris score - Cervical 3rd				0.014 (0.500)
1	10 (100.0)	5 (50.0)	15 (75.0)	
2	0 (0.0)	4 (40.0)	4 (20.0)	
3	0 (0.0)	1 (10.0)	1 (5.0)	
Smear layer score - Cervical 3rd				0.017 (0.590)
1	7 (70.0)	2 (20.0)	9 (45.0)	
2	3 (30.0)	5 (50.0)	8 (40.0)	
3	0 (0.0)	3 (30.0)	3 (15.0)	
Debris score - Middle 3rd				>0.999 (0.000)
1	7 (70.0)	7 (70.0)	14 (70.0)	
2	3 (30.0)	3 (30.0)	6 (30.0)	
Smear layer score - Middle 3rd				0.182 (0.320)
1	3 (30.0)	1 (10.0)	4 (20.0)	
2	6 (60.0)	6 (60.0)	12 (60.0)	
3	1 (10.0)	3 (30.0)	4 (20.0)	
Debris score - Apical 3rd				0.288 (0.263)
1	8 (80.0)	5 (50.0)	13 (65.0)	
2	2 (20.0)	4 (40.0)	6 (30.0)	
.	0 (0.0)	1 (10.0)	1 (5.0)	
Smear layer score - Apical 3rd				0.712 (0.100)
1	6 (60.0)	4 (40.0)	10 (50.0)	
2	3 (30.0)	5 (50.0)	8 (40.0)	
3	1 (10.0)	0 (0.0)	1 (5.0)	
.	0 (0.0)	1 (10.0)	1 (5.0)	

Table 4. Results by group - Examiner #3

	Group SAF N (%)	WaveOne N (%)	Overall N (%)	p (Kendall's τ)
Debris score - Cervical 3rd				0.034 (0.500)
1	10 (100.0)	6 (60.0)	16 (80.0)	
2	0 (0.0)	4 (40.0)	4 (20.0)	
Smear layer score - Cervical 3rd				0.185 (0.314)
1	5 (50.0)	2 (20.0)	7 (35.0)	
2	5 (50.0)	8 (80.0)	13 (65.0)	
Debris score - Middle 3rd				0.681 (0.105)
1	7 (70.0)	6 (60.0)	13 (65.0)	
2	3 (30.0)	4 (40.0)	7 (35.0)	
Smear layer score - Middle 3rd				0.213 (0.260)
1	2 (20.0)	1 (10.0)	3 (15.0)	
2	8 (80.0)	7 (70.0)	15 (75.0)	
3	0 (0.0)	2 (20.0)	2 (10.0)	
Debris score - Apical 3rd				0.294 (0.258)
1	7 (70.0)	4 (40.0)	11 (55.0)	
2	3 (30.0)	5 (50.0)	8 (40.0)	
.	0 (0.0)	1 (10.0)	1 (5.0)	
Smear layer score - Apical 3rd				>0.999 (0.000)
1	1 (10.0)	2 (20.0)	3 (15.0)	
2	8 (80.0)	5 (50.0)	13 (65.0)	
3	1 (10.0)	2 (20.0)	3 (15.0)	
.	0 (0.0)	1 (10.0)	1 (5.0)	

Chart 1. Results on 'Debris score - Cervical 3rd' by group - Examiners 1, 2 and 3

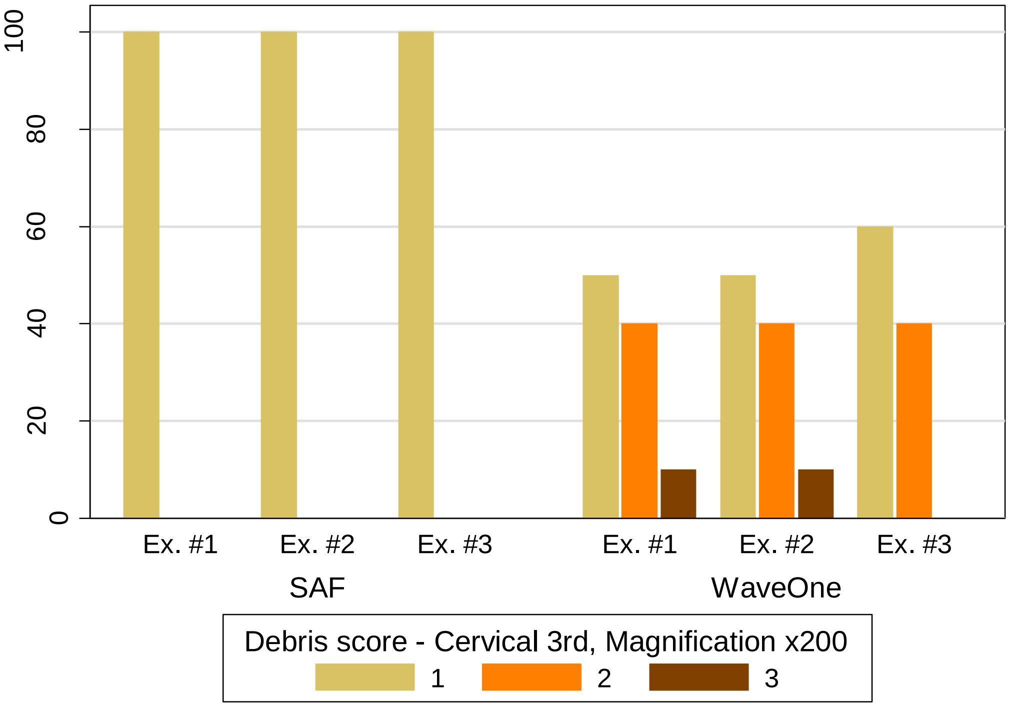
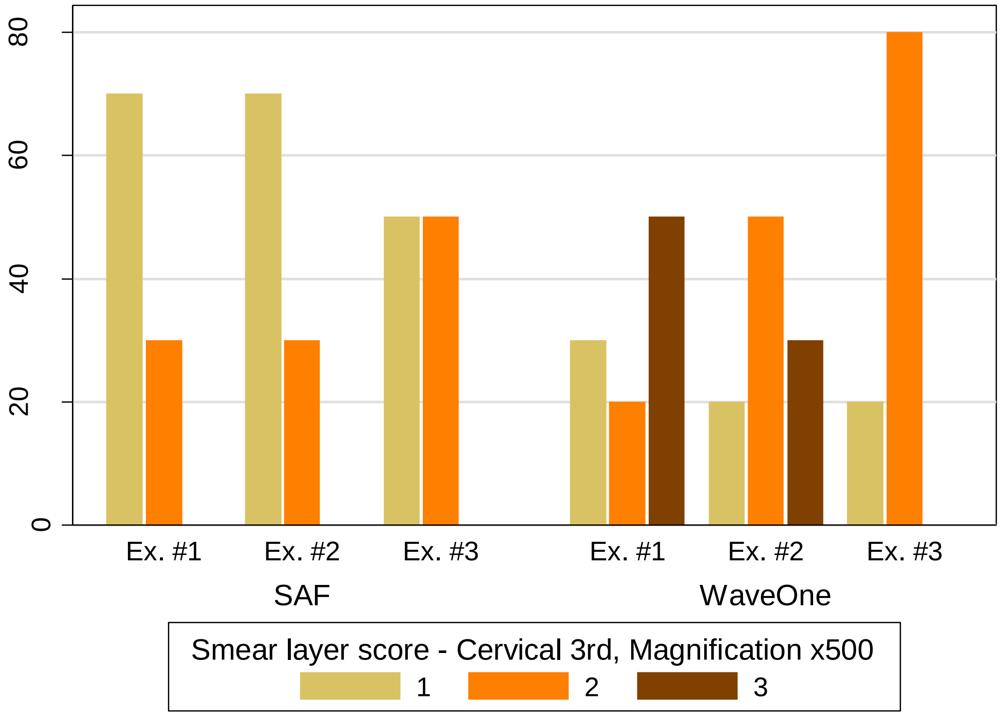


Chart 2. Results on 'Smear layer score - Cervical 3rd' by group -Examiners 1, 2 and 3



2

Chart 3. Results on 'Debris score - Middle 3rd' by group - Examiners 1, 2 and 3

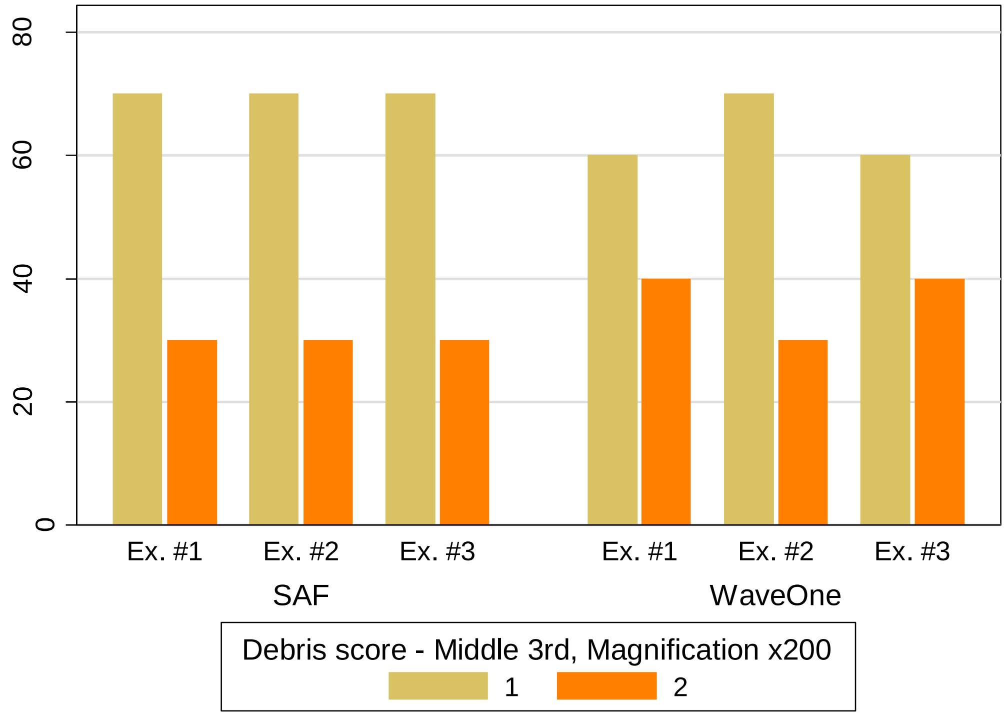


Chart 4. Results on 'Smear layer score - Middle 3rd' by group - Examiners 1, 2 and 3

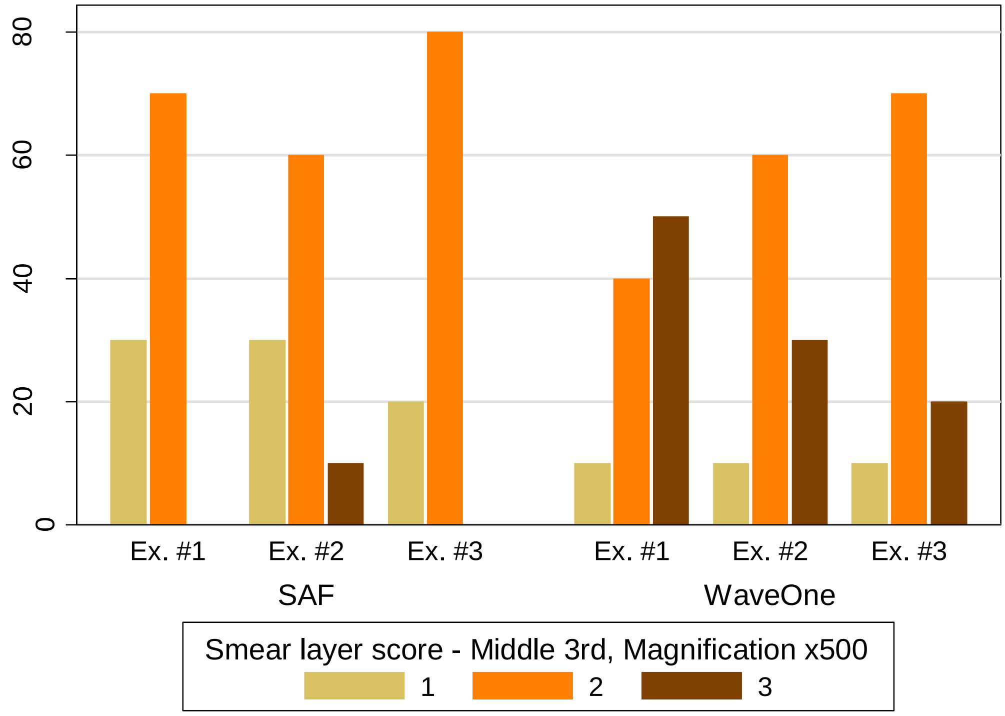


Chart 5. Results on 'Debris score - Apical 3rd' by group - Examiners 1, 2 and 3

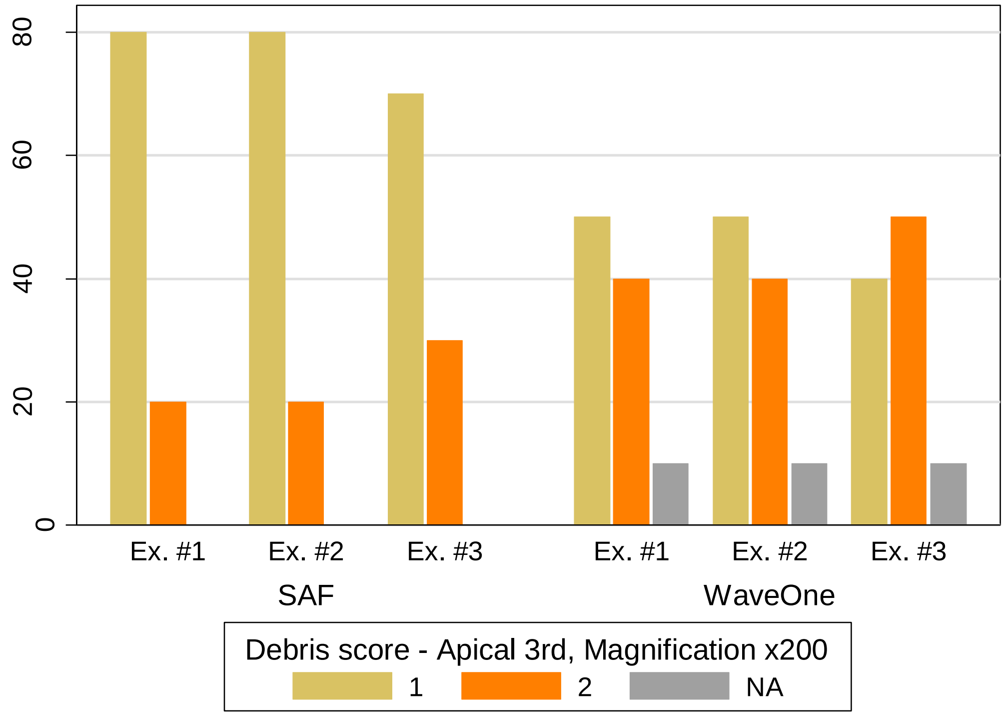


Chart 6. Results on 'Smear layer score - Apical 3rd' by group - Examiners 1, 2 and 3

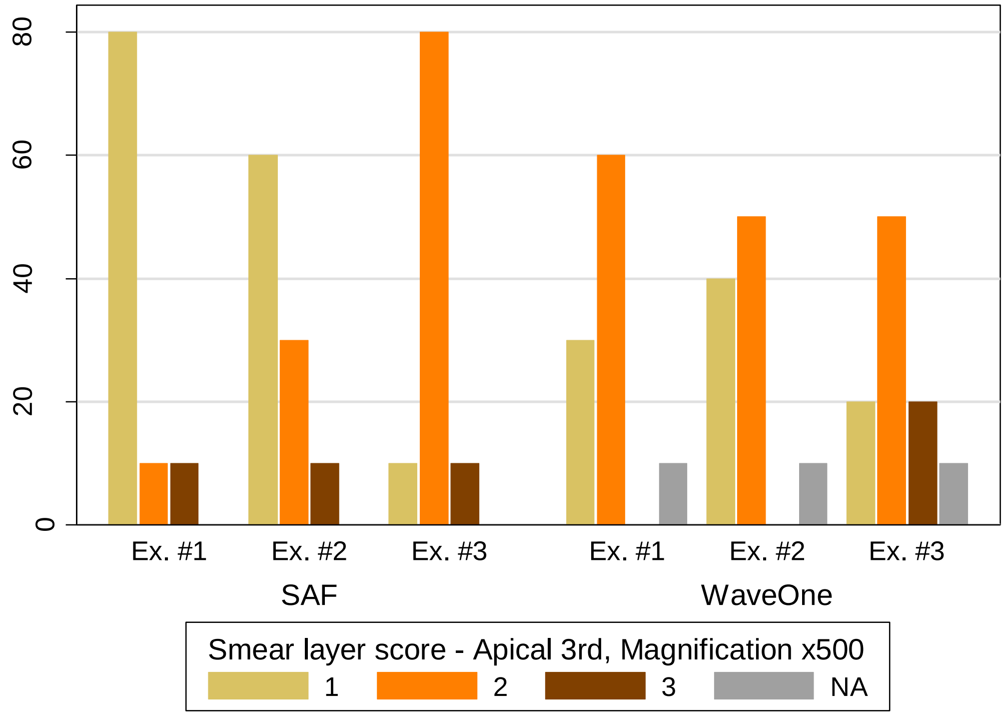


Table 5. Results by group averaged over 3 examiners

	Group SAF N (%)	WaveOne N (%)	Overall N (%)	p (Kendall's τ)
Debris score - Cervical 3rd				0.014 (0.500)
1	10 (100.0)	5 (50.0)	15 (75.0)	
2	0 (0.0)	4 (40.0)	4 (20.0)	
3	0 (0.0)	1 (10.0)	1 (5.0)	
Smear layer score - Cervical 3rd				0.007 (0.660)
1	8 (80.0)	2 (20.0)	10 (50.0)	
2	2 (20.0)	5 (50.0)	7 (35.0)	
3	0 (0.0)	3 (30.0)	3 (15.0)	
Debris score - Middle 3rd				0.681 (0.105)
1	7 (70.0)	6 (60.0)	13 (65.0)	
2	3 (30.0)	4 (40.0)	7 (35.0)	
Smear layer score - Middle 3rd				0.074 (0.410)
1	3 (30.0)	1 (10.0)	4 (20.0)	
2	7 (70.0)	6 (60.0)	13 (65.0)	
3	0 (0.0)	3 (30.0)	3 (15.0)	
Debris score - Apical 3rd				0.288 (0.263)
1	8 (80.0)	5 (50.0)	13 (65.0)	
2	2 (20.0)	4 (40.0)	6 (30.0)	
.	0 (0.0)	1 (10.0)	1 (5.0)	
Smear layer score - Apical 3rd				0.435 (0.199)
1	6 (60.0)	3 (30.0)	9 (45.0)	
2	3 (30.0)	6 (60.0)	9 (45.0)	
3	1 (10.0)	0 (0.0)	1 (5.0)	
.	0 (0.0)	1 (10.0)	1 (5.0)	

The distribution of debris scores at the cervical, middle and apical third are illustrated in **Table 5.** According to initial exploratory analyses by root tertile, the SAF-group showed statistically significant better scores (p=0.014) at the cervical third **(Chart 7).** At the middle and apical thirds no significant difference among the groups was observed **(Charts 9, 11).**

The distribution of smear layer scores at the cervical, middle and apical third are illustrated in **Table 5.** The SAF-group showed marginally better scores (p=0.074) at the cervical third **(Chart 8).** At the middle and apical thirds no significant difference among the groups was observed **(Charts 10, 12).**

Chart 7. Results on 'Debris score - Cervical 3rd' by group - averaged over 3 examiners

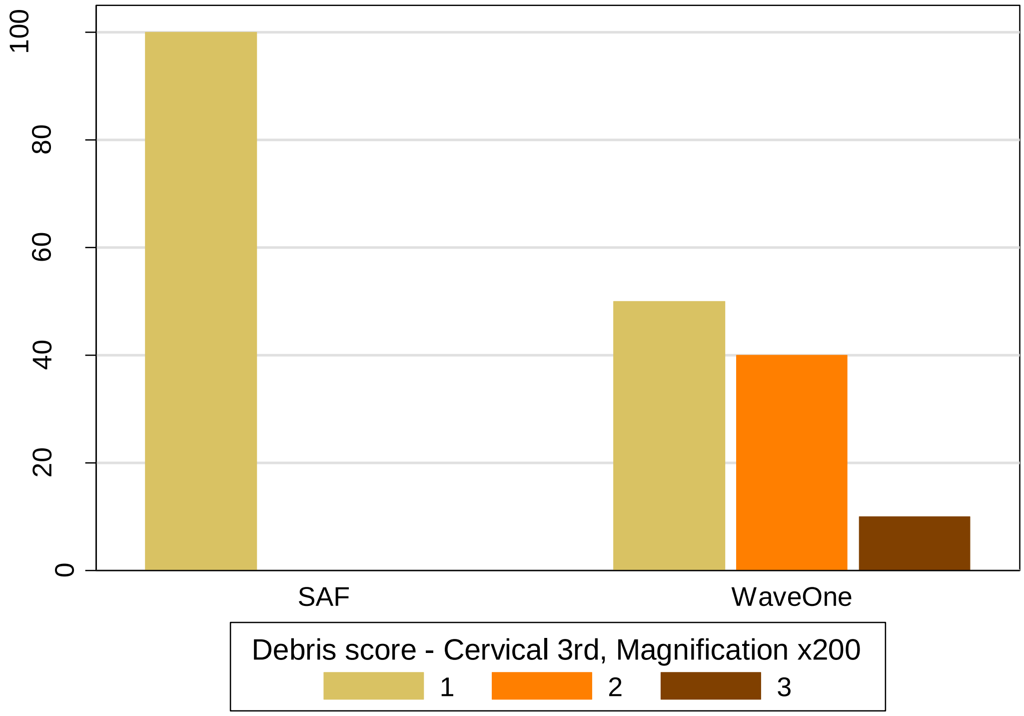


Chart 8. Results on 'Smear layer score - Cervical 3rd' by group - averaged over 3 examiners

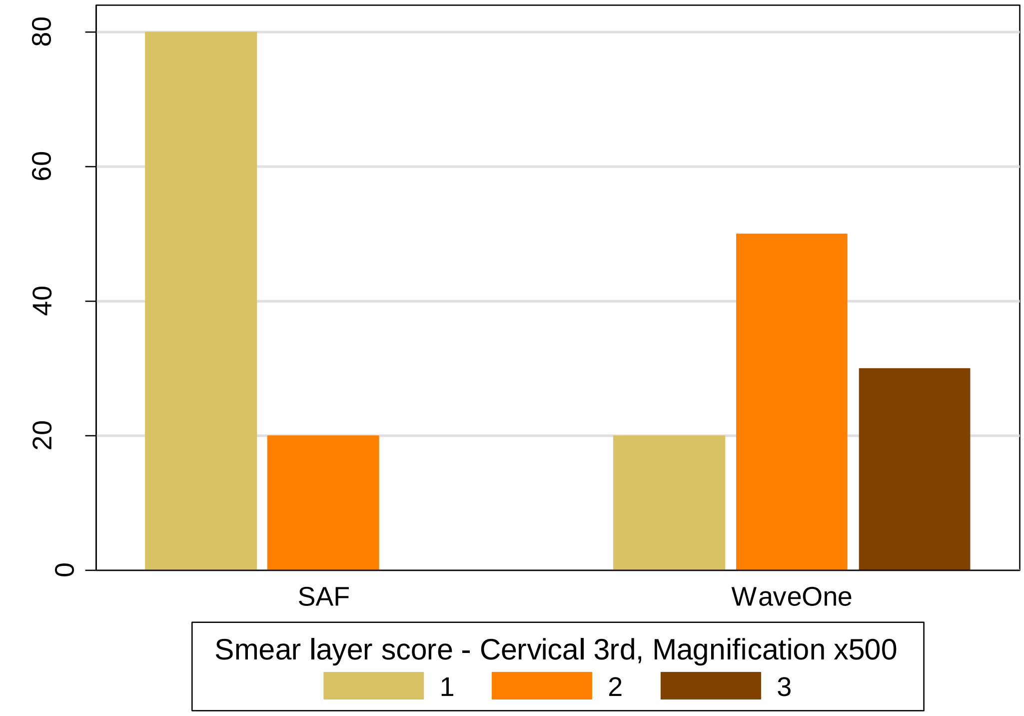


Chart 9. Results on 'Debris score - Middle 3rd' by group - averaged over 3 examiner

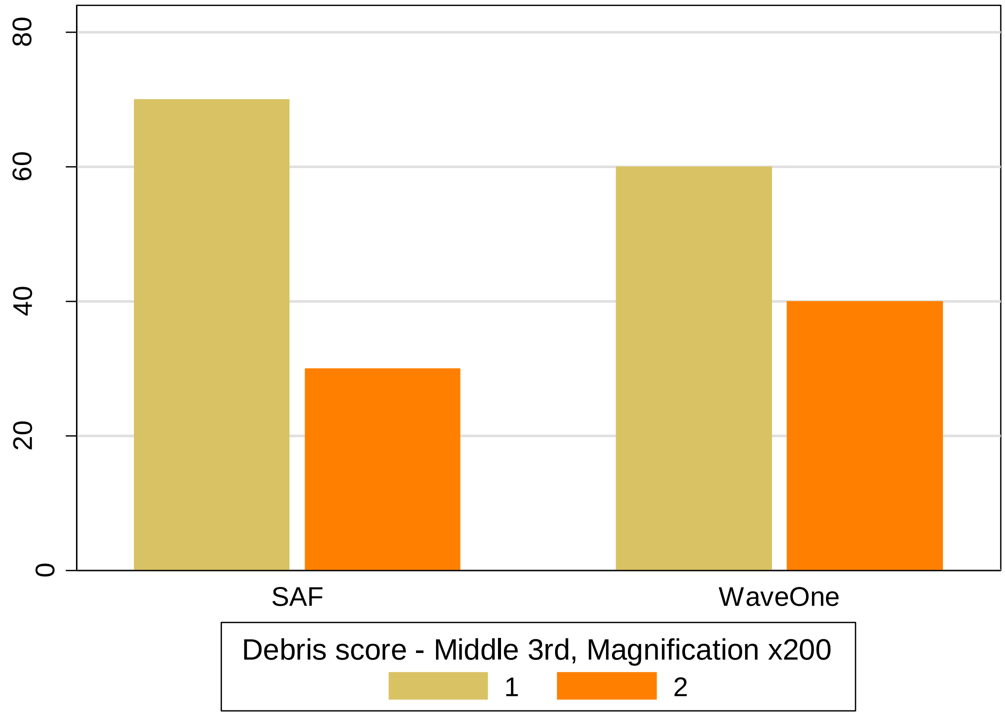


Chart 10. Results on 'Smear layer score - Middle 3rd' by group - averaged over 3 examiners

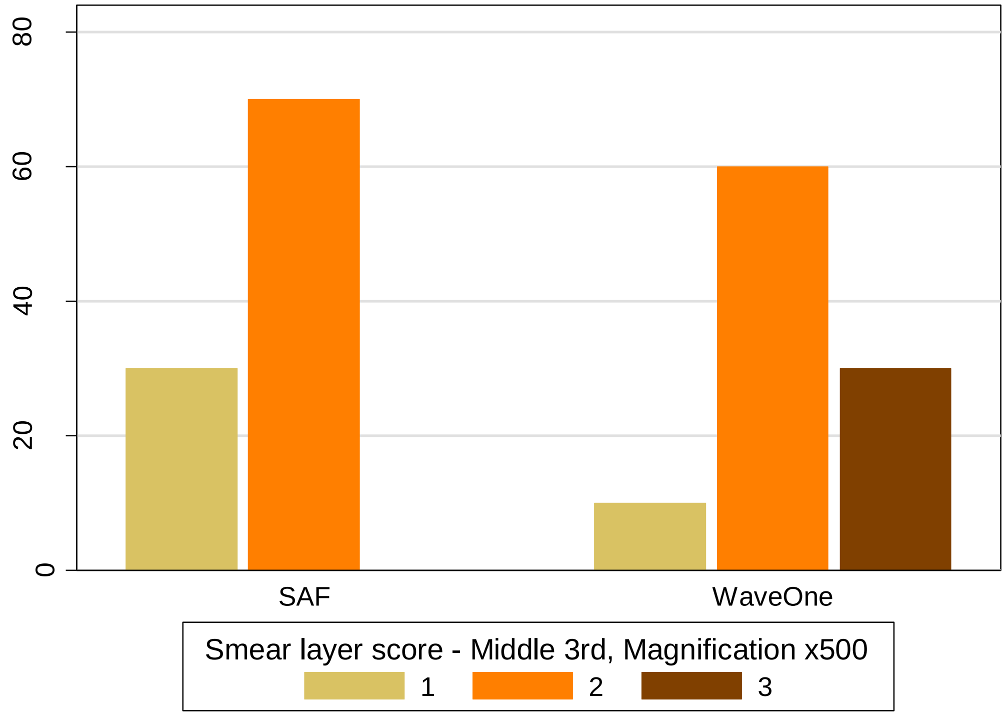


Chart 11. Results on 'Debris score - Apical 3rd' by group - averaged over 3 examiners

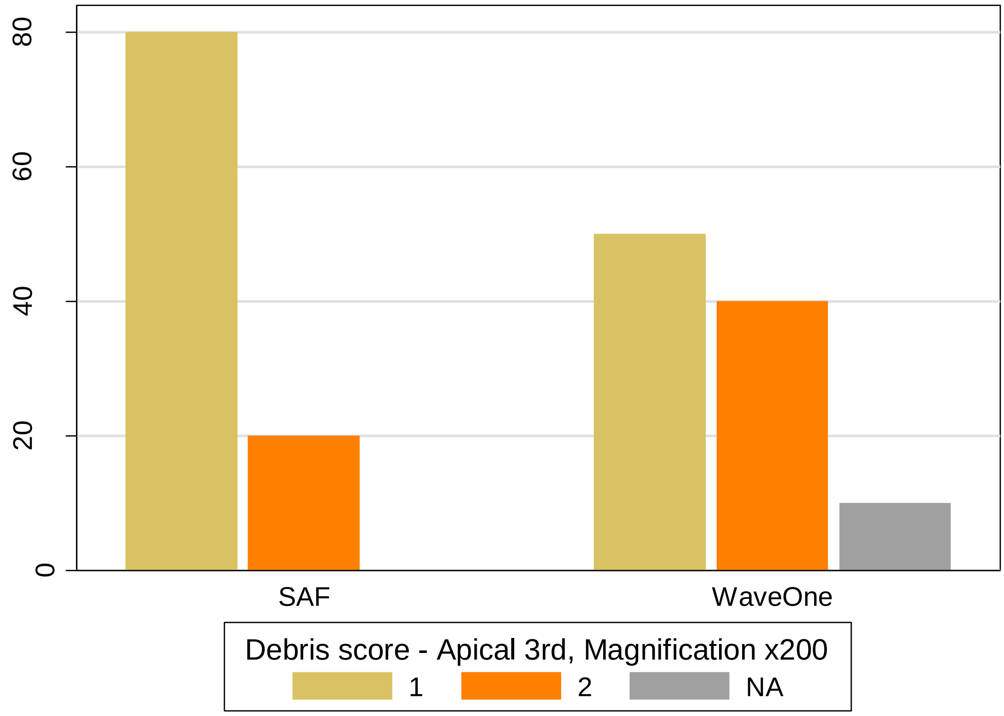


Chart 12. Results on 'Smear layer score - Apical 3rd' by group - averaged over 3 examiners

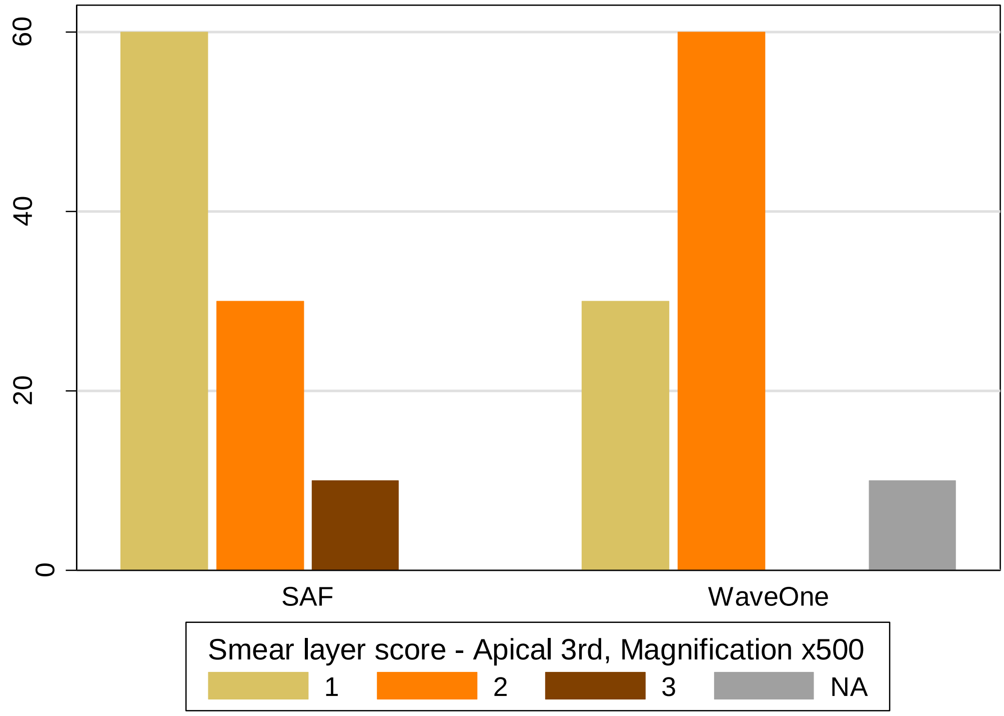


Table 6. Inter-examiner agreement

Examiner 1 vs. Examiner 2

	Examiner 2		
	1 **N (%)**	**2** **N (%)**	**3** **N (%)**
Examiner 1			
1	62 (95.4)	4 (9.1)	0 (0.0)
2	3 (4.6)	36 (81.8)	1 (11.1)
3	0 (0.0)	4 (9.1)	8 (88.9)

Agreement between examiners 1 and 2: 97.46%
Kappa coefficient of agreement: 0.881

Examiner 1 vs. Examiner 3

	Examiner 3		
	1 **N (%)**	**2** **N (%)**	**3** **N (%)**
Examiner 1			
1	50 (94.3)	16 (26.7)	0 (0.0)
2	3 (5.7)	35 (58.3)	2 (40.0)
3	0 (0.0)	9 (15.0)	3 (60.0)

Agreement between examiners 1 and 3: 93.64%
Kappa coefficient of agreement: 0.674

Examiner 2 vs. Examiner 3

	Examiner 3		
	1 **N (%)**	**2** **N (%)**	**3** **N (%)**
Examiner 2			
1	50 (94.3)	14 (23.3)	1 (20.0)
2	3 (5.7)	40 (66.7)	1 (20.0)
3	0 (0.0)	6 (10.0)	3 (60.0)

Agreement between examiners 2 and 3: 94.07%
Kappa coefficient of agreement: 0.676

Kappa coefficient of agreement results showed 88% inter-examiner agreement between examiners 1 and 2, 67% between examiner 1 and 3 and 68% between examiner 2 and 3. However, the percentage of agreement between examiners was higher than 93.64% in all cases **(Table 6).**

Table 7. Results from a multivariate ordinal logistic regression model

Data from all tertiles, magnifications and examiners used

Factor	Odds Ratio	95% C.I.	p-value
Group			
SAF*	1		
WaveOne	3.88	(1.51, 9.95)	0.005
Magnification			
x200 (Debris)*	1		
x500 (Smear layer)	5.77	(3.34, 9.94)	<0.001
Tertile			
Cervical*	1		
Middle	2.17	(0.89, 5.32)	0.090
Apical	1.23	(0.34, 4.52)	0.752
Examiner			
1*	1		
2	0.94	(0.69, 1.28)	0.691
3	1.29	(0.88, 1.89)	0.186

** Reference category*
- Global test for differences by tertile: p=0.089
- Global test for differences by examiner: p=0.182
- Test for interaction between group and magnification: p=0.818
(Test non significant i.e. group differences are similar in both x200 and x500)
- Test for interaction between group and tertile: p=0.139
(Test non significant i.e. group differences are similar in all tertiles)

According to **Table 7** the Odds Ratio of 3.88 for WaveOne vs SAF indicates that higher scores are almost 4-times more likely in the WaveOne group compared to the SAF group. The difference between the two groups independently from magnification and root third (tertile) was statistically significant (p=0.005), with the SAF group exhibiting better cleaning efficacy.

Overall, higher scores were observed for the smear layer (higher magnification, x500) than for debris (smaller magnification, x200), for both groups. The difference was statistically significant (p< 0.001).

There was also a tendency for higher scores at the middle third of the root but the difference was not statistically significant.

Table 8. Separate analyses by root canal third (tertile)
Cervical 3rd: Results from a multivariate ordinal logistic regression model
Data from all magnifications and examiners used

Factor	Odds Ratio	95% C.I.	p-value
Group			
SAF*	1		
WaveOne	12.59	(2.70, 58.85)	0.001
Magnification			
x200 (Debris)*	1		
x500 (Smear layer)	7.52	(2.45, 23.12)	<0.001
Examiner			
1*	1		
2	0.88	(0.51, 1.50)	0.627
3	0.69	(0.30, 1.58)	0.376

** Reference category*

Middle 3rd: Results from a multivariate ordinal logistic regression model
Data from all magnifications and examiners used

Factor	Odds Ratio	95% C.I.	p-value
Group			
SAF*	1		
WaveOne	2.87	(0.60, 13.74)	0.188
Magnification			
x200 (Debris)*	1		
x500 (Smear layer)	12.14	(3.56, 41.37)	<0.001
Examiner			
1*	1		
2	0.79	(0.47, 1.33)	0.384
3	0.82	(0.49, 1.36)	0.436

** Reference category*

Apical 3rd: Results from a multivariate ordinal logistic regression model
Data from all magnifications and examiners used

Factor	Odds Ratio	95% C.I.	p-value
Group			
SAF*	1		
WaveOne	2.41	(0.45, 12.78)	0.303
Magnification			
x200 (Debris)*	1		
x500 (Smear layer)	3.12	(1.39, 7.01)	0.006
Examiner			
1*	1		
2	1.13	(0.75, 1.70)	0.559
3	3.45	(1.55, 7.71)	0.003

** Reference category*

According to **Table 8** in the cervical root third (tertile) the SAF-group exhibited statistically significant better cleaning efficacy (p=0.001) compared to the WaveOne-group, with an odds ratio of 12.59, irrespectively of magnification level.

In the middle and apical third the difference was not statistically significant, though a tendency was noted, with lower scores for SAF-group (for both debris and smear layer). However, taking into consideration the Test for interaction between group and tertile **(Table 7)**, which revealed no statistically significant interaction; the SAF-group can be considered as having better cleaning efficacy in all tertiles.

Post-hoc power analysis

Results from a post-hoc power analysis showed that a study with the same design, similar distribution of results across examiners, root tertiles and magnification rates, similar intra-class correlation of scores obtained from the same tooth and the same sample size as the current study would have a power of at least 80% to detect a significant difference between techniques at an alpha level of 0.05 if the corresponding Odds Ratio was at least 4.3. The power of such a study to detect an Odds Ratio of 3.9 (as the one observed in our study) would be 72.9%. These results suggest that given the current sample size and design the power of our study is satisfactory but subgroup analyses might be underpowered.

DISCUSSION

Single rooted permanent human premolars were selected for this study. It is known that premolars with one root canal present an oval diameter, especially at the coronal and middle level (Vertucci 2005). 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 (Wu et al 2000). 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 (Wu et al 2000), the selected teeth presented a suitable choice for examining the cleaning efficacy of two relatively new single file systems, the recently introduced self-adjusting file (SAF) (ReDedent-Nova, Ra'anana, Israel) and the WaveOne. This is of great importance since a number of studies have shown that the SAF is superior in preparing oval canals (Metzger et al 2010, Siqueira et al 2010, De-Deus et al 2011, de Melo Ribeiro et al 2013).

1. SEM vs Optical Microscope

The present study addressed root canal cleanliness through observation on an optical microscope. Almost every other similar study in the past used a scanning electron microscope (SEM). The reason for using a SEM is that it offers a higher resolution (the minimum distance that can be separated as two distinguishable points in the SEM image) and is therefore also able of a higher magnification (Stewart 1985). In addition, scanning electron microscopy (SEM), has a greater depth of field compared to light microscopes due to the nature of electrons. These are desirable attributes when observing a small, usually curved surface like the inside of a root canal (Arends et al 1987).

However, SEM observation presents some major disadvantages which are often overlooked. De-Deus et al (2011), in a recent critical appraisal of smear layer-removal studies, roughly addressed some of these problems. Although, a detailed analysis of the working principles of a SEM are beyond the purpose of the present thesis, a brief mention of some of the aforementioned problems is needed in order to explain the utilization of optical microscopy:

Specimens observed on a SEM require a surface stain-coating with metals (usually gold) for electron conducting. This coating is necessary to prevent the charge-up on the specimen (tooth) surface by covering it with a conductive material, and to increase secondary electron emission by covering a specimen of low secondary electron emission with a metal of high secondary electron yield. If the coating is too thick, its particles become visible while at the same time the structures of interest may be obscured. Generally, surface coating "changes", to a certain degree, the characteristics of the surface observed. On the other hand, optical microscopy offers the advantage of observing a surface "as it really is", without altering its surface with another, however thin, material and in real colors (Allan-Wojtas et al 2008).

In scanning electron microscopy (SEM) an electron beam is focused into a small probe and is rastered across the surface of a specimen. Penetration depth of the electrons may vary depending on the accelerating voltage used. In SEM, finer surface structure images can generally be obtained with lower accelerating voltages. At higher accelerating voltages, the beam penetration and diffusion area become larger, resulting in unnecessary signals (e.g., backscattered electrons) being generated from within the specimen. These signals reduce the image contrast and veils fine surface structures. In other words, higher acceleration voltage results in more unclear surface structures. When observing a surface area like root dentin with small dentinal tubules in various sizes / depths, it can be assumed that such variations can interfere with the resulting image (Stewart 1985).

Images produced with the SEM will reveal a phenomenon termed the "edge effect", which is a result of the specimen's surface morphology. It means that edges and ridges of an observed sample emit more secondary electrons and thus appear brighter in the image taken. As already mentioned, an object like the inside of a root has many uneven surfaces. A high number of slants contribute most to the contrast of secondary electron images. As a result, areas inside the root canal, notably the circumference of dentinal tubules' openings may appear brighter than others thus interfering with the scoring process.

Specimen damage is possible by the electron beam: the loss of electron beam energy in the specimen occurs mostly in the form of heat generation at the irradiated point. The temperature increase at an irradiated point is dependent on: the electron beam accelerating voltage and dosage, scanning area, scanning time and the heat conductivity of the specimen. Traditional high-vacuum SEMs can cause damage to samples, i.e. teeth, by the loss of water that occurs as a result of heat generation (Fassel and Edmiston 1990, Little et al 1999). Hence, traditional SEM studies do not allow the observation of water-containing components of dentin, because the sample-chamber is under high vacuum most of the time (De-Deus et al 2011)

At this point, it should be mentioned that the environmental scanning electron microscopy (ESEM), an evolution of the SEM in the analysis of biological materials, can overcome some of the aforementioned problems. Namely, no metallization of samples is required and the damage caused is drastically minimized as they can be observed in low-vacuum conditions (De-Deus et al 2011).

The aforementioned disadvantages don't apply on optical microscopy. Rather, an optical microscope gives a direct, real color imaging with no need of sample pretreatment. As already mentioned an optical microscope has lower resolution (mainly due to the light diffraction limit) than a SEM and therefore is capable of smaller magnification. The actual power or magnification of a compound optical microscope is the product of the powers of the ocular (eyepiece) and the objective lens. The maximum normal magnifications of the ocular and objective are 10× and 100× respectively, giving a final magnification of 1,000×. Such levels of magnification are completely adequate for examining debris and smear layer, according to the majority of similar studies (Takeda et al 1999, Mayer et al 2002, Zmener et al 2005)

The main deficiency however, and the reason why optical microscopy isn't used in cleaning efficacy studies has to do with a term called "depth of focus". For the optical microscope, the depth of focus is the distance above and below the image plane over which the image appears in focus (Goldstein 1984). As the magnification increases in the optical microscope the depth of focus decreases. As a result and since the inside of a root canal is not a flat but rather a curved surface, with a variety of slants only a part of the image taken is in focus (sharp).

In our study, the use of a software called Helicon Focus (Helicon Soft Ltd) was advocated to overcome this problem, as it is a focus stacking program. Focus stacking is a post-processing technique that can extend the depth of field beyond what is available in one shot. Using this software in the way described at the Experimental

Part of the present thesis we were able to blend all the sharp areas of a number of pictures of the same root wall area and merge them into one sharp image.

De-Deus et al (2008) recently proposed an optical microscopy method for smear layer observation. This method however, requires a special preparation of the samples through grinding and polishing procedures, to render a flat surface. As the authors comment “this kind of specimen preparation does not reproduce the real smear layer obtained in clinical conditions”. To the best of our knowledge, the method used in the present study is the first that uses an optical microscope to examine root specimens in the “traditional” way normally applied for the SEM.

2. Scoring system

The standard method for evaluating post-operative root canal cleanliness is examining root segments under the microscope. Root sections can be longitudinal (Hulsmann and Stryga 1997, Bolanos et al 1998) or horizontal (Stamos et al 1987, Dietrich et al 2012). In our study we used longitudinal sections, because they allow nearly complete inspection of both halves of the entire root canal. Horizontal sections are preferable for investigating lateral recesses and isthmuses which was not the objective of this study.

To investigate debridement quality under the microscope (usually SEM) several different protocols have been described. Some of them are only of descriptive nature but most of them use predefined scores. The scoring systems used also vary and can include three scores (Walker and del Rio 1991, Wu et al 1995, Zmener et al 2005), four scores (Hulsmann and Stryga 1993, Prati et al 1994, Bertrand 1999), five scores (Hulsmann et al 1997, Peters 2000) or even seven scores (Cheung and Stock 1993).

The most commonly used scoring system used for evaluating canal cleanliness is the one by Hulsmann et al (1997). It is a 5-scale scoring system, using the SEM under x200 magnification for debris and x1000 magnification for smear layer assessment. For example, score 1 for debris means a clean root canal wall with only a few debris particles, score 3 agglomerations of debris covering up to 50% of canal wall and score 5 describes a completely, or nearly completely, debris covered canal wall. Accordingly, score 1 for smear layer means no presence of smear layer and open dentinal tubules, score 3 an homogeneous smear layer covering the canal wall and only a few dentinal tubules open, and score 5 describes a heavy, inhomogeneous smear layer covering the complete root canal wall. A problem with this scoring system is that it is qualitative and not *quantitative* in nature, meaning the observer roughly assesses the amount of debris, smear layer and open dentinal tubules in any given root canal wall area. In addition, criteria for scores 3 and 4 for the smear layer are almost indistinguishable.

In an attempt to quantify results and increase objectivity of observations, Peters et al (2000) suggested a scoring system that describes the percentage of dentin wall area that is covered with debris or smear layer. Thus, score 2 means debris covering less than 25% of canal wall, score 3 debris covering 25 to 50%, and score 5 more than 75% of canal wall covered with debris. Accordingly, score 2 describes less than 25% of canal area covered with smear layer and some open dentinal tubules, score 3 presence of smear layer along roughly 50% of the area, and score 5 a thick inhomogeneous smear layer covering the whole canal wall. The magnifications used are smaller, namely x10-15% for debris and x200-400 for smear layer, which offers the advantage of examining a bigger percentage of the root canal walls. However, there is no procedure by which the percentage of debris covered area can be calculated precisely, which is rather empirically assessed by the observer.

Other scoring systems have also been used, varying in the scoring scale number, magnification used and canal area on which they focus. Bechelli et al (1999) followed a similar technique by examining specimens under the SEM at x200 for debris and x780 for smear layer. The main difference is that their scoring system has 4 scores, thus being less precise on describing certain morphological features. As a result, a root canal wall with no smear layer at all will receive the same score (namely 1) as an area covered in less than 25% with smear layer. Zmener et al (2005) worked at x200 for debris and x400 for smear layer but used a 3-scale scoring system.

A different scoring system proposed by Prati et al (2004) is based on six microphotographs taken at each third (coronal, middle and apical) in standard positions at a standard magnification of x2,000 (SEM). As a result of such high magnification, smear layer scoring is largely based on the number of open dentinal tubules, which is not quite accurate, especially at the apical third where there is a decrease in dentinal tubules density (Mjor et al 2001). Finally, a more recent study proposed the use of an automated digital analysis method instead of the evaluators' traditional method (George et al 2008).

Based on all the above advantages and disadvantages of different scoring methods, the choice for this study was a combination of the protocols described by Peters et al (2000) and Mayer et al (2002). It employs a 5 scoring-scale for debris and smear, which was analytically described in the Experimental part of this study. We used an x200 magnification for debris and x500 for smear layer evaluation, in an optical microscope rather than a SEM nonetheless. An x500 scale magnification in the optical microscope was evidently adequate to examine and score features like dentinal tubule openings. The use of larger magnifications seen in other studies (Foschi et al 2004, Zand et al 2007) has the disadvantage of observing an even smaller area, thus being even less representative of the entire canal wall (Zmener et al 2005). The major advantage though of this technique is that, at each level of observation, horizontal serial micro-photographs are taken and placed adjacent to each other. This way we could examine a much more representative percentage of the root canal wall. Mayer et al (2002), in their study used a 200μm square grid which was superimposed onto the photographs in order to evaluate each assessment unit. This was not the case in our study. However, it is true that a grid scoring method, such as the one presented by Wu and Wesselink (1995) has the potential of yielding more accurate findings, when scoring debris and smear layer, due to the fact that they are often non-uniformly distributed.

3. Critical appraisal of our study

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 (Baumgartner and Mader 1987, Grawehr et al 2003).

<u>Volume and time of irrigants:</u> Studies have shown that larger volumes of NaOCl and EDTA can result in significantly cleaner root canal walls compared to smaller volumes (Yamada et al 1983). The duration of exposure has also been found to have

an effect on the tissue-dissolving ability of the NaOCl (Senia et al 1971). Volumes of irrigants and the time they were in contact with canal walls could not both be standardized along with following manufacturer's recommendations for each file system. A decision was made to ensure equal volumes of both irrigants at both groups: 22 mL 2.4% NaOCl (2 mL during glide path preparation, 10 ml during instrumentation, 10 mL final flushing) and 10 mL of 17% EDTA, and to follow the clinical protocols proposed by the manufacturers in an attempt to simulate clinical conditions. This resulted in different times of exposure and activation for the two irrigants between the two groups which may have an influence on final results.

Concentration of NaOCl: Studies have used NaOCl in concentrations varying from 0.5% to 5.25%. This is still a topic of controversy, even in terms of clinical use, although no significant differences between different NaOCl concentrations with regard to antimicrobial effectiveness have been found (Byström and Sundqvist 1985, Siqueira et al 2000). There is some evidence in vitro that a 1% NaOCl solution should suffice to dissolve the entire pulp tissue in the course of an endodontic treatment session (Sirtes et al 2005). Areas left unclean are most likely the result of the inability of solutions to physically reach these areas rather than their concentration (Senia et al 1971). In our study a 2.4% NaOCl solution was used, a concentration that seems adequate to dissolve the organic part of dentin wall and predentin on unprepared wall areas while not causing erosion of the peritubular dentin (Baumgartner and Cuenin 1992).

Patency: Each irrigation step was followed by recapitulation to ensure patency *(Brady et al 1985).* A #10 K-file was passed 0.5 mm beyond the WL to prevent apical plugging (Holland *et al* 1980, Souza 2006) and to prevent a vapor lock effect that could otherwise prevent effective irrigation at the apical third of the canal (Tay *et al.* 2010). The majority of root canal cleanliness studies do not clarify if patency was ensured during chemomechanical preparation, a factor that can most likely influence the results.

Chelating gels: Chelating gels, such as RC-Prep are routinely recommended for use with NiTi instruments to avoid breakage although there is no scientific evidence for this claim (Hulsmann et al 2003). There is a notion that they may significantly alter the nature of smear layer formed (Gulabivala et al 2005). Others claim that their action has no significant effect on the smear layer since their action is self-limiting (Hulsmann et al 2003). In any case no chelating gel was used in our study.

Sodium cloride crystals: a number of samples in our study, when examined under the microscope, revealed the presence of some distinctive cuboid particles **(Figure 7)** recognized as sodium chlorite crystals. It has been stated that such crystals will form and remain attached to dentinal walls when NaOCl is used for the final irrigation (Dotto et al 2007, Kuştarci et al 2011). For this reason there are studies were specimens are flushed with saline (Zmener et al 2005, Drukteinis and Balciuniene 2006, Zand et al 2007), tap water (Foschi et al 2004) or distilled water (Adiguzel et al 2011, Yigit Ozer et al 2011, Kaya et al 2011) as the final irrigant. A group of investigators advocated the use of sodium thiosulfate in a final flush in order to inactivate NaCl (Siqueira et al 2010, Alves et al 2011). In our study last irrigant was NaOCl solution. Interestingly though, a large number of studies also using NaOCl as the last irrigant (Wu and Wesselink 1995, Peters and Barbakow 2000, Hulsmann et al

2001, Versumer et al 2002, Mayer et al 2002, Metzger et al 2010) do not mention the presence of NaCl crystals in their findings.

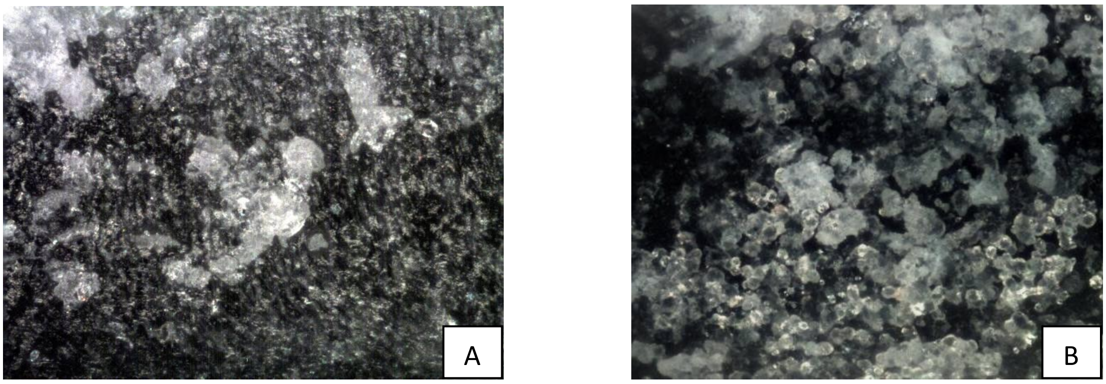


Figure 7 Sodium chloride (NaCl) crystals at A. 200x and B. 500x magnification

4. Critical appraisal of results

In our study SAF achieved much better results than WaveOne, especially at the coronal third (with an odds ratio of 12.59). A possible explanation of this finding could be the fact that the SAF exhibits the higher mean increase in root canal area and volume, along with the percentage of prepared walls, at the coronal third (Versiani et al 2011).

It has been shown that activation of final rinse has beneficial effects on canal cleanliness. Because of its unique design, the SAF has the advantage of allowing this activation throughout the whole cleaning and shaping process. As was suggested by De-Deus et al (2011), the vibration of the SAF file at 5 kHz, induces sonic activation of the irrigant (NaOCl or EDTA) during the whole procedure. Moreover, it is known that chlorine which is responsible for the dissolution of organic tissue and antimicrobial effect of NaOCl (Moorer and Wesselink 1982) becomes rapidly inactive when in contact with dentine (Haapasalo et al 2000, Van der Sluis et al 2006). The continuous replenishment of fresh NaOCl when using the SAF can presumably deliver sufficient free chlorine thus optimizing debridement. These two parameters may also have contributed to the better results achieved by SAF. It should be noted however, that according to de Gregorio et al (2012) the pecking motion of the SAF files allowed for further penetration of the irrigants but could not reach working length.

The EndoActivator (Advanced Endodontics, Santa Barbara CA) uses sonic energy (10 kHZ) to irrigate root canal systems. The EndoActivator tip was placed 2mm less than the working length, according to the manufacturers' protocol. In a recent study de Gregorio et al (2009), using sonic (EndoActivator) activation in simulated lateral canals, found better irrigation in the apical third (at 4.5 and 2mm from working length) than with traditional needle irrigation alone. The hydrodynamic phenomenon produced by vibrating the tip, in combination with moving the tip up and down in short vertical strokes (Ruddle 2002) may be more powerful near the tip (apical third), thus providing another possible explanation for our findings. Actually, the whiplash motion created, according to Newton's first law, accelerates the end of the tip more compared to the body of the plastic file, thus making it more efficient.

5. Comparison to other studies

In the present literature, there is no other study comparing these two systems directly to each other using a method like the one we used. The two studies comparing SAF to WaveOne used either horizontal sections in the mesial root of mandibular molars (Dietrich et al 2012) or a micro-computed tomography analysis (Versiani et al 2013). 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).

Metzger et al (2010) used single-rooted teeth that were prepared with the SAF for 4 minutes. The protocol used was the one that our study was based on; namely the SAF file was operated in two cycles of 2 minutes each. During the first minute of each cycle, NaOCl (3%) was used as the irrigant, whereas EDTA (17%) was used during the second cycle. The main difference was that, in their study, an additional irrigation with EDTA (17%) was performed for 0.5 minutes with the vibration mechanism turned off, before the final flush with NaOCl. Specimens were examined under the SEM (x200 magnification for debris and x1000 for smear layer) and scored based on the scoring system of Hulsmann et al (1997). Their results for debris evaluation showed scores of and 1 or 2, in 100% of the cases in the coronal, middle and apical thirds of the root canals. For smear layer, most samples also scored 1 or 2 for the cervical and middle third while 65% of the samples were reported as 1 or 2 and 35% with higher scores (3 or 4) in the apical third. This is in accordance to our findings were also all samples scored 1 or 2 for debris in the SAF group. For the smear layer all of our samples scored 1 or 2 for the cervical and middle thirds, while one sample had a higher score of 3. This last difference can possibly be attributed to the larger sample size used in the study by Metzger et al.

The next three studies come from the same group of investigators. Kaya et al (2011) investigated the ability of the SAF in the removal of smear layer at different concentrations of NaOCl in single rooted mandibular incisors. The teeth, much like in the previously mentioned study by Metzger were not selected for having oval canals which was the case in our study. The protocol of instrumentation was slightly different; the SAF file was used for 5 minutes total. The first 3 minutes with NaOCl at different concentrations (1.3%, 2.6% or 5.25%), were followed by 1 minute of 1% EDTA and 1 minute of NaOCl. A final rinse with distilled water was performed in this study, which differed from our study. The main difference however, was that the investigators used a closed-end canal system. The reason behind this was the notion that there is a difference between open and closed root canal systems when examining debris and smear layer removal due to the vapor lock effect caused by gas entrainment in the latter systems (Tay et al 2010). Specimens were examined under the SEM at x1000 magnification at the coronal (10mm to apex), middle (6mm to apex) and apical third (2mm to apex) for the smear layer. Scoring system was the one used by Hulsmann (1997). Smear layer scores were mostly 1 and 2, which is in accordance to our findings. Different concentrations of NaOCl did not have significantly different effects on smear layer removal on all thirds. The authors commented that no sample were characterized with scores 4 or 5 even at the apical third, which was also the case in our study concerning the SAF group.

The same group of investigators also examined the cleaning ability of the SAF using EDTA or MTAD on maxillary incisors (Adiguzel et al 2011). The apical foramen was also closed with boxing wax to create a closed system. A SAF operating protocol with two alternating operating cycles was used either with 17% EDTA or with MTAD used as the second irrigant and NaOCl as the first. A final rinse with distilled water was also performed in this study. Specimens were examined under the SEM at the coronal, middle and apical thirds (x200 magnification for debris and x2000 for smear layer). Scoring system was the one proposed by Hulsmann (1997). For debris, the vast majority of the samples scored 1 or 2, few were 3 and none 4 or 5. For smear layer, although again most samples were at the score of 1 or 2, there were more with 3, 4 and even some with a score of 5. This is a difference with the results of our study were none of the samples scored 4 or 5 for the smear layer even at the apical third. One can assume that this is due to the different root canal system; as mentioned a closed-end system was used in this study while an open root canal system, where patency was thoroughly maintained in our study. However, it is interesting that the same group of authors found no samples with 4 or 5 scoring in their previous study (Kaya et al 2011), despite the fact they used the same closed-end system and, in addition, an EDTA solution of 1% rather than the commonly used 17% which they used in the second study. It is possible that the higher magnification of x2000 resulted in the higher scoring.

Finally, Yigit Ozer et al (2011) compared the debridement capacity of the SAF with different operation times. They used the same files for each of the three groups examined, resulting in files used for 4, 8 and 12 minutes respectively. They used maxillary molars, but this time the investigators did not create a closed-end root canal system. The SAF operating mode was 3 minutes with 2.6% NaOCl as irrigant and 1 minute with 17% EDTA, followed by a final flush with NaOCl and then distilled water. They examined the apical thirds of the roots under the SEM (x 200 magnification for debris and x2000 for smear layer). Scoring system was the one proposed by Hulsmann (1997). Their results showed no significant differences between the three groups. In debris evaluation, 90% of the apical thirds scores 1 or 2, representing a clean root canal surface. In smear layer evaluation however, 64% of the samples were scored as 1 or 2 while 36% received a score of 3 to 5.

In contrast with our results for the SAF, as well as with the aforementioned studies, is the one by Paranjipe et al (2012). This study compared the cleaning efficacy of the SAF compared to the ProTaper rotary file system, in terms of both microbiological and SEM evaluation. Maxillary premolars with a single oval canal were selected, with the maximum diameter being more than 3 times bigger than the minimum. The preparation with the SAF consisted of 4 minutes with 6% NaOCl as irrigant. Final irrigation was completed in both groups, as follows: 3 ml of 6% NaOCl, 3 ml of 17% EDTA and 3 ml of 6% NaOCl at 2mm short of working length. The samples were prepared for SEM evaluation and examined at 1 and 3 mm from the apical foramen (x200 for debris and x1000 for smear layer) using the scoring criteria of Hulsmann (1997). For debris they reported significantly better results for the ProTaper group at the 3-mm level. For smear layer, ProTaper was better than the SAF group at both 1-mm and 3-mm levels. Interestingly, although no analytic results are given, scoring is at the scale of 4 to 5 for debris and above 4 for smear layer. Results for the ProTaper group are also at very high levels. This finding is not in accordance with the majority of the most recent cleaning efficacy studies, and no explanation is provided by the authors.

Concerning the WaveOne, a recent study (Kamel and Kataia 2014) compared its cleaning efficacy compared to the ProTaper system on single rooted teeth (but not strictly oval) under two different protocols. Each of the two systems was assessed with and without the use of a flexible microbrush (CanalBrush; Coltene Whaledent GmbH+ Co KG, Langenau, Germany) that supposedly helps in reducing the amount of remaining debris and smear layer (Garip et al 2010). After chemomechanical preparation teeth were split, examined under the SEM (x500 magnification for debris and x1500 for smear layer) at the approximate center of the coronal (10 mm to the apex), middle (6 mm to the apex) and apical thirds (2 mm to the apex). They were scored using the Schafer and Lohman criteria (Schafer et al 2002). Overall better results were achieved for the coronal than the middle and apical thirds, and for the group using the WaveOne along with CanalBrush. None of the groups achieved complete cleanliness.

In another study (Amaral et 2013), WaveOne was compared to Reciproc and Mtwo systems in terms smear layer removal on mandibular molars with root curvatures between 20-25°. Each group of teeth was prepared according to manufacturer's recommendations for each system. Examination under the SEM was performed under x500 magnification for smear layer only at the central area of coronal, middle and apical thirds. Scoring was based on the 3-level classification proposed by Torabinejad (Zand et al 2010). Results showed no significant differences between groups. For the WaveOne system results were: coronal third, 0 (40%), 1 (30%), 2 (30%); middle third 0 (20%), 1 (50%), 2 (30%); apical third, 0 (20%), 2 (80%).

Lastly, a study by Burklein et al (2012) compared the cleaning effectiveness of WaveOne and Reciproc compared to Mtwo and ProTaper in curved canals. Specimens were examined under the SEM at three levels (x200 magnification for debris and x1000 magnification for smear layer) and scored based on the scale of Hulsmann et al (1997). Overall WaveOne and ProTaper exhibited less clean canal walls in the apical part than Mtwo and Reciproc. Scores for all groups were rather high for both debris and smear layer evaluation. The authors acknowledge that this is probably a consequence of not using any chelating agent but only NaOCl solution (Calt and Serper 2000, O'Connell et al 2000).

In general, it is important to note that for all three aforementioned studies, major differences on many parameters (tooth type, preparation protocol, observation and scoring methods) do not allow comparisons to the results of our study.

6. Cleanliness studies: critical appraisal

Regardless of the technique and protocols that they follow, studies examining root canal cleanliness have some endogenous disadvantages.

Firstly, it is a well known fact that the formation of smear layer is the result of instrumentation on dentin. This means that a smear layer is not present were instruments did not contact dentin walls. Taking into consideration that the amounts of untouched dentin surfaces are reported as high as 35% (Peters et al 2003), or even 55 to 75% (Paque et al 2005) an important question is raised; how can one differentiate between smear-free areas because of the action of chelators and non instrumented areas? This can only be answered if there was a "mapping" of the pre-operative dentine status, of the same dentin area in the same sample. However difficulties involving specimens' preparation and mainly the fact that SEM evaluation can only focus on small areas of the whole dentin wall may be the reasons there are no studies following this procedure at the moment.

Concerning the SEM evaluation and scoring methods certain problems also arise. In many SEM studies it does not become clear if the specimens were coded and the examiner blinded before the SEM evaluation (Gulabivala et al 2005). In addition, in a small number of these studies is the reproducibility of the scoring described (Hulsmann et al 1997). To ensure a minimum standard of the subjective data deriving from score evaluations a minimum of 3 examiners with interexaminer reproducibility verification using Kappa statistics is required (George et al 2008). This requirement, which was met in our study, is rarely followed by most similar studies.

More importantly, however, the higher magnifications used under the SEM, especially for the smear layer assessment; result in only a small area of the root canal being observed. As there is no data to suggest that there is an even distribution of debris and smear layer after preparation, rather the opposite seems to be true (Gulabivala et al 2005), we can reach to the conclusion that results may be largely influenced by chance. Moreover, as Hulsmann et al (2005) point out “most SEM operators tend to select clean areas with open dentinal tubules rather than areas with large bulk of debris”. Taken into consideration that the selection of the canal area to be observed is nonrandomized and directly operator-dependent we can conclude that this kind of evaluation method is subjective and qualitative.

Despite variations in magnification and scoring method all studies assessing smear layer removal basically examine the percentage of dentinal tubules that are visible and patent. It is well known however, that dentin is a heterogeneous tissue, from a microstructural standpoint. The presence, density and diameter of dentinal tubules vary with the coronoapical site in the tooth and are subject to change because of age and insult (Carrigan et al 1984, Marshall 1993, Mjor et al 2001). In the apical third, dentinal tubules are irregular in density and direction (Mjor et al 2001). Mjor et al (2001) reported the presence of irregular secondary dentin as well as areas completely devoid of dentinal tubules in the apical third. Actually, sclerotic dentin is a common physiological phenomenon with tubules becoming sclerotic in the apical to coronal direction with aging (Vassiliadis et al 1983). The majority of smear layer removal studies do not take into consideration this sclerotic dentin in the examined teeth (Lottanti et al 2009). On that one unique study, where the effect of sclerotic dentin was actually took into account, the authors concluded that tubular sclerotic dentin is present along the whole root but more so in the apical third (Lottanti et al 2009). The authors commented that, as a result “it may not be the case that there is more smear layer in the apical than the middle and coronal areas, although this has been reported in many studies.”

The presence of debris and smear layer on the root canal walls after the completion of chemomechanical preparation has been investigated by a large number of studies. This extensive research is generated by the view that this residual debris and smear layer can have an impact on treatment quality (Czonstkowsky et al 1990, Wu et al 2001, Bowmann and Baumgartner 2002, Torabinejad et al 2002), as it may harbor bacteria (McComb and Smith 1975, Sen et al 1995), prevent or reduce the action of irrigants and intracanal medicaments into dentinal tubules (Bystrom and Sundqvist 1985, Orstavik and Haapasalo 1990) and reduce the adaptation of sealer and gutta-percha on the canal walls (Saunders and Saunders 1992, Oksan et al 1993). However, the role of “canal cleanliness” on treatment outcome has not yet been established on a high level of evidence. Indeed, some argue that body fluids such as blood, saliva or serum are the sources of growth for the causative bacteria rather than remaining pulp tissue or dentin components (Khot et al 2004).

Also it is of great interest for future projects, to compare results of cleanness studies to those of apical debris extrusion, especially in the same specimens, and see the influence of open/closed systems on the outcome of both parameters. No literature is published on that aspect.

In any case, root canal cleanliness in ex vivo studies is a poor outcome criterion for the adoption of a shaping system, as it has no established clinical relevance. A great need for properly designed randomized-controlled trials exists, in order to assess the effect of different techniques and protocols of chemomechanical preparation on endodontic treatment outcome.

Conclusions

Within the limitations and under the conditions of this study, it can be concluded that:

- Completely cleaned root canals were not found after instrumentation with any of the two systems.
- However, both the SAF system and the WaveOne + EndoActivator system exhibited scores ranging from 1 to 3 (on a 5-scale scoring system), meaning that in general root canal walls showed relatively minimal amounts of remaining debris and smear layer for both groups.
- In the cervical root third the SAF-group exhibited statistically significant better cleaning efficacy compared to the WaveOne-group.
- No significant differences between the two systems were found in the middle and apical third.
- Statistically significant higher scores were observed for the smear layer than for debris for both groups.

Summary

Research has shown that current methods of cleaning and shaping root canals produce dentine debris and a smear layer that covers the instrumented wall areas. This smear layer contains organic and inorganic substances, which include odontoblastic processes, microorganisms, and necrotic materials and can be compacted along the surface of canal walls, increasing the risk of bacteria 'contamination' or extrusion to the periapical tissues. Additionally, it reduces the adaptation of sealer and gutta-percha on the canal walls and inhibits the sealer penetration into the dentinal tubules. If tissue remnants and debris are not removed, the subsequent stage of root canal obturation may also be jeopardized, leading to a potential failure of treatment. Therefore, a great number of studies have examined the cleaning efficacy of different techniques, systems and protocols, i.e. the amount of debris and/or smear layer that remains inside root canal walls at the end of chemomechanical preparation.

The purpose of this study was to examine the cleaning efficacy of the WaveOne system and compare it with the SAF system in terms of debris and smear layer removal under optical microscopy.

Twenty four single rooted, intact permanent human premolars with one main apical foramen and mature apices were selected from a random collection of teeth that were extracted for orthodontic or prosthetic reasons. All teeth were checked for single-canal anatomy and for the presence of an oval canal, using mesio-distal and bucco-lingual digital radiographs. They were divided into two homogenous groups

(Group A and Group B), concerning total length, length of the root, bucco-lingual and mesio-distal dimensions at the cemento-enamel junction.

For all teeth endodontic access cavities were prepared and pulpal remnants were extirpated. Working length (WL) was determined by measuring the length of a #10 K file that emerged at the apical foramen, minus 1 mm. Only root canals with an initial apical gauging diameter of size 20.02 or less were used.

Group A was subjected to chemo-mechanical preparation with the SAF system, whereas Group B was processed using the WaveOne system.

For Group A, a SAF file *(ReDent- Nova,* diameter of 1.5 mm, length of 25 mm) was used, following a modification of the manufacturer's protocol. The SAF file was operated using an RDT handpiece-head with the motor rotating at 5000 rpm along with continuous simultaneous irrigation using the VATEA irrigation pump *(ReDent-Nova).* Irrigation was applied at 5 mL/min and alternated every minute between 2.4% NaOCl and 17% EDTA until 4 minutes were completed. Final irrigation was performed in all specimens with 10 mL of 2.4% NaOCl.

For Group B, the WaveOne primary file (25/08, 25 mm long) was used in a reciprocating and slow in and-out pecking motio generated by the WaveOne electric motor *(Dentsply-Maillefer, Ballaigues, Switzerland),* which was operated with a 6:1 reduction handpiece *(Sirona, Bensheim, Germany).* After three slow in-and-out movements (pecks), 2 mL of 2.4% NaOCl was used as an irrigant. During and until the completion of the WaveOne file operation, care was taken to ensure that a total volume of 10 mL of 2.4% NaOCl was used to irrigate each specimen's canal. Subsequently, a flushing with 10 mL of 17% EDTA solution that was activated with an Endo-Activator (15/.02 Yellow tip, *Dentsply-Maillefer, Ballaigues, Switzerland)* for one minute was performed. The final flushing was performed using 10 mL of 2.4% NaOCl solution that was activated again for 30 seconds, according to the manufacturers' protocol.

Following, specimens were split longitudinally, coded and blindly examined by one examiner under an optical microscope *(Nikon, Eclipse ME600 – camera Nikon FDX-35).* The obtained pictures were evaluated by 3 examiners for the presence of debris (x200) and smear layer (x500) at the cervical, middle and apical levels using a 5-scale scoring system. The recordings of the two groups were statistically analyzed in order to explore potential differences between techniques but also between examiners, root tertiles and magnification rates.

Only scores ranging from 1 to 3 were detected in this study. Completely cleaned root canals were not found after instrumentation with any of the two systems at any magnification (x200, x500).

In the cervical root third (tertile) the SAF-group exhibited statistically significant better cleaning efficacy ($p=0.001$) compared to the WaveOne-group, with an odds ratio of 12.59, irrespectively of magnification level. In the middle and apical third the difference was not statistically significant, though a tendency was noted, with lower scores for SAF-group (for both debris and smear layer). Overall, higher scores were observed for the smear layer (higher magnification, x500) than for debris (smaller magnification, x200), for both groups. The difference was statistically significant ($p< 0.001$).

Under the conditions of this study, the SAF system showed better cleaning efficacy in the cervical root third compared to the WaveOne system. No significant differences were detected in the middle and apical third.

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