Aus der Klinik für Mund-, Kiefer- und Gesichtschirurgie
(Prof. Dr. med. Dr. med. dent. H. Schliephake)
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Micro-morphometric study of the resected root surface after endoscope-supported apicoectomy

INAUGURAL – DISSERTATION

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Carolina Leiva Hernández
aus
Antofagasta, Chile

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Dekan:	Prof. Dr. rer. nat. H. K. Kroemer
Referent/in	Prof. Dr. med. Dr. med. dent. W. Engelke
Ko-Referent/in:	Prof. Dr. Michael Hülsmann
Drittreferent/in:	Prof. Dr. Martin Oppermann
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Abbreviations

BMP	Bitmap format
Cm	Centimeter
CVD	Chemical vapor deposition
DICOM	Digital imaging and communication in medicine
DOM	Dental operating microscope
dpi	Dots per inch
EBA	Ethoxy benzoic acid
EM	Endodontic microsurgery
Er:YAG	Erbium: Yittrium aluminium garnet
GIF	Graphics interchange format
HO:YAG	Holmium: Yittrium aluminium garnet
JPG	Joint photographic expert group
LD	Length of dentine
mm	Milimeter
MTA	Mineral trioxide aggregate
PNG	Portable network graphics
ppc	Points per centimeter
ppi	Pixel per inch
RRS	Resected root surface
SE	Support endoscopy
SIE	Support immersion endoscopy
TIFF	Tagged image file format
UMG	University Medical Center Göttingen
VCR	Video cassette recorder
VRF	Vertical root fracture

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1 Introduction

1.1 Historical development of apical surgery

Procedures of surgical treatment of the root apex were mentioned very early in the medical-dental literature, starting around 1750 when Heister submitted a method to cauterize granulation tissue using hot instruments (Heister 1750). In 1884, Farrar published the apicoectomy technique, practicing the first apicoectomy in molars (Farrar 1884).

After 1890, the studies carried out by Rhein increased the popularity of apical surgery (Rhein 1890). In 1899 Partsch and Kunert systematized the apicoectomy and through removal of the root tip it eliminated the risk of postoperative inflammation (Partsch and Kunert 1899). They described the particular difficulty of root resection of teeth located in the posterior area as this procedure was not usually performed in molars due to the lack of visibility in the area and the closeness of the apex to the maxillary sinus or to the mandibular nerve canal, meaning that there was a high risk of damage to these structures. Consequently for a long time apical surgery was limited to incisors and premolars (Marlette and Amen 1970; Selden 1971). Later, a number of studies emerged which included the performance of root end filling and compared as well the effectiveness of various materials for root end filling such as amalgam, gutta-percha and others (Barry et al. 1975).

Winstock (1980) reported the characteristics of persistent periapical radiolucent injuries and also published an extensive series of 9,804 apicected apices where it was possible to study the periapical injuries under an optical microscope. In addition, microbiological cultures were grown. In the late 1990s, improvements in techniques for root end cavity preparation began to emerge through the use of ultrasound and magnification system assisted surgery (Velvart 1996; Bernhart et al. 1999; von Arx et al. 2001 a).

One of the most common causes of treatment failure is related to the complex anatomy of the root which has not been sufficiently cleaned or it has been cleaned in such a manner that has allowed microbial flora to remain in the apical sections of the root canals (Nair et al. 1990). Apical surgery is currently considered the therapeutic alternative in those cases where periapical periodontitis persists since it can access the source of infection and provide a hermetic sealing of the apical area and when it is unfavorable or not possible to perform endodontic retreatment (von Arx 2011). Apical surgery offers the possibility of removing the inflamed periapical tissue and ensuring proper cleaning, preparation and sealing of the apical portion of the root canal (von Arx et al. 2001 b).

In the 90s, the appearance of microsurgical techniques (see 1.2 below for definition) and the application of ultrasound in endodontic surgery (Ishikawa et al. 2003), allowed a breakthrough in the performance, design and subsequent sealing of the root end cavity with different materials, which has allowed access to areas of the mouth where it was almost impossible in the past (Taschieri et al. 2004). The main advantages of microsurgery are the more accurate identification of apical structures, the performance of smaller osteotomies and the use of more superficial angles of resection, thus allowing better preservation of the cortical bone and the root structure. Perhaps the most important advantage of microsurgery if compared to conventional surgery is that microsurgery meets the biological and mechanical principles of endodontic surgery. In addition, scientific progress has allowed the use of magnification and lighting devices as well as the use of micro-instruments in apical surgery. This has given a more contemporary perspective, recognized as endodontic microsurgery (EM), whose practice has enabled more accurate and less traumatic procedures with a greater degree of predictability (Pecora and Andreana 1993; Sumi et al. 1996; Kim and Kratchman 2006; Setzer et al. 2010; Kang et al. 2015). EM has generated a new vision related to pre-, intra- and post-operative clinical factors, which has been the basis for the development of prognosis research in microsurgery in recent decades, with favorable results and a high success rate of around 85 - 94% (Sumi et al. 1996; Kim and Kratchman 2006). A recent study by Tsesis et al. (2013) has reported a satisfactory outcome with a success rate of 89% in patients undergoing endodontic surgery using modern techniques.

1.2 Microsurgical concepts in apical surgery

According to Blahuta and Stanko (2012), microsurgery is defined as a surgical procedure in exceptionally small and complex structures.
Microsurgery is based on three key elements: magnification, illumination and instruments. The magnification and illumination are provided by the microscope or endoscope (Blahuta and Stanko 2012), and the instruments are an adaptation of the conventional tools to be used in microsurgery (Carr 1997; Kim 1997). Some of these are miniature versions of the traditional instruments, but many were specially designed for microsurgical endodontics by Gary Carr (Carr 1992 a; Carr 1997) and others.

1.2.1 Microscopes and magnifying glasses

Several authors emphasize the importance of having good visibility of the operating field (Arens 2003; Geibel 2006). Using methods of visual magnification, such as magnifying glasses or optical microscopes facilitates the quality control that the surgeon executes on his work at the surgical site, achieving better long-term results (Bahcall et al. 1999; Kim and Kratchman 2006; Taschieri et al. 2008).

Magnifying glasses are devices with different types of binocular magnification:

1. Diopter system, consisting of a simple magnifier.
2. Surgical magnifiers with the Galileo system (two lens system), which offers a magnification range of x2 to x4.5.
3. Surgical magnifiers with the Kepler system using light-refracting prisms and providing magnification of up to x6 (Eichenberge et al. 2013).

In the diopter system, the working distance is 20 centimeters. Telescopes with Galileo or Kepler systems employ a working distance ranging from 28 to 51 centimeters. A further disadvantage of the magnifying glass is that the maximum magnification, in practice is x4.5 and those magnifiers that provide a higher magnification tend to be heavy and with a limited field of vision. Magnifying glasses may be connected to surgical lights in order to provide enhanced illumination, preventing the creation of shadows (Taschieri et al. 2013). The light sources have a fiber optic cable that is connected to the operator bond allowing any movement by the light to be followed, and they can increase the light up to four times the light provided by conventional lamps (Carr and Murgel 2010).

As well as having the disadvantage of being uncomfortable and heavy, magnifying glasses also have problems of image distortion, little depth of field due to the need for convergence of eyes to the object leading to eyestrain of the professional if used for long periods. The Dental Operating Microscope (DOM) was developed to overcome these disadvantages and to replace magnifying glasses.

In 1990 Baumann and Selden were the first to use a microscope in endodontics (Pecora and Andreana 1993). In the late 80s Gary Carr designed the basic microsurgical instruments: the first ultrasound tips and micro mirrors (Carr 1992 a; Carr 1992 b; Carr 1997), developing since then many variables and improvements (Layton et al. 1996; Zuolo et al. 1999; Peters et al. 2001; Navarre and Steiman 2002).

Rubinstein and Kim (1999) have reported many successful cases after apical surgery, stating that the use of the microscope can be a determining factor and that it can contribute to successful results. Also it has the great advantage of allowing work with stereoscopic vision, with an appropriate magnification in a perfectly illuminated operating field with coaxial light that improves diagnostic capacity and it enables work to be carried out more easily.

One of the main advantages of the microsurgical approach is the possibility of creating smaller osteotomies, bevels of lower angulation and conservation of more cortical bone and root. Additionally, the inspection of the resected root surface (RRS) with illumination and a large magnification makes it possible to easily observe anatomical details such as isthmus, accessory canals and lateral canals, and to control the quality of the root end filling (Saunders and Saunders 1997). The purpose of using the DOM for osteotomy is to clearly distinguish the root from the surrounding bone.

The size of the osteotomy depends mainly on the size of the instruments. This is because of the combination of the DOM, the micro mirrors and the use of small ultrasound tips of 3 mm bent at an angle of 90 degrees relative to the handle, make it possible to realize an almost flat apical preparation.

A small osteotomy and shallow bevels between 0° and 10° provide a minimum removal of the cortical bone, ensuring a correct and conservative root end cavity preparation**.**

Performing the resection of the root end under adequate illumination and magnification, it is easier to detect additional anatomical details as isthmus, microfractures and lateral canals (Blahuta and Stanko 2012; Tsesis et al. 2013). A simultaneous use of ultrasound makes it possible to realize more conservative root end cavity preparation, parallel to its longitudinal axis, and more precise root end fillings.

Perhaps the most important advantage of microsurgery over conventional surgery is the fact that microsurgery fulfills the biological and mechanic principles of endodontic surgery.

Kim and Kratchman (2006) recommend not to perform all surgical procedures on the highest magnification, since they consider that certain procedures are better performed with a smaller magnification, because, occasionally, the fields of view must be broad enough to align an ultrasonic tip. Thus they categorize the procedures according to the magnification needed (Fig. 1) and suggest that the low magnification (x4 to x8) should be used to achieve the guidance and inspection of the surgical site, as well as for the osteotomy.

The average magnification (x8 to x14) is used for most procedures, including apicoectomy, preparation and sealing of the root end cavity. High magnification (x14 to x26) is mainly used to observe in detail the anatomy of the RRS after resection and for documentation.

Magnification	Procedures
Low (x4 to x8)	Orientation, inspection of the surgical site, osteotomy, alignment of surgical tips, root end preparation and suturing
Midrange (x8 to x14)	Most surgical procedures including hemostasis. Removal of granulation tissue, detección of root tips, apicoectomy, root end preparation, root end filling
High (x14 to x26)	Inspection of resected root surface and root end filling, observation of fine anatomical details, documentation

Figure 1 Different magnifications used for different stages of endodontic surgery (Adapted from Kim and Kratchman 2006, p. 604)

1.2.2 Endoscopic systems

Held et al. (1996) have reported on endoscopic applications in conventional therapy and have described the first application of endoscopes as a supporting tool in apical surgery. A few years later, Bahcall et al. (1999) described an improved endoscopic technique for apical surgery and recommended the use of endoscopes with 6 cm length, a lens diameter of 4 mm and an angle of 30 degrees for this procedure. They described the use of endoscopes in the phases of root end resection for inspection of the RRS, as a supporting tool during ultrasonic root end cavity preparation and also during root end filling.

Von Arx et al. (2002) provided technical details on the use of endoscopes during endodontic surgical procedures. Bahcall and Barss (2003) reported on the use of an orascope with a 2.7 mm lens diameter, a vision angle of 70 degrees and a length of 3 cm, for visualization during endodontic surgery, and emphasized the need for having a hemostasis of the surgical field prior to the use of the endoscope. They also mentioned that the endoscope can provide assistance during ultrasonic instrumentation. At the same time von Arx et al. (2003 a) reported the use of a rigid endoscope of 3 mm lens diameter and a 70-degree angle to perform apicoectomy.

Taschieri et al. (2007) used a rigid endoscope of 3 mm lens diameter and a length of 6 cm with a 70 degrees angle to perform apical surgery. Here it was emphasized that it is necessary and important to keep the endoscopic lens free of blood. Later Taschieri et al. (2008) mentioned that the use of a rigid endoscope has a great influence on the result of endodontical surgical procedures.

Moshonov et al. (2009) reported that the use of flexible endoscopes of 0.9 mm and 0.55 mm lens diameter can also be used for endodontic therapy and not just for the visualization and the preoperative diagnosis. Likewise, Nahlieli et al. (2011) published a paper reporting on the use of semi-flexible endoscopic systems of small diameter with an integrated washing system that can be appropriate for some indications in oral surgery and implantology.

Von Arx et al. (2010) reported on the use of an endoscope in order to detect cracks in dentine after root end resection, and a year later, they performed an endoscopic evaluation after root end resection to report the frequency of present microstructures at the RRS (von Arx et al. 2011).

Support endoscopy technique (SE) is a technique that involves the use of a support sheath coupled to optics, making it possible to work at a short distance between the lens and the object. Alternatively, the support immersion endoscopy technique (SIE) reduces the risk of contaminating the optical system as a result of the short distance to the surgical site by the intermittent or continuous use of irrigation (Engelke 2002; Engelke and Beltran 2014).

1. 3 Phases of periapical surgery

Apical surgery includes the following most frequent and principal stages (Bernardes et al. 2009):

1.3.1 Incision technique and flap design

The flap design must be chosen according to clinical parameters, for example aesthetic and gingival biotypic parameters and the presence of any restoration on a marginal level, among others. Also to be considered are radiographic parameters such as location and extension of the periapical lesion and the condition of the marginal periodontium. At this stage it is common to use an operating microscope because it provides a general vision of the surgical field.

1.3.2 Osteotomy

Traditionally, relatively large instruments are used in conventional endodontic surgery, meaning that the size of the osteotomy is large too, having a diameter of about 10 millimeters, in order to allow the operator a proper visibility and to treat the tips with a conventional mirror and a handpiece (Luebke 1974; Laurichesse 1993). Excessive osteotomies and the removal of so much healthy tissue cause a slower, more painful and incomplete repair with greater risk of postoperative complications. In contrast, the microsurgical technique uses very small instruments that allow more conservative and precise preparations. The optimal size of the osteotomy should be at least 4 to 5 mm in diameter in order to allow the access of instruments of root end cavity preparation such as the ultrasound and apical shutters (Krastl and Filippi 2008; Blahuta and Stanko 2012) (Fig. 2 A).

During this stage, the periapical pathological tissue must be removed in order to achieve a better access and visibility of the surgical field (Fig. 2 B).

Using the endoscope after the osteotomy and locating the root apex allows the operator to observe the apical morphology and the presence of any foreign material (Blahuta and Stanko 2012).

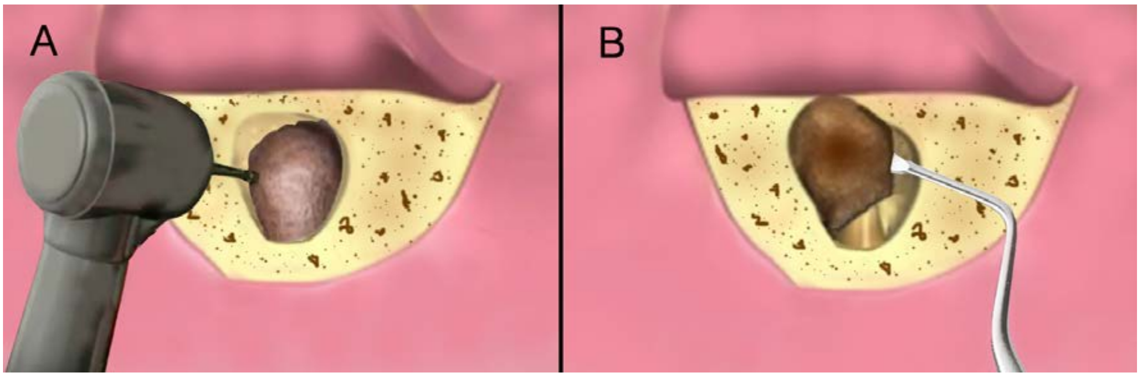

Figure 2 **A.** Scheme showing a osteotomy with round tungsten carbide drill to access the tooth roots. ; **B.** Elimination of the periapical pathological tissue

1.3.3 Root end resection

Root end resection must be performed as perpendicularly as possible in relation to the axis of the root.
Various techniques and instruments have been used to realize the resection of the root end, such as different types of burs, laser and ultrasound devices (Duarte et al. 2007; Ayranci et al. 2015). When comparing different burs, used at high and low speed, the Nedderman group reported that burs used at low speed and plain fissure burs produce smoother RRS when compared to crosscut fissure burs at both low and high speeds (Nedderman et al. 1988).

Bernardes et al. (2009) reported the use of ultrasonic tips for resection of the root end, where they found that the carbide burs produced more regularly RRS than did ultrasound activated CVD coated tips and that it also requires less time to perform the apicoectomy with burs at low speed than with CVD tips.

Er: YAG and Ho: YAG lasers have also been used to realize the root end resection. Studies by the Komori group showed that the Er: YAG laser produces cleaner and smoother surfaces without causing thermal damage (Komori et al. 1997). It also has advantages such as a reduced risk of trauma to the surrounding tissues, a reduced possibility of contamination of the surgical site and less vibration and discomfort (Komori et al. 1997). A recent study showed that tungsten carbide burs and laser

Er: YAG produce better cut root surfaces than the diamond-coated tip (Ayranci et al. 2015).

It is recommended that the last apical 3 mm be removed and, depending on the complexity of access to the root surface and the type of instruments employed, the resection be performed in the slightest possible depth of bevel (Fig. 3). If the periodontal ligament around the root cannot be seen properly, it is recommended that methylene blue be applied to aid its identification (Cambruzzi et al. 1985).

In conventional surgical technique a bevel of 45 degrees was recommended, so that the operator could view and identify the apex and thus could perform the root end cavity preparation in a controlled and precise way. Gilheany et al. (1994) showed that by increasing the angle of the bevel, the apical filtration increased, and also another study has demonstrated a dentinal exposure when the bevel angle increases (Gagliani et al. 1998). With the implementation of magnification and surgical instruments it is possible to achieve less deep resection bevel, allowing the conservation of more cortical bone and the crown-root length (Tsesis et al. 2013).

A critical step after root end resection is the inspection of the RRS under adequate illumination and magnification in order to visualize the morphology of the root surface, the number and configuration of the root canals, the possible presence of isthmus and cracks, and to detect the presence of any sealing gap between the filling material and the root canal wall (von Arx et al. 2010, von Arx et al. 2011). Using the conventional technique of inspection with the naked eye for this step makes it nearly impossible to adequately inspect and detect the critical structures in the RRS, yet an adequate inspection is decisive to the success of the therapy.

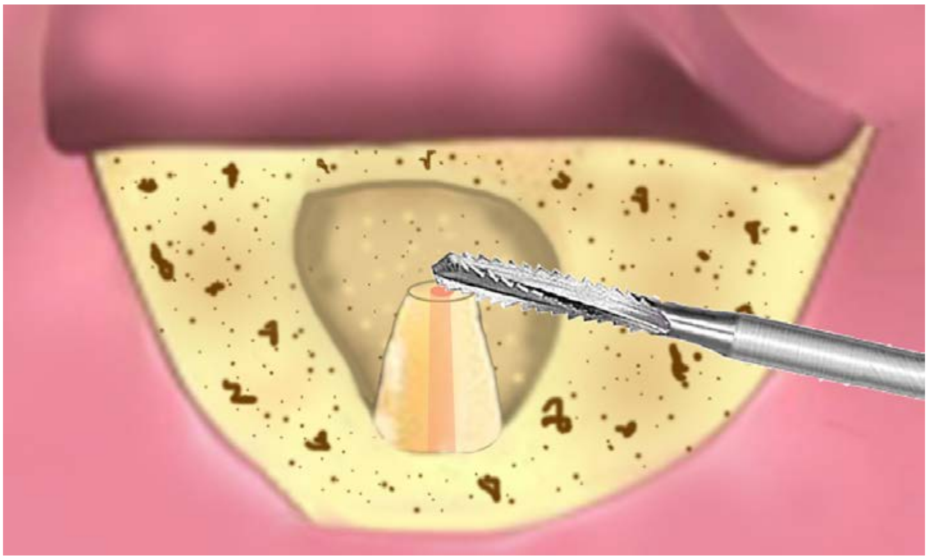

Figure 3 Scheme showing the root end resection with a Lindemann bur

1.3.4 Root end cavity preparation

The preparation of the root end cavity must have a depth of 3 mm and must follow the root canal's original trajectory. The cavity must also include the isthmus and the accessory canals if present. In the conventional technique, most of the preparations were made with straight hand pieces with small-headed angles or small spherical drills with inverted cones, which caused a problem regarding the root canal's depth and trajectory.

The current preparation technique using ultrasound, properly executed, is a simple procedure that fulfills all essential requirements for an ideal preparation, because it allows a root end cavity preparation with an adequate depth, with parallel walls to the axis, providing an optimal retention and keeping the mesial-distal dimension, which protects the root fragility at this level. A study by Gorman et al. (1995) has documented that the use of ultrasonic tips improves the cleaning of the cavity's walls compared to conventional tools, reducing the smear layer and the residues on the surface.

In addition, using microtips makes the preparation of sharp angles on the level of the resection unnecessary, in turn reducing the number of exposed dentinal tubules and minimizing the apical filtration (Taschieri et al. 2004). In fact, when using traditional techniques, a large bevel was inevitable, as the surgical tools were large, and the bevel was only realized for the surgeon's convenience in order to identify the apex and to realize the subsequent root end cavity preparation (Kim 1997).

1.3.5 Root end filling

Different materials have been used to fill the root end cavity, such as amalgam, Super EBA and glass ionomer cement. In the last decade, laboratory studies and clinical results classify Mineral Trioxide Aggregate (MTA) as the material that best fulfills the necessary requirements for a good sealing.

Histological studies on the response of the bone to MTA showed that this material is associated with significant bone regeneration (Torabinejad et al. 1995). It has been proved that its ability to achieve a hermetical seal exceeds the ability of amalgam or Super EBA. It also shows important advantages such as excellent biocompatibility (Camilleri and Pitt Ford 2006), it adheres well to the cavity's walls and has low solubility (Poggio et al. 2007), and the cement regeneration on the level of the sectioned root surface activates cementoblasts in order to produce a matrix of cement formation between the exposed dentine and the MTA's surface (Bernabé et al. 2007; von Arx 2011). This could be caused by its ability to seal hermetically, by its high pH or by the release of substances that cause the cementoblasts' activation in order to deposit a matrix where cementogenesis can take place (Baek et al. 2005).

At this stage the use of magnification permits an evaluation of retrograde sealing and makes it possible to inspect the marginal adaptation of the sealing material as well as detect the presence of deficiencies (Blahuta and Stanko 2012). (Fig. 4)

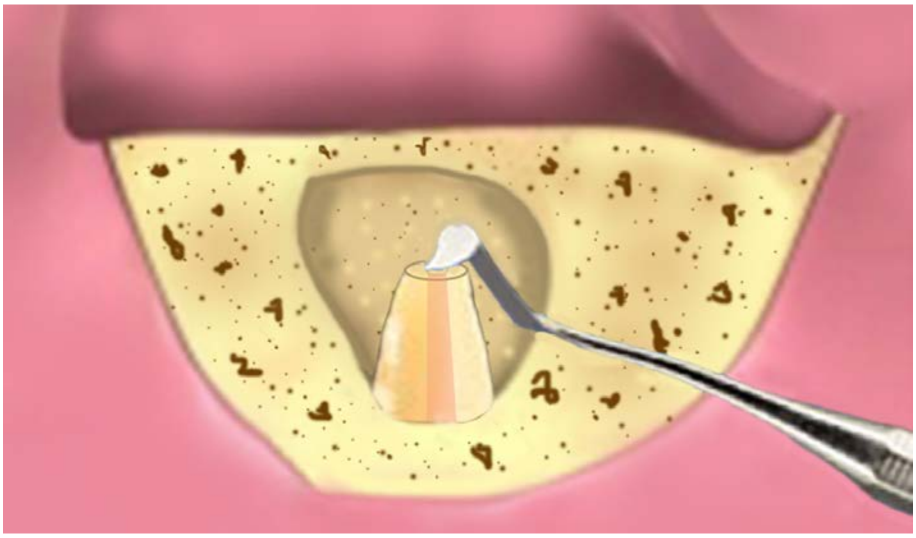

Figure 4 Schem showing a filling material placed in the root end cavity

1.3.6 Wound closure

Before carrying out wound closure it is necessary to review the surgical site and to clean it up. The reposition of the flap margins is carried out using simple individual sutures, preferably with a fine suture material. Von Arx (2011) recommended a slight compression with a dressing to bring the periosteal tissue in contact with the bone. During this stage of apical surgery, magnification is not used for the cleaning of the bone cavity, however it can be used in the case of special incisions that require very fine sutures as for example at the base of the dental papilla (Krastl and Filippi 2008).

1.3.7 Summary of microsurgical aspects

The risk that is associated with conventional endodontic surgery in the mandibular arch is the potential damage of the neurovascular bundles of the involved area (Blahuta and Stanko 2012). The following are the weaknesses of the classical periapical approaches (Gutmann 1984; Gutmann et al. 1994):

- Retrograde preparation divergent from longitudinal axis of the canal.
- Preparation with little retention.
- Preparation without a buccal-lingual extension for acceptable sealing.

- Preparations that weaken the apical area due to an unnecessary extension.
- Preparation that does not manage to include the isthmus area.
- Difficulty in detecting anatomical details at the RRS such as cracks.

The use of magnification devices, illumination and the use of micro-instruments during the stages of periapical surgery are considered to be important to minimize the trauma in the surgical area and to create optimal conditions to achieve the correct retrograde sealing (von Arx et al. 2010) and optimize the technique for this surgery in comparison with the conventional technique (Krastl and Filippi 2008, Blahuta and Stanko 2012). (Table 1)

Traditional Technique	Microsurgical Technique
Excessive Osteotomy	Small Osteotomy
Bigger loss of cortical bone	Smaller loss of cortical bone
Many dentinal tubules exposed	Few dentinal tubules exposed
Difficult identification of apices	Simple identification of apices
Difficult examination of the RRS	Simple examination of the RRS
Possibility of lingual perforation of the root	Minimum risk of lingual perforation

Table 1 Comparison between traditional and microsurgical technique. (Adapted from Krastl and Filippi 2008, p. 124 ; Blahuta and Stanko 2012, p. 311)

1.4 Findings at the resected root surface

After the section of the perpendicular root apex to the long axis of the tooth, it is important to remove the inflamed tissue and abrade the adjacent bone to achieve optimal conditions for the repair by sealing any path between the root canal and the periradicular tissues. To perform this step correctly and to identify the anatomical root details, a high magnification and illumination of the microsurgical technique are

necessary. In addition, complete and critical inspection of the RRS requires working the surface with a contrast dye such as methylene blue, which selectively dyes the periodontal ligament and the pulp tissue (Kim and Kratchman 2006).
In Figure 5 the phases of endodontic microsurgery and type of magnification used in each stage are described. For this study, findings at the RRS between root end resection stage and root cavity preparation stage were observed. Additionally the data at this level was collected.

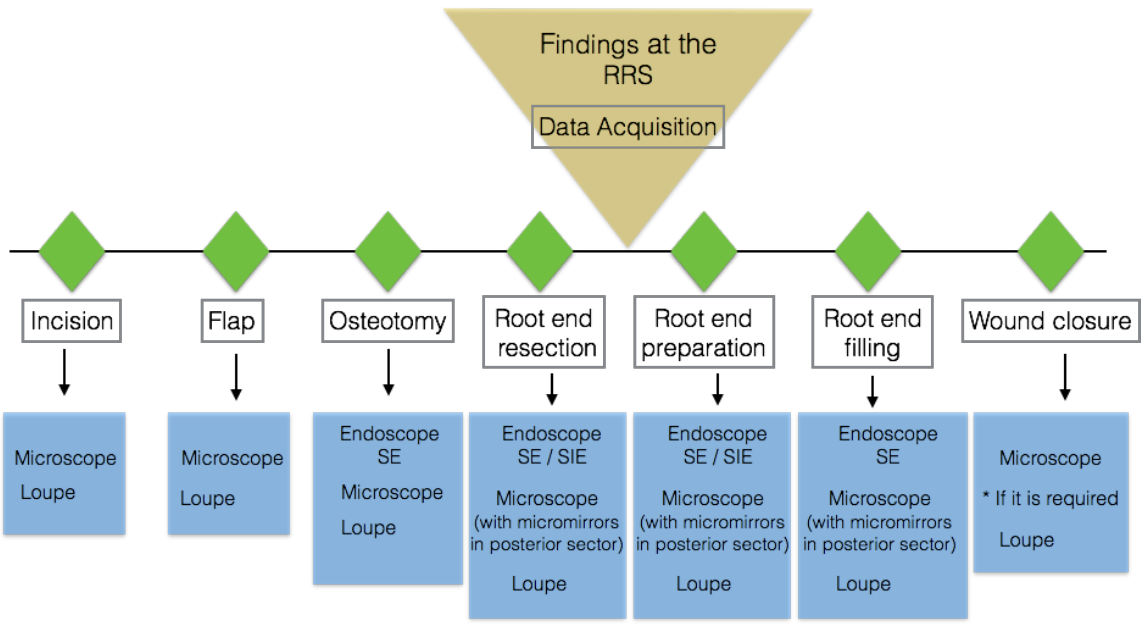

Figure 5 Stages of apical microsurgery and different magnification devices that can be used in each stage

Inspection of the RRS allows the detection of structures that might be areas of possible leaks, such as **isthmuses, accessory canals, dentinal cracks and craze lines, frosted dentine and sealing gaps**.

Weller et al. (1995) define the **isthmus** as a thin ribbon, linking two root canals and containing pulp tissue. The incidence of an isthmus varies and depends on the type of tooth and the level of the root (Vertucci 1984; al Shalabi et al. 2000). A high frequency of isthmuses has been reported in the mesial buccal root of the first maxillary molars and the mesial root of the first lower molars (Hsu and Kim 1997).

An accessory canal is defined as a canal, which has not been prepared during root canal treatment (von Arx et al. 2003 a). The accessory canals and the isthmuses between two canals can be considered as critical structures for the successful outcome of apical surgery (Weller et al. 1995; Hsu and Kim 1997), for this reason they must be recognized and properly handled.

One structure observed on the RRS and considered critical is the presence of **dentinal cracks** and **craze lines**. Cracks are defined as apparent fissures within the dentine while craze line was described as a dark line that appears to disrupt the integrity of the dentine (von Arx et al. 2011). Although the clinical significance of the cracks has not yet been well determined, it is speculated that they could be the precursor of future apical leakage or root fractures (de Bruyne and de Moor 2005).

Another frequent finding in the RRS is an opaque area within the root dentine called **frosted dentine**. The presence of this structure has been interpreted as regions of major tension. In a study by Slaton et al. (2003), it was speculated that the frosted dentine was caused by the formation of many microscopic cracks that had not yet melted into a greater crack. Such areas of frosted dentine could be a precursor in the formation of cracks; however, this relationship has not yet been clearly established.

Root fracture has been considered the cause for failure of many endodontically treated teeth. They have been defined as fractures that extend from root surface into canal space (Wilcox et al. 1997). Usually these fractures extend through the root canal to the periodontal ligament dividing the root into two fragments. Vertical root fractures are also a very important finding, whose prevalence, although not quite established, is believed to be found more commonly in teeth that have undergone a root canal treatment (Chang et al. 2016). A complete fracture expands in opposite directions of the root canal and involves the movement of two fragments of the root end (Walton et al. 1984). The presence of a **sealing gap** between the filling material and the dentinal walls of the root canal is related to a bacterial route of reinfection.

1.5 Digital imaging in dentistry

1.5.1 General concepts of digital image and digital imaging processing

A digital image is the two-dimensional representation of a physical object from a numerical matrix composed of binary digits. A binary number consists of digits called bits. A bit (binary digit) is the basic information unit and represents two possible states for a communication channel or an information storage system (Farman 2003). The binary system consists of only two numbers, namely 0 and 1. With a two-bit binary number only four numbers can be composed: 00, 01, 10 and 11. If a color corresponds to each of them, only four colors can be represented: black, dark gray, light gray and white (Krupinski et al. 2007). A digital image is composed of a finite number of elements, each with a specific place and value. These elements are called *pixels (picture elements)*. A pixel is represented by a code number in the computer and it is displayed on the monitor as a point of a specific color or intensity (Krupinski et al. 2007). Digital images are generated by the combination of pixels that contain information related to color or tones of gray at each pixel location (Farman 1994). In addition, the size of each pixel determines the spatial resolution of the digital image; the smaller the size, the better the resolution of the image.

Spatial resolution refers to the ability of a device to discriminate details or, more accurately, the ability of a device to distinguish between two points very close to each other that can appear as a single point (Workman and Brettle 1997; Williams et al. 2006). The resolution of a digital image depends on the number of pixels contained in the surface unit (expressed in cm^2 or in^2) (Rakhshan 2014). The greater the number of pixels per cm^2, the higher is the image resolution and the spatial definition. This depends not only on the number of pixels of the image but also on the characteristics of the output medium. If the image is projected onto a television screen, computer monitor, cameras or printed, the form of spatial resolution is called "DPI resolution" or "pixel density ". It is measured by the units DPI (dots per inch) or also PPI (pixels per inch) (Rakhshan 2014).

The final image resolution is directly proportional to the number of pixels of the matrix and it is defined as the number of pixels per square inch and its unit is dpi or ppi.

The color depth or bits per pixel (bpp) is a concept that refers to the number of bits of information needed to represent the color of a pixel in a digital image (Larobina and Murino 2014). In simple terms, it refers to the amount of colors that can be found in a digital picture. The higher the number of bits, the greater is the color definition of the image. For example, in 8-bit color mode, the color monitor uses 8 bits for each pixel, which allows a display of 2 raised to 8 (256) different colors or shades of gray, but it is important to note that the quality of a display system depends to a great extent on its resolution, i.e. the number of bits used to represent each pixel (Krupinski et al. 2007).

1.5.2 Applications using image processing software in dentistry

Digital processing or treatment of images involves algorithmic processes, which transform an image in order to highlight certain information of interest and / or mitigate or eliminate any information irrelevant for the application. The aim of these processes is to modify images to improve their quality or highlight the relevant details.

Digital tools have gradually become essential support elements for the diagnosis, registration and projection of treatments in all areas of health where visible physical changes and elements to manage body aesthetics are compromised. Such tools have been successfully used for many years in areas such as plastic surgery and dermatology through the use of indirect tools (image processing software) as well as specific programs for each area.

Informatics, through specific software and hardware, has provided considerable and decisive progress in the field of diagnostic imaging. Developments in information technology have helped to improve the quality and accuracy of the image, allowing the incorporation of new technologies or enhancing the existing ones, being of crucial importance for the development of diagnostic imaging methods (Panetta et al. 2015). The widespread use and availability of computers with higher capacity and faster calculation speed as well as the fast evolution of imaging systems originally not available in electronic form, pave the way for a promising field of research and technological development, generically referred to as Image Processing.

The technological development in recent years has introduced considerable computational progress into the scientific area and it is precisely in the area of medical imaging where scientific computing has been intensely involved. In this way, several computer programs for scientific processing and image display have been created and developed over the last two decades (Lehmann et al. 2002).

The use of software for image processing in dentistry was introduced in order to improve digital images and to make it possible to observe details of the image which cannot be visualized with the naked eye. Software has been used to identify carious lesions by measuring the gray value scale of image pixels (Carneiro et al. 2009) and also for measuring areas of demineralization on tooth surfaces (Murphy et al. 2007; Nassur et al. 2013).

In periodontology some of this software has been used to evaluate the formation of plaque in patients who were treated orthodontically (Klukowska et al. 2011) and also to evaluate the reduction of gingival inflammation by measuring changes in redness, using image analysis (Seshan and Shwetha 2012). In the area of orthodontics, it has also been used for quantification of white lesions and enamel demineralization of the surfaces with brackets (Benson et al. 2003: Livas et al. 2008)

In the area of endodontic the evaluation of gray levels and the use of tools to improve digital images have been very useful for the description and diagnosis of bone injuries in the apical zone when used for this purpose (Mol and van der Stelt 1992; Scarfe et al. 1999). Other studies have used image processing in order to better determine the working length of endodontic files inside the root canals (Piepenbring et al. 2000) (Li et al. 2004) (Oliveira et al 2012). Other studies that have used image processing, have improved visualization for early detection of external root reabsorption (Poleti et al. 2014).

It is also possible to analyze the conventional radiograph on a computer, and therefore it is necessary to convert radiograph into a digital image, which is called scanning or indirect digital radiography (Versteeg et al. 1997). The digitization is useful for the quantitative analysis of radiographs regarding the comparison of images and this is one of the biggest advantages of storing of radiographsin a computer, as you can perform the digital subtraction technique. When comparing two images one can get a new one, through the differences of density. This way a pattern of mineralization or healing of periapical lesions can be established, permitting one to observe the areas of lower mineralization of black color, and the areas of mineralization of white color (Brooks and Miles 1993).

The digital subtraction technique has been used to evaluate the diagnostic potential of digital subtraction at simulated apical resorption, comparing the conventional intraoral images with the images obtained by digital subtraction (Heo et al. 2001). Other studies report the use of digital subtraction technique to evaluate the effect of root canal treatment changing the size of the periapical lesions (Nicopolou-Karayianni et al. 2002, Carvalho et al. 2009).

1.5.3 Image J in dentistry

Image J software is a public domain computer program developed in Java programming language. It was created by Wayne Rasband in 1987 in the facilities of the US National Institutes of Health. Later an updated version of this software was developed in 1997 under the name of Image J, which was designed to manage different types of image data across several computing platforms. It has also been widely adopted by biologists due to its usefulness and ease of use. (Hartig 2013)

Image J provides a large number of tools for image editing, processing and analysis, which can be applied to 8-bit, 16-bit and 32 bit images, and images of multiple formats (jpg, bmp, png, gif, tiff, dicom). The tools used for image processing make it easy to calculate a given area and the distance and angle of any user-defined selection (Hartig 2013). In addition Image J has been able to establish connectivity with other computer programs such as IMARIS, CELL Profiles (Kamentsky et al. 2011) and KNIME (Lindenbaum et al. 2011).

In regard to editing tools, Image J makes it possible to convert images from one type to another, control advanced settings on brightness and contrast of images as well as modify their dimensions, check their properties, manage the specific characteristics of color images and indexed images.

Regarding analysis, it is important to mention that Image J is able to make a large number of measurements on the image or on specific areas of the image, for example histograms, profiles, area measurements, average brightness levels, standard deviations, maximum and minimum values, styles, etc. In addition, the program has tools for automatic analysis of objects in binary images and tools to calibrate such images (spatial and density calibrations) and offers a specific set of tools for the analysis of electrophoretic gels.

The functionality of Image J provides extensibility through an extended collection of macros and plug-ins. Macros are a series of instructions which are stored so they can be executed in a sequential manner through a single execution order, thus facilitating the automation of repetitive tasks. Plug-ins are external programs, most of them written in Java, which provide processing capabilities not found within the software basic capabilities (Abràmoff et al. 2004). Plug-ins allow more specific tasks to be performed by extending the program's own tools.

In odontology, Image J has been use to compare the efficacy of debridement of isthmus and root canals of two irrigation systems (Sarno et al. 2012, Adcock et al. 2011). Another study used Image J to compare the efficacy of cleaning by two types of endodontic files during and after instrumentation (Saghiri et al. 2012).
A study performed by Celik et al. (2015) used Image J to measure the amount of infiltration between adhesive systems and different surfaces of treatment. Image J has also been used to compare and measure the apical transportation induced by three types of rotary systems (Özer 2011).

1.6 Aims of study

The **first part** of the thesis provides an overview of the endoscopically-assisted apical surgery procedures that were performed in the UMG from 1998 to 2015.
The following questions will be addressed:

-What is the distribution of the apical surgical procedures related to gender and age of patients?

Null hypothesis:

There is no difference between the frequency of apical surgical procedures related to age and gender of patients

-Are there differences in the location of the apical surgical procedures in different oral regions?

Null hypothesis:

There is no difference between the frequency of apical surgical procedures in the anterior region and the posterior region.

The **second** part of the thesis provides a descriptive and comparative analysis of findings obtained from an examination of the RRS in the sample. The findings are catagorized according to von Arx (2011), namely craze lines, cracks, frosted dentine and sealing gaps. The following questions will be addressed:

-Is there a relationship between the frequency of microfindigs and age, tooth location, presence of root post and location per root segment?

Null hypothesis:

There is no a relationship between the frequency of microfindigs and age, tooth location, presence of root post and location per root segment

The **third part** of the thesis provides a micromorphometric analysis of findings at the RRS after the endoscopically assisted root resecction. The following questions will be addressed:

-Is there a relationship between frosted dentine area and extention of the crack/craze line?

Null hypothesis:

There is no a relationship between amount of frosted dentine area and extention of the craze lines/cracks

-Is there a relationship between the area or extention of findings and age of patients, tooth location and presence of a root post?

Null hypothesis:

The size or extention of findings is not related to the age of the patients, type of treated tooth, presence of a root post.

2 Materials and Methods

2.1 General aspects and selection of study cohort

From 1998 to 2015, a total of 237 apical endoscopically-assisted surgeries were performed at the Department of Maxillofacial Surgery, UMG.

A total of 191 patients underwent endodontic surgery: 84 male and 107 female. The average age was 40.7 years.

A rigid endoscopic system was used to perform all endoscopic procedures. The following inclusion and exclusion criteria were applied to select the study cohort:

Inclusion criteria:

- Availability of endoscopic procedures from the Department of Maxillofacial Surgery in the hospital's video archives.
- Video recordings of endoscopic surgeries of sufficient quality.
- Video recordings showing complete sequences of the root end resection phase including inspection of the resected root surface.
- Video recordings showing a view of the RRS after resection.
- RRS images displaying only one root canal.

Exclusion criteria:

- Unavailability of endoscopic procedures from the Department of Maxillofacial Surgery in the hospital's video archives.
- Video recordings of endoscopic surgeries of poor quality.
- Video recordings not showing complete sequences of the root end resection phase and/or not including the inspection of the RRS.

After applying the inclusion and exclusion criteria, 47 patients were selected for the **study cohort**, of which 21 were male and 26 were female with an average age of 44 years. 71 roots were evaluated within 56 teeth. A descriptive analysis of the interventions was performed according to demographic parameters and tooth location within the dental arch.

In the study cohort, findings at the RRS were analysed according to demographic parameters and tooth location. Micromorphometry of the RRS after endoscopically-assisted root resection in the study cohort was evaluated (Table 2).

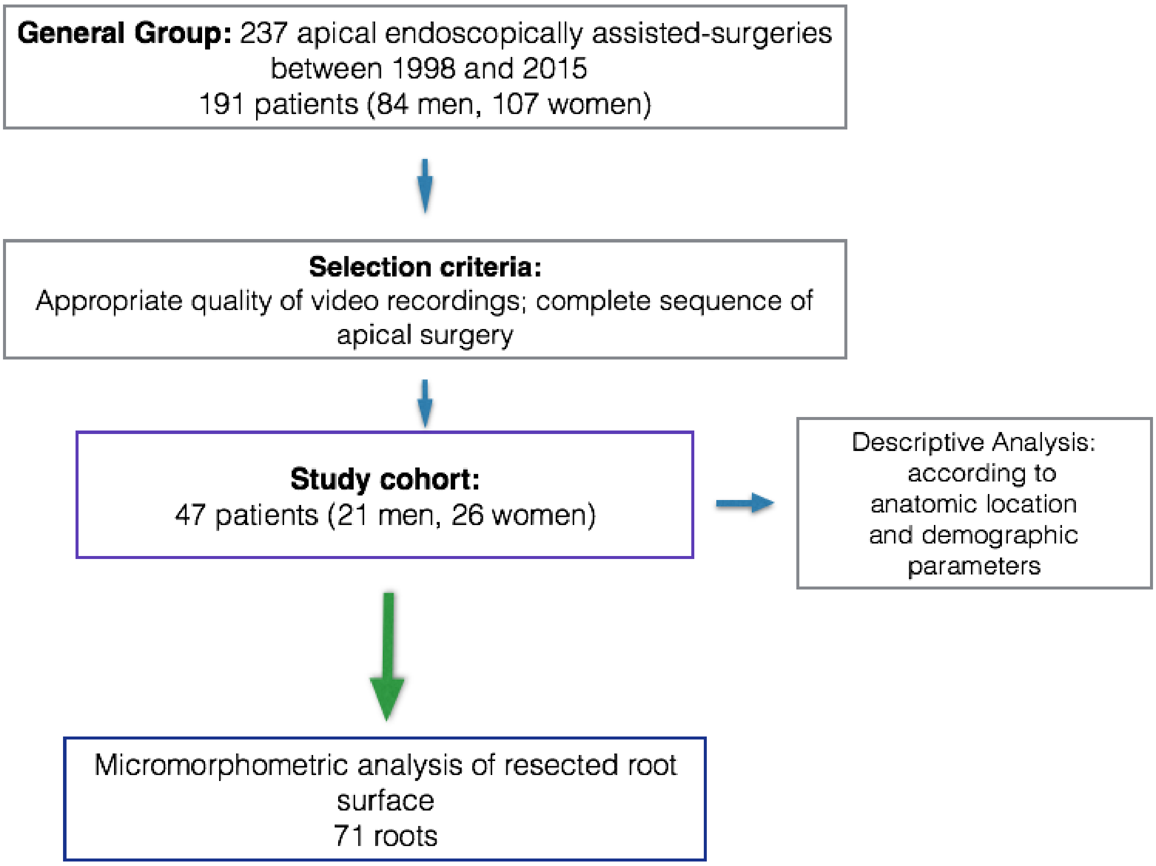

Table 2: Diagram of the selection criteria for study cohort

2.2 Diagnostic endoscopy

The endoscopic system used in all surgical procedures was the rigid Storz Hopkins endoscope with a 2.7 diameter and a support sheath (Karl Storz, Tuttlingen, Germany). The endoscopes were coupled to unit B 487 and a 300 W 6.000 K Xenon light source (Karl Storz, Tuttlingen, Germany) (Fig. 6).

For the support endoscopy (SE), a 2.7 mm diameter optical lens with a 30-degree view angle was used and inserted into a support sheath. The sheath's spatula tip enabled the endoscope to be supported and stabilised close to the surgical site.

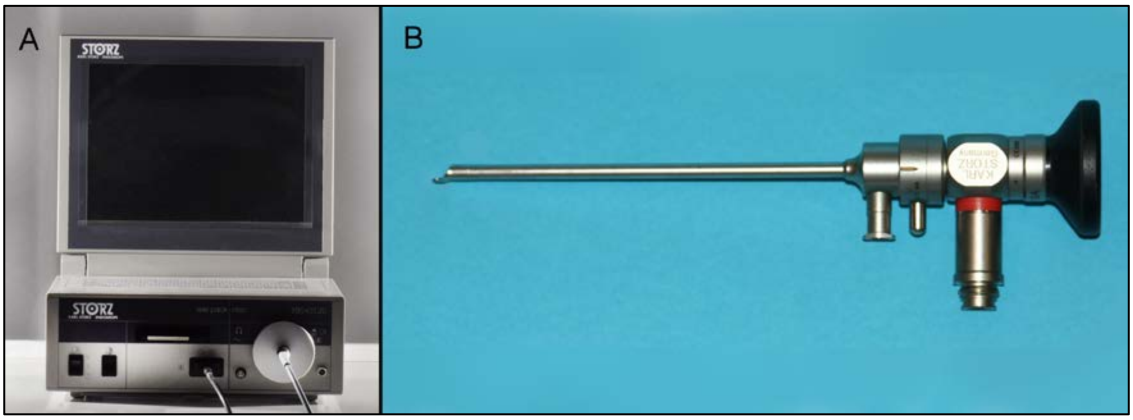

Figure 6 Karl Storz endoscope (Karl Storz, Tuttlingen, Germany) **A:** monitor, light source and archive system; **B:** Optic (Rigid) 2.7 mm with 30 degrees view angle inserted into the support irrigation sheath

Support endoscopy is generally performed at a variable distances and provides a panoramic view image during the diagnostic phase.
During root end resection and inspection of the RRS, the sheath's spatula tip can be supported against the bone surface, which allows a wide view of the surgical site and of the RRS (Fig. 7 A), or it can be supported as close as possible to the surface of the resected root (Fig. 7 B).

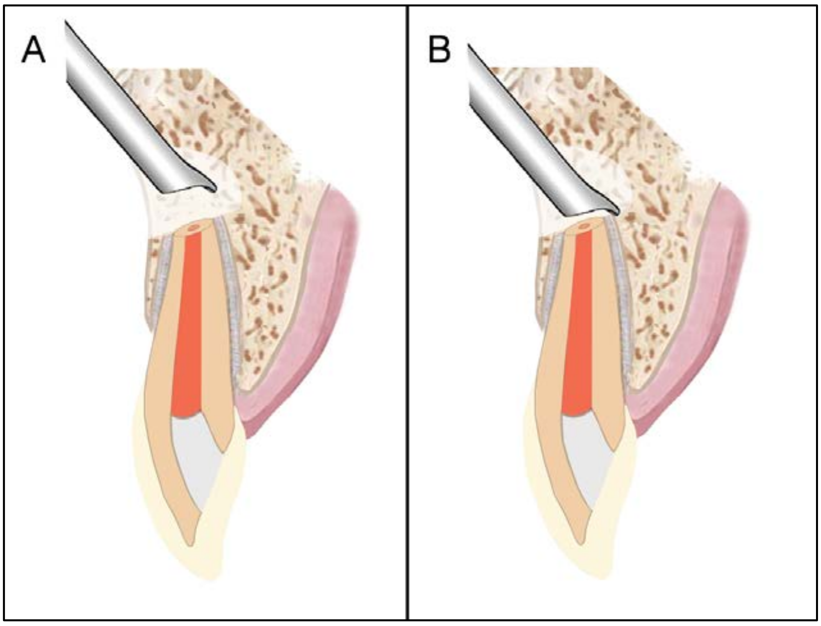

Figure 7 Scheme showing position of endoscope for observing the RRS. **A:** position at a short distance away from the RRS; **B:** position close to the RRS.

Figure 8 A shows the endoscopic view at a distance from the surgical field. Figure 8 B shows the endoscope´s position during observation of the RRS.

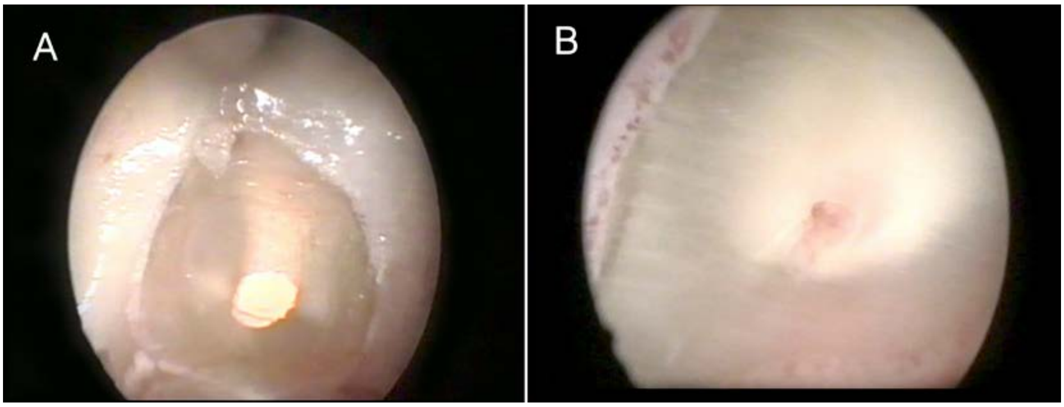

Figure 8 Clinical example of the visualisation of the RRS. **A.** Position of endoscope at a short distance from the RRS; **B.** Position in direct contact with the RRS.

2.3 Video footage preparation and selection

Selection of material: Classification of material was done on the basis of analogue video recordings SVHS of endoscopically-assisted apical surgeries. Videos were viewed with a video recorder and a colour monitor (SVHS AG 7350, Panasonic Corporation). Videos on file were selected according to the criteria set for this study. All video recordings showing either poor image quality, or technical faults were excluded. All videos with incomplete recordings were excluded.

Digitizing VHS videos: Videotapes in SVHS format were converted into digital images using a video capture device (USB 2.0 LogiLink VG0011). This device was connected via an adapter cable from the VCR's S-Video output (SVHAS AG 7350, Panasonic Corporation) to the USB port in the computer, allowing data transfer from the analogue video to the computer. The contents of the videotapes were transferred to a computer (Dell OptiPlex 780, Dell USA) and digitized using a video-editing program (ArcSoft ShowBitz 3.5) compatible with Microsoft Windows XP operating system. During digitalization, each tape was played in the VCR and watched simultaneously in a window of the computer's colour. Using the capture function of the video editing program, sequences of individual images were registered (final image size 870 x 480 pixels). These photos were selected and stored in JPG format on removable media (CD-ROM, Zip disks).

2.4 Clinical image assessment

Images of the root surface were selected immediately after apical resection during endodontic surgery, obtained from the video register. Images were used with a general view of the resected root surface with the support sheath placed at the border of the bone surface as close as possible to the root.

Structures in the images selected of every root surface were identified as follows (Fig. 9):

- **RRS**
- **Root canal**

- **Frosted dentine**: whitish or opaque dentine in contrast to the normal greyish or yellow dentine.
- **Sealing gap:** a space between the root canal filling material and the adjacent dentinal wall.
- **Craze line:** a dark line that appears to disrupt integrity of the dentine.
- **Crack:** an apparent fissure within the dentine.

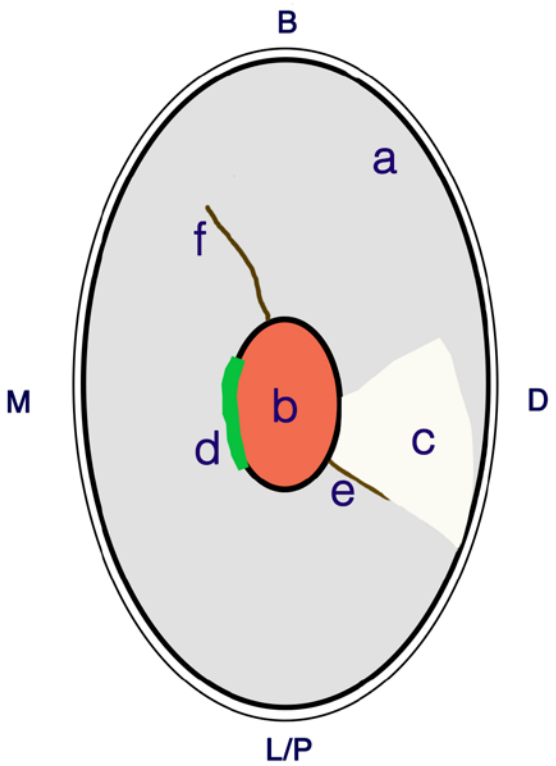

Figure 9 Structures that were assessed at the RRS. **a:** RRS; **b:** root canal; **c:** frosted dentine; **d:** sealing gap; **e:** craze line; **f:** crack; **M:** mesial ; **D:** distal; **B:** buccal; **L/P:** lingual/palatal

For the location of findings on the RRS, a four-sector grid was used based on the grid described in the study of von Arx et al. (2011). This matrix was superimposed on the static image obtained from the video screenshot and placed in the centre of the root canal, or in case of teeth with two canals in the middle of a line connecting the root canals.

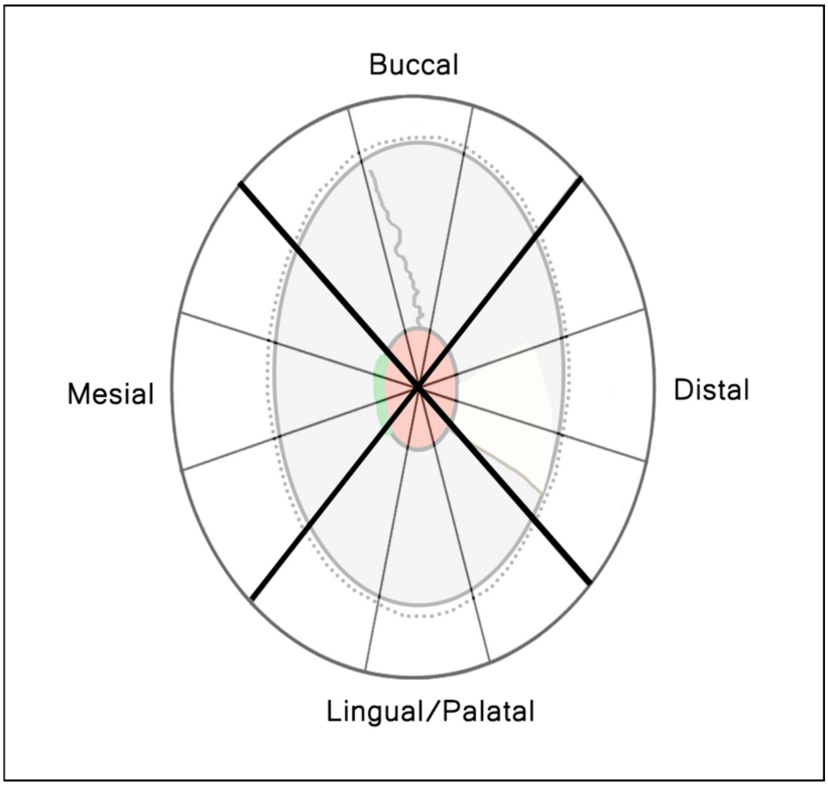

Figure 10 Schematic illustration of a root with 4 sector grid: mesial; buccal; distal and lingual/palatal

There were also secondary parameters included: the patient's age group (under 40 years, 40 to 64 years, and over 64 years); tooth location (anterior teeth, premolars and molars); and the presence or absence a root post.

2.5 Micromorphometric analysis

Digitized images were exported to a computer (Dell Optiplex 780, Dell USA) with a monitor (Dell 17 Inch LCD Monitor). An image analysis programme (Image J, V.64, National Institutes of Health, Bethesda, MD, USA) was used to open these images. Each original image was converted into an 8-bit greyscale and subsequently inverted using a function of the software (Fig. 11). The brightness and contrast values were adjusted individually for each image in order to improve their inspection. For a proper demarcation of the findings, images were used simultaneously in their original and inverted versions. Every image was laid out in so that the mesial aspect of the RRS was placed on the left-hand side of the image, the distal aspect on the right-hand side, the buccal aspect upwards and the lingual aspect downwards with respect to the image.

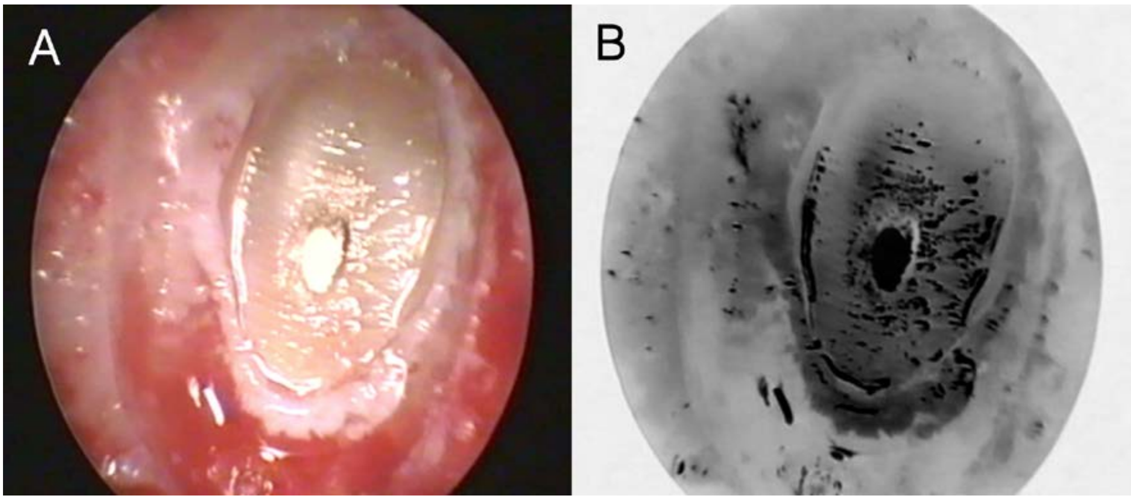

Figure 11 Image opened in program Image J using inverted function: **A.** original image; **B.** "inverted" function of image

1) The following measurements of the length of findings were done (Fig. 12):

- Length of craze line (5)
- Length of dentine (LD) along craze line (6)
- Length of crack (7)
- Length of dentine (LD) along crack (8)

The “straight line” function was selected from the menu of Image J to measure each image, which allowed tracing the line of the defined finding manually. The value was recorded and the number of pixels was read and exported to a spreadsheet (Excel 97, Microsoft Corp., Redmond, WA. USA).

2) The following width measurements of **findings** were performed:

- Width of craze line (5)

- Width of crack (7)

For measurements of findings' width in each image, the minimal width along the cracks and craze lines was measured. The value was recorded and the number of pixels was read and exported to a spreadsheet (Excel 97, Microsoft Corp., Redmond, WA, USA).

3) The following **areas** were measured:

- RRS area (1)
- Root canal area (2)
- Frosted dentine area (3)
- Sealing gap area (4)

For measurements of areas in each image, the "segmented line" function was selected from the menu in Image J, which allowed tracing the outline of the defined region manually. The value was recorded, the number of pixels was read for each area of interest, and was then exported to a spreadsheet (Excel 97, Microsoft Corp., Redmond, WA, USA).

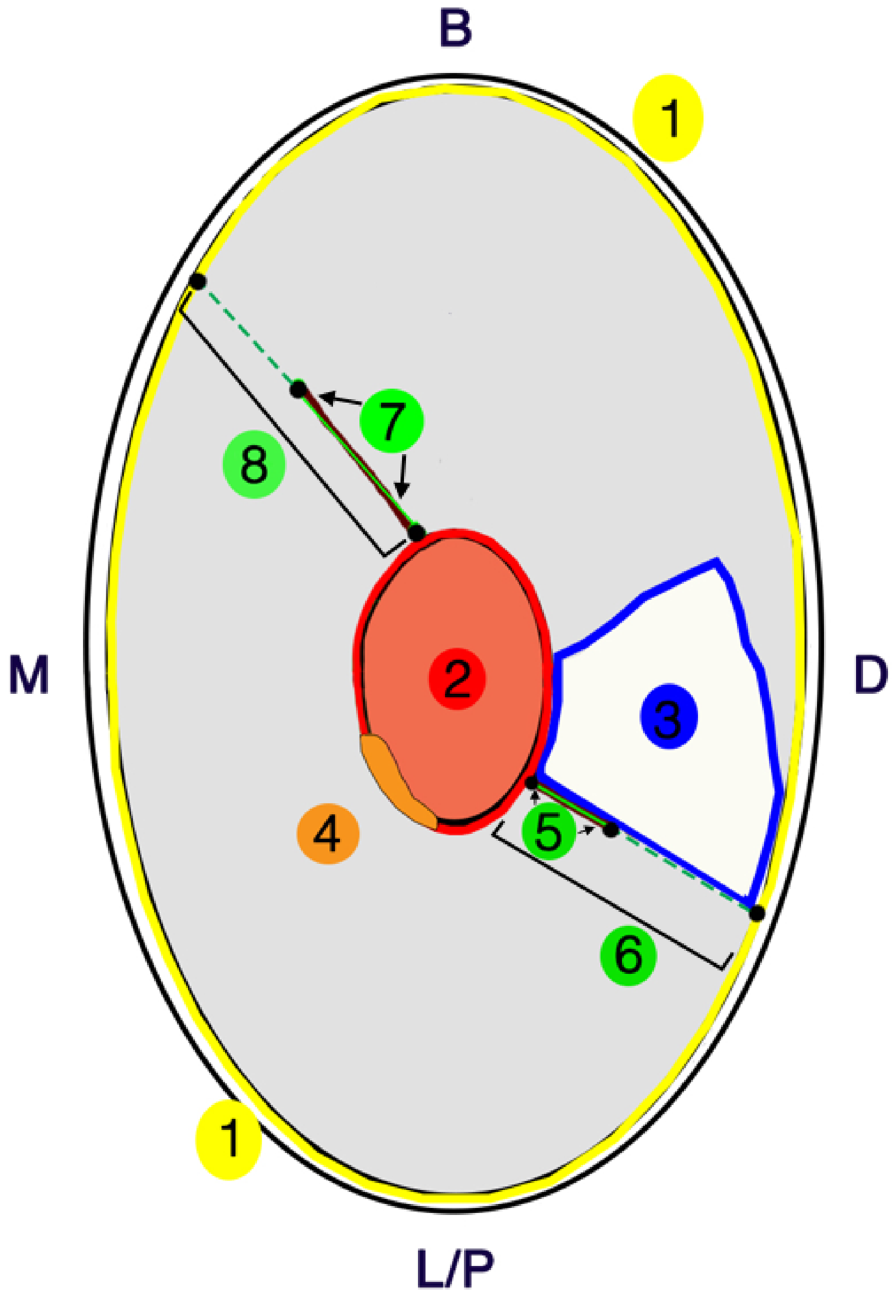

Figure 12 Scheme showing measurements at the RRS of each image: **1.** RRS area; **2.** Root canal area; **3.** Frosted dentine area; **4.** Sealing gap area; **5.** Line drawn along the craze line; **6.** Line drawn on the extension of dentine along craze line; **7.** Line drawn along the crack; **8.** Line drawn on the extension of dentine along crack

Secondary parameters were also included: the patient's age group (under 40 years, 40 to 64 years, and over 65 years); tooth location (anterior teeth, premolars and molars); and the presence or absence a root post.

2.6 Statistical analysis

The spreadsheets and graphs were created using Microsoft Excel 2013 (Microsoft Corporation, Redmond, USA). Further statistical analysis were performed with SPSS for Windows, version 16.0 (SPSS, Chicago, USA).

For the distribution of interventions of the study cohort related to demographic parameter (gender, age) and to anatomical localization in the arch, descriptive statistics were obtained and compared by chi square was tested .

The descriptive statistics of findings are presented as frequency tables. Associations in two-by-two tables were tested by Fisher's exact test. For proportions of segments per finding exact two-sided 95% confidence intervals (Clopper–Pearson) were computed and the null hypotheses of equal proportions within each findings were tested by chi-square test.

For the micro-morphometric analysis, the finding were compared with demographic parameters (age, tooth location, presence or absence of root post). All measured values were checked by Shapiro-Wilk test and Quantile-quantile plots for normality. For normally distributed outcomes a t-test for independent samples or One-way ANOVA was used. Descriptive statistics included mean, standard deviation, minimum and maximum values.

The possibility of increase of cracks lenght in the influence of increase of frosted dentine area was calculated by Pearson´s correlation. The significance level was uniformly set at 5%.

3 Results

3.1 Demographic aspects of apicoectomies

3.1.1 Distribution by gender

Of the patients studied, 21 were male (44.7%) and 26 female (55.3%). The distribution of patients by gender is shown in Fig. 1. The distribution by gender was assumed to be balanced. Given the small sample size no fundamental conclusions can be deduced (Chi-squared test, $p = 0.466$) (Fig.13).

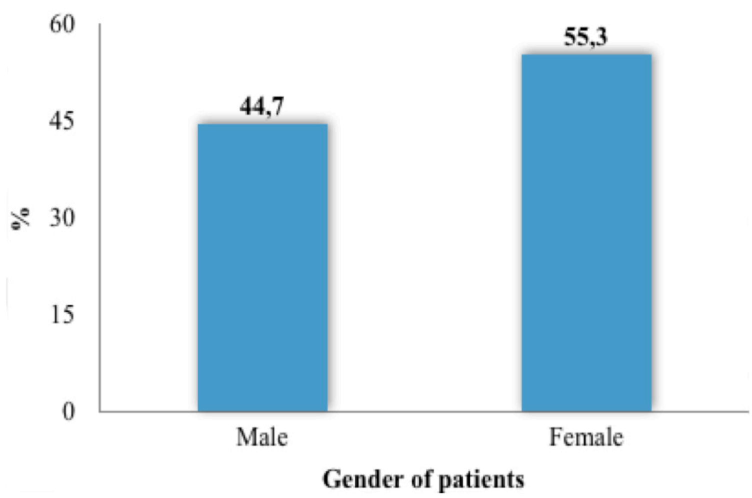

Figure 13 Distribution of apicoectomies by gender

3.1.2 Distribution by age

All three groups showed a normal distribution. The age of patients varied from 19 to 71 years with a mean of 40.7 years and a standard deviation of 14.1 years. The group of 40 to 64 year-olds had the highest number of patients (51.1%), followed by the group under 40 years (40.4%). Fewer patients were in the over 64 year-old group (8.5%) (Chi-squared test, $p = 0.001$). (Fig. 14)

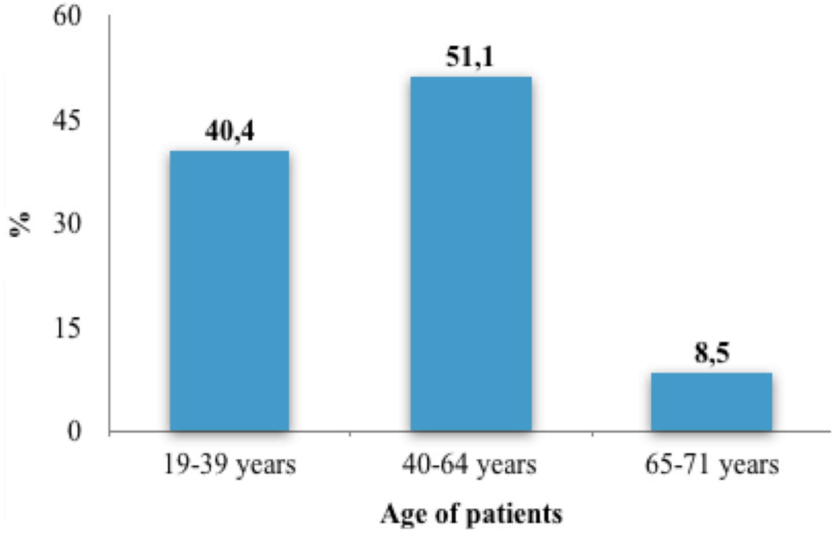

Figure 14 The figure shows a relationship between patient age and frequency of apicoectomies

3.1.3 Distribution by location

Fig. 15 shows the distribution of apical surgeries by dental arch and location of roots treated.

The frequency of intervention was found to be significantly higher in the maxilla (Chi-squared test, p = 0.01).

With respect to the location of roots treated, there were significant differences between the anterior teeth, premolars and molars. In the maxilla, arch apicectomies were performed mainly in posterior teeth (Chi-squared test, p = 0.02). In the mandible, the frequency of surgical treatment was significantly higher in posterior teeth (Chi-squared test, p = 0.000) (Fig. 15).

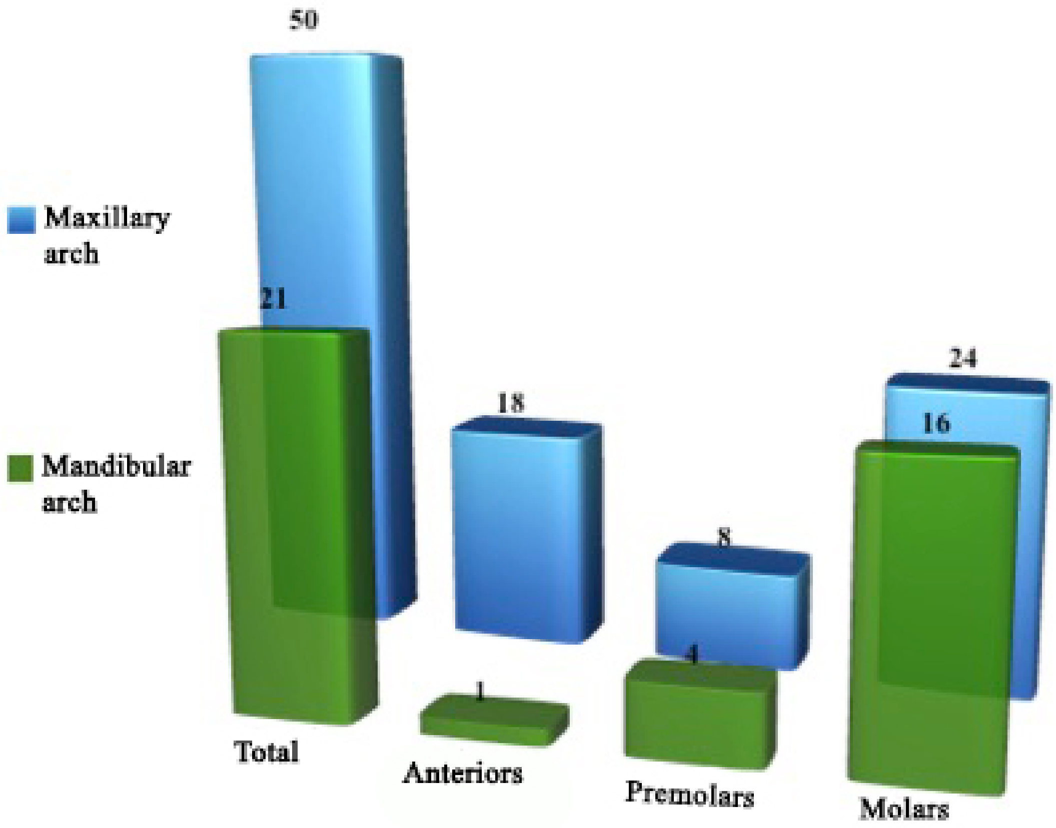

Figure 15 The figure shows the distribution of apicoectomies according to type of treated teeth and location in arches

3.2 Results of clinical assessment

3.2.1 Frequency and type of findings

Craze line: Craze lines were observed in 11 of the 71 roots with a total of 11 craze lines (one in each root). 5 craze lines were located in roots of anterior teeth and 6 in the root of molars. No craze lines were detected in premolars (Fig. 16).

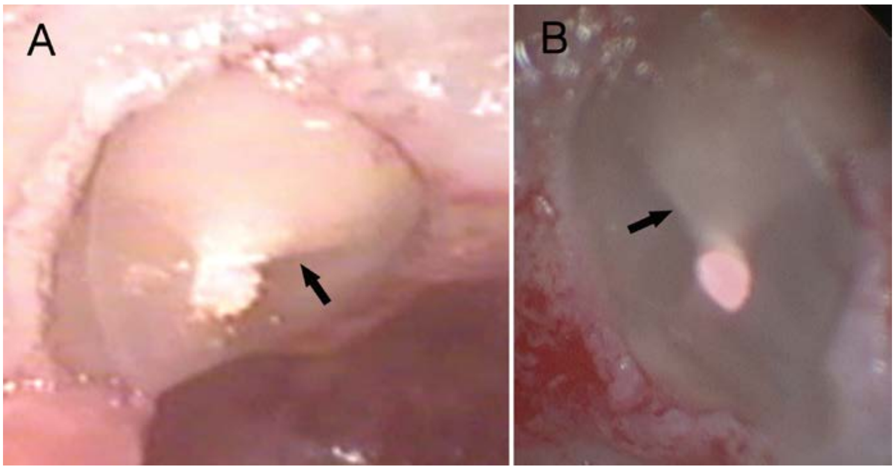

Figure 16 Endoscopic images of the RRS showing craze lines **A.** The palatal root of an upper first molar with a craze line. The craze line goes towards to the distal segment; **B.** Craze line in the distal RRS of a first maxillary molar that runs diagonally and delimits the frosted dentine

Cracks: 7 of 71 roots had a total of 7 cracks (one in each root). 2 cracks were located in roots of anterior teeth; 2 in roots of premolar teeth and 3 in roots of molars (Fig. 17).

During the clinical examination no root movement was observed in the area where the crack was present and therefore presence of fractures were excluded.

Fig. 17 RRS of a maxillary lateral incisor with crack, which starts from the root canal following the direction to the periodontal ligament space

Frosted dentine: Frosted dentine areas were observed in 67 of 71 roots, of which 17 were roots of anterior teeth, 11 roots of premolars and 25 roots of molars (Fig. 18).

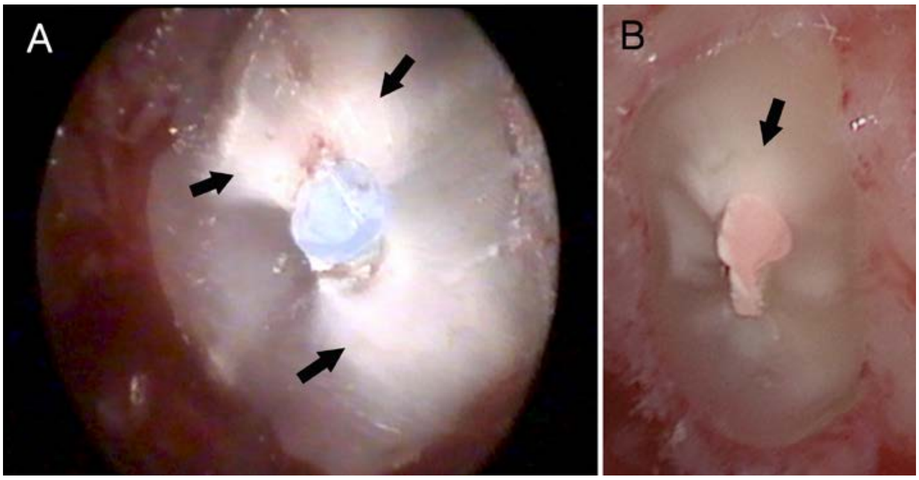

Figure 18 Endoscopic images showing frosted dentine areas **A** Root surface of maxillary central incisor with frosted dentine areas (black arrows) located in the buccal sector and palatal; **B.** Maxillary lateral incisor with of frosted dentine, which mainly expands the buccal segment

Sealing gaps: 12 sealing gaps were observed in 12 of 71 roots. 4 sealing gaps were located in roots of anterior teeth , 2 in roots of premolar teeth and 6 in roots of molars. (Fig. 19)

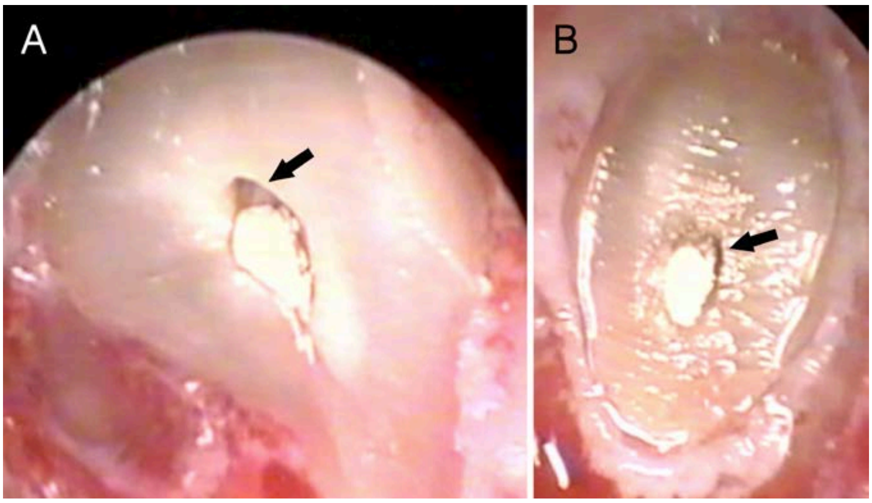

Figure 19 Sealing gaps at the RRS **A.** Resected root surface of a mesial root of a second molar with a sealing gap between the root canal wall and the filling material; **B.** Sealing gap observed at the RRS of maxillary central incisor

3.2.2 Findings related to patients' age, tooth location and presence of root posts

Descriptive statistics for findings are shown as frequency tables. Fisher's exact test was used to evaluate associations in two-by-two tables.

Craze lines and cracks were pooled in order to calculate the correlation of frequencies of craze lines or cracks with secondary parameters. The frequency of craze lines/cracks was 25.4 %, frosted dentine was seen in 94.4 % and sealing gaps in 17 % of the resected root surfaces.

Table 3 shows the occurrence of craze lines/cracks, frosted dentine and sealing gaps according to three different age groups (under 40 year-olds, 40-64 year-olds, over 64 year-olds). Sealing gaps were not found in the over 64 year-olds group.

There were no statistically significant differences amongst anterior teeth between the different age groups (Fisher's exact test: under 40, p = 0.409; 40-64, p = 0.189 and over 64 years-old, p = 0.445) and the presence of craze lines/cracks.

No significant difference was found between the different age groups (Fisher's exact test: under 40 year-olds, p = 0.594; 40-64 year-olds, p = 1.00 and over 64 year-olds, p = 0.595) and the presence of frosted dentine.

The occurrence of sealing gaps was similar (no statistically significant differences) for the age groups (Fisher's exact test; under 40, p = 0.166; 40-64 year-olds, p = 0.327).

	Findings at the RRS					
	Craze line /crack		Frosted dentine		Sealing gap	
	N	%	N	%	N	%
Total (n=71)	**18**	**25.4**	**67**	**94.4**	**12**	**17**
Age of patient						
Under 40 (n=33)	8	24.2	32	96.7	6	18.1
40-64 (n=33)	8	24.2	30	90.9	6	18.1
Over 64 (n=5)	2	40	5	100	0	0
Tooth location						
Front teeth (n=19)	7	36.8	17	89.5	4	21.1
Premolars teeth (n=11)	2	18.1	10	90.9	2	18.2
Molars teeth (n=41)	9	21.9	40	97.6	6	14.6
Presence of root post						
Roots with root post (n=15)	3	20	13	86.7	2	13.3
Roots without root post (n=56)	15	26.8	54	96.4	10	17.9

Table 3 Distribution of findings per group of roots related to secondary study parameters

There were no statistically significant differences amongst premolar teeth between the different age groups (Fisher's exact test: under 40, p = 0.615; 40-64, p = 1.00 and over 64 years-old, p = 1.00) and the presence of craze lines/cracks. No significant difference was found between the different age groups (Fisher's exact test: under 40, p = 0.758; 40-64, p = 0.541 and over 64 year-olds, p = 1.00) and the presence of frosted dentine. Also, no significant differences were found between the different age groups (Fisher's exact test: under 40, p = 1.00; 40-64 years-old, p = 1.00) and the presence of sealing gaps.

There were no statistically significant differences amongst molar teeth between the different age groups (Fisher's exact test: under 40, p = 0.284; 40-64, p = 0.284 and over 64 year-olds, p = 1.00) and the presence of craze lines/cracks. No significant differences were found between the different age groups (Fisher's exact test; under 40, p = 0.810; 40-64, p = 0.635 and over 64 year-olds, p = 0.647) and the presence of frosted dentine. The occurrence of sealing gaps was similar (no statistically significant differences) for the different age groups (Fisher's exact test: under 40, p = 0.393; 40-64 years-old, p = 0.690)

Regarding the presence or absence of root post, no significant differences were found for the occurrence of craze lines/cracks (Fisher's exact test, p = 0.745) and sealing gaps (Fisher's exact test, p =1.00). Regarding root posts, we found a tendency of their presence influencing that of frosted dentine (Fisher's exact test, p = 0.060).

3.2.3 Distribution of findings per root segments

The distribution of craze line/crack, frosted dentine and sealing gaps per root segment is shown in Table 4.

Findings at the RRS

	Observation of craze lines/cracks per segments		Observation of frosted dentine per segments		Observation of sealing gaps per segments	
	N	% [95% Confidence Interval]	N	% [95% Confidence Interval]	N	% [95% Confidence Interval]
Buccal Segment	8/71	11.3[5.0-21.1]	60/71	84.5 [73.9-91.2]	7/71	9.9 [4.1-19.3]
Mesial Segment	4/71	5.6[1.6-13.8]	40/71	56.3 [44.0-68.1]	4/71	5.6 [1.6-13.8]
Lingual Segment	5/71	7.0[2.3-15.7]	37/71	52.1 [39.9-64.1]	7/71	9.9 [4.1-19.3]
Distal Segment	3/71	4.2[0.9-11.9]	46/71	64.8 [52.5-75.8]	8/71	11.3 [5.0-21.1]
Total n=284	20/284	7.1/100	183/284	64.4 /100	26/284	9.2/100

Table 4 Distribution of findings per root segments

The observation of craze lines/cracks was in 20 of 284 segments (7.1 %). The majority of craze line/ crack was located in buccal segment (11.3 %) and lingual segment (7 %). The distribution of craze lines/cracks per root segment was not significant ($p = 0.459$)

Frosted dentine was observed in a 183 of 284 segments (64.4 %), mostly in the buccal segment (84.5 %) and the distal segment (64.8 %). The frequency distribution of frosted dentine per segment was statistically significant (Chi-squared test, $p = 0.0002$).

Sealing gaps were observed in 26 of 284 segments (9.2 %), mainly in the distal segment (11.3%). The distribution of sealing gaps per root segment was not significant ($p = 0.397$).

3.3 Micromorphometric analysis

3.3.1 Descriptive evaluation

The relative length of craze lines in relation to the length of dentine along the craze line was 43%. The relative length of cracks in relation to the length of dentine in the crack direction was 65%.

	Mean length (pixel)	**SD**	**Min value-Max value (pixel)**	**Relative length pixel (%)**
Length of dentine along craze line/crack (n=18)	166	70	64-308	100%
Craze line (n=11)	72	35	33-142	43%
Crack (n=7)	108	65	37-205	65%

Table 5 Relative length of craze lines and cracks in pixels

The measurement of width of craze line and crack calculated in pixels are presented in the table 6

	Mean width (pixel)	Minimal value (pixel)
Craze line	1.4	1
Crack	2.76	2.5

Table 6 Measurement of minimal width of craze lines and cracks in pixels

Table 7 shows measurements of the area of RRS and root canal area, frosted dentine area and sealing gap area in relation to the total area of RRS in pixels.

	Area pixel (mean)	SD	Min value-Max value (pixel)	Relativ measurement (%)
Resected root surface area (n=71)	142896	93603	31634-188904	100%
Frosted dentine area (n=67)	38365	35946	931-53672	26%
Root canal surface area (n=71)	8069	7253	1755-12755	5.8 %
Sealing gap area (n=12)	2593	1981	296 -5168	1,6%

Table 7 Areas at the RRS in pixels

The relative area of frosted dentine regarding the RRS area was 26.2% and the relative area of sealing gap in relation to RRS area was 1.6 %. The relative area of root canals regarding the RRS area was 5.8%.

Craze lines and cracks were pooled in order to calculate the correlation of morphometric analysis with secondary parameters. With regard to the correlation between findings, there was a positive correlation between the relative area of frosted dentine and the relative length of craze lines/cracks, therefore frosted dentine area has an influence on extension of the craze line/crack (**Pearson r = 0.008**).

3.3.2 Micromorphometric data and age

Micromorphometric analysis of craze lines/cracks related to age:

Following an **ANOVA** test, age showed an influence on the extension of craze lines/cracks (percentage in relation to the length of dentine along craze lines/cracks) on the analysed RRS (**p = 0.052**) (Fig. 20).

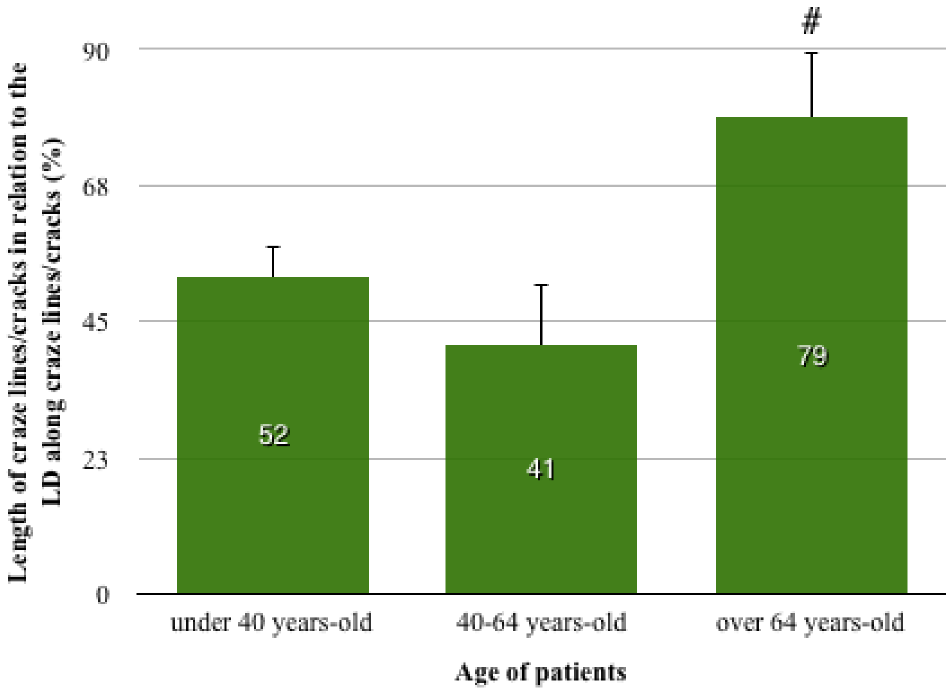

Figure 20 Length of craze lines/cracks in relation to the length of dentine along craze lines/cracks according to age of patients

Micromorphometric analysis of frosted dentine in relation to age:

Following an **ANOVA** test, no significant differences were found for frosted dentine areas (percentage in relation to total area of RRS) when comparing the three age groups (**p = 0.128**). Therefore, there was no influence of age on the area of frosted dentine analysed on the RRS. The area of frosted dentine showed a trend and decreased with age: the largest percentage was in the under 40-age group (28%), followed by the 40-64 age group (25%). It was considerably lower in the over 64-age group (15%) (Fig.21).

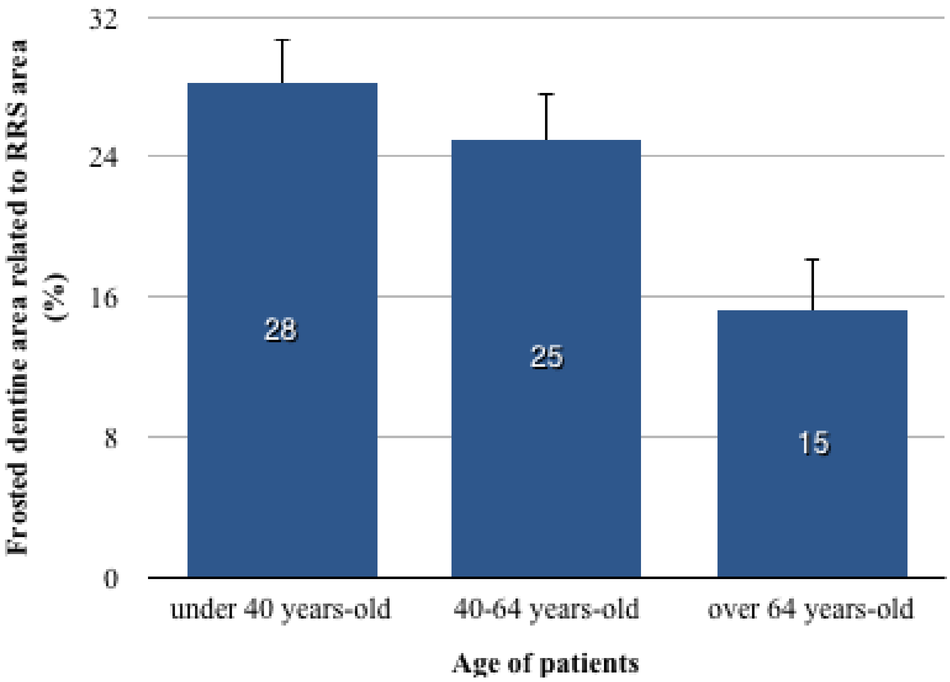

Figure 21 Frosted dentine areas in relation to total area of RRS according to age of patients

Micromorphometric analysis of sealing gaps in relation to age:

According to **t-test** analysis, no significant differences were found for sealing gaps areas (percentage in relation to total root canal area) when comparing the three age groups (**p = 0.523**). Therefore there was no influence of age on the sealing gaps area in the analysed RRS (Fig. 22).

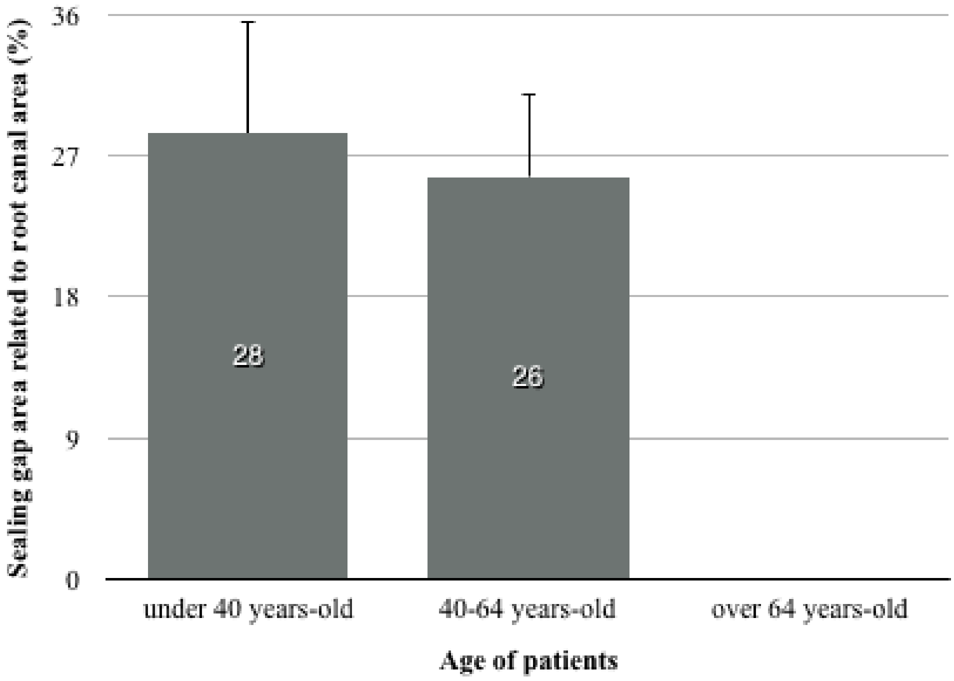

Figure 22 Sealing gap areas in relation to root canal area according to age of patients

3.3.3 Micromorphometric data and tooth location

Micromorphometric analysis of craze lines/cracks in relation to tooth location:

Following an **ANOVA** test, no significant differences were found for length of craze lines/cracks (percentage in relation to the length of dentine along craze lines/cracks) when correlated with tooth location (**p = 0.521**). Therefore, there is no influence of tooth location on extension of craze lines/cracks on the analysed RRS (Fig. 23).

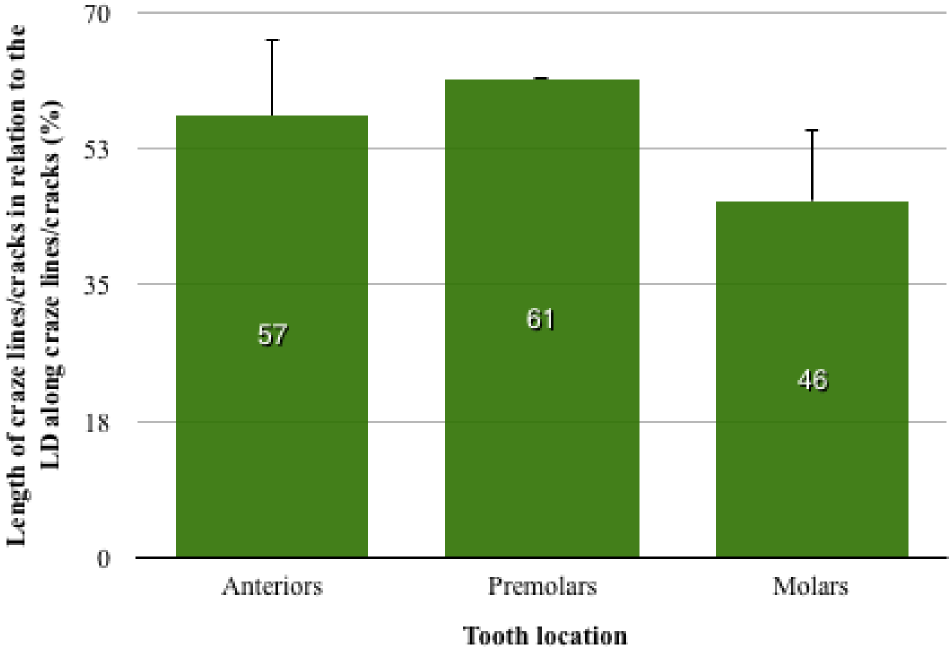

Figure 23 Length of craze lines/cracks in relation to the length of dentine along craze line/crack according to tooth location

Micromorphometric analysis of frosted dentine in relation to tooth location:

Following an **ANOVA** test, no significant differences were found for the frosted dentine area (percentage in relation to total area of RRS) when comparing anterior teeth, premolars and molars (**p = 0.625**). Therefore, there was no influence of tooth location on the area of frosted dentine that presents on the analysed RRS (Fig. 24).

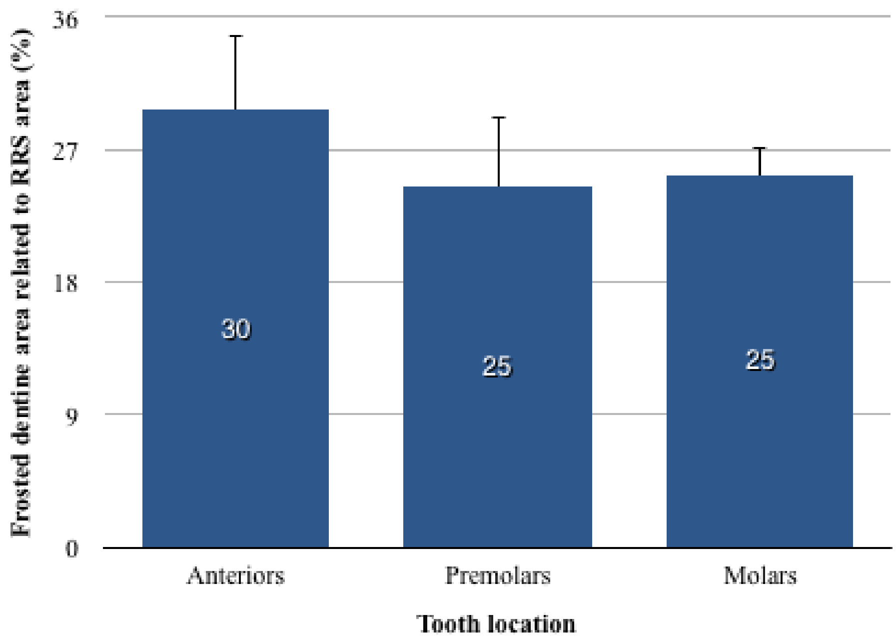

Figure 24 Frosted dentine areas in relation to total area of RRS according to tooth location

Micromorphometric analysis of sealing gaps in relation to tooth location:

Following an **ANOVA test,** no significant differences were found for the sealing gaps areas (percentage in relation to total root canal area) when comparing tooth location (**p = 0.145**). Therefore there was no influence of tooth location on the area of sealing gaps on the analysed RRS (Fig. 25).

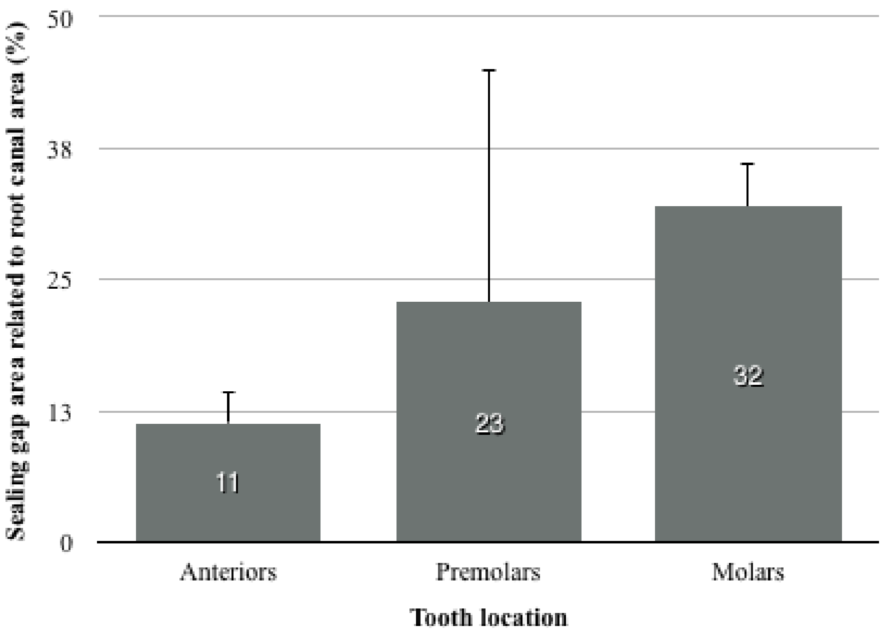

Figure 25 Sealing gap areas in relation to root canal area according to tooth location

3.3.4 Micromorphometric data and root posts

Micromorphometric analysis of the craze lines/cracks in relation to presence or absence of root posts:

From **t-test** analysis, the presence of a root post showed an influence on the length of craze lines/cracks (percentage in relation to the length of dentine along craze lines/cracks) on the analysed RRS (**p = 0.055**) (Fig. 26).

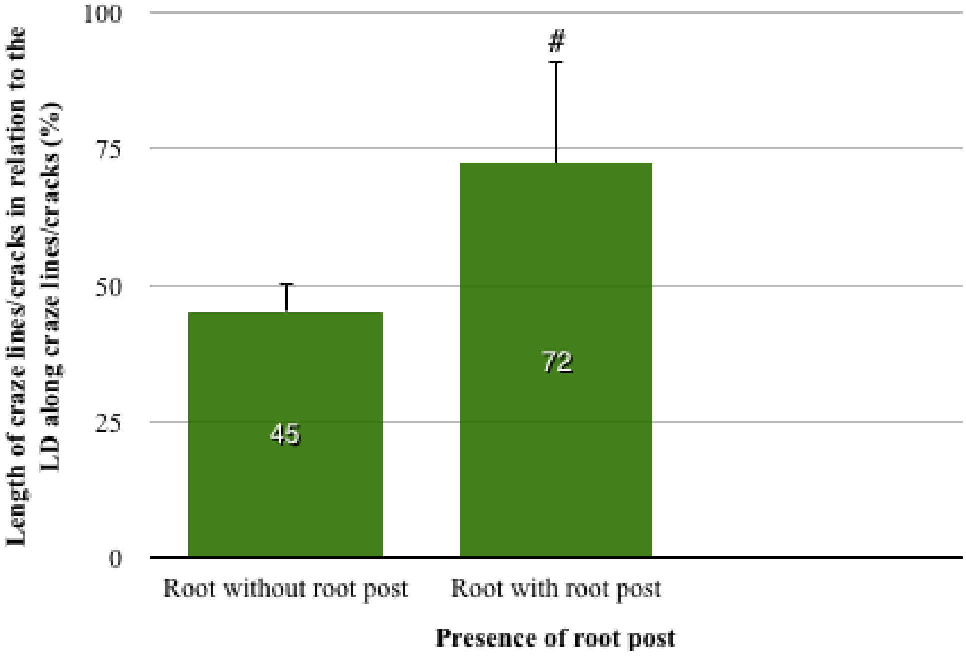

Figure 26 Length of craze lines/cracks in relation to the length of dentine along craze line/crack according to presence of root post

Micromorphometric analysis of frosted dentine in relation to presence or absence of root posts:

According to t-**test analysis**, no significant differences were found for frosted dentine area (percentage in relation to total area of RRS) when correlated with the presence or absence of root posts. (**p = 0.866**). Therefore, there is no influence of the presence of root posts and the areas of frosted dentine of the analysed RRS (Fig. 27).

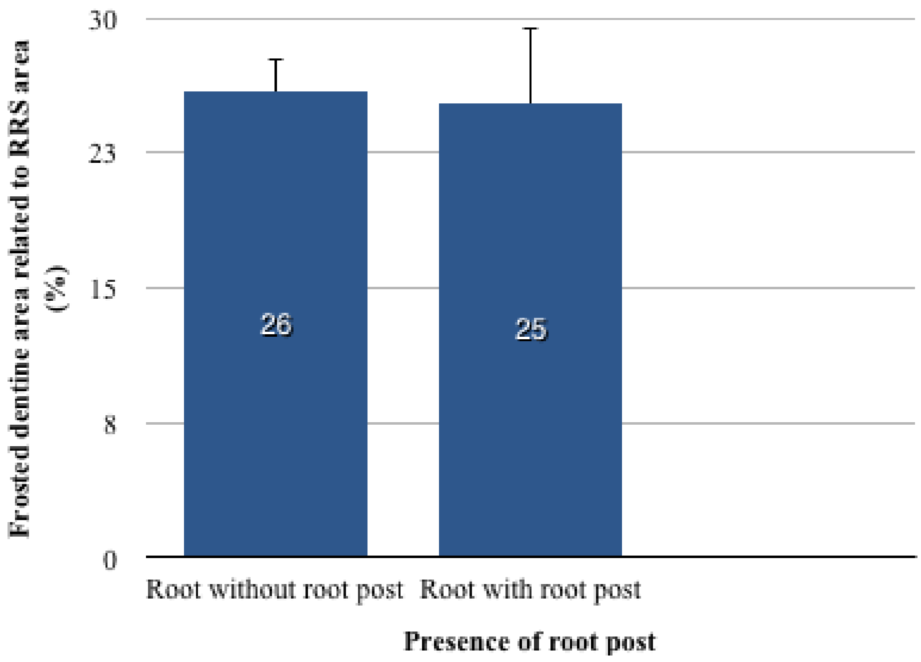

Figure 27 Frosted dentine areas in relation to total area of RRS according to presence of root post

Micromorphometric analysis of sealing gaps in relation to presence or absence of root posts:

According to **t-test analysis,** no significant differences were found in the areas of sealing gaps (percentage in relation to total root canal area) when comparing presence or absence of root posts (**p = 0.813**). Therefore, there was no influence of presence or absence of root posts in relation to sealing gap areas on the analysed RRS (Fig. 28).

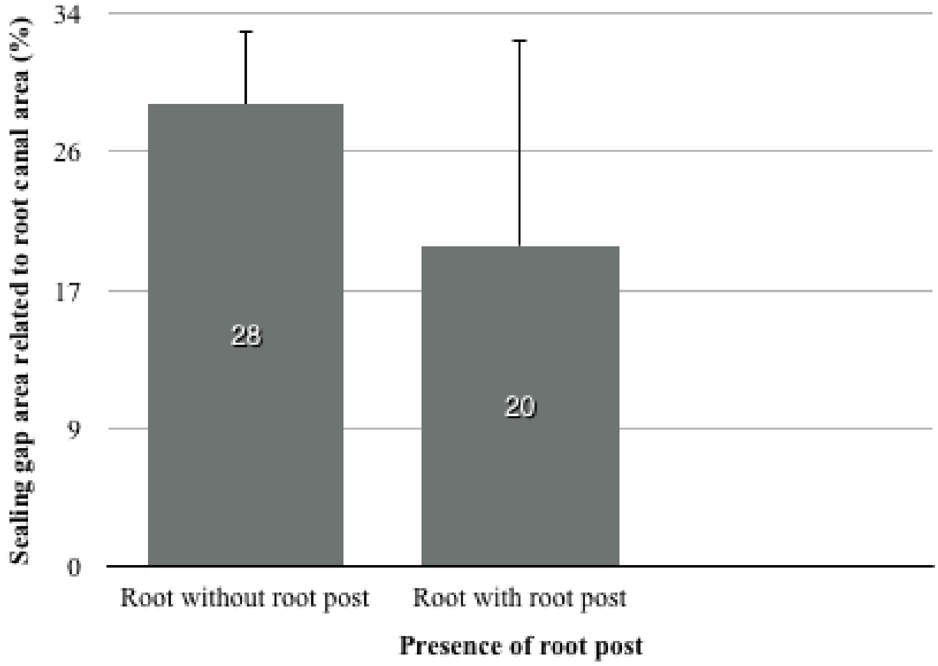

Figure 28 Sealing gap areas in relation to root canal area according to presence of root post

4 Discussion

4.1 Evaluation of the RRS – Methodical aspects

For the propose of scientific evaluation, findings at the RRS were evaluated by using either scanning electron microscopy (SEM), which is the most commonly used method for *ex vivo* studies, or a combination of an endoscope with a dental operating microscope for *in vivo* studies.

4.1.1 *Ex vivo* studies

The majority of evaluations of RRS have been performed on specimens *ex vivo* for ease of procedure and for better controlled experimental conditions for different purposes. SEM has been widely accepted and considered as the *gold standard* in the analysis of RRS, but there are also reports in the literature where operating microscopes have been used, and more recently, confocal laser scanning microscopy.

The following evaluations were focussed on the description of sealing gaps and crack findings; Nedderman et al. (1988) used SEM with magnifications of x24, x48 and x100 in order to observe the RRS of extracted maxillary molars and to observe the sealing gaps after performing the apicoectomy, using different configurations of drills at different speeds. Stabholz et al. (1992) used SEM in order to evaluate the effect of the Nd: YAG lasers on the root end, cutting the apices of 18 teeth. Gaglinani et al. (1998) evaluated RRS and sealing gaps, using a stereoscope at a magnification of x12.

Slaton et al. (2003) also evaluated the frequency of artificial dentinal cracks originating after apical resection using SEM at a magnification of x65. They identified them and compared the sensitivity, specificity and precision of three visualization techniques for their identification using x3.3 magnification loupes, x10 microscopes and x35 orascopes.

Another study also evaluated the frequency of cracks at the RRS and classified the type of crack formed after apical resection and the ultrasonic root end cavity preparation. To achieve this objective histological sections were cut and then analysed by SEM at a magnification of x48 (Taschieri et al. 2004).

A study by Adorno et al. (2013) evaluated the frequency of cracks after root end resection in order to relate the possible effect of the instrumentation and root canal filling on the formation and propagation of cracks in the apical dentine.

In a study with stereoscopes, the cracks were quantified on the root surface after resection and root end cavity preparation, using lasers, drills and ultrasonic tips of zirconium on 60 maxillary anterior teeth (Aydemir et al. 2014).

Confocal laser scanning microscopy at a magnification of x20 was used in a study by Kwak et al. (2014) in order to quantify the extent of the cracks after root end resection with ultrasonic and diamond tips at different speeds. They also quantified the size of the root end cavity and the remaining dentine thickness. A recent study by Ayranci et al. (2015) quantified the cracks after root end resection in a specific sample of mandibular premolars and used SEM at magnifications of x100 and x350 to quantify the roughness root surfaces comparing Er: YAG laser, carbide burs at low speed and ultrasonic diamond-coated tip.

Several authors have used replicas of RRS for further analysis with SEM to avoid the effects of drying. Morgan and Marshall (1999) made *in vivo* impressions of RRS with polysiloxane and later evaluated the frequency of cracks, analysing them by SEM at a magnification of x50.

In contrast, von Arx et al. (2003 a) evaluated the frequency of accessory canals, sealing gaps and the presence of chipping, but also quantified cracks in a sample of 22 extracted molars. SEM was also used to compare the accuracy of endoscopic diagnosis of RRS by analysis of the duplicates obtained after apical resection with tungsten carbide drills and after the root end cavity preparation with diamond-coated microtips. De Bruyne and De Moor (2005) used impressions of the root apices of cadaveric mandibular and maxillary teeth, extracted after root end resection and after root end cavity preparation using ultrasound with medium and low intensity. Results were subsequently analysed them by SEM at magnifications between x20 and x35 in order to evaluate the number and types of cracks.

Von Arx et al. (2010) evaluated replicas of resected root surfaces by SEM at a greater magnification (between x50 and x200) and compared the efficiency of visual assistance of endoscopes and microscopes at different magnifications in order to identify and classify dentinal cracks present on the RRS within a sample of 26 extracted molars.

In summary, the above-mentioned *ex vivo* studies of the RRS were carried out primarily: **1)** to evaluate the effect of the different drilling systems on the RRS or during root end cavity preparation and their correlation with the presence of findings at the RRS such as cracks and lines of partial or complete fractures; and **2)** to evaluate the effectiveness of different instrumental and filling systems and their correlation with the presence of cracks at the level of the cut root during apical surgery. A third approach of these studies was to evaluate the effectiveness of different magnification systems under *ex vivo* conditions in order to identify various findings on the RRS. In this case SEM was used as the most accurate method, compared to the use of other types of microscopes, endoscopes, or magnifying glasses. There are no studies in the literature with specific methods to evaluate frosted dentine.

4.1.2 *In vivo* studies

In vivo studies to evaluate RRS mainly at the phase of root end resection of apical surgery have been conducted in order to observe the anatomical structures of RRS in greater detail and to evaluate the short-term and long-term success of surgery. The most common techniques applied to these objectives have used endoscopes and dental operating microscopes.

Von Arx et al. (2011) evaluated the frequency and location of cracks, craze lines, sealing gaps and frosted dentine observed during the inspection of the RRS after root-end resection of 168 roots with endoscopic assistance at magnifications from x80 to x100.

So far there are few reports including a detailed inspection of RRS and quantifying the anatomical structures. The present study quantified findings observed *in vivo* on RRS during inspection after apical resection with support endoscopy. The focus was directed towards the situation directly after root end resection only, not on other stages of the procedure. In the future, other phases of apical surgery for the micro-morphometric analysis could be included as well as the quantification of other structures such as root canal variation and isthmus configuration as risk factors for treatment success.

4.1.3 Role of the Image J for structure quantification

Visualisation with high magnification is essential to identify accurately the presence of findings and provides the advantage of allowing a simultaneous quantification using image analysis software. The use of image analysis programmes helps to improve the observation of digital images by making it possible to adjust the brightness and contrast levels, and it is precisely this image processing which allows efficient extraction of necessary information to make a diagnosis while excluding irrelevant information. In this way the possibility of generating false positives decreases while identifying structures.

Image J is a well-known image analysis programme and is commonly used to process images in histomorphometry. However, in dentistry it has been used mainly as a quantifying method in order to determine degrees of apical root resorption and progression of bone repair as well as to compare the efficacy of irrigators in debridement removal from root canals.

Quantifying methods combine the use of image J software and digital images obtained directly from visualisation devices. Saghiri et al. (2012) compared the efficacy of removing detritus between two systems of irrigation during instrumentation quantifying the area where the detritus was removed using Image J software on an image obtained from SEM. Adcock et al. (2011) and later Sarno et al. (2012) used the same method. However, quatification was done on digital images obtained from light microscopy.

Özer (2011) compared the shape of root canals in curved canals and by means of Image J software determined the bending angle of three NiTi rotary instruments, comparing the pre- and post-instrumentation images obtained by computer tomography.

Celik et al. (2015) evaluated the effect of different adhesive systems. They quantified the resin-to-resin interface and the resin-to-tooth interface using image J software on images photographed with a digital camera.

A study by Engelke et al. (2015 a) presented an *in vivo* method to evaluate quantitatively the microstructure and vascularization of bone in dental extraction sites on endoscopic images.

In this study the programme Image J was chosen for image analysis. This coincides with Abràmoff et al. (2004) in that the programme's tools for image processing are easy to use and that it offers the advantage of a variety of image manipulation options. Similarly, the use of digital radiographs and the conversion of conventional radiographs to a digital format provide countless possibilities of image processing.

Image analysis allows estimating the quantification of findings in relation to the number of pixels that make up the digital image. This makes it possible to determine and quantify *in vivo* the size of a particular structure of RRS, and guided by this information clinicians can determine the dimensions of the findings and their importance in the prognosis of each case.

An important point is that digital images which are used for the analysis can be obtained from different sources such as digital camera, digital radiographs, conventional digitalised radiographs via a scanning process, or through high magnification equipment such as SEM, dental operating microscopes, endoscopes or HD endoscopes.

The application of digital image analysis in endodontics can currently provide many benefits and result in increased diagnostic accuracy, because it allows improving specific features in an area of interest. Clinically this could mean that endodontic failures associated with controllable factors, can be detected at a much earlier point in time. It also provides the possibility of *in vivo* diagnosis and allows quantifying and measuring pathological findings. This might have potential implications for case prognosis and might increase success rates of endodontic therapies.

4.2 General discussion of results

4.2.1 Diagnostic and demographic parameters

Cohort studies have allowed a large number of procedures to be quantified to improve the definition of diagnostic parameters. In the study of von Arx et al. (2011), the cohort study for assessment of the RRS included 114 patients.

Within the general group of the present study, only 47 out of a total of 190 patients could be evaluated according to the established selection criteria. This was because an endoscope was used in many cases only for certain specific procedures such as assisting in identifying granulation tissue residues, controlling those situations with anatomical difficulties, or for controlling the root end cavity filling, and not always for inspection of RRS after root end resection. Therefore, some patients were not suited to the systematic evaluation of findings. In order to perform a systematic evaluation in future studies, it may be necessary to use the criteria developed here with a quality control in prospective randomised trials, and also to evaluate the influence of certain therapeutic measures.

Compared to the number of 168 roots in the study of von Arx et al. (2011) our cohort was comparatively small due total selection criteria chosen.

Statistical analysis shows that all age groups were involved in endoscopically-assisted apical surgeries with the 40–64 years age group being the largest. Von Arx et al. (2011), found the highest frequency of apicoectomies in the ≥ 45 years age group.

Regarding patient gender, no difference was found between females and males. This coincides with the results of the ≥ 45 years age group in the von Arx et al. (2011) study.

The data in this study also shows that apical surgeries were mostly implemented in the maxilla and molar teeth. In contrast, von Arx et al. (2011) showed a higher frequency in mandibular molars.

The distribution of endoscopic apicectomies underlines the need for a better visualization of the posterior area. The anterior area could be an easily accessible area where the use of dental operating microscopes or magnifying glasses for visualisation does not imply a greater challenge. In relation to the assessed parameters, von Arx et al. (2011) included in his study craze lines, cracks, frosted dentine, sealing gaps and additionally the isthmus and roots with more than one root canal. In the present study only those roots with a single root canal were evaluated. With respect to the isthmus, it was not considered because it was not part of the objective of this study. Further studies could consider these two parameters.

4.2.2 Clinical assessment

4.2.2.1 Craze lines and cracks

Most of the craze lines and cracks observed in this study were unstained and entirely within the dentine. Aditionally, they did not communicate with the canal wall. After root end resection, an occlusal loading test was performed on the roots in order to detect movements and thus to exclude or confirm fractures.

The difference between crack and fracture is the evident separation of fragments from the root. In this study no teeth showed such characteristics, therefore discarding the presence of fractures.

In the present study, the craze lines and cracks were pooled for the distribution, location per root segment, and for the correlation with secondary parameters. Craze lines were observed in 11 of 71 roots and cracks were noticed in 7 out of 71 roots, meaning that a total of 18 craze lines/cracks were identified in 17 roots (25.4%). Von Arx et al. observed the presence of craze lines or cracks in 9.5% of their sample of 168 roots.

In the present study there was no relationship found between the occurrence of craze lines or cracks and the age of patients, presence of a root post, nor tooth location. In contrast, von Arx et al. (2011) found a high occurrence of cracks associated with the presence of root posts; however Altshul et al. (1997) showed there was no greater likelihood of root post placement causing root fracture than conventional endodontic therapy alone. Further investigations may provide more evidence regarding the relationship between cracks and the presence of root post and the relationship between cracks and frosted dentine are necessary.

Most craze lines/cracks were located in the buccal segment (9.9%). This situation has also been reported in the literature (von Arx 2011, Versluis et al. 2006, Lam et al. 2005). In this study a greater frequency of craze lines/cracks was observed in molars. This differs from the study of von Arx et al. (2011) who reported a high occurrence of craze lines/cracks in premolars; the reasons for this result are unknown.

Regarding the different drill systems for the resection of the apical root portion and its relationship with the formation of dentinal cracks, it is important to consider that irregularities produced by the use of drills for root resection may cause changes in the reflection of light and as a result of this create the illusion of cracks on the surface (Wright et al. 2004). For the cohort analysed in this study the preferred equipment consisted of Lindmann drills at high speeds, or ultrasonic systems, which might be a factor worth considering for future in *vivo* research studies.

4.2.2.2 Frosted dentine

In this study, frosted dentine was the finding most observed (67 out of 71 roots) and the high number of frosted dentine found in the present study is relevant (94.4 %). This result is in contrast with the results obtained by von Arx et al. (2011), where frosted dentine in 79.8% of evaluated roots was reported. One reason could be that because the effect of the resection angle was not evaluated in detail in this retrospective study, and although microsurgical techniques were used to perform apical resection, variations within the resection plane generated more areas of exposed tubules than when dried for subsequent inspection, thus generating more areas of frosted dentine. Another reason could be the misinterpretation of the frosted dentinal zones on the images, perhaps causing the results to be biased due to subjectivity of the examiner.

An association was found between the occurrence of frosted dentine and the presence of a root post, which could indicate that the root post exerts some kind of tension on the root. Neither patient´s age nor the type of treated tooth, were related to the occurrence of frosted dentine.

An interesting fact was found in this study: all RRS with craze lines or cracks displayed frosted dentine. In contrast, Onnink et al. (1994) found no correlation in their investigation between the occurrence of fractures and opaque or translucent dentine. The frequency of localisation for both findings was in the buccal segment. Data from this research could establish a new relationship between the presence of frosted dentine and the subsequent formation of cracks as was speculated by Slaton et al. (2003).

Frosted dentine was observed more in the buccal segment (84.5%) than in other segments and mainly in molars. This occurrence pattern coincides with the results of the study by von Arx et al. (2011), who attributed the high frequency in posterior teeth to the fact that these teeth contain more exposed dentinal tubules than the anterior teeth bucco-lingually. They observed this finding in 134 out of 168 evaluated roots: a lower occurrence than in this study.

The causes for the presence of frosted dentine have not yet been established with certainty. These areas in dentine have been mentioned in a previous study as areas caused by the formation of several microscopic cracks that have not yet coalesced to form a crack, being considered an area of great tension which increases the risk of developing cracks or root fractures (Slaton et al. 2003). However, von Arx et al. (2011) questioned this argumentation referring to the change in the refractive index of the exposed dental tubules as resulting from the fact that the drying of the surface generates a whitish appearance, thus such phenomenon should not occur in areas with sclerotic dentine since these tubules contain dentine patterns with a similar refractive index to the intertubular adjacent root dentine (von Arx et al. 2011).

More studies that focus on determining the causes of presence of frosted dentine and the function of structural and/or optical changes occurring in the dentinal tissue of RRS are necessary in order to evaluate their relationship with the presence of craze lines and cracks.

4.2.2.3 Sealing gaps

Sealing gaps are one of the main causes of failure of endodontic therapy and it is presumed that the formation of sealing gaps might have their origin in the procedures of root canal preparation and root filling during endodontic treatment. In this study 12 sealing gaps were observed (17%). In contrast, von Arx et al. (2011) found that the sealing gaps were the most observed finding on RRS (83.3%). The majority of patients were referred after sufficient retrograde root canal filling only to periapical revision, granuloma or cysts. Therefore a high incidence of sufficient root canal fillings without sealing gaps was found.

Neither patient age, nor type of tooth, nor presence of root posts showed any association with the occurrence of sealing gaps. These results agree with the results of von Arx et al. (2011).

Sealing gaps were found predominantly in the distal segment (11.3%). This differs from the results of von Arx et al. (2011), where these were located often in the buccal segment (29.7%). In this study the frequency of sealing gaps in the buccal segment was 9.9%.

One study shows that a possible cause explaining the presence of sealing gaps in the buccal or lingual segment would be that these areas of the root canal show areas with higher incidence of “wings” and that the rotating instruments probably generate round preparations within an oval shape of the root canal and therefore these areas are difficult to instrument (von Arx 2011). In this study, canal preparations were performed with conventional endodontic files, whereby the location could be explained by the influence of the technique the clinician used when performing endodontic therapy and also by the loss of filling material during the root end resection.

4.2.3 Micromorphometric aspects

4.2.3.1 Craze lines and cracks

The quantification of cracks and craze lines performed in the present study attempts to establish morphological properties in order to achieve a structural differentiation of the findings. Regarding the extension, the relative craze line extension is 43%, while the relative crack extension was 65%. That indicates that cracks had bigger extension than craze line, when they were compared with the remaining dentine of the location where the crack or craze line had been identified.

It is possible to conclude that in the case of cracks, the extension covers more than half of the remaining dentine thickness and this could be a critical aspect in the case of teeth with small thickness of remaining dentine. Abedi et al. (1995) reported in their study that more than 70% of cracks developed when the dentine walls were smaller than 1 mm.

In a study of Kwak et al. (2014) no association between the developments of crack with the thickness of remaining dentine was found, when it was more than 1 mm. In both studies the length of crack and minimal thickness of remaining dentine wall after preparation of root end cavity with ultrasound was measured.

For further studies it might be helpful to include the quantification of the extension of a crack and the remaining dentine during intraoperative procedures to determine its impact on the prognosis. It is important to consider that there are several types of dentinal cracks, one of them can cover all or a part of the thickness of the dentine or also can be within the dentine. Future quantification studies may be able to assess these aspects more in detail.

In relation to the thickness of craze lines and cracks, the minimal width of craze line was 1 pixel and the minimal width of crack was 2.5 pixels. This shows that the small size of the width of craze line could be unrecognizable to the naked eye. It also appears that the resolution of the visualisation system used in this study is limited. Thus it is necessary to enhance the visualisation technique and to optimize the amount of information being visualized in the display devices.

Future research would be valuable in supporting this observation and revealing the clinical significance of craze lines and cracks in the intra-operative assessment of RRS. More studies are also needed to clarify the structural differences between the two findings since most of the previous research has mainly focused on studying the formation of cracks and the role they play as a possible precursor of future vertical root fractures (Morgan and Marshall 1999). So far, there is few information in the literature related to the nature of craze lines and their clinical significance in crack formation.

This study found that the presence of a root post increased the extent of craze lines/cracks when present. Additionally, there was a positive correlation between the extent of the craze lines/cracks and the amount of frosted dentine. An association with patients' age was also found, whereby patients in the over 64 year-old group had longer cracks.

It is important to underline that the results of this study coincide with the study of von Arx et al. (2011) in which stated that aspects such as root canal centricity had not been evaluated because the canal might not have been in the centre of the root and other aspects such as the bevel of resection planes may vary and might influence in the quantification analysis. This includes the recommendation of further studies that should provide also a morphological analysis of the RRS at different levels.

Whether the nature of craze lines corresponds to the same nature of cracks, or if they could be caused by an optical phenomenon cannot be determinated from the present study. There is an additional need to determine if craze lines could be precursors of future cracks and the clinical impact of their presence.

The method of quantification of findings at RRS used in this study could be complemented with magnification devices to measure micro-movements intra-operatively *in vivo* (Engelke et al. 2004) this techniques and it could be very useful for differentiating fractures from cracks and craze lines.

4.2.3.2 Frosted dentine

In relation to quantification, the relative area of frosted dentine in this study was 26% in relation to RRS area. The areas of frosted dentine differed: there were very small as well as very large areas. It can therefore be concluded that there is a variable range in areas of frosted dentine. Taking into account this variability, in order to determine the role or impact of the size of findings further quantification studies are needed.

The presence of root posts did not affect the areas of frosted dentine. Regarding patients' age and tooth location, neither of these were related to the size of the frosted dentine area. However, it was found that frosted dentine areas had a tendency to decrease with age.

The present study showed also a positive correlation between the areas of frosted dentine and the length of the craze lines/cracks. Further studies are needed to establish this relationship with greater accuracy.

4.2.3.3 Sealing gaps

In this study, the relative area of sealing gap was 1.6 % in relation to RRS area.

The relative area of sealing gap in relation to root canal area was 27%. Before retrograde revision of the filling apparently the size of a sealing gap occupies a relatively great space within root canal. Nevertheless, in order to determine the real implication of the area of sealing gap more studies are required, because no other research apart from this study has quantified this finding. The intra-operative quantification of sealing gap area allows carrying out a quality control of the endodontic treatment. This should be applied in the future before and after revision of the RRS.

The sealing gaps represent a clinical challenge because their cause is associated with endodontic failure. Defects in canal obturation can result in bacterial contamination that can spreads towards the periapical area producing re-infection of this area. Hence, quantifying the size the sealing gap occupies within the root canal becomes clinically important because it could be considered as a factor with which to evaluate instrumentation techniques and root obturation in order to reduce the occurrence of sealing gaps in future. Also, the definition of a critical gap size could be an interesting task for futures RRS evaluations.

4.3 Clinical assessment using optical systems

The success of magnification-assisted endodontic surgical treatment (surgical microscopes with micromirrors and rigid endoscopes) can be explained by the fact that during surgery most causes of endodontic failure can be detected with adequate lighting and magnification, and corrected immediately. Held et al. (1996) mentioned that the inconspicuous root apices can be displayed using a rigid endoscope, which can also be used to reliably detect root fractures. On the other hand, Velvart (1996), using a surgical microscope with the help of micromirrorrs pointed out the need for a good visualisation of the operation field. He also referred to the possibility of improving root end cavity preparation using ultrasonic instruments.

Von Arx et al. (2001 b, 2002) emphasized that endoscopes, compared to surgical microscopes, provide a line of direct vision with a quick possibility to change the viewing angle and permitting visualisation of otherwise non-visible regions. They recommended the use of endoscopes for intra-operative diagnosis of RRS end and demonstrated that success rates of apical resections were lower without the use of endoscopes (von Arx et al. 2003 a; von Arx et al. 2003 b).

The intra-operative use of optical systems allows a proper and a homogeneous illumination of the surgical field. In addition, work areas can be overviewed directly, while a simultaneous view is attained of anatomical details with the use of different magnifications (Saunders and Saunders, 1997).

There is evidence to support that microsurgical techniques, compared to conventional techniques, provide better intraoperative diagnosis as well as the ability to document them by means of photos and video, thus allowing the direct intra-operative evaluation of the quality of procedures (von Arx et al. 2002: von Arx et al. 2003 a; von Arx et al. 2003 b).

The use of rigid endoscopes allows a safe control of the apical resection and the bone cavity during surgery. The use of a video-endoscope allows evaluation of obturation integrity in the existing root canal and permits identification of existing branching and canal areas without filling on the level of the resection (von Arx et al. 2003 b).

Several authors have argued that the use of magnification during removal of apical branching areas with bacterial contamination and during the removal of periapical pathological tissue is a great advantage to prevent re-infection (Pecora and Andreana 1993; Kim, 1997). On the other hand, existing sealing gaps or canal areas without endodontic filling, such as isthmuses between the root canals, can also be identified reliably with the use of rigid endoscopes (von Arx et al. 2003 b).

Regarding the effectiveness of visualisation methods for the identification of endodontic structures *ex vivo*, it has recently been proven that the combined use of different endoscope systems can deliver high superiority in the detection of findings even under difficult viewing conditions (Engelke et al. 2015 b). Compared to SEM, von Arx indicated that an endoscope presents a 100% sensitivity and specificity for identifying isthmuses or accessory canals. However, only 36% sensitivity was reported in identifying cracks (von Arx et al. 2003 a).

Von Arx concluded that a x64 magnification endoscope allows for greater visual accuracy in identifying cracks. But, this also delivers a great number of false positives cracks identification due to the short distance between the root end and optics when using a higher magnification (0.5 – 1mm) (von Arx et al. 2010). Slaton et al. (2003) showed that microscopes presented a high specificity in the identification of cracks (73%) compared to orascopes (63%). In addition, one study emphasised that the use of transillumination with magnification could be very useful in identifying cracks at the RRS (Wright et al. 2004).

Cracks and root fractures can be identified with high reliability through the magnified field view, making it possible to determine whether apical root resection, may still be indicated or extraction has to be performed.

4.4 Conclusions

The present evaluation intended to provide an improvement for the description of clinical findings at the RRS during periapical surgery by means of endoscopic imaging and subsequent digital image analysis. It could be demonstrated, that the video footage obtained during routine surgery allowed (a) a qualitative description of pathologic findings and (b) a quantitative assessment of the structural findings like craze line, cracks, frosted dentine and sealing gaps. The latter was obtained relative to anatomical distances (root canal diameter) and areas of the RRS (root surface area) and may be measured in the future in absolute values by using scaled video images.

Frosted dentine appears to be a very common phenomenon with a high incidence mostly in the buccal segment. The measurements reveal that frosted dentine has a variable relative area at the RRS, however is not possible to determine the impact of this variability.

Clinically detected cracks and craze lines show significantly different extension relative to the dentine thickness at their locations. The minimal thickness of craze line lies at the limits of the image resolution and requires further improvement of the visualisation tools. High-resolution visualization endoscopic could provide sufficient image quality suitable to pimprove for quantitative assessment. The present study could demonstrate a correlation of crack lenght and frosted dentine area, but further studies are required to clarify this relatioship.

Quantification of sealing gap defects may be used as an instrument of quality control for further studies.

5 Summary

Background:

Endoscopy combined with subsequent digital image analysis makes it possible to survey quantitative characteristics of clinical findings in dentistry. The aim of this study was to provide a quantitative description of diagnostic parameters of the resected root surface (RRS) during apicoectomy.

Method:

In a general group, the analogue video recordings of 237 apicoectomies in 191 patients (84 men, 107 women, with an average age of 40.7 years) at the Department of Oral and Maxillofacial Surgery of UMG were digitised and subsequently evaluated. 71 apicoectomies on 47 patients fulfilled the quality criteria of a complete representation of RRS without artefacts immediately after root end resection and were selected for the study cohort (n = 71). Evaluation was carried out in 3 steps: (1) Descriptive analysis of the interventions according to demographic parameters and location in the dental arch. (2) Assessment of findings at the RRS and correlation with parameters for age, tooth location, presence or absence of root posts and location per root segments.

(3) Micromorphometric analysis using Image J, with description of the relative length of craze lines and cracks and relative area of root canals, frosted dentine and sealing gaps. The complete RRS and the transverse root diameter served as references. The results were correlated with age, location of tooth and presence or absence of root posts. The distribution of interventions for the study cohort related to demographic parameters and to anatomical location, and were tested using the Chi-squared test. Descriptive statistics of findings were presented as frequency tables. Associations in two-by-two tables were tested using Fisher's exact test. For comparisons between proportions of segments per finding, exact two-sided 95% confidence intervals (Clopper–Pearson) were calculated and the null hypotheses were tested with the Chi-squared test. For the micro-morphometric analysis,

descriptive tables were presented. Values were tested for normal distribution and the outcomes tested for correlation with demographic parameters with a t-test for independent samples, or One-way ANOVA.

Results:

(1) In the study cohort there was no gender-specific distribution ($p = 0.466$) and the interventions were made mainly in the age group between 40 and 64 years (51.1%). Interventions were performed more frequently in the maxilla ($p = 0,01$), posterior areas dominated in the maxilla ($p = 0.02$) and in the mandible ($p < 0.001$).

(2) The frequency of craze lines or cracks was 25.4%, frosted dentine 94.4% and sealing gaps 17 % of the RRS analysed. There was no significant correlation of the findings of craze lines/ cracks, frosted dentine, or sealing gaps with patient age or tooth location ($p > 0.05$). The presence of a root post seemed to have an impact on the occurrence of the frosted dentine ($p = 0.06$). The observation of craze lines/cracks and frosted dentine was 7.1% and 64.4% of total segments respectively, mainly in the buccal segment (11.3% and 84.5% respectively). Th observation of sealing gaps was 9.2% of total segments, mainly distally (11.3%).

(3) In the micromorphometric analysis, the relative length of craze lines was 43% and that of cracks was 65% in relation to the extent of dentine along craze lines or cracks respectively. The minimal width of craze lines and cracks was 1 and 2.5 pixels respectively. The relative area of frosted dentine, root canals and sealing gaps in relation to the total area of RRS was 26%, 5.8% and 1.6% respectively. Borderline results arose for the length of cracks and craze lines depending on the age of the patient ($p = 0.05$), the presence of a root post ($p = 0.05$), but not on the type of tooth treated ($p > 0.05$). The frosted dentine area had an influence on the length of craze lines and cracks ($p = 0.008$).

Conclusion:

The present evaluation intended to provide an improvement for the description of clinical findings at the RRS during periapical surgery by means of endoscopic imaging and subsequent digital image analysis.

Morphometric analysis shows, that frosted dentine appears to be a very common phenomenon with a high incidence and variability mostly in the buccal segment. Craze lines and cracks show significantly different extension relative to the dentine thickness at their locations. The minimal thickness of craze line lies at the limits of the image resolution and requires further improvement of the visualisation tools. Quantification of sealing gap defects may be used as an instrument of quality control for further studies.

6 Appendix

6.1 Documents for Ethical Review

UNIVERSITÄTSMEDIZIN GÖTTINGEN **UMG**

Ethik-Kommission der Universitätsmedizin Göttingen, Von-Siebold-Straße 3, 37075 Göttingen

Ethik-Kommission der
Universitätsmedizin Göttingen
Vorsitzender: Prof. Dr. Jürgen Brockmöller
Referentin
Regierungsrätin Doris Wettschereck
0551 / 39-8644 **Telefon**

Von-Siebold-Straße 3, 37075 Göttingen
Adresse
0551 / 39-66629 **Telefon**
0551 / 39-9536 **Fax**
ethik@med.uni-goettingen.de **E-Mail**
www.ethikkommission.med.uni-goettingen.de

Herrn
Prof. Dr. med. Dr. med. dent. Wilfried Engelke
Klinik für Mund-, Kiefer- und Gesichtschirurgie
Robert-Koch-Straße 40

vorab per E-Mail: wengelke@med.uni-goettingen.de

09.09.2015 br – ha - gö Datum

Antragsnummer: **30/8/15 (bitte stets angeben)**

Studientitel: Morphometrische Untersuchung am Querschnitt resezierter Zahnwurzeln nach mikroendoskopischer Resektion

Antragsteller: Prof. Dr. med. Dr. med. dent. Wilfried Engelke, Klinik für Mund-, Kiefer- und Gesichtschirurgie, UMG

Doktorandin: Carolina Leiva Hernández

Zur Begutachtung lagen vor:
Anschreiben (Eingang am 07.08.2015)
Erhebungsprotokoll
Kurzprotokoll

Sehr geehrter Herr Prof. Dr. Engelke, sehr geehrte Damen und Herren,

die Ethik-Kommission der Universitätsmedizin Göttingen hat den oben genannten Antrag in der Sitzung vom 03.09.2015 beraten.

Die Ethik-Kommission hat keine ethischen oder rechtlichen Bedenken gegen das vorgelegte Studienvorhaben.

Bitte beachten Sie noch folgende Hinweise:

1. Bitte reichen Sie die Genehmigung des Klinikdirektors Prof. Dr. Dr. Henning Schliephake nach.
2. Die Angaben in der Verschwiegenheitserklärung und dem Studienprotokoll widersprechen sich hinsichtlich Pseudonymisierung/Anonymisierung der Daten:
    a. Erklärung: …(anbei die Anonymisierungstabelle)…Die gesammelten Daten für diese Studie werden mit Codes versehen, und nur der Untersucher dieser Studie kann diese Daten dem Patienten und dessen Krankengeschichte zuordnen.
    Der Datensatz ist anonymisiert. In diesem Sinne ist die Identifikation von einem individuellen Patienten nicht möglich und kann auch nicht durch einen Benutzer ermittelt werden. Sowohl von diesen Daten selbst als auch in Kombination mit anderen Informationen hat der Benutzer keinen Zugang zu diesen Daten. Jede Codierung steht speziell für einen Befund ohne dass man den Namen des Patienten erkennen kann.
    b. Biometrie: Die Daten werden auf Basis der Operationsbücher des ZMK-OP erhoben und unmittelbar nach vollständiger Erstellung der Excel Tabelle anonymisiert und nicht an Dritte weitergegeben.
    c. Datenschutz: Sämtliche Daten werden vertraulich behandelt und nur für diese Studie verwendet. Alle Daten werden pseudonymisiert und im Rahmen des Datenschutzes codiert. Dabei wird jedem Patienten eine Codenummer zugeordnet, welche auf dem internen Erhebungsbogen vermerkt wird. Auf einer separaten Zuordnungsliste, die geschützt bei Studienleiter verwahrt wird und nur für diesen und den Doktoranden zugänglich ist, werden die Zuordnung der Codenummer und des Patientennamens gespeichert. Außerdem werden die pseudonymisierten Daten an die medizinische Statistik zur Auswertung weitergegeben.

    Wir bitten um eine Vereinheitlichung der Angaben, wobei der Grundsatz zu beachten ist, dass soweit das Studiendesign es zulässt, möglichst mit anonymen Daten gearbeitet werden sollte.

Wir bitten geänderte Unterlagen **in einfacher Form** dem Sekretariat der Ethik-Kommission einzureichen und ein Anschreiben zu formulieren, indem Sie zu jedem Punkt unseres Schreibens eine kurze Stellungnahme formulieren und angeben, wo Sie in den Unterlagen Änderungen vorgenommen haben. Bitte kennzeichnen Sie die Änderungen in den Unterlagen durch Unterstreichung oder farbliche Hinterlegung.

Wir wünschen Ihnen viel Erfolg bei der Durchführung Ihres Projektes.

Unabhängig vom Beratungsergebnis macht die Ethik-Kommission darauf aufmerksam, dass die ethische und rechtliche Verantwortung für die Durchführung einer wissenschaftlichen Studie beim verantwortlichen Studienarzt und aller an der Studie beteiligten Ärzte liegt.

Alle Änderungen im Studienprotokoll müssen der Ethik-Kommission vorgelegt werden und dürfen erst nach der zustimmenden Bewertung umgesetzt werden.

Über alle schwerwiegenden unerwarteten unerwünschten Ereignisse, die während der Studie auftreten und die Sicherheit der Studienteilnehmer oder die Durchführung der Studie beeinträchtigen könnten, muss die Ethik-Kommission unterrichtet werden.

Der Abschluss/Abbruch der Studie ist mitzuteilen und ein Abschlussbericht vorzulegen.

Auf die Einhaltung einschlägiger Gesetze und Rechtsvorschriften wird hingewiesen. Die nach Rechtslage notwendigen Unterrichtungen (u. A. Änderung des Studienprotokolls, Meldung von Zwischenfällen, neue Datenlage, Nachmeldung von Prüfzentren, Abschlussbericht) sind der Ethik-Kommission unverzüglich vorzulegen.

Die Ethik-Kommission bestätigt, dass sie auf Grundlage nationaler Gesetze, Vorschriften sowie der GCP/ICH-Richtlinie arbeitet.

Mit freundlichen Grüßen

Prof. Dr. med. J. Brockmöller
Vorsitzender der Ethik-Kommission

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Curriculum vitae:

I was born on January 8th, 1982 in Antofagasta, Chile. I am the daughter of Resffa Hernández and Diego Leiva. My mother is an Elementary School Principal and my father worked for the Chilean Air Force and is now retired.

I started my Dental bachelor's degree in March 2002 and successfully finished it in December 2008.

From March 2010 to December 2011, I attended and obtained a Post Graduate degree in Endodontics at Universidad Andres Bello in Santiago. In 2012, I started working as a researcher of the Faculty of Medicine of Universidad La Frontera in Temuco and developed specialized activities for the under-graduate and post-graduate programs.

In 2013, I was part of a research project of the Faculty of Medicine of Georg-August-Universität Göttingen. This experience allowed me to live in Germany for the first time and most importantly helped me in bringing new ideas to our working group in Chile.

Since 2014 I've been living in Göttingen after earning a scholarship given by National Commission for Scientific Research and Technology in Chile (CONICYT) and by DAAD (Germany) to obtain my PhD in Dentistry/Georg-August-Universität.