QUALITY OF ROOT FILLING BY THREE DIFFERENT TECHNIQUES IN TEETH AFTER MINIMAL ROOT CANAL PREPARATION AND GENTLEWAVE CLEANING

by

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Abstract

Objective: This study aimed to examine the quality of root filling in canals obturated using three different filling techniques after minimal root canal preparation and GentleWave (GW) cleaning.

Methods: Root canals of 30 mandibular molars were instrumented to size 20/.04 taper using hand K-files, and a Vortex Blue NiTi rotary file. Sodium hypochlorite (NaOCl) 6% was used in-between each instrument insertion and the canals were finally cleaned using a 7.5 min irrigation by the GW. The samples were scanned with micro-CT using a voxel size of 30 µm after instrumentation (pre-GW), post-GW, and after obturation. The samples were randomly allocated into three groups (n = 10) with regard to the root filling method: (1) single-cone with AH Plus sealer (SC/AH+), (2) single-cone with GuttaFlow sealer (SC/GF), and (3) GuttaCore with AH Plus sealer (GC/AH+). The reconstructed 3D images were analyzed for the volumetric percentages of debris, filling materials, gaps, and voids at three canal levels. Data was analyzed statistically using Kruskal-Wallis and Mann-Whitney U tests. P values < 0.05 were considered to be significant.

Results: The proportion (volume %) gaps and voids proportions in multi-rooted canals obturated by using GC/AH+ and SC/GF was significantly lower than in canals filled with SC/AH+ (P = 0.000). Different values indicated a statistical difference between coronal, middle and apical gaps and voids volumes within each group. The mean volumes of coronal and middle gaps and voids were significantly different from the mean volume of apical gaps and voids in canals filled with SC/GF (P = 0.000), (P = 0.001). The quality of mesial fillings did not differ statistically from the
distal fillings amongst the three groups. After conventional irrigation, 4.58 % of canal volumes was filled with debris while no debris was found after GW cleaning.

**Conclusion:** A high quality of filling was achieved by GC/AH+ and SC/GF indicating that both these methods are suitable to fill the minimally instrumented root canals after GentleWave cleaning.
Preface

The research question and the study design were identified by Dr. Ala Zaghwan under the guidance of Dr. Markus Haapasalo and Dr. Ya Shen. Selection and preparation of the samples were accomplished by Dr. Ala Zaghwan. Micro-CT scans were performed by John Schipilow at the UBC Faculty of Dentistry Centre for High Throughput Phenogenomics (CHTP). The collected micro-CT data was analyzed by Dr. Ala Zaghwan under the supervision of Dr. Markus Haapasalo and Dr. Ya Shen. Dr. Ala Zaghwan prepared the Manuscript with editing by Dr. Markus Haapasalo, Dr. Ya Shen. The study was approved by the University of British Columbia Office of Research Services, Clinical Research Ethics Board (Certificate Number: H15-02793).
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List of Abbreviations

ASP ............................................................... apical size preparation
h ............................................................... hours
min ............................................................. minutes
NaOCl ........................................................ sodium hypochlorite
EDTA .......................................................... ethylenediamine tetra-acetic acid
µm .............................................................. micrometers
µA .............................................................. microampere
kVp ............................................................. peak kilovoltage
ms .............................................................. millisecond
sec ............................................................. seconds
ml .............................................................. milliliter
°C .............................................................. degree celsius
RCs ............................................................ root canals
GC ............................................................. GuttaCore
GF ............................................................. GuttaFlow
AH+ .......................................................... AH Plus
SC ............................................................ single-cone
gp .............................................................. gutta-percha
CSI ........................................................... conventional syringe irrigation
G .............................................................. gauge
ANP ........................................................ apical negative pressure
SNI .......................................................... syringe-needle irrigation

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PUI .......................................................... passive ultrasonic irrigation
CUI .......................................................... continuous ultrasonic irrigation
LAI .......................................................... laser activated irrigation
Er, Cr: YSGG ........................................... erbium chromium-yttrium-scandium-garnett
PIPS ......................................................... photon-initiated photoacoustic streaming
GW ........................................................ GentleWave™ system
% .......................................................................................................................... percentage
CLC ........................................................ cold lateral compaction
WVC ........................................................ warm vertical compaction
ISO ........................................................ International Organization for Standardization
MTA ........................................................ mineral trioxide aggregate
SEM ........................................................ scanning electron microscope
Micro-CT ................................................... micro-computed tomography
SD ........................................................... standard deviation
PGFA ......................................................... gutta-percha filled area
PSFA ........................................................... sealer filled area
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Dedication

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I dedicate this humble work to my:

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Chapter 1: Introduction

Kakehashi et al. (1) in 1965 studied the pathological changes in the exposed pulps of germ free laboratory animals before and after contaminating their oral cavities with a complex microflora. They established that the presence of microorganisms and their by-products caused pulp necrosis and formation of periapical granuloma and even abscess (1). Following caries or trauma, the microorganisms invade the root canal system through dentinal tubules and reach the area that eventually becomes necrotic and thereby a good environment for the microorganisms to multiply, colonize and release their by-products (2). Following this, the spread of bacterial antigens through the apical foramen and the lateral canals to the adjacent periradicular region causes apical periodontitis. The host immune system begins the repair process by starting the inflammatory reaction in order to kill or suppress the microbes, and to eliminate the necrotic tissue (3). The inflammatory processes cause periapical tissue destruction, which will continue as long as the microbial cause is not completely eradicated. After the root canal becomes necrotic, host defense system no longer can effectively attack the microbes inside the canal and the periapical inflammation continues (2). Hence, endodontic treatment is necessary in order to prevent the spreading of infectious microorganisms and their antigens and to start the healing process (4).
1.1 History of Endodontic Treatment

The word “endodontics” originates from two Greek words (endo means inside and odont means tooth). Pierre Fauchard was the first one to introduce it for the treatment of the root canal with an abscess in 1728 (5). The treatment included removal of pulp tissue using a pin, subsequently, filling the empty space using a lead foil. “Intentional endodontic treatment”, introduced by Bourdet in 1757, was an alternative treatment for the infected root canals (5). In that technique, the tooth was dislodged from its place in order to cut the nerve, then immediately returned back to its socket. Later, in 1766, Robert Woofendale used another procedure to treat the root canals. He cauterized the pulp with a hot tool to relieve the pain (5). In 1809, Edward Hudson introduced a gold foil as the first filling material used in endodontics (5). Many techniques and materials including paraffin, metal and amalgam were being used as filling materials following the use of gold foil material, and they produced satisfying results at that period of time (5). Baker was the first one who published a paper in the American Journal of Dental Science that described the pulpal tissues removal and root canal filling in 1839 (5).

In the 1850s, gutta-percha appeared as a material of choice for root fillings (5). The gutta-percha was mixed with other components to produce a cement called “Hill’s stopping”. The cement was used in conjunction with chloroform and a wooden plug to fill the root canal space (5). Consequently, several attempts were made to improve the adaptability of gutta-percha to the root canal walls. Dubuque carried out the first attempt when he softened the gutta-percha by using heat in 1865 (5). It had also been noticed that if the endodontically treated tooth was isolated from the
adjacent teeth, this would add more safety and control to the working area during root canal treatment. Therefore, Barnum introduced the rubber dam by using a thin sheet of rubber to insulate the tooth from the adjacent teeth and tissues (5).

In 1867, Magitot introduced the first pulp vitality test using electricity (5). It was believed for several years that the etiological factor of pulpal complications was the lack of vitality in the tooth, which was called the “vitality theory”. In 1879, Tomes revised the old vitality theory to a septic theory (6). He explained the septic theory as contamination from the infected dentine reaching the root canal of the non-vital tooth, which becomes infected, and subsequently the infection spreads into the periradicular area (6). The changes in the theories resulted in a belief that whether the pulp was vital made no difference if the pulp chamber and canals were cleaned with antiseptic agents. This concept encouraged the clinicians to use germicidal agents to clean the root canals and eliminate the bacteria (6). The basis of root canal obturation was also developed from this concept. In 1883, Mills and Brooklyn (6) designated a process in which an orangewood stick was introduced into a root canal after dipping the stick in creosote or carbolic acid solutions. Many practitioners were developing filling materials and techniques subsequently to introducing the wood points in market in 1895 (6). They also incorporated the use of x-ray radiation, first introduced by Kells in 1896, for the examination of the quality of the root fillings (6).
In order to use the appropriate antibacterial agent to sterilize the root canals, it was crucial to detect the different kinds of the bacteria that caused the pulpal diseases. A study carried out by Mayrhofer in 1909 showed that the pulp infection in 96% of the cases was caused by streptococci (7). The use of antimicrobial filling was therefore introduced by Hermann in 1920 (7). He advocated the use of calcium hydroxide (Calxyl™) in pulp capping, pulpotomy, pulpectomy, and the treatment of infected canals in 1930. He also showed that a secondary dentin could be formed when an amputated, vital pulp was covered with Calxyl (7). In 1925, Rickert suggested the use of a sealer with a gutta-percha cone. The gutta-percha was first fitted to reach the apical dentinocemental junction, then removed and coated with the sealer, consequently, inserted into the canal and pressed tightly to distribute the sealer apically and into the lateral canals (7). The technique was enhanced by allowing an instrument to be inserted into the canal to condense the gutta-percha and provide more space for additional points to be placed inside the canal, which is nowadays called “cold lateral condensation obturation technique”. At the same year, a new spiral shaped file was presented by Lentulo to facilitate the insertion of the sealer inside the root canal (7).

Rickert and Dixon in 1931 had a hypothesis of the “hollow tube effect”, i.e. if gap or void is present in the filling material, fluid may entrap into the spaces and undergo enzymatic breakdown (7). These products may reach the periapical area and cause an inflammatory reaction (7). Based on this concept, optimal root canal obturation is necessary for the success of the endodontic treatment.
1.2 Cleaning of the Root Canal System

The aim of endodontic treatment is achieved by thorough mechanical and chemical cleaning of the root canals to eradicate the microorganisms and their by-products (8). Several studies have shown that apical periodontitis is a consequence of penetration of microbes and their products to the periapical area (9, 10). Hence, apical periodontitis can be prevented or treated by endodontic treatment (11, 12). The failure of endodontic therapy can lead to a persistent periradicular lesion due to incomplete or improper elimination of bacteria inside the root canal system (12).

1.2.1 Mechanical Cleaning

Instrumentation is considered as one of the most essential steps of root canal treatment (13, 14, 15). It removes many/most of the bacteria inside the canals and thereby contributes to a favorable environment for the healing process (16). By shaping and enlargement of the root canals, instrumentation creates enough space for the irrigants to be delivered throughout the root canal system, and the filling material to be properly placed into the canal (17). The preparation of the root canals should be conservative with regard to the apex to avoid irritation of the periradicular tissue, and to reduce the susceptibility to tooth fracture (18).

Apical size preparation (ASP) is an important factor in achieving the goals of root canal preparation, by which the microorganisms and pathologic debris are eliminated (19, 20, 21, 22). There is a considerable variation in the literature regarding ASP, Tan and Messer (23) compared the quality of apical enlargement
achieved by both hand files (K files) and the nickel-titanium (Ni-Ti) rotary instruments in mandibular molars. They concluded that the mechanical instrumentation was relatively insufficient to reduce the debris in the root canal system. They did note, however, that more debris was eliminated from the apical third by enlarging the apical size preparation using the rotary files (23). Similarly, several other studies have proved that efficient irrigation and superior bacterial removal were achieved when larger apical size files were used to prepare the root canals (24, 25, 26, 27). The size and the taper of the apical preparation could also affect the delivery of the irrigating solution. Some literature suggested using #40/.04 as an ideal size and taper to allow the maximum irrigation volume to penetrate the apical third of anterior and posterior root canals (28, 29). Another study suggested that file size #30 is the minimum instrumentation size needed to delivering the irrigating solutions to the apical portion of the mandibular molar canals (30). Brunson et al. (28) examined the effect of both file size and taper on the volume of irrigants that reached the apical third. The authors used forty single rooted teeth and designed the study to include two aims. The first aim was to detect the smaller apical size that permits the irrigating solution to flow to the working length, while the second aim was to detect the ideal taper that lets more irrigants to penetrate to the working length. In order to achieve the first aim, they used the same taper of .06 with different file sizes ranged from #30 to #45, while they used the same file size of #40 with different tapers ranged .02-.08 to achieve the second aim. It was concluded from this study that the greater the apical preparation size and taper, the grater the irrigating solution delivered to the working length (28). On the other hand, it has been reported that “root canal preparation with GT rotary files
to apical preparation size 30 and final taper 0.04, 0.06, and 0.08 did not affect canal cleanliness” (31).

Extra caution should be taken when shaping canals especially those with external root concavities, as overpreparing these canals weakens roots and predisposes the tooth to fracture. It has been established that the thicker the remaining dentin, the greater the resistance to tooth fracture (32). Coldero et al. (33) used nickel-titanium rotary instruments with/without apical enlargement to compare the intracanal bacterial reduction. They found that using of rotary instruments in combination with irrigants and apical canal enlargement contributed to eliminating *E. faecalis* from the root canal system (33). Similarly, a study by Card et al. (26) showed that the greater the apical preparation size, the greater the intracanal bacteria elimination from the infected root canals. Moreover, Rollison et al. (27) suggested that instrumentation to an apical size of #35 was less effective in debriding and cleaning the root canals than when the apical preparation size was #50. In contrast, few studies found no significant difference in removing debris and bacteria from the root canals when using different apical preparation sizes. Albrecht et al. (34) in their study showed that when standardizing the taper to 0.10, there was no difference in debriding the root canal system by using ProFile G T instruments of 20 and 40 apical size preparation. Moreover, Yared and Dagher (35) found no difference between canals prepared to a size 25 and those prepared to a size 40 in terms of apical disinfection.
Anatomical complexity of the root canal system, lateral canals, isthmus area, apical ramification, and transverse anastomoses provide significant challenges for endodontic treatment (36). They are also reasons that 30-50% of the canal wall area remains untouched (37). Further, it has been shown that the level of bacteria in the root canal cannot be significantly reduced by only using mechanical preparation (38). Moreover, the endodontic irrigating solutions cannot be delivered to all complexities of the root canal system by conventional methods (39). Hence, remaining bacteria may develop into a biofilm that is even more difficult to be removed by the commonly used methods of instrumentation, irrigation and disinfection.

1.2.2 Chemomechanical Cleaning

1.2.2.1 Irrigation Solutions
The irrigating solutions are used with files or with special tools during cleaning of the root canal system to eliminate bacteria, dissolve necrotic tissue, facilitate dentin removal, remove the smear layer, dissolve inorganic tissue, and reduce the instrument friction during preparation (40, 41, 42). Sodium hypochlorite (NaOCl) is the most popular irrigant because of its highly antimicrobial reaction and capability to dissolve organic matter, necrotic tissue in particular (42, 43). The concentration of NaOCl commonly used in endodontics is between 0.5% and 6% (44). Tissue dissolution by NaOCl can be improved up to 50-fold by optimizing the concentration, temperature, flow (agitation) and surface tension (45).
The smear layer is a 1-2 µm-thick layer that is composed of organic and inorganic components including mineralized and unmineralized dentin, bacteria, and pulpal tissue remnants. It is amorphous layer with a flat surface and an inner layer that can infiltrate a few micrometers into the dentinal tubules (46). It has been suggested that the presence of inorganic constituents (dentin remnants) in the smear layer is responsible for the reduced killing effectiveness of NaOCl (1%) against *Enterococcus faecalis* (47). The smear layer cannot be removed by using NaOCl only. However, the organic portion in the smear layer is affected by NaOCl, which makes it more prone to the ethylenediamine tetra-acetic acid (EDTA). EDTA as a demineralizing agent reacts with the inorganic portion of the smear layer, and removes hydroxyapatite (48). In a systematic review, Shahravan *et al.* (49) showed that removal of the smear layer, improves the “fluid tight seal” regardless the filling technique or the sealer used. However, using of EDTA longer than the recommended time can cause dentinal tubular damage (50).

Chemomechanical cleaning is the integration of both mechanical flushing and chemical action of the irrigant to remove as much irritating material as possible. Irrigating solutions have been “activated” using different methods such as sonic and ultrasonic activation and laser.
1.2.2.2 Methods of Irrigation

1.2.2.2.1 Conventional Syringe Irrigation

A syringe attached to a needle has been the most widely used way to deliver irrigating solutions into the root canal; the irrigation needle placed optimally 2-3 mm short of the working length. Plastic syringes with different capacities (1-20 ml), needles in different sizes (25, 27, 30, 31 gauge), and various needle tip designs are used in this method. Large syringe size saves the time of solution refill, however, the apical pressure is more difficult to control than with small syringes sizes. Therefore, use of small size syringes of 1-5 mL is recommended (44). The use of needle size 30/31 gauge is preferred to bigger needles to enable the delivery of irrigants to the most apical canal. However, the high risk of the irrigating solution escaping beyond the apical foramen requires attention and caution during application (44). Needle tips are manufactured in different designs such as open and closed ended needles; some have the hole on the side (side vented needle), and the one or more outlets (51). During root canal irrigation the needle should be moved up and down while it is inside the canal. Size #30-35 is the minimum apical size preparation required for the placement of the needle to a maximum insertion depth to deliver the irrigating solutions to the apical root canal (51). Side vented (“safety”) needles can be placed at 0-1 mm short of the working length, whereas open-ended needles should be placed at 2-3 mm short of the working length to avoid apical extrusion of the irrigants (51).
1.2.2.2 EndoVac Negative Pressure System

Apical air bubbles that often form during the use of traditional methods of delivering the irrigating solutions to the apex can prevent the solutions reaching to the apical area of the root canal. The evacuation process of liquids through apical negative pressure is the method that secures a safe flow of irrigating solutions down to the depth of the EndoVac tip in the apical canal and then back (suction) through the needle. EndoVac negative pressure system is used to safely deliver the selected solution to the working length without further penetration to the periradicular area (52, 53, 54). It consists of three parts: the Master Delivery Tip, the macrocannula and the microcannula.

1.2.2.3 Sonic and Ultrasonic Irrigation

Although traditional syringe-needle irrigation has been considered as an effective cleaning method for the cleaning of the root canal system for many years, optimal cleanliness cannot be achieved in all parts of the root canal system by using this technique (55, 56). Therefore, various ways to improve the effects of syringe-needle irrigation have been developed. A cavitation phenomenon occurs when an ultrasonic wave is applied in a liquid, which creates vacuum bubbles (51). These bubbles become larger and unsteady till they explode and release high power waves and high temperature. The high-power stream generated has a strong cleaning effect on the rough surfaces and unreachable areas within the root canal system (51). Several studies compared the cleaning efficacy of conventional and ultrasonic agitation methods (57, 58, 59). Recent studies have examined the importance of
ultrasonic energy in maximizing the efficiency of the irrigants (55, 60). Greporio et al. (39) compared the penetration ability of NaOCl into the simulated working length and lateral canals. They found that the passive ultrasonic irrigation (PUI) method enhanced the ability of NaOCl to penetrate the lateral canals more than the apical negative pressure irrigation (ANP). However, the study also showed that ANP secured the delivery of NaOCl to working length better than passive ultrasonic irrigation (PUI) (39). Both PUI and Continuous Ultrasonic Irrigation (CUI) are examples of ultrasonic activation irrigation devices. PUI agitates the irrigants by using a metal file, which can have a smooth or rough surface and which is placed in the root canal without touching the canal wall during application. Therefore, this method may be inapplicable in curved and minimally instrumented canals (51). In some of the new CUI equipment such as ProUltra PiezoFlow (Dentsply Tulsa Dental Specialties, Tulsa, OK, USA), irrigant flows into the root canal through a metallic, hollow tip while the tip is oscillating at ultrasonic frequency (61). Several studies have suggested that this type of CUI can further improve canal cleanliness (62, 63). EndoActivator system is a model of sonic devices that attached to polymer tip (Dentsply Tulsa Dental Specialties). The sonic vibrations move NaOCl quickly around the canal to dissolve necrotic pulp tissues (45).

1.2.2.2.4 Laser Activated Irrigation (LAI)

Irrigation methods have also been developed using energy from different types of lasers. Use of an Er,Cr:YSGG (erbium chromium-yttrium-scandium-garnett) laser was first introduced by Blanken and Verdaasdonk (64). Matsumoto et al. (65) found that the liquid beside the laser tip evaporated and created bubbles. Once the laser
was stopped, the bubbles began to shrink and eventually collapsed due to the increase of the surrounding pressure. The explosion of the bubble develops a powerful liquid stream without rising the temperature.

**Photon-Induced Photoacoustic Streaming (PIPS)**

This technique is similar to LAI with a difference that the later system’s tip is placed inside the canals while the PIPS’s tip is placed at the orifice (66). The PIPS tip is placed in the pulp chamber with a continuous flow of the irrigants to keep the tip immersed in the solutions during the procedure. Six 30s cycles of laser activation are employed by this method (three 30s of NaOCl, two 30s of water, and one 30s of EDTA). A recent study showed that the samples irrigated using PIPS system with 6% NaOCl revealed the greatest eradication of bacterial biofilm in comparison to the samples cleaned by using either 6% NaOCl or PIPS with water (67). Some studies have shown the superior efficacy of PIPS in removing the dentine debris, bacteria, smear layer and biofilm compared with the other conventional syringe-needle irrigation (SNI), and sonic and ultrasonic irrigation (56, 67, 68, 69, 70). However, it has been shown by other studies that the removal of *E. faecalis* and smear layer by using PIPS with NaOCl was equal to that achieved by using conventional SNI with NaOCl + EDTA (71). Additionally, apical extrusion similar to that generated by conventional syringe-needle irrigation can occur also with the PIPS system (72).
1.2.2.2.5 GentleWave System (GW)

A novel technology for cleaning and disinfecting root canals (the GentleWave™ system) has recently been developed by Sonendo. The GentleWave™ system (Sonendo, Laguna Hills, Orange County, CA, USA) consists of console and treatment instrument, which resembles a handpiece (Figure 1.1). The console consists of numerous electrical circuits, containers for the different irrigating solutions/waste material, pressure producer, and degassing system. GentleWave treatment instrument delivers the irrigants at a high flow rate of 45 ml/min to the pulp chamber and consequently to the entire root canal system (73). When the irrigant flow hits the end plate of the tip of the nozzle in the pulp chamber, a stream of irrigating solution interacts with the static fluid in the pulp chamber and creates a shear force, which according to the manufacturer produces a hydrodynamic cavitation. High power waves of sounds are generated due to the strong hydrodynamic cavitation that propagates throughout the whole root canal system through the degassed fluids. The GentleWave™ system utilizes three irrigating solutions, NaOCl, EDTA and water (Figure 1.2), which are degassed inside the main console before being delivered to the pulp chamber. The degassing process of the irrigating solutions aims to remove microscopic air bubbles from the fluids and thereby eliminate the “vapor lock phenomenon” that often hinders the irrigants from reaching the apical third (74, 75). The tip of the handpiece is placed 1 mm above the pulp chamber; no or minimal instrumentation of size #15/0.04 taper is recommended by the manufacturer. While the GentleWave™ system is operating; the pulp chamber is completely sealed to prevent the escape of NaOCl to the adjacent working area. The excess of solutions and debris are evacuated by a five-
points vented suction on the inner surface of GentleWave treatment instrument creating a negative pressure within the root canal system.

Figure 1.1 (A) The GentleWave™ System and (B) the tip of the cleaning instrument in the pulp chamber of a molar. Image courtesy Sonendo Inc.

The efficacy of the GentleWave system in tissue dissolution and cleaning of the canals has been compared to a positive-pressure conventional syringe-needle irrigation, passive ultrasonic activation, and EndoVac. The studies showed that GW dissolved organic matter eight times faster than the next best system tested (73, 76). Haapasalo et al. (77) reported that negative apical pressure was created by the GentleWave™ system, which improves the safe use of this system in comparison to positive pressure syringe-needle irrigation. Another study compared the effectiveness of the GentleWave™ system in removing calcium hydroxide paste to Syringe-needle irrigation and PUI. It was concluded that 62.75%, 75.26%, 99.39% of Ca(OH)₂ were removed from the root canals by using conventional irrigation, PUI, and GentleWave, respectively. Furthermore, GentleWave™ system was reported to
have a high success rate in removing fractured files from root canals of molar teeth \textit{in vitro} (78).

![Diagram of the recommended protocol for the GentleWave cleaning](image)

**Figure 1.2 The recommended protocol for the GentleWave cleaning.**

### 1.3 Root Canal Obturation

The purpose of root canal obturation is to fill the canals with appropriate materials to fully occupy the space so that microorganisms cannot re-enter the root canal system. Additionally, the microbes that remain within the canals following the chemomechanical cleaning should be isolated from nutrients in the pulp chamber and in the tissue fluids by achieving a good seal with the filling materials (79, 80).
The obturation materials consist of a core material that fills most of the canal space, and a sealer that fills the interface between the core material and the canal wall (81). In addition, sealers often fill, at least partly, lateral canals. According to Grossman (82), the following properties are desirable for root filling materials:

- Provide a maximum sealing ability to the canal ramifications and the lateral canals, and allow for a fluid-tight seal
- Biocompatible with the periapical tissues, and doesn’t cause any irritation or discoloration to the tooth structure or the surrounding tissues
- Easy to be applied, and not difficult to be removed from the root canals when the endodontic retreatment is required
- Resist moisture (nonresorbable), and not shrink after being inserted into the canals
- Appear as a radiopaque material on the radiograph to be easily distinguished from the tooth structure
- Prevent the bacterial growth within the root canal through its bactericidal/bacteriostatic effect

1.3.1 Filling Materials

1.3.1.1 Gutta-Percha

The gutta-percha is the most widely used root filling material due to its thermomechanical, physical, and sealing properties (83, 84, 85, 86). It is derived from a tree of the Sapotaceae family (87). Alpha and Beta forms are the two crystalline phases of the gutta-percha; alpha is the natural form of gutta-percha.
while beta is the processed form of gutta-percha. Beta form is a result of cooling the warm alpha form at room temperature. Zinc oxide, which has some antimicrobial effect, is the major component of gutta-percha used in endodontics, it constitutes about 65% of gutta-percha. Gutta-percha constitutes 20%, and the rest 15% are natural waxes, (heavy) metals salts and plasticizers (88). Most of the gutta-percha used in endodontics is in beta form according to Goodman (89).

Gutta-percha is a non-toxic material, and no systemic reactions were reported by the clinical studies when using the gutta-percha as a root filling material (90). The gutta-percha is applied together with a sealer because of its poor adaptability to the canal walls if used alone (91). The gutta-percha-sealer as a filling material has superior properties in comparison to many other filling materials and produces good seal to the root canal system with few voids and gaps inside the filling and between the filling and the canal wall (92).

1.3.1.2 Root Canal Sealer

Root canal sealers are responsible for producing a fluid-tight seal when used with a core material or when used alone. Additionally, they can fill the interface between the core materials such as gutta-percha, and the canal walls. Moreover, they play an important role to fill the fins, lateral canals and the canal ramifications within the root canal systems. Several studies have shown that many sealers have a tendency to be dissolved over time leaving a gap that is a potential target for bacterial accumulation (93, 94, 95). Therefore, optimally the sealers should possess the
following properties to be used in endodontic therapy. Grossman (82), showed that the ideal root canal sealer should:

- Provide good seal when set
- Be an excellent adhesive to the root canal wall and the core material
- Be radiopaque
- Be dimensionally stable upon setting
- Be easily mixed and manipulated
- Not cause tooth structure discoloration
- Be bactericidal or at least bacteriostatic to depress the bacterial growth
- Set slowly to increase the working time
- Not be dissolved in tissue fluids
- Not irritate the periradicular tissues
- Be easily solved by a solvent if necessary

Sealers should be applied properly to not extrude to the periradicular tissues because most sealers have exhibited different degrees of toxicity, although the toxicity will be reduced or disappear over time (96). Sealers are classified according to their compositions into several categories; Zinc oxide-eugenol-based sealers, epoxy resin-based sealers, calcium hydroxide-based sealers, silicone-based sealers, and glass-ionomer cement sealers. AH Plus sealer (Dentsply Tulsa Dental Specialties) (Figure 1.3) is one of the most common epoxy resins cement that has been used in endodontic therapy for many years. It has been modified from AH-26 to reduce its cytotoxicity by preventing the release of formaldehyde during the
setting process (97). Although AH Plus sealer is less cytotoxic than AH-26, it still has a cytotoxic effect (98, 99, 100, 101). However, a recent study showed that after setting of AH+, fibroblast cells were able to grow on the surface of the sealer, similar to bioceramic sealers (102). AH Plus has a considerably low flowability, low film thickness, long working and setting time, low solubility, and slight shrinkage upon setting in comparison to other sealers (103).

GuttaFlow Sealer (GuttaFlow 2; Coltène/Whaledent, Langenau, Germany) is one of the Silicon-Based Sealers; it contains small gutta-percha particles, less than 30 µm in diameter in a silicone sealer. It is provided in a dual-barrel syringe with a mixing or delivery tip (Figure 1.4). It has been reported that the syringe-mixed endodontic sealers display significantly lower surface porosity than hand-mixed sealers (104). GuttaFlow sealer expands upon setting (103) and is characterized by a thixotropic property. This means that the sealer viscosity reduces under pressure. Hence, the
sealer flows into the small lateral canals and exhibits a good sealing ability in comparison to other sealers including AH Plus sealer (105, 106, 107, 108). Accordingly, a study by Brackett et al. (109) showed that “The use of GuttaFlow with a single gutta-percha master cone creates an apical seal that is equivalent to that produced with gutta-percha/AH Plus sealer using warm vertical compaction”. Although AH Plus sealer penetrates deeper in the dentinal tubules than GuttaFlow sealer when the smear layer is removed (110), the penetration in the dentinal tubule is affected by final irrigation method, root canal third and the root canal sealer used (111). AH Plus sealer is antibacterial and can kill bacteria in the infected dentin more efficiently than GuttaFlow sealer (112, 113, 114). However, the GuttaFlow sealer is biocompatible with the periradicular tissues (99, 115).

Figure 1.4 GuttaFlow 2 sealer in a dual-barrel syringe and a delivery tip.
1.3.2 Obturation Techniques

A high quality root canal filling may be accomplished using one of the various techniques. The selection of the technique depends on the final preparation size. However, the operator preference and the modern technologies may be factors in selecting the proper obturation technique (116). Cold lateral compaction (CLC), warm vertical compaction (WVC), single-cone obturation (SC), and carrier-based obturation are the most common methods to fill the root canal space (80).

1.3.2.1 Cold Lateral Compaction Obturation

Cold lateral compaction (CLC) is a widely used root filling technique (117). It is an uncomplicated method, inexpensive, can be used in different clinical situations, allows for working length control during compaction, and both retreatment procedures and post space preparation can be done easily in the root canals filled with this method (118). After final preparation and cleaning of the root canal, the previously selected master gutta-percha cone immersed in a sealer is inserted into the canal to the working length. A proper size finger spreader with a suitable size and taper is selected and inserted into the canal, maximally 1-2 mm short of the working length, to compact the gutta-percha apically and laterally. Immediately after removing the spreader, an accessory cone coated with sealer is placed in the space created by the spreader. Adding of accessory cones is continued until the canal is filled with gutta-percha cones, and the spreader insertion into the canals only extends 3 mm or less from the canal orifice. The gutta-percha cones are then severed at the level of the canal orifice by a heated plugger and condensed vertically.
to about 1 mm below the orifice (119). The size of the plugger used for cold vertical compaction should be slightly smaller than the coronal canal diameter to avoid potentially harmful forces against the canal wall dentin.

Cold lateral compaction technic may be difficult to use in severely curved canals, abnormal root canal anatomy or when there is internal resorption (80). The poor adaptation of the filling materials to the canal wall and formation of voids within the fillings are also drawbacks of using this technique (120). Additionally, in a study CLC exhibited lower filling mass in isthmus areas and in apical regions than continuous wave of condensation and Ortho MTA techniques (121). However, a recent study has shown that the sealing ability achieved by CLC is comparable to that achieved by injected gutta-percha, and single gutta-percha point techniques (122).

1.3.2.2 Warm Vertical Compaction Obturation

Warm vertical compaction technique (WVC) was first introduced by Schilder in 1967 (15). ‘Down-pack’ and ‘back-fill’ are the two steps of this technique. The root canals filled by WVC are first prepared to a continuously tapered shape while maintaining the original anatomy of the canal. Additionally, the apical foramen should be kept as small as feasible and maintained in its position. Prior to starting the obturation procedures, proper size pluggers are selected. Also, the master cone gutta-percha is trimmed to fit tightly 0.5–1 mm short of the working length and should show “tug back”. The master cone is coated with a sealer and placed in the canal until it fits the working length “tug-back”, and shows some resistance to withdrawal. A heated instrument is used to cut the cone at the canal orifice and the
gutta-percha cone is then compacted with a proper size plugger. The tip of the heat carrier is introduced inside 3 mm of the compacted core material and, subsequently, removed to pull out the coronal part of the gutta-percha from the canal. Smaller plugger sizes are used to compact the remaining gutta-percha, followed by removal of additional increments of the gutta-percha until the gutta-percha fills the apical 4-5 mm of the canal. The diameter of the used plugger should be always smaller than the root canal at the different canal levels to avoid interference with the canal walls. The coronal space is then filled with 3-4 mm increments of heat-softened gutta-percha using injection system (backfill). Next, the gutta-percha increments are compacted using a plugger of proper size sequentially till the whole canal space is filled with the filling material at the level of the canal orifice. A continuous wave condensation technique is a modified technique of WVC (123). The two techniques differ in the source of carrier heating system, where the electricity is employed in heating of the carrier tip of the continuous wave of condensation. The electric carrier tip’s temperature can be rapidly decreased and increased; therefore, it can be used as both heat carrier and a plugger.

It has been shown that the adaptability of WVC is better than CLC (124, 125). In addition, in CLC significantly gutta-percha and more sealer are present in the final filling than WVC filling. Also, a significantly higher percentage of voids are found in CLC fillings when compared to WVC (126). However, the long-term success rate achieved by warm vertical compaction technique is comparable to that accomplished by the cold lateral compaction method (127, 128). WVC is not an easy technique especially in curved canals where the rigid pluggers are difficult to be
introduced deep into the canal. In such cases, the curved canals may require more preparation and enlargement, which may increase the chance of tooth fracture because of the extensive tooth structure loss (129). The filling material extrusion, as a result of applied forces used in WVC, is also one of the disadvantages of the method (130). Despite the limited clinical signs of adverse periodontal effects following thermoplastic obturation methods (79), the heat produced from the heated carrier during the obturation can affect the periodontal ligament (131). Another disadvantage of using this technique is the difficulty of length control, and the dimensional changes of the filling upon cooling of the material.

1.3.2.3 Single-Cone Obturation

The single-cone technique was established and became popular with the initiation of standardization of filling cones and endodontic files (ISO) in the 1960s (132). The single-cone technique utilizes the use of a single GP point with a variable amount of sealer at room temperature and without using of condensation. The sealer is inserted into the prepared and cleaned canals by injecting it via special tips, or it introduced into the canals by using Lentulo or bidirectional spiral. Consequently, the pre-measured gutta-percha is inserted to fit with the tug-back. A heated instrument is used to cut the excess of the gutta-percha cone at the level of the canal orifice. The coronal portion of GP cone is then slightly vertically condensed using a non-heated plugger with a diameter smaller than the coronal canal diameter. Single-cone technic is an easy, simple and fast endodontic obturation technique (133, 134, 135). However, in terms of the obturation quality, bacterial penetration, marginal infiltration, and sealing ability, previous research has shown that the fillings done
with the single-cone technique have comparable or lower quality than fillings by other obturation techniques (136, 137, 138, 139).

1.3.2.4 Carrier-Based Obturation

In 1978, Johnson introduced a technique of delivering thermoplasticized gutta-percha into the root canal space (140). The idea of a carrier-based obturator started by using stainless steel file that was placed into the root canal about 1-2 mm from the working length. The file was marked at 2 mm away from the orifice level while it was still in the canal. The mark or the sign was made to facilitate the removal of the undesirable portion of the file after placing the filling material. The file was then coated with warmed gutta-percha and inserted in the canal after coating the canal with a sealer. Subsequently, the portion of the file above the mark was removed and the excess gutta-percha condensed. In recent years, (Thermafil®) was developed, and the metal core material was replaced with a plastic material. The clinical outcomes of this obturation technique are comparable to other techniques, and the sealing ability is satisfactory with less time consumed for the application (141). It is an effective technique even in curved canals, and the amount of core material and gutta-percha in the final filling by using this technique is significantly higher than that produced by both lateral compaction and warm vertical compaction (142, 143, 144, 145). Other studies have shown that the success rate of the treatment using Thermafil technique is comparable to CLC technique (143, 146, 147). However, complete removal of filling material from the root canal system filled with Thermafil is difficult, and sometimes impossible (148). Moreover, the filling material extrusion
and the difficulty of post space preparation are also drawbacks of using this technique (149, 150).

In 2010, GuttaCore obturator (Dentsply Tulsa Dental Specialties, Tulsa, Oklahoma, USA) was introduced. The GuttaCore obturator is made from proprietary cross-linked gutta-percha, which maintains its shape when heated, allows for the post space preparation easier than the Thermafil obturator, and can be removed easily if the root canal retreatment is indicated (151, 152).

In both GuttaCore and Thermafil techniques, the prepared canal is verified by a size verifier, which corresponds to the obturator size and taper. The size verifier is used to facilitate the insertion of the obturator into the root canal till it reaches the working length. A paper point is used to deliver a small amount of sealer into the coronal portion of the canal. The obturator is warmed in a special oven to a specific temperature as recommended by the manufacture. Subsequently, the obturator is removed and introduced directly into the canal with a continuous pushing motion over 5 seconds interval until it reaches the working length. The carrier is then cut off at the orifice level by using a sharp endodontic spoon excavator or long shank friction grip 1/2 round carbide bur mounted on a high-speed handpiece. Next, the gutta-percha is condensed vertically to reduce the dimensional changes resulting from the filling material cooling (153). A study by Li et al. (154) showed that the quality of obturation attained by GuttaCore was not differed from that achieved by WVC in oval-shaped canals. Moreover, the bacterial leakage that resulted by using GuttaCore technique was similar to teeth filled with the EndoSeal MTA technique.
Further, another study reported that GuttaCore technique produced homogenous fillings with a low incidence of voids and a high proportion of gutta-percha in comparison to lateral compaction and single-cone techniques (156). It was also concluded that the “push-out bond strength”, which is the strength of the bond between the carrier material and gutta-percha around it, is higher in GuttaCore obturator than in Thermafil obturator (157). As no technique covers all the requirements of an optimal root filling, the practitioner must recognize the advantages and limitations of each technique, and select the appropriate obturation method for each clinical situation.

1.4 Micro-Computed Tomography

Nielsen et al. (158) introduced the micro-CT into endodontic research in 1995. It is a “non-destructive method” to visualize the tooth morphology and root canal system without destroying the samples. Micro-CT (Figure 1.5) can be used to analyze the entire length of the tooth voxel by voxel and collect the data e.g. for calculating the volumes and relative proportions of different filling materials (159). It has also been used to measure changes in root canal morphology after instrumentation (160, 161, 162). Moreover, it has been used to calculate the hard tissue debris left in the root canals space after instrumentation (163, 164), to evaluate the quality of obturation (162, 165, 166), and to assess the quality of retreatment (167, 168). In micro-CT technology, the sample is exposed to x-ray radiation while rotating 360° around its vertical axis producing a large number of 2D images that are reconstructed to create a 3D image of the sample. Micro-CT images are based on threshold values, which
help to distinguish a specific structure from adjacent structure or material. The tooth enamel, dentin, cementum, and the empty/filled root canal space can be identified using correct threshold values. Micro-CT cannot be used in vivo studies due to the high radiation doses generated by this method and because of limited sample space. Micro-CT scanning is expensive and the scanning of each sample takes a long time (159).

In spite of the limitations mentioned above, micro-CT is a valuable method in endodontic research because of its accuracy and non-destructive features (169).

Figure 1.5 Micro-CT Specimen Scanner-Center for High-Throughput Phenogenomics. Courtesy Phenogenomics.dentistry.ubc.ca. http://www.phenogenomics.dentistry.ubc.ca/equipment/MicroCTSpecimenScanner/
Chapter 2 : Rationale and Hypothesis

The goal of endodontic treatment is to remove microorganisms, necrotic tissue and microbial by-products from the root canal system (170). A successful endodontic treatment can be achieved by a thorough canal debridement, effective disinfection and high quality obturation of the canal space (171). Instrumentation cleans the canal mechanically and creates space for irrigation, disinfection and the root filling. Root canal irrigation has an important role in chemical cleaning and disinfection of the root canal.

The minimum instrumentation size required for the optimal root canal therapy is still debatable (172). An in vitro study concluded that a #30 file is the minimum instrumentation size needed for the irrigating solutions to penetrate the apical third of the root canal (30). Another study showed that bacterial counts decreased with increasing size of the apical file (173). In contrast, contemporary studies support minimal root canal preparation in order to preserve the tooth structure and increase the fracture resistance of the tooth (32, 174).

Recently, a new multisonic ultracleaning (GentleWave™ System) for the cleaning of infected root canals chemically and mechanically has been developed. Several studies have established the greater ability of GW in obtaining complete cleanliness in minimally instrumented canals than other methods (73, 76, 175). Sigurdsson et al. (176) in a prospective clinical study also concluded that patients treated with the
GW had a high cumulative successful healing rate of 97.3% 12-months after the treatment.

No study so far has examined the quality of root filling in minimally instrumented root canals cleaned with the GW system. Thus, the specific aim of this study was to examine the root filling quality in canals obturated using each of the following methods: (1) single GP cone with AH Plus sealer (SC/AH+), (2) single GP cone with GuttaFlow sealer (SC/GF), and (3) GuttaCore with AH Plus sealer (GC/AH+) techniques after minimal root canal preparation and GW cleaning.

The null hypothesis (H0) is: There is no difference in the quality of root fillings amongst the three-obturation techniques in minimally instrumented root canals.
Chapter 3: Materials and Methods

3.1 Tooth Selection and Preparation

Thirty human permanent mandibular first and second molars (90 root canals) were used in this study. A stratified sampling was followed in this study by distributing the same number of mesial and distal root canals, mandibular 1st and 2nd molars and isthmuses into the experimental groups (Table 3.1). The sample size calculation for the current study was based on a pilot study with alpha-type error of 0.05 and power of 0.80. The means and standard deviations were entered into statistical software (G*Power 3.1.9.2 for Mac OS X; Heinrich Heine, University at Dusseldorf) (126) yielded a sample size of 10. Teeth extracted for reasons not related to this study, were collected from the faculty of Dentistry clinics at the University of British Columbia, and from different dental offices in Vancouver and Surrey, BC.

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<td>8 (6 mesial + 2 distal)</td>
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Table 3.1 Stratified sampling method.
After extraction, the teeth were placed in 0.01 % NaOCl solution and stored at room temperature. Exclusion criteria for teeth in the present study were: severely curved root canals (>30 degrees) according to Schneider (177), previously filled root canals, deep caries or restoration extending down to the root canal wall, internal or external resorption, incompletely formed roots or apical root fracture, extremely obstructed or calcified root canals, and wide (open) apical foramen. Teeth were inspected thoroughly and radiographed mesio-distally and bucco-lingually using digital radiography (Planmeca Intra, Helsinki, Finland) with size #2 Scan X Phosphor storage imaging plates (Air Techniques, Melville, NY). The radiographs were analyzed using digital radiography imaging software (Planmeca Romexis, Helsinki, Finland) to determine if the tooth met the inclusion/exclusion criteria.

Once the teeth were selected, calculus and soft tissue debris were removed from the outer surface using hand-scaling instruments. After gaining an access cavity with high speed bur under water cooling, endodontic hand K-files #8 and #10 were inserted in each root canal and advanced 1 mm through the apical foramen of the selected samples. Teeth with canals that could not be negotiated with these two sizes of endodontic files were excluded from the study. Selected teeth were assigned random sample numbers that were carved into the root surface using a long shank friction grip ¼ round carbide bur (Tulsa Dentsply) mounted on a high-speed handpiece.
3.2 Root Canal Instrumentation

The clinical procedures were performed under magnification using the dental operating microscope, and were all done by the same dentist (A.Z.) (Figure 3.1). A standard access cavity preparation was made in the teeth and the working length was determined by inserting size 10 stainless steel K file (Dentsply Maillefer) into the root canal until the file was just visible at the apical foramen, and subtracting 1 mm from this file length measurement. The root canals were cleaned and shaped using K-type endodontic hand files #8, #10, #15, #20 (Dentsply Tulsa Dental Specialties) sequentially. Before proceeding to the larger file size, it was crucial to ensure that each file fit in the canal loosely at the working length. Final canal preparation was performed with a size 20/.04 Profile Vortex Blue nickel-titanium rotary endodontic file (Dentsply Tulsa Dental Specialties). One ml of 6% NaOCl was used between each instrument by syringe-needle irrigation using a 31-gauge needle tip (178). Apical patency was maintained by using a #10 K-type file after each larger file.
After the instrumentation was finished, size 20/.045 verifier (Figure 3.2) was tried in the canal. In cases where the verifier could not reach the established working length, minor apical shaping was carried out using the same size verifier. This apical canal enlargement was performed to ensure that the filling material matches the exact apical diameter and the determined working length. After the correct working length was marked on the size verifier using a silicone stopper, the verifier was used in rotary clockwise motion with slight apical pressure at working length until the
instrument became loose in the canal and could be rotated 180° passively inside the root canal.

3.3 First Micro-CT Scan of the Instrumented Root Canals

Fifteen randomly selected samples were scanned using a Micro-CT 100 (SCANCO Medical AG, Bruettisellen, Switzerland) to measure the amount of dentin debris caused by the instrumentation with the following settings:

- Isotropic voxel size of 30 µm, tube current of 200 µA, energy of 90 kVp, integration time of 500 ms per projection with 2X frame averaging (1 sec total integration time) to minimize noise, and 0.1 mm Copper filter to minimize any beam hardening artifact. The scanning time per sample was 3 h and 30 min.
3.4 Root Canal Irrigation

The GentleWave™ system (GW; Sonendo Inc.) was used to clean all 30 samples. The GentleWave treatment instrument was applied onto the access opening of the sample, with the end of the nozzle 1 mm above the pulp chamber floor. The tip of the handpiece never touches the pulpal floor during the treatment procedures. GentleWave treatment was performed with 3% NaOCl for 5 minutes, distilled water for 15 seconds, 8% EDTA for 2 minutes, and distilled water for 15 seconds consequently for a total treatment time of 7.5 minutes as recommended by the manufacturer (73) (Figure 1.2). The irrigating solutions were released as a high speed flow (45 ml/min) onto the end plate of the nozzle tip and further into the pulp chamber and root canal system at a temperature of 40°C. During operation the pulp chamber was sealed from the surrounding working area to prevent the dispersion of the irrigating solutions. Excess NaOCl, water and EDTA were sucked from the pulp chamber through small holes in the GentleWave treatment instruments.

3.5 Second Micro-CT Scan of the Cleaned Root Canals

All 30 samples were scanned using a Micro-CT 100 (SCANCO Medical AG) with the same settings as in the first scan. The settings used in this study were tested in a number of pilot scans to optimize the quality of the scanned images. The data was analyzed using two software packages: Amira v. 6.0 (FEI Visualization Sciences Group, SAS, Burlington, MA), and MeVisLab 2.6 OS X Edition (MeVis Medical Solutions AG, Bremen, Germany) (179).
Amira was used to create animations and images, while MeVisLab was used to create images, and for matching and superimposing corresponding sample data from the same tooth but from different scans, and to analyze data quantitatively (Figure 3.3). The image volumes of both scans for all samples were matched in sagittal, coronal, and axial planes to facilitate visualization, identification, and comparison of the various parameters in the two images (Figure 3.4).

Figure 3.3 Matching process from three different projections using the MeVisLab software.
Figure 3.4 (A) Visualization of reconstructed images show the overlapping of post-GW (red color 2D slice) and pre-GW (grey color slice) from the axial section of the same sample, (B) matched pre/post-GW scans of the same sample.

The data of the first and second scans were analyzed to determine the volumetric percentage of debris before and after cleaning with the GW system: The following equation was used in the calculations of debris:

\[ D\% = \frac{Z-Y}{Z} \times 100 \]

D\% is the percentage of debris volume

Z is the volume of the empty canal space after GW cleaning.

Y is the volume of the empty canal space before GW cleaning.

The D\% was measured for the entire canal volume and for the three canal levels (coronal, middle, and apical thirds) using the same equation.
3.6 Assignment of Teeth

The teeth used in the study (mandibular first and second molars) had 2 – 4 root canals. The number of root canals for each group was equalized (stratified sampling) to avoid the effect the different morphology and the number of RCs on the filling quality. The teeth were then randomly divided into three groups according to the filling material and the obturation techniques.

3.7 Root Canal Obturation

After instrumentation the canals were dried using matching paper points (Dentsply Tulsa Dental Specialties) (Figure 3.6, B). Teeth in the three groups were filled with different filling materials and different techniques. Group I was obturated using Single GP cone with GuttaFlow sealer, Group II was filled using Single GP cone with AH Plus sealer, and Group III obturated using GuttaCore® (GC) (Dentsply Tulsa Dental Specialties) and AH Plus sealer.

3.7.1 Group I: Single GP Cone with GuttaFlow Sealer

A Vortex 20/.04 gutta-percha point (Dentsply Tulsa Dental Specialties) was used in canals where the apical size was 20/.04 and tug-back was observed when trying the cone. Bigger.04 taper GP cones were used when canal was naturally larger, the size was determined by inserting GP points of increasing size into WL until the correct size was detected. Larger GP points were used in the distal canals. GuttaFlow® 2 - Standard Set sealer was delivered into the prepared coronal canal from the 5 ml
Syringe and introduced deeper with Size #25 Lentulo spiral (Dentsply Maillefer) (Figure 3.5).

![Lentulo spiral](image)

**Figure 3.5 Lentulo spiral.**

The Lentulo spiral was mounted on a contra-angle low speed handpiece. The two thirds of the Lentulo was coated with GuttaFlow sealer (Figure 3.6, C), and inserted into the canal 2 mm short of the working length (Figure 3.6, D). The handpiece was operated when the Lentulo was inside the canal; the Lentulo was removed slowly out of the canal while the handpiece was still working. The master GP was inserted and pumped twice inside the canal to insure the optimal distribution of the sealer inside the canal (Figure 3.6, E). If necessary, additional amount of the sealer was added by using gutta-percha point (Figure 3.6, F). Before the insertion of master cone, the amount of the sealer was standardized for all samples by filling the root canal with a sealer up to 1mm short the canal orifice (Figure 3.6, F). The apical half of the pre-fitted master cone was coated with sealer and introduced into the canal and slowly placed to working length (Figure 3.6, G). The gutta-percha point was cut
using a hot instrument and the coronal part was condensed using 0.5, 0.6, 0.7, and 0.8 mm posterior Schilder plugger (Dentsply Maillefer, Ballaigues, Switzerland) (Figure 3.6 H, 3.7).

A) Pulp chamber after GW cleaning.

B) Close-up image of pulp chamber and canal orifice after drying of the canals.

C) Lentulo spiral coated with GuttaFlow.

D) Sealer-coated Lentulo inside the distal RC.

E) Distribution of sealer in the distal RC.

F) Filled distal canal with GF sealer.
3.7.2 Group II: Single GP Cone with AH Plus Sealer

A Vortex 20/.04 master gutta-percha point (Dentsply Tulsa Dental Specialties) was used in canals where the apical size was 20/.04 and tug-back was observed when trying the cone. Bigger .04 taper GP cones were used when canal was naturally larger, the size was determined by inserting GP points of increasing size into WL until the correct size was detected. Larger GP points were used in the distal canals. AH Plus sealer (Dentsply Tulsa Dental Specialties) was used and mixed following manufacturer's recommendation. The sealer was inserted into the prepared canal.
by using size 25 Lentulo spiral. The Lentulo was used in a similar manner as in group I, but with AH Plus sealer. The apical half of the pre-fitted master cone was coated with the sealer and introduced into the canal and slowly introduced to working length. The gutta-percha point was cut at the coronal end of the root canal using a heated instrument and compacted using 0.5, 0.6, 0.7, and 0.8 mm posterior Schilder plugger (Dentsply Maillefer, Ballaigues, Switzerland) (Figure 3.7).

![Figure 3.7 Color-coded Schilder plugger for posterior teeth.](image)

### 3.7.3 Group III: GuttaCore with AH Plus Sealer

A size 20 GuttaCore Size Verifier (Dentsply Tulsa Dental Specialties) was used to verify passive fit at working length. A GuttaCore endodontic obturator that corresponded to the Size Verifier size was selected. The working length was marked on the obturator by placing the silicon rubber stopper at the working length that coincides the size verifier's calibration mark. A small amount of AH Plus sealer (Dentsply Tulsa Dental Specialties) was spread into the coronal third of the canals by using paper point. The GuttaCore obturator was heated by using the GuttaCore
oven (Dentsply Tulsa Dental Specialties) at the settings recommended by the manufacturer. The warm obturator was removed from the oven and immediately placed into the canal pushing smoothly with a continuous motion over a period of 5 seconds until the desired length was reached. The handle was bent to opposite sides to break it off (180), and the GP was condensed with 0.5, 0.6, 0.7, and 0.8 mm Schilder plugger slightly below the pulp chamber floor.

The samples from all three groups were stored in an incubator at 100% humidity and 37°C for five days to allow the sealers to completely set before the micro-CT scanning.

3.8 Third Micro-CT Scan of the Teeth After Root Filling

All 30 teeth were scanned using a Micro-CT 100 (SCANCO Medical AG, Bruettisellen, Switzerland) with the same settings of the first and second scans. A resolution of 30 µm (voxel size) was chosen for the study as it has been used in several previous studies in endodontics and is sufficient for comparison of the quality of root fillings with different materials (175, 181, 182).
3.8.1 Reconstruction and Analysis of the Images

Voids were observed both in the 2D and 3D images within the region of interest. The apical 1 mm from the WL was excluded from the measurements of voids as in many teeth the root filling was slightly short and therefore this area was identified as unfilled instead of a large void. In other words, voids in the root filling can occur only in that area of the root canal that is filled. The volume of filling materials, gaps, and voids was determined by analyzing the data from the second and third scan. The difference between the volume of empty canal space and the volume of root fillings represented the volume of gaps and voids. The mean volume difference was calculated for each group and these values were compared using Kruskal-Wallis and Mann-Whitney tests. After importing the data to MeVisLab software in HDL format, the root canal volume was divided into three sections of equal length in each root in the apico-coronal direction using the Draw3DMacro module. The filling materials and the empty canal space were segmented with RegionGrowingMacro module. MeVisLab is a software program in which a lower threshold was selected to include the low grey scale values (empty canal space, gaps and voids), while an upper threshold was adjusted to include the high grey scale value material (root filling materials). These threshold values were standardized when analyzing all the samples (Figure 3.8).
Figure 3.8 Segmentation of root filling material using MeVisLab software. The highlighted area represents the filling materials (GP + sealer).
Chapter 4: Results

Two and three-dimensional reconstructed images of the root canal filling are shown from the coronal, axial and sagittal aspects in Figure 4.1. Voids, sealer, and gutta-percha were easily identified with their different grey scale levels, except for the GuttaFlow group. In this study, the volumes of the filling materials, gaps, and voids were measured, and the volumetric percentages were calculated. The calculated percentages were compared between the examined groups and within each group at three canal levels. Additionally, the volume of debris removed using the GentleWave system was measured and the percentages were calculated.

4.1 Gaps and Voids Analysis

The data for normal distribution and their equality of variance was evaluated and proper statistical tests were chosen to analyze the volumetric percentages difference of gaps and voids between groups and within each group at three canal levels. The data did not display normal distribution (Kolmogorov-Smirnov test and Shapiro-Wilk test; \( p < 0.05 \)) or equality of variances (Levene’s-test; \( p < 0.05 \)). Therefore, the data was analyzed with non-parametric tests. Kruskal-Wallis test indicated that there was a statistically significant difference between the groups \( (P = 0.000) \). Mann-Whitney U-test indicated statistical significances between SC/AH+ and SC/GF groups \( (P = 0.000) \), and between SC/AH+ and GC/AH+ groups \( (P = 0.000) \). However, there was no statistical difference between SC/GF and GC/AH+ groups \( (P = 0.100) \).
Figure 4.1 Reconstructed 2-D (left) and 3-D (right) images for a sample in the (SC/AH+) group, showing the distribution of gutta-percha, sealer, and voids inside the filled root canal space. (1 & 2) axial aspect, (3 & 4) coronal aspect, and (5 & 6) sagittal aspect.
The means and standard deviations (SD) of the overall proportion (% of the total canal volume) of filling material, gaps & voids are shown in Figure 4.2, and Tables 4.1, 4.2. The GC/AH+ (GuttaCore with AH Plus sealer) group showed the highest percentage of filling materials (98.79 %) (Table 4.1), and the lowest percentage of gaps and voids (1.21 %) (Table 4.2). The SC/AH+ (Single cone with AH Plus sealer) group had the lowest percentage of filling material (95.94 %) (Table 4.1), and highest percentage of gaps & voids (4.06 %) (Table 4.2).

<table>
<thead>
<tr>
<th>Group</th>
<th>Overall filling volume (%)</th>
<th>Mean and SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC/AH+</td>
<td>95.94 ± 3.32</td>
<td></td>
</tr>
<tr>
<td>SC/GF</td>
<td>98.66 ± 0.93</td>
<td></td>
</tr>
<tr>
<td>GC/AH+</td>
<td>98.79 ± 1.25</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.1 The proportion (% mean ± SD) of the root canal space filled with the three root filling materials.

<table>
<thead>
<tr>
<th>Group</th>
<th>Overall gaps and voids (%)</th>
<th>Mean and SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC/AH+</td>
<td>4.06 ± 3.32</td>
<td></td>
</tr>
<tr>
<td>SC/GF</td>
<td>1.34 ± 0.93</td>
<td></td>
</tr>
<tr>
<td>GC/AH+</td>
<td>1.21 ± 1.25</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.2 The proportion (% mean ± SD) of gaps & voids of the total root canal volume.
Overall filling volume (%)

![Bar chart showing mean volumes percentages of filling materials within three groups: SC/AH+, SC/GF, GC/AH+.](image)

Figure 4.2 Mean volumes percentages of the filling materials within three groups (1) Single-cone technique with AH Plus sealer (SC/AH+), Single-cone technique with GuttaFlow sealer (SC/GF), and GuttaCore technique with AH Plus sealer (GC/AH+). The bars represent the standard deviations.
4.1.1 Comparison of Gaps and Voids within Each Group

4.1.1.1 Comparison of Gaps and Voids in the Coronal, Middle and Apical Thirds of the Root Canals within Each Group

No statistical difference was found between the coronal, middle and apical thirds of the G&V volumes within the root canals obturated with the SC/AH+ technique (P = 0.170) (Figure 4.3).

![Diagram](image.png)

Figure 4.3 Proportion of gaps and voids (% mean) at the three canal levels in the SC/AH+ group.
The volumetric percentages of G&V that were detected in the apical third of the multi-rooted canals after using the SC/GF technique were significantly different from that detected in middle and coronal thirds within the same group (P = 0.001, P = 0.000) (Figure 4.4).

![Graph showing Gaps and voids (% mean) at three canal levels in the SC/GF group.](image)

**Figure 4.4** Proportion of gaps and voids (% mean) at the three canal levels in the SC/GF group.
The statistical analysis of the proportions of gaps and voids in the obturated canals using GC/AH+ group at the three levels showed no significant difference (P = 0.753) (Figure 4.5).

Figure 4.5 Proportion of gaps and voids (% mean) at the three-canal levels in the GC/AH+ group.
4.1.1.2 Comparison of Mesial and Distal Gaps and Voids within Each Group

The overall volume of gaps and voids in mesial and distal canals within each root filling group were compared using the Mann Whitney U-test. The test revealed no statistical difference between mesial and distal canals within each group (SC/AH+, SC/GF, and GC/AH+), with P values of 0.261, 0.433, and 0.487, respectively (Figures 4.6, 4.7, and 4.8).

![Graph showing comparison of gaps and voids in mesial and distal root canals filled with the SC/AH+ technique.]

Figure 4.6 Proportion of gaps and voids (% mean) in mesial and distal root canals filled with the SC/AH+ technique.
Figure 4.7 Proportion of gaps and voids (% mean) in mesial and distal root canals filled with the SC/GF technique.

Figure 4.8 Proportion of gaps and voids (% mean) in mesial and distal root canals filled with the GC/AH+ technique.
4.1.2 Comparison of Gaps and Voids between Groups

4.1.2.1 Comparison of Coronal, Middle and Apical Gaps and Voids between the Groups

Kruskal-Wallis and Mann-Whitney U tests were used to compare the total volume of gaps and voids in the coronal, middle, and apical thirds of the canals obturated with the three techniques. The coronal %G&V in canals filled by SC/AH+ differed statistically from the coronal %G&V found in canals obturated using SC/GF and GC/AH+ techniques (P = 0.001, P = 0.000). However, there was no statistical difference between the coronal %G&V between the SC/GF and GC/AH+ groups (P = 0.277) (Figure 4.9).

![Figure 4.9 Proportion of gaps and voids (% mean) in the coronal thirds of canals obturated with either SC/AH+, SC/GF, or GC/AH+ technique.](image)
The average volume of gaps and voids in the middle thirds of the SC/AH+ canals was five times greater than in canals filled with the GC/AH+ technique. Kruskal-Wallis test showed a difference between the three groups (P = 0.000) (Figure 4.10).

![Gaps & voids % at middle third](image)

Figure 4.10 Proportion of gaps and voids (% mean) found in the middle third of the canals obturated with either SC/AH+, SC/GF, or GC/AH+ technique.
The canals filled using the SC/GF method presented the lowest total volume of apical gaps and voids (0.68 %), followed by GC/AH+ (1.30 %). On the contrary, SC/AH+ group accounted for the highest volume of apical gaps and voids (3.26 %) (Figure 4.11). There was a statistically significant difference between the apical gaps and voids between the SC/AH+ group and both the SC/GF and GC/AH+ groups (P = 0.000). No statistically significant difference was found between the SC/GF and GC/AH+ groups (P = 0.429).

Figure 4.11 Proportion of gaps and voids (% mean) found in the apical third of the canals obturated with either SC/AH+, SC/GF, or GC/AH+ technique.
4.1.2.2 Comparison the Proportion of Gaps and Voids in the Mesial and Distal Canals between the Three Groups

The overall combined volume of gaps and voids in the mesial canals in the SC/AH+ group differed significantly from the mesial gaps and voids in the SC/GF and GC/AH+ groups (P = 0.000). However, no statistically significant difference was found between the SC/GF and GC/AH+ groups (P = 0.734) (Figure 4.12).

![Gaps & voids % in mesial root canals](image-url)

**Figure 4.12** Proportion of gaps and voids (% mean) in the mesial root canals filled by the SC/AH+, SC/GF, and GC/AH+ technique.
The total combined volume of gaps and voids in the distal canals of the SC/AH+ group differed significantly from the SC/GF and GC/AH+ groups (P = 0.000). However, no statistically significant difference in distal canal gaps & voids was found between the SC/GF and GC/AH+ groups (P = 0.084) (Figure 4.13).

![Gaps & voids % in distal root canals](image)

**Figure 4.13** Proportion of gaps and voids (% mean) found in distal canals obturated using the SC/AH+, SC/GF, and GC/AH+ technique.

The proportions of sealer and gutta-percha in the root canals from each group were also measured. The lowest amount of sealer and the highest amount of gutta-percha were found in the GC/AH+ group (Figure 4.14).
Figure 4.14 The proportions (%) of sealer and gutta-percha in root canals of three samples obturated using three different techniques; SC/AH+ (first row), SC/GF (second row), and GC/AH+ (third row). A, D and G views show segmented (sealer + GP) inside the root canals; B, E and H views show segmented GP inside the root canals; and C, F and I views show segmented sealer inside the root canals.
In Summary:

Tables 4.3, 4.4, and Figures 4.15, 4.16 summarize the results of the proportions of the root filling materials, gaps and voids in the different groups.

<table>
<thead>
<tr>
<th></th>
<th>SC/AH+</th>
<th>SC/GF</th>
<th>GC/AH+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesial</td>
<td>96.07 ± 3.44</td>
<td>98.47 ± 0.86</td>
<td>98.57 ± 1.51</td>
</tr>
<tr>
<td>Distal</td>
<td>94.53 ± 5.02</td>
<td>98.12 ± 1.36</td>
<td>98.49 ± 1.63</td>
</tr>
<tr>
<td>Mesial</td>
<td>96.09 ± 2.76</td>
<td>98.69 ± 1.34</td>
<td>98.02 ± 0.98</td>
</tr>
<tr>
<td>Distal</td>
<td>95.51 ± 2.97</td>
<td>99.03 ± 0.71</td>
<td>99.28 ± 0.47</td>
</tr>
<tr>
<td>Apical</td>
<td>97.11 ± 2.02</td>
<td>99.41 ± 0.51</td>
<td>98.59 ± 1.50</td>
</tr>
</tbody>
</table>

Table 4.3 The proportions (% mean ± SD) of the filling materials in the mesial and distal root canals obturated using the three techniques (SC/AH+, SC/GF, and GC/AH+), at three canal levels (coronal, middle, and apical thirds).

Figure 4.15 The proportion (Means ± SD) of filling materials in mesial and distal root canals obturated using the three techniques (SC/AH+, SC/GF, and GC/AH+), at three canal levels (coronal, middle, and apical thirds).
Table 4.4 The proportion (% mean) of the gaps and voids in mesial and distal root canals obturated using the three techniques (SC/AH+, SC/GF, and GC/AH+), at three canal levels (coronal, middle, and apical thirds).

<table>
<thead>
<tr>
<th>Technique</th>
<th>Mesial (%)</th>
<th>Distal (%)</th>
<th>Mesial (%)</th>
<th>Distal (%)</th>
<th>Mesial (%)</th>
<th>Distal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC/AH+</td>
<td>3.93%</td>
<td>5.47%</td>
<td>1.53%</td>
<td>1.88%</td>
<td>1.43%</td>
<td>1.51%</td>
</tr>
<tr>
<td>SC/GF</td>
<td>3.91%</td>
<td>4.49%</td>
<td>1.31%</td>
<td>1.98%</td>
<td>0.97%</td>
<td>0.72%</td>
</tr>
<tr>
<td>GC/AH+</td>
<td>2.89%</td>
<td>3.64%</td>
<td>0.76%</td>
<td>0.59%</td>
<td>1.41%</td>
<td>1.20%</td>
</tr>
</tbody>
</table>

Figure 4.16 The proportion (% mean) of the gaps and voids in mesial and distal canals obturated using the three techniques (SC/AH+, SC/GF, and GC/AH+), at three canal levels (coronal, middle, and apical thirds).
4.2 Debris Removal by the GentleWave System

The debris volumes that were measured before the GW cleaning system are presented in Table 4.5 and Figure 4.17. After conventional irrigation, 4.58 % of canal volumes were filled with debris while no debris was found after GW cleaning. The apical third contained the highest % of debris volumes before the use of the GentleWave™ system, followed by middle, and coronal thirds of the root canals.

<table>
<thead>
<tr>
<th></th>
<th>Coronal</th>
<th>Middle</th>
<th>Apical</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-D Debris %</td>
<td>3.83%</td>
<td>5.22%</td>
<td>5.83%</td>
<td>4.58%</td>
</tr>
</tbody>
</table>

Table 4.5 The proportion (% mean volume) of debris removed by the GentleWave system at the three canal levels.

Figure 4.17 The amount (% mean volume) of debris removed by using the GentleWave system at the three canal levels.
Chapter 5: Discussion

The current study was designed to compare the quality of root filling using a single-cone technique with either AH Plus or GuttaFlow sealers, and a thermoplastic GuttaCore technique with AH Plus sealer in teeth after minimal canal preparation and the GentleWave cleaning. As the total volume of gaps, voids and the filling materials between the three groups was statistically significantly different, the null hypothesis was rejected. Several studies have examined root canal debridement, apical pressure created during irrigation, and tissue dissolution by a new irrigation method, the GentleWave™ system, in minimally prepared canals (73, 76, 77). Our study was the first study to examine the quality of root filling in those minimally instrumented canals subjected to GW cleaning. The selection of the obturation techniques was a challenge since the final preparation size and taper were small (20/.04). Cold lateral compaction and warm vertical compaction were not applicable obturation techniques in this study because of the small final size of the prepared canals. The single-cone technique was selected to be used in this study based on various studies that have shown comparable results in terms of sealing ability between single-cone and both WVC and LC techniques (136, 137). In the current study, two different sealers were used, AH Plus and GuttaFlow sealers. The sealers were selected based upon their potential properties for the filling of minimally instrumented root canals (103, 105, 106, 107, 108). Both sealers are widely used by dentists worldwide. A recent study showed that after setting of AH+, fibroblast cells were able to grow on the surface of the sealer, similar to bioceramic
sealers (102). AH Plus has a considerably low flowability, low film thickness, and slight shrinkage upon setting in comparison to other sealers (103). GuttaFlow sealer expands upon setting (103) and is characterized by a thixotropic property. This means that the sealer viscosity reduces under pressure. Hence, the sealer flows into the small lateral canals and exhibits a good sealing ability in comparison to other sealers (105, 106, 107, 108). The GuttaCore technique was the other obturation method that was examined in this study. It was introduced six years ago as a further development of the Thermafil system and has been used since by many as a method of root canal obturation. However, it has not been used in minimally instrumented root canals. However, it has been proven that a filling with excellent ability in sealing the prepared root canals and a homogenous filling could be achieved by using GuttaCore technique (156). Based on the above-mentioned factors, the three filling techniques/materials were selected for the present study. It would have been possible to instrument the canals even smaller, to size 15/.04, however, the GuttaCore obturator is not available in size 15, the smallest available size is 20/.04 (180). Therefore, the apical master preparation that used in the current project was 20/.04.

In our study, the confounding factors that may affect the incidence of gaps and voids within the filling materials between the groups were minimized. The anatomical configuration of the root canal system such as isthmus area might contribute to creating a difference in the distribution of gaps and voids. Therefore, the stratified sampling was followed in this study by distributing the same number of mesial and distal root canals that have isthmuses amongst the experimental groups. Regarding
the quality of the canal preparation that could be another factor affecting the fillings quality, all samples were prepared by one operator (A. Z) following the same standardized procedures. In addition, the consistency and the volume of the sealer are important issues that should have been controlled and standardized amongst all samples. Hence, many pilot studies were done to develop a method for delivering the sealer into the canals in a way that allows for a complete fill of the canal space with the sealer. Therefore, a Lentulo spiral was used to deliver the sealer in both SC/AH+ and SC/GF groups into the root canals until the sealer filled the canal spaces up to 1 mm below the canals orifices. This method was followed to ensure the optimal delivery of the sealer into the root canals reaching the apical area and the lateral canals as shown in Figure 3.6. Although the amount of sealer was not equal within all root canals especially those that differ morphologically, it was possible to optimally fill the root canals and their ramifications with a sealer by using a Lentulo spiral. The obturation procedures were also carried out by one practitioner (A. Z).

In comparison with other studies that used decoronated teeth (76, 151, 165), the crowns of the samples in the present study were maintained to simulate a clinical scenario within which the clinical crown of the tooth is usually present. Moreover, the access cavity was prepared conservatively to maintain the tooth structure, and allow the placement of the GentleWave handpiece within the pulp chamber and allow its proper stabilization.

Multiple methods have been used in the literature to evaluate the quality of the obturation technique and the root fillings. Radiographs allow a limited assessment
of the filled canal contents by giving a two-dimensional view of a three-dimensional area (183). The invasive method of sectioning the roots with filled root canals and further viewing or scanning with a stereomicroscope or SEM is also used to assess the fillings quality. A loss of some parts of the filling materials, which may be mis-interpreted as a gap or a void, could be caused by such invasive methods. Clearing or demineralization technique is time-consuming (184), and the long immersion in alcohol may affect the flowability of gutta-percha and its ability to be compacted. Moreover, the tooth becomes weak against the forces of compaction, which may lead to its fracture during obturation procedures (185). Recently, a non-invasive (micro-CT) method has gained popularity as a research tool in various applications in dental research (158, 159, 160, 161, 162). In endodontics, micro-CT has been used in studying the root canal anatomy and morphology before and after instrumentation, measuring dentin debris, and assessing the quality of root fillings done using different filling materials and techniques (158, 159, 160, 161, 163). It is a relatively rapid method that provides highly accurate 3D images of the root fillings and voids. However, this technology is expensive, may not be available for all institutions, and cannot be used in-vivo due to the high dose of radiation utilized by this technique. In the present study, micro-CT was used as a method to detect gaps and voids within the filled root canals, calculate their relative proportions, and to measure the volume of debris removed by cleaning the root canals with the GentleWave™ system.

Despite using the same filling method for the two single-cone groups, SC/AH+ and SC/GF, in our study, the results revealed a statistically significant difference
between the groups. This might be explained by the findings of a recent study (103) that showed that GuttaFlow expands upon setting, which could result in a decrease in the incidence and volume of gaps and voids in the filled canals. In contrast to AH Plus sealer that shrinks upon setting possibly increasing the possibility of formation of gaps and voids. Another reason for the frequent presence of gaps and voids in canals filled with SC/AH+ may be the higher solubility of AH Plus sealer in comparison to GuttaFlow sealer (103, 186). A thixotropic property of GuttaFlow facilitates sealer penetration in narrow canals and its ramifications, as the sealer becomes less viscous under pressure. In support of our results, several studies have shown that the sealing ability of GuttaFlow sealer was significantly higher than that of AH Plus sealer with either single-cone or lateral compaction techniques (105, 106, 108).

Our study revealed a statistically significant difference between the GC/AH+ group and the SC/AH+ in the quality of filling and the incidence of gaps and voids. The root canals of these two groups were filled using two different obturation techniques (GuttaCore and Single-cone) with the same sealer (AH Plus). Similar findings were detected in a previous study when an invasive method together with a digital stereomicroscope were used to compare gutta-percha filled area (PGFA), sealer filled area (PSFA) and voids in root canals after filling using GuttaCore, single-cone and lateral compaction techniques (156). It was concluded from that study that the root filling material was very homogenous in the GuttaCore filled root canals with high PGFA and low occurrence of voids. As to the dimensional stability of gutta-percha after setting, the high proportion of gutta-percha in the filling material
maximizes the quality of fillings. Many sealers shrink upon setting and may become more soluble in tissue fluids over time (187, 188). Therefore, minimizing the sealer amount has been thought to result in better filling quality. Somma et al. (189) compared the carrier-based, system B, and single-point techniques in conjunction with AH Plus sealer using micro-CT and found similar results to our study. Although they did not find a statistically significant difference between the groups, the carrier-based filling technique had higher percentage of fillings and less gaps and voids than the single-cone technique.

In the present study, although the results were not different between SC/GF and GC/AH+ groups, the GuttaCore method exhibited the lowest incidence of gaps and voids. A recent study that compared the bacterial leakage between GuttaCore/AH Plus sealer (GC), and single-cone/GuttaFlow sealer (GF) in teeth with single root canals using confocal laser scanning microscopy found similar findings to our results (155). It was concluded from that study that GC group showed less bacterial leakage than the GF group.

Compared to SC/GF method, GC/AH Plus method:

1/ can be applied easily

2/ produces a homogenous filling material (156)

3/ utilizes little amount of sealer (156)

4/ can be easily removed, if necessary, in less time in comparison to other obturation techniques (152, 154). Therefore, GuttaCore technique might be the best technique to fill the minimally instrumented root canals compared with other obturation techniques that were examined in this study.
In a previously study (156), the evaluation of the PGFA and PSFA areas and the incidence of voids were performed at 2 mm, 4 mm, 6 mm, and 8 mm from the apex. Similarly, in the present work, we divided the root canals into three equal thirds (coronal, middle, apical), and measured the %G&V and filling materials volumes in each third, and compared the volumes amongst the different thirds. The results of these two studies are similar in terms of canal levels where the coronal, middle, and apical voids in root canals filled with SC/AH+ were higher than when GC/AH+ was used. This can be interpreted by the increase of sealer amount, and the decrease of GP quantity at different levels of the canals obturated using the single-cone technique. The incidence of coronal gaps and voids was higher than in the middle and apical thirds in all groups in our study. In addition, the apical thirds were associated with lower percentages of gaps and voids in all groups except for the GC/AH+ group, where the middle thirds had the lowest percentages of gaps and voids. Uneven coating of the GuttaCore points has been indicated to cause pushing of gutta-percha to one side, which can lead to uncovering of the core from the gutta-percha and make the carrier core “naked” especially in the apical third (93, 190, 191).

In the present study, the micro-CT was used also to measure the debris volume that was removed by the GentleWave system. The findings confirmed the high effectiveness of the device in cleaning the root canal system. After instrumentation and conventional irrigation, an average of 4.58 % of canal volume was filled with debris. The most debris was detected in the apical thirds of the canals, followed by middle and coronal thirds, while no debris was found after GW cleaning.
The cleaning of infected root canals by the elimination of bacteria, removal of necrotic pulp tissue, killing of planktonic and biofilms microorganisms, removing the smear layer, and dissolving the organic and inorganic tissues are goals of root canal irrigation (192, 193, 194). The anatomical complexity of the root canal system and the inaccessibility to the entire endodontic space prevent the completely cleaning by instruments and irrigants alone (44). Therefore, there was a necessity to use methods to agitate the irrigating solutions. Several previous studies have measured the effectiveness of different irrigation systems and irrigating solutions in root canal debridement (195, 196, 197). All methods have their limitations in different clinical situations.

Various assessment methods have been used to evaluate the effectiveness of root canal debridement. A microscope at a specific magnification with a digital camera has been used in some studies (198, 199). The debris amount was scored in a way that the higher the score, the larger the debris amount (198, 199). However, the low sensitivity is one of the drawbacks of this method in comparison to the micro-CT method. Scanning electron microscopy is also used after sectioning the root canals in order to establish the quality of cleaning and the quantity of remaining dentin debris, and to compare the effectiveness of different irrigation techniques (198, 200, 201, 202). These are invasive methods in which the sample can be used only one time to evaluate one irrigation technique. In contrast, when a non-invasive technique such as micro-CT is used, the sample can be examined and analyzed multiple times at various stages of the treatment.
In a recent study that aimed to compare the debridement efficacy of conventional syringe-needle irrigation, and GW system after minimal canal preparation, histological sections of the teeth were examined to compare the debridement efficacy of the two irrigation methods. After irrigation, each canal was filled with buffered formalin in order to fix the pulpal tissue and processed following standard procedures for tooth histological specimens (76). The GW system had significantly greater capacity in the cleaning of the root canals than syringe-needle irrigation method (76). In our study, the observation of complete removal of dentin debris also points toward the effectiveness of the GW system to completely remove tissue remnants (such as dentin debris), which may be important in facilitating the filling of hard-to-reach areas in the canal system with either sealer of warm gutta-percha. Micro-CT scanning has also been used by others to compare the efficacy of various irrigation methods in removing debris. Paqué et al. (163) established that micro-CT is an appropriate method for dentin debris assessment, and can be used for calculating the dentin debris quantitatively. Additionally, the three-dimensional reconstructed images of micro-CT scans enhance the qualitative observation of dentin debris (163). The technique of measuring the debris in our study was the same as the one used by Paqué, but with different resolution (203). Both studies used mandibular first and second molars because it has been shown that persistent apical periodontitis occurs most frequently in molars (204, 205). The anatomical complexity of molar root canal system that makes complete removal of biofilms and debris difficult is believed to be one of the most important factors of persistent endodontic infections (206). Paqué et al. (203) scanned the specimens four times, before instrumentation and after applying different irrigating solutions. After
registration of the outer contours of both scans, “Hard tissue debris was identified and calculated as follows: voxels that were identified as soft tissue, liquid, or air (canal volume) in the preoperative scan but then were filled with radiopaque material in the postoperative scan were assumed to be filled with hard tissue debris” (203). The study by Paqué et al. has some limitations; it was impossible to calculate the dentine debris that accumulated on the canal walls. However, in our study, we were able to calculate the dentin debris all through the canal because we were comparing the canal spaces of pre-GW scans (after instrumentation) versus the post-GW scans. Thus, no dimensional changes have occurred in the canal space except for the missing of debris voxels after using the GW system. Therefore, the difference between the empty canals volumes of both scans was considered as dentin debris volume that was removed by using the GW system. In the present study, the dentin debris volume was measured at three different canal levels in order to identify the most likely areas to be filled with dentin debris. We found that although the apical area of the root canal is the most difficult part to reach and clean by conventional needle irrigation, dentin debris was completely removed also in the apical canal by the GW cleaning.

The limitation of this study is that micro-CT can only be used to detect the hard tissue debris while the remaining soft tissue is not detectable by this method of assessment (163). In addition, the resolution used, 30 μm, does not allow detection of very thin layers of sealer or smallest amounts of dentin debris. On the other hand, higher resolution takes much longer time to scan the specimen, is expensive, and the resulting amount of image data is challenging to handle without very strong
processing power. It is possible that some areas identified as gaps may in fact have been voids, but the sealer at the area of the void next to canal wall dentin may have been too thin to be detected in the micro-CT scan. However, such errors, if present would not change the combined volume of gaps and voids. The use of 30 µm resolution may be not an optimal voxel size to detect the dentin debris. However, the results showed excellent filling of isthmus areas in most cases, which clearly indicates that GW effectively removed the debris and thereby made it possible to completely filled the root canals. Despite its potential shortcomings the resolution of 30 µm seems good enough to compare the quality of the three different root filling methods that were used in this study. The same micro-CT resolution is also used in many instrumentation studies (175, 181, 182).
Chapter 6: Conclusion

The overall proportion (%) of gaps, voids and the filling materials in the canal space was statistically significantly different between the three groups. Thus, the null hypothesis that there would be no difference in the quality of root fillings amongst single point technique with AH Plus sealer (SC/AH+), single point technique with GuttaFlow sealer (SC/GF), and GuttaCore® technique with AH Plus sealer (GC/AH+) was rejected.

The study outcome indicates that a high-quality obturation can be done in minimally prepared root canals by the GC/AH+ and SC/GF methods. However, the root canals obturated with GuttaCore technique had the lowest incidence and total volume of gaps & voids and the highest proportion of gutta-percha. GuttaCore exhibited a good adaptability and suitability to fill minimally instrumented root canals after GentleWave cleaning. Therefore, of the three techniques examined GuttaCore technique might be the best technique to fill the minimally instrumented root canals.

For an optimal root canal filling, the GentleWave system makes it possible to fill the entire root canal space due to its high efficacy to remove all dentin debris, also from the apical third, and completely clean minimally instrumented root canals.

This study was the first to examine the quality of fillings in minimally instrumented root canals after GW cleaning. It was carried out in conditions similar to clinical
situation. Therefore, in vivo, the root fillings in minimally instrumented, GW-cleaned and obturated root canals by two of the three root filling techniques examined in this study can be expected to have comparable quality to the fillings in our study. Future studies should investigate the quality of root fillings in minimally instrumented root canals using several different sealers, including bioceramic sealers. Further, the quality of root filling in uninstrumented, GW cleaned canals of different size should also be studied.
References


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Appendices:

Appendix A – Research Plan

Root canals of 30 mandibular 1st & 2nd molars were shaped and cleaned to Size #20/.04

15 samples scanned using micro-CT

Root canals of all 30 samples were cleaned using the GentleWave system and scanned for 2nd time

Group 1 (n=10)
Single-cone technique + AH Plus sealer (SC/AH+)

Group 2 (n=10)
Single-cone technique + GuttaFlow sealer (SC/GF)

Group 3 (n=10)
GuttaCore technique + AH Plus sealer (GC/AH+)

A third micro-CT scan of the teeth was carried out, and the reconstructed images were analyzed for the volumetric percentage of gaps and voids at three different canal levels (coronal, middle, apical).

Figure A.1 Research plan.
Appendix B – Micro-CT Settings

Figure B.1 Micro-CT settings.
Appendix C – Normal Distribution Tests

### Tests of Normality

<table>
<thead>
<tr>
<th>Groups</th>
<th>Kolmogorov-Smirnov&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Shapiro-Wilk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volumes values</td>
<td>St.</td>
<td>df</td>
</tr>
<tr>
<td>Single cone + AH Plus</td>
<td>.221</td>
<td>60</td>
</tr>
<tr>
<td>Single cone + GuttaFlow</td>
<td>.165</td>
<td>60</td>
</tr>
<tr>
<td>GuttaCore + AH Plus</td>
<td>.181</td>
<td>60</td>
</tr>
</tbody>
</table>

<sup>a</sup> Lilliefors Significance Correction

Table C.1 Kolmogorov-Smirnov, Shapiro-Wilk tests of data distribution normality.

### Test of Homogeneity of Variances

<table>
<thead>
<tr>
<th>Volumes_values</th>
<th>Levene Statistic</th>
<th>df1</th>
<th>df2</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14.517</td>
<td>2</td>
<td>177</td>
<td>.000</td>
</tr>
</tbody>
</table>

Table C.2 Levene statistics of data distribution normality.
Appendix D – Total Gaps and Voids Data Analysis Using Kruskal-Wallis Test

<table>
<thead>
<tr>
<th>Ranks</th>
<th>Groups</th>
<th>N</th>
<th>Mean Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volumes_values</td>
<td>Single cone + AH Plus</td>
<td>60</td>
<td>130.87</td>
</tr>
<tr>
<td></td>
<td>Single cone + GuttaFlow</td>
<td>60</td>
<td>76.08</td>
</tr>
<tr>
<td></td>
<td>GuttaCore + AH Plus</td>
<td>60</td>
<td>64.55</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>180</td>
<td></td>
</tr>
</tbody>
</table>

Table D.1 Total mean rank of the gaps and voids volumes in the three different groups (SC/AH+, SC/GF, and GC/AH+).

<table>
<thead>
<tr>
<th>Test Statistics(^{a,b})</th>
<th>Volumes_values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chi-Square df</td>
<td>55.485</td>
</tr>
<tr>
<td>Asymp. Sig.</td>
<td>2</td>
</tr>
</tbody>
</table>

Table D.2 Non-parametric statistical test (Kruskal Wallis Test) compares SC/AH+, SC/GF, and GC/AH+ groups.
Appendix E – Statistical Analysis of Gaps and Voids within and between the Three Tested Groups at Three Canal Levels

<table>
<thead>
<tr>
<th></th>
<th>SC/AH+</th>
<th>SC/GF</th>
<th>GC/AH+</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>M</td>
<td>A</td>
</tr>
<tr>
<td><strong>SC/AH+</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>C</strong></td>
<td>*</td>
<td>*</td>
<td>**</td>
</tr>
<tr>
<td><strong>M</strong></td>
<td></td>
<td>*</td>
<td>**</td>
</tr>
<tr>
<td><strong>A</strong></td>
<td></td>
<td>*</td>
<td>**</td>
</tr>
<tr>
<td><strong>SC/GF</strong></td>
<td>**</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>C</strong></td>
<td></td>
<td></td>
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<tr>
<td><strong>M</strong></td>
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<td>**</td>
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</tr>
<tr>
<td><strong>A</strong></td>
<td>**</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td><strong>GC/AH+</strong></td>
<td>**</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>C</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>M</strong></td>
<td></td>
<td></td>
<td>**</td>
</tr>
<tr>
<td><strong>A</strong></td>
<td></td>
<td></td>
<td>**</td>
</tr>
</tbody>
</table>

Table E.1 Statistical analysis of gaps and voids within and between the three different groups at three canal levels:

SC/AH+ = Single-cone technique with AH Plus sealer
SC/GF = Single-cone technique with GuttaFlow sealer
GC/AH+ = GuttaCore with AH Plus sealer
C = Coronal third
M = Middle third
A = Apical third
* = No statistical difference
** = Statistical difference