REMOVAL OF CALCIFICATIONS FROM DISTAL CANALS OF MANDIBULAR MOLARS WITH THE GENTLEWAVE™ SYSTEM

by

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submitted by Deborah Szabo in partial fulfillment of the requirements for the degree of M. Sc.

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Abstract

Objective: Radiographic examination of teeth often reveals the presence of calcified structures in pulp chambers. Many studies have also reported the presence of calcifications throughout the root canal system. These can complicate root canal therapy by obliterating the pulp chamber or blocking root canals. Many studies have proposed techniques to remove calcifications using instrumentation. GentleWave is a novel system for non-instrumentational cleaning of root canals, however, its ability to remove calcifications has not been established. The aim of our study was to determine the efficiency of the GentleWave system in removing calcifications from the root canal system.

Methods: Mandibular molars were accessed, and all canals identified. Patency of the distal canals was ensured with a size 10 K-File and the pulp chambers were irrigated with NaOCl. Micro-CT images were obtained to evaluate the initial volume of calcification in the distal canals. Teeth were then treated with the GentleWave system as recommended by the manufacturer and a second set of micro-CT images were obtained to assess for changes in the calcification volume. The Mann-Whitney test was used to compare calcification volumes before and after GentleWave treatment. The significance level was set at $P \leq .05$.

Results: All distal canals showed calcifications prior to GentleWave treatment. In teeth treated with the GentleWave system, distal canals showed statistically significant reduction in calcification volume when compared to the initial volume of calcification. Mean reduction in volume was 87.6%, with overall values ranging from 60-100%. Root canal volume prior to and after GentleWave treatment did not show any significant change.

Conclusions: The GentleWave system showed significant reduction in root canal calcification after treatment. Almost half (42%) of the distal canals showed complete elimination of calcification after GentleWave treatment.

Acknowledgements: Supported by the Canadian Academy of Endodontics. Sonendo provided the GentleWave unit for the cleaning of the teeth.

Lay Summary

The main goal of our study was to assess whether the GentleWave irrigation system could remove calcifications from the root canals of mandibular lower teeth. Calcifications can become impediments to proper cleaning and disinfection of the root canal system. To date, there is no specific protocol which allows for predictable removal of canal calcifications.

To answer our question, mandibular molars were accessed and then cleaned with the Gentle Wave system. Micro CT imaging was used to visualize and measure calcification volumes before and after GentleWave irrigation. Pre and post irrigation imaging revealed that the GentleWave system was able to result in significant reduction in root canal calcification.

Preface

The research question and study design were prepared by Dr. Deb Szabo, Dr. Markus Haapasalo and Dr. Ya Shen. Selection and preparation of samples were performed by Dr. Deb Szabo. Micro-CT scans were performed by Jingzhi Ma and Yan Yang. Teeth were cleaned with GentleWave by Dr. Duo Zhang. The collected micro-CT data was analyzed by Dr. Deb Szabo and Dr. Binwen Chen under the supervision of Dr. Markus Haapasalo and Dr. Ya Shen. The study was approved by the University of British Columbia Office of Research Services, Clinical Research Ethics Board (Certificate Number: H12-02430). The manuscript was prepared by Dr. Deb Szabo with editing by Dr. Markus Haapasalo and Dr. Ya Shen.

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List of Abbreviations

%.....percentage

%vol…… percent volume

°C.....degree Celsius

AP.....apical periodontitis

Ca…….calcium

$Ca(OH)_2$……..…..…….….calcium hydroxide

CBCT.....cone beam computed tomography

CHX……..……...…….……chlorhexidine digluconate

Cu……copper

EDTA.....ethylenediamine tetra-acetic acid

GP…….……..……....gutta percha

GW.....GentleWave™system

kVp.....peak kilovoltage

MDI……...……...manual dynamic irrigation

Micro-CT.....micro-computed tomography

ml.....milliliter

ms.....millisecond

NaOCl.....sodium hypochlorite

NCP..... non-collagenous proteins

P……...phosphorus

PCO .....pulp canal obliteration

PIPS.....photon-initiated photoacoustic streaming

PUI.....passive ultrasonic irrigation

SD.....standard deviation

sec.....second

SEM.....scanning electron microscope

SNI.....syringe-needle irrigation

Sr…….strontium

Zn……zinc

µA.....microampere

µm.....micrometer

Acknowledgements

This work would not be complete without the support and guidance of my supervisors Dr. Markus Haapasalo and Dr. Ya Shen. Their patience, dedication and devotion to endodontic research is truly commendable. I have tremendous respect both personally and professionally for Dr. Haapasalo and Dr. Shen.

It is with great pleasure that I would like to thank Dr. Haapasalo for his incredible ability to balance education and wit, making the research process both fun and challenging. As well, great thanks to Dr. Shen for always keeping her door open to receive my many questions and concerns.

I wanted to extend my deepest thanks to Dr. Binwen Chen. Her constant initiative and help allowed for smooth and timely progress of this research project.

Also, a huge thanks to my co-resident, Dr. Zhejun Wang. Being submitted to my constant research ideas and questions, Zhejun never shied away from my queries.

A very special thank you to my family. My mom, Lenke, and dad, Janos, for always supporting, loving and helping me throughout illness and health. My kids, Lindsay, Evan and Matthew for accepting their mom as a student, sharing with me challenges and hard work. For recognizing the effort required to balance motherhood and academia… and never making me feel like I didn't give them enough time and support.

And last, but by no means least, with all my love, a special thanks to my husband and best friend, Torin Barr. Thank you for understanding my constant desire to learn and advance myself. Thanks for making all of this possible.

Dedication

This dedication is for my dad who values the true importance of endless learning. Thanks for providing me with the privilege of education and opening its doors, which I know were often closed to you. Maybe one day I will achieve the wisdom you so genuinely demonstrate with complete charm and humility.

Chapter 1: Introduction

Endodontics is the dental specialty which focuses on the prevention and treatment of diseases of the dental pulp and periapical tissues. Over time, endodontics has had the goal of retaining the dentition by eliminating disease. Kakehashi's 1965 study on germ free rats provided the basis of endodontic disease: bacteria. Without bacteria, dental pulp is able to defend itself through the formation of dentin bridges (Kakehashi, 1965). However, bacteria are a part of the normal oral flora, and as such, present a constant threat to the dental pulp.

Through dental caries, bacteria are able to invade dentinal tubules and make their way to the dental pulp. This mounts the host's immune response in an attempt to protect tissue from bacterial invasion. Initially, the outward flow of dentinal fluid and intratubular immunoglobulins prevent progression of bacteria into the pulp (Okamura, 1985). When the bacteria and their by-products reach the odontoblasts, these respond by producing reactionary dentin to add further barrier to bacterial influx. The recognition of these antigens by odontoblasts and dendritic cells via their toll like receptors also initiate the innate immune response (Durand, 2006). This response to caries involves increased vascular permeability allowing the influx of natural killer cells, T-lymphocytes and their chemical mediators (Hahn, 2007). In addition to the cell mediated response, inflammatory mediators cause the release of neuropeptides resulting in neurogenic inflammation (Wakisaka, 1990). Together, these immune responses act to defend the pulp from bacteria and prevent their progression to the apical tissues.

If the infection persists, inflammation and its vascular effects can lead to increased intrapulpal pressure. As the dental pulp consists of a low compliance environment, pulp necrosis often ensues, and continued invasion of bacteria leads to eventual infection. The progression of

infection through the coronal pulp to the radicular pulp allows bacteria and their by-products to reach the apical portions of the root canal system (Nair, 2004). This leads to inflammation of the periapical tissues, resulting in apical periodontitis (Nair, 2004). At this point, the lack of vascularity within the root canal system inhibits the host from removing any source of inflammation. It is only after physically and chemically removing infected tissues that periapical periodontitis can be healed. This can be done through extraction of the entire tooth, ensuring complete removal of infected tissues, or through root canal treatment. Root canal treatment allows the patient to retain their teeth while eliminating infection. However, due to the complexity of the root canal system and its intimate relation with the apical tissues, disinfection of root canals is a complicated process. Historically, endodontics has seen the use of a variety of materials and systems involved in disinfecting canals. The common goal is to improve treatment success. As root canal treatment still cannot eliminate infection to the same success as dental extraction, the continued pursuit in finding the most predictable methods of disinfection is essential.

Chapter 2: Review of the literature

2.1 Root canal treatment

Periapical periodontitis is the host's inflammatory response that follows bacterial infection of the necrotic pulp tissues (Nair, 2004). As microbes invade the root canal system and progress through the radicular pulp, inflammation of the periapical tissues results in a periapical lesion that resorbs the periodontal ligament and surrounding hard tissues (Nair, 2004). The purpose of endodontic treatment is to eliminate and prevent the progression of periapical periodontitis. As currently the only treatment method to retain infected teeth, root canal treatment has success rates ranging from 80-94% (Friedman, 2008). The specialty of endodontics pursues excellence in treatment methods to advance the specialty allowing patients to keep their teeth. As bacteria have been shown to be the source of periapical inflammation in the human dentition (Kakehashi, 1965; Bergenholtz, 1974), progress in endodontics involves finding the best possible means to eliminate bacteria and their byproducts while resulting in an environment where bacteria cannot multiply or promote continued infection.

Current challenges in endodontic therapy include inability to completely reduce microbial load, anatomically inaccessible areas of the root canal system and both physiologically and iatrogenically generated blockages impeding access to infected areas of the root canal system (Peters, 2004). Without complete access to the entire root canal system, this system cannot be fully disinfected or sealed. Iatrogenically produced blockages include separated instruments, transported canals and perforations. Zipping and perforation have already been shown to reduce success of root canal treatment (Pettiette et al, 2001). The best method to manage these complications is prevention. Physiological impediments to the root canal system include severe

canal curvatures (Pruett, 1997), calcific metamorphosis (Cvek, 1982) and calcified structures within the pulp chamber and the pulp canal space (Langeland, 1967). The latter can exist as embedded structures in root dentin or as free calcified bodies in the pulp tissues. Attached tissues can become difficult to instrument past or may become detached and displaced. These and originally detached mineralizations can become displaced and block the canal space and access to its terminus.

Many methods have been proposed to manage calcified structures, yet none have presented predictable or definitive forms of treatment. GentleWave is a novel irrigation and cleaning system that has been developed to clean and disinfect root canal systems. The system uses irrigant flow dynamics and cavitation to accomplish this goal. As cavitation can be a powerful tissue elimination tool, GentleWave may have the potential to tackle such challenges as root canal calcifications while maintaining the goal of dentin preservation. GentleWave has been shown to effectively remove soft pulp tissues, calcium hydroxide paste and separated instruments from the root canal system (Wohlgemuth et al, 2015; Ma et al, 2015; Molina et al, 2015). As much as it cannot dissolve healthy, mineralized tissues, the combined activity of tissue dissolution and improved irrigant penetration may allow pulp canal calcifications to be accessed. Cavitation activity and irrigant flow may then be able to displace and potentially remove calcifications from their original locations. As there is a negative pressure component to the GentleWave system (Haapasalo et al, 2015), complete elimination of the calcified structures would be ideal as these would no longer present as impediments to proper shaping and cleaning.

2.1.1 Dentin-pulp complex

To understand the dynamics of root canal treatment and its challenges, an understanding of pulp dentin biology with its formative, reparative and defensive roles should be discussed.

Dentin comprises the bulk of tooth tissue. Surrounded by enamel coronally and cementum radicularly, it borders the dental pulp with which it serves many functions in response to injury. The dentin-pulp complex plays many roles in healing, regeneration and repair. It is also the biological space that may become invaded by bacteria leading to the progression of periapical periodontitis.

The dentin-pulp complex is composed of lined odontoblasts at the pulp periphery which extend their processes into predentin and eventually, dentin. Dentin is composed of 70% organic, 20% inorganic and 10% water contents by weight (Nanci, 2014). The inorganic portion of dentin includes hydroxyapatite plates while the organic portion mainly consists of Type I collagen, NCP's and lipids (Nanci, 2014). This composition varies throughout the tooth as the dentinal tubules fan out from the pulp chamber, with larger diameters of 1.5 – 2.5 μm at the dentin-pulp junction.

Dentin can be divided into primary, secondary and tertiary dentin (Kuttler, 1959).

2.1.1.1 Primary and secondary dentin

Primary dentin is formed during odontogenesis and composes the organized circumpulpal dentin formed by primary odontoblasts. Once crown and root formation are complete, secondary dentin is laid down at a much slower rate (Hargreaves et al, 2012). The dentinal tubules of secondary dentin are continuous with those of primary dentin as these are laid down by the same odontoblasts that form primary dentin. Over time, secondary dentin deposition results in reduction of pulp space

and in some areas also tubule sclerosis over time. These processes allow for reduced dentin permeability as well as protection of the pulp.

2.1.1.2 Tertiary dentin

Tertiary dentin is formed in response to external insult in the forms of thermal, mechanical or bacterial irritation. It differs from primary and secondary dentin in that it does not form along the entire dentin-pulp junction. Rather, it is formed directly in relation to the area of irritation as a defensive mechanism. As well, it tends to form a more irregular, amorphous structure, lacking the more organized tubular structure seen in secondary dentin (Smith, 2012; Ricucci et al, 2014).

Tertiary dentin can further be subdivided into reparative and reactionary dentin. Reactionary dentin is formed by existing odontoblasts while reparative dentin is formed by progenitor cells which differentiate into replacement odontoblasts. Reparative dentinogenesis is a more complex process as progenitor cells must be recruited and signaled to become odontoblast like cells in order to secrete and form the new dentin layer (Ricucci et al, 2014).

2.1.2 Reduced pulp canal space

Secondary and tertiary dentin cause narrowing of root canal spaces. This effect is more commonly seen in the coronal pulp where both secondary and tertiary dentin can be found. Tertiary dentin is formed in direct response to insult and is only deposited at the site of injury. Tertiary dentin can be identified as separate from secondary dentin histologically. While secondary dentin borders the pulp throughout the entire junction of these tissues, tertiary dentin is seen as a more irregular and atubular structure, asymmetrically deposited directly below areas of insult. An example of this

process can be seen when dentin bridges form below heavily restored teeth or below direct pulp caps (Sayegh, 1969; Murray et al 2000).

In addition to this coronal dentin deposition leading to reduced pulp chambers, heavily restored teeth demonstrate reduced radicular spaces (Fleig at al, 2016). This cannot be explained by tertiary dentin deposition in response to the restoration, as tertiary dentin is formed directly below those dentinal tubules invaded or destroyed by caries or restorative procedures (Fleig et al, 2016). The processes that may signal odontoblasts or progenitor cells to form calcifications in the radicular pulp is poorly understood.

In a CBCT analysis of contralateral restored and non-restored teeth, Fleig et al confirmed that restored teeth revealed significantly narrower radicular pulp spaces when compared to non-restored teeth (Fleig et al, 2016). As heavily restored teeth are more likely to require endodontic treatment if further insult occurs, this calcification process renders the root canal treatment more challenging than it would be on otherwise healthy teeth, with larger pulp spaces. In teeth with narrow or obstructed root canals, achieving patency becomes more difficult and smooth negotiation of the canal space is a more arduous task.

2.1.3 Pulp canal obliteration

Pulp canal obliteration (PCO) or calcific metamorphosis is the pulp's response to injury, commonly seen after trauma, and represents another method of dentin deposition. It results in the closing of pulp spaces with concurrent deposition of calcified tissues or reparative dentin. The mechanism of PCO is poorly understood but appears to result from disruption of the tooth's neurovascular supply (Robertson, 1998). More often seen after concussion and luxation injuries

(Oginni, 2007), PCO is found more in immature teeth and teeth that have evaded necrosis post injury (Andreasen et al, 1987). PCO has proven to be a challenge to endodontic treatment from both diagnostic and clinical perspectives as the pulp vitality is not always easy to discern (Robertson et al, 1996; Oginni et al, 2009), and when deemed necrotic, localization of canals can become very difficult. Only 7-16% of teeth with PCO end up requiring root canal treatment with relatively high success rates (Holocomb et al, 1976; Stalhane et al, 1975). However, it is recognized that the treatment itself can be more challenging due to the constriction and calcification of the pulp spaces (Cvek et al, 1982). This results in difficulties with orifice location and ability to reach the canal apex compromising complete disinfection of the root canal system.

2.1.4 Calcific structures in the pulp

It is not uncommon for pulp calcifications to occur in the absence of trauma. Generally, it is believed that at least 50% of teeth have some form of additional mineralized structures (Luukko et al, 2007). Prevalence of pulp calcifications have been reported to range from 4% to 78% (Goga et al, 2008). The discrepancy may lie in the different methodologies used to identify calcifications. Studies have used radiographic, histologic, X-ray diffraction and X-ray fluorescence methods to identify and describe pulp mineralizations. Calcifications identifiable by histology may not be readily seen on routine radiographic examination. Structures with diameters smaller than 200 μm may be too small to see or difficult to identify by two-dimensional imaging (Moss-Salentujn et al, 1983). This discrepancy in identifying calcifications presents clinical relevance as the presence of calcified bodies must be presumed even if they cannot be seen on pre-operative radiographs.

The calcified bodies that can be seen more readily as distinct entities on radiographic examination are pulp stones, also referred to as denticles (Figure 1.1). These tend to appear as well defined, concentric structures and are more commonly seen in the coronal pulp chamber (Figure 1.2). Pulp stones have been identified in pulp chambers of all teeth and in patients of all ages. It is believed that teeth in older patients tend to present more commonly with pulp stones due to age related changes, however calcifications have also been found in unerupted teeth (Nitzan et al, 1986).

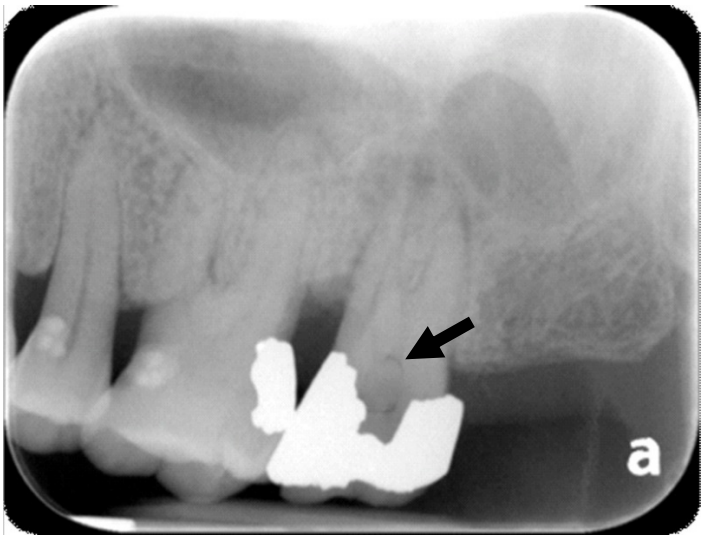

Figure 1.1 Pulp stone (black arrow) in pulp chamber of tooth #27

Figure 1.2 Conglomeration of pulp stones (black arrow) associated with extirpated pulp tissue

Calcifications in the radicular pulp tend to be more difficult to identify on radiographs and present as smaller, more diffuse and more irregular bodies. In addition to commonly being found near the apical foramen (Bernick et al, 1975), diffuse calcifications are also commonly found in association with neurovascular tissues (Bernick, 1967). Clinically, these calcifications can be challenging for endodontic treatment as they may impede access to infected areas of the root canal system (Figure 1.2).

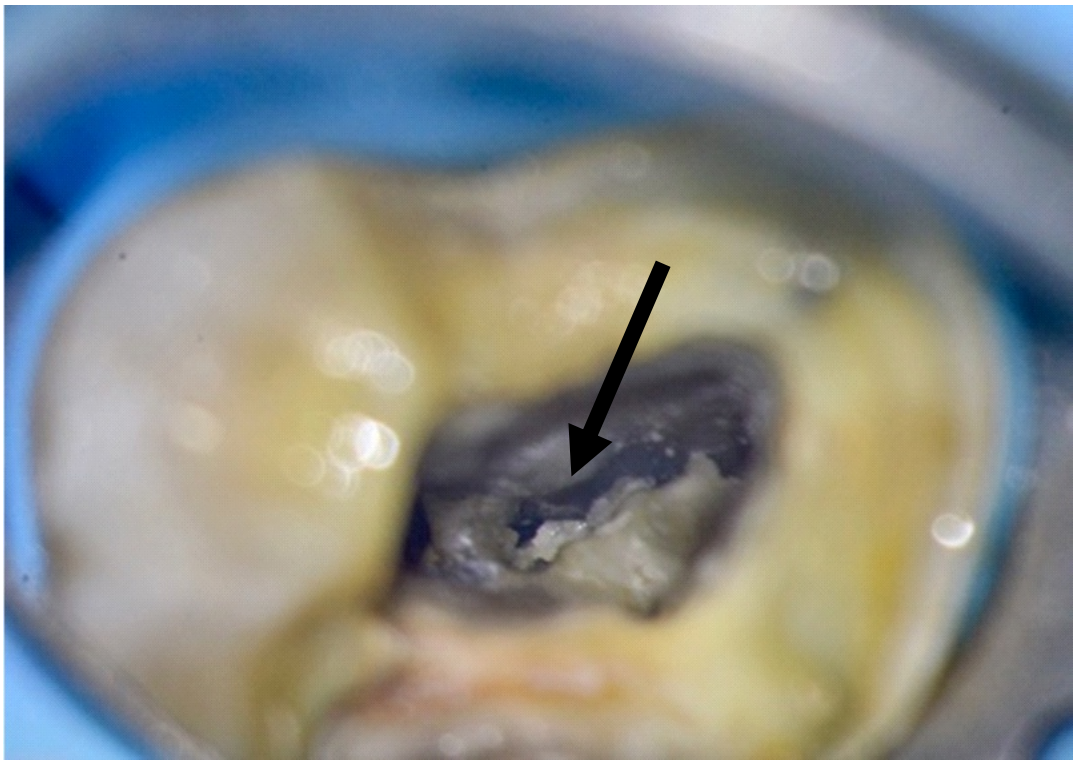

Figure 1.3 Pulp calcifications (black arrow) in the radicular root canal system. These can block access to the apex and impede complete cleaning and shaping of complex root canal systems.

Etiology of pulp stones is poorly understood. There are certain genetic conditions which seem to predispose teeth to formation of pulp stones. These include Van der Woude syndrome, dentin dysplasia and dentinogenesis imperfecta (Kantaputra et al. 2002). Recently, pulp stones have been found to appear commonly in patients with systemic diseases such as cardiovascular and renal diseases (Yeluri et al, 2015). Other etiological factors include pulp degeneration, orthodontic treatment, chronic inflammation, long standing irritation such as caries, cracks, restorations and periodontal disease (Goga et al, 2008).

Pulp calcifications have been classified on the basis of location, composition and structure. Kronfield classified pulp stones as “true”, “false” and “diffuse” based on their composition (Kronfield, 1933). “True” stones were considered to be composed of dentin while “false” stones

were considered to be composed of calcified material devoid of dentinal tissues. “Diffuse” calcifications were small calcified tissues distributed throughout the pulp tissue. This structural characterization may inherently imply the origin of the calcified bodies. However, the way these structures are formed, is still unknown. Moss-Salentijn and Klyvert (Moss-Salentijn et al, 1983) felt that, because stones could be composed of a conglomeration of dentin and non-dentin tissues, they would be better classified on their mode of development. These authors preferred the term “denticle” for stones composed of dentin whereas the term “pulp stone” was generally applied to any structure forming around calcified tissues (Moss-Salentijn et al, 1988). The latter terminology remains vague because the origin of calcification simply cannot be concluded with certainty.

Histological examination of pulp calcifications has shown the presence of smooth, spherical entities as well as amorphous structures. It appears that the rounder structures are formed by the addition of concentric layers whereas the irregular structures may be formed by simple mineralization of existing tissues (LeMay et al, 1991). In addition, mineralized tissues have been found to surround epithelial cells suggesting their source as remnants of Hertwig’s epithelial root sheath (Stenvik et al, 1970). These may differentiate into odontoblast like cells forming dentin like tissues.

Understanding the heterogeneity of pulp calcifications, Goga et al. presented a simplified table in their 2008 review paper, outlining the general terminology of pulp stones. The following terminology was presented in that review paper:

Pulp stone	True	Made of dentine and lined by odontoblasts
	False	Formed from degenerating cells which mineralize
	Free	Stone not related to pulp space wall, surrounded by soft tissue
	Adherent	Stone attached to wall of pulp space, not fully enclosed by dentin
	Embedded	Stone enclosed within canal wall, less attached than the above
Denticle		An alternative term for pulp stone, more usually a calcification filled with epithelial remnants surrounded by odontoblasts
Fibrodentin		Material produced by fibroblast-like cells against dentin prior to differentiation of a new generation of odontoblast like cells
Dystrophic calcification		Inappropriate biomineralization of the pulp in absence of mineral imbalance

Table 1.1 Goga's classification of pulp stones

The physicochemical composition of pulp stones has also been studied in an attempt to establish the process that lead to their formation (Berès et al, 2016). Studies have used X-ray diffraction and X-ray fluorescence to determine the precise constitution of pulp stones. These have revealed the presence of P, Ca, Cu, Zn and Sr in apatite structures (Berès et al, 2016).

Recently, Wang et al used GW to clean uninstrumented canals in premolars and identified mineralized structures such as pulp stones throughout the canal system (Wang et al, 2018). More mineralized tissues were detected in the middle and apical thirds of the root canals. The examined teeth in this study were intact and free of caries or restorations, implying that these structures consisted normal root canal anatomy.

Regardless of their composition, origin or etiology, pulp calcifications have in common their potential to hinder adequate root canal treatment (Ibarrola et al, 1997). Larger, free stones occurring in the pulp chamber (Figure 1.3), can be more readily removed with existing instruments and techniques; however, it is smaller or attached calcifications which can complicate root canal treatment. Because these calcifications are composed of crystalline, apatite structures, their mechanical removal is not as straightforward as tissue removal. As well, the calcified tissues cannot be dissolved by irrigants. It is established that pulp stones can pre-operatively exist in the apical portions of the root canal system (Bernick et al, 1975). However, they can be intra-operatively displaced into these more difficult to reach areas as well. In both scenarios, adequate dissolution and disinfection of necrotic tissues may not be possible. Complete obturation is also compromised and the success of endodontic therapy is reduced.

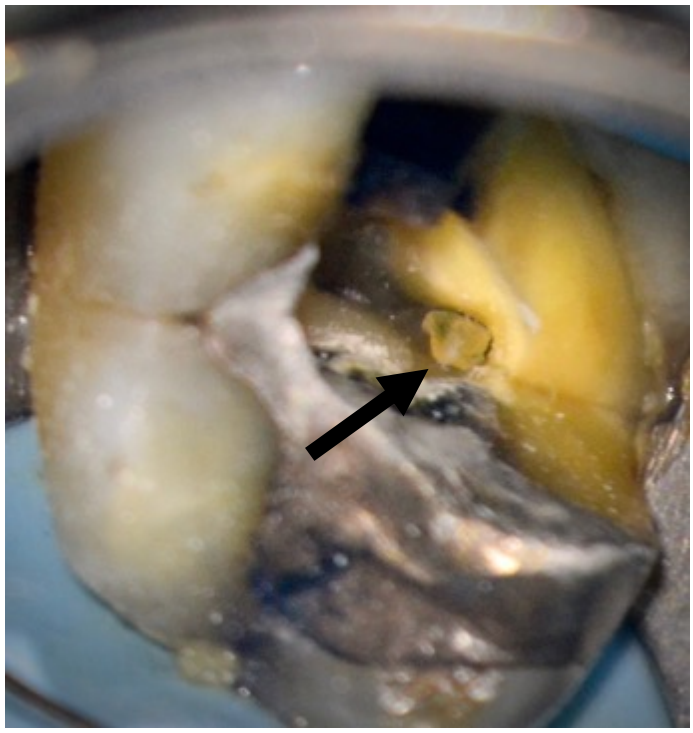

Figure 1.4 Pulp stone (black arrow) over the orifice of the palatal canal in a maxillary molar

Strategies to predictably remove pulp calcifications could improve the prognosis of root canal treatment. Not only would success be improved, but the treatment time could be further reduced benefitting both clinician and patient as well.

2.1.5 Managing calcified root canal systems

Many studies exist proposing methods to manage calcified root canal systems. Calcified systems result in smaller pulp spaces which can make orifice location and canal negotiation much more difficult during endodontic therapy.

In a study by Cvek et al (1982), healing of apical periodontitis in teeth with PCO was evaluated, and higher failure rates were detected in mandibular anterior teeth due to increased rates of iatrogenic errors caused by canal obliteration, such as root perforation (Cvek, 1982). In addition, it was speculated that the access required to locate obliterated canals further weakened cervical tooth structure, causing these teeth to be at increased risk of root fracture. Many techniques have been proposed to circumvent these complications. The surgical operating microscope has proven to be essential in locating obliterated canals (Seldon, 1989; da Cunha et al, 2009, Johnson, 2009; Reis et al, 2009). Krasner and Rankow have proposed rules for orifice location that may improve successful identification of canals (Krasner et al, 2004). In addition, a variety of burs and ultrasonic tips have been designed to locate canals blocked by calcification; methylene blue and the NaOCl "bubble test" have been proposed as well.

In teeth where mineralized tissue is laid down in the form of pulp stones, similar challenges to endodontic therapy are encountered as well. Success in endodontic treatment depends on successful negotiation of the root canal system. When a calcified body obstructs full access to the system, therapy becomes compromised. Langeland reviewed this clinical implication when discussing pulp stones (Langeland, 1967). Their presence could obscure the root canal space, further blocking or deflecting instruments from the root canal's natural path. Inability to achieve patency due to these blockages can compromise endodontic success (Ng et al, 2011).

Techniques have been suggested in managing pulp stones. UItrasonics are frequently recommended to dissect and remove larger calcifications from pulp chambers. When more diffuse calcifications are encountered in the radicular pulp, more coronally located bodies have enhanced opportunity for retrieval. Here, pulp stones are more readily located and accessed. Yet, it is known that pulp stones exist throughout the root canal spaces and can frequently occur in the apical

regions of the canal (Bernick et al, 1975). These can also occur via iatrogenic introduction of calcifications into the root canals. In order to locate and access these calcifications, dentin removal would be required. In addition, if an attempt is made to bypass the obstructions, additional dentin removal results, further compromising the integrity of the tooth. No system has yet been developed to tackle the challenge of removing pulp stones predictably and conservatively.

2.1.6 CBCT and calcifications

CBCT is becoming a more commonly used diagnostic tool in the clinical setting today. It adds tremendous value to endodontic diagnosis, treatment planning and follow-up (Patel et al, 2015). CBCT has the advantage of providing high resolution images in three dimensions, allowing for enhanced interpretation of canal anatomy, apical pathology and healing (Patel et al, 2015; Dutra et al, 2016). Another common use for CBCT is for the location of calcified canals that would otherwise be indiscernible on routine periapical radiographs (Ball et al, 2013). Allowing the clinician to see a reduced canal space in three dimensions provides the clinician a better sense and guide to conservatively access a contracted canal space. In a recent study by da Silva et al, CBCT was found to be a valuable tool in detecting pulp stones (da Silva et al, 2017). However, the authors could not support the use of CBCT for routine pulp stone identification. As well, the study seemed to focus on localisation of pulp chamber calcifications as CBCT provides minimal information on calcified structures within root canal spaces (da Silva et al, 2017).

Despite its limitations in locating specific calcified bodies within root canal spaces, CBCT can be very valuable in providing specific measurements that may guide the clinician to successfully negotiate a calcified root canal system (Yang et al, 2016). CBCT can provide the

clinician with information as to where, how deep and at what angle a canal may be obliterated by calcification. This allows access to calcified spaces to become a procedure that is more "quantitative" as opposed to "empirical" further improving the predictability and prognosis of root canal treatment (Yang et al, 2016).

Recent studies have gone so far as to use CBCT measurements of root canal systems obliterated by dystrophic calcifications to manufacture custom guides providing clinicians precise access to these otherwise challenging systems (van der Meer et al, 2016; Krastl et al, 2016).

2.1.7 Micro-CT studies on pulp stones

Many studies over the past decade have used micro-CT to analyze root canal anatomy and dentin debris removal by various irrigation and instrumentation systems (Paqué et al, 2009; Paqué et al, 2012). However, very few studies have used micro-CT to assess the presence of pulp calcifications. In a study by Markvart et al, 3-dimensional scanning by micro-CT and its ability to detect smaller volumes such as reduced pulp cavities and mineralized deposits was assessed (Markvart et al, 2012). They were able to identify largely reduced pulp chambers associated with calcifications covering canal orifices. As well, "sporadic" distributions of mineral deposits in all segments of the root canal were identified as "connected" to the canal walls or as separate entities. In younger teeth, with larger pulp chambers, mineralized areas were only seen in the apical portions of the root canal and as such, it was speculated that calcifications of root canals may begin in those regions. They also confirmed the commonly accepted notion that the presence of these calcifications complicated root canal therapy.

Micro-CT has proven to be a useful tool in allowing 3-dimensional analysis of root canal systems without destroying the integrity of the tooth under examination. Micro-CT cannot be used in the clinical setting as an adjunct to patient care, however it is valuable for ex vivo studies. Its high resolution allows the identification of canal contents that would otherwise not be identifiable during routine clinical radiographic examination. In addition, the 3-dimensional location of these contents can also be discerned.

2.1.8 Infection

Primary bacterial infection of the dentin-pulp complex occurs as bacteria multiply and invade dentinal tubules (Dahlen, 1991). The vital pulp exerts an outward pressure of fluids protecting itself from imminent insult (Tønder, 2009). However, when insult dominates, and odontoblasts and coronal pulp tissues are destroyed, bacteria advance more readily (Nagoaka, 1995). As inflammation of the pulp turns to necrosis, bacteria invade the root canal system, multiply and initiate the progression of the host's immune response leading to inflammation (Sundqvist, 1976). Once inflammation spreads to periapical tissues, the result is periapical periodontitis.

Root canal treatment addresses periapical periodontitis and comprises access to the infected dental tissues, chemomechanical preparation of the root canal system and finally obturation of the root canal system.

2.1.9 Chemomechanical preparation

The goals of chemomechanical preparation are to remove infected organic and inorganic tissues, to provide a space that can be readily disinfected and cleaned, to provide a space that can allow for sealing of the root canal system, preventing further invasion, and finally to respect and conserve dentin (Walton, 1976; Fabricius, 2006; Peters et al, 2011).

The careful balance of disinfection and dentin conservation is a challenging one to attain. Minimal coronal access and instrumentation have the advantage of dentin conservation; however, can limit access to infected areas (Moore et al, 2016) or increase the chances of iatrogenically generated mishaps (Rover et al, 2017). These include, transportation of canals, zipping, perforation and instrument separation. Mishaps can further impede complete access to the root canal system resulting in reduced success of treatment (Peters, 2004). As well, predictably obturating smaller preparations can present a challenge in itself.

On the other end, larger preparations improve access to anatomical complexities from both disinfection and obturation perspectives but at the risk of predisposing the tooth to fracture. Apical preparation to a size 30 allows irrigant access to the apical third of the canal (Khademi et al, 2006), however acceptable foraminal enlargement for maximum disinfection is still subject to debate. Adequate apical enlargement is a current focus of study for disinfection as there is no predictable means of chemically disinfecting the root terminus without providing access through mechanical preparation.

Different methods have been proposed to obtain the optimum balance between disinfection and dentin conservation. These range from non-instrumentation systems to instrumentation protocols with maximum tapers and apical diameters. Lussi et al introduced a technique where

instrumentation was eliminated with a hose, valve and pump system (Lussi et al, 2002). This system was placed over the access cavity and irrigants were delivered into the pulp chamber. It has not been shown to provide complete disinfection of root canals (Lussi et al, 2002) and therefore superior systems applying techniques of minimal preparation need to be developed.

More recently, GentleWave has been introduced as a novel technique to clean and disinfect the root canal system. Irrigation hydrodynamics of this system may provide a means of allowing more conservative preparations while allowing maximum disinfection as well (Sigurdsson et al, 2018).

2.1.10 Mechanical preparation

To date, endodontic files have been primarily used to mechanically remove tissues and prepare the root canal system. There are a variety of manual and rotary files with varied metallurgies and configurations. They all have in common, the mechanical removal of organic and inorganic tissues from root canals. Endodontic instruments are generally designed to provide a continually tapering root canal preparation allowing for maximum penetration of irrigants and predictable obturation. Schilder promoted the idea that root canal instrumentation create this continuously tapering shape from orifice to apex while following the original canal anatomy (Schilder, 1967). He suggested that this method of cleaning and shaping contribute to the three-dimensional filling of the root canal space (Schilder, 1967).

Mechanical preparation inadvertently removes dentin from the root canal walls and alters the original root canal anatomy. Weine et al described some of these alterations as straightening of the root canals, apical transportation, ledging, zipping and teardrop preparations (Weine et

al,1975). These procedural complications contribute to mechanical instruments' inability to completely rid the root canal of infected tissues. Furthermore, mechanical instruments are incapable of reaching tissues in apical recesses and ramifications, isthmuses and canal fins (Nair, 2006; Weller et al, 1995).

There is debate as to the ideal preparation size for optimal root canal disinfection. Khademi et al analyzed apical debris under SEM and concluded that the minimum apical preparation size required for penetration of irrigants was a size 30 (Khademi et al, 2006). The goal is to establish a conservative method of preparing canals so that a balance can be attained where minimal dentin is removed while maximum bacterial load is reduced. As endodontically treated teeth have been shown to be at higher risk of mechanical failure than vital teeth (Reeh et al, 1989), respecting the structural integrity of these teeth is critical in maintaining their success.

2.1.11 Chemical effects of irrigating solutions

The main purpose of chemical preparation is to dissolve and flush out organic tissue and debris while disinfecting any remaining infected tissues (Zhender, 2006; Sjogren et al, 1997). Endodontic irrigants are primarily used for these processes and are used in conjunction with different delivery and activating systems. The ideal irrigant has the following properties: ability to dissolve organic and inorganic tissues, bactericidal or bacteriostatic activity, ability to remove smear layer and penetrate anatomical complexities of the root canal, biocompatibility with the host's tissues, lubricating action during mechanical preparation and maintenance of the structural integrity of dentin (Haapasalo et al, 2010).

NaOCl is commonly used to irrigate root canals as it is an effective antimicrobial and tissue solvent. Concentration of NaOCl used during endodontic therapy ranges from 0.5 to 6% with greater concentrations leading to greater level of disinfection (Gomes et al, 2001). Despite NaOCl's potent activity, Haapasalo et al showed that the organic components of the root canal can buffer its effects (Haapasalo et al, 2000) . NaOCl cannot remove the inorganic component of the smear layer formed during instrumentation (Spangberg et al, 1973). In addition, NaOCl is toxic to vital tissues and can result in detrimental effects if extruded significantly beyond the root canal system (Pashley et al, 1985; Hulsmann et al, 2000). With these described limitations, adjunct irrigation techniques allowing controlled replenishing or enhanced activity of NaOCl, need to be developed.

Chlorhexidine is another potent antimicrobial irrigant and causes precipitation of bacterial cytoplasmic contents at higher concentrations (McDonnell, 1999). It also possesses substantivity allowing it to retain antimicrobial effect as it binds to hard tissues (Leonardo et al, 1999; Dametto et al, 2005; Carrilho et al, 2010). CHX differs from NaOCl in that it does not have the property of tissue dissolution. Without this desirable effect, CHX as an irrigant alone, may not remove all residual tissue from canals potentially affecting complete obturation of the root canal space (Haapasalo et al, 2010). Complementing CHX irrigation with NaOCl irrigation may provide improved tissue dissolution when compared to CHX irrigation alone, however the combination of these solutions has been shown to produce the dark red/brown precipitate PCA, a potential carcinogen when combined with NaOCl (Kolosowski et al, 2014).

Neither NaOCl nor CHX have the ability to remove smear layer. The smear layer is a 1-2 μm film formed during canal instrumentation composed of dentin debris, bacteria and necrotic tissues (Sen et al, 1995). It can prevent adequate disinfection of dentinal tubules by blocking

irrigant penetration to these areas and can provide potential for voids once dissolved post obturation. Chelating agents such as EDTA and citric acid have been added to NaOCl irrigation regimens during endodontic therapy to help remove smear layer. These acids have the ability to dissolve inorganic tissues and as such, the inorganic components of the smear layer (Baumgartner et al, 1984, 1987). When used in combination, NaOCl and chelating agents can effectively remove smear layer (Teixeira et al, 2005). Despite their advantage in aiding smear layer removal, EDTA and citric acid are weak antimicrobials. As such, these solutions on their own, are ineffective in disinfecting the root canal system.

2.1.12 Irrigation hydrodynamics

As the process of irrigation has the goal of disinfecting and cleaning root canals, systems designed to optimize the physical dynamics of fluids in addition to their chemical properties, have been developed over time (Siqueira et al, 2002; Gu et al, 2009; Caron et al, 2010; Halford et al, 2012). Hydrodynamics is the field of physics that studies fluid flow and the forces created by these fluids. These forces are used in endodontics to improve irrigant access to complex areas as well as to disrupt tissues that may otherwise be difficult to access by mechanical instrumentation alone (Cunningham et al, 1982; Cachovan et al, 2013).

2.1.13 Syringe needle irrigation (SNI)

Probably the most traditional method of irrigation is by needle irrigation. This method uses irrigant loaded in a syringe with an attached needle that is penetrated into the pulp chamber and root canals.

Positive pressure results as the clinician pushes the barrel of the syringe to extrude irrigant into the root canal space (Park et al, 2013). There is a variety of needle tips with differing gauges, exit portals and venting areas.

Many variables influence the efficiency of SNI. These include penetration depth, canal size, volume of irrigant, contact time, velocity of irrigant and needle type and size (Teplitsky et al, 1987). Chow suggested that for debris to be removed from the apical regions of the root canal, irrigant delivery needed to be as close to the apex as possible where it could create a current and allow removal of particles (Chow, 1983). The depth of needle penetration depends on apical enlargement and needle size (Chow, 1983). The smaller the apical preparation, the smaller the needle diameter needs to be in order to effectively debride the apical canal (Chow, 1983).

In their 1975 study, Baker et al found that the volume of irrigant was more important than type of irrigant when examining debris removal (Baker et al, 1975). In a recent study, Zorzin et al showed that greater irrigant volume was more effective in removing $Ca(OH)_2$ from canals than irrigant activation (Zorzin et al, 2016). It is also widely accepted that replenishing irrigant, and hence increasing volume, is critical to canal disinfection, as dentin can delay the antibacterial effect of conventional endodontic irrigants (Morgental et al, 2013).

Limitations of SNI are limited irrigant access to apical ramifications and isthmuses as well as vapor lock. Vapor lock is a phenomenon described by Gu et al referring to the entrapment of air in the apical 0-2 mm of the root canal (Gu et al, 2009). It has been shown that irrigant fails to progress further than 1-3 mm from the needle tip (Park et al 2012; Munoz et al, 2012; Boutsioukis et al, 2010) due to fluid dynamics. Vapor lock further limits irrigant advancement as it produces bubbles at the needle tip (Pesse et al, 2005; Tay et al, 2010). Manually lifting the needle up and

down into the root canal may agitate solution but newer irrigation methods have been developed to address the aforementioned limitations.

Finally, apical extrusion of solutions by SNI is a concern, especially in relation to caustic agents like NaOCl. As SNI involves positive pressures, there is risk of extruding solution past the apical foramen into surrounding vital tissues. Extrusion of NaOCl can lead to detrimental effects with significant impact to the patient (Pashley et al, 1985). Causing tissue oxidation, hemolysis, ulceration and cell destruction (Pashley et al, 1985), NaOCl contact with vital tissues can result in a "hypochlorite accident." Despite its rare incidence, the hypochlorite accident can lead to severe complications (Guivarc'h et al, 2017).

To circumvent potential adverse events, different needle designs have been developed. Needle design impacts the fluid flow beyond the needle vent. Open ended needles result in higher irrigant velocities, penetration, and turbulence when compared to side vented needles (Chen et al, 2014). Regardless, side vented needles are recommended as these are safer to position near the apical constriction where irrigant exchange is more localized and poses less risk of extrusion (Chen et al, 2014).

2.1.14 Manual dynamic irrigation (MDI)

A simple and non-invasive method of maximizing irrigant access into anatomical root canal complexities includes MDI. This process involves placing a well-fitting GP cone into the prepared root canal system flushed with irrigant. The GP cone is subsequently moved up and down in a pumping motion resulting in agitation of solution. The agitation hydrodynamically forces fluid into ramifications, isthmuses and accessory canals (Gulabivala et al, 2010). Studies have shown

that this method improves irrigant access to areas otherwise untouched by simple needle irrigation (Caron et al, 2010; Huang et al, 2008; McGill et al, 2008).

2.1.15 Sonic and Ultrasonic activation

Sonic and ultrasonic activation of irrigant are used to maximize root canal cleaning and disinfection. Acoustic streaming and cavitation are thought to be generated by these methods but whether these effects are produced in the root canal remains controversial. Sonic activation of irrigants involves placing a sonically driven instrument into the root canal system. Sonic devices, such as the EndoActivator system (Dentsply Tulsa Dental Specialties, Tulsa, OK), make us of the hydrodynamic phenomenon of oscillation where the instrument vibrates within the root canal at frequencies of 1–10 kHz (Gu et al, 2009). Sonic activation has been shown to be superior to non-agitation methods in producing cleanliness of the root canal system (Caron et al, 2010; Jiang et al, 2010), however some studies doubt the impact of sonic agitation as it has not yet been shown to produce cavitation (Macedo et al, 2014).

Ultrasonic activation works very much like sonic activation however the instruments are vibrated at higher, ultrasonic frequencies of 25-35 kHz. Ultrasonic activation was first proposed for root canal treatment by Richman in 1957. It has since been used specifically for activation of irrigants and the term "passive" has been added to ultrasonic activation (PUI). The confines of the root canal wall were found to reduce the effects of cavitation when US instruments contacted the canal walls (Ahmad et al, 1987). Passive ultrasonic activation allows tips to oscillate freely within the canal space maximizing oscillation amplitude. Ultrasonic activation of irrigants has been found to produce acoustic microstreaming (van der Sluis et al, 2007) while cavitation has been demonstrated with PUI as well (Macedo et al, 2014). When comparing sonic and ultrasonic

activation methods, ultrasonic agitation appears to be more effective in removing dentin debris (Jiang et al, 2010). Producing higher turbulence at the vibrating tip as well as enhancing fluid interaction with the root canal wall, US activation may have improved potential to disrupt root canal biofilm (Chen et al, 2014). Ultrasonic agitation has also been found to improve irrigant penetration into dentinal tubules at the apical third of the root canal when compared to manual dynamic irrigation, sonic activation, or rotary finishing file (Paragliola et al, 2010).

2.1.16 Negative pressure irrigation

Negative pressure systems like the EndoVac system (Discus Dental, Culver City, CA) rely on suction from the root canal to safely irrigate the apical portions of the root canal system (Miller et al, 2010). Rather than forcing irrigant to the apex, a microcannula is placed close to working length while irrigant is delivered to the pulp chamber. The irrigant and debris are evacuated from the canals by the microcannula through suction.

The EndoVac system has been shown to produce less irrigant extrusion (Mitchell et al, 2010) and at the same time increased irrigant penetration to the apical extent of the root canal when compared to needle syringe irrigation (Chen et al, 2014). However, whether the hydrodynamic forces produced by EndoVac are able to adequately clean and disinfect the root canal system, has been questioned (Chen et al, 2014). Nevertheless, Nielsen and Baumgartner showed that EndoVac was able to significantly better debride the apical 1 mm of the root canal when compared to needle irrigation (Nielsen et al, 2007). Mancini et al also showed superior smear layer removal from the apical extent of the root canal when EndoVac was compared to needle irrigation and sonic activation (Mancini et al, 2013). When the ability to reduce bacterial load was examined, an in

vivo randomized control clinical study failed to show superior bacterial elimination when EndoVac irrigation was compared to needle irrigation (Pawar et al, 2012).

2.1.17 Laser activated disinfection

Laser-activated irrigation (LAI) has been introduced as a powerful method for root canal irrigation (Blanken et al, 2007; George et al. 2008). The laser radiation produces transient cavitation in the liquid through optical breakdown by strong absorption of the laser energy (Blanken et al, 2007). Lasers have been used in conjunction with irrigants to optimize root canal disinfection. Delivered via a needle, wire and fibre placed to working length, lasers were originally shown to provide dentin debris removal (de Groot et al, 2009) and smear layer removal (Peters et al, 2010). More recently, a photon induced photoactivated streaming (PIPS) system has been developed to deliver pulsed energy through tips that need only be placed in the pulp chamber. This minimally invasive delivery method allows the clinician to provide irrigant access to apical portions of the root canal while reducing dentin removal otherwise required for this access.

In a study by Peters et al, PIPS was able to better reduce apical bacterial biofilm than US (Peters et al, 2011) . PIPS has also been shown to have superior dentin and smear layer removal when compared to non-agitation methods of irrigation (Gunesar et al, 2015, 2016). The fact that PIPS has been found to produce turbulent irrigant flow in uninstrumented areas of the root canal has several implications (Llyod et al, 2014). PIPS may generate improved cleaning of anatomical complexities such as isthmuses and fins. As well, the need for larger canal preparations otherwise created to improve irrigant access, may not be required allowing for the conservation of dentin.

2.1.18 Nanoparticles

Nanotechnology has taken advantage of nanoparticle properties to allow for medication delivery, antimicrobial action and disinfection applications. Nanoparticles are synthetic or natural particles whose external dimensions range in the size of 1-10 nm (Kishen et al, 2016). The small size may allow for dentinal tubule penetration where antimicrobial activity can be delivered to disinfect bacterial biofilm (Kishen et al, 2016).

Delivery of chitosan nanoparticles into dentinal tubules has been demonstrated but depends on cavitation for its delivery (Shrestha et al, 2009). Kishen et al have shown that cationic nanoparticles can reduce the adherence of certain bacterial species (Kishen et al, 2008). Silver nanoparticles have also been shown to eliminate bacterial biofilm but only when used in intracanal medicaments, not as irrigants (Wu et al, 2014). It is due to the need for prolonged contact time that nanoparticles have appeared more promising as intracanal medicaments or as components in sealers rather than as endodontic irrigants (Kishen et al, 2016). A recent study on iron oxide nanoparticles demonstrated an effective disinfection approach which may circumvent the temporal limitations of previously studied nanoparticles (Bukhari et al, 2018). In this study, a 10-minute application of nanoparticulate iron-oxide showed a peroxidase like antimicrobial effect that was more effective than conventional irrigants like CHX and NaOCl (Bukhari et al, 2018).

2.1.19 GentleWave

The GentleWave system is a novel root canal cleaning system that uses multisonic energy and complex fluid dynamics to remove tissue and debris from the complexities of the root canal system. GentleWave has the ability to achieve this debridement with minimal to no preparation of root canals while still delivering its action to complex apical regions (Molina et al, 2015). Multisonic energy and cavitation work in conjunction with chemical irrigant activity to provide clean root canal surfaces, and further allow penetration of irrigants to otherwise blocked or difficult to reach areas. Cavitation is the process that releases energy from imploding vacuum bubbles, allowing this force to dissolve and disintegrate tissues. This process has been implied as a main contributing factor to GentleWave's excellent tissue dissolution abilities (Haapasalo et al, 2104).

The GentleWave system consists of a console and treatment instrument that delivers irrigants into the accessed pulp chamber (Haapasalo et al, 2014). A custom tip is placed into the delivery instrument and sits 1 mm above the pulp chamber floor, never entering the root canals. The control panel regulates delivery of degassed irrigants, flowing at rates of 45 ml/min at temperatures of 45°C. To prevent leakage of fluids to the oral cavity, the pulp chamber is sealed with a block out resin (Kool-Dam; Pulpdent, Watertown, MA) which also takes an impression of the head of the instrument, ensuring an intimate fit.

GentleWave has been shown to provide superior tissue dissolution when compared to conventional irrigation methods. A study by Haapasalo et al showed GentleWave's ability to dissolve tissues at significantly higher rates than ultrasonic agitation, negative pressure systems and traditional needle irrigation (Haapasalo et al, 2014). GentleWave has the advantage of allowing deeper penetration of NaOCl into apical ramifications (Vandrangi et al, 2016) further

debriding the root canals of debris, smear layer and bacteria (Molina et al, 2015). GentleWave creates negative pressure at the apex (Haapasalo et al, 2016) with no extrusion of irrigant (Chakara et al, 2016), providing a safer means to apical disinfection as compared to needle irrigation. Wang et al further showed that GentleWave causes minimal dentin erosion (Wang et al, 2016).

In addition to superior cleaning, disinfection and debridement when compared to conventional irrigation methods, GentleWave has been shown to remove separated instruments (Wohlgemuth et al, 2015) as well as intracanal medicaments from the root canal system (Ma et al, 2015).

Perhaps most exciting is GentleWave's ability to produce superior cleaning results without the need for excess dentin removal. Sigurdsson's recent case series demonstrated a 12-month success rate of 97.7% in teeth with necrotic pulps and periapical lesions. In the study, canals were prepared with no orifice enlargement nor apical enlargement beyond a size 20 (Sigurdsson et al , 2018). With the possibility that GentleWave can provide clinically superior success rates while maintaining original root canal anatomy, the novel irrigation system may provide the utmost balance of dentin preservation and canal disinfection.

2.1.20 GentleWave fluid dynamics

GentleWave uses concepts of multisonic streaming and cavitation to clean and disinfect the root canal system. The application of acoustic energy for root canal debridement is not a new concept, however current systems require significant access to debride apical regions of the root canal. This significant access often occurs at the expense of dentin and eventually, integrity of tooth structure.

Application of sonic energy is termed sonication. Cavitation is the primary driving force of sonication and refers to the growth and collapse of microbubbles producing high energy. These microbubbles oscillate, grow and collapse producing shock waves, microjets, turbulence or shear forces (Ashokkumar, 2010; van Wijngaarden, 2015). Medical applications of ultrasound include emulsification, extraction and cleaning (Ashokkumar, 2010). Cavitation has been used to deactivate bacteria in wastewater and has been shown to be an effective cleaner of ultrafiltration membranes (Drakopoulou et al, 2009). These actions provide significant clinical relevance to root canal therapy. For one, bacteriostatic or bactericidal effects are ideal properties of endodontic irrigants, and second, the ability to clean surfaces otherwise accessible only by mechanical means has tremendous advantages. In industrial processes, the more current processes of cleaning membranes involve lengthy, non-productive and environmentally detrimental results (Yusof et al, 2015). This can be compared to the currently unfavourable effects of dentin removal which is necessary to access root canal surfaces during disinfection and debridement. The latter may result in the undesirable result of tooth fracture or tooth loss.

Chapter 3: Goals and Hypothesis

Root canal calcifications can be found in many forms including attached structures, embedded tissues or free-standing entities. These can lead to difficulty in locating orifices, instrumentation of canals or success in achieving patency. Many strategies have been proposed to manage these calcifications however few studies have examined the pre-operative location of calcifications and techniques for their subsequent removal prior to instrumentation. Free or attached calcifications are often displaced during instrumentation and can subsequently lead to blockage of canals. Calcification evacuation or removal, prior to mechanical instrumentation would be ideal so that any risk of impediment can be avoided. An unobstructed root canal system would be easier to access, clean and instrument and inherently obturate to the apical constriction. No study so far has examined the GW system's ability to remove calcifications from unprepared root canals.

The specific aim of this study was to examine the ability of GW to remove calcifications from unprepared root canal systems. Identification of a new method for calcification removal can have significant clinical relevance for root canal treatment. Removing impediments such as pulp calcifications can improve access to the apical constriction of root canals and aid in establishing patency. As success of orthograde root canal treatment is significantly influenced by achieving patency at the canal terminus (Ng et al, 2011), providing a means to unobstructed instrumentation may improve root canal outcomes. Furthermore, if GW can significantly remove pulp canal calcifications without the need for canal enlargement, the goal of dentin conservation can be maintained.

The null hypothesis is that the GW system does not have the ability to significantly remove calcifications from the root canal system.

Chapter 4: Materials and Methods

4.1 Tooth selection

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, BC. The study was approved by the University of British Columbia Office of Research Services, Clinical Research Ethics Board (Certificate Number: H12-02430).

Approximately eighty mandibular first and second molars with a single mesial and a single distal root were selected. Any teeth with decay or fractures below the cemento-enamel junction (CEJ), internal or external root resorption, open apices, or previous root canal therapy were excluded from the study. After exclusion, thirty molars were selected for sample preparation. Any presence of calcifications was unknown at this point in our study.

4.2 Pre-treatment sample preparation

Upon selection, calculus and soft tissue debris were removed from the outer surface of teeth with hand-scaling instruments. Any coronal areas destroyed by fracture or caries were restored with etch, prime and bond, and flowable composite resin. Access cavities were prepared with a high-speed handpiece and diamond burs with water coolant. Pulp tissue was removed from the pulp chambers with microdebriders, hand instruments and 6% NaOCl irrigation. Canal orifices were identified and #8 or #10 K-files, depending on canal size, were advanced into the canals to ensure patency. A size #6, 8 or 10 K-file was advanced 1 mm beyond the apical foramen, but no filing

was performed. Any teeth with inadequate access, unnegotiable distal canals or more than one distal orifice or canal were excluded from the study. The twenty teeth that remained were assigned random sample numbers that were carved into the coronal surface with a high-speed bur for identification purposes. Our final sample size (n=12) was a result of some samples being destroyed or lost during scanning or cleaning procedures.

4.3 First Micro-CT scan of the instrumented root canals

All selected samples were scanned using a Micro-CT 100 (SCANCO Medical AG, Basserdorf, Switzerland) to assess distal root canal anatomy and presence of calcifications. The following settings were used: isotropic voxel size of 15 μm, tube current of 88 μA and energy of 90 kVp. The settings used in this study were used to optimize the quality of the scanned images. The data was analyzed using the following software packages: ImageJ and MeVisLab 2.6 OS X Edition (MeVis Medical Solutions AG, Bremen, Germany). MeVis Lab was used to calculate canal volumes from pulp chamber floor to apical constriction. Attached and unattached calcifications from pulp chamber floor to apical constriction were identified based on differences in greyscale contrast relative to root dentin. Canal volume calculations involved adding attached or free calcification volumes to empty canal space volume as follows,

Total canal volume = calcification volume + empty canal volume

After calcification volumes and canal space volumes were calculated, the relative percent volume of canal calcification could be determined. The volume percent was thus the calcification volume in percent of the total canal space.

The following equation was used:

Volume percent = calcification volume/canal volume x 100%

Examples of calcifications are shown in figures 3.1, 3.2, 3.3 and 3.4. Free, attached and diffuse calcifications are illustrated.

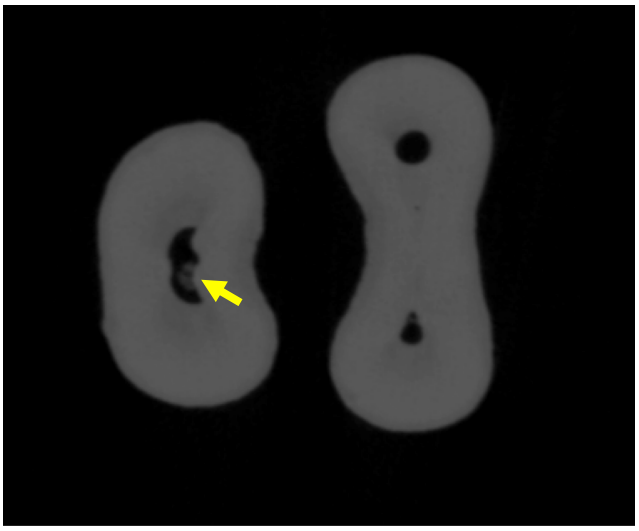

Figure 4.1 Yellow arrow shows attached calcification

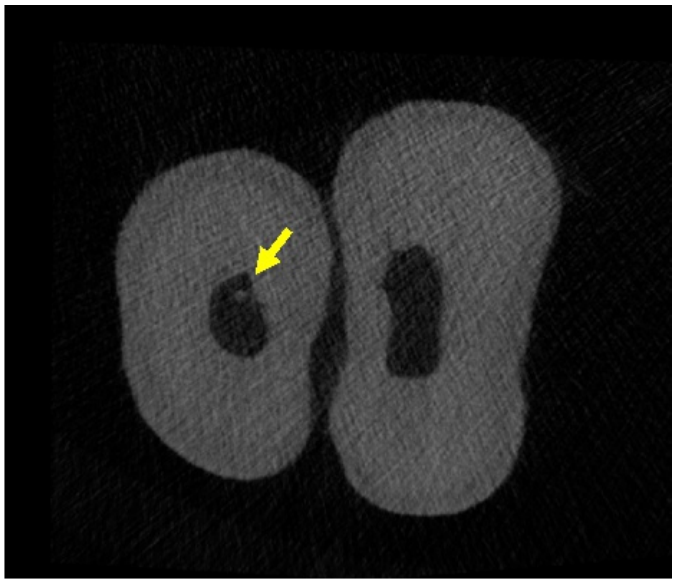

Figure 4.2 Yellow arrow shows unattached calcification

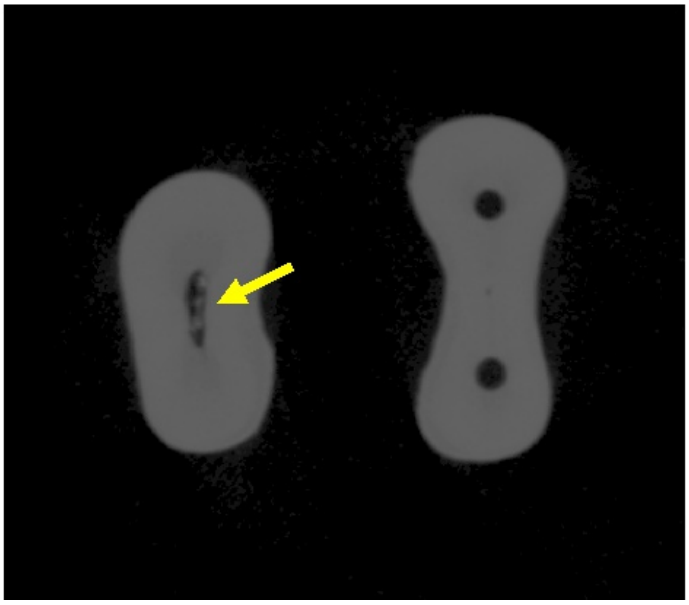

Figure 4.3 Yellow arrow shows attached calcification

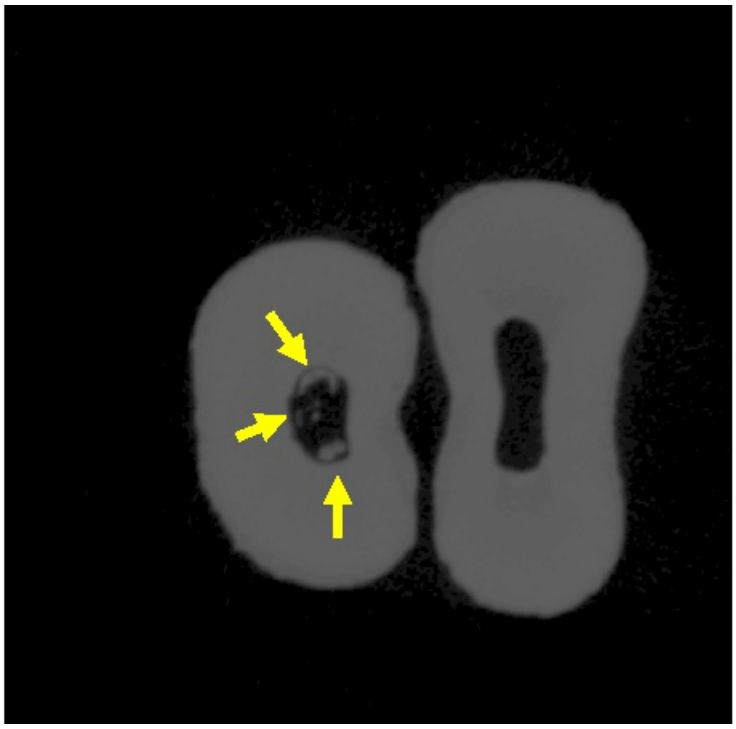

Figure 4.4 Yellow arrows shows attached and unattached calcifications

4.4 Root canal irrigation

After examining the initial micro CT images, samples were cleaned by the GentleWave™ system (Sonendo, Inc, Laguna Hills, CA) as per manufacturer recommendations (see below). The protocol for GentleWave treatment involves applying the GentleWave treatment instrument to the access opening of each tooth. Prior to placement, a flexible material is placed over the occlusal table followed by placement of the instrument head onto this material to ensure a custom fit. Once this impression is made, the composite material is cured so that the instrument can be replaced into the impression. The pulp chamber is then re-accessed through the composite platform. The platform ensures that the GW hand piece can properly seal the tooth's access cavity and prevent leakage of the irrigating solutions to the oral cavity. A nozzle placed into the handpiece hub is sized and then

placed into the access opening such that its tip is 1mm from the pulp chamber floor, ensuring that it does not touch the floor during treatment. GentleWave irrigation protocol is then started: a 3% NaOCl flush for 5 minutes (45 mL/min), followed by a distilled water flush for 15 seconds, an 8% EDTA cycle for 2 minutes, and finally a distilled water flush for 30 seconds. Total treatment time is 7 minutes and 45 seconds with irrigating solutions released at high speed flow (45 ml/min) and at a temperature of 45°C. During the irrigation protocol, irrigants are evacuated from the pulp chamber and root canal system through small suction holes present in GentleWave treatment instrument. The irrigation system creates negative pressure in the root canal which has been shown to prevent extrusion of irrigants past the apex (Haapasalo et al 2016; Chakara et al, 2016).

4.5 Second micro-CT scan of the cleaned root canals

After cleaning, samples were scanned using a Micro-CT 100 (SCANCO Medical AG) maintaining the same settings as in the first scan. Images were viewed with ImageJ and imported into MeVisLab for data analysis. Total canal volumes and calcification volumes were calculated as in the first sample data analysis.

4.6 Statistical analysis

Data was tested for normality and the appropriate statistical test was used to assess changes in canal volume and calcification volumes before and after GW treatment. Data demonstrating a normal distribution (Kolmogorov-Smirnov test $p>0.05$), was assessed by the student's t-test. Data that did not follow a normal distribution (Kolmogorov-Smirnov test $p<0.05$), was assessed with

the non-parametric Mann-Whitney test. Percent reduction of calcification was also calculated. All statistical tests were analysed by SPSS software (SPSS Inc, Chicago, IL).

Chapter 5: Results

Data was evaluated for normal distribution to determine the appropriate statistical test which could compare differences in canal volume and canal calcification before and after GW cleaning. As canal volumes demonstrated a normal distribution (Kolmogorov-Smirnov test $p>0.05$), the student's t-test was applied to assess changes before and after GW cleaning. Calcification volume and volume percent did not follow a normal distribution (Kolmogorov-Smirnov test $p<0.05$), therefore the non-parametric test, Mann-Whitney test, was applied to assess changes before and after GW cleaning.

The student's t-test showed no statistically significant change in canal volume before and after GW cleaning ($P>0.05$). Calcification volume and volume percent showed significant changes when comparing values before and after GentleWave cleaning ($p<0.05$). These changes are illustrated in Figures 4.1 and 4.2 which show images of samples pre and post GW cleaning. Mean values of canal volume, calcification volume and volume percent pre and post GW cleaning are shown in Table 4.1.

The values of canal volume, calcification volume and volume percent for each sample before and after GW cleaning are shown in Tables 4.2, 4.3, and 4.4 respectively. The changes in canal volume, calcification volume and volume percent are illustrated in figures 4.3-4.5, respectively.

The percent reduction of calcification ranged from 60-100% with a mean reduction of 87.60% (±14.90). Percent reduction values for each sample are shown in Table 4.5.

Free, attached and embedded calcifications were found at all levels within the root canal system before GentleWave cleaning. All types of calcification were found in the coronal, middle and

apical portions of the root canal and appeared in a random fashion. Pulp stone type did not seem to follow a particular pattern with respect to their occurrence throughout the root canal system.

After GentleWave cleaning, it was observed that most of the calcified bodies had been removed from the root canals in our sample. It was also observed that some calcifications had been displaced. Although not calculated specifically, it appeared that most of the displaced calcifications had been relocated to the apical portions of the root canals in our sample.

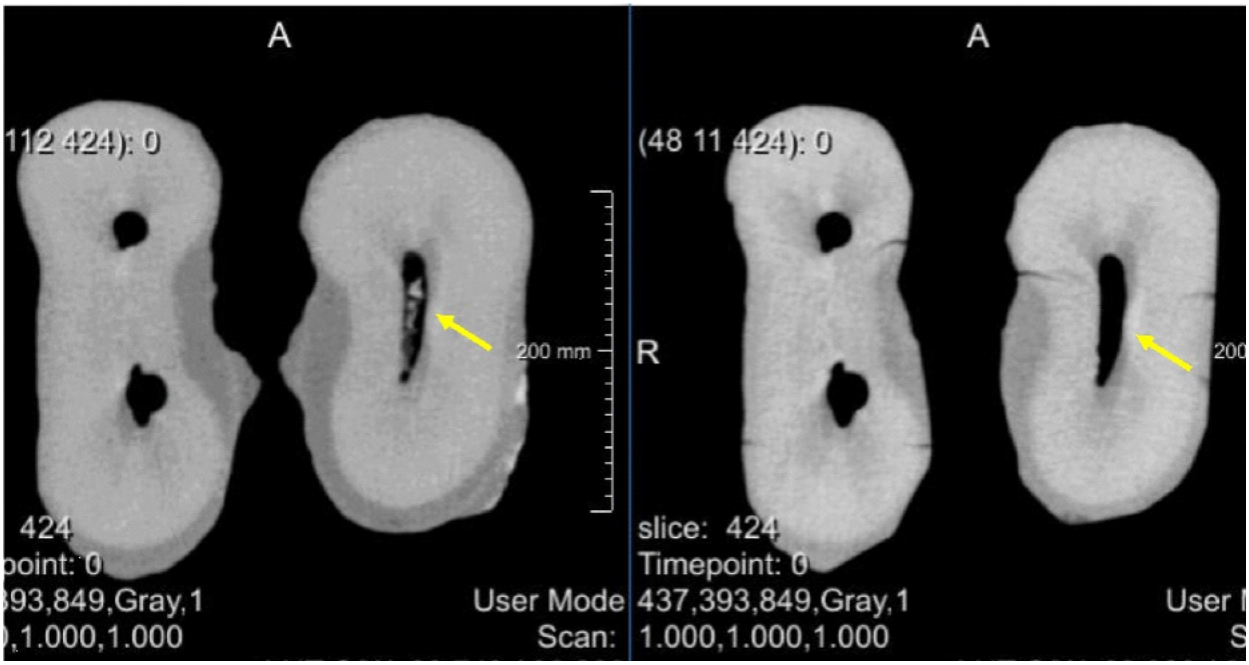

Figure 5.1 Canal calcification before and after GentleWave cleaning

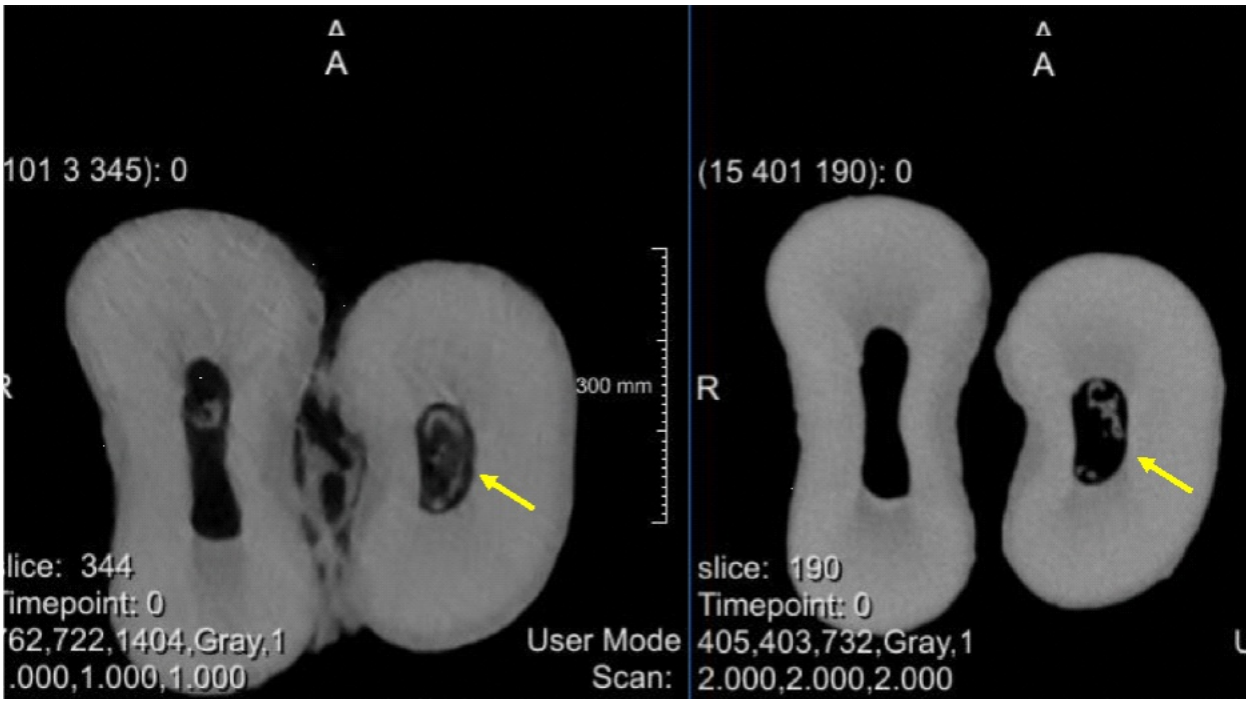

Figure 5.2 Canal calcification before and after GentleWave cleaning

	Mean values before GW cleaning	Mean values after GW cleaning
Canal volume (mm^3)	4.95 +/- 2.37	5.42 +/- 2.66
Calcification volume (mm^3)	0.48 +/- 0.40	0.06 +/- 0.11
Volume percent (vol%)	8.35 +/- 6.45	0.80 +/- 1.15

Table 5.1 Mean and standard deviation values of canal volume (mm^3), calcification volume (mm^3) and volume percent (vol%) before and after GW cleaning. Changes in calcification volume and volume percent were found to be statistically significant (Mann-Whitney test, $p < 0.05$). Changes in canal volume were found to be statistically insignificant (student t-test, $p > 0.05$).

Volume of each canal (mm^3)		
	before GW	after GW
1	8.70	10.30
2	3.38	3.70
3	9.11	9.11
4	4.68	4.69
5	5.61	5.58
6	3.97	3.99
7	3.77	5.54
8	6.33	7.73
9	1.96	2.20
10	2.26	2.21
11	2.91	2.94
12	6.66	7.11

Table 5.2 Volume of distal canals (mm^3) for each sample (n=12) before and after GW cleaning

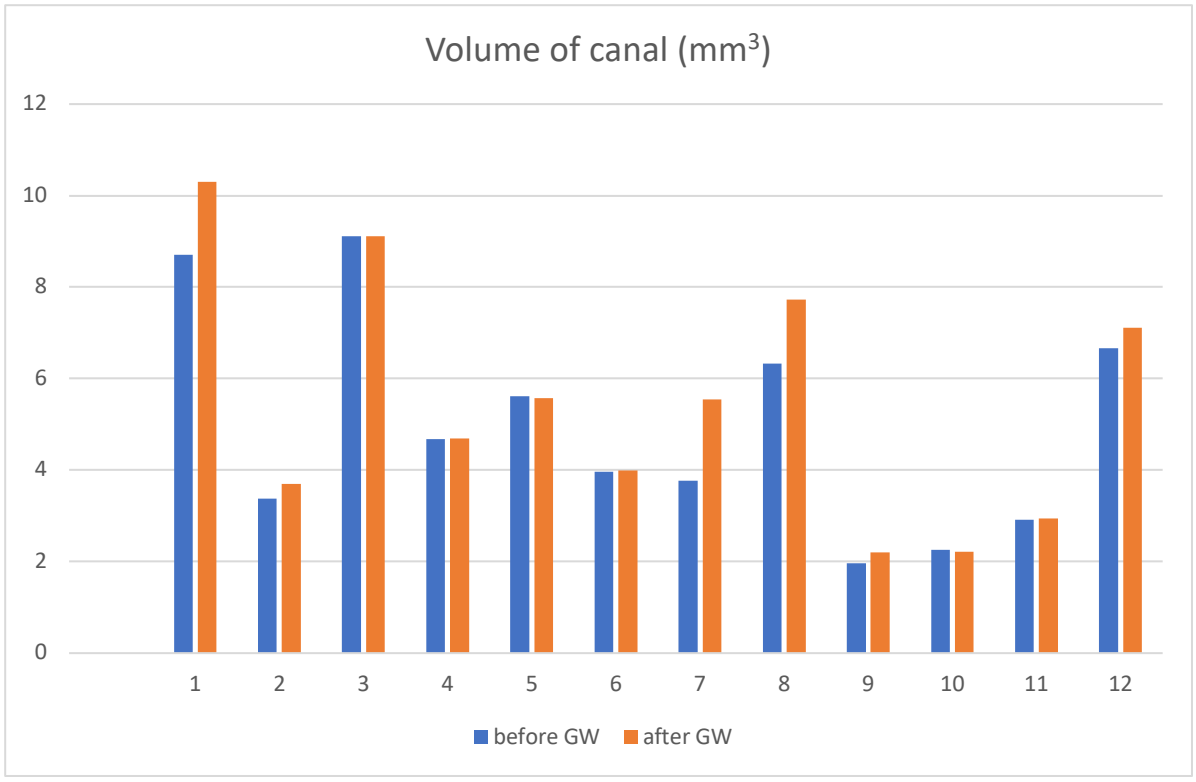

Figure 5.3 Volume of distal canals (mm^3) for each sample (n=12) before and after GW ($p > 0.05$)

Volume of calcification in each canal (mm^3)		
	before GW	after GW
1	0.86	0.38
2	0.11	0.02
3	0.36	0
4	0.14	0.02
5	0.66	0.12
6	1.19	0.07
7	0.64	0.07
8	1.07	0
9	0.18	0
10	0.04	0
11	0.09	0
12	0.45	0

Table 5.3 Volume of calcifications in distal canals (mm^3) for each sample (n=12) before and after GW cleaning

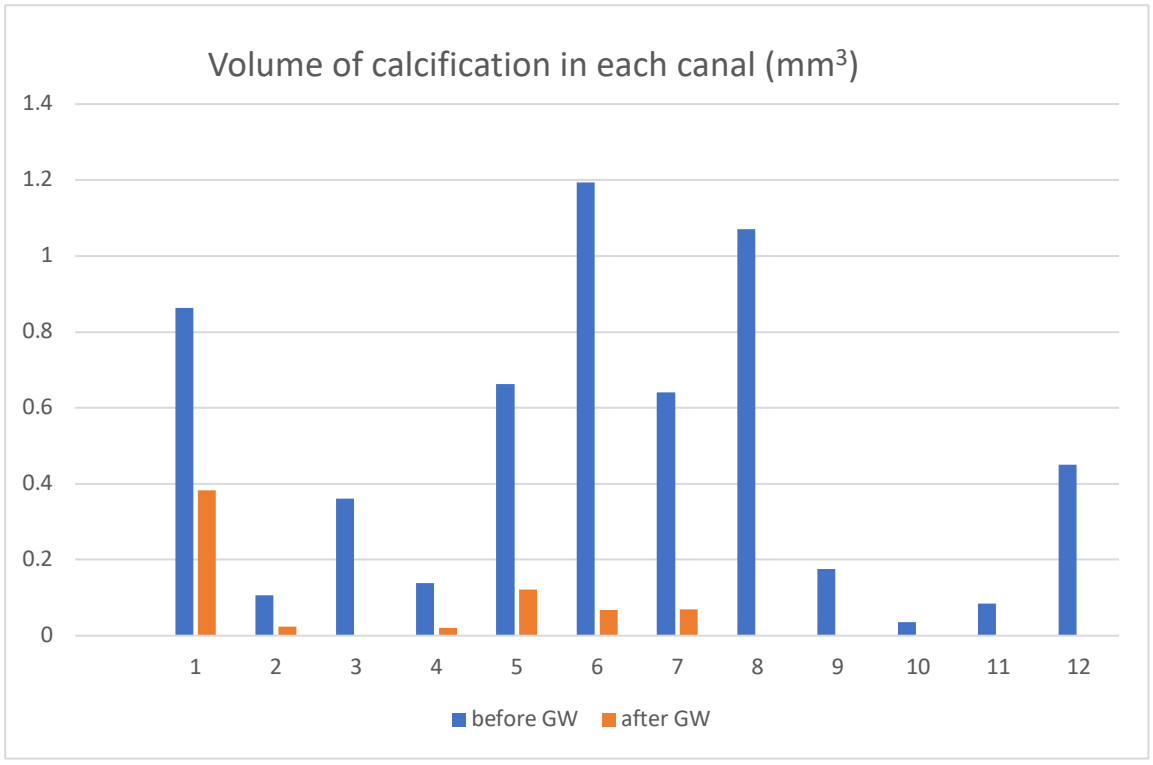

Figure 5.4 Volume of calcifications in distal canals (mm^3) for each sample (n=12) before and after GW cleaning ($p < 0.05$)

Percent volume in each canal (vol%)		
	before GW	after GW
1	9	3.6
2	3.1	0.6
3	3.8	0
4	2.9	0.4
5	10.5	2.1
6	23.1	1.7
7	14.5	1.2
8	14.5	0
9	8.2	0
10	1.5	0
11	2.8	0
12	6.3	0

Table 5.4 Calcification volume percent in distal canals (vol%) for each sample (n=12) before and after GW cleaning

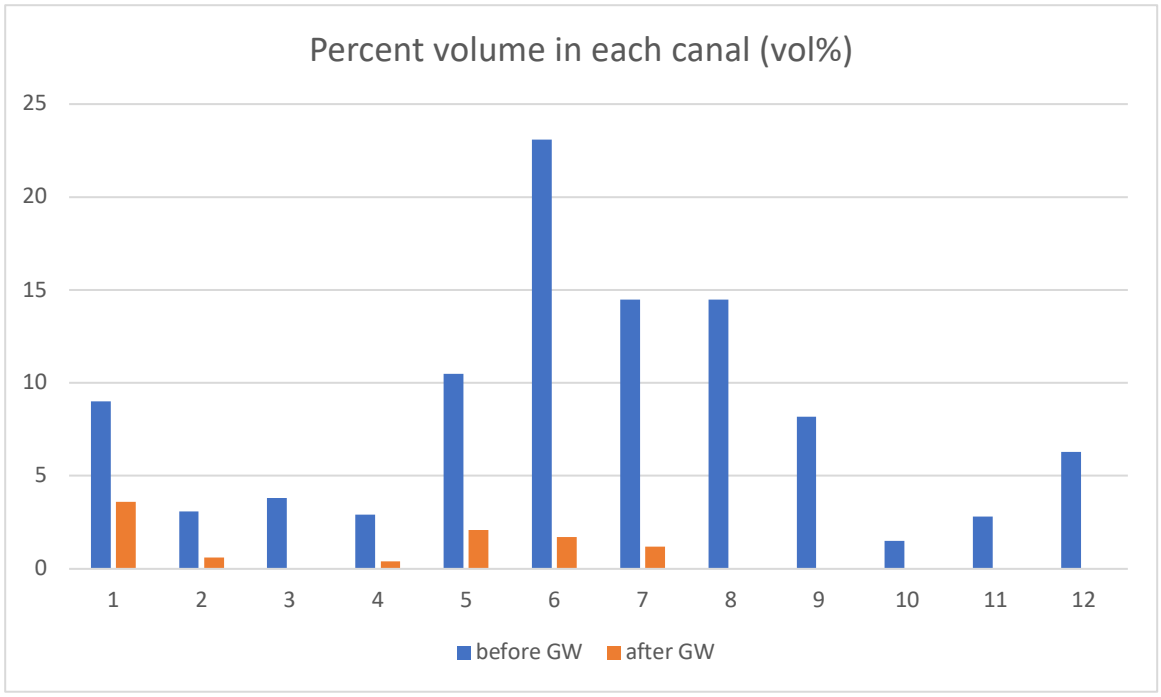

Figure 5.5 Calcification volume percent in distal canals (vol%) for each sample (n=12) before and after GW cleaning ($p < 0.05$)

Reduction in calcification volume in each canal (%)	
1	60
2	80.65
3	100
4	86.21
5	80
6	92.64
7	91.72
8	60
9	100
10	100
11	100
12	100

Table 5.5 Percent reduction in calcification volume in distal canals (%) for each sample (n=12)

Chapter 6: Discussion

The current study is the first one to establish GentleWave's ability to significantly remove calcifications from the distal root canals of mandibular molars without any mechanical instrumentation. Finding a novel method to remove calcifications from root canals is an exciting development as these blockages can present a considerable clinical challenge during root canal therapy. Furthermore, establishing a conservative method to remove pulp stones from canals, provides the clinician with a valuable tool for promotion of dentin conservation.

Micro-CT imaging showed significant reduction in calcification volume after GW treatment. In our study, root canals were examined before and after GentleWave cleaning and three-dimensional changes within the system were recorded. Prior to GentleWave treatment, we identified different types of calcifications. Free calcifications were easiest to detect due to their presence as separate entities within the canal space. Attached or embedded structures presented more of a challenge during data analysis. A grey scale was used to differentiate calcifications from canal walls and this would, at times, present difficulties in providing clear boundaries between these structures. This added a qualitative or subjective element to our data analysis when calculating volumes. Despite these challenges, we were able to confirm the presence of calcifications in all distal root canal systems of mandibular molars in our sample. Free and attached calcifications could be seen at coronal, middle and apical thirds of the root canal. There was no specific pattern to the distribution of calcified bodies and no conclusion could be drawn with respect to the level of the root canal they would most likely to be encountered.

Despite not being able to derive any conclusion as to the general distribution of calcifications within the distal canals, it was interesting to identify pulp stones in 100% of our samples. As much as CBCT is not a part of routine radiographic examination in the clinical setting,

it can help achieve a better understanding of the complexities that we may encounter during root canal treatment. Our micro-CT findings suggest that we can expect to encounter calcifications at any level within the root canal system, and on perhaps more common occasion than previously expected. Clinically, it is more conventional to radiographically identify pulp calcifications in the pulp chamber itself. Radicular calcifications cannot be seen with periapical radiography unless they present as larger structures or as extensions of pulp chamber calcifications.

Clinical examination of periapical radiographs is often used to differentiate a heavily calcified root canal system from a more open root canal system (Andreasen et al, 1987). The latter observation can only give the clinician a general sense of the difficulties that may be encountered during root canal preparation. Our micro-CT analysis of pulp calcifications had the advantage in that we were able to identify radicular calcifications in three dimensions. The specific locations of the calcified bodies could be seen more readily and their potential obstruction during mechanical preparation could be better visualized. As no pattern was detected with respect to type and location of calcification, it can be concluded that these calcified bodies may present as blockages at any point during instrumentation of distal canals of mandibular molars.

Identifying the precise location of pulp stones with micro-CT was very informative, however this method could not provide any additional information regarding their origin. We could not categorize our calcifications as described by Goga, as micro-CT is limited to evaluating their presence and location and not their constitution (Goga et al, 2008). Further use of micro-CT to examine pulp calcifications may help in determining their general concentration and perhaps lend ideas as to their physiologic or pathologic origins. It would be interesting to compare micro-CT images and their measurements with pre-operative radiographs of the same teeth to determine if

volume of calcification could be correlated with reduced radicular spaces. We were not able to do this comparison in our study.

After identifying calcification volumes, our samples were treated with the GW system. As GentleWave appears promising in cleaning root canal surfaces without the need for over preparation of canals (Molina et al, 2015), the current study evaluated GentleWave's ability to clear calcifications from uninstrumented canals. The goal was to determine if the irrigation dynamics of GentleWave, physical energy of possible cavitation, and the chemical effects of the irrigants had the ability to clean root canal spaces of calcified bodies. As cavitation has proven to be an effective means of cleaning surfaces in industrial processes (Nor Saadah et al, 2016), GentleWave's irrigation dynamics could potentially provide a minimally invasive method of debriding root canals of calcified bodies. GW's ability to debride the root canals of calcifications prior to instrumentation could have the added benefit of reducing their potential obstruction to proper shaping and cleaning. This could save the clinician time during instrumentation by removing a potential complication from the instrumentation process. Eliminating or reducing blockages would improve access to the root canal terminus, allowing patency to be achieved and finally, improving the prognosis of root canal treatment (Ng et al, 2011).

The present study showed that GW was able to remove calcifications from all parts of the root canal system without instrumentation of the canals. K-files were only introduced into the root canal systems to ensure patency of the canal. No filing was performed with the K-files, minimizing the impact of the file within the canal. The non-instrumentational method of GW is particularly exciting as no other method has been reported to have the ability to access all areas of the uninstrumented root canal system. Traditionally, apical portions of the root canal are only accessible by means of mechanical preparation. The root canal is enlarged to allow for irrigant

flow to the apical regions, ensuring proper debridement. The latter inherently results in the removal of dentin, alteration of original canal anatomy and the production of smear layer (Peters, 2004). GW proved capable of accessing calcifications in all segments of the root canals in our sample, thus circumventing the potential complications that could arise during root canal enlargement. These results suggest that GW could provide a conservative means to effectively clean root canals of calcified material that could otherwise obscure proper disinfection attempts.

Another advantage in comparing our root canal samples pre and post GW treatment in the absence of instrumentation was that we could be confident that we were indeed looking at original canal calcification. The instrumentation process has been shown to result in the accumulation of hard tissue debris (Abou-Rass et al, 1987; Paqué et al, 2009; Paqué et al, 2012; Lloyd et al, 2014). As such, canal preparation produces hard tissue debris which would be more difficult to differentiate from originally occurring pulp calcification. This hard tissue debris created by instruments could complicate calculations evaluating the removal of original calcified bodies only, as opposed to all hard tissues that may form within the root canal system.

In addition to the significant reduction in percent calcification volume, we did notice that some of the calcified bodies were displaced from their original location in the root canals. This could be confirmed when comparing the same micro-CT slices pre and post GW treatment. In these cases, the calcified body was identified only on the post-treatment image implying that it had been displaced from another location. Despite the fact that we could not confirm the original locations of these displaced bodies, most "new" post-treatment calcifications were found in the apical regions of the root canals. Namely, it was found that if a pulp stone had been displaced, it had been so to the more apical regions of the root canal system. Whether this relocation is clinically significant is questionable as the GW cleaning was performed with the teeth in an upright position

with the crown of the tooth located upward and apex downward. In a clinical setting, teeth are more likely to be worked on in a horizontal position as the patient is more often reclined. Whether gravitational forces had an impact on pulp stone relocation cannot be discounted in the present study.

Other than the observation of calcification relocation, it cannot be concluded from our study as to how the calcifications were removed from the root canal system. Since there was some evidence of displacement, it can be assumed that attached and free calcifications may have been agitated from their original locations and then evacuated through the GentleWave suction mechanism. It cannot be assessed whether GentleWave's irrigation dynamics also disintegrated the calcifications, further enhancing their removal. However, as the actual canal volumes did not change significantly after GentleWave treatment, it would be difficult to justify GentleWave's ability to completely break down calcifications as it does soft tissues. Having said that, in order to assess whether or not calcifications were possibly fragmented and removed by suction, we measured the greater diameters of larger calcifications in our axial slices and compared those to the diameter of the suction hole in the GentleWave handpiece. We found a range of calcification sizes with larger diameters ranging from 0.58 to 1.18 mm. The diameter of the suction mechanism was measured at 1.16 mm. With the smaller dimension of the evacuation hole, the larger calcifications may have been fragmented or dissolved in order to be removed. As well, as seen in figure 4.2, the calcifications that appeared pre GentleWave treatment were sometimes reduced in volume post GentleWave treatment (observed at the same slice). This would also imply some form of fragmentation of the calcified entities. This conclusion is simply speculation, however, as the precise method of calcification removal was not the focus of our study.

Our study did not have a control group to compare GW's ability to remove pulp stones with other currently suggested methods. However, there are no other methods in clinical or experimental use today that could be used to remove calcified bodies or any material from unprepared root canal systems. Instrumentation and enlarging the canal space is required for ultrasonic and other systems to be introduced into the canal. During this, most of the calcifications are likely to be lost; it is the instrumentation process that destroys the calcifications or displaces the pulp stones into more apical regions of canals. We chose to evaluate naturally occurring calcifications in root canals and assess the effect of GW on their removal. Had we added a control group with instrumented canals, some pre-existing pulp stones may have inadvertently been displaced, further complicating the traditional methods of pulp stone removal. Now that it has been established that GW is able to remove calcifications from the distal root canals of mandibular molars, perhaps an extension of the current study would involve comparing GW's and an ultrasonic agitation system's ability to debride the system of pulp stones in minimally prepared canals. As we did see that, in some cases, GW tended to displace calcifications in an apical direction as would instrumentation, the latter would provide a fair comparison.

Another limitation of our study was the sample size. Paqué et al have established sample size numbers for evaluating dentin debris removal by various irrigation systems (Paqué et al, 2006, 2009, 2012). Sample sizes of n=6 were shown to be effective in quantitatively comparing different instrumentation/irrigation regimens on the accumulation of dentin debris (Paqué et al, 2006). As no other study has determined adequate sample sizes for calculating calcification volumes, a power calculation from an initial group of samples was performed to determine a sample size that would show changes demonstrating significant power. A sample size of n=28 or 2 groups of 14 were calculated for power of 0.90. Our sample size of n=12 resulted from a loss of some samples during

treatment. Nevertheless, our calculations still demonstrated significant differences when assessing removal of calcification from distal root canals with the GentleWave system.

Chapter 7: Conclusion

The present study revealed some exciting findings related to GentleWave's ability to significantly reduce the volume of canal calcifications in uninstrumented distal root canals in mandibular molars. We hypothesized that GW would not show any significant difference in calcification volume before and after cleaning. The null hypothesis was thus rejected as the present study demonstrated a statistically significant reduction of calcification volume from root canals post GW cleaning. In fact, almost half of our samples were completely cleared of calcified structures. Of particular interest is that GW was able to significantly reduce calcification without any prior instrumentation of the root canals. Traditionally, some kind of orifice enlargement and canal instrumentation are required to access blockages, such as calcifications, encountered in root canals. We were surprised to find reduction of calcification from all levels of the root canal system. There is no clinically or experimentally available technique that has the ability to clean the entire root canal system without any prior instrumentation.

We also noted that there was no statistically significant change in canal volume before and after GW cleaning. This supports GW's capacity to clean root canals with maximum dentin conservation. The ability to maintain root canal volume during cleaning allows for preservation of original canal anatomy, a desirable outcome in endodontic therapy. For GW to rid the original root canal system of potential blockages while maintaining its natural form brings endodontic cleaning and shaping to a new level. For years, the delicate balance of dentin preservation and maximal canal disinfection has been sought. This study certainly demonstrated GW's ability to bring us closer to the ideal balance.

It should be noted however, that 100% reduction of calcification volume was not observed in all of our samples. Some samples retained calcified bodies and we observed that some remained attached to canal walls or were relocated to different parts of the root canal system. A limitation of our study was that GW cleaning was performed on our samples with the tooth in an upright position, crown up and apex down. This does not replicate the clinical setting and allows forces other than those provided by GW's irrigation hydrodynamics to influence the relocation of the calcified bodies. Future studies may want to reposition the teeth during GW treatment in a more clinically relevant position.

We did not have a control group in our experiment as this was the first study to evaluate a system's ability to remove calcifications from an uninstrumented root canal. As GW was not able to provide 100% reduction of calcification volume in our study, minimal instrumentation may enhance GW's ability to remove calcified bodies. A follow up study can compare GW's ability to remove pulp stones in a minimally instrumented canal with its ability to do so in an uninstrumented canal, or further compare the latter two with a more traditional method of irrigation, such as ultrasound.

It is an accepted idea that canal calcifications present significant challenges to the clinician during endodontic therapy, however their prevalence may be underestimated. The fact that calcifications were identified in 100% of our sample was unexpected. Micro-CT has the tremendous advantage of allowing us to observe what may not be obvious upon clinical examination. We did indeed observe that calcified structures may be present in root canals more often than not. This finding reinforces the need to establish clinically applicable methods for calcification removal.

We can conclude from our study that GW presents tremendous promise in removing calcifications from root canal systems. The fact that it has the ability to do so without dentin removal is an added advantage especially with regards to conserving the tooth's original structure.

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