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Effect of the GentleWave System on dentin microhardness and sealer penetration: an in vitro study

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THESIS

EFFECT OF THE GENTLEWAVE SYSTEM ON DENTIN MICROHARDNESS AND SEALER PENETRATION: AN IN VITRO STUDY

by

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In loving memory of Gazal Ince, Halil Ince, Gulcin Bilgic, and Ragip Yildir Bilgic. May their souls rest in eternal peace.

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EFFECT OF THE GENTLEWAVE SYSTEM ON DENTIN MICROHARDNESS AND SEALER PENETRATION: AN IN VITRO STUDY

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ABSTRACT

This study investigates the impact of the GentleWave (GW) System on dentin hardness and sealer penetration in root canal treatment, aiming to assess its potential advantages over conventional techniques. Conventional instrumentation can alter root canal anatomy, necessitating irrigation methods that minimize mechanical impact. The GW System, utilizing multisonic energy to activate irrigation solutions, has been lauded for its advantages in bacterial reduction, superior debris removal, and minimal dentin erosion.

Samples (n=10) were randomly assigned to three groups: control (Group C), traditional root canal treatment (Group T), and GW with minimal instrumentation (Group G). Following irrigation and obturation with AH Plus sealer and gutta percha using cold lateral compaction, transverse slices were obtained at 2-, 5-, and 8-mm distances from the apex. Assessment methods included microhardness testing and scanning electron microscopy (SEM).

Vickers Test results revealed significant differences in hardness values, with Group C exhibiting the highest and Group T the lowest values. In terms of sealer penetration, Group G demonstrated significantly higher penetration than Group T overall ($p = 0.004$). When considering slice locations, no significant differences were observed in the coronal sections. However, in the middle and apical thirds, Group T exhibited significantly lower sealer penetration levels ($p = 0.02$).

These findings suggest that the GW System maintains dentin hardness while promoting enhanced sealer penetration compared to traditional root canal treatment methods. By preserving dentin integrity and facilitating deeper sealer penetration, the GW System may contribute to improved treatment outcomes and long-term success rates in root canal therapy. These results support the adoption of innovative irrigation techniques, such as the GW System, in clinical practice to optimize patient care and treatment efficacy.

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LIST OF ABBREVIATIONS

amu	**Atomic Mass Units**
ANOVA	**Analysis of Variance**
CDJ	**Cemento-Dentinal Junction**
cps	**Counts Per Second**
EDS	**Energy Dispersive Spectrometer**
EDTA	**Ethylenediamine Tetra-Acetic Acid**
GW	**GentleWave**
ICP-MS	**Inductively Coupled Plasma Mass Spectrometry**
ISO	**International Standards Organization**
NaOCl	**Sodium Hypochlorite**
ppm	**Parts Per Million**
SEM	**Scanning Electron Microscope**
VHN	**Vickers Hardness Number**

Chapter 1. INTRODUCTION

1.1. Root Canal Therapy

Endodontic therapy is performed to save teeth with pulpal or periapical diseases caused by an infection or inflammation of the pulp or periapical tissues. The procedure involves removing the tooth's pulp through cleansing the root canal system followed by root canal filling.

1.1.1. Chemo-Mechanical Preparation

Irrigation and instrumentation form the two essential components of efficient root canal cleaning. Mechanical instrumentation plays a crucial role in enhancing cleaning efficiency by facilitating the irrigation solution's access to the apical regions. This expedites the removal of debris and contributes to achieving a higher quality of obturation. The synergy between shaping systems and the available filling materials on the market further ensures optimal outcomes in root canal procedures (1).

For the last couple of decades, new rotary files and systems have been introduced and are still being developed for more effective cleaning. But, even with the most recent techniques, more than 50% of the root canal walls remain untouched by endodontic files due to anatomical complexities such as apical ramifications, lateral canals, isthmuses, etc. (2). Debris accumulation into these inaccessible areas is an undesirable side effect of the mechanical preparation (3). To address this issue, irrigation becomes essential for lubrication, disinfection, debris dissolution, and debridement, particularly in these intricate regions. Various chemical solutions have been introduced as irrigation agents, and among them, sodium hypochlorite (NaOCl) stands out as one of the most widely used irrigants due to its superior antibacterial and organic debris dissolution activities.

The smear layer, defined by the American Association of Endodontists as a residual film of debris on the dentin surface following preparation, was previously considered for retention due to its believed role as a barrier against bacteria and irritants. However, it has been discovered that, in reality, the smear layer impedes sealer penetration and its intended effects (4). In a more contemporary approach, complete removal of this layer is preferred for several reasons, including enhanced diffusion of irrigation solutions, canal medicaments, and sealers, facilitated by a greater number of dentinal tubule openings. Increased sealer penetration into dentinal tubules is important for higher obturation quality, as it contributes to improved antimicrobial activity and mechanical retention, subsequently reducing microleakage, as previously mentioned. (5, 37). Therefore, sealer penetration serves as a reliable indicator of smear layer removal (6).

While NaOCl alone is insufficient for complete removal of the smear layer, Ethylenediamine Tetra-Acetic Acid (EDTA) is commonly employed to enhance smear layer removal. In addition to supporting the antibacterial efficiency of irrigation solutions as a chelating agent (7). EDTA plays a crucial role in this process. The disodium salt of EDTA anion binds to Calcium (Ca^{2+}) ions in dentin at a pH close to neutral, forming an equilibrium and ceasing its action once achieved. Traditional application techniques have shown that its capacity is self-limited. Ongoing investigations aim to discover more efficient ways to apply the solution from a clinical perspective (8).

Explorations into alterations in irrigation solution application, encompassing variations in solution concentration, volume, temperature, application duration, or delivery method, aim to enhance cleaning efficacy. Typically, NaOCl is employed with concentrations ranging from 0.5% to 6% for root canal disinfection, while the widely accepted concentration for EDTA is 17% (9). Increasing the concentration, volume, temperature, and duration of the irrigation solution has shown promise in improving effectiveness, but it comes with the caveat of heightened risks. These include increased toxicity, potential deterioration of living tissues, and adverse effects on dentin structure.

Irrigation solutions are traditionally administered to the root canal using a side-vented needle coupled to a syringe, which is considered the safest and most common method (10, 11). However, despite its widespread use, syringe irrigation is acknowledged as ineffective, particularly in the apical region. Studies have shown that when delivered with passive needle irrigation, solutions progress only 1 mm beyond the tip of the needle. While enlarged apical canals and finer needles may allow for deeper needle placement, improving debridement and disinfection, thorough cleaning of the most apical part of any preparation remains difficult, especially in narrow and curved canals. Additionally, this method poses a risk of having the irrigation solution forced out of the canal into the periapical area increasing the risk of flare-up or NaOCl accidents. Another limitation is that mechanical instrumentation alone cannot improve accessibility to lateral or accessory canals. Consequently, various alternative delivery methods have been introduced to the market, including sonic, ultrasonic, or laser-supported systems (12). These innovative irrigation methods and techniques have been proven to enhance the delivery and efficacy of solutions to lateral canals (13) and to other mechanically inaccessible areas (3).

The combined chemo-mechanical applications in root canal treatment can adversely affect the quality and quantity of dentin, critical for a successful outcome. While the use of rotary files is favored for its ability to expedite preparation and reduce NaOCl exposure time to dentin, it does not necessarily represent the most ideal procedure for the resilience of a tooth (14). Mechanically, the shaping process with endodontic files involves the removal of hard tissue to achieve a larger root canal, resulting in a thinner radicular dentin layer and, consequently, thinner root canal walls by the end of the procedure. Preserving the thickness of the root canal dentin is more desirable for a satisfactory prognosis, particularly in terms of avoiding root fractures (10).

The exposure of dentin structure to various chemicals during endodontic procedures may unfavorably alter its physical properties by causing a loss of collagen and minerals. Specifically, the removal of Magnesium (Mg) and Ca ions from dentin crystals through irrigation is noteworthy (14). Such alterations in dentin, including weight loss or a decreased Ca:P ratio, can have adverse effects on the elastic modulus and flexural strength of this hard tissue (8). Microhardness, another key physical property, may also be impacted by irrigation, given its sensitivity to the chemical composition of dentin (7). Considering this value is crucial when evaluating dentin quality with different treatment techniques, providing valuable insights for assessing the survival rates of endodontically treated teeth.

1.1.2. Root Canal Filling

The primary goal of root canal filling is to minimize microleakage post-treatment. Effective cleaning not only ensures disinfection but also enhances obturation. The penetration of filling materials into dentinal tubules is crucial for improving mechanical locking and the antibacterial effect by encapsulating any residual bacteria. A tight seal is imperative to prevent periapical diseases caused by persistent bacteria, and insufficient obturation can result in the failure of endodontic treatments (15, 16).

Gutta-percha, a commonly used core filling material, lacks bonding characteristics regardless of the application method (15, 17). Consequently, the use of a sealer becomes necessary to fill gaps and irregularities that gutta-percha alone cannot address (18, 19). While gutta-percha remains the gold standard as a core filling material, debates persist regarding the choice of sealer for filling voids.

Endodontic sealers function as lubricants and luting agents, filling spaces inaccessible to the core material and ensuring better adaptation to dentinal walls. Desirably, the sealer material should be adhesive to dentin, forming a hermetic seal. AH-Plus, widely used in endodontics, has been a favored sealer due to the historically higher standards associated with resin-based sealers (21, 22).

Both the obturation technique and the physical and chemical properties of the sealer play a significant role in determining sealer film thickness (23). Microleakage, with its negative impact on the prognosis of root canal treatment, can occur through interfaces like sealer-dentin and sealer-root canal filling material, or voids within the sealer. Hence, the quality of root canal filling hinges on the distribution and adhesion of the sealer to dentinal walls and gutta-percha (20).

Reducing sealer film thickness can help prevent microleakage by minimizing the chance of voids. Some studies suggest that conventional cold obturation techniques may fall short in providing fluid-tight sealing. However, novel rotary instrumentation systems with greater tapers enhance the adaptation of the master cone to the geometry of prepared root canals, allowing for single cone obturation in endodontic treatments (20). This advancement presents a promising approach to achieving more effective and reliable root canal seals.

1.2. GentleWave

Traditional instrumentation in endodontics faces inherent limitations and drawbacks, particularly when dealing with the intricate anatomy of root canals. Complexities in the root canal structure may lead to treatment errors such as root perforation, instrument separation, ledge formation, apical transportation, and overpreparation. These errors can significantly alter the original root canal anatomy, and widening of the root canal has been associated with a negative impact on tooth prognosis by lowering fracture resistance (10). To protect the radicular dentin thickness and avoid from the errors of this step of the treatment, novel irrigation techniques that require minimal mechanical instrumentation have been introduced. GentleWave (GW; Sonendo Inc, Laguna Hills, CA) is one of the systems that generates multisonic energy to activate irrigation solutions for increased efficiency (1, 18).

This device is designed to generate cavitation clouds that lead to the implosion of microbubbles triggered by shear forces during the delivery of the solution from the handpiece. These countless bubbles create a broad spectrum of sound waves within the irrigating solutions, effectively removing tissue, debris, and biofilms from the entire root canal system. The console controls advanced fluid dynamics and acoustics, enabling the fluid to be dispensed at a precise speed of 45 ml/min without the need for the solution to enter the root canal directly. The design of the GW device revolves around generating these cavitation clouds and microbubble implosions, ensuring that the tip of the handpiece is positioned without touching the pulp chamber floor, thereby maximizing efficiency and minimizing risk. Additionally, the system incorporates a 5-point vented suction system to collect excess solution, preventing apical extrusion of irrigants during the procedure (3, 10, 24). This innovative approach, based on providing negative pressure, not only enhances the cleaning process but also ensures that there is no apical extrusion of the irrigants, which is a significant benefit for patient safety and procedural effectiveness (10, 25). By integrating these advanced features, the device offers a sophisticated solution for endodontic irrigation, combining the principles of fluid dynamics, acoustics, and precision engineering to optimize root canal treatments.

Numerous studies in literature highlight the advantages of the GW system. Haapasalo et al. (2014) reported faster organic tissue dissolution with this system (26). Molina et al. compared GW with conventional root canal preparation, concluding that GW with minimal instrumentation showed better results in terms of canal cleaning and residual debris reduction (27). Wang et al. (2016) found minimal dentin erosion with both GW and syringe needle irrigation techniques (28). In 2018, they suggested the possibility of completely cleaning root canals without mechanical preparation using GW, as evidenced by the absence of dentin debris or organic tissue in their study (1). Zhong et al. (2019) also found superior debris removal with GW plus minimal preparation, suggesting the potential for achieving high-quality root fillings (18). Zhang et al. evaluated GW and an active ultrasonic system for their efficacy in root canal disinfection, concluding that both systems were effective, but GW with minimal instrumentation showed lower total amounts and variations of residual bacterial DNA (29). Additionally, GW demonstrated higher penetration of sodium hypochlorite (NaOCl), superior pulp tissue, calcification, smear layer, intracanal medication (10), and filling material removal (30). Remarkably, no extra short-term postoperative pain was reported with the use of GW (11). Clinical studies supporting GW's effectiveness are promising. A study with similar postoperative pain levels after GW treatment claimed a 97.7% success rate for periapical lesion healing determined at the 1-year follow-up (31).

Despite its evident benefits, it's worth noting that GW is currently available only in the United States and Canada, limiting its usage to a relatively small dental community. As the technology gains recognition, its potential for transforming endodontic practices on a broader scale remains an intriguing prospect.

1.3. Aim of the Study

Contradictory perspectives exist regarding the impact of sonic and ultrasonic devices on enhanced smear layer removal (2). Some studies propose limitations in the efficacy of activation methods, indicating challenges in reaching the apex or lateral canals (32). Additionally, certain investigations contend that previously introduced sonic activation devices exhibit no significant difference in sealer penetration compared to conventional irrigation techniques, attributing this to their insufficient capacity for increased smear layer removal (6, 33). Nonetheless, these findings are subject to further verification through SEM analysis for a more comprehensive evaluation (6).

On a different note, while some reports indicate that GW significantly enhances the penetration of irrigation solutions into dentinal tubules (34), there remains a knowledge gap regarding its potential positive effects on the sealing process and its impact on dentin microhardness.

Vickers hardness test is a method for measuring the resistance of a material to plastic deformation caused by indentation, and the hardness number provides a quantitative value for this resistance. The mechanical features of dentin may undergo changes with alterations in chemical composition resulting from irrigation protocols. Microhardness testing serves as a method for determining such changes in chemical structure (8).

This study aims to assess the effects of the GW System on dentin hardness through Vickers microhardness testing and sealer penetration through SEM and EDS evaluations. The null hypothesis posits that, as a novel irrigation technique, GW has no additional impact on sealer penetration levels and negatively affects dentin chemical composition when compared to the syringe irrigation technique.

Chapter 2. MATERIALS AND METHODS

30 freshly extracted and stored in a saline solution upper lateral teeth with mature apices without any decay/resorption were selected for this study (35). Samples were randomly divided into 3 groups (n=10): control group (Group C), traditional treatment group (Group T), GW with minimal instrumentation group (Group G). All the sample preparation steps, experiments, test and analysis were completed by a single examiner.

2.1. Mechanical Instrumentation

For Group C, no treatment was performed in order to get the microhardness values of intact teeth for a comparison.

During mechanical instrumentation and irrigation, Boston University Henry M. Goldman School of Dental Medicine Endodontics Department's conventional endodontic treatment protocol was followed. After the determination of the working length with a #15 K-file, the canals were mechanically prepared by using ProTaper Universal Rotary System (PTU; Dentsply Tulsa Dental Specialities, Tulsa, OK). 1 ml 5.25% NaOCl was used between files during canal preparation in all teeth for debridement.

For Group T, canals were traditionally instrumented with the rotary files up to the apical size of 0.3 mm with 0.09 taper (F3).

For Group G, canals were minimally instrumented with the rotary files up to the apical size of 0.2 mm with 0.07 taper (F1).

2.2. Final Irrigation and Obturation

Prior to the final irrigation, the apexes of all the treatment group teeth were covered by using a conforming resin material to create a closed environment (Figure 1).

Figure 1 Covered Apices of the Samples with a Resin Material

In Group T, 5 ml 17% EDTA and 5 ml 5.25% NaOCl for 1 minute each were used respectively as a final irrigation to remove smear layer by using a syringe needle with safe ended tip.

In Group G, GW irrigation protocol was applied to the prepared canals. In order to be able to perform this protocol, a platform was built with a resin material (SoundSeal, Sonendo Inc.) by placing an APM (Anterior-Premolar) matrix as described in the GW Training Manual as shown in Figure 2.

Figure 2 Resin Platform with a Direct Vision of the Root Canal Orifice

After ensuring there was no blockage in the access cavity by checking with inspection through the platform, the APM handpiece was placed onto the platform and the GW procedure was started. This final irrigation follows this order and time for solutions: 3% NaOCl for 5 minutes, distilled water for 30 seconds, 8% EDTA for 2 minutes, and distilled water for 15 seconds. Figure 3 shows the application of GW. The waste solution in the waste container of the GW console was collected as soon as finishing this protocol for the ion analysis.

Figure 3 Performing GW

Following irrigation protocols, canals were dried with paper points and obturated with an epoxy resin-based sealer (AH Plus Jet; Dentsply Caulk, Milford, DE) and gutta percha (PTU; Dentsply Tulsa Dental Specialities, Tulsa, OK) by single cone technique. Then, the access cavities were sealed with a piece of a Teflon type and temporary filling material (Cavit; 3M ESPE, Seefeld, Germany). During all the steps, all teeth were kept moist with the sterile gauze soaked in sterile water. The specimens were kept in an incubator (Precision Economy Incubator, Precision Scientific, Winchester, VA) at 37°C and 100% humidity for 2 weeks to allow the sealer setting.

2.3. Sample Preparation

Each tooth underwent embedding in resin and polymerized for 8 hours (Figure 4). Subsequently, the resin blocks were transversally sliced at distances of 2, 5, and 8 mm from the apex using a linear precision saw (Isomet 5000; Buehler, Lake Bluff, Illinois, USA) equipped with a 0.5 mm thick diamond blade.

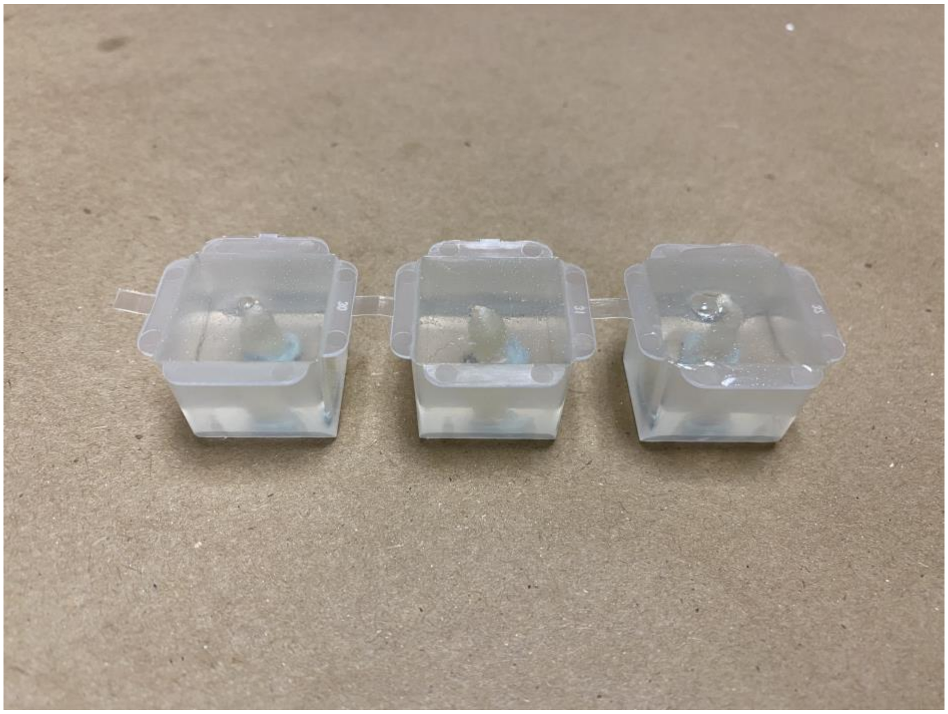

Figure 4 Embedment of the Samples in Resin Blocks

One surface of the dentin discs was designated for microhardness testing, while the opposite sides were allocated for sealer penetration assessments to safeguard the sealer tags from potential interference during the polishing procedure.

After sectioning, the posterior surfaces of the coronal and middle discs, positioned at 3- and 6- mm levels, were polished using a grinder polisher (EcoMet 250, Buehler in Lake Bluff, Illinois, USA). After polishing, the discs were rinsed with distilled water and allowed to air-dry in preparation for the microhardness test.

2.4. Microhardness Testing

The Vickers test was conducted using a microindentation hardness tester (Micromet 2003, Buehler, Lake Bluff, Illinois, USA) to measure the microhardness of the samples. Initially, the unpolished surfaces of the dentin discs were covered with weighing paper to protect the opposite surface designated for sealer penetration evaluation. Four indentations with a 100 g load for 10 seconds were made at 100 μm from the pulp-dentin interface in four directions, each 90 degrees apart, on the polished dentin surfaces as the application set-up can be seen in Figure 5.

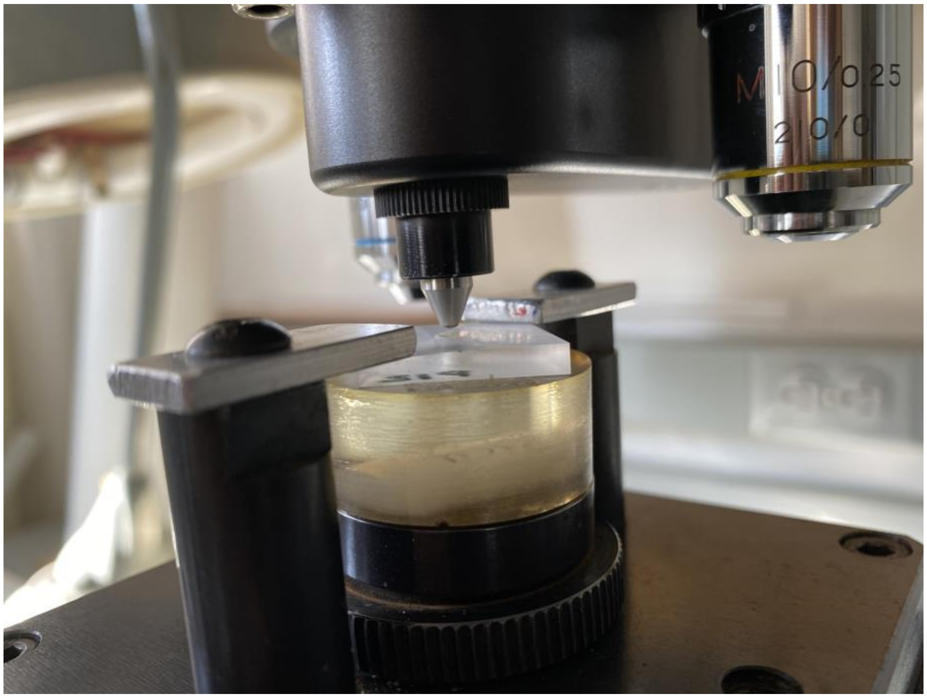

Figure 5 A Sample in a Microindentation Hardness Tester

The equation to calculate the Vickers hardness number (VHN) of a material involves dividing a constant value (1.854) by the square of the diagonal length of the indentation, multiplied by the applied force. This constant value is derived from the geometry of the indenter used in the Vickers hardness test. The resulting numerical value indicates the material's hardness or resistance to deformation under the specified testing conditions. In our analysis, we measured two dimensions of the indentations (Figure 6), obtained mean values for each section, and utilized them for subsequent statistical analysis.

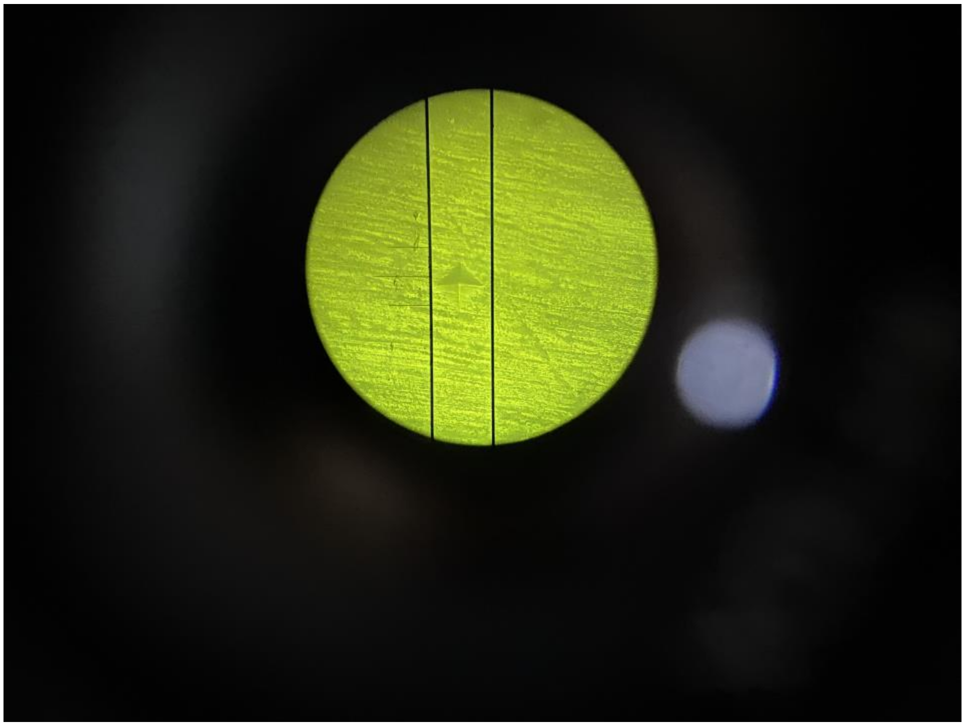

Figure 6 Measuring one Dimension of the Indentation Area

Post microhardness testing, the dentin surfaces underwent demineralization with a 30-second 5% HCl bath to eliminate the smear layer resulting from sectioning. This demineralization process was followed by a 30-minute 5.25% NaOCl bath to remove organic debris. Specimens were thoroughly washed with distilled water and dried with a gentle stream of air. Subsequently, they were placed in a vacuum oven overnight to ensure complete dehydration for subsequent Scanning Electron Microscope (SEM) analysis.

2.5. SEM Analysis

The dentin discs were mounted onto aluminum stubs using a conductive adhesive tape for sealer penetration evaluation. Low-magnification images (50x, 500x, and 1kx) were captured using a Field Emission Scanning Electron Microscope (FESEM) device (SU6600, Hitachi, Tokyo, Japan) at 15.0 kV and 60 Pa. Initially, an image was captured, encompassing the gutta-percha, to determine the x and y coordinates of the center of the root canal (Figure 7).

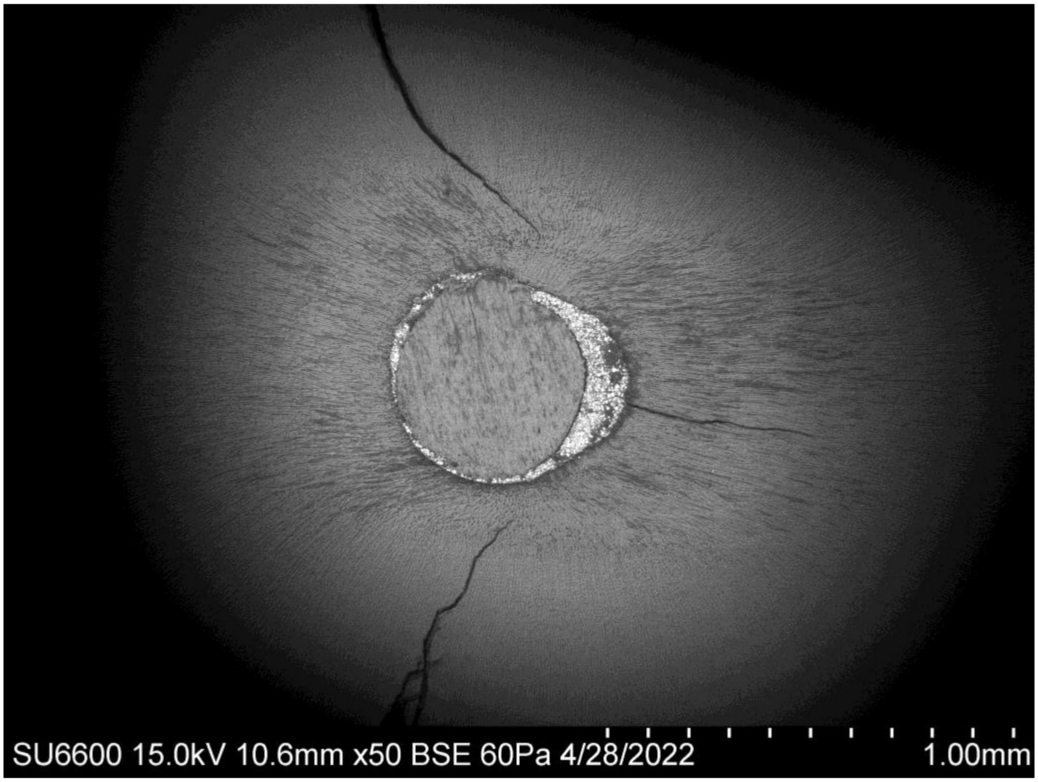

Figure 7 Image Taken for Determination of the Coordinates of the Root Canal Center

Subsequently, another image was taken, including the cemento-dentinal junction (CDJ), to determine the coordinates where dentin tubules terminate (Figure 8).

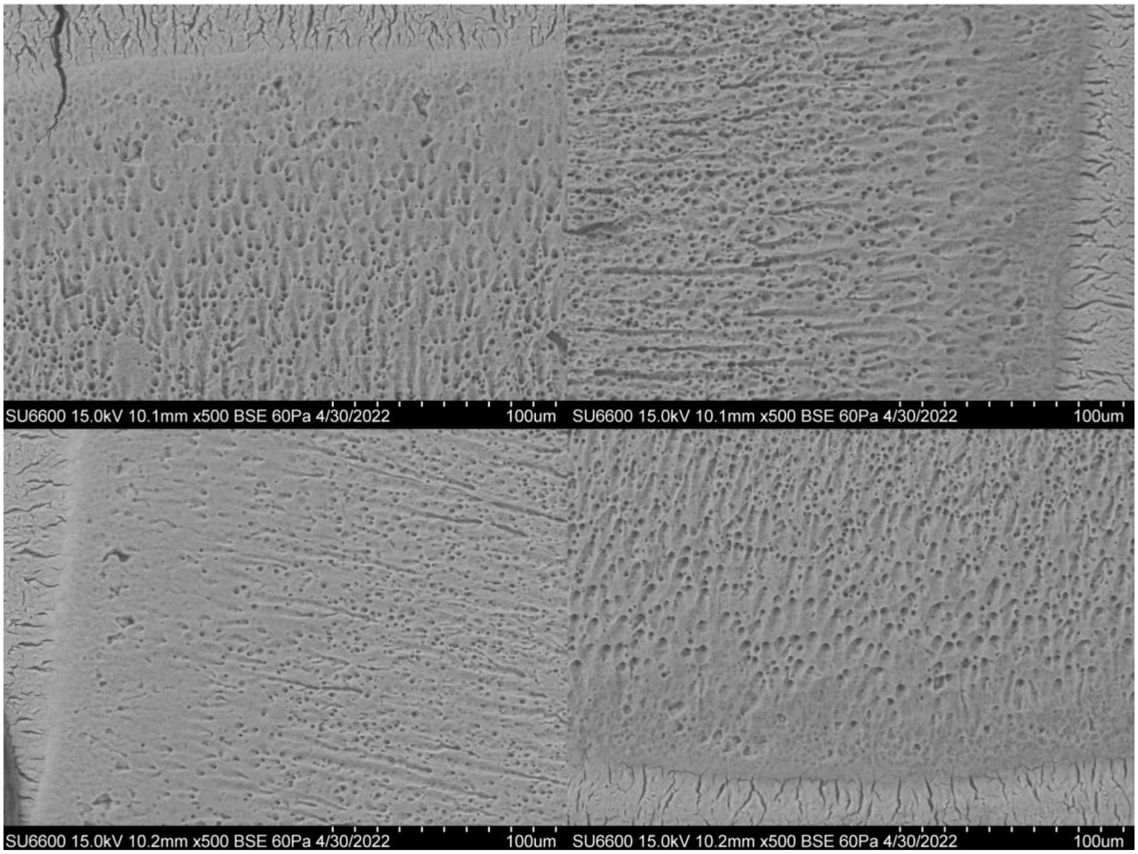

Figure 8 Images Taken for Determination of CDJ in 4 Directions

Finally, an image was captured, focusing on the resin tag at the furthest point towards CDJ (Figure 9). The saved coordinates on the x and y planes indicated the center of the picture. To accurately determine the location of the sealer tag, CDJ, or the center of the root canal, we utilized image processing software (ImageJ; Bethesda, MD).

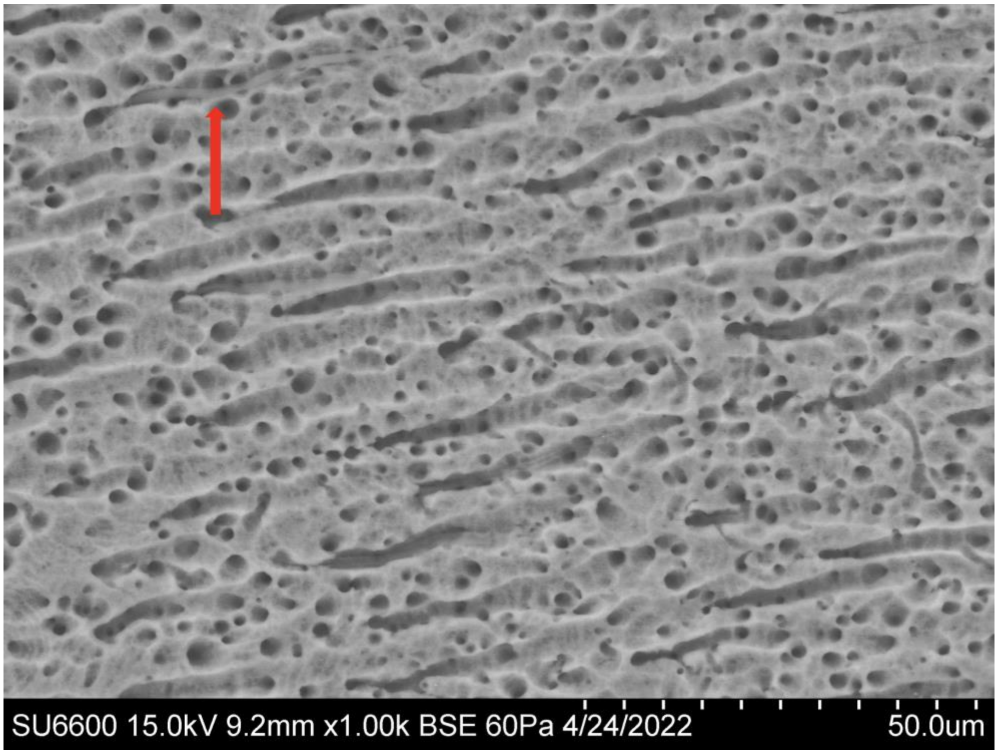

Figure 9 Capturing a Sealer Tag with a Higher Magnification

These CDJ and sealer tag images were systematically captured from four directions, 90 degrees apart, for every section. Utilizing the Euclidean distance formula, distances from the center to the CDJ and from the center to the resin tag were calculated. Penetration ratios were then determined by dividing these distances, and the results were recorded in four different directions for statistical analysis.

The elemental analysis of the region using Energy Dispersive X-ray Spectroscopy (EDS) served as a supportive test for confirming sealer penetration levels. This analysis was employed due to the presence of unique elements, namely zirconium and tungsten, in the sealer as specified in the product manual, which are not naturally found in dentin.

2.6. Ion Analysis

For the measurement of Ca, P, and Mg ions release, an Inductively Coupled Plasma Mass Spectrometry (ICP-MS) instrument (7800 ICP-MS, Agilent, Santa Clara, CA) was utilized. The waste solution collected from the GW treatment of a single tooth, approximately 400 ml in volume, underwent analysis to determine the concentrations of these ions originating from the dentin composition.

To establish standard calibration for each ion, respective stock solutions at a concentration of 1000 parts per million (ppm) were prepared. For Ca, calcium nitrate tetrahydrate was used, while for P, ammonium phosphate monobasic was employed. For Mg, magnesium chloride hexahydrate served as the compound.

The molecular weight of each compound was determined and then divided by the atomic mass of the corresponding ion to calculate the amount of compound required to prepare a stock solution. For example, the molecular weight of calcium nitrate tetrahydrate is 236.15 g/mol and the atomic mass of calcium is 40.07 amu. Dividing 236.15 by 40.07 results in 5.89 g. This amount of the compound was dissolved in 1 liter of distilled water to create a 1000 ppm stock solution.

Following the preparation of stock solutions, they were further diluted to generate standard solutions at concentrations of 0.01, 0.1, 0.5, 1, and 10 ppm. This dilution process was carried out by mixing the stock solution with distilled water in appropriate ratios, ensuring the desired concentrations were achieved.

The ICP-MS instrument, along with the Autosampler (SPS4, Agilent, Santa Clara, CA, USA), was configured with specific parameters for the measurement of each element. This included setting an uptake time, rinse time, and stabilization time for individual measurements of Ca, P, and Mg ions. These parameters ensure optimal performance and accuracy during the analysis.

Both the prepared standard solutions and the specimen were introduced into the Autosampler of the ICP-MS instrument. The Autosampler facilitated the automated introduction of samples into the instrument for subsequent measurements.

The ICP-MS instrument conducted measurements of each element in the standard solutions and the specimen. The obtained readings, representing the concentrations of Ca, P, and Mg ions, were recorded to provide insights into the dentin composition and any alterations resulting from the experimental procedures.

2.7. Data Analysis

Data was analyzed by creating data tables separate for microhardness testing and sealer penetration results in Microsoft 365 Excel (Microsoft Corporation, Redmond, WA) where means and standard deviations were calculated. Data was then transferred into JMP statistical program (JMP Pro 15, JMP Statistical Discovery LLC), where the graphs with means and standard deviations were created.

The microhardness and sealer penetration data was analyzed using one-way ANOVA (Analysis of Variance) and two-way ANOVA to detect existence of any difference between the obtained means. The significant level alpha was set as 0.05.

Chapter 3. RESULTS

3.1. Microhardness

Table 1 VHN for Groups

Group	**Significant Difference**		**N**	**Mean (kgf/mm^2)**	**Std Deviation**
Group C	**A**		80	44.67	8.44
Group G	**A**	**B**	80	42.29	9.49
Group T		**B**	80	40.77	6.56

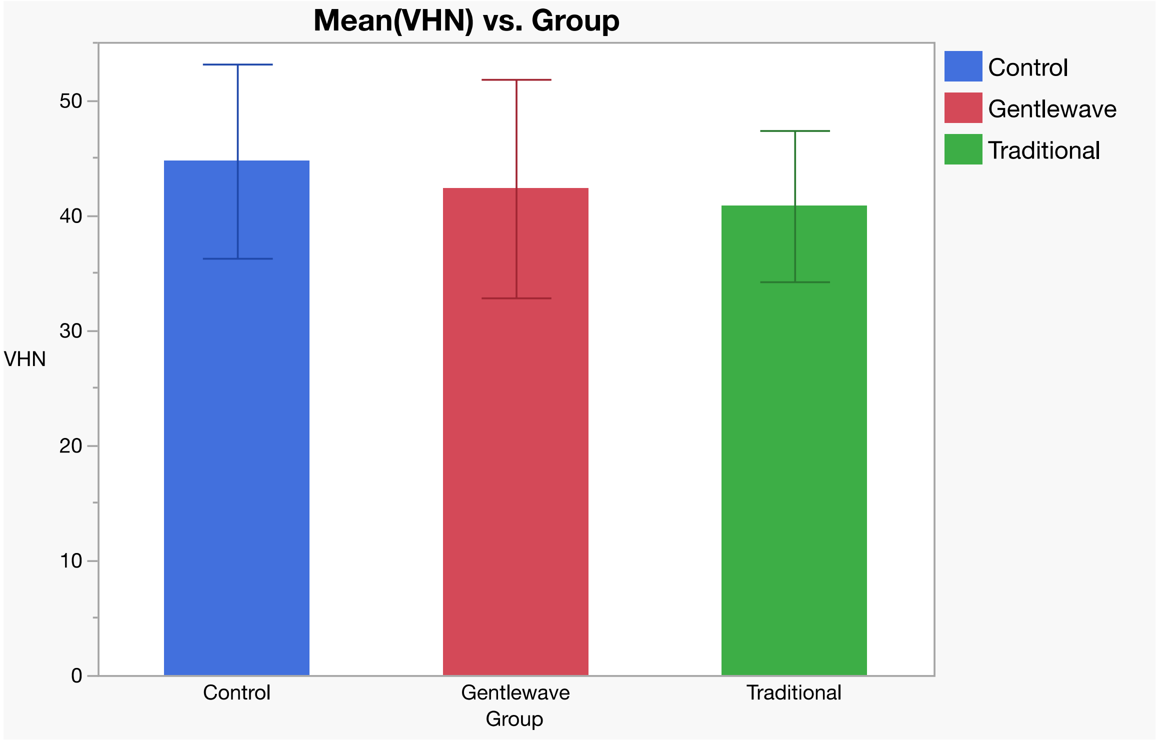

Figure 10 Mean Hardness Values of the Groups

Based on the Vickers Test results, it is evident that Group C exhibits the highest hardness values, as anticipated, while Group T demonstrates the lowest values. The difference between these two groups is statistically significant (p = 0.007). Group G, on the other hand, presents hardness values that fall between those of the other groups, but these differences are statistically insignificant, as illustrated in the provided Table 1 and Figure 10.

Table 2 Mean VHN for Groups and Slices

Group	**Slice**	**Significant Difference**		**N**	**Mean (kgf/mm^2)**	**Std Deviation**
Group C	**Apical**	**A**		40	49.15	6.28
	Coronal		**B**	40	40.20	7.97
Group G	**Apical**		**B**	40	43.44	8.38
	Coronal		**B**	40	41.15	10.46
Group T	**Apical**		**B**	40	41.41	4.99
	Coronal		**B**	40	40.12	7.85

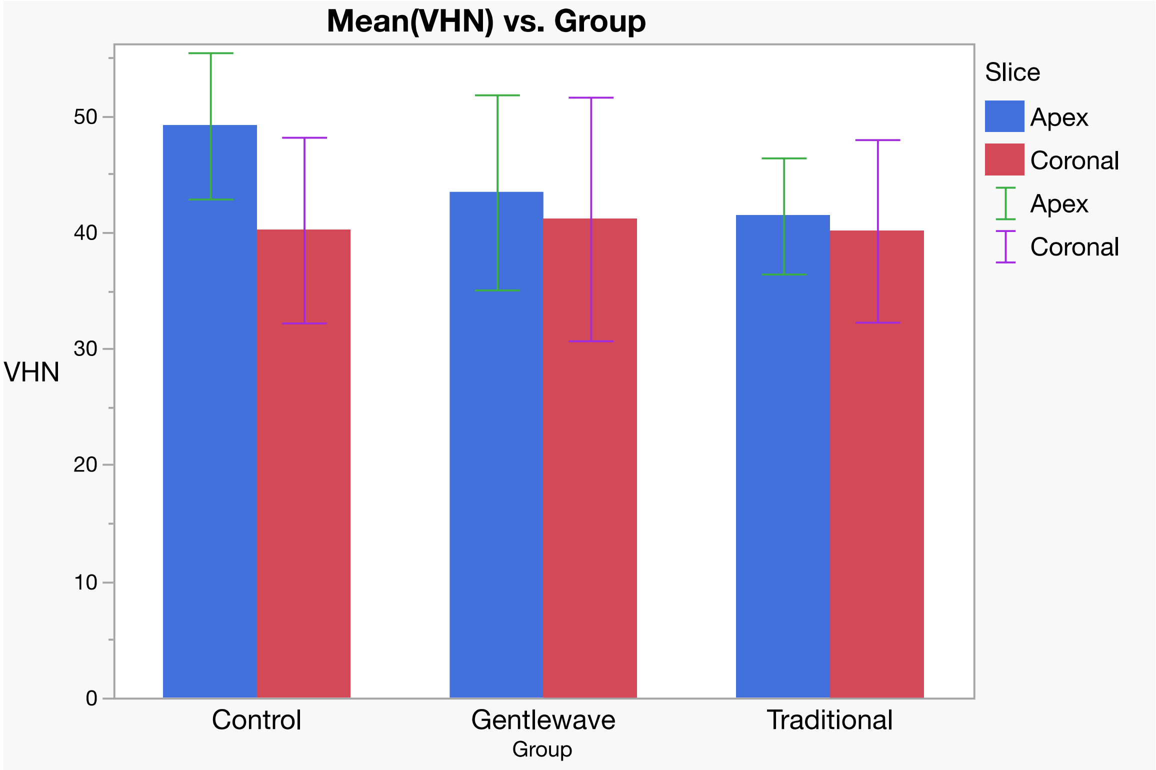

Figure 11 Mean Hardness Values of the Groups and Slices

The hardness test results indicate that, generally, the apical portion of the root exhibits higher values than the coronal portion. Specifically, within Group C, the apical section significantly demonstrates the highest hardness values compared to the other slices (p = 0.004), as detailed in Table 2 and Figure 11.

3.2. Sealer Penetration

Table 3 Sealer Penetration Ratios for Groups

Group	Significant Difference	N	Mean	Std Deviation
Group G	**A**	120	0.87	0.15
Group T	**B**	120	0.69	0.26

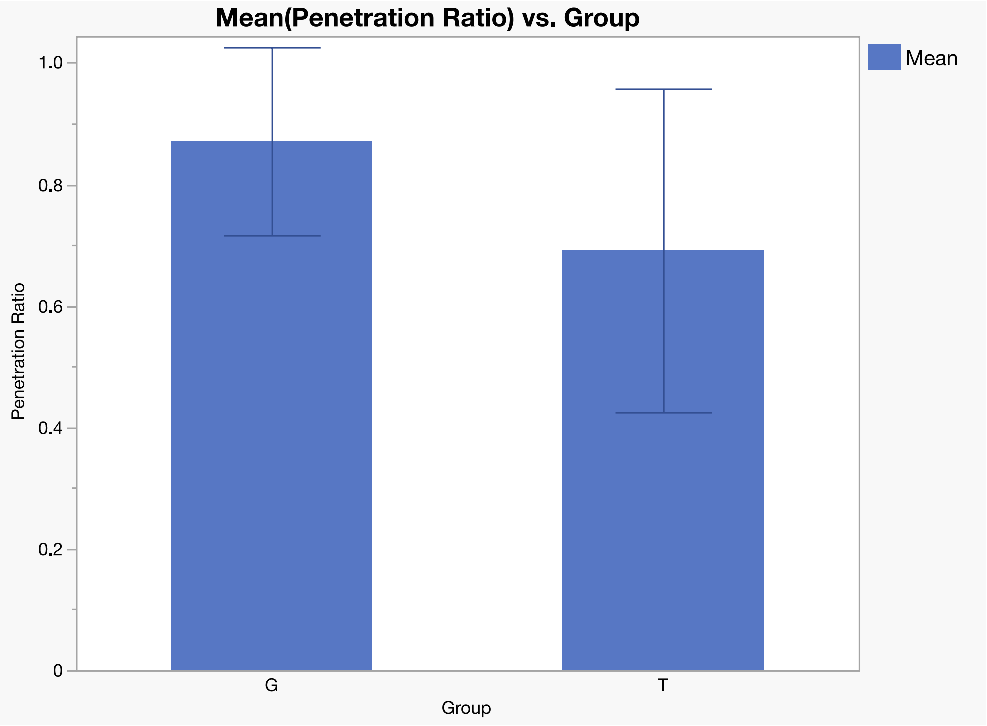

Figure 12 Mean Sealer Penetration Ratios for Groups

As shown in Table 3 and Figure 12, Group G shows significantly higher penetration than Group T when the slice location is not taken into consideration ($p < 0.05$).

Table 4 Penetration Ratios for Groups and Slices

Group	**Slice**	**Significant Difference**			**N**	**Mean**	**Std Deviation**
Group G	**Apical**	**A**			40	0.83	0.13
	Middle	**A**			40	0.89	0.11
	Coronal	**A**			40	0.88	0.19
Group T	**Apical**		**B**	**C**	40	0.68	0.23
	Middle			**C**	40	0.58	0.26
	Coronal	**A**	**B**		40	0.80	0.25

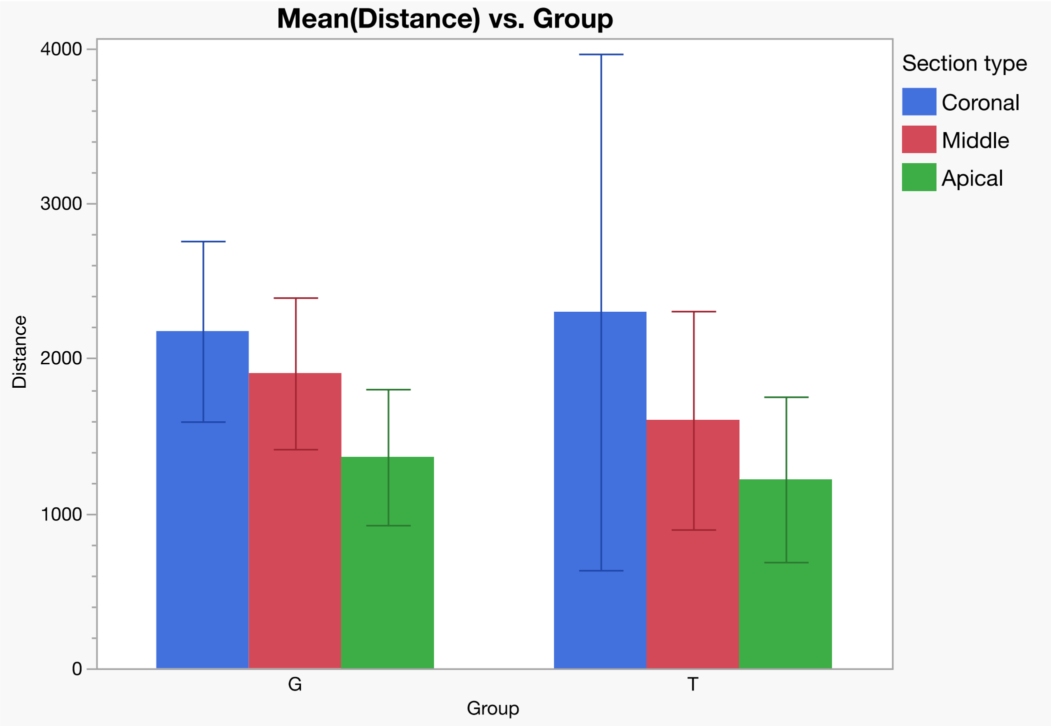

Figure 13 Sealer Penetration Ratios for Groups and Slices

Considering the locations, coronal sections showed significantly higher penetration levels in both groups (p = 0.04). No significant difference was found between all the sections of Group G and the coronal sections of Group T. Group T exhibited lower penetration levels in the middle and apical thirds, with the middle sections displaying the lowest penetration levels (p = 0.02). These findings are summarized in Table 4 and depicted in Figure 13.

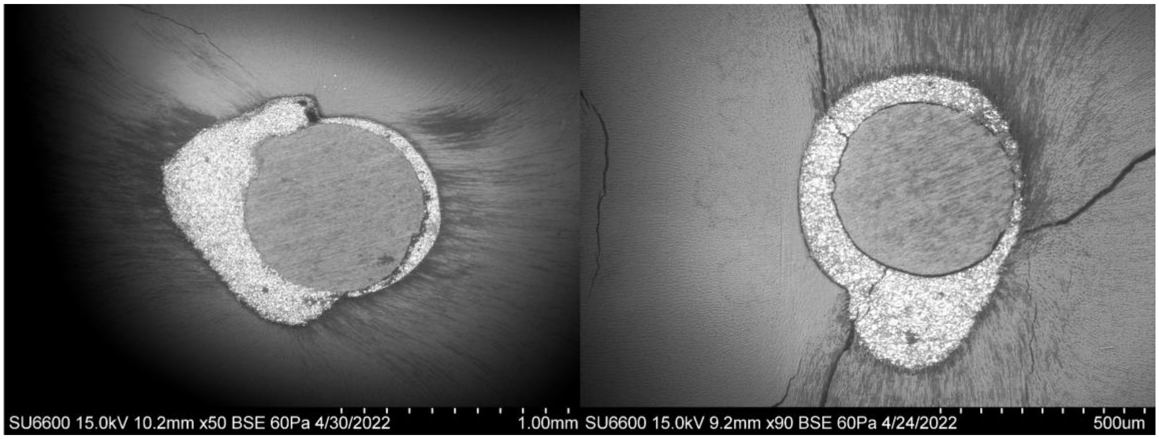

Figure 14 Images from Different Samples Showing the Sealer Behavior

Aside from the penetration levels, sealer penetration appeared clearer and more intense in two specific directions compared to the other two directions in the vertical cross-sections. This variation suggests a directional preference in how the sealer infiltrates the dentinal tubules. Figure 14 illustrates different samples exhibiting this distinctive sealer behavior. The images highlight the directional differences in sealer distribution within the dentinal structure.

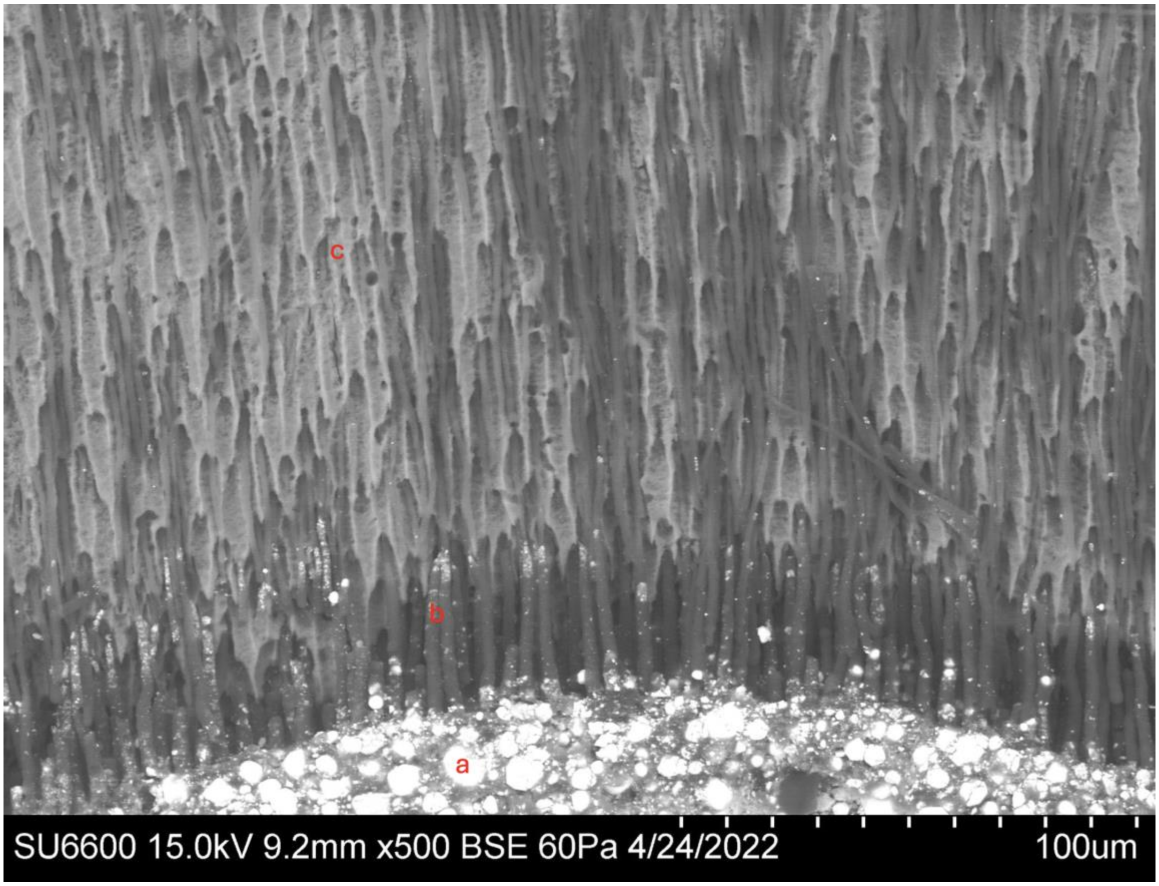

Figure 15 Large Sealer Particles in the Root Canal (a), and Sealer Tags (b) in Dentinal Tubules (c)

Additionally, SEM images revealed that after setting, the sealer contains large particles approximately 9-10 μm in size within the root canal space. These particles are too large to penetrate the dentinal tubules, which have dimensions of about 2-3 μm. Consequently, the bright crystals observed in the sealer do not extend towards the CDJ within the dentin (Figure 15).

3.3. Ion Analysis

Following the receipt of consistent data using different ppm standard solutions, the analysis of the GW waste solution revealed concentrations of 2 ppm for Ca (Figure 16), 0.5 ppm for P (Figure 17), and 0.2 ppm for Mg (Figure 18). In these figures, the x-axis represents counts per second (cps), and the y-axis represents ppm. Since none of the irrigation solutions used contain these elements in their chemical formulas, these findings support the idea that the detected elements originate from the tooth structure and serve as proof for the explaining the difference in microhardness values between Group C and G.

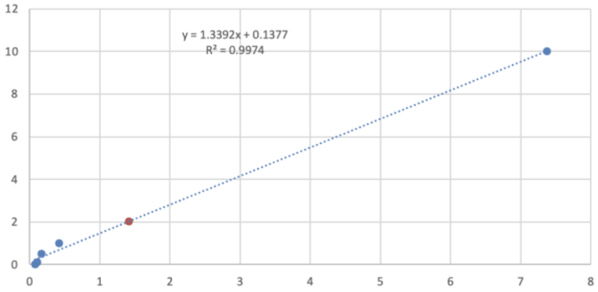

Figure 16 Ca Concentrations (Blue Represents Standard Solutions and Red Represents GW Waste Solution)

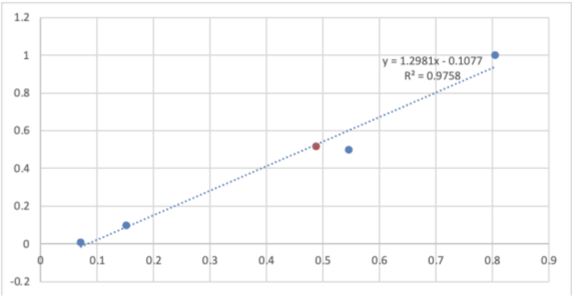

Figure 17 P Concentrations (Blue Represents Standard Solutions and Red Represents GW Waste Solution)

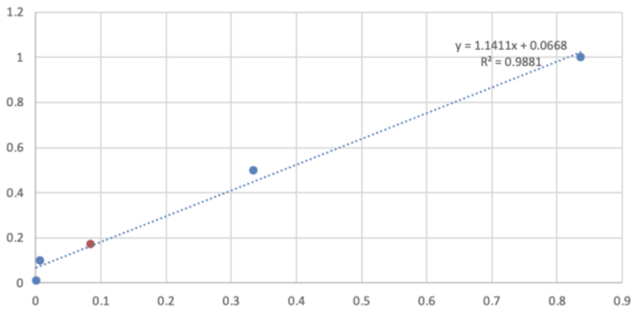

Figure 18 Mg Concentrations (Blue Represents Standard Solutions and Red Represents GW Waste Solution)

Chapter 4. DISCUSSION

Radicular dentin exhibits a distinctive characteristic known as the 'Butterfly Effect,' an optical phenomenon elucidated by Russell et al. (39). This effect arises from the higher density of dentinal tubules in the bucco-lingual direction compared to the mesio-distal direction around the root canal. In the mesio-distal direction, the dentin tends to become more sclerotic, leading to greater light transmission in one dimension than the other. Consequently, distinct shades form a butterfly shape on the dentin surface when observed in vertical sections. The significance of this phenomenon lies in its potential impact on sealer penetration patterns. Following obturation, vertical cross-sections are more likely to exhibit pronounced sealer tubule penetration in the bucco-lingual direction. This observed sealer behavior was corroborated in the present study (Figure 14), where sealer penetration appeared clearer and more intense in two directions, forming the distinctive butterfly image. Despite variations among dentin slices, resin tag intensity was not a focus of this study. To ensure robust analysis, penetration levels were measured in four directions, and their mean values were used in statistical analysis.

When considering obturation, factors such as the application method (obturation technique), sealer placement, and choice of material (sealer type) are important. In this study, the single cone technique was chosen to fill the root canals. While some studies suggest that the warm vertical technique may enhance deeper penetration into dentinal tubules, the penetration depth of gutta-percha remains unaffected by the obturation technique (21). However, the obturation technique may influence the penetration depth of the material into anatomical areas that are not easily accessible, such as lateral, secondary, and accessory canals.

Cold lateral compaction is regarded with high standards by authorities (16), but the single cone technique remains widely accepted due to its adaptability to innovations in endodontic files (20). Film thickness and sealer penetration are influenced by the obturation technique (23). In a study comparing obturation techniques, lateral compaction demonstrated better distribution in the mid-coronal areas compared to warm vertical compaction. Similarly, another study found that none of the five evaluated obturation techniques resulted in uniform sealer distribution along the entire length of the core obturation material. While the method of obturation does not significantly affect sealer distribution on the canal wall, it does impact sealer penetration into tubules. For instance, thermoplastic techniques have been shown to produce deeper sealer penetration into tubules.

Given that the primary aim of this study is to compare the effects of irrigation protocols and considering that the teeth used were limited to upper lateral incisors with single straight canals and no anatomically non-reachable areas such as isthmuses, the single cone technique was selected as the obturation technique for both groups. Besides its simplicity, cold techniques are still regarded as meeting high standards and are currently gaining popularity alongside novel instrumentation techniques (9, 16).

Another consideration is the sealer film thickness, which is more pertinent to microleakage than the level of sealer penetration (23). A thicker sealer film may contribute to increased microleakage due to its lower quality compared to gutta-percha in terms of dimensional stability following setting. Nevertheless, modern sealers address these concerns, as new technologies prioritize characteristics such as no shrinkage, a vital criterion for ideal sealers according to Grossman (9). Consequently, the use of gutta-percha primarily aims to provide retreatment opportunities rather than achieving dimensionally stable obturations, as contemporary sealers already fulfill this requirement.

While one study indicated that the depth of sealer penetration has not been sufficiently investigated (40) Aksel et al. reported a maximum sealer penetration depth of 1500 µm, with the majority of sealer distributed between 50-500 µm, irrespective of the irrigation method (4). Notably, a previous study found the maximum penetration level of NaOCl to be 300 µm (41). In our study, we observed a maximum penetration level of 3447 µm, with an average level across all teeth of 1755 µm. Additionally, in some obturated teeth, resin tags were detected in close proximity to the CDJ with a low density.

The penetration depth of sealers may also be influenced by their physical and chemical properties (15), including the particle size of the fillers used. In this study, the sealer contains finely ground calcium tungstate with an average particle size of 8 µm and finely ground zirconium oxide with an average particle size of 1.5 µm as fillers. AH Plus sealer was chosen for comparison due to its widespread use and convenience for evaluating sealing ability across various studies (22). It has been reported to exhibit optimal tubular penetration in some studies (19), although conflicting results exist (42), possibly due to variations in root canal morphology (21). By selecting AH Plus sealer, we aimed to use a liquid form sealer to eliminate the potential issue of large particles of powder-liquid sealer failing to penetrate due to their size. Additionally, we intended to standardize the consistency of the sealer by utilizing the mixing syringe tip provided with AH Plus sealer, ensuring equal consistency even when used at different times. However, SEM images revealed that in the following setting, the sealer contains large particles around 9-10 μm in size, which are unable to penetrate into the dentinal tubules with dimensions of 2-3 μm (Figure 15). Nonetheless, AH Plus has a film thickness of 26 μm, well below the threshold of less than 50 μm mandated by the ISO standard for root canal sealing materials (ISO 6876).

Various methods of sealer placement are available, including the master cone, lentulo spirals, files and reamers, and ultrasonics. According to the literature, the most variation in sealer coating occurs in the apical area, with no technique covering more than 62.5% of the canal wall surface. In this study, manual placement with a master cone was performed due to its availability , although ultrasonics produced the best sealer distribution when used circumferentially based on current information (9). Canals were dried with only paper points in this study, although there are leakage studies reporting that a better apical seal might be achieved by drying the canals with 95% ethanol prior to obturation. This is because better adhesion to the dentinal walls might be achieved by leaving the canals slightly moist for AH-Plus sealer, and the instructions in the manual were followed (38).

Different structural features within various locations of dentin can significantly influence sealer diffusion, as well as microhardness and elasticity. Among these, the anatomical variations in the apical third are the most prominent within the entire radicular dentin. In the apical third, primary dentinal tubules are fewer and exhibit irregular density and direction compared to the coronal region. Additionally, fine tubular branches with diameters ranging from 300 to 700 μm run at a 45-degree angle, while microbranches with diameters of 25 to 200 μm run perpendicular to the main tubules (9). These complex structural features are predominantly found in the apical portion, characterized by a higher presence of peritubular dentin but a lack of tubular dentin (4). Consequently, the morphology of the apical third poses greater challenges for endodontic treatments, requiring careful consideration and specialized techniques to achieve successful outcomes.

The variations in the configuration of dentinal tubules at different locations leaded us to assess microhardness values and sealer penetration levels at various depths within the root canal. To achieve this, three discs were obtained from each tooth representing the apical, middle, and coronal thirds of the root. Sealer penetration was evaluated on the surfaces corresponding to the levels of 2, 5, and 8 mm, while the hardness test was performed on the opposite side of the discs. This decision was made because polishing, a necessary step for the hardness test, could potentially interfere with sealer penetration evaluation by removing resin tags. Pilot tests revealed that polished surfaces exhibited tangled resin tags, affecting the accuracy of the assessment during SEM evaluation. To ensure a more precise evaluation of sealer penetration, it was decided to conduct the tests on separate sides of the discs. Additionally, only two discs were utilized for microhardness testing, as the remaining side of the apical discs corresponded to the physiologic apex, where dentin exposure was not present. Consequently, the roots were tested for hardness at the levels of 3 and 6 mm, deemed acceptable for comparison as representing the apical and coronal portions, respectively.

While there is a higher risk of iatrogenic errors, several studies advocate for increasing the apical preparation size to enhance cleaning efficiency (2). For traditional preparation, an F3 size can be deemed acceptable since the mean apex size for upper incisors is approximately 289.4 μm (9). Although the GW system can be utilized in traditionally instrumented canals, the primary focus of this study was to compare root canal preparation techniques rather than solely assessing irrigation activation effectiveness. Hence, selecting a size two steps down for apical enlargement provided minimal preparation and facilitated the intended difference between groups in terms of root canal width. Moreover, choosing the F1 size aligns with the guidelines provided in the GW manual for large canals such as those found in upper anterior teeth, adding another advantage to this size selection. Although some studies have explored the use of the GW system in uninstrumented canals, the updated manual suggests minimal preparation to establish a fluid path and facilitate root canal filling.

In the current study, NaOCl was used during instrumentation and as the final irrigation solution following EDTA in the traditional group. This approach aims to maximize antibacterial effectiveness and eliminate the unsupported organic layer after the smear layer is removed. However, recent research suggests that NaOCl following EDTA may lead to chemical weakening of dentin (28). The ion analysis results in this study indicate that the concentrations align with the microhardness values observed in the experimental groups. Specifically, the GW protocol appears to cause a loss of elements from the dentin structure, likely contributing to the lower microhardness values observed in Group G compared to Group C. This finding supports the idea that chemical composition plays a significant role in the microhardness of the dentin structure. In the GW protocol, NaOCl and EDTA are delivered in lower concentrations but with a much higher volume compared to the syringe irrigation technique. Furthermore, NaOCl is not used after EDTA in the GW protocol; instead, distilled water is used as the final irrigant. The intention behind this study by comparing the microhardness values of the different groups was to determine if the use of lower concentrations, and the specific sequence of irrigation solutions could potentially cause less damage to the dentin structure. This comparative analysis is crucial for understanding the impact of different irrigation protocols on the preservation of dentin integrity, ultimately guiding the selection of the most effective and least harmful endodontic treatment practices.

In the literature, alternative methods for evaluating microhardness and sealer penetration include maintaining horizontal sections instead of vertical. However, we chose vertical sections to replicate the force directions experienced by the tooth. Another common method involves using Rhodamine B dye to stain the sealer for confocal laser scanning microscopy evaluation. However, recent findings have revealed that this approach may not accurately indicate dentinal tubule penetration but rather merely display the presence of dentinal tubules in a root slice. This limitation raises questions about the validity of using Rhodamine B to assess penetration depth and challenges the concept of a sealer penetrating deep into the root dentin tubules to ensure a tight seal of the root canal system (21).

The penetration of sealer into dentinal tubules is desirable for improving prognosis, but it can pose challenges during retreatment. Kim et al. (43) reported that removing set filling materials from dentinal tubules requires the removal of 40-60% of extra-radicular dentin. Once the sealer penetrates the dentinal tubules, its removal during retreatment is physically impossible, and some materials, such as zinc oxide–eugenol-based sealers, may negatively affect the bond strength of another sealer during a retreatment filling procedure. However, a high degree of sealer remnants does not necessarily indicate treatment failure.

The findings of this study suggest that the GW System maintains dentin hardness without significantly affecting on dentin chemical composition, while promoting enhanced sealer penetration compared to traditional root canal treatment methods. By preserving dentin integrity and facilitating deeper sealer penetration, the GW System may contribute to improved treatment outcomes and long-term success rates in root canal therapy. These results reject the null hypothesis and support the adoption of innovative irrigation techniques, such as the GW System, in clinical practice to optimize patient care and treatment efficacy.

4.1. Limitations of the Study

While the use of irrigation activation systems has become increasingly popular among endodontists and is considered a standard approach for conventional treatment, the syringe delivery method remains widely accepted. Numerous studies in literature have compared activation techniques with syringe irrigation, making it a suitable choice for comparison as a standard irrigation technique (2, 12). However, it's important to note that this study does not assess the performance of the GW system in comparison to other activation devices or methods.

Conventional syringe irrigation may not effectively facilitate deep penetration of irrigants into the root canal system (4). When activation systems are compared to the conventional method, they generally demonstrate improved results in terms of smear layer removal, although these studies typically focus on teeth with straight canals (12). However, it's important to note that no activation technique, as reported in the literature, is able to completely eliminate debris and smear layer from curved root canals (2). Since this study was also conducted on straight canals, it does not demonstrate the potential performance of the GW system on curved canals.

Considering that microleakage can result from inadequate contacts between gutta-percha-sealer and sealer-dentin due to voids within the sealer (17), the density of the sealer within dentinal tubules is crucial for assessing obturation quality, along with sealer penetration depth (4). It's important to note that in this study, no homogeneity of resin tags was observed at the maximum sealer penetration level, indicating variability. Therefore, this study evaluates only one aspect of obturation quality.

4.2. Future Directions

Further investigations are needed to assess the efficacy of the GW system in curved canals and its comparison with other recent irrigation activation techniques and its clinical applications. Additionally, the comparison of microhardness between intact teeth and immediately after GW application was not explored in this study. The primary reason for obtaining results from intact teeth was to establish a baseline measurement. However, future studies focusing solely on the effect of irrigation techniques may benefit from examining this aspect in more detail.

Chapter 5. CONCLUSIONS

There is a significant difference in dentin microhardness between traditional root canal treatment and GW treatment. Traditional root canal treatment gives significantly lower values.

There is a significant difference in sealer penetration between traditional root canal treatment and GW treatment. GW improves the sealer penetration in dentinal tubules.

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