Effect of Moisture and Heat on Depth of Penetration of heat treated Bioceramic sealer versus MTA sealer

(An in vitro study)

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بسم الله الرحمن الرحيم

قالوا

سبحانك لا علم لنا
إلا ما علمتنا إنك أنت
العليم الحكيم

صدق الله العظيم

سورة البقرة الآية: ٣٢

Dedication

To my Father, Mother, Wife, Brother and my beloved daughter **Farida** whom after God's will, their help and endless support helped me through the journey to achieve my dreams, I am forever grateful.

I dedicate this work……

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List of Abbreviations

Abb.	Full term
°C	*Celsius*
BCSHF	*Bioceramic sealer HiFlow*
CBCT	*Cone beam computed tomography*
CLC	*Cold lateral compaction*
CLSM	*Confocal laser scanning microscopy*
CNI	*Conventional needle irrigation*
CSBs	*Calcium silicate-based sealers*
DOM	*Dental operating microscope*
EDTA	*Ethylenediaminetetraacetic acid*
kHz	*kilohertz*
MDA	*Manual dynamic activation*
MTA	*Mineral trioxide aggregate*
N/cm	*Newton centimeter*
NaOCl	*Sodium hypochlorite*
NiTi	*Nickel Titanium*
NPs	*Nano particles*
PBS	*Phosphate buffer solution*
PIPS	*photon-initiated photoacoustic streaming*
PUI	*Passive ultrasonic irrigation*
RCS	*Root canal sealer*
Rpm	*Rotation per minute*
SEM	*Scanning electron microscope*
SEM	*Scanning electron microscopy*
SMTSC	*Single matched tapered sized cone*
WVC	*Warm vertical compaction*

1. INTRODUCTION

Three-dimensional root canal filling is the aim of root canal treatment in order to obtain fluid tight seal inside the root canal. Sealers play a crucial role as it closes fins, irregularities and dentinal tubules, although it has important role sealers amount should be minimalized compared to gutta percha inside the root canal during obturation [(1)].

Traditional root canal sealers include zinc oxide eugenol, calcium hydroxide, and resin-based sealers. Although these sealers have been effective, there is still a quest for a sealer with better properties. Due to evolution of endodontics introduction of new techniques and materials was a must and hence bioceramic term appeared [(2)].

Bioceramic are ceramic materials that include silica, alumina, zirconia, bioactive glasses, ceramic glasses, calcium silicates, hydroxyapatite, and calcium phosphate. Their use in dental practice began with the introduction of Mineral Trioxide Aggregate (MTA), which led to the development of bioceramic based root canal sealers. These sealers represent a new category of root-end filling materials, they are classified into calcium silicate-based, MTA-based, and calcium phosphate-based types [(3)]. There are two major advantages associated with the use of bioceramic materials as root canal sealers. Firstly, their biocompatibility prevents rejection by the surrounding tissues. Secondly, bioceramic materials contain calcium phosphate which enhances the setting properties of bioceramic and results in a chemical composition and crystalline structure similar to tooth and bone apatite materials, thereby improving sealer-to-root dentin bonding [(4)]. The last generation known as premix bioceramic sealers, releases more calcium hydroxide during setting and requires no manipulation before use [(5)].

The sealing ability of the resin-based root canal depends on the degree of the remaining moisture content which is the main cause for failure in the bonding of resin-based sealer [(6)]. On the other hand moisture initiates setting of calcium silicate-based sealers with the hydration reaction [(7)]. Although clinicians may have varying perceptions of what constitutes an appropriate moisture level, many manufacturers advise keeping the root canal in a moist state to take advantage of their sealer's hydrophilic properties. However, they often do not provide specific clinical guidelines on the optimal degree of moisture required for their products [(8)].

Currently, a number of evaluation methods, such as stereomicroscopy, transmission electron microscopy, scanning electron microscopy (SEM), and confocal laser scanning microscopy (CLSM), are employed to assess the sealer/dentin interface. CLSM provides the advantage of detailed information about the presence and distribution of sealers inside dentinal tubules at a relatively low magnification through the use of fluorescent Rhodamine-marked sealers [(9)].

It is important to compare the penetrability of various types of sealers used in the routine dental clinic with different obturation methods to improve the quality of endodontic treatment and the success rate. This study spots the light on the penetration power of two bioceramic root canal sealers into the dentinal tubules at different moisture levels with different obturation techniques.

2. REVIEW OF LITERATURE

Section outline:

2.1 Obturation of the root canals.

2.1.1 Different obturation techniques.

2.1.2 Different root canal sealers.

2.2 Effect of moisture on depth of penetration of root canal sealers.

2.3 Effect of heat on depth of penetration of root canal sealers.

2.4 Methods of evaluation.

2.1. Obturation of the root canals:

The best obturation technique is the one that gives the best clinical outcome for the root canal treatment, simple to use in all types of cases, faster, with minimal complications and with easier learning curve for new users. The problem is that determining which obturation technique yields the best clinical outcome with highest success rate becomes very difficult, because there are so many variables which can affect the outcome of treatment. There are many different obturation techniques but no single technique has been identified which is superior in all aspects [(10)].

2.1.1. Different obturation techniques

Different root canal filling techniques have been proposed in an attempt to fill the root canal in three dimensions and reduce apical and coronal leakage[(11)]. Such as cold lateral compaction, warm vertical compaction, continuous wave compaction, warm lateral compaction, thermoplastic injection, carrier-based gutta-percha, thermo-mechanical compaction and single cone techniques [(12)].

Bowman in 1867 claimed the first use of Gutta Percha for canal filling in extracted first molar [(13)], then Perry in 1883 claimed the use of pointed gold wire wrapped with some soft gutta percha [(14)].

Elmer Jasper introduced silver points silver points at 1931[(15)]. Over the past few decades, the use of silver point has dramatically diminished. Silver points do not produce an acceptable three-dimensional seal of the root canal system; rather they simply produce a plug in the apical constriction, with poor adaptability to the root canal walls and do not seal the accessory canals that are frequently present. Also, silver points corrode over time which again compromises the apical seal [(16)].

Callahan [17] introduced Cold lateral obturation technique in the year of 1914. In this technique a master gutta-percha cone is placed with sealer and the spaces around it are adapted with the accessory cones of different sizes to get homogenous seal of the root canal[18]. Cold lateral compaction, which is the most widely used method for obturation of the root canals by many clinicians throughout the world due to its simplicity and adaptability to most cases [19]. This technique is easy to use, not requiring specific and expensive instruments and also has a low cost. Although there are many shortcomings of this technique which includes lack of gutta-percha homogeneity, high percentage of endodontic sealer at the apical portion of the root, risk of voids and spreader tracts formation, poor adaptation to the root canal walls, incomplete filling in some areas of the root canal system that are difficult to reach and possibility of apical extrusion of the gutta-percha [20,21].

In order to overcome the shortness of cold lateral technique warm lateral compaction technique was hopefully introduced in an effort to produce a more consistently dense, dimensionally stable, three-dimensional root canal filling new instruments have been introduced which provide electrically controlled, heated spreaders for use in warm lateral gutta-percha technique. One of the instruments (Endotec; Caulk, Milford, DE) reportedly maintains a temperature of 400°C. While the other (Touch 'n Heat model 5002; Analytic Technology, Redmond, WA) has a control knob which can vary the temperature from 0 to 816°C according to the manufacturer. The electrically heated spreaders, which maintain a constant temperature, could improve the traditional lateral compaction technique by achieving a more homogeneous mass of gutta-percha and a more complete obturation of the root canal system. Because these heated instruments are used within the root canal [22].

Luccy et al. **[22]** studied apical sealing ability of the standard lateral compaction technique in comparison to warm lateral compaction techniques. Sixty extracted human teeth with single canals were prepared and randomly assigned into one of three groups, Group 1 used standard lateral compaction, Group 2 used a warm lateral compaction technique with the Touch 'n Heat instrument and Group 3 employed the Endotec Thermal Endodontic Condenser with four additional teeth used as controls. Results showed that all experimental groups demonstrated leakage. The order of increasing leakage was group 1 (lateral condensation), group 3 (Endotec), and group 2 (Touch 'n Heat). The two negative controls showed no evidence of ink penetration whereas both positive control teeth showed ink penetration throughout the entire length of the canal.

Marshall & Massler **[23]** proposed the single cone obturation technique (SC), in 1961.

The SC obturation technique was introduced with the development of ISO standardization for endodontic instruments and filling points. After reaming a circular stop preparation in the apical 2mm of the canal, a single gutta-percha was selected to fit with tug-back to demonstrate inlay-like snugness of fit. The single-cone technique consists of a single cone filled at room temperature with a sealer layer whose thickness depends on the fit of the single cone to the walls of the canal [24].

On the basis of their excellent shaping ability, rotary nickel-titanium (NiTi) instruments are widely used for root canal instrumentation. This widespread use of these instruments has caused manufacturers to offer corresponding gutta-percha cones that match the taper and diameter of the instruments. It is claimed that these cones will match the taper and diameter of the canals prepared with the rotary NiTi instruments. Obturation with these cones used as a single-cone technique is alleged to provide a 3D obturation in less time than traditional obturation techniques and to ensure a high volume of gutta-percha in the canal, whereas the amount of sealer should be kept to a minimum [(25)].

The single cone obturation is simpler and faster than the cold lateral compaction which makes the operator less subjected to fatigue [(26)]. Since the compaction step during endodontic therapy is considered to be a contributing factor for vertical root fracture, single-cone filling technique is now regarded to be a simple and relatively safe method in canal obturation [(27)].

Pereira et al. [(28)] evaluated the effect of root canal cross-sectional shape on single-cone root filling bond strength, as well as to determine the percentage of gutta-percha-filled areas (PGFA) and sealer-filled areas (PSFA), establishing a relationship between these variables. Distal roots of mandibular molars were selected. Three groups were categorized according to canal shape, round, oval and long oval canals were prepared using R40 reciprocating instrument and filled with matching single-cone gutta-percha and AH Plus sealer. Results showed that round canals had higher bond strength in the coronal and middle thirds and exhibited more adhesive and mixed failure modes. Round canals also had higher PGFA and lower PSFA. A positive correlation was found between PGFA and bond strength, while PSFA showed a negative correlation. The study concluded that canal shape impacts bond strength, with higher PGFA leading to stronger bonding to dentine.

Herbert Schilder **[29]** introduced in 1967 Warm vertical compaction technique The procedure provides three dimensional seal at the terminus of the canal (4 to 5mm of the apical canal) .It fills the canal irregularities with the help of apical and lateral hydraulic forces by pressing the sealer and gutta percha along its path. It provides densely compacted obturation of the root canal system than the traditional obturation techniques [30].

The warm vertical compaction consists of two techniques which are Interrupted technique and Continuous wave technique. Interrupted technique which is also known as multiple wave technique, involves heating of the gutta percha inside the root canal with hot carrier for 2-3 sec then the softened coronal 3-4 mm of gutta percha s removed with the carrier, while the remaining plasticized gutta percha is condensed with cold plugger. This process is known as individual wave of compaction [31]. While continuous wave technique requires one individual wave of compaction rather than several multiple compactions. A single appropriate plugger is selected which fits the taper of the canal from 5-6mm of the canal terminus. The selected plugger is heat activated for 2-3 seconds and is moved apically in the canal so as to reach to its determined location. Once reached, the heat is inactivated and a vertical pressure for 10 seconds is applied by the same plugger. The heat is again activated for 1second and ceased for 1 second. This activation and inactivation of 1 second will help the plugger to be removed easily from the canal along with excess GP [32]

Yee et al. **[33]** introduced thermoplastic gutta-percha technique at (1977). It consists of injecting gutta-percha, heated by an electrical device, into a prepared root canal. The instrument has a gun-like shape whose cartridges are small gutta-percha cylinders that are heated to a temperature regulated by the user. Exerting pressure on the trigger activates a piston that presses the gutta-percha towards the tip of the instrument. The gutta-percha is then conveyed through a thin silver needle that is appropriately bent to allow better delivery of gutta percha into the appropriate length of the root canal operation in root canals of different teeth.

Christopher S Lea et al. (34) compared cold lateral technique to warm vertical compaction transparent 30-degree simulated root canals were used. They evaluated the quality of root canal filling based on pre and post obturation weight. Results showed that based on the weight continuous wave of compaction technique produced a significantly higher density compared to cold lateral compaction.

Gordon et al. (35) compared lateral compaction technique to single cone technique in acrylic resin blocks and mesiobuccal roots of maxillary first molar. The simulated root canals in the resin blocks were of 30 degree and 58 degree curvature. Results showed that there was no significant difference between the groups. They concluded that single cone technique was significantly faster than lateral compaction technique.

Krug et al. **(36)** performed a retrospective study comparing the quality of obturation techniques after root canal preparation using stainless steel manual files with matched single cone with greater taper NITI files. Results showed better length accuracy in the for molars and mandibular teeth, . However, results in the central and cervical thirds were less favorable, suggesting additional techniques may be needed for irregular canals. They concluded that NiTiR and mSC technique offered reliable canal shaping and improved outcomes in the apical third.

Putrianti et al. **(37)** in his study compared the push-out bond strength between cold lateral compaction and warm vertical compaction on the of

BioRoot, a calcium silicate-based sealer. Sixteen root canals were prepared and divided into two groups: one using cold lateral compaction and the other warm vertical compaction. After incubation, the dislodgement resistance was measured. Results showed that cold lateral compaction produced significantly higher push-out bond strength compared to warm vertical compaction. Contrary to expectations he concluded that cold lateral compaction provides better adhesion than warm vertical compaction for BioRoot obturation.

Öztürk et al.[38] compared shaping ability of reciprocal and rotational systems assessing the effectiveness of the single-cone filling technique in root canal, evaluation was done by Micro-CT imaging. Sixty mandibular molar canals were divided between the HyFlex EDM (rotational) and WaveOne Gold (reciprocal) systems, with fillings done using either lateral compaction or the single-cone technique. The single-cone method resulted in fewer voids, particularly in the middle and coronal thirds, with the best results observed in canals shaped with the WaveOne Gold system. Overall, the reciprocal system and single-cone technique proved more effective than their counterparts.

2.1.2 Different root canal sealers.

Over the last period of time, Sealers have been widely searched in order to reach to ideal formula that convey the ideal requirement that is biologically acceptable beside developing hermetic seal inside the Roat canal with the least drawbacks. Different categories of sealer had been developed include zinc oxide eugenol-based, calcium hydroxide-based, resin-based, glass ionomer-based, silicone-based, and recently calcium silicate based sealers which is considered a leap in the research of sealers.

Rickert et al.**[39]** in 1931 developed the zinc oxide-eugenol (ZOE) formula for root canal sealers which has been widely adopted and used globally. ZOE When mixed and placed in root canal, that contains a residual amorphous gel, which forms a rigid matrix [40,41]. However, ZOE-based sealers were less effective compared to other sealers due to their relatively high solubility [42]. Some of these powder-liquid sealers contain silver in the powder component which has caused darkening of the teeth. Silver-free formulas that avoid staining were developed to address this issue. [43].

In 1920, **Herman** introduced a calcium hydroxide-based sealer to endodontics for use in pulpal repair [44]. It is well known for its biocompatibility and high pH, attributed to the presence of hydroxyl ions, which promote hard tissue formation and provide antimicrobial activity[45]. Due to these advantages, calcium hydroxide-based materials are extensively utilized as pulp capping agents, intracanal medicaments, and root canal sealers. However, their physical reliability is compromised, as evidenced by substantial leakage [46].

Schroeder [47] introduced epoxy resin sealer In 1957. The AH series prototype, which exhibited exceptional physical properties and sealing capabilities. Today, epoxy resin-based sealers, derived from this development, remain the most commonly used root canal sealers in clinical practice., AH Plus has been widely used sealer in clinical practice due to its go. physicochemical properties such as appropriate setting time, flow, low viscosity, radio-opacity, appropriate particle size, low superficial tension, low solubility and good adhesion. Also, its antibacterial effect plays a major role in the widespread use of AH Plus [48]. However, AH Plus is a hydrophobic material and moisture from dentine wall may affect its adhesion to canal walls. The adhesiveness of AH Plus based on the formation of a covalent bond by an open epoxide ring to an exposed amino group in the collagen network [49].

Methacrylate resin-based sealers are designed to bond to both core materials and dentin, creating a cohesive structure known as a "monoblock." However, early generations of gutta-percha struggled to bond with these sealers without the application of a polybutadiene di-isocyanate methacrylate adhesive. Recent studies have indicated that methacrylate resin-based sealers contain more voids and gaps compared to traditional sealers and gutta-percha. Furthermore, these sealers demonstrate higher leakage rates due to the degradation of the polymers over time[50].***Nunes et al.***[51] compared the adhesion of two endodontic sealers, Epiphany and AH Plus, to human root dentin treated with different solutions. Root canals were treated with distilled water, sodium hypochlorite (NaOCl), or a combination of NaOCl and EDTA. The results showed that AH Plus had significantly stronger adhesion to dentin than Epiphany, regardless of the treatment applied to the root canal walls. Additionally, treating the dentin with NaOCl and EDTA enhanced the bond strength of both sealers, with the combination of NaOCl and EDTA providing the best results.

Portland cement which is derived from the calcination of mixture of the limestones from Portland and silicon-argillaceous materials. Portland cement exhibits both antibacterial and antifungal that are similar to MTA. But Portland cements produce amounts of lead and arsenic released from Portland cement added to its high solubility when compared to MTA and it concerns about the safety with respect to the surrounding tissues. Uncontrolled setting expansion of Portland cement could lead to crack formation on the tooth[52]. Older BC materials like Portland cements (PC) have many inherent drawbacks such as releasing high amounts of lead and arsenic, higher solubility, excessive setting expansion (jeopardizing the long- term seal of the material), lower com pressive strength, and reduced long- term efficacy of the material [53].

Torabinejad [54] introduced Mineral trioxide aggregate (MTA), a calcium silicate-based hydrophilic cement, was introduced to dentistry in the early 1990s as a material displaying superior biological and physical properties. With its good sealing ability, biocompatibility, and osteoconductivity, it was initially used as a root-end filling material, but is now widely used for various applications, such as root perforation repair, pulp-dentin regeneration, apical barrier formation, pulp capping, pulpotomy, and root canal filling [55]. With these excellent properties of calcium silicate-based cements, endodontic sealers based on calcium silicate have been introduced. This kind of sealer sets by reacting with water or under humid conditions [56].

Recent generation of sealers is now available in the market that being engineered to improve their ability to penetrate into dentinal tubules or bond to dentine and core filling material [57].

CSBS overall reported a shorter setting time compared to conventional formulations such as AH Plus. However, prolonged setting times were also highlighted, depending not only on formulation, but also on root canal moisture, as it has been noted that when the root canal is dry, setting time tends to increase. This explains why setting times vary between clinical trials and laboratory studies, and small amounts of fluids in contact with sealers may affect the latter [58].

MTA Fillapex (Angelus, Londrina, Parana) has been recently proposed as an endodontic filling material. The strong interest in developing mineral trioxide aggregate based endodontic materials is because of the excellent biocompatibility, bioactivity, and osteoconductivity of MTA (59). MTA Fillapex is a sealer that is composed of MTA, salicylate resin, natural resin, bismuth oxide, and silica. A recent study showed that this sealer has suitable physico chemical properties, such as good radiopacity, flow, and alkaline pH (60).

Bin (61) evaluated the cytotoxicity and genotoxicity of MTA-based canal sealer (Fillapex), white MTA cement, and AH Plus. Chinese hamster fibroblasts were exposed to these materials, and cell viability and genotoxicity were measured using the MTT assay and micronucleus formation test. Results showed that white MTA was the least cytotoxic, maintaining cell viability above 50%, while AH Plus and Fillapex MTA exhibited higher cytotoxicity and increased genotoxic effects. Both AH Plus and Fillapex MTA significantly reduced cell viability and increased micronucleus formation compared to the control group.

Zmener et al. (62) examined the subcutaneous connective tissue reactions in rats to an MTA-based endodontic sealer (Fillapex) and compared it with Grossman sealer. Medical-grade silicone tubes containing the materials were implanted in 24 Wistar rats, and tissue responses were analyzed at 10, 30, and 90 days. Results showed that Fillapex caused severe tissue reactions at all time points, while Grossman sealer had similar reactions initially, with a slight reduction after 90 days. Negative controls showed no adverse reactions. Statistically significant differences between Fillapex and Grossman sealer were found after 90 days, and both materials were more toxic compared to the controls throughout the study. The study concluded that both MTA-Fillapex and Grossman sealer remained toxic to subcutaneous tissues after 90 days.

Salles et al. [63] assessed the biocompatibility and bioactivity of MTA Fillapex (MTA-F), an MTA-based endodontic sealer, in human osteoblast-like cell cultures. The results showed that MTA-F caused increased cytotoxicity during the first three days of exposure, but cell viability improved by day seven. In contrast, Epiphany SE (EP-SE) and zinc oxide–eugenol (ZOE) sealers were cytotoxic at all time points. MTA-F also significantly increased alkaline phosphatase activity and demonstrated the highest levels of calcium deposit formation, promoting hydroxyapatite crystal nucleation. The study concluded that MTA-F reduces in cytotoxicity over time and exhibits good bioactivity for stimulating hydroxyapatite formation.

Nikhil et al. [64] This study examined the effectiveness of four endodontic sealers AH Plus, Pulp Canal Sealer EWT, Sealapex, and MTA Fillapex focusing on their filling ability and dentinal tubule penetration in root canals. Forty single-rooted teeth were analyzed at 2, 4, and 6 mm from the apex. At 2 mm, all sealers performed similarly in filling voids and tubule penetration. However, at 4 and 6 mm, MTA Fillapex had inferior filling effectiveness compared to the others, while Pulp Canal Sealer EWT showed poorer dentinal tubule penetration. Overall, MTA Fillapex had adaptation issues at 4 and 6 mm, and Pulp Canal Sealer EWT showed lower dentinal penetration at these levels.

TotalFill BC Sealer HiFlow is premixed single syringe which contains calcium silicates, calcium phosphate monobasic, zirconium oxide, tantalum oxide and less thickening agents than TotalFill BC Sealer [65].

HiFlow had low viscosity at different temperatures and fine particle size (<1µm) [66]. Suitable physicochemical properties that harden in the presence of moisture as found in wet locations such as dentinal tubules [67].

Antunes et al. **[68]** assessed the impact of heating on the chemical and physical properties of calcium silicate-based endodontic sealers, specifically EndoSequence BC Sealer HiFlow, Bio-C Sealer, BioRoot RCS, and AH Plus. Utilizing vibrational spectroscopy and Fourier-transform infrared spectroscopy, the researchers found that heating resulted in minimal chemical alterations, with inorganic components dominating the spectroscopic signals. Notably, changes in water absorption were observed for EndoSequence and Bio-C Sealers, while Bio-C Sealer showed signs of thermal decomposition at 100°C. The study also revealed that heating significantly affected the flowability and setting times of all sealers, with solubility levels exceeding ISO standards, which raises clinical concerns. AH Plus emerged as the only sealer exhibiting stability after heating, underscoring its potential suitability for clinical applications. Overall, the findings indicate that heating can compromise the properties of calcium silicate-based sealers, particularly regarding solubility and flowability.

Alegre et al. [69] this study investigated the intratubular penetration of a bioceramic sealer using various obturation techniques, including continuous wave (CW) and vertical compaction (VC) with conventional and bioceramic-coated gutta-percha, as well as a single cone (SC) technique. A total of 150 single-root human teeth were prepared and filled with TotalFill BC Sealer HiFlow before being sectioned and analyzed with confocal laser microscopy. The results showed significant differences in penetration areas among the techniques, with greater penetration in the coronal third compared to the apical third. Importantly, the findings indicated that the penetration of the bioceramic sealer was more influenced by the obturation technique than by the type of gutta-percha used, with warm obturation methods yielding superior results compared to the SC technique.

Reynolds et al. [70] compared the depth and percentage of dentinal tubule penetration using single-cone (SC) and warm vertical (WV) obturation techniques with two bioceramic sealers (BC Sealer and BC Sealer HiFlow) and an epoxy resin-based sealer (2Seal easymiX). Fifty canals were filled with either bioceramic or resin-based sealers, with the bioceramic groups using both SC and WV techniques, while the control group used only the WV technique. After sectioning the roots at 3 mm and 6 mm from the apex and analyzing them with a confocal laser microscope, the results showed significantly greater penetration at the 6 mm level compared to the 3 mm level. However, no significant differences were found between the types of sealers or obturation techniques at either level. Overall, the study concluded that dentinal tubule penetration was similar across the three sealers when using both obturation techniques.

Rodríguez-Lozano et al. [71] assessed the effects of Endosequence BC Sealer HiFlow (BCHiF), a calcium silicate-based sealer, on human periodontal ligament stem cells (hPDLSCs) in a laboratory setting. Eluates from BCHiF, EndoSequence BC Sealer (BCS), and an epoxy resin-based sealer (AH Plus) were evaluated for their chemical composition using scanning electron microscopy and energy-dispersive X-ray analysis, as well as ion release with inductively coupled plasma-mass spectrometry. Cell viability, migration, morphology, and adhesion were analyzed using MTT assays, wound healing techniques, and direct contact methods. The results showed that BCHiF contained more zirconium than BCS, while BCS had slightly higher calcium levels. Both BCHiF and BCS demonstrated similar cell viability, migration, morphology, and adhesion, with the epoxy resin group showing reduced adhesion. The Alizarin Red assay indicated significant mineralization in both BCS and BCHiF compared to the control group. Additionally, both sealers promoted the expression of osteogenic and cementogenic genes. In conclusion, BCHiF exhibited suitable biological properties for root canal filling and enhanced gene expression related to bone and cement formation in hPDLSCs.

Schmidt et al. [72] This study evaluated the effectiveness of endodontic sealers' penetration into dentinal tubules using scanning electron microscopy (SEM). Fifty-two human upper central incisors were instrumented with the ProTaper Gold NiTi system and filled with either the epoxy resin sealer AH Plus or the calcium silicate-based sealer TotalFill BC Sealer HiFlow (TFHF) via warm vertical compaction. Root slices were obtained at varying distances from the apex and analyzed for sealer penetration. Results showed that AH Plus penetrated significantly deeper into the dentinal tubules compared to TFHF at all levels measured. Additionally, penetration was greater in the bucco-oral direction than in the mesio-distal direction. Overall, the study found that warm vertical compaction did not significantly improve sealer penetration depth, with average penetration not exceeding 110 µm.

2.2 Effect of moisture on depth of penetration of root canal sealers

The hydrophilic characteristics might improve the penetration of the sealer into moist dentin and dentinal tubules, potentially reducing microleakage. Manufacturers recommend keeping the root canal walls moist, not dehydrated, to make the most of the hydrophilic properties of the sealers, thus enabling resin tag penetration and/or the formation of a hybrid layer. However, clear-cut clinical steps on how to achieve an ideal dentinal root canal surface are not provided to practitioners(73).

Zmener et al., DDS, Dr Odont et al. (21) conducted an in vitro study to investigate the impact of varying levels of root canal moisture on coronal seal effectiveness following filling with different materials: resin-coated gutta-percha cones/EndoRez (RGPC/ER), Resilon/Epiphany (RE/EP), and gutta-percha/Grossman's cement (GP/G). Seventy-six single-rooted human teeth were standardized to a length of 17mm and prepared according to specified protocols. The roots were filled with the respective obturation systems and subjected to dye penetration assessment after immersion in methylene blue dye for 7 days. Results showed that moisture levels significantly influenced dye leakage, with RGPC/ER and RE/EP demonstrating superior sealing under moist conditions.

Ozlek et al. **(74)** This study aimed to explore the influence of dentin moisture levels on the dislocation resistance of two bioactive root canal sealers (MTA Fillapex and GuttaFlow BioSeal) at 3 weeks and 3 months post-obturation. Mandibular premolars were divided into groups based on dentin condition (dry, moist, or wet) and filled with either MTA Fillapex or GuttaFlow BioSeal. Dislocation resistance was assessed by measuring push-out bond strength. Results showed that moist dentin yielded higher bond strength values for both materials at both time points, significantly outperforming wet and dry dentin, particularly at 3 months. Results suggested that maintaining dentin moisture, specifically moist conditions, may enhance the interaction between dentin and bioactive sealers, potentially improving their efficacy in root canal filling procedures.

Eymirli et al. **(75)** conducted a study aiming to evaluate the effects of intraradicular moisture conditions on the push-out bond strength of root canal sealers. They prepared eighty root canals using rotary instruments and categorized them into four groups based on moisture conditions: ethanol (dry), paper points (blot dried), moist (dried with low vacuum), and wet (flooded). Each group was further subdivided based on the sealer used: AH Plus, iRoot SP, MTA Fillapex, and Epiphany. Bond strength was measured using a push-out test setup. Results showed that iRoot SP displayed the highest bond strength to root dentin regardless of moisture conditions, followed by AH Plus, Epiphany, and MTA Fillapex. The study concluded that the degree of residual moisture significantly affects sealer adhesion to radicular dentin and suggested that leaving canals slightly moist before filling may be advantageous for the tested sealers.

Wang et al. **(76)** The study aimed to assess the impact of different dentin moisture conditions, resulting from various root canal drying protocols, on the push-out strength of a bioceramic root canal sealer. Twenty extracted human premolars were prepared and assigned to four groups based on drying protocols: normal moisture (paper point), ethanol dry, isopropanol dry, and complete dry. After obturation with iRoot SP sealer, push-out strength was measured using a universal testing machine. Results showed that push-out strength varied significantly among moisture groups, with the highest strength observed in the paper point group and the lowest in the complete dry group. Cohesive failures predominated in the coronal and middle thirds of the root, while mixed failures were common in the apical third. The study concluded that different drying protocols influenced the push-out strength between the bioceramic sealer and canal wall.

2.3 Effect of heat on depth of penetration:

Total Fill BC Sealer HiFlow is a calcium silicate-based sealer developed to be heat-resistant [77], but with some modifications in its composition that make it more suitable for warm obturation techniques. It has a lower viscosity than the original Total Fill BC Sealer formulation. For an adequate setting of the sealer, the right level of humidity of the dentinal tubules is essential since the setting reaction of calcium silicate-based materials is based on amount of moisture present [78]. The warm obturation technique may impact the physicochemical properties of the sealer and disrupt the setting reaction. Therefore, it is crucial to have a solid understanding of root obturation techniques when using these bioceramic sealers [79].

Eid et al. **[80]** evaluated the dentinal tubule penetration of two calcium silicate-based sealers using warm vertical compaction (WVC) versus the single cone (SC) obturation technique. Confocal laser scanning microscopy (CLSM) was used to measure sealer penetration at 1 mm and 5 mm from the apex in mandibular premolars. Results showed that WVC provided significantly better sealer penetration at 1 mm, with both techniques performing similarly at 5 mm. Overall, WVC enhanced sealer penetration at both levels compared to the SC technique.

Aksel et al. [81] investigated the impact of heat on the setting and chemical properties of HiFlow BC Sealer compared to other root canal sealers. Heat application prolonged the setting time for Endosequence and HiFlow, while it accelerated the setting for AH Plus and BioRoot. HiFlow showed higher flow and lower viscosity at elevated temperatures, whereas

BioRoot exhibited reduced flow, increased viscosity, and significant weight loss. Despite heat application, FTIR analysis indicated no significant changes to functional groups, except for increased water absorption in BioRoot. Overall, HiFlow maintained favorable flow and viscosity properties after heat exposure.

Ito et al. (82) investigated the effect of heating on the physical properties of a newly developed premixed calcium silicate-containing sealer (AH Plus Bioceramic Sealer; AHB), compared to EndoSequence BC Sealer (ES), AH Plus Jet (AH), and Pulp Canal Sealer. The setting time, flow, and film thickness were evaluated with and without heating at 100°C for 30 or 60 seconds, following ISO 6876:2012 standards. Ultrastructural and elemental analyses were conducted using scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS). Data were analyzed using one-way ANOVA and Tukey post hoc test. All sealers demonstrated significantly reduced setting time and flow after heating. AHB exhibited significantly greater film thickness compared to the other materials after heating. None of the heat-applied properties of AHB and ES met ISO standards, except for ES's setting time. SEM/EDS results for AHB and ES were unaffected by heating. These changes in physical properties could negatively affect the performance of premixed calcium silicate-containing sealers, especially AHB, during warm vertical compaction.

De-Deus et al. **(83)** The aim of this study was to compare the depth of tubular dentinal penetration of sealer among three different filling techniques. Seventy-two maxillary central incisors were instrumented and randomly assigned to three groups: Group A underwent lateral compaction, Group B utilized the single cone technique, and Group C employed warm vertical compaction of gutta-percha. Each sample was longitudinally sectioned and prepared for SEM analysis. Results found variations in tubular penetration depth among the groups. Group A had depths ranging from 19 to 81mm (average 34 ± 16mm), Group B ranged from 26 to 101mm (average 44 ± 28mm), and Group C ranged from 22 to 188mm (average 67 ± 37mm). Statistical analysis revealed significant differences between Group C and both Group A ($P = 0.021$) and Group B ($P = 0.009$). However, there were no significant differences between Group A and Group B ($P > .05$). Notably, samples filled using warm vertical compaction of gutta-percha showed significantly deeper tubular sealer penetration compared to lateral compaction and the single cone technique.

Ordinola-Zapata et al. **[84]** compared the percentage and depth of sealer penetration into dentinal tubules during obturation using Sealer 26, GuttaFlow, or Sealapex with the lateral compaction technique. Thirty root canals were filled with each sealer, and confocal microscopy was used for analysis. Teeth were sectioned at 3 and 5 mm from the apex, and statistical analysis was conducted using analysis of variance–Tukey test ($P < .05$).

Results showed that Sealapex exhibited the deepest sealer penetration at both levels evaluated. No statistically significant difference was found between Sealer 26 and GuttaFlow at either level. Additionally, there was no statistical significance in the percentage of penetration around the root canal wall among the three sealers evaluated. Therefore, while Sealapex displayed deeper penetration into the dentinal tubules, there was no difference in the percentage of adaptation to the root canal walls among the three sealers.

McMichael et al. **[85]** assessed tubular penetration using continuous wave (CW) and single-cone (SC) obturation methods with various sealers.

Eighty single-rooted teeth were divided into eight groups and filled with different sealers using CW or SC techniques. After sectioning, confocal laser microscopy was used to measure the percentage and maximum depth of sealer penetration. Results revealed that Tricalcium silicate sealers penetrated up to 2000 mm (2 mm) into tubules, with higher penetration observed at 5 mm from the apex. MTA Fillapex, a resin-based sealer with less than 20% MTA particles, showed significantly greater tubule penetration with the warm vertical technique compared to SC at the 1-mm level. The study concluded that CW and SC techniques resulted in similar tubule penetration levels for tricalcium silicate sealers.

2- Evaluation of sealer penetration in dentinal tubules:

Studies have applied different techniques to investigate sealer penetration into. dentinal tubules, Such as light microscope and digital image processing, scanning electron microscope and confocal laser scanning microscope.

De Deus et al. [86] evaluated the effect of the filling technique on the depth of tubular penetration of sealer using light microscopy and digital image processing and concluded that the thermoplasticized gutta-percha technique shows deeper penetration of root canal sealer into dentinal tubules.

Engel et al. **[87]** In the study, light microscopy was employed to quantify the penetration of Roth's 801 sealer into dentinal tubules following final rinses with 70% isopropyl alcohol, Peridex, or 6% sodium hypochlorite in instrumented, smear-free root canals. Despite the various rinsing agents, light microscopy revealed no significant differences in sealer penetration among the groups, indicating that neither isopropyl alcohol nor Peridex improved the sealer's ability to penetrate compared to the control group rinsed with sodium hypochlorite.

Schmidt et al. **[88]** assessed the penetration depth of two endodontic sealers, AH Plus and TotalFill BC Sealer HiFlow utilizing Scanning electron microscopy (SEM)), into dentinal tubules, addressing the limitations of traditional staining methods. The study involved 52 human upper central incisors, with root canals filled using the warm vertical compaction technique. The results indicated that AH Plus exhibited significantly greater penetration compared to TFHF at all levels examined, with enhanced penetration in the bucco-oral direction relative to the mesio-distal direction. Despite the application of high pressure during the compaction process, the maximum penetration depth of the sealers was limited, not exceeding a mean of 110 µm. AH Plus and Total Fill BC Sealer HiFlow (TFHF), into dentinal tubules using scanning electron microscopy (SEM) due to the inadequacy of traditional staining methods. Involving 52 human upper central incisors, root canals were filled using warm vertical compaction, and root slices were examined for sealer penetration. Findings revealed that AH Plus penetrated significantly deeper than TFHF at all levels, with greater penetration observed in the bucco-oral direction compared to the mesio-distal direction. Despite the high pressure applied during warm vertical compaction, the maximum sealer penetration depth did not exceed a mean of 110 µm.

Balguerie et al. [89] Utilized scanning electron microscopy (SEM) to evaluate the tubular penetration and adaptation of five different sealers used with softened gutta-percha cones in single-rooted teeth. Fifty-two teeth were prepared and filled with the selected sealers, followed by cross-sectioning the roots for SEM analysis. The assessment focused on the adaptation of the sealers to the root canal and tubular walls, as well as the extent of tubular penetration in the apical, middle, and coronal thirds of the root canal. The results revealed that AH Plus, an epoxy resin sealer, demonstrated superior tubular adaptation and penetration compared to the other tested sealers. The study concluded that the degree of tubular penetration and adaptation is influenced by the physical and chemical properties of the sealers, with AH Plus showing optimal performance in both metrics.

SEM has limited use when measuring the depth of sealer penetration inside dentinal tubules. It has two main shortcomes: the difficulty in obtaining homogeneous results and the artifacts produced during sample preparation [(90)].

To overcome the limitations of SEM, confocal laser scanning microscopy was used in the current study to accurately measure sealer penetration. CLSM allows the measurement of sealer penetration below the surface of the dentine, eliminating the need for destructive specimen preparation or smear layer removal which may result in loss of sealer [(91)].

CLSM is designed to be confocal with a point of light placed in front of the photodetector when the laser beam is concentrated on the sample. Thus, the photodetector receives only information from the focus plane of interest. The benefit of this approach is its ability to visualize structures on different layers within a biological specimen [(92)]. Chong 1990 first reported the use of confocal microscopy in endodontic research, and the usefulness of this microscope in evaluating adaptation and sealing ability has been well demonstrated [(93)].

To facilitate fluorescence under confocal microscopy, the sealers were labelled with Rhodamine B dye. Rhodamine B is a fluorescent dye that generates light following excitation by light. Rhodamine dyes are widely used for applications in biotechnology, including fluorescence microscopy, flow cytometry and ELISA [94].

Rhodamine B has been used successfully in many studies as an indicator for sealer penetration [95,96]. However, a study evaluated dentinal tubule penetration of calcium silicate-based sealer under CLSM which used Fluo-3 dye and concluded that Rhodamine B dye has a lower affinity for the calcium in the sealer composition compared to the Fluo-3[97].

Zapata et al. **[98]** with the help of confocal laser scanning microscope evaluated depth and percentage of endodontic sealers into dentinal tubules after root canal obturation using a lateral compaction technique and concluded that depth of penetration is affected by the type of sealer and root canal level.

McMichael et al. **[99]** evaluated dentinal tubule penetration of tricalcium silicate sealer using CLSM. He showed that the tricalcium silicate sealer penetrated into dentinal tubules as deep as 2000 um (2mm) and that the continuous wave and single cone techniques produced similar tubule penetration.

Jeong et al. **[100]** 110 by means of different obturation techniques using confocal microscopy evaluated the penetration of calcium silicate-based root canal sealer and showed that the sealer penetration into dentinal tubules occurs independently of the obturation technique. Also, another study evaluated epoxy resin sealer penetration by confocal laser scanning microscope and stated that the sealer penetration was not dependent on the root canal filling technique [101].

3. Aim of the Study

This study aimed to evaluate the effect of moisture and heat on the depth of penetration of two calcium silicate-based root canal sealers in extracted premolars using confocal scanning microscopic evaluation.

4. MATERIALS AND METHODS

Section outline

4.1: Study design and ethical committee approval.

4.2: Sample size calculation.

4.3: Selection and preparation of the teeth.

4.4: Chemo mechanical preparation of the samples.

4.5: Grouping of the samples.

4.6: Obturation of the samples.

4.7: Incubation of the samples.

4.8: Storage of the samples.

4.9: Sectioning of the root specimens.

4.10: Evaluation of the specimens.

4.11: Statistical analysis of the data.

4.1. Study design and ethical committee approval:

This is an in vitro randomized, interventional study. The study was accepted by ethical committee of the Faculty of Dental Medicine, Al Azhar University Cairo, boys with a code number (913/3691).

4.2. Sample size calculation:

Sample size calculation was done using G Power statistical power analysis program (version 3.1.9.4) to determine the appropriate sample size (102), based on outcomes of earlier study (103). A total sample size of 12 samples per group (6per each group) is deemed adequate to detect a substantial effective size (f) of 0.752, attaining an actual power (1-β error) of 0.95 (95%) and a significant level (α error) of 0.05 (5%) for a two-tailed hypothesis test. Although this result 10 samples for each subgroup were used.

4.3. Selection and preparation of the teeth:

A total of 120 freshly extracted single rooted mandibular premolar teeth were collected from the outpatient clinic of oral surgery department at faculty of dental medicine, Al-Azhar university from patients ranging between 18 and 40 years old. The teeth were extracted due to medical reasons not related to this study. Teeth were cleaned from soft and hard deposits using hand periodontal curette and scalpel blade, then disinfected by immersion in 5.25% Sodium hypochlorite (NaOCl) (Egyptian Company for household bleach, Cairo, Egypt) for 10 minutes then were rinsed with distilled water. The teeth were evaluated under a dental operating microscope (DOM) (S2350, Zumax Medical Company, Jiangsu, China) at 16x magnification to detect possible cracks. Initial radiographic evaluation was taken from buccolingual and mesiodistal directions using a digital sensor size 2 (Dabiatlante, Brazil) to detect if calcification, pulp stones or severe canal curvatures were present.

Mold preparation:

Fabrication of 8 artelion plastic molds were done as a modification of Alkhawas M. [104]. 17 mm in thickness, and contains 10 circular holes 5mm in diameter. Pink wax was used for teeth fixation inside the holes of the mold during radiographic examination. CBCT was done using Planmeca Promax 3D mid machine (Planmeca Promax 3D Mid Machine, Helsinki, Finland) with exposure time 15 seconds with 90 KV, 12 mA and voxel size 70 microns. CBCT scan was done to confirm type 1 root canal system according to vertucci classification. The values of image contrast and brightness were constantly adjusted using the software (Romexis) image-processing tool to ensure optimal standardization. Fig. (1), (2) and (3).

Figure (1): Photograph showing mold with 10 circular 5mm in diameter holes

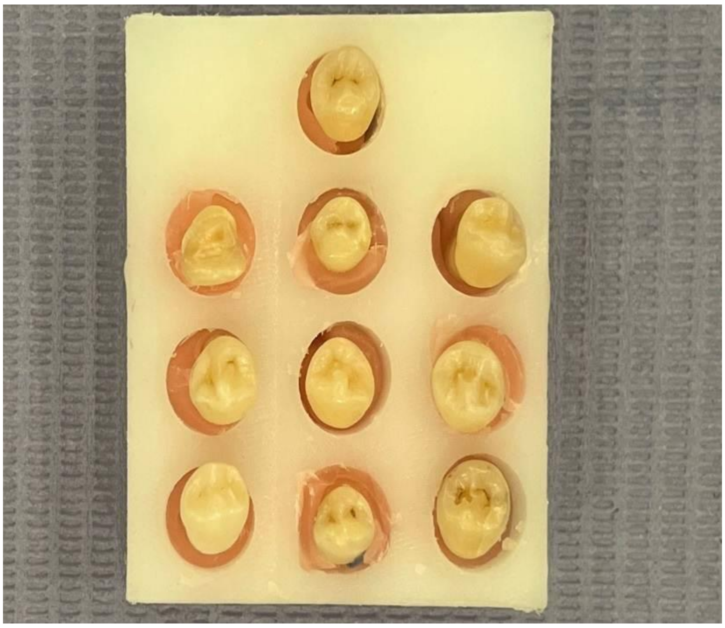


Figure (2): Photograph showing teeth held with wax in the mold at 17 mm.

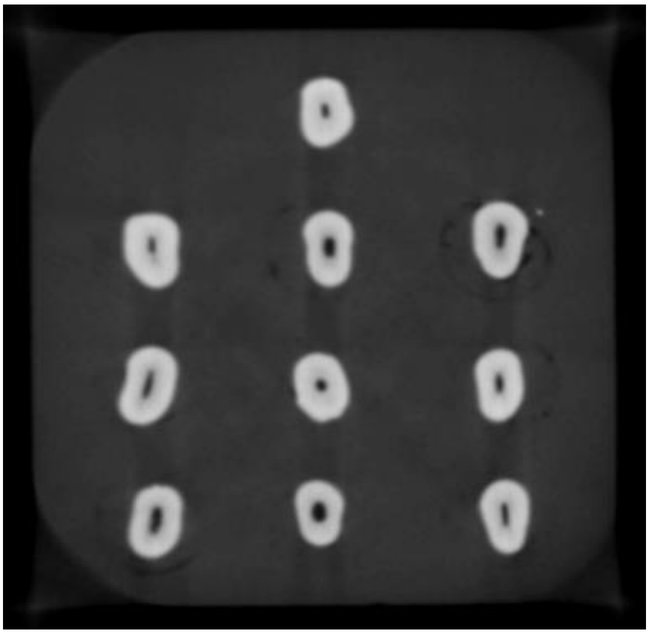


Figure (3): CBCT axial cut showing vertucci I root canal system

Out of 120 collected teeth, 80 freshly extracted teeth were selected to be used in the study according to the following inclusion criteria.

Inclusion criteria:

- Teeth with average length of 20-22 mm.
- Teeth with mature apices.
- Teeth with curvature ranging from 0 to 10 degree according to schnieder method in both mesiodistal and buccolingual direction.
- Teeth with type I single root canal according to vertucci classification.

Decoronation of the selected teeth was done at the coronal level of the plastic mold using low speed diamond saw of 0.3mm thickness under water coolant to be 17 mm root length. Each sample was stored in eppendorf containing normal saline solution at room temperature and finally each eppindorf was numbered from 1 to 80 using randomization software (http://www.randomized.org).

4.4 Chemo-mechanical preparation of the samples

Using #10 K file (Mani, Tochigi, Japan) patency of the root canal was performed by seeing the tip of the file at the apex then working length was adjusted by subtracting 1mm from the apex, Endostar E3 azure (Poledent, Warsaw, Poland) rotary file system was used for shaping of the root canal using NSK Endo-Mate DT motor (NSK, MIO, Tokyo, Japan) at settings speed 300 rpm and torque 2.1 Ncm according to the following sequence. Firstly #30 with taper 8% orifice opener was used for the coronal third at length 5mm, #20 with taper4%, #25with taper 4%, #30 with taper 4% and #40 with taper 4% to the full working length. Between each file, the Root canal was irrigated using 3 ml side vented needle (Side-vented, Demtsply-Maillefer, Balligues. Switzerland) with 5.25% sodium hypochlorite. The master cone was checked clinically and radiographically to confirm the cone fitting to the full working length. Finally, the root canals were irrigated by the following sequence: 3ml 5.25% sodium hypochlorite, 3ml distilled water, Ethylenediaminetetraacetic acid (EDTA) 17% (Pulpdent, USA) followed by 3 ml distilled water then 3ml 5.25% sodium hypochlorite and EDTA activated by passive ultrasonic tip irrisafe for 60 seconds, the canals were finally rinsed using distilled water.

4.5. Grouping of the samples:

Sample grouping was done based on the moisture condition, obturation technique and sealer type fig. (9):

Group 1 (n=10): samples were obturated using single matched cone technique utilizing BC sealer TotalFill BC Sealer HiFlow (FKG Dentaire, St. Maur de Fossés, Switzerland) in moist condition.

Group 2 (n=10): samples were obturated using single matched cone technique utilizing BC sealer TotalFill BC Sealer HiFlow in conventional dry condition.

Group 3 (n=10): samples were obturated using single matched cone technique utilizing MTA sealer Fillapex (Angelus, Londrina, Brazil) in moist condition.

Group 4 (n=10): samples were obturated using single matched cone technique utilizing MTA sealer Fillapex in conventional dry condition.

Group 5 (n=10): samples were obturated using warm vertical compaction technique utilizing BC sealer TotalFill BC Sealer HiFlow in moist condition.

Group 6 (n=10): samples were obturated with warm vertical compaction technique utilizing BC sealer TotalFill BC Sealer HiFlow in conventional dry condition.

Group 7 (n=10): samples were obturated using warm vertical compaction technique utilizing MTA sealer Fillapex in moist condition.

Group 8 (n=10): samples were obturated using warm vertical compaction technique utilizing MTA sealer Fillapex in conventional dry condition.

4.6. Moisture control:

- **Moist condition:** The moisture control was done according to the protocol submitted by Esin Ozlek, Hüseyin Gündüz, and Prasanna Neelakantan[105].in which the microcannula of the negative apical pressure irrigation system (EndoVac, SybronEndo, Orange, CA, USA) was placed into the root canal at the working length and moved in up-down motion for 5 seconds followed by 1 single paper point for 1 second.
- **Conventional dry condition*:*** The root canals were dried using #40 0.04 paper points. The moisture condition of the canal was done by a technique which depends on the weight difference of the paper point before insertion inside the root canal and after insertion inside the root canal. The weight was recorded for each paper point before insertion inside the canal and was recorded after removal from the canal. when weight before and after insertion was the same, the canal was assumed conventional dry. The paper points were weighted using (professional digital mini scale, China) Fig. (4).

Figure (4): Photograph showing weight balance of the paper point

4.7: Obturation of the samples:

Sealers were mixed with Rhodamine B dye for further analysis using confocal laser scanning microscope, each 1gm of the sealer was mixed with 0.001gm of 0.1% Rhodamine B dye measured by micropipette Fig. (5)

Figure (5): Photograph showing the micropipette used for Rodamine B application.

4.7.1. Single matched cone obturation technique:

Master cone fitting was checked clinically and radiographically. Sealer was loaded on Gutta percha #40 0.04(Meta Biomed, Chungbuk, Korea) and inserted into the canal. Excess gutta percha was removed by a heated condenser below the canal orifice level and the excess sealer was removed by moist cotton.

4.7.2 Warm vertical compaction:

In warm vertical compaction gutta percha #40 0.04 was used. Apical tug back of the gutta-percha was checked. #50/0.05 plugger size of (Fast pack, Eighteeth,China) was checked to reach 4 to 5 mm from apex with master cone fitted.. Master cone was embedded in the sealer at the last 3mm. After inserting master cone with sealer. The heat unit device (Fast pack, Eighteeth,China) was adjusted at heat temperature 200ºC and heat was applicated through the prefitted plugger to the coronal third. Then heat is turned off while maintaining pressure for 5 seconds. Heat is reapplied to the carrier and more apical pressure is applied to the middle third; heat is again turned off while maintaining apical pressure. A final one second burst of heat is applied to the heat carrier plugger then lateral movement to separate the master cone 4-5 mm of the working length. After cutting the gutta percha it was immediately condensed with a #50 Niti plugger (Eighteeth, Changzhou, China). Then back filling with warm gutta percha filling device Fast Fill (Eighteeth, Changzhou, China) was applied at 200ºC to fill the middle third and condensed with a #60 plugger, then a plugger #80 Niti to fill the coronal third until below the level of canal orifice by 1mm.

4.8 Incubation of the specimens:

The excess sealer was removed using a moist cotton pellet. The samples were kept at 100% humidity and at 37°C in an incubator (Memmeret, Schwabach, Germany) for two weeks to ensure complete setting of all specimens.

4.9 Sectioning of the specimens:

Each root was sectioned horizontally using low speed diamond saw with water coolant (IsoMet 4000 Precision Saw, Telangana, Secunderabad, India) at 3 mm, 6 mm and 9 mm from root apex. The dimensions of each slice were checked with a digital caliper to ensure their accuracy at 1±0.2 mm. All sections were polished with silicone carbide abrasive papers. For each slice was mounted onto glass coverslip 22X50 mm to be examined under confocal laser scanning microscope Fig. (6).

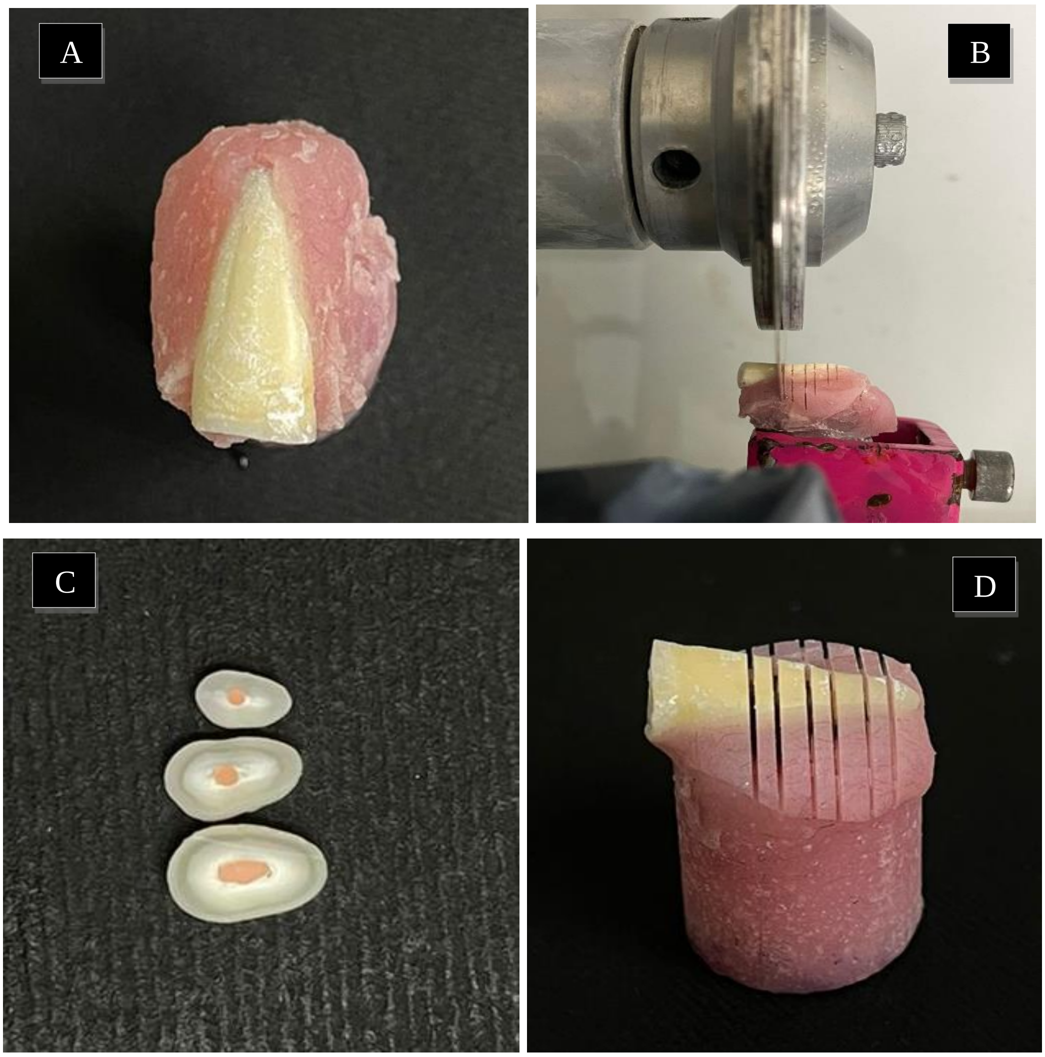


Figure (6): A photograph showing (A) the sample in the resin block, (B) isomet saw during horizontal cutting of the sample, (C) the sample after sectioning, (D) the root slices at the coronal, middle, and apical levels.

4.10 Confocal laser Microscopy of the specimens:

The slices were investigated under a Zeiss confocal laser scanning microscope LSM (Carl Zeiss, Jena, Germany) was set at the excitation wavelength 543 nm and the emission wavelengths of 546 – 735 nm in low light condition as the Rhodamine B dye is sensitive to light at digital zoom 1.2. The full sample acquisition was imaged with a 10x objective lens in the format of 1,024 × 1,024 pixels. While the 40x oil lenses confirm the content of the sealer inside the dentinal tubules Each sample was evaluated for a consistent fluorescent ring around the canal wall, indicating the sealer dye distribution. The penetration depth of the sealer into the dentinal tubules was illustrated by the fluorescence, which started from the outer surface of the canal until the maximum depth around the canal wall which serves as the starting point. The analysis of images acquired by CLSM were done by two equations: Fig. (7)

Figure (7): A photograph showing confocal laser scanning microscopy

The amount of sealer penetrated into the dentinal tubules was calculated by:

Dentin area = Total area – root canal area. Fig. (8)

$$percentage\ of\ sealer\ penetration = \frac{maximum\ area\ filled\ by\ sealer - root\ canal\ cross\ sectional\ area}{dentin\ area\ of\ root\ canal}$$

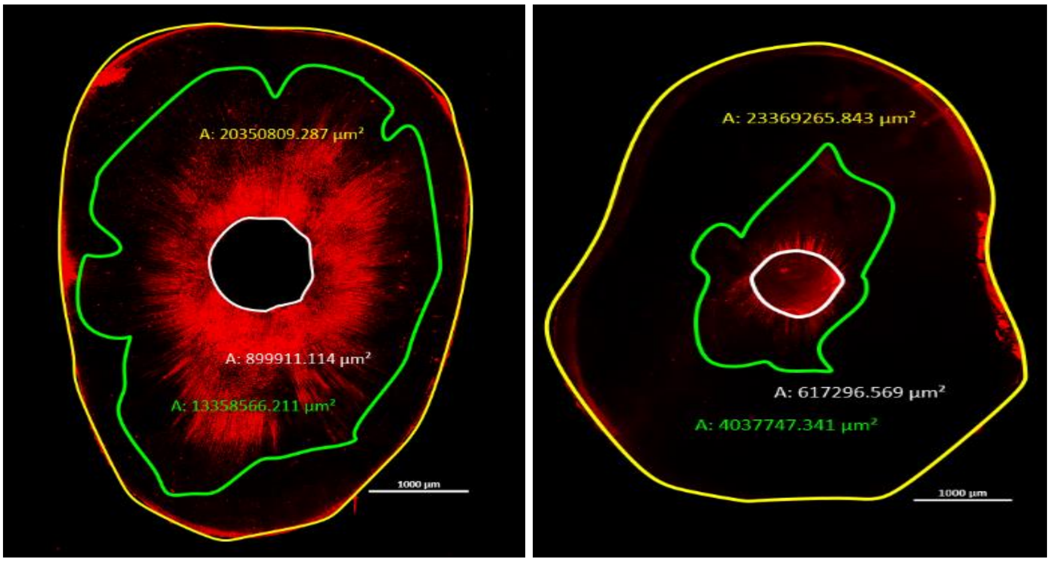


Figure (8): A micrograph representing sealer penetration into dentinal tubule, total cross section area of the root (yellow area), maximum area filled by the sealer (green area), root canal cross sectional area (white area)

4.11 Statistical analysis of the data:

Numerical data were explored for normality by checking the distribution of data and using tests of normality (Kolmogorov-Smirnov and Shapiro-Wilk tests). All data showed normal (parametric) distribution. Data were presented as mean and standard deviation (SD) values. Repeated measures ANOVA test was used to study the effect of condition, compaction technique, sealer type, root level and their interactions on different variables. Bonferroni's post-hoc test was used for pair-wise comparisons when ANOVA test is significant. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.

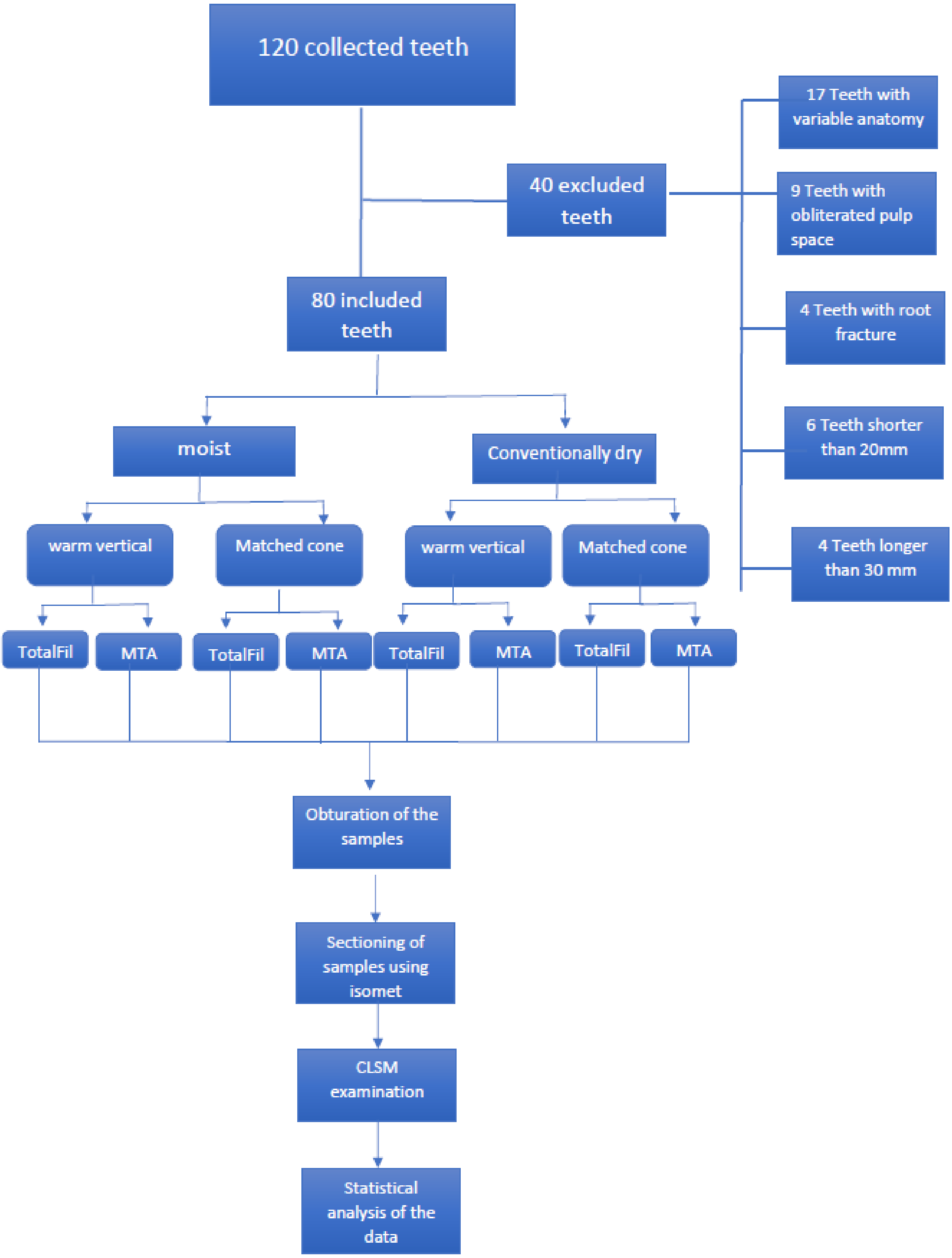


Figure (9): Selection of teeth and grouping of the samples

5. RESULTS

Repeated measures ANOVA results

The results showed that condition (regardless of other variables) had a statistically significant effect on mean sealer penetration. Compaction technique (regardless of other variables) had a statistically significant effect on mean sealer penetration. Sealer type (regardless of other variables) had a statistically significant effect on mean sealer penetration. Root level (regardless of other variables) had a statistically significant effect on mean sealer penetration. The interaction between variables had no statistically significant effect on mean sealer penetration. Since the interaction between the variables is non-statistically significant, the variables are independent from each other Table (1).

Table (1): Repeated measures ANOVA results for the effect of different variables on mean sealer penetration (%)

Source of variation	**Type III Sum of Squares**	**Df**	**Mean Square**	***F*-value**	***P*-value**	**Effect size *(Partial eta squared)***
Condition	834.931	1	834.931	14.794	<0.001*	0.170
Compaction technique	1026.482	1	1026.482	18.188	<0.001*	0.202
Sealer type	40717.810	1	40717.810	721.481	<0.001*	0.909
Root level	15618.046	2	7809.023	376.944	<0.001*	0.840
Condition x Compaction technique x Sealer type x Root level interaction	3.916	2	1.958	0.095	0.910	0.001

*df: degrees of freedom = (n-1), *: Significant at P ≤ 0.05*

a. <u>Effect of condition regardless of obturation technique, sealer type and root level</u>

Regardless of compaction technique, sealer type and root level, moist condition (31.6±18.7) showed statistically significantly higher mean sealer penetration % than dry condition (27.9±18.7) Table (2) Fig.(10).

Table (2): The mean, standard deviation (SD) values and results of repeated measures ANOVA test for comparison between sealer penetration (%) of the two conditions regardless of compaction technique, sealer type and root level

Dry		Moist		*P*-value	*Effect size (Partial Eta squared)*
Mean	**SD**	**Mean**	**SD**		
27.9	14.9	31.6	18.7	<0.001*	0.170

**: Significant at P ≤ 0.05*

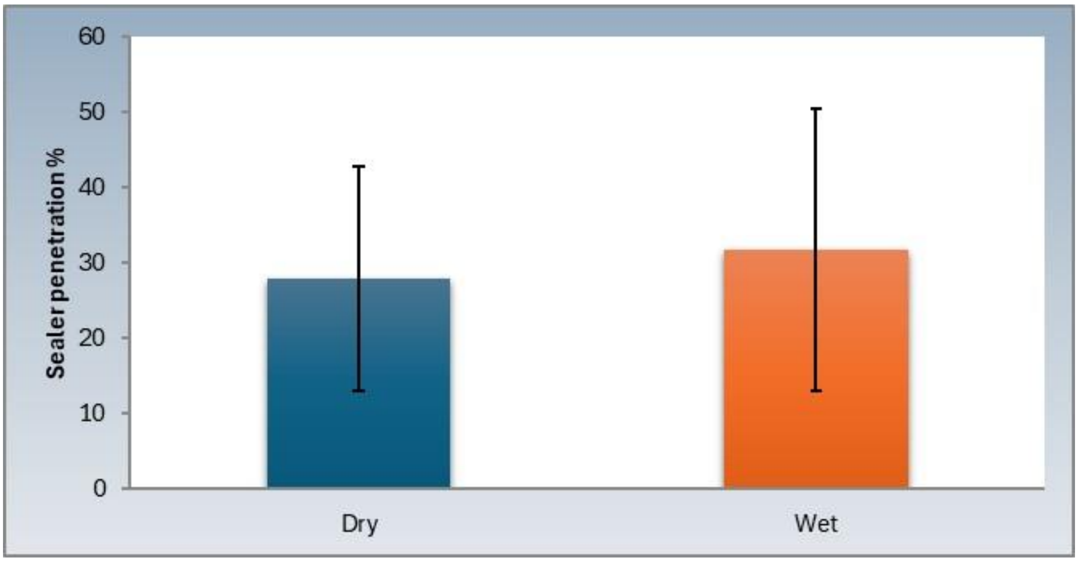


Figure (10): Bar chart representing mean and standard deviation values for sealer penetration of the two conditions regardless of compaction technique, sealer type and root level

b. Effect of obturation technique regardless of condition, sealer type and root level

Regardless of condition, sealer type and root level, warm vertical compaction (31.8±17.1) showed statistically significantly higher mean sealer penetration % than matched cone technique (27.7±16.7) Table (3) Fig. (11).

Table (3): The mean, standard deviation (SD) values and results of repeated measures ANOVA test for comparison between sealer penetration (%) of the two compaction techniques regardless of condition, sealer type and root level

Matched cone		Warm vertical compaction		*P*-value	*Effect size (Partial Eta squared)*
Mean	SD	Mean	SD		
27.7	16.7	31.8	17.1	<0.001*	0.202

**: Significant at P ≤ 0.05*

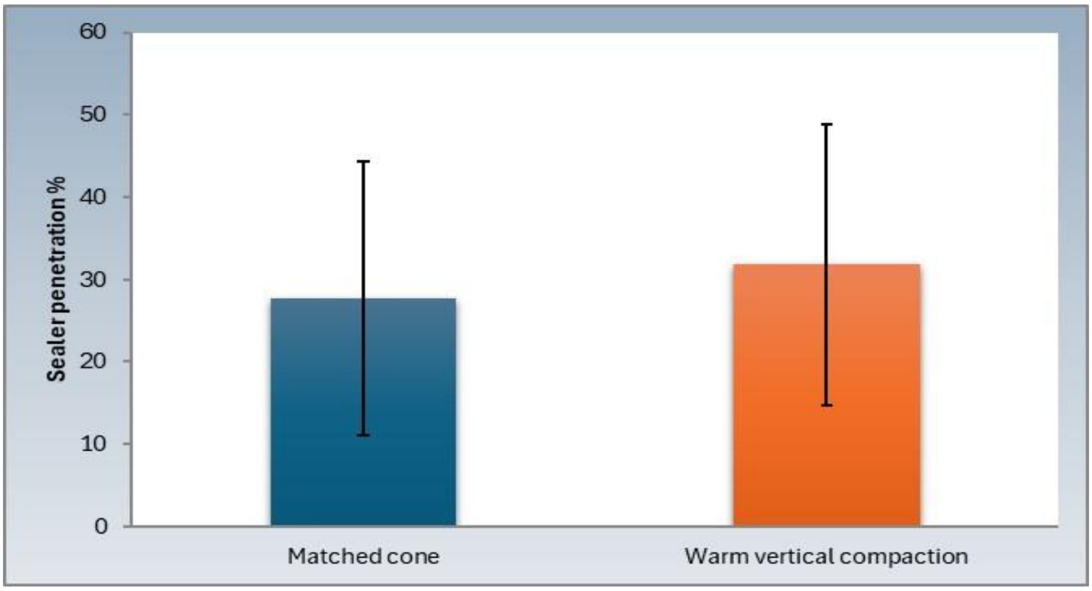


Figure (11): Bar chart representing mean and standard deviation values for sealer penetration of the two compaction techniques regardless of condition, sealer type and root level

c. *Effect of sealer type regardless of condition, compaction technique and root level*

Regardless of condition, compaction technique and root level, HiFlow (42.8±14.2) showed statistically significantly higher mean sealer penetration % than MTA (16.7±16.7) Table (4) Fig. (12).

Table (4): The mean, standard deviation (SD) values and results of repeated measures ANOVA test for comparison between sealer penetration (%) of the two sealer types regardless of condition, compaction technique and root level

MTA		HiFlow		*P*-value	*Effect size (Partial Eta squared)*
Mean	SD	Mean	SD		
16.7	6	42.8	14.2	<0.001*	0.909

**: Significant at P ≤ 0.05*

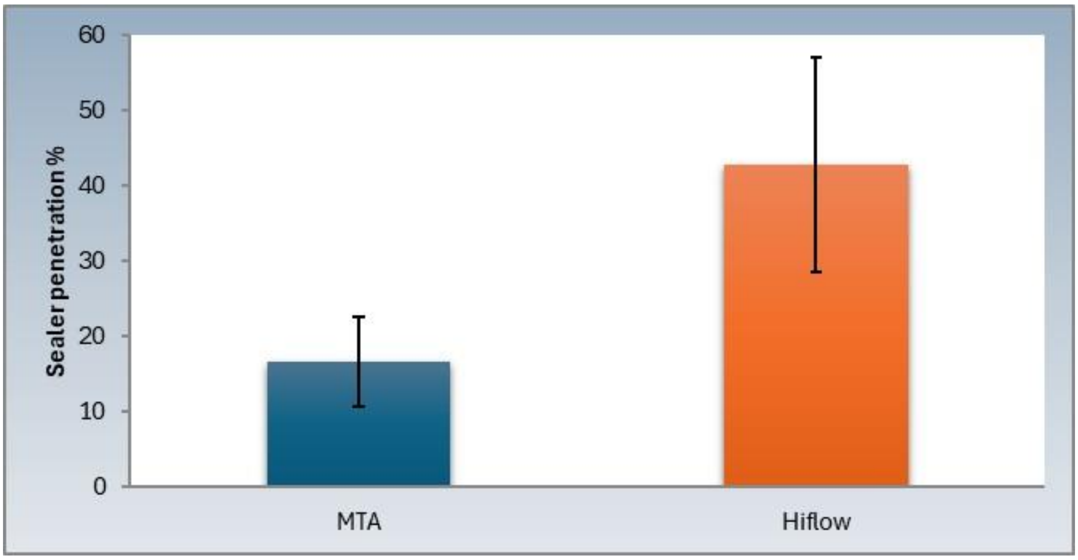


Figure (12): Bar chart representing mean and standard deviation values for sealer penetration of the two sealer types regardless of condition, compaction techniques and root level

d. Effect of root level regardless of condition, compaction technique and sealer type

Regardless of condition, compaction technique and sealer type, there was a statistically significant difference between root levels. Pair-wise comparisons revealed that coronal level (40.1 ± 18.4) showed the statistically significantly highest mean sealer penetration %. Middle root level (28.6 ± 14.5) showed statistically significantly lower mean value. Apical root level (20.5±11.4) showed the statistically significantly lowest mean sealer penetration %. Table (5) Fig. (13).

Table (5): The mean, standard deviation (SD) values and results of repeated measures ANOVA test for comparison between sealer penetration (%) at different root levels regardless of condition, compaction technique and sealer type

Coronal		Middle		Apical		*P*-value	*Effect size (Partial Eta squared)*
Mean	SD	Mean	SD	Mean	SD		
40.1 ^{A}	18.4	28.6 ^{B}	14.5	20.5 ^{C}	11.4	<0.001*	0.840

**: Significant at P ≤ 0.05, Different superscripts indicate statistically significant difference between root levels*

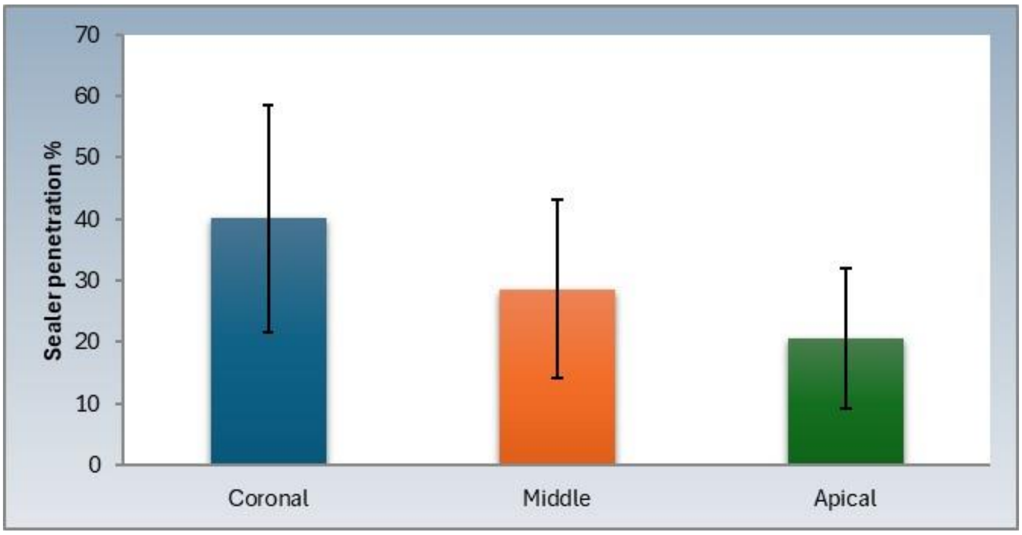


Figure (13): Bar chart representing mean and standard deviation values for sealer penetration (%) at different root levels regardless of condition, compaction technique and sealer type

<u>Effect of different interactions of variables on sealer penetration %</u>

1. ***Comparison between conditions with different interactions of variables***

Using matched cone technique with MTA, there was no statistically significant difference between dry and moist conditions at the coronal, middle as well as apical levels.

While for matched cone technique with HiFlow, moist condition showed statistically significantly higher mean sealer penetration % than conventional dry condition at the coronal and middle root levels. There was no statistically significant difference between conventional dry and moist conditions at the apical level.

Using warm vertical compaction technique with MTA, there was no statistically significant difference between conventional dry and moist conditions at the coronal, middle as well as apical levels.

While for warm vertical compaction technique with HiFlow, moist condition showed statistically significantly higher mean sealer penetration % than conventional dry condition at the coronal, middle as well as apical root levels. Table (6) Fig. (14).

Table (6): The mean, standard deviation (SD) values and results of repeated measures ANOVA test for comparison between sealer penetration (%) of the two conditions with different interactions of variables

Compaction technique	Sealer type	Root level	Dry		Moist		*P*-value	*Effect size (Partial Eta squared)*
			Mean	SD	Mean	SD		
Matched cone	MTA	Coronal	20.8	3.1	21.2	3.7	0.868	0.0004
		Middle	15.1	1.3	15.5	2	0.899	0.0002
		Apical	10.8	1.6	10.1	1.9	0.796	0.001
	HiFlow	Coronal	53	4.4	58.6	10.8	0.022*	0.071
		Middle	32.6	7.4	39.3	13.5	0.015*	0.079
		Apical	26.9	10.2	28.2	9.3	0.607	0.004
Warm vertical compaction	MTA	Coronal	22.9	2.2	27.1	5.2	0.081	0.042
		Middle	16.5	3.2	17.9	4.1	0.622	0.003
		Apical	11.7	2.5	10.9	1.8	0.736	0.002
	HiFlow	Coronal	53.2	5.3	64.3	3.1	<0.001*	0.232
		Middle	41	2.1	51	4	<0.001*	0.161
		Apical	30	3	35.3	5.3	0.045*	0.055

**: Significant at $P \leq 0.05$*

2. Comparison between compaction techniques with different interactions of variables

With dry condition and MTA, there was no statistically significant difference between the two compaction techniques at the coronal, middle as well as apical levels.

With conventional dry condition and HiFlow, there was no statistically significant difference between the two compaction techniques at the coronal as well as apical levels. At the middle level, warm vertical compaction showed statistically significantly higher mean sealer penetration % than matched cone technique.

With moist condition and MTA, warm vertical compaction showed statistically significantly higher mean sealer penetration % than matched cone technique at the coronal root level. At the middle as well as apical root levels, there was no statistically significant difference between the two compaction techniques.

While for moist condition and HiFlow, warm vertical compaction technique showed statistically significantly higher mean sealer penetration % than matched cone at the coronal, middle as well as apical root levels. Table (7) Fig. (14).

Table (7): The mean, standard deviation (SD) values and results of repeated measures ANOVA test for comparison between sealer penetration (%) of the two compaction techniques with different interactions of variables

Condition	**Sealer type**	**Root level**	**Matched cone**		**Warm vertical compaction**		***P*-value**	***Effect size (Partial Eta squared)***
			Mean	**SD**	**Mean**	**SD**		
Dry	MTA	Coronal	20.8	3.1	22.9	2.2	0.381	0.011
		Middle	15.1	1.3	16.5	3.2	0.613	0.004
		Apical	10.8	1.6	11.7	2.5	0.708	0.002
	HiFlow	Coronal	53	4.4	53.2	5.3	0.944	0.0001
		Middle	32.6	7.4	41	2.1	0.003*	0.119
		Apical	26.9	10.2	30	3	0.228	0.020
Moist	MTA	Coronal	21.2	3.7	27.1	5.2	0.015*	0.079
		Middle	15.5	2	17.9	4.1	0.385	0.011
		Apical	10.1	1.9	10.9	1.8	0.768	0.001
	HiFlow	Coronal	58.6	10.8	64.3	3.1	0.020*	0.073
		Middle	39.3	13.5	51	4	<0.001*	0.208
		Apical	28.2	9.3	35.3	5.3	0.008*	0.095

**: Significant at $P \leq 0.05$*

3. Comparison between sealer types with different interactions of variables

With all interactions of variables, HiFlow showed statistically significantly higher mean sealer penetration % than MTA at the coronal, middle as well as apical root levels. Table (8) Fig. (14).

Table (8): The mean, standard deviation (SD) values and results of repeated measures ANOVA test for comparison between sealer penetration (%) of the two sealer types with different interactions of variables

Condition	Compaction technique	Root level	MTA		HiFlow		*P*-value	*Effect size (Partial Eta squared)*
			Mean	SD	Mean	SD		
Dry	Matched cone	Coronal	20.8	3.1	53	4.4	<0.001*	0.716
		Middle	15.1	1.3	32.6	7.4	<0.001*	0.366
		Apical	10.8	1.6	26.9	10.2	<0.001*	0.354
	Warm vertical compaction	Coronal	22.9	2.2	53.2	5.3	<0.001*	0.690
		Middle	16.5	3.2	41	2.1	<0.001*	0.533
		Apical	11.7	2.5	30	3	<0.001*	0.413
Moist	Matched cone	Coronal	21.2	3.7	58.6	10.8	<0.001*	0.773
		Middle	15.5	2	39.3	13.5	<0.001*	0.519
		Apical	10.1	1.9	28.2	9.3	<0.001*	0.409
	Warm vertical compaction	Coronal	27.1	5.2	64.3	3.1	<0.001*	0.771
		Middle	17.9	4.1	51	4	<0.001*	0.677
		Apical	10.9	1.8	35.3	5.3	<0.001*	0.556

**: Significant at P ≤ 0.05*

4. Comparison between root levels with different interactions of variables

With all interactions of variables, there was a statistically significant difference between root levels. Pair-wise comparisons revealed that coronal level showed the statistically significantly highest mean sealer penetration (20.8±). Middle root level showed statistically significantly lower mean value (15.1±1.3). Apical root level showed the statistically significantly lowest mean sealer penetration (10.8±1.6). Table (9) Fig. (14).

Table (9): The mean, standard deviation (SD) values and results of repeated measures ANOVA test for comparison between sealer penetration (%) at different root levels with different interactions of variables

Condition	Compaction technique	Root level	MTA		HiFlow	
			Mean	SD	Mean	SD
Dry	Matched cone	Coronal	20.8 ^{A}	3.1	53 ^{A}	4.4
		Middle	15.1 ^{B}	1.3	32.6 ^{B}	7.4
		Apical	10.8 ^{C}	1.6	26.9 ^{C}	10.2
	P-value		<0.001*		<0.001*	
	Effect size (Partial Eta squared)		0.241		0.715	
	Warm vertical compaction	Coronal	22.9 ^{A}	2.2	53.2 ^{A}	5.3
		Middle	16.5 ^{B}	3.2	41 ^{B}	2.1
		Apical	11.7 ^{C}	2.5	30 ^{C}	3
	P-value		<0.001*		<0.001*	
	Effect size (Partial Eta squared)		0.283		0.625	
Moist	Matched cone	Coronal	21.2 ^{A}	3.7	58.6 ^{A}	10.8
		Middle	15.5 ^{B}	2	39.3 ^{B}	13.5
		Apical	10.1 ^{C}	1.9	28.2 ^{C}	9.3
	P-value		<0.001*		<0.001*	
	Effect size (Partial Eta squared)		0.277		0.751	
	Warm vertical compaction	Coronal	27.1 ^{A}	5.2	64.3 ^{A}	3.1
		Middle	17.9 ^{B}	4.1	51 ^{B}	4
		Apical	10.9 ^{C}	1.8	35.3 ^{C}	5.3

	P-value	<0.001*	<0.001*
	Effect size (Partial Eta squared)	0.456	0.721

**: Significant at P ≤ 0.05, Different superscripts in the same column indicate statistically significant difference between root levels*

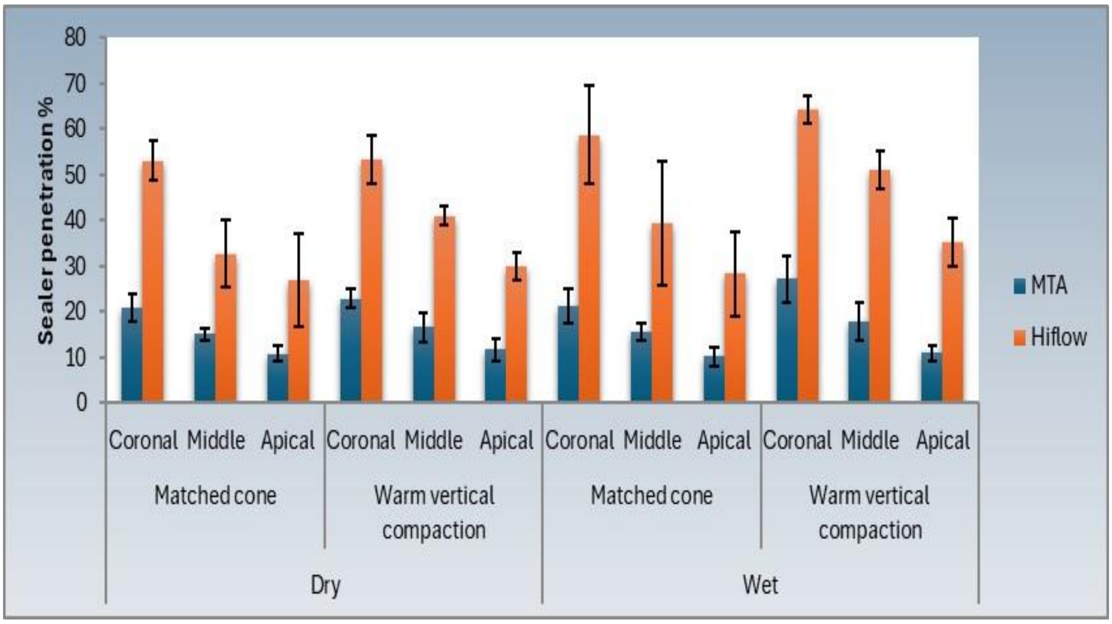
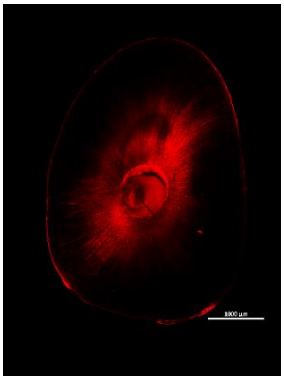
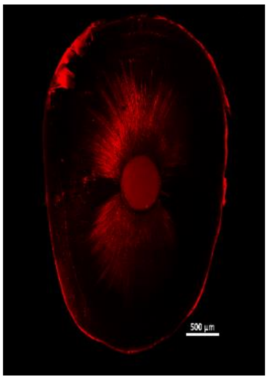
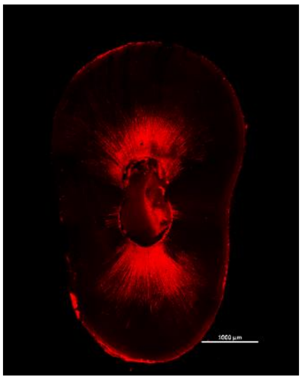
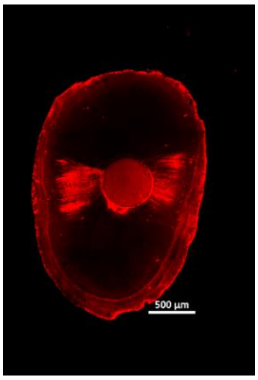
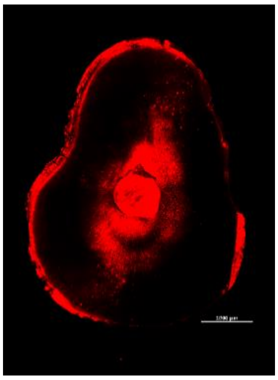
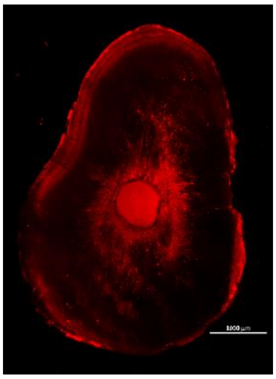


Figure (14): Bar chart representing mean and standard deviation values for sealer penetration (%) with different interactions of variables

Table (10): Micro photographs showing different sealer penetrations in dentinal tubules at different root levels

Root level **Group**	**Coronal**	**Middle**	**Apical**
Figure (15): HiFlow Moist Warm			
Figure (16): HiFlow Moist Matched Cone			
Figure (17): HiFlow Dry Warm			

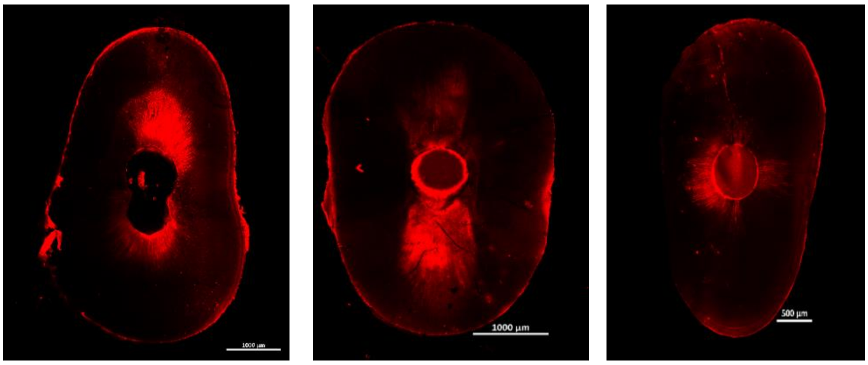


Figure (18): HiFlow Dry Matched Cone

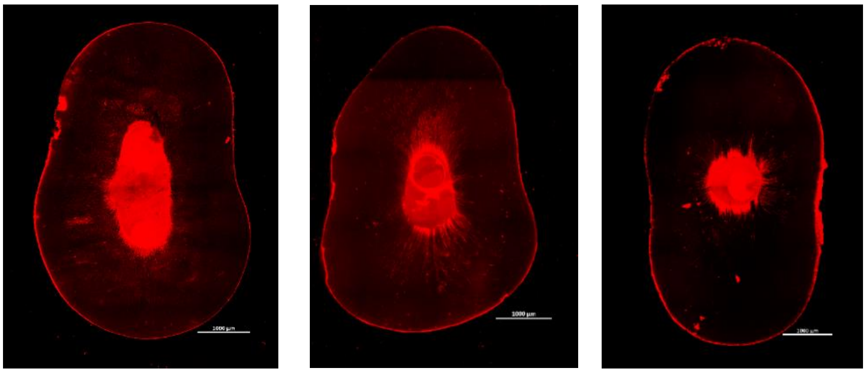


Figure (19): Mta Moist Warm

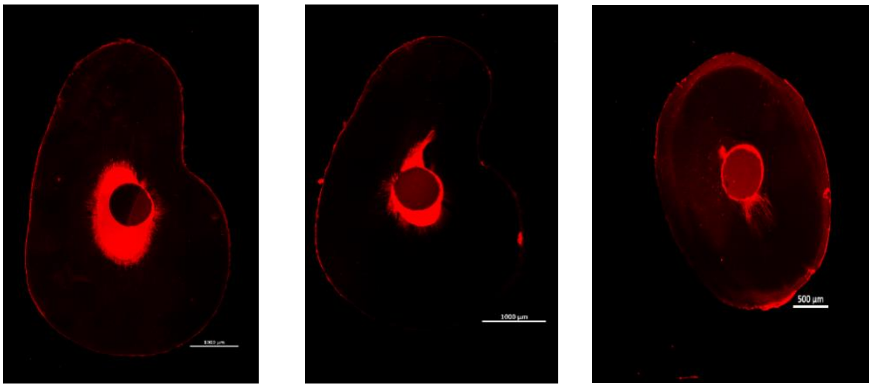


Figure (20): Mta Moist Matched Cone

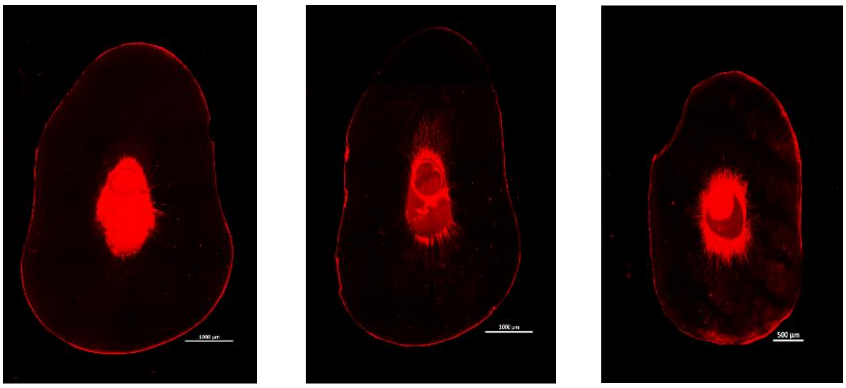
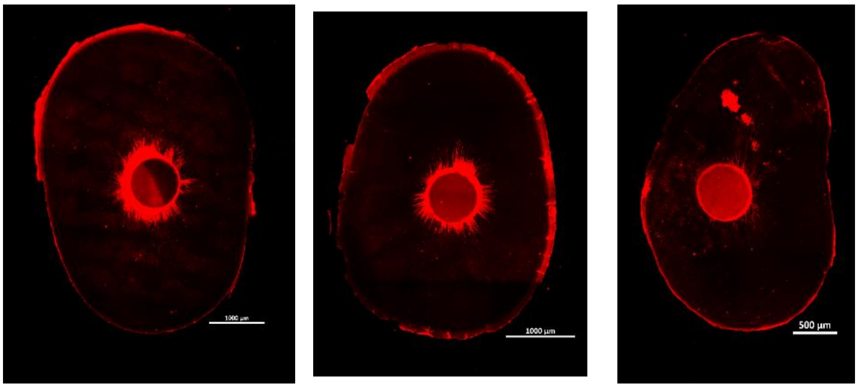


Figure (21): Mta Dry Warm

Figure (22): Mta Dry Matched Cone



6. DISCUSSION

Sealing of the root canals is one of the most important goals for successful endodontic treatment [106]. Different factors should be considered to obtain hermetic seal such as the obturation technique either warm or cold, sealer type and canal moisture condition [107]. Overcoming the drawbacks of cold lateral compaction technique, warm vertical compaction has a uniform obturation quality with less voids [108]. Unfortunately, application of heat may alter physio-chemical properties of the sealer [109]. To overcome such alterations of the sealer, matched cone obturation technique has been introduced utilizing calcium silicate-based sealers [110]. This randomized, controlled, interventional prospective study aimed to give a better understanding of the effect of canal moisture and heat application during the obturation on the depth of penetration of different calcium silicate-based sealers.

Teeth were collected from patients aged between 18 and 40 years old to minimize variability in dentin microstructure. Out of 120 recently extracted mandibular single rooted premolars, 80 teeth were selected to be used in the study. A pre-operative periapical X-ray was taken to assess the tooth anatomy, and CBCT was performed to confirm the root structure. All molds were prepared at a length of 17mm to allow for the future de-coronation of the embedded teeth, ensuring standardization [111]. A slow-speed diamond saw with water coolant was used for the de-coronation to prevent heat generation. Forty teeth were outcasted due to their deviation from the inclusion criteria including: 21 with variable anatomy other than Vertucci type I, 8 due to obliterated pulp chamber, 6 with root fracture, 5 teeth shorter than 20 mm and two teeth longer than 22 mm.

Chemo-mechanical preparation utilized crown down technique using Endostar E3 Azure system as it is highly flexible, heat-treated instruments able to transform from the martensite to the austenite phase at body temperature [112]. Concomitantly, root canal irrigation with 5,25% sodium hypochlorite solution between each file was done due to its superior antibacterial action and tissue dissolving power [113]. Reaching the master apical preparation to size 40/04 rotary file is sufficient to allow shaping the root canals while offering deeper penetration of the irrigating solution with resultant smear layer removal [114]. Final rinse with EDTA solution was done due to its superior chelating action acting on the inorganic components of the smear layer [115]. Final Passive ultrasonic irrigation (PUI) of both sodium hypochlorite and EDTA was done to improve the depth of penetration of the irrigating solutions with cavitation bubble implosions and acoustic streaming [116].

Among various methods that are used to evaluate sealer penetration into dentinal tubules (such as Light microscopy, stereomicroscopy, microcomputed tomography, scanning electron microscopy (SEM)), confocal laser scanning microscopy (CLSM) is selected in this study. CLSM stands out by allowing optical sectioning beneath the specimen’s surface without the need for preparation that could introduce artifacts. It provides comprehensive details on the sealer's penetration depth and fluorescence detection around the canal circumference [117]. Rhodamine B was used as a marker for sealer penetration [118,119]. As a fluorescent dye, it has been reported to have less drawbacks when used with calcium silicate-based sealers [120]. Both ***Akcay et al. and Wang et al***. utilized Rhodamine B dye into calcium silicate-based sealers without encountering any limitations in their studies related to its use [121,122].

Regarding the canal moisture condition, the presence of moist canal has deeper sealer penetration into the dentinal tubules than conventional dried canal. This finding is acceptable as the used calcium silicate-based sealers are flowable and hydrophilic in nature that set with hydration reaction in the presence of moisture. This reaction results in formation of an interfacial layer at the dentin wall known as the “mineral infiltration zone” due to calcium phosphate formation inducing apatite precursors and hydroxyapatite precipitation on the surface of the material improving the sealer penetration into the dentinal tubules [(123)].

Alternatively, another research Advocated using calcium silicate-based sealers at dry condition to improve its bond strength to the dentinal tubules. The discrepancy may be attributed to the concept of canal dryness in literature lacking the knowledge about its accurate definition among the researchers. While some research considered the canal is dry when it is dried with paper points, other research considered it a normal moist condition. Furthermore, excess moisture inside the root canals may result in incomplete setting of the sealer [(124)].

Regarding the obturation technique, warm vertical compaction could increase the depth of penetration of calcium silicate-based sealers than matched cone technique. The greater compressive forces applied coronally during the warm vertical compaction procedures improves sealer penetration into the dentinal tubules that allows for better adaptation to the canal wall resulting in homogenous obturation with less voids [(125)]. This is in accordance with another research that showed better penetration power of calcium silicate-based sealers, utilizing warm vertical compaction technique, into the simulated lateral canals compared to matched cone technique [(126)]. Furthermore, Heating and plugging the gutta-percha significantly increases apical sealing ability, adaptation to the root canal irregularities, apical adjustment of gutta-percha and the propulsion of filling materials into lateral or accessory canals during warm vertical compaction [127]. Improving the flowability of calcium silicate-based sealers is directly proportional with increasing the temperature during obturation of the root canals [128]. **Camilleri** upon investigating MTA Fillapex which is also resin based, the heat generated during warm vertical compaction did not affect the material chemistry and physical characteristics [129]. In contrast to the current study**,** other research has a comparable result between warm vertical compaction and single matched cone techniques. Furthermore, application of heat shortened the setting time and reduced flow of the sealers with increased film thickness [130]. Which contrasts with others showing reduced film thickness, accelerated setting time and decreased flowability of the sealer with application of heat [131].

The discrepancy among these researches may be attributed to the research model of each study such as presence or absence of irrigant activation, sealer type and the evaluation method used.

Regarding the sealer type, Total Fill BC HiFlow proved deeper penetration power into the dentinal tubules than MTA fillapex. Although both sealers are calcium silicate-based sealers, the unique characters of HiFlow including smaller particle size (<1 μm), volumetric expansion upon setting and basic pH allows for degradation of the collagen fibers forming a porous structure that facilitates the permeation of high concentrations of Ca (2+), OH (-), and CO (3) ions, leading to in tubular penetration and increased mineralization in this region [132,133]. In addition, Totalfill BC HiFlow Sealer exhibited better flowability when subjected to heat [134]. In accordance. A previous study concluded that BC Sealer had significantly more and deeper sealer penetration circumferentially than MTA Fillapex, the difference among these sealers is that MTA Fillapex shrinks 0.7% during setting, whereas the calcium silicate-based Sealer expands slightly (<0.1%), which may provide superiority [135]. Particle size of MTA Fillapex sealer is crucial factor for less sealer dentinal penetration due to its larger particle size (75 ±12 μm) in comparison to TotalFill BC Sealer HiFlow [136].

Overall viewing the different interactions among the tested groups revealed similar results as when evaluating each variable alone confirming the results of the study without repeating the possible causes. Based on the results of this study, the null hypothesis is rejected as there is a variability in the depth of penetration of the sealer among the tested groups.

7. SUMMARY

Three-dimensional obturation of the root canal system is essential for successful endodontic therapy. It prevents microleakage of periapical exudate into the canal space, stops reinfection, and creates a conducive environment for optimal healing. Endodontic sealers are crucial as they help seal the canal space by filling in re-entrances, irregular spaces, dentinal tubules and areas that are inaccessible to endodontic instruments. Moisture present in root canal or dentinal tubules affects obturation, it could occupy physical space in the root canal preventing adhesion between dentin and obturating material preventing the monoblock [137]. Moisture may prevent sealer setting by increasing or reducing its working or setting time, and sealer penetration as well bond strength. Eighty mandibular premolars were selected according to inclusion criteria and then prepared until file #40.04. According to the obturation technique either in moist or dry condition as follows: 1- matched cone compaction technique utilizing BC sealer TotalFill BC Sealer HiFlow in moist condition 2- matched cone compaction technique utilizing BC sealer TotalFill BC Sealer HiFlow in conventionaldry condition 3- matched cone compaction technique utilizing MTA sealer in moist condition 4- matched cone compaction technique utilizing MTA sealer in conventionaldry condition. 5- warm vertical compaction technique utilizing BC sealer TotalFill BC Sealer HiFlow in moist conditions 6- warm vertical compaction technique utilizing BC sealer TotalFill BC Sealer HiFlow in conventionaldry conditions 7- warm vertical compaction technique utilizing mta sealer in moist conditions 8- warm vertical compaction technique utilizing mta sealer in conventionaldry condition. All sealers were labelled with rhodamine B dye for evaluation under confocal laser scanning microscope. The samples

were sectioned horizontally into three slices at 3, 7, and 13 mm (apical, middle, and coronal) depths from the apex. The results showed that Total fill HiFlow sealer showed the statistically significantly highest mean sealer penetration %. MTA Fillapex showed statistically significantly lower mean value. And revealed that coronal level showed the statistically significantly highest mean sealer penetration %. Middle root level showed statistically significantly lower mean value. Apical root level showed the statistically significantly lowest mean sealer penetration % regardless of the moisture condition.

8. CONCLUSION

Total Fill HiFlow has deeper penetration power into the dentinal tubules than MTA Fillapex sealer at all root levels.

The tested bioceramic sealers have an affinity to penetrate the dentinal tubules in Moist condition.

Warm vertical compaction technique has better penetration ability into the dentinal tubules than single matched cone technique .

The penetration power of the bioceramic sealers increase in apical coronal direction.

9. RECOMMENDATIONS

1. Further research should be done to evaluate the effect of different degrees of moisture on the depth of penetration of bioceramic sealers.
2. Further research should be done to evaluate the effect of different degrees of heat on the depth of penetration of bioceramic sealers.
3. Further research should be done to evaluate the effect of different apical diameters and taper on the depth of penetration of bioceramic sealers.
4. Further research should be done to compare different methods of evaluation of depth of penetration of the sealers.
5. Further research should be done to evaluate the depth of penetration of bioceramic sealers with different dyes such as flou-3.
6. Further research should be done to evaluate the effect of different irrigating solutions on the depth of penetration of bioceramic sealers.
7. Further research should be done to evaluate the effect of different sealer activation methods on the depth of penetration of bioceramic sealers.

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الملخص العربى

يعد سد نظام قناة الجذر ثلاثي الأبعاد ضروريًا لنجاح العلاج اللبية. يمنع التسرب الدقيق للإفرازات المحيطة بالذروة في مساحة القناة، ويوقف الإصابة مرة أخرى، ويخلق بيئة مواتية للشفاء الأمثل. تعتبر المواد المانعة للتسرب اللبية ضرورية لأنها تساعد في إغلاق مساحة القناة عن طريق ملء إعادة المداخل، والمساحات غير المنتظمة، والأنابيب العاجية والمناطق التي لا يمكن الوصول إليها بواسطة أدوات المعالجة اللبية. تؤثر الرطوبة الموجودة في قناة الجذر أو الأنابيب السنية على الانسداد، ويمكن أن تشغل مساحة مادية في تمنع قناة الجذر الالتصاق بين العاج والمواد المسدودة مما يمنع الكتلة الأحادية. قد تمنع الرطوبة تثبيت مادة مانعة للتسرب عن طريق زيادة أو تقليل وقت عملها أو وقت إعدادها، واختراق مادة مانعة للتسرب بالإضافة إلى قوة الرابطة

تم اختيار أربعين من الضواحك السفلية وفقًا لمعايير الاشتمال ثم تم إعدادها حتى الملف رقم 40.04. وفقا لتقنية السد سواء في الحالة الرطبة أو الجافة على النحو التالي: 1- تقنية الضغط الجانبي البارد باستخدام سيلر **BC HiFlow** في الحالة الرطبة 2- تقنية الضغط الجانبي البارد باستخدام سيلر **BC HiFlow** في الحالة الجافة 3- تقنية الضغط الجانبي البارد باستخدام **MTA**سيلر في الحالة الرطبة 4- تقنية الضغط الجانبي البارد باستخدام سيلر **MTA** في الحالة الجافة. 5- تقنية الضغط العمودي الدافئ باستخدام سيلر **BC HIflow** في الظروف الرطبة 6- تقنية الضغط العمودي الدافئ باستخدام سيلر **BC HIflow** في الظروف الجافة 7- تقنية الضغط العمودي الدافئ باستخدام سيلر **MTA** في الظروف الرطبة 8- تقنية الضغط العمودي الدافئ باستخدام سيلر **MTA** في الظروف الرطبة حالة جافة . تم حشو العينات بماده لاصق علاج الجذور مخلوط بماده الرودامين بي مع وضع قمع وحيد من الجاتا بيركا للفحص تحت مجهر المسح بالليزر متحد البؤر بعد ان تم تقسيم العينات الي ثلاث شرائح بأطوال ٣-٧-١٣ (قمي و متوسط واكليلي) من ذروه السن. أظهرت النتائج أن كلاً من الكيتوزان وثاني أكسيد التيتانيوم النانوي أظهرا أعلى متوسط تغلغل للماده اللاصق بنسبة معنوية إحصائياً. أظهرت الجسيمات النانوية الفضية قيمة متوسطة أقل. و ان بشكل ملحوظ من الناحية الإحصائية. أظهرت بان ماده الاي دي تي ايه أقل متوسط تغلغل للماده اللاصق إحصائياً وكشفت أن المستوى الإكليلي أظهر أعلى متوسط تغلغل للماده لاصق القناه % بشكل معتد به إحصائياً. أظهر مستوى الجذر الأوسط قيمة متوسطة أقل بكثير من الناحية الإحصائية. أظهر مستوى الجذر القمي أقل متوسط تغلغل للسدادات بنسبة معنوية إحصائياً بغض النظر عن نوع الري.

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بحث مقدم كجزء من مقومات الحصول على درجة الماجستير في علاج الجذور

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