

Comparative Evaluation of Sealing Ability and Cytotoxicity of Ceraseal Versus AH Plus Sealers in Obturated Root Canals using Different Obturation Techniques (An in Vitro Study)

Sara M. Eliwa¹, Wael H. kamel², Ashraf S. Refa'at³, Marwa M. Abou Shadi⁴

¹ Endodontics Department, Faculty of Oral and Dental Medicine, Future University, Cairo, Egypt

² Professor and Head of Endodontic Department Faculty of Oral and Dental Medicine Future University in Egypt

³ Professor of Endodontics Department Faculty of Dentistry Al-Azhar University

⁴ Endodontics Department, Faculty of Oral and Dental Medicine, Future University, Cairo, Egypt. Division of Endodontics, School of Dentistry, University of Minnesota, Minneapolis, USA

ABSTRACT

Objectives: This study aimed to evaluate sealing ability of Ceraseal (BC) versus AH Plus (epoxy resin-based) sealers to root canals obturated with three different obturation techniques. and cytotoxicity of both sealers. **Methods:** 42 freshly extracted single-rooted teeth were divided into six groups (each n= 7). Group 1 (Ceraseal/CLC), Group 2 (Ceraseal/SC), Group 3 (Ceraseal/CW), Group 4 (AH Plus/ CLC), Group 5 (AH Plus/SC), and Group 6 (AH Plus/ CW). And instrumentation done. The sealer was fluorescently labeled by a fluorescent dye to allow analysis under confocal laser scanning microscopy (CLSM). Then specimens were obturated by different obturation techniques. Roots cross-sectioned perpendicular to their long axis, and then CLSM was employed to assess tubular penetration of different sealers. The cytotoxic effect of Ceraseal and AH Plus had been evaluated on the human gingival fibroblast cell line (HGF) using MTT assay test. One Way ANOVA test was used to compare different groups, followed by post hoc test for multiple section comparisons. **Results:** Ceraseal/LC 5.27 ± 2.84 group followed by Ceraseal/SC 3.29 ± 1.07 , AH+/CW 2.63 ± 0.62 , and AH+/LC 3.10 ± 1.43 , while the least were AH+/SC 2.40 ± 0.29 and Ceraseal/CW 1.86 ± 0.44 , with no significant differences. The viability of cells using AH Plus sealer showed 20.3% and 24.52%, and 25.79%, and Ceraseal sealer was 71.39% and 76.15% at 24 h and 48 h; it then increased rather substantially to 90.41% at 72 h. revealed significant differences. **Conclusion:** Obturation technique should be selected according to sealer type for a successful endodontic treatment. Ceraseal had higher ability of dentinal tubule penetration than AH Plus, reduced in apical part of root canal. Ceraseal expressed higher cell viability improved with time compared to AH Plus sealer.

Keywords: Bioceramic, Dentinal Tubules Penetration, Cytotoxicity

INTRODUCTION

Root canal obturation is crucial for preventing reinfection by providing an effective seal against bacterial ingress and toxins. Gutta-percha, in combination with root canal sealers, achieves this by sealing voids, accessory canals, and foramen; bonding the gutta-percha to root dentin; and encasing any remaining bacteria. Ideal sealers should possess properties such as tissue compatibility, slow setting time, minimal shrinkage, adhesiveness, radiopacity, bacteriostatic effects, and solubility in solvents while being insoluble in oral fluids [1]. Resin-based sealers are favored for their resorption resistance and dimensional stability, but they exhibit limitations, including cytotoxicity, inflammatory responses, and hydrophobicity, which hinder complete filling of the hydrophilic root canal. Bio-ceramic sealers, composed of calcium silicate and/or calcium phosphate, offer advantages such as an alkaline pH, no shrinkage, chemical stability, and superior bonding to root dentin, owing to their similarity to tooth and bone apatite. These sealers are highly biocompatible, non-toxic, and hydrophilic [2, 3]. The cold lateral condensation (CLC) technique is widely used for its simplicity but can be time-consuming and may induce microcracks or voids in the root. The Continuous Wave Technique (CWT), associated with better anatomical filling and less sealer use, requires special equipment and lacks longitudinal control. The bioceramic sealer is often paired with the single-cone technique, which offers faster root canal filling with minimized pressure on the canal walls, resulting in a more uniform mass and reduced risk of failure compared to multiple-cone techniques [4, 5]. Sealers are essential for creating a tight bond between dentin and the core

material, reducing microleakage, and enhancing sealer-to-dentin bonding through dentinal tubule penetration. Extrusion of sealers peri-apically can cause inflammation, leading to pain, tenderness, and delayed healing, underscoring the importance of biocompatibility and good tolerance by periapical tissues [6, 7].

MATERIALS AND METHODS

Dentinal Tubule Penetration (CLSM)

Sample Size

A total sample size of 32 was (16 per group) was sufficient to detect a large effect size (f) ranging from 0.64 to 0.81, with an actual power (1-β error) of 0.8 (80%) and a significance level (α error) 0.05 (5%) for two-sided hypothesis test. The number was increased to a sample size of 42 to allow for non-parametric distribution of the outcome variable. Sample size was calculated using G*Power statistical power Analysis program (version 3.1.9.4) for sample size determination [8]. Based on Chew et al. [9] for sealing ability, A sample size (n=42; divided into two main groups 21 in each, subdivided into three subgroups 7 in each group).

Specimen Preparation and Root Canal Instrumentation

The collected teeth were immersed in a 5.25% sodium hypochlorite (NaOCl) solution for 15 minutes to remove any soft tissue debris. Prior to root canal instrumentation, the teeth were decoronated

at the cemento-enamel junction (CEJ) using a diamond disc in a low-speed handpiece with copious water cooling, ensuring a standardized sample length of 17mm. This length was verified using a digital caliper [10, 11]. (fig.1). Canal patency was verified with a #15 K-file, and the working length was determined by subtracting 1 mm from the root length. Root canal preparation was performed using ProTaper NEXT rotary files up to size X3 (#30/0.04) in a crown-down manner with a 16:1 reduction handpiece driven by a torque-controlled electric motor, set to 300 rpm and 2 Ncm torque [12]. (fig.2). During instrumentation, the canals were irrigated with 2 ml of freshly prepared 2.6%NaOCl solution for 1 minute between each instrument, using a side-vented 30-gauge needle. The irrigant was passively delivered 2 mm short of the working length. After each file, a fixed volume of 2 ml of irrigation solution was used, followed by 2 ml of 17% ethylenediaminetetraacetic acid (EDTA) for 1 minute, and a final flush with saline to remove residual debris. The canals were then dried using a #30 paper point [13-15].

Root Canal Obturation

Prior to obturation, gutta-percha fitness was checked radiographically & for tug-back to the full working length of root canal.

a) Staining of Tested Sealers

TO allow analysis under the CLSM, the sealer was fluorescently labeled by adding rhodamine B at a 1:100 ratio by weight. Root canals were smeared with the rhodamine B labeled sealer using #30GP cone placed to entire working length. (fig.2). A specimen was randomly divided into six groups according to type of sealer and obturation technique applied (each group, n=7) as follows:

Group1: Ceraseal and cold lateral condensation (Ceraseal/CLC).

Group2: Ceraseal and single cone (Ceraseal/SC),

Group3: Ceraseal and continuous wave (Ceraseal/CW),

Group4: AH Plus and cold lateral condensation (AH Plus/ CLC).

Group 5: AH Plus and single cone (AH Plus/ SC).

Group 6: AH Plus and continuous wave (AH Plus/ CW).

b) Root Sectioning

Roots were cross sectioned perpendicular to their long axis using IsoMet mounting diamond disk 0.6 mm thickness with a slow speed (25,000 rpm) and feeding rate (10 mm/min) under water cooling. The apical third slice was obtained 3mm coronal to the apical tip of the root, the middle third slice was obtained 6mm coronal to apical tip of the root and the coronal slice was obtained 9mm coronal to apical tip of the root. The coronal slice was obtained 3mm apical to the coronal surface of the root, the middle slice was obtained 6mm apical to coronal surface of the root and the apical slice was obtained 9 mm apical to coronal surface of the root. (fig.3). The three sections of 2 mm thickness were obtained from coronal, middle and apical thirds of each sample (two slices in each third) respectively of the root. Each section was measured using digital caliper. (fig.4)

c) Confocal Laser Scanning Electron Microscopy Analysis of the Sample Sections

The penetration of the sealer into the dentinal tubules was measured using Leica software, which quantified the deepest sealer penetration in each sample. The software enabled visualization of a

fluorescent ring around the canal wall, representing the sealer-dentin interface, with the infiltrated areas displaying red fluorescence and un-infiltrated areas appearing dark. (fig.5). Penetration depth (PD max) was measured for each section, from the canal wall (sealer-dentin junction) to the maximum point of sealer penetration, using an electronic measuring tool. (fig.6) To obtain a single mean value for each section, the maximum penetration depth (in pixels) was measured at standardized points around the canal (12, 3, 6, and 9 o'clock positions) representing the buccal, mesial, distal, and lingual directions. Each measurement was repeated twice in each direction to ensure consistency and reproducibility. (fig.7)

Cytotoxicity Test (MTT Assay)

HGF-1 cells (human Gingival fibroblasts cell line) were obtained from American Type Culture Collection [ATCC] unit.

Chemicals Used: Dimethyl sulfoxide (DMSO), 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and trypan blue dye were purchased from Sigma. Fetal Bovine serum, HEPES buffer solution, L-glutamine, gentamycin and 0.25% Trypsin-EDTA were purchased from Lonza [16].

Cell Viability Assay

In a sterile environment, 5,000 HGF cells were seeded in each well of separate 96-well plates at a density of 5×10^4 cells/mL using 100 μ L of medium from 25 cm² T-flasks and incubated at 37°C with 5% CO₂. The experiments were conducted in triplicate for each root canal sealer on three 96-well plates. The HGF-1 cells were observed under an inverted microscope to ensure they were sufficiently mature before exposure to the materials. The growth medium, containing DMEM with FBS and PenStrep, was removed from the wells and replaced with 100 μ L of the corresponding extract solution, except for the positive control, which received 100 μ L of growth medium. A separate well containing only the growth medium served as a blank. The plates were incubated at 37°C with 5% CO₂ for 24, 48, and 72 hours, with one microplate used for each time point. After incubation, the extract solution was removed, and the samples were washed with 100 μ L of 0.1 M phosphate-buffered saline (pH 7.4). Then, 20 μ L of MTT solution was added, and the plates were incubated at 37°C for 4 hours. The MTT solution was drained, and 120 μ L of dimethyl sulfoxide was added to solubilize the purple formazan crystals. MTT is metabolized into a purple formazan product in viable cells with active metabolism, while non-dividing or dead cells are unable to metabolize it. The absorbance of each sample was measured at 570 nm (Abs570) using a microplate reader. Cell viability was calculated using the formula: $[(OD_t/OD_c)] \times 100\%$, where OD_t is the mean optical density of wells treated with the tested sample and OD_c is the mean optical density of untreated cells [17].

Statistical Analysis

The mean and standard deviation values were calculated for all tested groups. Data were explored for normality using Kolmogorov-Smirnov's and Shapiro-Wilk's tests, data showed parametric (normal) distribution. Two-way analysis of variance (ANOVA) was used to test the interaction between different variable. Repeated measures ANOVA test was used for comparing the three sections of the same root followed by Tukey's Post Hoc test for multiple comparisons. Independent sample t-test was used to compare between two groups in nonrelated samples. The significance level was set at $p \leq 0.05$. Statistical analysis was performed with IBM SPSS statistics version 25

for Windows. In all tests, a p -value of <0.05 was considered statistically significant.

Result

Laser Confocal Scanning Microscope (Penetration Depth)

a) Comparison Between the Six Groups at Each Level

The apical one third showed a significantly lower values in all groups compared to both the middle and coronal thirds ($p < 0.01$), while no significant differences were found between the values of the middle and coronal thirds in all groups ($p > 0.05$). In the apical level no significant differences between Ceraseal/LC, Ceraseal/SC while Significant difference was found in AH+/CW compared to them While the least mean value Ceraseal/CW, AH+/LC and AH+/SC. (table 1) (fig.8,9). In the middle level showed no significant difference between Ceraseal/LC, Ceraseal/CW and Ceraseal/SC However, groups showed significant difference with AH+/CW. While AH+/LC and AH+/SC was significantly the lowest. (table 2) (fig.10, 11). In the coronal level showed no significant difference between AH+/CW and Ceraseal/LC However, these groups showed significant difference with Ceraseal/SC, AH+/LC and Ceraseal/CW respectively had no differences between each other. While AH+/SC was significantly the lowest. (table 3) (fig.12,13)

b) Comparison Between Root Levels in the Same Group

In AH+/SC group, no significant difference between coronal and middle sections while apical section lowest significantly. (table 4) (fig.14,15)

In AH+/LC group, the coronal section the highest mean value. (table 5) (fig.15, 16)

In AH+/CW group, no significant difference between coronal and middle sections while apical section lowest significantly. (table 6) (fig.17, 18)

In Ceraseal/SC group, the coronal section highest mean value. (table7) (fig.19, 20)

In Ceraseal/LC group. the coronal section highest mean value. (table 8) (fig.21,22)

In Ceraseal/CW group, no significant difference between coronal and middle sections while apical section lowest significantly. (table 9) (fig.23, 24)

Cytotoxicity Test Result (Using MTT Assay):

Evaluation of Cytotoxicity Against HGF-1 Cell Line

There is a significant difference between AH plus and cell control ($p < 0.001$), between AH plus and Ceraseal ($p = 0.048$), between Ceraseal and cell control ($p = 0.048$). (table 10) (fig.25)

Discussion

The primary goal of endodontic treatment is to eliminate pulpal and peri-radicular pathosis while preserving natural dentition. Recent advancements in obturation materials and techniques have improved the success rates of root canal therapy [18, 19]. This study aimed to evaluate and compare the sealing ability of two different root canal sealers—epoxy resin-based AH Plus and bio-ceramic-based CeraSeal BC Sealer—using Confocal Laser Scanning Microscopy (CLSM), across various root canal sections. Additionally, the study assessed the

cytotoxicity of these materials using the MTT assay. Freshly extracted single-rooted teeth with fully formed apices were selected to allow for optimal instrumentation and sealer insertion, with an apical constriction to prevent the loss of intracanal irrigants. The study utilized NiTi rotary instruments to shape the canals, providing a tapered root without excessive risk of fracture or canal transportation. The sealing efficacy was tested across three obturation techniques: Lateral condensation, single cone, and continuous wave, to determine their impact on sealing and cytotoxicity [12]. AH Plus was used as a gold standard for comparison with other endodontic sealers. It exhibits higher bond strength through micro-mechanical locking via covalent bonds and minimal polymerization shrinkage, with smaller dimensional changes, ensuring stability. However, its sealing ability is limited by poor adhesion in the presence of moisture and difficulty in bonding to gutta-percha [20, 21]. Ceraseal, a bio-ceramic-based sealer, is a slow-setting, hydrophilic, water-based material composed of calcium silicate. In vitro studies highlight its strong antibacterial activity, excellent sealing ability, high bond strength, favorable flowability, low shrinkage, and insolubility. Its fine particle size and moisture-absorbing properties enable it to penetrate deeper into micro-irregularities and lateral root canals [22, 23]. Various obturation techniques aim to optimize sealing and minimize cement film thickness. Lateral condensation (LC) is commonly used in studies comparing new obturation systems, as it enhances adaptation to canal irregularities through pressure applied to both lateral and apical directions. However, LC results in poor compaction of gutta-percha and gaps between cones [24, 25]. The single cone (SC) technique, utilizing a larger master cone to match the geometry of nickel-titanium rotary systems, has become more common with advancements in rotary instrumentation. The modified single-cone technique, which uses accessory points to aid sealer penetration, improves canal filling with minimal pressure on canal walls [26-28]. The continuous wave obturation technique is also used, offering better anatomical filling and sealer adhesion to the radicular dentin but requiring special equipment and lacking longitudinal control [29]. Several methods, including dye penetration, electrochemical techniques, fluid filtration, radioisotope tracing, and microscopy, have been used to evaluate the sealing ability of endodontic materials. In this study, confocal laser scanning microscopy (CLSM) was employed to assess the depth of dentinal tubule penetration due to its advantages over other methods [30]. CLSM allows precise visualization of sealer penetration within the root canal system, offering the ability to control depth and reduce background interference by assembling multiple optical sections, as concluded by Chew et al. [9] Rhodamine B dye was used as a fluorescent marker to visualize penetration under CLSM, as it does not interfere with the physical or chemical properties of the tested sealers.

Sealing Ability Result

Role of Sealer

The study examined the impact of sealer type on dentinal tubule penetration depth, regardless of obturation technique or root level. The results showed that CeraSeal demonstrated the deepest penetration, while AH Plus exhibited the least, though no significant differences were found between the two. These findings align with previous studies indicating that bio-ceramic sealers, such as CeraSeal, provide greater tubular penetration due to their self-adhesive nature and chemical bond formation with dentin, facilitated by the production of hydroxyapatite during setting [31]. The alkaline nature of

bioceramic sealers, which degrades collagen in dentin, further promotes sealer penetration. Additionally, bio-ceramic sealers are hydrophilic, allowing deeper penetration compared to hydrophobic sealers, and their low contact angle enhances adaptation to canal walls. This was supported by studies showing bio-ceramic sealers' superior flow, reduced film thickness, and better marginal adaptation, resulting in reduced microleakage [32-36]. AH Plus, an epoxy resin-based sealer, demonstrated lower penetration into dentinal tubules due to its silicone oil content, which causes shrinkage and creates gaps at the sealer-dentin interface. This shrinkage can compromise the sealer's performance despite its good biocompatibility, sealing properties, and dimensional stability. In contrast, bio-ceramic sealers exhibited deeper tubular penetration, likely due to their smaller particle size (2 μm), which improves adaptation and reduces microleakage. The smaller particles of bio-ceramic sealers provide better sealing and penetration compared to the larger particles (8 μm) of epoxy resin sealers. Sealer penetration is influenced by factors such as particle size, material solubility, and viscosity [37-39].

Role of Obturation Technique

This study examined the effect of different obturation techniques on the penetration depth into dentinal tubules, irrespective of the sealer type and root level. The highest penetration depth was observed in the AH+/CW and Ceraseal/LC groups, with no significant differences between them. In contrast, AH+/LC, Ceraseal/CW, and Ceraseal/SC showed no significant differences, while AH+/SC had the lowest penetration depth. The cold lateral condensation (CLC) technique yielded the highest values, followed by the continuous wave technique (CWT), while the single cone (SC) technique exhibited the least penetration. This variation is likely due to the applied pressure during obturation, which helps the gutta-percha adapt to canal irregularities, particularly in the lateral and apical directions [40]. CWT showed superior marginal adaptation, with fewer voids at the sealer-dentin interface, likely due to the negative pressure enhancing the sealer's adaptation to dentin walls [41]. On the other hand, SC exhibited poor marginal adaptation, as the cone did not fit well in the middle and coronal thirds of the root canal, leading to sealer accumulation, poorer adaptation, and more voids. These findings align with previous studies by Al-Sabawi et al., Yilmaz et al., and Celikten et al., [42-44] who reported similar results regarding the lower adaptation and larger gaps associated with SC. However, some studies, such as those by Inan et al. and Ismail et al., [45] [46] found better adaptation with SC than with lateral compaction techniques, suggesting that obturation technique results can vary depending on the specific study conditions. Despite differences in findings, the CLC technique remains widely used for comparison with new obturation techniques [47].

The Continuous Wave (CW) obturation technique resulted in reduced penetration of bioceramic (BC) sealers compared to the Cold Lateral Condensation (CLC) technique [48]. This may be due to the temperature increase in the root canal, which reduces the humidity necessary for BC sealers, as they are hydrophilic and require moisture to properly flow and set. In contrast, premixed calcium silicate sealers, which have an inorganic matrix of calcium silicate hydrate with micro-spaces containing water, require several days to fully set. Heat application can cause water desorption, potentially affecting the sealer's properties. BC sealers are also sensitive to heat and exhibit high solubility, which raises concerns for their clinical use [49, 50]. However, studies have shown conflicting results, with some suggesting that the CW technique improves bond strength, while others report

fewer marginal gaps with CLC [51, 52]. AH Plus sealer, notably, remains stable after heat application and demonstrated the deepest tubular penetration in the apical third when used with the CW technique. Additionally, heat treatment has been found to reduce porosity in resin-based sealers, which can improve the sealing ability of root fillings [18, 49].

Role of Root Level

This study evaluated the impact of root level on tubular penetration depth, irrespective of obturation technique and sealer type. Results showed that both the coronal and middle sections exhibited greater tubular penetration compared to the apical section across all techniques and sealers. Gaps were more frequently observed at the apical level, which showed significantly lower values than the coronal and middle areas. This finding is attributed to the smaller diameter and lower density of dentinal tubules in the apical region, as well as challenges in smear layer removal, which hinder sealer adaptation [19]. Additionally, reduced irrigant delivery and the presence of sclerotic dentin in the apical third contribute to poor sealer penetration [53]. The differences in surface energy, cleanliness, and the physical properties of the sealer further explain these regional variations in sealer performance. These results emphasize the difficulty of achieving optimal sealer penetration in the apical region due to its unique structural and clinical challenges [54, 55].

Cytotoxicity of root canal sealers is a critical consideration for their clinical use, as these materials are in direct contact with periapical tissues. Sealing materials can release toxic substances during setting, potentially compromising their biocompatibility. Cytotoxicity testing of freshly mixed sealers is important because they are applied in an incompletely polymerized state, but it is also essential to assess the sealers' effects over extended periods (24, 48, and 72 hours) after setting, as toxic components may diffuse into the surrounding tissues over time, altering cytotoxicity levels [56]. The MTT assay, a widely accepted method for evaluating cell viability, was used in this study. This colorimetric assay measures cell activity and proliferation by quantifying the amount of formazan produced from the reduction of MTT, which correlates with the percentage of viable, metabolically active cells. The results are inversely proportional to cytotoxicity, providing a reliable and reproducible means of assessing biocompatibility. The study evaluated the cytotoxic effects of bioceramic root canal sealer (Ceraseal) and the gold standard epoxy resin sealer (AH Plus) on human gingival fibroblast cells (HGF). The MTT assay effectively measured cell viability and proliferation, offering valuable insights into the biocompatibility of these materials [57].

Cytotoxicity Test Result

Cell Viability Assay

This study evaluated the cytotoxicity of two root canal sealers, Ceraseal and AH Plus, at 24-hour, 48-hour, and 72-hour intervals. Ceraseal demonstrated the highest cell viability, showing mild cytotoxicity at 24 and 48 hours but becoming non-cytotoxic after 72 hours. This was attributed to its composition, including calcium silicate and zirconium oxide, which are known for their excellent durability, biocompatibility, and ability to stimulate fibroblast proliferation [58, 59].

In contrast, AH Plus exhibited the lowest cell viability across all time intervals, likely due to the toxic by-products of its polymerization

process, including bisphenol A diglycidyl and formaldehyde, which are known to have mutagenic and cytotoxic effects [60-62]. Cell viability for AH Plus was 20.3%, 24.52%, and 25.79% at 24, 48, and 72 hours, respectively, with a slight reduction in cytotoxicity over time, but no significant change. Conversely, Ceraseal's cell viability was 71.39% and 76.15% at 24 and 48 hours, increasing significantly to 90.41% at 72 hours, indicating a reduction in cytotoxicity over time. These results support the conclusion that Ceraseal is more biocompatible than AH Plus, with cytotoxicity decreasing over time for both materials [17]. In conclusion, the study rejected the null hypothesis regarding the differences in cytotoxicity between the two sealers, as significant differences were observed. However, the null hypothesis regarding

penetration depth was accepted, showing no differences between the two sealers across the tested obturation techniques.

Conclusions

Within the limitations of this study, it could be concluded that the bioceramic sealers are promising to increase sealing ability compared to resin-based sealers regardless of different obturation techniques used. The use of the thermoplasticized obturation technique is a reliable approach to enhance the penetration of resin-based sealer compared to bioceramic that is enhanced by the cold lateral condensation technique.

Abbreviations

Abbreviation	Long Term
ATCC	American Type Culture Collection
BC	Bio Ceramic
CEJ	Cemento-Enamel Junction
CLSM	Confocal Laser Scanning Microscope
CW	Continuous Wave Technique
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethyl Sulfoxide
EDTA	Ethylenediaminetetraacetic Acid
FBS	Fetal Bovine Serum
GP	Gutta Percha
HGF-1	Human Gingival Fibroblast Cell Line.
LC	Lateral Condensation Technique
NaOcl	Sodium Hypochlorite
PTN	Protaper Next Rotary File
PVC	Polyvinylchloride
RCT	Root Canal Treatment
SC	Single Cone Technique
SD	Standard Deviation
SVN	Side Vented Needle
ZOE	Zinc Oxide Eugenol

Declarations

Ethics Approval and Consent to Participate

This study was conducted in accordance with the principles and approved by Research Ethics Committee at Faculty of Oral and Dental Medicine, Future University (REC- FODM) with respect to scientific content a compliance with applicable research and human subject's regulations. The use of human-extracted teeth and human Gingival

fibroblasts cell was approved by the Research Ethics Committee at Faculty of Oral and Dental Medicine, Future University (REC- FODM) under reference number FUE.REC (4)/ 4-2022.

All teeth were collected from patients who provided informed consent for the use of their extracted teeth and cells for research purposes. Patient identifiers were removed to ensure anonymity, and no additional clinical interventions were performed solely for this study.

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