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Article

The efficacy of XP-endo retreatment system in removing bioceramic sealer from dentinal walls: An in vitro micro-computed tomography study

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Abstract:

Objectives: This study compared the efficacy of the XP-endo retreatment (XPER) system in removing TotalFill HiFlow BC (Bioceramic) and TotalFill BC sealers from dentinal walls to that of the ProTaper Universal retreatment (PTUR) system using micro-computed tomography (micro-CT) analysis. *Materials and methods:* Forty human-extracted single-rooted mandibular premolars were decoronated, and the roots were randomly divided into two main groups of 20 according to the retreatment system used (PTUR or XPER), and each group was divided again into two subgroups (n = 10) based on the sealer type used (TotalFill HiFlow BC or TotalFill BC). All root canals were instrumented and obturated. After two weeks, the retreatment procedure was performed using both systems. All specimens were scanned with a micro-CT device after obturation and again after retreatment, and the volume of root-filling material removed was measured. One-way analysis of variance and independent t-tests were used for statistical analysis. *Results:* XPER removed 88% ± 14.57% of the root canal filling material in the apical third, which was significantly (p = 0.000) more than that removed by PTUR (62.7% ± 24.95%). Overall, XPER removed significantly more HiFlow BC (94.66% ± 10.75%) than

conventional BC sealer (92.66% ± 10.31%). *Conclusions:* XPER was more efficient than PTUR in the BC sealer retreatment at the apical root third. Moreover, the use of HiFlow BC sealer with continuous wave obturation made the retreatment easier as compared with conventional BC sealer with single cone obturation.

Keywords: Bio-ceramic sealer; Retreatment; XP-endo Shaper; Endodontics

1. Introduction

The main objectives of nonsurgical endodontic retreatment are to regain access to the root canal system, remove previous root filling material, and provide space for potential root canal disinfection and reobturation [1,2]. In failed root canal treatments, microorganisms are usually present beneath the remnants of the filling material, especially in the canal walls, dentinal tubules, isthmus, and lateral canals. Therefore, it is important to use instruments that effectively and thoroughly remove filling material and debris from the dentinal walls [3].

Bioceramics (BCs) are ceramic materials that consist principally of alumina, zirconia, bioactive glass, calcium phosphate, and hydroxyapatite [4,5]. BC sealers have become favored by many endodontists because of their antibacterial properties, biocompatibility, ease of use, and excellent sealing properties [6]. Brasseler and FKG Dentaire, among other companies, offered two identical types of BC sealers. A standard BC sealer was produced for single cone obturation, while a heat-resistant (Hiflow) BC sealer was produced for warm vertical and continuous wave obturation.

A major drawback of BC sealers is the difficulty of retrieving them once they are set in the root canal system, which makes future procedures, including post-space preparation and retreatment, challenging [7]. Multiple studies indicate that no existing technique can completely remove filling material from a root canal system [8-11]. To enhance the removal of previous root filling material, a few supplementary techniques have been used in retreatment procedures, including passive ultrasonic irrigation, photon-initiated photoacoustic streaming, the GentleWave system, the ProTaper F3 and F4 files, and the XP-endo Finisher file (XPEF), which have exhibited various levels of effectiveness [12-16].

Several methods of removing BC sealer have been studied, including the use of retreatment reciprocating, and regular rotary files with or without solvents and with different irrigation materials, such as sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA) [17,18]. Hydrochloric acid is an effective solvent for removing BC sealers, but its safety is questionable [19].

Several types of rotary files with special designs have been used to remove different BC sealers, including the ProTaper Universal retreatment (PTUR; Dentsply Maillefer, Ballaigues, Switzerland), XP-endo Shaper (XPES; FKG Dentaire SA, La Chaux-de-Fonds, Switzerland), Reciproc and Reciproc Blue (VDW, Munich, Germany), R-Endo (Micro-Mega, Besancon, France), Mtwo (VDW, Munich, Germany), D-RaCe (FKG Dentaire, La Chaux-de-Fonds, Switzerland), and Vortex Blue (Dentsply Tulsa Dental, Tulsa, OK) files, which have proven efficient during retreatment procedures. However, based on longitudinal sectioning, cone-beam computed tomography (CBCT), or micro-computed tomography (micro-CT) testing, depending on the case, complete removal of residual root canal filling material was not achieved with any of these tools [9,10,20-24].

Researchers have not yet identified an efficacious protocol for completely removing BC sealer from the walls of root canals in clinical practice. Although the use of supplementary techniques reduces residual filling material, it is not considered ideal [17,18].

The XP-endo retreatment (XPER) system was introduced to the market for non-surgical retreatment. The present system integrates three distinct files derived from various sequences within FKG Dentaire's prior files, namely DR1 (derived from the D-Race system), XPES, and XP-endo finisher-R (XPEF-R). The initial file, denoted as DR1, consists of a cutting tip, a working end measuring 15 mm, a size of 30 ISO, and a taper of 0.1. The optimal speed range for DR1 is 800-1000 rpm. XPES is the subsequent file within the XPER system designed for utilization at greater depths within the root canal. It is a non-tapered file made of NiTi MaxWire alloy (Martensite-Austenite Electropolish Flex). It has a snake-like shape, six cutting blades, and a booster tip. This unique design maximizes the file’s capacity to access most of the canal walls, which can greatly improve cleaning and shaping procedures

[25,26]. This file is relatively straight in the martensite phase below 30°C. However, when inserted into the canal at body temperature, it shifts from the martensite to the austenite phase, and the file regains its inherent spiral shape. This shape increases the effectiveness of the tool in removing root canal filling material [27, 28]. The manufacturer recommended a higher speed range of 1000 to 2500 rpm when using XPES for retreatment procedures. Finally, XPEF-R completes the root-filling retrieval phase. XPEF-R is a non-tapered, small-core hook-like rotary file made of a MaxWire alloy that operates at a speed of 800-1000 rpm.

The ProTaper Universal Retreatment System (PTUR; Dentsply Maillefer, Ballaigues, Switzerland) is composed of three sequential files: D1, D2, and D3. D1 is the initial file with a cutting tip, 16mm working end, size 30 ISO, and 0.09 taper. It is used to remove filling material in the coronal third. D2 is the second file to be used with an 18-mm working end, a size 25 ISO, and a 0.08 taper. It is used to remove filling material in the middle third. D3 is the final file in the system, with a 22mm working end, a 20 ISO size, and a 0.07 taper used to remove filling material in the apical third. The speed range recommended for PTUR is 500–700 rpm for GP and carrier-based removal and 300 rpm for paste filling removal. According to the manufacturer, the heat and friction generated by the file will aid in the removal of the filling material.

The majority of published studies on the retreatability of BC sealers examined the use of XPEF and XPEF-R as supplementary tools, or XPES file alone, for retreating root canals filled with conventional BC sealers in a single cone obturation [29–32]. Only one published study, by Hassan et al., was identified in the literature that demonstrated the XPER system's ability to retreat root canals filled with heat-resistant (HiFlow) BC sealer in continuous wave obturation. However, Hassan et al. used scanning electron microscopy to assess the remaining filling material after longitudinal sectioning of the retreated teeth [33]. In the current study, we used micro-CT analysis to compare the efficacy of the XPER and PTUR systems in removing TotalFill HiFlow BC and TotalFill BC sealers from dentinal walls. The null hypothesis of our investigation would be as follows:

1. There is no difference between the XPER and PTUR systems in the efficiency of BC sealer retreatment.

2. There is no difference between TotalFill BC and TotalFill Hiflow BC sealers in their retreatability.

2. Materials and Methods

The study protocol—which was adapted from Crozeta et al. [11] with some modifications was approved by the Institutional Review Board of King Saud University, Riyadh, Saudi Arabia (Approval No. E-22-7112). This in vitro study was conducted in a laboratory at the Dental University Hospital, College of Dentistry, King Saud University.

2.1 Sample size calculation

The sample size calculation was performed with G*Power (G*Power 3.1.9.4 software for Windows; Franz Faul, Universität Kiel, Germany). At a level of significant (alpha) 0.05 with effect size (ES) 1 (large) and power of 87%, the total sample size should be at least 40, 20 randomly selected for each group.

2.2 Sample selection

Forty human extracted single-rooted mandibular premolars were selected. To standardize the canal shape and curvature, preoperative digital radiographs were obtained in two projections (mesiodistal diameter and buccolingual diameter). The inclusion criteria specified the presence of a single oval canal classified as Type I Vertucci with a curvature of 5° or less as measured using the Schneider method. [34, 35] The exclusion criteria ruled out teeth with more than one root canal, root caries, fractures, previous endodontic treatment, restorations, calcifications, internal or external resorption, or open apices.

2.3 Specimen preparation

The routine protocol for disinfecting teeth to ensure the researcher’s safety was administered. The teeth were thoroughly cleaned by removing the hard deposits using curettes and removing the soft deposits by soaking them in 5.25% NaOCl for 10 min. The teeth were then stored in a normal sterile saline solution at 37°C until use. The crowns were sectioned using a diamond disk (Keystone Industries,

Gibbstown, NJ, USA) at the cemento-enamel junction under water cooling, and the length of the roots was standardized at 16 mm. The root canals were then rinsed with 2.5% NaOCl (2.5 mL), and a size 10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) was inserted until its tip became visible at the apical foramen. The working length (WL) was determined to be 0.5 mm shorter than the apical foramen. If a tooth presented with an initial size smaller or larger than the (size 25) apical gauge, it was excluded from the study. Each root was mounted in a customized plastic container using polyvinyl siloxane impression material (Optosil base, Kulzer GmbH, Munich, Germany) to simulate the alveolar position and allow reproducible micro-CT scanning. [36]

2.4 Root canal preparation

The cleaning and shaping procedures for all samples were performed by one specialist. They used size 2 Gates–Glidden burs (Dentsply Maillefer, Ballaigues, Switzerland) at 5,000 rpm, followed by a K3 rotary nickel–titanium file system (SybronEndo, Orange, California) with a 0.04 taper up to size 30 coated with RC-Prep (Dentsply Maillefer, Ballaigues, Switzerland) under copious irrigation with 2.5% NaOCl. This was followed by final irrigation with 17% EDTA (SybronEndo, Orange, CA, USA) and drying with paper points (Vericom Co., Ltd., Korea). According to the retreatment system and type of sealer used, the roots were randomly divided into the following groups and subgroups:

- Group A (n = 20): The PTUR system was used for retreatment
    - Subgroup A-HiFlow (n = 10): TotalFill HiFlow BC sealer was used with continuous wave obturation.
    - Subgroup A-BC (n = 10): TotalFill BC sealer was used with single cone obturation.
- Group B (n = 20): The XPER system was used for retreatment
    - Subgroup B-HiFlow (n = 10): TotalFill HiFlow BC sealer was used with continuous wave obturation.
    - Subgroup B-BC (n = 10): TotalFill BC sealer was used with single cone obturation.

All roots were obturated by a single operator. Master cone periapical radiographs were obtained for all samples.

TotalFill HiFlow sealer obturation protocol

In this method, root canal filling was performed using the continuous-wave technique with a TotalFill BC gutta-percha (GP) point (FKG Dentaire, La Chaux-de-Fonds, Switzerland) and TotalFill HiFlow BC sealer (FKG Dentaire). The sealer was introduced into the canal using the plastic syringe tip provided by the manufacturer. The GP cone was then coated with the sealer and introduced into the canal to determine the WL. A 0.06 tip of the Alpha System (SybronEndo, Glendora, CA) was inserted into the canal to a depth of 5mm shorter than the WL for the down pack, followed by condensation with Dental Buchanan Hand Pluggers size 1 (SybronEndo, Glendora, CA). The canal was backfilled using a Beta Obturation Gun (B and L Biotech, Italy) and GP bars (Meta-Biomed Co. Ltd., Cheongju-si, South Korea).

TotalFill BC sealer obturation protocol

Here, root canal filling was performed using the single-cone technique with a TotalFill BC GP point (FKG Dentaire, La Chaux-de-Fonds, Switzerland) and TotalFill BC sealer (FKG Dentaire). The sealer was introduced into the canal using the plastic syringe tip provided by the manufacturer. The GP cone was then coated with the sealer and introduced into the canal to determine the WL. The Alpha System (SybronEndo, Glendora, CA, USA) was used to cut the cone at the root canal orifice level.

The coronal access points for all roots were sealed with customized plastic covers using a polyvinyl siloxane impression material (Optosil base, Kulzer GmbH, Munich, Germany), and the specimens were stored in 100% humidity at 37°C for 2 weeks.

Initial pre-retreatment micro-CT scanning

Each sample was individually embedded in a polypropylene tube (1.5 cm in height and diameter) containing 3M™ impression material (Minnesota, USA). The samples were loaded and mounted into the specimen chamber in a predetermined

position in the micro-stage to yield a reproducible representation of the pre- and post-treatment datasets. All roots were scanned using Bruker SkyScan 1172 high-resolution micro-CT (Bruker SkyScan, Kontich, Belgium) at 90 kV, a 102 µA anode current, a 316 ms exposure time, a 19.70 µm image pixel size, and a 0.4 rotation step for a 360° angle, with a frame-averaging value of 4 (to optimize the signal-to-noise ratio) and a random-movement value of 8 (to minimize ring artifacts). Flat-field correction was performed before the scanning procedure to correct for variations in the camera's pixel sensitivity. After the scanning, the projected images were reconstructed as cross-sectional images using NRecon reconstruction software (version 1.6.9.4; Bruker Skyscan, Kontich, Belgium). The numerical parameters required to produce the best image results were verified and adjusted. A ring artifact reduction setting of 5 was applied to prevent the non-uniformity of the background image taken by the X-ray camera, and a smoothing setting of 2 was implemented using a Gaussian kernel. The beam hardening compensation was set to 25% to prevent the specimen from appearing artificially denser on or near its surface and less dense in its central components. The images were saved in 16-bit TIF file format to to preserve the variety of specimen densities.

Retreatment procedures

PTUR (Dentsply Maillefer, Ballaigues, Switzerland) was performed at 500 rpm with a 1.5 Ncm torque as recommended by the manufacturer. The sequential crown-down technique was applied in brushing motions with sizes D1, D2, and D3 until the WL was reached and no further filling material could be removed without adding solvent. This was followed by irrigation with 2.5% NaOCl.

XPER was used in accordance with the sequence recommended by the manufacturer. The DR1 rotated at 1,000 rpm, and a torque of 1.5 Ncm was used in the obturation until a 3–4-mm coronal space was gained. The XP-endo Shaper was applied in a pecking motion with light pressure at 1,200 rpm and a 1 Ncm torque until the WL was reached. This was followed by 15 more long, gentle strokes until the WL was reached, and then by NaOCl irrigation. The XP-endo Finisher R was applied for 1 min in longitudinal movements at 1,000 rpm and 1 Ncm up to the WL, followed by a final copious 2.5% NaOCl irrigation. A single specialist performed

the retreatment procedures for all groups. Finally, post-retreatment micro-CT scanning was performed using the same parameters implemented in the initial scan.

2.5 Evaluation

For each sample, the total volume percentage of filling material was measured before (Pre-reTx vol%) and after (Post-reTx vol%) retreatment. The volume percentage of the removed filling material (removed filling vol%) was calculated using the following formula:

removed filling vol% = Pre-reTx vol% - Post-reTx vol%.

The volume of filling material removed was determined for the entire canal and for the coronal, middle, and apical thirds of the canal. To enable two- and three-dimensional evaluations and qualitative analysis of the presence and location of the remaining filling material, the reconstructed images were loaded into DataViewer Software (version 1.5.6.2; Bruker Skyscan, Kontich, Belgium) to coregister the initial and final scan data, determine the image quality, and reorient, resize, and visually inspect the 3D datasets. The co-registered dataset was then saved and loaded into CTAn software (version 1.20.8.0; BrukerSkyScan) for selective imaging and analysis, binarization, and quantification of the initial (post-obturation) and final (post-retreatment) scans of the filling material. Finally, CTVol (version 2.3.2.0; Bruker Skyscan, Kontich, Belgium) was used for 3D visualization and the production of color-coded images of the samples.

2.6 Statistical Analysis

Descriptive and inferential statistics were calculated. The descriptive statistics are presented as frequency, percentage, mean, and standard deviation of filling material removed. The normal distribution was tested using the Shapiro–Wilk test. One-way analysis of variance (followed by Tukey's multiple comparison test) and an independent t-test were used to compare the groups, subgroups, and locations. A p-value of ≤ 0.05 was taken to indicate statistical significance. The data collected were entered into IBM SPSS (version 25; IBM Corp., Armonk, NY, USA) and subjected to statistical analysis.

3. Results

All retreatment systems we tested were able to significantly remove root canal filling material with both types of sealer (Fig. 1). Tables 1 and 2 present the results as mean values and standard deviations of the root canal filling material removed by various systems in each root third. Overall, the XPER system removed significantly more root canal filling material than the PTUR system (Fig. 2) with both types of sealer, TotalFill BC and TotalFill Hiflow BC sealers (p=0.000, and p=0.003 respectively).

Regardless of the sealer used, XPER removed significantly more (88% ± 14.57%; p = 0.000) filling material in the apical third than PTUR (62.7% ± 24.95%), which means that XPER was able to remove 25.3% more material than PTUR in the apical third.

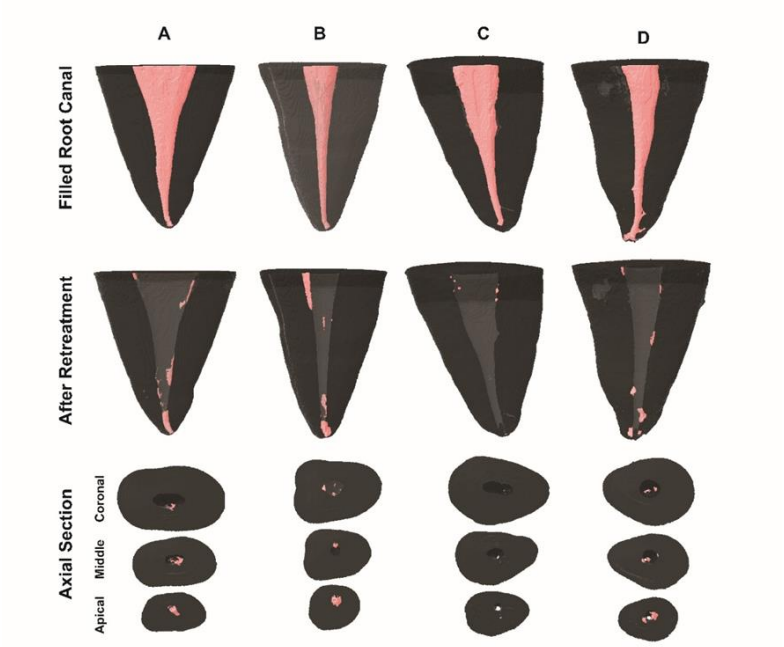


Figure 1. Representative micro-CT 3D reconstruction images of a single-rooted lower mandibular premolar. Gutta-percha and sealer are represented in pink. A represents roots obturated with TotalFill HiFlow sealer and retreated with PTUR system; B, roots obturated with TotalFill BC sealer and retreated with PTUR system; C, roots obturated with TotalFill HiFlow sealer and retreated with XPER system; and D, roots obturated with TotalFill BC sealer and retreated with XPER system. Representative cross-sections of the roots at the coronal, middle, and apical thirds after retreatment are shown for each group.

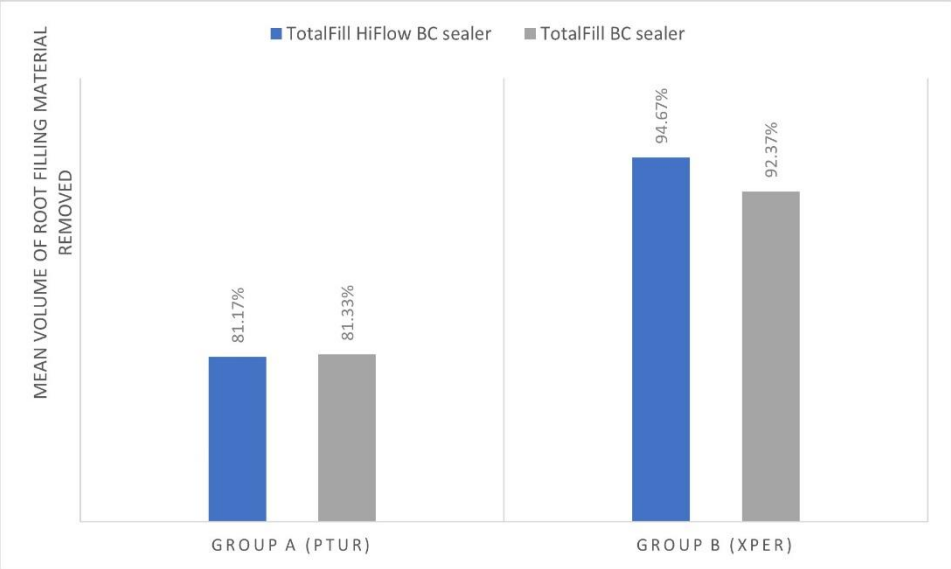


Figure 2. Mean volume of root canal filling material removed by different systems. PTUR: ProTaper Universal retreatment; XPER: XP-endo retreatment; BC: bioceramic.

3.1 ProTaper Universal Retreatment (Group A)

A significantly higher percentage (98.2% ± 2.29%) of filling material was removed from the coronal thirds of roots filled with continuous wave technique using TotalFill HiFlow sealer than from the apical (60% ± 29.28%) and middle thirds (p = 0.001). A significantly higher percentage (93.8% ± 6.47%) of material was removed from the coronal third of roots filled with the single cone technique using TotalFill BC (Group A: BC) sealer than from the apical (65.4% ± 21%) thirds (p = 0.002; Table 1).

Table 1. Mean and standard deviation (SD) of root canal filling material removed using the ProTaper Universal retreatment (PTUR) system in each third

File system	Sealer	Location	Mean	SD	P value	MCT		
						Coronal	Middle	Apical
PTUR (Group A)	TotalFill HiFlow	Coronal	98.2	2.289	0.001*	1		
		Middle	85.3	17.424		0.327	1	
		Apical	60.0	29.276		0.001*	0.021*	1
	TotalFill BC	Coronal	93.8	6.473	0.002*	1		
		Middle	84.8	17.727		0.448	1	
		Apical	65.4	21.001		0.002*	0.033*	1

* P < 0.05 indicates statistical significance. MCT: Measures of central tendency.

3.2 XP-endo Retreatment (Group B)

For the root canals retreated with the XPER system, the total volume of root canal filling material removed was significantly higher for roots filled with continuous wave technique using TotalFill HiFlow sealer (94.66% ± 10.75%) compared with those filled with the single cone technique using TotalFill BC sealer (92.66% ± 10.31%) (Figure 2).

For the roots filled with TotalFill HiFlow sealer, the volume of filling material removed was significantly higher in the coronal third (99.2% ± 1.26%) than in the middle and apical thirds. For those filled with TotalFill BC sealer, the volume removed was significantly higher (98% ± 3.68%) in the middle third compared with the coronal (91% ± 9.88%) and apical thirds (88.1% ± 13.15%), which were not significantly different (Table 2).

Table 2. Mean and standard deviation (SD) of root canal filling material volume removed using the XP-endo retreatment (XPER) system in each anatomical third.

File system	Sealer	Location	Mean	SD	P value	MCT		
						Coronal	Middle	Apical
XPER (Group B)	TotalFill HiFlow	Coronal	99.2	1.263	0.040*	1		
		Middle	96.9	4.115		0.866	1	
		Apical	87.9	16.579		0.043*	0.123	1
	TotalFill BC	Coronal	91.0	9.883	0.081	1		
		Middle	98.0	3.683		0.260	1	
		Apical	88.1	13.153		0.777	0.075	1

* P < 0.05 indicates statistical significance. MCT: Measures of central tendency.

4. Discussion

The main advantage of BC sealer is the strong chemical bond it forms with dentin. This is attributed to the calcium phosphate it contains, which produces a chemical composition comparable to that of teeth and bones. [37] The objective of this study was to assess the efficacy of the XPER and PTUR systems in removing conventional BC sealer (used with the single-cone obturation) and TotalFill HiFlow BC sealer (used with the continuous-wave obturation) from dentinal walls using micro-CT analysis.

We found that both retreatment systems were able to remove significant root canal filling material with both sealers (TotalFill HiFlow and TotalFill BC). The XPER

system removed significantly more material than the PTUR system. Therefore, the first null hypothesis was rejected.

Although XPER more efficiently removed root filling material, other factors should be considered before recommending the use of this system. During our study, we noticed that the XPER set was not easy to handle; it was challenging to insert the curved XPES and XPEF-R into the canal, making the procedure time-consuming. Therefore, it can be recommended as a backup for any other rotary retreatment file. Our findings confirm previous reports that the XPES effectively removes root filling material. It can remove approximately 97% of the obturation mass and is even more effective if used with XP-endo Finisher or Finisher-R [32,38-40]. However, all these investigations tested the efficiency of XPES, the supplementary use of XPEF, or XPEF-R to retreat the standard BC sealer used in the single-cone obturation. Previous studies did not evaluate the entire XPER system, as used in the current study. A standard BC sealer cannot withstand heat and thus cannot be used with warm vertical or continuous wave techniques. However, HiFlow BC sealers, such as EndoSequence BC Sealer HiFlow (Brasseler, USA) and TotalFill HiFlow BC (FKG Dentaire, Switzerland), are heat resistant, although they have the same chemical composition as the standard BC sealer, including tricalcium silicate, dicalcium silicate, and zirconium oxide. Moreover, HiFlow BC sealers demonstrate improved viscosity, flow, and radiopacity compared with other standard BC sealers. [41-43]

Furthermore, our findings indicated that retreatment was easier in the coronal thirds of roots filled with TotalFill HiFlow BC sealer than in those filled with TotalFill BC sealer. Therefore, the second null hypothesis was rejected. This may be attributed to the different techniques used in the two groups. A continuous wave technique was used with the TotalFill HiFlow BC sealer groups; therefore, most of the root filling material in the coronal third was GP, which was easier to retrieve during retreatment. In contrast, the single-cone technique was used with the conventional BC sealer groups, and the BC sealer layer in the coronal third was thick, making it more difficult to retrieve. No significant differences were found between the different types of sealers in the middle and apical thirds because the

thickness of the sealer was the same for both obturation techniques. All retreated samples showed sealer remnants in the dentinal walls. This is consistent with previous reports that no current technique can completely remove filling material from the root canal system. [8-11]

This in vitro study has some limitations, and the results do not reflect the complexity of clinical scenarios. The teeth selected had minimal curvature, and the roots were decoronated; therefore, it was expected that retreatment and material removal would be easier than in real clinical conditions. However, the decoronation was necessary to standardize the root length. [36]. According to the manufacturer, FKG, the XP-endo Shaper file changes shape and becomes more spiral when exposed to body temperature. However, temperature was not controlled in this study. This might have affected the shaping ability and removal efficiency of the XP-endo Shaper file.

In future investigations, we recommend the following: first, to use root canals with more complex anatomy to mimic challenging clinical conditions. Secondly, we recommend standardizing the use of solvents or not using them at all to control all variables that might affect the results. Lastly, we recommend using XPER at body temperature to maximize the advantages of the MaxWire alloy.

5. Conclusions

Both systems, XPER and PTUR, were able to remove root canal filling material. However, none of the tested systems were able to remove BC sealer completely from dentinal walls. Overall, the XPER system removed significantly more root canal filling material than the PTUR system with both types of sealer, TotalFill BC and TotalFill Hiflow BC sealers. Regardless of the sealer used, XPER system removed 25.3% more filling material than PTUR system in the apical third. XPER retreatment technique was found to be challenging. Using the TotalFill HiFlow BC sealer with continuous wave obturation made the retreatment easier than using the TotalFill BC sealer with single cone obturation.

Author Contributions

Conceptualization, S.A, B.A and A.A; Data curation, S.A, B.A, H.A.A, H. D.A, L.A.A and L.B; Formal analysis, S.A, B.A, H.A.A, H. D.A; Funding acquisition, A.A; Investigation, S.A, B.A, H.A.A, H. D.A, L.A.A and L.B; Methodology, S.A, B.A, L.B; Project administration, A.A.A; Resources, A.A.A and A.A; Software, L.B; Supervision, A. A; Validation, A.A; Writing – original draft, S.A, B.A, H.A.A, H. D.A, L.A.A and A.A; Writing – review & editing, S.A, B.A, H.A.A, H. D.A, L.A.A and A.A

Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of KING SAUD UNIVERSITY institutional review board (E-22-7112).

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

All data used and/or analyzed during this study are included in the manuscript

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Conflicts of Interest

The authors declare that they have no conflict of interest

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