

An In-vitro Evaluation of Marginal Adaptation of Various Root Canal Sealers at Dentin-Sealer, Sealer-Guttapercha Interfaces at 3-Levels of Root Canal Using Field Emission Scanning Electron Microscope (FESEM) Analysis

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Abstract

Aim: In-vitro evaluation of marginal adaptation of AH Plus, MTA Fillapex, Bio-C, GuttaFlow-2 root canal sealers at dentin-sealer, sealer-guttapercha interfaces in the coronal, middle and apical thirds of root canals using Field Emission Scanning Electron Microscope (FESEM) analysis.

Materials and methods: Eighty extracted human mandibular premolar teeth were used. All teeth were decoronated to standardize root length to 14mm. A round bur was used to gain access into canal orifices and working length was determined. Root canal instrumentation was done in crown-down technique using Protaper Next rotary files upto size X3 file along with the use of root canal irrigants. All specimens were then randomly divided into four groups with 20 specimens per group, depending upon root canal sealer used. Group A; AH Plus, Group B; MTA-Fillapex, Group C; Bio-C, Group D; GuttaFlow-2. All specimens were obturated with size X3 guttapercha point uniformly coated with respective sealers in single-cone technique and coronal access sealed with Glass ionomer cement. Specimens were incubated for 7 days and were then horizontally sectioned at coronal, middle and apical thirds of root canals. On each sample obtained, three points were randomly chosen and both sealer-dentin, sealer-guttapercha interfaces were examined under FESEM at 1000X. Marginal gaps of all four sealers at both interfaces, at 3-levels of root canals was measured in μm and values were recorded, tabulated and used for data analysis. One-way ANOVA and Post hoc Bonferroni tests were used for statistical analysis. A P value ≤ 0.05 was considered statistically significant.

Results: Compared to sealer-guttapercha interface, AH Plus, MTA-Fillapex, Bio-C sealer at all 3-levels of root canal showed more marginal gaps at sealer-dentin interface with significant difference ($P < 0.05$), However GuttaFlow-2 sealer showed no significant difference ($P > 0.05$).

Conclusion: The marginal adaptation of GuttaFlow-2 sealer is superior to both dentin and guttapercha at coronal, middle and apical thirds of root canals compared to other sealers used in the study.

Categories: Dentistry

Keywords: field emission scanning electron microscope, sealer-guttapercha interface, sealer-dentin interface, marginal adaptation, interfacial gaps

Introduction

Herbert Schilder in 1967 proposed the objective of endodontic therapy was to eliminate diseased root canal tissue and its contents to rectify periapical infection and inflammation. The breakdown of periapical tissues can be halted only when the root canal system is sealed from the periodontal ligament, and surrounding bone and this can be achieved with the use of instruments and antiseptics followed by three-dimensional filling or obturation of root canal space 0.5 mm to 1 mm from the radiographic apex [1]. Kuttler Y [2] defined ideal obturation as thoroughly filling the entire dentinal portion of root canals, sealing the cementodentinal junction, and stimulating the obliteration of the cemental portion of the canal with new cementum deposition.

Several materials were tried and tested as endodontic obturation material, of which Gutta-percha has been

most extensively used for years worldwide and has established itself as a gold standard [3]. The composition of gutta-percha cones used for root canal obturation is 56-66% zinc oxide fillers, 20% gutta-percha, 11% barium sulfate, and 3% waxes [4]. Gutta-percha alone cannot seal the root canal space, as it has no adhesion and only adapts to the root canal dentin. Root canal sealers are usually regarded as the seal-forming 'gasket' of the root canal filling, but can also act as a weak link in the root canal space, thus its volume should be minimized with more gutta-percha cones used as core filling material [5]. The rationale for the use of a sealer is to attain a fluid-tight seal apically, laterally, and coronally between the root canal dentin and gutta-percha. Root canal sealer acts as a lubricating agent helping in the proper seating of gutta-percha cones in root canals and also as a binding agent between the gutta-percha cones [6].

The ability of root canal sealers to penetrate deeper into the dentinal tubules consistently and effectively is one of the many factors influencing the choice of the sealer used in root canal obturation [7]. AH (Aethoxylinharz Hexamethylene tetramine) Plus (Dentsply, Maillefer, DeTray, Germany) root canal sealer is available in a 2-paste system. The Epoxide paste contains radiopaque fillers and aerosol and the Amine paste contains 1-adamantane amine, N, N-dibenzyl-5-oxa-nonandiamine-1,9, and TCD-Diamine [8].

MTA (Mineral Trioxide Aggregate) Fillapex (Angelus, Londrina, Parana, Brazil) is a Calcium silicate-based root canal sealer composed of salicylate resin, diluting resin, natural resin, bismuth trioxide, nano-particulate silica, MTA (tricalcium silicate, dicalcium silicate, tricalcium aluminate, tetra calcium aluminoferrite-silicate) and pigments [9]. Bio-C Sealer (Angelus, Londrina, PR, Brazil) is a new premixed bioceramic-based root canal sealer available in a single syringe form and is composed of calcium silicates, calcium aluminate, calcium oxide, zirconium oxide, iron oxide, silicon dioxide, dispersing agents [10]. GuttaFlow 2 (Coltene Whaledent Pvt. Ltd., Altstätten, Switzerland) is a silicone-based root canal sealer available in Automix syringe form. It contains guttapercha powder, polydimethylsiloxane, platinum catalyst, zirconium dioxide, and micro-silver [11].

The early Scanning Electron Microscope (SEM) used a heated tungsten filament cathode as an electron source, known as a thermionic emitter. Later in 1975, Lanthanum hexaboride cathodes replaced tungsten cathodes. Thermionic emitters emit high current with a beam size of 4 to 8nm, but it produces low brightness and also results in evaporation of filament called as Thermal drift, limiting optical performance at higher resolution. SEM that uses Field Emitter Gun (FEG) as Emitter type is called as Field Emission Scanning Electron Microscope (FESEM). FESEM produces higher electron density with a beam size focused to 2nm, resulting in higher resolution than SEM. The electron beam produced by FESEM is focused by the electromagnetic lenses and apertures to a tiny sharp spot of about 1000 times smaller than in SEM resulting in better image quality and very minute features, demarcations, presence of gaps, voids and adaptation of any 2 material surfaces can be more clearly examined [12].

Hence, this in-vitro study aimed to evaluate marginal adaptation or interfacial gaps of AH Plus, MTA Fillapex, Bio-C, GuttaFlow-2 root canal sealers at Dentin-Sealer and Sealer-Guttapercha interfaces in the coronal-third, middle-third, apical-third of root canals using Field Emission Scanning Electron Microscope (FESEM) analysis.

Materials And Methods

An in-vitro study was conducted in the Department of Conservative Dentistry and Endodontics after obtaining the Institutional Ethical Committee clearance certificate, TIDSHRC/IEC/2021/D006 of the Triveni Institute of Dental Sciences, Hospital and Research Center, Bilaspur, Chhattisgarh, India. Our study sample consisted of 80 human permanent mandibular single-rooted premolar teeth, extracted either for orthodontic reasons or periodontally compromised within the age group of 18-30 years following strict inclusion criteria. All teeth were examined under a stereomicroscope (Olympus SZ61, Olympus Optical Co., Tokyo, Japan) at 10X magnification to ensure that they were intact and without any caries or non-caries lesions, devoid of restorations, clinically detectable fractures, cracks, open root apices followed by Digital periapical radiographs of teeth were taken using Radiovisiography (RVG) (Kodak, Carestream Health India Pvt Ltd, Maharashtra, India) in buccolingual and mesiodistal directions to confirm the presence of a single root canal with angle of curvature less than 20° as assessed by Schneider's criteria [13].

All specimens utilized in our study were within one month of extraction. The collected teeth were cleaned of superficial debris, calculus, and residual tissue tags using ultrasonic instruments. Occupational Safety and Health Administration (OSHA), Center for Disease Control (CDC), and Prevention recommendations and guidelines were strictly followed during the collection, sterilization, and handling of extracted teeth to prevent any biohazard transmission. All teeth were then stored in 0.5% thymol at room temperature until used.

All specimens were then decoronated using a flexible diamond disc (Novo Dental products, Mumbai) attached to a straight micromotor handpiece (NSK, Japan) at low-speed under water coolant at the level of cemento-enamel junction perpendicular to the long axis of teeth to standardize root length of all specimens to 14mm measured by Vernier caliper. A no.4 Round bur (Mani, Japan) was attached to a contra-angle high-speed airtor handpiece (NSK, Japan) to gain access to root canal orifices of all specimens. A no.10 K(Kerr)-file (Dentsply, Maillefer, Switzerland) was placed into the root canal to establish the patency till the apical

foramen, and with the tip of the trail file just visible at the apical foramen, the working length was determined by subtracting 0.5mm from the length in all specimens.

All specimens were then numbered randomly from 1 to 80, with the numbers written on the coronal-third of roots in each specimen using a permanent marker pen (Kokuyo Camlin Pvt. Ltd) and were then individually embedded in cylindrical self-cure acrylic resin blocks (DPI RR Cold Cure, Dental Product of India, Mumbai, India) upto the level 3mm apical to cemento-enamel junction to facilitate ease of handling during root canal instrumentation.

Root canal instrumentation was done in all specimens in crown-down technique using ProTaper Next rotary file system (Dentsply, Maillefer, Switzerland) in file sequence of X1, X2 and X3, with X3 as Master Apical File (MAF) (Size 0.30, Taper 0.07) at 300 rpm, 2.8 Ncm of torque attached to torque-controlled Endomotor handpiece (CanalPro CL2, Coltene Endo, Coltene Whaledent Pvt. Ltd, Alstatten, Switzerland) following manufacturer's instructions. Each rotary file was used for instrumentation in only five root canals and were discarded, followed by the use of new files. In each root canal, during instrumentation 2ml of 17% EDTA (EthyleneDiamineTetraAcetic acid) solution (Prevest Denpro, Jammu, India), 2ml of 3% Sodium hypochlorite solution (Neelkaanth Health Care Pvt Ltd, Ahmedabad, India) were used as root canal irrigants with Max-I-Probe (Dentsply Maillefer, Switzerland) irrigation needles and each root canal was then finally rinsed with 2ml of distilled water (Sadbhavna Chemicals, Gujarat, India) to remove any remnants of root canal irrigants.

All specimens (n=80) were then randomly divided into four groups with 20 specimens per group depending upon the root canal sealer used and obturated with guttapercha in Single-cone technique as follows; Group A (n=20) and Group B (n=20); Root canals were completely dried with sterile absorbent paper points (Dia Dent International, Korea), AH Plus and MTA Fillapex sealers were manipulated on paper pad consistently following manufacturer's instructions respectively. ProTaper Next guttapercha points, Size X3 (Dentsply, Maillefer, Switzerland) were then uniformly coated with the respective sealers and slowly inserted into the root canals until it reached the predetermined working length and checked for tug-back.

Group C (n=20): Bio-C is a premixed sealer, so it was directly dispensed on a paper pad and following manufacturer instructions, root canals were not completely dried with sterile absorbent paper points and instead they were left slightly moist. ProTaper Next guttapercha points, Size X3 were then uniformly coated with the sealer and slowly inserted into the root canals until it reached the predetermined working length and checked for tug-back. Group D (n=20): Root canals were dried with sterile absorbent paper points and GuttaFlow-2 sealer was manipulated on a paper pad following manufacturer's instructions. ProTaper Next guttapercha points, Size X3 were then uniformly coated with the sealer and slowly inserted into root canals until it reached the predetermined working length and checked for tug-back.

In all specimens, guttapercha was seared off at the level of cemento-enamel junction using a heated plugger (BeeFill Heat Plugger, VDW GmbH, Munich, Germany) and vertically compacted 2mm below the cemento-enamel junction and the coronal access of root canals were sealed with Light-cure Glass Ionomer Cement (GC Light cure Universal Restorative, GC Corp, Tokyo, Japan). To prevent any inter-operator variability, root canal instrumentation and obturation in all specimens were done by a single endodontist. Later, to allow adequate time for complete setting of all four root canal sealers, all specimens were carefully removed from the acrylic resin blocks and placed in an Incubator (Zeal International, New Delhi, India) at 100% relative humidity, 37°C for 7 days.

All specimens were then horizontally sectioned using a flexible diamond disc attached to straight micromotor handpiece at low-speed under continuous water flow to prevent any frictional heat generation minimizing smearing of gutta-percha and root canal sealer to obtain 1mm thick cross-sections of roots at 3mm (apical-third), 6mm (middle-third), 9mm (coronal-third) from the predetermined working length. For ease of identification, distinctive labelling was done to all the cross-sections obtained from each specimen of Group A, B, C, D to differentiate them as apical-third, middle-third and coronal-third samples. The samples were then examined under Field Emission Scanning Electron Microscope (FESEM) [Quanta FEG 250, FEI Company (Thermo Fisher Scientific), Oregon, United States] at a magnification 1000X. Three randomly chosen points in coronal-third, middle-third and apical-third cross-sections of each root specimen were focussed under FESEM both at Dentin-Sealer and Sealer-Guttapercha interfaces. The marginal adaptation or interfacial gaps was measured using Image J software (Wayne Rasband, NIH, Bethesda, USA) and the average interfacial gaps at both Dentin-Sealer and Sealer-Guttapercha interfaces were calculated and the Mean (μm) was estimated for each sample. To prevent any inter-operator variability, FESEM photomicrographs of all specimens were analyzed by a single endodontist. The obtained data was recorded and tabulated.

IBM SPSS (Statistical Package for Social Sciences) software, Version 24 (IBM Corp., Armonk, NY) was used for data analysis. One-way ANOVA (Analysis of Variance) was used to compare the extent of interfacial gaps at dentin-sealer and sealer-guttapercha interfaces among the four experimental groups at three root levels. Post hoc Bonferroni test was done for pairwise comparison between the experimental groups. A P value ≤ 0.05 was considered statistically significant.

Results

One-way ANOVA test between the four groups for the presence of interfacial gaps both at dentin-sealer and sealer-guttapercha interfaces showed Group A (AH Plus sealer) with maximum interfacial gaps and Group D (GuttaFlow-2 sealer) with least interfacial gaps at coronal-third, middle-third and apical-third of root canals. A statistically significant difference was seen in the marginal adaptation or interfacial gaps expressed in μm between the 4 groups tested ($P \leq 0.05$) summarized in Table 1 and Table 2.

Interfacial gaps at the Dentin-Sealer Interface					
Levels of Root canal	Mean \pm S.D. (μm)				P value
	Group A (AH Plus sealer)	Group B (MTA Fillapex Sealer)	Group C (Bio-C Sealer)	Group D (GuttaFlow-2 Sealer)	
Coronal-third	7.68 \pm 5.47	8.58 \pm 6.06	7.22 \pm 5.14	6.34 \pm 3.68	P = 0.58 (Non-significant)
Middle-third	12.69 \pm 5.68	8.13 \pm 5.34	7.79 \pm 5.1	1.94 \pm 1.39	P = 0.001 (Significant)
Apical-third	6.12 \pm 5.45	4.82 \pm 3.93	5.75 \pm 5.98	2.35 \pm 1.26	P = 0.04 (Significant)

TABLE 1: One-way ANOVA test for Inter-group comparison of Interfacial gaps at Dentin-Sealer Interface in Group A, Group B, Group C and Group D specimens.

P: Probability, SD: Standard Deviation.

Interfacial gaps at Sealer-Guttapercha Interface					
Levels of Root canal	Mean \pm S.D. (μm)				P value
	Group A (AH Plus Sealer)	Group B (MTA Fillapex Sealer)	Group C (Bio-C Sealer)	Group D (GuttaFlow-2 Sealer)	
Coronal-third	42.80 \pm 22.69	2.84 \pm 2.52	3.21 \pm 2.28	0.60 \pm 0.85	P = 0.001 (Significant)
Middle- third	11.99 \pm 17.28	1.33 \pm 1.10	1.84 \pm 1.27	0.22 \pm 0.49	P = 0.001 (Significant)
Apical- third	9.27 \pm 19.73	3.18 \pm 2.59	2.63 \pm 2.40	0.09 \pm 0.17	P = 0.03 (Significant)

TABLE 2: One-way ANOVA test for Inter-group comparison of Interfacial gaps at Sealer-Guttapercha Interface in Group A, Group B, Group C and Group D specimens.

P: Probability, SD: Standard Deviation.

At the dentin-sealer interface; Group A (AH Plus) showed maximum interfacial gaps at middle-third (12.69 μm) followed by coronal-third (7.68 μm) and apical-third (6.12 μm). Group B (MTA Fillapex) showed maximum interfacial gaps at the coronal third (8.58 μm) followed by the middle third (8.13 μm) and apical-third (4.82 μm). Group C (Bio-C) showed maximum interfacial gaps at the middle third (7.79 μm) followed by the coronal third (7.22 μm) and apical third (5.75 μm). Group D (GuttaFlow-2) showed maximum interfacial gaps at the coronal third (6.34 μm) followed by the apical third (2.35 μm) and middle third (1.94 μm).

At the sealer-guttapercha interface; Group A (AH Plus) showed maximum interfacial gaps at middle-third (11.99 μm) followed by coronal-third (6.34 μm) and apical-third (9.27 μm). Group B (MTA Fillapex) showed maximum interfacial gaps at the apical third (3.18 μm) followed by the coronal third (2.84 μm) and middle third (1.33 μm). Group C (Bio-C) showed maximum interfacial gaps at the coronal third (3.21 μm) followed by the apical third (2.63 μm) and middle third (1.85 μm). Group D (GuttaFlow-2) showed maximum interfacial gaps at the coronal third (0.60 μm) followed by the middle third (0.22 μm) and apical third (0.09 μm). (Figure 1)

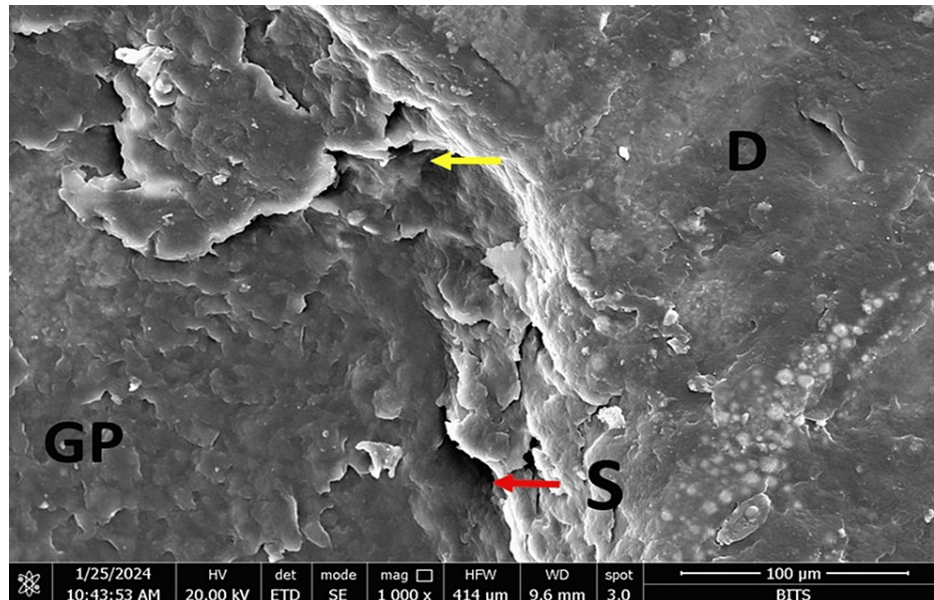


FIGURE 1: SEM photomicrograph of specimen of AH Plus sealer at coronal-third of root canal.

(D: Dentin, S: Sealer, GP: Gutta-percha, Red/Yellow Arrow marks denote Interface)

Post hoc Bonferroni analysis test was done to compare the significance in pair-wise groups for the interfacial gaps at dentin-sealer and sealer-gutta-percha interfaces at coronal-third, middle-third and apical-third of root canals of all specimens (Table 3 and Table 4).

Pair-wise comparison	P value	Significance
Pair-wise comparison of Interfacial gaps at Dentin-Sealer Interface in Coronal-third		
A vs B	0.62	Non-significant
A vs C	0.78	Non-significant
A vs D	0.37	Non-significant
B vs C	0.44	Non-significant
B vs D	0.16	Non-significant
C vs D	0.54	Non-significant
Pair-wise comparison of Interfacial gaps at Dentin-Sealer Interface in Middle-third		
A vs B	0.012	Non- significant
A vs C	0.006	Significant
A vs D	<0.00001	Significant
B vs C	0.83	Non- significant
B vs D	<0.00001	Significant
C vs D	<0.00001	Significant
Pair-wise comparison of Interfacial gaps at Dentin-Sealer Interface in Apical-third		
A vs B	0.39	Non- significant
A vs C	0.83	Non- significant
A vs D	0.004	Significant
B vs C	0.56	Non- significant
B vs D	0.01	Non- significant
C vs D	0.01	Non- significant

TABLE 3: Post hoc Bonferroni test in Pair-wise comparison of Interfacial gaps at Dentin-Sealer interface in Coronal, Middle and Apical thirds of root canals.

Bonferroni adjustment factor $\alpha = 0.008$; $P < \alpha$ = Significant, $P > \alpha$ = Non-significant

Pair-wise comparison	P value	Significance
Pair-wise comparison of Interfacial gaps at Sealer-Guttapercha Interface in Coronal-third		
A vs B	<0.0001	Significant
A vs C	<0.0001	Significant
A vs D	<0.0001	Significant
B vs C	0.63	Non-significant
B vs D	0.0005	Significant
C vs D	<0.0001	Significant
Pair-wise comparison of Interfacial gaps at Sealer-Guttapercha Interface in Middle-third		
A vs B	0.0089	Non-significant
A vs C	0.012	Non-significant
A vs D	0.004	Significant
B vs C	0.17	Non-significant
B vs D	0.0001	Significant
C vs D	<0.0001	Significant
Pair-wise comparison of Interfacial gaps at Sealer-Guttapercha Interface in Apical-third		
A vs B	0.17	Non-significant
A vs C	0.14	Non-significant
A vs D	0.04	Non-significant
B vs C	0.48	Non-significant
B vs D	<0.0001	Significant
C vs D	<0.0001	Significant

TABLE 4: Post hoc Bonferroni test in Pair-wise comparison of Interfacial gaps at Sealer-Guttapercha interface in Coronal, Middle and Apical thirds of root canals.

P: Probability; Bonferroni adjustment factor $\alpha = 0.008$; $P < \alpha$ = Significant, $P > \alpha$ = Non-significant

Post hoc Bonferroni analysis of dentin-sealer interface; at coronal-third no significant differences were seen in pair-wise comparison among the four groups, at middle-third statistically significant differences were seen in group pairs of A vs C, A vs D, B vs D and C vs D, at apical-third statistically significant difference was seen in group pair of A vs D.

Post hoc Bonferroni analysis of sealer-guttapercha interface; at coronal-third statistically significant differences were seen in group pairs of A vs B, A vs C, A vs D, B vs D and C vs D, at middle-third statistically significant differences were seen in group pairs of; A vs D, B vs D and C vs D, at apical-third statistically significant differences were seen in group pairs of A vs B, A vs C, A vs D and B vs C.

None of the groups showed complete marginal adaptation at both dentin-sealer and sealer-guttapercha interfaces at all 3 levels of root canal, there were both gap-free and gap-containing areas at three levels of root canal in all groups. However, GuttaFlow-2 sealer (Group D) exhibited maximum marginal adaptation and AH Plus sealer (Group A) showed minimum marginal adaptation at both dentin-sealer and sealer-guttapercha interfaces at all 3 levels of root canal. Compared to sealer-guttapercha interface, AH Plus, MTA-Fillapex, Bio-C sealer at all 3-levels of root canal showed more marginal gaps at sealer-dentin interface with significant difference ($P < 0.05$), However GuttaFlow-2 sealer showed no significant difference ($P > 0.05$).

At Dentin-Sealer interface:- In coronal-third of root canals; AH Plus and MTA Fillapex sealer showed maximum and GuttaFlow-2 sealer showed minimum interfacial gaps. In middle-third and apical-third of root canals; AH Plus sealer showed maximum and GuttaFlow-2 sealer showed minimum interfacial gaps.

At Sealer-Guttapercha interface:- In coronal-third, middle-third and apical-third of root canals; AH Plus sealer showed maximum interfacial gaps and GuttaFlow-2 sealer showed minimum interfacial gaps.

Discussion

The main objective of obturation is to provide a three-dimensional seal of root canal space, thus preventing root canal reinfection and preserving the health of periapical tissues an ideal root canal obturation is to have a greater volume of guttapercha as core filling material with minimal volume of root canal sealer penetrating deeper into the canal irregularities and dentinal tubules [14]. In the present study, single-canal mandibular premolar teeth were utilized, because they have approximately similar bucco-lingual and mesio-distal dimensions and the teeth were decoronated at the cemento-enamel junction to eliminate variations in endodontic access cavity preparation designs.

As guttapercha lacks any adhesive properties, a root canal sealer helps to adapt guttapercha to the root canal dentin and fills any canal imperfections, thus preventing microleakage, microbial contamination, and failure of endodontic therapy. An ideal root canal sealer should be biocompatible, and have low surface tension thereby having deeper penetration into dentinal tubules of the root canal with better wettability providing a fluid-tight seal and about 60% of endodontic failures are due to inadequate filling of root canal space. Because of the hydrophobic nature of gutta-percha, the sealer tends to pull away from it upon its setting leading to the formation of microscopic gaps at dentin-sealer and sealer-guttapercha interfaces and the marginal leakage through these interfacial gaps is a major reason for the failure of root canal therapy [15].

The use of the lateral condensation technique of obturation was reported to produce non-homogenous thick layers of sealers along the walls of the root canal, thus influencing the sealer penetration into the root dentin [16]. In the present study, all specimens were obturated with guttapercha in the Single-cone technique, as it is the commonly used method in clinical situations. With this technique, the volume of the sealer used is minimized and the use of calibrated X3 guttapercha point as supplied by the manufacturer for obturation corresponds to the size and taper of the X3 file, thus maintaining homogeneity among the specimens of all 4 groups. Single-cone obturation technique became very popular with the use of Rotary Ni-Ti (Nickel-Titanium) file systems for root canal instrumentation followed by the use of corresponding size and taper of gutta-percha point for obturation. This technique was stated to be time-saving compared to the commonly used lateral condensation technique of obturation [16]. In our study after root canal obturation, all specimens were incubated at 37°C for 7 days for the complete setting of sealers.

In the present study, Field Emission Scanning Electron Microscope (FESEM) was utilized for the assessment of marginal adaptation of AH Plus, MTA Fillapex, Bio-C, GuttaFlow-2 root canal sealers at dentin-sealer and sealer-guttapercha interfaces. FESEM provides topographical and elemental information at a magnification of 10X to 3000,000X with virtually unlimited depth of field. Compared with SEM, FESEM produces clear, less electrostatically distorted images with spatial resolution down to 11/2 nanometer three to six times better. FESEM allows a large amount of sample to be analyzed at a time and final evaluation can be done by preserving photomicrographs [17]. In the present study, all four root canal sealers showed marginal gaps at both sealer-dentin, sealer-guttapercha interfaces at the coronal, middle and apical thirds of root canals. However, maximum interfacial gaps were seen at the coronal-third, followed by middle-third, least in apical-third of root canals.

According to the FESEM findings of the present study, the samples obturated with GuttaFlow-2 sealer and guttapercha in single-cone technique showed better marginal adaptation both at both dentin-sealer and sealer-guttapercha interfaces at all 3 levels of root canal. These findings are in accordance with the studies of Sakshi Jain et al. [18,19]. GuttaFlow-2 system combines sealer and guttapercha in a single paste containing gutta-percha powder particles of size less than 30µm in polydimethylsiloxane matrix, highly biocompatible, homogeneous structure, expands 0.16% on setting [20], this expansion provides better sealing ability to create an impenetrable seal by adapting more intimately to both the guttapercha and root canal dentin. The findings of our study about the better marginal adaptation of GuttaFlow-2 to both root canal dentin and guttapercha is also supported by the studies of Bouillaguet S et al [21]. In the present study, GuttaFlow-2 sealer exhibited better marginal adaptation in the apical-third of root canals compared to other sealers used.

In our study, AH Plus sealer showed greater mean marginal gaps at both sealer-dentin and sealer-guttapercha interfaces at all 3-levels of root canal. This could be due to linear setting shrinkage of the sealer $0.034\% \pm 0.01\%$, as the sealer tends to pull away from guttapercha and root canal dentin. AH Plus sealer adapts by mechanical interlocking with root canal dentin due to its flow and long setting time [22]. The guttapercha-AH Plus sealer interface is highly hydrophobic and while dentin-AH Plus sealer interface is rather hydrophilic, creating gaps that act as major pathways of leakage as confirmed by SEM analysis [23].

MTA Fillapex sealer during its setting reaction hydrates inorganic oxide components leading to formation of Ca²⁺ hydroxide and Ca²⁺ hydrate phases forming a covalent bond with the amino group, resulting in the sealer expansion improving the overall sealing ability [24]. However in our study, MTA Fillapex sealer exhibited more marginal gaps at sealer-dentin interface compared to sealer-guttapercha interface.

The hydrophilic properties of Bio-C, a bio-ceramic based root canal sealer and the chemical bond it makes with root canal dentin with the tag-like formation of hydroxyapatite helps in achieving better adaptation, enhancing its sealing ability [25]. In contrast, in our study Bio-C sealer showed more marginal gaps at the sealer-dentin compared to sealer-guttapercha interfaces at all 3-levels of root canal. However, Bio-C sealer showed better marginal adaptation at both the interfaces and in all three thirds of root canals analysed compared to AH Plus sealer.

In the present study, FESEM was used for critical evaluation of genuine gaps at both sealer-dentin and sealer-guttapercha interfaces, from potential artifactual gaps generally happen with vacuum desiccation in conventional SEM studies. Marginal gaps at dentin-sealer and sealer-guttapercha interfaces may jeopardize the successful outcome of root canal treatment, So complete seal at both these two interfaces at all 3-levels of root canal is essential.

Conclusions

Under the conditions of our study, it was found that GuttaFlow-2 a silicone-based root canal sealer exhibited superior marginal adaptation with least gaps and AH Plus sealer showed poor marginal adaptation at both dentin-sealer and sealer-guttapercha interfaces at all 3-levels of root canal. GuttaFlow-2 adapted better to root canal dentin as well as to guttapercha at all 3-levels of root canal including the most critical apical-third and thus can be the most preferred root canal sealer among the four to be used in clinical practice. Nonetheless, further in-vivo studies are needed to confirm and correlate the findings of this in-vitro study.

Additional Information

Disclosures

Human subjects: Consent was obtained or waived by all participants in this study. Triveni Institute of Dental Sciences, Hospital and Research Centre issued approval TIDSHRC/IEC/2021/D006. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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