

Intratubular Penetration and Push-out Bond Strength of AH Plus, GuttaFlow 2 and GuttaFlow Bioseal Sealers: An In-vitro Study

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ABSTRACT

Introduction: The root canal obturating material provides a three-dimensional fluid-impervious seal. Root canal sealers play a major role in providing a seal between the core material and the tooth for the long-term success of endodontic treatment.

Aim: To evaluate the intratubular penetration and Bond Strength (BS) and push-out BS of AH Plus, GuttaFlow 2, and GuttaFlow Bioseal sealers.

Materials and Methods: The in-vitro study was conducted in the Department of Conservative Dentistry and Endodontics at Ranjeet Deshmukh Dental College and Research Centre, Nagpur, Maharashtra, India, from October 2019 to April 2021. A total of 75 human mandibular first premolars were decoronated at the Cemento-enamel Junction (CEJ) with a standardised length of 16 mm. The root canal was prepared using the crown-down technique with HyFlex Electrical Discharge Machining (EDM) rotary files and divided into three groups: Group-I: AH Plus, Group-II: GuttaFlow 2, and Group-III: GuttaFlow Bioseal. 0.1% Rhodamine B dye was added to the sealer and obturated using

the single cone technique. These samples were transversely sectioned into beams at the middle third of the root. A confocal laser scanning microscope was used to evaluate the depth of sealer penetration in the dentinal tubule, and a Universal Testing Machine was utilised to measure the push-out BS (MPa). The recorded data were subjected to statistical analysis {one-way Analysis of Variance (ANOVA) test, Tukey's post-hoc test, and Pearson's correlation coefficient}.

Results: The results obtained indicated that there was a significant difference in the depth of penetration and push-out BS of AH Plus and GuttaFlow 2 sealers ($p < 0.0001$). However, there was no significant difference found between AH Plus and GuttaFlow Bioseal sealers ($p = 0.206$). In addition, there was no significant difference in the correlation between intratubular penetration and push-out BS of the sealers (negative correlation).

Conclusion: Considering the results, the GuttaFlow Bioseal sealer was found to have comparable clinical performance with AH Plus sealer; hence, it can be used as an acceptable root canal sealer.

Keywords: Confocal laser scanning microscopy, Fluid impervious seal, Root canal filling materials, Root canal sealers, Root canal therapy

INTRODUCTION

Complete debridement of the infected root canals, shaping, cleaning, and three-dimensional filling with a biologically inert and dimensionally stable material are essential steps in successful root canal treatment. To establish a fluid-tight closure that lowers apical leakage and bacterial contamination, avoids apical periodontitis, and entombs the remaining irritants in the root canal, gutta-percha has been utilised in conjunction with endodontic sealers to accomplish this goal [1]. The obturating substance and the root dentinal wall should both be adhered to by the optimal root canal sealer [2].

Several endodontic sealers have been introduced to dentistry in the last ten years, including bioceramic calcium silicate-based sealers, zinc oxide eugenol-based sealers, calcium hydroxide-based sealers, silicone and methacrylate-based sealers, Glass Ionomer Cements (GIC)-based sealers, resin-based sealers, and Mineral Trioxide Aggregate (MTA)-based sealers. The ability of the sealer to enter dentinal tubules is measured by intratubular penetration [3]. This is a crucial component that improves the area of contact area between the filling material and the dentin, boosts sealing ability, and keeps microbes out of the dentinal tubule [4]. The intratubular penetration of the sealer is evaluated using a confocal laser scanning microscope since it makes it possible to recreation of three-dimensional structures [5]. Push-out BS is a mechanical test that measures the sealer material's interfacial bonding strength to the root dentin, which must be taken into account when assessing root canal sealer integrity [6]. The push-out BS of endodontic sealers has been previously evaluated [7], but there is a dearth of information about dentinal

tubule penetration. Moreover, substantial research on silicone-based sealers is required. Therefore, the current in-vitro investigation was undertaken to ascertain the push-out BS as a quantitative parameter and the intratubular depth penetration as a qualitative parameter of teeth obturated using three different types of root canal sealers.

MATERIALS AND METHODS

This in-vitro study was conducted in the Department of Conservative Dentistry and Endodontics at Ranjeet Deshmukh Dental College and Research Centre, Nagpur, Maharashtra, India, from October 2019 to April 2021. Before the study, approval was obtained from the Institutional Ethics Committee (IEC/VSPMDCRC/22/2019).

Inclusion and Exclusion criteria: Freshly extracted human mandibular first premolars extracted for orthodontic or periodontal purposes were selected for the study. Teeth with caries, trauma, fractures, or other defects such as root calcification, root resorption, incompletely formed apices, developmental anomalies, and severe curvatures were excluded. The samples were cleaned, disinfected, and stored as per the guidelines laid down by Occupational Safety and Health Administration (OSHA) and Centre for Disease Control and Prevention (CDC) in phosphate-buffered saline solution during entire study duration [8].

Sample size calculation: It was based on a previous study [9] where only the push-out bond strength of the sealers was evaluated, and the difference between the groups was statistically significant with a sample size of 10 teeth per group. Thus, considering a study power of 80% and a confidence interval of 95%, 25 samples were allocated per group, with a total sample size of 75 teeth taken for the study.

Study Procedure

Standardisation of samples: All samples were decoronated at 16±1 mm from the apex using a double-sided diamond disc (Dentsply Maillefer, Ballaiques, Switzerland) under copious water irrigation, and the coronal third of the canal was enlarged using Gates Glidden drills. The radiographic working length was determined using a #15 K-file, and biomechanical preparation of all the samples was done using HyFlex EDM rotary files (Coltene/Whaledent AG, Altstatten, Switzerland) up to size #40, 0.04 taper, and were irrigated with 1 mL of 5.25% Sodium Hypochlorite (NaOCl) between each file size. After preparation, the root canals were irrigated with 1 mL of 17% Ethylenediamine Tetraacetic Acid (EDTA) for one minute, followed by final rinsing with 10 mL of saline for the removal of all chemicals.

Study groups: These teeth were further divided into three groups with twenty-five teeth each based on the type of sealer used for obturation. The three groups were:

- Group-I: AH Plus (n=25)
- Group-II: GuttaFlow 2 (n=25)
- Group-III: GuttaFlow Bioseal (n=25)

Group-I served as the control group, while Group-II and III were the experimental groups.

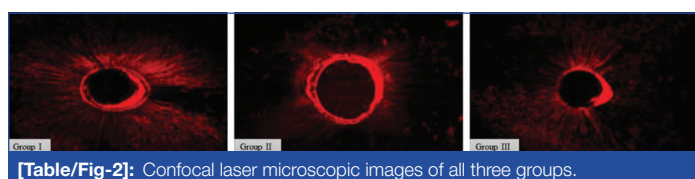
The sealers were manipulated according to the manufacturer's instructions and were further mixed with rhodamine B isothiocyanate dye and introduced into the canal orifice with a lentulo spiral (Dentsply, Maillefer, Switzerland). All the samples for each group were obturated using the single cone technique. After the complete setting of the sealers, all samples were stored at 37°C with 100% relative humidity for one week.

Evaluation of Intratubular penetration and push-out bond strength:

The root samples were sectioned with a microtome precision saw (Isomet, Beuhler, Germany) at 4 mm below the Cemento-enamel Junction (CEJ), resulting in sections 1±0.1 mm thick, represented the middle part of the root canal [Table/Fig-1]. This was done to evaluate the intratubular penetration of sealers into root dentin using Confocal Laser Scanning Microscopy (CLSM) (ZEISS with LSM Software ZEN 2007, Germany, Europe). Images from CLSM were recorded at four standardised areas (mesial, distal, buccal, and lingual) of each sample. To quantify the depth of penetration, measurements were performed at four different locations on each image, and the data was recorded in micrometers [Table/Fig-2].

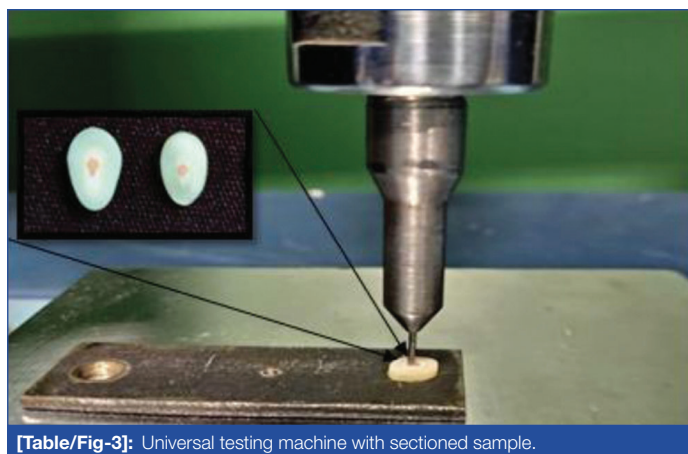


[Table/Fig-1]: Sectioned samples showing middle third of the root.



[Table/Fig-2]: Confocal laser microscopic images of all three groups.

All the samples were then subjected to testing under a Universal Testing Machine (ACME Engineers, India) to evaluate the push-out bond strength by applying an axial load to the sealer, and it was evaluated using the formula by Dem K et al., [Table/Fig-3] [9].



[Table/Fig-3]: Universal testing machine with sectioned sample.

Formula for Push-out bond strength (MPa)=

$$\frac{\text{Push-out load (N)}}{\text{Area of bonded interface (sq/mm)}}$$

Where, area of bonded interface (sq/mm)=2πrh

$$\pi=3.1416,$$

r=Radius of cross section,

h=Thickness of cross section

The maximum failure load was recorded in Newtons (N) and converted into Megapascals (MPa).

STATISTICAL ANALYSIS

The data of push-out bond strength (MPa) was collected, and intratubular penetration (µm) was subjected to descriptive statistical analysis using Statistical Package for Social Sciences (SPSS) 26.0 (IBM Corp). The comparison of these two parameters for each sealer was done using one-way ANOVA [Table/Fig-4,5], and the paired comparison was done using Tukey's post-hoc test [Table/Fig-5,6]. The correlation between intratubular penetration and push-out bond strength was determined using Pearson's correlation coefficient [Table/Fig-7].

| Sealers | No. of samples (N) | Mean±Standard Deviation (µm) | p-value* |
|-----------------------------|--------------------|------------------------------|-------------|
| Group-I AH Plus | 25 | 1143.12±349.18 | <0.0001 (S) |
| Group-II GuttaFlow 2 | 25 | 587.74±223.85 | |
| Group-III GuttaFlow Bioseal | 25 | 1004.45±281.51 | |

[Table/Fig-4]: Descriptive statistics for depth of penetration of the sealers.

*Obtained using one-way analysis of variance; S: Significant; Dentinal tubule penetration in γm

| Comparison of sealers | Mean difference (I-J) | p-value* | 95% confidence interval | |
|----------------------------------|-----------------------|-------------|-------------------------|-------------|
| | | | Lower bound | Upper bound |
| AH Plus vs GuttaFlow2 | 0.8536 | <0.0001 (S) | 0.4000 | 1.3072 |
| AH Plus vs GuttaFlow Bioseal | 0.3256 | 0.206 (NS) | -0.1280 | 0.7792 |
| GuttaFlow 2 vs GuttaFlow Bioseal | -0.5280 | 0.018 (S) | -0.9816 | -0.0744 |

[Table/Fig-5]: Pair-wise comparison of push-out bond strength of the sealers.

*Pair-wise comparison using Tukey's post-hoc test; S: Significant; NS: Not significant

| Comparison of sealers | Mean difference | p-value* | 95% confidence interval | |
|----------------------------------|-----------------|----------|-------------------------|-------------|
| | | | Lower bound | Upper bound |
| AH Plus vs GuttaFlow 2 | 555.384 | <0.0001 | 359.4820 | 751.2860 |
| AH Plus vs GuttaFlow Bioseal | 138.668 | 0.214 | -57.2340 | 334.5700 |
| GuttaFlow 2 vs GuttaFlow Bioseal | -416.716 | <0.0001 | -612.6180 | -220.8130 |

[Table/Fig-6]: Pair-wise comparison of depth of penetration between sealers.

Pair-wise comparison using Tukey's post-hoc test; Dentinal tubule penetration in γm

| Sealers | Parameters | |
|-------------------------------------|-------------------------|------------|
| | Correlation coefficient | p-value |
| Group-I AH plus (n=25) | -0.241 | 0.246 (NS) |
| Group-II Gutta Flow 2 (n=25) | -0.007 | 0.973 (NS) |
| Group-III Gutta Flow Bioseal (n=25) | -0.067 | 0.749 (NS) |

[Table/Fig-7]: Correlation of push-out bond strength and depth of penetration according to sealer types.

RESULTS

The mean values and standard deviations of intratubular penetration for the three sealers (Group-I, Group-II, Group-III) at the middle level are presented in [Table/Fig-4]. Group-I exhibited the maximum intratubular penetration ($1143.12 \pm 349.18 \mu\text{m}$), while Group-II showed the minimum intratubular penetration of $587.74 \pm 223.85 \mu\text{m}$. The mean difference in intratubular penetration of the sealer between AH Plus and GuttaFlow Bioseal was 138.668 mm , which was not statistically significant ($p=0.214$) [Table/Fig-6]. However, a significant difference was seen between AH Plus and GuttaFlow 2 ($p<0.0001$) and between GuttaFlow 2 and GuttaFlow Bioseal ($p<0.0001$) [Table/Fig-6].

The AH Plus also exhibited the maximum push-out bond strength (1.67 ± 0.76), while Group-II showed the minimum push-out bond strength of 0.82 ± 0.42 [Table/Fig-8]. There was a significant difference in push-out bond strength between AH Plus and GuttaFlow 2 ($p<0.0001$). GuttaFlow 2 exhibited significantly lower push-out bond strength than GuttaFlow Bioseal ($p=0.018$) [Table/Fig-5].

| Sealers | No. of samples (N) | Mean±Standard Deviation (MPa) | p-value |
|-----------------------------|--------------------|-------------------------------|-------------|
| Group-I AH Plus | 25 | 1.67 ± 0.76 | <0.0001 (S) |
| Group-II GuttaFlow 2 | 25 | 0.82 ± 0.42 | |
| Group-III GuttaFlow Bioseal | 25 | 1.35 ± 0.76 | |

[Table/Fig-8]: Descriptive statistics for push-out bond strength of the sealers.

*Obtained using one-way analysis of variance; S: Significant; Push-out bond strength in MPa

The correlation between intratubular penetration and push-out bond strength was assessed using Pearson's correlation coefficient. A negligible correlation was observed in all the groups ($p>0.05$) [Table/Fig-7].

DISCUSSION

The push-out bond strength and intratubular penetration of three distinct types of sealers were assessed in the present investigation. The results obtained indicated that there was a significant difference in the depth of penetration and push-out bond strength of AH Plus and GuttaFlow 2 sealers. However, no significant difference was found between AH Plus and GuttaFlow Bioseal sealers, and furthermore, no significant correlation between intratubular penetration and push-out bond strength of the sealers was reported. The study's null hypothesis was that there would be no appreciable variation in the push-out bond strength and intratubular penetration between AH Plus, GuttaFlow 2, and GuttaFlow Bioseal sealers. The recent findings led to the rejection of the null hypothesis.

A three-dimensional, fluid-impermeable closure of the root canal system depended critically on the root canal obturating material. Gutta-percha is considered the gold standard for obturating materials. The use of root canal sealers is essential for effectively filling the gaps between dentin surfaces and the core material, ensuring a fluid-tight seal [10]. Since the development of epoxy resin-based sealers in 1984, AH Plus has been a reliable sealer compared to other traditional sealers [11]. It possesses good mechanical qualities, is radiopaque, has reduced solubility, thin film thickness, quick setting time, and is biocompatible [12]. As it cures chemically, it also exhibits long-term dimensional stability and less polymerisation stress [7].

GuttaFlow 2, an upgraded version of GuttaFlow, a polydimethylsiloxane sealer, was introduced. This cold flowable filling solution combines

gutta-percha and sealer into a single product, offering superior sealing capabilities, biocompatibility, reduced curing shrinkage, and outstanding adhesion [13]. Recently, GuttaFlow Bioseal, a silicone-based, cold-filling sealer with GP powder and bioactive glass, was created. It has better dentin penetration, appropriate physical and biological qualities, and higher cytocompatibility than AH Plus. It also exhibits both osteointegrative and osteoconductive effects [14].

Controlled Low-strength Material (CLSM) allowed for visualising both the surface and subsurface of a specimen, assessing the depth of intratubular penetration, and providing comprehensive data on the presence and distribution of dental adhesives inside dentinal tubules throughout the entire circumference of the root canal walls [15], which is why it was applied in this investigation. Due to reports from other studies indicating that this area exhibited bigger diameters and a higher number of tubules, the middle half of the tooth section after obturation was examined in this study, with samples sectioned at a distance of 4 mm from the cephalic outlet [16].

The AH Plus sealer's maximum depth of penetration ($1876.13 \mu\text{m}$) and push-out bond strength (2.77 MPa) of the AH Plus sealer were noted in this study, which was indicative of the sealer and its ability to be pulled into tubules by capillary action and form a covalent bond, thereby strengthening the sealer's resistance to pressure and stress. The findings of the present study were consistent with previous research by Karobari MI et al., and Kurup D et al., who assessed push-out bond strength and discovered that teeth obturated with gutta-percha and AH Plus sealer had stronger bond strengths ($p<0.05$) [17,18]. Because of their outstanding flow ability, expandability, and insoluble nature, silicone based sealers have demonstrated good sealing performance.

Among all sealers, GuttaFlow 2 ($p=0.973$) demonstrated the lowest depth of penetration and binding strength in the current investigation. The composition's silicone content resulted in strong surface tension pressures [19], making it difficult to propagate on the intratubular dentin. Additionally, because its film thickness was greater than that of AH Plus, the flow became more problematic, and the mechanical bond strength decreased [20]. The findings for GuttaFlow Bioseal were consistent with those of Taddei P et al., and Lee SH et al., where the penetration into dentinal tubules was significant ($p=0.749$) and similar to the dentinal tubule penetration of AH Plus sealer [21,22]. The calcium silicate particles in this sealer mechanically connected to bone tissue by forming hydroxyapatite crystals, enhancing the push-out bond strength [23]. The push-out bond strength of AH Plus and GuttaFlow Bioseal sealers was comparable in the current study.

In the current investigation, no statistically significant differences were found in the push-out bond strengths of AH Plus, GuttaFlow 2, and GuttaFlow Bioseal sealers, and in the correlation of sealer penetration depth. However, the outcomes were consistent with Tedesco M et al., who examined the relationship between bond strength and depth of intratubular penetration using push-out bond strength and CLSM and found no connection between bond strength and depth of intratubular penetration [24]. Therefore, the acquisition and assessment of the results from the current study suggest that the quality of the sealer in the root canal was more important than the penetration depth.

Limitation(s)

The study had some limitations despite using extreme caution at each stage of the root canal process. Since this was an in-vitro investigation, it was not feasible to precisely simulate the oral state. Furthermore, the tooth experienced continuously increasing static stress, which differs from the load encountered in the actual oral environment. However, more research is required to provide a definitive statement on the long-term impact of various sealers on the binding strength and penetration depth of the sealer to root dentin. Researchers should conduct more studies on the newly developed sealers, including those based on calcium silicate.

CONCLUSION(S)

Within the limitations of the study, a similar depth of penetration and bond strength was observed for both AH Plus and GuttaFlow Bioseal sealers ($p=0.206$). Whereas GuttaFlow 2 showed the lowest depth of penetration and bond strength. Considering the biocompatibility and better performance of GuttaFlow Bioseal sealer, it can be recommended as a choice of material for routine clinical use to enhance the prognosis of endodontic therapy.

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