

**QUALITATIVE AND QUANTITATIVE ASSESSMENT OF ADHESIVE  
BOND OF AH PLUS, GUTTAFLOW 2 AND GUTTAFLOW BIOSEAL  
SEALERS: AN IN-VITRO CONFOCAL AND PUSH OUT BOND  
STRENGTH STUDY.**

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## LIST OF ABBREVIATIONS



Sr. No.	Abbreviations	Full form
01.	SEM	Scanning Electron Microscope
02.	TEM	Transmission Electron Microscope
03.	CLSM	Confocal Laser Scanning Microscope
04.	EDTA	Ethylene Diamine Tetra-acetic Acid
06.	NaOCl	Sodium Hypochlorite
07.	RDIZ	Resin Dentin Interdiffusion Zone
08.	Min	Minutes
09.	ml	Milli litres
10.	Ni-Ti	Nickle Titanium
11.	RITC	Rhodamine B IsoThioCynate
12.	OSHA	Occupational Safety and Health Administration
13.	CDC	Centre for Disease Control
14.	RVG	Radio Visio Graphy
15.	CEJ	Cemento-Enamel Junction
16.	WL	Working Length
17.	sec	Seconds
18.	SPSS	Statistical Package for the Social Sciences
19.	µm	Micro meter
20.	ANOVA	Analysis of Variance
21.	HSD	Honest Significant Difference

<b>Sr. No.</b>	<b>Abbreviations</b>	<b>Full form</b>
22.	SD	Standard Deviation
23.	S	Significant
24.	NS	Not Significant
25.	HS	Highly Significant
26.	N	Number of specimens
27.	p-value	Probability of obtaining a test statistic at least as extreme as the one that was actually observed
28.	Max.	Maximum
29.	Min.	Minimum
30.	No.	Number
31.	CI	Confidence Interval
32.	CHX	Chlorhexidine
33.	Mm	Milli Meter
34.	UTM	Universal Testing Machine
35.	AWL	Actual Working Length
36.	EHP	Electric Heat Plugger

## **INTRODUCTION**

*“It is by logic that we prove, but by intuition that we discover”*

*- Henri Poincare*

In contemporary endodontics, the main objective of root canal treatment is to complement proper cleaning and shaping of the root canal system, 3-dimensional filling with a biologically inert and dimensionally stable material.<sup>1,2</sup> To serve this aim, gutta-percha has been used in combination with various endodontic sealers.<sup>3</sup> Numerous endodontic materials have been developed for complete and impermeable fillings.

Root canal sealers plays a pivotal role in achieving the fluid impervious seal. An ideal root canal sealer should adhere to both root dentin wall and the obturating material.<sup>4,5</sup> They should seal the root canal apically and laterally, and also fill voids and irregularities. These sealers along with obturating materials forms a fluid-tight seal

which reduces apical leakage and bacterial contamination, prevents apical periodontitis, and entombs the remaining irritants in the root canal.<sup>6</sup>

The most frequent root canal sealer used earlier was Zinc Oxide Eugenol. However, this conventional sealer has a number of drawbacks, including the fact that it is non-adhesive, irritates periapical tissue, and is carcinogenic. Furthermore, **Schroeder et al**<sup>8</sup> were the first to use epoxy resin-based sealers in 1984, and in 2003 advanced silicon-based sealers introduced a new revolution in dentistry by providing a reliable substitute for traditional sealers. These sealers, according to **Harpreet singh et al. (2014)**<sup>9</sup>, have outstanding physical qualities, effective apical sealing, and assure acceptable biological performance.

Newer generation sealers are being engineered to improve their ability to penetrate into the dentinal tubule or to bond to both dentin and core material surfaces. Dentinal tubule penetration of the sealer creates a micro-mechanical lock and therefore enhancing the retention and resistance to dislocation of the sealer<sup>10</sup>. Also, the strong bond between the root canal sealer and the root dentin is essential for maintaining the integrity of the sealer-dentin interface during the preparation of post-spaces and during tooth flexure.<sup>11</sup>

Advances made in adhesive endodontics has led to the introduction of different resin-based root canal sealers. Resin-based root canal sealers have conventionally been used, offering the advantages of reduced solubility, tight apical sealing, and micro retention to the root dentin.<sup>12</sup> Many researchers have frequently used AH Plus an epoxy resin-based sealers as a control material in conjunction with gutta-percha (GP) in various root filling techniques.<sup>13</sup> AH Plus is characterized by

very good mechanical properties, high radio opacity, less polymerization shrinkage, reduced solubility, and long term dimensional stability on storage. However, its sealing ability remains controversial because AH Plus does not bond to gutta-percha.<sup>14</sup> The toxicity of these sealers is reduced after setting; however, they exhibit toxicity when freshly mixed.<sup>15</sup> To overcome the problem of sealing ability and toxicity, new silicon-based sealers have been developed.

Furthermore, silicone-based sealers have exhibited good sealing performance due to their insoluble nature, expandability, and excellent flow ability. GuttaFlow 2, a polydimethylsiloxane sealer, was introduced as an improved version of GuttaFlow. GuttaFlow 2 comprises gutta-percha powder of less than 30 µm in size, and micro-silver particles with poly-dimethylsiloxane, platinum catalyst and zirconium dioxide.<sup>16</sup> It has extraordinary chemical and physical properties that offer maximum sealing quality and biocompatibility.<sup>17</sup> Also, material has good adaptability because of increased flowability and the fact that it does not shrink, but expands slightly by 0.2% and retains some elasticity even after it has cured.<sup>16</sup> The slight expansion of the material leads to an excellent seal. According to manufacturer, GuttaFlow2 also shows excellent adhesion to the gutta-percha point as well as to the dentine wall.

GuttaFlow Bioseal (GFB) sealer is a polydimethylsiloxane sealer which is composed of Gutta-percha powder particles, polydimethylsiloxane, a platinum catalyst, zirconium dioxide, calcium salicylate, nanosilver particles, colouring, and bioactive glass-ceramic.<sup>18</sup> It also includes calcium silicate, which, upon contact with biological tissues, releases natural repair constituents and aids in the regeneration of periapical tissues.<sup>16</sup> GuttaFlow Bioseal has adequate physical and biological properties, good dentin penetrability, and a higher cytocompatibility than AH Plus.<sup>19</sup>

GuttaFlow 2 and GuttaFlow Bioseal have similar composition except that the GuttaFlow 2 contains micro silvers while GuttaFlow Bioseal has nano silvers and calcium silicate.<sup>16</sup> GuttaFlow Bioseal has ensured the ability to regenerate and heal tissues in the root canal. GuttaFlow Bioseal has both osteointegrative and osteoconductive effects and it is said to bond mechanically to bone tissue through the formation of hydroxyapatite crystals.<sup>20</sup>

GuttaFlow 2 has been shown to be more biocompatible than AH Plus sealer and less toxic to human gingival fibroblasts cells than AH Plus as suggested by **Accardo C et al 2014**)<sup>21</sup>. **Santos JM et al (2019)**<sup>22</sup> studied the biocompatibility of a GuttaFlow Bioseal, GuttaFlow 2 with AH plus sealer and observed that GuttaFlow Bioseal showed excellent biocompatibility in both the fresh and set states.

**Akçay et al (2016)**<sup>23</sup> evaluated the penetration of GuttaFlow Bioseal into the dentinal tubules using confocal laser scanning microscopy. They concluded that GuttaFlow Bioseal has similar dentinal tubule penetration to that of AH Plus sealer. **Majumdar TK (2021)**<sup>24</sup> assessed and compared the sealer penetration depth of AH Plus, Apexit Plus, and GuttaFlow Bioseal under confocal laser scanning microscope. They observed that GuttaFlow Bioseal has shown higher sealer penetration than Apexit Plus and AH Plus sealer.

Recently, **Dem K et al, (2019)**<sup>16</sup> studied the push out bond strength of AH plus, GuttaFlow2 and GuttaFlow Bioseal and stated that AH plus exhibits higher push-out bond strength when compared to GuttaFlow2 and GuttaFlow Bioseal. However, there is literature scarcity analysing the relationship of intratubular penetration and push out bond strength of AH plus, GuttaFlow 2 and GuttaFlow

Bioseal sealer.

The null hypothesis was that there is no significant difference on intratubular penetration and push out bond strength of AH plus, GuttaFlow 2 and GuttaFlow Bioseal sealer.

## **AIM & OBJECTIVES**

### **Aim**

Qualitative and Quantitative assessment of adhesive bond of AH plus, GuttaFlow 2 and GuttaFlow Bioseal Sealers: A confocal and push out bond strength of sealers

### **Objectives: -**

1. To evaluate the Intratubular penetration of AH plus, GuttaFlow 2 and GuttaFlow Bioseal sealers.
2. To compare the Intratubular penetration of AH plus, GuttaFlow 2 and GuttaFlow Bioseal sealers.
3. To evaluate the push out bond strength of AH plus, GuttaFlow 2 and GuttaFlow Bioseal sealers

4. To compare the push out bond strength of AH plus, GuttaFlow 2 and GuttaFlow Bioseal sealers
5. To analyse the relationship between Intratubular penetration and Push out bond strength of AH plus, GuttaFlow 2 and GuttaFlow Bioseal sealers.

## **REVIEW OF LITERATURE**

The current strategies for achieving three-dimensional impervious sealing of the root canal system based on the intratubular penetration of sealer and its relation to bond strength of sealer. Therefore, it is imperative to understand the mechanisms, principles and strategies of sealers that have evolved over time; and the challenges encountered by researchers and clinicians that are associated with it. As it is rightly stated by Confucius “Study the past, if you would define the future.”

**Kimura Y, Wilder-Smith P, Krasieva T, Arrastia-Jitosho A, Liaw L, Matsumoto K. (1997)** <sup>25</sup> studied the visualization and quantification of dentin structure using thirty extracted teeth using confocal laser scanning microscopy and stated that confocal microscopy is well suited for the observation of dental and material surfaces and to monitor the effects of various agents and factors on their microstructure.

**Balguerie E, Van der Sluis L, Vallaeyts K, Gurgel-Georgelin M, Diemer F (2011)** <sup>26</sup> in an invitro study assessed tubular adaptation, penetration depth and adaptation to the root canal walls in the apical, middle and coronal third of the root canal of 5 different sealers used in combination with softened gutta-percha cones by Scanning Electron Microscopy (SEM). The 5 different sealers used were Acroseal, Endobtur, Ketac-Endo, RSA and AH Plus. It was observed that the AH Plus showed the best adaptation to the root canal wall, tubular penetration and adaptation to the peritubular dentin, followed by Acroseal. They stated that epoxy resin sealers like AH Plus shows higher adhesion to the dentin and gutta percha and the flow of AH Plus is significantly higher than other sealers tested. The penetration in the dentinal tubules was significantly greater in the coronal and middle of the root canal than the apical part of the root canal.

**Sagsen B, Ustün Y, Demirbuga S, Pala K (2011)** <sup>27</sup> in an invitro study assessed the push-out bond strength of two new calcium silicate-based endodontic sealers in the root canals of thirty extracted teeth. After instrumentation and irrigation using 5 mL 2.5% NaOCl between each instrument, and the smear layer removal using 5 mL 17% EDTA, teeth were randomly distributed to three groups (n = 10). The canals were filled with three different sealers using a cold lateral compaction technique: group 1: AH Plus + gutta-percha, group 2: I Root SP + gutta-percha and group 3: MTA Fillapex + gutta-percha. Three horizontal sections were prepared at a thickness of 1 mm  $\pm$ 0.1 in the apical, middle and coronal parts of each root. They stated that I Root SP and AH Plus had significantly higher bond strength values than the MTA Fillapex (P < 0.05). Within the limitation of study, they concluded that push-out bond strengths in the middle and apical specimens were significantly higher

than those of the coronal specimens. There were no significant differences between the push-out bond strengths in the middle and apical specimens.

**Gurgel-Filho E, Leite F, Lima J, Montenegro J, Saavedra F, Silva E. (2014)** <sup>28</sup> in an invitro study evaluated the push out bond strength of three root canal sealer, a mineral trioxide aggregate (MTA)-based sealer (MTA Fillapex®), an epoxy resin-based sealer (AH Plus®), and a zinc oxide eugenol-based sealer (EndoFill®) to root dentin using thirty extracted single-root human teeth. The roots were randomly assigned to 3 groups for obturation with one of the three sealers: AH Plus, EndoFill or MTA Fillapex. The samples were sectioned horizontally into eight 1±0.1 mm-thick serial slices and then the push-out test was carried out. The results showed that AH Plus presented significantly higher bond strengths than the other sealers, while MTA Fillapex showed the lowest bond strengths and concluded that EndoFill sealer and MTA FillApex core combination was not superior to AH Plus® sealer and gutta-percha core combination.

**Oliveira D, Cardoso M, Queiroz T, Silva E, Souza E, De-Deus G (2014)** <sup>29</sup> in an invitro study evaluated and compared the push-out bond strength of MTA Fillapex (Angelus, Londrina, Brazil), iRoot SP (Innovative BioCeramix Inc., Vancouver, Canada) and AH Plus to the dentine walls of root canals using sixty extracted human single-rooted teeth. After standardized canal preparation and irrigation, the canals were dried with paper points and filled with one of four sealers: AH Plus, iRoot SP, MTA and MTA Fillapex. Roots were sectioned, and push-out tests were performed. The results showed that AH Plus had significantly higher bond strength than both MTA Fillapex and iRoot SP. They concluded that the adhesion to root dentine associated with newer calcium silicate-based sealers was compromised

even in a well-monitored laboratory conditions when compared to AH Plus sealer.

**Silva R, Silveira F, Horta M, Duarte M, Cavenago B, Morais I et al (2015)**<sup>30</sup> in an invitro study evaluated the root canal filling and dentinal tubule penetration of MTA Fillapex in comparison to AH Plus, Pulp Canal Sealer EWT and Sealapex by using stereomicroscope and confocal laser microscopy using forty single-rooted teeth. Based on the findings obtained in the study they concluded that the AH Plus, Pulp Canal Sealer EWT and Sealapex were found to have a good and similar performance regarding filling material adaptation to the root canal walls, except for MTA Fillapex, which showed more failures at 4 and 6 mm from the root apex. These sealers penetrated deeply into the dentinal tubules, except for the Pulp Canal Sealer EWT, which had a shorter penetration than MTA Fillapex and AH Plus at 4 and 6 mm from the root apex, respectively. But, none of the sealer was able to penetrate the whole extension of the dentinal tubules.

**Sungur D, Purali N, Coşgun E, Calt S. (2016)**<sup>31</sup> in an invitro study assessed the push-out bond strength and dentinal tubule penetration of tertiary monoblock units consisting of resin- and glass ionomer-based sealers used with coated Gutta Percha (GP), to a secondary monoblock unit consisting of a resin-based sealer used with conventional GP using seventy-two single-rooted human mandibular incisors. The teeth were divided into 4 groups corresponding to the sealer and gutta percha used: Group 1, EndoRez sealer and a size 40/0.04 EndoRez point; Group 2, Activ GP sealer and a size 40/0.04 Activ GP point; Group 3, Smartpaste sealer and a size 40/0.04 Smartpoint point; Group 4, AH 26 sealer (Dentsply de Trey, Konstanz, Germany) and a size 40/0.04 GP point. The push out bond strength test showed that the use of coated core materials with resin- and glass ionomer- based sealers did not enhance the bond

strength of the root canal obturation material. The confocal laser scanning microscope showed no significant difference amongst the percentage of sealer penetration. SmartSeal showed the least area and depth of tubule penetration while no difference was found amongst the others. Within the limitations of the study, they concluded that dentinal tubule penetration has limited effect on push-out bond strength of the root canal sealers.

**Sonu KR, Girish TN, Ponnappa KC, Kishan KV, Thameem PK (2016)** <sup>32</sup>

in an invitro study investigated the dentinal tubule penetration of mineral trioxide aggregate (MTA) Fillapex, GuttaFlow 2 sealer with standard sealer AH Plus in instrumented root canals obturated by using cold lateral compaction techniques in either the presence or absence of the smear layer in sixty extracted human mandibular premolars by using scanning electron microscope. Results showed that AH plus showed deeper penetration, followed by MTA Fillapex and GuttaFlow® 2. The penetration depth of the sealers at the cervical and middle third of root was significantly more than those at apical third. They concluded that GuttaFlow 2 sealer showed the minimum penetration and AH Plus sealers showed the maximum sealer penetration depth at coronal middle and cervical levels.

**Patil P, Rathore VP, Hotkar C, Savgave SS, Raghavendra K, Ingale P**

**(2016)** <sup>33</sup> in an invitro study investigated the apical sealing ability between GuttaFlow and AH Plus using eighty extracted human maxillary anterior teeth. The root canals were cleaned and shaped using a standard step back preparation to size 60# master apical file at the established working length and divided into four groups Group 1: GuttaFlow sealer with gutta-percha; Group 2 : AH Plus sealer with gutta-percha; Group 3: positive control group (Teeth were instrumented and left without

obturation); Group 4: negative control group (Teeth were totally coated with nail varnish). Results showed that the GuttaFlow group had a mean leakage of 1.38 mm whereas AH Plus had a mean of 1.425 mm. They concluded that none of the sealers used in the study could completely seal the apical foramen. GuttaFlow and AH Plus showed no statistically significant difference in microleakage but the better result was shown by GuttaFlow.

**Piazza B, Rivera-Peña M, Alcalde M, De Vasconcelos B, Duarte M, De Moraes I et al. (2017)** <sup>34</sup> in an invitro study analysed the influence of humidity on the intra-tubular penetration, bond strength and type of failure mode associated with endodontic sealers. Sixty human mandibular premolar teeth were randomly divided into 4 groups Group 1 - AH plus /moist, Group 2 - AH plus/dry, Group 3 - MTA Fillapex /moist and Group 4 - MTA Fillapex /dry. Results showed that MTA Fillapex sealer exhibited higher intra-tubular penetration values compared to AH Plus ( $P < 0.05$ ) sealer. AH Plus showed the highest bond strength values They concluded that humid conditions did not influence the intra-tubular penetration, bond strength and failure mode of AH Plus and MTA Fillapex sealers. Consequently, MTA Fillapex exhibited higher intra-tubular penetration values when compared to AH Plus, irrespective of the moisture conditions. Moreover, AH Plus showed bond strength values higher than MTA Fillapex, and was not being influenced by the moisture conditions. The failure mode analysis identified a majority of cohesive failures.

**Camargo RV, Silva-Sousa YT, Rosa RP, Mazzi-Chaves JF, Lopes FC, Steier L, Sousa-Neto MD (2017)** <sup>35</sup> in an invitro study assessed the physicochemical properties of AH Plus, GuttaFlow 2, GuttaFlow BioSeal, and MM Seal, five samples of each root canal sealer were evaluated to determine their setting time (ST),

dimensional change (DC), solubility (SL), flow (FL), and radiopacity (RD) according to American National Standards Institute/American Dental Association (ANSI/ADA) Specification 57. According to the methodology employed and the results obtained in this study, AH Plus can be concluded to be the only sealer that fulfills ANSI/ ADA specifications.

**Bharath N, Thillainayagam S, Srikanth D (2017)** <sup>36</sup> in an invitro study evaluate and compare penetrability of epoxy resin based sealer, Polydimethylsiloxane based sealer, calcium silicate based sealer and zinc oxide eugenol using scanning electron microscope in eighty freshly extracted mandibular premolars. Group 1 used epoxy resin based sealer, Group 2 used polydimethylsiloxane based sealer, Group 3 used calcium based sealer and Group 4 used zinc oxide based sealer. It was found that Group 1 had higher depth of sealer penetration than the other group. Within the limitations of the present study it can be concluded that AH plus sealer Group 1 had better penetration when compared with the other sealers evaluated in this study.

**Piai G, Duarte M, Nascimento A, Rosa R, Só M, Vivan R (2018)** <sup>37</sup> in an invitro study evaluated the penetrability of a new epoxy-based sealer (Sealer Plus) into dentinal tubules compared to the gold standard sealer (AH Plus) using twenty single rooted teeth. They were divided into 2 groups and instrumented using Ni-Ti rotary files, irrigated with NaOCl + EDTA and obturated with cold lateral condensation (CLC). The roots were transversally cleaved under water cooling at 2, 4, and 6 mm from the apex. They concluded that root canal level affected the penetration of the sealer, but no statistically significant differences were found between the two experimental groups ( $p > .05$ ). Sealer plus presented similar dentinal penetration and perimeter integrity to the gold standard AH Plus sealer.

**Donnermeyer D, Dornseifer P, Schäfer E, Dammaschke T (2018)**<sup>38</sup> in an invitro study evaluated and compared the push out bond strength of calcium silicate-based sealers with an epoxy resin-based sealer in eighty extracted human mandibular premolars. The samples were randomly divided into four experimental groups. Group A: Total Fill BC Sealer; Group B: Endo CPM Sealer; Group C: BioRoot RCS; control Group D: AH Plus. Samples were sectioned and evaluated for dislodgement resistance and mode of failure. Within the limitations of this study, the following conclusions were drawn; The push-out bond strength of the investigated calcium silicate-based sealers was lower than the push-out bond strength of AH Plus. Total FillBC showed the highest push-out bond strength of the calcium silicate-based sealers followed by BioRoot RCS and Endo CPM Sealer respectively.

**Türker S, Uzunoglu E, Purali N (2018)**<sup>39</sup> evaluated and compared the effect of smear layer on the penetration depth and push-out bond strength of various root canal sealers using ninety extracted human mandibular premolars. These samples were randomly assigned into 2 groups: smear layer preserved and smear layer removed. Then the roots were further divided into 3 subgroups according to the sealer used: AH 26, BioRoot RCS and MTA Plus. Results of the study showed that retention of MTA Plus and BioRoot RCS was higher than that of AH 26 when the smear layer was preserved. Within the limitations of this in vitro study, they concluded that Smear layer removal adversely affects the adhesion of MTA Plus; however, the same did not hold for AH26 and BioRoot RCS. Additionally, smear layer removal or preservation did not affect the penetration depth and percentage of any root canal sealer. Dentinal tubule penetration had limited effect on the push-out bond strength of the root canal sealers.

**Dabaj P, Kalender A, Unverdi Eldeniz A (2018)**<sup>40</sup> in an invitro study evaluated and compared the push-out bond strengths of root canals obturated with Endosequence BC Sealer, along with the thermo-plasticized injectable technique, while comparing it against AH Plus, and cold lateral compaction technique using eighty mandibular premolars. Roots were instrumented using Protaper Universal rotary files and were randomly divided into four experimental groups Group 1: AH-Plus + cold-lateral-compaction technique; Group 2 : Endosequence-BC-Sealer + cold-lateral-compaction technique; Group 3 : AH-Plus + thermo-plasticized injectable technique; and Group 4 : Endosequence-BC-Sealer + thermo-plasticized injectable technique They results showed that AH Plus had significantly higher push-out bond strength amongst all experimental groups, regardless of the obturation techniques used and also observed that thermo-plasticized injectable technique with Calamus Flow - Delivery - System lowered the bond strengths of the sealers, especially Endosequence - BC - Sealer. Therefore, this technique is not recommended to calcium-silicate-based sealers.

**Jain S, Adhikari HD (2018)**<sup>41</sup> in an invitro scanning electron microscopic (SEM) study was to determine which of the commonly used root canal sealer among AH-Plus, GuttaFlow, and RealSeal provides a superior marginal adaptation with the dentin (Part-I) in the apical third region of root canals using thirty humans freshly extracted maxillary central incisors. Teeth were biomechanically prepared, then divided equally into three groups and obturated with AH-Plus, GuttaFlow, and RealSeal using single-cone obturation technique. Results showed that GuttaFlow exhibited least average marginal gap of  $2.38 \pm 1.43 \mu\text{m}$ , followed by AH-Plus ( $4.11 \pm 2.85 \mu\text{m}$ ) and RealSeal ( $4.65 \pm 3.91 \mu\text{m}$ ) with no significant difference among the

groups. They concluded that GuttaFlow exhibited better adaptation at dentin-sealer interface at apical third of root canal when compared with RealSeal and AH-Plus sealers.

**Jain S, Adhikari HD (2018)** <sup>42</sup> in an invitro scanning electron microscopic (SEM) study was to determine which root canal sealer among AH-Plus, GuttaFlow, and RealSeal provides a superior marginal adaptation with the core obturating material(Part-I) in the apical third region of root canal in thirty humans freshly extracted maxillary central incisors. Teeth were biomechanically prepared, then divided equally into three groups and obturated with AH-Plus, GuttaFlow, and RealSeal using single-cone obturation technique. Results showed that mean average gap was significantly higher ( $P < 0.05$ ) for AH-Plus ( $15.65 \pm 10.48 \mu\text{m}$ ), when compared to GuttaFlow ( $3.51 \pm 1.81 \mu\text{m}$ ) and RealSeal ( $6.01 \pm 2.51 \mu\text{m}$ ). Between RealSeal and GuttaFlow, the latter showed least marginal gap They concluded that GuttaFlow is better adapted than RealSeal and AH-Plus to core obturating material in the apical third of root canals.

**Kim S, Kwak S, Lee J, Goo H, Ha J, Kim H (2019)** <sup>43</sup>, in an invitro study compared the penetration ability of calcium silicate root canal sealers and conventional resin-based sealer using confocal laser scanning microscopy (CLSM). Within the limitation of study, they concluded that the highest sum fluorescence intensity (SFI) level was found in the coronal third, and the lowest in the apical third in all experimental groups, AH Plus showed the highest SFI in apical and coronal areas, whereas the BioRoot RCS group showed a relatively higher intensity level in the middle area, similar to AH Plus, and the maximum sealer penetration distance was higher at the apical third in the AH Plus group compared with BioRoot RCS and

Endoseal MTA.

**Khalil MM, Abdelrahman MH, El-Mallah S (2019)**<sup>44</sup> in an invitro study evaluated the bond strength and solubility of a novel polydimethylsiloxane-gutta-percha calcium silicate-containing root canal sealer (GuttaFlow® bioseal) and compare it with the zinc oxide and eugenol sealer using fourteen single-rooted human teeth. The bond strength was assessed using push-out bond strength test in 3 root segments: coronal, middle and apical. The solubility was tested according to the American National Standards Institute / American Dental Association (ANSI/ADA) specification No. 57 at 3 different time intervals: 1, 7 and 14 days. Results of the study showed the push-out bond strength in all root segments was significantly higher in Zical compared to GuttaFlow bioseal. Within the limitations of this study, they concluded that the endodontic sealer GuttaFlow bioseal showed low bond strength values compared to zinc oxide and eugenol sealer. The solubility of the set GuttaFlow bioseal and zinc oxide and eugenol sealer were both within the recommended ANSI/ADA levels.

**El Hachem R, Khalil I, Le Brun G, Pellen F, Le Jeune B, Daou M et al. (2019)**<sup>45</sup> in an invitro study assessed the dentinal tubule penetration of three different sealers, AH Plus, BC Sealer and a novel tricalcium silicate sealer (NTS) to root dentin. Eighty six extracted human maxillary central incisors were endodontically treated and randomly assigned in three groups, Group1- AH Plus, group2- BC Sealer and group3- NTS. Confocal laser scanning microscopic analysis was performed to examine the depth of penetration of sealers. The results showed that the maximum and mean penetration depths were significantly higher at 5 mm compared to 1 mm from the apex in the AH Plus, BC Sealer and NTS groups. Within the study

limitations, they concluded that the BC Sealer and novel tricalcium silicate sealer (NTS) demonstrated better dentinal tubule penetration results compared to AH Plus.

**Dem K, Wu Y, Kaminga AC, Dai Z, Cao X, Zhu B (2019)**<sup>16</sup> in their invitro study assessed the push out bond strength of AH plus, GuttaFlow2 and GuttaFlow Bioseal. Thirty root slices were prepared from the middle third of 10 mandibular premolars. Each slice was  $1 \pm 0.1$  mm thick. Three holes, 0.8 mm wide each, were drilled on the axial side of each root slice and each hole was filled with three root canal sealers. Then, the universal testing machine was used to measure the push out bond strength. The results showed that the AH Plus had a significantly superior push out bond strength than GuttaFlow 2 and GuttaFlow Bioseal, while the push out bond strength of GuttaFlow Bioseal was significantly stronger than that of GuttaFlow 2. Within the study limitations, they concluded that AH Plus is a better root canal sealer than GuttaFlow 2 and GuttaFlow Bioseal.

**Elmuttalibi HF, Mahdi JA (2019)**<sup>46</sup> in their invitro study assessed the push out bond strength of three different obturation materials GuttaFlow 2, Therma-fil and GuttaCore in thirty extracted upper molars. The first group was obturated with GuttaFlow 2, the second group was obturated with Thermafil and the third group was obturated with GuttaCore obturating materials then each slice introduced to the push out testing using a universal testing machine at a cross head speed of 0.5 mm/min. Results showed that Push-out bond strengths were significantly higher when canals were filled with GuttaCore than those filled with Thermafil and GuttaFlow 2 and Thermafil showed a higher significant difference than the GuttaFlow 2. Within the limitation of study, they concluded that Push-out bond strengths were higher when canals were filled with GuttaCore than those filled with Thermafil and GuttaFlow 2.

**Tedesco M, Chain MC, Felipe WT, Alves AM, Garcia LD, Bortoluzzi EA et al (2019)** <sup>47</sup> in an invitro study correlation between bond strength to dentin and depth of penetration of three root canal sealers of different bases: Endofill, AH Plus and MTA Fillapex, by push-out test and analysis of the adhesive interface using CLSM using eighty freshly extracted human mandibular premolars. Root canals were prepared according to the crown-down technique and filled with guttapercha associated to the following sealers : Group 1: Endofill, Group 2 : AH Plus and Group 3 : MTA Fillapex. The Bond strength results of Endofill and MTA Fillapex were statistically similar, however, they were statistically different from AH Plus. CLSM analysis verified tags formation in all groups and higher penetration of the specimens filled with AH Plus. Within the limitations of an study, it is possible to conclude that the epoxy resin-based sealer showed higher bond strength and sealer intratubular penetration than the other tested sealers. Considering the different compositions and properties of the sealers, there is no correlation between bond strength and intratubular penetration.

**Dsouza AP, Suvarna N, Shetty KH, Farhana F, Syed AA (2020)** <sup>48</sup> in an invitro study evaluated and compared the depth of penetration of various root canal sealers in sixty-four extracted human teeth with single canals. The samples were divided into four groups. GROUP A: Root canals sealed with gutta percha and GuttaFlow Bioseal. GROUP B: Root canals sealed with gutta percha and BioRoot RCS. GROUP C: Root canals sealed with gutta percha and MTA Fillapex. GROUP D: Root canals sealed with gutta percha and AH Plus. Sections were taken 3 mm from the apex and assessed using Confocal Laser Scanning Microscope (CLSM). Results showed that BioRoot RCS showed the best depth of penetration into the radicular

dentine amongst the four sealers with a mean value of  $1369.096 \pm 189.590 \mu\text{m}$ . With the limitations of this study, they concluded that BioRoot RCS has superior sealing properties and higher depth of penetration into the dentinal tubules.

**Lee SH, Oh S, Al-Ghamdi AS, Mandorah AO, Kum KY, Chang SW (2020)**<sup>49</sup> in an invitro study assessed the sealing ability of GuttaFlow bioseal with the AH Plus root canal sealer using a subnanoliter-scaled fluid-flow measuring device in extracted human mandibular premolars. The fluid flow in root canal-filled teeth using either gutta-percha cone (GP) with AH (GAR; n = 10) or GP with GB (GBR; n = 10) and in GP inserted in AH blocks (GA; n = 10) or GP inserted in GB blocks (GB; n = 10) was measured. In addition, fluid flow in sealer blocks of AH (A; n = 10) and GB (B; n = 10), which served as negative controls, was measured. Root canal-filled teeth using GP without any sealer (GR) acted as positive controls (n = 10). The mean leakage in the GBR group was lower than that in the GAR group ( $=0.001$ ), while the mean leakages in the GA and GB groups were not significantly different. Within the limitation of this study, GuttaFlow bioseal provided more fluid-tight seal than AH Plus when used with the single-cone obturation technique.

**Kanwar SS, Taneja S, Kumar P, Dudeja C (2020)**<sup>50</sup> in their invitro study evaluated the effect of final irrigant on depth of tubular penetration of resin based root canal sealer and bioactive sealers using Confocal Laser Scanning Microscope in ninety- six freshly extracted human mandibular first premolar teeth. The sample were divided into three groups according to final irrigant used : Group A (17 % EDTA), Group B (QMix 2 in 1), Group C (Distilled water). The final irrigation in each respective group was performed with EndoVac system. The samples were further subdivided into 4 subgroups according to the type of sealer used for obturation with

6% guttapercha cones - Subgroup I (AH Plus), Subgroup II (Gutta Flow Bioseal), Subgroup III ( Endosequence BC ), Subgroup IV (EndoSeal MTA).Results showed that Endosequence BC showed maximum depth of penetration and penetrated percentage perimeter, while Gutta Flow Bioseal showed least values. Q Mix 2 in 1 showed better penetration values than EDTA and distilled water. They concluded that within the limitations of the present study, it can be concluded that type of irrigant, nature of sealer and level of root canal affected sealer penetration. Endosequence BC showed the maximum depth of dentin tubular penetration among all the sealers used in the study. Q Mix 2 in 1 was the most effective final irrigating solution compared to EDTA and Distilled water

**Majumdar TK, Mukherjee S, Mazumdar P (2021)** <sup>24</sup> in their in vitro study assessed the sealer penetration depth and interfacial adaptation of AH Plus, Apexit Plus, and GuttaFlow Bioseal sealer to root dentin in sixty extracted single-rooted premolar teeth. The samples were randomly divided into three groups consistent with the sort of sealer used for obturation. After obturation with lateral compaction technique, half of the samples were sectioned transversely for measuring tubular depth penetration under a confocal laser scanning microscope and longitudinal sections were obtained for the rest half the samples to gauge the difference of sealer using the scanning electron microscope. Results showed that GuttaFlow Bioseal has shown a significantly higher depth of sealer penetration and minimum interfacial adaptation than resin-based sealer and calcium hydroxide-based sealer. They concluded that the GuttaFlow Bioseal sealer exhibited more sealer penetration and minimum interfacial adaptation whereas the Apexit Plus sealer exhibited less sealer penetration and maximum interfacial adaptation.

**Kaul S, Kumar A, Badiyani BK, Sukhtankar L, Madhumitha M, Kumar A (2021)** <sup>51</sup> in an invitro study evaluated and compared the sealing ability of Bioceramic sealer, AH plus, and GuttaFlow in conservatively prepared curved root canals obturated with single-cone technique in one hundred and twenty-five curved roots of maxillary and mandibular third molar teeth. The root canals were cleaned and shaped using a standard single-cone preparation to file at the established working length and divided into five groups of 25 each. Group A: with GP, using EndoSequence BC sealer , Group B: with ceramic coated , Group C: with GP, using AH Plus sealer Group D: with GuttaFlow bioseal Group E is a negative control group. Dye penetration was observed in all the specimens. Results showed that the group AH Plus showed more leakage values than the GuttaFlow group and of two groups of BC sealer and negative control. They concluded that none of the sealers used in the study could completely seal the apical foramen to have a fluid-tight seal but better result was shown by BC sealer.

**Naji, A.N., & Al-Gharrawi, H.A. (2021)** <sup>52</sup> in an invitro study evaluate and compare the sealing ability of GuttaFlow bioseal with different obturation systems (GuttaFlow 2, AH plus, Bioceramic sealer) of single cone techniques in sixty four freshly extracted maxillary first molars. Four experimental groups according to the obturation system, Group I: AH plus sealer, Group II: GuttaFlow 2, Group III: Bioceramic sealer, Group IV: GuttaFlow bioseal sealer, and 2 control groups. Apical leakage evaluation was done by dye extraction methods. The results revealed that GuttaFlow bioseal had significantly lower leakage than GuttaFlow 2 and Bioceramic sealer, while it had no significant difference in the mean leakage when compared with AH Plus. They concluded that GuttaFlow bioseal sealer ( Bioactive glass based

sealer) showed the best sealing ability compared to GuttaFlow 2 and Bioceramic sealers.

**Mustaffa M, Nordin N, Embong SN, Ibrahim M (2021)** <sup>53</sup> in an invitro study compared the obturated surface area, the extrusion of root filling material beyond the apical foramen and the duration of obturation procedure in single-rooted mandibular premolar using monocone obturation technique. The root canal of twenty single-rooted mandibular premolars were prepared, and then divided into two groups; Group 1 (GuttaFlow Bioseal (GFB) and a gutta-percha (GP) cone) and Group 2 (RoekoSeal Automix root canal sealer and a GP cone). The roots were sectioned perpendicularly and the surfaces were observed under scanning electron microscope. Results of the study showed that The median score of obturated surface area in Group 1 and 2 at the apical was 86.51 and 83.00, at the middle was 90.48 and 87.35 and at the coronal was 93.00 and 83.39, respectively. The extrusion of root filling material between two groups did not show statistically significant difference. Within the limitations of the present study, the conclusions that could be made are: The obturated surface area at the apical and middle regions using GuttaFlow Bioseal and RoekoSeal Automix root canal sealer was comparable, however at the coronal region, GuttaFlow Bioseal showed 11.5% better coverage compared to the RoekoSeal Automix root canal sealer ; The extrusion of root filling material beyond the apical foramen using GuttaFlow Bioseal and RoekoSeal Automix root canal sealer was equivalent; The obturation procedure using GuttaFlow Bioseal took 8.6% longer than the RoekoSeal Automix root canal sealer.

## **MATERIALS AND METHODS**

Approval from the Institutional ethics committee was taken for the study.

Seventy-five freshly extracted human mandibular permanent first premolars with single root and single canal were selected for the study. Presence of single canal was assessed by using RVG. The teeth were cleaned, disinfected and stored as per the recommendations and guidelines laid down by OSHA and CDC (2003 report 17). All the selected teeth were stored in phosphate buffer saline solution (Severn, Biotech).

### **METHOD OF SELECTION OF STUDY SUBJECT:**

#### **INCLUSION CRITERIA:**

1. Sound Permanent Mandibular First Premolars with single canal.
2. Teeth extracted either for orthodontic or periodontal purpose.

**EXCLUSION CRITERIA:**

1. Teeth with caries, trauma, fracture or other defects such as root calcification, root resorption.
2. Teeth with incompletely formed apices.
3. Teeth with developmental anomalies.
4. Teeth with severe curvatures.

**ARMAMENTARIUM:**

**Instruments Used:**

- Straight probe (PLATE I)
- Explorer (PLATE I)
- Pair of Tweezers (PLATE I)
- Excavator (PLATE I)
- Hand Scaler (Satelec P5 Newtron Worktop Scaler, Satelec Acteon)
- Digital Vernier calliper (WorkZone Hand Tools, Germany) (PLATE I)
- Cotton holder (PLATE I)
- Waste receiver (PLATE I)
- Mixing spatula (PLATE II)
- Mixing pad (PLATE II)
- Straight hand piece (NSK, Japan) (PLATE II)
- Mini Endo Block (DENTSPLY, Maillefer, Switzerland) (PLATE II)
- Double sided diamond disc (DFS, Germany) (PLATE II)
- Gates Glidden drills (Mani, Japan) (PLATE III)
- Standard 2% K & H files # 10-80 (Mani, Japan) (PLATE III)
- Reamers (MANI, Japan) (PLATE-IV)

- Lentulospirals (MANI, Japan) (PLATE-IV)
- HyFlex EDM-rotary files. (Coltene, Switzerland) (PLATE IV)

### **Equipment Used**

- X-Smart Endomotor (DENTSPLY, Maillefer, Switzerland) (PLATE III)
- Digital Radiovisiography System (Kodak 5100 RVG, France)
- Calamus Dual 3D Obturation System (DENTSPLY, Maillefer, Switzerland) (PLATE-V)
- Precision cutting saw (IsoMet 5000, Buehler, Germany) (PLATE-XIII)
- Grinder polisher (Buehler, Germany) (PLATE-XIII)
- Confocal laser scanning microscope (ZEISS with LSM Software ZEN 2007) (PLATE-XIII)
- Universal testing machine (ACME Engineers, Model no. UNITEST-10) (PLATE-XIV)

### **Materials Used:**

- Root canal irrigation solutions (PLATE-VI)
  - Sodium hypochlorite (NaOCl)(5.25%) (Hyposept UPS Hygienes, India)
  - Normal saline (0.9 % w/v, Nirlife, India)
- 5ml syringe with 30gauge side vented needle (Nirlife, India) (PLATE-VI)
- RC Help 17% EDTA (Prime Dental Products, India) (PLATE-VI)
- Paper points (size#0.04.40) (DENTSPLY, Maillefer, Switzerland) (PLATE-VII)

- AH Plus root canal sealer (DENTSPLY, Maillefer, Switzerland) (PLATE-VIII)
- GuttaFlow 2 (Coltene, Switzerland) (PLATE-VIII)
- GuttaFlow Bioseal (Coltene, Switzerland) (PLATE-VIII)
- Gutta Percha points (DENTSPLY, Maillefer, Switzerland) (PLATE-IX)
- Rhodamine B dye (Loba Chemie, India) (PLATE-VII)

### **SAMPLING TECHNIQUE:**

For sample size estimation, a study by **Dem K. et al. (2019)<sup>16</sup>** was referred.

They found higher bond-strength with AH plus sealer group (12.20MPa) compared to GuttaFlow 2 group (0.43MPa) at middle level. Assuming that similar differences could be obtained in the present study, the estimated sample size that could provide 80% power and 95% confidence interval, 25 samples were allocated per group. Therefore, the total sample size of 75 was taken for the current study.

### **The formulation used was:**

The null hypothesis was:

H0:  $\mu_1 = \mu_2 = \mu_3$  i.e.

the three mean push out bond strengths are equal

Against the alternative that

H1:  $\mu_i \neq \mu_j$  ( $i \neq j = 1, 2, 3$ )

Accordingly, the sample size was obtained using formula:

$$n = \frac{(z_{1-\alpha/2} + z_{1-\beta})^2}{ES^2}$$

where  $\tau$  is the number of possible comparisons ( $2 \times 3C = 3$  in the present case),  $z_{1-\alpha/2\tau}$  (2.395) is the standardized value for 5% error and for 3 paired comparisons,  $z_{1-\beta}$  (0.842) is the value for 80% power and ES is the effect size (0.4).

## **SAMPLE PREPARATION**

**Decoronation** - All teeth were decoronated  $16 \pm 1$  mm from the apex under copious water irrigation with a double-sided diamond disc (DFS, Germany) to obtain a standardized length of 16mm

**Determination of the working length** - The working length was determined at the point at which the #15 K-file (Mani, Japan) was detected in apex with the help of radiograph.

**Cleaning and shaping** - All roots were shaped uniformly at full working lengths with HyFlex EDM rotary files to size #40, 0.04 taper using the X-Smart-Endo-motor (Dentsply, Maillefer, Switzerland) in a crown down technique at 500 rpm speed and torque of 2.5 Ncm. The root canals were irrigated with 1mL 5.25% sodium hypochlorite (NaOCl) between each file size using a 30-gauge side vented needle. After preparation, the root canals were irrigated with 1 ml of 17% EDTA for 1minute followed by rinsing with 10ml saline to remove all chemicals. Flooding of saline was confirmed by visual inspection at the coronal access and by extrusion through the apical foramen.

## **DISTRIBUTION OF STUDY GROUPS:**

According to the sealer used, the samples were randomly assigned in 3 groups as follows:

Groups	Sealers	No. of Samples
Control - Group-I	AH Plus	25
Experimental - Group-II	GuttaFlow 2	25
Experimental - Group-III	GuttaFlow Bioseal	25

## **Obturation**

Master Cone Gutta Percha (size #40, 0.04 taper) was selected and snug fit was checked. The sealers, were mixed with rhodamine B isothiocyanate dye. The mixed sealers were introduced into the canal orifice with a Lentulo spiral (Dentsply, Maillefer, Switzerland) rotated at 500 rpm in a clockwise direction with a slow-speed handpiece inserted up to 1 mm short of the Working length. All the samples for each group were obturated using single cone technique. After complete setting of sealers, all samples were stored at 37 °C and 100% relative humidity for 1 week.

## **SECTIONING OF TOOTH**

The root samples were sectioned with a microtome precision saw (Isomet, Buehler, Germany) at 4mm below the cemento-enamel junction (CEJ). The resulting sections of each tooth was  $1\pm 0.1$ mm thick. Each section represented the middle part of root canal. The sectioned surfaces were polished with a series of silicon carbide abrasive papers (upto 2400 grit) using running tap water as a lubricant on MetaServ 2000 Grinder polisher machine. (Buehler, Germany). The samples were kept in humidifier to maintain humidity throughout the study period.

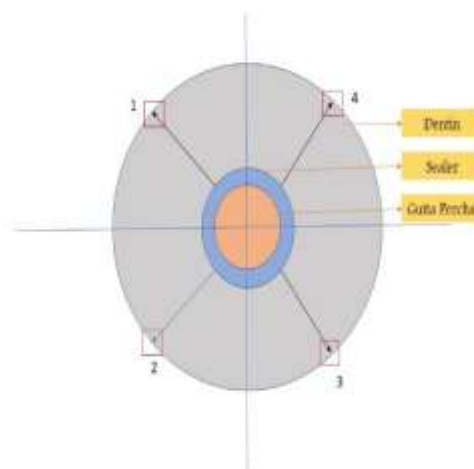
## METHODS OF MEASUREMENTS

### Two parameters were measured:

- i. **Intratubular penetration of Sealers into root dentin.**
- ii. **Push-out bond strength of Sealers to root dentin.**

### i) **Intratubular penetration of sealers into root dentin**

Confocal Laser Scanning Microscopy (CLSM) was performed with a 'ZEISS Microscope' with LSM Software ZEN 2007. An Ar/Kr mixed gas laser was used as the light source. Excitation light had a wavelength maximum at 568 nm. The intensity of the excitation light as well as the amplification of the photomultiplier was kept constant during the investigation period. CLSM images were recorded in fluorescent mode. The detected light was conducted through a 590 nm long-pass filter, thus, fluorescent light emitted from the specimen was discriminated from reflected and scattered light. The visualized layer was selected 10  $\mu\text{m}$  below the sample surface and images were recorded with an oil immersion objective (40x, numerical aperture 1.25). The size of the images recorded was 62.5 x 62.5  $\mu\text{m}^2$ , and the resolution was 512 x 512 pixel.



Images were recorded at four standardized areas of each sample. In order to quantify the depth of penetration, the measurements were performed at four different locations on each image, and a mean was calculated in  $\mu\text{m}$ .

**ii. Push-out bond strength of Sealers to root dentin.**

To evaluate the push-out bond strength same samples were subjected under Universal testing machine (ACME Engineers, India. Model No. UNITEST-10, Accuracy of the machine:  $\pm 1\%$ ) by applying an axial load to the sealer at a Crosshead speed: 1 mm/minute. The maximum failure load was recorded in Newtons (N) and converted into Megapascals (MPa) using following formula in accordance with Ferrari et al (2009):

**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) =  $2\pi rh$

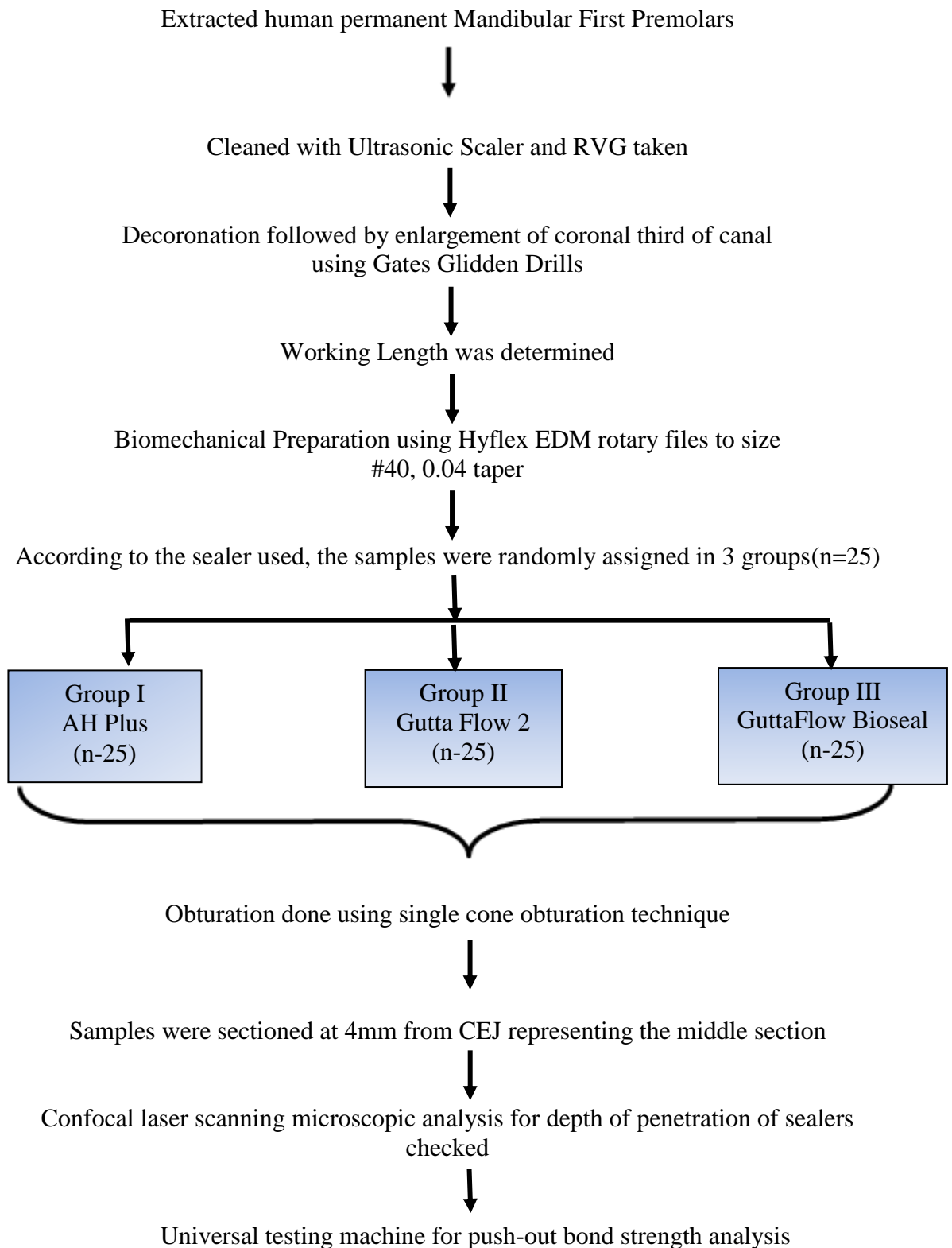
$\pi = 3.1416$ ,

r = Radius of perforated cross section,

h = Height of perforation

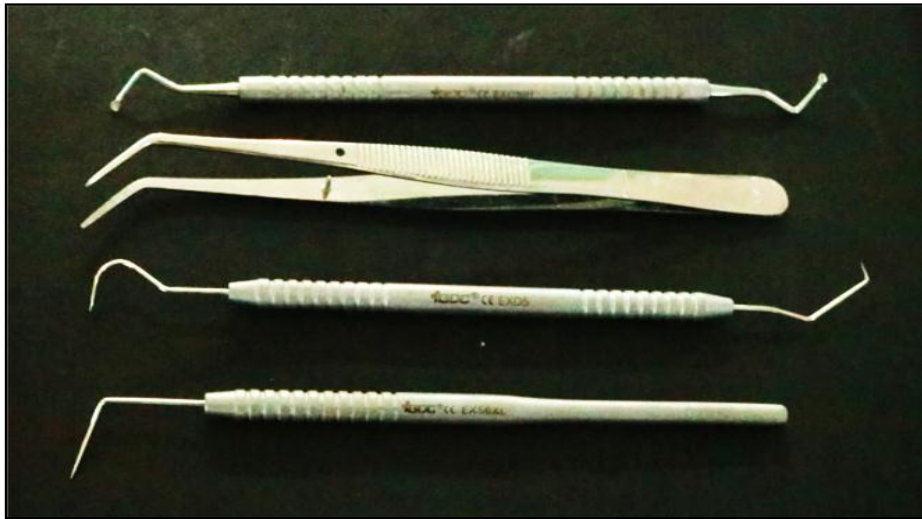
The data of push out bond strength was collected in Mpa and tabulated using an excel sheet (Microsoft Office 2010). This data was then subjected to statistical analysis using a licensed version of SPSS 26.0 (IBM Corp).

**ALGORITHM FOR METHODOLOGY**



## PLATE -I

# ARMAMENTARIUM



**Hand Instruments (GDC,India)**



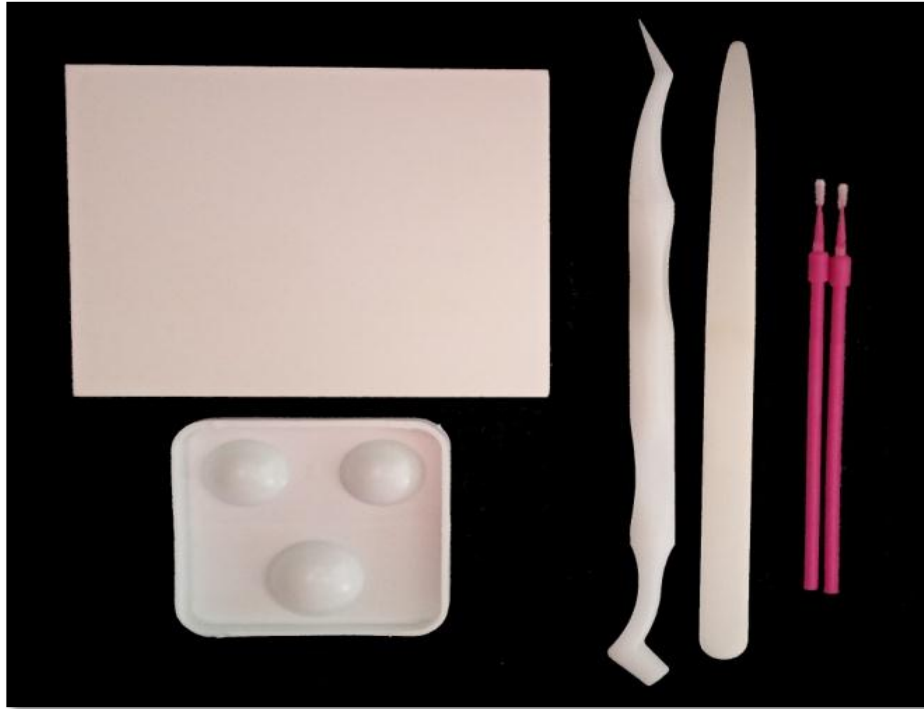
**Cotton Holder & Waste Receiver (GDC,India)**



**Digital Vernier Caliper (Workzone Tools,Germany)**

PLATE -II

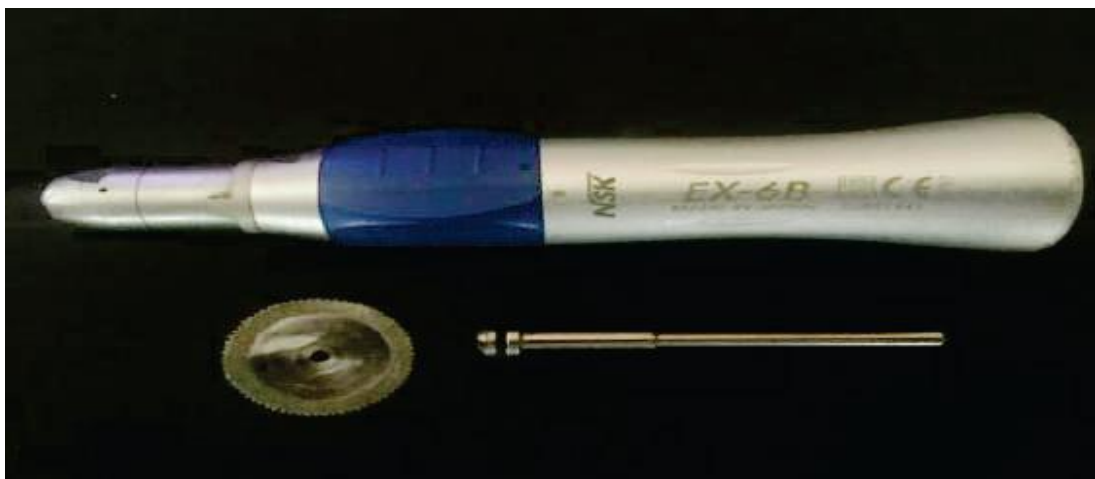
ARMAMENTARIUM



**Mixing pad, Spatula, Microbrushes, Dispenser**



**Endobloc (DENTSPLY, Maillefer, Switzerland)**



**Straight Handpiece (NSK, Japan), Double Sided Diamond Disc (DSF, Germany)**

PLATE -III

ARMAMENTARIUM



**Endodontic Motor X-Smart**  
(DENTSPLY, Maillefer, Switzerland)



**Gates Glidden Drills**  
(MANI, Japan)



**Standard 2% K & H Files (#15-80)** (MANI, Japan)

# PLATE -IV

## ARMAMENTARIUM



**Reamers** (MANI, Japan)



**LentuloSpiral** (MANI, Japan)



**HyFlex EDM-rotary files**  
(COLTENE, Switzerland)

ARMAMENTARIUM



**Calamus Dual 3D Obturation System**  
( DENTSPLY, Maillefer, Switzerland)

## PLATE - VI

# MATERIALS



**Sodium Hypochlorite (Hyposept UPS Hygienes)**  
**Normal Saline (Nirlife)**



**Irrigation Syringe , Side Venting Needle (Nirlife)**



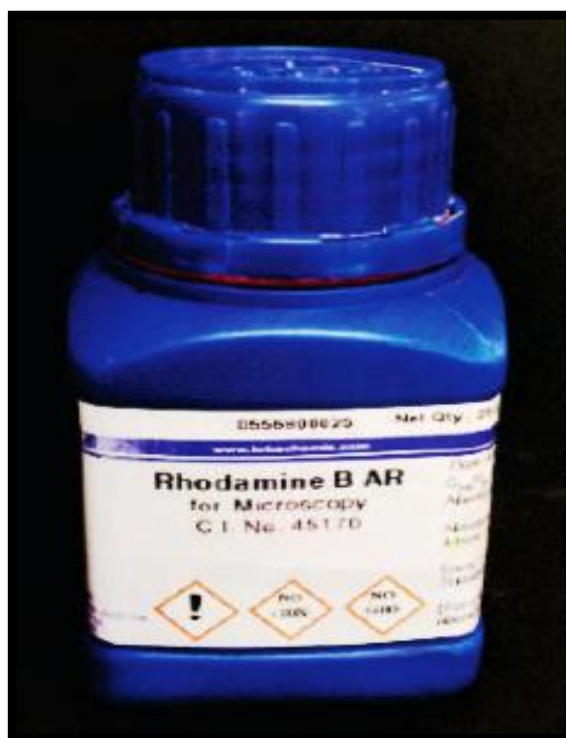
**RC Help 17% EDTA (Prime Dental Products)**

## PLATE -VII

# MATERIALS



**Absorbent Paper Points**  
(DENTSPLY, Maillefer, Switzerland)



**Rhodamine B dye**  
(Loba Chemie, India)

MATERIALS



AH Plus Resin Sealer (DENTSPLY, Maillefer, Switzerland)



GuttaFlow Bioseal (COLTENE, Switzerland)



GuttaFlow 2 (COLTENE, Switzerland)

## PLATE -IX

# MATERIALS

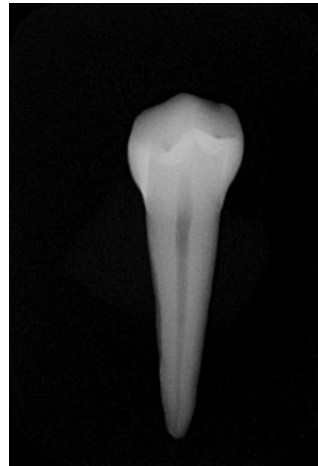


**Gutta Percha Points** (DENTSPLY, Maillefer, Switzerland)

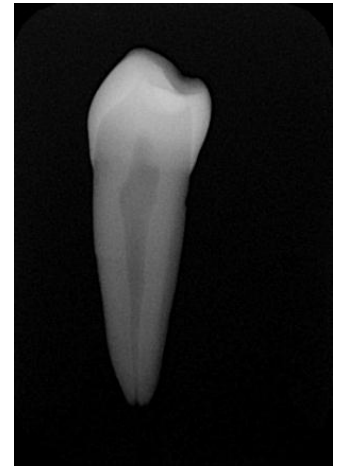
**METHODOLOGY**



**Sample Size (N=75)**



**Mesiodistal**

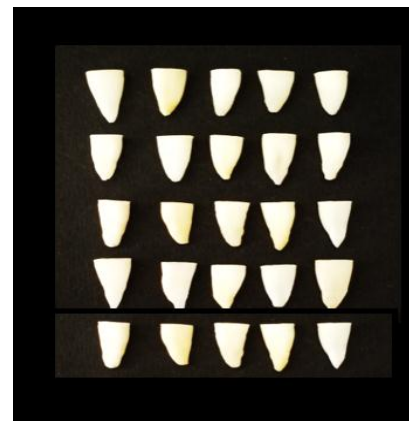


**Buccolingual**

**Pre Operative Radiographs**



**Decoronation of Samples**

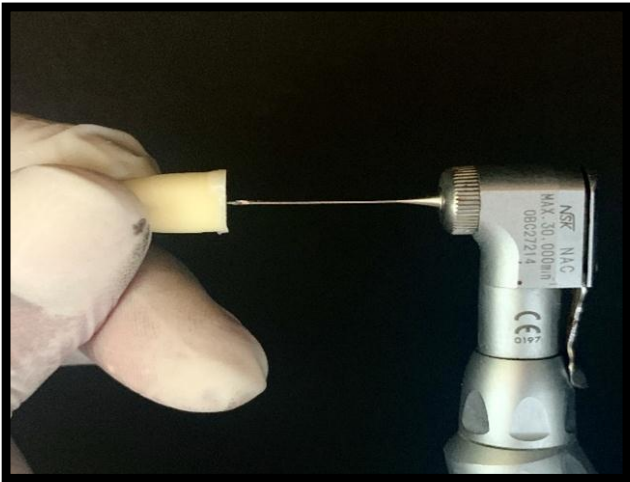


**Decoronated Samples(n=25)**



**Length Measured with Digital Vernier Caliper**

**METHODOLOGY**



**Coronal Enlargement with  
Gates Glidden Drill**



**Working Length Determination**

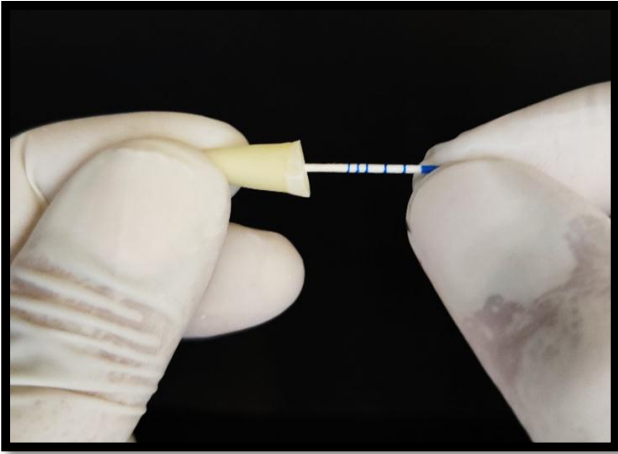


**Cleaning & Shaping**

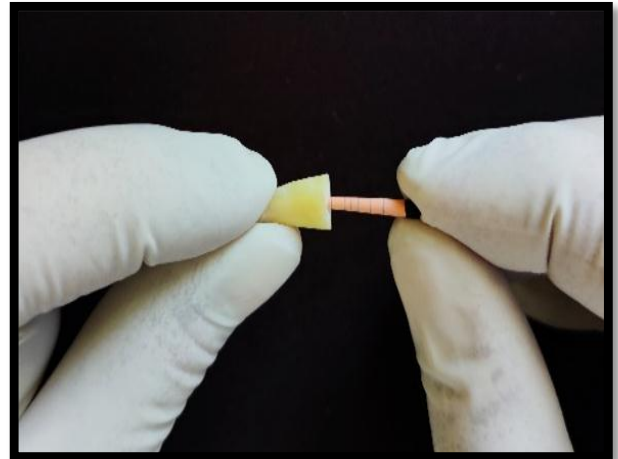


**Irrigation of Root Canal**

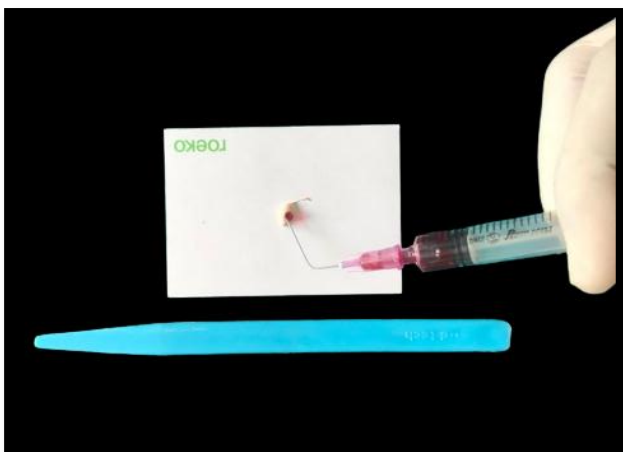
METHODOLOGY



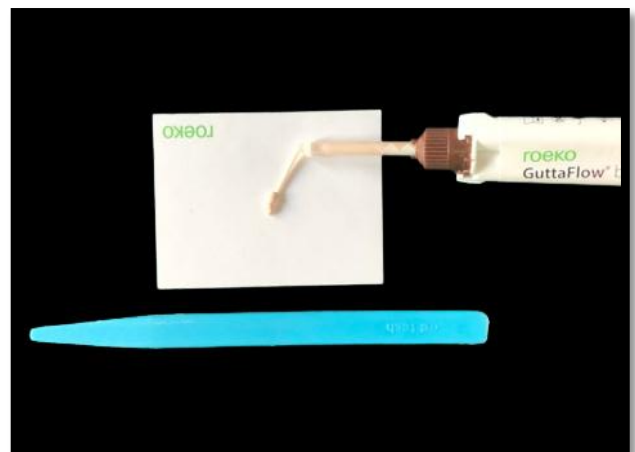
Canal Drying with Paper Point



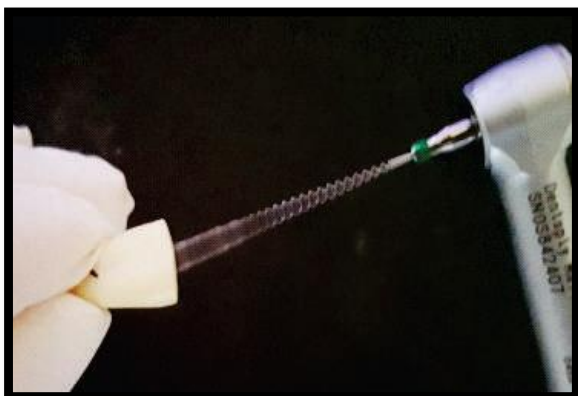
Master Cone Selection



Labelling with Rhodamine  
B Dye



Manipulation of Sealer



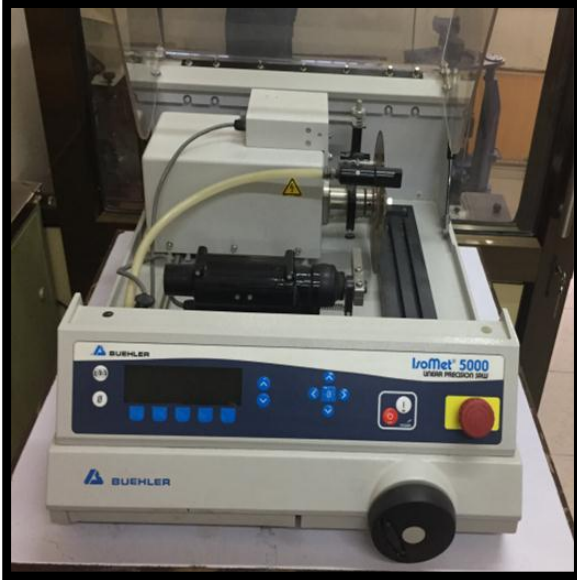
Application of Sealer with  
Lentulo Drill



Obturated Root Canal

# PLATE -XIII

## METHODOLOGY



**Sectioning of samples with Precision saw  
(IsoMet 5000, Buehler)**



**Polishing of Samples on  
Grinder & Polisher (Buehler)**



**Sectioned Samples**



**Confocal Laser Scanning Microscope  
(ZEISS with LSM Software ZEN 2007)**

## PLATE -XIV

# METHODOLOGY



**Universal Testing Machine** (ACME Engineers, Model no. UNITEST-10)

CONFOCAL LASER MICROSCOPIC IMAGES

**GROUP - I**

**AH Plus (MIDDLE)**

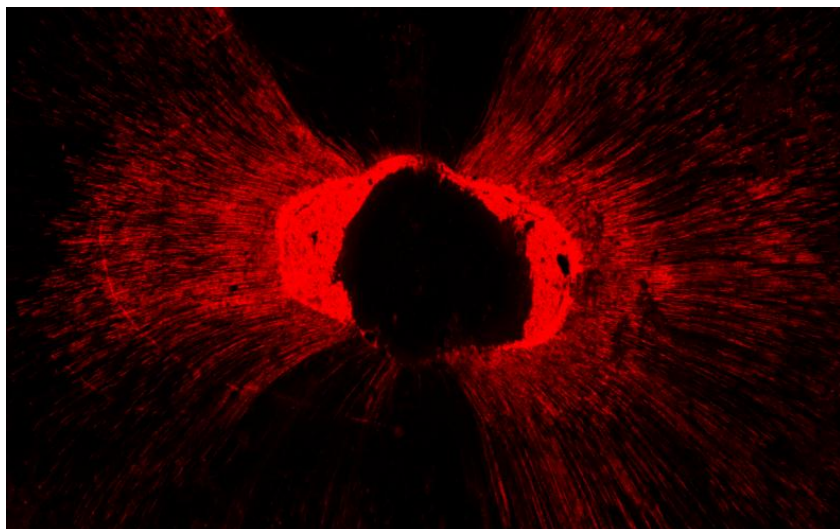
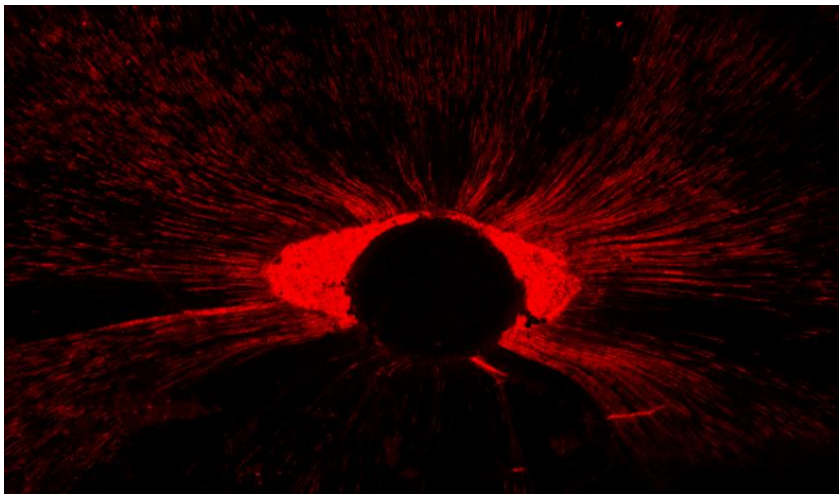
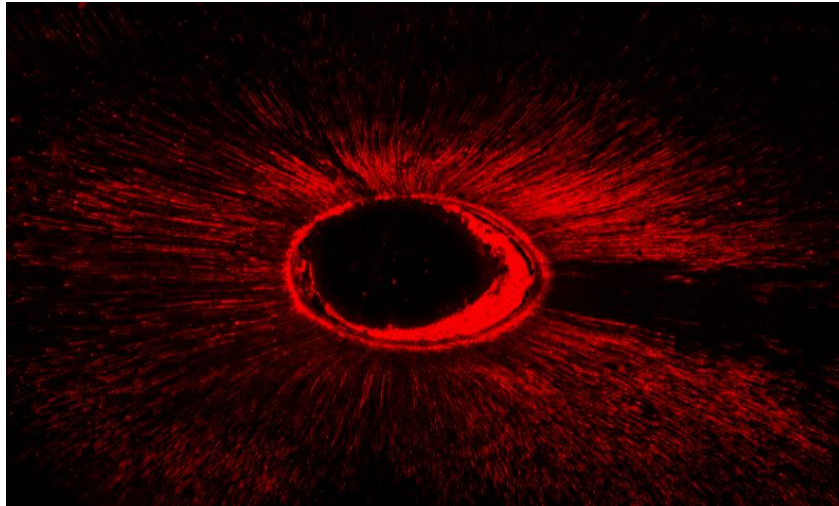


PLATE -XVI

CONFOCAL LASER MICROSCOPIC IMAGES

**GROUP - II**

**GuttaFlow 2 (MIDDLE)**

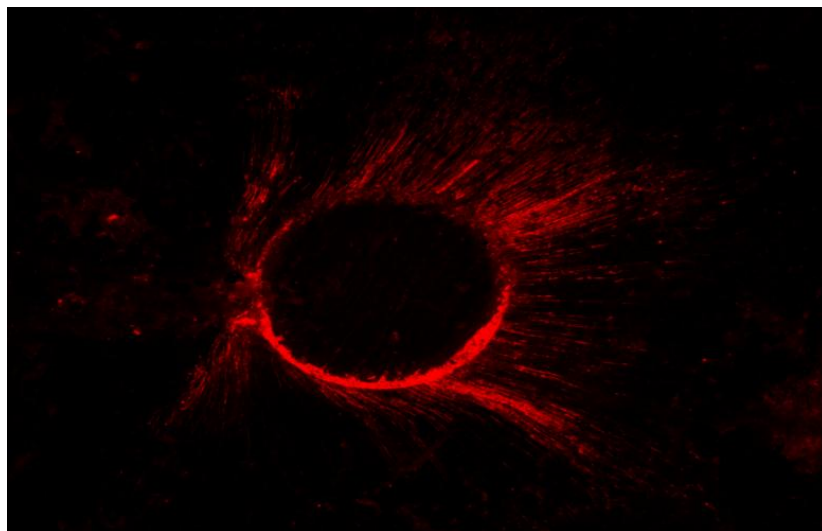
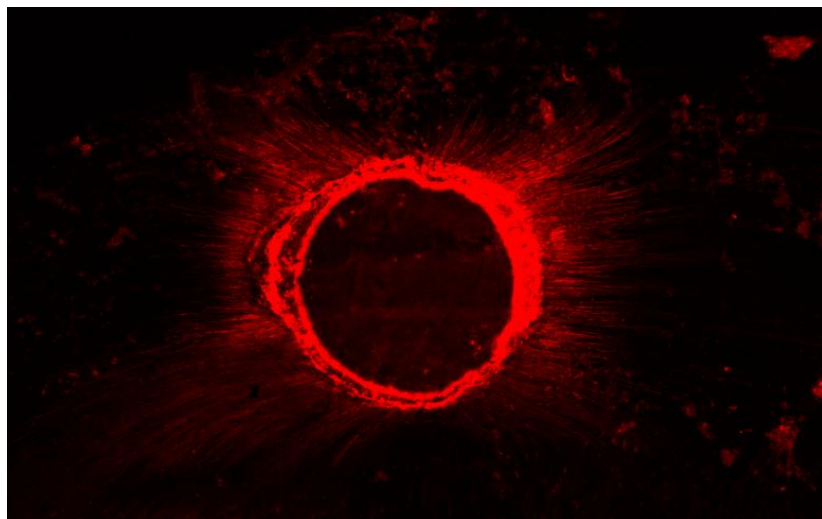
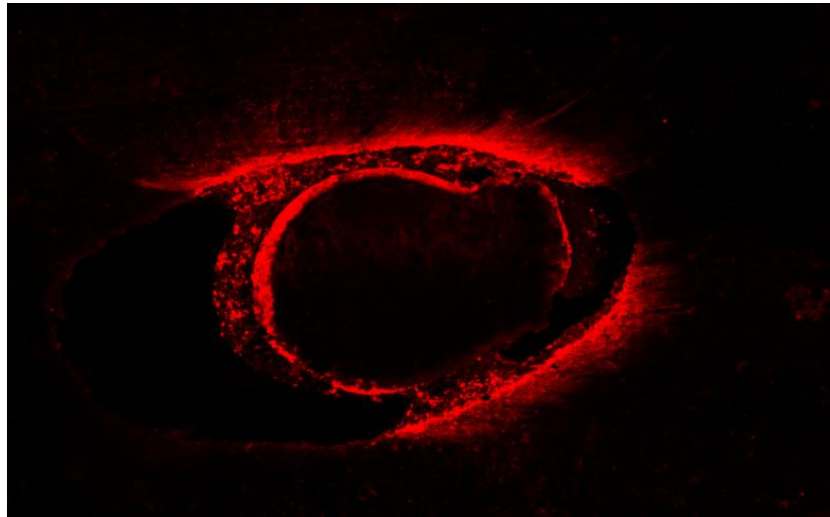
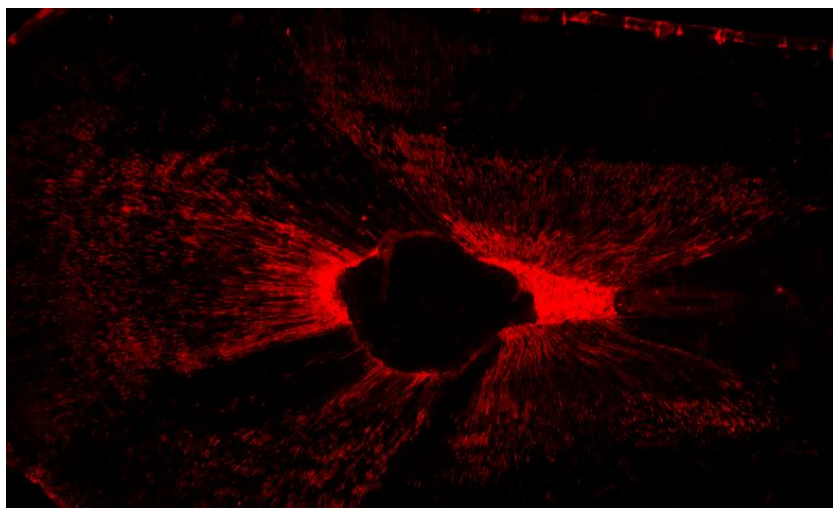
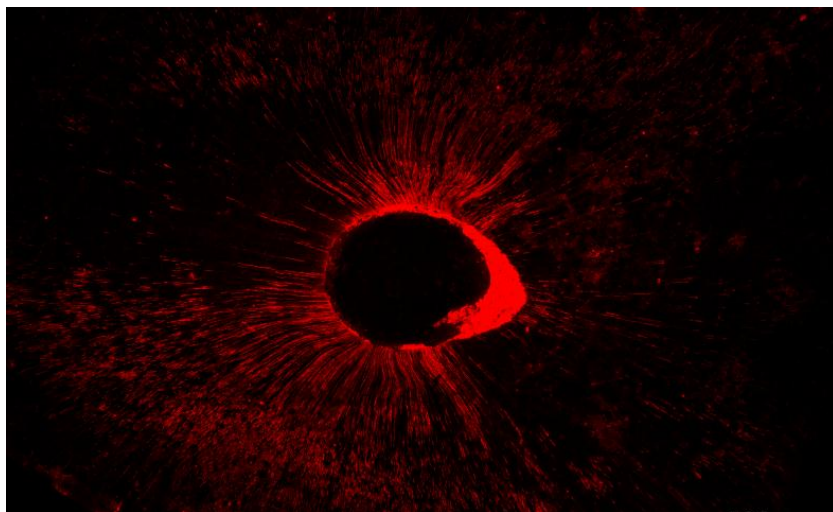
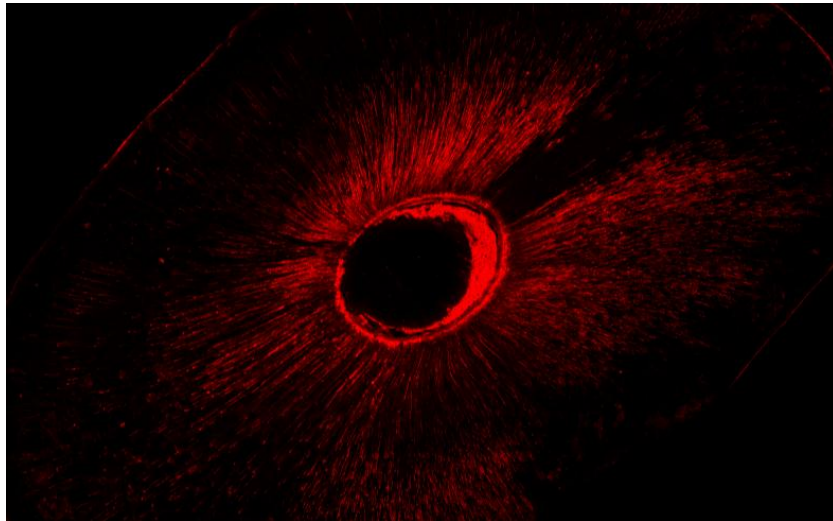


PLATE -XVII

CONFOCAL LASER MICROSCOPIC IMAGES

**GROUP - III**  
**GuttaFlow Bioseal (MIDDLE)**



## **RESULTS**

The present in vitro study was conducted to evaluate the Intratubular penetration of AH plus, GuttaFlow 2 and GuttaFlow Bioseal sealers by confocal laser scanning microscope and its relation to Push out bond strength.

Depending on the sealer used, the samples were randomly assigned in groups as follows:

**CONTROL GROUP I - AH PLUS (n=25)**

**EXPERIMENTAL GROUP II - GUTTAFLOW 2(n=25)**

**EXPERIMENTAL GROUP III – GUTTAFLOW BIOSEAL(n=25)**

### **Statistical Analysis**

Licensed version of SPSS 26.0 (IBM Crop USA) was used for statistical analysis. The data on intratubular penetration and ( $\mu\text{m}$ ) and push out bond strength (Mpa) were obtained on samples according to three sealer types at middle sections.

The statistical summaries like mean and standard deviation were obtained for each sealer type and at each section. The summaries were obtained for both intratubular penetration and push out bond strength. The comparison of mean intratubular penetration and push out bond strength between two sealer types for each canal drying protocol was performed using t-test for independent samples. Further, the comparison of these two parameters for each sealer type was performed using one-way analysis of variance (ANOVA). The paired comparison between two protocols was done using Tukey's post-hoc test. Also, the correlation between intratubular penetration and push out bond strength was determined using Pearson's correlation coefficient. The analysis was performed for each protocol and each sealer type.

All the statistical analyses were performed using SPSS ver 26.0 (IBM Corp, USA) and the statistical significance was tested at 5% level of significance.

### **The formulations used in the study are as below:**

#### **1. Measures of central tendency**

##### **Arithmetic Mean**

If  $x_1, x_2, \dots, x_n$  are the observations on a random variable X, then following measures of central tendency can be obtained:

Mean for a set of observations is given by

$$\bar{x} = \frac{1}{n} \sum_{i=1}^n x_i$$

## 2. Measures of dispersion

### Standard deviation

Standard deviation for a set of observations is given by

$$s = \sqrt{\frac{1}{(n-1)} \sum_{i=1}^n (x_i - \bar{x})^2}$$

Where,  $x_i$  = observation on each object ( $i=1,2,\dots,n$ ) and  $n$  = number of objects

### One-way Analysis of variance

Analysis of variance (ANOVA) is used to test the significance of difference in the mean of three or more groups. The basic assumption is that the variable of interest is normally distributed in the population under study.

Here the interest is to test the null hypothesis that the population means are same,  $H_0: \mu_1 = \mu_2 = \dots \mu_m$  i.e.

against the alternative  $H_1$  that they are not same.

Some of the statistics computed to test the hypothesis are as below:

- i) **Grand mean:** It is the mean of set of all observations in the studied groups and is given by:

$$\bar{x}_{GM} = \frac{1}{N} \sum_{i=1}^N x_i$$

- ii) **Total sum of squares:** It is the sum of squares of each observation from the grand mean and is given by:

$$TSS = \sum_{i=1}^N (x_i - \bar{x}_{GM})^2$$

Total sums of squares is the sum of two components i.e., variation between groups and within groups.

**iii) Between group sum of squares**

$$SSB = \sum_{j=1}^m n_j (\bar{x}_j - \bar{x}_{GM})^2$$

**iv) Within group sum of squares**

$$SSW = \sum_{j=1}^m \sum_{i=1}^n (x_{ij} - \bar{x}_j)^2$$

The mean sum of squares is obtained by dividing the above sum of squares with the respective degrees of freedom, i.e.  $N-1$ ,  $p-1$  and  $p(n-1)$ .

**v) F-statistic:** It is the ratio of between and within mean sum of squares

$$F = \frac{MS_{Between}}{MS_{Within}}$$

If the  $p$ -value based on F-statistic is greater than 0.05,  $H_0$  is accepted, otherwise  $H_1$  is accepted.

**vi) Tukey's post-hoc test**

After performing ANOVA, if alternative hypothesis  $H_1$  is accepted, then the subsequent interest is to determine the pair wise significance of difference in the means of study groups. This could be carried using Tukey's post-hoc test. The difference between the means of all groups are determined and compared with this critical difference called the honest significant difference (HSD). It is given by:

$$HSD = q \sqrt{\frac{MS_{within}}{n}}$$

where,  $q$  is the studentized range statistic derived from the tables,  $n$  is the sample size and the mean square value is from the ANOVA analysis. If the critical difference exceeds the absolute difference between any two sample means, then the corresponding means differ significantly.

## Correlation analysis

### Pearson's correlation

Pearson's correlation coefficient quantifies the relationship between two measurable variables. It measures the linear relationship between two variables. Thus, if  $X$  and  $Y$  are two variables taking values  $x_1, x_2, \dots, x_n$  and  $y_1, y_2, \dots, y_n$ , then the correlation coefficient ( $r$ ) between the two variables is given by:

$$r = \frac{n(\sum xy) - (\sum x)(\sum y)}{\sqrt{[n\sum x^2 - (\sum x)^2][n\sum y^2 - (\sum y)^2]}}$$

The value of  $r$  lies between -1 to +1, with -1 indicating perfect negative correlation and +1 indicating perfect positive correlation.

## Overall Results

The mean values and standard deviations of intratubular penetration and pushout bond strength for three different sealers namely AH Plus (Group I), GuttaFlow2 (group II), Guttaflow Bioseal (Group III) Middle section have been described in Table.1 and Table.3 respectively.

The maximum intratubular penetration (1876.13) was observed in Group I at middle level whereas the minimum intratubular penetration (255.75) was observed in Group II at middle level. The maximum push-out bond strength (2.77) was observed

in Group I at middle level whereas the minimum pushout bond strength (0.26) was observed in Group II at middle level.

Higher values for intratubular penetration and push-out bond strength were obtained for group in which AH Plus sealer was used as compared to those where GuttaFlow 2 and GuttaFlow Bioseal sealers were used.

### **Analysis for Intratubular Penetration:**

In order to determine the intratubular penetration of sealers, one-way analysis of variance was performed with groups. (Table1, Figure 1, Annexure I,II,III)

The pair wise comparison of depth of penetration between groups was performed using Tukey's post-hoc test. The mean difference of penetration depth between AH plus and GuttaFlow 2 was 555.384 [95% CI: 359.4820, 751.2860] mm and was significant with a p-value < 0.0001. The mean difference between AH plus and GuttaFlow Bioseal was 138.668 [95% CI: -57.234, 334.57] mm and was statistically insignificant (p=0.214). The mean difference between GuttaFlow 2 and GuttaFlow Bioseal was -416.716 [95% CI: -612.618, -220.813] and was statistically significant with a p-value < 0.0001. (Table2, Figure 2, Annexure I,II,III).

### **Analysis for Push-Out Bond Strength:**

In order to determine the push-out bond strength of sealers, one-way analysis of variance was performed with groups, The difference of mean push out bond strength across groups was statistically significant with a p-value < 0.0001. (Table 3, Figure 3, Annexure IV,V,VI)

The pair wise comparison of push out between groups was performed using

Tukey's post-hoc test. The mean difference of push out bond strength between AH plus and GuttaFlow 2 was 0.8536 [95% CI: 0.4000, 1.3072] Mpa and was significant with a p-value < 0.0001. The mean difference between AH plus and GuttaFlow Bioseal was 0.3256 [95% CI: -0.1280, 0.7792] Mpa and was statistically insignificant (p=0.206). The mean difference between GuttaFlow 2 and GuttaFlow Bioseal was -0.5280 [95% CI: -0.9816, -0.0744] Mpa and was statistically significant with a p-value of 0.018. (Table 4, Figure 4, Annexure IV,V,VI)

### **Analysis of correlation between intratubular penetration and pushout bond strength:**

In order to determine the Correlation between intratubular penetration and pushout bond strength for each sealer type, Pearson's correlation coefficient quantifies the relationship between two parameters. (Table 5)

For AH plus type, the correlation between the two parameters was negligible negative with a coefficient of -0.241, which was statistically insignificant (p=0.246).

GuttaFlow 2 type, the correlation between the two parameters was negligible negative with a coefficient value -0.007, which was statistically insignificant (p=0.973)

For GuttaFlow Bioseal type, the correlation between two parameters was -0.067, which was negligible negative and statistically insignificant (p=0.749).

## **DISCUSSION**

The root canal obturating material plays a pivotal role in establishing a three-dimensional fluid impervious sealing of the root canal system for the long-term success of endodontic treatment<sup>54</sup>. Gutta percha a gold standard obturating material comprises of a solid or semisolid core along with sealer.<sup>55</sup> Gutta-percha occupies bulk of the canal space and has no adhesive qualities towards dentin regardless of the obturation techniques used. Therefore, root canal sealers plays a major role in achieving the fluid tight seal by filling the interface between the core material and the dentin wall, accessory & lateral canals, voids, spaces and irregularities. The choice of sealer is not only dependent on its ability to create a sound seal, but it must also be biocompatible to surrounding peri radicular tissues.<sup>56</sup>

Traditionally, Zinc Oxide Eugenol was the most commonly used root canal sealer. However, there are several disadvantages associated with this conventional sealer such as: non adhesive, irritant to periapical tissue and carcinogenic. Further,

**Schroeder et al**<sup>8</sup> were the first to use epoxy resin based in 1984 and now a days with advance silicon-based sealers in 2003 brought a new revolution in the field of dentistry by providing a reliable substitute for conventional sealers. **Harpreet singh et al. (2014)**<sup>9</sup> stated that these sealers possess very good physical properties, excellent apical sealing and ensure adequate biological performance. Newer generation sealers are being engineered to improve their ability to penetrate into the dentinal tubule or bond to both dentin and core material surfaces. Dentinal tubule penetration of the sealer creates a micro-mechanical lock and therefore enhancing the retention and resistance to dislocation of the sealer.

However, there is literature scarcity analyzing the relationship of intratubular penetration and push out bond strength of AH plus, GuttaFlow 2 and GuttaFlow Bioseal sealer. Hence, the present study was carried out to evaluate qualitative and quantitative adhesive bond of AH Plus, GuttaFlow 2 and GuttaFlow Bioseal Sealers using Confocal Laser Scanning Microscopy and its relation to push-out bond strength.

Seventy-five freshly extracted human mandibular first premolars were selected for the study. The teeth were cleaned, disinfected and stored as per the recommendations and guidelines laid down by OSHA and CDC (2003 report 17)<sup>57</sup>. The presence of single canal was confirmed by bucco-lingual and mesio-distal RVG of the sample. Teeth with caries, trauma, fracture or other defects such as root calcification, root resorption, incompletely formed apices, developmental anomalies and severe curvatures were excluded. The teeth were stored in phosphate-buffered saline for not more than 12 weeks as suggested by **Jameson MW et al. (1994)**<sup>58</sup>. They advocated the use of phosphate-buffered saline as storage media which shows the best compatibility in maintaining the hydration of the extracted teeth during its

storage period.<sup>58</sup>

In our study, Mandibular First Premolars were selected as they present a unique combination of occlusal dynamics, structural loading and anatomical design and they are most frequently extracted for orthodontic purposes, which enables their availability easier for in-vitro research.<sup>59</sup>

The data published on push out bond strength by **Dem K. et al. (2019)**<sup>16</sup> was referred to obtain the sample size for the proposed study. They found higher bond-strength with AH plus sealer group (12.20Mpa) compared to GuttaFlow 2 group (0.43Mpa) at middle level. Assuming that similar differences could be obtained in the present study, the estimated sample size that could provide 80% power and 95% confidence interval, 25 samples were allocated per group. Therefore, the total sample size of 75 was taken for the current study.

**The formulation used was:**

$$n = \frac{(z_{1-\alpha/2\tau} + z_{1-\beta})^2}{ES^2}$$

where  $\tau$  is the number of possible comparisons,  $z_{1-\alpha/2\tau}$  (2.409) is the standardized value for 5% error and for 3 paired comparisons,  $z_{1-\beta}$  (1.282) is the value for 90% power and ES is the effect size ( $f$ ).

The selected teeth were standardized by decoronating them at predetermined distance i.e.,  $15 \pm 1$  mm from apex using water cooled diamond disc perpendicular to long axis of tooth and the cut coronal surface was ground flat using an abrasive paper.<sup>60</sup>

Working length estimation was achieved as per the protocol suggested by **Shanmugaraj M et al**<sup>61</sup> in which they inserted an endodontic file into the root canal until the tip of the file was just visible at the apical foramen. The stopper was adjusted to the reference point and the file was withdrawn. The canal length was determined and the working length was established by deducting 1 mm from this length; these readings were registered as actual working length (AWL).

Cleaning and shaping is recognized as one of the most important steps in root canal treatment. **Dafalla A et al. (2010)**<sup>62</sup> found that NiTi rotary files prepare canals more rapidly, and shows low incidences of blockage, and only limited loss of working length. Canal preparation with K-file was time consuming and showed higher incidence of deformed instruments probably due to low elasticity of the stainless-steel metal. **Ataide I et al.**<sup>63</sup> concluded that Nickel-titanium rotary instruments demonstrated a superior quality of canal preparation compared to stainless steel K files, with respect to canal cleanliness, canal transportation and canal shape. HyFlex EDM rotary instruments demonstrated a comparatively better quality of canal preparation in the apical region of teeth. Therefore, in the present study, the root canals were prepared by HyFlex EDM rotary instruments till size (40/.04) under constant irrigation with 5.25% NaOCl using a 30-gauge needle.

Irrigation regimen described by **Zehnder et al. 2006**<sup>64</sup> was employed in the study. The canals were instrumented and irrigated using copious amounts of the 5.25% Sodium hypochlorite solution and once the shaping procedure was completed, canals were thoroughly rinsed using aqueous 17% EDTA followed by final rinse with 5ml saline.

In the present study, after irrigation, block randomization of 75 samples was done into three groups (25 samples per group) and corresponding treatment with sealers was employed as control Group AH Plus, and experimental group GuttaFlow 2, GuttaFlow Bioseal respectively.

Root canal sealers are used in combination with gutta percha for an endodontic obturation. **Borges H A et al. (2014)**<sup>65</sup> studied physicochemical property of AH plus as compared to other conventional sealers and concluded AH Plus as gold standard. Although resin-based sealers have good properties, some researchers have shown increasing interest in materials capable of enhancing periapical tissue repair.

**E. Assmann et al (2012)**<sup>66</sup> stated that the Conventional Gutta percha (GP) with sealer did not provide an impervious seal of the root canal system; therefore, new obturation materials and techniques have been developed over the past decades to obtain an optimum seal in the root canal system<sup>51</sup>. **Bouillaguet S et al (2008)**<sup>67</sup> reported GuttaFlow2 sealer a cold flowable filling system, combining sealer and gutta-percha in one product. GuttaFlow 2 has exceptional chemical and physical properties; also material has good adaptability because of increased flowability and it expands slightly.

**Collado-González M et al (2017)**<sup>68</sup> stated that GuttaFlow Bioseal is a recently developed, silicone-based, cold-filling sealer containing GP powder and bioactive glass which can induce an osteogenic and osteoconductive properties. GuttaFlow Bioseal has adequate physical and biological properties, good dentin penetrability, and a higher cytocompatibility than AH Plus. So, in present study GuttaFlow 2, GuttaFlow Bioseal sealers was used in comparison with AH Plus sealer

to evaluate the intratubular penetration of sealers and its relation to push out bond strength.

**Oliveira et al. (2017)**<sup>69</sup> in their invitro study reported that 0.3 mg/mL rhodamine/sealer concentration is appropriate concentration for endodontic sealer studies. So, in present study 0.3 mg/mL rhodamine/sealer concentration was mixed with AH Plus, GuttaFlow 2 and GuttaFlow Bioseal. Mixing of the sealers was done according to manufacturer's manual. **Monticelli F et al. (2006)**<sup>70</sup> emphasized that it is very important to follow the manufacturer's recommendation to achieve an optimum bonding to root canal dentin with any type of dentin adhesives.

In present study, Sealer was introduced into the canal orifice with a Lentulo spiral (Dentsply Maillefer, Switzerland) rotated at 500 rpm in a clockwise direction with a slow-speed handpiece inserted up to 1 mm short of the WL. **Dash K A et al. (2017)**<sup>71</sup> reported that the depth and percentage of sealer penetration are influenced by the type of placement technique and by the root canal level, with penetration decreasing apically. Lentulo spiral has shown better penetration of sealer than the bidirectional file and ultrasonics.

**Lee SH et al (2020)**<sup>49</sup> & **Singla MG et al (2021)**<sup>72</sup> stated that GuttaFlow Bioseal sealer has shown better apical seal and less leakage as compared to other sealers when obturated with single-cone technique. So, in our present study root canals were obturated by means of single cone obturation technique.

As studies on the morphology of root canal dentin showed that number of tubules decreases from the crown to the apex, the response to irrigation and consequently dentine bonding can vary among different areas of the same root canal.

<sup>73,74</sup> **Mjör A et al. (2000)**<sup>75</sup> reported a higher number and larger diameters of the tubules in the middle thirds could cause more sealer penetration in these areas as compared to other third. So, in our study, after obturation, only middle portion was considered and samples were sectioned at 4mm from CEJ.

Several microscopy techniques are currently used to evaluate the intratubular penetration of sealers, including stereomicroscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM) and confocal laser scanning microscopy (CLSM). In comparison to conventional SEM, CLSM has the advantage of providing detailed information about the presence and distribution of dental adhesives inside dentinal tubules in the total circumference of the root canal walls at relative low magnification as 100× than that of SEM because of its non-destructive nature. Also, CLSM offers improved rejection of out-of-focus noise and provides greater resolution than conventional imaging, yielding greatly enhanced images of biological structures. Further, drying of samples is not necessary with CLSM, which is indispensable for conventional microscopic technique which leads to decrease risk of shrinking or other drying artefacts. An additional feature of the confocal principle is that it permits visualization of not only a specimen surface, but also its subsurface. Thus, in the current research CLSM was preferred over SEM and TEM and the sections were observed under the confocal laser scanning microscope.<sup>76</sup>

For determining the bond strength of sealer to root dentin, push-out bond strength test has been considered as a reliable technique to measure the bond strength of root canal filling materials to root dentin. Thus, the push out bond strength approach was adopted in the present study and the same specimens were subjected to axial compression force until fracture under universal testing machine at the cross-

head speed of 1mm/min. This speed was used by various authors in previous studies to measure the fracture resistance of teeth using the UTM<sup>77</sup>.

To reduce the variability, all the samples were prepared and investigated by one operator using the standard technique.

**I) Intratubular penetration of AH Plus, GuttaFlow 2, GuttaFlow Bioseal Sealers :**

Dentinal tubule penetration depth is considered as performance measure of a root canal sealer. Previous studies have shown that the penetration of sealer into the dentinal tubules forms a physical barrier and entombs residual bacteria and improves retention of the root filling.<sup>78,79</sup> **Goracci C et al. (2004)**<sup>80</sup> showed that the penetration of root canal sealers into dentinal tubules decreases the interface between the core material and dentin, and retention of the core material might be improved by mechanical interlocking.

In this study, maximum depth of penetration (**1876.13**  $\mu\text{m}$ ) was observed in AH Plus Sealer (Group I) as compared to other groups and the mean depth of penetration was lowest for GuttaFlow 2 sealer (Group II) (**255.75**  $\mu\text{m}$ ). Statistically significant difference was observed between AH Plus and GuttaFlow 2 with a p-value  $< 0.0001$ ; where as insignificant difference observed between AH Plus and GuttaFlow Bioseal with a p = 0.214.

Higher intratubular penetration was observed for AH Plus sealer attributing to sealer integrity as well as the property of sealer being drawn into the tubules by capillary action. On the other hand, AH Plus being chemically cured allows for compensation of polymerization shrinkage and exhibits zero polymerization stress as compared to other sealers.<sup>81,82</sup>

In the present study, GuttaFlow 2 showed lowest depth of penetration among all sealers. The presence of silicone in the polydimethylsiloxane-based GuttaFlow 2 sealer, which possibly produces high surface tension forces. Thus, it is difficult for it to spread on the intratubular dentin. Besides, its film thickness is higher than AH Plus, this makes GuttaFlow 2 penetrate lesser into dentinal tubules.<sup>83</sup>

The results of our study are in accordance to the study done by **Sonu KR et al (2016)**<sup>32</sup> where they evaluated dentinal tubule penetration of mineral trioxide aggregate (MTA) Fillapex, GuttaFlow2 sealer and AH Plus sealers and stated that GuttaFlow 2 showed minimum penetration and AH Plus showed maximum sealer penetration attributing to its thin film thickness.

However, the result of our study showed that the GuttaFlow Bioseal and AH Plus sealers having similar dentinal tubule penetration. GuttaFlow Bioseal sealer has shown low surface tension, high flow, limited dimensional change on setting (0.6%–0.2%), low water sorption, and having a particle size of (2–10) µm, which enhances the penetration of the particles into the dentinal tubules<sup>84</sup>.

The results of our study are similar to **Pereira et al and Akcay et al (2016)**<sup>23</sup> where they evaluated the penetration of GuttaFlow Bioseal into the dentinal tubules using confocal laser scanning microscopy. They concluded that GuttaFlow Bioseal has similar dentinal tubule penetration to that of AH Plus sealer.

## **II) Push-out bond strength of AH Plus, GuttaFlow 2, GuttaFlow Bioseal Sealers :**

When assessing an endodontic sealer, the bonding strength of the obturating material to the intraradicular dentin is a key factor to be considered. The ability

of a root canal sealer to adhere to the dentin is essential in maintaining the integrity of the sealer–dentin interface while undergoing mechanical stresses.

The maximum push-out bond strength (2.77Mpa) was observed in AH Plus Sealer (Group I) as compared to other groups and the minimum pushout bond strength (0.26Mpa) was observed in GuttaFlow 2 (Group II) Statistically significant difference observed between AH Plus and GuttaFlow 2 with a p-value < 0.0001; where as insignificant difference observed between AH Plus and GuttaFlow Bioseal with a p = 0.206.

In present study, better results for AH Plus sealer could be justified as AH Plus forms a covalent bond and they forms epoxide rings when it is exposed to amino groups available in the collagen linkage, hence making the push out bond strength more resilient to pressure/ stress. In addition, AH Plus has been shown to have a long-standing dimensional stability and lower polymerization stress.<sup>85</sup>

**Dabaj P et al (2018)**<sup>40</sup> assessed the push-out bond strength of root canals obturated with Endosequence BC Sealer, and AH Plus sealer and they concluded that AH Plus had significantly higher push-out bond strength amongst all experimental groups.

In the present study, GuttaFlow 2 showed lowest push out bond strength among all sealers. The lower push out bond strength of GuttaFlow 2 is due to the presence of silicone resin in its composition, which cause an increase in the surface tension, hence making the flow of materials difficult and ultimately leads to decrease mechanical bond strength.<sup>86</sup>

**Elmuttalibi Haidar T. F. et al (2019)**<sup>46</sup> studied the push out bond strength of three different obturation materials and they observed that GuttaFlow 2 showed the minimum bond strength attributing to the minute voids, within the core of GuttaFlow and smaller contact angle of GuttaFlow 2 sealers as compared to other sealers.

The result of our study showed that the GuttaFlow Bioseal and AH Plus sealers have similar push out bond strength. GuttaFlow Bioseal sealer doped with calcium silicate particles has both osteointegrative and osteoconductive effects which bond mechanically to bone tissue through the formation of hydroxyapatite crystals and hence increases the push out bond strength.<sup>19,87</sup>

**Gandolfi MG (2016)**<sup>87</sup> studied that properties of a novel polysiloxane guttapercha calcium silicate-bioglass-containing root canal sealer and the observed that with slight calcium release, low solubility and alkalizing activity of the calcium ions and phosphate ions stimulate the development of a superficial layer of calcium phosphate, which can fill out the voids and improve the push out bond strength.

These results are in accordance with the study done by **Dem K et al (2019)**<sup>16</sup> where they studied the push out bond strength of AH plus, GuttaFlow2, GuttaFlow Bioseal and concluded that AH Plus exhibits higher push out bond strength when compared to GuttaFlow Bioseal and GuttaFlow 2 sealers. They also concluded that GuttaFlow Bioseal has slightly better push out bond strength than GuttaFlow 2 sealers.

### **III. Correlation between intratubular penetration and push-out bond strength:**

The depth of penetration and push out bond strengths for the AH plus, GuttaFlow2 and GuttaFlow Bioseal sealers used in this study differed.

In the present study, statistically no significant differences were observed between correlation of depth of sealer penetration and push out bond strength of AH plus, GuttaFlow2 and GuttaFlow Bioseal sealers.

However, the results are also in accordance with **Tedesco M et al (2019)**<sup>47</sup> evaluated the correlation of bond strength (BS) and dentin penetration of Endofill, AH Plus and MTA Fillapex sealers by push-out bond strength and Confocal Laser Scanning Microscopy (CLSM) and considering the different compositions and properties of the sealers, they concluded that there is no correlation between bond strength and intratubular penetration.

The results are in accordance to study of **Sungur DD et al (2016)**<sup>31</sup> evaluated and compared the push-out bond strength and dentinal tubule penetration of root canal sealers used with coated core materials and conventional gutta-percha. They concluded that dentinal tubule penetration has limited effect on push-out bond strength of the root canal sealers.

Thus, showing a close relationship between intratubular penetration and push-out bond strength. There is limited research available on intratubular penetration and push-out bond strength of AH Plus, GuttaFlow 2, GuttaFlow Bioseal Sealers and further more dedicated research is needed to be carried out in this area of correlation between intratubular penetration and push out bond strength.

Thus, the null hypothesis of this study that there is no significant difference on intratubular penetration and push out bond strength of AH plus, GuttaFlow 2 and GuttaFlow Bioseal sealer was accepted.

## **LIMITATIONS**

In spite of stringent care taken in every step of the root canal procedure certain limitations are present in the study

1. Even a slight variation in tooth dimensions can influence the results of the bond strength.
2. As this was an in vitro study, exact simulation of the oral conditions was not possible. Therefore, the results cannot be directly extrapolated to the clinical situation.
3. In this study a continually increasing static load was applied to the tooth which is not the type of load that occurs in natural oral environment.

## **SUMMARY AND CONCLUSION**

In the past decade, several experimental and clinical studies established that different sealers in the root canal have shown to alter quality of adhesion between root canal dentin and sealers. However, several researchers have recommended various sealers for proper intratubular penetration and bond strength of sealers.

The present in vitro study was carried out to evaluate the effect of various sealers on Intratubular penetration and its relation to Push out bond strength of Resin based sealer and silicone-based sealer to root dentin.

In present study, 75 freshly extracted human mandibular first premolars, which fulfilled the inclusion criteria, were selected. The teeth were decoronated, prepared and irrigated using standard techniques.

After irrigation protocol, teeth were divided into three groups according to sealers used in the study.

**GROUP I:- AH Plus (n=25)**

**GROUP II: - GuttaFlow 2 (n=25)**

**GROUP III:- GuttaFlow Bioseal sealers (n=25)**

The samples were obturated with single cone Obturation technique. After complete setting of three sealers, all samples were stored at 37 °C and 100% relative humidity for 1 week. All samples were then sectioned using a precision saw at 4mm. These sections were evaluated for the depth of penetration under confocal laser scanning microscope and the same samples were evaluated for pushout bond strength under universal testing machine.

The results obtained indicated that there is a significant difference in depth of penetration and push-out bond strength of AH Plus and GuttaFlow2 sealers. But, there is no significant difference found between AH Plus and GuttaFlow bioseal sealers.

**Within the limitations of the study, following conclusions can be drawn:**

1. There is no significant difference on intratubular penetration of AH Plus, GuttaFlow 2 and GuttaFlow Bioseal sealers.
2. There is no significant difference on push out bond strength of AH Plus, GuttaFlow 2 and GuttaFlow Bioseal sealers.
3. There is no significant difference on correlation between intratubular penetration and push out bond strength of AH plus, GuttaFlow 2 and GuttaFlow Bioseal sealers.

Taking into consideration the findings of the present study, it can be concluded that under experimental conditions, similar depth of penetration and bond strength of AH Plus and GuttaFlow Bioseal sealers occurred. However, further investigations which could give a conclusive remark on the long-term effect of different sealers on the depth of penetration and bond strength of sealer to root dentin are needed.

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## TABLES

**Table 1: Descriptive statistics for depth of penetration of the sealers**

Sealers	No of Samples (N)	Mean	Standard Deviation	Minimum	Maximum	P-value*
<b>Group - I AH Plus</b>	25	1143.12	349.18	476.17	1876.14	< 0.0001 (S)
<b>Group - II GuttaFlow 2</b>	25	587.74	223.85	255.76	1001.50	< 0.0001 (S)
<b>Group – III GuttaFlow Bioseal</b>	25	1004.45	281.51	533.26	1545.23	< 0.0001 (S)

\*Obtained using one-way analysis of variance;

S: Significant

Dentinal tubule penetration in  $\mu\text{m}$

**Table 2 - Pair wise comparison of depth of penetration between sealers**

Comparison of sealers	Mean Difference	P-value*	95% Confidence Interval	
			Lower Bound	Upper Bound
<b>AH Plus vs GuttaFlow 2</b>	555.384	< 0.0001	359.4820	751.2860
<b>AH Plus vs GuttaFlow Bioseal</b>	138.668	0.214	-57.2340	334.5700
<b>GuttaFlow 2 vs GuttaFlow Bioseal</b>	-416.716	< 0.0001	-612.6180	-220.8130

Pair wise comparison using Tukey's post-hoc test

Dentinal tubule penetration in  $\mu\text{m}$

**Table 3 - Descriptive statistics for push out bond strength of the sealers**

Sealers	No of Samples (N)	Mean	Standard Deviation	Minimum	Maximum	P-value
<b>Group - I AH Plus</b>	25	1.67	0.76	0.56	2.90	< 0.0001 (S)
<b>Group - II GuttaFlow 2</b>	25	0.82	0.42	0.26	1.84 1001.50	< 0.0001 (S)
<b>Group – III GuttaFlow Bioseal</b>	25	1.35	0.76	0.26	2.90	< 0.0001 (S)

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

**Table 4 - Pair wise comparison of push out bond strength of the sealers**

Comparison of sealers	Mean Difference (I-J)	P-value*	95% Confidence Interval	
			Lower Bound	Upper Bound
<b>AH Plus vs GuttaFlow2</b>	0.8536	< 0.0001 (S)	0.4000	1.3072
<b>AH Plus vs GuttaFlow Bioseal</b>	0.3256	0.206 (NS)	-0.1280	0.7792
<b>GuttaFlow 2 vs GuttaFlow Bioseal</b>	-0.5280	0.018 (S)	-0.9816	-0.0744

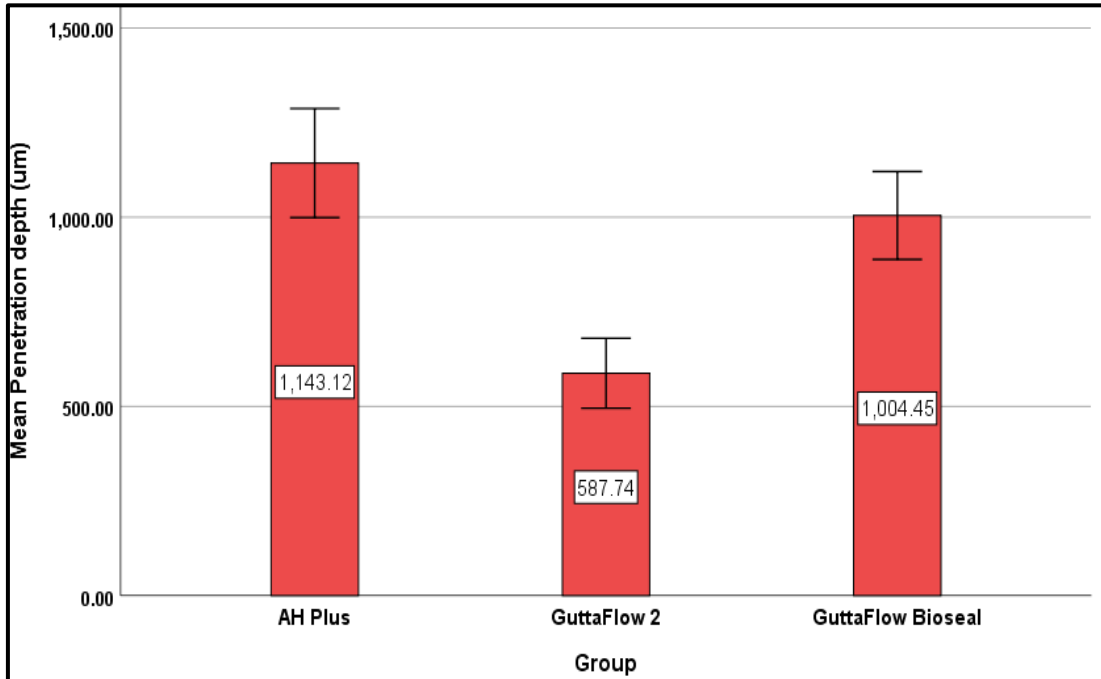
\*Pair wise comparison using Tukey’s post-hoc test;

S: Significant; NS: Not significant

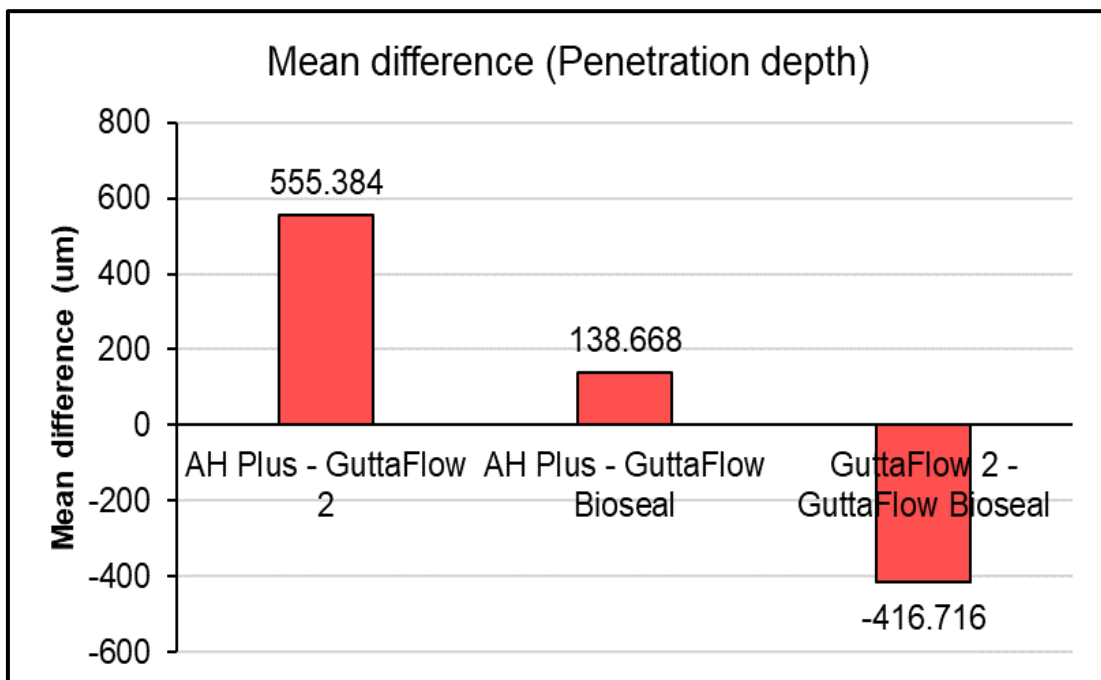
**Table 5: Correlation of push out bond strength and depth of penetration according to sealer types**

Sealers	Parameter		Interpretation
	Correlation coefficient	P-value	
<b>Group - I</b> <b>AH plus (n=25)</b>	-0.241	0.246 (NS)	Negligible negative correlation
<b>Group - II</b> <b>Gutta Flow 2(n=25)</b>	-0.007	0.973 (NS)	Negligible negative correlation
<b>Group - III</b> <b>Gutta Flow Bioseal (n=25)</b>	-0.067	0.749 (NS)	Negligible negative correlation

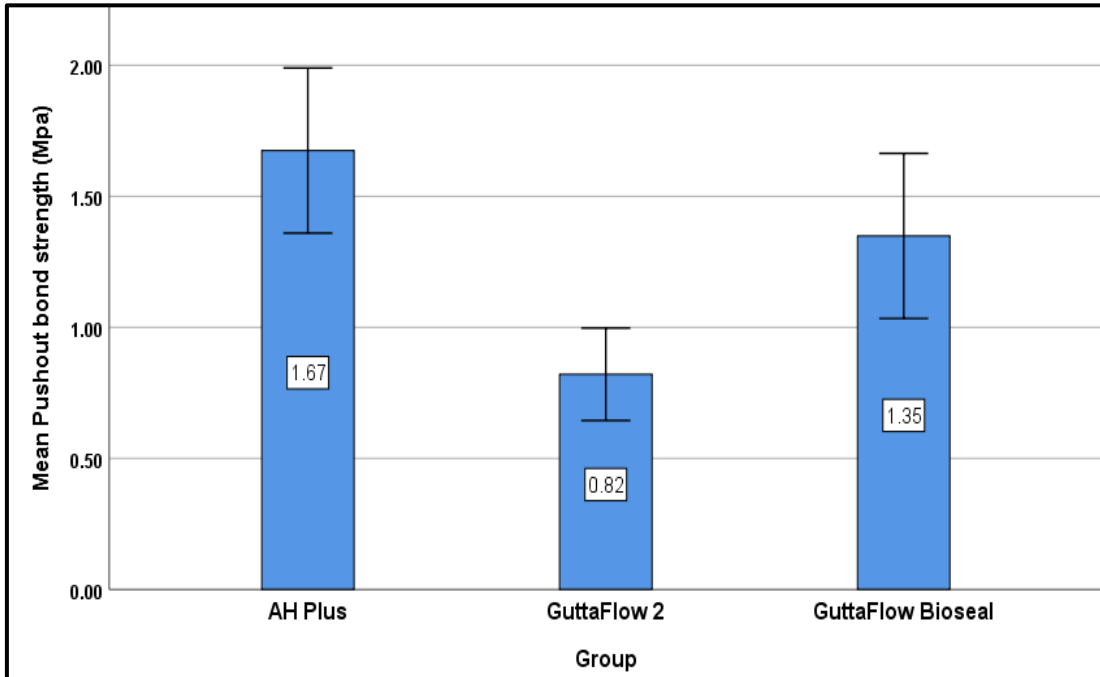
**Figure 1: Column chart with 95% error bars showing mean penetration depth for samples treated with three sealers**



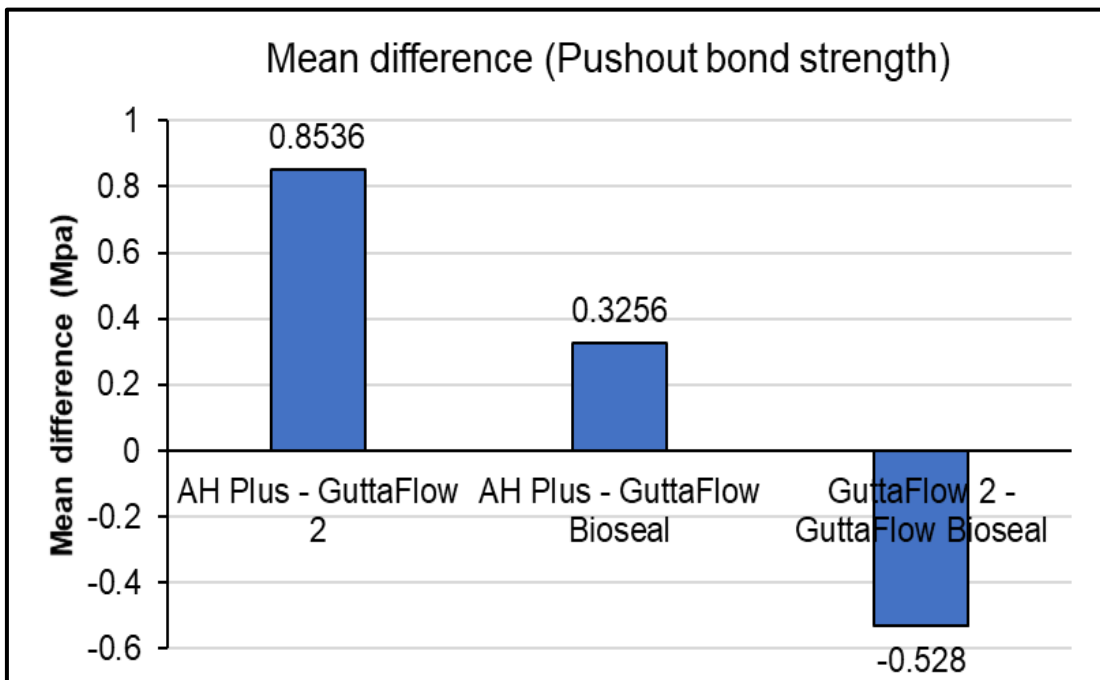
**Figure 2: Column chart showing mean difference of penetration depth between two sealers**



**Figure 3: Column chart with 95% error bars showing mean pushout bond strength for samples treated with three sealers**



**Figure 4: Column chart showing mean difference of pushout bond strength between two sealers**



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**ANNEXURE I**  
**Intratubular sealer penetration in Group I - AH Plus**

<b>Sample No.</b>	<b>Deepest penetration of sealer in middle section (<math>\mu\text{m}</math>)</b>
1	480.441 $\mu\text{m}$
2	1876.136 $\mu\text{m}$
3	1370.578 $\mu\text{m}$
4	1035.872 $\mu\text{m}$
5	1253.838 $\mu\text{m}$
6	1347.288 $\mu\text{m}$
7	938.192 $\mu\text{m}$
8	1419.439 $\mu\text{m}$
9	1055.979 $\mu\text{m}$
10	1157.625 $\mu\text{m}$
11	476.171 $\mu\text{m}$
12	1201.931 $\mu\text{m}$
13	1311.260 $\mu\text{m}$
14	951.744 $\mu\text{m}$
15	1511.751 $\mu\text{m}$
16	1020.303 $\mu\text{m}$
17	883.503 $\mu\text{m}$
18	1313.586 $\mu\text{m}$
19	1220.501 $\mu\text{m}$
20	789.833 $\mu\text{m}$
21	930.900 $\mu\text{m}$
22	1101.111 $\mu\text{m}$
23	885.302 $\mu\text{m}$
24	909.333 $\mu\text{m}$
25	1735.435 $\mu\text{m}$

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**ANNEXURE II**  
**Intratubular sealer penetration in Group II – GuttaFlow 2**

<b>Sample No.</b>	<b>Deepest penetration of sealer in middle section (<math>\mu\text{m}</math>)</b>
1	533.263 $\mu\text{m}$
2	1354.804 $\mu\text{m}$
3	951.730 $\mu\text{m}$
4	1273.264 $\mu\text{m}$
5	1311.260 $\mu\text{m}$
6	1605.111 $\mu\text{m}$
7	1405.279 $\mu\text{m}$
8	666.865 $\mu\text{m}$
9	1779.707 $\mu\text{m}$
10	1360.566 $\mu\text{m}$
11	638.856 $\mu\text{m}$
12	585.357 $\mu\text{m}$
13	778.504 $\mu\text{m}$
14	1108.304 $\mu\text{m}$
15	1201.204 $\mu\text{m}$
16	890.333 $\mu\text{m}$
17	1411.677 $\mu\text{m}$
18	752.533 $\mu\text{m}$
19	995.444 $\mu\text{m}$
20	670.553 $\mu\text{m}$
21	990.930 $\mu\text{m}$
22	1312.133 $\mu\text{m}$
23	778.531 $\mu\text{m}$
24	993.504 $\mu\text{m}$
25	1011.101 $\mu\text{m}$

## ANNEXURE III

## Intratubular sealer penetration in Group III – GuttaFlow Bioseal

Sample No.	Deepest penetration of sealer in middle section ( $\mu\text{m}$ )
1	375.115 $\mu\text{m}$
2	394.710 $\mu\text{m}$
3	255.758 $\mu\text{m}$
4	480.365 $\mu\text{m}$
5	511.002 $\mu\text{m}$
6	505.129 $\mu\text{m}$
7	792.751 $\mu\text{m}$
8	388.911 $\mu\text{m}$
9	366.258 $\mu\text{m}$
10	400.457 $\mu\text{m}$
11	578.562 $\mu\text{m}$
12	531.991 $\mu\text{m}$
13	709.301 $\mu\text{m}$
14	502.505 $\mu\text{m}$
15	901.303 $\mu\text{m}$
16	404.555 $\mu\text{m}$
17	399.931 $\mu\text{m}$
18	693.901 $\mu\text{m}$
19	798.701 $\mu\text{m}$
20	889.888 $\mu\text{m}$
21	901.303 $\mu\text{m}$
22	1001.503 $\mu\text{m}$
23	505.503 $\mu\text{m}$
24	1000.533 $\mu\text{m}$
25	403.501 $\mu\text{m}$

**ANNEXURE IV**  
**Push out bond strength of sealer to root dentin in Group I-AH Plus**

<b>Group : I</b>			
<b>Sr. No.</b>	<b>Sample No.</b>	<b>Maximum Load (N)</b>	<b>Pushout Bond Strength (MPa)</b>
1	No.1	2.94	0.56
2	No.2	3.03	0.57
3	No.3	13.82	2.61
4	No.4	10.68	2.02
5	No.5	5.07	0.96
6	No.6	3.88	0.73
7	No.7	13.32	2.52
8	No.8	4.19	0.79
9	No.9	6.10	1.15
10	No.10	9.70	1.84
11	No.11	14.60	2.77
12	No.12	13.32	2.52
13	No.13	11.60	2.20
14	No.14	9.70	1.84
15	No.15	6.10	1.15
16	No.16	15.30	2.90
17	No.17	4.11	0.78
18	No.18	5.70	1.08
19	No.19	8.13	1.54
20	No.20	10.68	2.02
21	No.21	5.07	0.96
22	No.22	9.70	1.84
23	No.23	14.60	2.77
24	No.24	8.13	1.54
25	No.25	11.60	2.20

**ANNEXURE V**  
**Push out bond strength of sealer to root dentin in Group II-GuttaFlow 2**

<b>Sr. No.</b>	<b>Sample No.</b>	<b>Maximum Load (N)</b>	<b>Pushout Bond Strength (MPa)</b>
1	No.1	9.60	1.82
2	No.2	7.50	1.42
3	No.3	11.60	2.20
4	No.4	6.10	1.15
5	No.5	15.30	2.90
6	No.6	6.76	1.28
7	No.7	9.70	1.84
8	No.8	14.60	2.77
9	No.9	3.03	0.57
10	No.10	3.88	0.73
11	No.11	6.76	1.28
12	No.12	5.07	0.96
13	No.13	9.70	1.84
14	No.14	14.60	2.77
15	No.15	10.68	2.02
16	No.16	6.10	1.15
17	No.17	3.53	0.67
18	No.18	8.13	1.54
19	No.19	1.76	0.33
20	No.20	4.19	0.79
21	No.21	6.10	1.15
22	No.22	2.25	0.42
23	No.23	6.27	1.19
24	No.24	1.37	0.26
25	No.25	3.53	0.67

**ANNEXURE VI**  
**Push out bond strength of sealer to root dentin in Group III-GuttaFlow Bioseal**

<b>Sr. No.</b>	<b>Sample No.</b>	<b>Maximum Load (N)</b>	<b>Pushout Bond Strength (MPa)</b>
1	No.1	2.25	0.42
2	No.2	6.27	1.19
3	No.3	1.37	0.26
4	No.4	3.53	0.67
5	No.5	8.13	1.54
6	No.6	1.76	0.33
7	No.7	4.11	0.78
8	No.8	5.70	1.08
9	No.9	3.03	0.57
10	No.10	3.88	0.73
11	No.11	6.76	1.28
12	No.12	5.07	0.96
13	No.13	9.70	1.84
14	No.14	2.94	0.56
15	No.15	1.76	0.33
16	No.16	4.11	0.78
17	No.17	4.11	0.78
18	No.18	5.70	1.08
19	No.19	8.13	1.54
20	No.20	3.03	0.57
21	No.21	3.88	0.73
22	No.22	2.94	0.56
23	No.23	1.76	0.33
24	No.24	2.25	0.42
25	No.25	6.27	1.19