Volume 6 (2024) | Issue 1| Pages 181-192

**Original Article**

# Evaluation of the Push-Out Bond Strength and Penetration of Guttaflow bioseal, Endoseal MTA and Guttaflow 2 versus AH-Plus Root Canal Sealers into the Dentinal Tubules in Mandibular Premolar Teeth (A Randomized in vitro comparative study)

**Mariam Hesham Eissa[1], Randa El Boghdadi[1], Marwa Bedier[1]**

[1]Department of Endodontics, Faculty of Dentistry, Cairo University.

**Email:** mariam.abdelfattah@dentistry.cu.edu.eg

**Submitted:** 08-2-2023
**Accepted:** 30-4-2023
**Publish Date** : 01-01-2024

## Abstract

**Aim:** To compare between Guttaflow Bioseal, Endoseal MTA, Guttaflow 2 and AH-Plus root canal sealers, regarding the Push-Out Bond Strength and Sealer Penetration into the Dentinal Tubules.
**Methodology:** Eighty Single-canalled mandibular premolars were instrumented using M-Pro-dental file system and irrigated with NaOCl and 17% EDTA, then obturation was performed using single cone technique and 4 distinct sealers. For push-out bond strength, 40 specimens were assigned randomly to one of four groups (n=10) based on the sealer used: Group (I) Guttaflow Bioseal, Group (II) Endoseal MTA, Group (III) Guttaflow 2 and Group (IV) AH-Plus. Specimens were sectioned horizontally into 2-mm slices from the apical, middle, and coronal thirds. For sealer penetration, the remaining 40 roots were allocated into 4 groups (n=10). 5mm sections from the apical segment were scanned using a confocal laser scanning electron microscope (CLSM). Sealer penetration was measured and statistical analysis included the ANOVA test and the Tukey post hoc test was used.
**Results:** AH-Plus showed significantly greater push-out bond strength than the other groups in the apical, middle, and coronal thirds ($p$<0.001). Endoseal MTA demonstrated a maximal penetration depth that was significantly higher than that of the other groups, followed by AH-Plus, which demonstrated a maximal penetration depth that was significantly higher than that of Guttaflow Bioseal and Guttaflow 2 with no difference between the latter two (p>0.05)..
**Conclusion:** AH-Plus showed better push-out bond strength, while Endoseal MTA exhibited better dentinal tubule penetration. The Push-out bond strength showed a significant moderate correlation with the Maximum depth of sealer penetration for all sealers.

**Keywords:** CLSM; Endoseal MTA; Guttaflow Bioseal; Guttaflow 2 ; Push-Out bond strength; Sealer penetration.

## I. INTRODUCTION

The primary target of root canal treatment, is to obturate the root canal system in three dimensions. A fluid-tight seal, prevents apical periodontitis, lessen coronal leakage and bacterial contamination, and encapsulates any leftover irritants in the root canal. Root canal sealers are required to fill the space between the root dentin wall and the obturating material. It is crucial for the sealer to be able to enter the dentinal tubules to establish a fluid-tight seal and prevent the entry of toxins (Al-Haddad & Aziz 2016).

The epoxy resin-based sealer AH-Plus (Dentsply, DeTrey, Konstanz, Germany), has been employed due to its improved apical seal, reduced solubility, enhanced micro-retention to root canal dentin, as well as its low shrinkage and great dimensional stability, but it also exhibits cytotoxic effects (Kim et al. 2019).

Guttaflow 2 (Roeko-ColtenelWhaledent, Langenau, Germany) is a silicone-based root canal sealer also includes gutta-percha powder, microsilver, platinum catalyst, and zirconium dioxide. According to Saygili et al. (2017), Guttaflow 2 has superior biocompatibility than AH-Plus and exhibits a small expansion after mixing, which improves sealing.

Endodontics, has only had access to bioceramic-based sealers for the past thirty years. They consist of alumina, zirconia, bioactive glass, glass ceramics, hydroxyapatite, and calcium phosphates. They are extremely biocompatible, contain calcium phosphate, which improve the setting characteristics and produces materials with a chemical build and crystalline structure resembling those found in bone and tooth apatite, strengthening the sealer and root dentin bond. A major drawback is how difficult it is to remove from the root canal for a subsequent retreatment or post-space preparation, according to Hench (1991), Cherng et al. (2001), Koch & Brave (2009), Al-Haddad &Aziz (2016).

Recently, Guttaflow Bioseal (Coltene/Whaledent AG, Alstatten, Switzerland) was developed. The bioactive ingredients in this sealer are supposed to support tissue regeneration and repair. Studies show that Guttaflow Bioseal works and cures more quickly than Guttaflow 2 and has the advantage of being superior when used as directed by the manufacturer's recommendations (Saygili et al. 2017).

As per our knowledge, no studies in literature evaluated the Push-Out Bond Strength and Sealer Penetration into the Dentinal Tubules of Guttaflow Bioseal, Endoseal MTA, Guttaflow 2 and AH-Plus root canal sealers, thus the target of the study was to compare between them regarding the Push-Out Bond Strength and Sealer Penetration into the Dentinal Tubules. The null hypothesis assumed that there would be no difference among the effects of different groups.

## MATERIALS AND METHODS

### 1) Ethical approval

The ethical committee (EC), Faculty of Dentistry, Cairo University, examined and approved the protocol of this in vitro study in terms of scientific substance and conformity with applicable research.

### 2) Sample size calculation

Based on a previous study by **Donnermeyer et al. (2018)**, the sample size was estimated using the (PS program). Each subject group's response was normally distributed, with a standard deviation of 2.41. If the true difference between the experimental and control means is 3.23, we will need to study 10 experimental sample and 10 control sample to reject the null hypothesis, with power of 80% and error probability of 0.05. The total number of subjects is 40.

### 3) Sample selection

Eighty single-canalled, recently extracted premolar teeth with completely formed apices and oval-shaped canals were gathered. For the push out bond strength test, forty premolar teeth were employed, and forty more were used to evaluate the sealer's penetration of the dentinal tubules. All teeth were inspected, and those that had received previous root canal therapy, root caries, root resorption, or root fractures or cracks were excluded.

### 4) Samples preparation:

A total of 15mm (1mm) of the root length from the apex to the cement-enamel junction was left after all teeth were decoronated at the CEJ level. The working length was next adjusted manually with K-file #15.

**5) Root canals preparation and obturation:**

Following the manufacturer's instructions, the 3 file Dental MPro System (IMD, China) was used to prepare the root canals using the crown-down approach in a continuous rotation motion (MPro opener size 18/taper 0.04, second file MPro size 20/taper 0.04, third file MPro size 25/taper 0.06). Manual files up to size 40 K-file (Mani, Inc., Utsunomiya, Japan) were used for preparation. Master cones were selected according to the last K-file used. Following that, radiograph was performed to confirm proper extension of the master cone (size 40, taper 0.04); and the single cone method of obturation was employed.

**Irrigation protocol during preparation:**

A 30-gauge, side-vented needle (NaviTip; Ultradent South Jordan, UT, US) was used to provide irrigation using 2 ml of (NaOCl) solution between each rotating instrument. Following this, the remaining smear layer was removed with 2 ml of a 17% (EDTA) solution for 1 minute, and any leftover irrigating solutions were flushed away using 10 ml of distilled water. After chemomechanical preparation, all of the canals were dried using size 40 points of sterile absorbent paper (Diadent Group International Inc. in Chongju, Korea).

**A- Push-out Bond strength**

Based on the root canal sealer, 40 samples were randomly allocated into four groups; Group (I) Guttaflow Bioseal, Group (II) Endoseal MTA, Group (III) Guttaflow 2 and Group (IV) AH-Plus. The root canal was entirly filled with the master cone size 40 and sealer as per the manufacturer's recommendations. The excess guttapercha was eliminated with a hot plugger, and then access cavities were sealed with provisional temporary filling. All samples were radiographed to evaluate the quality of the root filling. Samples were then kept in an incubator for two weeks to achieve complete sealer setting.

**Evaluation of Push-out Bond strength:**

Specimen was sectioned horizontally, perpendicular to the tooth's long axis, that were 2.0 ± 0.1 mm thick. Each specimen was fastened to a support jig on the Universal Testing Machine's base with room for the dislodged material. The coronal end of each specimen faced the support jig, while the apical end faced the load cell. The applied stress quickly dropped as the punch fell at a speed of 0.5 mm per minute once the obturation started to protrude from the specimen. A universal testing device was connected to a computer software that calculated the bonding strength. The filling material was subjected to a maximum load in newtons prior to debonding. According to Mishra P et al. (2017), the bond strength was calculated by dividing the load at failure, which is represented in newtons (N), by the interfacial area (MPa).

**B- Sealer Penetration into dentinal tubules**

For evaluating the sealer's penetration into the dentinal tubules, forty samples were divided randomly into four groups based on the root canal sealer; Group (I) Guttaflow Bioseal, Group (II) Endoseal MTA, Group (III) Guttaflow 2 and Group (IV) AH-Plus. In order to add fluorescence to enable Confocal laser scanning microscopy analysis, Rhodamine B (Sigma-Aldrich, St. Louis, MO, USA) was added to the sealer and labeled at a concentration of 0.1%. After coating, the master cone was pushed into the canal to its maximum working length. The excess cone was removed with a hot plugger, and Cavit provisional temporary filling was utilized to temporarily fill and seal the access cavities. All samples were radiographed then teeth were kept in an incubator for two weeks at 37 °C and 100% relative humidity to achieve complete sealer setting.

**Evaluation of Sealer Penetration using CLSM:**

To obtain 2.0 ± 0.1 mm thick section, each test sample was horizontally sectioned, 5 mm from the apex. The samples' coronal surfaces were then oriented toward the laser beam. The samples were examined with a CLSM (LSM 800 with Airyscan; Carl Zeiss Microscopy

GmbH, Jena, Germany). Rhodamine B was set to excite and emit at 558 and 575 nm, respectively. The images were examined using the Image Examiner Software (Carl Zeiss Micro; Imaging GmbH, Jena, Germany). Measurements in square micrometers (m2) were taken along the periphery of the root canal wall and throughout the area where the sealer had penetrated. By dividing the highlighted region with sealer penetration by the circumference of the canal wall, the percentage of sealer covering the root canal wall (in percent) was calculated. The maximum depth of penetration (m) was determined by measuring the distance between the canal wall and the point where the sealer penetrated the deepest. The sealer penetration area was computed by subtracting the quantity of root canal space from the total amount of sealer penetration area, **Gawdat and Bedier (2021)**.

### 6) Statistical Analysis:

All data was collected and analyzed statistically. The mean and standard deviation values were determined for each group in each test. The data indicated a parametric (normal) distribution when the normality of the data was determined using the Shapiro-Wilk and Kolmogorov-Smirnov tests. The repeated measures ANOVA test was used to compare more than two groups in similar samples. The paired sample t-test was used to compare two sets of related samples. One-way ANOVA was used to compare samples with more than two groups from unrelated populations, then Tukey post-hoc test. The Pearson test was used to correlate the maximum depth of sealer penetration with the push out bond strength.

## *II. RESULTS*

A) Push-out bond strength:

Data for the push-out bond strength are shown in Table (1) and Figure (1).

1) Effect of different root canal thirds:

In all sealer groups, the coronal third had the highest value, followed by the middle third, and the apical third had the lowest value.

In Group I (Guttaflow Bioseal), a statistically significant difference existed between (Coronal) and each of (Middle) and (Apical) thirds ($p<0.005$), with no difference between (Middle) and (Apical) thirds, while in Group II (Endoseal MTA) and Group IV (AH Plus), there was a statistically significant difference between (Apical) and each of (Coronal) and (Middle) thirds where ($p<0.005$), with no significant difference in the latter two thirds, and Group III (Guttaflow 2), showed a statistically significant difference between all thirds ($p<0.005$).

2) Effect of different root canal sealers:

In the Coronal third, the highest value was detected in Group IV (AH-Plus), followed by Group II (Endoseal MTA) and Group I ( Guttaflow Bioseal), while the least value was rported in Group III (Guttaflow 2).A statistically significant difference between Group IV (AH-Plus) and each of Group I (Guttaflow Bioseal), Group II ( Endoseal MTA) and Group III ( Guttaflow 2) ($p<0.005$) was detected.

The highest mean value in the Middle and Apical thirds was discovered in Group IV (AH-Plus), followed by Group II (Endoseal MTA), then Group III (Guttaflow 2), and the lowest value in Group I (Guttaflow Bioseal). A statistically significant difference in the middle third, between Group IV (AH-Plus) and each of Group I (Guttaflow Bioseal), Group II ( Endoseal MTA) and Group III ( Guttaflow 2)

**Table (1): Values of push-out bond strength of the different root canal sealer groups at different root canal thirds.**

| Variables | Push-out bond strength | | | | | | |
|---|---|---|---|---|---|---|---|
| | Coronal | | Middle | | Apical | | p-value |
| | Mean | SD | Mean | SD | Mean | SD | |
| Group I (Guttaflow bioseal) | 2.92 bA | 1.05 | 1.27 cB | 0.40 | 1.01 cB | 0.39 | <0.001* |
| Group II (Endoseal MTA) | 3.36 bA | 1.09 | 3.04 bA | 1.13 | 1.87 bB | 0.48 | <0.001* |
| Group III (Guttaflow 2) | 2.77 bA | 1.06 | 2.38 bcB | 0.93 | 1.71 bC | 0.64 | 0.001* |
| Group IV (AH Plus) | 5.51 aA | 1.89 | 5.02 aA | 1.70 | 2.63 aB | 0.74 | <0.001* |
| *p-value* | <0.001* | | <0.001* | | <0.001* | | |

**Means with various capital letters in the same row indicate a significant difference between root sections, while means with different small letters in the same column indicate a significant difference between sealers. *; significant (p<0.05)**

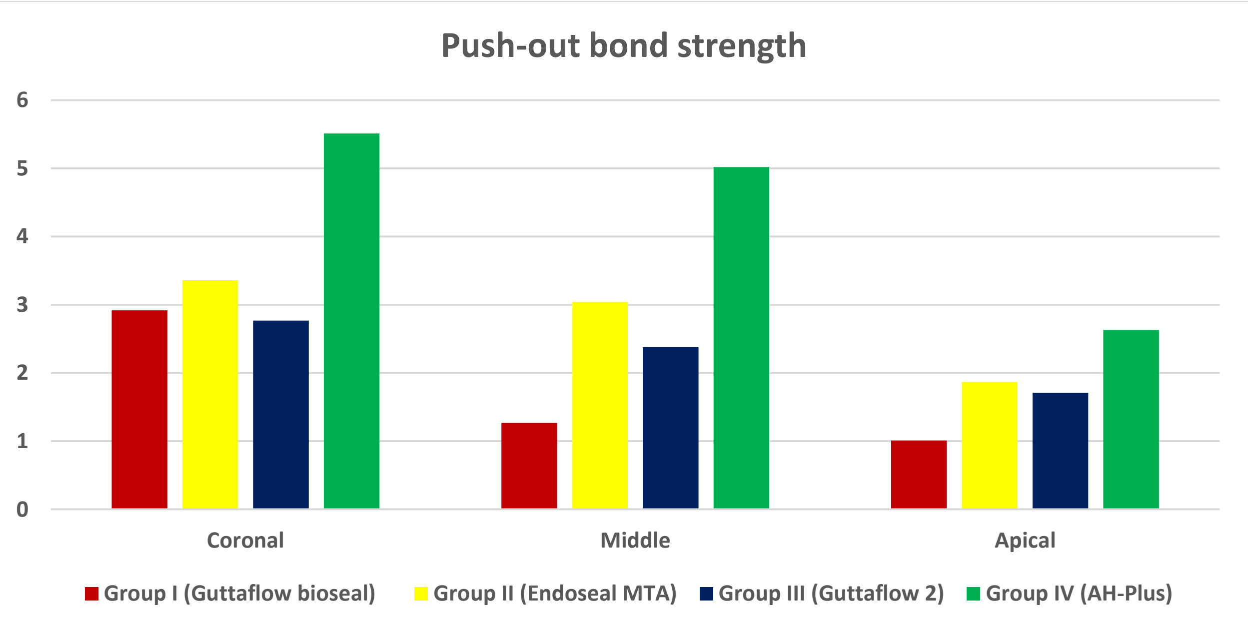


Figure (1): Bar chart representing the push-out bond strength of different sealers at different root canal thirds

Sealer penetration into the Dentinal tubules:
The mean and standard deviation values for sealer prenetration are presented in Table (2,3 and 4 ) and Figure (2).

Percentage of sealer covering the canal wall:

The highest value was observed in Group II ( Endoseal MTA), followed by Group IV (AH-Plus), then Group III (Guttaflow 2), while the lowest value was observed in Group I (Guttaflow Bioseal). A statistically significant difference was reported between Group II (Endoseal MTA) and the three other groups and between Group IV (AH-Plus) and other groups ($p<0.005$) with no statistically significant difference between Group I (Guttaflow Bioseal) and Group III (Guttaflow 2).

**Table (2): Values of Percentage of sealer covering the canal wall of the different root canal sealers.**

| Variables | Percentage of sealer covering the canal wall | |
|---|---|---|
| | Mean | SD |
| **Group I (Guttaflow bioseal)** | 1.40 [c] | 0.07 |
| **Group II (Endoseal MTA)** | 4.01 [a] | 0.37 |
| **Group III (Guttaflow 2)** | 1.49 [c] | 0.08 |
| **Group IV (AH Plus)** | 2.90 [b] | 0.18 |
| ***p-value*** | **<0.001*** | |

1) **The dentinal tubule penetration area:**

Group II (Endoseal MTA) had the highest value, followed by Group IV (AH-Plus), then Group III (Guttaflow 2), and Group I (Guttaflow Bioseal) had the lowest value. A statistically significant difference was reported between Group II (Endoseal MTA) and the three other groups and between Group IV (AH-Plus) and other groups ($p<0.005$) with no statistically significant difference between Group I (Guttaflow Bioseal) and Group III (Guttaflow 2).

**Table (3): Values of the dentinal tubules penetration area for different root canal sealers.**

| Variables | The dentinal tubule penetration area | |
|---|---|---|
| | Mean | SD |
| **Group I (Guttaflow bioseal)** | 1422.21 [c] | 248.62 |
| **Group II (Endoseal MTA)** | 11055.33 [a] | 1151.33 |
| **Group III (Guttaflow 2)** | 2073.63 [c] | 259.14 |
| **Group IV (AH Plus)** | 7179.91 [b] | 1023.91 |
| ***p-value*** | **<0.001*** | |

**1) Maximum depth of sealer penetration:**

Group II (Endoseal MTA) had the highest value, followed by Group IV (AH-Plus), then Group III (Guttaflow 2), and Group I (Guttaflow Bioseal) had the lowest value. There was a statistically significant difference between Group II (Endoseal MTA) and the other three groups ($p<0.005$) and between Group IV (AH-Plus) and other groups ($p<0.005$), with no statistically significant difference between Group I (Guttaflow Bioseal) and Group III (Guttaflow 2).

**Table (4): Values of maximum depth of sealer penetration of the different root canal sealers**

| Variables | Maximum depth of sealer penetration | |
|---|---|---|
| | **Mean** | **SD** |
| **Group I (Guttaflow bioseal)** | 456.81 $^{c}$ | 37.25 |
| **Group II (Endoseal MTA)** | 2117.03 $^{a}$ | 175.43 |
| **Group III (Guttaflow 2)** | 654.00 $^{c}$ | 60.74 |
| **Group IV (AH Plus)** | 1580.85 $^{b}$ | 173.13 |
| ***p-value*** | **<0.001*** | |

**B) Correlations between Push-out bond strength and the maximum depth of sealer penetration for different root canal sealers:**

In terms of correlations between Push-out bond strength and Maximum depth of sealer penetration for all sealers, the Push-out bond strength shown a substantial weak-moderate association with the Maximum depth of sealer penetration. Table (5)

**Table (5): Total Correlations between all root canal sealers and maximum depth of sealer penetration**

| | | Push-out bond strength | Maximum depth of sealer penetration |
|---|---|---|---|
| **Push-out bond strength** | **Pearson Correlation** | 1 | .429$^{**}$ |
| | ***p*-value** | | 0.006 |

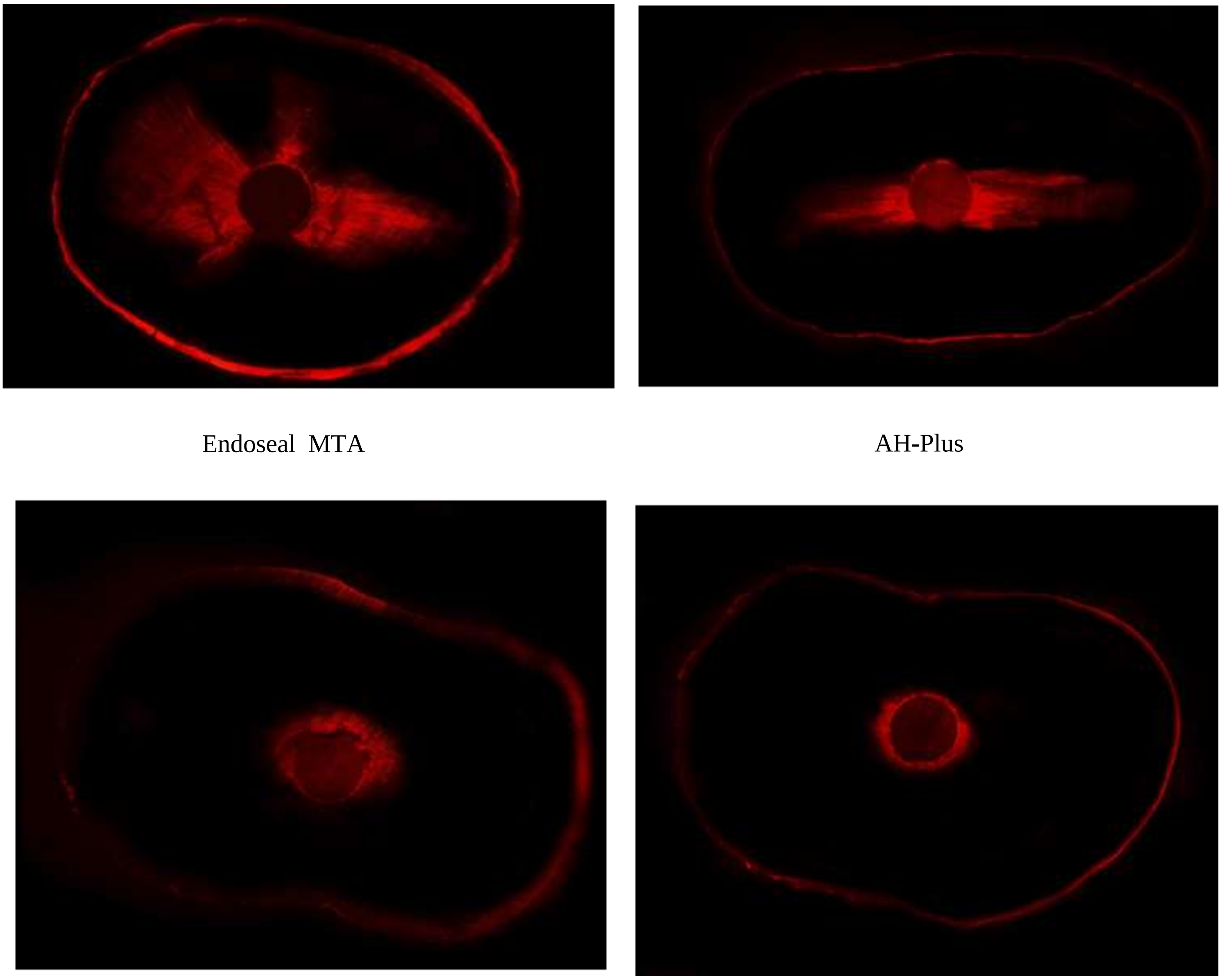


Figure.(2): Representative confocal laser microscopy images showing sealer penetration of the four different sealers

## *III. DISCUSSION*

Endodontic sealers can interact with dentine both chemically and physically. Material penetration inside dentinal tubules causes mechanical retentions, resulting in physical interaction. The chemical interaction is defined by tag production along the sealer-dentine interface, according to Roizenblit et al. (2020). The objective of current study was examine push-out bond strength as well as dentinal tubule penetration since a strong bond between the sealer and the dentine walls does not ensure sealer penetration depth inside the dentinal tubules (Tedesco & colleagues 2019).

High bond strength to dentine is regarded as a critical characteristic of sealer because it helps create a gap-free interface between dentine and root canal filling material, which is essential for creating a fluid tight seal and offering resistance to root filling dislocation during tooth flexure, clinical stresses are simulated by applying parallel load to dentin-sealer interface (chen et al. 2013).

The findings of the push out bond strength at different root thirds revealed a substantial difference between groups in the coronal area. The coronal region of the AH-Plus group had a stronger bond strength than Endoseal MTA, Guttaflow Bioseal, and Guttaflow 2, but there was no statistically significant difference between them. This is consistent with prior research, which found that AH-Plus had a significantly higher bond strength in the coronal portion than Epiphany and Sealer 26 (Carneiro et al. 2012).

It has been hypothesized that histological and anatomical factors, such as the density and diameter of dentinal tubules, the amount of intertubular dentin, and the sealer's failure to sufficiently wet the apical dentin as much as the coronal dentin, affect root dentine bonding. Furthermore, as tubule density decreases from coronal to apical, sealer penetration into the smaller tubule diameter in the apical thirds decreases, and lack of irrigation solution access to the apical region, resulting in incomplete removal of the smear layer, may reduce sealer penetration into dentinal tubules and thus affect adhesion in the apical region (Marshall et al. 1997, Mjör et al. 2001, Collares et al.2015). Furthermore, vapour lock-effect in the apical area may avert irrigant and sealer penetration depth (Topçuoğlu et al. 2013).

The bond strength of the AH-Plus sealer was significantly higher than that of Endoseal MTA, Guttaflow 2, and Guttaflow bioseal. This was consistent with the findings of prior studies by Eid et al. (2021). Dem et al. (2019) also noted that AH-Plus had a better bonding strength than Guttaflow Bioseal and Guttaflow 2. Silva et al. (2016) discovered that AH-Plus had significantly stronger bond strength than Endoseal MTA and MTA Fillapex.

According to De-Deus et al. (2009), the improved adhesiveness of AH-Plus may be due to the formation of covalent bond between the exposed amino groups in the collagen network and an open epoxide ring. Furthermore, the sealer's large creep range and extended setting time increased mechanical adherence and resistance to displacement and/or removal from dentine. (Nunes et al. 2008). Furthermore, Lee et al. (2002) and Neelakantan et al. (2011) revealed that low polymerization stress, long-term dimensional stability, and effective intermolecular cohesion all boosted AH-Plus root dentin micro retention.

Dentinal tubule penetration by a sealant, on the other hand, establishes a physical barrier, improves root filling retention, and encapsulates residual microorganisms, according to Nunes et al. (2008). Kok et al. 2014 and Wang et al. 2018 employed confocal laser scanning microscopes to investigate the depth of intratubular sealer penetration in this work. One slice was cut from each tooth at a depth of 5 mm from the apical root segment and a thickness of around 2 0.1 mm (Kok et al. 2012, Jardine et al. 2016 and Gawdat & Bedier 2021), and analyzed under CLSM at 10x with a wavelength of 560-600 nm.

The percentage of sealer covering the canal wall, dentinal tubule penetration area, and maximum depth of sealer penetration were measured. The results showed that Endoseal MTA had a substantially larger dentinal tubule penetration area and of maximum depth of penetration of the sealer area then the other three sealers tested. Other investigations have identified Endoseal MTA to have remarkable flowability, connected to material penetration into dentinal tubules, anatomical abnormalities, or auxiliary canals, boosting sealing ability and bond strength, according to Khatib et al. (2019).

The results of this investigation demonstrated a weak-moderate Pearson connection between numerous metrics for all the groups, including push out bond strength and sealer penetration depth into dentinal tubules. This was in consistence with De-Deus et al. (2012), who reported no connection between sealer penetration into dentinal tubules and sealing ability, Tedesco et al. (2019) where no significant correlation between bond strength and intratubular penetration of the tested sealers was reported, Deniz Sungur et al. (2016), Who detected Dentinal tubule penetration has limited effect on bond strength and

Oksan et al. (1993) demonstrated that the micromechanical retention caused by sealer tag penetration into the tubules may not have a significant impact on the ability of root canal sealers to adhere.

According to the findings of various investigations, the adhesiveness of sealants to dentin walls cannot be explained only by the extent to which they penetrate dentinal tubules. The process of adhesion in the dentin of the root canal is complicated because it is dependent not only on the chemical and physical interactions between the dentinal walls and the molecules in the sealers, but also on the frictional resistance of the filling material at the root canal's surrounding walls (Fisher et al. 2007, Tedesco et al. 2014).

Within the constraints of this investigation, it is possible to conclude that the AH-Plus sealer displayed higher Push-out bond strength than the other three sealers, with the strongest in the coronal third, followed by the middle third, and the lowest in the apical third. Furthermore, the Endoseal MTA sealer penetrated dentinal tubules better than the other three sealers. Thus, the maximum depth of sealer penetration was shown to have a weak-moderate connection with push-out bond strength.

**Conflict of Interest:**

The authors declare no conflict of interest.

**Funding:**

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors

**Ethics:**

This study protocol was approved by the ethical committee of the faculty of dentistry-Cairo university on: 28-07-2020, approval number: 25720

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