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To Compare And Evaluate The Sealing Ability Of GuttaFlow Bioseal, BioRoot RCS And MTA Fillapex With AH Plus: An In Vitro Study.

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Abstract:
Background:*A successful endodontic treatment requires thorough debridement of the canal space and a 3-dimensional obturation. This is facilitated by placement of a good obturating material and a sealerto prevent ingress or survival of bacteria. The aim of this study was to determine the depth of penetration of GuttaFlow Bioseal, BioRoot RCS, MTA Fillapex and AH Plus into the radicular dentine using Confocal Laser Scanning Microscope.*
Materials and Methods:*64 teeth with single canals were decoronated and instrumented with ProTaper system (Dentsply, Maillefer, Ballaigues, Switzerland) until a F5 (50/05) instrument. 2.5% sodium hypochlorite and 17% EDTA were used for 3 minutes to irrigate the canals and were then washed with 3 ml of distilled water.The samples were divived 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 CLSM.*
Results:*BioRoot RCS showed the best depth of penetration into the radicular dentine amongst the four sealers with a mean value of 1369.096 ± 189.590 µm.*
Conclusion:*With the limitations of this study, it was proved that BioRoot RCS has superior sealing properties and higher depth of penetration into the dentinal tubules.*
Key words: *BioRoot RCS, GuttaFlow Bioseal, MTA Fillapex, AH Plus, sealer, Confocal Laser Scanning Microscope, depth of penetration.*

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I. Introduction

A successful root canal treatment aims at thoroughly debriding the root canal system by elimination of pathogenic organisms and sealing the root canal space using gutta percha points. For a fluid tight seal to be present, root canal sealers should be placed in between the dentine and gutta percha to prevent ingress of bacteria from the oral environment. It should be able to create an accurate 3-dimensional obturation throughout the length of the endodontic space. [1]

Root canal sealers fill the root canal system; entomb the remaining bacteria; and favor periapical healing. They are flowable and hence, fill the spaces that cannot be reached by the gutta-percha. This prevents leakage and avoids the entrance of inflammatory exudates, bacteria, saliva, and chemical fluids inside the canal (Ersahan and Aydin, 2013). [2] An important parameter in assessing each new sealer is evaluating its depth of penetration into the radicular dentine. [3] This increases the interface between material and dentin thus improving the sealing ability and retention of the material by mechanical locking. [4]

The sealers that are now available are classified according to their chemical components as: Zinc oxide eugenol sealers, sealers containing calcium hydroxide, and resin based, glass ionomer based, silicone based, and bioceramic sealers.[5] Several microscopy techniques are available to evaluate the sealer/dentin interface, including stereomicroscopy, scanning electron microscopy, transmission electron microscopy, and confocal laser scanning microscopy (CLSM).In this study, GuttaFlowBioseal (Coltene/Whaledent AG, Altstatten, Switzerland), BioRoot RCS (Septodont, Saint-Maur-des-Fosses, France), MTA Fillapex (Angelus, Londrina, Brazil) and AH Plus (Dentsply International), have been used. They were evaluated using confocal laser scanning microscopy.

II. Materials and Methods

Inclusion Criteria

- Human teeth with single canals.

Exclusion Criteria

- Grossly decayed teeth.
- Teeth with root caries.
- Teeth with anomalies.
- Teeth with immature apices.

Procedure:

64 teeth with single canals were first decoronated to standardize the root length to 15 mm. The root canals were then instrumented with ProTaper system (Dentsply, Maillefer, Ballaigues, Switzerland) until a F5 (50/05) instrument. The working length was established with a #10-K file at 1 mm from the apical foramen. A volume of 1ml of 2.5% sodium hypochlorite (NaOCl) was used to irrigate the canal after the use of each instrument. After irrigation, the canals were activated with endo-activator to effectively remove the smear layer. Next, the canals were irrigated with 3 ml of 2.5% NaOCl and 17% EDTA for 3 minutes and washed with 3 ml of distilled water. Finally, the canals were dried with paper points and the specimens were randomly divided into four groups.

Group A: Root canals sealed with GuttaFlowBioseal and filled with gutta-percha cone.
Group B: Root canals sealed with BioRoot RCS and filled with gutta-percha cone.
Group C: Root canals sealed with MTA Fillapex and filled with gutta-percha cone.
Group D: Root canals sealed with AH Plus and filled with gutta-percha cone.

The sealers were mixed with Rhodamine-B dye to allow visualization on a confocal laser-scanning microscope (CLSM). Next, the canals were filled using single cone technique. The access cavities were sealed with composite resin (Tetric n ceram). The teeth were maintained at 100% humidity for 7 days at 37°C to allow the sealer to completely set. After setting, all samples were sectioned at 3 mm from the apex and assessed by confocal laser-scanning microscope.

Confocal laser scanning microscopy:

2mm thick samples were submitted to confocal laser scanning microscopy under ×10 magnification. The microscope used was LEICA microsystems DMi8, Germany. Argon laser with an intensity of 545-609 nm was used. The greatest depth of penetration the root canal sealer was recorded for each sample in that respective group using a linear measurement. The canal wall served as the starting point and sealer penetration into dentinal tubules was measured to a maximum depth for each tooth section of each group and the values were recorded.

Statistical Analysis

The statistical parameters such as mean and SD of the depth of penetration of each sealer were obtained for each group as shown in Table 1. The mean depth of penetration for BioRoot RCS group was the highest 1369.096 ± 189.590 µm, followed by GuttaFlowBioseal group 1026.615 ± 128.028 µm and AH Plus group 835.232 ± 131.085 µm while that of MTA Fillapex group was the lowest 338.973 ± 52.004 µm.

Analysis of variants was highly significant with the $P=0.000 \leq 0.001$.Further Post hoc or Pairwise analysis by TUKEY HSD test was carried out as shown in Table 2.

DEPTH OF PENETRATION OF SEALER INTO DENTINE **(Table 1)**

GROUPS	N[a]	Mean	Std. Deviation	Minimum	Maximum
BIOROOT RCS	16	1369.096	189.590	1084.650	1664.830
GUTTAFLOW BIOSEAL	16	1026.615	128.028	808.960	1256.130
AH PLUS	16	835.232	131.085	622.650	1076.490
MTA FILLAPEX	16	338.973	52.004	241.560	421.520

PAIRWISE ANALYSIS OF DIFFERENT GROUPS **(Table 2)**

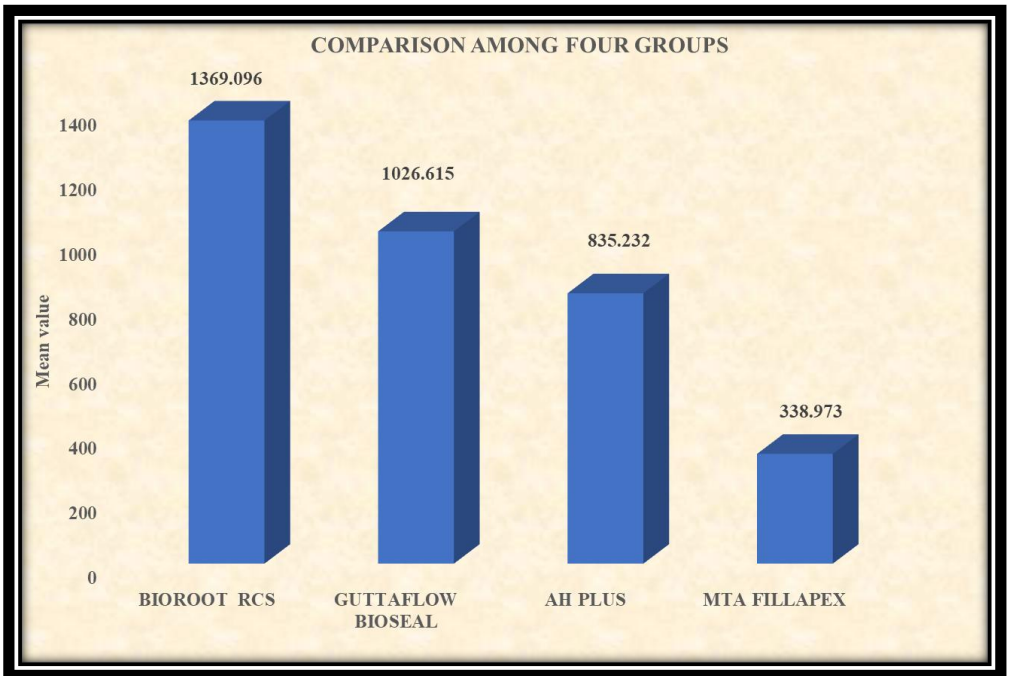
Multiple Comparisons
Dependent Variable: VALUE1
Tukey HSD

I) Group	(J) Group	Mean Difference (I-J)	P
BIOROOT RCS	GUTTAFLOW BIOSEAL	342.481	.000
	AH PLUS	533.864	.000
	MTA FILLAPEX	1030.123	.000
GUTTAFLOW BIOSEAL	AH PLUS	191.383	.001
	MTA FILLAPEX	687.642	.000
AH PLUS	MTA FILLAPEX	496.259	.000

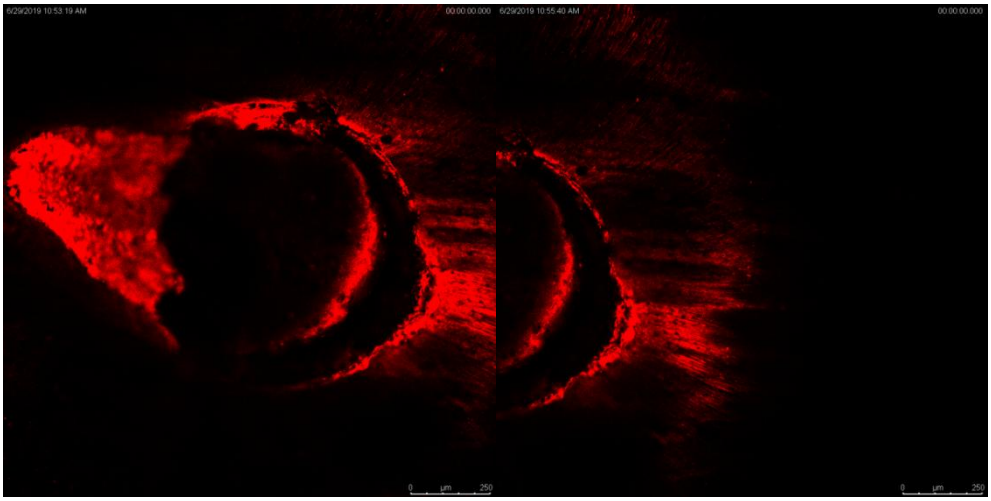
III. Result

In the present study, the depth of penetration of sealer into the dentinal tubules was considered as an ideal requirement of a good sealer. AH Plus showed a mean depth of penetration of 835.232 ± 131.085 µm. This sealer has been considered as a gold standard throughout literature and in the present study.BioRoot RCS showed the best dentinal penetration amongst all the other sealers with a mean value of 1369.096 ± 189.590 µm. The depth of penetration of GuttaFlowBioseal sealer was slightly better than AH Plus sealer with a mean value of 1026.615 ± 128.028 µm and had a slightly lower value than BioRoot RCS sealer. For MTA Fillapex, the mean depth of penetration was 338.973 ± 52.004 µm, which was the least amongst all sealers.

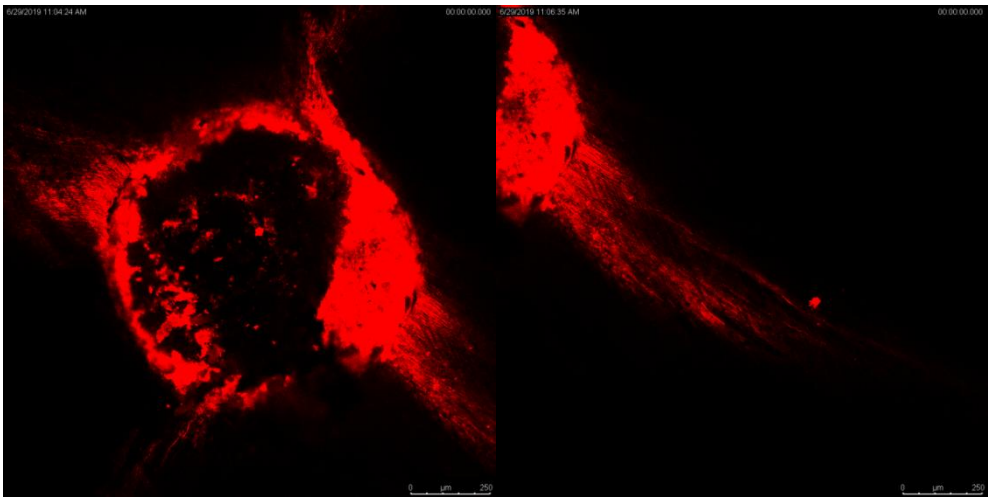
BAR DIAGRAM SHOWING A COMPARISON AMONGST THE FOUR GROUPS



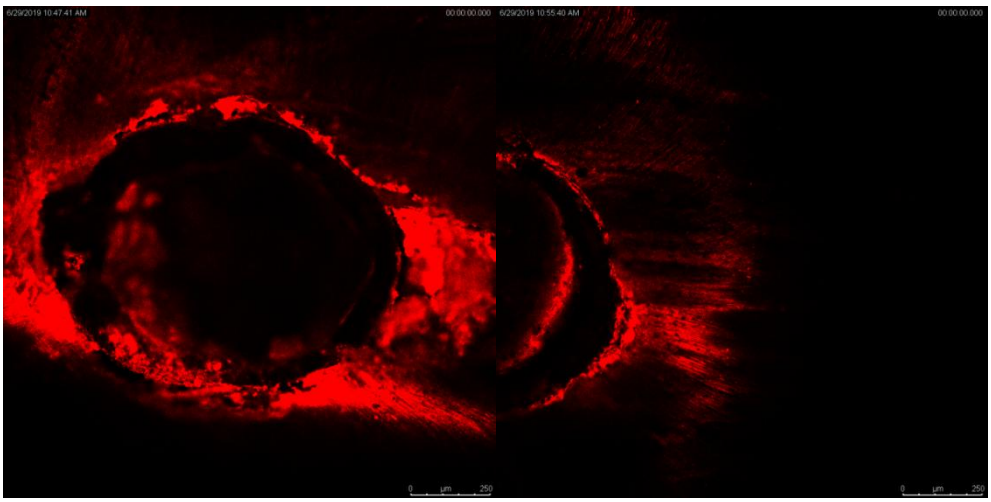
CONFOCAL LASER SCANNING IMAGES FOR BIOROOT RCS



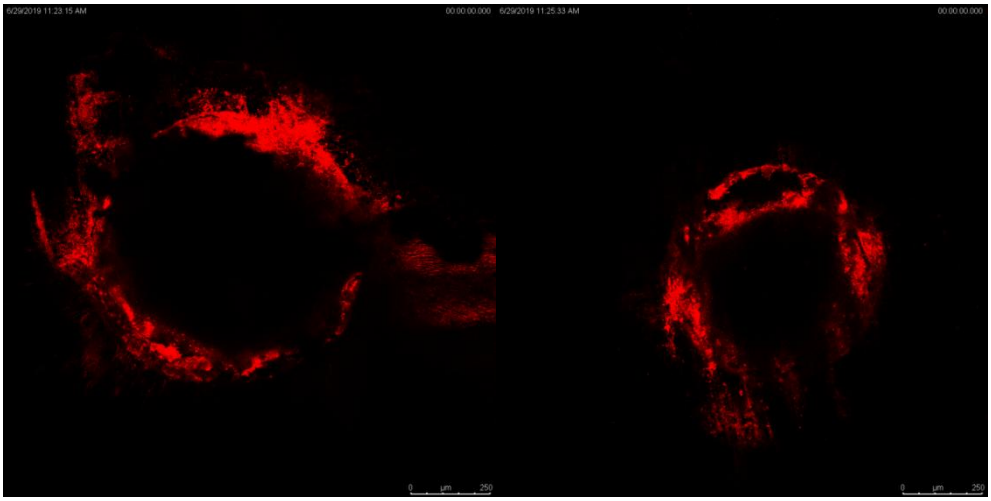
CONFOCAL LASER SCANNING IMAGES FOR AH PLUS



CONFOCAL LASER SCANNING IMAGES FOR GUTTAFLOW BIOSEAL



CONFOCAL LASER SCANNING IMAGES FOR MTA FILLAPEX



IV. Discussion

The major goal of a successful root canal filling is to prevent any exchange between the oral cavity, the root canal system, and the peri radicular tissues, providing a barrier to the existing canal infection and any possible reinfection by the effective usage of gutta-percha and sealers.[4]

The most common failure of the root canal obturation is the presence of gaps and porosities at the sealer-dentin interface. This can lead to re-colonization of microorganisms leading to failure of the endodontic treatment. Gutta percha has commonly been used as the material of choice for obturation. It possesses many properties such as biocompatibility, chemical stability, radiopacity, non-porosity & the ability to be manipulated. However, Gutta percha does not bond to the internal tooth structure due to its hydrophobic nature, resulting in an incomplete seal with the root canal. Therefore, a good sealer with an excellent sealing ability is important to combat the drawbacks of Gutta Percha. [6]

AH Plus sealer has been considered a gold standard reference while considering desirable physicochemical properties of a sealer for root canal filling.This sealer when used with Gutta percha has gained popularity due to its biocompatibility, availability, radiopacity and ease of use. AH Plus is an epoxy bis phenol resin-based sealer. It contains adamantine and has good adhesion to the root canal wall. It contains calcium tungstate and zirconium oxide in its composition and this association produces superior radiopacity. [5] But a study done by Pawar et al showed that there was inadequate bonding between the sealer and the gutta-percha point, allowing leakage at this interface. Since it contains resin and has faster setting time, AH plus tends to shrink and cause early debonding from the root canal. [7] In this study, AH Plus showed to have satisfactory penetration with a mean of 835.232 µm.

MTA Fillapex is a calcium silicate-based sealer marketed in 2011. It is a salicylate resin-based sealer containing calcium silicate particles (mineral trioxide aggregate, MTA) and silicon dioxide. [1]According to a study by Torabinejad M et al (2010), relatively high levels of biocompatibility, antimicrobial activity and sealing ability were reported for this material. [8] The pH of MTA Fillapex is high. These cements are rich in calcium ions which are converted to calcium hydroxide upon contact with water, which then dissociates into calcium and hydroxyl ions, thereby increasing the pH of the solution. A high pH activates alkaline phosphatase which plays an active role in the mineralization process, and neutralizes the acids secreted by osteoclasts, preventing further destruction of mineralized tissue. [5]

In the present study, the depth of penetration of MTA Fillapex was the least at 338.973 µm, when compared to AH plus and showed significantly less penetration than BioRoot RCS sealer.Orstavik et al (2001) [2] reported that during the setting reaction, sealers containing salicylate in its composition showed initial volumetric shrinkage which caused an overall contraction of the sealer which could also be a contributing factor in the present study for minimal sealing of the dentinal walls by MTA Fillapex.

Recently a new class of bio-ceramic material has been introduced in the market, BioRoot RCS.This tricalcium based sealer has a property of reacting with the tissue fluids when in contact with it and reduces the chance of micro leakage by the formation of calcium hydroxide at the sealer and dentin interface (Camilleri 2015). It leaches high levels of calcium which is twice that of EndoSequence BC Sealer. This was observed in a study conducted by Xuereb et al (2015).[3]These calcium ions form a calcium phosphate phase which leads to the formation of a mineral infiltration zone between the sealer and the root canal dentin, on contact with the

physiological fluids. This zone helps in the bio mineralization activity of the sealer. Thus, this sealer exhibits its higher sealing property.[9] Also, a higher fracture resistance was observed in BioRoot RCS which may be explained by the interaction of this sealer with root canal dentinal walls with regard to its bio-mineralization activity. This property may have the potential to reinforce the instrumented root canals against vertical root fracture. [10]

BioRoot RCS is highly biocompatible and has less cytotoxicity than other materials as proved by Jean Camps et al (2015). The study proved that BioRoot RCS induces angiogenic and osteogenic growth factors secretion. The results of this study show that the viability of PDL cells after direct contact with BioRoot RCS and their capacity to secrete significant levels of osteogenic and angiogenic factors such as BMP-2, VEGF, and FGF-2 show that BioRoot RCS has bioactive properties.[11] Another study was conducted by Susanne Jung et al (2018) where the PDL cell viability was analyzed by living-cell-count, MTT assay, and living/dead-staining, cytotoxicity by LDH-assay.In a study conducted by HemalathaParanthaman and PriyadharshiniTheetharappan (2019), it was seen that Bioroot RCS group possessed greater sealing ability when compared to Guttaflow II and AH Plus. [9] The findings of the present study are consistent with the study by Viapiana et al where the author compared the sealing ability of Bioroot RCS and AH Plus and concluded that Bioroot RCS showed a better sealing ability. [3]

GuttaFlowBioseal is a novel formulation of polydimethylsiloxane-gutta percha doped with calcium silicate particles.[1]It is a silicone-based endodontic sealer. It has both osteointegrative and osteoconductive effects and it is said to bond mechanically to bone tissue through the formation of hydroxyapatite crystals. [12] Very few studies have been conducted to assess the dentinal tubule penetration of this sealer. Studies by Pereira *et al* and Akcay et al (2016) have been conducted to determine the penetration of GuttaFlowBioseal into the dentinal tubules using confocal laser scanning microscopy. They showed that GuttaFlowBioseal has similar dentinal tubule penetration to that of MTA Fillapex and AHPlus.[12] This finding is true of the present study as well where the dentinal penetration of GuttaFlowBioseal is similar toAH plus.

In a study by Gandolfi MG et al (2016) it was observed that GuttaFlowbioseal showed high water sorption and porosity but low solubility. It also showed the ability to nucleate deposits of hydroxyapatite which enhances its bio-mineralization properties.[1]As there have been very few references in literature regarding the properties of this material, future studies should be conducted to assess whether the bioactive glass in GuttaFlowBioseal has a positive effect on bone tissue as well. In the present study, the depth of penetration of GuttaFlowBiosealsealer was slightly better than AH Plus sealer with a mean value of 1026.615 µm making it slightly lower than BioRoot RCS sealer which was at 1369.096 ± 189.590 µm. Nevertheless, GuttaFlowBiosealhas the potential to produce ideal sealing between root canal wall and the obturation material.

The present study was evaluated using CLSM. Confocal Laser Scanning Microscope offers several advantages like the use of non-decalcified or hard tissue samples that do not require a specific section technique (sputter coating). It also provides detailed information about the presence and distribution of sealers at relative low magnification through the use of fluorescent Rhodamine-marked sealers and allows the exclusion of artifacts from the sample.[4] It does not require special specimen processing, and observations can be made under near normal conditions.[13] The CLSM used in the present study was the LEICA Microsystems DMi8, Germany. The scanning speed was 200Hz. All the images were analyzed using the LEICA Application Suite X (LAS X) software.

V. Conclusion

Within the limitations of the present study, the sealing ability of all four sealers was determined using Confocal Laser Scanning Microscope and visualized using a Rhodamine B dye. The extent of sealer penetration into the dentine was measured which concluded that BioRoot RCS besides being biocompatible also has excellent sealing properties making it an ideal endodontic sealer followed closely by GuttaFlowBioseal.

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Conflict of interest : Nil

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